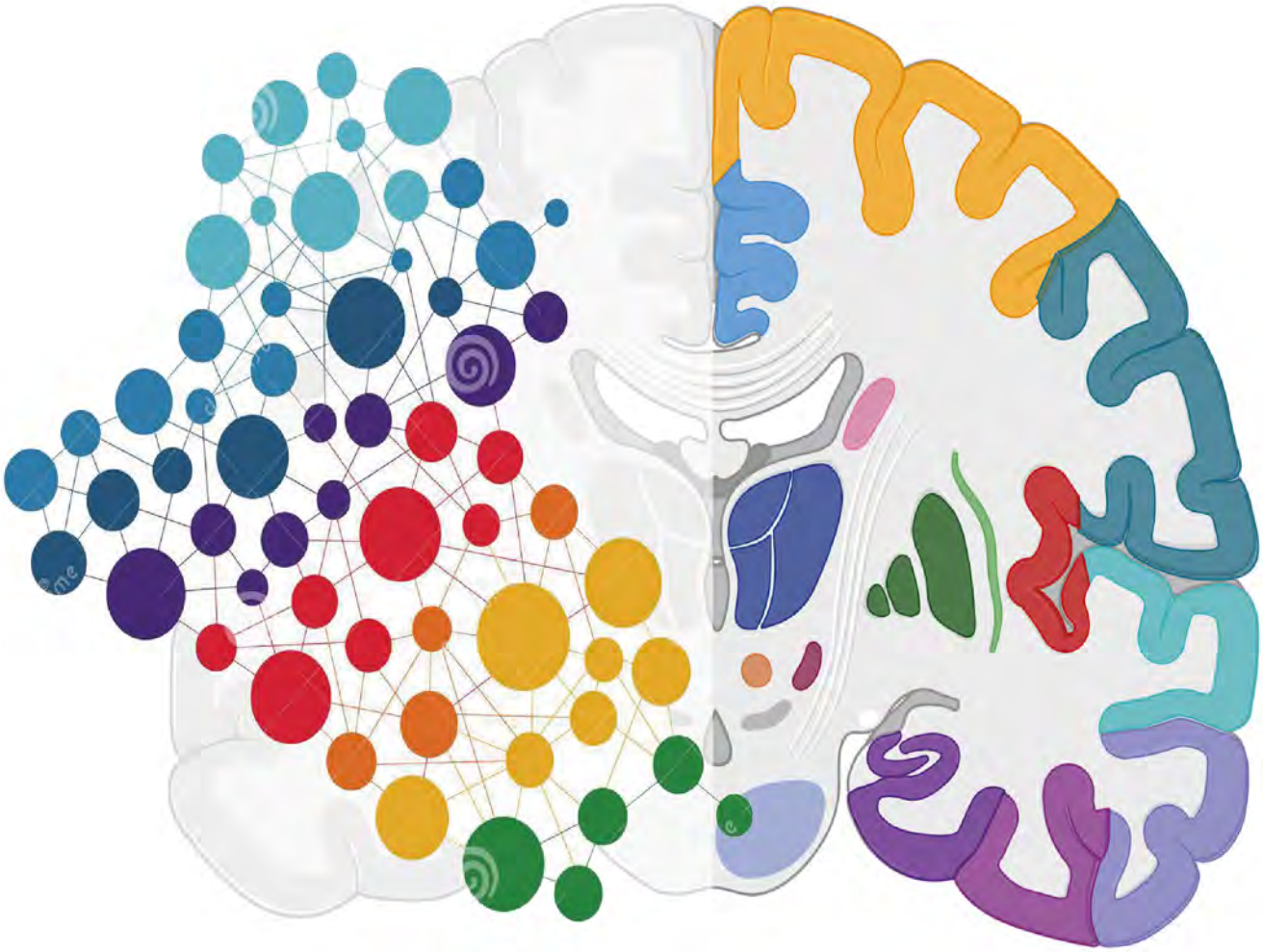
Vol. 7 No. 4 2022
ISSN:1390-9347

Bionatura

Latin American journal of Biotechnology and Life Sciences



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La Revista Bionatura publica trimestral en español o inglés trabajos inéditos de investigaciones básicas y aplicadas en el campo de la Biotecnología, la Inmunología, la Bioquímica, Ensayos Clínicos y otras disciplinas afines a las ciencias biologícas, dirigidas a la obtención de nuevos conocimientos, evaluación y desarrollo de nuevas tecnologías, productos y procedimientos de trabajo con un impacto a nivel mundial.

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Bionatura

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ISSN: 1390-9347 (Versión impresa)
Formato: 21 x 29,7 cm
ISSN: 1390-9355 (Versión electrónica)
Sitio web: **http://www.revistabionatura.com**

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ÍNDICE / INDEX

EDITORIAL

The 2nd International blended Virtual Conference of Agricultural Sciences

Hassan Hadi Mustafa

DOI. 10.21931/RB/2022.07.04.1

Dean of College of the Agriculture /University of Diyala.Iraq
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Diyala University is an Iraqi public university located in the city of Baquba, the center of Diyala Governorate, northeast of Baghdad's capital. The scientific and civilized march of the province.

The establishment of the College of Agriculture at the University of Diyala was an urgent necessity due to the great potential of the Diyala governorate in the agricultural sector and the vast fertile lands. It is an agricultural governorate with distinction, and its orchards are famous for citrus fruits, as they were called Baquba, the city of oranges. It is also characterized by rare types of dates and citrus fruits. Livestock in Diyala Governorate has the largest share in the agricultural sector, as it is one of the essential economic pillars; where there are three large cow stations and many poultry breeding halls, whether for the production of table eggs or white meat, where Diyala Governorate came first after Baghdad, the number of halls and hatcheries in Iraq.

After the success of the First International Scientific Conference on Agricultural Research, which was held under the logo(The Role of Scientific Research in the Prosperity of Agricultural Reality in Iraq and the World) on December 25-26, 2019, we had to prepare for the Second Virtual International Conference on Agricultural Research.

The 2nd International blended Virtual Conference of Agricultural Sciences is a prestigious event organized by the College of Agriculture, University of Diyala, with a motivation to provide an excellent international platform for academicians, researchers, engineers, industrial participants and budding students around the world to share their research findings with the global experts.

IBVCAS (International blended Virtual Conference of Agricultural Sciences) was held basically via physical attendance for two days, August 17 -18, 2022, at the University of Diyala. Only researchers who were unable to travel restrictions, particularly the international participants, were allowed to participate virtually via Free Conference Call (ZOOM) platform. Each participant was given approximately 10 minutes to view the most important findings of their research and 5 minutes to discuss these results.

The 2nd International Virtual Conference of Agricultural Sciences (IBVCAS 2022)Finding practical solutions to agricultural problems, improving the management and productivity of farms and Supporting the Iraqi national product in raising international quality standards Enhancing researchers to raise the quality of scientific research that serves the local agricultural sector to achieve the food security in

Figure 1. Iraqi maps Administrative map of Diyala Governorate.

Citation: Henao-Ramírez, AM.; Palacio- Hajduk, DH.; Urrea-Trujillo, AI. Cost Analysis of Cacao (Theobroma cacao L.) Plant Propagation through the Somatic Embryogenesis Method. Revis Bionatura 2022;7(2) 2. http://dx.doi.org/10.21931/RB/2022.07.02.2
Received: 14 July 2021 / **Accepted:** 10 December 2021 / **Published:** 15 May 2022

Figure 2. Agriculture in Diyala governoratey.

Iraq Providing an opportunity to exchange ideas and experience among researchers, academics and production sectors, well as the public sectors who are engaged in efforts to under set and the agricultural sciences in fields of Animal Science and Zoology, Agronomy, Soil Science and Water Resources, Horticulture Science, Agricultural Economy, Ecology, Food Science.

This event offers the chance for a world gathering of scientists, practitioners, engineers, industrial participants, and educators from totally different professional views who share common interests to explore and become aware of the developments in these fields. We are delighted to have over 48 participants from all over the world join the IBV-CAS conference, share their research findings, and enlighten their new ideas to make this event grow from strength to strength. We believe that this conference outcome will contribute significantly to the knowledge base in these up-to-date scientific fields.

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2. University of Diyala
3. College of Agriculture

ARTICLE / INVESTIGACIÓN

Determination of IFN-y in Patients with *Pseudomonas aeruginosa*-Inflicted Burn and Wound

Mohammed A. Jwad[1], Wasan A. Gharbi[2]

DOI. 10.21931/RB/2022.07.04.2

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Abstract: The sample collection was carried out from 1/12/2021 to 1/4/2022 at the burns hospital, Baghdad teaching hospital in Baghdad city, and Alhussain teaching hospital in Almuthana city. Samples were collected from pa-tients' sera collection samples from (69) patients, from burn patients 53 and 16 samples from wound patients infected with pseudomonas aeruginosa; 41 patients were males, and 28 were females. This group used the oth-er 69 patients that were not infected by bacteria as a control group. The level of IFN-y was investigated by ELISA assay in the teaching Laboratories in al Sawawah city. The results showed a decrease in IFN-y levels were 35.2±2.6 for burn patients and 35.4±2.3 for wound patients compared to the control group of 46±2.5. The current study reported a highly significant difference in IFN-y levels between the burn and wound patients and the control group (P<0.0001).

Key words: *Pseudomonas aeruginosa*, Burn, wound, Interferon – Gamma.

Introduction

Pseudomonas aeruginosa is a common pathogenic germ that can cause significant oppor-tunistic infections, especially in immunocompromised people. The spread of this organism in healthcare facilities is highly harmful, as it infiltrates the human host's primary defensive line and enters the body through the skin, resulting in nosocomial infections, particularly in hospital inten-sive care units (ICUs). Due to the availability of various mechanisms of natural resistance to most antibiotics, the pathogenesis of *P.aeruginosa* is multifactorial, resulting in the creation of a diverse set of cellular structures and extracellular chemicals that play a crucial role in increasing pathogenicity[1]. A rod-shaped GN bacteria *P.aeruginosa* is a common cause for hospital-acquired illnesses. While HPV usually does not affect healthy people, it can colonize any part of the hu-man body that has enough moisture to form a niche. *P. aeruginosa* is responsible for roughly 8-10% of all healthcare-associated infections in the United States (51,000 cases in 2013). Burns is one of the most common types of trauma destruction[2].

Burns compromise skin integrity and the skin's immune system, which protects against pathogenic organisms' activity[3]. Nosocomial infection is a significant concern for burn patients. Infection is a primary source of morbidity and mortality in hospital burn patients. Because of their weakened state and the nature of the damage, nosocomial infection is more common in burn patients[4]. Wound, by definition, breaks in skin epithelial integrity and may cause further dis-ruption in skin anatomy, physiology, and functions. There are two types of wounds known as acute and chronic[5]. In response to harmful agents such as bacteria, tumor cells, viruses, and par-asites, vertebral cells produce interferon-gamma (IFN-y). IFN-y is a critical cytokine in adaptive and innate immunity to intracellular microorganisms. IFN-y increases T and B cell development, activates macrophage microbicidal activity, and boosts cytotoxic T cells[4].

Materials and methods

Species study

The present work includes 138 the collection of serum from (53) burn patients and (16) wound patients that infection with pseudomonas aeruginosa; 41 patients were males, and 28 were fe-males; the other 69 patients were not used patients not infected by bacteria as a control group. The samples were collected from burns hospitals in Baghdad and Almuthanna city from 1/12/2021 to 1/4/2022. These samples were used to investigate the IFN-y in the patients and control group. The sera were collected, brought to the teaching laboratories in Almuthanna and tested. This study used the IFN-y kits to perform the assay using the ELISA instrument. The company that accoutered the kits was Shanghai YL Biont / China.

Sample Collection and Storage

10mls of venous blood was carefully drawn into appropriate sample bottles and spun to separate the serum. The serum was separated into a plain sterile sample bottle and stored at -20o C for analysis.

Data Management and Analysis

The study was designed by a Completely randomized design (CRD) that was used in the analysis of variance for data of gamma interferon values by using a one-way ANOVA test, independent t-test, and Dunnett's test at a 5% level

Citation: Jwad M A , Gharbi W A. Determination of IFN-y in Patients with Pseudomonas aeruginosa-Inflicted Burn and Wound. Revis Bionatura 2022;7(4) 2. http://dx.doi.org/10.21931/RB/2022.07.04.2
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

of significance. Moreover, All frequency data were analyzed by Pearson's chi-squared test and Fisher's exact test. Data were processed and analyzed by using statistical program social science (SPSS 22), and the results were expressed as Mean±SD or percentages[6].

Laboratory Procedure

In vitro test for the quantitative determination of ELISA serum gamma interferon . The blood was drawn through a syringe, added to a gel tube, then transferred to a centrifuge to separate the blood components from the serum, and examined in a machine on ELISA system and Elisys uno Germany gamma interferon Test.

Assay procedure

Standard solutions: (This kit has a standard original concentration, which could be diluted in small tubes by the user independently following the instruction.):

The number of stripes needed is determined by the tested samples and standards. It is suggested that each standard solution and each blank well should be arranged with three or more wells as much as possible.

Sample injection

Blank well: Add only Chromogen solutions A and B, and stop the solution.

Standard solution well: Add 50µl standard and streptavidin-HRP 50µl. 3) Sample well to be tested: Add 40µl sample and then 10µl IFN-GAMMA antibodies, 50µl streptavidin-HRP. Then cover it with a seal plate membrane. Shake gently to mix them up. Incubate at 37°C for 60 minutes.

Preparation of washing solution: Dilute the washing concentration (30X) with distilled water for later use.

Washing: Remove the seal plate membrane carefully, and drain and shake off the remaining liquid. Fill each well with washing solution. Drain the liquid after 30 seconds of standing. Then repeat this procedure five times and blot the plate.

Color development: Add 50µl chromogen solution A to each well and then add 50µl chromogen solution B to each well. Shake gently to mix them up. Incubate for 10 minutes at 37°C away from light for color development.

Stop: Add 50µl Stop Solution to each well to stop the reaction (the blue color changes into yellow immediately at that moment).

Assay: Take a blank well as zero, and measure the absorbance (OD) of each well one by one under 450nm wavelength, which should be carried out within 10 minutes after having added the stop solution.

According to standards' concentrations and the corresponding OD values, calculate the linear regression equation of the standard curve. Then according to the OD value of samples, calculate the concentration of the corresponding sample. Special software could be employed to calculate as well.

Results and discussion

Table 3 show a decrease in the level concentration of Gamma interferon for burn and wound patient with *p. aeruginosa* infection, where the score mean 35.2±2.6 pg/ml and 35.4±2.3 pg/ml, respectively, when compared with healthy people (control group) score 46±2.5 pg/ml

The current study agrees with (4) showing the decrease of gamma interferon in a patient infected with *Pseudomonas Aeruginosa*, and also agrees with (7) when it shows

240ng/ml	Standard No.5	120µl Original Standard + 120µl Standard diluents
120ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard diluents
60ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard diluent
30ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard diluent
15ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard diluent

Table 1. Standard solutions: (This kit has a standard original concentration).

Stock standard						
Tube	standard	S5	S4	S3	S2	S1
ng/ml	480	240	120	60	30	15

Table 2. Stock standard.

Parameters	Control	Burn	Wound
Gamma INF	46±2.5	35.2±2.6	35.4±2.3
Number	50	53	16
P Value		a- <0.0001* b- <0.0001* c- <0.0001* d- 0.788	

Table 3. Comparison of Gamma INF means among studied groups (Control, Burn and Wound) * repre-sent a significant difference at p<0.05. letters represent the type of statistical analysis: a; the sta-tistical analysis among all studied groups (Control, Burn and wound), b; the statistical analysis between the Burn group and Control group; c: the statistical analysis between the Wound group and Control group; d: the statistical analysis between Burn group and wound group.

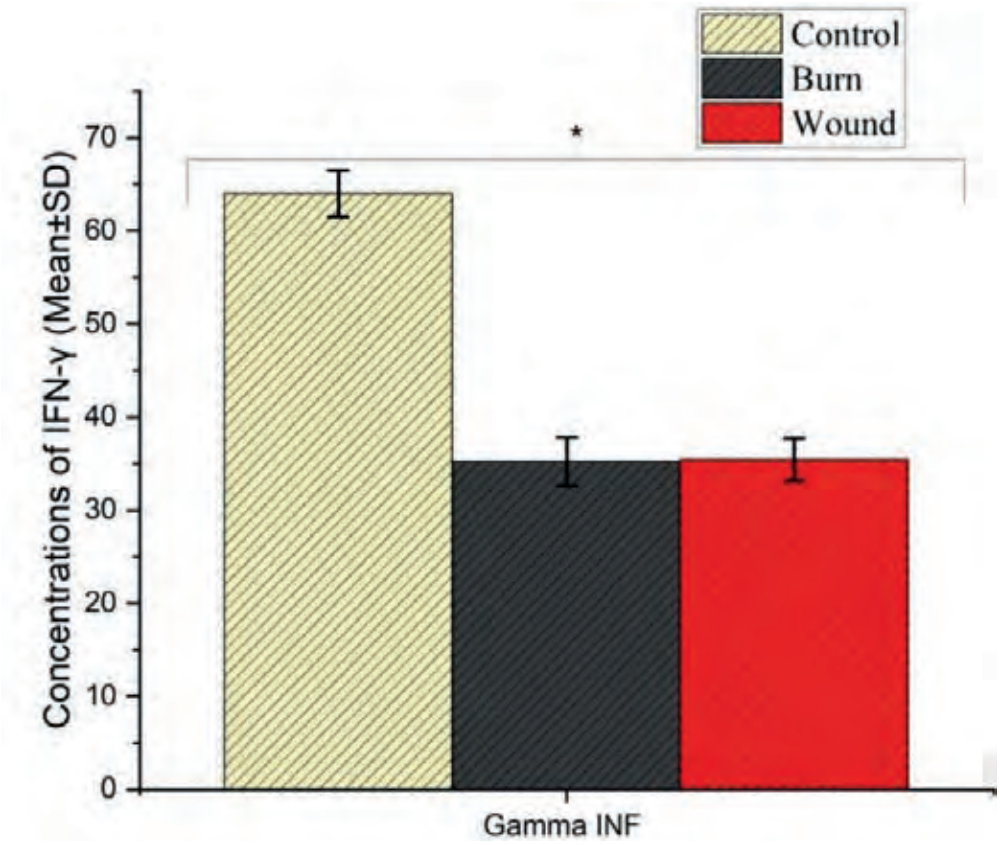

Figure 1. Comparison of Gamma INF means among studied groups (Control, Burn and Wound).

the decrease in interferon – γ level with a patient infected with (PA) compared with no infection; our study also agrees with (8, 9) that reported the decrease in IFN- y in patients with *pseudomonas* infection. This decrease may result in a suppression of immune system in burn and wound patients afflicted with *p.aeruginosa*. The immunosuppression includes inhibiting NK cells that produce IFN- y[10]; IFN- y are proteins produced by most cells of vertebrates. IFN- y is a critical cytokine for adaptive and innate immunity[4]. The current study disagrees with (11). That reported no significant difference be-tween burn patients and the control group.

Conclusions

This study showed the decreased production of gamma interferon As a result of a reaction to in-fection caused by the *Pseudomonas aeruginosa* in burn and wound patients due to immunosup-pression by bacteria study due to virulence factor.

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ARTICLE / INVESTIGACIÓN

The Babylon River's common carp (*Cyprinus carpio*) gills were used for the histopathological examination and PCR detection of Koi herpesvirus disease (KHVD)

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DOI. 10.21931/RB/2022.07.04.3
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Abstract: The first outbreak of koi herpesvirus in Iraq occurred in Babylon Province in the floating cages in the Euphrates River from the Musayyib thermal electric power station to the Al-Hindiya Dam. Fish were suffering from gills rot that did not respond to treatment and lesions and ulcers on the fish's body. The fatalities reached 80%. was water temperature 24°C, pH 6.85, Salinity 760 ppt. So the present study aims to highlight the pathological changes in gills after the Koi herpes virus infection in Babylon Province. The study started after the detection of (KHV) by polymerase chain reaction (PCR) from the suspected samples (30) for the detection of virus nucleic acid. Also, study the water characterization during the periods of outbreaks. The gills specimen was collected from the positive cases for gross and histopathological examination. The result showed that ten samples were positive for (KHV) infection. The gross and histopathological examination results showed severe congestion with necrotic foci and sloughing of secondary lamella with increased mucus secretion. In addition, necrosis in the primary lamellae, Edam and inflammatory cells infiltration with hyperplasia of the secondary lamellae with the presence of intranuclear inclusion body in all exanimated slides.

Key words: Koi Herpes, Cyprinus Carpio, Euphrates River, KHV, secondary lamellae, floating cages.

Introduction

Koi herpesvirus disease (KHVD) is a rare virus that can infect koi and carp quickly and widely[1]. The first report of the koi herpes disease outbreak in Iraq occurred in Babylon Province (north of the Province) in the floating cages in the Euphrates River in the area extending from the Musayyib thermal electric power station to the Al-Hindiya Dam. The first death report was on 10/28/2018, and the death continued until 27/11/2018. Affected fish lost their appetite and exhibited abnormal swimming patterns before they died. Discoloration, elevated respiratory rate[2], swollen gills, and grayish and patchy skin lesions are the most constant signs of the sickness[3]. The koi herpes virus is very contagious and causes massive koi (*Cyprinus carpio koi*) and common carp mortality[4]. For at least four hours, the virus is contagious in water. Which explains why it is so contagious in lakes[5]. It's unclear if The virus enters the fish's body through its gills. Then, it multiplies and causes necrosis and mucosal sloughing or enters the intestine[5,6]. During overt infection, KHV is most prominent in the gill, kidney, and spleen[7]. Gill injury is most likely a major factor in fish mortality. The virus replicates in the infected gills before being released into the water and transmitted to the kidney by white blood cells. The virus causes severe interstitial nephritis in the kidneys. This idea fits with the contagious disease's rapid spread and tends to be similar to respiratory viruses in mammals[8].

Materials and methods

Samples collection

Thirty live fish suspected samples were collected randomly from fish breeding cages in the Hilla River in October 2021. The project consists of ten floating cages with a capacity of ten thousand fish. The fish were suffering from gills rot that did not respond to treatment and lesions and ulcers on the fish's body. The fatalities were more than 80%. The samples were sent directly to the central laboratory of the Veterinary Department for PCR detection.

PCR detection

The real-time PCR detection of the KHV nucleic acid was done in the laboratory of the Veterinary Directorate by using Microboss Hightech GmbH /Germany) kit and the following primers:

Water characteristics

Some characteristics of water were measured over five days from sample collection, such as Sa-linity, Ph and Temperature.

Gross and Histopathological examination

All collected samples were examined grossly for recording the gross pathological changes and for taking the

Citation: Al-Haider S, Alneamah G, Alshkarchy S, Farhood A. The Babylon River's common carp (Cyprinus carpio) gills were used for the histopathological examination and PCR detection of Koi herpesvirus disease (KHVD). Revis Bionatura 2022;7(4) 3. http://dx.doi.org/10.21931/RB/2022.07.04.3
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022
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specimen for histopathological section; the gills were fixed in 10 % formaldehyde pro-cessed routinely by histokinette, embedded in paraffin, sectioned for 5µm stained by hema-toxylin and eosin stain as described by (8).

Results

PCR Detections

The Real-time PCR detection of KHV nucleic acid showed positive results (ct 30.35) according to the manu-facturing kit (up to 30-40 Ct should be taken positive results) as shown in figure (1).

Water characteristics

The mismeasurement of some water characteristics du-ring the study is illustrated in the table(1). The mean result of temperature was (24 ˚c), Ph (6.85), and salinity (760).

Gross and histopathological examination

The grossest observation in most cases was severe congestion with necrotic foci and sloughing of secondary lamella with an increase in mucus secretion (figure2). However, the primary histo-pathological lesion in most exa-mination sections was necrosis in the primary lamellae, with de-generative changes in the epithelial cells lining the se-condary lamellae, with the presence of an in-tranuclear in-clusion body (figure 3). The presence of viral inclusion was shown in most sections characterized by chromatin migra-tion with vacuolation in the lamellar epithelium (figure 4). Edam inflammatory cell infiltration and hyperplasia of the secondary lamellae are also seen (figure 5). Many exami-ned sections showed congestion of the central venous si-nus with fusion and des-quamation of secondary lamellae (figure 6,7).

Discussion

In poikilothermic species, temperature dramatically in-fluences how the disease progresses[3]. Water temperature has been found to influence the initiation and severity of viral infection by influencing virus multiplication and indirectly by improving the efficacy of the host immune re-sponse[9]. Tem-peratures in the water have a direct impact on cellular and humeral immunity. KHV outbreaks in koi and common carp are influenced by water temperature. Viral load in the en-vi-ronment is not as crucial for infection outbreaks when the temperature of infected fish shifts from lower to warmer tem-peratures (e.g., 23°C); a death occurs quickly (7–12 days from onset to death). This finding matched that of (6), who discovered that water temperature, virus pathogenicity, fish age and condition, high population, and stress conditions all influence sickness patterns (e.g., trans-portation, spawning, poor water quality). Other research has suggested that the infection is tem-perature dependant, occurring between 16 and 25 ° C.[5,6,10,11]. Gilad *et al.*; Ilouze *et al.*[7,12] found that the disease caused high mortality under experimental condi-tions at 28°C but not at 29°C or 30°C, nor at 13°C.

Target	Primer	Sequence (5'→3')
KHV-DNA	KHV-86f	5 '- GAC GCC GGA GAC CTT GTG-3 '
	KHV-109p	5'-CGG GTT CTT ATT TTT GTC CTT GTT-3'
	probe 78 bp	5'-FAM-CTT CCT CTG CTC GGC GAG CAC G-TAMRA3 '

Table 1. Oligonucleotides used in PCR.

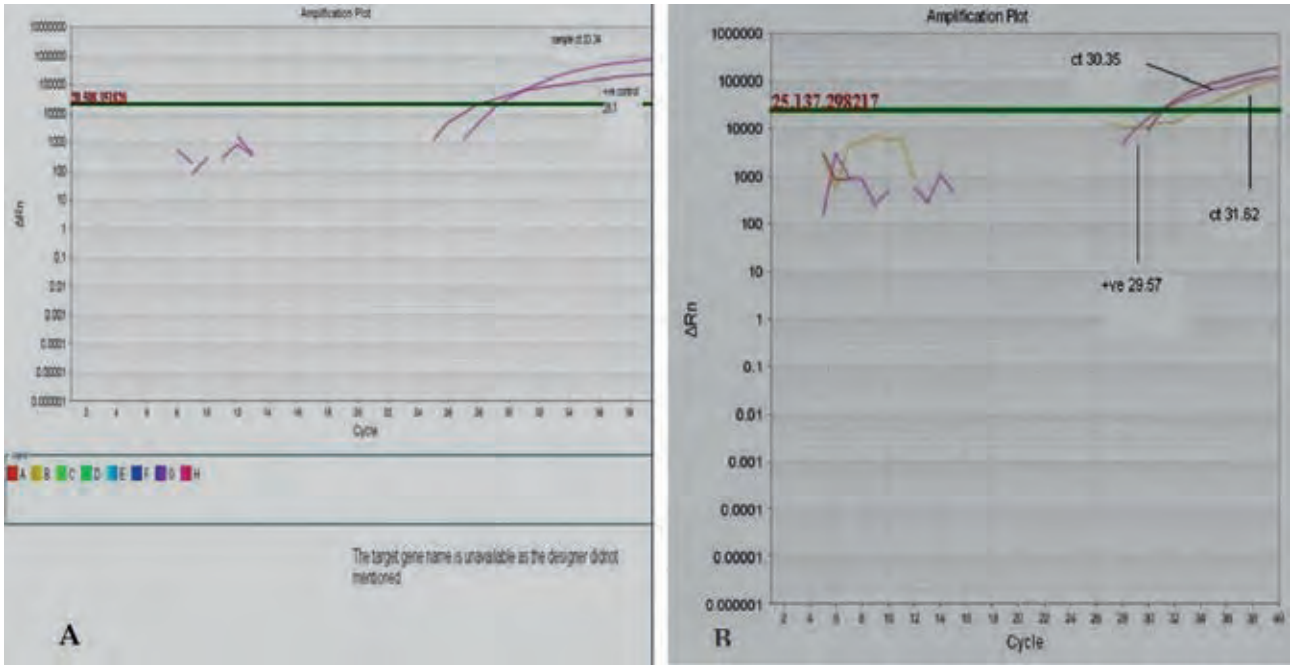

Figure 1. Amplification plot for Real time detection of KHV (A)for control(Ct33.34) and(B) for collected samples(Ct 30.35).

Day	Temperatures(˚c),	Ph	Salinity (ppt)
1st day	23.9	6.9	757
2nd day	23.8	7	760
3rd day	24.1	6.75	762
4th day	24.2	6.8	759
5th day	24	6.8	762
mean	24	6.85	760

Table 2. Measurement of the Temperatures (˚c), Ph, and Salinity (ppt) during the sample collection period.

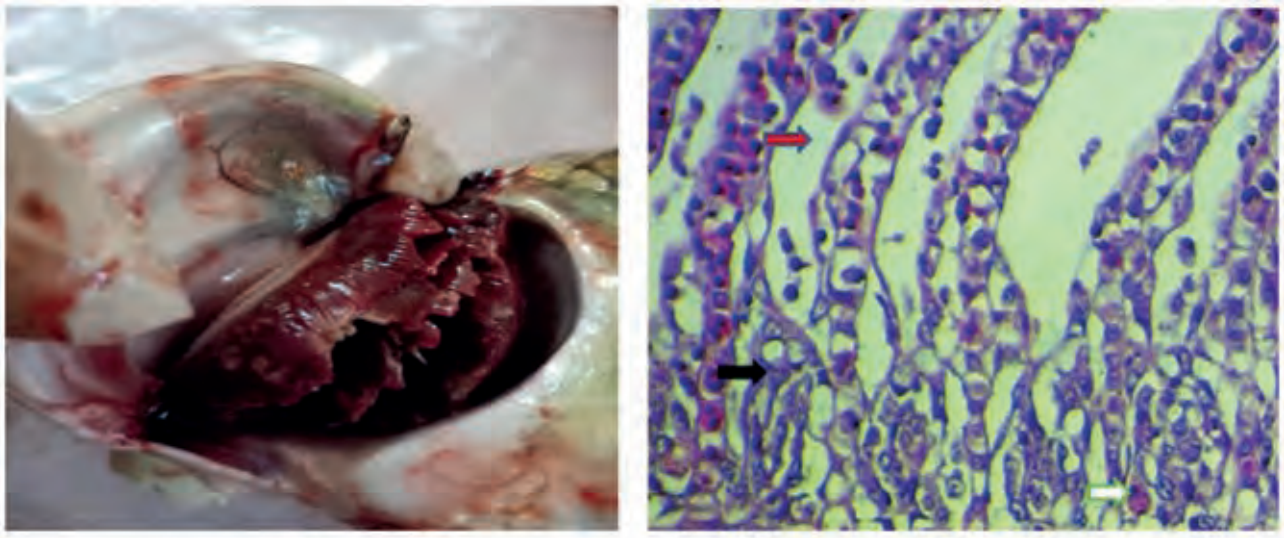

Figure 2. (a) Gross photograph of the fish gills infected with (KHV) shows severe congestion with necrotic foci and sloughing of secondary lamella with the increase in mucus secretion.; (b) Histopathological section of the fish gills shows necrosis in the primary lamellae (black arrow), with degenerative changes in the epithelial cells lining the secondary lamellae (red arrow), with the presence of intranuclear inclusion body (white arrow). H&E stain, 400X).

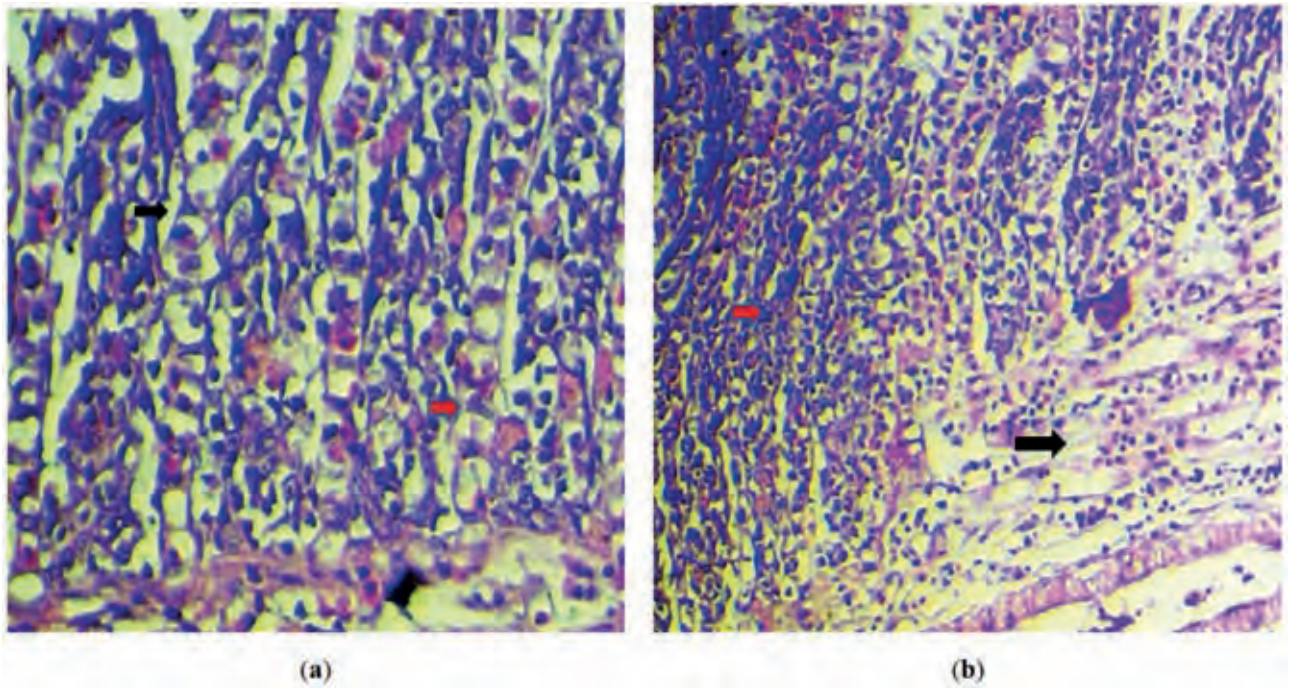

Figure 3. (a) Histopathological section of the fish gills showing intranuclear inclusion body with chromatin migration (black arrow) with vacuolation in the lamellar epithelium(red arrow). H&E stain, 400X).; (b) Histopathological section of the fish gills showed edam inflammatory cells infiltration (black arrow) and hyperplasia of the secondary lamellae (red arrow). (H&E stain, 200X).

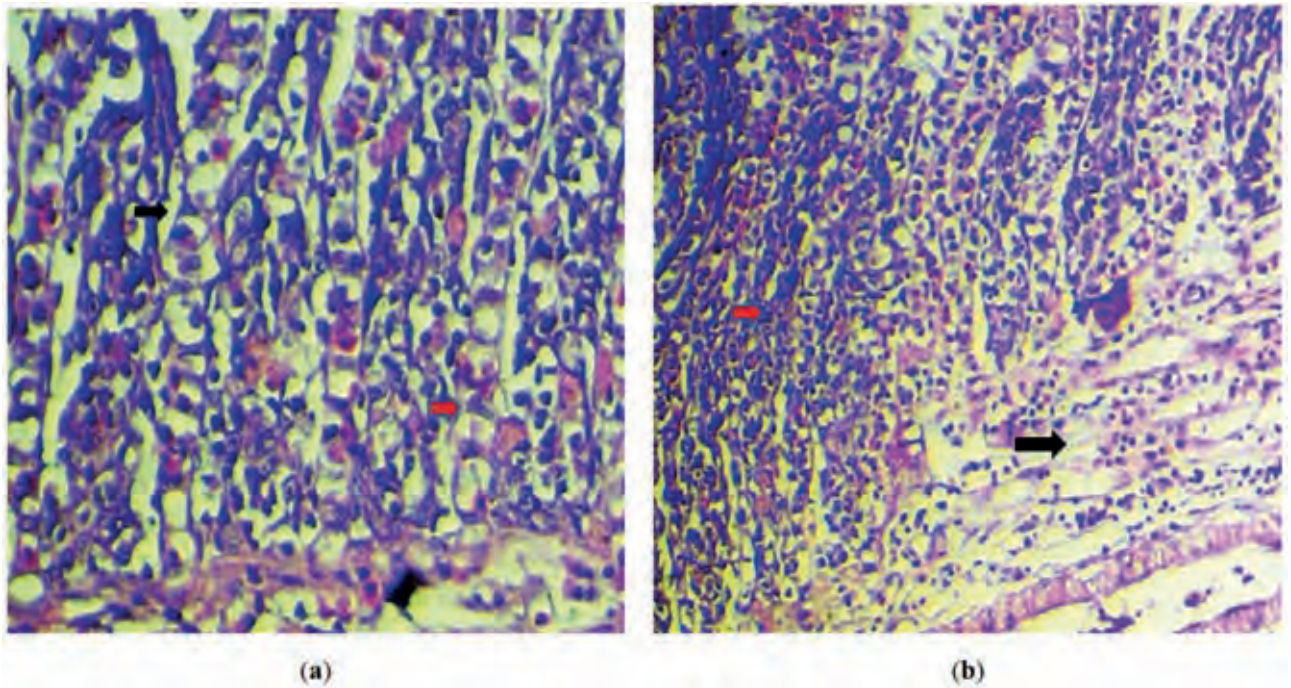

Figure 4. (a) Histopathological section of the fish gills shows fusion of the secondary lamellae (black arrow) with sloughing of the others (red arrow). (H&E stain, 400X).; (b) Histopathological section of the fish gills shows severe congestion of the central venous sinus (black arrow) with fusion and desquamation of secondary lamellae (red arrow). (H&E stain, 200X).

However, viral DNA was identified in the fish by PCR at 13°C, suggesting that virus reservoirs may exist among infected fish surviving at low temperatures[7].

The primary histological abnormalities in gill filaments were confirmed by (13). He discovered that most of the gill lamellae's respiratory epithelial cells were enlarged or vacuolated, with nuclear degeneration. Among the nuclear alterations seen were pale coloration, karyorrhexis, and the formation of an intranuclear inclusion body (IIB), defined as basophilic material within the nucleus with marginal hyperchromatic generated by heterochromatin deposition on the inner nuclear membrane. The injured respiratory epithelial cells seemed to have combined with cells from nearby lamellae, causing lamellar fusion and gill filament clubbing. According to to (5–7), the primary target cells in the per-gill infection were respiratory epithelial cells of the gill lamellae, where nuclear degradation and IIB were indicators of infection. The formation of IIB was shown to be caused by an increase in filamentous nucleoproteins and the construction of multiple viral capsids and nu-cleocapsids.

Conclusions

We concluded that the disease eruptions occurred during seasonal and sudden temperature changes and caused a severe mortality rate of fish of various weights, especially in marketing weight. Also, KHVD can cause severe pathological changes in fish gills with the presence of an intranuclear inclusion body in histological exanimated sections.

Author Contributions

Conceptualization, Sadeq. Al-Haider. Ghusoon .Alneamah And Samer Alshkarchy.; methodology, Ghusoon A. A. Alneamah; software, Samer .Alshkarchy; validation, Samer. Alshkarchy, Sadeq. Al-Haider. and Ghusoon .Alneamah; formal analysis, Sadeq. Al-Haider. Ghusoon .Alneamah And Sa-mer Alshkarchy; investigation, Ahmed Farhood.; resources, Ahmed Farhood.; data curation, Samer Alshkarchy.; writing—original draft preparation, Ghusoon .Alneamah.; writing—review and editing, Ghusoon .Alneamah. Samer. Alshkarchy; All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Acknowledgments

We would like to thank Laboratory of Plant Physiology and Plant Tissue Culture of the Universidad de Antioquia. A special acknowledgment to Universidad de Antioquia's Research Development Committee (CODI) and Granja Yariguíes – Compañia Nacional de Chocolates.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Acknowledgments

We also want to thank Babylon Veterinary Hospital for their assistance with the live fish care during the trial.

Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results".

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ARTICLE / INVESTIGACIÓN

Leg cuts from Awaasi lambs fed a diet with varying levels of *Rhus coriaria L.*, Physical dissection and chemical composition

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DOI. 10.21931/RB/2022.07.04.4

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Abstract: This research was conducted in a private slaughterhouse in Samarra from 1/5/2019 to 1/8/2019. Sixteen Awassi lambs were raised with average body weight (of 20±25) kg and an age of about (5.5 to 6) months. We divided the lambs into four treatments. The first treatment was without additives, called control; the second treatment was 1% Sumac, the third treatment was 3% Sumac, and the last one was the fourth treatment with 5% Sumac. The results showed significant differences between treatments in biceps femoris and semimembranosus muscle (treatment 3 the female) were (180.9±10.66)g, (168.4±5.72)g respectively. And a highly significant difference in adductor muscle (treatment 3, the female) was (75.6±2.58)g compared with the control (62.85±5.93)g. significant differences in treatment 3 in Semitendinosus muscle between males and females were(57.2±3.65) and (74.4±3.02)g, respectively. The rectus femurs showed significant differences between treat-ments 3 and 4 in females were(99.6±8.76) and (76.3±6.98)g, respectively. In physical dissection, a low significant difference in treatment 4 in lean was (56.3+3.66)%. The lean percentage in treatment 3 of the female was (59.2±0.99) %. About chemical analysis, the high percentage of moisture in the male in treatments 2,3 and 4 were (72.7±0.14),(72.8±0.21),(and 72.8±0.21)%, respectively.

Key words: Leg cut, physical dissection, *Rhus Coriaria L.*

Introduction

Because medical plants have demonstrated the effect on human and animal life activities, the world has moved towards using them as a therapeutic material instead of chemical compounds.

The most important source of red meat in Iraq is sheep, so they are raised in big farms by breeders; the ease with which they are raised increases where it is known by their short and rapid production cycle; it also has a high growth rate compared with other ruminants[1,2]. and raising the income of societies caused changes in the requirements of consumers they started looking forward to getting wholesome and healthy quality meat[3].

As we know, nutrition is the essential factor causing the excellent performance of raised animals[4].

Because medical plants have demonstrated the effect on human and animal life activities, the world has moved towards using them as a restorative material instead of chemical compounds[5,6].

Their use is harmful side effects, especially on some body organs such as the liver, pancreas, kidneys, etc., along with its apparent effect on the body's immune system; after the experiments on poultry, the researchers moved to ruminants to study these medicinal plants on the performance of ruminants, tiny ones like sheep and goats[7,8] on of these plants is *Rhus Coriaria L.* it was used in this research as natural growth stimuli.

Materials and methods

This research was conducted in a private slaughterhouse in Samarra called (good land farm) sixteen Awassi lambs were raised (individual cages) with average body weight (of 20±25)kg their age (of 5.5-6) months. The lambs have divided into four treatments according to the additive: the first treatment was control without sumac, the second treatment was 1% sumac, the third treatment was 3% Sumac, and the last treatment was the fourth 5% Sumac. The cages were supplemented with a feeder and utensils of drinking water; the mineral salt block was in front of the lambs.

Dietary treatment

A typical diet given to the lambs consisted of barley, wheat bran, soybeans, yellow corn, salts and minerals. The barley was the most significant percentage, then the wheat bran.

	T1	T2	T3	T4
Barley	40	40	40	39
Wheat bran	39	40	40	39
Soybean	10	10	11	11
Corn	10	8	5	5
Sumac	0	1	3	5
Mineral and vitamins	1	1	1	1

Table 1. Experiment with diet ingredients percentage.

Citation: Ibraheem M W, Muhaimeed A R, Mohammed Th T. Leg cuts from Awaasi lambs fed a diet with varying levels of Rhus coriaria L., Physical dissection and chemical composition. Revis Bionatura 2022;7(4) 4. http://dx.doi.org/10.21931/RB/2022.07.04.4
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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Lamb slaughter

At the end of the experiment, the lambs fasted from food only for 12 hours, then they were slaughtered, and we put the carcass in the fridge at 4 C for 24 hours; the next day, we took the leg cut off the carcass right half to do the measurements.

Physical dissection

It was performed in the laboratory, where the lean was separated from the fat and bone, also the leg muscles were separated according to the scientific method of separating using the dissection substances.

Chemical examination

Determination of moisture, protein content, fat and ash was done according to (9).

Panel test

Sensory evaluation of the leg cut was performed by the method of (10). A group of people was selected from the faculty of Agriculture in the department of animal production who were experienced in judging meat samples and evaluating them in terms of tenderness, juiciness, flavor and general acceptance by cooking the samples at 165º C in the oven for 20 minutes. The judges were provided with detailed information about the grade of each trait before the test began.

Statistical analysis

The statistical analysis of the data was done using the complete random design (CRD) to study the effect of parameters on different characteristics[11]. The significant differences between the average were compared by using the Duncan test[12] in the following linear additive model:

$$Yij = \mu + Ti + eij \qquad (1)$$

Where:

Yij: the observation of additives

μ : is the overall mean effect

Tj : effect of treatment

eijk : is independent normally distributed random error term zero mean and variance δ^2

Results

Leg weight and muscles

The results in a table (2) showed significant differences between treatment in biceps femoris and semimembranosus muscle (treatment 3 the female) were (180.9±10.66)g,(168.4±5.72)respectively. And high significant difference in adductor muscle (treatment 3, the female) was (75.6±2.58) g compared with the control (62.85±5.93)g; the reason may be due to the high weight of the leg in treatment 3 compared with the other treatments. At the same time, gracilis muscle did not show significant differences between the four treatments.

Leg muscle weight

The results in(table 3)showed significant differences in treatment 3 in Semitendinosus muscle between males and females (57.2±3.65) and (74.4±3.02)g, respectively; the reason may be due to the chemical composition. Also, the rectus femurs showed significant differences between treatments 3 and 4 in females were(99.6±8.76) and (76.3±6.98) g, respectively.

Physical dissection

The results of physical dissection in(table 4) showed a low significant difference in treatment 4 in lean, it was (56.3±3.66)%, while the lean percentage in treatment 3 the female was (59.2±0.99)%; the reason may be due to the high percentage of fat in the same treatments and that is because of the inverse relationship between protein and fat, and that would explain the high percentage of fat in treatment 4 the female it was (23.8+ 3.77) % also it is may be due to the Sumac additives in this treatment.

For bone, the best percentage was conducted by treatment3and 4 the female; it was the minor percentage between the four treatments were(19.7±1.12) and (19.9±0.24) %, respectively.

Chemical composition

The results of chemical composition explain a lot. (table 5) the high percentage of moisture in the male in treatments 2,3 and 4 were (72.7±0.14),(72.8±0.21),(and 72.8±0.21)% respectively, while the low percentage was in the female in the same treatments they were (72.1±0.17), (72.0±0.05), (72.0±0.03)% respectively the reason may be due to the differences in genetic structures between male and female sheep.

Regarding protein, the significant differences were minor for treatment 4, the male; it was (22.7±0.20) %; as we know, protein is the least variable in the chemical composition of meat.

We noticed the differences were evident in fat percentage, especially in treatment 2,3; the female was (3.6) %; the reason may be due to the reverse between fat and moisture.

Ash showed highly significant differences in treatment 4; the male was the best conducted(0.6±0.11) % while the female in the same treatment conducted (1.6±0.07)%.

Panel test

The sensory evaluation of the semimembranosus muscle from the leg cut showed significant differences in flavor in treatments 3 and 4 in males (4.3). Also, juiciness was conducted a highly significant difference in the same treatments (4.6±0.33)

Regarding the acceptability and tenderness treatment 4, the female was the best. It conducted 4.6 and(4.0) respectively, may be that the addition of sumac affected the sensory evaluation in treatments 3 and 4

Discussion

Leg weight and muscles

From the results of table 2, we noticed that adding 3% sumac to ewes diets could be helpful to increase semimembranosus muscle, which is very useful for predictors and consumers because both prefer an increase in lean percentage rather than fat and bone ratio. The rise of the semimembranosus muscle increased leg muscle because of a high rate of the mentioned muscle compared with the others, so it is a significant and valuable increase[2]. Maybe this addition of sumac to the diets would be helpful too because it involves a reasonable amount of sumac, as it doesn't negatively affect other treatments.

TRT	SEX	Leg Weight	Biceps Femoris	Gracilis	Semimem-branosus	Adducter
		gm				
T1 control	M	1717.3±71.32 b	138.9±8.78 b	32.7±3.61 a	131±6.92 b	**62.85±5.93 bc**
	F	1666±61.07 b	146.7±6.74 ab	38.8±2.7 a	133.8±7.03 b	**57.34±3.13 c**
T2 1%sumac	M	1793.3±58.02 b	141.9±0.33 b	31.5±0.84 a	132.2±15.11 b	**66.9±0.52 ab**
	F	1845.3±17.6 b	137.4±3.36 b	31.4±0.77 a	132.9±4.90 b	**71.9±2.96 ab**
T3 3% sumac	M	1831±58.19 b	152.3±15.29 ab	34.3±4.07 a	134.7±7.07 b	**69.6±0.57 ab**
	F	2149±81.28 a	180.9±10.66 a	41.82±1.32 a	168.4±5.72 a	**75.6±2.58 a**
T4 5% sumac	M	1924.7±33.65 ab	160.7±10.49 ab	38.52±4.87 a	134.9±16.72 b	**70.15±1.36 ab**
	F	1718.3±87.78 b	134.63±19.41 b	37.8±8.30 a	127.1±6.48 b	**63.4±1.99 bc**
		*	*	N.S.	*	**

Different letters within the column refer to significant differences ($p \leq 0.05$) between means.

Table 2. Effect of different treatments and sex on leg weight and muscles (mean ± S.E).

TRT	SEX	Semitendinosus	Rectus Femoris	Vstus Lateralis	Vastus Intermedialis
		gm			
T1 control	M	59.4±1.85 ab	89.9±0.43 ab	85.5±3.51 a	**15.9±0.02 a**
	F	62±2.98 ab	86.7±3.45 ab	73.1±3.93 a	**16.7±1.62 a**
T2 1%sumac	M	66.9±9.57 ab	78.1±2.12 ab	73.4±4.02 a	**15.4±0.97 a**
	F	66.3±0.06 ab	93.5±1.86 ab	74.1±2.18 a	**16.2±1.47 a**
T3 3% sumac	M	57.2±3.65 b	90.7±10.98 ab	73.3±6.89 a	**14.9±0.54 a**
	F	74.4±3.02 a	99.6±8.76 a	79.9±3.23 a	**15.4±1.46 a**
T4 5% sumac	M	59.5±5.23 ab	90.2±9.08 ab	77.9±6.59 a	**17.1±1.95 a**
	F	56.1±4.91 b	76.3±6.98 b	65.3±11.93 a	**15.2±0.62 a**
		*	*	NS	**NS**

Different letters within the column refer to significant differences ($p \leq 0.05$) between means.

Table 3. Effect of different treatments and sex on leg muscles weights (mean ± S.E).

TRT	SEX	Lean	Fat	Bone
		%		
T1 control	M	62.2±0.80 a	14.5±1.43 cd	**23.3±0.63 ab**
	F	60.5±2.03 ab	18.2±1.44 abcd	**21.1±0.59 cd**
T2 1%sumac	M	58.1±1.42 bc	18.2±1.44 abcd	**21.2±0.59 cd**
	F	58.1±1.42 bc	18.4±2.01 abcd	**23.6±0.59 ab**
T3 3% sumac	M	60.8±0.84 ab	15.5±1.06 bcd	**23.7±0.22 a**
	F	59.2±0.99 ab	21.1±0.16 ab	**19.7±1.12 d**
T4 5% sumac	M	58.6±0.92 ab	19.9±0.49 abc	**21.5±0.43 bcd**
	F	56.3±3.66 c	23.8±3.77 a	**19.9±0.24 d**
		*	**	**

Different letters within the column refer to significant differences ($p \leq 0.05$) between means.

Table 4. Effect of different treatments and sex on physical dissection components (mean ± S.E).

TRT	SEX	Moisture	Protein	Fat	Ash
		%			
T1 control	M	72.9±0.21 a	22.3±0.26 ab	3.0±0.17 b	**1.2±0.07 b**
	F	72.7±0.07 a	22.3±0.20 ab	3.3±0.18 ab	**1.5±0.06 ab**
T2 1%sumac	M	72.7±0.14 a	22.3±0.28 ab	3.0±0.05 b	**1.3±0.17 ab**
	F	72.1±0.17 b	22.0±0.20 b	3.6±0.16 a	**1.4±0.03 ab**
T3 3% sumac	M	72.8±0.21 a	22.0±0.17 b	3.0±0.18 b	**1.2±0.14 b**
	F	72.0±0.05 b	22.0±0.06 b	3.6±0.08 a	**1.3±0.03 ab**
T4 5% sumac	M	72.8±0.21 a	22.7±0.20 a	3.0±0.08 b	**0.6±0.11 c**
	F	72.0±0.03 b	22.0±0.14 b	3.3±0.08 ab	**1.6±0.07 a**
		**	*	**	**

Different letters within the column refer to significant differences ($p \leq 0.05$) between means.

Table 5. Effect of different treatments and sex on semimembranosus chemical composition (mean ± S.E)

TRT	SEX	Flavor	Tenderness	Juiciness	Acceptability
T1 control	M	2.7±0.33 cd	2.3±0.33 b	2.7±0.33 bc	**3.7±0.38 ab**
	F	2.0±0.0 d	2.3±0.33 b	2.3±0.33 bc	**3.3±0.33 b**
T2 1%sumac	M	3.6±0.34 abc	3.0±0.57 ab	3.3±0.67 abc	**3.6±0.35 ab**
	F	3.0±0.58 bcd	3.3±0.35 ab	2.6±0.88 bc	**3.3±0.66 b**
T3 3% sumac	M	4.3±0.33 a	3.3±0.66 ab	4.6±0.33 a	**4.0±0.33 ab**
	F	3.3±0.33 abc	3.0±0.04 ab	4.0±0.57 abc	**3.6±0.33 ab**
T4 5% sumac	M	4.3±0.36 a	3.6±0.36 ab	4.6±0.61 a	**4.3±0.37 ab**
	F	4.0±0.57 ab	4.0±0.51 a	4.3±0.33 ab	**4.6±0.33 a**
		**	**	**	*

Different letters within the column refer to significant differences ($p \leq 0.05$) between means.

Table 6. Effect of different treatments and sex on semimembranosus sensory evaluation (mean ± S.E).

Leg muscle weight

As for the rest of the thigh muscles, adding 3% sumac to the ewe's diets positively affected the semitendinosus and rectus femoris muscles. The high proportion of each muscle in the leg cut is economically essential[4], as the leg is one of the primary cuts of the carcass, and the high proportion of meat is always the goal of the breeders and the consumer.

Physical dissection

By physical dissection of the leg, it can be inferred that the addition of sumac affected the third and fourth treatments, which resulted in an increase in fat percentage in the fourth treatment, which is a non-positive indicator[4]. The permanent goal is to increase the proportion of lean in the carcass, not fat. Adding sumac may cause undesirable variations in the overall physical dissection. However, expanding the tissue of some leg muscles in the female and male sheep is positively affected by the sumac adding, not the physical dissection.

Chemical composition

On the chemical analysis of the leg, the differences in each characteristic between the parameters and even between the animal gender are unmistakable. But in general, we saw high moisture in all treatments with high protein. Because of the inverted relationship between moisture and fat, it was shallow in most treatments. This is a reassuring indicator of the possibility of using sumac in general animals[2]. The primary desire of both producer and consumer is a high protein, low-fat meat. This is evident from the results of this research.

Panel test

The second and third treatments had the best results in the panel test, although the proportion of meat composition varied between treatments. This differentiation of the third and fourth treatments is reassuring since the juiciness and tenderness are critical qualities on which meat can be classified and graded. The high tenderness of the two treatments above is a good indicator[10] and may contribute even a tiny part to improving the qualities of the sensory meat.

Conclusions

This research concludes that sumac can be used in male and female sheep diets, as it contributes to a lower fat content, increases the nutritional value of the meat also, indirectly preserves it from decay and oxidation, and improves the sensory qualities of the meat, meaning its smoothness and juices.

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ARTICLE / INVESTIGACIÓN

Effect of flaxseed oil dosing on fertility, growth characteristics and some physical, biochemical, and hormonal blood parameters during the early pregnancy of Awassi ewes

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DOI. 10.21931/RB/2022.07.04.5

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Abstract: This study aims to complete the direction of experimental research in determining the effect of flaxseed oil doses on the fertility of Awassi ewes, as well as some important blood parameters to clarify the animal's physiological state during the duration of the experiment. This study was conducted in the animal field (College of Agriculture - Tikrit University) for 76 days (16 days before and 60 days after fertilization). 2-3 years have an average live weight of 51.74 kg, and the ewes were randomly divided into three treatments (each treatment was nine ewes). The three treatments were given doses of flaxseed oil at the rate of 0, 6 and 8% / kg of feed, respectively. The results showed that there was no significant difference (P≤0.05) between the treatments in the ewes' weights and physical blood characteristics during the experiment period, while it was noted that the fertility rate of the second and third treatments amounted to 66.66% for each of them, over the first treatment (55.55%). In addition, the second treatment was significantly (P≤0.05) superior in blood globulin concentration over the rest. In contrast, the third treatment was significantly (P≤0.05) superior in triglyceride concentration (62.66) mg/dL, and the first treatment showed a significant (P≤0.05) superiority. In glucose concentration (70.33) mg/dL. The following characteristics were not significantly different in total protein, al-bumin, urea, creatinine, ALT and AST enzymes, as well as no significant difference between treatments in the concentration of estrogen and progesterone hormones.

Key words: Flaxseed oil, Awassi ewes, fertility .

Introduction

Adding fats to diets is receiving great attention as a mechanism for increasing the unsaturated fatty acids rich in omega 3 and 6 fatty acids in animal products(that are not manufactured in the body, as well as reducing the percentage of saturated fatty acids in them, so they must be included in diets, and we can find these essential fatty acids in some vegetable oils such as flaxseed oil[1]. A lot of recent research recommends reducing the content of short and medium-chain saturated fatty acids and increasing the proportion of long-chain unsaturated fatty acids such as linoleic acid in particular[2]. Therefore, unsaturated oils extracted from the seeds of oilseeds were used in the diet components[3]. Flaxseed oil is one of the vegetable oils rich in unsaturated fatty acids[4,5], in addition to containing tocopherols (vitamin E) and high amounts of phenolic compounds antioxidants. Flaxseed oil also contains about 60% of the omega-3 fatty acids that have vital benefits to the body[6]. It also includes a large proportion of vitamin B complex, lecithin, and zinc, in addition to the necessary minerals for the body, such as magnesium and potassium. Scientific studies have proven that flaxseed oil helps reduce harmful fats such as LDL, VLDL and triglycerides[7]. (8) indicated that adding omega-3 to cow diets led to improved fertility and attributed the reason to an increase in the energy level. This positively affected the level of progesterone and estrogen necessary in maintaining pregnancy and the initial growth of the fetus by reducing the level of prostaglandin release from the uterine wall and thus reducing Fetal mortality rate[9]. This study was conducted to know the biological effect that the addition of fats that contain a high percentage of unsaturated fatty acids and the use of flaxseed oil can have on some productive and physiological traits.

Materials and methods

The study was conducted in the animal field (College of Agriculture - Tikrit University) for 76 days (16 days before and 60 days after fertilization). 2-3 years have an average live weight of 51.74 kg. The ewes were divided into three groups according to their weight (each group had nine ewes). The ewes were placed in a semi-enclosed enclosure under the same environmental conditions. The ewes were allowed to graze in the morning and provided a supplementary ration of 2.5% of their body weight, divided into two morning and evening meals, and hay was provided freely. Barley, soybean, corn and bran as shown in Table (1) as a preliminary period, then the transactions were distributed to the totals as follows:

1- Control group without addition.

2- The second group (dose) with flaxseed oil (6%) / kg of dry matter was ingested.

Citation: Noaman A I, Khalaf R M, Emad GH, Al-Abbasy, Mohammed Th. T. Effect of flaxseed oil dosing on fertility, growth characteristics and some physical, biochemical, and hormonal blood parameters during the early pregnancy of Awassi ewes. Revis Bionatura 2022;7(4) 5. http://dx.doi.org/10.21931/RB/2022.07.04.5

Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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the ingredients	Third transaction	second transaction	first treatment
Barley	52	52	52
Bran	20	20	20
Maize	15	15	15
Soybean	11	11	11
salts and minerals	2	2	2
The chemical composition of the relationships%			
dry matter	89.71	89.71	89.71
Organic matter	87.40	87.40	87.40
raw protein	14.98	14.98	14.98
raw fiber	3.01	3.01	3.01
ether extract	2.34	2.34	2.34
soluble carbohydrates	61.57	61.57	61.57
Metabolites (MJ/kg dry matter)	11.29	11.29	11.29

* The diet was analyzed in the laboratories of Erbil Feed Company - Erbil
** The represented energy was estimated according to the following equation[10] :ME (MJ/kg DM) = 0.31CP + 0.12EE + 0.05CF + 0.14NFE

Table 1. Ingredients and chemical composition of the experimental ratio %.

3- The third group (dosing) with flaxseed oil (8%) for each kg of dry weight consumed.

Vaginal sponges were used to unify the molars and were raised after 14 days of laying. Then all ewes were injected with PMSG hormone to stimulate ovulation. A pregnancy test was carried out with an ultrasound (sonar) device to confirm pregnancy by a specialized veterinarian. 10 ml of blood was withdrawn from the jugular vein, of which 2 ml were taken and placed in plastic tubes containing the anticoagulant Ethylene Diamine Tetra Acetic acid (EDTA) for use in physical blood tests. The remaining 8 ml was placed in glass tubes to separate the blood serum in a centrifuge (3000 cycles/minute and for 15 minutes), after which the serum was kept in the freezer (-20°C) until biochemical tests were performed on it. Glucose, cholesterol, triglycerides, total protein, albumin, globulin, blood urea and creatinine. The activity of aspartate aminotransferase (AST) and alanine (ALT) enzymes in serum was estimated using the ready-made diagnostic kit (kit) supplied by the French company Biolabo, and based on the information installed on the standard solution container.The physical characteristics of blood were calculated using the MYTHIC device from the Swiss company ORPHEE, which works on the principle of electrical impedance, to calculate the red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), rate of the combined blood cell volume (PCV) and the number of platelets. The second section was taken from the frozen blood sample (-20 m). The blood samples were analyzed in the accuracy laboratory using a device (Cobas E 411), a German-origin device from the German company Roche. The device works with chemiluminescence technology to estimate the concentration of the hormones estrogen and progesterone. The results were analyzed by the SAS statistical analysis program[11] using a complete random design (CRD), and the means were compared using Duncan's multiple range test[12] according to the following mathematical model:

$$yij=\mu + ti + eij$$

Since:
yij = value of views.
μ = overall average of views.
ti = the effect of the treatment.
eij = effect of experimental error.

Results

Effect of flaxseed oil dosing on fertility

The results in Table (2) indicate that there is an increase in the fertility rate in favor of the second and third treatments, 6 and 8% of flaxseed oil of body weight, reaching 66.66% for each, over the first treatment (control) 0% flaxseed oil, which was 55.55%. The reason can be attributed to the fact that adding fat directly affects fertility by modifying the energy status[13]. This was confirmed by (14), that the use of flaxseed oil by 10% and 12% led to an increase in the concentration of progesterone in the blood.

Adjective	T1 0% FO*	T2v 6% FO	T3 8% FO
Fertility	55.55	66.66	66.66

Table 2. The effect of flaxseed oil dosing on fertility.

Ewe weights

The results in Table (3) indicate no significant differences between the treatments in the weights of ewes during the experiment period, as they ranged between 50.13 - 53.78 kg. The reason may be that the treatments dealt with the same quantity of the ration with the uniformity of the per-

centage of crude protein in the ration, which did not cause differences in the weight gain.

Physical blood characteristics

The results in Table (4) indicate no significant differences between the treatments in all the studied physical characteristics, which gives us evidence that the ewes were in good health throughout the experiment. From infection, maintenance of cell membranes, improvement in the synthesis of white blood cells, and improvement in the vital immune functions of the body.

Biochemical blood parameters

The results in Table (5) showed that there were no significant differences (p≤ 0.05) between treatments in total blood protein and albumin, which indicates the minimum nutritional effects and protein catabolism in muscles[15]. The results also showed no significant differences (p≤ 0.05) between the treatments in the concentration of cholesterol, although there was a clear arithmetic increase in the third treatment and that the reason for the increase in animals fed flax oil could be due to the increase in the concentration of fatty acids in the small intestine and their absorption and conversion to lipoprotein, as well as the increase in cholesterol can be attributed to an increase the level of fat in the diet[16]. The high level of cholesterol when adding fat to the diet may be due to the increase in the level of digestion of unsaturated fatty acids contained in these fats compared to saturated fatty acids[17].

The oil treatments did not affect urea and creatinine, indicating that they did not affect kidney function because both urea and creatine in the blood are indicators of glomerular filtration in the kidneys[20], on the integrity of liver function. At the same time, there was a significant (p≤0.05) superiority for the second treatment of globulin (2.13 g/dL) compared to the first and third treatments (1.00 and 1.80) g/dL, respectively. The reason may be that flaxseed oil has a role in improving the immune system and preventing infection. Many serious diseases and detoxification of the body[5]. Consequently, it led to an increase in globulin, which performs several functions in the blood plasma, the most important of which is the enzymatic role that gives acquired immunity to the body and is a carrier of hormones. Innovative to destroy pathogenic organisms[18].

While there were no significant differences (p≤ 0.05) between treatments in the triglycerides, the results showed an apparent arithmetic increase for flax oil treatments, which amounted to 58.66 and 58.33 mg/dL in the second and third treatments, respectively, compared to the control treatment, which amounted to 50.66 mg/dL. The increase in triglycerides can be attributed to the rise in the level of fats in the diet[16]; the high level of triglycerides when adding fats to the diet may be due to the increase in the level of digestion of unsaturated fatty acids contained in these fats compared to saturated fatty acids[17]. In comparison, there was a significant superiority (p≤ 0.05) for the first treatment of glucose, which reached 70.33 mg/dL, compared to the second and third treatments, which amounted to 66.33 and 62.50 mg/dL, respectively. In ruminants, hepatic glucose is formed from proteins[19].

Treatments / Adjectives	T1 0% FO*	T2 6% FO	T3 8% FO
initial weight (kg)	50.25 ±1.98a	51.05 ±1.99a	**51.5 ±2.06a**
First month of pregnancy(kg)	50.57 ±1.42a	50.13 ±1.55a	**50.63 ±1.75a**
Second month of pregnancy (kg)	52.40 ±2.21a	53.78 ±2.21a	**53.28 ±2.16a**

*FO= Flaxseed oil

Table 3. Effect of flaxseed oil dosing on live weight of ewes (mean ± standard error). The values that carry different letters within the same row indicate the presence of significant differences at the probability level (p ≤ 0.05).

Treatments / Adjectives	T3 8% FO	T2 6% FO	T1 0% FO*
Total protein (g/dL)	4.86 ±0.21a	5.00 ±0.05a	4.86 ±0.63a
Albumin (g/dL)	3.86 ±0.12a	2.86 ±0.24a	3.06 ±0.52a
Globulin (g/dL)	1.00 ± 0.26 b	2.13 ±0.23a	1.80 ±0.30ab
Triglycerides (mg/dL)	50.66 ±4.37 a	58.66 ±5.92 a	58.33 ±5.45a
Cholesterol (mg/dL)	82.00±4.50 a	79.00±1.15a	87.00±7.00a
Glucose (mg/dL)	70.33±0.33 a	66.33 ±1.66 ab	62.50 ±2.50 b

Table 4. Effect of flaxseed oil dosing on the physical characteristics of blood (mean ± standard error). The values that carry different letters within the same row indicate the presence of significant differences at the probability level (p ≤ 0.05).

Treatments / Adjectives	T3 8% FO	T2 6% FO	T1 0% FO*
Total protein (g/dL)	4.86 ±0.21a	5.00 ±0.05a	4.86 ±0.63a
Albumin (g/dL)	3.86 ±0.12a	2.86 ±0.24a	3.06 ±0.52a
Globulin (g/dL)	1.00 ± 0.26 b	2.13 ±0.23a	1.80 ±0.30ab
Triglycerides (mg/dL)	50.66 ±4.37 a	58.66 ±5.92 a	58.33 ±5.45a
Cholesterol (mg/dL)	82.00±4.50 a	79.00±1.15a	87.00±7.00a
Glucose (mg/dL)	70.33±0.33 a	66.33 ±1.66 ab	62.50 ±2.50 b
Urea (mg/dL)	36.66±1.76a	43.33±5.84a	36.50±0.86a
Creatinine (mg/dL)	1.20±0.10a	1.23±0.03 a	1.23±0.08 a
AST (U/L)	68.33±11.68a	59.66±2.02a	66.00±0.57a
ALT (U/L)	19.00±1.73a	17.33±1.85a	19.66±2.18a

Table 5. Effect of flaxseed oil treatment on blood biochemical parameters in early pregnancy (mean ± standard error).

Hormonal influence

The results in Table (6) indicate that there were no significant differences (p≤ 0.05) between treatments for estrogen and progesterone, with an apparent arithmetic increase for the second and third treatments over the control treatment for estrogen and progesterone. Fats or oils prepared in food increase the concentration of cholesterol, which is the basis for synthesizing steroid hormones. And that its high level in the blood may lead to a rise in the level of the hormones estrogen and progesterone[21].

Discussion

When returning to what the sources have shown about the effect of adding oils, it can be said that dosing with flaxseed oil may have improved the hormonal response level in raising fertility. Un-saturated fatty acids significantly reduced stress, although they did not appear substantial because of the small number of animals[22].

We can say the decrease of triglycerides concentration in blood for the third treatment may be due to the effect of omega-3, which increases the absorption of large amounts of triglycerides in fat cells, on the other side while reducing the concentration of cholesterol in the blood, which limits the change in the level of sex hormones (estrogen and progesterone), because cholesterol is the main component in the synthesis of steroid hormones.

An increase in glucose level is also observed, which may be due to the high concentration of long-chain fatty acids formed in the rumen, which leads to increased production of propionic acid, which in the blood is converted into glucose.

The omega-3 found in flaxseed oil is very effective in reducing the effectiveness of liver enzymes in the blood because it contains a high percentage of omega-3, which has a significant role in reducing liver damage and thus maintaining the average ratio of these enzymes in the blood[23].

Treatments / Adjectives	T3 8% FO	T2 6% FO	T1 0% FO*
Estrogen(pg/ml blood)	6.63±0.37a	8.33±0.52a	7.50±0.75a
Progesterone (ng/ml blood)	2.33±0.24a	2.36±0.26a	2.73±0.21a

Table 6. Effect of treatment with flaxseed oil on the hormones estrogen and progesterone in blood serum (mean ± standard error). The values that carry different letters within the same row indicate the presence of significant differences at the probability level (p ≤ 0.05).

Conclusions

There is no significant indication of the importance of flaxseed oil dosing in the characteristics of weight and blood studied in the experiment. However, the ewes dosed with oil were more fertile than the treatment not dosed with oil. But it is impossible to give a definitive result because the small numbers used do not give a clear picture of the breed's fertility as important as the Iraqi Awassi sheep.

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ARTICLE / INVESTIGACIÓN

Role of ascorbic acid and appetite stimulants on a few blood serum biochemical characteristics in pregnant Iraqi ewes under heat stress

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DOI. 10.21931/RB/2022.07.04.6

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Abstract: This study evaluated the effect of VêO® premium (2 or 4)g and ascorbic acid on pregnant ewes on some minerals. Twenty Iraqi ewes are aged 2-4 years in the Fallujah, Al-Anbar Government regions from August 5th, 2019, to February 9th, 2020. The ewes were divided randomly into four groups. It was fed naturally and on one diet, and the (G1) was given VêO® premium 4 g, (G2) was given VêO® premium 2 g, (G3) was given ascorbic acid 40 mg, and (G4) was treated as the control group. Blood samples were taken monthly via the external jugular vein before and during pregnancy. The serum samples were liquated in tubes and immediately stored at -20 °C until assay for analysis of calcium, phosphorus, potassium, sodium and magnesium. The results showed the effect of VêO® Premium and ascorbic acid on calcium concentration in G2 before pregnancy. Phosphorous concentration was significantly higher (P≤0.05) in the (G1, G2, and G4) before pregnancy, but no significant difference in potassium between the groups before and during pregnancy. Sodium concentration was significantly higher (P≤0.05) in the (G1, G2, G3, and G4) before pregnancy. Magnesium concentration was significantly higher (P≤0.05) in the (G2) during pregnancy and (G3) before pregnancy. It was concluded from the current study that the addition of VêO® premium (2 or 4)g and ascorbic acid (40 mg) to the feed increased some minerals before and during pregnancy in Iraqi ewes.

Key words: VêO® premium, ascorbic acid, minerals, pregnancy, Iraqi ewes.

Introduction

Reproduction is closely related to nutrition through the level of energy and protein, especially at the beginning of puberty. There are many nutrients and minerals whose deficiency leads to reduced reproduction[1,2,3,7].

Several studies have shown the benefit of adding nutritional supplements to animal diets on fertility and that their deficiency leads to a reduction in enzyme activity affecting energy; protein metabolism; synthesis of hormones; integrity of rapidly dividing cells within the reproductive system; the activity of rumen microflora, and the role of micronutrients as depression antioxidants[16].

Minerals are found in components and metabolites of the follicular fluid that exhibit physiologic functions and chemical constituents of semen from farm animals[7,8]. These minerals also may have specific roles and requirements in reproductive tissues. The conditions of a mineral in reproductive tissue and cell type may change with the physiological state of the tissue during reproductive cycling and pregnancy[10].

Therefore, the current study aimed to evaluate the effect of VêO® premium and ascorbic acid on Iraqi ewes in pregnancy on some minerals.

Materials and methods

The experimental work was carried out in Fallujah, Al-Anbar Government regions from August 5th, 2019, to February 9th, 2020. 20 Iraqi ewes aged 2-4 years were divided randomly into four groups. It was fed naturally and on one diet, and the (G1) was given VêO® premium 4 g, (G2) was given VêO® premium 2 g, (G3) was given ascorbic acid 40 mg, and the (G4) was treated as a control group. VêO® premium supplementation components: Orange sweet, calcium carbonate, silicon dioxide, Vit. E and wheat flour (Produced by the Phodé, French). The sponges were withdrawn 14 days after they were placed, and the male was inserted for five days. Blood samples were taken monthly via the external jugular vein before and during pregnancy. The blood sample was centrifuged at (3000 RPM for 10 minutes), and the serum samples obtained were liquated in tubes and immediately stored at -20 °C until assay. Using commercially available kits, we used the serum samples to determine the concentrations of calcium, phosphorus, potassium, sodium and magnesium. A spectrophotometric analyzer was used for the mineral analytes (APLI. The kit was provided by SPINREACT, S.A./S.A.U. Ctra. Santa Coloma, SPAIN).

Analysis of variance (GLM; SPSS program/ version 25) was used to determine the effects of groups (control, VêO® premium and ascorbic acid) and mineral concentration.

Citation: A I, Mohammed Th T, Abdulateef S M, Jebril N 4 Mahmud KI. Role of ascorbic acid and appetite stimulants on a few blood serum biochemical characteristics in pregnant Iraqi ewes under heat stress. Al-Rawi F T, Abdul-Rahaman Y T, Noaman Revis Bionatura 2022;7(4) 6. http://dx.doi.org/10.21931/RB/2022.07.04.6
Received: 20 July 2022 / **Accepted:** 15 October 2021 / **Published:** 15 November 2022
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Comparison of means was carried out by the Least Significant Differences test (LSD) according to (23). Differences were considered to be significant at ($p<0.05$).

Results

Calcium and Phosphorous

The effect of VêO® premium and ascorbic acid on Iraqi ewes in pregnancy on calcium and Phosphorous are presented in Table (1). G2 before pregnancy was significant ($P\leq0.05$) in calcium concentration than during pregnancy, and no significant difference in the other groups. Phosphorous concentration was significantly higher ($P\leq0.05$) in the (G1, G2 and G4) before pregnancy. There was no significant difference before and during pregnancy (G3).

Potassium, Sodium and Magnesium

The effect of VêO® premium and ascorbic acid on Iraqi ewes in pregnancy on Potassium, Sodium and Magnesium are presented in Table (2). There was no significant difference in potassium before and during pregnancy. Sodium concentration increased significantly ($P\leq0.05$) in the (G1, G2, G3, and G4) before pregnancy. Magnesium concentration was significantly higher ($P\leq0.05$) in the (G2) during pregnancy and (G3) before pregnancy. But no significant differences in (G1 and G4). Potassium and sodium are also necessary for maintaining normal energy metabolism and reproductive physiology to reduce complications of excess sodium intake on blood pressure and keep blood pressure levels, though excess consumption of potassium can cause a problem[5,14].

Minerals	Groups	Periods	
		Before pregnancy	During pregnancy
Calcium (mmol/l)	G1	9.37 ± 0.49	9.12 ± 0.175
	G2	9.275 ± 0.58 a	8.975 ± 0.255 b
	G3	9.12 ± 0.19	9.28 ± 0.18
	G4	8.82 ± 0.23	8.995 ± 0.35
Phosphorus (mmol/l)	G1	4.28 ± 0.4 a	3.94 ± 0.52 b
	G2	5.26 ± 1.22 a	4.035 ± 0.185 b
	G3	4.3 ± 0.665	4.38 ± 0.175
	G4	5.09 ± 0.885 a	3.88 ± 0.11 b

Different superscript letters denote statistical differences in rows ($P<0.05$)

Table 1. Effect of VêO® premium and ascorbic acid to Iraqi ewes in pregnancy on Calcium and Phosphorus.

Minerals	Groups	Periods	
		Before pregnancy	During pregnancy
Potassium (mmol/l)	G1	4.28 ± 0.185	4.475 ± 0.22
	G2	4.185 ± 0.125	4.43 ± 0.165
	G3	4.015 ± 0.07	4.135 ± 0.155
	G4	4.345 ± 0.165	4.11 ± 0.105
Sodium (mmol/l)	G1	140.01 ± 3.28 a	138.125 ± 2.21 b
	G2	135.195 ± 3.085 a	134.67 ± 2.87 b
	G3	137.875 ± 4.735 a	136.125 ± 2.055 b
	G4	140.15 ± 3.9 a	135.463 ± 2.335 b
Magnesium (mmol/l)	G1	2.66 ± 0.24	2.56 ± 0.135
	G2	2.29 ± 0.12 b	2.52 ± 0.095 a
	G3	2.79 ± 0.11 a	2.54 ± 0.1 b
	G4	2.525 ± 0.19	2.64 ± 0.08

Different superscript letters denote statistical differences in rows ($P<0.05$)

Table 2. Effect of VêO® premium and ascorbic acid to Iraqi ewes in pregnancy on Potassium, Sodium and Magnesium.

Discussion

Calcium carbonate improves bone strength because of the bone calcium granules supply[22]. Calcium absorption and metabolism are regulated by calcitriol, which is the presence of ascorbic acid. It increases calcium absorption in early pregnancy to structure the skeleton of the fetus[15,19]. Calcium affects reproduction in animals through a decrease in milk production and fertility. It has also been found that low calcium affects uterine reflux, which leads to fertility problems in females[21]. Calcium ion works to suppress the immunity of the female reproductive system to regulate the movement of sperm for fertilization and implantation of the embryo in the uterus by stimulating estrogen hormone secretion[12,18].

Pregnant females need phosphorus to sustain and sustainable of pregnancy[22]. The decreased concentration of phosphorus in the stages of pregnancy is due to the stresses occurring in these stages[13]. A decreased phosphorous level in the feed leads to decreased fertility and ovarian activity, irregular estrous cycles, higher occurrence of cystic ovaries, delayed sexual maturity and reduced conception rates[4].

It may be the cause of low phosphorus during the stages of pregnancy in the current experience of its absence within VêO® premium components.

Some studies have shown that increasing potassium and sodium levels in an animal's diet can delay puberty and ovulation, impair corpus luteum development and increase the incidence of anestrous in heifers[4,21]. Gestational magnesium deficiency may cause hematological and teratogenic damage and low birth weight[9,14]. Magnesium deficiency affects ruminants because of its low in food and high potassium in green pastures, which inhibits magnesium absorption in the rumen. This deficiency affects the survival and growth of the fetus and the high rate of fetal malformations[24]. The study showed that the magnesium level was not affected during pregnancy in ewes.

Conclusions

Our study demonstrated that the addition of VêO® premium 2 g or 4 g to the diet improves some minerals before and during pregnancy in Iraqi ewes.

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ARTICLE / INVESTIGACIÓN

Chemotherapy's impact on a few blood parameters

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DOI. 10.21931/RB/2022.07.04.7

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Abstract: Cyclophosphamide (CP), Ifosfamide (IFO), Paclitaxel (PTX) and Doxorubicin (DOX) are commonly used cytostatic drugs. The present study investigates the ecotoxicity and genotoxicity of CP, DOX, and PTX, their human metabolites/ transformation products (TPs) cyclophosphamide (NCP) as individual compounds and as a mixture. The three-parent compounds (Further ecotoxicity studies of metabolites. The measured toxicity of the cross was lower than the toxicity predicted by the concentration addition model indicating potentiating effects of the CPCOOH toxicity. Revealed genotoxic activity of CP and the mixture in the presence The degradation study with UV irradiation of samples containing (CP, cyclo, and DOX) showed efficient degradation of compounds and remained toxic. They are suggesting that no stable with adverse effects was formed. This is the first study describing the ecotoxicity and genotoxicity of the commonly used cytostatics CP, cyclo, and DOX, their known metabolites, and their mixture. The results indicate the importance of toxicological evaluation and monitoring drug metabolites as they may be more hazardous to humans than parent compounds.

Key words: Paclitaxel, Doxorubicin, Cyclophosphamide, toxicity.

Introduction

The most common reason cancer patients experience low blood counts is as a side effect of chemotherapy. Chemotherapy involves the use of drugs to destroy cancer cells. Chemotherapy works by destroying cells that proliferate, a characteristic of cancer cells. Unfortunately, chemotherapy also affects normal cells that overgrow, such as cells in the bone marrow that produce red blood cells, white blood cells, and platelets.Cancer is the second leading cause of death worldwide, with approximately 9.6 million cancer-related deaths in 2018[1]. Cancer is a generic term for a large group of diseases that can affect any body part. Other terms used are malignant tumors and neoplasms. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, which can invade adjoining body parts and spread to other organs; the latter process is referred to as metastasis. Metastases are the primary cause of death from cancer[2]. In compliance with this trend of increasing cancer prevalence, new ANP drugs are also being designed, tested, and manufactured at an increasing rate[3]. More than over the past few years, 70 new ANPs (antineoplastic drugs) drugs have been released to treat 20 variants of tumors (cancerous growths), and the number of antineoplastic drugs (ANP) drugs has expanded by more than 60%. More than 500 companies are currently pursuing ANP drug development, with 300 companies having cancer drugs under clinical development stages[4], so the chemotherapy or antineoplastic agents are designed to interact directly or indirectly with DNA by damaging its structure, inhibiting, altering and disrupting mechanisms of its transcription, replication, and synthesis. Derivatives of nitrogen mustards were developed, including the DNA alkylating agents cyclophosphamide, chlorambucil, and melphalan, widely used in clinical therapeutics[6,7], and chemotherapy may be administered to cancer patients through usual routes of intravenous, intramuscular or subcutaneous injection. In general, the fate of chemical biotransformation includes the generation of metabolites, which may be desired for the therapeutic purpose or not, due to toxicity. Briefly, drug pharmacokinetics occurs in two phases of biotransformation, comprised of the functionalization (Phase I) and conjugation (Phase II) of the compound load, to increase its polarity and facilitate its elimination through excretion[8]. Phase I relies on an enzymatic system of defense against most of the xenobiotic compounds, composed of members of the cytochrome 450 gene family (CYP 450), flavin-containing monooxygenases (FMO), monoamine oxidases (MAOs), and xanthine oxidase/aldehyde oxidase. Those enzymes are differentially expressed in tissues but broadly distributed in the liver, kidney, and intestine, employing introducing, modifying, or unmasking reactive functional groups at the parent drug. and the Phase II reactions occur with the introduction of acetyl, sulfate, glucuronide acid, glutathione, and amino acids functional groups, either in the parent molecule or in a phase I metabolite structurally changed, enabling binding sites for conjugation. These reactions are mostly catalyzed by the enzyme uridine 5'- diphosphate (UDP)-glucuronosyltransferase (UGTs), sulfotransferases (SULTs), glutathione S-transferase (GSTs), and N-acetyltransferases (NATs). Biotransformation mechanisms present low specificity to pharmaceuticals and, in general, enhance the polarity of compounds, thus favoring excretion. Nevertheless, this change is often incomplete, with parent compounds excreted together with the metabolites in a variable proportion[14]. So The pharmaceuticals elected for the toxicity assessment in the present thesis were: cisplatin, cyclophosphamide, and tamoxifen. They were chosen according to a combi-

Citation: Al-Shibli Q, Al-Haidarey M. Chemotherapy's impact on a few blood parameters.Revis Bionatura 2022;7(4) 7. http://dx.doi.org/10.21931/RB/2022.07.04.7

Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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nation of 50 criteria regarding their consumption and environmental occurrence. Despite the increase in the number and quality of innovative new drugs currently released in the pharmaceutical market[5], the first drug(CP), The advent of chemotherapy fundamentals, emerged in the first four decades of the 20th century. Breakthrough advances undertaken in World War II after an accidental spill of sulfur mustards led to the marked depletion of bone marrow and lymph nodes in men exposed to those chemicals, following discoveries of potential anticancer therapeutic[9]. In this course, cyclophosphamide (CP) was developed as a mustard prodrug with cytotoxic and alkylating purposes[10]. The CP dosage commonly administered in humans varies widely depending on the clinical indication, and can be defined as low (1–3 mg kg^{-1} or 40–120 mg m-2), daily orally administered; intermediate (15–40 mg kg^{-1} or 600–1,500 mg m-2), via intravenous, every 3 to 4 weeks; and high (> 120 mg kg^{-1} or > 5,000 mg m-2), every two days. After consumption, CP undergoes subsequent activation and transformation by the CYP 450 enzymes to yield the major cytotoxic species activation, the phosphoramide mustard (PAM) and acrolein. These species form labile covalent DNA adducts and inter-strand crosslinks, accountable for blocking DNA replication and avoiding cell proliferation[11]. According to Bagley *et al.*[12], not more than 20% of injected CP is excreted intact in urine at any dose level. Chemotherapy-induced alterations and incomplete blood count may cause significant problems in clinical practice. Anemia, neutropenia, and thrombocytopenia induced by chemotherapy regimens may cause life-threatening complications such as severe infections and hemorrhagic complications. Moreover, these side effects may also necessitate dose reduction and delay in schedules of chemotherapy treatment[13], and the reason for choosing this study is to know the toxicity of chemotherapy drugs and their effect on some blood parameters (WBC, HB, PLT) Table1

Materials and methods

In this study, samples were collected from the Middle Euphrates cancer center in Al-Najaf city, Iraq collected 40 samples were from patients 20 patients treated with different types of chemotherapy-treated withe(Cyclophosphamide, DOX rubicin, paclitaxel) take the sample in (Nov and Dec -2021) Serum hemoglobin Table 6 (HGB) levels, white blood cell Table4 (WBC), platelet Table5 (PLT) count, and 20 patients considered as control (Table 1,2). And work (UV) to know the wavelength of three drugs (figure 1, 2,3)

Results

After working in UV spectrophotometer measure, the wavelength of each drug is taken urine and serum after and before taking drugs so camper between this process the rate before took drugs and this test show the percentage of drugs in the patient's body like this (figure 1) after and before taking the drugs and measure samples by (UV) to know the wavelength to three drugs.

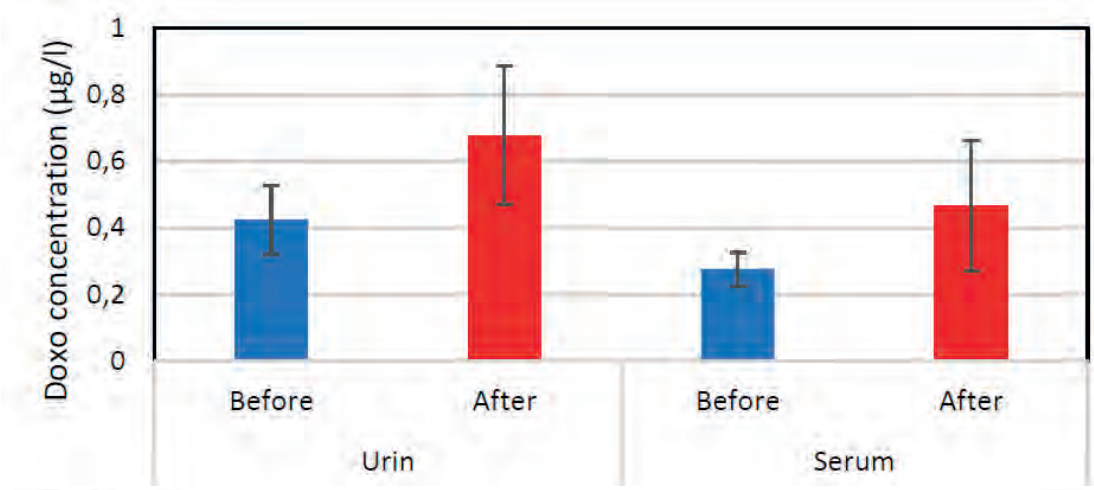

Figure 1. Shows the process of measuring serum and urine samples by (UV) spectrophotometer to know the wavelength of drugs and, after learning the wavelength according to published research, the toxicity percentage for each drug.

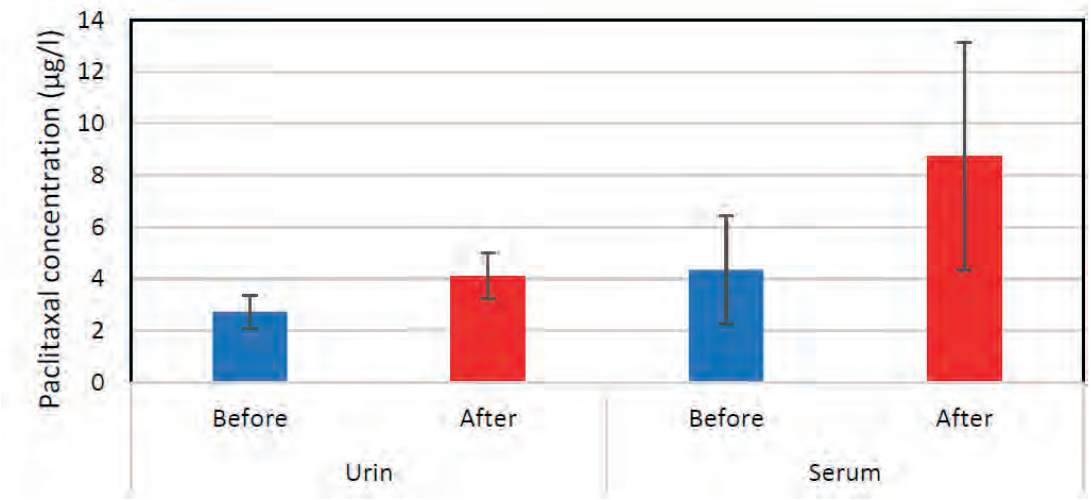

Figure 2. Paclitaxel drugs(urine, serum) after and before taking the drugs show an increased rate of drugs after taking the drug as a result of the difficulty of breaking down these compounds inside the patient's body.

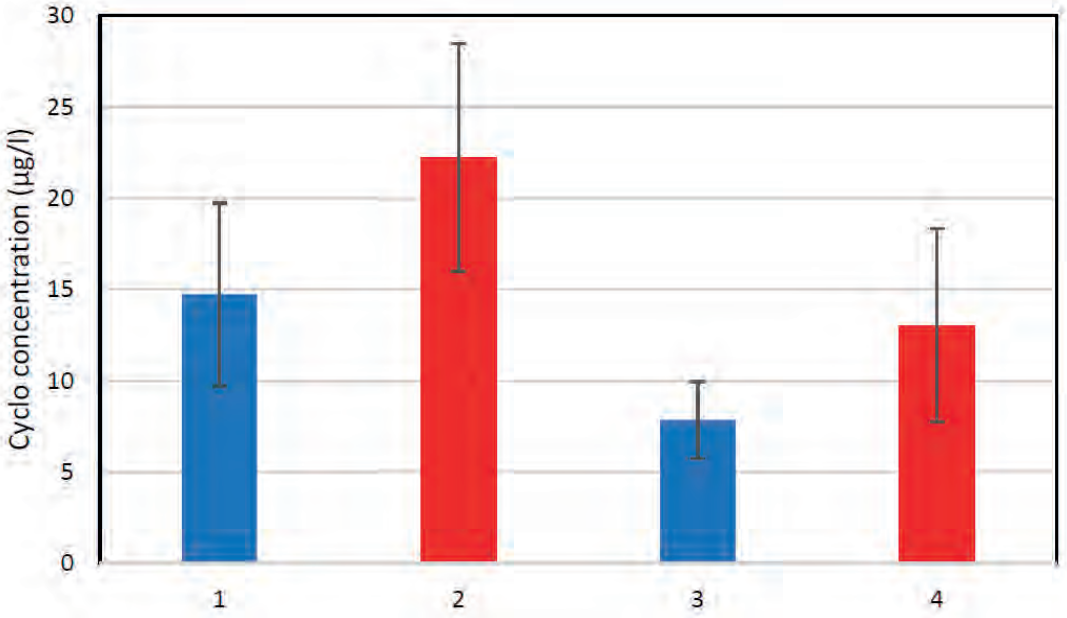

Figure 3. Cyclophosphamide drugs (urine, serum) after and before taking the drugs.

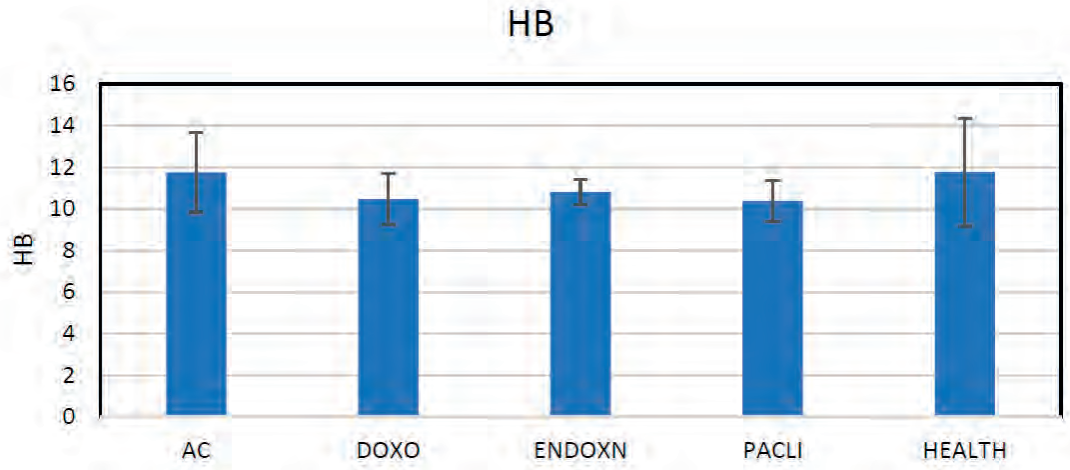

Figure 4. Shown patients receiving (AC) drugs, meaning (endoxan and DOX rubicin drugs) and influence or minimize the percentage of hemoglobin.

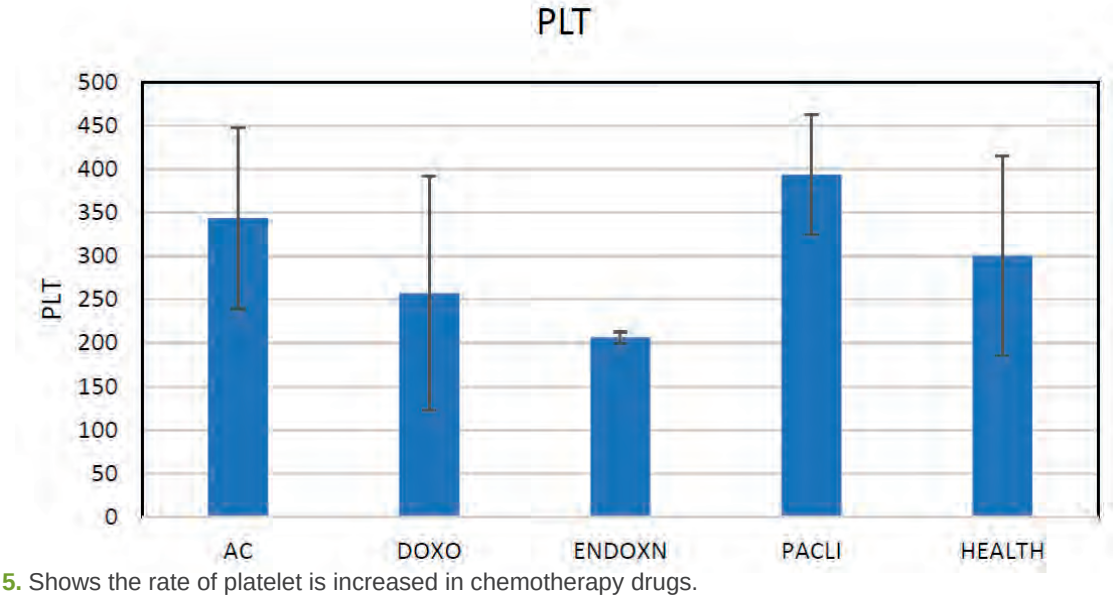

Figure 5. Shows the rate of platelet is increased in chemotherapy drugs.

ANOVA		
	Sum of Squares	Df
WBC	Between Groups	127.340
data	Within Groups	748.945
data	Total	876.285
HB	Between Groups	14.674
data	Within Groups	172.245
data	Total	186.919
PLT	Between Groups	89632.067
data	Within Groups	427438.907
data	Total	517070.974
	Sum of Squares	Df

Table 1. Explain the sum and mean square between the WBC, HB, PLT.

Descriptives								
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
WBC	AC	6	7.2500	3.41921	1.39589	3.6618	10.8382	4.40	13.90
	DOX	4	12.7100	7.59897	3.79949	.6183	24.8017	4.20	20.60
	ENDOXN	2	11.1000	1.55563	1.10000	-2.8768	25.0768	10.00	12.20
	PACLI	7	6.8314	2.47437	.93522	4.5430	9.1198	3.84	10.20
	No Medication	20	9.8700	5.01630	1.12168	7.5223	12.2177	.00	24.10
	Total	39	9.2759	4.80210	.76895	7.7192	10.8326	00	24.10
HB	AC	6	11.7500	2.17509	.88798	9.4674	14.0326	8.80	14.10
	DOX	4	10.4750	1.43149	.71575	8.1972	12.7528	8.40	11.60
	ENDOXN	2	10.8000	.84853	.60000	3.1763	18.4237	10.20	11.40
	PACLI	7	10.3714	1.05469	.39864	9.3960	11.3469	9.00	12.20
	No Medication	20	11.7600	2.66604	.59615	10.5123	13.0077	5.90	15.20
	Total	39	11.3282	2.21786	.35514	10.6093	12.0472	5.90	15.20
PLT	AC	6	344.0000	106.75205	43.58134	231.9706	456.0294	213.00	509.00
	DOX	4	257.5000	155.44024	77.72012	10.1599	504.8401	26.00	352.00
	ENDOXN	2	206.5000	9.19239	6.50000	123.9097	289.0903	200.00	213.00
	PACLI	7	393.8571	74.22809	28.05558	325.2076	462.5067	307.00	527.00
	No Medication	20	300.3500	118.06121	26.39929	245.0957	355.6043	92.00	545.00
	Total	39	314.6410	116.64961	18.67889	276.8276	352.4545	26.00	545.00

Table 2. Explains the descriptives statistics between the three drugs and other drugs, not chemotherapy.

Discussion

The Ministry of Health should import cancer treatments from internationally accredited companies. With this study, it was possible to analyze cancer treatments to know their effect on patients and the degree of benefit to the patient after taking medicine. It also allowed us to see the influence of antitumor drugs and to reduce them by combining them with a special diet for patients to increase their immunity. Cancer patients should take the necessary preventive measures (wear masks and do not enter crowded places. The arrangement is made By comparing these concentrations with a set of experiments. The measured toxicity of the crossover was lower than predicted by the concentration addition model, indicating potentiating effects of CPCOOH toxicity. They revealed the genotoxic activity of CP and the mixture. UV irradiation degradation study of the samples containing (CP, cyclo, and DOX) showed efficient degradation of the compounds and they remained toxic.The results of the current study were estimated, as they revealed the effect of these treatments on a decrease in WBC, as well as a decrease in PLT as a result of taking this treatment and its effect on the patient's immunity because it is classified as toxic solid chemotherapy as in the table of WBC, PLT, and HB.

Multiple Comparisons

Dependent Variable		(I) Factors	(J) Factors	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
WBC	LSD	AC	DOXO	-5.46000-	3.02956	.080	-11.6168-	.6968
			ENDOXN	-3.85000-	3.83213	.322	-11.6378-	3.9378
			PACLI	.41857	2.61115	.874	-4.8879-	5.7251
			No Medication	-2.62000-	2.18465	.239	-7.0597-	1.8197
		DOXO	AC	5.46000	3.02956	.080	-.6968-	11.6168
			ENDOXN	1.61000	4.06459	.695	-6.6502-	9.8702
			PACLI	5.87857	2.94173	.054	-.0997-	11.8569
			No Medication	2.84000	2.57067	.277	-2.3842-	8.0642
		ENDOXN	AC	3.85000	3.83213	.322	-3.9378-	11.6378
			DOXO	-1.61000-	4.06459	.695	-9.8702-	6.6502
			PACLI	4.26857	3.76307	.265	-3.3789-	11.9161
			No Medication	1.23000	3.48070	.726	-5.8436-	8.3036
		PACLI	AC	-.41857-	2.61115	.874	-5.7251-	4.8879
			DOXO	-5.87857-	2.94173	.054	-11.8569-	.0997
			ENDOXN	-4.26857-	3.76307	.265	-11.9161-	3.3789
			No Medication	-3.03857-	2.06112	.150	-7.2273-	1.1501
		No Medication	AC	2.62000	2.18465	.239	-1.8197-	7.0597
			DOXO	-2.84000-	2.57067	.277	-8.0642-	2.3842
			ENDOXN	-1.23000-	3.48070	.726	-8.3036-	5.8436
			PACLI	3.03857	2.06112	.150	-1.1501-	7.2273
HB	LSD	AC	DOXO	1.27500	1.45287	.386	-1.6776-	4.2276
			ENDOXN	.95000	1.83776	.609	-2.7848-	4.6848
			PACLI	1.37857	1.25222	.279	-1.1662-	3.9234
			No Medication	-.01000-	1.04768	.992	-2.1391-	2.1191
		DOXO	AC	-1.27500-	1.45287	.386	-4.2276-	1.6776
			ENDOXN	-.32500-	1.94924	.869	-4.2863-	3.6363
			PACLI	.10357	1.41075	.942	-2.7634-	2.9706
			No Medication	-1.28500-	1.23280	.305	-3.7904-	1.2204
		ENDOXN	AC	-.95000-	1.83776	.609	-4.6848-	2.7848
			DOXO	.32500	1.94924	.869	-3.6363-	4.2863
			PACLI	.42857	1.80464	.814	-3.2389-	4.0960
			No Medication	-.96000-	1.66923	.569	-4.3523-	2.4323
		PACLI	AC	-1.37857-	1.25222	.279	-3.9234-	1.1662
			DOXO	-.10357-	1.41075	.942	-2.9706-	2.7634
			ENDOXN	-.42857-	1.80464	.814	-4.0960-	3.2389
			No Medication	-1.38857-	.98844	.169	-3.3973-	.6202
		No Medication	AC	.01000	1.04768	.992	-2.1191-	2.1391
			DOXO	1.28500	1.23280	.305	-1.2204-	3.7904
			ENDOXN	.96000	1.66923	.569	-2.4323-	4.3523
			PACLI	1.38857	.98844	.169	-.6202-	3.3973

Table 3. The difference between the three drugs means the difference. *. The mean difference is significant at the 0.05 level.

PLT	LSD	AC	DOXO	86.50000	72.37556	.240	-60.5848-	233.5848
			ENDOXN	137.50000	91.54865	.142	-48.5492-	323.5492
			PACLI	-49.85714-	62.37989	.430	-176.6283-	76.9140
			No Medication	43.65000	52.19076	.409	-62.4144-	149.7144
		DOXO	AC	-86.50000-	72.37556	.240	-233.5848-	60.5848
			ENDOXN	51.00000	97.10201	.603	-146.3350-	248.3350
			PACLI	-136.35714-	70.27727	.061	-279.1777-	6.4635
			No Medication	-42.85000-	61.41270	.490	-167.6556-	81.9556
		ENDOXN	AC	-137.50000-	91.54865	.142	-323.5492-	48.5492
			DOXO	-51.00000-	97.10201	.603	-248.3350-	146.3350
			No Medication	-93.85000-	83.15319	.267	-262.8376-	75.1376
		PACLI	AC	49.85714	62.37989	.430	-76.9140-	176.6283
			DOXO	136.35714	70.27727	.061	-6.4635-	279.1777
			ENDOXN	187.35714*	89.89899	.045	4.6604	370.0539
			No Medication	93.50714	49.23970	.066	-6.5600-	193.5743
		No Medication	AC	-43.65000-	52.19076	.409	-149.7144-	62.4144
			DOXO	42.85000	61.41270	.490	-81.9556-	167.6556
			ENDOXN	93.85000	83.15319	.267	-75.1376-	262.8376
			PACLI	-93.50714-	49.23970	.066	-193.5743-	6.5600

*. The mean difference is significant at the 0.05 level.

Table 3. The difference between the three drugs means the difference. *. The mean difference is significant at the 0.05 level.

HB			
	Factors	N	Subset for alpha = 0.05
			1
Duncan[a,b]	PACLI	7	10.3714
	DOX	4	10.4750
	ENDOXN	2	10.8000
	AC	6	11.7500
	No Medication	20	11.7600
	Sig.		.417
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 4.506.			
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.			

Table 4. Shows the rate of HB in three drugs.

Conclusions

This study suggests that no stable compounds with adverse effects were formed. This is the first study to describe the ecotoxicity and genotoxicity of the commonly used cytostatics CP, Cyclo and DOX, their known metabolites and their mixture. The results indicate the importance of toxicological evaluation and monitoring drug metabolites, as they may be more hazardous to humans than the parent compounds.

Funding

This research received no external funding.

PLT				
	Factors	N	Subset for alpha = 0.05	
			1	2
Duncan[a,b]	ENDOXN	2	206.5000	
	DOX	4	257.5000	257.5000
	No Medication	20	300.3500	300.3500
	AC	6	344.0000	344.0000
	PACLI	7		393.8571
	Sig.		.101	.103
Means for groups in homogeneous subsets are displayed.				
a. Uses Harmonic Mean Sample Size = 4.506.				
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.				

Table 5. Different rates of PLT between three drugs.

Institutional Review Board Statement

The study was conducted according to the guidelines (or Ethics Committee) of the university of Kufa (protocol code 58799and 2021/12/16.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

At the tumors hospital in the middle Euphrates, Iraq.

Acknowledgments

In this study, three types of chemotherapy were used, which are Doxorubicin, paclitaxel and cyclophosphamide. The devices include (ultraviolet rays and centrifuges), and samples include blood, urine, and sewage.

Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ARTICLE / INVESTIGACIÓN

The impact of nitrogen on oat *Avena sativa L*. development and output under the effects of repeated cutting stress

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DOI. 10.21931/RB/2022.07.04.8

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Abstract: A field experiment was carried out during season 2018-2019 at one of the Warka fields to study the effect of nitrogen levels and the number of cuts on the growth and yield of oat grains. The experiment was carried out according to the split-plot design using a Randomized Complete Block Design (RCBD), with three replicates for each treatment. The nitrogen levels of agriculture (0, 60, 120 and 180) kg N. ha^{-1} were the main plots, as for the secondary properties, they were represented by the number of times of cutting (without a cut, one and two-time cutting). The results showed significant superiority in the level of fertilizer 180 kg ha^{-1}, on the qualities of the plant height (100.98 cm), the number of the grains (49.55 grains. $dahlias^{-1}$), dahlias number (424.7 clusters m^{-2}), stem to leaf ratio (6.71), green fodder yields (25.08 tons ha^{-1}) and the biological yield (3.667 tons of ha^{-1}). The results of the cutting showed that it was superior to the treatment of the two-time cutting on green fodder yield (22.17 tons ha^{-1}), treatment without cutting on grain yield (3.53 tons ha^{-1}) , the combination (180 kg ha^{-1} × two-time cutting) showed significant superiority on plant height (107 cm), green fodder yield (25.27 kg ha^{-1}) and the number of the grains in the dahlias (50.26 grain. $dahlias^{-1}$), also (120 kg ha^{-1} × without cutting) outperformed on the dahlias number (392.9 m2).

Key words: Nitrogen, oats *Avena sativa L*., repeated cutting stress.

Introduction

The Oats *Avena sativa L*. belongs to the family Poaceae; it was grown as a dual-purpose crop for grain and fodder; it ranks sixth among the world's cereal crops in production, comes after wheat, rice, barley, sorghum and millet. Its leaves contain a high nutritional value that includes vitamins and nutrients; it is considered an important forage crop and is palatable to animals.

In addition to owning six species scattered around the world, four of them are classified as jungles (for fodder production), while the other two species were *A. sativa* (white oat) and *A. byzantina* (red oat), they were dual-purpose: (producing feed and grain as food)[1].

The cultivation of oats is successful in various types of soils. However, it thrives in well-drained, fertile, loamy soils and tolerates soil acidity more than wheat and barley[2].

The productive efficiency of fodder and grain yield resulting from growing the crop is raised by many agricultural operations; one of these processes is the use of nitrogen fertilizers, the quantities of seeds and the appropriate planting distances, which was considered one of the critical agricultural factors, affecting the production and quality of various crops, including winter grain crops. Nitrogen is the primary nutrient that determines the production of field crops; using the right amount of seed that achieves the appropriate numerical density for the efficient investment of growth factors, with the use of adequate nitrogen levels, may lead to increased production of cereal crops[3].

The areas used for oat cultivation in Iraq are still few; Oats are often grown in irrigated areas with a moderately cold climate. In addition, oats are a winter crop that cannot tolerate excessive drought; as for its cultivation in rainy areas, he chooses sites in which the rain rate is not less than 811 mm[4]. The cutting (Mowing) has a benefit in reducing the phenomenon of recumbency, suffering from some species and the increase in the number of ribs; it provides a high amount of green fodder in the winter season, which was characterized by a lack of fodder[5]. Due to the importance of the oats crop in providing insects at a time of scarcity of fodder, this study was prepared.

Materials and methods

The A field experiment was carried out in Al-Muthanna Governorate / Warka during the season 2018-2019 to determine the effect of different nitrogen levels and the number of cutting times on the yield of green fodder and grain yield of oats, Shifa variety. Some of its chemical and physical properties in soil are shown in Table (1).

Nitrogen ($kg\ ha^{-1}$)	Cutting number			Mean
	without	One time	Two time	
0	89.36	88.49	92.92	90.26
60	97.64	83.50	91.85	91.00
120	97.86	101.15	89.97	96.33
180	94.73	101.15	107.06	100.98
Mean	94.90	93.57	95.45	
$L.S.D_{0.05}$	N	C	N×C	
	2.98	NS	4.15	

Table 1. Some chemical and physical properties of field soil.

Citation: Al-Jayashi M T, Adham A, Hanaf M. The impact of nitrogen on oat Avena sativa L. development and output under the effects of repeated cutting stress. Revis Bionatura 2022;7(4) 8. http://dx.doi.org/10.21931/RB/2022.07.04.8
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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The experiment was carried out according to the split-plot design using the Randomized Complete Block Design (RCBD) with three replicates. The nitrogen levels represented the cultivation (0, 60, 120 and 180) kg N. ha^{-1} of the main plots; as for the secondary stories, they were represented by the number of mowing times (without a cutting, one and two times cutting) and then leaving the crop to form grains.

The cutting process was carried out when the height of the plant became 35-40 cm; the experi-mental land was prepared by plowing two orthogonal plows using the inverted plow. After per-forming her tracheostomy, the soil was smoothed with disc harrows. The straightening machine settled it; the land was divided according to the design used into plots with an area of 2 × 2 m, and the number of experimental units reached 36. The planting was carried out on lines with a distance between one line and another 20 cm. The secondary plots were separated from each other (0.5 m).

Stages of Cacao Plant Production via SE

Plant height (cm):

After the plants reached the flowering stage, the size of the plants was measured using a metric ruler; from the surface of the soil to the top of the plant, ten plants were taken randomly from each experimental unit.

The ratio of the leaves to the stem

Calculated as an average number of leaves for ten stems randomly from each experimental unit.

The yield of green fodder tons ha^{-1}

According to the result of green fodder for each cutting, during randomly cut a midline (2 m) from each experimental unit, taking into account the start of the cutting process after the dew has been removed from the leaves of the plants, then weigh the fodder by electronic scale to avoid moisture loss, then adjust the weight on the basis of tons.ha^{-1}, with the entire experimental unit tampered with when taking the reading.

The grain number in the dahlia (grain Dalia^{-1})

Was calculated as the average number of grains of ten dahlias randomly selected from the middle lines.

Yield ton ha^{-1}

A measure of the weight of midline grains from each experimental unit and about based on ton ha^{-1}.

Results

Table (2) shows a significant increase with the increased levels of added nitrogen, as the nitrogen treatment recorded 180 kg ha^{-1}, and the highest plant height was 100.98 cm. Significantly superior to the rest of the other treatments, while the comparison treatment gave the lowest average plant height of 90.26 cm. The results of table (2) showed a signifi-cant effect of the interaction between nitrogen levels and the number of cutting times, the combination (180 kg ha^{-1} × two time cutting) was significantly superior to the rest of the other treatments by giving the highest average plant height (107.06 cm), whereas, the combination (60 kg ha^{-1} X one-time cutting) gave the lowest average plant height of (83.50 cm).

Green fodder yield (ton ha^{-1})

Table (3) shows significant differences in the levels of added nitrogen, the number of hay, and the interaction between them in the trait of green forage yield. The nitrogen treatment of 180 kg ha^{-1} recorded the highest gain of green fodder (25.08 tons ha^{-1}), significantly su-perior to the rest of the other treatments. In contrast, the comparison treatment gave the lowest average result of green fodder (15.86 tons ha^{-1}).

Two time-cutting treatments were significantly superior to the other treatments in the yield of green fodder. It gave the highest result for green grass (22.17 tons ha^{-1}).

The table shows the interaction between nitrogen levels and the number of weeds in the yield of green forage, the combination (180 kg ha^{-1} × two time cutting) was significantly superior to the rest of the other treatments by giving the highest average yield of green fodder; it reached 25.27 tons ha^{-1}. The combination (0 kg ha^{-1} × without cutting) showed the lowest average work of green grass, 12.64 tons ha^{-1}.

The ratio of leaves to the stem of the oat plant

Table (4) shows a significant increase with increasing levels of added nitrogen, the nitro-gen treatment of 180 kg ha^{-1} recorded a leaf-to-stem ratio of 6.71, significantly superior to the rest of the other treatments. In contrast, the comparison treatment gave the lowest av-erage leaf-to-stem percentage of 6.31.

The number of grains in the dahlia (grain dahlia^{-1})

Table (5) shows a significant increase with increasing levels of added nitrogen. In contrast, the nitrogen treatment of 180 kg ha^{-1} recorded the highest number of grains in the dahlia, 49.55 grains dahlia^{-1}, significantly superior to the rest of the other treatments. At the same time, the comparison treatment gave the lowest mean number of grains, 44.89 grains of dahlia^{-1}.

Table (5) shows a significant effect of the interaction

Nitrogen (kg ha^{-1})	Cutting number			Mean
	without	One time	Two time	
0	89.36	88.49	92.92	90.26
60	97.64	83.50	91.85	91.00
120	97.86	101.15	89.97	96.33
180	94.73	101.15	107.06	100.98
Mean	94.90	93.57	95.45	
L.S.D$_{0.05}$	N	C	N×C	
	2.98	NS	4.15	

Table 2. Effect of nitrogen levels and the number of cutting on oats plant height (cm).

Nitrogen (kg ha^{-1})	Cutting number			Mean
	without	One time	Two time	
0	12.64	17.25	17.70	15.86
60	20.98	21.14	20.39	20.84
120	20.73	21.90	24.80	22.48
180	24.48	24.98	25.78	25.08
Mean	19.71	21.32	22.17	
L.S.D$_{0.05}$	N	C	N×C	
	0.90	0.71	1.37	

Table 3. Effect of nitrogen levels and the number of cutting on the yield of green fodder for oats (tons ha^{-1}).

Nitrogen (kg ha^{-1})	Cutting number			Mean
	without	One time	Two time	
0	6.28	6.22	6.41	6.31
60	6.83	6.87	6.68	6.80
120	6.42	6.64	6.77	6.62
180	6.54	6.86	6.72	6.71
Mean	6.52	6.65	6.65	
L.S.D$_{0.05}$	N	C	N×C	
	0.21	NS	NS	

Table 4. Effect of nitrogen levels and the number of cutting on the ratio of leaves to stems of oats.

between nitrogen levels and the number of cutting times. The combination (180 kg ha^{-1} × twice cutting) significantly out-performed the rest of the treatments by giving the highest mean (50.26 dahlia grains^{-1}), while the mixture (0 kg ha^{-1} × single cutting) gave the lowest mean (43.71 dahlia grains^{-1}).

Yield (ton ha^{-1})

Table (6) show significant differences between the levels of added nitrogen and the cutting time on the yield, as the nitrogen treatment of 180 kg ha^{-1} recorded the highest product of 3.67 tons ha^{-1}, significantly superior to the rest of the other treatments. In contrast, the comparison treatment gave the lowest mean of 3.12 tons ha^{-1}. Also, the treatment without tamping was significantly superior to the rest of the other therapies in seed yield and showed the highest yield (3.53 tons ha^{-1}), while the treatment of two-time cutting gave the lowest yield (3.19 tons ha^{-1}).

Discussion

The significant increase in the height of oats Table (2) was because nitrogen is an essential element in all biological processes taking place within the plant. It dramatically affects cell division and the meristematic activity of cells. Nitrogen fertilizer leads to an increase in cell size and the speed of cell division. The increase in amino acids, including tryptophan, forms the basis for auxin construction, affecting cell division and the growth in plant height; this result agrees with (6). At the same time, the number of cutting treatments did not show a significant effect on plant height.

The significant increase in green forage yield is consistent with the findings of (7), which resulted in an increase in green forage yield in oats in the second cut over the first cut yield. Attributing this reason to increased oat root establishment and its production of more branches, hence more

Nitrogen (kg ha^{-1})	Cutting number			Mean
	without	One time	Two time	
0	3.53	3.06	2.76	3.12
60	3.26	3.34	3.14	3.24
120	3.53	3.42	3.35	3.43
180	3.81	3.68	3.51	3.67
Mean	3.53	3.37	3.19	
L.S.D$_{0.05}$	N	C	N×C	
	0.28	0.16	NS	

Table 6. Effect of nitrogen levels and the number of cutting on yield, ton ha^{-1}.

Nitrogen (kg ha^{-1})	Cutting number			Mean
	without	One time	Two time	
0	46.07	43.71	44.89	44.89
60	47.30	47.68	45.92	46.97
120	48.56	48.42	46.83	47.94
180	48.50	49.88	50.26	49.55
Mean	47.61	47.42	46.98	
L.S.D$_{0.05}$	N	C	N×C	
	0.83	NS	1.24	

Table 5. Effect of nitrogen levels and the number of cutting on the number of grains in the dahlia (grain dahlia^{-1}).

forage yield in the second cut.

The increase in the leaf-to-stem ratio of the oat plant table (4); was due to the fact that ni-trogen is an essential element in all biological processes taking place within the plant. By drastically affecting cell division, the meristematic activity of cells, the leaf surface ex-pands accordingly. Increased nitrogen will also increase the chlorophyll pigment in the leaves. Therefore, the efficiency of photosynthesis is increased, which is positively reflect-ed in the leaf area of plant[6].

The reason for the increase in the number of grains in the inflorescence in the flowering table (5) and may be attributed to the increased levels of nitrogen; the availability of nitro-gen in the growth stages of the crop raises the efficiency of the photosynthesis process, in-creasing its products as well as the chlorophyll content, leading to an increase in the number of spikelets, which form the grains and find a suitable opportunity, to reduce the incidence of abortion in the flower clusters, by reducing the state of competition On the food produced, then, the number of grains increased by delta, and these results are con-sistent with (8).

At the same time, the treatment of the number of cuttings showed no significant effect on the number of grains of dahlia (dahlia^{-1} grain). The considerable increase in yield (ton ha^{-1}) in the table (6) may be attributed to the role of nitrogen in increasing the number of flowering buds per unit area and the number of grains in the flowering inflorescence worked together to improve the grain yield. With the increase of the two components (grain and straw), the biological yield and yield increased; these results agreed with (9-11), who showed a significant increase in biological yield and yield by increasing nitrogen levels.

Conclusions

The results showed that adding the fertilizer quantity 180 kg/ha gave the highest marks, so we recommend using it with other crops. The second cutting gave the highest yield of green fodder, enhancing the crop yield value. The cutting process also clearly reduced the sluggish operations that the oat crop suffers from.

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ARTICLE / INVESTIGACIÓN

Evaluating the serum ferritin levels of COVID-19 patients from Basra

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DOI. 10.21931/RB/2022.07.04.9

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Abstract: Coronavirus disease 19, "COVID-19, "is occurred by a coronavirus called (SARS CoV-2), which causes severe infection in many infected persons. Early Identifying risk factors for this disease can significantly help manage critical cases and save patients' lives. This study aimed to assess the predictive value of the ferritin, the erythrocyte sedimentation rate ″ESR″, the C-reactive protein ″CRP ″, and white blood cell ″WBC″. Positive cases of COVID-19 were confirmed by "real-time polymerase chain reaction." From the patient's records were obtained demographic data and laboratory investigations were. According to clinical syndromes, patients were categorized into two groups, including COVID -19 patients with severe and non-severe diseases. Of 305 COVID-19 patients, they have a mean age of 42.73 ± 16.37 years, 59.01% of patients are female, and 40.99% are male. The levels of ferritin were variable in COVID-19 patients, our results revealed that18.68% had increased serum ferritin in patients, and the ESR, as well as CRP, were high in most patients; it's above the normal range. 4.91% of patients had decreased WBC, and the result showed lymphopenia in 1.96%. Neutrophils were above the normal range in 14.75% of patients, and 2.95% of patients had decreased serum platelets, a significant difference in WBC, Lymphocytes, Neutrophils and Basophils between severe and non-severe COVID-19 patients ($p<0.05$). A positive correlation was observed between the levels of ferritin and the severity of the disease.

Key words: COVID-19, Ferritin, ESR, CRP, WBC.

Introduction

In 2019, a novel coronavirus was spread in Wuhan, China[1]; subsequently, this disease spread worldwide, and WHO declared coronavirus 2019 (COVID- 19) a global pandemic in March 2020[2]. From asymptomatic infection to the development of interstitial pneumonia, the severity of disease and clinical presentation of novel disease vary, and additionally, failure in respiratory and acute respiratory syndromes[3]. Pro-inflammatory mediators are released, and the immune system is ac-tivated in this disease, which has a complex pathophysiology[4]. The cytokine storm considers the leading cause of COVID-19 mortality.

Ferritin is a representative of body iron stores, and high levels of it are related to COVID-19. Previous studies observed that increased levels of it were linked to a cytokine storm in severe COVID-19 patients, and an increase in ferritin was associated with the inter and spread of the viral in the human body as well as affected iron metabolism[5,6].

Many studies confirmed significant changes in several laboratory parameters involving WBC (Lymphocytes, Neutrophils, monocyte, Basophils), ferritin, CRP, and platelets in COVID-19 cases[7-10].

The current study evaluates the correlation of ferritin with many laboratory parameters and the severity of the disease.

Materials and methods

A retrospective study was conducted at Basra Teaching Hospital, which treats COVID-19 patients. Between 1 and 30 April 2020, this center confirmed the cases by naso-pharyngeal/throat swab specimens, and RT-PCR was used to diagnose 305 cases, ranging from18 -85 years.

A manual perusal of patients' case sheets was used to review patient medical records. A data collection checklist was used to collect data from electronic medical records on epidemiological, clinical, laboratory, and outcome measures. In addition, patient information, such as past medical history, symptoms, and indicators, as well as laboratory tests, such as CRP, ESR, serum ferritin, WBCs, and platelet count, were collected.

SPSS version 16 (IBM, Chicago, USA) was used to calculate the data. The median and percent were used to represent data and compare variables, and These statistical tests were conducted using″ Mann–Whitney U-tests″ and″ Student's t-tests″.

Results

Three hundred five patients were examined; the mean age of patients was 42.73 ± 16.37 years,182 (59.01%) were females, and 123 (40.99%) were males. 22 (7.23%) patients had severe syndrome, and 281 (92.13%) had non-severe.

Serial ferritin data shows a significant difference between both sexes ($p<0.05$). In most of the variables in table 1, no significant difference was found between male and female participants.

The levels of ferritin were variable in COVID-19 patients; our results revealed that 23.6% of patients had decreased serum ferritin and 18.68% had increased serum

Citation: Saleh A A, Waheed S. Evaluating the serum ferritin levels of COVID-19 patients from Basra. Revis Bionatura 2022;7(4) 9. http://dx.doi.org/10.21931/RB/2022.07.04.9
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

ferritin, and the levels of ESR and CRP were high than the normal range in most patients.

The blood counts showed 15 (4.91%) patients had decreased white blood cells (< $4*10^9$ g/L), and results were observed lymphopenia in 6 patients (1.96%), neutrophils were high than the normal range in 45 (14.75%) patients as in Table 2.

The results revealed that significant difference in WBC, Lymphocytes, Neutrophils, and Basophils between the disease severe and non-severe ($p<0.05$), but no significant difference between the two groups in monocyte ($p > 0.05$) in table 3.

There was a positive relation R= 0.12 between Ferritin levels and Neutrophils and a negative relation R=-0.3, R=-0.05 between Ferritin levels and Lymphocytes, monocytes respectively, as in figure 1.

A positive relationship was observed between Ferritin levels and the severity of the disease, R= 0.385, as in figure 2.

Discussion

Our results appear that female was more susceptible than male; both sexes are affected by infec-tious diseases differently. Among the differences are socioeconomic status, gender inequities, occupational exposure and a sex gap in immune responses to sex hormones. According to a study from China, males and females are at risk of contracting the disease[11,12].

In this study, ferritin level was higher in males than females, maybe due to the presence of Hepcidin; Hepcidin regulates ferritin levels and is related to sex hormones involving estrogens and testos-terone[13]. As well as high serum ferritin levels as is associated with some diseases, including insulin resistance, cardiovascular disease, and COVID-19[14-16].

In the current study, the counts of WBC were within the normal range at 78.03% of patients, in-creased at 17.05% of patients, and decreased at 4.92% of patients; leukopenia has been changed according to the severity of the disease[17]. The count of WBC was less elevated among COVID-19 patients who needed intensive care unit admission or died compared with other patients[18]. Early studies did not observe changes in eosinophils because of a relatively small number among WBCs[19]; however, our results appear that the counts of eosinophils in severe COVID-19 patients were low but not significant compared to patients in another group. This indicates the critical role of eosinophils in COVID-19 infection.

Non-severe COVID-19 infection was observed to be related to decreased counts of neutrophils, monocytes, and basophils compared to severe disease; inflammatory cell depletion occurred in patients during the recovery phase but may worsen as the disease progress. Previous studies showed the decreased count of peripheral inflammatory cells throughout the movement of it from pe-ripheral blood towards the lungs; these cells have a role in tissue repair[20] and immune defense against infection of virus[21].On the other hand, many studies reported that the differences in the count either increased or decreased in monocyte[22] and neutrophil[23] in COVID-19; levels of neu-trophil were increased on the first days of infection; in contrast, the levels were slightly reduced after the treatment[24], other researchers indicated that the count of neutrophils, could help predict severe cases of COVID-19[25].

Blood routine	*Males (n=123)*	*Females (n=182)*	*P-vale*
Serum ferritin ng/ml	243.82±226.60	104.33±140.96	0.005
*Platelets *10 ^{4}/µL*	267.93±110.82	301.83*10 ^{4}±96.21	0.85
ESR mm/h	34.01±35.87	38.46±34.89	0.46
*Lymphocytes *10 ^{9} g/L*	2.25 ± 1.20	2.59±1.06	0.53
*Neutrophils*10 ^{9} g/L*	4.63*10 ^{9}±2.36	5.18±2.33	0.53
*Monocyte *10 ^{9} g/L*	0.76*10 ^{9}±0.38	0.82±1.14	0.17
*Eosinophils*10 ^{9} g/L*	0.19±0.19	0.21±0.25	0.62
*Basophils *10 ^{9} g/L*	0.033±0.07	0.037±0.09	0.55

Table 1. Laboratory data between Males and Females COVID-19 patients.

Blood routine	Patients n(%)		
	normal range	*Increased*	*Decreased*
Serum ferritin	176(57.71%)	57(18.68%)	72(23.60%)
Platelets	222(72.78%)	74(24.26%)	9(2.95%)
ESR	136(44.59%)	169(55.41%)	---
C-reactive protein	139(45.57%)	166(54.42%)	---
White blood cell	238 (78.03%)	52 (17.05%)	15 (4.92%)
Lymphocytes	274(89.83%)	24(8.19%)	6(1.96%)
Neutrophils	239 (78.36%)	45 (14.75.%)	21(6.88%)
Monocyte	265 (86.88%)	24 (7.86%)	16(5.24%)
Eosinophils	243(79.67%)	23(7.54%)	39(12.78%)
Basophils	282 (92.45%)	23 (7.54%)	----

Table 2. Laboratory findings of COVID-19 patients.

Blood routine	Severe (n=22)	Non-severe (n=283)	P -value
White blood cell*10^9 g/L	9.16±5.71	7.65±3.14	0.045
Lymphocytes *10^9 g/L	1.48 ±0.77	2.52±1.11	0.001
Neutrophils *10^9 g/L	6.45±4.78	4.82±2.02	0.002
Monocyte*10^9 g/L	0.83±0.47	0.79±0.93	0.848
Eosinophils *10^9 g/L	0.13±0.11	0.21±0.23	0.125
Basophils*10^9 g/L	0.07±0.19	0.032±0.06	0.021

Table 3. Laboratory data between patients with severe and non-severe disease.

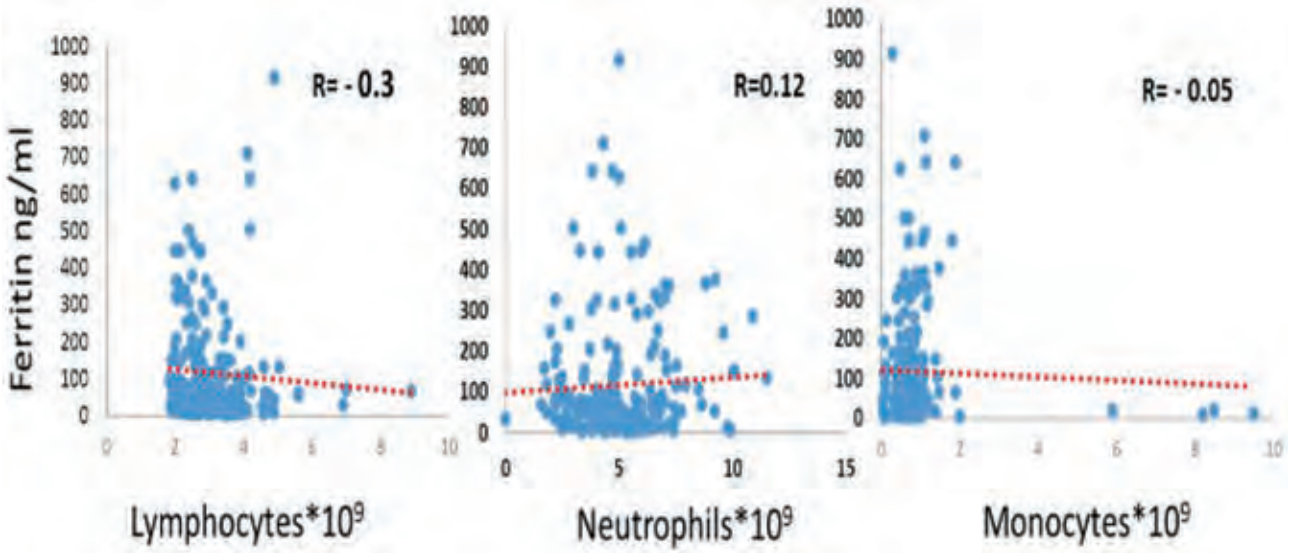

Figure 1. Correlation between ferritin and (Neutrophils, Lymphocytes, and monocyte) levels.

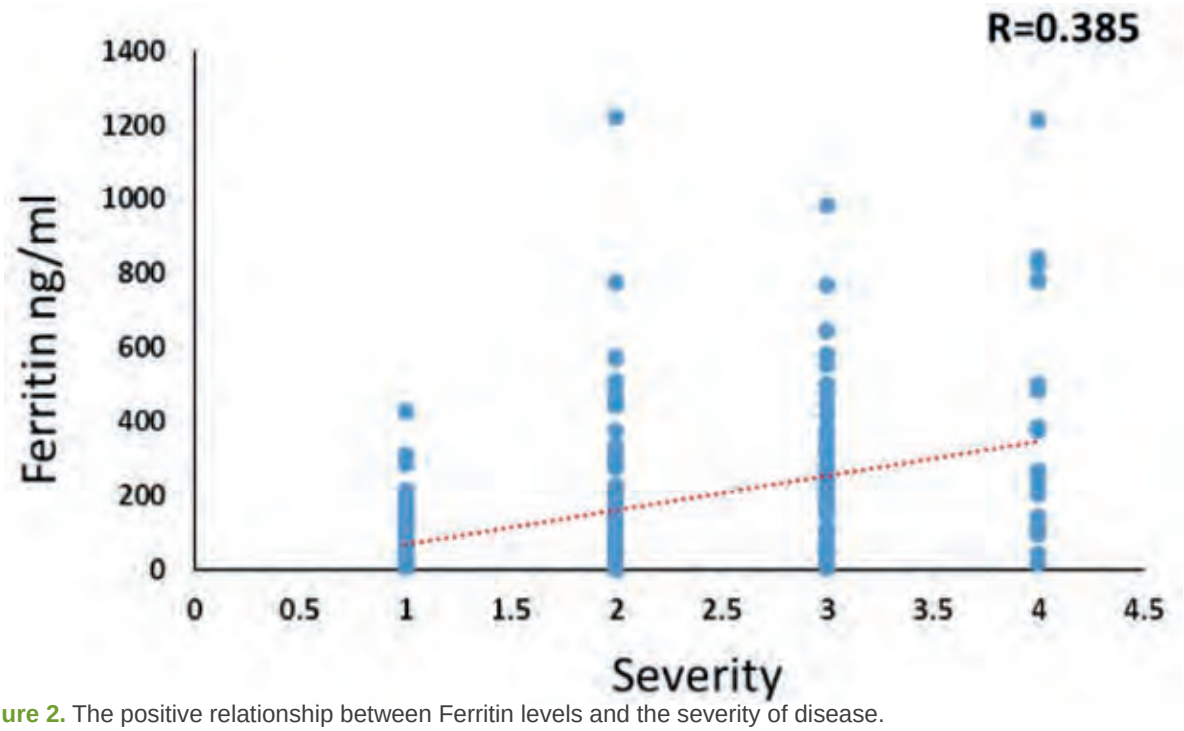

Figure 2. The positive relationship between Ferritin levels and the severity of disease.

In our study, the ferritin level was highly significant in COVID-19 patients with severe disease, and the relationship between levels of ferritin and disease severity was positive. So, the current study supports the correlation between ferritin levels and severe COVID-19. Ferritin is an iron storage protein and an inflammatory biomarker in acute and chronic inflammation. Many factors can cause an increase in the level of ferritins, such as some cytokines that stimulate ferritin synthesis and cellular damage in inflammation that leads to leakage of intracellular ferritin[26]. In this study, ferritin levels were 365 ng/mL in severe COVID-19 patients, which was above the normal ferritin range. Another study found that ferritin levels in infection with severe disease of COVID-19 were generally above the normal range[16].

Conclusions

Ferritin is correlated with the severity of the disease. Therefore, ferritin can be used for the as-sessment of the state of disease and. treatment of COVID-19 patients.

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ARTICLE / INVESTIGACIÓN

A study comparing the oncogenic microRNA-21-5p and the CA15-3 characteristics as an effective tumor marker in breast cancer patients from Iraq

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DOI. 10.21931/RB/2022.07.04.10

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Abstract: Breast cancer (BC) is a genetic disease in the mammary glands' ducts and lobules, with ductal cancers comprising most of the malignancies. Biomarkers can provide an assessment of cancer diagnosis and prediction. The study aims to compare the expression of serum (miR-21-5p) and CA 15-3 expression in the Iraqi population as more efficient biomarkers, then checked MiR-NA-21 main characters as a biomarker comparison with (CA15-3) levels. Circulating serum miRNA-21 expression was measured using (the quantitative Real Time-PCR technique) in 50 patients at various stages of breast cancer compared to 27 healthy controls. Meanwhile, CA 15-3 levels were quantified using electro-chemo luminescence immunoassay (ECLIA) methods. The results show the expression of miRNA-21 and the concentration of CA15-3 increased signifi-cantly ($p>0.01$) in patients as compared to control, but the higher median level of MiRNA-21 than of CA15-3. The ROC curve analysis shows that the accuracy, Overall Model Quality, AUC, sensitivity and specificity of miRNA-21 as a biomarker is much higher than the CA 15-3. In conclusion, miRNA-21 may fill the gap that CA 15-3 still lacks in detecting breast cancer at an early stage.

Key words: Breast cancer, microRNA-21, CA15-3, gene expression, RT-q PCR.

Introduction

Breast cancer is the most common malignancy among women globally[1]. Breast cancer is the primary cause among women and the leading cancer-related female mortality in Iraq[2]. The malignancy of the breast tissues results from the un-controlled proliferation of breast ductal and lobular epithelial cells, with ductal cancers accounting for most cases[3]. Early detection and treatment have been proven to be the most effective techniques, and early screening programs have dramatically improved BC outcomes and survival rates[4]. Many studies routinely measured serologically CA 15-3 frequently raised in BC patients[5]. There is low sensitivity and specificity of the CA15-3 marker, so we need to develop a more sensitive approach for early breast cancer diagnosis[6]. The key to delivering preventive healthcare is the clinical novel assays that enable early disease detection. Such assays are based on biomarkers such as (microRNAs) from tissue or liquid biopsy[7]. Circulating MiRNAs are emerging as new diagnostic and prognostic biomarkers for BC; there help predict tumor response to specific chemotherapeutic drugs[8]. MicroRNA-21 has been described as one of the most significantly up-regulated miRNAs in human breast cancer regardless of pre-vious exposure to chemotherapy treatment which reinforces its role as an "oncomiR" and a potential biomarker[9]. The study aims to analyze the expression quantity of serum (miR-21-5p) and compare it with one of the most widely used serum markers (CA15-3), then check sensitivity, specificity, accuracy, quality and discrimination power comparison for both miRNA-21 and CA15-3 markers.

Materials and methods

The study enrolled from November 2021 to January 2022 in the laboratories of the Baghdad university's genetic engineering and biotechnology institute. The sam-ples were collected from patients who were first diagnosed with breast cancer and consulted Al-Andalus Specialist Oncology Hospital in Baghdad and Al Anbar Spe-cialized Center for Cancer treatment.

Venous blood was taken from patients and healthy groups five milliliters (mL); all patients diagnosed with primary BC by histology were placed in gel tubes for 30 minutes at room temperature, then centrifuged for 10 minutes to obtain serum. The separated sera were divided into two tubes, one for biochemical assay and the other for molecular assay.

Biochemical assay measurement of Serum (CA15.3) concentration

The "ECLIA" electrochemiluminescence immunochemical assay technology is de-signed for the quantitative measurement of CA 15-3 in human serum, obtained from an automated quantification process using the COBAS ECLIA immunoassay analyzer (COBAS E 411) (Roche Diagnostics, Basel, Switzerland). This procedure was performed according to Elecsys CA 15-3 II ECLIA kit[10].

Protocol of microRNA extraction from the serum blood samples

Total RNA, including microRNA, was isolated from the sample according to the protocol of TRIzol™ Reagent, 0.2 mL of chloroform add to the aqueous phase containing RNA, 0.5 mL of isopropanol was added for RNA precipitated

Citation: Abbas R A R, Aziz I H. Study comparing the oncogenic microRNA-21-5p and the CA15-3 characteristics as an effective tumor marker in breast cancer patients from Iraq. Revis Bionatura 2022;7(4) 10. http://dx.doi.org/10.21931/RB/2022.07.04.10
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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as white gel-like Pellet, 0.5mL of 70% ethanol was added for RNA washing. Finally, Pellet was rehydrated in 50µl of Nuclease Free Water and then incubated in a water bath at 55–60°C for 10–15 minutes.

Reverse Transcription for complementary DNA (c DNA) synthesis

RNA sample 4 µl was mixed with 1 µl stem-loop RT primers of miR-21. The Primers for miR-21 were designed in this study using (The Sanger Center miR database Registry) and (27). The cDNAs were synthesized by reverse transcription of miRNA using a script cDNA synthesis kit, "5GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACCAACA 3".

Quantitative Real-Time Polymerase Chain Reaction (RT-q PCR)

The PCR master mix preparation is shown in table (1), and Real-Time PCR Program, and thermal cycling conditions for miR-21, are in table (2).

Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to detect the effect of different factors on study parameters. T-test used to compare between means. The diagnostic accuracy, sensitivity and specificity were performed using the (ROC) curve analysis. The cut-off values and area under the ROC curve (AUC) were then de-termined were done using (SPSS) package software version 24[11].

Results

Molecular Analyses of circulating miRNA-21 expression level

The RT-q PCR results for miR-21 were analyzed by the relative quantification of gene expression levels (folding changes) based on the (Ct) values. All patients show a high level of miR-21 level which was significantly elevated (**P>0.01) among BC patients (5.27 times increase) than healthy control (1.00) Table (3).

Master mix components	Volume (µl)
SYBR Green Master Mix	5
Forward primer	0.5
Reverse primer	0.5
Nuclease Free Water	3
miRNA-21 cDNA template	1
Total volume	10
Aliquot per single rxn	9µl of Master mix per tube and add 1µl of Template

Table 1. The PCR master mix preparation for miR-21.

Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	**1**
Denaturation	95	00:20	**50**
Annealing	55	00:20 Acquiring on Green	
Extension	72	00:20	

Table 2. Real-Time PCR Program.

Descriptive Statistics	Breast Cancer	Healthy Control
C_t mature miR-21	31.13 ±4.05	33.39 ±3.63
C_t RNU- 43	20.53 ±2.91	20.39 ±3.08
Relative expression $\Delta C_t = (C_{t\,21} - C_{t\,RNU})$	10.60 ±1.07	13.00 ±1.59
Relative expression (2^-Δ Ct)	6.4429 E-0.4 ± 0.00083	1.2207 E-0.4 ± 0.000077
Experimental / control	6.4429 E-0.4 / 1.2207 E-0.4	1.2207 E-0.4 / 1.2207 E-0.4
Median Fold change	5.27803 ± 0.93	1.00 ± 0.00
P-value		(0.0001**)
The Statistical analyses		** (P≤0.01)

Table 3. Comparison expression of miRNA21 between both study groups.

Biochemical analyses of CA 15-3 concentration Level

The mean ± SEM of the serum CA15-3 concentration was (23.17 ± 0.78 U/ml) in the BC group and (9.15 ± 0.70 U/ml) in the control group shown in Table 4.

The significant increase in the mean values of CA15-3 concentration in breast cancer patients for (2.36 times increase) compared to control groups that gave a high signifi-cantly statically analysis as (**p>0.01) in BC patients.

The Diagnostics Performance of miRNA-21 marker in the Studied Groups

Receiver operator characteristics curve (ROC) analysis of miRNA-21 in the serum of breast cancer patients recorded high sensitivity (96%) and specificity (92.6%) at a cut-off value of (1.04) with high AUC values as (0.981) in discriminately the breast cancer pa-tients. Table (5) & Fig. (1).

The Diagnostics Performance of CA15-3 marker in the Studied Groups

Receiver Operator of Characteristics (ROC) curves analysis of serum CA 15-3 was found with low sensitivity and specificity of (72% and 70.4%) respectively, at the low area under the curve (AUC= 0.563) and already known cut-off (25 U/mL).

Comparison of miRNA-21 and CA15-3 and their diagnostic accuracy and discrimination power (AUC)

The calculation of diagnostic accuracy for each marker and the value of area under the curve (AUC) as a discrimination power for the selected miRNA-21 compared to the AUC value of the traditional BC biomarker CA15-3 by using receiver operating characteristic (ROC) curve analyses that show in the table (7).

Descriptive Statistics	The serum concentration of CA15-3 (U/ml)	
	BC patients	HC groups
Mean ± SEM	23.17 ± 0.78	9.15 ± 0.70
median fold change	2.365 ± 0.74	
P-value	** (0.0001)	
The Statistical analyses	** (P≤0.01)	

Table 4. Mean serum concentration of CA15-3 between study groups.

Parameters	Cut off value	Sensitivity	Specificity	Area under the curve (AUC)
miRNA-21 (2 – ΔΔCt)	1.04	96 %	92.6 %	0.981

Table 5. ROC curve analysis of miRNA-21 to distinguish between BC and HC.

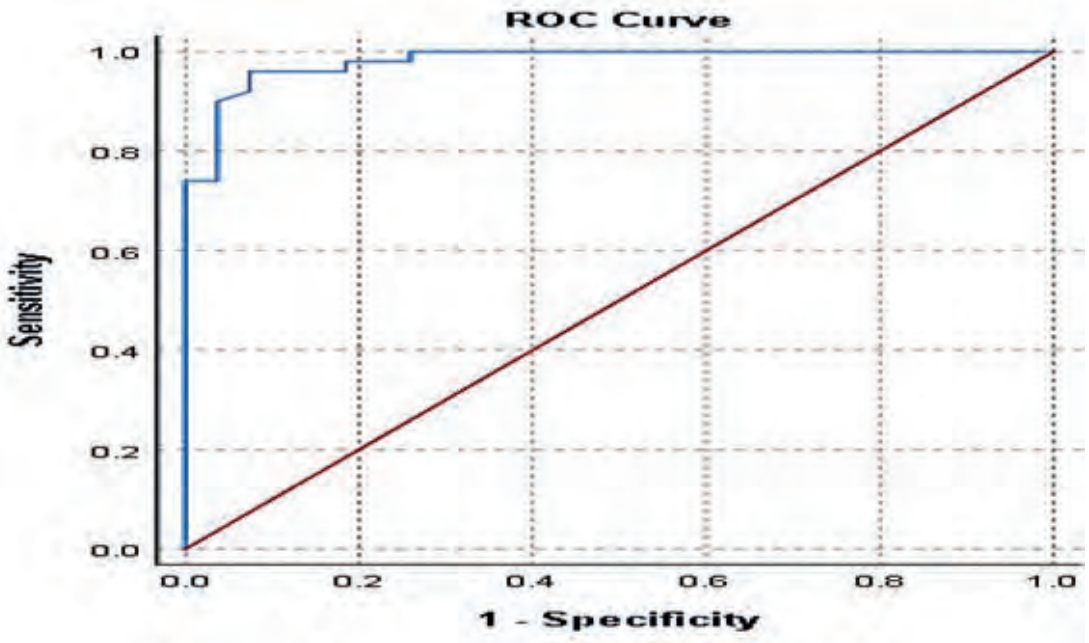

Figure 1. MiRNA-21 Sensitivity and Specificity by ROC curve.

Parameter	Cut off value	Sensitivity	Specificity	Area under the curve (AUC)
CA15-3 (BC with control)	25.1	72 %	70.4 %	0.563

Table 6. ROC curve of CA 15-3 in distinguishing between breast cancer and healthy sub-jects.

The diagnostic accuracy of both biomarkers was calculated; among the analyses molecules, the miR-21-5p marker showed the highest diagnostic accuracy (0.94) than the low diagnostic accuracy (0.71) of the CA15-3 marker.

The Statistical analyses were carried out using (ROC-AUC) curve to determine the discrimination biomarkers power of both markers to discriminate BC patients from HC groups; we compared the area value under the curve (AUC) of both markers. The results show that the miRNA-21 had sig-nificantly higher AUC values of discriminately the BC group (0.981), while a lower AUC of CA15-3 is recorded only (0.563). Show in table (7) & fig. (3) & fig. (4).

Comparison of miRNA-21 and CA15-3 Overall Model Quality

The Overall Model Quality of tumor biomarkers is represented in the chart that generally measures the model quality of molecular miRNA-21 and biochemical CA15-3 biomarkers samples and median expression level in breast cancer group folds in all study groups (patients and control).

The result of MiRNA-21 model quality was significantly higher at (0.95) than CA15-3 with (0.70), shown in tables (8) and (9) & figure (5).

Discussion

The results of the present study show that microRNA was successfully extracted from the serum samples of the patient and control groups. Statistical analysis revealed a significant increase in miRNA-21 expression in the serum of BC patients; this was higher than in standard breast samples. This evaluation of miRNA-21 makes it act as a diagnostic indicator to discuss its role as a serum marker in BC diagnosis and treatment monitoring. This result agrees with many novel studies[10,12].

Several studies have the same result mention the primary underlying mechanism for the connection of miRNA-21 and BC is the location of the miRNA-21 gene on chromosome 17q23.2. This region is frequently amplified in BC and correlated with high expression of miRNA-21, the miRNA expression regulated by epigenetic machinery. Hypo-methylated of CpG island in the promoter region of mature miRNA-21 sequence in BC causing up-regulation of MiRNA-21 ex-pression[13,14].

Many studies are similar to the present result. A significant up-regulation of MiRNA-21 in the BC group as an oncogenic microRNA. This is due to its ability to promote tumor growth, invasion, angiogenesis, and metastasis by targeting and suppressing several apoptotic and tumor suppressor genes in post-transcriptional, including PDCD4, PTEN and TP53[15-17].

The diagnostic performance of miRNA-21 was studied by analysis (ROC curve) which showed that circulating mature miRNA-21 has high sensitivity and specificity. This made it a su-perior indicator of the high-risk group in the early phase of breast cancer screening and was con-sidered an effective marker in breast cancer patients compared to the healthy control group.

The same result shown in Iraqi studies mentions the high

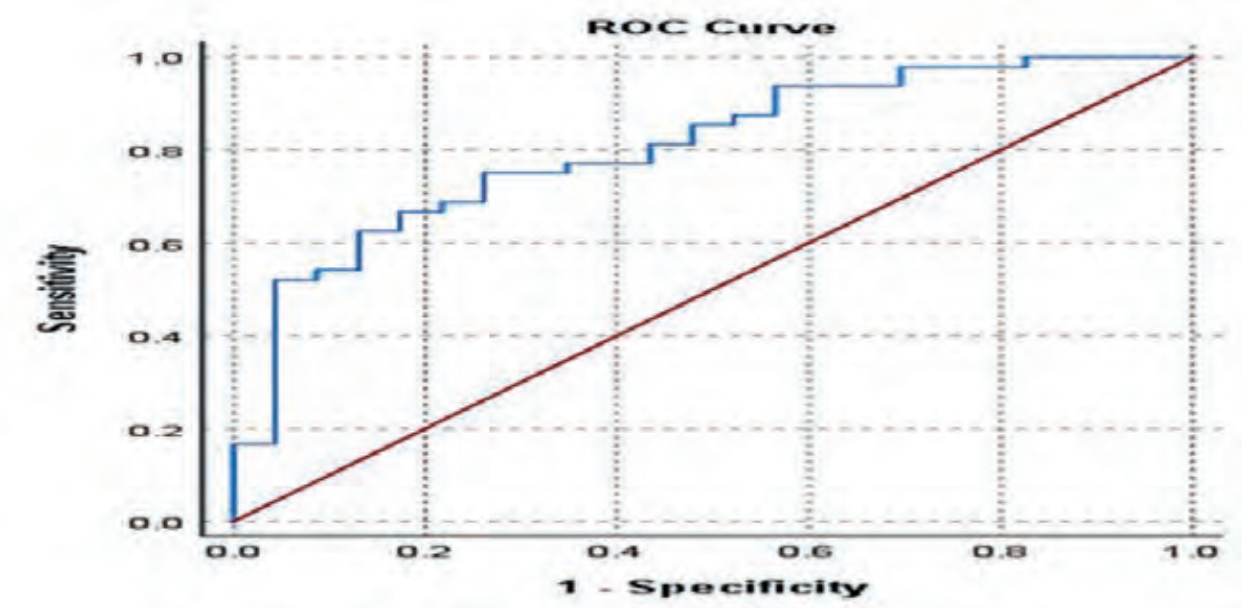

Figure 2. CA 15-3 Sensitivity and Specificity by ROC curve.

Parameters	Average diagnostic accuracy	Discrimination power (AUC) ± SEM	P-value	Statistical analyses	Interpretation
miRNA-21 (2 – ΔΔCt)	94 %	0.981 ± 0.0121	0.0001**	**(P≤0.001)	(0.94) Excellent
CA15-3 (U/mL)	71 %	0.563 ± 0.0345	0.070	NS	(0.71) Good

Table 7. Diagnostic accuracy and AUC of both circulation biomarkers.

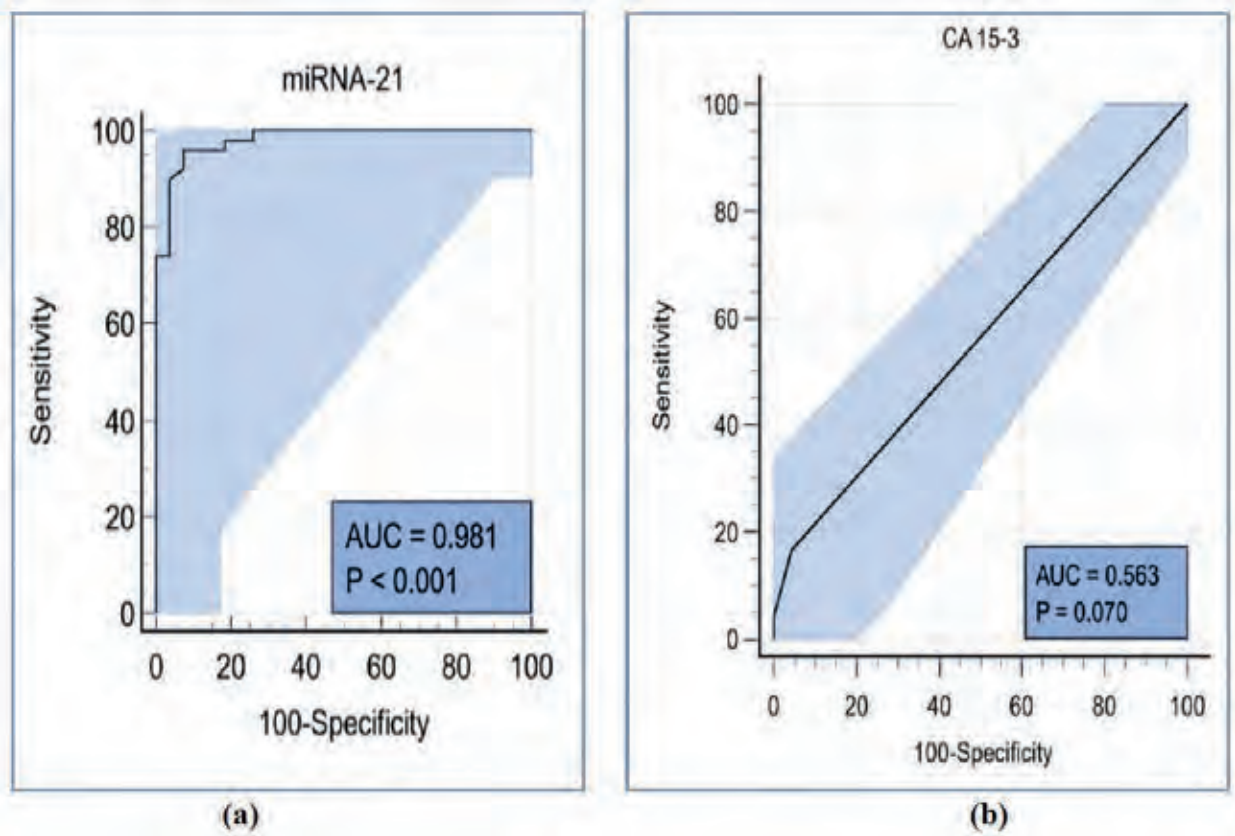

Figure 3. This figure includes: (a) the AUC value of MiRNA-21-5P ; (b) the AUC value of CA 15-3

Variable	miRNA-21 N. Frequency	Valid Percent	CA 15-3 N. Frequency	Valid Percent
Marker Sample size	77 samples	100%	77 samples	100%
Process group	77	100%	71	92%
TP group	50	(64.94%)	48	(67.61%)
TN group	27	(35.06%)	23	(32.39%)
Missing group	0	(0 %)	6	(8.45%)

TP: True Positive, BC patients +, TN: True Negative, BC patients –

Table 8. Statically database for study samples processing summary.

Parameters	PPV- samples %	NPV- samples %	Overall Model Quality %
MicroRNA-21-5P	96.5 %	92.5 %	94.5 ≈ (95%)
CA 15-3	81.8 %	57.6 %	69.7 ≈ (70%)

PPV: Positive predictive Value, NPV: Negative predictive Value.

Table 9. Statically database for sample model quality in both biomarkers.

sensitivity and specificity of rel-ative expression of circulating miRNA-21 in the BC patients compared with healthy control group[17,18]. Their agreement with the Iranian study showed higher sensitivity and specificity values for microRNA-21[19].

CA 15-3 expression on the luminal surface of the normal glandular breast secretary epithe-lium and its expression and secretion are increased with malignant cell transformation. A novel Iraqi study mentioned that a high CA15-3 concentration is an indicator to help physicians assess breast cancer disease progression and determine adjuvant treatment for a better outcome when CA 15.3 concentrations are elevated. During the early course of therapy, this is due to disease pro-gression or ineffective treatments[20].

This observation result agrees with several novel Iraqi studies that found higher serum of CA 15-3 levels is more likely to have breast cancer[1,17]. This high concentration level of CA15-3 is similar to several new studies making it a predictive, diagnostic and prognostic biomarker[21-23].

The diagnostic performance of serum CA 15-3 in the present study by ROC curve shows low sensitivity and specificity to easy detection of BC patients of newly diagnosed, with these common characteristics making it not enough

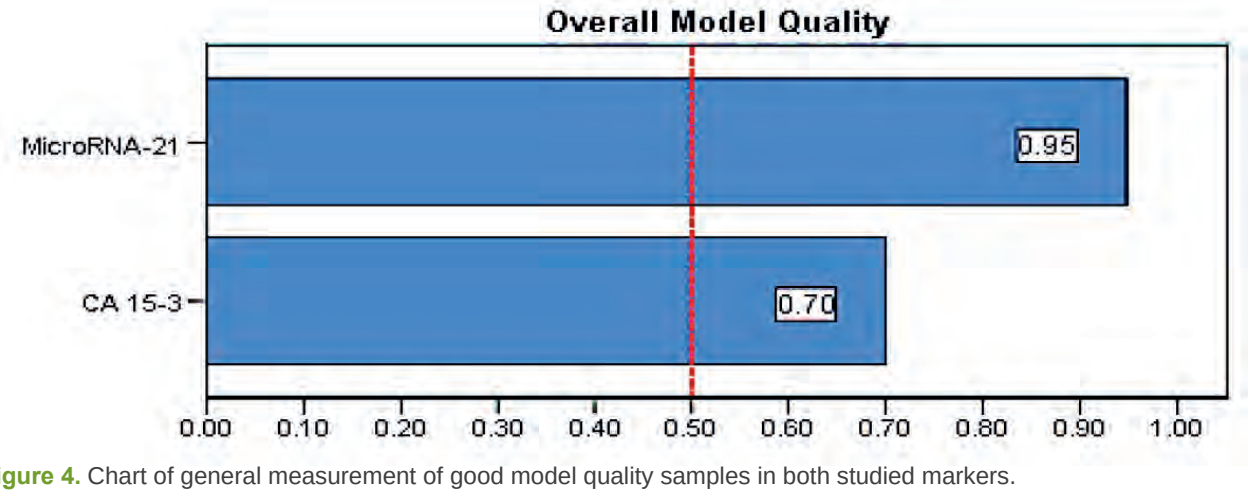

Figure 4. Chart of general measurement of good model quality samples in both studied markers.

used at early diagnosis of breast cancer stages.

The present study result regarding novel Iraqi summary studies shows the ROC curve yields low sensitivity of CA153, merely 22.47%[18]. The same line with a new study in 2021 showed that the low value of the sensitivity of CA15-3 serum marker in BC patients was only (59.06%)[24].

Many reports documented that CA 15-3 with low sensitivity is not recommended as a screening tool for early detection of BC and remains an important asset to monitor the efficacy of medical therapies[25].

The diagnostic value of CA15-3 is relatively low specificity, with increased serum values that can be detected in the presence of other neoplasms, such as lung, liver, pancreatic, and ovarian cancers, making it lack specificity[26].

The static analysis of diagnostic accuracy for both biomarkers was calculated, showing that the miR-21-5p marker has the highest diagnostic accuracy than the CA15-3 marker.

The statistical analyses were carried out by (ROC-AUC) curve to determine the discrim-ination biomarkers power of both miR-21 and CA15-3 markers by calculation of the value of area under the curve (AUC), showing that miRNA-21 has a more comprehensive and more meaningful AUC than CA 15-3.

This result agrees with the Iraqi study found high folding change expression of serum miR-21and high specificity and sensitivity has high accuracy (100%) with excellent (100%) Interpretation[27].

In Iran new (ROC-AUC) curve analyses study reported miR-21 was valuable in higher distinguishing power (higher AUCs) of early breast cancer disease and recurrent breast cancer (82% for early diagnosis and 86% for recurrent) respectively than the average healthy control group[19]. Also, it is similar to the Italian study, mentioning high miR-125 AUC was (85%) able to discriminate BC patients from healthy donors than the traditional BC biomarkers CA15-3 only (70%) and showed a high diagnostic accuracy of miRNA-125 (79%) than accuracy in CA15-3 (68%)[28].

A similar result in a study identified the type of tumor, whether malignant or not, providing a possible result in terms of AUC and accuracy in the more challenging case of breast cancer. When using augmented data sets, their area under the curve (AUC) reached (92.9%) with accuracy (96.7%) of case BC[29].

The overall model quality can be considered a "good model" when the correct prediction rate for positive responses meets the specified minimum probability. Overall, Model Quality was good model when its value was above (0.50), but a value less than (0.50) indicates the model is no better than random prediction.

According to the present quality result, the circulating miRNA-21 with high model quality was considered an efficient blood circulation sample used in molecular analysis to the diagnostic, predictive and prognostic marker of breast cancer patients than healthy controls.

The novel study has the same result mentioning tumor biomarkers are sample molecules that are measured in tissue and other body fluids, being considered efficient blood circulation samples that can predict the risk of getting cancer (predictive biomarkers) and signal early stages of cancer (diagnostic biomarkers) and thus assess the risk of cancer progression or possible response to therapy (prognostic biomarkers)[22].

Conclusions

The statistical analyses of miRNA-21 expression and CA 15-3 levels were significantly in-creased in the BC group compared to the control group. However, CA15-3 had a lower median concentration level (2.3 times) than miRNA-21 (5.2 times), making the miRNA-21 a superior marker for detecting the high□risk BC group at the early BC diagnosis stage.

The ROC curve was plotted for the investigated markers, and a cut-off point was detected that miRNA-21 had higher sensitivities, specificities, diagnostic accuracy, discrimination power AUC and diagnostic overall model quality than the tumor marker (CA15-3), making the MiRNA21 a more vivid diagnostic, predictive and prognostic breast cancer marker compared to CA 15-3 biomarker.

The higher characteristics of circulating mature miRNA-21 make it a practical test and po-tential diagnostic indicator to compare BC patients with healthy controls. Thus, their usefulness as noninvasive markers helps minimize the unnecessary breast biopsies used for the early detection of breast cancer.

Funding

Self-funding

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

https://gco.iarc.fr/today/home,

http://atlasgeneticsoncology.org/,
https://www.ncbi.nlm.nih.gov/gene .

Acknowledgments

We acknowledge any person who supports me in completing this project.

Conflicts of Interest

The authors declare no conflict of interest.

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ARTICLE / INVESTIGACIÓN

Biological and chemical control of *Ectophoma multirostrata* causing root-rot and seedling death of *Celosia argentea* in Karbala/Iraq

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DOI. 10.21931/RB/2022.07.04.11

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Abstract: This study was conducted in the College of the Agriculture/University of Karbala to control the fungus *Ectophoma multirostrata* that causes root rot of *Celosia argentea* by using *Azotobacter chrocooccum*, Salicylic acid and the chemical pesticide Beltanol. The pathogenic *E. multirostrata* was isolated for the first time in Iraq and showed a reduction in seed germination by 16.66% and 16.00%. The results showed that the bio-control bacteria *A. chrocooccum*, Salicylic acid and Beltanol effectively reduced the infection rate and severity of Celosia argentea root rot disease and increased the growth parameters. Among the treatments, Beltanol was the highest in reducing the infection rate and severity down to 0.00%, followed by the treatment of integration between *A. chrocooccum* and Salicylic acid to minimize infection and severity to 16.33% and 8.00%, compared to the infected untreated that showed 80%, 62.00% respectively. In addition, the *A. chrocooccum* and Salicylic acid integration improved plant growth, including shoot length, shoot and root dry weight to be 22.50 cm, 0.423 g and 0.133 g, compared to the untreated infected treatment that resulted in 5.00 cm, 0.090 g, and 0.003g, respectively.

Key words: *Celosia argentea*, *Ectophoma multirostrata*, *Azotobacter chrocooccum*, Root rot.

Introduction

Celosia argentea is exposed to several pathogens, such as leaf spot, root and stem rot, blight and root knot nematodes[1]. Root rot and seedling death are among the most important of these diseases, as they pose a significant threat where the damage begins indistinguishable underground. The disease can't be controlled primarily when the symptoms appear on the upper part of the plant, usually combined with advanced stages of injury. Symptoms associated with root rot diseases are the transformation of roots color to brown with soft affected tissues; they become tender and decomposed. The spots on the roots vary in number, size and color - from reddish to brown and black, with root splintering. The infected plants show leaf yellowing, plant stunting and low yield[2,3]. The progression of root disease pathogens depends on the availability of favorable conditions or recurrent and other factors that contribute to plant stress. Many soil-borne pathogens can cause these diseases, some of which are host-specific and others are of a broader range of plant hosts[4]. *Ectophoma multirostrata* was reported as a cause of root rot disease on a limited number of plants and was recorded on chickpeas in India[5]. Because of the importance of this disease and the absence of previous studies in Iraq on root rot disease on *Celosia argentea*, and to reduce the damage caused by chemical pesticides, the research was conducted to evaluate using safe control methods such as bacteria, *Azotobacter chroococcum* and Salicylic acid in comparison to chemical pesticide Beltanol.

Materials and methods

The fungi accompanying the roots of *Celosia argenta* were isolated, which had symptoms of weak growth, yellowing of the vegetative system and rotting of the root system. Infected samples were collected from some plant nurseries located in Karbala Province. The roots were washed well, cut into small pieces (1-1.5 cm), sterilized with sodium hypochlorite solution, transferred to Petri dishes containing more dextrose (PDA) medium, and incubated at 25 ± 2°C for three days. Fungi were purified and initially diagnosed based on phenotypic traits using the taxonomic keys described previously[6-8]. In addition to their molecular diagnosis in a previous study by analyzing the sequences of the nitrogenous bases of the DNA products by PCR for selected genetic markers and using the BLAST (Basic Local Alignment Search Tool) program, these isolates from the roots of *Celosia argentea*, are *Ectophoma multirostrata* (Ec2) and are registered in the NCBI under accession number ON025673[1].

Pathogenicity of fungal isolates on red radish seeds on Water Agar media

The pathogenicity of the three isolates of the fungus was tested (Table 1). The fungi were isolated from the roots of infected *Celosia argenta* plants by using plastic plates[9] and using a water agar medium. The susceptibility of radish seeds to infection with fungal isolates was tested in comparison to the control treatment based on the percentage of germination[2]. As well as calculating the percentage of inhibition following Abbott's (1925)[10] equation[11].

Citation: Sheehan, S. J.; Abdalmoohsin, R. G. Biological and chemical control of Ectophoma multirostrata causing root-rot and seedling death of Celosia argentea in Karbala/Iraq. Revis Bionatura 2022;7(4) 11. http://dx.doi.org/10.21931/RB/2022.07.04.11
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

Pathogenicity of fungal isolates on *Celosia argentea* seeds in plastic pots under greenhouse conditions

This experiment was carried out by mixing soil mixture with peat-moss (1:2) and sterilizing it using an autoclave at a temperature of ˚121°C and a pressure of 15 psi/ing2 for 60 minutes. Then the soil was inoculated with the fungus-bearing millet seeds (*Panicum miliacem*) at a percentage of 1% for 48 hours[12], then planted with *Celosia argenta* seeds at a rate of 10 seeds/pot and watered carefully as needed. The germination rate was calculated 30 days after planting.

Effect of the three experimental factors on root-rot fungus *E. multirostrata*

Antagonistic of *Azotobacter chroococcum* to *E. multirostrata* on PDA culture medium was evaluated. Several dilutions of *A. chroococcum* were used to determine the best concentration against the pathogenic fungus *E. multirostrata*, and the percentage of inhibition was calculated[13]. The efficiency of fungicide Beltanol against *E. multirostrata* in PDA was also evaluated at three concentrations, including the recommended by the manufacturer, 0.5 and 0.75% of the recommended concentration. The percentage of pathogen inhibition was determined. Salicylic acid at 0.5, 1.0, and 1.5 $g.L^{-1}$ was also tested against *E. multirostrata* on PDA culture media and the percentage of inhibition was calculated after seven days of inoculation.

A greenhouse pot experiment

The efficiency of *A. chroococcum*, salicylic acid and Beltanol and their integration was evaluated in controlling *E. multirostrata* on *Celosia argentea* under greenhouse conditions.

The inoculum of the pathogenic *Ectophoma multirostrata* loaded on millet seeds was added to the plastic pots at a rate of 1% / pot and with three replications for each treatment. In contrast, the bacterial biological agent *Azotobacter chroococcum* was added to the pot at a rate of 10 ml/pot. The chemical pesticide Beltanol was added at a concentration of 1 ml/liter in addition to a treatment A comparison in which the seeds were sown without addition and with a number. After 48 hours, the seeds were planted in the soil, while salicylic acid was added ten days after the emergence of seedlings. Nine treatments were implemented as the factors were used single and overlapping with the presence of a comparison treatment. After 60 days of applying the experiment, the infection rate was calculated. The pathological key of 6 degrees (0-5) was used to assess the severity of root rot disease[2,14]. Infection severity was also calculated according to Mckinney equation (1923)[15], following Jaber (2020)[11] and Dkhyl (2021)[2]. At the end of the experiment, the plants of each treatment were uprooted to measure shoot length, fresh weight and dry weight.

Treatments and experimental units were distributed as a Completely Randomized Design (CRD) with three replications as a one-factor experiment. Data analysis, analysis of variance ANOVA, and the Least Significant Difference (LSD) among treatments were performed using the GenStat program, 10th edition.

Results and discussion

Three isolates of *Ectophoma multirostrata* were obtained from plants that showed symptoms of infection (Fig. 1-C), as they were characterized by their formation of olive-brown fungal colonies(Fig.1-B). Microscopic examination showed the presence of a divided mycelium, as well as the presence of single-celled oval conidia (Fig. 1-A) and spirochetal C. The shape is dark in color; these results are consistent with what was found by Chobe *et al.* (2020)[5] and Kashyap *et al.* (2022)[16], who isolated this fungus for the first time in the world and recorded it as a cause of root rot and seedling death of chickpea seedlings.

Testing the pathogenicity of the fungi isolated in this study

Pathogenicity of fungal isolates on red radish seeds on Water Agar media

The results (Table 2 and Figure 2) showed that all tested isolates of mushrooms led to a significant reduction in germination percentage, compared to the comparison treatment in which the seed germination ratio was 100%. The other isolates led to a reduction in the rate of seed germination to 16.66%. The isolates varied among themselves in the decrease in germination percentage, which may be attributed to genetic differences within the same species collected from different regions or differences in their ability to secrete pectin- and cellulose-degrading enzymes in the early stages of infection. Enzymes such as pectinase, phosphatase, cellulase, methyl esterase, and methyl hydrolase are involved in host penetration, which significantly influences the pathogenicity of the fungus, in addition to the ability of these fungi to produce some toxins of phenolic and glycoside nature[2].

Three isolates of *Ectophoma multirostrata* were obtained from plants that showed symptoms of infection (Fig. 1-C), as they were characterized by their formation of olive-brown fungal colonies(Fig.1-B). Microscopic examination showed the presence of a divided mycelium, as well as the pres.

Pathogenicity of fungal isolates on *Celosia argentea* seeds in plastic pots under greenhouse conditions

The results (Table 3) indicated that the addition of isolates of the fungus *Ectophoma multirostrata* led to a reduction in the germination of seeds compared to the comparison treatment, which had a percentage of germination, which was 100%. The seed germination rate is 16.00%, and the percentage of inhibition is 84.00%, followed by a difference in the Significance of isolate Ec1, which reached 20.2% and 79.8%, respectively. Based on these results, and agreeing with the previous studies[17] isolate, Ec2 was chosen for use in subsequent experiments.

Location of collection	Isolate symbol (abb.)	Location of collection
Karbala-Hussainiya	Ec1	Karbala-Hussainiya
Karbala-Alhur	Ec2	Karbala-Alhur
Karbala-Ibrahimiya	Ec3	Karbala-Ibrahimiya

Table 1. Fungal isolates from *Celosia argenta* infected roots and dead seedlings collected from different Karbala areas.

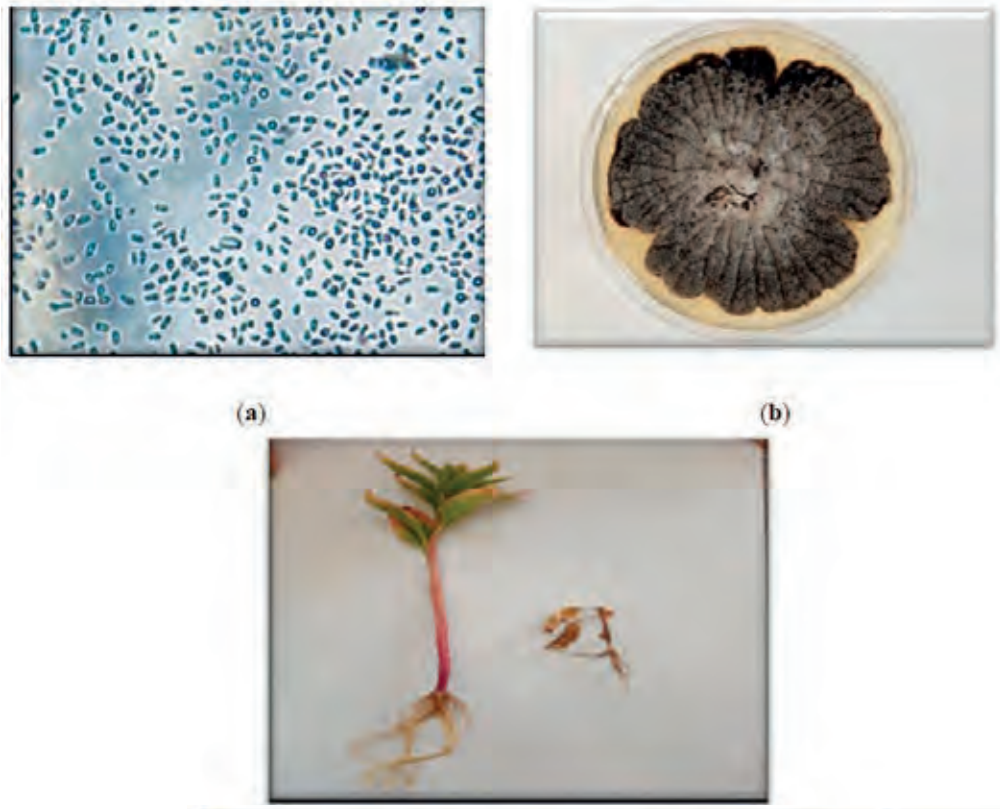

Figure 1. Phenotypic characteristics of Ectophoma multirostrata E2 isolated from *Celsia argentea* plants. (a) A pure culture of *E. multirostrata* on PDA, (b) a Micrograph (40X) of the spores of *Ectophoma multirostrata*, and (c) healthy (right) and infected (left) plants.

Table 2. Detection of pathogenic isolates using red radish seeds on Water Agar.

Isolation symbol	% for germination	% to inhibit
Control	100.0	00.00
Ec1	53.33	46.67
Ec2	16.66	83.34
Ec3	60.00	40.00
LSD 0.05	2.478	2.609

[1]Each number in the table represents an average of three replicates, Ec= *Ectophoma multirostrata*

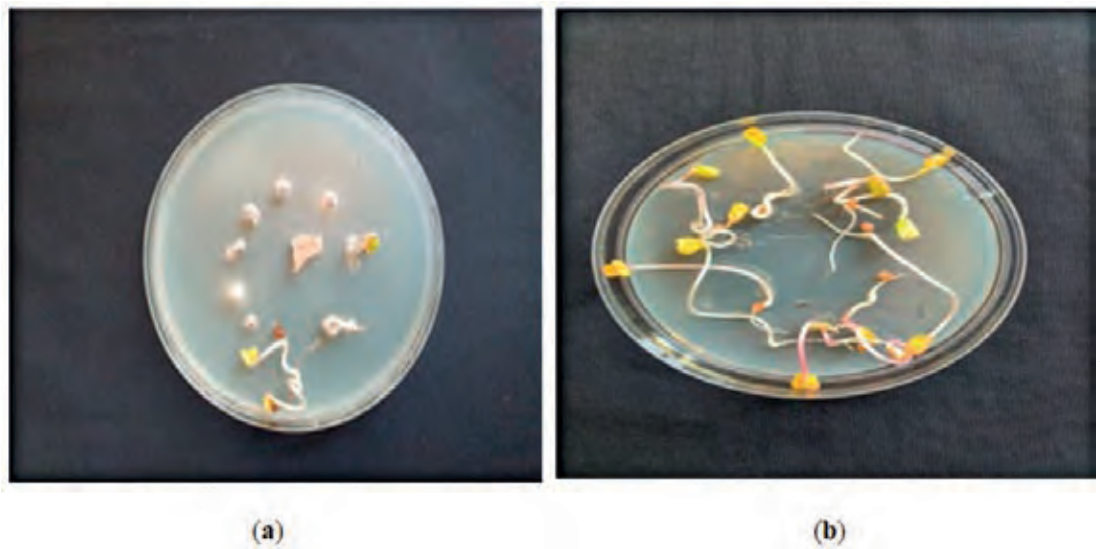

Figure 2. Shows the pathogenicity test using red radish seeds on a Water agar medium (WA). *Ectophoma multirostrata* (Ec2), A=Control*

Isolation symbol	% for germination	% to inhibit
Control	100.0	0.00
Ec1	20.2	79.8
Ec2	16.00	84.00
Ec3	22.6	77.4
L.S.D. (P≤0.05)	1.3055	1.3055

[1]Values are means of three replicates, Ec= *Ectophoma multirostrata*.

Table 3. Pathogenicity of *Ectophoma multirostrata* isolates on seed germination of *Celosia argentea* in plastic pots under greenhouse conditions.

Control of the fungus *Ectophoma multirostrata*, the causes of root rot and damping off on *Celosia argenta*

Antagonistic ability of *Azotobacter chroococcum* against *Ectophoma multirostrata*

The results (Table 4 and Figure 3) showed the ability of *A.chroococcum* bacteria to inhibit the growth of the pathogenic fungi *Ectophama multirostrata* (Ec2) isolated in this study on PDA culture media. % compared with the treatment of pathogenic fungi alone, which amounted to 0.00%. The results showed that there is a direct proportion to the percentage of inhibition by increasing the concentration of bacteria, as it caused a significant reduction in the growth of isolate Ec2. The higher the concentration of *A. chroococcum* bacteria, the higher the inhibition percentage compared to the fungus treatment alone, which amounted to 0.00%. herbicolin[18]. In addition, *A. chroococcum* can produce low molecular weight compounds that function to resist pathogenic fungi, including hydrogen cyanide (HCN). The presence of this compound in high concentrations inhibits the growth of pathogenic fungi[19], and it has a strong ability to compete with pathogens for iron through its production of siderophores[20]. The production of many compounds useful for plant growth, such as ammonia, vitamins and growth regulators such as indole acetic acid, gibberellin and cytokinin, promote seed germination and plant growth[21,22].

These results agree with the results of other studies that found the inhibitory ability of *A. chroococcum* bacteria for many plant pathogens. It showed its high antagonistic ability when used directly or the filtrate to inhibit the growth of the fungi *R. solani* and *F. solani* that cause the death of tomato seedlings[19]. It has also been shown to inhibit *Marcelleina persoonia, Fusarium oxysporum, fungi Lasiodiplodia theobromae, Fusarium equiseti, Curvularia lunata , Cochliobolus, Trichocladium griseum* Causes root rot and damping off several ornamental plants on PDA culture media2.

The results showed (Figure 4) that the chemical pesticide Beltanol. It led to the inhibition of the fungus *Ectophoma multirostrata* (Ec2) by 100% using the concentrations recommended by the producing company and 0.5 and 0.75% of the recommended concentration. These concentrations did not differ significantly among themselves in the percentage of inhibition of pathogenic fungi, which amounted to 100%. The effect of the chemical pesticide Beltanol on pathogenic fungi may be attributed to its ability to form chelating compounds with copper inside its host tissues, thus facilitating the process of its passage into the pathogen's cells and then liberating and killing the pathogen[2,23]. Such effects of the pesticide may also be due to the active substance (8-Hydroxyquinoline), which is known for its effectiveness against a wide range of plant pathogenic fungi. And the effect of this substance on fungi is due to causing ab-

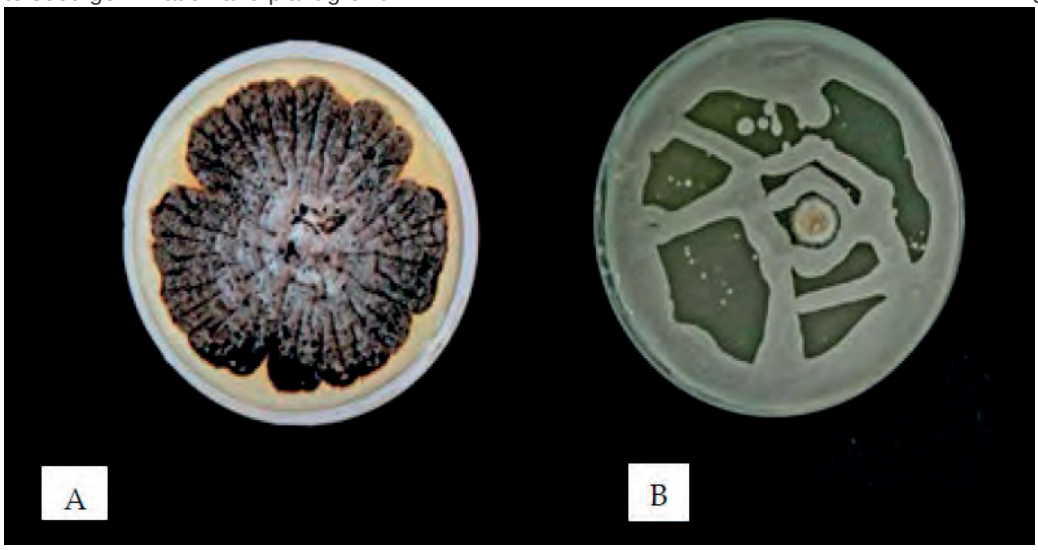

Figure 3. The antagonistic ability of the biological agent *A. chroococcum* against the fungus *E. multirostrata* (Ec2), A=Control B= *Ectophoma multirostrata*+ *A.chroococcum*.

Treatment	Dilution	Average Fungi colony diameter (cm)	% to inhibit
Ec2+Azo	100	9.00	0.00
	10-1	0.00	100.00
	10-2	0.00	100.00
	10-3	0.00	100.00
	10-4	0.50	94.44
	10-5	1.00	88.88
	10-6	3.00	66.66
	10-7	5.00	44.44
	10-8	6.50	27.77
LSD. (P≤0.05)		0.338	4.5593

[1] Values are means of three replicates, Ec= Ectophoma multirostrata

Table 4. The antagonistic ability of *A.chroococcum* against the fungi that cause root rot and damping off *Celosia argentea* on PDA culture medium.

normalities in fungal cells, changing the permeability of cell membranes, leaking their contents to the outside, and inhibiting the formation and germination of Sclerotia bodies[24,25].

Evaluation of the efficacy of salicylic acid against fungi causing root rot and damping off *Celosia argentea* in PDA culture medium

The results showed (Table 5 and Figure 5) the effectiveness of all used concentrations of salicylic acid in inhibiting the growth of the fungus *Ectophoma multirostrata* (E2). The comparison treatment, in which the diagonal growth rate was 9.0 cm and the concentration of 1.5 g / liter, significantly exceeded the other concentrations of *Ectophama multirostrata* (Ec2) inhibitor 2.5 cm, with a growth inhibition rate of 72.2%. The concentration of 0.5 gave the slightest effect in inhibiting the pathogenic fungus. Still, it differed significantly from the control treatment and reached a growth rate of the fungus at 6.2 cm and an inhibition rate of 33.11%. The results show a positive relationship between the increase in acid concentration and the increase in the percentage of inhibition. The effectiveness of salicylic acid may be attributed to its inhibition of many vital processes in pathogenic fungi, such as the action of enzymes and amino acids, and then affecting the activity and growth of pathogens[26,27]. These results agree with the findings of Hassan (2005)[28] indicating complete growth inhibition of *Pythium aphanidermatum* on PSA culture media when SA was used at 400 ppm. Similarly, a direct relationship was found between the concentration of salicylic acid and the percentage of growth inhibition of the fungus *Pythium aphanidermatum* that causes seed rot disease and cucumber death[29].

Effect of *A. chroococcum*, salicylic acid and Beltanol in controlling *E. multirostrata* on *Celosia argentea* under greenhouse conditions

The results showed (Table 6) that all the factors used effectively reduced the percentage of infection and its severity and increased growth parameters compared to the infected untreated control. Beltane fungicide was superior in reducing the affection rate and its seriousness to 0.00%, followed by Az+Sal+Ec2 treatment, which amounted to 16.33%, and 8.00%, respectively. Shoot length and dry wei-

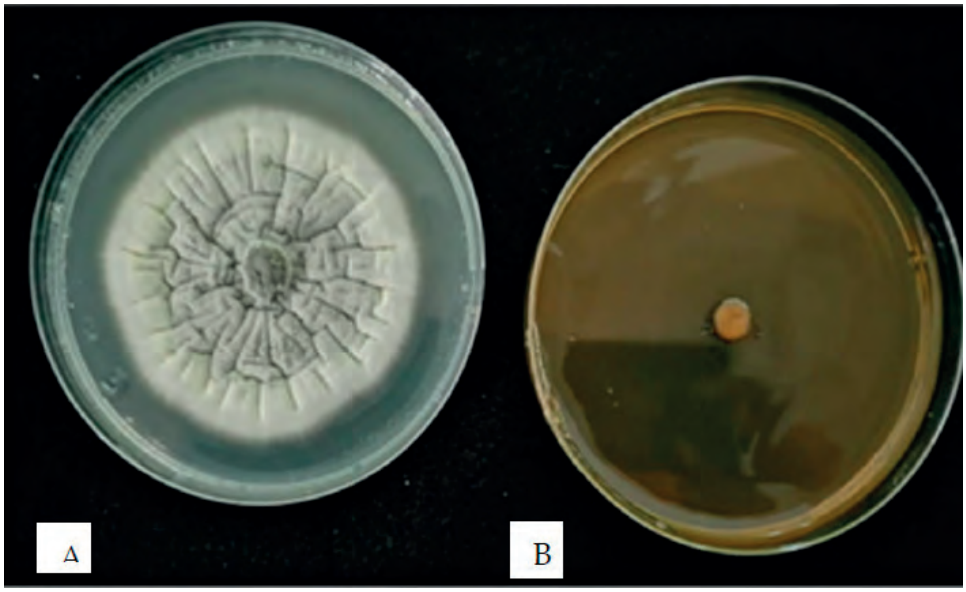

Figure 4. Antagonistic ability of the chemical pesticide Beltanol against the fungus that causes root rot and seedling death of *Celosia argentea* on PDA medium. A= *E. multirostrata* (control) and B= *E. multirostrata*+Beltanol.

Concentration g/L	Average Fungi colony diameter (cm)	% inhibition
Control	90.0	0.00
0.5	6.2	3.11
1	3.5	61.1
1.5	2.5	72.2
LSD. (P≤0.05)	0.8804	0.5111

[1]Values are means of three replicates, Ec= *Ectophoma multirostrata*.

Table 5. Salicylic acid effect on *E. multirostrata* growth on PDA culture media

Figure 5. Salicylic acid (SA) Efficiency against the pathogenic *Ectophoma multirostrata* in PDA culture medium. A= *E. multirostrata* (control), B= *E. multirostrata*+SA

ght of vegetative and root groups 22.50 cm, 0.423 g, and 0.133 g, respectively, as well as all factors used without the presence of the causes to increase growth parameters[2,23]. The pesticide is converted to the active substance (8-Hydroxyquinoline), which is known for its effectiveness against many plant pathogenic fungi. One of the substances derived from this active substance proved its inhibitory efficiency against the fungi *Sclerotinia sclerotiorum, Fusarium graminearum, Magnaporthe oryzae* and *Ilyonectria liriodendra*. And it was inhibiting the formation and germination of Sclerotia bodies[24,25].

The effectiveness of salicylic acid may be due to its inhibition of many vital processes in pathogenic fungi, such as the action of enzymes and amino acids, and then affecting the activity and growth of these pathogens[26,27].

Conclusions

The study showed the presence of the fungus *Ectophoma multirostrata* accompanying the symptoms of root rot on the *Celosia argentia*. This plant host recorded the pathogenic fungus for the first time in Iraq. The study also showed the possibility of reducing infection by using *Azotobacter chrocooccum*, salicylic acid and the chemical pesticide Beltanol. In general, the chemical pesticide had the highest effect in reducing the rate and severity of infection, followed by the combination treatment of *A. chrocooccum* and salicylic acid. The contrast of fungal isolates, in their ability to sicken and their inhibition of *Celosia argentea* seed germination, may be attributed to the genetic difference between fungal isolates of the same species that were collected from different regions or due to the difference of isolates in their ability to secrete pectin- and cellulose-degrading enzymes in the early stages of infection. These enzymes play a role In penetrating the family, and from it, Pectinase, Phosphatase, Cellulase, Methylesterase, Pectinmethylhydrase, and Protase, which have a significant effect on the pathogenicity of fungi, as well as the ability of these fungi to produce some toxins of a phenolic and glycoside nature

The effect of *A. chroococcum* bacteria may be attributed to its high ability to produce some antifungal compounds, metabolites, organic compounds, and several enzymes that can degrade the cell walls of pathogenic fungi, including

Treatment	Infestation rate (%)	Infection severity (%)	Plant height/ cm	Shoot Dry weight (g)	Root Dry weight (g)
Control	0.00	0.00	21.00	1.32	0.012
Ec2	80.00	62.00	5.00	0.090	0.003
Az	0.00	0.00	24.00	2.03	0.051
Sal	0.00	0.00	24.00	2.02	0.051
Bel	0.00	0.00	22.00	2.10	0.041
Az+Sal	0.00	0.00	34.00	2.45	0.693
Az+Ec2	45.33	37.66	16.50	1.09	0.067
Sal+Ec2	46.00	38.00	16.33	1.09	0.067
Bel+Ec2	0.00	0.00	21.83	2.02	0.312
Az+Sal+Ec2	16.33	8.00	22.50	0.423	0.133
LSD0.05	0.6189	0.5054	0.8047	0.0059	0.0949

[1] Values are means of three replicates, Sal=Salicylic acid, Bel=Beltanol, Ec=*E. multirostrata*, Az=*A. chroococcum*.

Table 6. Effect of *A. chroococcum*, salicylic acid and Beltanol and on pathogenicity of *E. multirostrata* on *Celosia argentea* under greenhouse conditions

Glucanase, Chitinase, laminarinase, and the production of several antibiotics such as Phenazine, Pyoluteorin.

The effect of the chemical pesticide Beltanol on pathogenic fungi may be attributed to its ability to form chelating compounds with copper within its host tissues, thus facilitating the process of its passage into the pathogen's cells and then liberating and killing the pathogen. The same combination also led to a clear improvement in the studied indicators of plant growth.

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ARTICLE / INVESTIGACIÓN

Effect of adding natural zeolite and vitamin E to diets of laying hens (*Lohman Brown*) on some physiological traits and productive per-formance during hot weather

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DOI. 10.21931/RB/2022.07.04.12

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Abstract: This study was conducted in a poultry farm of the Department of Animal Production / College of the Agriculture/ University of Anbar, from 20/7/2021 to 12/10/2021, aiming to study the effect of adding zeolite to the diets on productive performance and some physiological qualities character-istics of laying hens. Seventy-two laying hens of Lohman Brown were used in this experiment at the age of 43 weeks and distributed randomly to six treatments with four replications (3 hens/ replicate). The birds were fed with the diet with the additives, and the treatments were as follows: T1 (Vit E 0.06), T2, T3, T4 and T5 with an addition of 0.25, 0.50, 0.75 and 1.0% relay, normal zeolite and T6 control treatment. Results showed moral differences (P≤0.05) in the egg mass, the ratio of egg production and the number of cumulative eggs for T3, T4 and T5 treatments during the productive duration of the experiment.

Key words: Natural zeolites, productive performance, heat stress, laying hens.

Introduction

Eggs are considered an essential food item worldwide as they provide the human body with good quality proteins, fats, vitamins and minerals compared to other protein sources.

However, modern and intensive breeding has led to the emergence of many problems, in-cluding heat stress, which leads to oxidative stress as a result of high concentrations of Free radi-cals inside the bird's body which leads to weak resistance to diseases, deterioration of production, and then death. In the end, the production performance of hens is soft, so researchers must find solutions to these problems. Zeolite is one of the natural volcanic minerals that was formed after the eruption of lava millions of years ago. The basic building unit of the zeolite structure has a central atom of tetravalent silicon and trivalent aluminum. This leads to a negative charge on aluminum and silicon that is saturated with a positive ion. Replacing it with another positive ion, in this way, the zeolite acquires the well-known ion exchange property. It carries a negative charge consisting of hydrated aluminum silicate and alkaline earth elements. Silica, aluminum and oxygen are linked together to make the tetrahedra unit. The zeolite is light in weight and is brittle with a very light yellow or green hue[20]. Many stressors impair the growth performance and health status of chickens. Redox homeostasis is the usual denominator of responses to these stresses that is maintained through a balance between the production of reactive oxygen species (ROS) and reactive nitrogen species and the antioxidant defense system. Oxidative stress results when ROS production exceeds the ability of the antioxidant defense system to remove these tox-ic molecules[13,22]. Several studies have shown that there is a significant difference in the presence of zeolite in the diet[5], the effect of zeolite improves digestion and intestinal absorption, which contributes to weight gain and the provision of feed use as well as reducing production costs[21] as well as noted[7] significant differences in production Eggs, average egg weight, egg mass, and the amount of feed consumed in the zeolite treatment compared to the control treatment, (16) indicat-ed a positive effect of zeolite on the characteristics of production performance and eggshell qual-ity. Therefore, the study aimed to show the impact of adding natural zeolite as an antioxidant on the productive performance of laying hens and some physiological characteristics.

Materials and methods

This study was conducted on the farm of the poultry Department of Animal Production/College of the Agriculture/ University of Anbar from 20/7/2021 to 13/10/2021 to study the effect of add-ing zeolite to the diet on the productive performance and some physiological characteristics of laying hens. 72 hens were used in this experiment(Lohman Brown), 43 weeks old. They were randomly distributed to six treatments and four replicates per treatment (3 hens/ replicate). The birds were fed a normal diet (Table 1) with supplements, and the treatments were as follows: T1(Vit E 0.06), T2, T3, T4 and T5 by adding 0.25, 0.50, 0.75 and 1.0% sequentially natural zeo-lite and T6 to the control treatment. At the same time, water was provided according to the water nipple system, and the illumination period was given to 15.5 hours per day. The percentage of egg production was calculated According to HD%, the average weight of eggs,

Citation: E H H Alsalmany, Mohammed T H T. Effect of adding natural zeolite and vitamin E to diets of laying hens (Lohman Brown) on some physiological traits and productive performance during hot weather. Revis Bionatura 2022;7(4) 12. http://dx.doi.org/10.21931/RB/2022.07.04.12

Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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feed consumed, the mass of eggs produced and provided conversion factor (gm feed/gm egg). The data were statistically analyzed using the statistical program for (4) to study the effect of different treatments on the traits studied in the experiment according to a completely randomized design (CRD) and comparison of significant differences between means with Duncan's polynomial test[16].

Ingredient	%
Yellow corn	35.4
Wheat	30
Soybean meal (44% CP)	23
Premix*	2.5
Vegetable Oil	0.5
Limestone	7.5
Salt	0.1
Di calcium phosphate	1
Total	100
Chemical analysis **	
ME, kcal/kg	2737
CP, %	17.527
Fat %	2.287
Fib.%	2.553
Ca, %	3.153
Ava. Phosphor %	0.518
Lysine, %	0.825
Met.%	0.294
Cys. %	0.305
Met. + Cys. %	0.599
*premix provided per kilogram of diet:7.8 % crude protein. 2930 kcal metabolizable energy. 23.1% Ca. 3.8% Ava. P %.7.7% Methionine+ Cysteine . 2.4% Lysine. **Chemical analysis according to NRC (1994)[15]	

Table 1. Shows the components of the ratio used in the experiment.

Results and discussion

Productive traits

Table 2 showed no significant differences (P<0.05) in the average egg weight for the total produc-tion period when different levels of zeolite and vitamin E were added to the laying hens' diets. It is also noted from the table that there are significant (P<0.05) differences between the experi-mental treatments in the mass of eggs produced for the total period, where the treatments T3, T4 and T5 were significantly (P<0.05) superior compared to the rest of the experimental treatments. The table also shows a significant superiority (P<0.05) for the T5 addiction treatment (1% zeolite) in the percentage of egg production on the remaining experimental treatments compared to the control treatment, and we notice a significant superiority (P<0.05) for the T5 addiction treatment over the rest of the experimental treatments during the productive period. In the cumulative number of eggs compared to the control treatment T6, We also note from the same table that there is a significant improvement for the treatment T5 (1% natural zeolite) compared with T2 and T6 in the feed conversion factor. These results are in agreement with findings[1,9,11]. The re-sults of the experiment did not agree with the findings of (18) and (8), which indicated no significant differences when adding zeolite to laying hens' diets. It played an indirectly effective role in pre-serving liver cells from oxidative damage caused by heat stress[7]. Zeolite improves digestion and intestinal absorption, which contributes to saving the use of feed, as well as reducing production costs[6]. The discrepancy in the content of these effective vehicle transactions and their influence is in restraining free radicals, increasing antioxidant activity in the body, inhibiting lipid peroxida-tion and reducing oxidative stimuli. Antioxidants work to perpetuate the raw materials needed for the growth of ovarian follicles, most of which are fatty substances.

The role of treatments as antioxidants in poultry diets works to protect lipoproteins and fat-ty compounds. The other substances enter into the yolk formation from oxidation, which leads to an abundance of these substances, and then the maturation of the ovarian follicles in a short-er time than those in chickens that did not take levels of antioxidants in the diet. The role of anti-oxidants is to reduce the formation of free radicals and protect the membranes. Thus, the cells have preserved them from exposure to harmful damage due to free radicals, protecting lipopro-teins from breakdown, regulating the representation of body fats and encouraging the deposition of materials necessary for ovarian follicle growth. Thus the cells continue to carry out vital activi-ties, which results in higher production performance and quality improvement[3,12,14].

Blood traits

Measurement of PCV

The results are illustrated in Figure 1. There are statistically significant differences between the experi-mental treatments for cellular blood traits at the end of the experimental period. We note the superiority of the T6 control treatment in the volume of compressed blood cells, which amounted to 30.1% over the rest of the ad-ditional treatments.

L/H . ratio

We notice from Figure 2. A significant decrease (P<0.05) in treatment T1, T2, T3 and T5 compared to treatment T4 and control treatment T6 in the ratio of heterophil to lymphocytes L H^{-1}. The reason for the superi-ority of the additional treatments may be due to the variation in the content of these treatments from the active compounds and their effect in reducing the impact of heat stress through restricting free radicals, increasing the activity of antioxidants in the body, inhibiting lipid peroxidation and reducing oxidation catalysts[12].

Treatment	average egg weight (gm)	egg mass (gm)	Egg production% HD%	HD (egg)	feed conversion ratio
T1 VitE 0.06%	56.3	14562 b	86.4 abc	72.6 abc	1.37 abc
T2 Zeo 0.25%	58.9	14046 b	79.1 c	66.4 c	1.51 a
T3 Zeo 0.50%	60.6	16542 a	90 ab	76.8 ab	1.27 c
T4 Zeo 0.75%	59.6	1350 a	91.6 ab	77.0 ab	1.29 bc
T5 Zeo 1.0%	59.2	16755 a	94.1 a	79.0 a	1.27 c
T6 Control	56.30	14358 b	84.4 bc	69.3 ab	1.44 ab
SEM	58.527	97.61	1.497	1.346	0.0275
Adj. average	58.527	5137.2	87.77	73.56	1.36
Morale level	NS	*	*	**	**

*The different letters within the same columns are significant differences at (P<0.05).
**The different letters within the same columns are significant differences at (P<0. 05).
NS = Non-Significant.

Table 2. Effect of adding different levels of Natural Zeolite and vitamin E to the diets Of laying hens (*Lohman Brown*) on productive performance of laying hens.

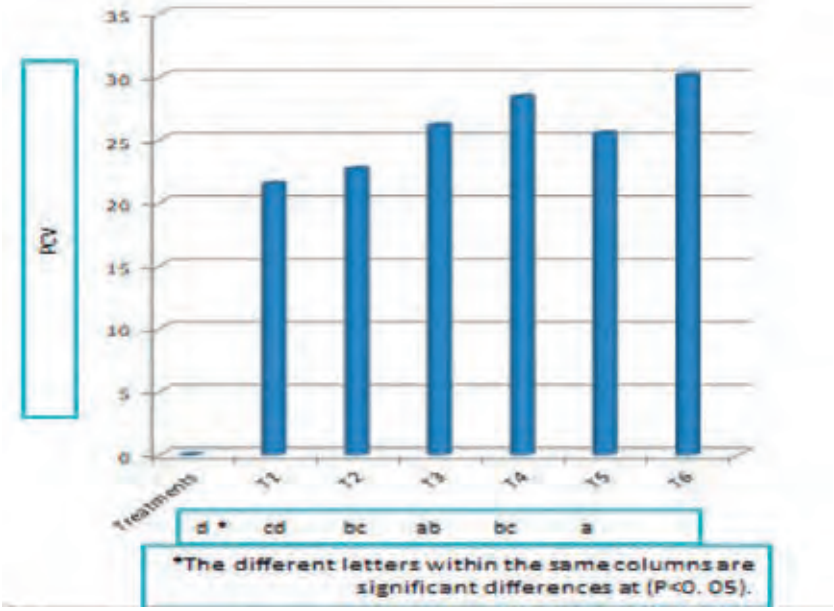

Figure 1. Effect of adding different levels of natural zeolite and vitamin E to the diets of laying hens (*Lohman Brown*) on the volume of packed blood cells.

Glucose and blood proteins

The results of Table 3. indicated that there were no significant differences (P<0.05) between the experi-mental treatments at the end of the total experimental period, as the table shows that there was no significant difference in the concentrations of (glucose, total protein and globulin) for all the different experimental treat-ments. These results agreed with what was reached by (2,5,10,17), who indicated no significant differences when adding zeolite to chicken diets. The results of the statistical analysis in the same table showed that there was a significant (P<0.05) superiority for the T4 supplement (0.75% natural zeolite) treatment in the serum albumin concentration, which did not differ significantly from the T3 supplement (0.5% natural zeolite) treatment com-pared to the rest of the experimental treatments. These results agreed with the findings[17]. Albumin is the main part of the total protein in the blood. It is manufactured by the liver and is considered an indicator of the liver's ability to produce proteins. Low albumin is evidence that the liver's manufacturing function has decreased as a result of damage to liver tissues and cells. As for globulin, which includes antibodies present in blood plasma, it is considered an indicator of immunity and gives evidence of the extent of liver tissue damage[13]. Therefore, the T4 addition treatment showed a significant improvement in liver function during hot weather, followed by the T3 treatment, which did not differ significantly from T4.

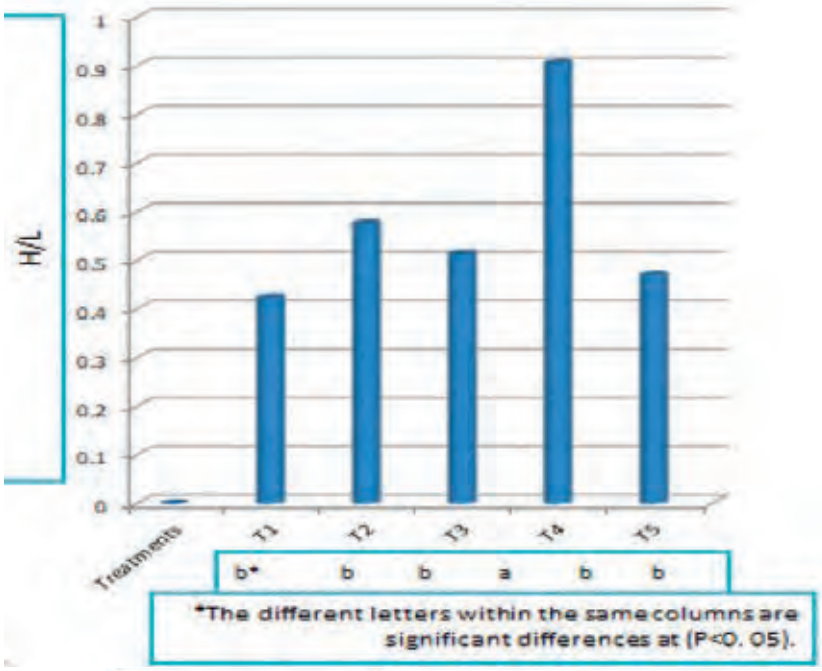

Figure 2. A significant decrease (P<0.05) in treatment T1, T2, T3 and T5 compared to treatment T4 and control treatment T6 in the ratio of heterophil to lymphocytes

Treatment	Glucose	Total protein	Albumins	Globulin
T1 VitE 0.06%	281	4.5	2.25 b*	1.95
T2 Zeo 0.25%	179	4.5	2.09 b	2.11
T3 Zeo 0.50%	167	4.61	2.51 ab	2.09
T4 Zeo 0.75%	87.5	6.38	3.06 A	3.31
T5 Zeo 1.0%	160	4.24	2.19 B	2.05
T6 Control	157	4.06	2.17 B	1.89
SEM*	21.4	2.45	0.103	2.474
Adj. average	172.3	4.23	2.38	6.84
morale level	Ns**	Ns	0.029	Ns

* SEM: Standard Error Mean.

** GM: It means that there are no significant differences between the mean of the transactions at the level of significance (P≤0.05).

a, b, c: the different letters within the same row indicate the presence of significant differences between the treatments at the level of significance (P≤0.05).

Table 3. The effect of adding different levels of natural zeolite and vitamin E to the diets of laying hens (*Lohman Brown*) on blood biochemical characteristics (HD) during the productive period (43-54) weeks (20/7/2021-13). 10/2021).

Conclusions

We conclude from this study that adding zeolite to laying hens' diets at a rate of 1% led to an in-crease in egg production, egg mass and a decrease in the feed conversion ratio (gm of feed/gm of eggs).

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ARTICLE / INVESTIGACIÓN

Hepatoprotective effect of *Thyme aqueous* extract in Acetamino-phen induces hepatotoxicity in male rats

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DOI. 10.21931/RB/2022.07.04.13

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Abstract: *Thyme vulgaris* is effective in treating acetaminophen toxicity in clinical trials. The present study investigates Thyme aqueous extract's effect on rats poisoned with Acetaminophen. In this study, the data were obtained from male Wister rats. Animals were divided into three groups: distilled water, acetaminophen (1mg/ kg), and aqueous thyme extract (400 mg/kg). All animals were orally treated for seven days respectively. The animal was sacrificed on the eighth day. ALT, AST, GSH, TAC, and Caspase3 were all measured in plasma obtained from heart derived blood samples centrifuged to determine plasma levels of these enzymes and other antioxidants, malondialdehyde precursors (MDA). Liver enzyme levels were reduced, total antioxidant levels were in creased, and an aqueous extract of thyme compensated for glutathione levels. Caspase3 levels were also reduced. Acetaminophen induced liver tissue damage and inflammatory cell damage were considerably lessened by Aqueous Thyme extract treatment. To protect the liver from Acetaminophen induced hepatotoxicity, aqueous Thyme extract was found to be beneficial.

Key words: Acetaminophen, Hepatotoxicity, Thyme aqueous extract, Histopathology.

Introduction

One of the primary causes for a drug's removal from the market is its hepatotoxicity. Hepatotoxicity caused by drugs accounts for 50% of all acute liver failure cases and 5% of hospitali-zations[1]. It is common to see nonspecific symptoms, including stomach pain, nausea, vomiting, diarrhea, and pruritus, with the nonspecific hepatotoxicity symptoms, such as abdominal pain, jaundice, fever, and rash in patients who have been exposed to hepatotoxicity[2]. Acetaminophen (APAP) is a potent analgesic and antipyretic that has been around for a long time. If you're look-ing for personal medication, you can buy it over the counter or through your doctor's office[3]. Acetaminophen is rapidly absorbed from the gastrointestinal tract after oral administration[4]. Peak plasma concentrations are noticed between thirty minutes to two hours, with protein binding varying from 20 to 50% at traditional therapeutic doses. Acetaminophen is metabolized primarily within the liver into non toxic products[5]. Acetaminophen induced liver damage is charac-terized by the extensive release of cellular contents (liver enzymes), nuclear degradation and inflammatory response. These are typical features of oncotic necrosis. Cell death in vivo and in vitro is caused by oncotic necrosis[6]. The thyme plant belongs to the family Labiatae and is an herbaceous plant spread in the Mediterranean region. The medicinal part of thyme is concentrated in the leaves and the entire plant. It has been widely used as an antiseptic, carminative, anti-spasmodic, rheumatic, dermatological, antifungal, anthelmintic, and analgesic[7]. Thyme is also used in treating cold cases, bronchitis and whooping cough, and it has broad uses in veterinary medicine as an antiseptic for the intestines and an antihookworm. It improves heart rate and lowers blood pressure. It was used as an antibacterial and improves Nutritional efficiency, leads to increased body weight, obesity and appetite in rabbits, and is considered a non toxic plant[8]. Thyme contains many chemicals, including volatile oil, where thyme oil contains 55% of phenols, the most important of which are thymol and carvacrol, and resinous materials such as rais-ing and tannin. It is also a source of thiamine[9]. As for the effects of thyme on reducing sugar, studies have indicated that, injecting thyme extract into rabbits leads to lowering blood glucose, and adding it to the rabbit diet at concentrations of 150-300 mg/kg diet leads to an improvement in food intake, weight gain and food conversion factor[10].

Materials and methods

Chemicals

From the GSK firm in the United Kingdom, we acquired a 500 mg acetaminophen tablet, BioMerieux, France, and supplied the reagent kits for the assay of transaminases. Elisa kits for determining total antioxidant capacity were bought from BT LAB, China, for tissue malondialdehyde, glutathione (GSH), caspase 3, and full antioxidant capacity (TAC). Methods for each diagnostic kit's work were followed precisely.

Thyme Aqueous Extraction

In the Al-Najaf Province, Kufa City, dried leaves of *Thymus vulgaris* were purchased and identified at the Kufa University Herbarium. Using commercially available equipment,

Citation: Mohammed R A, Fadheel Q J. Hepatoprotective effect of Thyme aqueous extract in Acetaminophen induces hepatotoxicity in male rats.Revis Bionatura 2022;7(4) 13. http://dx.doi.org/10.21931/RB/2022.07.04.13
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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T. vulgaris leaves were pulverized into a fine powder in a grinder. It took 100 grams of fine powder in 200 milliliters of denatured alcohol for 30 minutes of continuous infusion. It was then centrifuged for 10 minutes at 3000 revolutions per minute, then dried in an oven at 60 degrees Celsius. Sterilized bottles were used to hold the dry material[11].

Experimental Animals

The rats were obtained from a College of Science/ University of Kufa animal house and ranged in age from 10 to 14 weeks and weight from 180 to 200 g. Plastic cages were used to house the animals. Wooden shelves were inserted into the cage, and it was kept at a temperature of (23–25°C) and a humidity level of (60–65%), allowing the animals to drink tap water and consume a standard chow diet. This experiment required using rats that had first spent two weeks acclimated in an animal house to laboratory conditions. This allowed the rats to better cope with the stress of being moved into a new environment. Twentyone male rats were divided into three equal groups and given seven days of treatment in each group.

•Group one: Rats administrated distilled water 1 ml/kg/day orally

•Group two: Rats administrated only distilled water and Acetaminophen at a dose of 1g/kg/day orally for seven days.

•Group three: Rats administrated aqueous thyme extract orally at 400 mg/kg/day for seven days + Acetaminophen at 1 g/kg/day orally on the seventh day. All animals were sacrificed on the nine day.

Tissue Sampling for Histopathology

The apical portion was preserved and fixed in 10% neutral formalin, then embedded in a paraffin block and cut into sections with a thickness of 5 micrometers for histopathological examinations. Sections stained with hematoxylin and eosin blue were examined under light microscopy[12].

Statistical Analysis

SPSS version 27 was used for the statistical analysis. (Means SD) was used to represent continuous variables. Three or more groups were compared using an ANOVA test. A p-value of less than 0.05 was deemed significant in this study.

Results

It is shown in Table 1 that the thyme extract has an effect on liver function tests as well as on biochemical, oxidative, and apoptosis parameters. Aspartate and alanine aminotransferase enzyme activity increased significantly compared to the negative control after an oral dose of 1 mg of Acetaminophen per kilogram of body weight. When used as a treatment, thyme extract significantly reduced serum levels of these enzymes (table 1). Accumulation of lipid peroxidation in liver tissue (MDA level) and depletion of antioxidant defense mechanisms (GSH level) were dramatically ameliorated by treatment with thyme extract (TAC). In the grand scheme of things.

Effects of Thyme Extract on Liver Histopathology

The liver slices from the positive control group showed extended necrosis, significant hydropic degeneration, and increased Kupffer cell proliferation; thyme extract resulted in only mild degeneration and no necrosis (table 1, figure 1).

The results are presented as mean and standard deviation, with Gp1 representing the control group (no treatment), Gp2 representing the Acetaminophen-treated group, and Gp3 repre-senting the Acetaminophen-treated group (with thymus extract). S. ALT and S. AST represent se-rum alanine aminotransferase and aspartate aminotransferase, respectively. T. GSH represents tissue glutathione, and T. MDA represents tissue.

In this study, the histological evaluation of the liver male rat section of the control group shows normal histological structures. The results are shown in Figure (1).

Histopathological examination of the liver male rat section of group 2 treated with Acetaminophen at a dose (1g/kg, day) showed activation of kupffer cells, irregular and enlarged portal tract, necrosis with the appearance of newly formed bile ductules as shown in Figure (2) compared with the control group. The histopathological score of rat liver group 2 is shown in Table (2).

The results showed that there was damage noted in hydropic degeneration, congestion of the cen-tral vein, no harm in the mitotic figure, and no damage in perisinusoidal fibrosis. Mild toxicity was seen as kupffer cell proliferation. Moderate toxicity was seen as apoptosis and portal In-flammation, and severe toxicity was caught in congestion of the central vein, lobular Inflamma-tion and bile ducts, necrosis, and dilation of the sinusoids. Histopathological examination of the liver male rat section of group 3 treated with thyme aqueous at a dose (of 400 mg/kg per day) shows dilation of the sinusoids with mild mononuclear cells infiltration and an increase in the number of kupffer cells Figure (3) compared with the control group.

The histopathological score of rat liver group 3 is shown in Table (2). The results showed that there was no fibrosis, no congestion of the central vein, no bile duct injury, no necrosis, and no congestion of the central vein. During moderate symptoms of Inflammation and dilation of the sinusoids and mitotic figure, mild symptoms of apoptosis and hydropic degeneration were seen.

Variables	Gp1 (N=7)	Gp2 (N=7)	Gp3 (N=7)	P value
AST (U/L)	15.963±6.117	24.693 ± 7.408	17.796 ± 2.215	<0.698
ALT (U/L)	62.025 ± 25.351	105.342 ± 23.850	29.599 ± 14.141	<0.018
MDA (µmol/g)	3.123 ± 0.521	5.266 ± 1.757	3.340 ± 0.227	<0.159
TAC (mmol/g)	4.038 ± 0.695	5.371 ± 0.347	8.214 ± 0.779	<0.005
GSH (mmol/g)	69.767 ± 3.889	55.695 ± 5.845	61.399 ± 7.918	<0.691
Caspase 3 (ng/ml)	0.235 ± 0.014	0.418 ± 0.123	0.248 ± 0.012	<0.121

Table 1. Using thyme extract, liver function tests, biochemical and oxidative stress were compared to Acetaminophen and the control group.

Group 3	Group 2	Group 1	Groups	Histopathological changes
1	2	0	Hydropic degeneration	**Hepatocellular changes**
0	3	0	necrosis	
1	2	0	Apoptosis	
2	0	0	Mitotic figure	
0	3	0	Congestion of the central vein	
2	3	0	Dilation of the sinusoids	
2	2	0	Portal	**Inflammation**
2	3	0	lobular	
2	1	1	Kupfer cells proliferation	
0	3	0	bile duct injury	**Bile ducts**
0	2	0	bile duct hyperplasia	
0	2	0	portal	**Fibrosis**
0	1	0	Septal	
0	0	0	perisinusoidal	

Table 2. Represents the Histopathological changes that were observed representing (0) no symptoms, (1) mild symptoms, (2) moderate symptoms, (3) severe symptoms (4) acute symptoms.

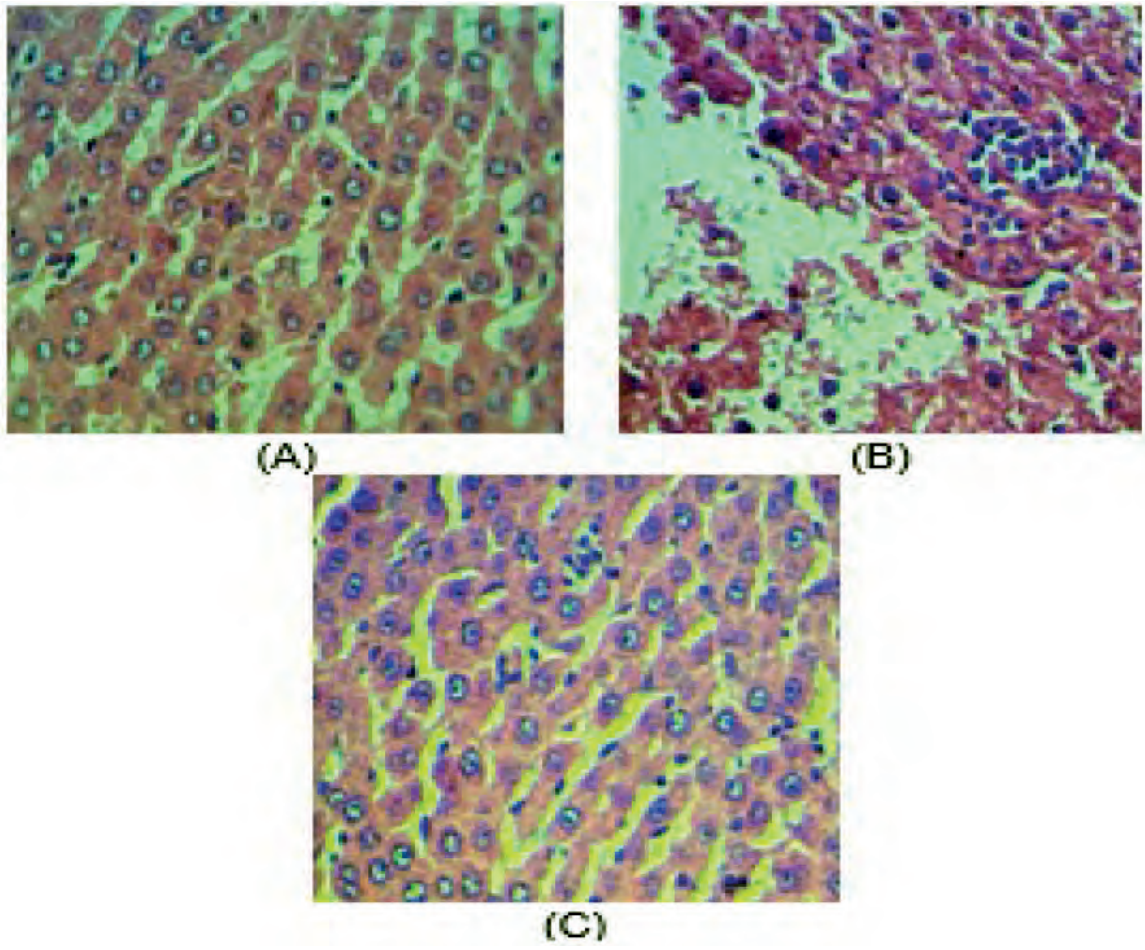

Figure 1. Section of liver of albino male rat of study groups (A: Normal group, B: Acetaminophen group, C: Thyme group) on day 8 of the experiment. 400X, H&E.

Discussion

Even though Acetaminophen has long been considered a generally safe medicine, the general safety of the drug in therapeutic, permitted levels has lately been questioned because multiple studies have shown alanine aminotransferase increases with more than five days of therapeutic dosing. Even though Acetaminophen is widely used and there have been no instances of severe liver injury, the likelihood of developing liver damage is still relatively low[15]. Acetaminophen, a common over-the-counter painkiller and antipyretic. Users include patients suffering from hypertension, migraines or myocardial infarction who frequently and chronically use Acetaminophen to minimize headaches or illdefined pain associated with these conditions[16]. On the other hand, antioxidants can be used in these patients as the central therapeutics or, concomitantly, as a precaution. Acetaminophen can be presented as a drug that can impair liver function as it has a liver-damaging potential[17]. There were no deaths in any of the groups of rats who received APAP or distilled water for 24 hours. Histopathology examination of the control group reported normal morphological features, while Acetaminophen administrated rats' morphological features show hepatotoxicity; activation of kupffer cells and slight congestion of central vein, portal venopathy and the epithelium in the bile duct is irregular, and associated Inflammation is minimal. According to the histopathological severity score, the Acetaminophen group revealed severe toxicity, significantly different from the Control group, which revealed zero histopathological severity score. This histopathological result agrees with a study by Muhammad-Azam *et al.*[18]. Studies by (19). Muhammad-Azam *et al.*[20], agree with the current research, and the study of (21) reported the critical protective role of aqueous thyme extract in hepatocyte morphology and prevention of Acetaminophenmediated apoptosis through the antioxidation and antiinflammatory effects of aqueous thyme extract. The histopathological examination of Acetaminophen revealed severe hepatotoxicity according to histopathological severity score due to Acetaminophen mediated apoptosis, Inflammation and oxidative stress, Histopathological examination of aqueous thyme extract was shown to activate kupffer cells, irregular and enlarged portal tract, scholangitis with the appearance of newly formed bile ductules induced by Aceta-minophen. The effect of aqueous thyme extract on liver enzymes was small and not uniformly consistent.

These findings are consistent with a previously reported conclusion that aqueous thyme extract has cytoprotective effects against oxidative injury caused by acute Acetaminophen toxicity in rat liver and restored the enzyme level[22].

Thyme aqueous extract may exert hepatoprotection through various mechanisms. Free radical scavenging properties and inhibition of lipid peroxidation *in vitro*[23], is one mechanism involved. In the present study, malondialdehyde (MDA) levels, both in serum and liver homoge-nate, which is a marker of oxidative stress, were reduced, and serum glutathione, another charac-teristic of oxidative stress, was marginally increased.

This may suggest that hepatoprotection by Thyme aqueous extract may involve an antiox-idant mechanism.

APAP-induced hepatotoxicity is thought to be caused in part by apoptotic cell death and in-flammatory reactions[24]. According to earlier studies, thyme contains anti-apoptotic and anti-inflammatory properties. In liver slices from lead exposed rats, thyme therapy reduced necrosis, inflammatory cell infiltrations, and hemorrhage. More human clinical investigations are needed to verify the effectiveness of this treatment[25,26].

Conclusions

Thyme aqueous extract is protective against Acetaminophen-induced hepatotoxicity in rats. The treatment results in a positive result in oxidative stress and apoptosis marker with reten-tion of the average level of liver enzyme and prevents changes in the histopathological section.

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ARTICLE / INVESTIGACIÓN

Evaluation of ejection fraction in patients with Acute Myocardial Infarction in Mosul City, Iraq

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DOI. 10.21931/RB/2022.07.04.14

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Abstract: Between 1 January 2021 and 1 July 2021, 120 blood samples from acute myocar-dial infarction (AMI) patients—60 as controls and 60 patients—of both sexes who were accepted to the cardiac surgery unit at AL- Salam teaching hospital, the in-tensive cardiac care unit, and outpatient clinics in Mosul City/Iraq, were taken. In the presented research, the ejection fraction regarding patients with AMI was evaluated by echocardiography, and the blood-liver enzyme levels (ALP and AST \ ALT ratio) were examined in those patients. The findings indicate that patients with AMI in the age range of 35 to 45 years have a (52.41) % ejection fraction (EF) rate, which is closer to (51.344%) than it is in samples from healthy controls. In addition to that, findings indicate that patients in an age range of 46 to 56 years have heart failure with a mid-range EF (HFmrEF), with a (48.13%) percentage, whereas this heart failure type in healthy controls has been preserved ejection fraction (HFpEF) with (53.368 %). In the case of the comparison of the AST/ALT ratio and EF across all of the age groups that have been tested, the results suggest a significant level (P ≤ 0.050) relation between the ALP enzyme and EF for the pa-tients within the (35-45 years) age group additionally, as there has been a positive and weak linear connection (r = 0.10650) and a nominal p-value (p = 0.47240) at a threshold of probability ≤ P 0.050, the link and correlation between the AST/ALT ratio and cardiac EF have been investigated.

Key words: ALP enzyme, AMI patients, Ejection Fraction, AST \ ALT ratio.

Introduction

Worldwide, one of the significant causes of mortality is AMI[1]. The patient's age is one of the essential factors diagnosed as a risk factor in AMI patients, as the death rate increases among patients, especially within the older age groups[2]. Elevated levels of liver enzymes are closely related to the development of the risk of car-diovascular disease (CVD). Their increase may lead to a rise in stenosis and thus lead to blood clots[3]. Elevated levels of ALP enzyme in the blood serum lead to vascular calcification and affect the heart's blood supply[4]. The increase in the rate of these calcifications increases the risk of developing cardiovascular diseases in the general population, especially in patients with acute myocardial infarction[5]. The AST/ALT ratio is considered a strong indicator of myocardial damage. It can be relied upon after a heart infarction as a rapid measurement vital sign available in clinical laboratories. It can be easily performed to diagnose patients at risk of de-veloping CVD and can help prevent complications. After an MI[6], AST/ALT ratio is commonly used as a marker of liver disease; in MI patients, elevated AST/ALT is often an indicator of myocardial damage[7]. Ejection fraction or left ventricular ejection fraction (LVEF) is a medical term that refers to the ratio of the amount of blood ejected from the ventricle to the amount of blood remaining in the ventricle during a one-time ventricular contraction. The ejection fraction is usually measured only in the left ventricle as it pumps oxygen-rich blood to all cells of the body[8]; various patients are suffering from left ventricular dysfunction and post-heart at-tack heart failure, a high-risk long-term condition[1]. Left Ventricular Ejection Fraction (LVEF) after AMI is an important and reliable indicator for assessing the heart's integrity and ability to function properly[9]. The present study aims to de-termine the relationship and correlation between EF on echo-cardiography and liver function in acute myocardial infarction patients.

Materials and methods

Study Samples

Between 1 January 2021 and 1 July 2021, 120 blood samples from AMI patients—60 as controls and 60 patients—of both sexes who were accepted to the cardiac surgery unit at AL- Salam teaching hospital, the intensive cardiac care unit, and outpatient clinics in Mosul City/Iraq, were collected. Samples have been divided into two groups (control and patients) and three age groups (35-45), (46-56) and (57-80) years for both genders (5 ml) of intravenous blood has been withdrawn for patients and healthy by medical syringes capacity (5ml). The sam-ples were placed in a centrifuge quickly (3000 rpm) for 12 minutes for serum purposes. The serum was divided into several Eppendroff tubes and kept freezing at a temperature of (-20c) until the physiological and chemical tests were carried out.

Citation: Al-Nafoly D M, Alameen A M, Alshamaa S D. Evaluation of ejection fraction in patients with Acute Myocardial Infarction in Mosul City, Iraq. Revis Bionatura 2022;7(4) 14. http://dx.doi.org/10.21931/RB/2022.07.04.14
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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Determination of Aspartate Transaminase (AST)

Principle

The concentration of (AST) enzyme activity in the blood serum has been measured using a kit supplied by the Italian company (Giesse Diagnostics) using a chemistry analyzer.

Determination of Alanine Aminotransferase Transaminase (ALT)

Principle

The concentration of the enzyme activity (ALT) in the blood serum has been measured using a ready-made estimation kit (Kit) supplied by the Italian company (Giesse Diagnostics) using a chemistry analyzer.

Determination of Alkaline Phosphatase (ALP)

Principle

The concentration of the enzyme activity (ALP) in the blood serum has been measured using a ready-made estimation kit (Kit) supplied by the Italian company (Giesse Diagnostics) using a chemistry analyzer. The alkaline Phosphatase enzyme is hydrolyzed to p-nitro phenyl phosphate (4-NPP) to produce (4-np) P-nitro phenol. The enzyme activity in the sample has been measured at a wavelength of 305 nm.

Assessment of Ejection Fraction in AMI Patients

Ejection fraction in acute myocardial infarction patients has been evaluated by Echocardiography (ECO) according to Simpson's method by measuring the area in simplicity and estimating the volume of content, and then calculating the area in contraction and then evaluating the size of the remaining ventricle by applying the following formula[10].

$$EF = \frac{\text{diastolic volume} - \text{systolic volume}}{\text{diastolic volume}}$$

Statistical Analysis

The SPSS v. 19 from IBM Company, US, has been used for all statistical analysis. P ≤ 0.05 are considered significant when using the x2 test to compare groups sta-tistically.

Results

According to Table 1's findings and Figure 1, patients with myocardial infarction who are between the ages of 35 and 45 had an EF rate of 52.41 %, which is close to the healthy control samples' EF rate of 51.344 %. In other words, heart failure in patients and healthy control samples has a preserved ejection fraction (HFpEF), whereas heart failure in elderly patients aged 57 to 80 years has a mid-range ejec-tion fraction (HFmrEF). The rates between healthy samples and patients are also similar (43.048 % and 43.76 %, respectively).

The patients in the 46 to 56-year-old age range have an EF of heart failure with the mid-range EF type (HFmrEF), with a (48.13%) percentage, whereas the healthy control sample's ejection fracture is of the preserved ejection fraction type (HFpEF), with a percentage of (53.368 %).

Relationship Between Liver Enzymes and Ejection Fraction

In the case when comparing the ratio of AST/ALT enzymes and the heart's ejection fraction across all age groups tested, the results, as presented in Table (2) demon-strate a significant level (P ≤ 0.050) association between the ALP enzyme and EF for the patients who are the age group (35-45 years). Results for the correlation and relationship between cardiac ejection fraction and AST/ALT enzymes are depicted in Figure 2. They reveal a weak and positive linear correlation (r = 0.10650) and no statistically significant differences (p = 0.4724) at a probability level of (P ≤ 0.050).

Discussion

The results of our study agree to some extent with many studies conducted to assess ejection fraction in patients, including patients with myocardial infarction[11-13]. Another study conducted by (14) to evaluate EF in patients with heart failure revealed that the elderly patients have preserved EF HFpEF, while the el-derly patients in our study have a midrange EF type HFmrEF. If the ejection fraction of the heart's left ventricle is ≤ 40%, it is considered heart failure with reduced ejection fraction (HFrEF). If the ejection fraction of the left ventricle of the heart is 40% and <50%, it is considered heart failure with mid-range ejection fraction (HFmrEF), but if the is LVEF ≤ 50%, it is considered a preserved ejection fraction HFpEF[11]. so far, at present it remains ejection fraction EF is the first measure for diagnosing heart failure HF, one of the most common causes of which is a weakness or defect in the left ventricular muscle of the heart, which often occurs in patients with acute myocardial infarction[12,15]. HF and liver disease are among the most common mortality causes worldwide[16]. The heart's inability ex-plains heart failure to sufficiently pump blood to all body organs due to a synthetic and functional heart disorder. Heart failure is measured by assessing the EF of the left ventricle of the heart LVEF[15]. An elevated AST/ALT ratio leads to an in-creased ejection fraction, and this convergence may be caused by the fact that patients are in the early stages of the disease. Many researchers in different countries[17-19] have studied the correlation between liver defect and heart failure. The researcher (20) reveals

Variable	Age groups		
(EF)	Age 35-45	Age 46-56	Age 57-80
Patients	1.18 ± 52.41*	1.17 ± 48.13*	1.41 ± 43.76*
Control	1.00 ± 51.34	1.23 ± 53.46	0.93 ± 43.04

Table 1. Ejection fraction of patients and healthy people (control) for all age groups.

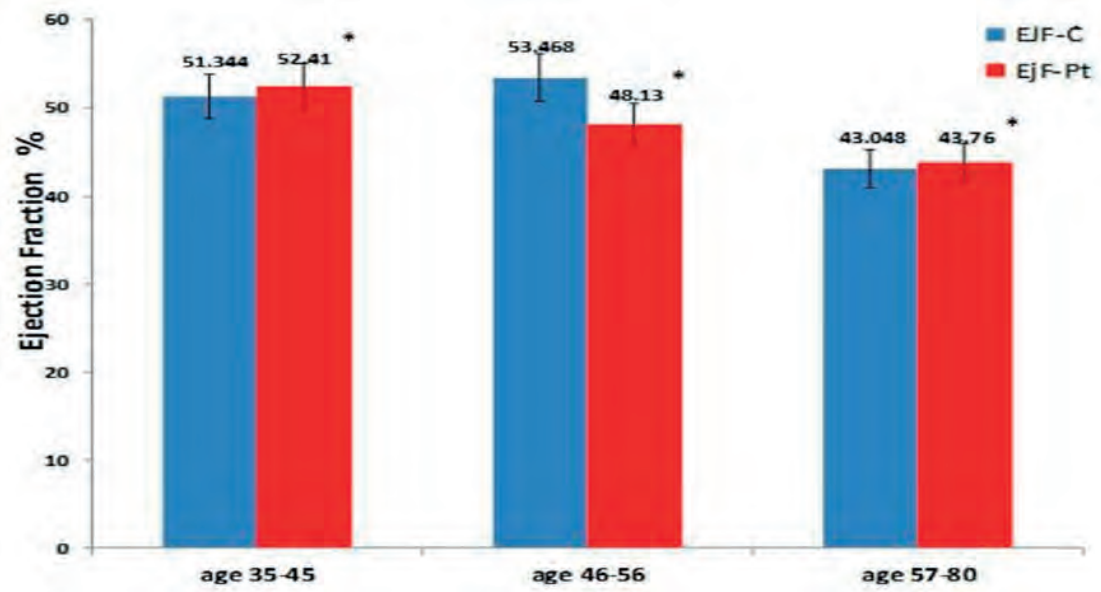

Figure 1. Ejection fracture of cardiac and healthy patients (control) for all age groups.

Liver Function	Age groups	Ejection Fraction		P-VALUE
		EF< 50	EF >50	
ALP>150U/L	35 - 45	0	23	0.024
	46 - 56	24	0	0.463
	57 -80	13	0	0.0761
AST/ALT ≥2	35- 45	0	23	0.4432
	46 - 56	24	0	0.364
	57 - 80	13	0	0.610

P≤ 0.05 Significant, EF, Ejection Fraction

Table 2. Correlation between liver enzymes and ejection fraction.

that the deficiency in liver enzymes and the increased AST enzyme levels indicate heart failure that requires medical attention for hospital stewards. In the study conducted by researcher[21], there are normal levels of ALP enzyme or moderately high serum in patients, which disagrees with our study's results, as shown in Table (2). In a study conducted by the researcher (19) to find correlation between EF ejection fracture and liver functions in HF patients, he concludes that patients with ≤ 40% ejection fraction have an increase in the process of the liver by 92.50%, whereas there is an increase in the function of the liver by 61.7% in patients with ejection fraction > 40%.

The interaction between liver and heart diseases is predictable. There is a close interconnectedness between them, and the reason is that the liver receives 25% of the blood pumped by the heart[22] and not only cardiovascular system is damaged by heart failure in the long term but the damage is done to the rest of the body organs and various tissues[23,24]. In a study conducted by the researcher (15), it has been intended to create a relationship between EF and liver function in patients who have heart failure, by which it is concluded that the levels of ALP, ALT and AST en-zymes are elevated in patients' serum who have an average ejection fraction ≤ 40% and lower levels in patients with an average ejection fraction > 40%. This is somewhat consistent with our findings, as shown in Table (2). Many studies have shown an increase or rise in ALP enzyme levels in the blood serum of HFpEF patients[17,19,25]. People with liver diseases related to heart failure are often not symp-tomatic for prolonged periods and are accompanied by a defect in liver enzymes or abnormal levels, which is the first sign of liver disease[26-29].

Conclusions

The findings suggest that liver function and ejection fraction are related in those with AMI.

Author Contributions

The experiments were designed and carried out by all authors. The data were examined, and Duaa Mohammed Al-Nafoly wrote the manuscript. The article was read and approved by all the authors.

Funding

This research received no external funding.

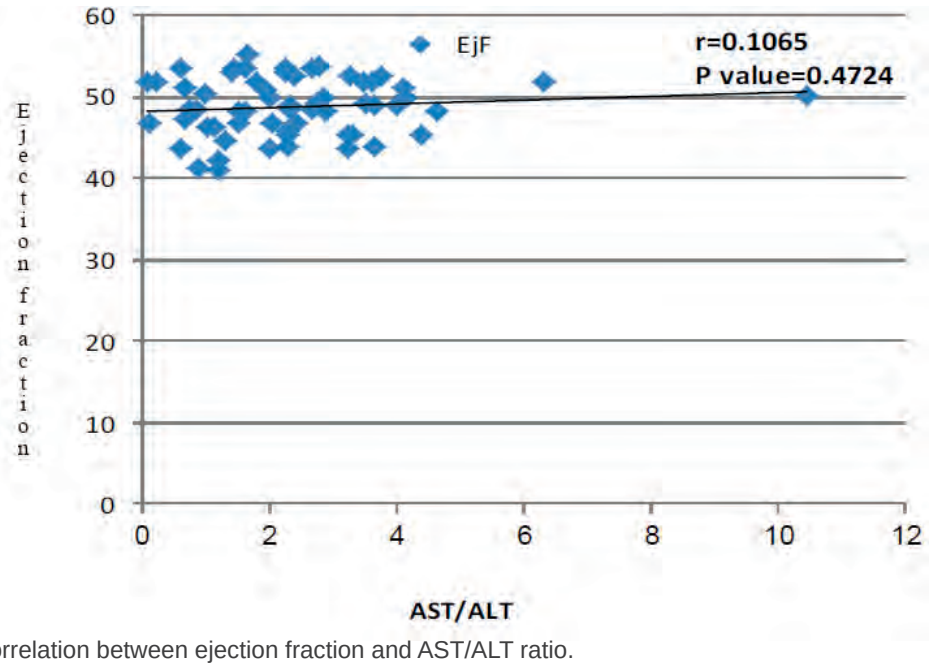

Figure 2. The correlation between ejection fraction and AST/ALT ratio.

Institutional Review Board Statement:

The Medical Research Ethics Committee at Mosul Univ. and Iraqi Medical Hos-pitals approved this work. The research's blood sample collection and all patient consent requirements have been approved. All patients' identities have been kept private as necessary.

Acknowledgments

The Dept. of Biology, College of Science, Univ. of Mosul, support this research. Many thanks to the nurses and doctors at the cardiac surgery unit at AL- Salam teaching hospital, the intensive cardiac care unit, and outpatient clinics in Mosul City/Iraq, who helped to complete the present research.

Conflicts of Interest

The funders did not have any impact on the design of this study; in the collection, analyses, or interpretation of data, manuscript writing, or decision to publish re-sults.

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ARTICLE / INVESTIGACIÓN

Wheat seed deterioration stimulated by plant extracts

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DOI. 10.21931/RB/2022.07.04.15
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Abstract: During 2019-2020, the experiment was conducted in the laboratory of the Department of Field Crop Sciences, Faculty of Agricultural Engineering Sciences - Baghdad University, to investigate the impact of soaking wheat seeds produced during the 2016 agricultural season with three plant extracts (licorice root extract 2%, 4% and 6%, Acadian and Humic(500, 1000, & 1500 mg L^{-1}). Aside from the two control treatments (soaking in distilled water with dried seeds). The results show that the soaking treatment with licorice root extract outper-formed the other therapies in conventional laboratory germination, root length, and seedling vigor index (95 percent and 3.42 cm 1207) compared to the two control treatments (soaking with distilled water and dry seeds). While all the Humic and Acadian soaking treatments at the concentrations (500 and 1000) mg L^{-1} did not significantly differ with the distilled water soaking treatment.The characteristics of standard laboratory germination percentage, root length, coleoptile length and seedling vigor index. Thus, we conclude that soaking wheat seeds with high concentrations of Acadian (more than 1000 mg L^{-1}) leads to a deterioration in the vitality of the seeds. While soaking with licorice root extract enhances the vibrancy and ac-tivity of wheat seeds compared to the other extracts used. As a result, we propose soaking the somewhat old and low-vital wheat seeds in a concentration of at least 2% licorice root ex-tract.

Key words: Radicle dry weight, Seedling vigor, seed germination, seed storage.

Introduction

Regarding nutritional, industrial, and economic importance, planted areas, and overall output, wheat is the first cereal crop in Iraq and the globe. However, Iraqi production of this crop is modest compared to world production and does not meet the country's needs. The reason for this may be due to poor management as well as environmental conditions during the growing season. Because of this, it is necessary to search for suitable means that may help raise the productivity of this vital crop, starting with the seed and field establishment, because of its great importance in establishing the final seed yield. Seed priming technology is one of the most important ways to improve the vitality of seeds and give solid and homogeneous seedlings, and then a good and early field establishment[1]. Soaking the seeds with plant extracts can stimulate growth as a result of containing several natural compounds that encourage vegetative and flowering growth and yield characteristics[2,3]. In addition, it is cheap, readily available, easy to use and does not pollute the environment; the most important of these extracts is licorice (*Glycyrrhiza glabra L.*)[4]. The behavior of licorice root extract is similar to that of gibberellin because it contains the primer of gibberellin biosynthesis (the intermediate lion compound of mevalonic acid), which stimulates an increase in germination rate and aids cell division and elongation. This increases the size of the vegetative system and improves flowering and yield characteristics[5]. Wheat seeds were irrigated with three concentrations of licorice root extract (1, 3 and 5%) in addition to the control treatment (distilled water), and a beneficial effect on viability and germination vigor was found[1]. Priming the seeds of three types of bread wheat (Abu Ghraib 3, IPA 99 and Al-Fath) by soaking them with concentrations of growth regulators and plant extracts, including licorice root extract, which improved the vitality and activity of seedlings of wheat cultivars compared to the control treatment. Seaweed extracts have many benefits for plant growth in general and enhance growth characteristics, as they improve the seed germination process and emergence in the early stages of growth[6]. Most extracts of medicinal plants, including marine plants, include chemicals that can activate the proteinase enzyme, which works to break down proteins during the onset of germination[7].

Furthermore, soaking crop seeds, particularly wheat, in aqueous solutions containing seaweed extracts improve the physiological processes within the seed. This adds to the success of seed germination and field emergence by shortening seed germination time and producing vigorous seedlings[8-10]. Among the most critical materials used in the seed priming process are organic nutrients such as Humic[11,12]. The importance of priming seeds with humic acid in achieving the highest germination rate and speed of germination d may be due to their absorption of the nutrients present in the organic solution), which provides a rapid source of energy and materials needed in the vital construction of the developing embryo. This helps the embryo inside the seed to rush from the heterotrophic to the autotrophic stage. Patil 201013 found a significant relationship between the amount of humic used, germination percentage, coleoptile and radicle length. As a result, this study aimed to investigate the

Citation: Al-Khafajy M J, Majeed H A, Mutlag N A, Cheyed S H. Wheat seed deterioration stimulated by plant extracts. Revis Bionatura 2022;7(4) 15. http://dx.doi.org/10.21931/RB/2022.07.04.15
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022
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effect of seed priming of degraded wheat seeds using certain plant extracts on the vegetative characteristics of wheat germ.

Materials and methods

During 2019-2020, the experiment was carried out in the Seed Technology Laboratory of the Department of Field Crop Science, College of Agricultural Engineering Sciences - University of Baghdad, to study the effect of seed priming of wheat (IPA 99) produced in 2016 with three types of organic extracts (Acadian, Humic and Licorice Root Extract), as well as two control treatments (soaking with distilled water, and dry seeds). The experiment was carried out using a complete randomized design (CRD) with four replications.

To make licorice root extract, 200 gm of root powder was soaked in one liter of water for 24 hours, then the extract was filtered using filter paper, and the filtrate was diluted to prepare three concentrations of the extract, namely (2, 4 and 6%). Three concentrations of acadium (500, 1000 and 1500) mg L-1 were also prepared, and three concentrations of humic (5, 10 and 15) mg L-1. Wheat seeds were steeped in the extracts and concentrations described above for 12 hours before germinating in plates in four replications with 50 seeds per replicate using the germinator at 25°C and 85 percent humidity for ten days.

Characters studied

Standard laboratory germination (%): The number of normal seedlings was calculated on the tenth day of the examination, and the percentage of standard laboratory germination was calculated using the following equation[14]:

$$\text{Standard laboratory germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds}} \times 100$$

On the tenth day of the examination, ten normal seedlings were taken from each experimental unit, and the following characteristics were measured:

•Germination Speed: The following law was used to compute the rate of germination[15].

$$\text{Germination Speed} = \frac{\text{Σ (The number of seeds germinated in each count x the number of the day)}}{\text{Germination rate at the end of the examination period}}$$

Radicle and coleoptile length, radicle and coleoptile dry weight

The radicle and coleoptile were removed from their contact regions with the seed and dried in a dry-ing oven at 65 °C for 72 hours before being weighed using a four-decimal sensitive scale to compute the dry weight of each of the radicle and coleoptile.

Seedling vigor index

The Seedling vigor index was determined using the equation below[16].

The data for the examined qualities were statistically analyzed using a completely randomized design (CRD). The averages were compared using the least significant difference (LSD) test at a threshold of significance of 5% using the Genstat v12.1 software.

Results and discussion

Most of the seed quality characteristics show substantial changes among the treatments, according to the data (Table 1); in the laboratory standard germination rate, the licorice root extract soaking treatment was outperformed with a germination rate of 95%, while the Acadian soaking gave the lowest laboratory standard germination rate of 74.58%, which were not statistically different from the two control treatments (distilled water soaking and dry seeds). For germination speed rate and radicle length rate, the results showed no significant variations among treatments. The results showed that soaking with the extracts resulted in a considerable increase in the mean coleoptile length compared to the two control treatments (distilled water wash and dry seeds), which resulted in the lowest mean coleoptile length of 8.44 cm and 8.22 cm, respectively. While the Humic soaking treatment resulted in the most extended average coleoptile length (9.35 cm), it did not differ substantially from the licorice root extract and Acadian soaking treatments.

Compared to the other treatments, the soaking with Humic worked much better, with the root's most significant average dry weight (3.45 mg). While the two soaking treatments of licorice root extract and Acadian were not substantially different from the control treatments (soaking with distilled water and dry seeds). While the Acadian and Humic soaking treatments were considerably superior in terms of the coleoptile average dry weight (7.38 mg and 7.31 mg, respectively), they did not vary significantly. When compared to the two control treatments, the soaking treatment with licorice root extract did not substantially increase the average dry weight of the coleoptile. Regarding seedling vigour index, the soaking treatment with licorice root extract yielded the highest rate of 1207, followed by the wet treatment with Humic, which produced 1073. This might be because Acadian has the most significant standard laboratory germination rates. Still, the data show that soaking with Acadian had no signifi-cant changes compared to the two control treatments[17,18].

The results show considerable changes in the percentage of laboratory germination across treatments (Table 2). When compared to the two control treatments (soaking with distilled water and dry seeds) and the rest of the soaking treatments with Acadian and Humic, all soaking treatments with licorice root extract were significantly superior and gave the highest rates of laboratory germination (93.75 percent, 96.25 percent, and 95.00 percent) for concentrations of 2%, 4%, and 6%, respectively. While soaking with Acadian at a dosage of 1500 mg L^{-1} resulted in the lowest average of the usual laboratory germination rate of 56.25 percent. Soaking treatments in Acadian at 500 mg L^{-1} and 1000 mg L^{-1} concentrations did not differ appreciably from the soaking treatment with purified water.

The analysis of variance results showed significant differences in germination speed between treatments. It is noted that the speed of germination increases with the concentration of Acadian, with the concentration of 1500 mg L^{-1} giving the highest rate of germination speed at 7.46 seedlings day^{-1}. The soaking treatment with Humic at a dosage of 1500 mg L^{-1} produced the lowest germination rate of 6.72 seedlings day^{-1}, which did not differ substantially from the dry seeds treatment. The findings also show no significant variations in average radicle and coleoptile length across treatments. The data also show that soaking in licorice root extract at 2% and Acadian at 500 mg L^{-1} resulted in a substantial decrease in radicle dry weight, which was 1.93 mg and 1.97 mg, respectively, as compared to the two control treatments (soaking with distilled water and dry seeds).

Treatments	Seed quality characters						
	Germination %	Germination speed (seedling day-1)	Radicle Length Cm	Coleoptile Length cm	Radicle Dry wt Mg	Coleoptile Dry wt mg	Seedling vigor index
Licorice root extract	95.00	3.58	3.42	9.29	2.65	5.50	1207
Acadian	74.58	3.51	3.31	9.02	2.68	7.38	893
Humic	84.59	3.53	3.29	9.35	3.45	7.31	1073
Distilled water	80.00	3.54	3.18	8.44	2.25	5.50	932
Dry seeds	75.00	3.41	2.88	8.22	2.08	5.18	833
LSD $_{0.05}$	5.48	N.S	N.S	0.67	0.58	0.67	118

Table 1. Soaking wheat seed in licorice root extract, Acadian, and Humic affects various quality attributes.

While increasing the concentrations of such extracts resulted in a considerable rise in the radicle's dry weight rates. Soaking with Humic raised the dry weight of the radicle at a rate of 4.40 mg at a concentration of 1000 mg L^{-1}. Subsequently, it dropped at a concentration of 1500 mg L^{-1}, yielding an average dry weight of the radicle of 2.38 mg with no significant differences between the two control treatments (soaking with distilled water and dry seeds).

The results also show that the average dry weight of the coleoptile behaved similarly to the average dry weight of the radicle due to the effect of soaking the extracts and their different concentrations, as the soaking treatment with licorice root extract at a concentration of 2% gave the lowest average dry weight of the coleoptile (4.28 mg). In contrast, the soaking treatment with Acadian gave the highest rate of 8.33 mg.

The seedling vigour index improved dramatically as the concentrations of licorice root extract increased, with the concentration of 6 percent producing the highest mean seedling vigour index of 1264 (without any significant differences between the concentrations). Whereas soaking with Acadian produced the maximum rate of seed-ling vigour index at a concentration of 500 mg L^{-1}, it dropped with increasing extract concentration to produce the lowest rate of seedling vigour index at a concentration of 1500 mg L^{-1}. It is also reported that soaking with Humic at doses (500 and 1000) mg L^{-1} increased the mean seedling vigour index to 1101 and 1139, respectively, as compared to the two control treatments (there is no significant differences between the two concentrations). However, increasing the concentration of Humic to 1500 mg L^{-1} resulted in a drop in seedling vigour index, which reached 979. There were no significant differences between the different concentrations of licorice extract and the two Humic (500 and 1000) mg L^{-1} doses.

Conclusions

This study shows that soaking degraded wheat seeds in storage with licorice root extract, Acadian and Humic significantly improved the percentage of standard laboratory germination, speed of germination and seedling vigor. However, high Acadian concentrations (over 1000 mg L^{-1}) negatively influenced wheat seed germination. When humic concentrations approach 1000 mg L^{-1}, the rates of wheat seed quality features to increase and then decline with growing attention.

Treatments		Seed quality characters						
Extract	Conc.	Germination %	Germination speed seedling) (day-1	Radicle Length Cm	Coleoptile Length cm	Radicle Dry wt mg	Coleoptile Dry wt mg	Seedling vigor index
licorice root extract	%2	93.75	7.24	3.43	8.66	1.93	4.28	1130
	%4	96.25	7.16	3.11	9.65	2.48	5.15	1228
	%6	95.00	7.08	3.74	9.56	3.55	6.80	1264
Acadian	500	85.00	6.74	2.74	9.29	1.97	5.85	1001
	1000	85.50	6.88	3.23	8.32	2.95	7.95	951
	1500	56.25	7.46	3.49	9.44	3.13	8.33	727
Humic	500	86.25	7.18	2.98	9.72	3.58	7.63	1101
	1000	85.00	7.26	3.54	9.85	4.40	8.10	1139
	1500	82.50	6.72	3.36	8.46	2.38	6.20	979
Distilled water		80.00	7.08	3.10	8.44	2.25	5.50	932
Dry seeds		75.00	6.82	2.88	8.21	2.08	5.18	833
LSD $_{0.05}$		6.97	0.24	NS	NS	0.66	0.86	167

Table 2. Soaking wheat seed in various doses of licorice root extract, Acadian, and Humic affects several quality characteristics.

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ARTICLE / INVESTIGACIÓN

Analyzing the nutritional value of *Acanthopagrus latus* and *Coptodon zilli* muscles from the Al-Hindiya River in the Iraqi region of Karbala

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DOI. 10.21931/RB/2022.07.04.16

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Abstract: The present study dealt with the determination of the protein, fat, moisture and ash chemical content for two species of bony fish (Teleosts) that belonged to two different families, namely *Acanthopagrus latus* (Sparidea) and *Coptodon zilli* (Cichlidae). Also, the total energy (calories) to the protein and fat contents in the muscles of the two studied fish and for two regions for each species. Samples were collected from the Al-Hindiya river in Karbala Province between August and October 2020. The current results noted an apparent difference in the mean values of chemical contents for the studied length groups, which ranged between 100-400 mm, and for two regions of the body, as the rates of protein content in A. latus were 17.42-18.60% while their rates ranged in C. zilli between 17.20-18.54%. The rates of fat content in A. latus were 6.40-7.50%, whereas ranged between 5.70-6.30% in C. zilli and the rates of moisture content ranged from 74.60 to 70.16%, while In C. zilli fish, the rates ranged between 75.40-72.40%, and the ash content ranged from 1.42 to1.80% and 1.14-1.34% in A. latus and C. zilli fish respectively. Both fish were considered medium-fat content fish depending on its value in their muscles.

Key words: Nutritional value, fish muscles, *Coptodon zilli*, *Acanthopagrus latus*.

Introduction

Fish is one of the oldest natural resources that man has exploited and benefited from, thus in-creasing its demand for its flavor and high nutritional value in addition to its ease of adaptation and suitability as an essential food component[1]. The study of the chemical composition of fish rep-resents an important aspect, as it can determine its nutritional value. Plans have been drawn up to study the possibilities of its use since it contains a high percentage of proteins. These proteins range from 15-20% and include all the essential amino acids. They meet the organism's needs and contain fats ranging from (15-22%)[2]. These fats contain essential fatty acids that the body needs[3]. They are rich in mineral elements such as phosphorus, sulfur, copper, iron, and fluorine, which are included in forming hormones and enzymes that benefit human health[4]. Oda[5] explains that by studying the chemical composition of the fat content, we can know what the fish contains from them, thus giving us their actual nutritional value. On this basis, the fish are divided into three sections based on their fat content, namely: Fatty fish in which the lipid content is more than 10% and medium-fat fish with a fat content ranging between (2.5-10%), and non-fat or meaty fish with a fat content of less than (2.5%). The ash content of fish consists of many salts found in the soft part of the tank. In a way that the body can benefit from it when consuming it as food[6]. As the ash content is the accurate indicator of the mineral content of fish, we notice a higher percentage in marine fish compared to river fish[7].

Materials and methods

Sampling

Two locally important fish species (*Acanthopagrus latus* and *Coptodon zilli*) were chosen and collected for the determination of the chemical components of muscles in. Samples of fish collected from the Al-Hindiya River in Karbala province – Iraq, from August to October 2020. They were transported to the laboratory to perform the tests associated with estimating the chemical com-position of the studied fish muscles. Two body regions from the studied fish were chosen, the first being the trunk region below the dorsal fin (R1) and the second region representing the caudal peduncle (R2). Protein, fat, moisture and ash contents were determined in each specimen's muscles according to (8),(9). The caloric value energy for protein and fat content of fish's muscles was es-timated according to the method of (10).

Statistical Analysis

Statistical analyses were conducted using the IBM SPSS Statistical 25 software. The data of chemical components obtained from muscle fibers for two different regions in the studied fish were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Variations were considered to be significant when the P value < 0.05.

Citation: AL-Humairi K, AL-Muhanna M, Mansour A. Analyzing the nutritional value of Acanthopagrus latus and Coptodon zilli muscles from the Al-Hindiya River in the Iraqi region of Karbala. Revis Bionatura 2022;7(4) 16. http://dx.doi.org/10.21931/RB/2022.07.04.16
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

Results

The results of the chemical content analysis for calculating the levels of protein, lipid, moisture and ash content of the lateral muscles in the studied body regions R1 and R2, and in both studied species, showed apparent differences in the values of their rates and the studied length groups, whose length ranged between (100-300 ml). As in Tables (1) (2), the levels of protein content ranged in *A. latus* fish (17.42-18.60%), while those of *C .zilli* fish ranged (17.20-18.54%), while the levels of lipid content in *A. latus* fish (17.42-18.60%), while the rates ranged in *C. zilli* fish (5.60-6.30%). The rates of moisture content in *A. latus* ranged between (74.60-70.16%), while it was (75.40-72.40%) in *C. zilli* fish, while the ash contents were (1.42% -1.80%) in *A. latus* fish, while in *C .zilli* fish, its rates ranged between (1.14-1.34%). The statistical analysis of the results to clarify the recorded differences proved the presence of significant differences when studying the protein, fat, ash and other contents. Differences in moisture content were not significant (P>0.05) in the R1 region in the two studied fish. Still, when looking at protein, fat and ash contents, the differences were significant (P<0.05) in the same region in the studied species(Table 7). While in the R2 area, the differences in protein and moisture content were non-significant (P >0.05), whereas they were significant (P < 0.05) in fat and ash contents in the two studied fish(Table 7). The results of the total energy of protein and fat content in the two regions and both species showed a difference in the values of their rates and length groups searched. The energy values of protein content ranged from (69.86-47.40% kcal / g) in *A. latus* fish, while their total values ranged from (68.80-74.16 % Kcal / g) in *C. zilli* fish. While the energy values for fat content in *A. latus* were (57.60-67.50% kcal / g) while in *C. zilli*, the values ranged between (50.40-56.70% kcal / g). The total energy of protein and fat content for the two studied regions ranged between (127.28-141.90% kcal/g) in *A. latus* fish, while it ranged (119.20-130.86% kcal / g) in *C. zilli* (Tables 3,4,5,6).

	R1				R2			
Length	Protein %	Fat %	Moisture %	Ash %	Protein %	Fat %	Moisture %	Ash%
100 – 300 mm	17.42 ± 0.21	6.40 ± 0.28	74.60 ± 0.32	1.42 ± 0.03	18.20 ± 0.19	6.64 ± 0.28	73.40 ± 0.26	1.50 ± 0.04
	17.66 ± 0.22	6.54 ± 0.20	74.20 ± 0.31	1.50 ± 0.02	18.40 ± 0.18	6.80 ± 0.21	73.24 ± 0.31	1.62 ± 0.03
	18.16 ± 0.18	6.60 ± 0.21	73.58 ± 0.27	1.54 ± 0.05	18.48 ± 0.22	6.86± 0.24	72.62 ± 0.28	1.70 ± 0.02
	18.20 ± 0.14	7.08 ± 0.23	72.48 ± 0.31	1.60 ± 0.04	18.56 ± 0.22	7.40 ± 0.23	71.20 ± 0.30	1.74 ± 0.05
	18.30 ± 0.24	7.30 ± 0.25	72.25 ± 0.28	1.72 ± 0.03	18.60 ± 0.18	7.50 ± 0.29	70.16 ± 0.34	1.80 ± 0.03

Standard error ±

Table 1. Chemical composition of the muscles in(R1, R2) regions in *A. latus*.

	R1				R2			
Length	Protein %	Fat %	Moisture %	Ash %	Protein %	Fat %	Moisture %	Ash %
100 – 300 mm	17.26 ± 0.16	5.60 ± 0.17	75.40 ± 0.31	1.22 ± 0.02	18.16 ± 0.18	5.80 ± 0.26	73.60 ± 0.33	1.14 ± 0.03
	17.30 ± 0.22	5.64 ± 0.21	74.60 ± 0.25	1.26 ± 0.03	18.20 ± 0.22	5.92 ± 0.23	73.50 ± 0.30	1.18 ± 0.04
	17.50 ± 0.18	5.72 ± 0.23	74.40 ± 0.33	1.28 ± 0.04	18.28 ± 0.19	6.12 ± 0.20	72.82 ± 0.31	1.24 ± 0.05
	17.52 ± 0.24	5.78 ± 0.22	74.20 ± 0.35	1.32 ± 0.07	18.40 ± 0.20	6.26 ± 0.22	72.60 ± 0.27	1.28 ± 0.02
	17.70 ± 0.26	5.82 ± 0.24	74.14 ± 0.30	1.34 ± 0.05	18.54 ± 0.17	6.30 ± 0.25	72.40 ± 0.29	1.32 ± 0.04

Standard error ±

Table 2. Chemical composition of the muscles in (R1, R2) regions in *C. zilli*.

Protein %	Energy kcal/g	Fat %	Energy kcal/g	Total energy kcal/g
17.42	69.68	6.40	57.60	127.28
17.66	70.64	6.54	58.86	129.50
18.16	72.64	6.60	59.40	132.04
18.20	72.80	7.08	63.72	136.52
18.30	73.20	7.30	65.70	138.90

Table 3. Total energy of the protein and fat content in muscles R1 regions in *A. latus*.

Protein %	Energy kcal/g	Fat %	Energy kcal/g	Total energy kcal/g
18.20	72.80	6.64	59.76	132.56
18.40	73.60	6.80	61.20	134.80
18.48	73.92	6.86	61.74	135.66
18.56	74.24	7.40	66.60	140.84
18.60	74.40	7.50	67.50	141.90

Table 4. Total energy of the protein and fat content in musclesR1 regions in *C. zilli*.

Protein %	Energy kcal/g	Fat %	Energy kcal/g	Total energy kcal/g
17.20	68.80	5.60	50.40	119.20
17.30	69.20	5.64	50.76	119.96
17.50	70.00	5.72	51.48	121.48
17.62	70.48	5.78	52.02	122.50
17.70	70.80	5.82	52.38	123.18

Table 5. Total energy of the protein and fat content in musclesR2 regions in *A. latus*.

Protein %	Energy kcal/g	Fat %	Energy kcal/g	Total energy kcal/g
18.16	72.64	5.80	52.20	124.84
18.20	72.80	5.92	53.28	126.08
18.28	73.12	6.12	55.08	128.20
18.40	73.60	6.26	56.34	129.94
18.54	74.16	6.30	56.70	130.86

Table 6. Total energy of the protein and fat content in musclesR2 regions in *C. zilli*.

Region	Parameters	F. value	Sig. value	Differences type
R1	Protein %	6.069	0.039	Significant
	Fat %	36.552	0.000	Significant
	Moisture %	4.685	0.062	Non-Significant
	Ash %	24.727	0.001	Significant
R2	Protein %	1.771	0.220	Non-Significant
	Fat %	23.704	0.001	Significant
	Moisture %	1.645	0.236	Non-Significant
	Ash %	51.380	0.000	Significant

Table 7. Statistical analysis of the chemical composition of the muscle regions (R1, R2) in *A. latus* and *C. zilli*.

Discussion

Temperature is one of the essential and influencing environmental factors in fish life, and water temperature is affected by air temperature. The first directly affects the physical and chemical characteristics and the living conditions of the water surface[2,6,11]). The variation is attributed to the chemical composition of the fish as well as the fish being studied, the sex, the nature of the food, the feeding, or the fishing spot[12-14].

The study showed a clear difference in the values of the chemical composition of protein, lipid, moisture and ash in the study fish, in addition to the difference between the studied body areas. The protein and water in the fish's anterior region (R1) are more significant than in the posterior region (R2). At the same time, the proportion of fat and ash

in the rear part is more critical than in the front area, which is consistent with (15,16,17).

The results showed that there is a difference in the percentage of fat in the two studied types of muscles of the study fish, and this may be due to the difference in the type, sex, age, location of muscle fibers and specific factors related to fish feeding and migration[1,18,19].

The study's results showed that the percentage of moisture in the muscles of the study fish was inversely proportional to the proportion of fat, that is, the more moisture in the fish, the lower the fat percentage in it[6,8,20].

The difference between the contents of the ash percentage in the fish is due to the body's metabolism or nutrition[5,15,21]. The calories in fish are directly proportional to the rate of fat in their muscles, so the more calories, the percentage of fat increases because one gram of fat gives nine calories when oxidized. In contrast, protein and carbohydrates contain four calories[7,16,19].

Conclusions

The nutritional values of fish muscles depend on their protein and fat content, and these all change according to the conditions accompanying them, such as nutrition, seasonal variations, and physiological status. On this basis, The *A. latus* and *C. zilli* are considered medium-fat fish de-pending on the fat content in present fish muscles.

Author Contributions

All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

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ARTICLE / INVESTIGACIÓN

Nutritional values and phytochemical analysis of *Allium calocephalum* Wendelbo, a valuable endemic wild garlic to Zagros mountains

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DOI. 10.21931/RB/2022.07.04.17

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Abstract: Wild edible plants provide the local people with food and medicines and are considered one of the natural eco-system services. These wild edible diets and herbal medicine always reflect local communities' regional identity and their traditional ecological knowledge. In the new global economy, the natural product field has become a central issue for preserving the traditional culture related to nature, particularly in the context of a sustainable environment. This research study aims to determine the nutritional value and phytochemical contents in a wild population of *Allium calocephalum*. This wild edible garlic, endemic to the Zagros mountains, is overharvested by Kurdish rural people to enhance their food security at a household level and to perpetuate the preservation of their natural heritage. Here, we estimated the total phenols, flavonoids, carbohydrates, protein, fibers, ash, oil yield, and significant mineral content in both leaves and bulbs of *A. calocephalum*. Phytochemical analyses were conducted at the Faculty of Agricultural Engineering Sciences (University of Duhok) and the environ-mental directory of Duhok, Kurdistan Region of Iraq, to get an overview of its nutrients and phytochemical values. Interestingly, a high level of phenolic compounds was obtained from bulbs (0.684 mg gallic acid equivalents/g of dry extract, eq.100g-1). The lowest level of phenolic compounds was found in leaves (0.522 mg gallic acid equivalents/g of dry extract, eq.100g-1). Simultaneously, the bulbs extract gave higher content of flavonoid compounds than the leaves extract (6.31 and 4.73 µg quercetin equivalents/g of dry extract, eq.100g^{-1}, respec-tively, for the bulbs and leaves). The highest dry weight basis of total carbohydrates, energy value (Kcal), oil content, and moisture content were observed in bulbous parts, and the values were 71.75, 408.86 (Kcal), 9.52, and 92.37, respectively. On the other side, the highest dry weight basis of total protein, fibers, and Ash content was observed in shoot parts, and the values were 15.93, 13.89, and 9.32, respectively. The evidence from this research study supports the idea that this Zagrosian endemic wild garlic enhances the food security and the nu-trient diet values of the rural Kurdish people.

Key words: Wild garlic; ethnobotany; edible plants; food security; natural resources; herbal medicine.

Introduction

Our knowledge of environmental conservation and human well-being is fundamentally shaped by the linkages and interactions between human cultures and plants[1-3]. The production of food, shelter, clothes, transportation, fertilizers, flavors and scents, and medicines have all been basic human needs that have always been met by nature[4-6]. In this sense, sophisticated traditional medical systems that have existed for thousands of years and continue to offer humanity novel treatments have their roots in wild edible plants (WEPs)[7,8]. Despite some claims that plants pos-sess therapeutic properties turning out to be incorrect, medicinal plant therapy is founded on ac-tual data from hundreds, if not thousands, of years of use[8,9]. The practice of herbal medicine has gained credibility and acceptability within the local and medical communities in recent years, and there has been an increase in interest in the field[9,10]. The search for possible chemotherapeu-tic agents in nature continues, and over the past 40 years, numerous powerful medications have been produced from wild plants[7].

In addition to their therapeutic properties, these WEPs have significant nutritional value and are a crucial component of the diets of rural communities worldwide. These WEPs offer the human body high dietary benefits, including vitamins (A, B, C, etc.), a good number of minerals, carbo-hydrates, protein, fat, antioxidants, etc.[11,12]. They have actively aided in ensuring food security, particularly during times of crisis[13]. Due to their few side effects, the wide variety of options, and high nutritional value, local communities tend to turn to natural resources for treatment and regu-lar foods[2,11]. Any portion of the plant with active ingredients, in-

Citation: Hussein W I, Faizy H S, Youssef S M A. Nutritional values and phytochemical analysis of Allium calocephalum Wendelbo, a valuable endemic wild garlic to Zagros mountains. Revis Bionatura 2022;7(4) 17. http://dx.doi.org/10.21931/RB/2022.07.04.17
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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cluding the roots, bulbs, stems, bark, leaves, flowers, fruits, seeds, bulbs, gum, etc., can be used to make these wild foods or wild edible diets[14]. The *Allium* genus serves as a well known illustration of these WEP usages. The nutritional value of many Allium taxa has been examined globally[15], as well as their ethnobotanical aspect[16-18] and phytochemistry[19,20]. For illustration, in traditional medicine, the *Allium* taxa are widely used for treating common health problems like wounds, bacterial infections, and worms[21,22].

On the other hand, current research in contemporary medicine has demonstrated the healing properties of their active ingredients, including their ability to fight cancer[23], anti-inflammatory, cardio-protective[24], hepato-protective[25], antithrombotic[26], etc. Furthermore, the phytochemical characteristics of Allium taxa include essential oil, steroidal sapogenins, saponins, tannins, carbohydrates, proteins, vitamins, flavonoid glycosides, organic acids, amino acids, fatty acids, alkaloids, etc., have been well studied[19,20,27,28]. In addition, from the perspective of natural services, it is expected that many significant new treatments will be found and made commercially available in the future, just as they have up until now, by following the cues given by traditional knowledge and experiences.

People in daily life have used wild plants since the beginning of civilization[6]. These wild plants provide a significant portion of the natural ecosystem services regarded as edible for human use (i.e., food and dishes)[29,30]. The regional identity of the indigenous populations and their tradition-al ecological knowledge is always reflected in these wild edible diets[31-33]. The traditional wild food knowledge has been documented in numerous nations, regions, and continents around the world and has occasionally been referred to as a diet for local/regional communities: In Europe[33], Mediterranean Region[34,35], Iran[36], Turkey[18,37], Kurdistan Region of Iraq[16,38-40] etc. As a result, it is getting harder to disregard the significance of the WEPs in modern human society in the con-text of a sustainable environment. The market for natural products has emerged as a critical concern in the new global economy for maintaining the traditional culture associated with nature. Even though wild edible plants have always played a key role in local and regional folk tradi-tions, they are gradually losing position regarding plant usage patterns and behaviors[41,42]. The expansion of the agroindustry, urbanization, changes in lifestyle and land use decrease and/or loss of the local traditional ecological knowledge, and decreased interaction with nature are the key factors contributing to the changes in wild edible plant use patterns[31,33].

Additionally, some native plant species may be threatened by the current ongoing overharvesting and growing demand for edible wild plants by local people in urban areas. Therefore, it is essen-tial to implement the appropriate modifications to traditional cultural traditions to help the local populace comprehend the significance of preserving the WEPs and their natural habitat. Addi-tionally, wild edible plants are being included in both modern and traditional innovation models for local healthy diets and food security. Therefore, one of the most important justifications for protecting natural ecosystem resources is the potential for unknown pharmacological properties. This research study's objective was to offer information on the nutritional value and phytochem-ical components of wild edible garlic, *Allium calocephalum* Wendelbo, from Liliaceae family. This study has provided a broad view of its potential traditional use in current wild diets from the perspective of ethnobotany. Wild garlic from this particular *Allium* species is endemic to Zagros Mountains in NE Iraq and SE Turkey[18,43]. It is primarily found between 1200 and 2500 meters above sea level in the Mateen, Gara, Bradost, Hindreen, Sherîne, and Pîrês mountains among oak trees and scrub. Traditional uses include eating its leaves and young stem, frequently fried or combined with other dishes 16,18. However, little is known about this wild edible garlic, and no studies have been done on its nutritional benefits or phytochemical properties.

Materials and methods

Species study

Allium calocephalum is endemic wild garlic to the Zagros mountains of NE Iraq and SE Turkey[43]. It typically occurs in the upper forest zone of mountains like the Mateen, Gara, Bradost, Hin-dreen, Sherîne, and Pîrês ranges. Kurdish rural communities refer to it as "Soriyaz" locally. This garlic is typically collected from the wild between early spring and summer, frequently sold in traditional Kurdish markets[16,18]. Due to the increasing demand brought on by the rising human population, this wild edible garlic is severely threatened in this context of overharvesting. The ecology of seed germination and seedling growth of *A. calocephalum* species have recently undergone extensive research from the standpoint of natural regeneration[44]. From the perspective of biological conservation, it has been suggested that ethno-domesticating and cultivating this species will help prevent the removal of it from its natural habitats by giving many small farmers in rural areas a source of income[44].

Plant Material collection

Fresh plant material, including leaves and bulbs of the *A. calocephalum* species of Zagrosian endemic garlic, was taken from the north side of Gara Mountain (Hariké Village, Deralok District; latitude: 37.007705°; longitude: 43.689504°; elevation 1240 m). To estimate the phytochemical components and nutritional value correctly, We took the plant material in April 2018 when the species was in its complete development cycle. The harvested plant materials were placed in a polyethylene bag to protect against moisture loss while being transported in the field. Taxonomi-cally, the plant species were identified using the Flora of Iraq[43] as a source. Various specimens were deposited in the herbarium of the University of Duhok's Forestry Department (DPUH), College of Agricultural Engineering Sciences.

Sample Preparation

The shoot materials (leaves with stems) and bulbs were carefully cleaned by cutting the bulb roots and removing their outer tunics. After this cleaning procedure, each plant material was washed under running water and placed in the plastic basket to drain the remaining moisture. The samples were then split into smaller pieces and shade dried at room temperature for around 20 days. The dried parts were then pulverized using an electric household blender. For additional analysis, the powdered samples were kept in a glass jar and refrigerated (approximately 5 °C). The laboratory facility at the College of Agricultural Engineering Sciences, University of Duhok, was used to estimate the various sample parameters, including moisture content, total ash, crude oil, crude fiber, crude protein, energy value (kcal), and total carbohydrate on a dry weight basis, using 100g of a dried powdered sample.

Determination of Total Moisture Content

According to a technique provided in (45) Method No. 44-15A, the moisture content was measured: *A. calocephalum* fresh samples weighing 3 g each (for both leaves and bulbs) were put into a pre-weighed crucible (W0) and weighed (W1) before being heated in a forced draft oven for 24 hours at 98–100 °C. The crucibles were taken out, let to cool in a desiccator, and then weighed. The procedure was performed several times until the crucible, and wild garlic sample had a constant weight (W2). This was done to guarantee that the crucibles were dry. The equation below was used to determine moisture content:

%Moisture =(w1-w2)/(w1-w0)×100
Where:
W0 = Weight (g) of the empty crucible
W1 = Weight (g) of fresh garlic sample + empty crucible
W2 = Weight (g) of dried sample + empty crucible

Determination of Crude Proteins (Kjeldahl nitrogen method)

According to Kjeldahl's procedure, which included protein digestion, distillation, and titration[46], the dried powdered sample was examined for crude protein concentration.

Protein Digestion: By combining 0.5 g of each bulb and leaf sample with 10 ml of concentrated H2SO4 (98% conc.) and adding 2 ml of perchloride acid (70%) before heating, the protein con-tent was ascertained. The mixture was then heated in the digestion chamber until transparent res-idue contents were obtained. Then it was given time to cool. The digest was cooled before diluting it with the proper amount of distilled water to make it 100 ml.

Protein Distillation: The Markham distillation equipment was first steamed for 15 minutes. The condenser was then positioned beneath a 50 ml conical flask containing 5 ml of 2% boric acid to submerge the condenser tip in the solution. Through a tiny funnel hole, 5.0 ml of the digested ma-terial was pipetted into the apparatus's main body. After washing the digest with distilled water, 5 ml of (10 N) NaOH solution was added. The condenser's digest was boiled until enough ammoni-um salt was obtained. The green color of the boric acid plus indicator solution indicated that all of the ammonia released had been captured.

Protein Titration: By determining how much HCl was used in the process, the concentration of 0.01 N HCl was added to the solution in the receiving flask until it changed color to red. Following titration, the formula was used to determine the % nitrogen:

%N=(V.HCl*N HCl(0.01)*Nitrogen Atomic Mass(14))/1000*(100ml D.Water)/(5ml Sample So-lution)*(%100)/(0.5 Wt.of Sample)
Where, %N = Nitrogen percentage
V. HCl = Volume (ml) of acid required to titrate
N HCl = Normality of the HCl
D. Water = Distilled water added
Wt.= Weight of a sample (g).

The formula was then used to determine the sample's crude protein content as a percentage.:
%Crude Protein =%N*F (6.25)
Where F= Conversion factor is equivalent to (6.25)

Determination of Crude Oil

Soxhlet extraction for six hours was used to evaluate the crude fat content of the powdered sample. Approximately 3.0 g of samples were precisely weighed and placed in labeled thimbles. 100 ml of hexane 96% was added to the dried boiling flasks (250 ml), which had been considered in accordance. Cotton wool was used to cover the extraction thimbles securely. After putting the Soxhlet apparatus together, the flask was attached to the extractor, set on the heating mantle, and allowed to reflux for 6 hours. After the extraction was finished, the thimble was taken off, and the boiling flask was heated in a hot air oven at 70 ºC until the solvent evaporated and the hexane was almost eliminated. The flask was dried, cooled in a desiccator, and weighed again[45].

The percentage of crude oil was calculated using the formula:

Crude oil (%) = (Wt. of oil (g) / Wt. of the original sample (g)) × 100

Determination of Crude Fiber

The percentage of crude fiber was calculated using the literature recommended method of (47). Following this, 2 g of a dried, fat-free sample of *A. calocephalum* powder was weighed (W0) into a 1000 ml conical flask. H_2SO_4 (20 ml with 20%) and distilled water (100 ml) were combined, and the mixture was gently heated for 30 minutes. The content was filtered through Whatman No.1 filter paper. A spatula was used to scrape the residue back into the flask. After that, 100 ml of distilled water and 20 ml of 10% NaOH were added, and the mixture was allowed to boil gently for 30 minutes. The contents were filtered, and the residue was extensively treated with hot dis-tilled water, 10% HCl once, ethanol twice, and petroleum ether three times. It was allowed to dry, scraped into the crucible, and then dried in an oven for an additional night at 105 ºC. Following removal, the sample was cooled in a desiccator. The sample was weighed (W1), then ashed in a muffle furnace for 90 minutes at 600 ºC, removed, cooled in a desiccator, and weighed once again (W2). The percentage of crude fiber was calculated using the equation:

%Crude Fiber=(w1-w2)/w0×100
Where:
W0 = Weight of sample (g)
W1 = Weight of dried sample (g)
W2 = Weight of ash sample (g)

Determination of Ash Content

Ash is the inorganic residue that is left after material has either been wholly burned at a high temperature of 550 °C in a muffle furnace or completely oxidized. It is the totality of all non-volatile inorganic elements. The ash content determination was followed by a method according to (48). In this experiment, 2 g of the dry powder sample was weighed (W1) into pre-weighed empty porcelain crucibles (W0), where it was then burned for 5 hours at 55 °C in an ash-muffling fur-nace to produce ash. Afterward, the ash crucibles were removed, cooled in a desiccator, and re-weighed (W2).

The % ash content was calculated as follows:

%Ash=(w2-w0)/(w1-w0)×100
Where:
W0 = Weight of empty crucible (g)
W1 =Weight of crucible + powdered sample (g)
W2 = Weight of crucible + ash sample (g)

Determination of Total Carbohydrate

The total percentage carbohydrate content at dry weight bases (DWB) in the *A. calocephalum* sample was followed by method[48]. As a result, the carbohydrate content was determined by sub-tracting 100 from the difference between 100 g of the dry mass of a sample and the total values of its crude protein, crude fat, natural fiber, and ash constituents[46,49,50]. The following equation estimated the total carbohydrate value:

%Carbohydrate (DWB)=100-(% crude fiber + % protein + % lipid + % ash)

Determination of Energy Value of Samples

Following the method of (51,52), the energy value (kcal/100 g) of the dried samples was calculated by multiplying the values of carbohydrate content by 4, protein content by 4, and fat content by 9. This equation estimated the energy value (kcal/100 g):

Energy Value = (Crude protein × 4) + (Total carbohydrate × 4) + (Crude fat × 9)

Minerals Analysis

To determine P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Cd, Pb, Al, and Cr, plant material was digested in a di-acid solution (wet digestion). HNO_3 and $HClO_4$ were used in a 9:4 ratio for the procedure. A 100 ml volumetric flask containing 1.0 g of the dried sample powder was filled with 10 ml of ni-tric acid (HNO3) and left overnight for pre-digestion. Add 8 ml of HClO4 the following day and gently stir with a magnetic stirrer. The flask was put on a hot plate and heated to a temperature of about 100 °C. After that, it was heated to a temperature of about 260 °C for more than an hour to cause the red NO2 vapors to disappear. The volume of the flask's contents was reduced to 3 to 4 ml through continued evaporation without drying up the mixture. The digestion process continued until the solution color altered and became colorless. The flask was filled with 20 ml of deionized water after cooling. Deionized water was used to make up the volume and dilute it to the proper concentration, while Whatman No.1 filter paper was used to filter the sample. The prepared solution was used for the determination of K, Ca, Mg, S, Fe, Mn, Zn, Cu, Cd, Pb, Al, and Cr. Atomic absorption spectroscopy (AAS) was used to estimate these mineral nutrients. While phosphorus (P) assessment was performed using a spectrophotometer using a colorimetric meth-od, with the absorbance being measured at 882 nm[52-54]. To measure Na in *A. calocephalum*, the sample was prepared using the dry ashing method. After digestion, sodium (Na) was estimated using flame photometry[46].

Total phenolics and Flavonoids content analysis

Sample preparation and extraction

Extracted crude plant material, which was prepared by combining the dried powder with 500 ml of 96% ethanol in a conical flask, was utilized to estimate the total phenolic and flavonoid content. This extraction process took roughly 24 hours. Then, plant extracts were filtered through layers of folded filter paper, and ethanol extracts were concentrated using a Rotary Vacuum Evaporator at 40 °C. The solvent was eliminated using a rotary evaporator. This method in-volved carefully controlling the solvent removal while using a vacuum. The water bath was heat-ed to 40 °C while the distillation flask was rotated between 150 and 200 rpm with the distillation flask filled to 50% with ethanol plant extracts. Following the evaporation of ethanol, the con-densed crude plant material was collected and scraped off the wall of the distillate flask using a spatula. The remaining plant stock was scraped off the flask's wall using a small amount of ethanol solvent, and it was added together before being allowed to evaporate at room temperature overnight. The obtained crude plant material weight was then recorded and stored in a freezer at a temperature of -5 °C for further analysis.

Total phenol content (TPC)

Folin-Ciocalteu's method was used to determine the total phenolic content in the ethanolic extract of *A. calocephalum* according to (55,56). Folin-Ciocalteu's phenol reagent and concentrated ex-tract material solution were mixed in a test tube with 1 mL each. The mixture was then left in the dark for about 5 minutes, at which 10 ml of a 7% sodium carbonate (Na_2CO_3) solution was added, followed by 13 ml of deionized distilled water, and the mixture was gently shaken until well mixed. The mixture was left in the dark for about 30 minutes at room temperature (20–23 °C) to allow the reaction to complete. Then the absorbance of the mixture's blue color was determined at 760 nm using a UV spectrophotometer. The total phenol content was calculated us-ing a gallic acid solution standard curve. The results were represented as milligrams of gallic acid equivalents (mg GAE/100 g) of extracted dried samples. The estimation of the total phenolic compounds in triplicate was performed.

Total flavonoid content (TFC) determination

Total flavonoid content was determined according to the method described by (57). Consequently, a half milliliter of each extract (0.1 gm/ml) in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, then 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled wa-ter were added. The combination then stayed at room temperature for 30 minutes. A UV/visible spectrophotometer was used to detect the reaction mixture's absorbance at 415 nm. Every sam-ple was tested twice. Following the process for all traditional quercetin solutions (12.5–100 µg /ml), a standard curve was created in methanol. Results were stated as milligrams of quercetin equivalent per gram (mg QE/g) of extract.

Results

According to the study's findings in Figure 1, the number of phenolic compounds was significant-ly influenced by the plant part used. Interestingly, bulbous sections showed a high level of phe-nolic compounds (0.684 mg gallic acid equivalents/g of dry extract, eq.$100g^{-1}$); While the shoot sections, including the leaves and young stems, had the lowest concentration of phenolic compounds (0.522 mg gallic acid equivalents/g of dry extract, eq.$100g^{-1}$). These findings strongly suggested that the portion used impacted the number of phenolic compounds overall.

The total flavonoids of Allium calocephalum extracts were estimated using the aluminum chlo-ride colorimetric method Figure 2. The number of flavonoids in the bulbous parts extract was higher than that in the shoot parts extract the values were (6.31 and 4.73 µg quercetin equiva-lents/g

of dry extract, eq.100g^{-1}) respectively, for both bulbous and shoot parts.

The results of proximate composition in studied sample materials are given in Table 1; the com-parative analysis of the two portions of *Allium calocephalum* was obtained. The bulbous parts had the most significant dry weight basis of total carbohydrates (71.75%), while the shoot parts had the lowest percentage (54.33%). There was a substantial difference between the oil content of the two parts, with bulbous legs having a more excellent value (9.52%) than shoot parts (6.53%), and moisture content was highest in bulbous parts of Allium calocephalum (92.56%) as compared to shoot details (90.24%). Also, the highest energy value (Kcal) found in bulbous parts (408.86 Kcal) was comparable with the (339.84 Kcal) value found in shoot parts. Contrarily, the crude protein, crude fiber, and natural ash content were higher in the shoot sections, with discounts of 15.93%, 13.89%, and 9.32%, respectively. In contrast, the bulbous areas had the low-est values, at 9.05%, 6.84%, and 2.83 %, respectively.

The results in Table 2 showed that there was some variation in the macro-mineral contents of the two plant parts for calcium, phosphorus, magnesium, and sulfur; the shoot parts had the highest contents (384.82, 329.59, 154.549, and 349.8 mg/100g) respectively, as opposed to the bulbous parts (309.66, 46.35, 107.692, and 284.5 mg/100g). However, the bulbous sections had the highest potassium and sodium content (1995.902 and 372.402 mg/100g), respectively, while the shoot parts had the lowest potassium and sodium content (1343.6 and 159.6 mg/100g), respec-tively.

The data in Table 3 show no significant changes between the micro-mineral contents analyses in the two parts of the plant. According to the study, the high concentrations of zinc, iron, manganese, lead, aluminum, and chromium identified in shoot parts were (3.857, 11.514, 0.714, 2.131, and 13.846 mg/100g) respectively, while the low concentrations detected in bulbous parts were (3.108, 3.5, ND, 1.668, 8.869, and 0.1 mg/100g). Unexpectedly, it was discovered that bulbous portions (0.8 mg/100g) had a higher level of copper content than shoot parts (0.582 mg/100g).

Overall, differences in the chemical composition of *Allium calocephalum* Wendelbo and other plant tissues may be caused by various influences, including soil composition, growing conditions, temperature, precipitation, sun exposure, and interactions with other plants and animals in the ecosystem. Wide intraspecific variability was found mainly in carbohydrates, which are closely involved in plant metabolism. Hence, slight variations in the growth stage can lead to differences in carbohydrate and mineral element content, which are strongly influenced by environmental circumstances, including soil composition, among other aspects[74].

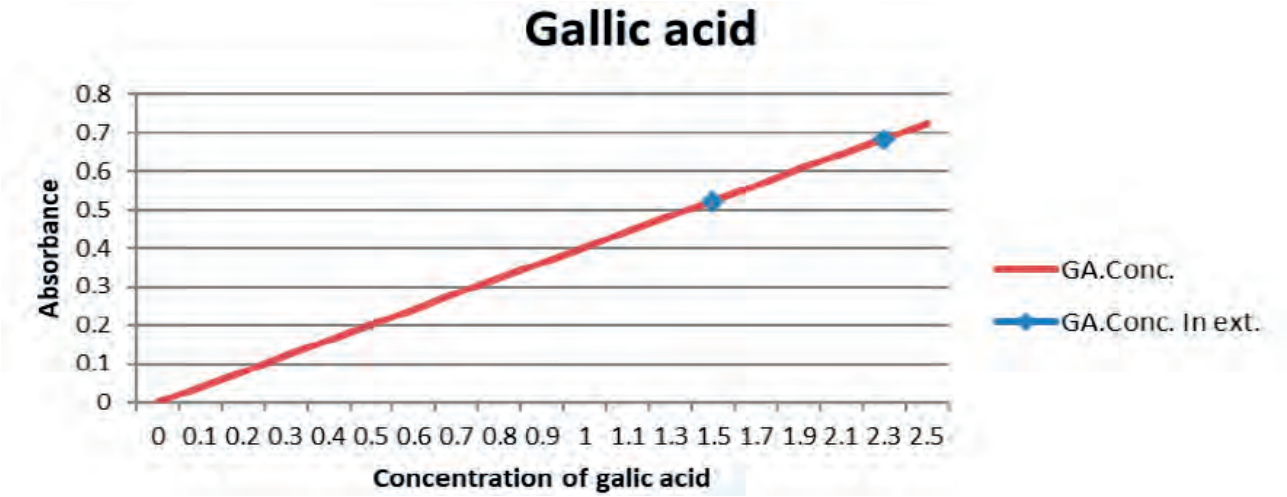

Figure 1. Compare phenolic compounds for plant materials of *Allium calocephalum* of the shoot and bulbous parts.

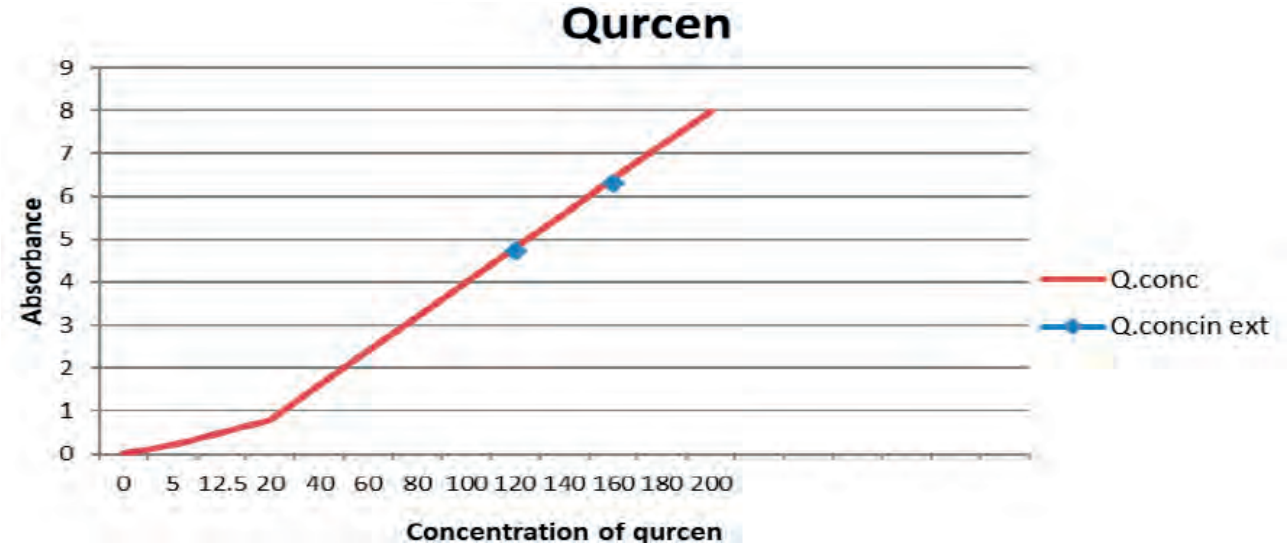

Figure 2. Comparison of flavonoid compounds in plant materials of *Allium calocephalum* of the shoot and bulbous parts.

The parameters	Shoot parts	Bulbous parts
%Total Carbohydrate (DWB)	54.33±0.52	71.75±0.95
%Crude oil (DWB)	6.53±0.13	9.52±1.68
% Fresh Moisture Content	90.24±1.13	92.37±0.92
Energy value kcal/100g	339.84±7.01	408.86±19.76
%Crude Protein (DWB)	15.93±0.94	9.05±0.21
% Crude Fibre (DWB)	13.89±0.74	6.84±0.59
% Ash (DWB)	9.32±0.27	2.83±1.34

[1] Values are mean on a dry weight basis ± standard deviation for three replications

Table 1. Proximate composition of *Allium calocephalum* plant in both shoot and bulbous parts.

Macro-elements composition (mg/100g)	Shoot parts	Bulbous parts
Calcium (Ca)	384.82±4.3	309.66±3.6
Phosphorus (P)	329.59±5.1	46.35±4.2
Magnesium (Mg)	154.549±3.2	107.692±1.5
Sulfur (S)	349.8±3.7	284.5±2.9
Potassium (K)	1343.675±7.3	1995.902±9
Sodium (Na)	159.621±1.4	372.402±1.7

[2] Nutritional value per 100 g of dry plant material ± standard deviation for three replications.

Table 2. Macro-mineral elements of the *Allium calocephalum* plant were studied in both shoot and bulbous parts.

Micro-elements composition (mg/100g)	Shoot parts	Bulbous parts
Zinc (Zn)	3.857±0.05	3.108±0.05
Iron (Fe)	11.514±0.07	3.5±0.06
Manganese (Mn)	0.714±0.003	ND
Lead (Pb)	2.131±0.05	1.668±0.04
Aluminium (Al)	13.846±0.2	8.869±0.1
Chromium (Cr)	0.418±0.005	0.1±0.003
Copper (Cu)	0.582±0.008	0.8±0.005
Cadmium (Cd)	0.0151±0.004	0.0308±0.005

[3] Nutritional value per 100 g of dry plant material ± standard deviation for three replications. ND: Not Detectable

Table 3. Micro-mineral composition of *Allium calocephalum* plant in both shoot and bulbous parts.

Discussion

Previous research has highlighted the significance of the *Allium* taxa in terms of its pharmacological and nutritional properties[15,58]. The indigenous communities have historically harvested these low-toxic edible vegetable plant species in large quantities for their daily diets, excellent flavor (which is rich in sulfur content), and medicinal properties (anticancer, antioxidant, antimicrobial, etc.)[19,20]. Due to its numerous medical applications, which have a wide range of benefits against ailments like cancer, arthritis, and asthma, these natural phytochemical compounds are becoming more common in today's society. In contrast to pharmaceutical chemicals, these natural phytochemicals are human-friendly medications since they can treat diseases without harming or endangering humans[59]. The metabolic products are known as polyphones, and phenolic compounds are found in plant-based meals. These compounds had numerous biological and pharmacological properties that could offer protection from chronic illness[60]. They are

more active antioxidants than vitamins. They can neutralize oxidative free radicals[61].

The results of phenolic compounds are consistent with (62) observed for all tested cultivated garlic *Allium sativum L* extracts. Various levels of phenolics (0.05–0.98 mg gallic acid equivalents/g of dry extract). Also, agreement with (63) when they found that the total phenolic content in cultivated garlic *Allium sativum L.* varied from 3.4 mg gallic acid equivalents (GAE)/g of dry matter (dm) to 10.8 mg GAE/g of dm with a mean value of 6.5 mg GAE/g of dm in the bulbous parts. The average total phenolics and flavonoids of the wild leek are 5.77 mg GAE/g extract, and 0.86[64] reported entire phenolic contents for Allium porrum (0.369 mg GAE/g extract).

On the other hand, the results of total flavonoids coincide with those reported[65] when evaluating the phenolic and flavonoid contents and the antibacterial and antioxidant properties of onion (*Allium* cepa) and garlic (*Allium sativum*). The maximum value of total flavonoids was 17.64 mg EC/100 g, obtained from an 80% ethanolic garlic extract. The lowest value was 0.41 mg EC/100 g for aqueous oak extracts. According to studies in (66), the phenolic components of *Allium sativum* were lower (0.56 mg EC/g extract), while *Allium* cepa had higher levels of flavonoids (1.31 mg EC/g extract). The antioxidant activity of garlic was strongly correlated with its phenol content, which had antioxidant properties and flavonoid content. Phenol content, antioxidant activity and differences among garlic, onion and *Allium calocephalum* cultivars would facilitate the choice of cultivars with medicinal advantages[67].

These results of the dry weight basis of total carbohydrates, energy, oil content, the moisture con-tent in bulbous parts dry weight basis of complete protein, fibers and ash that were observed agreed with the outcomes were consistent with those[68]. It revealed a moisture content of about 78%, which was between the cultivated leek *Allium porrum* (86%) and garlic *Allium sativum* (64%). 69 found that wild leek has an average value of (4.23%), making it an attractive source of dietary fiber. According to the Food Nutrition Board's recommendations, a 100 g portion can give (20.29%) of the daily amount needed for women and (11.21%) of the required daily amount for men. Additionally, compared to cultivated species, wild leeks had a more meaningful average fi-ber content (2.9%). Regarding protein content, *A. sativum* and *A. porrum* have (0.9 g/100 g and 2.1 g/100 g), respectively, but the average values found in wild leeks are (1.67 g/100 g) 68. 70 found that *Allium sativa* had the highest carbohydrate composition (16.60 g/100 g), but the wild *Allium* cepa had a higher carbohydrate composition (16.60 g/100 g). The primary macronutrient of the bulb has an energy value of (78.92 kcal/100 g) in *Allium sativum*, while *Allium sativum* and *Allium porrum* have an energy value of (139 kcal/100 g)[68]. On the other side, the findings of macro-elements composition are consistent with those of (71), who reports that Chrysanthemum coronari-um had a K content of roughly 1800 mg/100g, which was comparable to other culinary plants like savory, black thyme, and oregano, with values of about (1366, 1654.6, and 1962.5 mg/100g) respectively[72]. Additionally, Capsicum's Ca, P, and Mg contents (633, 148.5, and 443.2 mg/100g), respectively, were comparable to those of *Reseda alba*, which has average Ca, P, and Mg contents of about (1210, 250, and 220 mg/100g) respectively[71].

The results of the micro-elements composition showed that, contrary to expectations, there was no substantial difference between the two parts of the plant in the comparable cadmium concentrations. This result agrees with (73) findings, which indicated that the Zn contents of *Zea mays* L. and *Ipomoea batatas* (L.) Lam. were (3.8 and 3 mg/100 g), respectively. Additionally, (72) noted that cumin, caraway, and fennel each had a Fe concentration of (12.9, 4.67, and 9.72 mg/100g) respectively. It is widely known that the presence of some calcium-binding compounds, such as oxalic acid, which encourages the production of insoluble calcium oxalates and may be present in high concentrations in leafy vegetables, interferes with the absorption of calcium of plant origin.

The highest secondary metabolite contents, such as total phenolic and flavonoids, were found in the aerial parts and in plants collected from high altitude areas. These results confirm the effects of different geographical and environmental regions[66]. The plants that are collected from high altitudes contain high secondary metabolites.

Environmental conditions are important factors affecting plant growth and chemical compound levels[68], reported photoperiod (light and day lengths) affected plants content, essential oils and mineral nutrients[71]. Other studies have also noted the influence of altitude, drought and light intensity on plants' growth and their contents[73]; this confirmed the differences in the chemical composition affected by various altitudes and geographical positions. The researchers concluded that the ecological factors of habitat, such as altitude and soil physiochemical properties, could affect plant vegetative growth and change the quality and quantity of essential oils and chemical compounds in aromatic and medicinal plants. These changes happened as resistance to environmental stress[70,72].

Conclusions

This research study has given an account of the reason for the traditional uses of *Allium calocephalum* by the rural communities in the Kurdistan Region of Iraq to enhance their food security and preserve their natural heritage. The results of this investigation show that this zagrosian wild endemic garlic has excellent nutritional value and phytochemical prosperities and is considered one of the natural ecosystem services. Interestingly, the phytochemical compound levels were significantly affected by the plant parts. Indeed, the highest level of phenolic and fla-vonoid compounds, total carbohydrates, energy value, and fat content were obtained from bulbous parts. In contrast, total protein, fibers, and Ash content were observed in shoot parts. Concerning the macro-mineral contents analysis (Calcium, Phosphorus, Magnesium, Sulphur, potassium, and sodium), both bulbs and leaves details showed variation in content level. The cur-rent findings add substantially to our understanding of the nutritional value and phytochemical prosperities of this wild edible garlic and will serve as a base for future ethnobotanical and phar-macological studies.

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ARTICLE / INVESTIGACIÓN

Assessment of Thepax and Bio Boost for promoting microbial growth in common carp intestines *Cyprinus carpio*

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DOI. 10.21931/RB/2022.07.04.18

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Abstract: The study aimed to evaluate the effect of Thepax and BioBoost as food additives on the microorganisms in the intestines of fish. From March 4 to October 22, the total number of common carp was 900 fish with an average weight of 163.41 ±10.16 g and a density of 100 fish/cage; three replicates were used for each treatment. The fish were fed three times daily. The included T1(0%additive), T2(1g/kgThepax)and T3 (1 g/kgBioBoost). The highest final weight value is Thepax treatment (2209.34 g), followed by Bio Boost and control. Microorganisms showed significant differences (P<0.05) in T2 for Lactobacillus sp. (10^2 x 65 CFU/ml) followed by T3 (10^2 x 55 CFU/ml) and control T1 (10^2 x 23 CFU/ml), also for Cellulomonas sp. in T2 (10^2 x 54 CFU/ml) followed by T3 (10^2 x 39 CFU/ml) and control T1 (10^2 x 7 CFU/ml). At the same time, *Aeromonas sp.* bacteria was higher in T1 (10^2 x 34 CFU/ml) over the treatments of T2 and T3 (10^2 x 2 CFU/ml) for both. We concluded the best additive was 1 g of Thepax / kg of feed in the recommended diets for common carp.

Key words: Thepax, Additives, Microorganisms, Intestines, Bacteria, Lactobacillus, Endo Bio Boost.

Introduction

Aquaculture is the fastest-growing food production and supports nearly 50% of all aquatic food for human consumption[1]. The main objective of aquaculture is to increase production[2]. During cultivation, fish may be exposed to various diseases, especially if they are stressed due to the deterioration of water quality and exposure to stressful conditions, which leads to significant economic losses[3].

Antibiotics in aquaculture lead to many severe problems, such as antibiotic-resistant bacterial strains and antibiotic residues in fish muscles, which would cause serious public health effects on human consumers and environmental pollution[4,5]. Therefore, finding safe and environmentally friendly alternatives is necessary to avoid the negative consequences of antibiotic use[6]. Most non-nutritive feed additives include antibiotics, immunostimulants, antioxidants, probiotics and prebiotics[7,8], which were added to improve diet quality, health, performance and feeding efficiency of fish[9]. Prebiotics are present in the cell wall of bacteria, yeasts and molds[10,11]. Prebiotics primarily affect the host by stimulating the selective growth of one or a limited number of non-pathogenic bacteria in the fish's gut, resulting in improved host health. Lactic acid bacteria are the most important, as they are part of the natural microorganisms of the fish gut. All their strains are non-pathogenic, in addition to their antagonism against many types of pathogenic organisms through their production of lactic acid[12,13]. The current study was conducted to evaluate the effect of Thepax and Bio Boost as feed additives on the microorganisms in the intestine of the common carp Cyprinus carpio.

Materials and methods

Study Site

The current study was accomplished in the ponds belonging to the Aquaculture Unit, which is located in the Agricultural Research and Experiment Station in Basrah Governorate (College of the Agriculture / University of Basrah), in Al-Hartha District, 16 km north of Basrah (300 65' 64.6"N, 470 74' 79.5"E). The current experiment was conducted in a large pond with 2500 m^2. The water was provided with electric pumps from one branch of the Shatt Al-Arab river.

Fishes and Experimental Cages

Experimental cages were made of polyethylene with dimensions of length × width × height (3 × 1.7 × 1.8 m) and enclosed by external nets (10 × 10 mm). The cages were placed inside the earthen pond which was filled with water at the height of 1.5m; ventilation fans were employed to avoid the shortage of oxygen, especially during high temperatures. There were 600 common carp fish with an average weight of 163.41±10.16 g distributed in the cages at 100 fish/cage density; two replications for each treatment were used.

Feeding Management

Experimental diets were produced at the feed production plant of the Agricultural Advisory Office, Faculty of Agriculture, University of Basra. Three diets were formulated, which included a control T1 (0% additive), T2 (1g/kg Thepax) and T3 (1g/kg BioBoost). The pellet size was 6 mm.

Citation: Al-Janabi M, Al-Noor J, Al-Dubakel A Y. Assessment of Thepax and Bio Boost for promoting microbial growth in common carp intestines Cyprinus carpio. Revis Bionatura 2022;7(4) 18. http://dx.doi.org/10.21931/RB/2022.07.04.18
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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The ingredients used in the experimental diets are presented in Table (1), and the daily amount of feed for each cage was calculated based on body weight. Sampling was repeated every 20 days to monitor growth and adjust the amount of diet. The experiment lasted 233 days (from March 4 to October 22). The fish were fed daily, and the daily amount of feed for each treatment was divided into three equal meals; the first was in the morning (7 A.M.), the second was during noon, and the third was in the afternoon (4 P.M.).

Environmental factors

Water's most significant environmental features were measured during the study period on the days of sampling. All measurements were taken within nine A.M., including water temperature, pH, salinity, and dissolved oxygen.

Growth performance

Growth criteria were used to describe the growth performance of common carp fed the experimental diets as follows:

Weight gain:

WG =Final weight (g) – Initial weight (g)

Survival rate (%):

Survival rate = (No. of fish at the end / Total No. of fish) ×100

Hematology

Blood was drawn from different groups of fish before and after the experiment. After cutting the caudal peduncle, the fish were held vertically with the head up to help blood flow by pressing on the caudal vein. Capillaries and tubes containing an anticoagulant were used. They were transferred to the laboratory to test the hematocrit, red blood cells (RBC) and white blood cells (WBC), according to Witeska *et al.*(2022).

Gut Sampling

Before and after the experiment, three fish were randomly sampled from each treatment and transported alive to the laboratory. The intestines were isolated from the body cavity using sterile scissors and forceps, and the contents were collected into sterile 2 ml tubes by squeezing with forceps.

Principle of the pour plate method

In this method, serial dilutions of the inoculum (serially diluting the primary specimen) are added within sterile Petri plates to which is poured melted and cooled (42-45°C) agar medium and completely mixed by revolving the plates, which are then left to solidify.

After incubation, the plates are observed for the appearance of individual colonies growing everywhere in the medium. The pure colonies of varying sizes, shapes and colors may be isolated/transported into test tube culture media to prepare pure cultures.

Tested bacteria

- *Lactobacillus spp.*
- *Falvobacterium spp.*
- *Aeromonas spp.*
- *Cellulomonas spp.*

Chemical analysis

The experimental diets were analyzed according to AOAC (1990), moisture, protein, lipid, ash and fiber were determined, while nitrogen-free extract was calculated according to New (1987) as follows:

%NFE=%DM-(% EE+%CP+%ASH+%CF)

Where:

NFE = Nitrogen-free extract

DM = Dry matter

EE = Ether extract or crude lipid

CP = Crude protein

CF = Crude fiber Statistical analysis

The experiment data were analyzed with a completely randomized design (CRD). The differences between the treatment means were tested by analysis of variance (ANOVA), and the significant differences were tested by LSD test at 0.05 probability level using SPSS program Ver. 26.

Results

Environmental factors

Table 2 shows the values of environmental factors during the study period, the highest water temperature in July

Ingredients	Control (T1)	Thepax (T2)	BioBosst (T3)
Fish meal	250	250	250
Soybean meal	200	200	200
Wheat flour	310	310	310
Wheat bran	200	200	200
Vit. & mineral premix	20	20	20
Vegetable oil	20	20	20
Thepax	-	1	-
Bio Boost	-	-	1

Table 1. Ingredients of the experimental diets (g/kg).

Date	Temp.(°C)	pH	DO (mg/ l)	Salinity (PSU)
4/3/2020	21	7.5	7.2	3.13
23/3	23	7.6	7.1	3.52
13/5	26	7.7	7.0	4.17
2/6	27	7.9	6.9	4.52
22/6	28	7.8	6.7	4.42
12/7	30	8.1	6.5	4.84
16/8	28	7.8	6.6	4.21
8/9	28	7.9	6.6	3.91
28/9	27	7.7	6.7	3.28
22/10/2020	24	7.6	7.1	3.61

Table 2. The environmental factors during the study period.

(30°C) and the lowest in March (21°C), while changes in pH were limited (7.5 -8.1), the concentration of dissolved oxygen ranged between 7.2 mg / l in March to 6.5 mg / l in July. Water salinity fluctuated from 3.13 PSU in March to 4.84 PSU in July.

Chemical analysis

The chemical composition of the experimental diet was presented in Table (3); the percentage of protein was 27.84% ±0.82, the lipid content was 6.39%±2.47, NFE was 44.20% ±2.35, and the Gross energy was 4272.50 Kcal.kg-1±60.10.

Table (4) shows the averages of initial and final weight and the total weight gain of common carp for the different treatments. The highest value of the final weight was recorded in the Thepax treatment (2209.34 g), followed by Bio Boost (1918.27 g) and the lowest in the control treatment (1631.62 g). This was similar for weight gain, where the highest value was in Thepax (2035.65g), and the lowest value was recorded in the Control treatment (1478.23g).

The blood parameters of common carp for different treatments showed in Table (5). It was noted that the hematocrit value did not differ significantly (P> 0.05) between the two treatments and the control. In contrast, all the hematocrit values for all the treatments were quite different (P<0.05) from the value of the pre-experiment fishes. Regarding the importance of red blood cells (RBC), it was significantly different (P<0.05) of the treatments of Thepax and BioBoost over the control treatment, which scored 1.543 and 1.533, respectively, then followed by the control treatments 1.257. While two treatments were significantly different (P<0.05) to the control treatment in the values of white blood cells (WBC) as Thepax and BioBoost treatments recorded 91.200 and 96.033, respectively, while the control treatment and pre-experiment recorded the least and amounted to 78.967 and 78.150 respectively.

Proximate composition (%)	
Moisture	7.51±0.58
Crud protein	27.84±0.82
Crud lipid	6.39±2.47
Ash	9.86±0.54
Fiber	4.20±0.36
NFE	44.20±2.35
Gross energy (Kcal.kg^{-1})	4272.50±60.10

Table 3. Proximate composition of the experimental diet.

Parameters	Treatments		
	Control	Thepax	Bio Boost
IW (g)	153.39 ±0.22	173.70 ±11.09	158.55 ±3.54
FW (g)	1631.62 b ±82.38	2209.34 a ±9.21	1918.27 b ±146.69
WG (g)	1478.23 b ±82.60	2035.65 a ±20.31	1759.73 b ±143.15

Table 4. Growth performances of common carp in different treatments.

Parameters	Before experiment	After experiment		
		Control	Thepax	BioBoost
HCT	8.300 b ±2.404	16.333 a 1.380 ±	16.733 a 2.250±	19.867 a 1.301±
RBC	1.365 b 0.177±	1.257 c 0.112±	1.543 a 0.177±	1.533 a 0.215±
WBC	78.150 b 9.546±	78.967 b 10.081±	91.200 a 7.702±	96.033 a 4.021±

Data in each row with different letters are significantly different (P≤0.05).

Table 5. Growth performances of common carp in different treatments.

Gut microorganisms

Table (6) presented the numbers of bacteria in the intestines of common carp in different treatments. Where the number of colonies of *Lactobacillus* bacteria increased significantly (P<0.05) in the treatment of Thepax compared with the BioBoost treatment and control, as it recorded 10^2 x 65 cfu/ml and the BioBoost treatment recorded 10^2 x 55 cfu/ml followed by the control treatment which recorded 10^2 x 23 cfu/ml . In comparison, the highest rate of Aeromonas colonies was recorded in the intestines of fish in control, as it reached 10^2 x 34 CFU/ml. The colony numbers of these bacteria were significantly (P<0.05) reduced in the treatment of Thepax and BioBoost as it was recorded 10^2 x 2 CFU/ml for both treatments. The numbers of Flavobacterium increased significantly (P<0.05) in the control treatment, recording 10^2 x 33 CFU/ml. At the same time, it decreased in the Thepax and BioBoost therapy, as it recorded 10^2 X 3 CFU/ml and 10^2 X 7 CFU/ml, respectively. From Table (6), it was also noted that the number of colonies of cellulose-degrading bacteria Cellolomonas was significantly higher (P<0.05) in the Thepax treatment, recording 10^2 x 54 CFU/ml, followed by the BioBoost treatment with 10^2 X 39 CFU/ml, while the control treatment recorded 10^2 X 7 CFU/ml.

Discussion

Water quality was considered the main factor for the success of fish culture projects[14]; the water The temperature ranged from 21 to 30°C during the experiment, i.e., March to October. Hwang and Lin[15] found the optimum growth and FCR in common carp compared at 25°C. Song-bo *et al.*[16] observed the maximum daily feed intake at 28°C, which was in the range of the present study. The pH during the experimental period was within the appropriate limits for common carp culture, according to (16). The water salinity in the present study was within the applicable limits for common carp, which can tolerate a wide range of salinities[17,18]. Dissolved oxygen values in the entire study period were above 3 mg/l, and common carp optimal ranges between 4-8 mg/l[19,20].

The results obtained in the present study for growth parameters were in agreement with study[21], which found that the Thepax treatment was significantly higher than the control treatment. Al-Mhanawi *et al.*[21] found that 1 g/kg Thepax has great potential as an important component for improving nutrient utilization and metabolism by increasing the surface area of the intestine in common carp and has a role in improving the digestive system compared to the con-

Bacteria	Treatments	Mean (cfu/ml)	Std. Deviation (±)
Lactobacillus	Control	10^2 x 23 c	10^2 x 5.00
	Thepax	10^2 x 65 a	10^2 x 6.55
	Bio boost	10^2 x 55 ab	10^2 x 6.24
Aeromonas	Control	10^2 x 34 a	10^2 x 11.13
	Thepax	10^2 x 2 b	10^2 x 1.73
	Bio boost	10^2 x 2 b	10^2 x 1.00
Flavobacterium	Control	10^2 x 33 a	10^2 x 8.00
	Thepax	10^2 x 3 b	10^2 x 2.00
	Bio boost	10^2 x 7 b	10^2 x 2.00
Cellolomonas	Control	10^2 x 7 c	10^2 x 3.60
	Thepax	10^2 x 54 a	10^2 x 10.14
	Bio boost	10^2 x 39 b	10^2 x 6.55

Data in each row with different letters are significantly other ($P \leq 0.05$).

Table 6. Numbers of bacteria in the intestine of common carp in different treatments.

trol. The results were similar to those of study[22] by adding the prebiotic Mannan Oligosaccharide to trout feed, which showed a superior increase compared to other diets in terms of final weight. Al-Faragi[23] also increased weight indicator values by adding the prebiotic beta-glucan to common carp feed for eight weeks. While the results of the present study did not agree with those of Taher *et al.*[24] in grass carp (*Ctenopharyngodon Idella*) when adding 1 g/kg Thepax and 1 g/kg vitamin C to the diets, as no statistically significant differences were recorded with the control treatment in final weight and weight gain.

The survival rate of fish of the experimental therapies significantly increased compared to the control treatment. The best survival rate was recorded for the treatment of Thepax and BioBoost. A similar result was observed in the survival rate in the study of Tejpal *et al.*[25] on tilapia, where he confirmed that diets based on probiotics have a more positive effect on survival rate.

Blood parameters are considered one way to determine a fish's health in terms of stress and disease[26,27]. The present study's results agree with Akrami *et al.*[28], which revealed that common carp fed 1, 2, and 3 g/kg of the prebiotic MOS diet had significant superiority compared to the control in terms of hematocrit and white blood cells. The reason for this increase is due to the effect of the prebiotic on immune stimulation in fish. Modulation of the immune system is one of the generally proposed benefits of prebiotics and their ability to stimulate systemic immunity. Notably, several prebiotic supplements can even increase the rates of phagocytic cells, lysozyme cells and erythrocytes[27].

The results of the current study indicate a clear superiority of the additive treatments over the control treatment in the intestinal content of colonies of beneficial bacteria Lactobacillus and Cellolomonas, especially the treatment of Thepax. These results also indicate a decrease in the number of colonies of pathogenic and harmful bacteria Aeromonas and Flavobacterium for the additive treatments compared to the control treatment. The results of the current study agree with Ringo[28], in which probiotics were used in fish feed to enhance the immune response and increase fish resistance to diseases by increasing beneficial microorganisms in the intestine. Also, in the study of Yun Xia *et al.*[29] in which probiotics from Bacillus cereus and Bacillus subtilis were used as food additives for tilapia, the results showed a stimulating effect on the group of beneficial microorganisms in the intestine. After stopping the intake of probiotics by the fish for one week, the beneficial bacteria in the intestine were very few. As for the results of Lv *et al.*[30] study, it was in agreement with the results of the current research, as FOS was used, which reduced Aeromonas bacteria in the intestines of hybrid Nile tilapia. And the study of Dimitroglou *et al.*[1] where nutritional supplements (MOS) were used, as a result, gave a significant reduction in the number of harmful bacteria Aeromonas in the intestines of rainbow trout.

Conclusions

The addition of Thepax enhanced growth performance, feeding efficiency, survival rate, increased Lactobacillus count and low Aeromonas bacteria in the intestines of common carp.

Author Contributions

A short paragraph specifying their individual contributions must be provided for research articles with several authors. The following statements should be used "Conceptualization, Jalal M. Al-Noor. and Adel Y. Al-Dubakel.; methodology, Mohamed F. Al-Janabi.; software, Jalal M. Al-Noor.; validation, Mohamed F. Al-Janabi, Jalal M. Al-Noor. and Adel Y. Al-Dubakel.; formal analysis, Adel Y. Al-Dubakel; investigation, Mohamed F. Al-Janabi; resources, Jalal M. Al-Noor; data curation, Adel Y. Al-Dubakel.; writing—original draft preparation, Mohamed F. Al-Janabi.; writing—review and editing, Jalal M. Al-Noor.; visualization, Adel Y. Al-Dubakel.; supervision, Adel Y. Al-Dubakel.; project administration, Jalal M. Al-Noor; All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ARTICLE / INVESTIGACIÓN

Use of Ginger Essential Oil with Cephalosporin antibiotics as Beta-Lactamase inhibitors in pharmaceutical design to fight *Escherichia coli* UTI

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DOI. 10.21931/RB/2022.07.04.19

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Abstract: This research aimed to investigate multi-target inhibitors against the Beta-Lactamases protein of urinary tract infections (UTI) *Escherichia coli*, which is considered the main virulence factor of this bacterium. Drug design is regarded as a new approach to drug discovery and industry. The combination of Ginger Essential Oil (GEO) and Cefepime (FEP) showed effective results against *Beta*-Lactamase enzymes of UTI *E.coli*, 512 FEP+ 100% GEO and 1024 FEP + 100% GEO for (20 mm and 26 mm) inhibition zone respectively. The present study concluded that the isolates of *E.coli* of UTI from Iraqi hospitals were MDR and XDR, and their virulence was due to the presence of bla_{TEM} genes. *In silico* screening, servers have been used to design an inhibitor model for *Beta*-Lactamases from the natural product of GEO. Cefepime and Ginger's essential oil showed a strong synergistic effect on these bacteria.

Key words: *Escherichia coli*, ESBLs, Ginger Essential Oil, Cefepime, UTI.

Introduction

Escherichia coli producing enzymes Extended Spectrum *Beta*-Lactamases (ESBLs) has appeared as a significant reason for UTI. ESBLs are the enzymes that hydrolyze all Penicillins, Cephalosporins and Monobactams and cause cross-resistance to co-trimoxazole, fluoroquinolones and amino acids glycosides, all of which are commonly favored in UTI management[1].

Essential oils and their constituents have been used to treat many human diseases since ancient times. Today, EOs are an alternative source with their oral, topical and aromatherapy treatment. Essential oils are compounds from spices, aromatic herbs, fruits, and flowers. Extract of Ginger was confirmed effective against four test organisms – two drugs resistant and two non drug resistant bacteria. Thus, it is remarkable to recognize the potential use of Ginger Extract in treating infections caused by *Staphylococcus aureus*, *E.coli*, Methicillin Resistant *Staphylococcus aureus*, and ESBLEC[2].

The prevalence of ESBL-producing *E.coli* urinary tract infections (UTIs) is increasing worldwide. The impact and risk factors were investigated[3].

The study aimed to determine the bactericidal property of Ginger Essential Oil against Extended Spectrum *Beta*-Lactamase enzymes producing *Escherichia coli* (ESBLEC) in combination with Cephalosporin antibiotic.

Materials and methods

Patients Specimens Collection

Through the period extending from November /2021 to February /2022, 100 Clinical specimens comprising; patients suffering from (UTI) of all ages and genders. Urine samples were collected in sterilized containers from inpatients admitted in hospitals in Baghdad in Al-Karkh.

Laboratory Prepared Culture Media

All media, including MacConkey agar, Muller Hinton agar and broth, Nutrient agar and broth and Eosin Methylene Blue (EMB) agar, were prepared according to the manufacturing company instruction; the constituents were dissolved in distilled water (DW), pH was adjusted to 7.2± 0.2 then boiled in water to dissolve all branches completely. The sterilization of media was done by autoclaving at 121ºC for 15min at 15 pounds/inch^2, then distributed into sterile Petri dishes; otherwise, the media were incubated at 37 ºC for 24 hours to ensure sterility.

Genes Selection

This study used conventional PCR to detect the following genes: Uniplex PCR was used to amplify ESBL, including bla_{TEM} gene.

Minimum inhibitory concentration (MIC) was determined by agar dilution and broth dilution method against antibiotic Ceftazidime (CAZ), Ceftriaxone (CRO), and Cefepime (FEP) of antibiotic powders. A loop entire (1µl) of culture was streaked on MH broth plates containing antibiotics at a concentration of (64, 128, 256, 512, and 1024 µg/ml); these plates were incubated for 24 hours at 37°C. Growing colonies were macroscopically observed after 24 hours[4,5].

Minimum Inhibitory Concentration (MIC) of Ginger Essential Oil (GEO)

Minimum Inhibitory Concentration (MIC) was determined by agar well diffusion method, loop full (1µl) growths from bacterial isolate that were inoculated into nutrient agar and then incubated at 37 °C for 24 hours. The bacterial suspensions were diluted with normal saline. Adjust the turbidity and compare with the standard tube (McFarland number 0.5) to yield a uniform suspension containing

Citation: Khalil, YJ.; Saadedin, SMK. Pharmaceutical Design Using a Combination of Cephalosporin Antibiotic and Ginger Essential Oil as Beta-Lactamase Inhibitors Against UTI Escherichia coli.Revis Bionatura 2022;7(4) 19. http://dx.doi.org/10.21931/RB/2022.07.04.19
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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Name of primer	Sequence' 5----------3'	Product Size(bp)	Primers Design
TEM_F	TCTCAGAATGACTT-GGTTGAG	566	Designed in current study
TEM_R	TTAATCAGTGAGGCACC-TATC		

Table 1. Listed the Sequences of the Primers Used for Conventional PCR to Detect *Beta*-lactamase Gene.

1.5×10^8 CFU/ml. A cotton swab was dipped and streaked into adjustment suspension the entire Mueller-Hinton agar (for all tested bacteria) surface of plates. Media were cut into four wells (6mm diameter) by cork borer, diluting the oil with 10 % DMSO. 100 µL of GEO were added into wells (The plates were performed in triplicates) at 100%, 50%, 25%, and 12.5%. All plate of the tested organisms was then allowed to incubate at 37°C overnight. After 24 hours of incubation, each plate was noted for the zone of inhibition for all isolates. The inhibitions zone's diameter was measured by measuring scale in millimeters (mm)[6].

Estimation of Antibacterial Effect

Depending on the MIC value of fourth antibiotic generation of cephalosporin (Cefepime). The optimum MIC value was selected according to the growth of bacteria; the concentration of inhibition was 1024 µg / ml of Cefepime, and the focus taken below was 512 µg / ml. The highest concentration of Ginger oil was taken 100 %. The same wells method was used for antibiotic FEP and GEO[7], Depending on who indicated that quercetin showed high antibacterial activities and additive or synergistic effects with the antibiotic.

Zingiber officinale (Ginger)

Zingiber officinale (Ginger) rhizomes were bought from the market of Baghdad city and classified in the College of Science, Department of Life Biology, the University of Baghdad as *Zingiber officinale* Roscoe.

Activation of Bacteria

Bacteria were activated in brain-heart infusion broth and then incubated at 37°C for 24 hrs.

Pre – Treatment

100 *E.coli* clinical isolates DNA were extracted after growing in brain-heart infusion broth (BHIB) (without antibiotic and GEO), then incubated at 37°C to detect bla_{TEM}.

Essential Oil Extraction

The essential oil was extracted from the Ginger plant rhizomes using a specialized Clevenger device to remove the light oil connected with a volumetric flask of 1000 ml, as 200 gm. Fresh Ginger rhizomes were mixed with 500 ml of (DW), then the distillation process was carried with 80-90 □, and distillation lasted 2 hours. As a result, the oil yield obtained for every run was calculated using (1)[8].

Isolation and Identification of *Escherichia coli*

In CHROMagar, isolates of *E.coli* appeared as pink-red colonies at 37°C for 24 hours, as shown in Figure (1); this medium also has selectivity for other urinary tract pathogens with a specific color for each bacterial genus. Chromogenic agars are reliable for detecting aerobic Gram-negative bacteria by easier recognizing different colonies on these media.

Minimum Inhibitory Concentration (MICs) Susceptibility Test

Minimum inhibitory concentration (MICs) is defined as the lowest concentration of an antimicrobial agent that will inhibit the visible growth of microorganisms after incubation. The antibiotic susceptibilities of clinical isolates of Multi-Drug Resistant *E.coli* (MDREC) were determined in terms of MICs of antibiotics against the isolates, using the agar dilution method. The MICs that have been investigated in the present study are shown in Figure (2).

Cefepime (FEP), CRO: (64 µg/mL -1024 µg/mL) Resistance, CAZ: (64 µg/mL) Sensitive, FEP:(1024 µg/mL) Sensitive.

This indicated that the MIC value of Ceftazidime (CAZ) was at a concentration (64 µg/mL), and of Cefepime (FEP) was at a concentration (1024 µg/mL). Finally, *E.coli* isolates exhibit a high resistance to Ceftriaxone (CRO).

Minimum Inhibitory Consecration (MICs) OF Ginger Essential Oil (GEO)

Wells method was utilized after the 24 hours incubation period, for 40 samples (containing genes bla_{TEM}) qualified as the final study sample (each sample was repeated three times). The GEO exhibited varying degrees of inhibitory activity against the ESBL *E.coli*. As expected, higher GEO concentration produced wider zones of inhibition. Table (2) Shown antibacterial activity of GEO against ESBL (UTI) *E. coli.*

Estimation of Antibacterial Effect

Depending on the MIC value of the fourth antibiotic generation of cephalosporin (Cefepime) for ten isolates, the optimum of MIC value was 1024 µg/ml of Cefepime, and the concentration was taken below 512 µg/ml. The highest MIC of GEO was 100%. The same wells method was used, as shown in table (3).

$$\text{Essential oil (\%)} = \frac{\text{amount of essential oil (g) obtained}}{\text{Fresh ginger rhizomes (g)used}} \text{ x } 100\ \% \qquad (1)$$

Results

Isolation and Identification of *Escherichia coli*

The isolates that were obtained from the 100 samples were identified according to the following characteristics observed.

In the table (3), the optimal MIC value of the GEO that appeared in this study is 100% (15 mm), the optimum MIC value of the Cefepime 512 and 1024 (15 mm and 20 mm inhibition zone, respectively), while the mixed of GEO and FEP (100% + 512 and 100% + 1024) was (20 mm and 26 mm inhibition zone respectively). Compared with CLSI, the bacteria were resistant and became sensitive when treated with GEO and FEP.

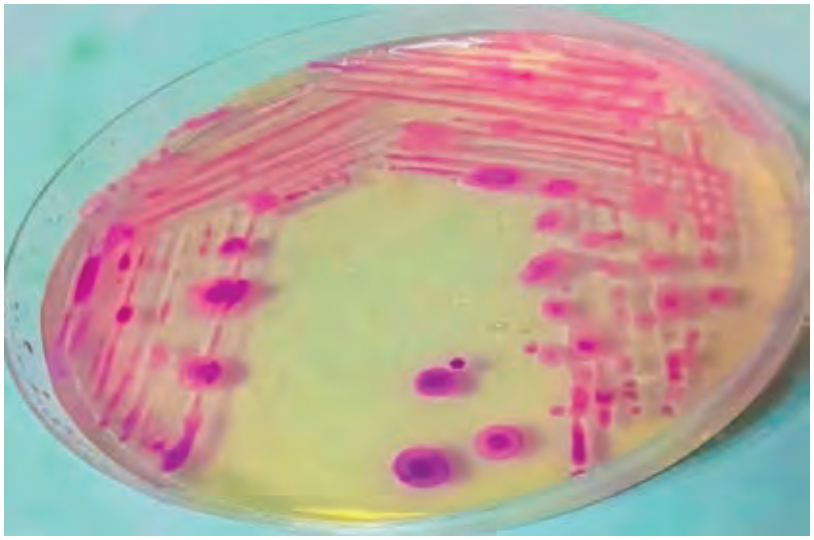

Figure 1. Growth of *Escherichia coli* on CHROMagar after incubation at 37°C for 24 hours.

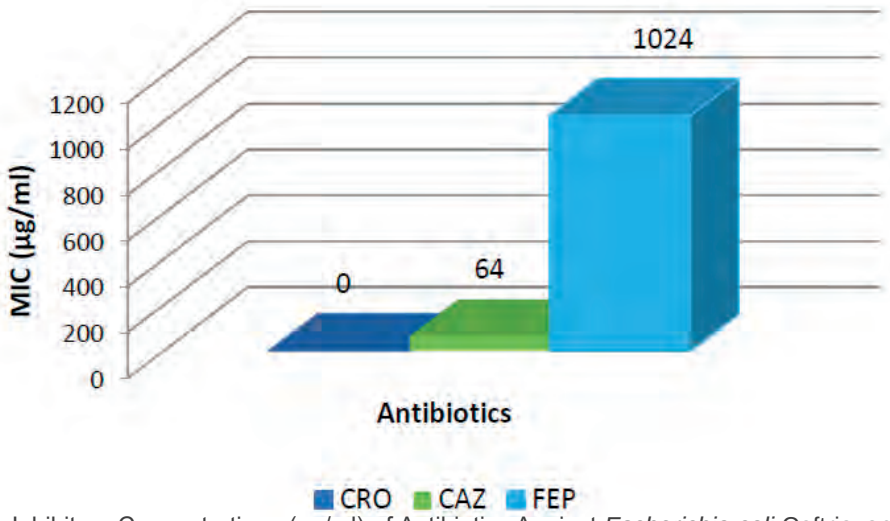

Figure 2. Minimum Inhibitory Concentrations (µg/ml) of Antibiotics Against *Escherichia coli* Ceftriaxone (CRO), Ceftazidime (CAZ).

Treatments	No	Diameter of inhibition zone (mm)
100%	40	14.51 ± 0.10 a
50%	40	9.72 ± 0.06 b
25%	40	9.18 ± 0.11 bc
12.5%	40	7.95 ± 0.07 c
Control DMSO	40	6.00 ±0.00 d
LSD value	----	1.377 **
Means having different letters in the same column differed significantly. ** (P≤0.01)		

Table 2. The Antibacterial activity of Ginger Essential Oil against Extended Spectrum *Beta*-Lactamase *Escherichia coli*.

Molecular Detection *bla*$_{TEM}$ gene

The prevalence of *Beta*-Lactamases producing genes (*bla*$_{TEM}$) was detected and determined for each *E.coli* clinical isolate in the present study shown in Figure (3).

In this study, the optimal annealing temperature of the reaction was 50 °C. However, the annealing temperature of the reaction started at 67 °C and decreased to about 1°C every second cycle until the optimum annealing temperature of primer was reached, followed by 15 additional cycles at 50 °C. The minimum number of processes needed during the earlier part of the TD program to eliminate nonspecific priming would depend on the amplification's efficiency during high-temperature cycling.

Discussion

CHROMagar Orientation medium is preferred medium because of the high accuracy and the `rapid identification with meager false favorable rates[9,10]. This media is regarded as highly selective and sensitive media for *E.coli*, and this media contains agents which inhibit the growth of most gram-positive organisms. It incorporates substrates enabling color-based preliminary identification of colonies recovered within 24 h of inoculation. 71 of the 100 sample

No	ESBL *Escherichia coli*	Diameter of inhibition zone (mm)				
		GEO 100%	FEP 512	FEP 1024	GEO 100% + FEP 512	GEO 100% + FEP 1024
1	ESBL *E. coli*	15	15	20	20	26
2	ESBL *E. coli*	15	15	20	20	26
3	ESBL *E. coli*	15	15	20	20	26
4	ESBL *E. coli*	15	15	20	20	26
5	ESBL *E. coli*	15	15	20	20	26
6	ESBL *E. coli*	15	15	20	20	26
7	ESBL *E. coli*	15	15	20	20	26
8	ESBL *E. coli*	15	15	20	20	26
9	ESBL *E. coli*	15	15	20	20	26
10	ESBL *E. coli*	15	15	20	20	26

GEO: Ginger Essential Oil, FEP: Cefepime

Table 3. Estimation of Antibacterial Effect of Ginger Essential Oil and Cefepime antibiotics against Extended Spectrum *Beta*-Lactamase *Escherichia coli*.

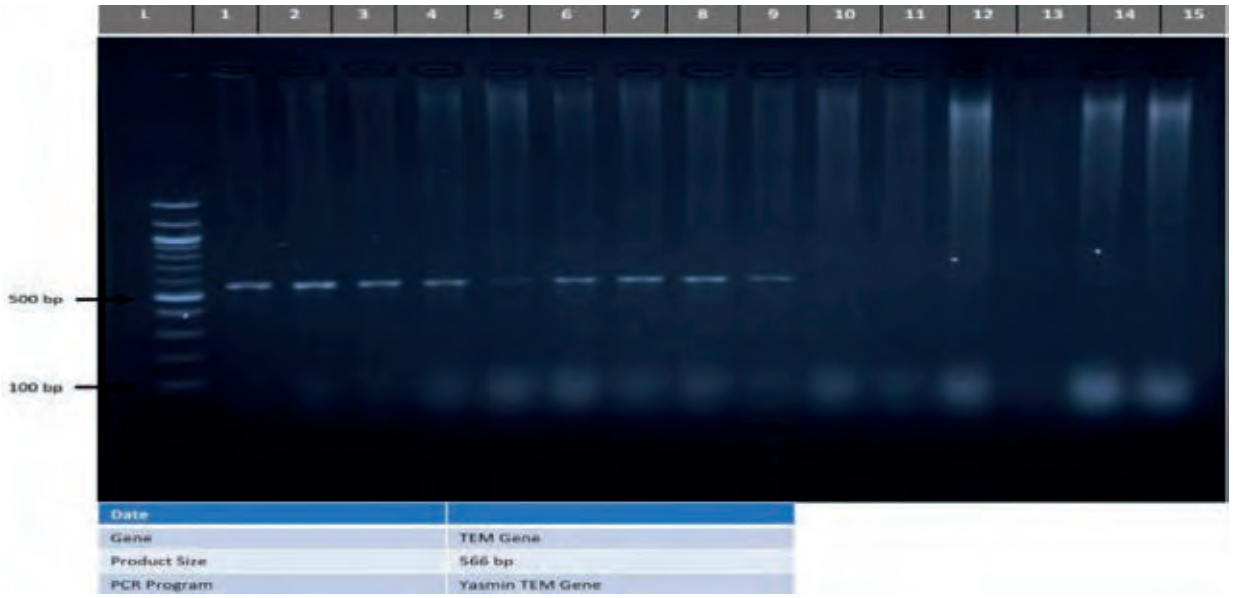

Figure 3. Gel Electrophoresis profile of bla_{TEM} Gene PCR Product (566bp) on 2%Agarose gel with ethidium bromide (5V/cm, 80 mins).

isolates were suspected to be *E.coli*; these results were similar to (11).

Clinical laboratories use MICs mainly to confirm resistance; they are also employed as a research tool for determining the activity of new antimicrobial agent and their MIC breakpoints[12].

The result of the present study for Cefepime and cefotaxime MIC agreed with the result of (13), which showed higher catalytic efficiency observed of ceftazidime and Cefepime. *Beta*-lactam antibiotics are the most prescribed antimicrobial class. The efficacy of *Beta*-lactams is threatened by the production of *Beta*-lactamase enzymes, the predominant resistance mechanism impacting these agents in Gram-negative bacterial pathogens. The *Beta*-lactam antibiotic cefepime inhibits Broad-Spectrum *Beta*-Lactamase in *E.coli*[14]. Antibiotic resistance is a growing concern when treating bacterial infections. The number of resistance mechanisms acquired by bacteria is increasing each year and the prevalence of multidrug-resistant (MDR) bacteria is also increasing[15]. Few treatment options remain as the rate of discovery and development of new antibiotics is outpaced by bacterial resistance development[16].

GEO substances act individually or in synergy with one another, including but not limited to sesquiterpene compounds like bisapolene, zingiberene, zingiberol, sesquiphellandrene, curcurmene, phenolic compounds like shogaols and gingerols, and other compounds like 6-dihydrogingerdione, galanolactone, gingesulfonic acid, zingerone, geraniol, neral, monoacyldigalactosylglycerols and gingerglycolipis[17-19]. The extract may have caused irreparable damage to the gram-negative bacteria's outer membrane, causing their eventual death. Gram-negative bacteria have hydrophilic outer membranes owing to lipopolysaccharide molecules permitting only lipophilic compounds and macromolecules. If these molecules have antibacterial activity, they can penetrate the middle layer (which comprises a skinny peptidoglycan layer) and disturb cellular function, metabolism, and loss of cellular constituents, leading to bacterial death[20,21]; also reported a similar explanation in their studies.

Analyses show that GEO, combined with other antimicrobials, exhibits different effects (additive, synergistic, indifferent and antagonistic) against *E.coli*. The result above in agreement with (7), who indicate that quercetin showed

high antibacterial activity, additive or synergistic effect with antibiotic against *E.coli*.

The production of *beta*-lactamases, a family of enzymes that hydrolyze the *beta*-lactam ring, thereby inactivating the antibiotic molecule before binding with PBP's, is the principal mechanism of resistance to *Beta*-Lactam antibiotics. They also play a significant role in bacteria's intrinsic and acquired resistance, mainly in gram-negative[22]. Comparing the inhibitory activity shows that the GEOs are more effective against ESBL *E.coli* when added to the antibiotic Cefepime (FEP). The extract may have caused irreparable damage to the gram-negative bacteria's outer membrane, causing their eventual death. Gram-negative bacteria have hydrophilic outer membranes owing to the presence of lipopolysaccharide molecules permitting only lipophilic compounds and macromolecules. If these molecules have antibacterial activity, they can penetrate the middle layer of gram-negative bacteria (which comprises a skinny peptidoglycan layer) and disturb cellular function, metabolism, and loss of cellular constituents, leading to bacterial death[20,21]. reported a similar explanation in their studies. The absence of bla_{SHV} and the presence of bla_{TEM} were seen in (23), similar to the finding in the present study.

Conclusions

Escherichia coli identified by molecular technique gives more accurate bacterial identification than traditional methods. Most local clinical isolates of *E.coli* had multidrug resistance to most antibiotics used to treat these bacteria in our hospitals. According to molecular techniques, all of the present study's local clinical isolates of *E.coli* were bla_{TEM} genotypes. Using the antibacterial test, Ginger Essential Oil gave a synergistic effect when added to the antibiotic, which led to breaking the resistance of *E.coli*.

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ARTICLE / INVESTIGACIÓN

Characterization of the influence of diet on Japanese quail

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DOI. 10.21931/RB/2022.07.04.21

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Abstract: The experiments were conducted in fields of the department of animal production- agriculture college – Tikrit University by adding different levels of onion and sumac to the quail dietary and know its effect on the chemical composition of the thigh and the percentage of some saturated and unsaturated fatty acids and measures the peroxide value. The quail was divided into five treatments: Treatment 1 was controlled without adding, treatment 2 (3.5)g/kg of onion added, Treatment 3 (7)g/kg of onion added, treatment 4 (5)g/kg of sumac added. Treatment 5 (10)g/kg of sumac added. The results showed a high percentage of moisture in treatment 3 (73.08) % compared with the others; also, treatment 4 showed significant differences in fat percentage (7.50)% to the others. No significant differences led to protein and ash percentages between the five treatments. Regarding saturated fatty acids, treatments 1 and 4 showed significant differences in palmitic acid (7.98, 7.01)%, respectively. Stearic acid was high in treatment one than the others (9.10)%. Regarding saturated fatty acids didn't notice significant differences between the five treatments, but about peroxide, the values in treatments 2, 3, 4 and 5 were lower than the control.

Key words: Natural anti-oxidants, medical plants, meat rancidity.

Introduction

Meat is a high nutritional value as it is a significant source of amino acids and a source of vitamins, and minerals[1]. Due to the chemical and biological nature of the meat, it is damaged by storage because of oxidation of fat, which causes deterioration of the strength, flavor and test meat of meat[2]. Several industrial anti-oxidants have been used, but recently the focus has been on utilizing some plant sources suitable for consumption as natural oxidants because they are cheap and available in the markets. These contain phenolic compounds, which are anti-oxidants available in most plants[3]. Onion has been used in different countries since ancient times for food and health purposes. It was mentioned by ancient physicians such as ibn al-Bitar and al-Razi, and recently onion has been used in dermatological diseases because it contains organic sulfur[4,5]. It was also used to treat hyperglycemia in humans and to treat flu, cough, stomach and regulate blood pressure[6]. In recent years, interest in onion and some medicinal plants has increased, and it has been introduced into poultry diets in place of antibiotics that have been widely used recently[7]. Onion was selected among all plants added to diets because it contains antimicrobial substances and antioxidants[8,9]. The same is valid for sumac, which includes many active substances with anti-oxidant and medicinal benefits[10]. Therefore, the present study aimed to determine the effect of onion and sumac in preserving quail meat.

Materials and methods

The experiment was conducted in the poultry halls in the fields of the faculty of agriculture, department of animal production, from 14-11-2017 to 17-1-2018. Twenty Quail used in this experiment were divided into five treatments, each one contain four quail:

Treatment1 was controlled without adding treatment 2 (3.5)g/kg of onion added.

Treatment 3 (7)g/kg of onion added, treatment 4 (5)g/kg of sumac added.

Treatment 5 (10)g/kg of sumac added.

Moisture content determination

Moisture content was determined in sheep meat samples according to (11) by drying about 15 gm of the model at 120°C until a constant weight was recorded. Then calculate, the weight difference and the moisture was determined by the difference in weight before and after drying.

Protein determination

Total nitrogen was measured according to (11) procedures using (micro-Kjeldahl) techniques, and a conversion factor of 6.25 extract protein percent in the meat sample was used.

The percentage of fat in meat was measured by using soxhlet extraction units according to (11) procedures.

Ash determination

According to *AOAC 1980*[11] procedures, Ash is determined: 2 gm. of meat was weighed, put in a silica platinum dish, and transferred to a muffle furnace maintained at (500-600°C)

Citation: Ibraheem M W, AL Mjbel A A, Abdulwahid A S, Mohammed Th T. Characterization of the influence of diet on Japanese quail. Revis Bionatura 2022;7(4) 21. http://dx.doi.org/10.21931/RB/2022.07.04.21
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

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for 6 hours until grey ash was obtained. It was left to be cooled, then weighted, and the ash percent was calculated.

Free fatty acids

Free fatty acids are estimated according to (12). Weigh 3g of meat and added 50ml of ethyl alcohol concentrate 98% and added to the sample drops of phenolphthalein after heating in a water path till boiling then squeeze the mixture with potassium hydroxide 0.1 standards till converted to pink then estimate the percentage by the equation:

Free fatty acids %= titration(A-B)×N×282/ 1000×W.t of sample(g) ×100

Peroxide value

Peroxide was estimated by (12). The weight of 3g of meat was increased by 30 ml of a mixture containing three parts of ice acetic acid, two pieces of chloroform,5ml of saturated potassium iodide and 20 ml of water dice and a few drops of starch, and then scraped the mixture with sodium thiosulfate solution standard 0.001. until the disappearance of the blue color and the amount of peroxide using the equation:

Peroxide(ml) = numbers of milliliters of sodium thiolate × 0.01 × 1000/weigh of sample (g).

Statistical analysis

The results were statistically analyzed using (SPSS) and full random design according to the mathematical model[13]:

Yij = μ + Ti + eij

Yij= the observation I to treatment j

μ = the overall mean effect

Ti = effect of treatment I

eij = : is an independent normally distributed random error term with zero mean and variance .σ^2

difference between averages was compared using Duncan,s Multiple Range Test[14].

Results

Chemical composition of the thigh

The results of the chemical analysis in the table (1) showed significant differences in moisture content in treatment 3 (onion 7g/kg) it was (73.08) compared with the rest treatments, the higher percentage of moisture in the third treatment (7% onion) compared with the rest treatments may be because of the high rate of moisture in onion which is positively affected the therapy. In general, water was high in all treatments. Protein didn't notice significant differences between the five treatments except for some account differences.

Fat content showed highly significant differences in treatment five, which was 7.50% compared with the other treatments, and that may be due to the reverse relationship between fat and moisture. Also, we noticed that the lowest percentage of fat in treatments 2 and 3 was (4.99, 5.01) % (the onion additives); the reason may be due to the low rate of fat in the onion—also, the difference in the chemical composition between onion and sumac.

No significant differences were noticed between the five treatments in ash percentage; it was low values in all treatments, which is a good indicator of the quality of meat because heavy metals are always found in Ash.

Saturated Fatty acids

In saturated fatty acids (palmitic), in table (2), the control treatment conducted a high level(7.98)%, it was higher than the rest treatments (6.99, 5.98,7.01, and 5.97)%, respectively. And that may be due to the effectiveness of the substance added to the other four treatments (onion and sumac). Also, we noticed the lower percentage of saturated free fatty acids in treatment 3(7g/kg onion and 5 g/kg sumac) was (5.98, 5.97)%, respectively; the high level of additives used in these treatments than the other treatments also the high percentage of palmitic cames from the other source of it is the amino acids grounded in diets and carbohydrates

About citric acid, the same has happened. Also, the control was higher than the others (9.10)% because management didn't have any anti-oxidant additives. Treatments 4 and 5 conducted the lowest percentage of citric (5.09, 5.12)%.

Unsaturated fatty acids

The results table (3) about palmitoleic acid showed no significant differences between 1,2,3and 5 treatments it was (25.45, 25.98, 25.98, 25.63)%, respectively and that is an accepted range not more than the limits, but treatment four has significantly differences lower than the rest treatments it was (24.98)%. About linoleic acid, we noticed treatment 1 (control) has significant differences (17.04) % from the other treatments it was (16.09, 14.87, 15.99, 15.87)%, respectively is may be due to the natural anti-oxidant substance added to the Quail diets with different percentage. Also, significant oleic differences noticed in treatment 4 was (43.08)%, but the other treatments were (42.01 42.68, 42.98, 42.01)% for treatment 1,2,3,5, respectively.

The three unsaturated fatty acids results did not differ highly from the control. That may be due to the anti-oxidant effectiveness of additives onion and sumac; everyone is different from the other in its contents and effects on diets.

Peroxide value

The results in a table (4) effecting adding different levels of onion and sumac on peroxide showed highly significant differences between treatment 1 (control) was (1.00) and treatments 2, 3, 4, and 5 were (0.8, 0.6, 0.7, 0.6) respectively this low in peroxide in treatments 2, 3, 4 and 5 then the treatment one may be due to the additives used in this research as we now onion and sumac have an anti-oxidant effect, so it is known the peroxide values will be decreased when we use these additives that is very important for us to know the effect of natural anti-oxidant on meat quality and if they affected by it or not. And that it will be clear in stored meat the fresh.

Panel test

The sensory evaluation of the femur cut showed highly significant differences in flavor in treatment 4 (5g/ kg sumac) (4.4±0.82) compared with control and treatment 3 (10g/kg sumac). They were (1.87±0.32 and 3.3±0.21) respectively. Also, juiciness and acceptability were conducted high significant differences in the same treatments; they were (4.6±0.35 and 4.87±0.54) respectively, compared with control and treatment two, were (2.8±0.32 and 2.65±0.88) respectively, in juiciness and control in acceptability was (3.12±0.12). About the tenderness treatment, 2 (7g/kg onion) was the best it conducted (3.8±0.35) compared with control was (1.3±0.54); the tenderness affected by moisture and juiciness these three traits are incredibly related, and they influenced each other and also they affected on the acceptability.

Discussion

Chemical composition of the thigh

The higher percentage of moisture in the third treatment (7% onion) compared with the rest of the treatments may be because of the high rate of water in the onion, which positively affected the therapy. In general, moisture was increased in all treatments, which may be due to the age of the quail, so the meat of the younger quail has more water than the oldest quail. Protein didn't notice significant differences between the five treatments except for some account differences, which may be due to the early age of quail, so it converted the diet to protein rather than fat.

Regarding the fat content, the reason may be due to the reverse relationship between fat and moisture. Also, we noticed that the lowest percentage of fat was in treatments 2 and 3 (the onion additives); the reason may be due to the low rate of fat in the onion. Also, the difference in the chemical composition between onion and sumac may cause a difference in the chemical composition of Quail meat between treatments.

Low values in all treatments in ash content are an excellent indicater of the quality of meat because heavy metals are always found in Ash.

Saturated Fatty acids

In saturated fatty acids (palmitic), the control treatment conducted high level was higher than the rest treatments. That may be due to the effectiveness of the substance added to the other four treatments (onion and sumac). Also, we noticed the lower percentage of saturated free fatty acids was in treatment 3, which may be due to the high level of additives used in these treatments than the other treatments. Also, the high percentage of palmitic may be cames by the other source of it, the amino acids grounded in diets and carbohydrates

About citric acid, the same happened. Also, the control was higher than the others, and that is because the power didn't have any anti-oxidant additives. Treatment 4 and 5 conducted the lowest percentage of citric that is may be due to the high anti-oxidant effectiveness of sumac; the presence of citric acid is significant because it considers a natural anti-oxidant helps the substance from boiling and also protect the meat from a long time.

Unsaturated fatty acids

About palmitoleic acid showed no significant differences between 1,2,3and 5 treatments, which is an accepted range that exceeds the limits. Still, treatment four has significant differences lower than the rest treatments. About linoleic acid, we noticed treatment 1 (control) has significant differences that may be due to the natural anti-oxidant substance added to the Quail diets with different percentages. Also, significant oleic differences were noticed in treatment four, that is may be due to the additional effect of the chemical composition of Sumac and Onion on the Quail diets

In general, the results of the three unsaturated fatty acids did not highly differ from the control. They may be due to the anti-oxidant effectiveness of additives onion and sumac. Everyone is different than others in their contents and their effects on diets.

Peroxide value

Adding different levels of onion and sumac to peroxide showed highly significant differences between treatment 1 and treatments 2, and 3. This low peroxide in treatments 2, 3, 4 and 5 and then the treatment 1 may be due to the additives used in this research; as we know, onion and sumac have anti-oxidant effects, so it is known the peroxide values will decrease when we use these additives that is very important for us to know the effect of natural anti-oxidant on meat quality and if they affected by it or not. And that it will be transparent in stored fresh meat.

Panel test

The sensory evaluation of the femur cut showed highly significant differences in flavor in treatment 4 compared with control. Treatment 3 may be due to the fine addition of sumac to treatment 4 and the lovely taste of sumac compared with onion; many people reject the onion flavor. Also, juiciness and acceptability were conducted with highly significant differences in the same treatments compared with control and treatment 2 in juiciness and control in acceptability; that may be due to the moisture percentage because juiciness depends on the moisture in the meat cut. Regarding the tenderness treatment, 2 was the best compared with control of the tenderness affected by moisture and juiciness; these three traits are incredibly related, and they affected each other and acceptability.

Conclusions

The addition of these natural anti-oxidant (Onion, Sumac) to Quail diets improved the chemical and sensory qualities of Quail meat; this is a good indication of the trend towards using natural additives in Quail and Avian diets to help improve the quality of meat consumed by human and in addition to the health benefit, it also benefits the consumer and the producer because of the lower cost compared to the other synthetic anti-oxidant used.

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Treatments	Percentage	Moisture%	Protein%	Fat%	Ash%
Control	0	71.98± 2.0ab	22.07± 3.20 a	5.600± 0.7 b	0.3± 0.002 a
T2	3.5 g/kg	72.06± 1.2ab	22.32± 0.01 a	4.99± 0.8bc	0.4± 0.001 a
Onion	7g/kg	73.08± 1.1 a	21.65± 0.01 a	5.01± 0.9 b	0.5± 0.003 a
T3 sumac	5g/kg	70.08± 1.3b	21.09± 0.06 a	7.50± 0.8 a	0.3± 0.001 a
	10g/kg	69.49± 1.0c	22.32± 0.08 a	6.99± 0.4 ab	0.4± 0.001 a

Different letters within the column refer to significant differences ($p \leq 0.05$) between means.

Table 1. Adding a Combination of Onion and Sumac Powder to the Japanese quail Diet on chemical composition (mean ± SE).

Treatments	Percentage	Palmatic	Citiaric
Control	0	7.98± 0.06 a	9.10± 0.04 a
T2 onion	3.5 g/kg	6.99± 0.02 ab	8.94± 0.05 b
	7g/kg	5.98± 0.09 b	8.12± 0.07 b
T3 sumac	5g/kg	7.01± 0.09 a	5.09± 0.01 c
	3.5 g/kg	5.97± 0.05 b	5.12± 0.02 c

Different letters within the column refer to significant differences (p ≤0.05) between means.

Table 2. Adding a Combination of Onion and Sumac Powder to the Japanese quail Diet on some saturated fatty acid (mean ±).

Treatments	percentage	Palmitoleic C16:1	Linoleic C18:2	Oleic C18:1
Control	0	25.45± 0.05 a	17.04± 0.12 a	42.01± 0.2 ab
T2 onion	3.5 g/kg	25.98± 0.01 a	16.09± 0.05 b	42.68± 0.1 ab
	7g/kg	25.98± 0.90 a	14.87± 0.02 c	42.98± 0.6 ab
T3 sumac	5g/kg	24.98± 0.03 ab	15.99± 0.06 bc	43.08± 0.7 a
	3.5 g/kg	25.63± +0.06 a	15.87± 0.80 bc	42.01± 0.4 ab

Different letters within the column refer to significant differences (p ≤0.05) between means.

Table 3. Adding a Combination of Onion and Sumac Powder to the Japanese quail Diet on some unsaturated fatty acid (mean ± SE).

Treatments	percentage	Peroxide
Control	0	1.00± 0.008 a
T2 onion	3.5 g/kg	0.8± 0.003 b
	7g/kg	0.6± 0.002 c
T3 sumac	5g/kg	0.7± 0.003 b
	3.5 g/kg	0.6± 0.003 c

Different letters within the column refer to significant differences (p ≤0.05) between means.

Table 4. Adding a Combination of Onion and Sumac Powder to the Japanese quail Diet on peroxide value (mean ± SE).

Treatments	Percentage	Flavor	Tenderness	Juiciness	Acceptability
Control	0	1.87±0.32 c	1.3±0.54 c	2.8±0.32 c	2.7±0.54 bc
T2 onion	3.5 g/kg	3.7±0.65 ab	3.01±0.62 ab	3.53±0.93 bc	4.6±0.95 ab
	7g/kg	3.4±0.58 ab	3.8±0.35 a	2.65±0.88 c	3.12±0.12 b
T3 sumac	5g/kg	4.4±0.82 a	3.1±0.22 ab	4.6±0.35 a	4.87±0.54 a
	3.5 g/kg	3.3±0.21 b	3.36±0.76 ab	4.1±0.88 ab	4.6±0.65 ab

Different letters within the column refer to significant differences (p ≤0.05) between means

Table 5. Adding a Combination of Onion and Sumac Powder to the Japanese quail Diet on panel test (mean ± SE).

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ARTICLE / INVESTIGACIÓN

Effect of the prolactin gene polymorphism on quantity and quality of milk in Iraqi goats

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DOI. 10.21931/RB/2022.07.04.22

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Abstract: Domestic goats in Iraq are one of the oldest domesticated animals in Mesopotamia. Their adaptation characterizes them to harsh environmental conditions such as heat in the summer and cold in the winter. Still, they suffer from neglect and the lack of genetic research that would improve the production of this animal, which is considered a multi-production (milk production, meat production, hair production). And because the prolactin gene is crucial, the current study focused on how the prolactin gene (PRL) affects milk yield and its composition. We obtained three genotypes of the prolactin gene exon 5: AA, AB, and BB. The hybrid genotype AB had the highest daily milk yield in domestic goats, although the recessive type BB had a more significant fat percentage. The genetic approach is advised if breeding aims to enhance milk output daily. So select hybrid genotype AB to exon 5 for the (PRL) gene.

Key words: Domestic Iraqi goats, prolactin gene, milk production.

Introduction

Livestock output in Iraq typically falls behind agricultural production in terms of development, management standards, and husbandry; crop farming and raising livestock have not always been combined[1]. The native goat breeds in Iraq are extensively distributed throughout the country and bred with sheep as a mixed flock. They are noted for their determination to endure the challenging conditions of poor pastures and high disease resistance. Typically, goats graze intensively on natural grassland and agricultural wastes with sheep for more than six months out of the year. There is relatively little forage produced[2]. Goats of the Iraqi strain are all different shades of white, black, and brown. Each color spreads with the other colors mentioned in varied ratios[2]. The native goats in Iraq have a population of roughly 1.3 million. It is dispersed throughout the nation, with l2.5, 44.2, and 43.3 percent being in the country's southern, central, and northern regions, respectively[1].

Production of meat and milk is the primary goal of goat farming, but hair production is still a secondary goal. Through utilizing advancements in genetic and biological sciences and identifying numerous genetic markers that were strongly correlated with the phenotype variance of significant traits like milk production, meat production, reproductive traits, and behavioral performance, numerous attempts were made to improve native goat performance[3]. Prolactin (PRL) is a protein hormone that controls breastfeeding and reproductive processes in animals[4,5]. And is mainly released by eosino-phils in the anterior pituitary gland. Prolactin regulates the differentiation and proliferation of mammary gland cells to encourage milk production throughout the development of animal mammary glands[6].

Additionally, PRL regulates milk's protein and lipid concentrations and the production of lactose, among other critical functions[7]. Many studies have found that PRL polymorphisms are linked to wool or cashmere traits in goats and sheep[8]. Others investigated the relationship between PRL polymorphism and dairy traits such as benefit yield and protein milk yield in cattle breeds[9]. Furthermore, many researchers have suggested that the PRL gene improves prolificacy in various sheep breeds. Since there are few studies on the prolactin gene in local Iraqi goats, our research chose to shed light on how the polymorphism of the prolactin gene in local goats relates to milk production and its constituent parts.

Materials and methods

The 50 female goat samples included in the current investigations are from Iraq. Fifty female goats, mostly from Al-Mahawil, were found in numerous commercial farms in Babylon City between October 1, 2020, and June 1, 2021. The animals were raised in open fields and fed a concentrated diet (450–550 grams per head per day. Hand milking involves manually collecting the milk from the ewes during the preweaning stage once a week (until 115 days). The milk is then kept in ice until it is transported to the lab to be calculated, and its various components are examined in the morning and evening. After milk filtration, a public health lab in Baghdad utilized Lacto flash solution to evaluate the milk's composition.

To determine the genetic structures of the studied gene segment, each female goat had approximately 5 ml of blood drawn from the jugular vein, which was kept in a sterile container at 4°C for DNA extraction[2,16]. The following PCR conditions were used: 3 l of genomic DNA, primer, 10 mM Tris-HCl (pH 9.0), 30 mM KCl, 250 M of each dNTP ("dATP,

Citation: Alsarai T M. Effect of the prolactin gene polymorphism on quantity and quality of milk in Iraqi goats.Revis Bionatura 2022;7(4) 22. http://dx.doi.org/10.21931/RB/2022.07.04.22
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

dGTP, dTTP, and dCTP"), 1 U Top DNA 2 polymerase, and 1.5 mM MgCl (BioNeer Company, Korea). The generated DNA fragment was amplified using one primer from Integrated DNA Technologies. As mentioned below about the details of the primer sequence for exon 511 :

F: ATTCCTGGAGCCAAAGAG

R: TGTGGGCTTAGCAGTTGT

Product Length: 655pb

Restriction Enzyme: Eco 241

As a result, the study used restriction enzyme (2U/ Rsal I) for two hours at 38°C to reveal the DNA fragment sizes between experimental ewes. Restriction fragment length polymorphism (RFLP) identifies specific restriction enzymes that show a pattern difference between the DNA fragment sizes in individual organisms[10]. The study used gel electrophoresis and the restriction enzyme to digest the product with roughly 1.5% from gel agars. Then it estimated the frequency of alleles and genotype of the prolactin gene in female goats.

Statistical Analysis

The data, according to the SAS application[11].

Were examined.

Yij = μ +αi +eij

Where: Where: μ: is an overall means, αi: the genotype of the prolactin gene.

eij: is a random error, and genes (AA, AB, and BB) and their effect and eij: is an unexpected error.

Calculations were done using the genotype and allele frequencies Formula[12].

Gene frequency =2D + H /2N

If H is the number of animals with heterozygosis, D is the number of homozygous animals for specific alleles

N: the total number of animals. Chi-square (χ) test was used to predict the essential variations between individuals

X = 2 Σ(Observed No. – Expected No.)2/ Expected No.

GLM, a general linear model, was utilized in the study, and Duncan's numerous ranges Test[13].

To predict the significant difference between groups.

Results

Milk production and composition are examples of some quantitative traits loci (QTL) in Iraqi goats. Our study was about the genetic pattern of exon 5 for (PRL) gene and its relationship to the Daily productive performance of the milk production recipe and its milk components.

Genetic polymorphism

Our data analysis shows that RFLP-PCR is a valuable approach for estimating the genetic variability of Iraqi goats. In an Iraqi goat, a 420 bp PRL gene fragment was amplified using a specific primer (Fig 1).

The genetic polymorphism and allele frequency for the PRL gene were measured in the current investigation. As shown in table(1), the percentages of the AA, AB, and BB genotypes were each (46, 34, and 20)%, respectively.

The relationship of genotypes to milk production and its components

In our study, when we compared the AB, AA and BB genotypes in Iraqi goats of the PRL gene, we found the height level of fat composition connected to the BB genotype was 5.16, and the lowest level was 3.81. While the proportion of proteins related to the BB genotype was 2.66, and the balance to the AB genotype was 1.28, respectively. Moreover, as explained in the table, the high ratio of solids nonfat (SNF) connected BB was 7.10, and the lowest with AB was 3.47; table (2).

Discussion

Fifty blood samples in our current study using PCR-RFLP methods, the results of genetic polymorphism were three genotypes (AA, AB, and BB. The percentages of their genotypes, as given in table (2), were each (46, 34, and 20)%, respectively. Also, the percentages of allele frequency were 0.36 for A and 0.37 for B. This is different from the study on Egyptian goats, as the ratio of hybrids is higher than other genotypes[14]. But it agrees with what was stated in the study about Chinese goats since the AA type is the most numerous[8]. It is possible to attribute the results to the in-field breeding strategy.

This gene was selected due to its direct contribution to milk synthesis, secretion maintenance, and mammary gland growth and development[7,15]. However, there is little evidence of the relationship between this marker and milk yield and composition in Iraqi dairy goats. As shown in table(2), our study discovered that the height level of fat composition associated with the BB genotype was 5.16, and the lowest level was 3.81. Furthermore, 2.66 percent of proteins were linked to the BB genotype, while 1.28 percent were related to the AB genotype. Additionally, as shown in the table, BB and AB had the highest and lowest ratios of solids nonfat

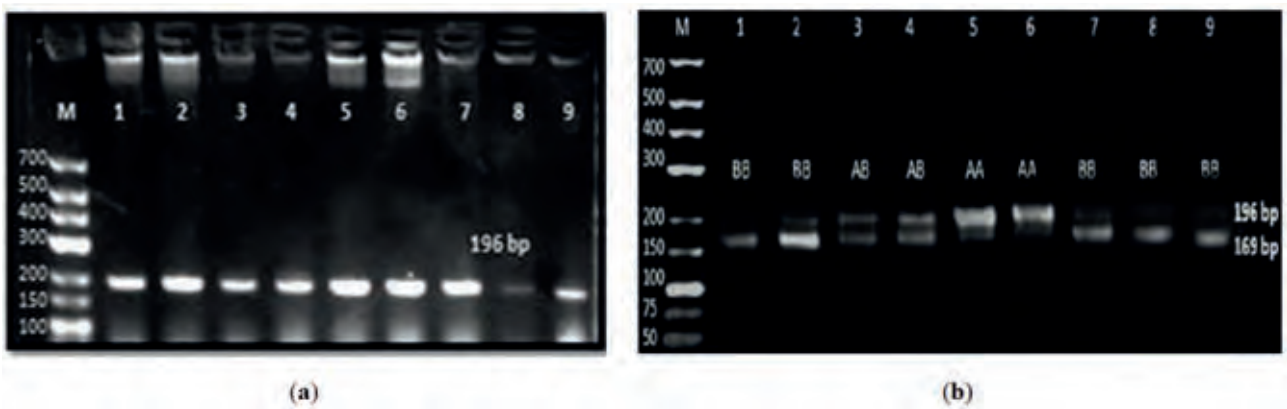

Figure 1. (a): Agarose gel electrophoresis of PRL-PCR fragment (196 bp). Lane M, 50 bp DNA ladder (b): Agarose gel electrophoresis of PRL- Eco24I/PCR-RFLP fragments. Lane M, 50 bp DNA ladder, lanes (5, 6) Genotype AA (196 bp), lanes (3, 4) Genotype AB (169 to196 bp) and lanes (1, 2, 7, 8, 9) Genotype BB (169 bp).

Genotype	No	Percentage (%)
AA	23	46.00
AB	17	34.00
BB	10	20.00
Allele	Frequency	---
A	0.36	---
T	0.37	---
** (P≤0.01).		

Table 1. Genetic polymorphism and allele frequency for the PRL gene.

	AA	AB	BB
Daily milk yield (gm)	420.56 ±26.20	601.22 ±30.22	400.22 ±22.23
Fat	3.81 ±0.01	4.27 ±0.02	5.16 ±0.10
Protein	2.22 ±0.03	1.28 ±0.01	2.66 ±0.22
SNF	5.99 ±0.11	3.47 ±0.12	7.10 ±0.21
Relative density	1.014 ±0.21	1.00 ±0.01	1.017 ±0.23
Freezing point	-0.89 ±0.03	-0.22 ±0.13	-0.46 ±0.04

Table 2. The relationship of PRL genotypes to milk production and its components in Iraqi domestic goats.

(SNF), respectively, at 7.10 and 3.47; some studies confirm the importance of paying attention to this trait because it is linked to health benefits such as bone mineral density. It also reduces the risk of developing type 2 diabetes and coronary heart disease[16]; our results contrast the study which found that goats with the dominant Model AA of the exon five segment for prolactin gene produced the most milk daily[15]. Also, we found that the relative density was higher in the BB genotype in this study and in Table 2, which could be due to the correlation of density with the fat percentage in milk[17], as the mentioned genotype has the highest rate of fat. Furthermore,

Our findings show that when this piece of the prolactin gene is studied, it becomes clear that if we want to select based on milk production, we choose the hybrid genotype AB. I hypothesize that the strength of the hybrid for this trait may affect the output level. Still, if we want to select based on fat and SNF proportion, we choose animals with the genotype BB that is the highest in these traits.

Conclusions

Goats are one of Mesopotamia's oldest domesticated animals. They are distinguished by their ability to adapt to harsh environmental conditions, such as heat in the summer and cold in the winter. Domestic goats in Iraq are neglected, and genetic research is lacking to improve their production. The prolactin gene (PRL) is a potential candidate gene for goat milk traits in marker-assisted selection. As a result, this research aimed to identify PRL gene polymorphism and its relationship to milk traits. So, if the goal of breeding is to increase milk output daily, we recommend for the (PRL) gene, choose hybrid genotype AB of exon 5. The hybrid genotype AB had the highest daily milk yield in domestic goats. However, if we want to select fat and SNF proportion, we choose animals with the genotype BB, which is the highest in these traits.

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ARTICLE / INVESTIGACIÓN

NDRG1 is being investigated as a possible bladder cancer biomarker in the Iraqi population

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DOI. 10.21931/RB/2022.07.04.23

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Abstract: With 549,393 new cases recorded in 2018, bladder cancer is one of the most common malignancies worldwide. Urinary bladder cancer is the cause of about 3 percent of all new cancer diagnoses and 2.1 percent of all cancer deaths. This study aims to evaluate the efficiency of the N-myc downstream-regulated gene 1(NDRG1) as a biomarker for bladder cancer patients in the Iraqi population. One hundred individuals in the case-control study were enrolled and divided into two groups. The first group included 50 patients diagnosed with a bladder mass and investigated by undergoing cystoscopy examination for transurethral resection of bladder tumor (TURB). The second group included 50 healthy individuals who had normal bladder tissue. The results of the present study showed the highest level of (NDRG1) among cases with statically significant association (p=0.001). The ROC curve demonstrated that the protein level of (NDRG1) could distinguish disease patients from healthy individuals with a sensitivity of 96% and a specificity of 92%. Serum (NDRG1) protein is an efficient and noninvasive tumor marker for diagnosing bladder cancer.

Key words: N-myc downstream-regulated gene 1 (NDRG1), non-muscle-invasive bladder cancer (NMIBC), transurethral resection of bladder tumor (TURB).

Introduction

The primary purpose of the bladder is to store urine because it is a hollow muscular organ that lies in the anterior section of the pelvic cavity and has a spherical shape when it is filled with pee[1]. Bladder cancer can develop from epithelial (urothelium) cells that line the inner surface of the bladder[2]. Or from nonepithelial (mesenchymal) cells that differentiate between developing smooth muscles of the bladder[3]. However, 90 to 95 percent of bladder cancer cases are urothelial carcinomas, with transitional cell carcinomas accounting for most of these cases and being considered the most common type of bladder cancer[4]. The four members of the N-myc downstream-regulated gene (NDRG) protein family, NDRG1, NDRG2, NDRG3, and NDRG4, share 57-65 percent of the same amino acids. Except for the C-terminal 5-amino acid residues, which are totally maintained in all four NDRG proteins, the sequence variations amongst family members are primarily found in the N- and C-terminal regions. Three 10-aa (GTRSRSHTSE) tandem repeats can be found in the C-terminal part of NDRG1, which are lacking in the other family members and are thought to be essential for the protein's function[5]. A 43-KDa protein of 394 amino acids called N-myc downstream-regulated gene 1 (NDRG1), also known as Cap43, RTP, Rit42, Drg1, or PROXY-16[6]. It was first cloned and identified in 1997 and has been associated with several diseases, including esophageal squamous cell carcinoma, prostate cancer, and pancreatic cancer[7].

Multiple biological processes, including cell division, growth, stress reactions, differentiation, and immunology, are influenced by NDRG1[8,9]. The NDRG1 protein is generally cytoplasmic in origin at the cellular level. But this localization can differ between various cell types. For example, breast and intestinal epithelia express a membrane-associated protein, while prostate epithelial cells demonstrate nuclear localization of NDRG1. Additionally, mitochondrial localization is seen in specific cell types[10,11]. These results collectively show that NDRG1 acts in a tissue-specific way[12]. In breast, colorectal cancer, and glioma, NDRG1 has been tested as a prognostic marker, and many studies have revealed a link between NDRG1 expression levels and survival. While in other types of cancer, such as hepatocellular carcinoma, cervical cancer, and bladder cancer, NDRG1 is upregulated. These contradictory outcomes for NDRG1 expression show that this protein operates differently in different types of cancer depending on the tissue[13]. The high levels of NDRG1 typically indicate that cells have been exposed to elements that can cause DNA damage, oxidative stress, hypoxia, and heavy metal toxicity. NDRG1 regulates cellular proliferation, differentiation, angiogenesis, tumor growth, apoptosis, and metastasis by responding to those stimuli and heavy metal and hypoxia sensing[14]. Bladder cancer is intimately associated with smoking and is mainly brought on by chemical compounds that result in hypoxia and DNA damage. According to several studies, bladder cancer patients with high HIF-1 expression have a bad prognosis. HIF-1 is an indication of hypoxia and is extensively expressed in bladder cancer. Additionally, research suggests that NDRG1 is elevated by HIF-1, so its protein level may contribute to the development of bladder cancer and serve as a possible biomarker for the disease[9].

Citation: Henao-Ramírez, AM.; Palacio- Hajduk, DH.; Urrea-Trujillo, AI. Cost Analysis of Cacao (Theobroma cacao L.) Plant Propagation through the Somatic Embryogenesis Method. Revis Bionatura 2022;7(2) 2. http://dx.doi.org/10.21931/RB/2022.07.02.2
Received: 14 July 2021 / **Accepted:** 10 December 2021 / **Published:** 15 May 2022

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Materials and methods

All patients in this case-control study were recruited from Ghazi Hariri Hospital for surgical specialties in Baghdad from December 2021 to June 2022. One hundred individuals were enrolled in this study and divided into two groups. The first group included 50 patients (46 males, 4 females) with an age range of (16-79) years. Nearly thirty-five patients smoked cigarettes for more than 5 years and were defined as smokers. All patients were first diagnosed with a bladder mass, investigated by transurethral resection of bladder tumor (TURBT), and sent to the histopathological examination for biopsy of bladder lesion. The second group included 50 healthy individuals (46 males, 4 females) with normal bladder tissue and no previous history of other renal systemic diseases. All the patients and the healthy individuals participating in this study were informed, and their consent was obtained before any action related to this study took place. In addition, approval has been taken by the Ethical Committee of the College of Medicine/ University of Baghdad.

•Inclusion criteria:

Patients who were represented with single superficial bladder mass.

•Exclusion criteria:

Patients with health disorders that make them unfit for general anesthesia.

Results

The results of the present study showed the highest level of NDRG1 among cases with statically significant association (p=0.001). And regarding biopsy, the result showed the highest positive among cases while the highest negative among controls with statically significant association (p=0.001). as shown in (figure 1) and (table1):

The current study showed a positive relationship between the mean of NDRG1 and age through increased level of NDRG1 with increased generation of more than 60 years with statical significance (p=0.045). In addition to that, we see the same result among patients with smoking and hematuria with a statical difference (p=0.001). as shown in (figure2) and (table2):

Next, the receiver operating characteristic (ROC) curve based on the ELISA results was plotted to evaluate the potential of NDRG1 as a noninvasive biomarker for diagnosing disease. The ROC curve demonstrated that the protein level of NDRG1 could distinguish disease patients from healthy controls. The optimum cut-off value for the diagnosis of disease patients was 294.94 ng/mg. The area under the curve (AUC) of NDRG1 expression to diagnose disease was 0.942 (95% CI, 0.890–0.995), with a sensitivity of 96% and a specificity of 92%. as shown in (figure3) and (table3):

Discussion

Bladder cancer represents a spectrum of illnesses, from chronically recurrent, noninvasive tumors that can be controlled to aggressive, advanced-stage diseases requiring invasive and multimodal treatment[15]. Regarding tumor suppression, metastatic suppression, and oncogenesis, N-myc downstream-regulated gene 1 (NDRG1) is essential for understanding cancer biology. NDRG1, typically found in human epithelial cells, regulates the biological processes of several cancer cells, including bladder cancer[16]. Depending on our results, we can recognize that the levels of NDRG1 protein were the highest among the bladder cancer patients group with a statically significant association (p=0.001). With a sensitivity of 96% and a specificity of 92% and these results are in agreement with the results of other studies such as Aiwei Li *et al.* They revealed that the expression level of NDRG1 mRNA and protein is considerably increased in bladder cancer tissues with an association for NDRG1 protein (P=0.002) and they were an independent prognostic factor. In NMIBC patients, high NDRG1 expression was linked to poor overall survival and a higher likelihood of developing MIBC. And they discovered that NDRG1, which has a sensitivity of 84.6 percent and a specificity of 86.7 percent, maybe a potential biomarker for the diagnosis of bladder cancer[9]. One hundred nineteen bladder cancer patients and 65 patients with non-cancerous bladder diseases were included in the study. According to Sun, Y.J., *et al.*, the expression of NDRG1/Cr was significantly higher in bladder cancer patients than in non-cancerous bladder disease patients (P=0.009), with a sensitivity of 63.8 percent and a specificity of 73.4 percent[17]. Using ELISA, it was discovered

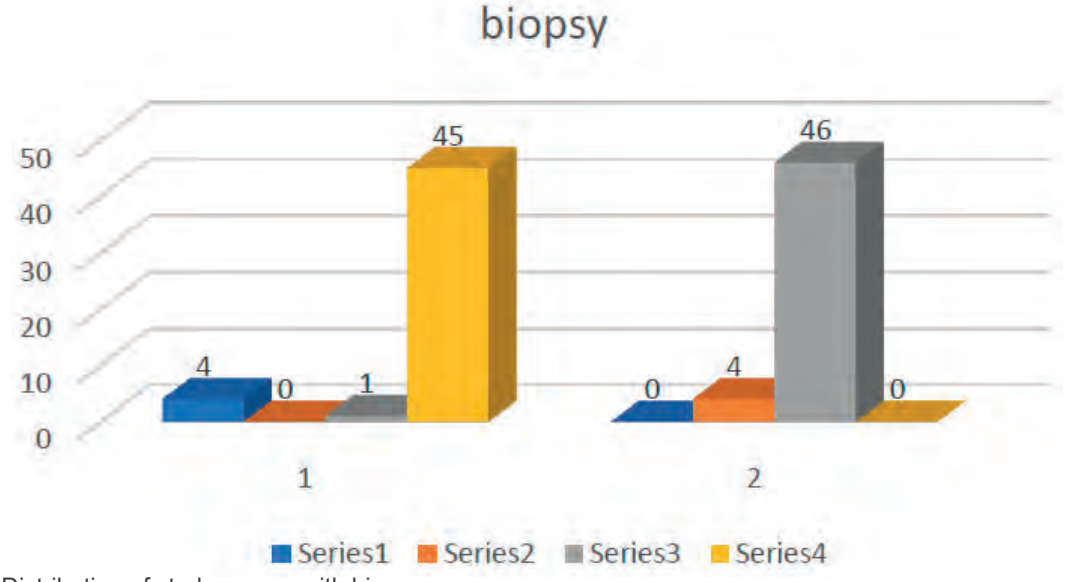

Figure 1. Distribution of study groups with biopsy.

biopsy	cases	controls	Total	P-value
false negative	4	0	4	0.001*
	8.00%	0.00%	4.00%	
false positive	0	4	4	
	0.00%	8.00%	4.00%	
negative	1	46	47	
	2.00%	92.00%	47.00%	
positive	45	0	45	
	90.00%	0.00%	45.00%	
Total	50	50	100	
	100.00%	100.00%	100.00%	
[1] Pearson Chi-Square		[2] statically significant *		

Table 1. Correlation of study groups with biopsy.

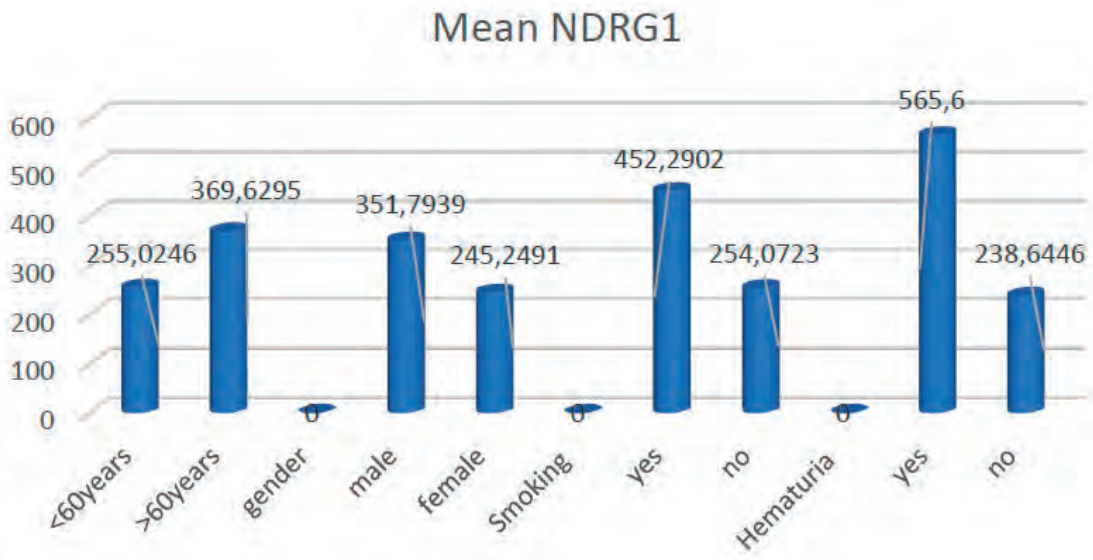

Figure 2. Distribution of study sample according to NDRG1 mean.

Age	N	Mean NDRG1	Std. Deviation	P-VALUE
<60years	23	255.0246	199.8733	0.045*
>60years	77	369.6295	247.1395	
gender	N	Mean NDRG1	Std. Deviation	P-value
male	92	351.7939	246.1707	0.170
female	8	245.2491	148.9325	
Smoking	N	Mean NDRG1	Std. Deviation	P-value
yes	45	452.2902	247.1432	0.001*
no	55	254.0723	196.8959	
Hematuria	N	Mean NDRG1	Std. Deviation	P-value
yes	30	565.6	236.2081	0.001*
no	68	238.6446	159.1796	
		[1] Independent t-test	[2] statically significant *	

Table 2. Distribution of study sample according to NDRG1 mean.

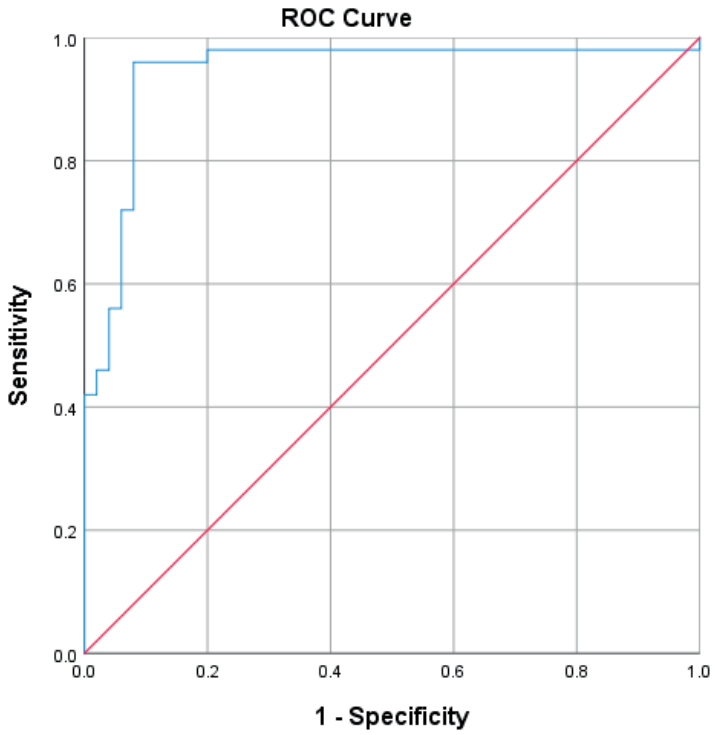

Figure 3. The receiver operating characteristic (ROC) curve.

Test Result Variable(s): NDRG1				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.942	.027	.000	.890	.995
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

Table 3. Area under the curve. Test Result Variable(s): NDRG1.

that NDRG1 could be identified in the serum of bladder cancer patients as a differentiating marker[18]. If smoking is one of the causes that lead to hypoxia and is strongly related to bladder cancer, and as is represented in our results, there is a positive relationship between the mean of NDRG1 and smoking with a statical difference (p=0.001). This ensures Cangul, H.'s study offers convincing proof that HIF-1-dependent and –independent pathways regulate the expression of the NDRG1 gene at both the protein and RNA levels[19,20]. In addition, hematuria showed a positive relationship with NDRG1 in the current study with statically significant (p=0.001). These results are consistent with other studies: In the cohort of more than 2,000 patients who experienced asymptomatic microscopic hematuria, Gonzalez, N.A. *et al*. revealed that 25 patients (1.2%) had bladder cancer discovered by cystoscopy; these cases were noninvasive and were found in patients older than 50 years[21]. According to Samson, P. *et al.*, only 6 of the 1049 patients who experienced asymptomatic microscopic hematuria, with a mean age of 77 years, and the youngest patient diagnosed at 59 years, had bladder cancer[22]. As shown in the above studies, we have only disagreed on the mean and prevalence of hematuria among patients with bladder cancer, which is higher in our study. That may be due to our small sampling group compared to the cohorts born in previous studies. Despite all the positive aspects of NDRG1 as a biomarker for bladder cancer mentioned above, there is a need for further investigations and research in the future which makes it not suitable for routine work yet.

Conclusions

Serum NDRG1 protein is an efficient and noninvasive tumor marker for the diagnosis of bladder cancer. Its levels were found in the patient's group to be significantly higher than in the healthy individual's group. The NDRG1 protein was tested by ELISA technique for the quantitative detection of NDRG1 protein by determining its concentration in serum. It is readily available and causes no patient discomfort or health complication.

Funding

This research received no external funding.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the College of the Medicine/ University of Baghdad. (protocol code 1573 and date of approval 2021/11/23).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest

The authors declare no conflict of interest.

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ARTICLE / INVESTIGACIÓN

Effect of adding and in ovo injecting hatching eggs produced with omega-3 on some hatching traits and body weight of Japanese quail

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Abstract: This study was conducted at a private hatchery in Thi-Qar Governorate to determine the effect of adding omega-3 fatty acid to quail's diet with the outcome of ovo injecting the resulting hatching eggs with omega-3 on some hatching traits and body weight of Japanese quail. A total of 540 eggs were used. The experiment treatments were as follows: T1: Negative control (without adding oil); T2: Positive control (adding 0.01% sunflower oil); T3: Feed the parent with 0.01% omega-3 oil; T4: Feed the parent with 0.01% omega-3 oil, and the resulting eggs were in ovo injected with 0.01 ml omega-3; T5: Feed the parent by 0.01% omega-3 oil, and the resultant eggs were in ovo injected with 0.01 ml sunflower; T6: Feed the parent by 0.01% sunflower oil, and the resulting eggs were in ovo injected with 0.01 ml sunflower; T7: Feed the parent by 0.01% sunflower oil, and the resultant eggs were in ovo injected with 0.01 ml omega-3; T8: Feed the parent by free diet, and the resulting eggs were in ovo injected with 0.01 ml omega-3; T9: Feed the parent by free diet, and the resultant eggs were in ovo injected with 0.01 ml sunflower. The results showed a significant improvement in T4 (the treatment whose parents were fed omega-3 and in ovo injected with omega-3 oil) compared to the control treatment on hatching rate and fertility rate of whole eggs, with a significant decrease in the percentage of embryonic mortality and pipped eggs for the hatched chicks. Feeding Japanese quail mothers with omega-3 hatching egg injections led to a substantial increase in the average weekly body weight.

Key words: In ovo injecting, hatching eggs, with omega-3, hatching traits, body weight, Japanese quail.

Introduction

The quail bird occupies a prominent position as one of the smallest types of domestic birds[1], for its contribution to meeting the needs of animal protein has unique characteristics that distinguish it from other domestic birds[2,3]; among these characteristics that determine the quail bird from other domestic birds, which made him popular for scientific research, including the short range of the generation, where the period of hatching ranges between 16-18 days, it takes up little space per unit area, small size, ease of handling, consumes less feed compared to chicken, considered an economic bird, it is a dual-purpose bird raised to produce eggs and meat, characterized by being a highly efficient biological machine in food conversion, its abundant production of eggs[4].

The fertilized egg is an isolated environment; it has characteristics that aim to produce new chicks when providing this environment with some nutrients by using the early feeding technique, which can improve the growth and development of fetuses, positively reflecting on the performance of chicks after hatching[5].

Recently, attention has focused on the process of injecting eggs with nutrients to provide the fetus with additional amounts of nutrients because the reserve of nutrients for the fetus decreases as it grows to reduce the stress that the chick is exposed to when hatching, supporting the development of the immune system of hatched chicks[6,7].

Including the use of one of the types of unsaturated fatty acids, especially omega-3, in ovo injection by air cell. Omega-3 acts as an energy source that supports the growth of fetuses and enhances the ability to digest and metabolize[8]. Omega-3 enhances the absorption of fat-soluble vitamins, increases the palatability of the feed materials and improves the utilization of the consumed energy; it also reduces the rate at which food passes through the gastrointestinal tract, leading to better absorption of nutrients[9].

The study aimed to determine the effect of adding omega-3 oil to quail's diet and injecting the resulting eggs with omega-3 on hatching traits and body weight.

Materials and methods

This study was conducted from 20/11/2021 to 11/12/2021 in a private hatchery affiliated with Thi-Qar Governorate. A total of 540 360 fertile eggs from Japanese quail parents was reared at the poultry field of the Department of Animal Production, College of Agriculture and Marshlands, Thi Qar University. The experiment treatments were as follows:

T1: Negative control (without adding oil).
T2: Positive control (adding 0.01% sunflower oil).
T3: Feed the parent by 0.01% omega-3 oil.
T4: Feed the parent by 0.01% omega-3 oil, and the resulting eggs were in ovo injected with 0.01 ml omega-3.
T5: Feed the parent by 0.01% omega-3 oil, and the resulting eggs were in ovo injected with 0.01 ml sunflower.

Citation: Al-Aboudi A S A, Shahid, M. J, Al-Gharawi, J.K. Effect of adding and in ovo injecting hatching eggs produced with omega-3 on some hatching traits and body weight of Japanese quail.Revis Bionatura 2022;7(4) 24. http://dx.doi.org/10.21931/RB/2022.07.04.24
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

T6: Feed the parent by 0.01% sunflower oil, and the resulting eggs were in ovo injected with 0.01 ml sunflower.

T7: Feed the parent by 0.01% sunflower oil, and the resulting eggs were in ovo injected with 0.01 ml omega-3.

T8: Feed the parent by free diet, and the resulting eggs were in ovo injected with 0.01 ml omega-3.

T9: Feed the parent by free diet, and the resulting eggs were in ovo injected with 0.01 ml sunflower.

The eggs were in ovo injected on day zero after sterilization with cotton and ethyl alcohol at a concentration of 70%. The injection hole was closed with nail polish, placed the eggs in the incubator; after setting the temperature at 37.7°C and humidity at 65%, the eggs hatched after 18 days of hatching and incubation. The following hatching traits were studied:

Fertility

The fertility was calculated after breaking the unhatched eggs and identifying dead embryos in each hatch, where the fertility and hatchability ratios were measured for each replicate, as indicated by Yoo and Wientjes[10] by the following equation:

Fertility=(Fertile eggs)/(Total eggs)×100

Hatchability of fertile eggs

The hatchability of fertilized eggs is calculated according to the following equation:

Hatchability of fertile eggs=(Hatched chick)/(Fertile egg)×100

Hatchability of whole eggs

The hatchability of total eggs was calculated, was calculated from the whole eggs after breaking the non-hatched eggs due to the difficulty of optically examining fertilized quail eggs because of the spotted eggshell color and according to the following equation[11].

Hatchability of fertile eggs=(Hatched chick)/(Total egg)×100

Pipped Eggs

All unhatched eggs were individually broken after the hatching period, and the number of pipped eggs was recorded, according to what was mentioned by Al-Zujaji and Ibrahim[12]. As in the following equation:

Pipped eggs perecetnt=(Pipped eggs)/(Fertile egg)×100

Embryonic mortality

Embryonic mortality was calculated for each of the experimental treatments according to the equation mentioned[13]:

Embryonic mortality=(Embryo dead)/(Fertile egg)×100

Average weekly live body weight

The birds were weighed weekly for each replicate of the experiment using a scale with a capacity of 50 kg.

Results

Table (1) indicates the effect of feeding Japanese quail parents. In ovo, injecting the resulting eggs with omega-3 fatty acid and sunflower oil on the fertility and hatchability of fertilized and total eggs, embryonic mortality, and pipped eggs of hatched chicks, the results indicate that there was a significant effect of (P≤0.01) of omega oil on the fertility, as the superiority of treatment T2, T3, T4, T5 and T6 compared to the rest of the treatments, its values were recorded as 93.36, 96.32, 96.26, 96.33 and 93.41%, followed by the treatments that significantly outperform T1 and T9, its values were recorded at 90.33 and 90.33%, respectively.

The results showed a highly significant (P≤0.01) for the hatching of whole eggs in the T3 treatment, it did not differ

Treatments	Fertility (%)	Total eggs hatchability (%)	Fertile eggs hatchability (%)	Embryonic mortality (%)	Pipped eggs (%)
T1	90.33±0.38 ^{b}	86.48±0.43 ^{c}	96.37±0.43 ^{a}	3.71±0.11 ^{c}	0.00±0.00 ^{e}
T2	93.36±0.55 ^{a}	82.97±1.38 ^{d}	89.17±0.44 ^{c}	3.53±0.12 ^{c}	7.24±0.55 ^{c}
T3	96.32±0.62 ^{a}	92.68±1.33 ^{a}	96.44±0.72 ^{a}	0.00±0.00 ^{d}	4.08±1.12 ^{d}
T4	96.26±0.57 ^{a}	90.40±0.42 ^{ab}	96.37±0.43 ^{a}	0.00±0.00 ^{d}	7.23±0.10 ^{c}
T5	96.33±0.61 ^{a}	89.66±1.45 ^{b}	96.37±0.66 ^{a}	0.00 ±0.00 ^{d}	6.79±0.15 ^{c}
T6	93.41±0.38 ^{a}	86.36±0.55 ^{c}	92.78±0.54 ^{b}	7.32±7.32 ^{b}	0.00±0.00 ^{e}
T7	86.48±0.72 ^{c}	76.54±0.79 ^{e}	88.27±0.54 ^{c}	0.00±0.00 ^{d}	7.66±0.20 ^{c}
T8	84.82±2.35 ^{c}	70.38±0.84 ^{f}	80.31±0.58 ^{d}	7.48±0.10 ^{b}	11.47±0.49 ^{b}
T9	90.33±0.38 ^{b}	66.52±0.77 ^{g}	74.35±0.50 ^{e}	11.28±0.51 ^{a}	14.80±0.11 ^{a}
Sig.	**	**	**	**	**

Table 1. Effect of Japanese quail parents feeding and the resulting eggs being in ovo injected with omega-3 fatty acid and sunflower oil on some hatching traits (mean ± standard error).

with treatment T4 followed by treatment T5 in comparison with the rest of the experimental treatments, the values of the therapy were 92.68, 90.40 and 89.66%, respectively.

The results of the hatchability percentage of fertilized eggs indicated a significant increase was observed in favor of treatment T1, T3, T4 and T5 compared to the rest of the experimental treatments; its values were recorded as 96.37, 96.44, 96.37, and 96.37%, respectively.

The results showed a significant superiority of the T6 treatment compared to the rest; its value was 92.78%.

The results of the table indicated the percentage of embryonic mortality, and there was a decrease in the rate of embryonic mortality for the treatments in which omega-3 oil has been added, while the results recorded a highly significant increase in treatment T9, its value was 11.28 compared to the rest of the experimental treatments.

Table (2) shows the effect of feeding Japanese quail mothers and the resulting eggs injected with omega-3 fatty acid and sunflower oil on the weekly body weight of the resulting chicks. There was a significant increase (P≤0.01) for treatments T4 and T5 compared to the positive and negative control treatments T1, T2 and the rest of the treatments, whose value reached 28.09 and 24.62 gm, respectively, for the first week of rearing. While there were no significant differences between the two control treatments and the rest of the treatments (T3, T6, T7, T8 and T9). While it was noted that there was a significant increase (P≤0.01 for treatment T4 compared with the negative and positive control treatments and the rest of the treatments, as its value reached 53.82 g and a significant increase for treatment T6 compared to treatments T2, T3 and T9 for the second week of life of the birds. In the third week of life, it was found that there was a significant increase For treatment T4 compared to the control treatment and the rest of the treatments the value was 91.02 g and a significant increase for treatments T5, T6 and T7 compared with the rest of the treatments and their value was 80.68, 82.05 and 1.418 g. While no significant difference was observed between the two control treatments and T3, T8 and T9, and their value was 78.28 and 76.26 and 76.70 g, respectively. It was found that there was a significant increase in treatment T6 compared with the control treatment, and the rest of the treatments were valued at 124.31 and did not differ with treatments T7, T8 and T9, and the values were 121.05, 121.44 and 118.70 gm, respectively, at the fourth week of bird life. It indicated a significant increase in T4 compared to the control treatment, and its value was 152.80 gm; it did not differ from T2, T3, and T7 values, which were 147.93, 148.75 and 148.78 gm, respectively, at the fifth week of life. While it was observed that there was a significant increase for treatments T4, T5, T6 and 8T compared with the control treatments and the rest of the treatments and a significant rise in the treatments, as the values of treatments were recorded as 231.71, 232.12, 229.67 and 231.25 gm, respectively. So, no significant difference was observed between the control and the rest of the treatments, T3, T4, T7, T8 and T9, in the sixth week of the age.

Discussion

The reason is that omega-3 oil contains unsaturated fatty acids, which have a role in increasing the rate of fertility[14].

As for the hatchability of the total eggs, it was observed that there is a significant superiority of the omega-3 treatment, a decrease in fetal mortality was also observed in favor of omega-3 treatment, because it is a fatty acid, and this agrees with Botsoglu *et al.*[15]. Omega contains a wide range of nutrients, minerals, chemical compounds, and vitamins, which has a positive role in improving production performance[16]. Thus, the egg's internal components were the main source of growth and embryonic development. Omega is an antioxidant because it contains many phenolic compounds, vitamins C and E.

The results showed a significant decrease in the percentage of pipped eggs for all treatments, a highly significant increase was recorded for the treatment T9, as its value was 14.80%; as for the characteristic of pipped eggs, a decrease in the percentage of captive eggs was observed with omega-3 treatment, the increased activity and vitality of chicks is due to the presence of unsaturated fatty acids, supportive of Growth[17,] which was the result of increased

Treatments	Bird age (week)					
	1	2	3	4	5	6
T1	23.53±^{c} 0.21	41.97±0.54 ^{cd}	76.87± ^{c} 0.20	115.78± ^{bc}3.12	144.12±0.29 ^{bc}	225.11±3.09 ^{b}
T2	23.51± ^{c} 0.25	41.11±0.06 ^{d}	76.17± 0.53 ^{c}	111.10±2.41 ^{cd}	147.93±^{ab} 1.13	208.93±0.32 ^{c}
T3	23.94±^{bc}0.19	40.10± 0.51 ^{d}	78.28±0.08 ^{c}	109.18±0.44 ^{cd}	148.75±^{ab}2.73	209.08±0.54 ^{c}
T4	28.09± ^{a} 0.29	53.82±2.56 ^{a}	91.02±0.12 ^{a}	122.45±0.90 ^{bc}	152.80±0.95 ^{a}	231.71±3.39 ^{a}
T5	24.62±^{b} 0.09	49.18±0.19 ^{b}	80.68±0.86 ^{b}	105.64± ^{d}0.76	141.60±2.12 ^{c}	232.12± ^{a} 0.60
T6	23.47±0.17 ^{c}	45.34±1.39 ^{c}	82.05± ^{b} 0.65	124.31±3.59 ^{a}	143.43±^{bc} 2.54	229.67±0.78 ^{ab}
T7	23.17±0.52 ^{c}	44.09±1.10 ^{cd}	81.41±0.88 ^{b}	121.05±2.17 ^{ab}	148.78±2.36 ^{ab}	224.89±3.20 ^{b}
T8	23.82±0.40 ^{bc}	42.63±1.55 ^{cd}	76.26±0.88 ^{c}	121.44±3.62 ^{ab}	146.51±1.70 ^{bc}	231.25±0.52 ^{ab}
T9	23.56±0.37 ^{c}	40.31±1.16 ^{d}	76.70±0.85 ^{c}	118.70±1.88 ^{ab}	142.72±1.44 ^{bc}	225.07±1.05 ^{b}
Sig.	**	**	**	**	**	**

Table 2. Effect of Japanese quail parents feeding and the resulting eggs being in ovo injected with omega-3 fatty acid and sunflower oil on body weight (mean ± standard error).

accumulation of glycogen stores in the liver in the last period of hatching, it gives the chick energy that enables it to make the hatching process faster[18]. Its effect may be attributed to the analysis of red blood cells or affect membrane permeability and gas exchange; thus, it disrupts the general biological system, which appeared with a high percentage of sucker eggs and dead pecking chicks[19].

The reason for the weight gain of the treatment to which omega-3 was added is due to the presence of unsaturated fatty acids, especially omega-3, which is present in cod liver oil and its addition to the diets of plant sources converts the carbohydrates and glycogen present in abundance in the diets of plant sources into glucose and transmitted by insulin, which binds With unsaturated fatty acids and converting them to triglycerides in the adipose tissue, thus achieving superiority in weight gain, as well as working to supply the chicks with fat-soluble vitamins that enter the composition of cells or due to a decrease in the speed of food passage through the alimentary canal and thus allowing food to be absorbed well as confirmed[20], agreed with Abdullah and Bahaa El Din[21] when adding olive oil and argan oil to the diet did not agree with Nobakht *et al.*(2011), as no significant differences were observed between the treatments to which the source of fat was added in the weight gain for broiler meat.

Conclusions

We conclude that omega-3 has an essential role in improving the hatching characteristics of Japanese quail; injecting hatching eggs with omega-3 improves the attributes of hatching, which is represented by an increase in the rate of hatching and a decrease in the percentage of embryonic deaths, which is reflected in a financial return on the hatchery owners, and thus they can be encouraged to use omega-3 injections for hatching eggs on a commercial scale. Injection of early hatching eggs for broilers with different percentages of omega-3 improved the final weight rate during the period of rearing, which is one of the indicators of improved health of birds.

Supplementary Materials

The following are available in this PDF, Table S1: Composition culture medium, Sheet 1 S2: Total cost, Sheet 2 S2: Stages of production, Sheet 3 S2: Direct and indirect labor, Sheet 4 S2: Culture medium, Sheet 5 S2: IMC, Sheet 6 S2: 6. Assumptions.

Author Contributions

Conceptualization, Ana María Henao Ramírez and Aura Inés Urrea Trujillo; methodology and software, Hernando David Palacio Hajduck and Ana María Henao Ramírez; validation anf formal analysis, Ana María Henao Ramírez; investigation, resources, data curation, writing—original draft prepara-tion, Ana María Henao Ramírez; writing—review and editing and supervision, Aura Inés Urrea Trujillo. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by General Royalties System - Science, Technology, and Innovation Fund with the Center of Agrobiotechnological Development and Innovation– CEDAIT- BPIN 2016000100060, National Planning Department, Office of the Governor of Antioquia, Universidad de Antioquia, Universidad Católica de Oriente and Compañía Nacional de Chocolates.

Acknowledgments

We would like to thank Laboratory of Plant Physiology and Plant Tissue Culture of the Universidad de Antioquia. A special acknowledgment to Universidad de Antioquia's Research Development Committee (CODI) and Granja Yariguíes – Compañia Nacional de Chocolates.

Conflicts of Interest

The authors declare no conflict of interest.

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ARTICLE / INVESTIGACIÓN

Impact of varying amounts of Moringa oleifera seed powder in the diet on a few aspects of common carp growth *L. Cyprinus carpio*

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DOI. 10.21931/RB/2022.07.04.25
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Abstract: The current study was conducted in experimental cages in a mud pond, First Agricultural Research and Experiment Station, Agriculture College, Al-Muthanna University, to determine the effect of different levels of Moringa oleifera seed powder on the diets of common carp. A total of 75 common carp fish with an average weight of 65.08±0.42 g were used; it was randomly distributed to 5 treatments with three replicates (5 fish for each replicate). The fish that were fed on experimental diets was divided into five equal therapies in terms of protein percentages, different in the proportions of adding Moringa seed powder; the rate of seeds added to the treatments was 0, 0.5, 1, 1.5 and 2%, respectively, the fish were fed on the experimental diets at 5% of the live weight, divided into four meals a day. The results showed a significant superiority of T2 and T3 treatments compared with other therapies on growth parameters (final weight, weight gain, daily growth rate, specific and relative), and give the best feed conversion ratio, the highest food conversion and protein efficiency ratio. Indicates that adding Moringa seed powder to diets at rates of 0.5 and 1% led to fish growth promotion and increased utilization of feed intake.

Key words: Moringa oleifera, growth parameters, common carp *Cyprinus carpio L*.

Introduction

The world faces significant challenges in increasing food production, eradicating famine, and achieving food security in developing countries and the poorest in the world, coinciding with the increasing population growth[1]. The world population is expected to reach 9.8 billion in 2050[2]. Aquaculture, one of the fastest growing and developing sectors of animal food production, plays an essential role in providing a cheap and continuous source of animal protein to meet human nutritional needs worldwide[3-5].

Fish farming is one of the developing food industries; despite the progress made in recent years, it suffers from many problems and obstacles that limit production and profits. It causes substantial economic losses, such as the manufacture of fish feed and its high cost, because feeding fish represents 60% of the fish production cost of fish farms[6]. Putting specialists in this field before a significant challenge in selecting feed ingredients should be suitable, available, naturally available, environmentally friendly and low cost[7]. Most traditional feed sources, whether plant or animal, such as fishmeal, soybeans, peanuts, etc., face competition in other uses such as direct human consumption or as feed for other animal species[8,9].

These problems and obstacles affecting the development and sustainability of fish farming have opened new perspectives for researchers to test and use other medicinal plants as less competitive alternatives to traditional and low-priced feed ingredients. It promotes growth and increases production. One of the most famous plants that have been used is Moringa oleifera; it is called the tree of life or the miracle tree because of its importance and versatility[10]. Foidl *et al.*[11] indicated that its original habitat is in the Himalayan regions of northwestern India; it grows in many tropical and subtropical countries. Their rapid growth and adaptability characterize Africa, Arabia and Southeast Asia to drought. Known to be of high nutritional value, every part of it can be used in different medical and industrial applications, such as the pharmaceutical industry or as food for humans and animals[12].

Moringa seeds are characterized by high nutritional value because of their high protein content, characterized by their range of important essential amino acids. They are also a good source of fats, such as unsaturated fatty acids. In addition to carbohydrates, crude fiber and essential minerals contain a mixture of critical active compounds, such as phenols, flavonoids, tannins and saponins[13]. Moringa seeds enter the diets of many animals, especially fish, as a functional protein and nutritional supplement or as substitutes for fishmeal or soybeans; it promotes growth and improves digestibility[14,15].

This study was conducted due to the scarcity of other studies related to Moringa seeds on its use in the diets of common carp *Cyprinus Carpio L.*, one of the most important, commercialized and consumed fish species in the world16. Hence the importance of shedding light on the knowledge of the effect of this plant on fish diets. The present study aims to show the impact of Moringa seed powder in the diets of common carp fish on some parameters of their growth.

Citation: Al-Rawashi, J.B.Z.; Salman, A.H.; Al-Gharawi, J.K. Effect of different levels of Moringa oleifera seed powder to the diet on some growth parameters of common carp Cyprinus carpio L. Revis Bionatura 2022;7(4) 25. http://dx.doi.org/10.21931/RB/2022.07.04.25
Received: 20 July 2022 / **Accepted:** 15 October 2022 / **Published:** 15 November 2022

Materials and methods

The experiment was conducted at the first agricultural research and experiment station, Umm Al-Akf area, Al-Muthanna Governorate, from 1/10/2120 to 20/12/2021, in dugout ponds, 45 m long, 35 m wide and 1.5 m deep, it was about 570 m away from the Euphrates River, Atshan river. The experiment used fish farming cages consisting of two rectangular pieces of wood, 244 cm long and 122 cm wide. Eight circles were drilled in each piece with a diameter of 45 cm in two parallel rows, and the circular holes in the wooden cages were filled by installing 15 clip-on plastic cylindrical troughs with a diameter of 45 cm and a depth of 65 cm.

A total of Seventy-five Common carp *Cyprinus carpio L.*, with an average weight of 65.08±0.42 g, were used, distributed randomly and evenly to the experimental cages (5 fish in each tank).

Moringa seeds were bought from local markets; after drying well by the sun, it was ground using a home grinding machine, then a sample was drawn from them to know their chemical composition (Table 1). Then it was added to other experimental diet components and distributed to treatments at rates 0, 0.5, 1, 1.5 and 2%, respectively (Table 2).

Content	Percent (%)
Humidity	1.62
Crude Protein	23.18
Ether extract	9.90
Crude fiber	22.82
Ash	3.54
Nitrogen Free Extract (NFE)	38.94
Total	100.00

Table 1. The chemical composition of Moringa seeds.

Field experience

The experiment lasted for 82 days with localization; five different experimental formulas were used for the proportions of adding Moringa seed powder. The five experimental diets were T1: as the control diet, T2: 0.5%, T3: 1%, T4: 1.5% and T5: 2% Moringa seed powder, with a crude protein percentage of 28% in all diets, the experimental fish were fed 5% of their live weight, served four meals a day, the amount of feed was adjusted according to the periodic weight of the fish every ten days, weighing was done for the experimental fish, using a sensitive scale 500 g type DIGITAL SCALE, after drying it with a cotton cloth.

Growth parameters

Total weight gain (WG): WG = F.W-I.W (FW: Final weight, IW: Initial weight)

Daily Growth Rate (DGR): = (W2 – W1)/(T2 – T1) D.G.R

(W1: Initial weight, W2: final w eight (second), T2–T1: The duration of the trial or between the two weights).

Relative Growth Rate (RGR): RGR=(W2-W1)/W1×100

Specific Growth Rate (SGR): SGR=(Ln W2-LnW1)/(T2-T1)×100

Feed Conversion Ratio (FCR): FCR=(R(gm))/(G (gm))

(R: weight of food intake (gm), G: weight gain of fish (gm)).

Feed conversion efficiency (FCE): FCE=G/R×100

Protein Efficiency Ratio (PER): PER=(T.W.G)/(P.I)

(TWG: total weight gain (kg), PI: protein intake (kg)).

Statistical analysis

Randomized Complete Design (CRD) was used to study the effect of treatments on the studied traits; significant differences between means were tested using Duncan's[17] multiple range test at a significance level of 0.05. The ready-made statistical program SPSS[18] was used to analyze the data.

Items	T1 Control	T2 0.5% Moringa seeds	T3 1.0% Moringa seeds	T4 1.5% Moringa seeds	T5 2.0% Moringa seeds
Concentrated protein	20.00	20.00	20.00	20.00	20.00
Soybean	35.00	35.00	35.00	35.00	35.00
Wheat bran	15.00	15.00	15.00	15.00	15.00
Maize	15.00	15.50	15.00	15.00	15.00
Barley	10.00	10.00	10.00	10.00	10.00
Flour	3.00	3.00	3.00	3.00	3.00
Oil	1.00	1.00	1.00	1.00	1.00
Premix	1.00	1.00	1.00	1.00	1.00
Moringa seeds powder*	0	0.50	1.00	1.50	2.00
Total	100.00	100.00	100.00	100.00	100.00
Chemical analysis					
Humidity (%)	Protein (%)	Ether extract (%)	Ash (%)	Fiber (%)	NFE (%)	Energy (kcal)
7.94	28.00	5.66	8.53	4.69	45.18	390.73

* Moringa seed powder was added to the rations in 5, 10, 15 and 20 g per 1 kg of the ration after raising an amount of the mixture equal to the amount added and according to each treatment.

Table 2. The composition of the diets in the experiment.

Results

Table (3) shows that there were significant differences (P≤0.05) between the experimental treatments in the studied growth criteria, the fish of T3 significantly outperformed compare the other experimental treatments in the final weight, the highest rate was recorded and reached 186.80 g, the highest rate of weight gain was 121.78 g, it also recorded the highest daily growth rate of 1.74 g/day. T2 and T3 recorded the highest relative growth rate of 187.29 and 180.86%, respectively, and they recorded the highest specific growth rate of 1.47 and 1.50% / day for T2 and T3, respectively. The fish of T2 and T3 outperformed compare with the other experimental treatments on the feed conversion ratio, which amounted to 2.39 and 2.42 gm of feed/gm of weight gain, respectively, it also outperformed the feed conversion efficiency, which amounted to 41.38and 41.91% for T2 and T3, respectively, T2 and T3 were superiority on the percentage of protein efficiency, with values of 1.49 and 1.47 gm of weight gain/gm of protein intake, respectively.

Discussion

The results in Table (3) show that the fishes of T2 and T3 treatments were superior in growth parameters (final weight, total weight gain, daily, relative and qualitative growth rates) compared to the other experimental treatments. The reason for this superiority and the positive results obtained may be due to the addition of Moringa at low and safe rates, have a clear impact on the growth parameters mentioned above, the chemical composition of Moringa seeds contains a good mix of essential amino acids important in the growth of the body, as well as being a good source of carbohydrates and raw fibers, it has a rich blend of vitamins such as vitamin C and E, and essential minerals such as calcium, phosphorous, magnesium and iron[13,19]. The high percentage of fat in it is one of the body's most important sources of energy, mainly unsaturated fatty acids, topped by oleic acid[20]. The results of the present study were close to the results of the study by Ayoola *et al.*[21]. These results were also in agreement with the findings of Yuangsoi and

Growth parameters	Treatments					Sig.
	T1	T2	T3	T4	T5	
Initial Weight (IW) (gm/fish)	65.250.34±	65.180.52±	65.020.29±	0.67±65.19	64.770.11±	NS
Final Weight (FW) (gm/fish)	±154.38 0.41 d	±183.05 1.63 b	±186.80 0.74 a	±158.78 0.83 c	153.91 1.002± d	*
Weight Gain (WG) (gm/fish)	0.65±89.13 d	±117.87 1.55 b	±121.78 0.50 a	1.11±93.58 c	89.140.88± d	*
Daily Growth Rate (DGR) (g/day)	0.010±1.27 d	0.023±1.68 b	0.006±1.74 a	0.015±1.34 c	1.270.012± d	*
Relative Growth Rate (RGR) (%)	±136.61 1.66 c	±180.86 2.81 a	±187.29 0.61 a	±143.60 2.94 b	137.611.12± bc	*
Specific Growth Rate (SGR) (%/day)	0.010±1.23 c	0.012±1.47 a	0.003±1.50 a	0.017±1.27 b	1.230.008± bc	*
Feed Conversion Ratio (FCR) (gm of feed/gm of weight gain)	0.026±2.92 c	0.030±2.42 a	0.030±2.39 a	0.040±2.80 b	2.850.14± bc	*
Feed conversion efficiency (FCE) (%)	±0.3034.26 c	±0.4941.38 a	±0.5541.91 a	±0.4935.71 b	±0.1935.02 bc	*
(PER) Protein Efficiency Ratio (gm gain/gm protein intake)	8±1.220.00 c	±1.470.018 a	±1.490.021 a	±1.270.017 b	±1.250.005 bc	*

* The different letters indicate the presence of significant differences within the same grade at the level of significance (P<0.05).
NS indicates no significant differences within the same grade at the level of significance (P<0.05).

Table 3. Some growth parameters (mean ± standard error) of fish for experimental treatments.

Masumoto[22] in their study of common carp, which showed that the addition of Moringa leaves at 2%, or 20 g/kg, to the diets did not positively or negatively affect growth and digestion parameters.

Fish in treatments T2 and T3 were superior by recording the highest significant difference (P<0.05) in feed conversion ratio, feed conversion efficiency and protein efficiency ratio compared to other experimental treatments. We believe that the inclusion of moringa seed powder in the experimental diets at rates of 0. 5, and 1%, was the best in terms of its content of adequate, balanced and varied concentrations of nutrients, such as amino acids and active sub-stances essential for fish growth and health. It also enhanced the work and activity of the fish digestive system, improving feed digestion by stimulating the action of protein and fat-digesting enzymes, such as proteases, pepsin and lipases. It increased the ability to absorb and assimilate nutrients by increasing the length of the villi inside the intestine[23]. Thus, this leads to an improvement in the efficiency of feed consumption and conversion by increasing body weight and growth, as well as raising the digestibility and efficiency of the protein[24], while the fifth treatment fish fed on a ration containing Moringa increased by 2%, the lowest values were obtained in the feed conversion ratio, feed conversion efficiency and protein efficiency ratio compared to the rest of the addition treatments, no significant differences were recorded between it and the control treatment, this may be due to the high levels of growth-inhibiting substances and anti-nutrients, with higher percentages of addition, as the presence of phytic acid in relatively high proportions, it has a direct effect on reducing the efficiency of the fish gut in splitting and digesting protein, thus, reducing the use of protein intake, during the formation of complexes with the protein as a result of its association with the enzyme trypsin, as well as the negative effect of phytic on reducing the availability and absorption of some nutrients necessary for growth[25,26]. We also believe that the reason is due to the lack of palatability of the feed due to the high concentration of saponins and alkaloids in diets containing relatively high percentages of Moringa seeds[27,28]. This irritates the lining of the mouth, leading to reduced appetite. In addition to the high fiber content in Moringa seeds, its presence at high rates hinders food consumption and digestion efficiency, creating a high bulk density within the intestines, and leading to reduced appetite[29]. The results of our current study were close to the findings of Akin-Obasola and Ajewole[30]; who showed that the addition of Moringa seed powder to African catfish rations in amounts of 6.25 and 9.37 g/kg of diet gave superiority in protein efficiency ratio and feed conversion ratio over the rest of the experimental treatments.

Conclusions

This study showed that Moringa seeds added to the diets of common carp fish at low rates, not exceeding 1.5%, had a positive and significant effect on all growth parameters studied in the experiment.

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ARTICLE / INVESTIGACIÓN

Effect of humic acid addition and spraying with ginger rhizome extract on the growth and some chemical contents of apricot seedlings *Prunus armeniaca L.* cv.

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DOI. 10.21931/RB/2022.07.04.26

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Abstract: This study was conducted at the slat house of the Department of Horticulture and Landscaping - College of Agriculture / Anbar University, during the growing season of 2021. The effect of soil application of humic acid (H) at 0, 4, 8 ml L^{-1} and ginger rhizome extract (Z) spraying at 0, 5, 10 g L^{-1} on some growth characteristics and some chemical contents of apricot seedlings, cultivar Hamawi was studied. Eighty-one two-year-old seedlings were selected that grafted on the stock of the apricot seed. A two-factor experiment (3 x 3) was carried out according to a randomized complete block design (RCBD). The investigation included nine treatments, three replications, and three seedlings for the experimental unit. The results indicated the significant effect of adding humic acid to seedlings in all the studied traits, especially the high-level H2 (8 ml L^{-1}), which achieved the best values for the traits (Branch number, Branch diameter, Branch dry matter, Leaf area, Nitrogen, Phosphor, Potassium). Treatment Z2 (10 g L^{-1}) of spraying with ginger rhizome extract was characterized by giving it the best significant effect of the characters (Branch number, Branch dry matter, Leaf area, Nitrogen, Phosphor, Potassium). The interaction of the study factors was significant for all the studied traits except for the diameter of the branch. The highest values were for the treatments H2Z2 (8 ml L^{-1} and 10 g L^{-1}) and H2Z1 (8 ml L^{-1} and 5 g L^{-1}), where the lowest values were in the control treatment (H0Z0) for all the studied traits.

Key words: Apricot, Humic acid, Ginger, Vegetative growth, Chemical content.

Introduction

Apricot *Prunus armeniaca L.* belongs to the Rosaceae family; it may also be called Armeniaca Vulgaris[1]. Some references indicate that Apricot trees originated in northern China, where it was planted 4000 years ago[2]. There are wild types of it, the cultivation of which extends from Japan to Afghanistan, and the Romans called it the Armenian apple. That is why some scholars believe that the apricot's origin is Armenia, which is why this name[3] is called it. The word Apricot goes back to the Greeks, called AL-Praecox, which means early fruit[4].

One of the most important positive aspects of the use of natural and manufactured organic fertilizers in fertilization is their great effectiveness in plant growth and development and that they do not cause damage to the environment since they do not add substances that the plant cannot use, and that is toxic to water and soil[5]. Humic acids are among the most essential manufactured organic fertilizers, which have proven their high efficiency in increasing the growth and production of plants because they contain organic compounds, amino acids and mineral elements[6]. Humic acid improves physical, chemical and biological soil properties[7]. It also contributes to increasing the permeability of cell membranes and the absorption of nutrients. In addition, it contributes to the activation of the formation of chlorophyll pigment and the assembly of sugars, amino acids and enzymes[8]. Activate cell division, increase growth rates, shoot and root system development, and increase dry matter in plant tissues[9,10]. It also increases the ability of plants to resist diseases, and it reduces the stresses resulting from high heat and salinity that cause poisoning, which gives the plant a kind of resistance, which reflects positively on the continuation of vital processes[11-13]. (14) and (15) reported that humic acid increases photosynthesis efficiency, synthesizes carbohydrates and proteins, and reduces the breakdown of amino acids caused by stress. Thus, these effects contribute to an increase in vegetative growth rates, which is positively reflected in the yield. Plant extracts are among the materials used recently to stimulate and encourage the vegetative and flowering growth of many plants as a source of nutrients and natural growth regulators, as well as being easy to absorb and contain effective substances and natural chemical compounds.

That differs according to the species and plant parts[16-20], in addition to its significant role in the biological control of many pests that infect plants, which contributes to improving vegetative growth and thus increasing production[21,22].

Ginger (*Zingiber officinale Roscoe*) belongs to Zingiberaceae and the genus Zingiber. It is a perennial herb that is multiplied by rhizomes[23]. It is a medicinal plant that grows in hot areas, is used as a stimulant and a gas repellent, increases sweating, hypnotic, anti-emetic, cough suppressant, and is anti-inflammatory. Recent studies have also proven that it acts as an anti-cancer and is added to pickles, pastries and various foods[24,25]. Ginger contains volatile oil with

Citation: Hardan M E, Al-Dulaimy A F Z. Effect of humic acid addition and spraying with ginger rhizome extract on the growth and some chemical contents of apricot seedlings Prunus armeniaca L . cv. Revis Bionatura 2022;7(4) 26. http://dx.doi.org/10.21931/RB/2022.07.04.26
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

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a pungent odor and a pungent taste that includes its main compounds (Geraniol, Neral, Curcumene, Zingiberene, Zingeberol, Linallol, beta-phelanddrine and Camphor-D). It also contains the Aryl alkanes group, and the most important compounds of this group are Gingerols, which includes gingenol, the compound to which the spicy taste is attributed[26,27]. The researchers pointed to the influential role played by the extract of ginger rhizomes when sprayed on the shoots of plants in improving the vegetative growth of these plants[28-30]. Researcher[31] explained that each 100 g of ginger rhizome contains (Moisture 15.02%, Protein 5.087 g, Fat 3.72 g, soluble fiber 23.5%, In Soluble fiber 25.5%, Carbohydrate 38.35 g, Vitamin C 9.33 mg, Total carotenoids 79 mg, Ash 3.85 g, Calcium 88.4 mg, Phosphorous 174 mg, Iron 8.0 mg, Zinc 0.92 mg, Copper 0.545 and Manganese 9.13 mg).

Despite the efficiency of chemical fertilizers in improving plant growth, it has been scientifically proven that these substances are dangerous to the environment and human health. Hence, the current agricultural policy seeks to provide nutrients to improve plant growth, not harm the environment, and increase plants' tolerance of unsuitable environmental conditions[32]. Many nutrient solutions were used for development to achieve these goals, including amino acids, organic acids, plant hormones, and others. They have proven their efficiency in improving the growth and productivity of various horticultural plants[33,34].

Due to the above-mentioned and as a result of the lack of studies on the effect of both manufactured organic fertilizers and plant extracts, especially on seedlings grown in the conditions of Anbar Governorate, we decided to implement this study to investigate the effect of humic acid addition and spraying with ginger rhizome extract on some vegetative growth characteristics and some chemical contents of apricot seedlings cv. Hamawi produces vigorous seedlings with a solid structure, especially in the first years of cultivation, and the possibility of reaching the production stage early, since seedlings in the first years of their life deplete large amounts of nutrients that are used in their various vital processes. Also, both factors of the study are considered environmentally friendly materials preferred to be used in plant nutrition, especially after discovering the harmful effect of using chemical fertilizers in agriculture, both on the environment and human health.

Materials and methods

The study was conducted on Apricot seedlings cv in the lath house of the Department of Horticulture and Landscaping - College of Agriculture / University of Anbar during the growing season 2021. Hamawi, to study the effect of humic acid application and spraying with ginger rhizome extract on some vegetative growth characteristics and chemical content of seedlings. Eighty-one of the two-year-old seedlings were selected. The field operations of pest control and irrigation (drip irrigation) were conducted equally for all the treatments under study. A two-factor experiment (3 x 3) was carried out according to a randomized complete block design (RCBD). The investigation included nine treatments, three replications, and three seedlings for each experimental unit. Thus, the total number of seedlings used in the experiment was 81. In the experiment, two factors were used, the first was the addition of humic acid (0, 4, 8 ml L^{-1}) two times (1/4 and 1/6), and the above concentrations were symbolized by the symbols (H0, H1 and H2) respectively, the second factor, spraying with ginger rhizome extract in concentrations (0, 5, 10 g l^{-1}) and spraying time (1/4, 1/5, 1/6, 1/7, 1/8 and 1/10). The above extract concentrations were symbolized by the symbols (Z0, Z1 and Z2) respectively. The studied traits were: branch number (branch $plant^{-1}$), brunch diameter (mm), dry brunch matter (%), leaf area (cm^2), percentage of Nitrogen phosphor and potassium. The data were statistically analyzed, the averages were compared using the least significant difference (LSD) test at a probability level of 5%[35], and the Genstat V12.1 software did the analysis.

Results

Branch number (branch $plant^{-1}$)

The application of humic acid had a substantial impact on the rate of branch number increase (Figure 1A), particularly in treatment H2, which differed significantly from the other two treatments, H0 and H1 and yielded the highest value of 18.03 branch $plant^{-1}$. Treatment H0 had the lowest value, 13.92 branch $plant^{-1}$. The results, on the other hand, showed that spraying with ginger rhizome extract had a significant effect on the studied trait, particularly the treatment Z2, which showed a substantial difference from treatment Z0 and Z1 and achieved the highest rate of increase in branch number 17.23 branch $plant^{-1}$. At the same time, treatment Z0 had the lowest rate, 14.31 branch $plant^{-1}$. The significant effect of the interaction of the study factors followed the same path, especially for treatment H2Z2, which achieved the most critical value of 21.24 branch $plant^{-1}$. In comparison, the control treatment H0Z0 achieved the lowest value of 12.72 branch $plant^{-1}$(Table 1).

Branch diameter (mm)

The results show that the application of humic acid to apricot seedlings increased the diameter of the branch significantly, reaching a maximum of 5.79 mm for treatment H2 (Figure 1B), which differed considerably from the lowest value of 4.62 mm for treatment H0. On the other hand, spraying with ginger rhizome extract and both factors' interaction hasn't shown a significant effect in the studied trait (Table 1).

Branch dry matter (%)

The results show that the application of humic acid to apricot seedlings increased the dry matter of the branch significantly (Figure 1C), with the treatment H2 having the most significant values of 63.59% and being substantially better than treatment (H0), which had the lowest value of 51.87%. Spraying ginger rhizome extract had a substantial effect; two treatments Z2 and Z1 generated the most outstanding discounts of 60.12 and 59.51%, correspondingly, and they differed significantly from treatment Z0, which gave the lowest value of 52.97%. The interaction of the study factors had a significant effect, particularly with treatment H2Z1, which resulted in the maximum rate of increase in stem diameter of 68.75%, while the value fell to the lowest level of 48.23% when the control H0Z0 was used (Table 1). Leaf area (cm^2)

The results show that the application of humic acid to apricot seedlings increased the leaf area significantly (Figure 1D), particularly for treatment H2, which differed considerably from the other two treatments, H0 and H1 and had the highest value of 15.38 cm2. In contrast, treatment H0 had

the lowest value of 9.12cm^2. Spraying with ginger rhizome extract had a substantial effect, with the treatments Z2 and Z1 producing the maximum value of 13.94 and 13.10 cm^2, a significant difference from the treatment Z0, which made the lowest value of 9.82 cm2. The interaction of the study factors had a substantial impact, particularly with treatment H2Z1, which had the maximum value of 17.14 cm^2, while the control treatment achieved the lowest value of 7.83 cm^2 H0Z0 (Table 1).

Nitrogen (%)

The addition of humic acid had a substantial impact on the rate of Nitrogen percentage (Figure 2A), particularly in treatment H2, which differed significantly from the other two treatments, H0 and H1 and yielded the highest value of 1.94%. Treatment H0 had the lowest value of 1.79%. Spraying ginger rhizome extract had a substantial effect; the treatment Z2 had the maximum value of 1.92%, significantly different from the treatment Z0, which had the lowest value of 1.78%. The interaction of the study components had a significant effect, particularly with treatment H2Z1, which had a maximum weight of 1.97%. In comparison, the percentage fell to the lowest in the control treatment H0Z0, 1.62% (Table 2).

Phosphorus (%)

The results demonstrated that when applied to apricot seedlings, humic acid contributed to substantial changes in phosphorus percentage. Treatment H2 had the highest value of 0.46% compared with the other two treatments, H0 and H1. On the other hand, it had the lowest value of the examined characteristic (Figure 2B). Spraying with ginger rhizome extract Z2 was much better than Z0 and Z1 treatments, with the most significant value of 0.45% and the lowest value of 0.37%. The interaction of the study factors had a substantial effect, especially for treatment H2Z2, which had the maximum percentage of phosphorus at 0.51%. In comparison, the value dropped to its lowest level of 0.33% for the control treatment H0Z0 (Table 2).

Potassium (%)

The results in Figure 2C show that adding humic acid to apricot seedlings significantly increased the potassium content of leaves, particularly treatment H2, which showed a significant difference from treatment H0 and H1 and achieved the highest percentage of 1.39%. While the treatment H0 had the lowest rate, 1.29%. Spraying ginger rhizome extract had a substantial influence on the examined cha-

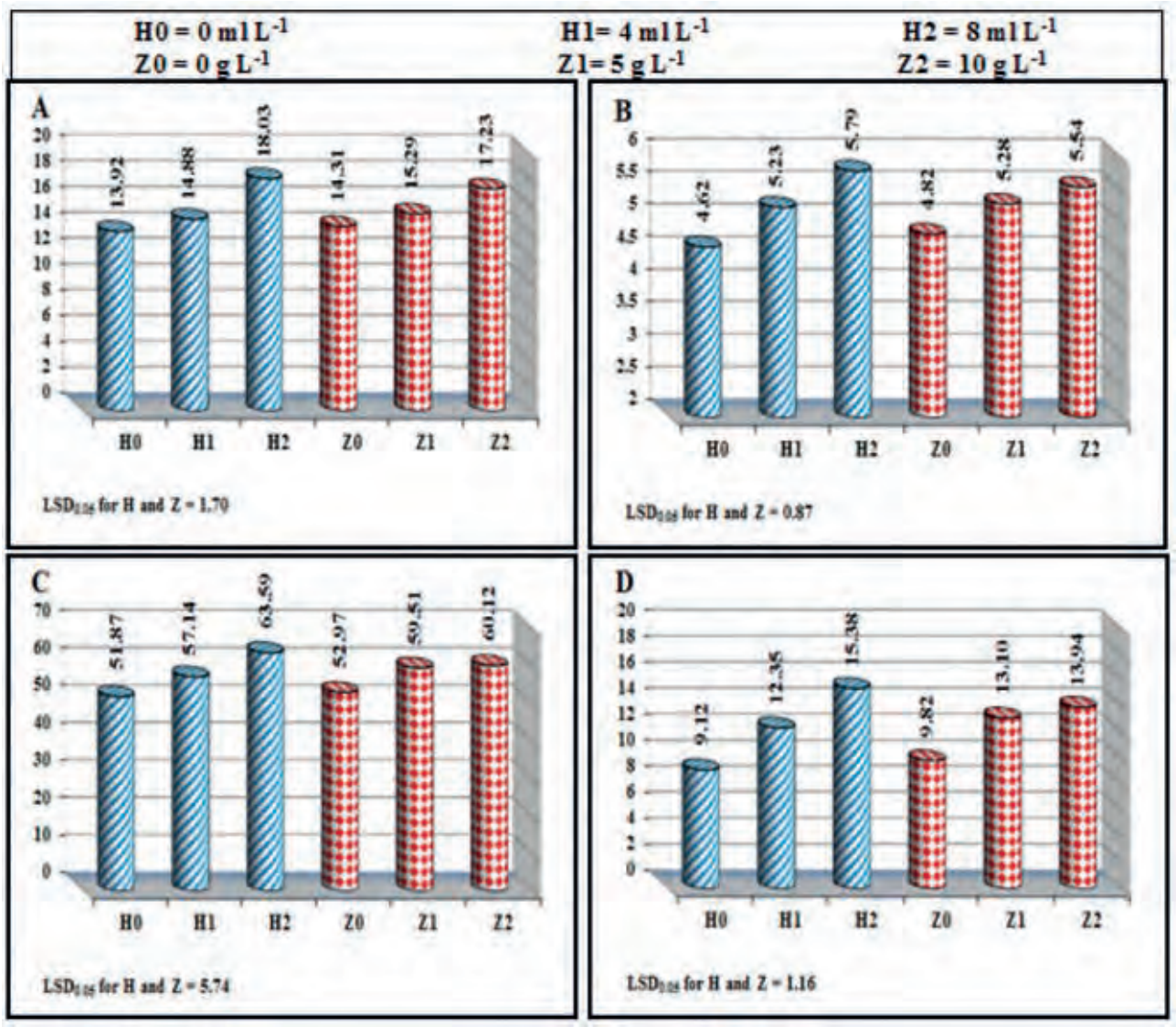

Figure 1. Effect of humic acid application and spraying with extract of ginger rhizomes on some vegetative growth traits of Apricot seedlings cv. Hamawi (A: Branch number, B: Branch diameter, C: Branch dry matter, D: Leaf area).

racteristic, as evidenced by the considerable superiority of treatment Z2 over non-spray treatment Z0, and it yielded the highest percentage of 1.38%, compared with treatments Z0 and Z1, which caused the lowest value of 1.31%. The interaction of the study components had a substantial effect, especially for treatment H2Z2, which gave the highest potassium content of 1.47%, while the value for the control treatment H0Z0 was 1.23% (Table 2).

Humic acid (ml L^{-1})	Extract of ginger rhizomes (g L^{-1})	Branch number (branch $plant^{-1}$)	Branch diameter (cm)	Branch dry matter (%)	Leaf area (cm^2)
0	0	12.72	4.46	48.23	7.83
	0	14.87	4.54	56.91	8.48
	10	14.16	4.87	50.47	11.05
4	0	12.90	5.17	57.36	9.12
	5	15.46	5.08	52.87	13.67
	10	16.29	5.44	61.18	14.26
8	0	17.31	4.82	53.30	12.51
	5	15.53	6.23	68.75	17.14
	10	21.24	6.31	68.72	16.50
LSD 5%		2.94	NS	9.93	2.00

Table 1. Effect of humic acid application and spraying with extract of ginger rhizomes interaction on some vegetative growth traits of Apricot seedlings cv. Hamawi.

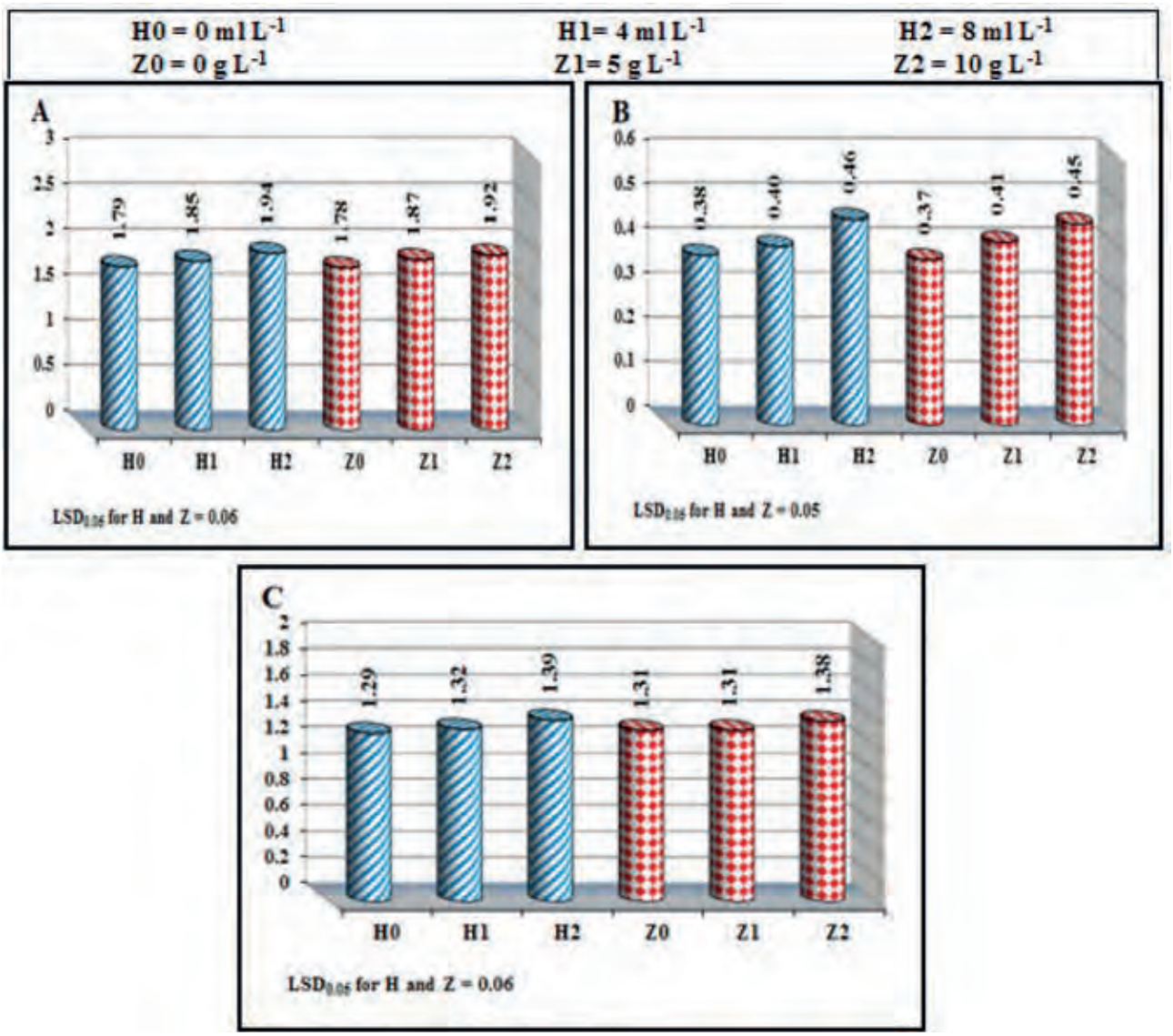

Figure 2. Effect of humic acid application and spraying with extract of ginger rhizomes on (Nitrogen, Phosphorand potassium) in leaves of Apricot seedlings cv. Hamawi. (A: Nitrogen, B: Phosphor, C: Potassium).

Humic acid (ml L^{-1})	Extract of ginger rhizomes (g L^{-1})	Nitrogen (%)	Phosphor (%)	Potassium (%)
0	0	1.62	0.33	1.23
	0	1.84	0.36	1.35
	10	1.91	0.44	1.30
4	0	1.83	0.42	1.32
	5	1.80	0.38	1.26
	10	1.92	0.40	1.38
8	0	1.89	0.37	1.37
	5	1.97	0.49	1.32
	10	1.94	0.51	1.47
LSD 5%		0.10	0.09	0.07

Table 2. Effect of humic acid application and spraying with extract of ginger rhizomes interaction on (Nitrogen, Phosphor and potassium) in leaves of Apricot seedlings Cv. Hamawi.

Discussion

The addition of humic acid significantly affected all vegetative characteristics and chemicals under the study of apricot seedlings (Cv. Hamawi); the reasons for this may be attributed to its role in increasing the leaf area (Figure 2D), which is positively reflected in the increase in the content of chlorophyll in leaves and thus the production of larger quantities of carbohydrates. Humic acid also contributes to the rise in the development of chlorophyll and the assembly of sugars, amino acids and enzymes[36]. It also has a similar role to auxins in increasing cell division, improving root system development, and increasing the amount of dry matter, which encourages plant growth and improves vegetative growth[37]. In addition, humic acid contributes to an increase in the formation of energy-rich compounds (ATP) and the formation of proteins within plant tissues[38]. Humic acid also plays a positive role through its content of organic acids and nutrients that contribute to plant growth[39]. Humic acid also plays an essential role in improving soil properties, root growth, increasing microbial community activity and increasing soil water retention[40,41], as well as its role in improving the chemical and physical properties of the growth medium and expanding the availability of nutrients and thus increasing plant growth[42,43]. Humic acid also contributes to increasing plant resistance to pest infestation[44]. It is also a complementary source of polyphenol, which acts as a respiratory chemical medium. This, in turn, leads to an increase in the biological activity of the plant, as the enzymatic system increases in activity and dry matter production increases[45,46].

The positive effects of spraying with ginger rhizome extract, in improving the features of vegetative growth and increasing the chemical content of seedlings, can be attributed to its role in increasing the leaf area and maybe the chlorophyll content in the leaves. This significantly impacts the manufacture of carbohydrates in the process of photosynthesis, which is used to supply the energy needed for vital processes taking place within plants. As well as the extracted content of macro- and micronutrients, vitamins, proteins and carbohydrates have an effective role in activating various physiological processes within plants[47].

Conclusions

After reviewing the study results, it can be concluded that apricot seedlings, cv. Hamawi responded by applying humic acid and spraying with ginger rhizome extract, especially at the high concentrations. Therefore, we recommend fertilizing apricot seedlings with both factors of the study, as improving the vegetative growth of plants depends primarily on balanced nutrition. That hunger has a detrimental effect on the development of seedlings.

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ARTICLE / INVESTIGACIÓN

Evaluation of the water quality of the Sirwan River in the Garmian region for irrigation purposes using the Irrigation Water Quality Guidelines (IWQG)

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DOI. 10.21931/RB/2022.07.04.27

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Abstract: The water quality index (WQI) is one of the simplest ways of converting complex water quality data into an individual value that expresses the state of water quality. The present study aims to assess and classify the quality of water in the Sirwan River within Garmian Region for irrigation uses through using the Irrigation Water Quality Guideline (IWQG). The IWQG determines the risks of soil salinity and sodicity as well as the risks of water toxicity to various types of crops. The water samples were collected from (24) sampling stations in the Sirwan River downstream of Darbandikhan Dam and Jalawlaa Sub-district in December 2021. All the samples were analyzed in terms of physicochemical parameters, including (Ca^{+2}), (Mg^{+2}), (Na^{+}), (HCO_3^{-}), (Cl^{-}) and (EC). The results indicated that the (IWQI) values ranged from (42.34) to (56.70) with an average of (53.7), and most of the stations fall within the high restriction category. This indicates that the water quality of Sirwan River is suited for plants with moderate to high salt tolerance, and it can be used in high permeability soils. Salinity control practices should be implemented, except in water that contains low concentrations of (Na^{+}, Cl^{-} and HCO_3^{-}).

Key words: Irrigation, Irrigation Water Quality Guideline (IWQG), Sirwan River, Garmian Region, Restrictions.

Introduction

Water is considered the most invaluable natural resource, and it plays an indispensable role in the existence of life on our planet. Global pollution has increased due to increasing population, urbanization, industrialization and traffic. So, there is a need for accurate monitoring, database and information regarding water quality. The condition of the water is described by the term water quality, which includes the biological, physical and chemical characteristics regarding its suitability for different purposes.

Domestic waste is responsible for approximately 80% of water pollution in developing countries[1]. Also, mismanagement of water resources causes significant issues in the quantity and quality of water[2]. The quality of water resources has been deteriorating because of natural and anthropogenic activities. The anthropogenic factors include mining, waste disposal, animal production and agricultural processes, and fertilizers applications[3,4]. It is significant to study surface water bodies in terms of quality. Surface water worldwide is used for different purposes such as drinking, irrigation and industrial needs. Water quality monitoring is a significant part of the water management process. Also, data analysis is essential to identify and describe water quality problems. Moreover, the water quality monitoring process by the water quality index (WQI) is regarded as the foundation of water quality management[5].

Researchers in the past years proposed many irrigation water quality guidelines and classifications. These guidelines are essential; none have been satisfactory due to the variety of field conditions. Also, each does not give a comprehensive water quality in the rivers or reservoirs. A practical solution to this issue is utilizing an index that automatically combines all water quality parameters and provides an understood and readily description of water. The water quality index is considered a straightforward and effective tool for evaluating water quality by combining different parameters[6,7]. Thus, it converts the parameters to a single value which ultimately expresses the condition of water quality[8]. The water quality index has been used widely to evaluate different water sources, including surface and groundwater quality in various areas[9,10]. The concept of WQI relies on the comparison of parameters with standard values[11]. The primary aim of this study is to apply Irrigation Water Quality Guideline (IWQG) to the Sirwan River to assess its quality for irrigation uses. The IWQG has been developed recently to evaluate the quality of Iraqi water resources and help Iraqi people properly manage water resources.

Materials and methods

Study area

The Sirwan River is considered one of the main tributaries of the Tigris River, and it covers a distance of more than 445 km. The study area includes a distance of 80 km, starting from the downstream of Darbandikhan Dam to Jalawlaa Sub-district and passing through Kalar City, one of the largest districts in Sulaymaniyah Governorate. Twenty-four sampling stations were selected along the study area, as shown in figure (1).

Citation: Sarhat A R, Al-Obaidi B S. Evaluation of the water quality of the Sirwan River in the Garmian region for irrigation purposes using the Irrigation Water Quality Guidelines (IWQG). Revis Bionatura 2022;7(4) 27. http://dx.doi.org/10.21931/RB/2022.07.04.27
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

Publisher's Note: Bionatura stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

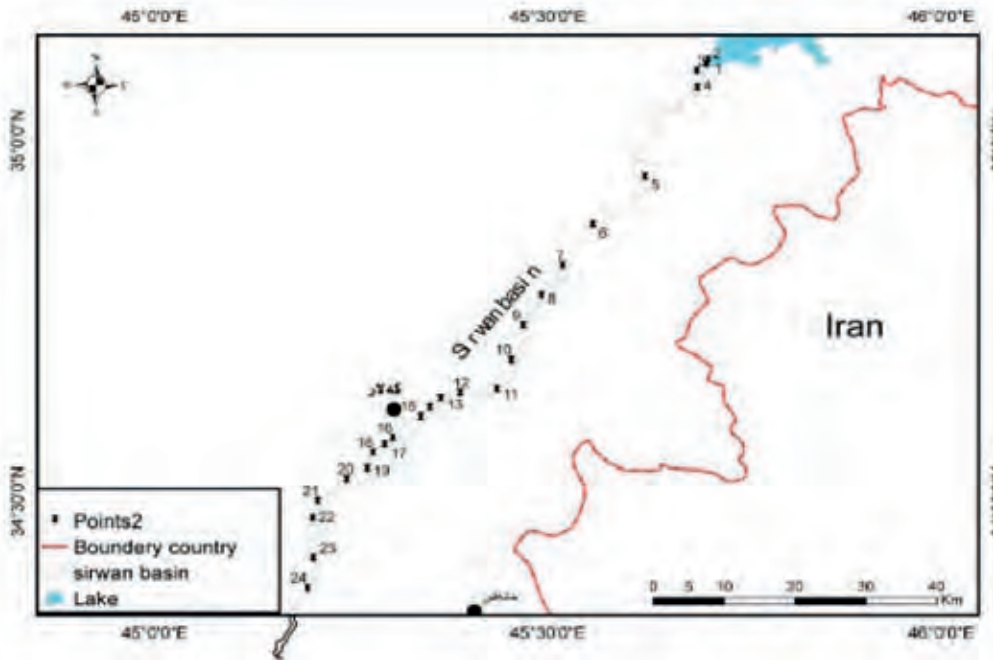

Figure 1. Sampling stations in the study area.

Sample collection and analysis

The samples were collected from each mentioned station in figure (1) in December 2021 in 1-L Polyethylene bottles, which were rinsed several times before filling. The pH and EC were immediately measured on-site using a portable pH meter and WTW conductometer. Some anions, including sulfite (SO_4^{-2}) and chloride (Cl^-), were measured by ion selective electrodes (SENTEK) after 24 hours from the date of sample collection; however, (HCO_3^-) was measured by titration. The cations (Ca^{+2}, Mg^{+2}, K^+ and Na^+) were measured using induced coupled plasma optical emission spectroscopy (ICPOES). The values of cations and anions were converted by the (IWQG) into some indices to evaluate water quality for irrigation uses.

IWQG software

Irrigation Water Quality Guidelines (IWQG) is a program that is set to assess water for irrigation uses according to the typical ranges of parameters and indexes according to FAO standard 1994[12] as shown in table (1). It was developed in 2014 at The National Center for Water Resources Management and was lately approved by the Ministry of Water Resources[13,14]. This program calculates water quality indicators for irrigation, as shown in table (2).

Applying the irrigation water quality index (IWQG) includes two main steps. The first is the statistical method or analysis of the main parameters, including HCO_3^-, Cl^-, Na^+, EC and SAR as the most critical indicators for water quality assessment.

The other step is allocating the sub-index quality (Qi) and the weight (Wi) for each parameter (ith). The (Wi) values were determined according to the values of the parameters in the studied area and the criteria according to Ayers and Westcot (1994), as shown in table (3). The total summation of (Wi) values must equal (1). The following equation was applied to calculate the values of (Qi) based on the tolerance limits shown in table (4)[24].

$$Qi = qi\ max - [(Xij - Xinf) * qiamp / Xamp] \quad (I)$$

Where; (Qimax) is each category's maximum value of (qi). (Xij) is the monitored value of the parameter, and (Xinf) is the minimum value of the same parameter's category.

Parameters	Unit	Normal Ranges
Electric Conductivity (ECw)	$dS.m^{-1}$	0 – 3
Total Dissolved Solids (TDS)	$mg.l^{-1}$	0 – 2000
Calcium (Ca^{+2})	$mg.l^{-1}$	0 – 20
Magnesium (Mg^{+2})	$mg.l^{-1}$	0 – 5
Sodium (Na^+)	$mg.l^{-1}$	0 – 40
Carbonate (CO_3^-)	$mg.l^{-1}$	0 - 0.1
Bicarbonate (HCO_3^-)	$mg.l^{-1}$	0 – 10
Chloride (Cl^-)	$mg.l^{-1}$	0 – 30
Sulphate (SO_4^{-2})	$mg.l^{-1}$	0 – 20
Potassium (K^+)	$mg.l^{-1}$	0 – 2
Sodium Adsorption Ratio (SAR)	$Meq.l^{-1}$	0 – 15
Potential Hydrogen (pH)		6.5 - 8.5
Parameters	Unit	Normal Ranges

Table 1. Normal ranges of chemical parameters in irrigation water [15]

Index	Equation	Unit	References
Sodium Adsorption Ratio SAR	SAR= $Na/\sqrt{Ca+Mg/2}$	meq.l^{-1}	[15]
Adjusted Sodium Adsorption Ratio adj.SAR	$adj.SAR=(Na/\sqrt{Ca+Mg/2})*\{1+(8.4+PHc\}$	meq.l^{-1}	[15]
Sodium Percentage Na%	Na%=$(Na*100)/(Ca+Mg+Na+K)$	meq.l^{-1}	[16]
Potential Salinity	$PS= Cl-+0.5*SO4-2$	meq.l^{-1}	[17]
Permeability Index (PI)	$PI=(Na+\sqrt{HCO3})x100/(Ca+Mg+Na)$	meq.l^{-1}	[17]
Kelley Index (KI)	$KR= Na/(Ca+Mg)$ KR values =<1 indicate suitability for irrigation; However, KR values >1 indicate unsuitability for irrigation uses.	meq.l^{-1}	[18], [19]
Magnesium hazard (MH)	MH=$(Mg/Ca+Mg)\times100$ Values of MH =<50 indicate suitability for irrigation; However, MH values >50 indicate unsuitability for irrigation uses.	meq.l^{-1}	[20]
Soluble sodium percentage (SSP)	SSP= $((Na+k)*100)/(Na+Ca+K+Mg)$	meq.l^{-1}	[21]
Residual Sodium Carbonate (RSC)	RSC=$(CO_3^{-2}+HCO_3^-)$ - $(Ca^{+2}+Mg^{+2})$	meq.l^{-1}	[22], [23]

Table 2. Irrigation water quality indexes.

Qi	EC (µs/cm)	SAR	Na^+	Cl^-	HCO_3^-
			meq/l		
0 – 35	EC < 200 or	SAR < 2 or	Na+ < 2 or	Cl− <1 or	HCO3− < 1 or
35 – 60	EC ≥ 3000	SAR ≥ 12	Na+ ≥ 9	Cl− ≥ 10	HCO3− ≥ 8.5
60 – 85	1500 ≤ EC < 3000	6 ≤ SAR < 12	6 ≤ Na+ < 9	7 ≤ Cl− < 10	4.5 ≤ HCO3− < 8.5
85 – 100	750 ≤EC < 1500	3 ≤ SAR < 6	3 ≤ Na+ < 6	4 ≤ Cl− < 7	1.5 ≤ HCO3− < 4.5
Wi	200 ≤ EC < 750	2 ≤ SAR < 3	2 ≤ Na+ < 3	1 ≤ Cl− < 4	1 ≤ HCO3− < 1.5

Table 3. The values of sub-index quality (Qi) and their weights (Wi) to calculate (IWQG), EC in (µs/cm) and all others in (meq.l^{-1}) [24].

Potential issue of irrigation		Restriction Degree		
		None	Slight to Moderate	Severe
Salinity	EC(ds.m^{-1}) at 25 ◦C	< 0.7	0.7 – 3	> 3
	TDS(mg.l^{-1})	< 450	450 – 2000	> 2000
Sodicity	SAR = 0 - 3 and EC	> 0.7	0.7 - 0.2	< 0.2
	SAR = 3 - 6 and EC	> 1.2	1.2 - 0.3	< 0.3
	SAR = 6 - 12 and EC	> 1.9	1.9 - 0.5	< 0.5
	SAR = 12 - 20 and EC	> 2.9	2.9 - 1.3	< 1.3
	SAR = 20 - 40 and EC	> 5	5 - 2.9	< 2.9
SAR		< 3	3 – 9	> 9
Chloride(Cl)(meq.l^{-1})		< 4	4 – 10	> 10
HCO_3(meq.l^{-1})		< 1.5	1.5-8.5	> 8.5
pH			6.5 - 8.5	

Table 4. The characteristics of water quality for irrigation [15]

(Qiamp) is the normal range of the category. (Xamp) is the normal range of each parameter within a category.

Then, the final step is calculating the (IWQI) as the following equation. The (IWQI) is ranged between (0-100), and its value is unitless.

$$IWQI = \sum_{i=1}^{n} W_i * Q_i \quad \text{(II)}$$

The IWQI values indicate the acceptability of the water for irrigation uses, and its values are divided into five categories, as shown in table (5). The program calculates the ionic accuracy between cations and anions, and the values should not exceed 5%[25].

I

This is only suitable for plants with high salt tolerance, except for water that contains low concentrations of (Na, Cl and HCO_3).

It must not be used for irrigation in normal conditions. However, it can be used occasionally in some exceptional cases. Gypsum application is required for water with low salt concentrations and high SAR values. This type of water must be used only in high-permeability soils. To avoid salt accumulation, excess amounts of water should be applied.

IWQI value	Restriction type	Recommendations	
		Types of plants	Soil
85 - 100	No Restriction (NR)	It is suitable for plants, and there is no risk of toxicity.	It is recommended to be used for most types of soil with a low chance of causing sodicity and salinity issues. Also, It is recommended to be used for leaching within irrigation processes, except for deficient permeability soils.
70 - 85	Low Restriction (LR)	Suitable for most plants except salt-sensitive plants.	It is recommended to be used in light texture soils or moderate permeability, and it can be used for salt leaching. It can cause sodicity, especially in soils with heavy texture. So, it should not be used in soils with high clay content.
55 - 70	Moderate Restriction (MR)	Suitable for Plants that have moderate tolerance to salts.	It could be used in moderate to high permeability soils. It is recommended to be used for medium leaching of salts.
40 - 55	High Restriction (HR)	Suitable for Plants that have moderate to high tolerance to salts. Also, salinity control practices should be implemented, except in water that contains low concentrations of (Na, Cl and HCO_3).	It can be used in high-permeability soils. For water with EC > 2000 dS/m and SAR > 7, a schedule of frequency irrigation should be implemented.
0 - 40	Severe Restriction (SR)	This is only suitable for plants with high salt tolerance, except for water that contains low concentrations of (Na, Cl and HCO_3).	It must not be used for irrigation in normal conditions. However, it can be used occasionally in some exceptional cases. Gypsum application is required for water with low salt concentrations and high SAR values. This type of water must be used only in high-permeability soils. To avoid salt accumulation, excess amounts of water should be applied.

Table 5. Categories and characteristics of IWQG

Results and discussion

The results of the studied chemical parameters are shown below in table (6) and figure (2).

Potential Hydrogen (pH) is an essential factor that works as an indicator of contamination. Changing the pH of water increases the pollutants' toxicity; therefore, measuring the pH of water is very important. The values of pH in the study area varied between (7.19) (and 8.37) with a mean of (7.89).) Based on FAO standards, the acceptable limit of pH value for irrigation water is set between (6.5) (and 8.5). The results were found to be approximately neutral to slightly alkaline.

The results show that the electrical conductivity (EC) values of the Sirwan River ranged between (0.042) to (0.68) $ds.m^{-1}$. The lowest value of (EC) is at location (1), wherever the river enters the Kalar City border. Then, the values gradually increase until the river exits the city border, knowing that this increase is not a dangerous indicator. Also, this slight increase is attributed to wastewater containing higher concentrations of salt directly discharged into the Sirwan River. The EC mean value is (0.46) ds/m which indicates no saline condition of the river water[25] and no detrimental effects on plants.

The values of Ca^{+2}, Mg^{+2}, K^{+} and Na^{+} ranged between (61.7), (18.9), (9.8) and (1.1) to (110.3), (50.2), (13.2), (2.1) $mg.l^{-1}$, with average values of (91.58), (32.53), (11.13) and (1.74) $mg.l^{-1}$ respectively. The abundance of the cations order is as follows $Ca^{+2} > Mg^{+2} > Na^{+} > K^{+}$, where the Ca^{+2} and Mg^{+2} are the most dominant cations in Sirwan River.

The values of Cl^{-}, SO_4^{-2} and HCO_3^{-} ranged between (45), (74) and (130) to (88.3), (150.4) and (201.4) $mg.l^{-1}$, with average values of (71.62), (120.99) and (181.92) mg/l respectively. However, the importance of CO_3 was found to be very low, so it was neglected. The abundance of the anions order is as follows $HCO_3^{-} > SO_4^{-2} > Cl^{-}$. The results indicated that bicarbonate and sulfite are the dominant anions in the study area. A high concentration of bicarbonate is related to rock and water interaction. The cations and anions concentrations in the study area were within the normal ranges based on (FAO) guidelines.

The results also showed that the values of total hardness (TH) in the study area ranged between (236.75) (and 438) $mg.l^{-1}$. The lowest value was recorded at a station (1) due to the good quality of water that flows from the Darbandikhan dam and the absence of factories or large cities in that area. The highest concentration was recorded in the station (21) due to the high concentrations of calcium and magnesium. Most effluent dump sites are located within this area, which discharges directly without treatment. The mean value of total hardness (TH) was (365.45) $mg.l^{-1}$; therefore, the IWQG classified the water river as very hard. Hard water may cause soil organic matter dissolution. Also, it causes clay dispersion which ultimately causes poor soil structure, and water movement through the soil will be very slowly[26].

The cations and anions values were converted into some indexes regarding irrigation water quality, as shown in table (7) and figure (3).

The SAR is the relative concentration values of calcium, magnesium and sodium. It indicates the influence of Ca^{+2} and Mg^{+2} concentrations on Na^{+} accumulation in the soil. Thus, a high concentration of Na^{+} with a low concentration of Ca^{+2} may lead to a decline in the water within the root zone15,27. The values of SAR varied between (0.22) to (0.33) $mg.l^{-1}$ with a mean value of (0.26) $mg.l^{-1}$; so, Sirwan River water is classified under excellent class, and it is suitable for irrigation uses. Based on the IWQG software results, the sodium adsorption ratio (SAR) values indicate a low sodium hazard of the Sirwan River water.

Adjusted Sodium Adsorption Ratio (adj.SAR) is an index that is usually used to determine the risk of alkalinity in irrigation water. The adj.SAR values with less than (3) are considered a good category; however, the bet will appear when the values range between (3-9). The risk becomes severe when the values are above (9)[15]. The results of the

No.	Location	pH	EC	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	Cl^{-}	$SO4^{-2}$	HCO_3^{-}	Ion balance
			$ds.m^{-1}$				$mg.l^{-1}$				%
1	35°06'37.2"N 45°42'12.1"E	7.61	0.409	61.70	19.60	11.10	1.12	45.00	74.0	130.00	3.6
2	35°06'17.2"N 45°42'06.0"E	8.25	0.598	80.40	18.90	10.60	1.3	48.90	86.6	155.40	1.5
3	35°05'42.1"N 45°41'21.9"E	7.99	0.359	81.20	19.88	10.70	1.16	50.30	90.4	160.30	1.3
4	35°04'26.6"N 45°41'22.3"E	7.86	0.392	90.35	19.39	9.87	1.1	50.32	95.9	178.40	1.1
5	34°56'59.9"N 45°37'21.3"E	8.13	0.358	91.90	19.50	13.20	2.01	58.95	98.8	180.90	1.16
6	34°53'00.7"N 45°33'17.8"E	8.1	0.391	91.56	20.35	11.88	1.99	60.80	97.8	180.55	1.22
7	34°49'32.3"N 45°31'07.4"E	8.37	0.611	95.95	22.60	9.99	1.8	70.60	96.0	183.33	1.28
8	34°47'04.1"N 45°29'31.6"E	8.12	0. 43	100.54	23.66	9.90	1.67	76.45	100.6	188.50	1.08
9	34°44'33.3"N 45°28'11.9"E	8.2	0.42	110.30	26.80	9.80	1.18	78.76	120.4	195.65	2.08
10	34°41'41.1"N 45°27'24.3"E	8.36	0.412	99.60	30.33	11.11	1.78	77.90	125.8	180.00	1.06
11	34°39'15.3"N 45°26'05.8"E	7.25	0. 42	95.30	31.46	10.45	1.88	76.77	130.5	177.59	1
12	34°38'57.5"N 45°23'19.0"E	7.67	0.419	95.00	33.20	11.15	1.90	80.40	129.4	180.30	1.01
13	34°38'29.5"N 45°21'50.0"E	8.05	0.429	90.90	37.55	11.09	1.65	80.90	125.9	188.40	1.23
14	34°37'44.0"N 45°21'01.2"E	7.44	0.385	95.96	39.60	11.13	1.59	88.30	130.4	190.60	1.37
15	34°36'57.0"N 45°20'20.4"E	7.89	0.62	90.87	35.00	12.12	1.90	78.77	120.5	180.50	1.45
16	34°35'11.2"N 45°18'10.0"E	7.95	0.631	91.55	40.40	10.30	1.80	79.00	130.6	178.99	2.4
17	34°34'40.1"N 45°17'27.3"E	7.49	0.617	100.01	39.50	12.80	2.10	78.99	142.5	190.50	3.03
18	34°33'56.4"N 45°16'46.5"E	8	0.516	89.65	39.95	12.65	1.99	69.90	141.7	200.30	1.55
19	34°32'45.1"N 45°16'02.7"E	8.08	0.436	90.67	43.50	11.01	2.00	74.50	145.8	201.40	1.66
20	34°31'45.6"N 45°14'39.2"E	7.56	0.684	95.67	44.33	10.90	1.95	78.60	150.4	200.54	1.87
21	34°29'59.6"N 45°12'27.6"E	7.19	0.593	90.98	50.2	11.01	2.01	80	148.9	199.3	3.1
22	34°28'31.0"N 45°12'02.3"E	8.1	0.515	89.98	39.95	11.00	1.95	78.60	133.8	188.50	1.09
23	34°25'11.2"N 45°12'07.2"E	7.89	0.677	88.93	39.88	11.30	1.98	76.56	140.0	175.50	2.36
24	34°22'43.5"N 45°11'38.9"E	7.9	0.602	88.87	45.30	12.01	2.00	79.60	147.1	180.54	3.33

Table 6. The studied physicochemical parameters.

present area showed that the adj.SAR values are varied between (0.26) to (0.39) with an average of (0.31). Therefore, the Sirwan River water is classified under the excellent category, and there is no risk associated with adj.SAR.

The values of sodium percentage (Na%) ranged between (5.6%) (and 9.73%). These values indicated excellent conditions which never impact soil structure, and the plants can increase. However, excess amounts of sodium in irrigation water adversely affect soil structure, and the plants cannot survive growth easily[28].

All irrigation water 'regardless of its sources' contains several dissolved salts. These dissolved salts are the leading indicators determining irrigation water quality due to their role in raising the osmotic pressure of soil solution and the deterioration of soil properties over the long term of irrigation. A table (2) mentioned, the potential salinity index (PS) is defined as the summation of chloride and half of the sulfate concentrations. The PS values of all the water samples ranged between (2.14) (and 4.23) with an average of (3.53). The values of PS in all the water samples of Sirwan River are classified under the safe category. So, it is suitable for all types of soil textures.

Each of (Na^{+}, Ca^{+2}, Mg^{+2} and HCO_3^{-}) concentrations affects water's permeability index (PI) for irrigation uses. The PI values of the water samples ranged between (5.2) (and 9.21) with a mean value of (6.34). The PI values of all the samples are classified under the suitable category. Also, these results indicate that the water in the Sirwan River is ideal for soil permeability which is not influenced by long-term irrigation.

Soluble sodium percentage (SSP) is also one of the most critical parameters implemented to evaluate irrigation water's hazard. The soluble sodium percentage (SSP) values ranged from (5.6) to (9.73) with a mean value of (6.86). The results of (SSP) reflect that all the water samples in the river are classified under the good category.

The magnesium hazard (MH) is expressed in terms of magnesium hazard or magnesium adsorption rate (MAR), which is mentioned in table (2). The magnesium hazard computed values of the water in the studied dam ranged between (26.16) (and 47.89) with an average of (36.46). Based on the results, the water samples are less than (50), thus safe and suitable for irrigation.

The Kelly's ratio index (KR) is another way to determine the risk of sodium on water quality for irrigation uses. It also illustrates the potential impacts of sodium on irrigation water. According to the results, the Kelly ratio values of the water samples varied between (0.05) to (0.1) with a mean value of (0.07). These results fall under the (<1) limit and are considered suitable for irrigation usage.

Increased the concentration of Na^{+} in irrigation water, thus, causing soil dispersion, and Na^{+} is replaced with Ca^{+2} [25]. Therefore, the values of Kelly ration for irrigation water must not exceed (1) [29] [30]. Based on the results of the studied area, all water samples fall under suitable water for irrigation, and there is no significant excess of Na in the studied area.

Residual sodium carbonate (RSC) is one of the most significant parameters in determining the suitability of water for irrigation. According to the results of the present study, all the RSC values are less than (0). Therefore, the water samples of Sirwan River are classified under the excellent category for irrigation uses as there are no RSC-associated problems.

Irrigation water with high residual sodium carbonate (RSC) causes high sodium hazards. Also, alkaline soil (high pH) causes infertility in the soil due to Na_2CO_3 deposition[31]. Excessive values of HCO_3 and CO_3 in irrigation water causes reactions with Ca^{+2} and Mg^{+2} in the soil solution. This will allow the adsorbed Na^{+} to dominate the clay surfaces.

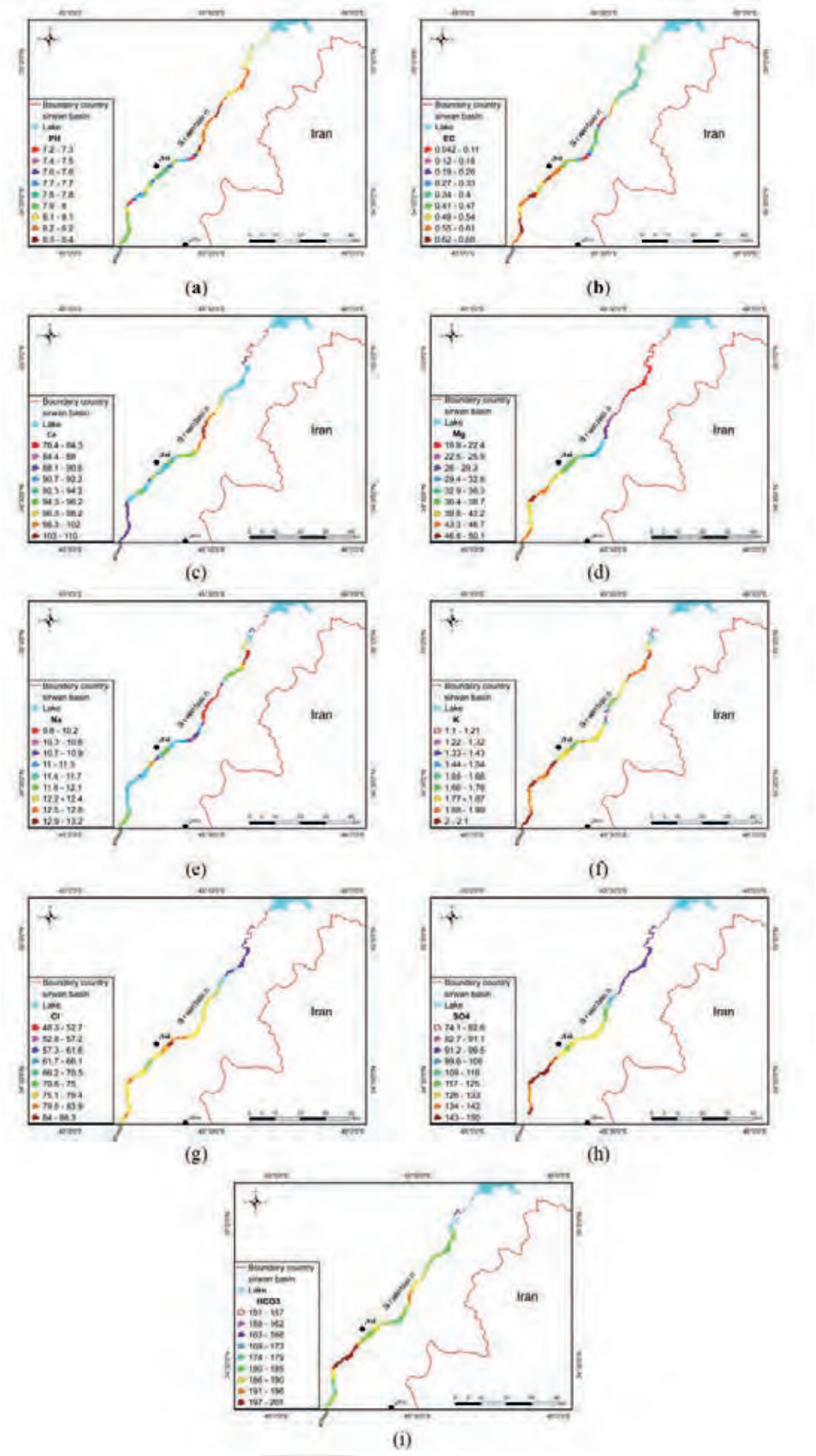

Figure 2. Distribution of (pH, EC, Ca^{+2}, Mg^{+2}, Na^{+}, K^{+}, Cl^{-}, SO_4^{-2} and HCO_3^{-}) values within the study area.

The results of IWQG in more than (58.3%) of the studied sampling stations have high limitations in use for most crops, as shown in table (8) and figure (4). Most of the sampling stations located between Kalar District and Jalawalaa Sub-district have high restrictions due to discharging significant amounts of effluent wastewater into the river daily without any treatment. Kalar District is the largest city within the study area, and its population is over (250) thousand people. Irrigation water with high restriction is suitable for plants with moderate to high salt tolerance.

However, approximately (41.7%) of the samples have moderate limitations. So, irrigation water with reasonable regulations is suitable for plants that have moderate tolerance to salts and could be used in average to high permeability soils. It is recommended to be used for medium leaching of salts. Also, salinity control practices should be implemented, except in water that contains low concentrations of (Na^+, Cl^- and HCO_3^-). For water with an EC value of more than 2000 dS/m and SAR value above (7), a schedule of frequency irrigation should be implemented.This is example 2 of an equation:

Conclusions

The Iraqi Irrigation Water Quality Guide (IWQG) has been used to evaluate and prove the suitability of water in the Sirwan River for irrigation uses. The results of this program will help the decision-makers, researchers, experts and farmers to design and calculate water quality quickly and properly. The values of electrical conductivity (EC) and the concentrations of cations and anions in all the study locations were within the permissible limits according (to FAO). Still, there were slight increases in these values within the Kalar City border due to disposing of large amounts of wastewater into the river. Also, the results revealed that the total hardness exceeded the permissible limits and reached the degree of extreme hardness in most of the sites, and it got more than (350) mg/l in most of the sampling stations. Moreover, the results of IWQG in most of the studied stations have high limitations. Therefore, the water quality of Sirwan River is suited to be used in irrigating crops with high or moderate tolerance to salt; thus, the farmers in the study area must avoid culturing salts-sensitive plants.

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Station	Na%	SAR	Adj. SAR	PS	TH	MH	PI	KI	SSP	RSC
1	9.73	0.31	0.37	2.14	236.75	34.67	9.21	0.1	9.73	<0
2	8.03	0.27	0.32	2.49	280.78	28.16	7.58	0.08	8.03	<0
3	8.03	0.28	0.33	2.61	286.79	28.97	7.58	0.08	8.03	<0
4	6.97	0.25	0.3	2.71	307.3	26.38	6.54	0.07	6.97	<0
5	9.18	0.33	0.39	2.94	311.81	26.16	8.52	0.09	9.18	<0
6	8.32	0.29	0.35	2.86	314.31	27.07	7.65	0.08	8.32	<0
7	6.69	0.24	0.29	3	334.83	28.25	6.17	0.07	6.69	<0
8	6.29	0.23	0.27	3.18	350.35	28.14	5.79	0.06	6.29	<0
9	5.6	0.22	0.26	3.61	388.39	28.87	5.25	0.06	5.6	<0
10	6.47	0.25	0.3	3.7	376.39	33.64	6	0.06	6.47	<0
11	6.45	0.24	0.29	3.79	370.39	35.54	5.85	0.06	6.45	<0
12	6.69	0.25	0.3	3.84	376.9	36.79	6.11	0.07	6.69	<0
13	6.34	0.24	0.29	3.75	384.42	40.76	5.88	0.06	6.34	<0
14	6.02	0.24	0.29	3.95	406.44	40.76	5.58	0.06	6.02	<0
15	7.2	0.27	0.32	3.62	373.9	39.09	6.63	0.07	7.2	<0
16	5.81	0.23	0.27	3.82	397.94	42.39	5.36	0.06	5.81	<0
17	6.84	0.27	0.32	4.12	415.95	39.71	6.31	0.07	6.84	<0
18	7.13	0.28	0.33	3.99	391.43	42.58	6.57	0.07	7.13	<0
19	6.09	0.24	0.29	4.05	408.95	44.43	5.55	0.06	6.09	<0
20	5.77	0.23	0.27	4.21	424.97	43.58	5.25	0.06	5.77	<0
21	5.71	0.23	0.27	4.23	438	47.89	5.2	0.05	5.71	<0
22	6.33	0.24	0.29	3.91	392.43	42.47	5.77	0.06	6.33	<0
23	6.49	0.25	0.3	3.98	389.43	42.8	5.93	0.06	6.49	<0
24	6.48	0.26	0.31	4.23	411.96	45.93	5.94	0.06	6.48	<0

Table 7. This is a table. Tables should be placed in the main text near to the first time they are cited.

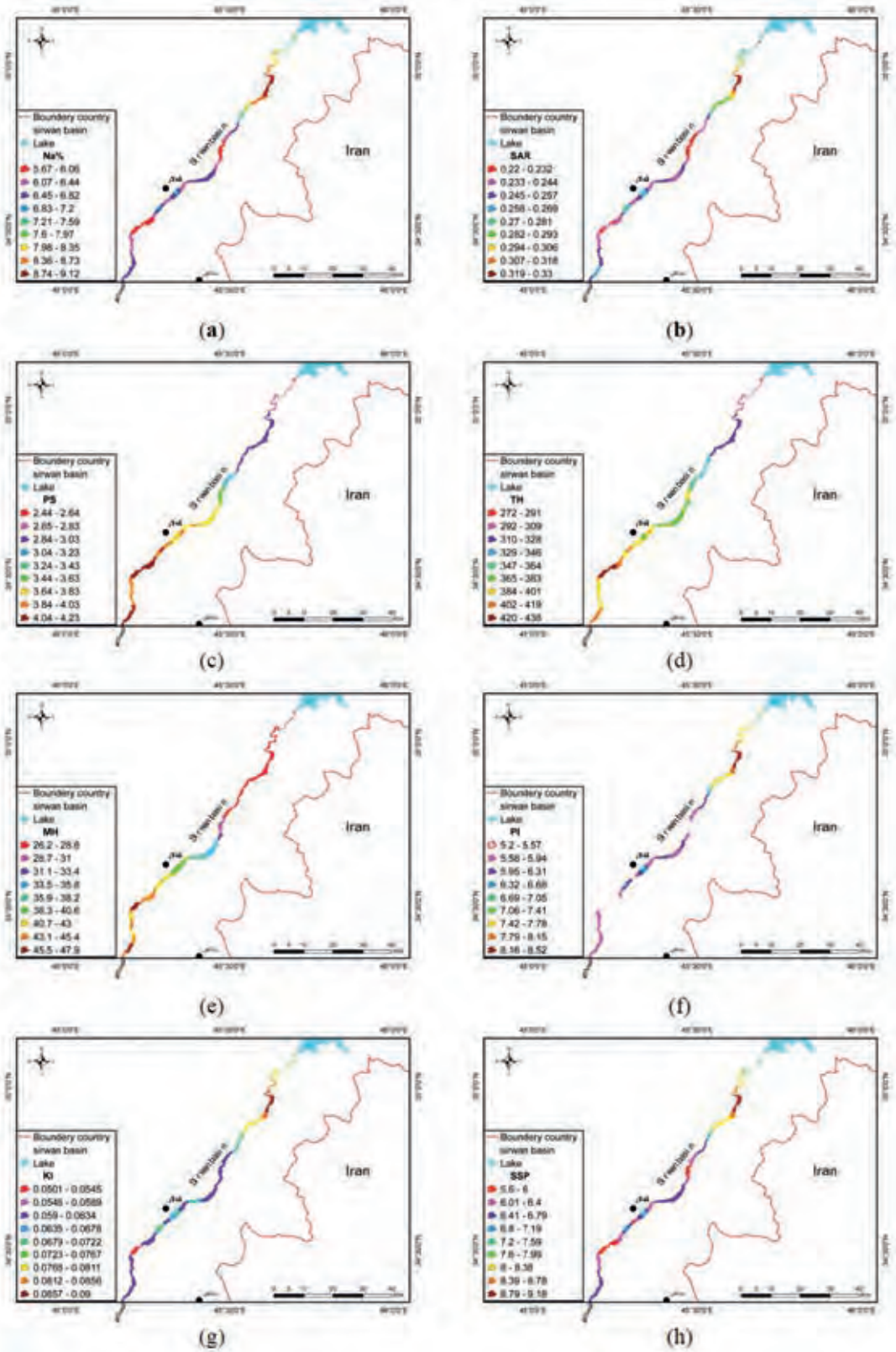

Figure 3. Distribution of (Na%, SAR, PS, TH, MH, PI, KI and SSP) values in the study area.

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No.	WQI	Restriction type
1	56.49	Moderate Restriction (MR)
2	54.58	High Restriction (HR)
3	56.7	Moderate Restriction (MR)
4	56.53	Moderate Restriction (MR)
5	55.96	Moderate Restriction (MR)
6	56.08	Moderate Restriction (MR)
7	54.43	High Restriction (HR)
8	43	High Restriction (HR)
9	55.87	Moderate Restriction (MR)
10	55.57	Moderate Restriction (MR)
11	42.34	High Restriction (HR)
12	55.37	Moderate Restriction (MR)
13	55.44	Moderate Restriction (MR)
14	55.75	Moderate Restriction (MR)
15	53.43	High Restriction (HR)
16	53.53	High Restriction (HR)
17	52.85	High Restriction (HR)
18	53.82	High Restriction (HR)
19	54.98	High Restriction (HR)
20	52.55	High Restriction (HR)
21	53.39	High Restriction (HR)
22	54.41	High Restriction (HR)
23	52.7	High Restriction (HR)
24	53.06	High Restriction (HR)
Average	53.7	High Restriction (HR)

Table 8. This is a table. Tables should be placed in the main text near to the first time they are cited.

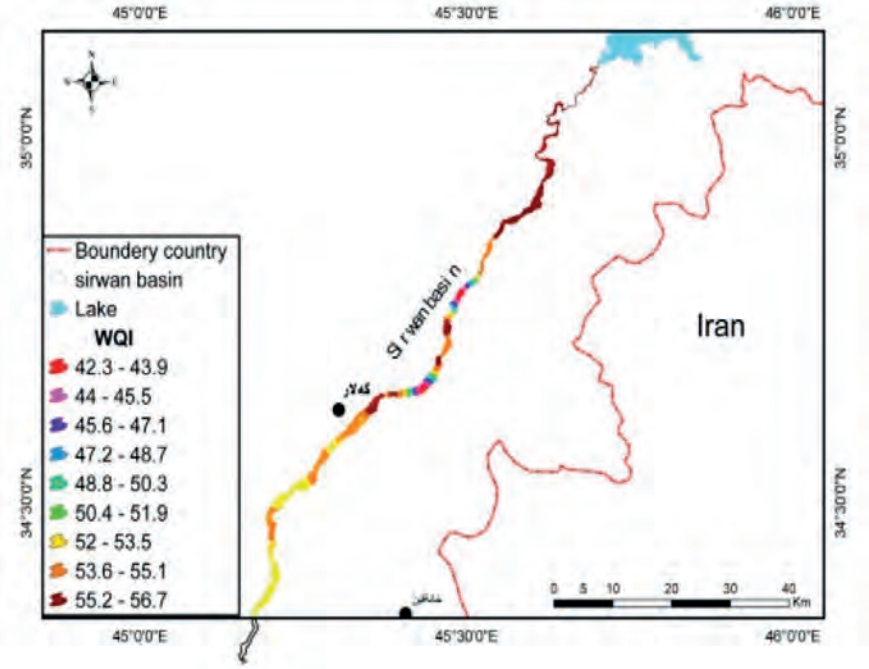

Figure 4. Distribution of WQIG values within the study area.

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ARTICLE / INVESTIGACIÓN

Effect of a gradual increase in the intensity of lighting on the physiological performance of broilers

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DOI. 10.21931/RB/2022.07.04.28

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Abstract: Birds are exposed when the light is turned on by using the gradual increase of the light system. This study used 224 chicks (Ross 308) at one day old. Those chicks were randomly distributed into four treatments with four replicates for each treatment, i.e. (14 chicks/replicate). The treatments include the following: T1 the lighting program according to the guide for 308 ROSS company (Control Treatment), T2 the lighting program according to the guide with a gradual increase of lighting intensity every 20 minutes, T3 used the same method with an increase of lighting intensity every 40 minutes, T4 used lighting program with a gradual increase of lighting intensity every 60 minutes. The blood biochemical and hematological characteristics of 22 and 37 days of the age of chicks were studied. The results showed that females of T2 and T3 were significantly superior in blood sugar levels over the females of T1 and T4. Besides, it was observed that there was a significant decrease in the value of ALP enzyme in the blood of chicks males of all treatments compared with the control treatment. The H/L stress indicator was significantly decreased in T2, T3 and T4 compared with the control treatment. At the same time, there were no significant differences in other blood characteristics among all treatments. Finally, at the age of 37 days, it was noted that there was no significant difference among all blood characteristics at this age. It can be concluded that there were no significant effects of the gradual lighting intensity on the physiological performance of broilers. Still, at the period of 22 days, there was a reduction in stress levels, particularly H/L and very low-density lipoproteins. This experiment was conducted in the College of Agriculture, the University of Anbar's poultry fields for (37 days) the period from 18/10/2021 to 21/11/2021. the research was undertaken to reduce the effect of stress resulting from the sudden and high light intensity that birds are exposed to when the light is turned on by using the gradual increase of the light system. This study used 224 chicks (Ross 308) at one day old. Those chicks were randomly distributed into four treatments with four replicates for each treatment, i.e. (14 chicks/replicate). The treatments include the following: T1 the lighting program according to the guide for 308 ROSS company (Control Treatment), T2 the lighting program according to the guide with a gradual increase of lighting intensity every 20 minutes, T3 used the same method with an increase of lighting intensity every 40 minutes, T4 used lighting program with a gradual increase of lighting intensity every 60 minutes The blood biochemical of 22 and 37 days of the age of chicks were studied. The results showed that females of T2 and T3 were significantly superior in blood sugar levels over the females of T1 and T4. Besides, it was observed that there was a significant decrease in the value of ALP enzyme in the blood of chicks males of all treatments compared with the control treatment. The H/L ratio stress indicator was significantly decreased in T2, T3 and T4 compared with the control treatment. In comparison, there were no significant differences in other blood characteristics among all treatments. Finally, at the age of 37 days, it was noted that there was no significant difference among all blood characteristics at this age. It can be concluded that there were no significant effects of the gradual lighting intensity on the physiological performance of broilers. Still, at 22 days, there was a reduction in stress levels, particularly H/L ratio and very low-density lipoproteins.

Key words: Broiler, ROSS 308, Light Intensity, Lux, Blood characteristics.

Introduction

Lighting is an essential element in broiler production because it is the most crucial external factor since it controls physiological and behavioral processes in birds[1]. Therefore, light management has emerged as one of the critical management tools for broiler[2]. The effect of light on the pineal gland helps synchronize the circadian rhythm and inhibits melatonin secretion[3]. Light intensity has a strong influence on the behavior of broilers in general. General bright light will increase activity while decreasing the intensity effects on controlling the aggressive actions that lead to predation[4].

The use of intermittent lighting programs positively affects the immunity of broilers as it affects the circadian rhythms, which in turn regulates melatonin production and thus improves the general health of birds. The effect of light on the pineal gland helps synchronize the circadian rhythm and inhibits the secretion of melatonin[5]. The blocking of light is regarded as a form of moderate feeding restriction, as these programs work on reducing the early growth rate as well as reducing feed consumption. It also improves the feed conversion factor and reduces electrical energy costs, as lighting is a critical environmental and administrative factor affecting broiler flocks' performance, welfare and

Citation: Jassim A A, Al-Jugif WI. Effect of a gradual increase in the intensity of lighting on the physiological performance of broilers.Revis Bionatura 2022;7(4) 28. http://dx.doi.org/10.21931/RB/2022.07.04.28
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

production[6]. Continuous lighting exposure of birds leads to a severe decrease in the production of the melatonin hormone, which negatively affects the health and well-being of broilers as a result of stress. Many studies refer to the increase of stress indicators when using 22-23 hours lighting systems besides using high light intensity which causes a decrease in the level of the melatonin hormone as well as the stress hormone that leads to weakening the immunity of birds and deterioration their physiological state[7,8].

Therefore, this study aimed to lessen the stress a bird experiences every day when the lights are turned on for the first time to an unexpectedly high intensity.

Materials and methods

The experiment was conducted in the poultry field of the Department of Animal Production at the College of Agriculture, University of Anbar. The experiment period of 37 days in the period from 18/10/2021 to 23/11/2021. The experiment was assigned to the batteries hall, with four batteries and four floors of each local battery. The dimensions of each floor were 120 x 1 m. equipped with lighting. The lighting program uses 20 hours of light and 4 hours of darkness, according to the ROSS 308 company manual. An electric device was used to grade the intensity of the homemade lighting, as it works on 220 volts and a frequency of 50

Hz. It also works on a gradual light intensity with four graded degrees (4 lux, 8 lux, 14 lux and 20 lux), respectively. Accordingly, this device can control the intensity times through its timer counters. A "Luxmeter" is a device used to measure the intensity of lighting.

Unsexed broiler chicks (ROSS 308) one-day-old were used in this experiment. Those chicks were brought from the Al-Waha modern hatchery in Kubaisa to the west of Anbar province.

In this study, 224 unsexed chicks of the Ross 308 strain were used, with an average weight of 40 g. The chicks were distributed randomly into four experimental treatments with four replicates of each treatment (14 birds/replicate).

The four treatments were the following:

T1: - Lighting program according to the guide of 308 ROSS (control treatment).

T2: - Lighting program according to 308 ROSS company guide with the gradual increase in the lighting intensity every 20 minutes.

T3: - Lighting program according to 308 ROSS company guide with the gradual increase in the lighting intensity every 40 minutes.

T4: - Lighting program according to 308 ROSS company guide with the gradual increase in the lighting intensity every 60 minutes.

Three types of feeds were given during the period of the experiment; these types were; 1. (starter feed) from the age of 1 day to day 14, 2. (grower feed) from day 14 to day 27, 3. (finisher feed) from day 27 to the end of the experiment 37 days, according to the chemical composition of the feeding guide as stated in (13). Blood was collected from birds at 22 days of age and 37 days of age. The studied features include the following: heterophile and lymphocytes blood cells (heterophile/lymphocytes, H/L ratio), packed cell volume (PCV), glucose level, total protein, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cholesterol, triglycerides,high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) in broiler blood plasma[9-14].

The results of the study were analyzed using Complete Random Design (CRD) to investigate the effect of different treatments on the studied features as well as using Duncan's multivariate test (7), and the differences between the means at the mean level of 0.05 and 0.01 were examined using the statistical analysis system (SAS, 2002)[15].

Results

Heterophile and lymphocytes blood cells, H/L ratio, and PCV in broiler blood

The results in Table 1 show the effect of lighting intensity on heterophile, lymphocytes H/L ratio, and PCV of broiler chicks. Lymphocytes (H/L ratio), which represents the stress level of birds; thus, T1 ($P < 0.05$) records the highest value with a significant difference among other treatments. Likewise, it was observed that there were differences among treatments in the number of heterogeneous cells, where the two treatments, T1 and T4, outperform the other two treatments T2 and treatment T2 was superior to treatment T3 at the age of 22 days (first blood draw). Regarding the lymphocytes, the results show that treatment T3 has a significant superiority over other treatments, i.e., T1, T2 and T4.On the other hand, treatment T2 was superior to treatment T1, which was overtaken by treatment T1 at the age of 22 days (first draw).

As for the results of the H/L ratio, it was observed that there were significant differences among the treatments, where treatment T1 outperforms all other treatments, T2, T3, and T4. Whereas treatment T4 overtakes the two treatments, i.e., T2 and T3, at 22 days (first draw). As concerns PCV, there were no significant differences among the treatments for both males and females at the age of (22 days).

As regards the second draw, which was at the age of 37 days, it was noted that there was no significant difference among the treatments in all the studied characteristics shown in table No. (1) included heterogeneous blood cells, lymphocytes, H/L ratio and PCV (for both sexes, males and females).

Accordingly, the results of this study agree with (16) as they use incandescent light (60 watts) as a control treatment, while the other treatments like; LED light, red light, blue light, green light, white light, and mixed light on Ross 308 broilers show that there was no significant difference of the packed cell volume (PCV henceforth) and the percentage of heterophile cells (H/L) when compared with the control treatment. The results of this study also agree with (17) when the light intensity of 5 lux and 20 lux was used for Coop broilers as it was not considerably affected the PCV and ousheterophil/lymphocyte (H/L) ratio among experiment parameters.

The H/L ratio (T2, T3, T4) decrease in all the experimental treatments compared with the control treatment may be due to the reduction of stress to which the birds were exposed when the lights were turned on at a high intensity. Elevation of the H/L ratio value in birds directly exposed to high light intensity compared to birds exposed to a gradual light intensity on white blood cells and the immune system.

Glucose Level, total protein, albumin and globulin in broiler blood plasma

The results in table 2 show the effect of the gradient

Treatments		Heterogeneous cells	Lymphatic cells	H/L	PCV%	
					Male	Female
The first draw at the age of 22 days	T1	0.380 a ± 0.008	0.547 d ± 0.010	0.695 a ± 0.020	33.4 ± 0.668	33.9 ± 1.03
	T2	0.295 b ± 0.005	0.637 b ± 0.012	0.463 c ± 0.006	34.3 ± 0.760	35.0 ± 0.476
	T3	0.262 c ± 0.004	0.682 a ± 0.006	0.384 c ± 0.008	33.4 ± 1.37	36.5 ± 1.53
	T4	0.350 a ± 0.017	0.592 c ± 0.022	0.597 b ± 0.055	32.0 ± 2.04	32.5 ± 2.25
	Probability value	0.0001	0.0001	0.0001	N.S	N.S
The second draw at the age of 37 days	T1	0.332 ± 0.028	0.615 ± 0.029	0.551 ± 0.071	32.2 ± 1.70	32.1 ± 1.52
	T2	0.300 ± 0.004	0.632 ± 0.012	0.474 ± 0.003	35.6 ± 1.73	35.1 2.23±
	T3	0.262 ± 0.004	0.692 ± 0.004	0.379 ± 0.005	30.4 ± 1.12	31.5 ± 1.84
	T4	0.327 ± 0.030	0.602 ± 0.039	0.559 ± 0.084	31.8 ± 1.19	31.0 ± 1.08
	Probability value	N.S	N.S	N.S	N.S	N.S

*Arithmetic mean ± standard error.
- The different letters within the same column indicate the presence of significant differences among the treatments.

Table 1. The effect of a gradual increase in the intensity of lighting on some blood characteristics.

in the intensity of lighting on the glucose levels, total protein, albumin and globulin in the blood plasma of male and female broilers. No significant differences were observed between treatments for males in the level of glucose in the blood plasma. At the same time, it was noted that treatment T2 ($P< 0.01$) was superior to treatments T1 and T4, and it doesn't differ significantly from the between treatments for females at the age of 22 days.

As far as the total protein was concerned, no significant differences were perceived between the treatments of both males and females at 22 days (the first draw). Concerning albumin, there was a considerable decrease in males in all the treatments of the experiment compared to the control treatment. In females, no significant differences were observed among all treatments at the age of 22 days (first draw). Likewise, for globulin, there were also no significant differences between the treatments of both males and females at the age of 22 days (the first draw). As for the second draw, which was at the age of 37 days, it was observed that there was no significant difference among all treatments in all the studied features shown in the table. No. (2), i.e., glucose, total protein, albumin, globulin, and for both sexes (males and females).

Treatments		Globulin Mg/100ml		Protein Mg/100ml		Albumin Mg/100ml		Globulin Mg/100ml	
		Males	Females	Males	Females	Males	Females	Males	Females
The first draw at the age of 22 days	T1	123 ± 13.2	175 b ± 22.0	65.5 ± 9.72	37.2 ± 4.42	9.29 a ± 1.14	5.12 ± 1.04	56.2 ± 9.82	32.1 ± 4.99
	T2	155 ± 21.0	295 a ± 6.51	70.9 ± 25.5	39.0 ± 3.30	3.89 b ± 1.30	7.14 ± 1.63	67.0 ± 24.3	31.8 1.98±
	T3	219 ± 34.7	251 ab ± 35.7	66.9 ± 14.0	44.5 ± 7.98	4.72 b ± 1.21	5.22 ± 1.16	62.1 ± 15.1	39.3 ± 7.57
	T4	192 ± 44.5	192 b ± 22.1	41.8 ± 6.21	35.0 ± 2.05	3.97 b ± 1.34	7.00 ± 1.22	37.8 ± 7.24	28.0 ± 2.83
	Probability value	N.S	0.014	N.S	N.S	0.029	N.S	N.S	N.S
The second draw at the age of 37 days	T1	236 ± 32.3	196 ± 39.3	29.5 ± 7.12	31.1 ± 1.19	3.60 ± 0.342	4.90 ± 1.29	25.9 ± 6.98	26.2 ± 2.41
	T2	229 ± 27.7	275 ± 68.3	31.4 ± 1.16	37.8 ± 5.28	4.36 ± 1.00	7.34 ± 0.553	27.0 ± 1.16	30.5 ±4.75
	T3	325 ± 60.1	183 ± 35.8	36.1 ± 0.92	25.3 ± 1.58	5.98 ± 1.05	6.55 ± 1.85	30.2 ± 0.795	18.7 ± 2.94
	T4	169 ± 15.9	125 ± 13.0	30.2 ± 0.73	36.2 ± 9.12	6.40 ± 1.96	7.58 ± 0.80	23.8 ± 1.99	28.7 ± 8.40
	Probability value	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

*Arithmetic mean ± standard error.
- The different letters within the same column indicate the presence of significant differences among the treatments.

Table 2. Effect of a gradual increase in the intensity of lighting on some characteristics of blood plasma.

Treatments		AST IU/liter		ALT IU/liter		ALP IU/liter	
		Males	Females	Males	Females	Males	Females
The first draw at the age of 22 days	T1	3.54 ± 0.407	5.46 ± 0.419	5.30 ± 0.493	6.49 ± 0.491	5.80 a ± 1.49	Z3.76 ±0.452
	T2	3.89 ± 0.430	4.95 ± 0.887	5.84 ± 0.681	6.57 ± 0.175	7.12 a ± 1.36	7.08 ± 1.43
	T3	3.90 ± 0.344	4.89 ± 0.524	6.13 ± 0.724	4.73 ± 0.949	4.75 ab ± 0.450	8.03 ± 0.686
	T4	3.33 ± 0.264	4.41 ± 0.425	5.08 ± 0.769	5.66 ± 0.299	2.31 b ± 0.546	5.36 ± 1.90
	Probability value	N.S	N.S	N.S	N.S	0.045	N.S
The second draw at the age of 37 days	T1	3.84 ± 0.821	2.03 ± 0.296	4.86 ± 0.496	3.04 ± 0.791	3.21 ± 1.41	1.77 ±0.337
	T2	2.32 ± 0.239	3.55 ± 0.833	4.32 ± 0.750	3.63 ± 0.595	2.91 ± 0.683	1.77 ± 0.174
	T3	4.77 ± 0.943	2.83 ± 0.617	4.57 ± 1.20	3.91 ± 1.08	4.56 ± 0.814	1.36 ± 0.428
	T4	4.33 ± 1.10	2.75 ± 0.712	5.15 ± 1.15	3.24 ± 0.497	2.66 ± 0.404	1.77 ± 0.263
	Probability value	N.S	N.S	N.S	N.S	N.S	N.S

*Arithmetic mean ± standard error.

Table 3. The effect of the gradient in light intensity on liver enzymes in broiler blood plasma.

The reason that there was no effect of lighting intensity when increasing the age of the bird to the age of 37 days was the ability of the bird to consume large quantities of feed when providing light, which removes the effect of lack of light that prevents the availability or consumption of food in times of darkness. In line with (18), the results of the study agree that when using two types of lighting, the first was bright and the second was dim when raising Roos 308 broilers; it does not significantly affect glucose, cholesterol and total protein among all experiment treatments when studying these characteristics of the broiler at the age of 37 days.

Liver enzymes in broiler blood plasma

The results summarized in Table 3 show us the effect of light intensity on AST, ALT and ALP for males and females of broilers. There were no significant differences among treatments in both males and females for AST and ALT enzymes. As for ALP, the results of males were the highest ($P < 0.05$) among the T1 and T2 and on treatment T4, and it does not significantly differ from treatment T3. Concerning the results of females, no significant differences were recorded among the treatments at 22 days.

For the second draw at 37 days, there were no significant differences among all treatments in all the studied characteristics in table No. (3), e.g., AST, ALT and ALP enzymes for both sexes (males and females).

The results of this study go in line with (17) who used 5 lux and 20 lux for Coop broilers which was not significantly affected by the enzyme Aspartate Amino Transfers (AST) and Alkaline phosphatase (ALP) in the experimental treatments. The results of the study also agree with (19) who use light intensity either bright (20 lux from day 1 to day 42) or (dim 5 lux from day 1 to day 8, 2.5 lux from day 9 to 15 day, and 1.25 Lux from 16 days to 42). Ross308 broilers did not significantly affect the enzyme Aspartate Amino transfers (AST) among the experimental treatments.

Lipid profile in broiler blood plasma

The results summarized in Table 4 refer to the effect of light intensity on cholesterol, triglycerides, HDL, LDL, and VLDL; thus, there were no significant differences among all the treatments of the given features for males and females at the age of 22 days. As for the second draw at 37 days, the results indicate no significant differences among the different treatments in cholesterol, HDL and LDL for males and females. Regarding triglycerides, it was noticed that the two treatments and T2 were higher than the two treatments and T4 for males, while females had no significant differences among the treatments.

Moreover, it was observed that the treatment of T1 and T2 in the value of VLDL have higher superiority over the other two treatments, T3 and T4, for males. In contrast, females have no significant differences among the treatments.

Thus, the fat values in the blood plasma of birds under the experiment indicate a highly significant decrease in

Treatments		cholesterol Mg/100ml		Triglycerides Mg/100ml		HDL Mg/100ml		LDL Mg/100ml		VLDL Mg/100ml	
		Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
The first draw at the age of 22 days	T1	106 ± 8.53	115 ± 10.1	46.9 ± 6.57	30.3 ± 6.74	29.0 ± 3.08	47.9 ± 4.23	67.8 ± 9.69	61.7 ± 8.98	9.37 ± 1.31	6.06 ± 1.34
	T2	118 ± 29.0	131 ± 34.7	38.6 ± 5.79	40.6 ± 12.5	31.4 ± 4.49	53.6 ± 2.31	79.1 ± 29.7	69.5 ± 36.3	7.72 ± 1.15	8.13 ± 2.50
	T3	110 ± 13.5	156 ± 13.4	73.3 ± 20.4	46.3 ± 5.66	22.9 ± 8.89	40.7 ± 11.1	72.5 ± 19.4	106 ± 11.4	14.6 ± 4.08	9.27 ± 1.13
	T4	145 ± 24.9	149 ± 12.6	46.8 ± 7.70	57.2 ± 8.59	18.9 ± 3.50	41.1 ± 3.51	117 ± 23.8	97.3 ± 14.5	9.37 ± 1.54	11.4 ± 1.71
	Probability value	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
The second draw at the age of 37 days	T1	*174 ± 32.2	138 ± 34.4	87.0 a ± 13.8	90.6 ± 19.8	2.82 ± 1.21	16.9 ± 7.28	154 ± 31.6	102 ± 38.7	17.4 a ± 2.76	18.1 ± 3.96
	T2	238 ± 8.29	169 ± 13.3	79.2 a ± 18.9	95.3 ± 14.1	5.64 ± 2.50	18.5 ± 4.18	217 ± 11.4	131 ± 15.9	15.8 a ± 3.79	19.0 ± 2.83
	T3	202 ± 4.19	117 ± 34.6	23.3 b ± 6.16	108 ± 9.59	17.7 ± 6.61	13.3 ± 6.97	179 ± 8.75	82.1 ± 35.4	4.66 b ± 1.23	21.6 ± 1.92
	T4	194 ± 3.92	215 ± 11.9	32.1 b ± 10.4	96.1 ± 7.10	8.06 ± 2.63	18.5 ± 9.25	180 ± 6.99	177 ± 12.3	6.42 b ± 2.09	19.2 ± 1.42
	Probability value	N.S	N.S	0.009	N.S	N.S	N.S	N.S	N.S	0.009	

*Arithmetic mean ± standard error.
- The different letters within the same column indicate the presence of significant differences among the treatments.

Table 4. The effect of the gradient in the lighting intensity on the lipid profile in the broiler's blood plasma.

treatment T3 and 4T, respectively, in the two features, i.e., triglycerides and very low-density lipoproteins. These two features were considered bad cholesterol, which causes strokes. They were increased significantly in T1 and. This increase may be due to the decrease in birds' stress caused by the high light intensity, as shown in table No. (1) which shows the H/L ratio value, achieving a good health condition that leads to very low-density lipoproteins.

The outcomes of the study agree with (20), who use two types of light intensity (5 lux, 50 lux) when breeding Roos 308 broilers, as they do not significantly affect cholesterol, high-density lipids (HDL) and low-density lipids (LDL) among the experiment parameters.

The significant superiority in blood images may be attributed to the fact that light allows birds to settle down and synchronizes many functions, including body temperature and metabolic steps that facilitate feeding and digestion. Likewise, light stimulates the secretion of patterns of several hormones that significantly control growth, maturation and (21). The research concluded a significant decrease in stress values, especially at 22 days. These values were H/L ratio and very low-density lipoproteins, which indicates a reduction in the stressful effect of the direct intensity of light on birds when the lights were turned on using the system of gradual increase in the power of light on the physiological performance of broilers (Ross 308).

Conclusions

This study concluded a significant decrease in stress values, especially at 22 days. These values were H/L and very low-density lipoproteins, which indicates a reduction in the stressful effect of the direct intensity of light on birds when the lights were turned on using the system of gradual increase in the intensity of light on the physiological performance of broilers (Ross 308).

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ARTICLE / INVESTIGACIÓN

Response of buckthorn seedlings to foliar spraying with Kelamyth Fe and algae mixture on vegetative growth traits for cultivar AL-Tafahi

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DOI. 10.21931/RB/2022.07.04.29

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Abstract: This study was done in the lath house of the Horticulture and Gardening Dept. / College of Agriculture / University of Anbar during the growth season 2021 to study the effect of leaf spraying kelamyth chelated Fe in three levels (0, 50, 100 mg.L^{-1}), with three levels Alga Mix of (0, 1, 1.5 g.L^{-1}) and their interaction on vegetative growth traits and leaf content of minerals for buckthorn seedlings AL-Tafahi cultivar. The results showed that the interaction between chelated kelamitic Fe and F2S2 algae mixture at a concentration (100 mg.L^{-1} kelamitic Fe + 1.5 g.L^{-1} algae mixture) had given a significant increase in plant height, number of branches, leaf area, chlorophyll, carbohydrates, Fe and saponins. Successively with values (75. 057cm, 19.183 units.seedling-1, 23.833cm2, 41.537 mg. g-1 fresh weight, 3.800%, 194.000 ppm, 1.545 c.ml-1), compared to control treatments.

Key words: Ziziphus mauritiana, chelated iron, seaweed extract, foliar spraying, Buckthorn.

Introduction

Buckthorn, *Ziziphus mauritiana* Lam belongs to the genus *Ziziphus* and grapes family Rhamnaceae includes 58 geniuses and more than 900 species and contains trees, standing or climbing bushes and rarely grasses. The original home of buckthorn trees in South Europe regions, the Himalayan mountains, North China, Sudan, the Arab Peninsula, Iraq, South America and maybe North Africa[8]. In Iraq, it's cultured in central and southern areas[1]. Buckthorn fruits are featured with delicious taste and attractive colors. They're widely consumed for their high nutrient content as they contain saccharides, proteins, organic acids, amino acids, vitamins like vitamin C and carotene, suitable concentrations of calcium, phosphorus, Fe iron, lipids, fibers mineral salts and antioxidants[4,7,16,17]. Fertilizing is a necessary process that influences the growth of fruit trees in general, and to increase fertilizing efficiency, the plant is supported via leaf fertilizing (foliar application), especially Fe iron element that is considered a microelement that is important in plant growth and development as it plays a fundamental role in nucleic acid and plastid assimilation; so, it participates manufacturing chlorophyll though it isn't included in its structure. It also acts in building cytochromes important for photosynthesis and respiration processes. It also enters plant protein manufacture[21]. Alga mixes are organic sources used for agricultural production and are fertilizer complements, not substitute[24]. They're used, therefore, in massive amounts and could reach 15 million tons in the agricultural field worldwide. These extracts induce plant growth, enhancing plant physical and chemical characteristics by containing macro and microelements, amino acids, organic acids and growth regulators like oxins and cytokinins, hormones, vitamins and polysaccharides. It functions by increasing plant resistance to salinity and draught[15]. Due to the lack of studies interested in the effect of foliar spraying of essential nutrients to buckthorn seedlings, in this study, spraying of Kelamyth Fe element and algal mixture on the shoot system of buckthorn seedlings cultivar AL-Tafahi was carried out to determine the effect of Kelamyth Fe and algal mixture on the growth of buckthorn seedlings cultivar AL-Tafahi.

Materials and methods

This study was carried out on buckthorn seedlings AL-Tatahi cultivar, inoculated on the seedy origin of two years old, cultured in black plastic anvils of 10kg capacity (soil + bitmus) in (1:1) ratio. They were treated by spraying the shoot system with Kelamyth Fe in three levels (0, 50, 100 mg.L^{-1}) and alga mix in three levels (0, 1, 1.5 mg.L^{-1}) and the trees were sprayed to wet on the following dates: April 11, 2021, May 11, 2021, June 11, 2021, September 11, 2021, and October 11, 2021. A factorial experiment (3X3) was done according to Randomized Complete Block Design (RCBD); so, the investigation included nine treatments in three replications, and every four seedlings were isolated as a single experiment unit, and the data were analyzed according to the statistical apparatus (Genstat). Mean values have been compared using the least significant difference (LSD), and subsequent studies and measurements were made.

Average Increase in Plant Height (cm)

Seedlings' height was measured using metric measuring tape starting from the stem-soil surface joint spot to the top apical meristem on the main seedling stem at the beginning and end of the experiment; the difference between the two readings is the increased value.

Citation: El- Hamdani, H AL-Ali H. Response of buckthorn seedlings to foliar spraying with Kelamyth Fe and algae mixture on vegetative growth traits for cultivar AL-Tafahi. Revis Bionatura 2022;7(4) 29. http://dx.doi.org/10.21931/RB/2022.07.04.29
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

Publisher's Note: Bionatura stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Average Increase in Vegetative Branch Number (branch.seedling^{-1})

The number of components per seedling was counted according to the average number of vegetative features for each replication, then the average number of vegetative branches was extracted for each treatment.

Leaf Area (cm^2)

Measuring was done using a specified computer software where leaves were photo-scanned via a scanner with a measuring ruler to determine the space and measure the leaf area[2].

Leaf Chlorophyll Content (mg. g^{-1} fresh weight)

Total chlorophyll was estimated depending on the model of (19).

Estimating Leaf Total (Structural) Carbohydrate Content (%)

Leaf total carbohydrate content was estimated based on what's mentioned by (10).

Leaf Fe Content (ppm): leaf Fe content was estimated by the model of (11).

Estimating Leaf saponins Content (g.ml^{-1})

A 20g sample was taken, 80ml ethanol was added to the precipitant, and then re-extraction was made from 20 ml di-ether. This operation is repeated many times, and then 60ml butanol is added and filtered, and the precipitant is washed with (5% NaCl). Then, evaporate the extract and weigh the precipitant that represents saponins in sample[23].

Results

Plant height (cm)

The results in Table 1 showed the effect of foliar spraying of buckthorn seedlings, cultivar AL-Tafahi, with kelamyth Fe and seaweed mixture extract, separately or shared on the average increase in plant height. Thus, treatment F_2 at 100 mg. L^{-1} has significantly dominated, giving the highest value of 72.52cm. Followed by treatment F_1 at a concentration of 50 mg.L^{-1}, which, in turn, has dramatically dominated over F_0 and reached 69.82cm, while the lowest value was in the control treatment F_0, which obtained 65.73cm. Besides, spraying alga mix extract has led to a significant increase in plant height; so, the treatment S_2 at concentration 1.5 g.L^{-1} with the highest value of 72.47cm, followed by treatment S_1 at concentration 1 g.L^{-1} significantly dominated on S_0 and reached 69.87cm, while the most negligible value was at treatment S_0 as 65.74cm. Regarding the study factors interaction, a significant effect in the trait was pronounced by achieving the treatment F_2S_2, the highest value of 75.05cm, compared to the control treatment F_0S_0, which made the most diminutive average plant height 60.11cm.

Table 2 results showed significant differences in the average increase of branch number; so, the treatment F_2 has significantly dominated the other two treatments, giving the largest number of vegetative branches as 15.13.seedling^{-1}, followed by treatment F_1 that reached 11.16 branch.seedling^{-1}, which in turn has significantly dominated on F_0 that reached 9.42 branch.seedling^{-1}. Turning to alga mix spray caused a significant increase in this trait, especially for treatment S_2 that reached 13.50 branch.seedling^{-1}, followed by treatment S_1 that reached 11.69 branch.seedling^{-1} that did not differ significantly from S_0; while the least value was at the control treatment S_0 that reached 10.52 branch.seedling^{-1}. While the interaction between both study factors has shown a significant effect in this trait via achieving the treatment F_2S_2, the highest value is 19.18 branch.seedling^{-1}, compared to control treatment F_0S_0 made the least average vegetative branches number as 9.05 branches seedling^{-1}.

Leaf Area (cm^2)

Results of Table 3 showed that study treatments had significantly influenced leaf area. So, the treatment F_2 made the largest leaf area at 22.62cm^2, followed by treatment F_1, which reached 22.05cm^2, which didn't differ significantly from the first one, while the most negligible value was with the control treatment F_0, reached 19.66cm^2. Also, spraying the alga mix caused a significant increase in leaf area, especially at treatment S_2, which came 22.47cm^2, then the treatment S_1, which reached 21.81 cm^2, which didn't differ significantly from it, while the most negligible value was at the control treatment S_0 that went 20.05cm^2. Considering the interaction between both study factors, it showed its effect in this trait through the treatment F_2S_2, which made the highest value as 23.83cm^2, while the control treatment S_0F_0 made a minor average leaf area at 17.10cm^2.

The Leaf Chlorophyll Content (mg. g^{-1} fresh weight)

Results of Table 4 showed that leaf spraying kelamyth Fe on buckthorn seedlings significantly affects leaf chloro-

Alga mix 1.5 g.L^{-1}	Iron chelate (Kelamyth Fe) mg.L^{-1}			Average effect of Alga mix
	F_0	F_1	F_2	
S_0	60.11	67.54	69.57	**65.74**
S_1	66.58	70.08	72.95	**69.87**
S_2	70.51	71.86	75.05	**72.47**
	65.73	**69.82**	**72.52**	**Average effect of Iron chelate**
LSD F*S=2.76	**LSD S=1.59**			**LSD F=1.59**

Table 1. Effect of Leaf Spraying Kelamyth Fe and Alga mix and their interactions in Average Increase of Plant Height (cm).

Alga mix 1.5 g .L^{-1}	Iron chelate (Kelamyth Fe) mg.L^{-1}			Average effect of Alga mix
	F_0	F_1	F_2	
S_0	9.05	10.50	12.03	**10.52**
S_1	9.38	11.50	14.18	**11.69**
S_2	9.83	11.49	19.18	**13.50**
	9.42	**11.16**	**15.13**	**Average effect of Iron chelate**
LSD F*S=2.89	**LSD S=1.67**			**LSD F=1.67**

Table 2. Effect of Leaf Spraying Kelamyth Fe and Alga mix and their interactions in Average Number of Vegetative Branches (branch.seedling^{-1}).

Alga mix 1.5 g .L^{-1}	Iron chelate (Kelamyth Fe) mg.L^{-1}			Average effect of Alga mix
	F_0	F_1	F_2	
S_0	17.10	21.74	21.32	**20.05**
S_1	20.20	22.52	22.72	**21.81**
S_2	21.68	21.90	23.83	**22.47**
	22.62	**22.05**	**19.66**	**Average effect of Iron chelate**
LSD F*S=1.81	**LSD S=1.04**			**LSD F=1.04**

Table 3. Effect of Leaf Spraying Kelamyth Fe and Alga mix and their interactions in Average Leaf Area (cm^2).

phyll content. So, the treatment F_2 especially dominated the two other treatments with 41.09 mg. g^{-1} fresh weight significant increase that differed substantially from F_1 reached 39.09 mg. g^{-1} new weight, which didn't vary significantly from F_0, while the most negligible value was with the control treatment F_0 that went 38.02 mg. g^{-1} fresh weight. Considering the alga mix spray, the table shows significant differences, especially at treatment S_2, which reached 41.20 mg. g^{-1} new weight, followed by treatment S_1, which didn't differ significantly from the latter, as it gained 39.83 mg. g^{-1} fresh importance. In comparison, the most negligible value was with treatment S_0 which reached 37.16 mg. g^{-1} new weight. Considering the interaction between both study factors, it showed its significant effect in this trait through the treatment F_2S_2 that made the highest value as 41.53 mg. g^{-1} fresh weight. In comparison, the control treatment F_0S_0 has made the most negligible chlorophyll content as 34.59 mg. g^{-1} new weight.

Leaf Total (Structural) Carbohydrate Estimation (%)

Results of Table 5 show that leaf total carbohydrate content has been influenced significantly by study treatments. So, treatment F_2 made the highest carbohydrate content at 2.42%, then treatment F_1 reached 1.91%, while the lowest value was at the control treatment F_0, which was 1.82%. Also, spraying with algal extract has caused a significant increase in leaf carbohydrate content, especially with treatment S_2, which reached 2.45%, followed by treatment S_1, which gained 1.89%, while the most negligible value was at control treatment S_0 got 1.80%. Considering the interaction between the two study factors, it showed its significant effect on this trait through the treatment F_2S_2, which made the highest value at 3.80%. In contrast, the control treatment F_0S_0 made the minor carbohydrate content at 1.45%.

Leaf Fe iron Content (ppm)

Results of Table 6 showed that leaf spraying kelamyth Fe had increased leaf Fe iron percentage; so, the treatment F_2 significantly dominated the other treatments to reach 190.66ppm. They were followed by treatment F_1, which gained 187.88 ppm, which, in turn, significantly dominated treatment F_0, which went 185.66 ppm as the small leaf Fe iron percentage. In return to alga mix spray, the table shows that treatment S_2 also significantly increased leaf Fe iron

Alga mix 1.5 g .L^{-1}	Iron chelate (Kelamyth Fe) mg.L^{-1}			Average effect of Alga mix
	F0	F1	F2	
S_0	34.59	36.06	40.84	**37.16**
S_1	38.00	40.60	40.91	**39.83**
S_2	41.47	40.61	41.53	**41.20**
	38.02	**39.09**	**41.09**	**Average effect of Iron chelate**
LSD F*S=2.77	**LSD S=1.60**			**LSD F=1.60**

Table 4. Effect of Leaf Spraying Kelamyth Fe and Alga mix and their interactions in Leaf Chlorophyll Con.

Alga mix 1.5 g .L^{-1}	Iron chelate (Kelamyth Fe) mg.L^{-1}			Average effect of Alga mix
	F_0	F_1	F_2	
S_0	1.45	2.23	1.73	**1.80**
S_1	2.23	1.70	1.73	**1.89**
S_2	1.77	1.79	3.80	**2.45**
	1.82	**1.91**	**2.42**	**Average effect of Iron chelate**
LSD F*S=0.78	**LSD S=0.45**			**LSD F=0.45**

Table 5. Effect of Leaf Spraying Kelamyth Fe and Alga mix and their interactions in Leaf Total (Structural) Carbohydrate Content Estimation (%).

Alga mix 1.5 g .L^{-1}	Iron chelate (Kelamyth Fe) mg.L^{-1}			Average effect of Alga mix
	F_0	F_1	F_2	
S_0	184.00	185.33	188.00	**185.77**
S_1	186.66	189.66	190.00	**188.77**
S_2	186.33	188.66	194.00	**189.66**
	185.66	**187.88**	**190.66**	**Average effect of Iron chelate**
LSD F*S=2.47	**LSD S=1.42**			**LSD F=1.42**

Table 6. Effect of Leaf Spraying Kelamyth Fe and Alga mix and their interactions in Leaf Fe Content (ppm).

percentage to 189.66ppm, followed by treatment S_1, which reached 188.77 ppm, which didn't differ considerably from S_2. In contrast, the control treatment S_0 has made the minor Fe percentage at 185.77ppm. In return, both study factors' interaction influenced this trait by making the treatment F_2S_2 the highest leaf Fe percentage as 194.00. In contrast, the control treatment F_0S_0 has reached the most negligible value at 184.00%.

Leaf Saponins Content (g.ml^{-1})

Results of Table 7 confirmed that study treatments had significantly influenced leaf saponins content. So, the treatment F_2 has made the highest saponins content at 1.21 g.ml^{-1}, dominated substantially the two other medicines, followed by treatment F_1, which reached 1.02 g.ml^{-1}, did not significantly differ from F_0, while the most negligible value was at the control treatment F_0 that was 0.96 g.ml-1. Likewi-

Alga mix 1.5 g .L^{-1}	Iron chelate (Kelamyth Fe) mg.L^{-1}			Average effect of Alga mix
	F_0	F_1	F_2	
S_0	0.92	1.00	1.04	**0.99**
S_1	0.97	1.03	1.05	**1.02**
S_2	0.98	1.03	1.54	**1.18**
	0.96	1.02	1.21	**Average effect of Iron chelate**
LSD F*S=0.27		LSD S=0.15		LSD F=0.15

Table 7. Effect of Leaf Spraying Kelamyth Fe and Alga mix and their interactions in Leaf Saponins Content (g.ml^{-1}).

se, spraying the algae mixture led to a significant increase in leaf saponin content, especially with treatment S_2, which reached 1.18 g.ml^{-1}, with a behavior similar to that of Kelamyth Fe in its effect on this trait, followed by treatment S_1, which gained 1.02 g.ml^{-1}, which did not differ significantly from S_0, while the lowest value was in the control treatment S_0, which reached 0.99 g.ml^{-1}. When we return to both study factors' interaction, it showed its significant effect on this trait by achieving the treatment F_2S_2 the highest value as 1.54 g.ml^{-1}, while the control treatment F_0S_0 has mad the most miniature leaf saponins content as 0.92 g.ml^{-1}.

Discussion

The reason for the height of the plant is due to the role of the element iron, which enters into the representation of nucleic acids, DNA and RNA necessary for cell division. It also joins as a catalyst in forming chlorophyll and enzymes that promote the construction of materials needed for the plant. Therefore, it increases the height of the plant[14]. The reason can be attributed to its role in the structure of chlorophyll. However, it did not enter in its formation, as it was found that (70%) of the total iron is present in chloroplasts, in addition to its entry in the form of cytochromes important in the process of photosynthesis. And respiration[5] explains the increase in the number of branches and leaf area and is consistent with what was found in 9 in *Hibiscus sabdariffa*, were spraying with chelated iron increased the number of components. Therefore, it agrees with (20) strawberry seedlings, cultivar Winter dawn, were spraying with chelated iron rose leaf area. Glutamate to Y-aminolevulinic acid to Y-aminolevulinic acid and the process of converting the complex Mg-protoporphyrin 1x methyl ester to Proto-chlorophyllide are two essential steps in building chlorophyll (18) that caused the increase in carbohydrate content in Buckthorn leaves when sprayed with chelated iron To the role of iron in activating the process of respiration and photosynthesis, as it participates in the formation of protein and the manufacture of chlorophyll, which has an essential role in the process of building carbon and increasing the stomata delivery of carbon dioxide, which leads to an increase in the accumulation of processed nutrients in plants that leads to the collection of carbohydrates in Leaves[12] increase in the percentage of iron in the leaves when spraying with chelated iron is due to the increase in the leaves' absorption of this element to increase its share in the spray solution and may be due in the rise in the vegetative growth of seedlings as it contributes to the chlorophyll synthesis processes[6]. The effect of seaweed extract spraying on most vegetative growth characteristics is that it contains many nutrients that play an essential role in increasing the plant's metabolic activities. Potassium activates enzymes to synthesize amino acids and proteins, as well as helps to synthesize the necessary chlorophyll in the process of photosynthesis and the formation of proteins, sugars and ATP energy compounds, leading to an increase in plant growth and size and, therefore, an increase in vegetative growth[13]. Perhaps the increase in chlorophyll content in leaves treated with seaweed extract is due to the effect of seaweed extract in inhibiting the decomposition of chlorophyll by Betaines compounds. Glycine betaines, which led to the continuation of photosynthesis as mentioned, (3) and this may be due to the marine algae extract containing organic acids that can increase the permeability of cell membranes and facilitate the transport of nutrients that have an influential role in activating metabolism for proteins and enzymes that accompany carbohydrate metabolism[22].

Conclusions

The F_2 concentration was superior in most indicators of vegetative growth and the content of mineral elements in leaves. The effect of foliar spraying with marine algae extract, especially at a concentration of 1.5 S2 g/L^{-1}, on all indicators of vegetative growth and the content of mineral elements in the leaves.

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ARTICLE / INVESTIGACIÓN

Effects of Leptin antagonist treatments on testosterone and testis histological characteristics of immature male mice

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DOI. 10.21931/RB/2022.07.04.30
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Abstract: The present study aimed to ascertain how leptin antagonist injection affected testis weights, testis morphology and testosterone levels in immature male Swiss mice. Animals were administered with anti-leptin antibody subcutaneously, with or without equine chorionic gonadotropin (eCG). Control animals were treated with non-immune serum. Blood and testis were collected. The Androgen profile was analyzed in serum and tissue homogenates, and testes were histologically examined. Compared to controls, mice treated with an anti-leptin antibody with or without gonadotropins had a significant ($p<0.05$) increase in testis weight. Testosterone concentrations in the testis were significantly ($p<0.05$) higher in mice administered with anti-leptin antibody compared to control, but testosterone concentrations in blood were not affected. The diameter of seminiferous tubules, the diameter of the lumen and the width of spermatogenic cells were significantly ($p<0.05$) higher in mice in treatment groups compared to controls. We conclude that anti-leptin antibody administration in immature male mice increased testosterone concentrations in the testis and improved testis histological characteristics.

Key words: Leptin, mouse, histology, testis, testosterone, immature male.

Introduction

The testis is an essential sexual organ in male animals. Testicular growth directly influences semen quality in humans and male mammals, including rats and boars, which is necessary for reproductive functions[1,2]. Testicular development occurs primarily in the seminiferous tubules after birth, including the Sertoli cell, germ cell, and Leydig cell proliferation. Most human testes development occurs during adolescence between the ages 2 to 14 years[3]. Testis volume, testis weight, Sertoli cells, and testosterone levels in the testes gradually rise from 2 to 8 years of age, and the number of germ cells grows by 3 to 6 times[4]. As a result, the spermatogonial cells proliferate rapidly.

In late 1994, a significant publication by Zhang *et al.* on the structure of the mouse obese (*ob*) gene and its human equivalent using positional cloning was described[5]. The ob gene results in the protein known as leptin. The *ob/ob* mouse's obesity is caused by a gene mutation that prevents leptin from being secreted by adipocytes, which leads to obesity. Leptin reduces food intake and increases energy expenditure, which leads to a loss in body weight in mice[6,7]. Additionally, *ob* gene expression is elevated in many animal models of obesity[8] and human obesity[9]. Leptin may be crucial for regulating body weight, according to accumulating research. The *ob/ob* mouse has atrophic reproductive organs and is infertile[10]. Much like in prepubertal animals, gonadotropin secretion is impaired and extremely sensitive to the negative feedback of gonadal steroids in the *ob/ob* mouse[11]. It has been demonstrated that long-term administration of leptin can restore fertility and the growth and function of the reproductive system in *ob/ob* mice[12] by boosting the release of gonadotropins[13]. According to the crucial weight hypothesis, puberty starts when body weight reaches a certain threshold[14]. Since underfed rats postpone puberty, the original premise is untrue. However, when given access to food, rapid weight gain causes rats to reach puberty at weights far lower than the weight required for normal nutritional conditions[15]. As a result, when body fat comes to a specific level, puberty is likely to occur[14,15].

By altering kisspeptin production in the arcuate nucleus, leptin can indirectly control gonadotropin secretion from the hypothalamus[16]. In addition to its stimulatory actions on the hypothalamus, leptin directly affects the anterior pituitary[17]. Gonadotropin-releasing hormone (GnRH), which is produced by the hypothalamic-pituitary-gonadal axis and regulates the activity of the testicles during reproduction, stimulates the pituitary gland to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH)[18]. Luteinizing hormone and FSH control steroidogenesis and spermatogenesis in the testis[19]. Leptin is present in men's spermatocytes, and high levels of leptin in the testicles have been associated with defective spermatogenesis[20]. Male fertility may be hampered by obesity. In humans and rodents, obesity can impair spermatic function and reduce sperm motility, viability, and concentration[21,22]. Obese people have a higher BMI and higher leptin levels, contributing to poor sperm quality[23], lower sperm counts[24], and a higher DNA fragmentation index[25]. Leptin and leptin receptor levels are higher in infertile males, implying that leptin has local effects on spermatogenesis and testis function[26]. This study aimed to see how antagonist leptin affects testis histology and testosterone levels in immature male mice.

Citation: Al-Aqbi, Mohammed. Effects of Leptin antagonist treatments on testosterone and testis histological characteristics of immature male mice. Revis Bionatura 2022;7(4) 30. http://dx.doi.org/10.21931/RB/2022.07.04.30
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

Materials and methods

Animal Maintenance

Twenty immature male Swiss random-bred male mice (3 weeks old) with an average weight of 15–20 g were used in this experiment. Mice were housed in an air-conditioned room under a 12-h light-dark cycle (lights on 0700–1900 hours). Water and food in the form of standard pellets were given ad libitum to the mice. Wood shavings were used as bedding, covering cages' bottoms to absorb urine. The bedding would be changed on average every three days to maintain a clean environment for the mice and reduce unnecessary infection.

Experimental Design

Four groups of mice (n = 5/group) were given subcutaneous (sc) injections (100 µl) every 48 h of the following treatments for three times: (i) non-immune Ig (50 µg) as the control group; (ii) anti-leptin antibody (JMCK#16, 50 µg); (iii) ECG (40 IU) with non-immune Ig (50 µg) and (iv) anti-leptin antibody (50 µg) with ECG (40 IU). On the morning of the day, 6 of treatment (0900 h), animals were euthanized by CO_2 asphyxiation and testes, dissected and weighed, and blood was collected. Testes were stored at –20°C for homogenization or fixed in Bouin's solution.

Tissue and Sample Preparation

Testis tissues were homogenized on iv in a handhold homogenizer (IKA® T 10 Basic) for 30 seconds in homogenizing buffer containing (EDTA 5mMol/L, EGTA 5mMol/L, and 0.02% sodium azide). Homogenates were stored at -20°C for required to assay hormones. Mice were euthanized by CO2 asphyxiation, and blood was collected immediately and allowed to clot for 30 minutes before being centrifuged and the serum removed and frozen at -20C. The testes were weighed and placed in 5ml polypropylene vials (Tube 5ml*16pp+Cap NAT). In each animal, one testis was put on ice and then stored at -20C until processed further, and the other testis was fixed in Bouin's solution for 48 hours at room temperature. All the animal experimentation was approved by the University of New England Animal Ethics Committee and is under the NH&MRC Code of Practice for the Care and Use of Animals for Experimental Purposes.

Steroid Assays

Testosterone concentration in serum or tissue homogenate was determined in duplicate by LC/MS/MS using the method of (27) with minor modifications. A Shimadzu UPLC and 8050 triple quadrupole mass spectrometer equipped with a heated electrospray ionization source (HESI) operating in positive ion mode were used. The samples were separated using a Kintetex 2.6u Evo C18 column (2.1 x 50mm 2u particle size) (Phenomenex). Samples were eluted with a gradient from 10% to 100% methanol with 0.2mM ammonium fluoride over 6 minutes. Testosterone was eluted with a retention time of 2.1 and 2.5 minutes, respectively and quantitative analysis was performed in multiple reaction monitoring modes (MRM) of the two most abundant product ions (m/z 361 > 163.1 & 121.1 and 363.2 > 121.2 & 309.3). The inter and intra-assay coefficients of variation were determined to be 4.9% and 2.7%, respectively. The sample results were calculated in serum as pg/ml and pg/mg in tissue homogenates.

Testis Histology

The abstracted testis was fixed in Bouin's solution for 48 hours at room temperature. Fixed testes were trimmed transversely into three parts. The middle part was then immersed in 70% alcohol, followed by immersions in a series of alcohol solutions with ascending concentrations. After dehydration, the tissue samples were processed further before being sectioned using a rotary microtome. A small drop of Mayer's Albumin was placed at the center of the glass slide and spread evenly using a cleaned finger. A drop of distilled water was then placed on the same glass slide, and tissue sections were transferred onto the slide. The glass slides were dried and kept in a slide box. The hematoxylin and eosin (H&E) staining technique would stain the nucleus purple and the cytoplasm pink. The steps of the H&E staining technique included deparaffinization, hydration, hematoxylin and eosin staining, dehydration, and clearing. The features evaluated were the diameter of seminiferous tubules, the diameter of the lumen, and the width of the spermatogonia layer, spermatocytes layer, and spermatid-sperm layer.

Statistical analysis

Two-way analysis of variance followed by the Student-Newmen-Keules multiple range tests was performed using the SAS computer software package (SAS Institute Inx., Cary, NC USA). Data are presented and expressed as means ± standard error of the mean (SEM). Unless otherwise stated, $p < 0.05$ was considered significant.

Results

Testis weights

The relative testicular weights (mg) are shown in figure1. The testicular weights of mice treated with anti-leptin, ECG and anti-leptin with ECG (96.29± 7.32 mg, 99± 11.66 mg and 132.63± 12.92 mg, respectively) were significantly ($p< 0.05$) heavier than the control group (74.87± 5.04 mg). The anti-leptin with ECG group was considerably more severe than the anti-leptin and ECG groups.

Testis Histological characteristics

Relative testis histological characteristics of the mature male mice treated with anti-leptin, ECG, anti-leptin with ECG and the control group are shown in figure 2 and table 1. The seminiferous tubules lumen diameter was significantly ($p<0.05$) more significant in anti-leptin(199.4±11.29 μm), ECG(207.2±13.04 μm), and anti-leptin with ECG (219.4±16.45 µm) than the control group (187.7±12.14 μm).

The diameter of seminiferous tubules lumen of mice treated with anti-leptin and anti-leptin with ECG groups (51.6±5.18 μm and 53.9±9.88 μm respectively) was significantly ($p<0.05$) more significant than the control group (49.3±5.85 μm).

The width of the spermatogonia layer of mice treated with anti-leptin, ECG and anti-leptin with ECG (19.2±5.33 μm, 20.1±4.74 μm and 22.8±6.38 μm respectively) was significantly ($p<0.05$) more significant than the control group (17.1±4.24 μm), while were no significant ($p<0.05$) differences between anti-leptin group and eCG group.

The width of the spermatocytes layer of mice had no significant ($p<0.05$) differences between the anti-leptin group (29.4±4.87 μm) and control group (30.1±4.37 μm). However, the width of the spermatocytes layer of mice trea-

ted with eCG and anti-leptin with eCG (33.2±6.24 μm and 33.5±9.55 μm respectively) was significantly more significant than the control group.

The width of the spermatid-sperm layer of mice treated with anti-leptin, eCG and anti-leptin with eCG (24.9±3.58 μm, 25.3±5.30 μm and 27.8±7.94 μm respectively) was significantly ($p<0.05$) more significant than the control group (23.2±2.26 μm).

Testosterone levels in serum and testis

Testosterone concentrations in the serum of immature male mice treated with anti-leptin, eCG, anti-leptin with eCG and the control group are shown in Figure 3 (a). Testosterone concentrations in the serum of mice treated with anti-leptin and control group were significantly ($p<0.05$) lower than eCG and eCG supplemented with anti-leptin groups. Testosterone concentrations in the serum of mice treated with eCG (4.47± 1.46 ng/ml) were the highest and more significant than the mice that were treated with eCG supplemented with anti-leptin (3.92± 1.32 ng/ml), control group (0.77± 0.14 ng/ml) and anti-leptin (1.02± 0.22 ng/ml).

Testosterone concentrations in the testis of treatment groups were significantly ($p<0.05$) higher than the control group (Fig.3b). Testosterone concentrations in the testis of mice treated with eCG supplemented with anti-leptin (527.69±0.88 ng/testis) were significantly higher than control group (63.28±0.35 ng/testis), anti-leptin group (237.77±0.18 ng/testis) and eCG group (242.87±0.11 ng/testis).

Discussion

Leptin, an ob gene hormone released by adipocytes, is a key player in weight control and serves as a neuroendocrine mediator for reproductive function[17,28]. Leptin acts on the central nervous system (CNS) as a neuroendocrine hormone to modulate the HPG axis's production as a nutritional signal[29]. Leptin seems to play a role in both males' and females' onset of puberty and the maturation of their reproductive systems[30,31]. Compared to controls, leptin administration to *ob/ob* male mice boosted serum levels of FSH and increased testicular weights. In contrast, leptin administration had an inhibitory effect on reproduction in normal males[13]. Exogenous leptin treatment is detrimental to testis morphology and sperm count in normal rats[32,33], and in normal mice[34]. The contribution of leptin to the function of the male reproductive system has been less clear. Therefore, in the present work, we investigated for the first time the effects of *in vivo* anti-leptin treatment on testis morphology and testosterone concentrations in normal immature mice. The current results show testis weights in mice-treated groups were significantly heavier than in the control group.

Moreover, our study showed that the testicular testosterone concentrations were significantly higher in anti-leptin-treated mice than in the control group. These results suggest that anti-leptin treatment positively affects testis weights and testosterone concentrations in the testis. These results correspond with prior studies, which showed that

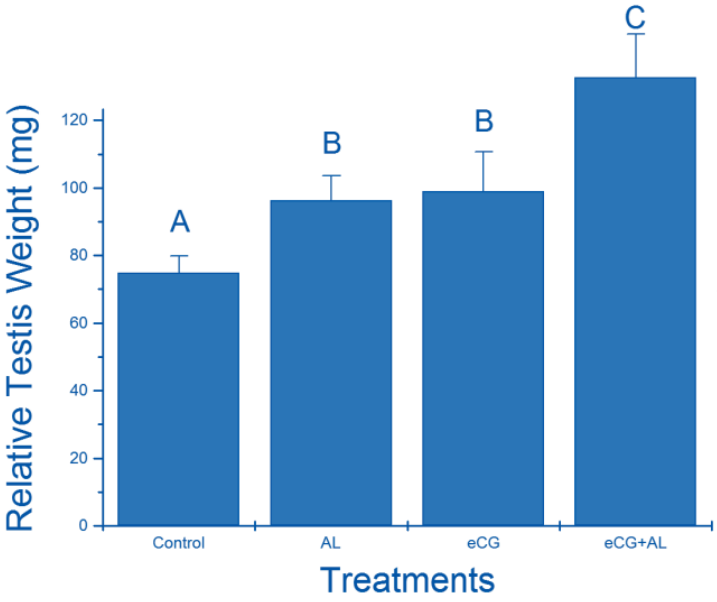

Figure 1. The relative effect of treatment with anti-leptin (AL), eCG, and a combination of AL and eCG on testis weight.

Treatments	Diameter of seminiferous tubules (μm) (mean ± SE)	Diameter of lumen (μm) (mean ± SE)	Width of spermatogonia layer (μm) (mean ± SE)	Width of spermatocytes layer (μm) (mean ± SE)	Width of spermatid-sperm layer (μm) (mean ± SE)
Control (n=5)	187.7±12.14 [a]	49.3±5.85 [a]	17.1±4.24 [a]	29.4±4.87 [a]	23.2±2.26 [a]
AL (n=5)	199.4±11.29 [b]	51.6±5.18 [b]	19.2±5.33 [b]	30.1±4.37 [a]	24.9±3.58 [b]
eCG (n=5)	207.2±13.04 [c]	49.9±7.11 [a]	20.1±4.74 [b]	33.2±6.24 [b]	25.3±5.30 [c]
eCG + AL (n=5)	219.4±16.45 [d]	53.9±9.88 [c]	22.8±6.38 [c]	33.5±9.55 [b]	27.8±7.94 [d]

Different letters denote the level of significance ($P<0.05$)

Table 1. Testis histological characteristics (mean ± SE).

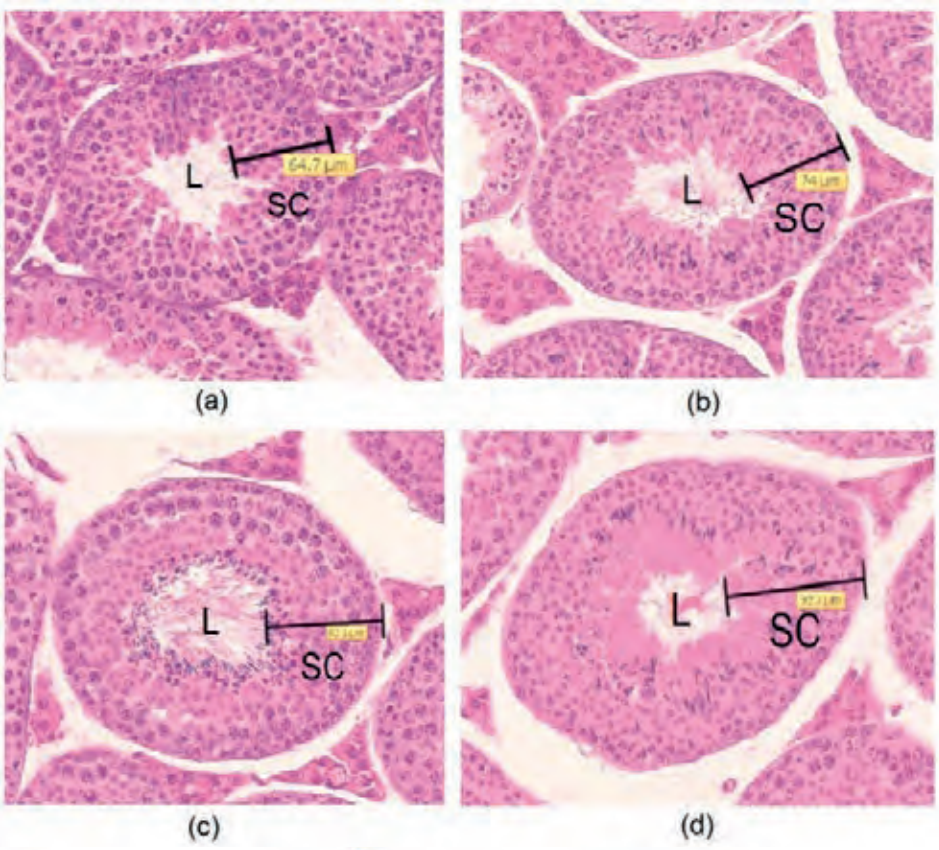

Figure 2. Seminiferous tubules (200x magnification) for (a) C group, (b) anti-leptin (AL) group, (c) eCG group, and (d) eCG+AL group. Note L = lumen of the seminiferous tubule, and SC = spermatogenic cells.

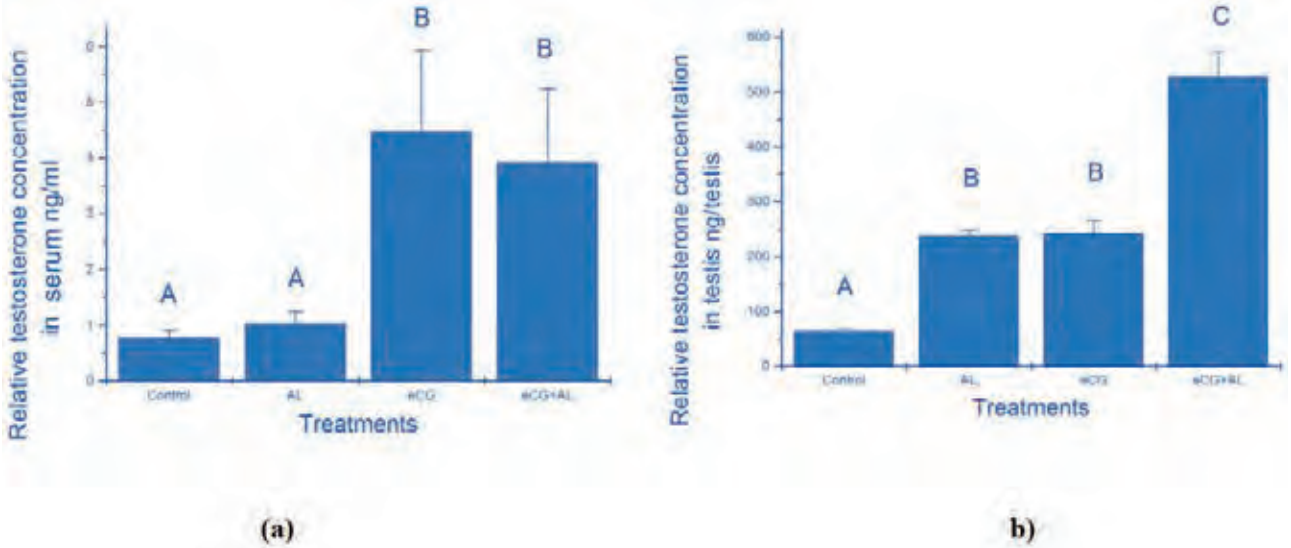

Figure 3. (a) The relative effect of treatment with anti-leptin (AL), eCG, and a combination of AL and eCG on testosterone concentrations in serum; (b) The relative effect of treatment with anti-leptin (AL), eCG, and a combination of AL and eCG on testosterone concentrations in testis.

leptin inhibits the reproductive axis[33,35], claiming that reducing leptin could stimulate reproductive function. Moreover, testosterone concentrations in the testis of eCG with anti-leptin treated mice were significantly higher compared to anti-leptin, eCG and control groups. Anti-leptin and eCG treatment have a synergetic effect on the local concentrations of testosterone in the testis of immature male mice, which increase testosterone concentrations, suggesting that anti-leptin treatment increase the consumption of testosterone that is used for reproductive activities. This hypothesis could explain the non-significant differences in circulating testosterone concentrations between anti-leptin-treated mice and control. Leptin appears to negatively impact male fertility when serum leptin levels are higher than usual in lean mice treated with exogenous leptin. According to our research, anti-leptin treatment induces and increases testis weight and gives better testis histological characteristics in immature mice.

The exogenous treatments of anti-leptin to normal immature male mice increased the diameter of seminiferous tubules and the diameter of the lumen compared to the control. In addition, rustles also showed improvement in the width of spermatogenic cells, which includes the spermatogonia layer, spermatocytes layer and spermatid-sperm layer in the seminiferous tubules. In all of the previous layers, increasing their thickness has been shown in our results.

To maintain ongoing male fertility, the testes continuously create millions of spermatozoa each day during steady-state spermatogenesis[36]. The self-renewal and differentiation of spermatogonial stem cells, a tissue-specific stem cell population recognized in mammals, are necessary for this process[37,38]. Testis weights and seminiferous tubule diameter were increased by the reduction of leptin levels in normal immature male mice. The reproductive pathways of the male mouse are significantly influenced by leptin[39]. The results of testis histological characteristics research revealed the process of spermatogenesis. Spermatogenesis and male fertility are maintained in part by testosterone signaling[40]. In hypothesis, the reduction of leptin in males induces Leydig cells to produce more testosterone in the testis. Leydig cells located in the interstitial compartment are the source of testosterone in the testes. Therefore, it is not surprising that testicular testosterone levels are much higher than blood serum levels[41], suggesting a boost of sperm counts and testosterone concentrations in the testis and improving fertility in immature mice. Moreover, results also showed a synergistic effect of anti-leptin and eCG on the diameter of seminiferous tubules, the diameter of the lumen, and the width of spermatogenic cells. All these parameters were significantly higher in treatment groups compared to controls. eCG is known to have LH-like action[42], leading to increased testosterone. Baker et al. demonstrated that dihydrotestosterone (DHT) is the primary active androgen in immature testis, whereas, in mature testis, the dominant androgen is testosterone[43]. Dihydrotestosterone is converted to testosterone by testicular 5α-reductase activity[44]. The increased weight of testis in mice treated with anti-leptin plus eCG may be attributed to the action of DHT and mesenchymal/epithelial interactions.

Conclusions

Our study implicates the effect of leptin antagonist treatments on testosterone and testis histological features of immature male mice. The treatment of anti-leptin with or without eCG in juvenile male mice improves testis histological features and increases testis weights and testicular testosterone concentrations.

Funding

This research received no external funding.

Institutional Review Board Statement

All animal experimentation was approved by the University of New England Animal Ethics Committee and followed the NH&MRC Code of Practice for the Care and Use of Animals for Experimental Purposes.

Data Availability Statement

This study's study data and materials are available upon request.

Acknowledgments

The author acknowledges the assistance of the school of Science and Technology at the University of New England, Armidale, NSW, Australia. Thanks to Professor James R McFarlane for his collaboration in the research process.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ARTICLE / INVESTIGACIÓN

The impact of different potassium concentrations on the yield of mungbean (*Vigna radiata L.*)

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DOI. 10.21931/RB/2022.07.04.31

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Abstract: To investigate potassium's impact on mungbean yield, a field experiment was carried out in the Kut that was the Agriculture Directorate of Wasit in season 2018- 2019. This experiment compared four different potassium amounts (0, 1000, 2000 and 3000 mg.L^{-1}), symboled K0, K1, K2 and K3, respectively. The findings of this research indicated that there were significant effects on pod length, the total number of pods per plant, seeds per pod, biological yield and grain yield, with treatment K3 achieving the highest average of 10.49 cm, 35.83 pods plant^{-1}, 10.60 seeds pod^{-1}, 1.430 ton ha^{-1}, and 2.026 ton ha^{-1}, respectively. At the same time, neither the ha harvest index nor the 100-seed weight was significantly impacted.

Key words: Potassium, seed yield, biological yield, harvest index, mungbean.

Introduction

Not only does the mung bean (*Vigna radiata L.*), an essential leguminous crop with a high nutritional value, play a significant role in humans' food. They also contribute to the enhancement of soil fertility through Nitrogen fixation by the bacterial node in their roots. In comparison to other types of leguminous, its seed is more delicious, nutritious, readily digestible, and does not cause flatulence[10]. Mung bean is a type of legume that matures in a relatively short amount of time. This crop is well-known and widely grown in the south and Southeast Asia, and it is a highly significant source of protein[6].

This plant prevents soil erosion as a covering plant, enriches and fertilizes the soil through biological nitrogen fixation, and produces green feed[4].

Mung bean is one of the most extensively farmed food legumes in tropical areas worldwide. It is also one of the most popular food legumes[3].

Mung bean's growth, development, and output are significantly impacted by how fertilizers are administered, making this one of the most crucial aspects of crop production[5].

The third macronutrient is potassium, which comes after phosphorus and nitrogen, and is the macronutrient that plants need for growth and military transport. It plays an essential role as a macronutrient in plants and crop production in a sustainable way[9]. It is also currently second only to nitrogen. Regarding the importance of this crop, the root system facilitates the transport of the products of photosynthesis to the economic part of the plant, which improves yield in terms of quantity and quality[17].

Potassium plays a significant role in forming protein and deepening the root system; potassium is considered one of the most important and influential elements in harvesting productivity[13]. This is because it activates more than 75 enzymes that contribute to completing multiple critical biological activities in the plant. Additionally, potassium contributes to the process of photosynthesis, and it helps the plant complete numerous important physical activities[2].

Hussain *et al.* (2011)[7] stated that potassium displayed an unusual reaction in growth and production when they did a field experiment on mung bean crops at the Faisalabad University of Agriculture; the investigation was conducted on mung bean crops According to Salih *et al.* (2012)[15], the use of potassium fertilizer had a substantial impact on the amount of dry matter and grain produced. According to Al-Shaheen *et al.* (2016)[2], the Mung bean had a favorable response to applying potassium fertilizer.

The information presented above, This study was conducted to know the best concentration for spraying the mung crop, which achieves the highest yield and the best quality and also reduces the ground application of potassium fertilizer, reducing economic costs.

Materials and methods

Location of the experiment

The central Kut, where the experiment was carried out, is located in the Kut district of the Wasit Province. This area is located at (Longitude 32'-44o and 36'-46o East and latitudes 31o-57' and 31'-32' North). Before the planting of the crop, composite soil samples were gathered from the area being experimented. Before planting the crop, soil samples were collected from 0 to 40 cm depths and then tested using various soil analysis methods to evaluate the soil's chemical and physical characteristics. These results are found in the table (1)[14].

Citation: Al-Wardy M, Khuit S, Al khafagi, Haidar K K. The impact of different potassium concentrations on the yield of mungbean (Vigna radiata L.). Revis Bionatura 2022;7(4) 31. http://dx.doi.org/10.21931/RB/2022.07.04.31
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

Publisher's Note: Bionatura stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Measured Character		Value	measuring unit
pH		7.9	
Electrical conductivity (EC)		1.6	dS m^{-1}
Nitrogen		31	mg kg^{-1}
Phosphorus		12	
Potassium		40	
Soil components	Sand	31	gm kg^{-1}
	Silt	26	
	Clay	43	
Soil texture		Clay	

Table 1. Chemical and physical characteristics.

Study treatments and design

A randomized complete block design was utilized in the experiment, and each plot had an area of 4 m^2, with three replications. Foliar potassium spraying at four different concentrations (K_2O 43 %), as follows: control (K0), 1000 (K1), 2000 (K2), and 3000 (K3) $Mg.L^{-1}$.

Agronomic practices

On August 9, during the 2018 growing season, mung bean seeds were planted, and there was a distance of 50 cm between rows. Phosphate fertilizer was applied all at once before planting, considering the possibility of (75 Kg. ha^{-1}). While the nitrogen fertilizer in the 40 kg ha^{-1} was added after 16 days had passed since seeding the seeds.

In contrast, the foliar fertilizer potassium spraying (K_2O 43 %) was applied when flowering first began in three separate applications of equal strength.

Using a basin irrigation approach, plots were irrigated simultaneously right after planting using the same irrigation schedule. The plants' needs and the environment's characteristics were considered while determining the length of time between irrigations. Hoeing was used to keep the field free of weeds so we could plant. At harvest time (the last week of October 2019), plants were counted in each net experimental unit. In addition, five plants were randomly selected from each experimental unit to conduct further research. The following information was kept in the log: length of the pod (cm), number of pods per plant (pod $plant^{-1}$), number of seeds per pod (seed pod^{-1}), 100-grain weight (g), grain yield (ton h^{-1}), biological yield (ton h^{-1}), and harvest index.

Statistical analysis

The data were analyzed using GenStat, and the means were compared using the LSD test at 0.05 probability levels[16].

Results

K3 treatment supplied the highest average of the pod length (10.49 cm), and the treatment K0 resulted in the lowest average (8.85 cm). These results go along with what is shown in (Table 2), which demonstrates that there is a significant relationship between potassium levels and pod length[13]. who stated the effect of positive potassium in raising the pod length might be related to the relevance of concentration nutrients processed during photosynthesis, which resulted in higher division and elongation of the pod cells.

Significant impacts on the number of plant pods were observed when potassium levels were varied (Table 2). When administered at a concentration of 3000 mg/L, potassium produced the most significant number of pods per plant (35.83). The control treatment required a minimum number of pods, which was 29.25. These findings are consistent with (8) as well as (9). The number of pods can be attributed to the element potassium in the composition of many plant compounds. Potassium is one of the components of proteins, enzymes, and chlorophyll; as such, it is incorporated into all biological processes, including enzymatic reactions and photosynthesis[13].

There was a substantial correlation between the different potassium concentrations and the number of seeds produced by each pod (Table 2). When sprayed at a level of 3000 $mg.L^{-1}$, potassium gave the highest quantity of seeds per pod, which came out to be 10.60. In plots where potassium was not given to the soil, the lowest number of seeds per pod (9.0 seeds per pod-1) was achieved. These results are consistent with (9), (11), respectively[13]. In addition to the increase in the availability of other nutrients, the K treatment also facilitated the movement of photosynthates; the primary reason for the rise in the number of seeds may be because protein synthesis from source to sink was increased. The seeds number per pod was significantly altered after potassium was administered to the plants[7].

When varied amounts of potassium were used, the potassium levels in 100 seeds' worth of mung bean did not significantly differ. (Table 2).

The results are shown in (Table 3) that there is a significant relationship between the potassium fertilizer concentrations and the seed yield. The K3 treatment produced the highest rate of seed yield (1.430 tons ha-1), while treatment K0 gave the lowest rate of seed yield (1.127 tons ha-1). The results of (9), (11) and (13) led to the same conclusion[13]. This may be owing to the high nutrient content of the leaves, which has enhanced the effectiveness of photosynthesis and raised the quantity of glucose needed to create the protein and fat stored in the seeds, hence increasing seed production[12].

Significant influence on the biological yield caused by potassium concentrations of 3000 mg L^{-1}. The highest possible biological yield (2.026 ton ha^{-1}) was achieved with an application of potassium at a concentration of 3000 mg L^{-1} (Table 3)

Control treatment had the lowest overall biological yield (1.673 ton ha^{-1}) of all the effects. These results were in agreement with those of (1), who observed the positive role of potassium levels in the provision of sufficient quanti-

ties of photosynthesis products, their transfer and collection within the plant, which exploits its high efficiency in the production of dry matter, which led to an increase in biological yield. Potassium levels were found to play a role in providing sufficient quantities of photosynthesis products. This is indicated by (17), who confirmed that increasing potassium levels increase grain yield and biological yield, transfer, and collection within the plant. There was not a significant difference in the harvest index of mung bean between different K Concentrations. (Table 3).

Treatments	Pod length (cm)	No. of pods	No. of seed	100 seed weight (gm)	
K_0	8.85	29.25	9.00	7.61	
K_1	9.27	31.67	9.50	7.98	
K_2	10.37	35.40	10.57	8.07	
K_3	10.49	35.83	10.60	8.26	
LSD (0.05)	0.541	0.630	0.750	N.S	K =

Table 2. Effect of fertilizer potassium on Pod length, number of pods, seeds number and 100 seed weight of mung bean (Season 2018-2019).

Treatments	Seed yield (ton ha^{-1})	Biological yield (ton ha^{-1})	Harvest index	
K_0	1.127	1.673	67.38	
K_1	1.324	1.894	70.01	
K_2	1.418	2.022	70.12	
K_3	1.430	2.026	70.62	
LSD (0.05)	0.051	0.079	N.S	K =

Table 3. Effect of fertilizer potassium on Seed yield, Biological yield and Harvest index of mung bean (Season 2018-2019).

Conclusions

Among different potassium Concentrations, the application of potassium is 2000 and 3000 mg. L^{-1} was found to be the most effective, which exhibited significant yield attributes and productivity in terms of grain, and biological yield.

Using potassium fertilizer resulted in a favorable response from the mung bean plant. We have concluded that, compared to the other macronutrients, potassium (K) is of comparable importance and should be added to the mungbean crop. The experiment demonstrated a reaction in terms of yield due to potassium administration levels of 2000 and 3000 mg. L^{-1}.

Concentrations of K, which are based on one season experiment, influenced the parameters mentioned above of mung bean; additional tests may be required to evaluate the product's effectiveness in various environmental conditions and types of soil.

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ARTICLE / INVESTIGACIÓN

Nano fertilizer spraying affects the yield of Sorghum (*Sorghum bicolor L.*) varieties.

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DOI. 10.21931/RB/2022.07.04.32

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Abstract: The field experiment was carried out at the Abu Ghraib Agricultural Research Station Iraq (AGARSI), affiliated with the Agricultural Research body-Ministry of Agricultural of Iraq, to study the spraying with nano fertilizers and the stages of the yield and yield components of varieties of Sorghum. The experiment was designed as a spilled-spilled-plot according to the (RCBD) of three replicates. The main plots involved three varieties of Sorghum (Rabih, Lilo, and Inqath). In contrast, the subplots involved spraying the nano-fertilizers (Nano-Iron and Nano-Zinc) and the control treatment (distilled water). The sub-sub-plots involved the spray stages (Spraying at emerging five leaves, spraying at emerging the last folded leaf, and spraying at 50% flowering stage). The results showed that there were significant differences in all traits, where the variety Inqath had the highest was superior in panicle weight (g), the number of grains per panicle, (CGR)($gm^{-2}.day^{-1}$), and grain yield ($tons.ha^{-1}$). Nano iron was also superior in four traits, panicle weight (g), the number of grains per panicle (CGR)($g.m^{-2}.day^{-1}$), and grain yield ($tons.ha^{-1}$). For the spray stage, the stage of the emergence of five true leaves (S1) was significantly superior in the traits of panicle weight (g), the number of grains per panicle, (CGR)($g.m^{-2}.day^{-1}$), and grain yield ($tons.ha^{-1}$).

Key words: Nano fertilizers, Nano-Iron, Nano-Zinc, grain yield.

Introduction

Sorghum is a cereal and summer forage crop that ranks fifth in the world in terms of cultivated area and production behind wheat, rice, maize, and barley[10]. Sorghum grain contains 65% starch, 12-10% protein, and 3% fat and contains vitamins, salts, and minerals necessary for body growth[8]. The importance of this crop lies in the fact that it is a genotype tolerant of drought and heat. It is a staple crop in areas not suitable for the cultivation of wheat. Despite many varied uses of this crop, its grain is accompanied by several problems, including weak field establishment. This is a significant reason for the yield decline, especially in arid and semi-arid areas, including Iraq[2]. However, the appropriate variety was not the only factor in achieving the highest productivity; there are other factors, including the use of chemicals fertilizers, despite their importance and indispensability, have adverse effects on the environment and humans and in addition to their high costs when added in large quantities to the soil. Therefore, we must reduce the risks of these fertilizers on the one hand and increase the efficiency of plant growth and productivity, on the other hand, at the lowest economic cost. Foliar nourishment is the best method that has these advantages.

The foliar fertilization technique with nano iron and nano zinc is an easy and economical method and is environmentally friendly. Despite that, it is a complement to soil fertilization and not a substitute for it. Iron is essential for plant growth and plays a substantial role in chlorophyll constitution[9]. Despite that, it is not included in its composition. It plays a critical role in synthesizing enzymes responsible for chlorophyll assimilation. It also participates in the design of many enzymes necessary for respiration[11]. Zinc also has an essential role in constituting tryptophan, the amino acid from which indole acetic acid IAA is formed[3]. Zinc also contributes to the construction of each of the glycol dehydrogenases necessary for the assimilation of the proteins, and it is necessary for the chlorophyll formation and plays an essential role in the pollen grain germination on the stigmas[13]. Nano fertilizers are nutrient carriers of 1-100 nm in size. They are various, involving the macronutrients such as nitrogen, phosphorous, and potassium fertilizers, singly or in combination, mini-nutrients, and micro-nutrients, which are either coated or encapsulated, or loaded on carriers, which means that the fertilizer will be slow to release[14]. And that the nano-encapsulation process reduces costs and increases the productivity of the crop; that is, it increases the farmer's profitability.

Materials and methods

The field experiment was carried out at the Abu Ghraib Agricultural Research Station Iraq, affiliated with the Agricultural Research body - Ministry of Agriculture of Iraq, during the spring season of 2021-2022. In a silty clay mixture soil, some of its chemical and physical properties are shown in Table 1.

The experiment was designed as a spilled _spilled_ plot according to the (RCBD) of three replicates. The main fields involved three varieties of Sorghum (Rabih (V1), Lilo (V2), and Inqath (V3)), while the subplots involved spraying

Citation: Al-Rubaie M A J, Mohammed A R, Ali H S. Nano fertilizer spraying affects the yield of Sorghum (Sorghum bicolor L.) varieties. Revis Bionatura 2022;7(4) 32. http://dx.doi.org/10.21931/RB/2022.07.04.32
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

the nano-fertilizers (Nano Iron at a concentration of 1000 mg. L-1 (N1) and Nano-Zinc at a concentration of 1000 mg. L-1 (N2)), in addition to the control treatment (spray with distilled water) (N0). The sub_sub_ plots were involved in the spray stages (Spraying at emerging five leaves(S1), spraying at emerging the last folded leaf (S2), and spraying at 50% flowering stage (S3)[19].

Studied traits

1- Panicle weight (g)
2- Number of grains per panicle
3-100-grains weight (g)
4-Crop growth rate for the period from 100% inflorescence to the physiological maturity ($gm^{-2}.day^{-1}$) (CGR)
5-Grain yield ($tons.ha^{-1}$).

Results

Panicle weight

Results in tables (2) and (3) show a significant difference between the varieties in the panicle weight. The variety Inqath (V2) produced the highest trait average, amounting to 164.54 and 162.78g, compared to Lilo (V3), achieving the lowest panicle weight averaging 127.13 and 116.58g for the two study seasons, respectively. The difference between the varieties in panicle weight may be due to their difference in the number of grains in the panicle (Tables 2 and 3), which is consistent with the findings of Al-Kubaisi and Najm[5,15] who indicated that sorghum varieties differed significantly in panicle weight.

Results listed in Tables (2) and (3) illustrate a significant difference in the panicle weight due to the effect of the nano-fertilizer spraying treatments. Spraying nano-iron (N1) achieved the highest character average, amounting to 154.79 and 153.40g, compared to the control treatment (N0), reaching the lowest panicle weight averages of 134.27 and 125.84 for the two study seasons, respectively.

The binary interaction between the varieties and the nano-fertilizer spraying was significant in Tables (2) and (3) and the panicle weight. The plants of the variety Inqath (V2) sprayed with nano-iron (N1) achieved the highest value of the interaction, which was 173.22 and 173.61g for the two seasons, respectively, which did not differ significantly from the plants of the same variety sprayed with nano-zinc (N2) in the second seasons which achieved 169.63g. While the plants of the Lilo variety (V3) sprayed with distilled water only (N0) achieved the lowest interaction value of 118.48 and 104.48g for the two seasons, respectively.

The interaction between the varieties and the stages of spraying the nano-fertilizers affected the panicle weight significantly in Tables (2) and (3). The plants of the variety Inqath (V2) spread at the stage of the emergence of five true leaves (S1) gave the highest interaction values wof170.52 and 171. On the contrary, plants of the variety Lilo (V3) sprayed at the stage of 50% of inflorescence (S3) reached the lowest values of the interaction, which were 123.94 and 110.43g for the two seasons of the study. Those did not differ significantly from plants of the same variety (V3) sprayed at the stage of the last folded leaf (S2) emergence, giving 126.50g in the first season of the study.

Concerning the binary interaction between the spraying of nano-fertilizers and the spray stages, the effect on the head weight was significant in Tables (2) and (3), as the nano-iron (N1) spraying at the location of the emergence of five true leaves (S1) attained the highest value of the interaction, amounted to 164.46 and 165. In contrast, the control treatment (N0) at the three stages (S1, S2, and S3) achieved the lowest values of the interaction, which were 135.54, 132.80 and 134.48g in the first season and 127.22,

Variable		2021	2022	Units
Soil texture		Alluvial clay mixture		
Soil Separators	Sand	15.1	14.5	%
	Silt	53.2	54.3	
	Clay	31.7	31.2	
Electrical Connection (EC)		4.25	4.14	Desmol M-1
CEC		21.37	20.87	Desmol Kg soil - 1
Ph		7.49	7.54	-
Dissolved ions	Co_3	Nill	Nill	mEq L-1
	$CaCO_3$	22.94	23.56	
	Ca	18.02	18.63	
	K	1.11	1.06	
	Mg	11.12	10.49	
	Na	8.23	8.66	
	CI	22.3	22.73	
	HCO_3	5.27	5.71	
	SO_4	9.13	8.39	
N		33.58	36.68	Mg Kg soil-1
P		13.95	14.11	
K		127.14	131.56	

Table 1. Some chemical and physical properties of field soil for the years 2021- 2022.

varieties (V)	Nano Fertilizer (N)	Stages (S)			V x N
		S1	S2	S3	
V1	N0	132.75	132.25	133.54	132.85
	N1	171.46	160.62	141.26	157.78
	N2	147.69	152.29	138.43	146.14
V2	N0	153.74	150.68	150.06	151.5
	N1	183.06	175.79	160.81	173.22
	N2	174.75	173.56	158.36	168.89
V3	N0	120.12	115.47	119.84	118.48
	N1	138.85	133.28	127.95	133.36
	N2	133.88	130.75	124.03	129.55
LSD 0.05			5.78		3.85
S × V					
Var.		S1	S2	S3	average Var.
V1		150.64	148.39	137.74	145.59
V2		170.52	166.68	156.41	164.54
V3		130.95	126.5	123.94	127.13
L.S.D 0.05			3.62		3.16
S × N					
(N)		S1	S2	S3	average Nano Fer.
N0		135.54	132.8	134.48	134.27
N1		164.46	156.57	143.34	154.79
N2		152.11	152.2	140.27	148.19
LSD 0.05			3.28		2.15
average stages		150.7	147.19	139.36	
LSD 0.05			1.86		

Table 2. Shows the effect of spraying nano fertilizers and the y stage on the Panicle weight of Sorghum varieties (The first season).

125.99 and 124.30g in the second season at the three stages respectively without significant difference between them.

The triple interaction between the study factors was significant in the panicle weight in Tables (2) and (3). The plants of the variety Inqath (V2) sprayed with nano-iron (N1) at the stage of the emergence of five true leaves (S1) gave the highest values of the interaction, which were 183.06 and 186.32g for the two study seasons. They did not differ significantly from the plants of the same variety sprayed with nano-zinc at the emergence stage of five true leaves (V2N2S1) that gave 179.68g in the second season. While the plants of the variety Lilo (V3) sprayed with distilled water only (N0) gave, at the three stages (S1, S2, and S3), the lowest values of the interaction reached 120.12, 115.47, and 119.84g in the first season and 105.70, 106.84 and 100.89g in the second season and for the three stages respectively without significant difference between them.

The results above show the positive role of spraying nano iron at the stage of the emergence of five true leaves in increasing the percentage of fertilized florets and then increasing the number of grains in the panicle, which led to an increase in the weight of the panicle.

Number of grains per panicle

Results in tables (4) and (5) show a significant difference between the sorghum varieties in the number of grains per panicle. Variety Inqath (V2) attained the highest average of 2681.9 and 26849.8 grains. panicle^{-1}. The difference may be due to the genetic nature. This result is consistent with those found by (4) and (18).

Spraying nano-iron (N1) was significantly superior and

varieties (V)	Nano (N) Fertilizer	Stages (S)			V x N
		S1	S2	S3	
V1	N0	127.82	127.13	128.88	127.94
	N1	178.95	165.73	139.39	161.36
	N2	148.66	154.4	135.54	146.2
V2	N0	148.15	143.99	143.13	145.09
	N1	186.32	176.73	157.77	173.61
	N2	179.68	175.11	154.11	169.63
V3	N0	105.7	106.84	100.89	104.48
	N1	132.71	125.13	117.88	125.24
	N2	125.92	121.67	112.53	120.04
LSD 0.05		6.91			4.49
S × V					
Var.		S1	S2	S3	average Var.
V1		151.81	149.08	134.6	145.17
V2		171.38	165.28	151.67	162.78
V3		121.44	117.88	110.43	116.58
L.S.D 0.05		4.13			3.4
S × N					
(N)		S1	S2	S3	average Nano Fer.
N0		127.22	125.99	124.3	125.84
N1		165.99	155.86	138.35	153.4
N2		151.42	150.39	134.06	145.29
LSD 0.05		3.99			2.65
average stages		148.21	144.08	132.23	
LSD 0.05		2.25			

Table 3. Shows the effect of spraying Nano fertilizers at the spray stage on the Panicle weight of varieties of Sorghum (The second season) .

attained the highest average number of grains per panicle, which amounted to 2524.9 and 2471.2 grains. panicle^{-1}, compared to the control treatment (N0), achieved the lowest average for the trait of 21111.3 and 2121.9 grains.head^{-1} for the two study seasons, respectively. The superiority of nano-iron spraying may be due to an increase in the products of photosynthesis, their transmission and accumulation in the reproductive parts, and the reduction of competition between them. This increases the percentage increase in the level of necessary elements that help in the success of pollination and double fertilization. Then, the growth of the number of grains in the panicle is in line with the results obtained by (12) in his study on wheat. That indicated a significant effect of nano iron spraying on the number of grains in the ear.

The results in Tables (4) and (5) show that nano fertilizer spraying at the five true leaf emergence stages (S1) was significantly higher than the highest average number of grains per panicle, which amounted to 2465, 3 and 2414.6 grains panicle^{-1}, compared to spraying at the 50% flowering stage (S3), which reached the lowest mean for the trait, amounting to 2189.2 and 2191.5 grains panicle^{-1} for the two study seasons, respectively.

The number of grains per panicle is affected by several factors, including the availability of nutrients at the appropriate stage of plant growth. So the superiority of plants sprayed at the growth stage of five actual leaf emergence may be due to the essential role of feeding these elements at the early stages of plant growth in stimulating vegetative growth.

The effect of binary interaction between varieties and nano fertilizer spraying was significant on the number of grains per panicle in tables (4) and (5). The plants of the variety Inqath (V2) sprayed with nano-iron (N1) achieved the highest interaction value of 2846.0 and 2788.3 grains of panicle^{-1}, respectively; however, they did not differ significantly from the plants of the same variety (V2) sprayed with nano-zinc (N2) which gave 2788.9 and 2739.5 grains. panicle^{-1}.

While Lilo plants sprayed with distilled water only (N0) achieved the lowest interaction value of 1814.4 and 1859.7 panicle^{-1} for two seasons, respectively.

As for the binary interaction between the varieties and the stages of spraying the nano-fertilizers, it had a significant effect on the number of grains per panicle Tables (4) and (5), as the plants of the variety Inqath (V2) sprayed at the stage of the emergence of five true leaves (S1) gave the highest value of the interaction, which was 2813.1 and

varieties (V)	Nano (N) Fertilizer	Stages (S)			V x N
		S1	S2	S3	
V1	N0	2157	2097	2072.8	2108.9
	N1	2938.7	2663.1	2226.8	2609.5
	N2	2462.9	2497	2170.5	2376.8
V2	N0	2455.6	2394.6	2381.9	2410.7
	N1	3016	2925.1	2597	2846
	N2	2967.7	2851.4	2547.6	2788.9
V3	N0	1832.1	1848.9	1762.2	1814.4
	N1	2228.5	2117.6	2011.3	2119.1
	N2	2129.1	2067.1	1933	2043.1
LSD 0.05		106.4			71.1
S × V					
Var.		S1	S2	S3	average Var.
V1		2519.5	2419	2156.7	2365.1
V2		2813.1	2723.7	2508.8	2681.9
V3		2063.2	2011.2	1902.2	1992.2
L.S.D 0.05		66.7			58.3
S × N					
(N)		S1	S2	S3	average Nano Fer.
N0		2148.2	2113.5	2072.3	2111.3
N1		2727.7	2568.6	2278.4	2524.9
N2		2519.9	2471.8	2217	2402.9
LSD 0.05		60.3			39.7
average stages		2465.3	2384.6	2189.2	
LSD 0.05		34.1			

Table 4. Shows the effect of spraying nano fertilizers and spray stage on the number of grains per panicle of varieties of Sorghum (The first season).

2767.1 grain. panicle^{-1}, while the plants of the variety Lilo (V3) sprayed at (S3) achieved the lowest value of the interaction, which was 1902.2 and 1932.3 grains.panicle^{-1} for the two seasons, respectively.

Concerning the binary interaction between spraying nano-fertilizers and the spray stages, it had a significant effect on the number of grains per panicle Tables (4) and (5), as the spraying of nano-iron (N1) at the stage of the emergence of five true leaves (S1) achieved the highest value of the interaction, which amounted to 2727.7 and 2644.3 grains. panicle^{-1}, while the control treatment (N0) at the three stages (S1, S2, and S3) achieved the lowest value of the interaction, which in the first season was 2148.2, 2113.5, and 2072.3 grains.panicle^{-1}, and in the second season 2147.9, 2110.3 and 2107.6 grains. panicle^{-1} for the three stages, respectively and without significant difference between them.

The effect of the triple interaction between the study factors was significant in the number of grains per panicle in Tables (4) and (5), the plants of the variety Inqath (V2) that were sprayed with nano-iron (N1) at the stage of the emergence of five true leaves (S1) gave the highest value of the interaction which was 3016.0 and 2962.7 grains.panicle^{-1}.

It did not differ significantly from plants of the same variety sprayed with nano-zinc at the stage of the emergen-

varieties (V)	Nano Fertilizer (N)	Stages (S)			V x N
		S1	S2	S3	
V1	N0	2100.4	2091.4	2063.8	2085.2
	N1	2765.2	2598.3	2196.4	2519.9
	N2	2356.7	2435.6	2147.7	2313.3
V2	N0	2460.2	2407.6	2396.9	2421.6
	N1	2962.7	2820.6	2581.6	2788.3
	N2	2878.5	2800.5	2539.6	2739.5
V3	N0	1883.2	1831.8	1862	1859.7
	N1	2205	2109.3	2001.3	2105.2
	N2	2119.4	2065.7	1933.6	2039.6
LSD 0.05			99		63.4
S × V					
Var.		S1	S2	S3	average Var.
V1		2407.4	2375.1	2136	2306.2
V2		2767.1	2676.2	2506.1	2649.8
V3		2069.2	2002.2	1932.3	2001.3
L.S.D 0.05			61.6		52.2
S × N					
(N)		S1	S2	S3	average Nano Fer.
N0		2147.9	2110.3	2107.6	2121.9
N1		2644.3	2509.4	2259.8	2471.2
N2		2451.5	2433.9	2207	2364.1
LSD 0.05			56.2		35.3
average stages		2414.6	2351.2	2191.5	
LSD 0.05			32.6		

Table 5. Shows the effect of spraying Nano fertilizers and spray stage Number of grains per panicle of varieties of Sorghum (The second season).

ce of five true leaves (V2N2S1), which gave 2967.7 and 2878.5 grains.panicle^{-1}, and the plants of the same variety sprayed with nano-iron at the stage of the emergence of the last folded leaf (V2N1S2) in the first season. While plants of variety Lilo (V3) sprayed with distilled water only (N0) in the three stages (S1, S2, and S3) achieved the lowest interaction value of 1832.1, 1848.9, and 1762.2 grains. panicle^{-1} in the first season, 11.62, 11.72 and 11.17 grains.panicle^{-1} in the second season for the three stages, respectively, without significant difference between them.

1000-grain weight

Results in the two tables (6) and (7) show significant differences between the sorghum varieties in 1000-grain weight. The variety Rabih (V1) gave the highest average of this trait, amounting to 27.96 and 30.89g, which did not differ significantly from the variety Inqath giving 27.63 and 30.32g. Variety Lilo (V3) produced the lowest 1000-grain weight averaging 24.50 and 24.17g in the two seasons. The difference between the sorghum varieties in the 1000-grain weight is due to the difference in the genetics of each variety's nature. This result agrees with the findings of Al-Maini and Shammari[6,18].

Spraying nano-zinc (N2) was significantly superior in 1000-grain weight achieving the highest weight amounted to 28.62 and 30.86g, compared to the control treatment (N0), which produced the lowest average for the trait of 24.17 and 25.65g for the two study seasons Tables (6) and (7). The superiority of spraying nano-Zn in grain weight may be due to the physiological effect of zinc on the formation of amino acids and carbohydrates (7). This result agrees with the findings (17) about Sorghum, indicating a significant increase in Grain weight when spraying with nano-zinc.

The results of Tables (6) and (7) show that spraying nano-fertilizers in the stage of the emergence of the last folded leaf (S2) was significantly superior in 1000-grain weight giving the highest value of which amounted to 27.58 and 29.51g, compared to spraying it at the stage of (S3), which achieved the lowest average of 25.63 and 27.14g for the two study seasons respectively. This shows that spraying nano-micronutrients at the stage of the emergence of the last folded leaf (S2) was the most effective in transferring the photosynthetic products to the fertilized florets.

The effect of the binary interaction between the varieties and spraying the nano-fertilizer was significant in the 1000-grain weight Tables (6) and (7). The Rabeh variety plants (V1) spread with nano-zinc (N2) achieved the highest interaction values of 30.06 and 33.62g. They did not differ significantly from the plants of the variety Inqath (V2) sprayed with nano-zinc (N2), which gave 29.72 and 33.50g, while plants of the variety Lilo (V1) sprayed with distilled water only (N0) achieved the lowest interaction values of 22.13 and 22.39g for the two seasons respectively.

The binary interaction between the varieties and the stages of spraying nano-fertilizers affected the 1000-grains weight significantly in Tables (6) and (7). The variety Rabeh's plants (V1) spread at the stage of the emergence of the last folded leaf (S2) gave the highest value of the interaction, which was 28.85 and 32.20g for the two seasons of the study, respectively. They did not differ significantly from the plants of the variety Inqath (V2) sprayed in the stage of the emergence of the last folded leaf (S2) that gave 28.64 gm in the first season. While the plants of the variety Lilo (V3) sprayed at the stage of the emergence of the last folded leaf (S2) achieved 50% inflorescence (S3) and had the lowest interaction values reaching 23.82 and 23.51g for the two seasons, respectively. It did not differ significantly from the plants of the same variety sprayed at the emergence stage of five true leaves (S1), which achieved 24.04 g in the second season.

The binary interaction between spraying nano-fertilizers and the spray stages significantly affected the 1000-grain weight Tables (6) and (7). Spraying nano-zinc (N2) at the location of the appearance of the last folded leaf (S2) gave the highest values of the interaction, which were 30.49 and 32.88g. While the control treatment (N0) at the three stages (S1, S2, and S3) gave the lowest interaction values, which in the first season were 24.08, 24.25, and 24.18g, and in the second season were 25.34, 25.91, and 25.69g for the three stages respectively without significant difference between them.

The effect of the triple interaction between the study factors was significant in the 1000-grain weight Tables (6) and (7). The plants of the variety Inqath (V2) sprayed with nano-zinc (N1) at the stage of the emergence of the last folded leaf (S2) gave the highest values of the interaction, which were 32.55 and 36 for the two seasons of the study respectively, which did not differ significantly from the plants of the variety Rabah sprayed with nano-zinc at the stage of the emergence of the last folded leaf (V1N2S2), which gave 31.80g in the first season. While the plants of the variety Lilo (V3) that were sprayed with distilled water only achieved (N0) at the three stages (S1, S2, and S3), the lowest interaction value were 21.62, 22.46, and 22.30g in the first season and 21.77, 22.73 and 22.67g in the second season at the three stages respectively without significant difference between them.

Crop growth rate for the period from 100% inflorescence to physiological maturity (g.m^{-2}.day^{-1})

The results of tables (8) and (9) show a significant difference between sorghum varieties in the crop growth rate. The variety Inqath (V2) gave the highest average for the trait of 15.79 and 16.93g.m^{-2}.day^{-1} compared to the variety Rabeh (V1), which achieved the lowest standard of 11.94 and 12.63g.m^{-2}.day^{-1} for the two study seasons, respectively. This result is consistent with the findings of (1) and (4), indicating that sorghum varieties differed significantly in the growth rate of their plants.

The results of tables (8) and (9) indicate that spraying nano iron (N1) was significantly superior and achieved the highest growth rate of the crop, which reached 14.78 and 16.08g.m^{-2}.day^{-1}, compared to the control treatment (N0), which attained the lowest average of 12.69 and 13.49g.m^{-2}.day^{-1} for the two study seasons, respectively.

The results of Tables (8) and (9) show that spraying nano-fertilizers at the stage of the emergence of five true leaves (S1) was significantly superior. It attained the highest crop growth rate reaching 14.39 and 15.60g.m^{-2}.day^{-1} compared to spraying it at the stage of 50% inflorescence (S3), which achieved the lowest average, reaching 13.19 and 14.09g.m^{-2}.day^{-1} for the two study seasons, respectively, showing that the role of spraying micronutrients at the early stages of plant growth in stimulating vegetative growth and increasing the plant growth rate for the period from 100% inflorescence until physiological maturity.

The effect of the binary interaction between the varieties and spraying nano-fertilizer was significant on the growth rate of the crop Tables (8) and (9). The plants of the variety Inqath (V2) sprayed with nano-iron (N1) achieved

varieties (V)	Nano (N) Fertilizer	Stages (S)			V x N
		S1	S2	S3	
V1	N0	25.18	25.09	24.97	25.08
	N1	29	29.65	27.57	28.74
	N2	30.33	31.8	28.05	30.06
V2	N0	25.44	25.19	25.29	25.31
	N1	29.06	28.18	26.39	27.88
	N2	29.67	32.55	26.93	29.72
V3	N0	21.62	22.46	22.3	22.13
	N1	25.57	26.17	24.12	25.29
	N2	26.06	27.14	25.03	26.08
LSD 0.05			0.97		0.63
S × V					
Var.		S1	S2	S3	average Var.
V1		28.17	28.85	26.86	27.96
V2		28.06	28.64	26.2	27.63
V3		24.42	25.26	23.82	24.5
LSD 0.05			0.58		0.47
S × N					
(N)		S1	S2	S3	average Nano Fer.
N0		24.08	24.25	24.18	24.17
N1		27.88	28	26.03	27.3
N2		28.69	30.49	26.67	28.62
LSD 0.05			0.56		0.37
average stages		26.88	27.58	25.63	
LSD 0.05			0.32		

Table 6. Shows the effect of spraying nano fertilizers and the spray stage on the 1000-grain weight of Sorghum varieties (The first season).

varieties (V)	Nano (N) Fertilizer	Stages (S)			V x N
		S1	S2	S3	
V1	N0	27.03	27.67	26.91	27.2
	N1	32.75	33.21	29.62	31.86
	N2	34.01	35.71	31.12	33.62
V2	N0	27.23	27.33	27.5	27.35
	N1	31.36	30.42	28.51	30.1
	N2	34.09	36.32	30.09	33.5
V3	N0	21.77	22.73	22.67	22.39
	N1	24.92	25.57	23.53	24.67
	N2	25.44	26.59	24.34	25.46
LSD 0.05			1.22		0.77
S × V					
Var.		S1	S2	S3	average Var.
V1		31.27	32.2	29.22	30.89
V2		30.89	31.36	28.7	30.32
V3		24.04	24.96	23.51	24.17
LSD 0.05			0.74		0.61
S × N					
(N)		S1	S2	S3	average Nano Fer.
N0		25.34	25.91	25.69	25.65
N1		29.68	29.73	27.22	28.88
N2		31.18	32.88	28.52	30.86
LSD 0.05			0.7		0.44
average stages		28.73	29.51	27.14	
LSD 0.05			0.4		

Table 7. Shows the effect of spraying nano fertilizers and spray stage on 1000-grain weight of varieties of Sorghum (The second season).

varieties (V)	Nano (N) Fertilizer	Stages (S)			V x N
		S1	S2	S3	
V1	N0	11.25	10.78	11.13	11.05
	N1	13.17	12.6	11.96	12.58
	N2	12.66	12.34	11.55	12.18
V2	N0	14.69	14.38	14.31	14.46
	N1	17.68	16.84	15.41	16.64
	N2	16.94	16.72	15.16	16.27
V3	N0	12.55	12.49	12.63	12.56
	N1	16.51	15.39	13.42	15.11
	N2	14.07	14.54	13.13	13.91
LSD 0.05			0.59		0.38
S × V					
Var.		S1	S2	S3	average Var.
V1		12.36	11.91	11.55	11.94
V2		16.44	15.98	14.96	15.79
V3		14.38	14.14	13.06	13.86
LSD 0.05			0.37		0.32
S × N					
(N)		S1	S2	S3	average Nano Fer.
N0		12.83	12.55	12.69	12.69
N1		15.79	14.94	13.6	14.78
N2		14.56	14.53	13.28	14.12
LSD 0.05			0.33		0.21
average stages		14.39	14.01	13.19	
LSD 0.05			0.19		

Table 8. Shows the effect of spraying nano fertilizers and spray stage on (CGR) of varieties of Sorghum (The first season).

varieties (V)	Nano (N) Fertilizer	Stages (S)			V x N
		S1	S2	S3	
V1	N0	11.62	11.72	11.17	11.5
	N1	14.13	13.43	12.75	13.44
	N2	13.5	13.11	12.26	12.95
V2	N0	15.57	15.18	15.1	15.29
	N1	19.12	18.23	16.47	17.94
	N2	18.5	18.08	16.15	17.58
V3	N0	13.68	13.61	13.78	13.69
	N1	18.63	17.2	14.75	16.86
	N2	15.62	16.15	14.4	15.39
LSD 0.05			0.64		0.42
S × V					
Var.		S1	S2	S3	average Var.
V1		13.08	12.75	12.06	12.63
V2		17.73	17.16	15.91	16.93
V3		15.98	15.66	14.31	15.31
LSD 0.05			0.39		0.32
S × N					
(N)		S1	S2	S3	average Nano Fer.
N0		13.62	13.51	13.35	13.49
N1		17.73	16.29	14.66	16.08
N2		15.87	15.78	14.27	15.31
LSD 0.05			0.37		0.24
average stages		15.6	15.19	14.09	
LSD 0.05			0.21		

Table 9. Shows the effect of spraying nano fertilizers and spray stage on (CGR) of varieties of Sorghum (The second season).

the highest interaction values of 16.64 and 17.94g.m^{-2}.day^{-1}, which did not differ significantly from the plants of the same variety that were sprayed with nano-zinc (N2) which gave 16.27 and 17.58 g.m^{-2}.day^{-1}. While the plants of the variety Rabih (V1) sprayed with distilled water only (N0) achieved the lowest interaction values of 11.05 and 11. 50 g.m^{-2}.day^{-1} for the two study seasons, respectively.

For the binary interaction between the varieties and the stages of spraying nano-fertilizers, it had a significant effect on the growth rate of the crop Tables (8) and (9). The plants of the variety Inqath (V2) that were sprayed at the stage of the emergence of five true leaves (S1) gave the highest interaction values, which were 16.44 and 17.73 g.m^{-2}.day^{-1}, while the plants of the variety Rabih (V1) sprayed at 50% inflorescence stage (S3) achieved the lowest interaction values of 11.55 and 12.06 g.m^{-2}.day^{-1} for the two study seasons, respectively.

Concerning the binary interaction between the spraying nano-fertilizers and the spraying stages, it affected the growth rate of the crop significantly Tables (8) and (9). Spraying nano-iron (N1) at the stage of the emergence of five true leaves (S1) achieved the highest interaction values, which were 15.79 and 17.73 g.m^{-2}.day^{-1}, while the control treatment (N0) at the three stages (S1, S2, and S3) achieved the lowest values of the interaction, reaching in the first season 12.83, 12.55, and 12.69 g.m^{-2}.day^{-1} and 13.62, 13.51, and 13.35 g.m^{-2}.day^{-1} in the second season for the three stages, respectively, without significant difference between them.

The effect of the triple interaction between the study factors was significant on the growth rate of the crop Tables (8) and (9). The plants of the variety Inqath (V2) sprayed with nano-iron (N1) at the stage of the emergence of five true leaves (S1) gave the highest values of the interaction, which were 17.68 and 19.12 g.m^{-2}.day^{-1}. The plants of the same variety sprayed with nano-zinc in the stage of the emergence of five true leaves (V2N2S1) were not significantly different for the two study seasons, which gave 18.50 g.m^{-2}.day^{-1} in the second season. While the plants of the variety Rabih (V1) were sprayed with water, only distilled water (N0) in the three stages (S1, S2, and S3) achieved the lowest interaction values of 11.25, 10.78, and 11.13g.m^{-2}.day^{-1} in the first season and 11.62, 11.72, and 11.17 g.m^{-2}.day^{-1} in the second season for the three stages respectively without any significant difference between them.

Grain yield (t.ha^{-1})

It is noticed from the results of tables(10) and that there is a significant difference between sorghum varieties in grain yield. The variety Inqath (V2) achieved the highest average for the trait amounting to 5.344 and 5.079 tons.ha^{-1} compared to the variety Lilo (V1), which attained the lowest grain yield average of 3.943 and 3 .588 tons.ha^{-1} for the two study seasons, respectively.

The reason for the superiority of the variety Inqath in grain yield may be due to its prominence in panicle weight Tables (2) and (3), the number of grains per panicle Tables (4) and (5), and the crop growth rate for the period from 100% inflorescence to physiological maturity Tables (8) and (9). These results are consistent with the findings of (4) and (18), who indicated that sorghum varieties differed significantly in grain yield.

Spraying nano-iron (N1) was significantly superior. It achieved the highest average grain yield of 5.023 and 4.828 tons.ha^{-1} compared to the control treatment (N0), which attained the lowest average of 4.203 and 3.872 tons.ha^{-1} for the two study seasons, respectively Tables (10) and (11). The reason behind the superiority of spraying nano-iron in the grain yield may be due to its dominance in panicle weight Tables (2) and (3), the number of grains per panicle Tables (4) and (5), and the growth rate of the crop for the period from 100% inflorescence to physiological maturity Tables (8) and (9). In the same direction, the results of (16) and (9) in maize yield showed a significant increase in grain yield due to spraying nano iron.

The results of the two tables (10) and (11) show that spraying nano-fertilizers in the stage of the emergence of five true leaves (S1) was significantly superior to the highest grain yield average of 4.911 and 4.655 tons.ha^{-1} compared to spraying it in the stage of 50% inflorescence (S3), which produced the lowest yield average of 4.350 and 4.081 tons.ha^{-1} for the two study seasons sequentially.

The effect of the binary interaction between the varieties and the nano-fertilizer spraying was significant on the grain yield in Tables (10) and (11). The plants of the variety Inqath (V2) sprayed with nano-iron (N1) achieved the highest values of the interaction, which were 5.703 and 5.475 grains of tons.ha^{-1}, and it did not differ significantly from the plants of the same variety that were sprayed with nano-zinc (N2) achieving 5.562 and 5.316 tons.ha^{-1}. The plants of the variety Lilo (V3) sprayed with distilled water only (N0) achieved the lowest interaction values of 3.609 and 3.157 tons.ha^{-1} for the two study seasons, respectively

The binary interaction between the varieties and the stages of spraying the nano-fertilizers affected the grain yield significantly in Tables (10) and (11). The plants of the variety Inqath (V2), sprayed nano fertilizer at the stage of the emergence of five true leaves (S1), gave the highest interaction values, which were 5.641 and 5.395 tons.ha^{-1}, while the plants of the variety Lilo (V3) sprayed at the 50% inflorescence stage (S3) gave the lowest interaction values, which were 3.774 and 3.372 tons.ha^{-1}, for the two seasons, respectively.

As for the binary interaction between the spraying of nano-fertilizers and the stages of spraying, it significantly affected the grain yield in Tables (10) and (11). Spraying nano-iron (N1) at the stage of the emergence of five true leaves (S1) achieved the highest value of the interaction, amounting to 5.437 and 5.271 tons.ha^{-1}, while the control treatment (N0) at the three stages (S1, S2, and S3) achieved the lowest value of the interaction that in the first season were 4.282, 4.213, and 4.113 tons.ha^{-1} and in the second season were 3.935, 3.889 and 3.793 tons.ha^{-1} for the three stages respectively without significant difference between them.

The triple effect of the interaction between the study factors was significant in the grain yield Tables (10) and (11). The plants of the variety Inath (V2) sprayed with nano-iron (N1) at the stage of the emergence of five true leaves (S1) gave the highest values of the interaction, which were 6.122 and 5.971 tons.h^{-1} for the two study seasons respectively. Yet, they did not differ significantly from the plants of the same variety sprayed with nano-zinc in the emergence stage of five true leaves (V2N2S1), which gave 5.931 tons.ha^{-1} in the first season. In contrast, the plants of the variety Lilo (V3) sprayed with distilled water only (N0) achieved in the three stages (S1, S2, and S3), the lowest interaction values were 3.642, 3.674 and 3.511 tons.ha^{-1} in the first season and 3.233, 3.196 and 3.042 tons.ha^{-1} in the second season at the three stages respectively without any significant difference between them.

Varieties (V)	Nano (N) Fertilizer	Stages (S)			V x N
		S1	S2	S3	
V1	N0	4.335	4.212	4.146	4.231
	N1	5.804	5.286	4.466	5.185
	N2	4.909	4.974	4.36	4.748
V2	N0	4.869	4.754	4.681	4.768
	N1	6.122	5.851	5.135	5.703
	N2	5.931	5.713	5.042	5.562
V3	N0	3.642	3.674	3.511	3.609
	N1	4.387	4.179	3.979	4.182
	N2	4.201	4.084	3.832	4.039
LSD 0.05		0.225			0.157
S × V					
Var.		S1	S2	S3	average Var.
V1		5.016	4.824	4.324	4.721
V2		5.641	5.439	4.952	5.344
V3		4.077	3.979	3.774	3.943
LSD 0.05		0.145			0.132
S × N					
(N)		S1	S2	S3	average Nano Fer.
N0		4.282	4.213	4.113	4.203
N1		5.437	5.105	4.527	5.023
N2		5.014	4.924	4.411	4.783
LSD 0.05		0.126			0.085
average stages		4.911	4.747	4.35	

Table 10. Shows the effect of spraying nano fertilizers and spray stage on Grain yield (t.ha-1)of varieties of Sorghum (The first season).

It appears from the results of the triple interaction that the variety Inqath, sprayed with nano iron at the stage of the emergence of five true leaves, invested its genetic energy and physiological efficiency and directed them towards increasing panicle weight and the number of grains in the panicle and the (Tables 2, 3, 4 and 5), positively reflected on its superiority in grain yield, which is the outcome of the biological processes that take place within the plant system.

Varieties (V)	Nano (N) Fertilizer	Stages (S)			V x N
		S1	S2	S3	
V1	N0	4.008	4.042	3.986	4.012
	N1	5.677	5.228	4.381	5.095
	N2	4.679	4.863	4.257	4.6
V2	N0	4.562	4.429	4.351	4.448
	N1	5.971	5.582	4.872	5.475
	N2	5.653	5.53	4.765	5.316
V3	N0	3.233	3.196	3.042	3.157
	N1	4.166	3.922	3.658	3.915
	N2	3.947	3.71	3.416	3.691
LSD 0.05			0.255		0.178
S × V					
Var.		S1	S2	S3	average Var.
V1		4.788	4.711	4.208	4.569
V2		5.395	5.18	4.663	5.079
V3		3.782	3.61	3.372	3.588
LSD 0.05			0.173		0.161
S × N					
(N)		S1	S2	S3	average Nano Fer.
N0		3.935	3.889	3.793	3.872
N1		5.271	4.911	4.304	4.828
N2		4.76	4.701	4.146	4.536
LSD 0.05			0.139		0.088
average stages		4.655	4.5	4.081	
LSD 0.05			0.08		

Table 11. Shows the effect of spraying nano fertilizers and spray stage on Grain yield (t.ha-1)of varieties of Sorghum (The second season).

It is also apparent that the grain yield of the three sorghum varieties was more responsive to spraying nano iron at the emergence stage of five real leaves. Still, the size of the response was different according to the difference in the genetic material of each variety and its response to environmental conditions, which explains the reason for the significant effect of the interaction between the study factors.

Conclusions

Nano-fertilizers are of economic benefit because the quantities used are minimal compared to conventional fertilizers, as well as with a high and quick response by the plant due to the small size of their particles; through the study, it was found that fertilizer iron and nano-zinc has a positive effect on yield traits, as well as cultivars differed significantly among themselves in yield traits and cultivar superiority (V3) Also, the spraying stages differed considerably among themselves. The stage (S1) was significantly superior compared to the other stages.

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ARTICLE / INVESTIGACIÓN

Role of Salicylic acid in stay green, growth and yield of two purposes maize hybrid

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DOI. 10.21931/RB/2022.07.04.33

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Abstract: An experiment was conducted at Al- Hamidhia Research Fields, Faculty of Agriculture / Al- Anbar University in North Ramadi city to study the levels of salicylic acid 0, 200 and 400 mg. L^{-1} and fifteen genotypes (5 inbred lines + 10 hybrids) of maize. A randomized complete block design was used in a slit table arrangement; thus, the main treatments are for salicylic spray, and the secondary tables are for genotypes (inbred lines and hybrids). Three replications were made to produce two-purpose hybrids (grain and forage yield) by introducing some inbred lines and maize hybrids grown by the semi-co-breeding program. Results have shown the dominance of hybrid BK104× Zm6 giving the highest product of leaves staying green that reached 222.1g and 8.9 leaves, successively in the spring season. In comparison, the hybrid Inb-27× BK104 has dominated in the fall season, giving yield and leaves stay green as 247.01g and 9.45 leaves successively. The reason for these hybrids dominance returned to their dominance with grains numbered a row, grain weight and dominance of salicylic acid concentration 400 mg.L^{-1} for both seasons, giving the highest yield as 179.10 and 177.33g and didn't differ significantly from salicylic acid concentration 200 mg.L^{-1}. The interaction treatments were significant for all the traits, except the trait of 300 grains weight for the spring season. So, we recommend using 200 mg.L^{-1} concentration of salicylic acid and the dominant hybrids in yield for both seasons.

Key words: AS, Inbred lines, Diallel crops, plant growth regulator, grains yield, grain weight.

Introduction

Maize (*Zea mays L.*) is considered the life vein for many human communities, and the grain yield increase is the goal of plant breeders in general, especially for maize breeders. The quality and quantity of plant production generally, and farm crops specifically depend on their genetic material nature and growth environment, as well as the value of their interaction that could be managed through the growth factors management to suit the farmer's goal. Despite the global increase of maize that reached about 1670 million tons annually in 2020, the need for more production is still necessary because of the increased uses of maize with the tremendous increase in population[1].

In Iraq, the total area cultured with this crop for 2020 is about 13.95 thousand hectares, with an average productivity of 4.5 tons.hec^{-1}. The reason for low productivity compared to global productivity is maybe the farmer's carefree to culture highly productive genotypes seeds, especially the dominant single hybrids that adapt to local environmental conditions due to the high expense of imported hybrids seeds, the absence of modern techniques and weakness of soil and crop maintenance services because of the limited support of agricultural section. Hybridization and selection are the essential bases of maize improvement and generate a broad genetic framework that helps breeders to exploit genetic storage in different breeding programs. In addition to genetic prospecting techniques and variable parameters for their study to increase their production efficiency per unit area and improve their specific traits[2-4].

Salicylic is a phenolic compound and an important plant hormone that regulates ion absorption, hormonal balance, division rate, flower induction, stomata motion and photosynthesis[5]. It has important physiologic roles in enhancing plant growth and increasing the efficiency of photosynthesis and flowering[6]. It improves plant tolerance via activating the un-enzymatic antioxidants as superoxide demonize, catalase and prior oxidase enzymes[7]. The external salicylic effect on plants depends on many factors like the used dosage, plant type where the regulator is used, plant growth stage and salicylic way of addition, and of the reasons that cause productivity decrease in not fulling the grains that urge using salicylic, which the main feature is delaying leaves aging and increases photosynthesis activity; thus, it prolongs the grains fulling period that reflects on increasing grains yield[8], also delaying plant aging could happen by ethylene prohibition, increasing enzymes activity, increased division of meristematic root cells and keeping chloroplasts safe of degradation because of free roots; thus, it activates the production of the antioxidants for their effect on cells free roots[9].

Salicylic acid alone is insufficient to raise crop productivity unless it is gathered with an efficient genetic enhancer.

The study aims to produce dual-purpose hybrids (grain and fodder yield) by introducing a group of maize inbred lines and hybrids resulting from half diallel cross program.

Citation: Hamad, H.; Abdulhamed, Z.; Role of Salicylic Acid in stay green, Growth and Yield of Two Purpose Maize Hybrid. Revis Bionatura 2022;7(4) 33. http://dx.doi.org/10.21931/RB/2022.07.04.33
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

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Materials and methods

Experiments were done in the farms of Al- Hamidhiya Research Station of the College of Agriculture / Al- Anbar University to the north of Ramadi City, which lies on latitude 33° 27' 10.8" N to the north and longitude 43° 23' .2.4" E to the east with collected mixed texture in two seasons: spring and fall 2021. All the culturing operations like spading, softening and leveling were done. The soil was then fertilized with dap in an average of 300 kg.hec^{-1}. On soil preparation, urea fertilizer was added with 46% nitrogen at a rate 300 kg.hec^{-1} on two loads: the first when plant height reached 30cm and the second before the inflorescences germinated. In all seasons, weed control was made after culturing and before germination with brobonite herbicide[11]. all the crop service operations were made, like irrigation, grassing, spading and weed control.

In the spring and fall seasons of 2021, an experiment was done among the individual hybrids in the company of parental inbred lines; so, the number of genotypes included in the control experiment is ten derived hybrids + 5 parental inbred lines = 15 genotypes and three salicylic level (0, 200, 400) mg.L^{-1} using the randomized complete block design in slit boards arrangement; so, the primary treatment is for salicylic spray and secondary boards for genotypes (inbred lines and hybrids) in three replications.

Salicylic Acid Preparation

A standard solution of salicylic acid (SA) weighing 1000 mg was prepared to a concentration of 1000 mg l^{-1} then resolved in several ethyl alcohol drops, and later the size was completed with distilled water to prepare 200 mg.L^{-1}. After that, two groups of concentrations (200 and 400) mg.L^{-1} are prepared and sprayed on the shoot system 30 days after culture, and the second spray is two weeks after the first. So, the first spray was made in the early morning for complete wetting, while the control plants were sprayed with distilled water only.

Studied Traits

- Days of Silks emergence (day):

Counted for the period from planting to silks emergence in 50% of plants per experiment unit.

- Leaf Area (cm^2): counted from the square length of the leaf that lies under the central tip leaf crossed with constant (0.75)
- Row Grains Number. It was determined by counting the rows in the primary ear at the harvest stage on a sample of five plants taken from the middle of each experimental unit.
- Stay Green by counting the number of green leaves at maturity above the upper ear.
- 300 Grains weight (gm): 300 grains were counted, then the final weight was corrected based on the ideal water content
- Plant Grain Yield (g): by the average weight of five plants' yield.

Statistical Analysis

Statistical analysis was made for each trait using the randomized complete block design (RCBD) according to slit boards arrangement; the comparison was made between the average genotypes using the least significant difference (LSD) at a likelihood level 5%.

Results

Days from culture to 50% Ear Flowering

Statistical analysis results in a table (2) showed significant differences among the average concentrations of salicylic acid and genotypes (parents and their co-hybrids) for both seasons and the cooperative interaction of both study factors of 50% ear flowering trait for both spring and fall seasons. The plants of two inbred lines 3 and 4 lasted the shortest duration of ear flowering at 62.00 and 62.09 days, successively, while parent 5 lasted the most prolonged period of ear flowering at 63.78 days for the spring season. In return to the fall season, inbred line 1 and 2 required the shortest number of days to reach the stage of 50% ear flowering, 59.37 and 59.42 days, successively, while the inbred line 3 plants needed the longest time to get this stage as 61.31 days. This would indicate that hybrids have tended to early flowering compared to their parents; so, the co-crossing 1X3 of the spring season has lasted the least duration of ear flowering as 59.10 days, which has differed significantly with seven hybrids and the longest days number to reach flowering has been made by hybrid 4X3 as 62.77 days. During the fall season, the cooperative hybrid 3X2 lasted the shortest duration of ear flowering at 55.42 days and didn't differ significantly from the two cooperative hybrids. Also, the hybrid 5X1 lasted 58.35 days, indicating the general tendency of hybrids for early ear flowering. These results agreed with (12,13) when they referred to significant differences among genotypes in the trait of days number from culture to 50% ear flowering. The same table results also refer to the dominance of plants cultured with 400 salicylic in ear flowering to give a shorter number of days as 61.11 and 56.95 days for spring and fall seasons, successively, which, in turn, differed significantly from 200 mg.L^{-1} concentration for both seasons successively as 62.26 and 58.26 with the difference in trait mean values for spraying concentrations 400 mg.L^{-1} of salicylic acid for both seasons to give the lowest flowering duration in days number. The decrease of days number from culture to 50% ear flowering with spray concentrations 400 mg.L^{-1} salicylic acid and the early appearance of ear node is attributed to its role in increasing the Auxin content that acts vitally to increase photosynthetic products and so the plant nutrients to gain excessive sac-

Seq.	Inbred line Name	Source
1.	**BK 116**	Locally derived 106 research
2.	**Zm 6**	Locally derived
3.	**ABS 6**	Locally derived
4.	**BK 104**	Locally derived 106 research
5.	**Inb-27**	Locally derived

Table 1. Pure inbred lines of maize, brought by the supervisor doctor, have been used and input in a semi-diallel-crosse program according to the second model of (10).

Genotypes	Spring Season				Fall Season			
	Salicylic Concentrations mg.L^{-1}				Salicylic Concentrations mg.L^{-1}			
	0	200	400	Average	0	200	400	Average
1	64.77	64.66	61.66	63.70	60.11	59.68	58.47	59.42
2	64.66	63.66	62.66	63.66	60.57	60.20	57.33	59.37
3	62.33	63.35	60.33	62.00	63.27	62.33	58.33	61.31
4	63.47	62.66	60.14	62.09	62.09	60.53	59.16	60.60
5	64.00	64.70	62.66	63.78	60.80	60.15	60.20	60.38
2×1	61.21	59.82	58.33	59.79	58.37	57.25	55.98	57.20
3×1	60.92	58.73	57.66	59.10	57.29	56.25	56.07	56.53
4×1	61.33	62.33	61.11	61.59	57.46	55.15	55.13	55.91
5×1	62.66	62.66	61.33	62.22	58.33	59.01	57.70	58.35
3×2	60.81	59.84	60.66	60.44	57.33	55.10	53.84	55.42
4×2	62.21	62.69	60.66	61.85	58.47	57.97	56.51	57.65
5×2	63.61	63.00	61.66	62.75	57.23	57.67	56.49	57.13
4×3	62.33	63.57	62.40	62.77	58.78	58.18	56.30	57.75
5×3	61.00	60.33	62.66	61.33	57.98	57.55	56.80	57.44
5×4	61.66	61.85	62.66	62.06	57.96	56.90	56.00	56.95
Average	62.46	62.26	61.11		59.07	58.26	56.95	
LSD 5%	salicylic		*0.94*			*1.50*		
	Genotypes		*1.02*			*1.27*		
	Interaction		*1.84*			*2.41*		

Table 2. Effect of Salicylic Concentrations, their Hybrids and Interaction on Average ear Flowering (Day) for Spring and Fall Seasons 2021.

charides ready to support shoot system growth and cause late flowering, this result agrees with what's found by (14), who suggested that the plants sprayed with salicylic acid would early reach ear flowering. The interaction between spray treatments with salicylic and genotypes concentration has a significant effect; the same table shows the dominance of 2X1 hybrid plant cultured in 400 salicylic concentration with an average rate of 58.33 in the spring season with no difference from two cooperative hybrids. Still, in the fall season, the hybrid 4X1 plants cultured with 400 mg.L^{-1} salicylic have last the least ear flowering time at 55.13 days and didn't differ significantly from the five cooperative hybrids.

Leaf Area (cm^2)

The statistical analysis results of table (3) showed significant differences among the level concentrations of salicylic acid and genotypes (parents and their cooperative hybrids) for both seasons and the co-interference of both study factors in leaf area traits for seasons spring and fall. Inbred line 2 plants have given the highest average value of this trait as 4967cm^2, which has not differed significantly from parents 3 and 4, while parent 1 made the lowest trait average value of 4467cm^2 for the spring season. While in the fall season, inbred line 3 produced the highest middle leaf area at 5724cm^2 with no significant difference from inbred line 2. The least value has been made by inbred line 5 plants as 4722cm^2; the hybrids resulting from co-crossing had a tremendously considerable effect, so they exceeded parents in this trait, and the cooperative hybrid 4X1 achieved the highest average value as 5966 and 6425cm^2, successively for both culture seasons. The reason behind that is the nature of genes each genetic enhancer carries that reflect their difference in average leaf width and height and leaves number in addition to the length of vegetable growth duration. These results have come similar to whats obtained by Abdulhamed. *et al.*, 2021, when indicated significant differences among genotypes in the traits of leaf area and carbon assimilation maintenance during the growth time. From the same table, it is noticed the dominance of plants cultured with 400 mg.L^{-1} salicylic for leaf area cm^2 gave the highest average value as 5480 and 5513cm^2 day for spring and fall seasons successively, with no significant difference from 200 mg.L^{-1} for both seasons. Perhaps the reason for the leaf area cm^2 increase in spray concentrations 400 mg.L^{-1} salicylic acid is its role in increasing Auxin content, which plays an essential role in increasing photosynthesis products and thus increases the plant nutrients to gain excessive saccharides ready to support the shoot system growth, leading to late flowering. This result meets the one obtained by (15,16), who referred to an increase in each of CO_2 assimilation and photosynthesis, and this photosynthesis support would increase cellular juice production in middle lamella that saves suitable water content in the leaf, leading to better growth configured with increased leaf area. In return to salicylic and genotypes spray interaction, it's noticed in the same table that in the spring season, the hybrid 5X3 cultured in 400 mg.L^{-1} dominated to reach 6562cm^2, so it didn't differ significantly from some cooperative hybrid, while in the fall season, the plants of hybrid 4X1 cultured in 400 mg.L^{-1} salicylic has made the highest value as 6639cm^2 and didn't differ significantly with 3 cooperative hybrids. This result agreed with what was obtained by (17) when they

Genotypes	Spring Season				Fall Season			
	Salicylic Concentrations mg.L^{-1}				Salicylic Concentrations mg.L^{-1}			
	0	200	400	Average	0	200	400	Average
1	*4341*	*4577*	4482	4467	*4637*	*4942*	*5086*	4888
2	*5282*	*5312*	*4307*	4967	*5167*	*5661*	*5760*	5529
3	*5490*	*4748*	*4186*	4808	*5485*	*5701*	*5985*	5724
4	*5198*	*4464*	*4638*	4767	*5340*	*4945*	*4968*	5084
5	*4310*	*4712*	*4853*	4625	*4585*	*5200*	*4382*	4722
2×1	5314	5243	5764	5441	5003	5354	5779	5379
3×1	5291	5503	5729	5508	5333	5767	5707	5602
4×1	5797	5963	6139	5966	6352	6285	6639	6425
5×1	5301	5628	5838	5589	6240	5634	5580	5818
3×2	5315	5605	5694	5538	6084	6128	5713	5975
4×2	5529	5607	5761	5633	5439	5659	5179	5426
5×2	5273	5514	5994	5594	5216	5020	5485	5240
4×3	5591	5828	6318	5913	5097	5290	5561	5316
5×3	5426	5732	6562	5907	4996	5467	5518	5327
5×4	4926	5673	5937	5512	5558	4919	5361	5279
Average	5226	5341	5480		5369	5465	5513	
LSD 5%	salicylic	*172.5*			*85.6*			
	Genotypes	*209.2*			*263.5*			
	Interaction	*371.4*			*445.0*			

Table 3. Effect of Salicylic Concentrations, their Hybrids and Interaction on Average Leaf Area (cm^2) for Spring and Fall Seasons 2021.

used salicylic acid at concentrations 0 and 200ppm leaf spray on maize in two stages the first after two weeks and the second after three weeks of germination, to obtain dominated leaf area.

Stay Green

The statistical analysis results of table (4) referred to the presence of significant differences among the average concentrations of salicylic acid and genotypes (parents and their cooperative hybrids) for both seasons and the co-interference of both study factors for the trait of leave stay significant duration for spring and fall seasons. The plants of inbred lines 1 and 2 have the highest average value of the attribute at 7.36, which didn't differ significantly from the three inbred lines. In contrast, parent one has given the lowest duration of this trait as 6.35 for the spring season. While in the fall season, inbred line 5 achieved the highest value of 7.4, while inbred line 1 plants gave given the lowest duration of 6.31, while the hybrids resulted from co-crossing for the spring season, the cooperative combination 4X2 provided the highest rate of 8.90 and didn't differ significantly from hybrid 3X2. The cooperative hybrid 5X3 gives the least duration, 6.96. but in the fall season, the hybrid 5X4 made 9.45 and didn't differ significantly from 4 cooperative hybrids. Had obtained similar results when they referred to significant differences in genotypes for the trait of leaves staying green. It's noticed from the same table results the dominance of plants cultured in 400 salicylic concentration in this trait, giving the highest value as 8.28 and 8.22 for spring and fall seasons successively, and it differed significantly with concentrations of 0 and 200 mg.L^{-1} for both seasons. The table shows the difference of average trait values for spray concentrations 400 mg.L^{-1} salicylic for two seasons, giving the most extended duration of leaves staying green. This trait probably increased due to spray concentrations of 400 mg.L^{-1} salicylic. This result agrees with (18,19), who suggested that the plants sprayed with salicylic acid.

While for the interaction among the spray treatments of salicylic and genotypes for the spring season, the same table stated the dominance of hybrid 4X2 with a value of 8.90 that differed significantly with five hybrids this season. While for the fall season, the plants of hybrid 5X4, cultured in 400 mg.L^{-1} salicylic, have made the highest trait interference as 10.13, which differed significantly from the other hybrids in this season.

Grains Number a R

Results of statistical analysis in the table (5) refer to significant differences among the concentrations of salicylic and genotypes (parents and their cooperative parents and their cooperative hybrids) and the co-interference of both study factors for the trait of grains number a row in two seasons. Genotypes have significantly differentiated among themselves, so inbred line 1 has the highest average value of 26.49, which differed considerably from the other inbred lines. In contrast, inbred line 3 has the most negligible average value of 24.71 for the spring season. While in the fall season, inbred line 2 has the highest average value of

Genotypes	Spring Season				Fall Season			
	Salicylic Concentrations mg.L^{-1}				Salicylic Concentrations mg.L^{-1}			
	0	200	400	Average	0	200	400	Average
1	5.73	6.33	6.98	6.35	6.00	6.20	6.73	6.31
2	7.09	7.26	7.73	7.36	5.73	6.53	6.93	6.40
3	6.40	7.24	7.46	7.03	5.50	6.67	6.86	6.34
4	6.40	7.07	8.53	7.33	6.26	6.52	6.91	6.56
5	6.39	7.20	7.60	7.06	6.40	7.50	8.33	7.41
2×1	7.26	8.20	9.20	8.22	7.66	8.93	9.13	8.57
3×1	7.31	7.66	9.06	8.01	8.10	7.26	8.26	7.87
4×1	7.66	8.00	8.46	8.04	6.80	7.73	8.44	7.65
5×1	8.20	8.60	8.62	8.47	7.66	7.90	8.40	7.99
3×2	8.10	8.60	8.78	8.49	7.73	7.90	8.62	8.08
4×2	8.26	9.01	9.44	8.90	8.80	9.40	9.71	9.30
5×2	7.46	8.13	8.26	7.95	8.00	8.50	8.90	8.46
4×3	6.56	7.20	7.82	7.19	5.40	7.10	7.83	6.77
5×3	6.30	7.00	7.58	6.96	6.10	7.76	8.17	7.34
5×4	8.01	8.23	8.72	8.32	8.60	9.64	10.13	9.45
Average	7.14	6.33	8.28		6.98	7.70	8.22	
LSD 5%	salicylic		0.22				1.19	
	Genotypes		0.46				1.47	
	Interaction		0.79				4.08	

Table 4. Effect of Salicylic Concentrations, their Hybrids and Interaction on Average Leaves Stay Green for Spring and Fall Seasons 2021.

27.97, with no significant difference from inbred line 1, whereas the most negligible average value was made by inbred line 5 at 24.17. in return, hybrids produced by inbred lines could exceed the hybrid 4X2 giving the highest average value of this trait as 40.37 in the spring season.

In contrast, the hybrid 5X2 gave the most negligible value of grains number a row as 35.10, which differed significantly from the other cooperative hybrids. While in the fall season, the co-hybrid 2X1 gave the highest trait value of 37.82 and the least average value was given by hybrid 3X1 (29.48), the reason for this difference in row grains number could be the presence of differences in oval number that later transformed to grains to reflect this on inheritance and increase of grains number, compared to its generation of dominant hybrids, and these results have agreed with both of (20) who referred to significant differences among genotypes in row grains number. It's noticed from the same table results, the considerable effect of salicylic addition in different concentrations on row grains number, figured by the salicylic increase in two seasons above; so, the plants cultured in concentration 400 mg.L^{-1} salicylic have dominated in this trait, giving the highest average value as 33.60 and 32.15 for spring and fall seasons successively with no significant difference from spraying concentrations 200 mg.L^{-1} for the fall season only, compared with the control treatment that gave 32.64 and 30.69 for spring and fall seasons, successively, when he used salicylic acid in different concentrations. The leaf plant spraying a plant leads to early plant flowering and increases the average plant flower number that reflects, in turn, on the number of the grain produced later.

300 Grains weight (g)

Statistical analysis results in table (6) showed significant differences among the concentrations of salicylic acid and genotypes (parents and their co-hybrids) for spring and fall seasons and the co-interference of both study factors for 300 grins weight in the spring season. The plants of the inbred line (4) have made the highest value of 300 grains weight trait as 78.82g and didn't differ significantly from the other inbred lines, while inbred line 1 made the lowest value of 76.46g for the spring season. While in the fall season, inbred line 5 produced the highest average value, 85.53g, which differed significantly from other parental inbred lines. The most negligible average value was made by inbred line 2 plants at 72.15g. The cooperative hybrid 4X2 in the spring season made the highest average value at 97.89g, which didn't differ significantly from some combinations. The most negligible average value was created by hybrid 5X3 as 74.97g. while in the fall season, the co-hybrid 4X2 made the highest value of this trait at 99.99g with no significant difference from some co-hybrid, hybrid 3X1 has produced the lowest average value of this value at 81.94g, indicating that hybrids have exceeded their parents in this trait. Grain weight is one of the most critical crop components where the degree of grain fulfilling, bulk and specific density. The reason for hybrids' dominance in this trait is maybe the early flowering and plant leaf area. This result agrees with Sharif *et al* .,2020 who stated that the genetic nature affects this trait. The results of the same table also refer to the dominance of plants cultured in 400 mg.L^{-1} concentration salicylic in this trait; so, it gave the highest average value as 84.26g. for the spring season and didn't

Genotypes	Spring Season				Fall Season			
	Salicylic Concentrations mg.L^{-1}				Salicylic Concentrations mg.L^{-1}			
	0	200	400	Average	0	200	400	Average
1	*26.15*	*26.86*	27.80	26.94	*27.73*	*25.73*	*25.66*	26.37
2	*25.40*	*25.53*	*25.93*	25.62	*26.53*	*28.46*	*28.93*	27.97
3	*24.20*	*25.06*	*24.86*	24.71	*24.00*	*25.33*	*26.06*	25.13
4	*23.20*	*23.73*	*23.46*	23.46	*23.33*	*23.86*	*23.68*	23.62
5	*24.53*	*24.60*	*25.60*	24.91	*23.13*	*24.73*	*24.64*	24.17
2×1	33.00	37.93	38.40	36.44	37.33	37.73	38.40	37.82
3×1	36.77	35.26	36.23	36.08	28.26	29.73	30.46	29.48
4×1	38.66	37.40	37.96	38.00	29.00	31.13	33.20	31.11
5×1	37.50	37.53	39.06	38.03	33.20	34.13	35.20	34.17
3×2	38.00	38.30	39.00	38.43	30.40	33.20	32.86	32.15
4×2	40.10	40.50	40.50	40.37	37.26	37.16	36.53	36.98
5×2	35.00	35.00	35.30	35.10	36.53	38.13	35.73	36.80
4×3	36.60	36.80	36.56	36.65	32.66	33.20	34.33	33.40
5×3	35.00	36.46	36.62	36.03	35.46	36.73	37.00	36.40
5×4	35.46	36.00	36.77	36.07	35.60	37.90	39.60	37.70
Average	32.64	33.13	33.60		30.69	31.81	32.15	
LSD 5%	salicylic		*0.43*			*1.10*		
	Genotypes		*1.03*			*0.93*		
	Interaction		*1.75*			*1.77*		

Table 5. Effect of Salicylic Concentrations, their Hybrids and Interaction on Average Grains Number a Row for spring and fall Seasons 2021.

differ significantly from 200 mg.L^{-1} concentration, while the most negligible value was made by 0 mg.L^{-1} as 42.34g. also, the average trait values have variated for interaction between genotypes and salicylic acid spray levels for the spring season; so, the plants of hybrid 4X2 cultured gave 97.93g with no significant difference from 6 co-hybrids. While in the fall season, there weren't significant differences between genotypes and salicylic acid spray levels. The increase in this trait is, perhaps, because the leaf support of maize with salicylic acid has improved most of the vegetative growth indicators and thus reflected in grains' weight. This result has agreed with each of (21).

The reason is that spraying salicylic acid concentration of 150 mg.L^{-1} has supported the increase of products of photosynthesis in vegetative growth traits of maize sugary yields, certainly the leaf area increase; so, crop salicylic acid spraying operation has decreased high temperatures and abortion during inoculation and fertilization to increase the weight and number of grains in the tip.

These results agree with the results, and the cause is that salicylic acid concentration 150 mg.L^{-1} has dominated in plant height and leaf area, the evidence is significantly 500 grains.

Plant Yield (g)

Grain yield is considered the final result of growth and evolution processes connected complicatedly with its essential components (tip grains number and grain weight), which are affected by genetic and environmental factors and their interaction. The statistical analysis results in table (7) refer to significant differences among the average values of salicylic acid and genotypes (parents and their co-hybrids) and the co-interference of study two factors for individual plant yield traits for both seasons. Table (7) states the variance of genotypes significantly among themselves; so, inbred line (3) gave the highest average value of 128.2g with no significant difference from stain 2, while inbred line 1 gave the most negligible average value (111.4g) for the spring season. For the fall season, inbred line 5 gave the highest average value of 126.95g, and parent bequeathed their variance to their co-hybrids; so, in the spring season, the co-hybrid 2X4 dominated, giving the highest average value of plant grains yield trait as 222.1g with no significant difference with co-hybrids 2X3 and 1X5. In the fall season, the co-hybrid 4X5 made the highest value of trait as 247.01g. the reason for these hybrids' dominance and abundant yield is the crop components' dominance. These differences among inbred lines and their hybrids reflect the genetic differences and their dominance in one or more crop components (grains number, row grains number and 300 grains weight). (22-24) had obtained close results when they referred to significant differences among genotypes in

Genotypes	Spring season				Fall season			
	Salicylic Concentrations mg.L^{-1}				Salicylic Concentrations mg.L^{-1}			
	0	200	400	Average	0	200	400	Average
1	76.00	75.93	77.53	76.49	75.00	75.15	75.67	75.27
2	75.60	75.60	79.67	76.96	69.74	72.88	73.83	72.15
3	76.63	80.60	78.27	78.50	69.83	73.41	73.67	72.30
4	80.20	77.67	78.60	78.82	80.17	81.83	83.67	81.89
5	78.91	76.87	79.54	78.44	83.50	86.75	86.33	85.53
2×1	78.40	78.48	79.84	78.91	96.38	94.37	98.33	96.36
3×1	87.64	88.40	88.60	88.21	79.83	83.00	83.00	81.94
4×1	86.53	93.82	88.70	89.68	88.33	92.58	90.50	90.47
5×1	95.04	96.74	95.87	95.88	93.83	90.33	88.67	90.94
3×2	81.44	81.71	87.69	83.61	79.83	82.01	84.00	81.95
4×2	96.97	97.93	98.76	97.89	98.87	100.10	101.00	99.99
5×2	81.30	90.07	90.31	87.23	85.42	86.33	90.50	87.42
4×3	79.10	80.24	79.93	79.76	88.42	88.50	90.67	89.19
5×3	79.70	72.44	72.77	74.97	90.33	92.83	96.00	93.06
5×4	81.60	85.20	87.76	84.85	97.75	98.52	100.45	98.91
Average	82.34	83.45	84.26		85.15	86.57	87.75	
LSD 5%	salicylic	1.29			2.02			
	Genotypes	2.74			2.66			
	Interaction	4.68			NS			

Table 6. Effect of Salicylic Concentrations, their Hybrids and Interaction on Average (300) Grains Weight (g) for Spring and Fall Seasons 2021.

individual plant yield. It's noticed from the same table results in the considerable effect of adding salicylic in different concentrations on individual plant yield, figured with the increase of average grains yield with increased salicylic concentrations in both study seasons; so, the plants cultured with 400 mg.L^{-1} salicylic have dominated with individual plant yield, giving average yield as 179.10 and 177.13g for the two seasons, spring and fall, successively, and they didn't differ significantly with spray concentrations 220 mg.L^{-1}, compared with the control treatment that reached 164.69 and 169.26g for spring and fall seasons, successively. This yield increase could be attributed to the positive effect of salicylic growth regulator to raise photosynthesis efficiency, plant growth enhancement, dry matter accumulation increase, and thus increasing grains production rate and salicylic impact to increase grains number through transporting synthetic products from source to sink. These results have come supporting to what's found by (8), who suggested that maize grains yield would increase by salicylic acid spray.

Genotypes have significantly responded to salicylic spray, so the co-hybrid 2X4 has exploited salicylic spray concentrations through a yield of 222.1g at spray treatment 400 mg.L^{-1} salicylic, with no significant difference from 11 co-hybrids in the spring season. In contrast, for the fall season, co-hybrid 4X5 exploited spraying at a concentration of 400 mg.L^{-1} salicylic with high efficiency, when it made the highest value of co-interference with a yield of 254.67g. these results have met the results of past studies done by (25).

This was because of many factors like plant capacity to store assimilating materials because of salicylic acid that provides assimilating products during the fulfilling grain time, increased readiness of nutrients for the plant, their absorption and then increasing vegetative growth and metabolic processes and photosynthetic products and then increasing the yield that reflected positively on grains yield increase.

Due to the application of salicylic acid treatments that supported catalase and peroxidase content and phenols accumulation and photosynthesis efficiency and reduced hydrogen peroxide content in plants with salicylic acid increased concentration (26) to increase grains weight.

Genotypes	Spring season				Fall season			
	Salicylic Concentrations mg.L^{-1}				Salicylic Concentrations mg.L^{-1}			
	0	200	400	Average	0	200	400	Average
1	109.7	103.5	120.9	111.4	108.33	110.67	113.97	110.99
2	114.3	119.1	122.3	118.6	122.62	126.72	131.50	126.95
3	126.3	128.0	130.3	128.2	100.00	103.16	108.71	103.96
4	107.4	112.7	115.5	111.9	94.67	100.55	102.41	99.21
5	101.3	109.6	111.6	107.5	110.68	113.67	115.93	113.43
2×1	177.8	190.5	197.1	188.5	218.91	221.00	231.36	223.76
3×1	198.8	200.9	207.4	202.4	173.33	178.70	184.52	178.85
4×1	186.8	198.7	203.0	196.2	165.33	172.67	176.92	171.64
5×1	204.8	209.7	214.9	209.8	197.67	206.97	215.18	206.61
3×2	209.6	212.2	215.7	212.5	180.67	186.67	195.49	187.61
4×2	218.2	219.3	228.7	222.1	223.67	231.37	236.33	230.46
5×2	191.1	204.1	221.8	205.7	214.69	224.67	160.83	200.06
4×3	151.4	172.9	208.1	177.5	180.33	192.00	204.47	192.27
5×3	168.6	170.1	176.3	171.7	207.33	218.50	224.61	216.82
5×4	203.6	208.4	213.3	208.4	240.67	245.70	254.67	247.01
Average	164.60	170.60	179.10		169.26	175.53	177.13	
LSD 5%	salicylic	*10.31*			*5.43*			
	Genotypes	*12.77*			*6.34*			
	Interaction	*22.62*			*11.32*			

Table 7. Effect of Salicylic Concentrations, their Hybrids and Interaction on Average Individual Plant Yield (g) for Spring and Fall Seasons 2021.

Conclusions

We can conclude from previously mentioned information that parent ABS6 for the spring season and parent 6Zm for the fall season is the best parent because of the high number of row grains number and plant grains yield, and they could be invested to produce various hybrids and classes. The crossing (BK104× Zm6) for the spring season and crossing (Inb-27× BK104) for the fall season is the best crossings for giving a high plant grains yield (222.1 and 247.01 g.plant^{-1}), and a number for leaves stay green (8.9 and 9.45 leaves) for spring and fall seasons, successively. In the time that concentration 200 mg.L^{-1} was featured, giving the highest yield for both seasons, and the hybrids featured with high yield and duration of leaves stay green could be invested to produce another hybrid or double purpose structural class that could be used for grains and making fodder plant.

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ARTICLE / INVESTIGACIÓN

Improved micropropagation and salinity tolerance of strawberry (*Fragaria X ananssa L*) cv. Albion

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DOI. 10.21931/RB/2022.07.04.34

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Abstract: Gamma-ray has been used to increase genetic variation to obtain salt-tolerant plants in strawberries.The protocol was established to multiply strawberry cv. Albion from runner segments cultured on multiplication Murashige and Skoog (MS) medium contain 0.5 mg l^{-1} of 6-benzyl adenine (BA) and 0.1 mg l^{-1} of Naphthaleneacetic acid (NAA). Cultures were irradiated with gamma rays at (0, 20, 50, 100) Gy after 30 days, and the irradiated and unirradiated shoots were exposed to different concentrations of Sodium Chloride (NaCl) (6,10,14, 22) dS m^{-1}. The results showed the superiority of doses 20 and 50 Gy in giving the highest rate of the number of shoots reached (9.25 and 8.44) shoot explant^{-1}. The treatment 6 dS m^{-1} NaCl with 20 Gy was superior in giving the highest fresh 4.75 g and dry weight 0.36 g. A significant increase of proline was observed in the shoots irradiated with a dose of 50 Gy and grown on a medium with 22 mg l^{-1} of NaCl, as it reached 34.36 (µm proline g^{-1} fresh weight) compared 6 dS m^{-1} and unirradiated media and the highest enzyme activity of (POD) was)263.50 units g^{-1} FW (when treated with 100 Gy grown on a medium with 22 ds m^{-1} of salt. While the dose exceeded 20 Gy without adding salt, as it gave the highest activity of (CAT) enzyme, reaching)4.042 units g^{-1} FW(. It was observed that multiplication was generally restricted, depending on the increase in salt applications and gamma rays.

Key words: BA, NAA, Fragaria, Micropropagation, mutation gamma ray. Salt tolerance.

Introduction

Strawberry (Fragaria x ananassa) is a hybrid of the genus Fragaria, cultivated worldwide for its fruits. It is distinguished by its red color, juicy texture, aromatic smell and sweet taste[1,2]. Salinity is one of the significant environmental stresses that affect the biological processes of many plants and have different effects on the plant's physiological processes, such as an increase in respiration rate, ion toxicity and a decrease in the amount of net CO_2 assimilation rate[3]. High salinity stimulates ROS (reactive oxygen species) and their accumulation in plant cells[4]. Oxidative stress defenses occur through an enzymatic anti-oxidant mechanism that includes catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD)[5]. based on in various studies, salinity stimulates the plant to produce free radicals reactive oxygen species (ROS)[6], the increase in the production of free radicals in plants encourages the processes of demolition. It develops the strategies that cause necrosis and plant damage[7]. In vitro mutagenesis techniques is an alternative to induce genetic variation[8,9]. Genetic variations resulting from in vitro mutagenesis are free of regulatory limitations and allow the regeneration of genetic variations in a short period and at a low cost. The system provides easy manipulation of explants in confined and controlled spaces under aseptic conditions[10,11]. Mutation with cobalt 60 gamma rays has a high penetration potential and no risk to the environment and can be used to irradiate cells, tissues, organs and whole plants[12,13]. The objective of the present work was to obtain a salt-tolerant clone in strawberries using an in vitro mutation method.

Materials and methods

The current study was conducted at the Ministry of Science and Technology/ Directorate of Agricultural Research, Genetic Engineering Department, in 2021. Fragaria x ananassa Duch. cv. Albion was used in this experiment. As the seedlings were taken from the Municipality of Baghdad, the runner was separated from the mother plant in the greenhouse and transferred to the laboratory; the top of the runner was cut with a length of approximately 1 cm[14]. Then they were cleaned well with liquid soap and washed with running water for an hour.(to reduce phenolic substances); then, the plant parts were kept in flasks containing an anti-oxidant solution consisting of a mixture of 150 mg.l^{-1} citric acid and 100 mg.l^{-1} of ascorbic acid 15. Plant parts are kept in the refrigerator at a temperature of 4°C for 24 hours before planting it and then transferred inside the laminar air flow cabinet for surface sterilization. Subjected ethyl alcohol (70%) for 1 minute, then treated with mercuric chloride HgCl2 (0.1%) for 7 minutes with a few drops of liquid soap as a diffuser to reduce its surface tension[16]. The explants were washed 3 times with sterile distilled water for 5 minutes. Micropropagation: runners explant were cultured on MS medium[17] supplemented with 0.5 mg l^{-1} of BA and 0.1 mg l^{-1} of NAA. The cultures were incubated in a culture room under 16-h light and 8-h dark photoperiod at 23±1°C.Radiation: To study gamma radiation's effect on plants propagated by tissue culture, the plants were irradiated with different doses (0,20,50,100) Gy of gamma ray (source Co60)at the University of Baghdad / College of Science / Department of Physics. Salinity treatment: The irradiated and unirradiated

Citation: Alhamza Juameer R A, Assi Obaid A, Ayed Yousif S.Improved micropropagation and salinity tolerance of strawberry (Fragaria X ananssa L) cv. Albion. Revis Bionatura 2022;7(4) 34. http://dx.doi.org/10.21931/RB/2022.07.04.34
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

shoots were grown on MS medium containing 0.5 mg l^{-1} of BA and 0.1 mg l^{-1} NAA and supplemented with sodium chloride (NaCl) at concentrations of (6, 10, 14, 22) dS m^{-1}.The culture was incubated at the same conditions previously mentioned above. Thirty days later, shoots numbers, shoot length (cm), vegetative fresh and dry weight (g) were recorded, and amino acid proline was estimated according to 18 as well as the peroxidase activity was evaluated according to the method19 with some modifications and the activity of catalase enzyme was evaluated according to the method of 20 with some changes. The experiment was designed as a factorial experiment based on a Completely Randomized Design (CRD). The factors included gamma irradiation and salt levels with 4 replications for each treatment. DATA analyzed by the Genstat program and means were separated at 5% probability using Duncan's test.

Results and discussion

Effect of salinity levels and radiation dose

Average shoots number

The results in Table 1, figure 1 showed the superiority of the concentration 6 dS m^{-1} and 10 dS m^{-1} in giving the highest average number of shoots, reaching 8.688 and 9.000 shoots, respectively, and no significant differences were found between both treatments. As for the effect of gamma irradiation on the number of projections, the results showed that 20 Gy significantly affected the highest average number of nodes, reaching 9.875 shoots. For the Interaction between gamma radiation and NaCl, results in Table 1 and Figure 1 revealed that the highest number of shoots came at 13.75 shoots at 20 Gy in media stressed with 10 dS m^{-1} NaCl. In contrast, the lowest shoots number 1.250 shoots in 100 Gy interaction with 22 dS m^{-1} NaCl.

Average length of shoots

Table (2) results for shoot length indicated that 6 dS m^{-1} gave the highest average shoot length, reaching 2.311 cm compared to other NaCl treatments. Moreover, (0, 20) Gy were superior in providing the highest average of shoot length (2.053, 1.933) cm, respectively, and no significant differences were found between both treatments. For the Interaction between different radiation doses and saline concentrations, the results showed that 6 dS m^{-1} gave the highest average shoots length, reaching 3.510 cm, which was significantly superior as compared to the other salts treatments, while 22 dS m^{-1} of NaCl with 100 Gy gave the lowest length of shoots reached 0.150 cm. The effect of salts on inhibiting plant growth is due to the increased concentration of salts with many physiological and biochemical processes, causing problems in ionic imbalance, lack of nutrient absorption, osmotic stress, ion toxicity and oxidative stress. These processes interfere with cellular components, including DNA, proteins, lipids, and pigments, and thus affect the overall operations of growth and development[21].

As for the effect of radiation levels on the studied traits, the increase in the number of shoots at a dose of 20 Gy

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	8.750 c	12.00 ab	11.25 b	2.750 efgh	8.688 A
10	1.750 gh	13.75 a	10.25 bc	10.25 bc	9.000 A
14	4.750 de	10.00 bc	4.000 def	5.750 d	6.125 B
22	1.250 h	3.750 defg	2.000 fgh	1.250 h	2.062 C
mean	4.125 C	9.875 A	6.875 B	5.000 C	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly diferent (P<0.05).

Table 1. Effect of gamma rays and salinity on shoots number of strawberry cv. Albion after four weeks from cultured on MS medium.

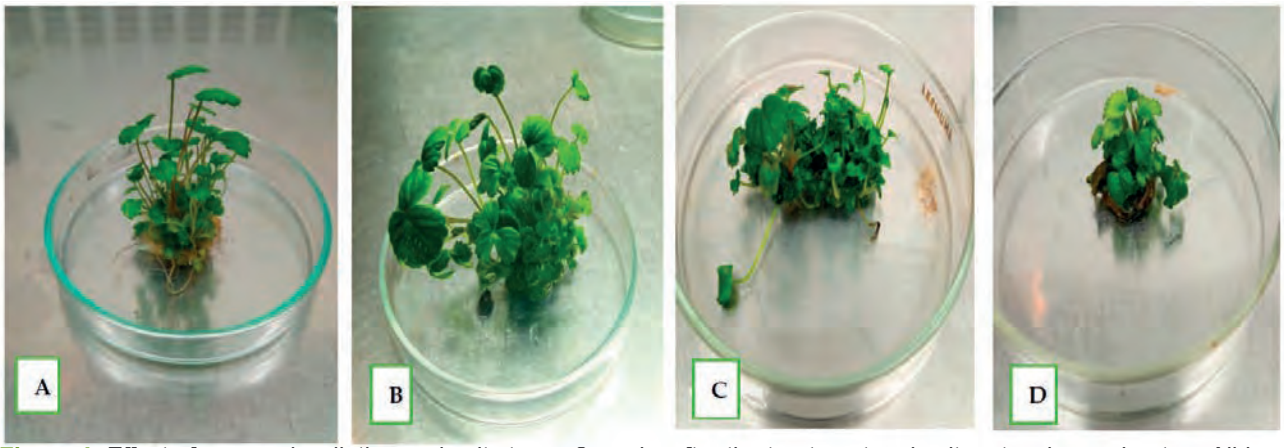

Figure 1. Effect of gamma irradiation and salt stress, 8 weeks after the treatment on in vitro strawberry shoot cv. Albion. A-6 dS m^{-1} .B- 20 Gy and 10 dS m^{-1} NaCl. C- 50 Gy and 10 dS m^{-1} NaCl D- 100 Gy and 10 dS m^{-1}.

could be associated with a hormetic result. According to 22 the hormetic effect is characterized by beneficial or stimulation of development at low doses; and toxicity and inhibition at high doses. According to 23, In this study, doses higher than 100 Gy caused a reduction in the number of shoots per explant and shoot length. The reduced development and increased mortality rate at high doses could be associated with prolonged exposure to 60Co. The high dosage of gamma-ray causes the production and accumulation of ROS, which are toxic to plant tissues[24,25].

Average of fresh weight (g)

The results shown in Table (3) revealed that 6 dS m^{-1} gave the highest average of fresh weight, reaching (2.36 g), which was significantly superior as compared to the other salts treatments, while 22 dS m^{-1} of NaCl gave the lowest fresh weight got 0.37 g. Regarding the gamma effect, the dose 20 Gy showed the highest new importance, reaching 2.82 g, which differed significantly from the other doses, and 100 Gy gave the lowest fresh weight of 0.74 g. Concerning the Interaction between salt levels and gamma doses, 20 Gy with 6 dS m^{-1} NaCl treatment showed the highest fresh weight, reaching 4.75 g compared to the lowest new weight of 0.12 g in 22 dS m^{-1} of NaCl with 100 Gy treatment.

Average of dry weight (g)

The result shown in table (4) revealed 6 dS m^{-1} NaCl gave the highest dry weight average, reaching 0.21 g, which was significantly superior to the other salts treatments, while 22 dS m^{-1} of NaCl gave the lowest dry weight, going 0.04 g. Regarding the gamma effect, the dose 20 Gy gave the highest dry weight, getting 0.19 g, which differed significantly from the other doses, and 50 and 100 Gy gave the lowest dry weight (0.08, 0.07)g respectively, and no significant differences were found between both treatment Concerning to the Interaction between salt levels and gamma doses, 20 Gy with 6 dS m^{-1} gave the highest dry weight reached 0.36 g compared to lowest dry weight 0.02 g in media supplemented with 22 dS m^{-1} of NaCl interaction with 100 Gy.

It is noted from the results that salinity caused a decrease in the fresh and dry weight of the shoots may be due to an increase in salt levels resulting in a reduction of the storage of carbohydrates[26], which consequently decreases the growth. As for the inhibitory effect of irradiation, whether as a single factor or its Interaction with salt levels, it has been indicated by several studies[27]. In another study, it was found that the culture media containing l.0 mg l-1 Kin + 1.0 mg l-1 2,4-D gave the highest average fresh weight was 1.0.2 [28].

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	3.510 a	2.700 b	1.243 def	1.790 cd	2.311 A
10	2.698 b	2.223 bc	0.703 fghi	1.050 efg	1.668 B
14	1.460 de	2.095 bc	1.018 efg	0.850 efgh	1.356 C
22	0.542 ghi	0.713 fghi	0.300 hi	0.150 i	0.426 D
mean	2.053 A	1.933 A	0.816 B	0.960 B	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 2. Effect of gamma rays and salinity on strawberry cv shoots length (cm). Albion after 4 weeks from cultured on MS medium.

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	2.80 c	4.75 a	1.40 e	0.50 ij	2.36 A
10	1.14 f	4.22 b	0.94 g	1.59 e	1.97 B
14	0.56 ij	1.82 d	0.64 hi	0.77 gh	0.95 C
22	0.39 j	0.49 ij	0.47 ij	0.12 k	0.37 D
mean	1.22 B	2.82 A	0.86 C	0.74 D	

According to Ducan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 3. Effect of gamma rays and salinity on shoots fresh weight of strawberry cv. Albion after 4 weeks from cultured on MS medium.

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	0.27 b	0.36 a	0.14 d	0.05 fgh	0.21 A
10	0.10 e	0.22 c	0.07 f	0.14 d	1.13 B
14	0.06 fg	0.14 d	0.05 fghi	0.07 f	0.08 C
22	0.03 ghi	0.03 hi	0.06 fg	0.02 i	0.04 D
mean	0.12 B	0.19 A	0.08 C	0.07 C	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 4. Effect of gamma rays and salinity on shoots dry weight (g) of strawberry cv. Albion after 4 weeks from cultured on MS medium.

Proline content in shoots

Data in table (5) clarified that 22 dS m^{-1} positively affected the highest accumulation of amino acid proline, reaching 21.18 µm. g^{-1} FW, compared to other treatments. As for gamma effects, data in the same table revealed 50 Gy gave the highest proline accumulation, reaching 18.02 µm. g^{-1} FW. Interaction between irradiation and salt levels showed a significant increase of proline came 34.36 µm. g^{-1} FW at the dose 50 Gy in the presence 22 dS m^{-1} of NaCl.

The proline content in leaves generally increases with the salinity level; proline has essential functions in regulating and reducing the undesirable effect of reactive oxygen species (ROS)under saline stress. Therefore, high proline accumulation in plants can induce high tolerance under saline conditions[29,30]. This may be due to the osmotic regulation at different salinity levels. Several studies have suggested that the production of these osmotic alterations may be a common occurrence in response plants to salinity conditions. The role of proline is to increase the adaptation and survival of plants[31,32]. The results of the study showed an increase in the content of proline in irradiated plants; these results are consistent with what was found by 33, who concluded that different doses of gamma rays had other effects on plant biochemical properties such as increasing proline, chlorophyll content and stimulating germination and growth. This technique can be used to produce mutant plants that have the capability of withstanding environmental stresses, it was also found that spraying proline at a concentration of 200 ppm and interfering with irrigation water salinity 2 dS/ m gave the highest total chlorophyll[34].

Activity of peroxidase

The results in table (6) revealed the superiority of the concentration of 22 dS m^{-1} NaCl in giving the highest activity of POD enzyme reached 255.6 units g^{-1} FW, compared to the other salt treatments. Regarding the gamma effect, the dose 100 Gy gave the most increased activities of POD reaching 85.79 units g^{-1} FW, and the control treatment (0 Gy) gave the lowest activities of POD, running 78.81 units g^{-1} FW. Interaction between irradiation and salt levels showed a significant increase of activities of POD got 263.50 units g^{-1} FW compared to the lowest activities of POD 2.92 units g^{-1} FW in 10 dS m^{-1} of NaCl with 20 Gy.

Activity of catalase

The results in a table (7) revealed that 6 dS m^{-1} of NaCl gave the highest average of activities of CAT reaching 3.222 units g^{-1} FW, which was significantly superior as com-

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	4.89 f	7.11 ef	4.83 f	7.96 ef	6.20 C
10	5.40 f	6.76 ef	8.25 ef	11.57 de	8.00 C
14	8.48 ef	6.89 ef	24.64 b	7.08 ef	11.77 B
22	20.95 bc	13.14 d	34.36 a	16.27 cd	21.18 A
mean	9.93 B	8.48 B	18.02 A	10.72 B	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 5. Effect of gamma rays and salinity on proline content(µm. g^{-1} FW) in strawberry CV. Albion after 4 weeks from cultured on MS medium..

NaCl level (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	14.08 h	9.58 hi	9.00 hij	4.75 ij	9.35 C
10	25.67 g	2.92 j	8.00 hij	11.58 h	12.04 C
14	29.17 g	75.25 d	53.75 f	63.33 e	55.38 B
22	246.33 c	252.3 b	260.4 a	263.5 a	255.6 A
mean	78.81 C	85.00 AB	82.79 B	85.79 A	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 6. Effect of salinity levels and radiation doses on the activity of peroxidase (POD) (unit g^{-1} FW) of strawberry, CV Albion after 4 weeks from cultured on MS medium.

NaCl levels (dS m^{-1})	Radiation dose (Gy)				
	0	20	50	100	mean
6	2.788 cd	4.042 a	3.143 c	2.913 c	3.222 A
10	1.611 i	2.742 de	2.151 g	1.834 h	2.085 B
14	2.624 e	1.217 j	1.577 i	2.315 f	1.933 C
22	1.258 j	0.647 l	0.937 k	1.217 j	1.015 D
mean	2.070 B	2.162 A	1.952 C	2.070 B	

According to Duncan's test, means in the same column or their interactions followed by the same letters are not significantly different (P<0.05).

Table 7. Effect of salinity levels and radiation doses on the activity of Catalase (CAT) (unit g^{-1} FW) of strawberry, CV Albion after 4 weeks from cultured on MS medium.

pared to the other salts treatments, while 22 dS m^{-1} of NaCl gave the lowest activities of CAT reached 1.015 units g^{-1} FW. At the same time, the effect of irradiation was superior to the treatment 20 Gy, which gave 2.16 units g^{-1} FW, which was significantly superior compared to the other treatments. Concerning the Interaction between irradiation and salinity levels, the dose 20 Gy with 6 dS m^{-1} of NaCl gave the highest activity CAT reaching 4.042 units g^{-1} FW. In contrast, the dose 20 Gy with 22 dS m^{-1} NaCl delivered the lowest activities reaching 0.647 units g^{-1} FW. The increase in the activity of the peroxidase enzyme POD with an increase in salinity level may be due to the increase in anti-oxidants due to iron deficiency, as well as the disruption of the electron transport chain in chloroplast[35] due to the increase in sodium or chloride ions, which increases the effectiveness of some enzymes, including POD[36]. While the results of table (7) showed that salinity caused a decrease in the activity of the CAT enzyme by increasing the salt levels, it may be due to the highly dynamic system of anti-oxidants, where the enzyme activity varies in different species and also depends on the intensity or duration of the stress. The activity of the CAT enzyme was inhibited under exposure to salt stress for a long time, which leads to the inhibition of the primary product of stress for the expression of the corresponding gene, which is consistent with what (37) found. Still, a decrease in the activity of CAT enzyme may occur due to exposure to less time to stress, as shown in other experiments[38]. The mechanism of the effect of gamma rays lies in its stimulation of many genetic changes[39], phenotypic and biochemical changes[40] and physiological changes in plant cells and tisues[41].

Conclusions

Gamma radiation 20, 50 and 100Gy were chosen to induce variations in vitro. The result proved that 20 Gy had a good efficiency for strawberry improvement, giving the highest shoots number, fresh and dry weight and activity of peroxidase and catalase. The increased salinity increased proline and peroxidase activity.

Funding

This research received no external funding

Acknowledgments

We would like to thank Laboratory of Plant Physiology and Plant Tissue Culture of the Universidad de Antioquia. A special acknowledgment to Universidad de Antioquia's

Research Development Committee (CODI) and Granja Yariguíes – Compañia Nacional de Chocolates.

Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ARTICLE / INVESTIGACIÓN

Impact of organic, biological and mineral fertilizations on the mineral content of potato sprouts and tubers planted in gypsum soils

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Abstract: The study was carried out during the 2021 spring planting season at the Fallujah palm station belonging to the Department of Horticulture, the Ministry of Agriculture, in gypsum soil to study the Impact of organic Fertilization and inoculation with P.sudomonas bacteria, *Trichoderma harzanium* and Mineral fertilization on the growth and yield of potatoes of Burren variety, Experiment included three factors. The addition of fertilization recommendation by 100% (M100) led to a significant increase in the content of major nutrients (N, P, K) in the vegetative part, which amounted to 1.35, 0.25 and 1.42 percent, respectively, and in the tubers which amounted to 1.22, 0.21 and 1.28%, respectively. And the starch in the tubers reached 12.89 g. plant-1, while adding half of the Fertilization recommendation (M50) gave the lowest rates for the traits mentioned earlier. The double and triple interactions gave significant results in growth characteristics, yield and mineral content.

Key words: Potatoes, Organic fertilization, Bio-fertilizations, Mineral fertilization, yield.

Introduction

The potatoes (*Solanum tuberosum L.*), which belong to the Solanaceae family, are the third food Yield after wheat and rice. It is also the most critical type of yield because it contains a quantity of protein, starch, and vitamins[4]. Adding organic matter to soil increases the activity of soil biology, as microorganisms decompose these wastes in the process of mineralizing the organic matter consisting of carbonic acid, which increases the readiness of the elements and reduces the degree of soil interaction, and organic matter may accumulate in the soil (SOM) as a result of the decomposition of waste The plant material that is added to the field soil from the residues of agricultural Yields, and organic materials can be added that are secretions from tree leaves, root secretions and the remains of decomposing soil organisms[2].

Bio-fertilizations are one of the modern agricultural applications, as they contain many microorganisms and have an essential role in the production of growth regulators in addition to improving the physical and mineral properties of the soil, which increases the Impactiveness of bio-fertilizations by adding them with organic Fertilizations and this leads to an increase in yield production[3]. The presence of organic and Bio-fertilizations and the addition of organic matter in the soil increases the activity of microorganisms on the earth. Bacteria that stimulate plant growth (PGPR) are essential for many agricultural Yields[20] as different types of (PGPR) eliminate many plant diseases. It facilitates plant growth directly or indirectly, as well as the decomposition of organic matter[16].

Pseudomonas bacteria secrete plant hormones that encourage plant growth, such as auxins, including IAA (Indol acetic acid) and cytokinin. They also secrete the Enzyme (ACC-deaminase) that inhibits the presence of ethylene[5]. *Trichoderma harzanium* fungus is a valuable living throw fungus that is used in biocontrol on a large scale. Experiments in many countries have also shown that Trichoderma mushrooms have a substantial impact in the agricultural field, especially increasing the readiness of nutrients such as nitrogen, phosphorus and potassium through its excretion of some enzymes and its high ability to degrade existing organic substances or soil additives, as well as its high ability to give the plant breadwinner resistance against pathogens[5,13,18] found that the addition of mushrooms *Trichoderma spp*. It gave the highest level of nitrogen and phosphorus at each level of organic matter, and the addition of mushrooms gave the highest values to the weights of dry matter of the tomato plant.

Showed[22] when adding organic matter (sheep, poultry and horse manures) in interaction with bio-fertilization (*Azotobactre chroococcum*, *Pseudomonas florecienis* (that the organic matter contributed either alone or in interaction with growth-stimulating bacteria in increasing the growth and production of potato plants.

The macronutrients in general and the macronutrients in particular are among the factors that affect the yield of the plant due to their importance in the formation of the vegetative, root and yield Among the most important of these macronutrients are N, P, K These nutrients come from mineral Fertilizations that are added to the soil[11,12] found in their study conducted to find out the impact of the interaction between organic and mineral fertilization and for two seasons on the growth and yield of potatoes when adding urea fertilization at four levels (0, 50%, 75%, 100%) of the Fertilization recommendation approved in his study[24]. Results of the spring season showed that the 100% treatment outperformed the concentration of nitrogen and potassium in the lea-

Citation: Alhamadani, Najm.; Al Ma'amery, A. A. K.; Lastname, F. Impact of Organic, Bio and Mineral Fertilizations on the Mineral content of Shoots and potato tubers planted in Gypsum Soils. Revis Bionat a 2022;7(4) 35. http://dx.doi.org/10.21931/RB/2022.07.04.35
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

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ves as it reached 2.07% and 3.65% respectively, compared to the comparison treatment which gave the lowest rates for the aforementioned traits[25] found in his study, which was conducted to find out the impact of the level of mineral and organic Fertilizations added to the soil on the characteristics of the Mineral content, to the presence of superiority in the aspects of the study when adding nitrogen Fertilization with an amount of 240 kg N H^{-1}, compared to the comparison treatment, which gave the content of leaves at the maturity stage of Nitrogen, Phosphorous and Potassium were 4.77, 0.45 and 4.67%, respectively, compared to the comparison treatment, which gave the lowest average for the aforementioned traits.

The study aimed to know the impact of organic, Bio and Mineral Fertilizations, with the interaction between them on characteristics of Mineral content of the shoots and tubers of potato plants.

Materials and methods

The study was carried out during the 2021 spring planting season at the Fallujah palm station belonging to the Department of Horticulture, the Ministry of Agriculture, on gypsum soil to study the impact of organic matter and bio-fertilization with *P.Seudomonas florecienis* bacteria, *Trichoderma harzanium* and mineral Fertilization on vegetative growth characteristics, Mineral content and yield traits. RCBD randomized complete blocks with three replications. The area required to carry out the experiment was determined, and the process of preparing the soil for cultivation was carried out by performing orthogonal plowing, smoothing and leveling operations. Soil samples were taken from a depth of 0.3-0 m from different sites of the field. They were mixed well, dried aerobically, smoothed and passed through a sieve with holes 2 mm in diameter, from which samples were taken to conduct some physical and mineral analyses and the results of which are presented in table (1). A field was divided into three sectors, and each sector was divided into 15 experimental units, and each experimental unit was into three lines (each 2.50 m long and the distance between line and the other 0.75 m) left a distance of (1 m) between the experimental units and (2 m) between the sectors.

Study factors

The first factor: Organic Fertilization

We are adding two levels of fermented organic fertilization: (1) without adding C0 organic Fertilization (2) Adding organic Fertilization C1.

The second factor:

Bio-fertilization(1) comparison and symbolized with the symbol (B0)

(2) adding the bacterial vaccine Pseudomonas and symbolized by the symbol (B1)

(3) Adding the Trichoderma fungal vaccine and symbolizing it with the symbol (B2)

(4) Adding the bacterial vaccine Pseudomonas and the Trichoderma fungal vaccine and symbolizing it with the symbol (B3).

The third factor: Mineral fertilization:-

(1) Add half of the Fertilization recommendation (120 kg N, 60 kg P and 200 kg. K h^{-1}), and its symbol is M50

(2) Add 3/4 of the quantity at a rate of 75% (180 kg N, 90 kg P and 300 kg . K h^{-1}) from the Fertilization recommendation and its symbol is M75

(3) Add the total amount of fertilization: (240 kg. N, 120 kg. P and 400 kg .K h^{-1}), and its symbol is M100.

Knowing that the recommended Fertilization recommendation is 250 kg $N.h^{-1}$ and 50 kg $N.h^{-1}$ and potassium is 400 kg, depending on the source[7].

Property	Unit		Value
PH	-		7.30
E.C	ds m^{-1}		4.2
N	Mg.kg-1		62.0
P			16.2
K			149.0
O.M	$g.kg^{-1}$		1.2
C.E.C			36.18
	Ca++	20.95	
	Na+	6.25	
	K+	1.6	
	Mg++	10.75	
	$Co^{=}_3$	Nil	
	HCO^{-}_3	1.15	
	CL^{-}	10.50	
Bulk density	g/cm		1.10
Microbial density	Cfu.10^5 g^{-1}soil		2.21
Gypsum	$g.kg^{-1}$. soil		330
Clay	$g.kg^{-1}$. soil		10
Silt	$g.kg^{-1}$. soil		860
Sand	$g.kg^{-1}$. soil		130
Calcium carbonate	$g.kg^{-1}$. soil		44
Texture Class	-		Lomay Sand

Table 1. Some Mineral and physical properties of field soil before planting.

The Bacterial inoculum was multiplied by taking a weight of 15 g of the nutritional medium (Nutrient Broth) dissolved in a liter of distilled water. The pH of the solution was adjusted to 7.5 -7, then sterilized by the autoclave, the solution was cooled, and the liquid culture was prepared by taking a smear of Pseudomonas bacteria by means of a loop, the lube was inserted into the container flask. The nutrient solution must be carefully designed to prevent other organisms from entering the food environment and contaminating it. This process was carried out inside a Hood device sterilized by alcohol; after the culture process was carried out, the bacterial culture was placed in the incubator at a temperature of 28 °C for Pseudomonas bacteria for 72 hours until signs of growth appeared if Discoloration and turbidity appeared in the solution. The density of Pseudomonas inoculum was 2.3×108 CFU.

After preparing the bacterial inoculum from Pseudomonas bacteria, the bacterial inoculum was added. The tubers were contaminated with the liquid nutritional medium prepared in advance in the laboratory by adding 10% gum arabic and immersing the tubers in the nutrient medium for half an hour to ensure cell adhesion to the tubers. After placing the vaccine, the tubers were directly covered with soil to avoid the impact of sunlight and drought on the vaccine; the Agricultural Research Department prepared local isolates of the bio-vaccine. Ministry of Science and Technology[15].

Sheep residues were added to the soil after they were obtained from the place near the implementation site. The search was done in a pit of dimensions (2 x 3 x 0.5) m.

Characteristics of Mineral content of the shoots and potato tubers

Determination of some elements in leaves and tubers: The fourth leaf was taken from the developing top of the main stem of five plants randomly from the middle line for each experimental unit in the maturity stage according to (Sanderson and White 1983), it was washed with distilled water to remove dirt and dust, and dried in an electric oven containing a vacuum on Temperature is 70 degrees Celsius until the weight is stable[26] then it is Crush and sieved.

Percentage of total nitrogen:- The percentage of total nitrogen was estimated after adding a base acid of NaOH at a ratio of 10 molary by evaporation and distillation method by Micro Kieldahl device after titration with 0.04 standard hydrochloric acid[14].

Percentage of phosphorous:- Percentage of phosphorous was estimated using ammonium molybdate by taking 5 ml of the digested sample, adding to it 10 ml of ammonium molybdate solution, then leaving the samples for 10 minutes, after which the resulting blue intensity was measured by spectrophotometer at a wavelength (620 nm)[28].

Percentage of potassium:- The percentage of potassium is estimated by means of a flame photometer, according to the method used by (19).

Percentage of starch in Tubers-: The percentage of starch (%) in tubers was calculated according to the equation shown in[1] as follows: Starch percentage = 17.55 + 0.89 (% dry matter - 24.18).

Results

Nitrogen content of leaves (%)

The results of table (2, 3) show that the addition of organic fertilization significantly impacted the nitrogen percentage in the leaves and tubers, as the treatment O1 gave the highest nitrogen content in the leaves and tubers, which amounted to 1.38%, and 1.26% respectively. In contrast, the comparison treatment O0 showed the lowest percentage, which amounted to 1.13% and 0.99%, respectively.

While the results of the same tables indicated the significant Impacts as a result of adding bio-fertilization in the nitrogen content of leaves and tubers, as treatment B3 achieved the highest percentage of 1.57% and 1.45% sequentially, then followed by a significant difference between treatment B2 by giving it a rate of 1.33%, 1.20% sequentially, which It differed from treatment B1, which provided a percentage of 1.19% and 1.05%, while treatment B0 achieved the lowest rate of 0.93% and 0.79%, respectively.

Results of table (2, 3) also show the moral Impacts as a result of adding mineral Fertilization, as the M100 treatment recorded the highest percentage of 1.35% and 1.22% sequentially, then followed by a significant difference by the M75 therapy by giving it 1.25%, 1.13% sequentially, while the M50 treatment achieved the lowest percentage. They reached 1.16% and 1.02%, respectively. It was found that the interaction of organic and biological Fertilizations achieved a significant increase in the rate of nitrogen in leaves and tubers, as the treatment O1B3 gave the highest percentage of 1.70% and 1.57%, respectively. In comparison, the treatment O0B0 gave the lowest percentage of 0.84% and 0.67%, respectively.

The percentage of nitrogen also increased significantly as a result of the interaction of organic and Mineral Fertilizations, especially the treatment O1M100, which gave the highest percentage of nitrogen amounted to 1.47% and 1.34%, respectively, while the treatment O0M50 gave the lowest percentage of 1.05% and 0.89% respectively. The bilateral interaction between bio-fertilization and mineral fertilization significantly increased the percentage of nitrogen, as the treatment B3M100 achieved the highest percentage of 1.70% and 1.57%, respectively. In comparison, the treatment B0M50 gave the lowest percentage of 0.74% and 0.58%, respectively.

As for the triple interaction between organic,bio-fertilization and Mineral Fertilizations, the results of table (2, 3) showed significant Impacts due to the tripartite interaction of the research treatments, as the treatment O1B3M100 achieved the highest percentage of 1.77%, 1.64% respectively, while the treatment O0B1M50 performed the lowest percentage of 0.71%, 0.45 % sequentially.

Phosphorous content of leaves and tubers (%)

The results of Table (4, 5) indicated that the phosphorous content of leaves and tubers was significantly affected by the addition of organic Fertilizations, as treatment O1 gave the highest content of 0.26% and 0.22%, respectively, while treatment O0 gave the lowest phosphorous content of 0.19% and 0.15% respectively. It was also shown from the results of the same table that the phosphorous content of leaves and tubers increased as a result of the addition of bio-fertilization, as treatment B3 gave the highest concentration of 0.34% and 0.29% sequentially, followed by a significant difference by treatment B2 with a percentage of 0.27%, 0.23% respectively. In comparison, treatment B0 gave The lowest concentration, 0.12% and 0.08%, respectively.

It was found that the treatment with Mineral fertilization achieved a significant increase in the phosphorous content of leaves and tubers, as the M100 treatment gave the highest concentration of 0.25%, and 0.21% sequentially, then followed by a significant difference by the treatment M75 with a percentage of 0.22%, 0.18% sequentially, while the M50 treatment gave the lowest concentration 0.20%, 0.16% sequentially. The bilateral interaction between organic and biological fertilization had a significant impact in increasing the phosphorous content of leaves and tubers, as the O1B3 treatment showed the highest percentage of 0.39% and 0.34%, respectively, compared to the comparison treatment, which showed the lowest percentage of 0.10% and 0.07%, respectively.

As for the bilateral interaction between organic and mineral fertilization, the results of table (4, 5) showed that it caused a significant increase in the phosphorous content of the leaves, as the treatment O0M100 gave the highest percentage of 0.29% and 0.25%, respectively, while the treatment O0M50 gave the lowest percentage of 0.16%, 0.13% sequentially.

We also note from the results of table (4, 5) that the second interaction between biological and mineral fertilization has made a significant difference, as treatment B3M100 gave the highest percentage of 0.36%, 0.31%, respectively, while treatment B0M50 gave the least significant difference in phosphorous content of leaves and tubers, which amounted to 0.10%. 0.07% sequentially.

(O) Organic Fertilization	(B) Bio- Fertilization	(M) Mineral Fertilization			B × O
		M_{50}	M_{75}	B_{100}	
O_0	B_0	0.71	0.83	0.98	0.84
	B_1	1.04	1.06	1.14	1.08
	B_2	1.14	1.14	1.17	1.15
	B_3	1.30	1.42	1.62	1.45
O_1	B_0	0.77	1.10	1.16	1.01
	B_1	1.24	1.28	1.36	1.30
	B_2	1.46	1.49	1.58	1.51
	B_3	1.62	1.69	1.77	1.70
Impact of Organic Fertilization (O)					
M × O	O_0	1.05	1.11	1.23	1.13
	O_1	1.27	1.39	1.47	1.38
Impact of Bio- Fertilization (B)					
M × B	B_0	0.74	0.97	1.07	0.93
	B_1	1.14	1.17	1.25	1.19
	B_2	1.30	1.32	1.37	1.33
	B_3	1.46	1.56	1.70	1.57
Impact of Mineral fertilization(M)		1.16	1.25	1.35	
L . S . D 5 %					
O	B	M	B × O	M × O	M × B	O × M × B
0.07	0.10	0.09	0.14	0.12	0.17	0.25

Table 2. Impact of organic,bio-fertilization and Mineral Fertilizations on the nitrogen content of leaves of potato plant (%).

(O) Organic Fertilization	(B) Bio- Fertilization	(M) Mineral Fertilization			B × O
		M_{50}	M_{75}	B_{100}	
O_0	B_0	0.45	0.70	0.85	0.67
	B_1	0.90	0.93	0.98	0.94
	B_2	0.99	1.00	1.03	1.01
	B_3	1.20	1.30	1.50	1.33
O_1	B_0	0.70	0.98	1.03	0.90
	B_1	1.11	1.15	1.25	1.17
	B_2	1.33	1.40	1.45	1.39
	B_3	1.50	1.56	1.64	1.57
Impact of Organic Fertilization (O)					
M × O	O_0	0.89	0.98	1.09	0.99
	O_1	1.16	1.27	1.34	1.26
Impact of Bio- Fertilization (B)					
M × B	B_0	0.58	0.84	0.94	0.79
	B_1	1.01	1.04	1.12	1.05
	B_2	1.16	1.20	1.24	1.20
	B_3	1.35	1.43	1.57	1.45
Impact of Mineral fertilization(M)		1.02	1.13	1.22	
L . S . D 5 %					
O	B	M	B × O	M × O	M × B	O × M × B
0.07	0.10	0.09	0.14	0.12	0.18	0.25

Table 3. Impact of organic , bio-fertilization and Mineral Fertilizations on the nitrogen content of tubers (%).

As for the triple interaction, the results shown in Tables (4, 5) indicate that the research treatments have significantly increased the phosphorous content of leaves and tubers, as the O1B3M100 treatment achieved the highest percentage of 0.42%, and 0.36%, respectively. In comparison, the O0B0M50 interaction treatment achieved the lowest percentage of 0.08%, 0.05% sequentially.

Potassium content of leaves and tubers (%)

The results shown in table (6, 7) show that the addition of organic fertilization significantly increased the potassium content of leaves and tubers, as the O1 treatment achieved the highest concentration of 1.45% and 1.32%, respectively. In comparison, the comparison treatment O0 gave the lowest potassium content of 1.19%, 1.08 % sequentially.

We note from the results of table (15) the significant differences as a result of adding bio-fertilization, where treatment B3 gave the highest concentration of potassium, as it reached 1.69%, 1.50%, respectively. Treatment B2 followed by giving it the percentage of 1.43%, 1.28%, while treatment B0 gave the lowest percentage The potassium content of leaves and tubers was 0.94% and 0.93%, respectively.

It was also found that the addition of Mineral Fertilization has achieved a moral increase in the content of leaves and tubers of potassium, with the M100 transaction achieving the highest ratio of 1.42%, 1.28% sequentially, followed by the M75 transaction by giving it a ratio of 1.33%, 1.20% sequentially. In contrast, the M50 transaction gave the lowest ratio to the content of papers and tubers.

Binary overlap between organic and bio-fertilization had a moral Impact, giving the O1B3 transaction the highest ratio of 1.78%, 1.61% sequentially, while the trade gave the lowest proportion O0B0 the content of leaves and tubers of potassium 0.87%, 0.89% sequentially. The results of the same table also indicate the moral impact of adding organic and metallic compost, giving the O1M100 transaction the highest ratio of 1.55%, and 1.39% sequentially, while the O0M50 transaction gave the lowest rate of 1.07%, 0.99% sequentially.

As for the bilateral interaction between bio and Mineral fertilization, Table (6 , 7) showed a positive Impact in increasing the potassium content of leaves and tubers. The B3M100 treatment achieved the highest percentage of 1.83% and 1.64%, while the O0M50 treatment gave the lowest percentage of 0.80%, and 0.87. % sequentially.

As for the triple interaction of the research treatments, results shown in table (6 , 7) showed that the tripartite interaction achieved a significant increase in potassium content of leaves and tubers, as the treatment O1B3M100 gave the highest percentage amounting to 1.90%, 1.71% respectively. In contrast, the treatment O0B0M50 showed the lowest percentage. They reached 0.74% and 0.82%, respectively.

Starch in tubers

Table 8 shows the impact of fertilization on the proportion of growing up in potato plant tubers since the addition of organic fertilization has given a slight increase in the proportion of growing up to tubers compared to the level of non-additivity, where the balance of growing up in tubers is 12.82%. A moral difference has been observed in biomedicine treatment by bacteria and fungi. The addition of Trichoderma fungi with pseudomonas bacteria gave the ideal

(O) Organic Fertilization	(B) Bio- Fertilization	(M) Mineral Fertilization			B × O
		M_{50}	M_{75}	B_{100}	
O_0	B_0	0.08	0.09	0.11	0.10
	B_1	0.13	0.15	0.18	0.15
	B_2	0.19	0.22	0.27	0.23
	B_3	0.25	0.30	0.31	0.28
O_1	B_0	0.12	0.13	0.15	0.13
	B_1	0.18	0.22	0.25	0.22
	B_2	0.28	0.31	0.34	0.31
	B_3	0.37	0.37	0.42	0.39
Impact of Organic Fertilization (O)					
M × O	O_0	0.16	0.19	0.22	0.19
	O_1	0.24	0.26	0.29	0.26
Impact of Bio- Fertilization (B)					
M × B	B_0	0.10	0.11	0.13	0.12
	B_1	0.16	0.18	0.21	0.18
	B_2	0.24	0.27	0.31	0.27
	B_3	0.31	0.33	0.36	0.34
Impact of Mineral fertilization(M)		0.20	0.22	0.25	
L . S . D 5 %					
O	B	M	B × O	M × O	M × B	O × M × B
0.01	0.02	0.02	0.02	0.02	0.03	0.04

Table 4. Impact of organic, bio-fertilization and mineral fertilizations on the phosphorous content of leaves of potato plant (%).

(O) Organic Fertilization	(B) Bio- Fertilization	(M) Mineral Fertilization			B × O
		M50	M75	B100	
O_0	B_0	0.05	0.07	0.08	0.07
	B_1	0.09	0.11	0.13	0.11
	B_2	0.15	0.18	0.22	0.18
	B_3	0.20	0.24	0.26	0.23
O_1	B_0	0.08	0.10	0.12	0.10
	B_1	0.15	0.18	0.21	0.18
	B_2	0.25	0.28	0.31	0.28
	B_3	0.33	0.32	0.36	0.34
Impact of Organic Fertilization (O)					
M × O	O_0	0.13	0.15	0.17	0.15
	O_1	0.20	0.22	0.25	0.22
Impact of Bio- Fertilization (B)					
M × B	B_0	0.07	0.08	0.10	0.08
	B_1	0.12	0.15	0.17	0.15
	B_2	0.20	0.23	0.27	0.23
	B_3	0.27	0.28	0.31	0.29
Impact of Mineral fertilization(M)		0.16	0.18	0.21	
L . S . D 5 %					
O	B	M	B × O	M × O	M × B	O × M × B
0.01	0.02	0.01	0.02	0.02	0.03	0.01

Table 5. Impact of organic, bio-fertilization and mineral fertilizations on the phosphorous content of tubers (%).

ratio for the highest average of 14.85% tuberculosis-formed growing up compared to the comparison treatment that offered the lowest starch rate of 9.23%.

As for the Impact of Mineral Fertilization, the same table results indicate moral differences, as the M100 transaction gave the highest potassium concentration at 12.89%. In comparison, the M50 transaction showed the lowest concentration at 11.44%.

As for the bilateral overlap between organic composting and bio-fertilization, it recorded a moral difference where the O1B3 transaction excelled and gave the highest rate of 15.96%, while the non-additionality transaction gave O0B0 8.81%

One of the results mentioned in the table shows us that there are moral differences in the treatment of organic Fertilization and Mineral fertilization in the percentage of origin found in tubers, giving the O1M100 transaction the highest rate of 13.65%. In comparison, the O0M50 transaction gave the lowest percentage of 10.95%.

The bilateral overlap between bio-fertilization and Mineral Fertilization has had a moral Impact on increasing the proportion of starch material in tubers, giving the B3M100 the best result of 16.01%, compared to the comparison treatment B0M50, which gave the lowest result of 8.46%. Concerning the triple overlap of the study's factors, the table results indicated a moral increase in the percentage of starchy substances in potato tubers, giving B3O1M100 the highest percentage of 17.30% compared to the comparison treatment B0O0M50 which showed the lowest rate of 8.30 %.

Discussion

The main reason for the increase in the characteristics mentioned in tables (2, 3, 4, 5, 6, 7, 8 and 9) may be due to the components of the added organic fertilization containing many nutrients, in addition to the fact that its components are decomposed and thus stimulates the growth of the plant by providing macro and micronutrients Which the plant needs to increase the Mineral content of the potato plant. Organic fertilization improves the physical properties of the soil, as it helps the roots to spread and increase of absorption of elements and water. These two factors contribute to forming a good root system reflected in the nutrient content of the plant and tubers[17].

The reason for the increase in the concentration of nutrients N, P and K in the soil may be due to bio-fertilization contains nitrogen-fixing organisms and phosphate-dissolving organisms.

The role of these organisms in the secretion of organic acids, growth regulators and chelators leads to an increase in the concentration of remaining nutrients in the soil. It may be due to the rise in the surface area of the roots, which increases the absorption capacity of the roots and, thus, the concentration of the remaining elements in the soil. The rest of the nutrients with bio-fertilization was more apparent when added with mineral fertilization because of what the latter provides of ready-made nutrients and easy to lose by volatilization, washing, sedimentation and fixation in the soil. Also, the integrated fertilization between the three vital, mineral and organic Fertilizations has an essential role

(O) Organic Fertilization	(B) Bio- Fertilization	(M) Mineral Fertilization			B × O
		M50	M75	B100	
O_0	B_0	0.74	0.90	0.98	0.87
	B_1	1.03	1.06	1.05	1.05
	B_2	1.07	1.32	1.35	1.24
	B_3	1.43	1.60	1.75	1.59
O_1	B_0	0.85	1.00	1.18	1.01
	B_1	1.32	1.41	1.48	1.40
	B_2	1.58	1.62	1.65	1.62
	B_3	1.69	1.75	1.90	1.78
Impact of Organic Fertilization (O)					
M × O	O_0	1.07	1.22	1.28	1.07
	O_1	1.36	1.45	1.55	1.36
act of Bio- Fertilization (B)					
M × B	B_0	0.80	0.95	1.08	0.80
	B_1	1.18	1.23	1.27	1.18
	B_2	1.32	1.47	1.50	1.32
	B_3	1.56	1.68	1.83	1.56
Impact of Mineral fertilization(M)		1.21	1.33	1.42	
L . S . D 5 %					
O	B	M	B × O	M × O	M × B	O × M × B
0.03	0.04	0.03	0.05	0.05	0.07	0.09

Table 6. Impact of organic, bio-fertilization and Mineral Fertilizations on the potassium content of leaves of potato plant (%).

(O) Organic Fertilization	(B) Bio- Fertilization	(M) Mineral Fertilization			B × O
		M50	M75	B100	
O_0	B_0	0.82	0.89	0.95	0.89
	B_1	0.91	0.93	0.94	0.93
	B_2	0.97	1.15	1.20	1.11
	B_3	1.24	1.40	1.57	1.40
O_1	B_0	0.92	0.93	1.08	0.98
	B_1	1.19	1.25	1.31	1.25
	B_2	1.41	1.46	1.48	1.45
	B_3	1.53	1.58	1.71	1.61
act of Organic Fertilization (O)					
M × O	O_0	0.99	1.09	1.17	0.99
	O_1	1.26	1.31	1.39	1.26
Impact of Bio- Fertilization (B)					
M × B	B_0	0.87	0.91	1.02	0.87
	B_1	1.05	1.09	1.12	1.05
	B_2	1.19	1.31	1.34	1.19
	B_3	1.38	1.49	1.64	1.38
Impact of Mineral fertilization(M)		1.12	1.20	1.28	
L. S . D 5 %					
O	B	M	B × O	M × O	M × B	O × M × B
0.02	0.04	0.03	0.05	0.04	0.06	0.09

Table 7. Impact of organic, bio-fertilization and mineral fertilizations on the potassium content of tubers (%)

(O) Organic Fertilization	(B) Bio- Fertilization	(M) Mineral Fertilization			
		M50	M75	B100	B × O
O_0	B_0	0.82	0.89	0.95	0.89
	B_1	0.91	0.93	0.94	0.93
	B_2	0.97	1.15	1.20	1.11
	B_3	1.24	1.40	1.57	1.40
O_1	B_0	0.92	0.93	1.08	0.98
	B_1	1.19	1.25	1.31	1.25
	B_2	1.41	1.46	1.48	1.45
	B_3	1.53	1.58	1.71	1.61
act of Organic Fertilization (O)					
M × O	O_0	0.99	1.09	1.17	0.99
	O_1	1.26	1.31	1.39	1.26
Impact of Bio- Fertilization (B)					
M × B	B_0	0.87	0.91	1.02	0.87
	B_1	1.05	1.09	1.12	1.05
	B_2	1.19	1.31	1.34	1.19
	B_3	1.38	1.49	1.64	1.38
Impact of Mineral fertilization(M)		1.12	1.20	1.28	
L. S . D 5 %					
O	B	M	B × O	M × O	M × B	O × M × B
0.02	0.04	0.03	0.05	0.04	0.06	0.09

Table 8. Impact of organic, bio-fertilization and mineral fertilizations on the starch content of tubers (%).

in increasing the concentration of nutrients remaining in the soil[8,10]. The impact of bio-fertilization on the absorbed nitrogen, phosphorous and potassium came from the critical role of bio-fertilization in improving some of the soil's physical, mineral and fertility properties and then improving the absorption of those elements. Nutrients by plants, as the response to bio-fertilization, were clearer when added with Mineral fertilization because of what mineral fertilization provides from nutrients ready for absorption[21].

The increase in the concentration of nutrients in part before the plant may be attributed to converting the unready formula to its ready-to-absorb formula, which is in line with what was mentioned by (9).

That bio-fertilizations are essential in increasing the average concentration of nutrients nitrogen, phosphorous and potassium in plants. The superiority is due to the availability of nutrients in a balanced and integrated manner as a result of adding mineral, organic and biological Fertilizations together. Thus bio-fertilization is distinguished by providing growth stimulants and regulators that increase the growth of the root system, as well as the availability of nitrogen in the soil solution and organic fertilization. Provide a balanced growth medium of nutrients, followed by mineral Fertilization, in which the plant is equipped with the necessary elements.

Conclusions

All of this contributes to improving the plant's content of nutrients, especially nitrogen, to build a good root and vegetative group that can benefit from the elements available in the soil and a vegetable group that performs its vital activities that help increase the concentration of elements in the tubers, and these results are consistent with (27) who showed that the plant yield is of high value from the plant's nitrogen content when integrated fertilization is available, which helps the plant to grow actively, manufacture nutrients and transfer them to the yield

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ARTICLE / INVESTIGACIÓN

Effect of D-Aspartic acid on the level of some sex hormones and the biochemical parameters of the blood of Shami Bucks

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DOI. 10.21931/RB/2022.07.04.36

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Abstract: This study was conducted at the Animal Production Department/ College of the Agriculture/ University of Diyala from 15/9/2021 to 15/10/2021 to investigate the effect of injecting D-aspartic amino acid in Shami Bucks on some blood biochemical and hormonal characteristics. Twelve's Shami Bucks aged between 1.5-2 years, and body weight ranged between 35-40 kg. The animals were divided into four groups (treatments) with three replicates among each group as follows, T_1 (control group) was injected with normal saline only, T_2, T_3 and T_4 groups were injected i.m. with D-aspartic acid as follows, 125 mg, 250 mg and 375 mg for T_2, T_3 and T_4 groups respectively, every 48 hours in the afternoon. Blood samples were collected from the jugular vein, and serum was taken and stored at -20 ° C until analyzed. The results of the present study also indicated significant differences (P<0.05) of FSH (1.37±2.59, 1.45±0.89, 1.87±1.76 and 0.77±0.45) and LH (1.96±1.56, 2.19 ± 0.22, 2.22±1.44 and 1.11±1.30) respectively for the T_2, T_3 and T_4 treatments as compared with the T_1 (control group). The results showed a significant increase (P<0.05) of total protein (6.23±0.02, 6.26±0.39, 6.46±1.23 and 4.35±0.12), albumin (4.36±1.24, 4.56±1.00, 4.75±1.34 and 3.34±0.11), globulin (1.87±1.33, 1.70±0.11, 1.71±0.01 and 1.01±1.22) and blood urea (6.45±0.23, 6.43±1.39, 6.56±1.56 and 5.22±1.25) respectively for the T_2, T_3 and T_4 treatments as compared with the T_1 (control group). While no significant differences between all experimental treatments in the concentrations of thyroid hormones (T_4, T_3), cholesterol and triglycerides. It can be concluded from the present study that injection of D-aspartic acid had a significant effect on some biochemical blood traits and the level of pituitary sex hormones.

Key words: D-Aspartic acid, Shami Bucks, biochemical parameters, sex hormones.

Introduction

Goats are considered one of the critical economic animals in Iraq, as they are raised for meat, milk, skin and hair, but they live outside pastoralism and agriculture and feed on waste and jungles. Their fertility and productivity are low, so they need to be cared for and their fertility improved[1]. The Shami goat originated in the Sham[2] and was introduced to Cyprus from Syria more than 70 years ago to improve the performance of their goats. Thus, it was called the Cypriot goat. This type of goat was introduced to Iraq (Ruminant Research Station/ General Authority for Agricultural Research/ Ministry of Agriculture) in the name of the Cypriot Shami goats after they were imported from Cyprus through the Ministry of Agriculture in 2006[3]. Several studies have indicated that environmental factors significantly affect the reproductive efficiency of farm animals, such as increasing the daily light duration and high temperatures[4-5]. The measurement of hematological and biochemical parameters is essential in evaluating animal activity. It indicates animal health, reflected in the production characteristics, as the blood and biochemical parameters are affected by breed, age, physiological condition, season, nutrition, etc. The animal body's physiological regulation works well, which is reflected in its ability to adapt to circumstances and prevailing environmental conditions[6-8]. (9) indicated that amino acids such as arginine and aspartic affect the circulatory system by increasing the heart rate without affecting the number of heartbeats and increasing blood flow to the blood vessels, leading to increased blood circulation. Because there is no study in Iraq to our knowledge showing the effect of injecting aspartic acid on some biochemical parameters of Shami Bucks. This study was conducted to find out the impact of injecting the amino acid L-aspartic by measuring the following characteristics, Total protein, Albumin, Globulin, blood urea, Cholesterol, Triglycerides, Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), T_3 (Tri-iodothyronine) and T_4 (Thyroxine).

Materials and methods

This study was conducted at the Animal Production Department/ College of the Agriculture/University of Diyala from 15/9/2021 to 15/10/2021. In the present experiment, twelve Shami Bucks of 1.5-2 years of age and 35-40 kg weight, placed inside semi-open pens in individual cages, were used. The animals were fed with the 1 kg concentrate feed, in addition to giving them hay and green fodder, according to the animal's need (in an open manner) with the availability of drinking water throughout the experiment. The animals were divided into four treated groups with three replicates, T_1 (control group) was injected with normal saline i.m., T_2, T_3 and T_4 were injected with aspartic acid with a concentration

Citation: Ahmad, Ali.; Atiyah, Safaa. Effect of D-Aspartic acid on the level of some sex hormones and the biochemical parameters of the blood of Shami Bucks. Revis Bionat a 2022;7(4) 36. http://dx.doi.org/10.21931/RB/2022.07.04.36
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

of 125 mg, 250 mg and 375 mg i.m. respectively every 48 hours in the afternoon. At the end of the experiment, blood samples were collected from the jugular vein using sterile 8ml syringes with a test tube free of anticoagulant, centrifuged at a speed of 3000 rpm for 15 minutes. Serum was withdrawn using a special pipette, numbered and kept in the freezer at -20 °C until all the analyzes of the biochemical components were completed.

Total protein

The measurement depends on the occurrence of a color change due to the interaction of copper in a base solution of the measuring kit (prepared by the German Human company) with the peptide bonds of the protein that is found in the blood serum. The solution is mixed and left at room temperature for 10 minutes; a spectrophotometer extracts it at a wavelength of (520) nm. The following equation is applied to obtain the total protein level[10].

Blood urea

The hydrolysis of urea to ammonium ions and carbon dioxide is the basis for measuring urea in the blood, which was approved by the Human German company manufacture of urea measurement kits. Ammonium ions with chlorine and salicylate produce a blue-green complex. This complex and its concentration are measured by a spectrophotometer on A wavelength (600), which indicates the amount of urea in the blood.

Cholesterol

The level of cholesterol was measured in the blood serum using a spectrophotometer with a wavelength of (500) nm using ready solutions (Kit)[11].

Triglycerides

The triglyceride concentration was estimated using a measuring kit supplied by the Human German company, based on the method of (12), and by a spectrophotometer at a wavelength of (500) nm.

Hormonal assays

The levels of T_3, T_4, FSH, and LH hormones were measured using an Enzyme-linked immunosorbent assay (ELISA) and a kit to measure each hormone supplied by Novormon company.

Statistical Analysis

The experiment was carried out using a completely random design (CRD), and data were analyzed using the statistical program SPSS (SPSS, 2011); the averages of the coefficients for each treatment were compared using Duncan's polynomial test[13] to determine the significance of differences between the means. According to the following mathematical model: Yij = + ti+ eij.

Results and discussion

The results of the present study indicated a significant ($P<0.05$) effect of FSH and LH hormones for T_2 (125 mg), T_3 (250 mg) and T_4 (375 mg) compared to T_1 (control group), which were 1.37±2.59, 1.45±0.89, 1.87±1.76 and 0.77±0.45 for FSH and 1. 96±1.56, 2.19±0.22, 2.22±1.44 and 1.11±1.30 for LH respectively (Table 1). There were no significant differences between all treatments of the experiment and the control group in thyroid hormone concentrations (T_4, T_3) and the control group (Table 1). D'Aniello *et al.*[14] indicated that the level of aspartic acid was elevated in the hypothalamus, pituitary, and blood serum, which reached its highest level after one hour of injection. When comparing them, the highest concentration was found in the hypothalamic glands for the presence of enzymes that convert L-Asp to D-Asp in the gland tissue. These results were also confirmed by Di Fiore *et al.*[15] of the increase in the activity of Neuro-steroidogenic enzymes in the brains of mice injected with aspartic acid.

Sheffield *et al.*[16] found a decrease in the concentration of aspartic acid in sheep's blood plasma and body tissues fed a low concentration of aspartic acid. Still, if fed a diet rich in aspartic acid, the concentration will increase in both body tissues, the brain and endocrine glands. Boni *et al.*[17] showed a high percentage of aspartic acid in the tissues of both the pineal gland and the pituitary gland. Still, the pituitary gland was the most efficient in storing aspartic acid in sheep. It recorded that the concentration of aspartic acid tripled more than the average concentration in the pituitary gland, ovary, brain and blood serum after 12 hours of aspartic acid treatment. There are more than 700 amino acids, but only 20, including aspartic acid, are the building units of proteins in cells[18].

The results showed a significant increase ($P<0.05$) of total protein, albumin, globulin and blood urea for the T_2 (125 mg), T_3 (250 mg) and T_4 (375 mg) treatments compared with the T_1 (control group) (table 2). While no significant differences among the treatments group (T_2, T_3 and T_4). The results also showed no significant differences among all experiment treatments and control groups in the concentrations of cholesterol and triglycerides (Table 2).

Amino acids are of great physiological importance, serving as building units for proteins and substrates to synthesize low-molecular-weight substances. Depending on growth or nitrogen balance, amino acids have traditionally been classified as nutritionally essential or non-essential for animals[19]. Abdelhamed *et al.*[20] mentioned that the concentration of aspartate was the lowest in the local goat breed, while it was the highest in the hybrid goat breed, and this may be due to the breed effect on amino acid production.

Conclusions

The present study showed that injection of D-aspartic acid significantly affected some sex hormones and the biochemical parameters of the blood of local Shami Bucks.

Funding

Self-funding.

Conflicts of Interest

There is no conflict.

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Hormones concentration	Treatments			
	T_1 (control)	T_2 (125 mg)	T_3 (250 mg)	T_4 (375 mg)
FSH (ng/ml)	0.77±0.45 b	1.37±2.59 a	1.45±0.89 a	1.87±1.76 a
LH (ng/ml)	1.11±1.30 b	1.96±1.56 a	2.19±0.22 a	2.22±1.44 a
Thyroxin (T_4) (ng/ml)	61.38±1.23 a	61.59±1.2 a	61.55±0.55 a	63.66±0.94 a
Triiodothyronine (T_3) (ng/ml)	2.08±1.16 a	2.47±0.44 a	2.49±1.66 a	2.43±1.70[1] a

[1]Means with different letters within one row indicate significant differences ($p < 0.05$).

Table 1. Effect of aspartic acid injection on thyroid and some pituitary hormones of Shami Bucks (mean ± standard error).

Blood Parameters	Treatments			
	T_1 (control)	T_2 (125 mg)	T_3 (250 mg)	T_4 (375 mg)
Total protein g/dl	4.35±0.12 b	6.23±0.02 a	6.26±0.39 a	6.46±1.23 a
Albumin g/dl	3.34±0.11 b	4.36±1.24 a	4.56±1.00 a	4.75±1.34 a
Globulin g/dl	1.01±1.22 b	1.87±1.33 a	1.70±0.11 a	1.71±0.01 a
Blood urea mg/dl	5.22±1.25 b	6.45±0.23 a	6.43±1.39 a	6.56±1.56 a
Cholesterol mg/dl	19.99±2.22 a	21.11±1.00 a	20.48±1.33 a	21.44±0.76 a
Triglyceride mg/dl	89.88±0.76 a	91.32±2.15 a	90.79±1.00 a	90.76±1.44[1] a

Table 2. Effect of aspartic acid injection on some blood biochemical traits of Shami Bucks (mean ± standard error).

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ARTICLE / INVESTIGACIÓN

Response of three broad bean varieties (*Vicia faba* L.) to boron, iron, and zinc nano fertilizers

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DOI. 10.21931/RB/2022.07.04.37

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Abstract: This work was carried out at the Research Station of the Seed Technology Center of the Agricultural Department / Ministry of Science and Technology during the season 2020-2021 to investigate the effect of three nano fertilizers (boron, iron, zinc) at a rate of 2 mg. l-1 on three wide been varieties (Syrian, Spanish, Taqa). The experiment was carried out in a randomized complete block design (R. C. B. D.) with three replicates. The results showed that the Syrian variety gave the highest values of plant height and number of pods per plant, while the Spanish type gave the highest values of pod length, number of seeds per pod and seed weight per plant. Taqa variety recorded the highest number of branches, plant and total seed yield weight of 100 seeds, the lowest number of days to 50% flowering and the first pod formation. Nano fertilizers increased some growth and yield properties. B-nano fertilizer gave the highest values of the number of pods per plant, pods weight per plant, pod length and the number of seeds per pod, while Zn- nano fertilizer increased plant height, plant and total seeds yield.

Key words: Broad been, pods, yield, seeds, nano fertilizers.

Introduction

Faba bean (*Vicia faba* L.) is a winter-growing food legume crop, growing for three main reasons as a component of a rotation based on winter or summer cereals or cotton, green manure where soils have been degraded in organic and physical fertility. And cash crops through marketing dry seeds.

Many studies on faba bean show significant differences among varieties concerning yield and yield components[1,2]. Growing three faba bean cultivars (Nubaria 1, Nubaria 2 and Nubaria 3), they found that Nubaria 2 recorded the highest value of plant height (cm), pod length (cm), number of pods/plant, number of seeds/pod, 100- seed weight (g), grain, straw, and biological yield (tons/fed.)[3].

In a study to evaluate three faba bean cultivars (Reina, Giza and Local), the results showed that the Reina cultivar significantly increased (No. of branches, Pod Yield g. plant^{-1}, Total Yield ton. Donum^{-1}, No. of seed per pod, no. of seed. Plant^{-1}, seed weight (g), seed, yield.plant^{-1} total seed yield ton. donum^{-1} and weight of 100 seed g). While the Giza cultivar significantly increased (plant high, pod length cm, pod weight g.)[4]. A study to evaluate three faba bean varieties (Gora, Moti and Degaga) found that the highest seed yield was from Degaga, Moti and Gora varieties, respectively[5].

Nanotechnology can present solutions for increasing the value of agricultural products and reducing environmental problems. Using Nano-particles and nanopowders can be delayed releasing fertilizers. Nanoparticles have high reactivity because of more specific surface area, more density of the reactive regions, or increased reactivity of these areas on the particle surfaces. The Nano fertilization technique depends on minimizing bulk materials to get at least one dimension smaller than 100 nm[6].

The use of nano fertilizers causes an increase in their efficiency, reduces soil toxicity, minimizes the potential adverse effects associated with overdosage and reduces the frequency of application. Nano fertilizers mainly delay the release of nutrients and extend the fertilizer effect period. Hence, nanotechnology has a high potential for sustainable agriculture, especially in developing countries[7]. In a study of the effect of nanomaterial on faba bean, the results indicated that zinc nanomaterials (50ppm) caused a significant increase in overall bean length, number of branches, and number of branches compared with sulfur concentrations and the control[8]. Spraying faba bean plants with iron as iron nano oxide (Fe_3O_4), 150 mg Fe L^{-1}, and 300 mg Fe L^{-1} showed the highest plant height, the number of pods per plant, and seed yield by the high nano-Fe 300 followed by 150 and 300 mg Fe L^{-1} (as Fe-EDTA) respectively[9]. Studied the response of faba planted to the foliar spray of ZnO NPs concentrations (0, 50, and 100 mg L^{-1}), while an enhancement was recorded in response to foliar treatment with ZnO NPs at 50 and 100 mg L^{-1}. The highest amounts of chlorophyll a, b, carotenoids, and total pigments were recorded in plants that received 50 mg L^{-1} ZnO NPs compared to the alternative control. Secondary metabolites (phenols, flavonoids, and tannins) were accumulated in response to ZnO NPs treatment was noticed[10]. This study investigates the effect of three nano fertilizers (Fe, B and Zinc) on the growth and yield of three faba bean varieties.

Citation: Alabade A, Al-Khashab S, Kahlel A. Response of three broad bean varieties (Vicia faba L.) to boron, iron, zinc nano fertilizers. Revis Bionat a 2022;7(4) 37. http://dx.doi.org/10.21931/RB/2022.07.04.37
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

Materials and methods

This study was carried out at the Research Station of Seed Technology Center of the Agricultural Department of Science and Technology Ministry during the season 2020-2021 to investigate the effect of spraying three types of nano-fertilizers (boron, iron, zinc) on growth and yield of three varieties of broad bean (Syrian, Spanish, Taqa) at a concentration of 2 g L^{-1}.

The experiment was carried out using a randomized complete block design (R. C. B. D.), and each treatment was repeated three times.

Studied traits: plant height (cm), Number of branches/plant, Number of days until 50% flowering, The number of days for the appearance of the first pod, Number of pods/plant, Weight of pods/plant (gm), Pod length (cm), Number of seeds/pod, Weight of seeds/plant (gm) and Weight of 100 seeds (gm)

The results were statistically analyzed according to the statistical analysis system (SAS) and compared with the means by the Duncan multiple range test at 0.05 level[11].

Results

Data in table (1) revealed the effect of foliar spraying of nano fertilizers in the vegetative growth of three broad bean varieties. It was found that the Syrian variety gave the highest Plant height (70.5 cm), number of days to 50% flowering (63.5 days), number of days to first pod (82.1 days), and number of pods/plant (14.6). Meanwhile, the highest number of branches per plant (9.6) was from the Taqa variety. Also, table 1 showed that spraying with nano fertilizers increased the vegetative growth parameters, the highest plant height (61.6 cm), and the number of branches per plant (8.2). The number of pods/plant (13.1) was from spraying with Zn- nano fertilizer, while the highest number of days to 50% flower (63.5 days), number of days to first pod (81.3 days) was from spraying Fe- nano fertilizer.

Table 2 showed that combination treatments between varieties and nano fertilizers significantly affected the vegetative growth parameters.The highest plant height (72.6 cm) was obtained from a combination treatment between the Spanish type and Zn nano fertilizer. At the same time, the highest number of branches per plant (10.5) was from a combination treatment between the Taqa variety and control. The combination treatment between the Spanish variety and control gave the highest number of days to 50% flowering (64.6 days). Moreover, the highest value of the number of days to the first pod (83.6 days) and the number of pods per plant (18.9 pods) was recorded from a combination treatment between the Syrian variety and Fe –nano fertilizer.

Table (3) result illustrates the effect of foliar spray of nano fertilizers in yield components of three broad bean varieties. It was found that the Taga variety gave the highest weight of pods/plant (340.8 gm) and weight of 100 seeds (126.7 gm). While the Spanish variety recorded the most increased pod length (22.9 cm), the number of seeds per pod (5.8), and seed weight per plant (60.3 gm).

Table 3 also showed that spraying nano fertilizers increased the yield component of broad bean, B- nano fertilizer recorded the highest weight of pods/plant (320.3 gm), pod length (19.6 cm), and the number of seeds per pod (4.8). While Zn- nano fertilizer recorded the highest seed weight/plant (49.8 gm) and weight of 100 seeds (120.2 gm).

Table 4 illustrates the effect of combination treatments between varieties and nano fertilizers in yield components. It was found that combination treatment between the Taqa variety and B – nano obtained the highest value of the weight of pods per plant (389.1 gm), in regards to pod length (cm), the highest value (24.5 cm) recorded from combination treatment between Spanish variety and B-nano. At the same time, the highest value of the number of seeds per pod (6.3) and seed weight per plant (72.5 gm) was recorded from a combination treatment between the Spanish variety and Zn-nano fertilizer. The combination treatment between the Taqa variety and Zn-nano fertilizer recorded the highest weight value of 100 seeds (138.6 gm).

Discussion

Increasing vegetative growth among varsities can be attributed to their genetic differentiation, which may allow higher plant capacity to absorb more nutrients from the soil, more photosynthetic surfaces, and, therefore, better photosynthetic capacity[12,13]. The significant increase in growth and yield of broad bean plants due to the application of micronutrients and micronutrients might be attributed to improvement in growth parameters and yield attributes. The

Treatments	Plant height (cm.)	No. of branches per plant	No. of days to 50% flowering	No. of days to first pod	No. of pods per plant
Varieties					
Syrian	70.5a	7.5b	63.5a	82.1a	**14.6a**
Spanish	69.8a	6.2b	63.1a	81.2a	**11.6b**
Taqa	38.9b	9.6 a	61.6b	78.5b	**11.7b**
Types of nano fertilizers					
control	58.4a	7.7a	63.3a	80.1b	**12.7ab**
B- nano	58.8a	7.7a	62.7a	80.5ab	**13.4a**
Fe- nano	60.0a	7.4a	62.5a	81.3a	**11.3b**
Zn- nano	61.6a	8.2a	62.4a	80.5ab	**13.1a**

The averages with the same letter for each factor have non-significant differences according to Duncan's multiple range tests under level 0.05

Table 1. Effect of foliar spraying of nano fertilizers in vegetative growth of three broad bean varieties.

Treatments		Plant height (cm)	No. of branches Per plant	No. of days to 50% flow-ering	No. of days to first pod	No. of pods per plant
Syrian	Control	72.3a	6.9cd	64.0 a	82.3ab	14.5bc
	B- nano	69.3a	7.8bcd	63.3a-c	82.3ab	12.0c
	Fe –nano	71.3a	7.8bcd	63.6 ab	83.6a	18.9a
	Zn- nano	69.3a	7.6bcd	63.3a-d	81.6ab	13.1c
Spanish	Control	68.6a	5.8d	64.6a	81.3bc	11.6cd
	B- nano	68.6a	6.2d	63.3a-c	82.3ab	12.1c
	Fe- nano	70.0a	5.7d	62.6a-d	81.6ab	9.1d
	Zn- nano	72.6a	7.1bcd	62.6a-d	80.3bc	13.5bc
Taqa	Control	42.3b	10.5a	62.3b-d	77.3e	12.1c
	B - nano	34.3c	9.2ab	61.6cd	77.6de	16.1b
	Fe- nano	38.6bc	8.6abc	61.3d	79.3cd	6.1e
	Zn- nano	44.6b	10.1a	61.6cd	79.6c	12.7c

The averages with the same letter for each factor have non-significant differences according to Duncan's multiple range tests under level 0.05.

Table 2. Effect of combination treatment between varieties and nano fertilizers in vegetative growth of three broad bean varieties.

Treatments	Weight of pods per plant (gm)	Pod length (cm)	No. of seeds per pod	seed weight per plant (gm)	Weight of 100 seeds (gm)
Varieties					
Syrian	251.3b	13.6c	3.4c	33.1b	**114.3a**
Spanish	301.5ab	22.9a	5.8a	60.3a	**108.2a**
Taqa	340.8a	17.1b	4.1b	31.2b	**126.7a**
Types of nano fertilizers					
control	317.4a	17.1b	4.2a	39.6b	**112.7b**
B- nano	320.3a	19.6a	4.8a	39.9b	**115.7ab**
Fe- nano	289ab	16.9b	4.2a	36.1b	**117ab**
Zn- nano	274.7b	18.8b	4.5a	49.8a	**120.2a**

The averages with the same letter for each factor have non-significant differences according to Duncan's multiple range tests under level 0.05.

Table 3. Effect of foliar spraying of nano fertilizers in yield component of three broad bean varieties.

product of a plant is an outcome of improvement in growth-attributing parameters. The improved growth and yield components observed in the present investigation might be due to higher uptake of nutrients due to an enhanced supply of nutrients with addition primarily through foliar application. The adequate supply of all essential nutrients might have helped improve photosynthesis and translocation of photosynthates from source to sink.

Vegetative growth enhancement may be due to the role of nano micronutrient stimulatory effects on the production of chlorophyll, photosynthesis, mitochondrial respiration, and hormone biosynthesis[14]. The positive impact of foliar spraying of zinc nano-fertilizer on vegetative growth parameters comes along with results reported on snap bean plants[15]. This could be explained by the fact that the foliar application of micronutrients has led to an increase in vegetative growth, consequently, higher production capacity, which is reflected in the quality. These results are in accordance[3] on faba bean. As one of the essential micronutrients, zinc is involved in many vital physiological processes, such as hormone biosynthesis (auxin, abscisic acid, gibberellin and cytokinin), chlorophyll synthesis, chloroplast development, protection and maintenance of the stability of the cell membrane structure. It is also a cofactor of many enzymes associated with transferases, hydrolases, ligases and isomerases that regulate the ionic balance and stomatal conduction when different nanoparticles pass through the roots by the apoplastic pathway and are transported in the shoots through the vascular system[16].

Conclusions

From this study, it can be concluded that the Syrian variety gave the highest values of plant height and number of pods per plant, while the Spanish variety gave the highest values of pod length, number of seeds per pod and seed weight per plant. B-nano fertilizer gave the highest impor-

Treatments		Weight of pods per plant (gm)	Pod length (cm)	No. of seeds per pod	seed weight per plant (gm)	Weight of 100 seeds (gm)
Syrian	Control	280.3cd	12.4e	2.7f	22.4e	119.7cd
	B- nano	265.6cd	16.6c	4.6bcd	35.2cde	107.2f
	Fe –nano	247.3cd	13.4de	3.5def	40.8cd	118.0cde
	Zn- nano	211.9d	11.8e	2.9e f	34.0cde	112.3def
Spanish	Control	297.6 bcd	22ab	5.7ab	65.5ab	106.5f
	B- nano	306.3abc	24.5a	5.9a	57.2b	108.9ef
	Fe- nano	317.2abc	21.2b	5.2abc	44.6c	107.8f
	Zn- nano	285.0 bcd	23.8ab	6.3a	72.5a	109.8ef
Taqa	Control	374.2ab	17.9c	4.1cde	31.0de	112.0def
	B – nano	389.1a	17.7c	3.8def	27.3e	131.0ab
	Fe- nano	302.4a-d	16.1cd	4.1cde	22.8e	125.2bc
	Zn- nano	297.4bcd	17.8c	4.4cd	43.0cd	138.6a

The averages with the same letter for each factor have non-significant differences according to Duncan's multiple range tests under level 0.05.

Table 4. Effect of combination treatment between varieties and nano fertilizers in yield component of three broad bean varieties.

tance for the number of pods per plant, pods weight per plant, pod length and the number of seeds per pod, while Zn- nano fertilizer increased plant height, plant and whole grains yield.

Author Contributions

Conceptualization Abdelmonnem, methodology, Ahmed; software, Safwan; validation, Abdelmonnem and Safwan, formal analysis, Ahmed; investigation, Abdelmonnem; resources, Safwan; writing—original draft preparation, Ahmed and Safwan and Abdelmonnem, writing—review and editing, Safwan and Abdelmonnem; visualization, Ahmed: project administration, Abdelmonnem, All authors have read and agreed to the published version of the manuscript.

Funding

Self-funding.

Conflicts of Interest

There is no conflict.

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ARTICLE / INVESTIGACIÓN

Allelopathic effect of sunflower residues on some soil properties and growth parameters of wheat, bean and flax crops

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DOI. 10.21931/RB/2022.07.04.38

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Abstract: Allelopathic effects of the sunflower verities *Helianthus annuus* residues were evaluated on some soil properties and their potential against growth parameters of the proposed successive crops Wheat *Triticum aestivum* L., Broad Bean *Vicia fabe* L. and Flax *Linum Ustatissmim* L. Sunflower plants were chopped and incorporated with field soil after getting seed, and then successive crops were cultivated. The sunflower residues have reinforced the soil with the macronutrients considered essential for the germination of any crop. The soil organic matter content and the percentage of organic carbon in the ground were increased. Significant suppression of broad bean and flax crops was observed, while the sunflower residues did not affect wheat growth. The results obtained showed that broad bean and flax crops are not recommended to grow after the sunflower crop to avoid losses due to the negative allelopathic potential of these crops. Sunflower residue incorporation may provide multidimensional benefits for better weed control, enhanced soil health, and higher seed yield of wheat.

Key words: Allelopathic effect, Sunflower residues, Succssesive crops, Crop injury symptoms.

Introduction

Allelopathy is the chemical interaction between plants or plants with microorganisms that lead to either positive or negative effects on the performance of neighboring organisms. Allelopathy plays a significant role in natural ecosystems by determining vegetation patterning, plant dominance, plant succession, and plant biodiversity, preventing seed decay, and causing seed dormancy[1]. Allelopathy plays an essential role in the study of suitable agricultural systems as well as in weed control[2]. Also, allelopathy has a significant role in agricultural ecosystems. It plays a substantial role in weed-crop, crop-weed, crop-crop, forestry and nutrient cycling[3]. Sunflower is an annual dicotyledonous plant that can be grown throughout the year in subtropical and tropical regions for its seeds. The allelopathic potential of sunflowers is presented to inhibit the growth and development of other plants. The allelopathic effects of sunflower plants on successive crops and soil properties vary from stimulation to inhibition depending on the quantity and quality of the bioactive compounds in this plant and their residues.

Over 200 natural bioactive compounds include allelopathic compounds characterized in sunflower plants. Most of these compounds inhibit or stimulate the germination and growth of organisms. Heliannuols, terpenoids, flavonoids, chlorogenic acid, chlorogenic acid, and scopoletin were the most bioactive compounds found in sunflower plants[4]. Moreover, sunflower residues contribute to increasing the soil's organic matter, and thus becoming a natural organic fertilizer provides the soil with the necessary elements without the need for chemical fertilizers. The demeanor of sunflower residues towards successive crops is unclear and needs more in-depth studies. Hence, the current study aims to screen the allelopathic effect of three sunflower varieties on the growth parameters of successive crops and their content from essential nutrients in trying to minimize the use of conventional chemical fertilizers. Purvis *et al.*, 1990[5] reported that sunflower crop residues reduced wheat germination and growth by 4-33%. As for the remaining sunflower residues on the surface of the soil and leaving them without plowing, it often reduced the development of the crops that were planted after them compared to the removal of residues or traditional plowing. At the same time, wheat productivity increased in the non-cultivated treatments, reaching 5.2 tons. ha^{-1} When mixed with soil and plowing, the productivity decreased to 4.9 tons/ha. The sunflower crop residue in the soil reduced harmful weed growth early and, when mixed with soil, reduced the yield of later crops[6].

Materials and methods

The field experiment was conducted in the agro-station belonging to the college of Agriculture- University of Anbar-Iraq, which is located at 43.39 latitudes and 33.44 longitudes. In brief, three varieties of sunflowers, Sakha, Akmar and Ishaki, were grown in plots with a 70 cm distance between rows and 20 cm between plants inside the row. When plants reached the maturity stages, plans were chopped after getting the seeds and mixed well with the field soil. The proposed successive crops, Wheat *Triticum aestivum*, Broad bean *Vicia fabe* and Flax *Linum Ustatissmim*, were cultivated in the same plots of sunflower plants parts were mixed. Seeds of the Ibaa 99 wheat variety were grown in 3m rows with 20 cm between each other.

Regarding flax plants, using local variety with 3m row length and 25 cm between each other. Broad bean seeds

Citation: Flayyih T M, Almarie A A. Allelopathic effect of sunflower residues on some soil properties and growth parameters of wheat, bean and flax crops. Revis Bionat a 2022;7(4) 38. http://dx.doi.org/10.21931/RB/2022.07.04.38
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

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were cultivated in 3 m rows with 25 cm in between holes. Weedy check and free weed treatments of successive crops are used as controls. Weed plants were continuously removed from treatments throughout the growing season to ensure that just the sunflower's allelopathic potential affected the consecutive crops.

Soil Nutrient Analysis

Samples were taken from the field's soil from a depth of 0 to 30 cm after mixing the sunflower crop for four weeks to evaluate the changes in the soil nutrients compared to the control treatment. The analysis was conducted in the central laboratory belonging to the college of Agriculture- at the University of Anbar.

Successive crops growth parameters

The injury score of successive crops was estimated beginning from cultivation until the fifth week from germination by eye observation using a numerical scale from (0–5) as 0: no harm, 1: slight yellowing, 2: yellowing or burning, or visible dwarfing, 3: yellowing, burning or severe spotting, 4: semi-dead plants and 5: complete plant death. When successive crops reached maturity, growth parameters were taken.

Experimantal analysis

Experimental data were subjected to one analysis of variance (ANOVA) using the SASS system, version (9). The experiment was conducted in (RCBD) design with four replications. The differences between mean values were determined using Dunkin's range test (P≤ 0.05)[7].

Results

Soil Nutrient Analysis

Data is presented in Table 1. Showed some soil properties for the experimental field according to the samples taken at 2, 4 and 6 weeks after mixing sunflower residues compared with the controls. As can be seen, most treatments have differed significantly compared to the management. The sunflower residues enhanced soil properties by increasing the essential nutrients, NPK. Moreover, organic matter increased in soil and growing ranges from 44 to 143%. The same is true for the organic carbon and C/ N ratio, which witnessed a significant increase in all treatments.

Crop Injury Score

The injury score of the proposed successive field crops is presented in Figure1. Wheat plants were represented by changing the green color to a pale green color due to the damage that occurred to the plant due to sunflower residues. Regarding the Broad bean crop, the injury score in this plant affected by sunflower residues behaved the same as wheat plants except for slight dwarfing, but the general condition of the plant is rather good. Regarding flax crops, plants showed more sensitivity to the sunflower residues in all three varieties.

Crop Growth Parameters

As can be seen from the data presented in table 2., most of the study growth parameters of the wheat crop were enhanced by the residues of the three sunflower varieties. Although plant high and flag leaf area treats of wheat crop did not show a significant difference within the studies treatments, the weedy check recorded the highest value of plant high, which was 91.6 cm and the most negligible value of flag leaf area, which was only 29.36 cm. Also, the weedy check treatment recorded the lowest values compared to the other studied traits.

Table 3 shows the negative effect of all studies' treatments regarding the broad bean crop. The wide bean plant cultivated after mixing sunflower residues suffers from these treatments. The high of broad bean plants did not reach significant variations in all treatments. However, the plants growing in the weedy check treatment recorded the highest value of plant high, which was 85.33 cm. The other studied traits presented in Table 3. appeared that the free weed treatment was the highest in terms of the superiority of the studied features, which differed significantly from the sunflower residues treatment, which came less than expected as a result of the negative effect of sunflower residues on this crop.

As for the flax plants grown after the sunflower resi-

Treatments	Duration	K mg.kg^{-1}	p mg.kg^{-1}	N mg.kg^{-1}	Organic Matter	Organic Carbon	C/N Ratio
Sakha	2 Weeks	434	50.0	1843	1.11	5.16	19.1
Akmar		343	50.0	2396	1.16	4.53	18.5
Ishaqi		401	64.6	2796	1.40	6.1	39.5
Sakha	4 Weeks	521	49.7	1256	1.19	5.6	39.8
Akmar		405	26.5	1555	2.00	5.0	32.0
Ishaqi		394	29.0	1549	2.10	8.3	31.3
Sakha	6 Weeks	432	43.0	2422	1.30	5.6	31.0
Akmar		419	70.0	1430	1.54	7.9	35.0
Ishaqi		418	71.0	1177	1.61	6.2	37.3
Control		17.6	16.2	92	0.67	2.42	14.5
L.S.D. : 0.05		174	24.2	361	0.38	1.48	14.3

Table 1. Soil properties of the field according to the samples taken at 2, 4 and 6 weeks after mixing sunflower residues compared with the controls.

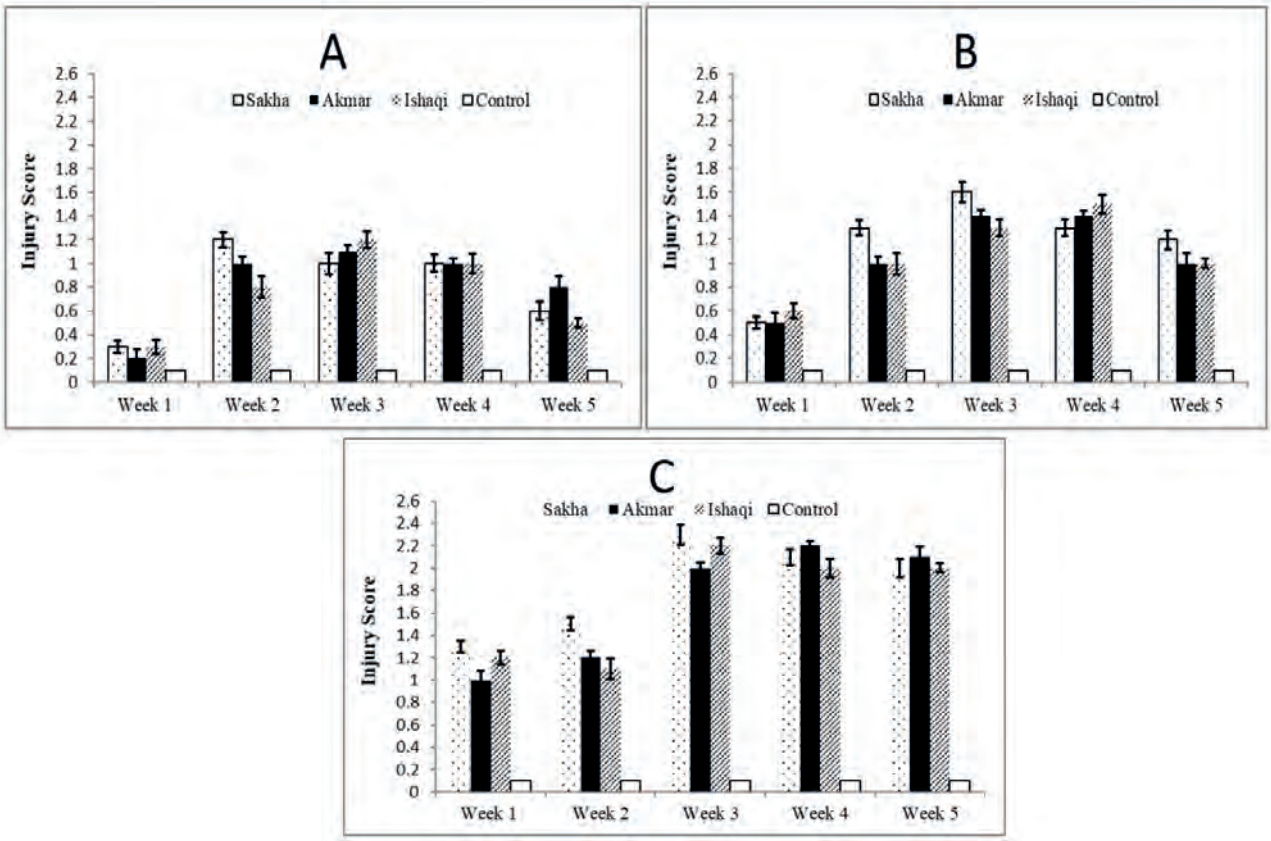

Figure 1. Injury symptoms of proposed successive crops affected by three varieties of sunflower residues mixed with soil field A: Wheat, B: Broad bean, C: Flax. Scale from (0–5) 0: no harm, 1: slight yellowing, 2: yellowing or burning, or visible dwarfing, 3: yellowing, burning or severe spotting, 4: semi-dead plants and 5: complete plant death.

Treatment	Plant High cm	Flag leaf Area cm^2	Dry matter of vegetative part (g)	Total yield Ton.ha^{-1}
Sakha	76.70	31.43	10.81	5.32
Akmar	74.17	33.23	10.75	5.31
Ishaki	80.43	31.73	11.86	5.07
Weedy Check	91.60	29.36	7.98	4.11
Free Weed	79.49	34.03	12.57	4.97
L.S.D: 0.05	N.S.	N.S.	2.1	0.823

Table 2. Allelopathic effects of sunflower residues on growth parameters of wheat.

Treatment	Plant High cm	leaf Area cm^2	Dry matter of vegetative part (g)	Total yield Ton.ha^{-1}
Sakha	62.33	2277	11.98	2.95
Akmar	57.80	2390	7.75	3.86
Ishaki	76.26	2328	7.01	4.63
Weedy Check	85.33	2162	6.44	4.20
Free Weed	76.86	2669	7.62	5.30
L.S.D: 0.05	N.S.	218.6	1.32	1.20

Table 3. Allelopathic effects of sunflower residues on growth parameters of Broad bean.

Treatment	Plant High cm	Number of leaves per plant	Dry matter of vegetative part (g)	Total yield Ton.ha^{-1}
Sakha	62.3	85.0	2.35	0.837
Akmar	58.1	94.0	2.40	0.979
Ishaki	55.8	84.3	2.47	0.843
Weedy Check	96.0	114.0	3.73	0.754
Free Weed	104.3	166.7	11.33	1.380
L.S.D: 0.05	13.65	20.94	0.70	0.406

Table 4. Allelopathic effects of sunflower residues on growth parameters of Flax.

dues, the same trend of the broad bean plants was sculpted regarding the negative impact of these residues in all the studied traits. The results shown in Table 4 showed that the treatments were significantly different from the free weed treatment, which come in the highest values of characteristics.

Discussion

Soil Nutrient Analysis

The sunflower residues increased the amount of the essential nutrients for plant growth (NPK) and those necessary nutrients beneficial for the activity of microorganisms and their proportions. The results align with the results of Ullah *et al.*, 2018[9] which confirmed that sunflower residue incorporation at 6 ton ha^{-1} improved soil health, suppressed weeds and resulted in better seed yield of spring-planted mung bean. Returning soil fertility naturally is desirable as it is one of the promising strategic solutions to provide it naturally without using chemical fertilizers, which negatively affect the properties of the soil[10]. The extreme extravagance in adding chemical fertilizers to the soil in quantities that exceed the needs of the plant and at unsuitable dates for the growth stage of the crop leads to the destruction of the balance in the soil between the elements of the plant's food and creates an unbalanced environment for the growing plants in addition to the possibility of an imbalance that harms the biological diversity in the soil.

Crop Injury Score

Various crop sensitivity to the sunflower residues is due to genetic factors related to genetic variation. Many researchers concluded that the field crops grown after sunflowers were affected diversely, and the degree of the effect depended upon the extract's concentration and the sunflower biomass in the field soil[11].

Crop Growth Parameters

The results in Table 2. indicated the positive effect of sunflower residues on growth characteristics and seed yield of the wheat crop as a result of the positive response to those residues. This comes as an expected result of the response to improving the soil with the necessary elements needed by the wheat crop, which helped in its healthy development and growth, thus improving yield and quality. The current results are consistent with (12,13), who mentioned that the wheat crop is suitable for cultivation under sunflower allelopathic stress. The results of the broad bean and flax crop response came to the exact opposite of the results achieved in the wheat crop, as shown in Tables 3 and 4. Despite the revitalization of the soil with the necessary elements by the sunflower residues, the allelopathic effect was negative by reducing the growth parameters, which affected the reduction of seed yield. Over 200 natural bioactive compounds include allelopathic compounds characterized in sunflower plants. Most of these compounds are involved in inhibiting or stimulating germination and growth of organisms, such as heliannuols, terpenoids, flavonoids, chlorogenic acid, chlorogenic acid, and scopoletin, most bioactive compounds found in the sunflower plants[14].

Conclusions

Sunflower residues are a valuable source of nutrition and play an efficient role in sustainable crop production. Not all successive crop shows a positive response to sunflower residues because the allelopathic substances which secrete them, in turn, play a positive or negative role on the subsequent crops. The improvement in soil properties and suppression resulted in better wheat yield. On the contrary, it negatively affected the growth parameters and yield of bean and flax crops. In short, sunflower residue incorporation may provide multidimensional benefits for better weed control, enhanced soil health and higher seed yield of wheat. More studies are needed to determine the allelopathic effects of sunflower residues on different successive crops.

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ARTICLE / INVESTIGACIÓN

Estimation of path coefficient analysis for some quantitative traits in Rice (*Oryza sativa* L.) genotypes

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DOI. 10.21931/RB/2022.07.04.39

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Abstract: A field experiment was conducted at the Al Mushkab Rice Research Station (AMRR), Najaf, Iraq, during the rice growing season of 2018-2019 in Randomized Complete Block Design (RCBD) for the aim of estimating the path coefficient in 15 introduced and local genotypes of rice. The path coefficient was estimated for the number of days from planting to 50% flowering, the number of days from flowering to physiological maturity, plant height, leaf area index, number of branches/panicle, number of panicles / m^2, number of grains/panicles, infertility percentage (sterility %), 1000 grains weight (gm), biological yield (kg. ha^{-1}) and harvest index (HI %) with grain yield kg, $ha.^{-1}$. The results of the study concluded that the trait of harvest index is an effective selection criterion for improving Rice grain yield because it achieved the highest overall positive effect, i.e., the highest positive genotypic correlation amounted to 0.761, and this trait also achieved a high direct positive effect on grain yield amounted to 0.83833.

Key words: Rice, Genotypes, Path, Coefficient, Harvest Index.

Introduction

Rice (*Oryza sativa* L.) is one of the food crops whose average consumption is constantly increasing, especially in the Arab world. It was cultivated with an area of 55525 hectares, an annual production of (265900) tons, and a productivity rate of 4.78 tons.ha^{-1}. This yield in the United States of America and China is 8.11 and 6.86 t. ha^{-1}, respectively[1]. Rice is the principal food for over half the world's humans. About 480 million metric tons of milled rice are produced every year. China and India alone have 50% of the rice grown and consumed. Rice is critical for food security and gives up to 50% of the nutritional caloric supply for millions in Asia, Latin America, and Africa[2,3]. Nearly 90% of the world's rice crop is produced in Asian countries. It plays a crucial role in food security in Iraq and different countries[4].

In Iraq, rice is an essential summer crop. It comes in third place after wheat and barley in terms of cultivated area and production. Still, Iraq is a country that imports cereals and its rice production are not enough to meet the needs of its population (1.1 million tons was imported in 2017)[5,6]. Genetic improvements, primarily through selection, are an essential means in the hands of plant breeders to increase the yield. Plant breeders need to carefully select the most critical associated traits that are phenotypically and genetically related, directly or indirectly, to the grain yield to use them as selection indices. Since the simple correlation measures the relationship in its abstract form, the path coefficient determines traits' direct and indirect effects on the yield based on genetic correlations[7]. The path-coefficient analysis is simply a standardized partial regression coefficient, which measures one variable's direct and indirect impact upon another and permits the separation of the correlation coefficient into components of direct and indirect effect[8]. In agriculture, path analysis has been used by plant breeders to assist in identifying traits that are useful as selection criteria to improve crop yield[9,10]. Path coefficient analyses evaluate each trait's direct and indirect contribution to the product could be estimated by picking up appropriate features for indirect selection[11]. Based on the preceding, this research aimed to determine the traits most related to grain yield and count them as selective indices for plant breeders to use in improving grain yield by analyzing the path factor.

Materials and methods

A field experiment was conducted during the summer agricultural season of 2018 at Al-Mashkhab Rise Research Station (AMRRS) affiliated with the Agricultural Research Department / Ministry of Agriculture, which is (22 km southeast of the center of Najaf governorate) and located within 44.31 east longitude. It is 31.89 in north latitude and 70 m above sea level. Rice seedlings were prepared in cultivation and by seedling method on 17/6/2018 for all the genotypes shown in Table (1), which were obtained from the genome bank at the Rice Research Station in Al-Mishkhab. The experiment was carried out using a Randomized Complete Block Design (RCBD) with three replications. The path coefficient was estimated for the number of days from planting to 50%, the number of days from planting to physiological maturity, plant height, leaf area index, number of branches/panicle, number of panicle /m^2, number of grains/panicle, infertility percentage (%), the weight of 1000 grains (gm), biological yield and harvest index (%) with grain yield kg. $ha.^{-1}$. The experiment was fertilized with the full amount

Citation: Al-Musawi, Balqees.; Al- Anbari, Mohammed. Estimating path coefficient analysis for some quantitative traits in Rice (Oryza sativa L.) genotypes. Revis Bionat a 2022;7(4) 39. http://dx.doi.org/10.21931/RB/2022.07.04.39
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

of fertilizer to the crop, as Dab fertilizer (P_2O_5 46% N 18%) was added at a rate of 120 kg. ha.$^{-1}$ mixed with soil before planting, urea (46%) N fertilizer was added with an amount of 280 kg. ha.$^{-1}$, in two batches, the first 12 days after seedlings, and the second batch a month after the first batch, and for all experimental units[12]. The rest of the soil and crop service operations were conducted as needed. After confirming the existence of genetic correlations between the studied traits, the path coefficient analyzes were entered into. The path coefficient is the standardized partial regression coefficient corresponding to the results of the regression analysis that (13) established in segmenting the correlation coefficient between two variables into direct effects (Cause) in effect (Effect) and indirect effects of the cause in effect, through other causes, as figure 1. The Path analysis was done according to Dewey and Singh[8,14]. The following scale was adopted to clarify the importance of direct and indirect effects, based on (15): Table 2.

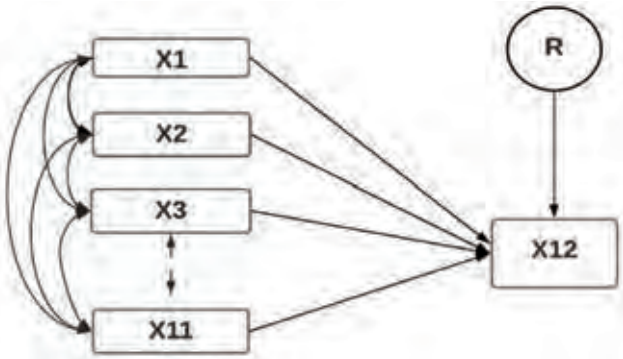

Figure 1. Path Coefficient diagram depicting interrelationships among traits.

Results

The path coefficient analysis was carried out at the level of genotypic correlation coefficients for the eleven studied traits to partition the correlation coefficient between each of the studied traits and the yield into its direct and indirect effects to determine the traits that most influence the grain yield and describe them as selectin on indices to improve the yield. This is illustrated in Table (3).

Number of days from planting to 50% flowering

The number of days from planting to 50% flowering achieved a little negative direct effect with grain yield of -0.15505 and positive indirect effects that ranged from 0.78733 through plant height, which has a high impact, to 0.05041 through non-fertility percentage (%), which was unimportant It is neglected. The number of days from planting to 50% flowering also achieved adverse indirect effects ranging from -1.47859 for the number of days from cultivation to physiological maturity, in which the product was very high, to -0.03332, which was unimportant and neglected. As for the value of the total effect, the genetic correlation coefficient was Few and negative as it reached -0.188.

Number of days to physiological maturity

The trait of the number of days from planting to physiological maturity achieved the value of the total effect; that is, the genetic correlation coefficient was few and negative, reaching -0.225 with grain yield .it is also achieved a very high negative direct impact with grain yield of -1.52125 this agree with Jeke[16]. The positive indirect effects ranged from 0.77503 through plant height, in which the product was

Genotypes' name	Pedigree
1. Amber 33	Local (Iraqi)
2. Amber al-Baraka	Introduced from India
3. Amber Furat	Technology& Science Ministry/ Baghdad
4. Amber Baghdad	Technology& Science Ministry/ Baghdad
5. Amber Menathera	Technology& Science Ministry/ Baghdad
6. Sumar	Technology& Science Ministry/ Baghdad
7. Dijlah	Introduced from China
8. Ghadeer	Introduced from IRRI (Philippines).
9. Brnamge -4	Introduced from IRRI(Philippines).
10. Dorfak	Sepidrood/Salari- Iran
11. Gohar	Pusa1238-1/Pusa1238-81-6-Iran
12. Khazar	IR2071-625-1-52/TANU7456-Iran
13. Shiroudi	Khazar / Deylamani – Iran
14. Neda	Amol3/Hassansarayee/sangetarom-Iran
15. Nemat	Amol3/sangetarom-Iran

Table 1. Rice genotypes used in the study.

The value of direct and indirect effects	Importance
From zero – 0.09	**Not essential and neglects (Negligible)**
from 0.10 - 0.19	**Low**
from 0.20 - 0.29	**Moderate**
from 0.30 - 0.99	**High**
from one or more	**Very high**

Table 2. The importance of direct and indirect influences.

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	Total (genotypic correlations)
X1	-0.15505	-1.47859	0.78733	0.32093	0.41121	0.08025	-0.18299	0.05041	-0.03332	0.32204	-0.30973	**-0.188**
X2	-0.15070	-1.52125	0.77503	0.31489	0.42352	0.07917	-0.20247	0.07441	-0.02848	0.44019	-0.42963	**-0.225**
X3	-0.13392	-1.29337	0.91158	0.38273	-0.05960	0.04012	-0.05335	-0.00801	-0.00775	0.44876	-0.38717	**-0.160**
X4	-0.11750	-1.13109	0.82381	0.42351	-0.14500	0.01869	-0.09568	0.03614	-0.07251	0.43036	-0.40887	**-0.238**
X5	-0.06625	-0.66944	-0.05646	-0.06380	0.96241	0.09921	-0.31828	0.16253	-0.01456	-0.04982	0.09037	**0.076**
X6	0.07838	0.75864	-0.23037	-0.04985	-0.60142	-0.15875	0.10302	0.03052	0.03055	0.31765	0.07965	**0.358***
X7	-0.06999	-0.75984	0.11997	0.09997	0.75567	0.04035	-0.40536	0.26310	-0.03828	0.31260	0.17094	**0.489****
X8	0.02582	0.37399	0.02412	-0.05056	-0.51677	0.01601	0.35235	-0.30268	0.03796	-0.42802	-0.29716	**-0.765****
X9	0.02820	0.23642	-0.03854	-0.16759	-0.07647	-0.02647	0.08468	-0.06270	0.18323	-0.20086	0.13573	**0.096**
X10	-0.05615	-0.75296	0.45999	0.20494	-0.05391	-0.05670	-0.14248	0.14568	-0.04138	0.88934	-0.40526	**0.191**
X11	0.05729	0.77963	-0.42101	-0.20655	0.10375	-0.01508	-0.08265	0.10729	0.02967	-0.42992	0.83833	**0.761****

Residual effect =0.00013* and ** indicate significance at 5% and 1% level of probability, respectively.X1= Days from planting to 50 % flowering, X2 = Days from growing to physiological maturity, X3 = Plant height, X4 = Leaf Area Index, X5 = Branches/panicle, X6 = N. panicles / m2, X7 = N. grains/panicle, X8 = Sterility %, X9=1000 grain weight, X10= Biological yield, X11 = Harvest Index and X12 = Grain yield

Table 3. Estimates of direct and indirect effects and the total effect of yield attributing traits on grain yield.

high, to 0.42352 through the number of branches/panicles, which was also increased. The characteristic of the number of days from planting to physiological maturity, adverse indirect effects, ranging from -1.52125 through the number of days from planting to 50% flowering, in which the product was very high, to -0.02848 through the weight of 1000 grains (g), which was unimportant and neglected. As for the value of the total effect, the genetic correlation coefficient was few and negative, reaching -0.225.

Plant height (cm)

The direct effect of plant height was high, amounting to 0.91158. There were positive and negative indirect effects, the highest of which was achieved through the characteristic of biological yield, which was a high and positive effect on grain yield and amounted to 0.44876 and a very high and negative indirect effect on grain yield through the characteristic of the number of days from cultivation to physiological maturity reached -1.29337. Still, the plant height trait achieved a small total effect of -0.160.

Leaf Area Index (LAI)

The direct effect of the leaf area index was high and amounted to 0.42351. There were positive and negative indirect effects, which were highly positive, on the grain yield through the characteristic of plant height, which amounted to 0.82381 and negative and very high in its impact on the grain yield through the part of the number of days from planting to. The physiological maturity reached -1.13109, and the leaf area index trait had a small negative effect of -0.238.

Number of branches/panicles

The direct effect of the number of branches/panicles was high, amounting to 0.96241. There were positive and negative indirect effects, which were highly positive, on the yield of grains through the characteristic of the biological substance, which amounted to 0.9037, and negative and high impact on the grain yield through the feature of the number of days from cultivation to physiological maturity. It reached -0.66944, and the characteristic of the number of branches/panicles achieved a total effect that was not important and neglected, which amounted to 0.076.

Number of panicles, / m²

The direct effect of the number of panicles / m² was low and negative, amounting to -0.15875. Still, there was a positive and high indirect effect on the grain yield through the characteristic of the number of days from planting to physiological maturity, which amounted to 0.75864 and a negative and high indirect effect on the grain yield through the feature of the number the branches/ panicle, reached -0.60142, in addition to other positive and negative effects. The element of the number of panicles/m² achieved a total result of 0.358.

Number of grains /panicles

The trait of the number of grains/panicle achieved a positive indirect effect through the number of branches/panicle, high with a grain yield of 0.75567 and indirect, negative and high -0.75984 through the number of days from planting to physiological maturity. The trait of the number of grains/ panicles also had a high direct negative effect of -0.40536 And other positive and negative effects. The value of the total impact, the value of the genetic correlation coefficient, was tall and positive, reaching 0.489.

Infertility percentage (Sterility %)

The direct effect of the percentage of infertility (%) was high and harmful, amounting to -0.30268. There were positive and negative indirect effects for the rest of the traits. They were highly influential and favorable on the grain yield through the days from cultivation to physiological maturity, which amounted to 0.37399 and negative and high. The grain yield through the trait number of branches/panicle reached -0.51677, and the trait of infertility percentage (%) achieved a high, negative total effect of -0.765.

1000 grain weight (gm)

The direct effect of the weight of 1000 grains (gm) was low, amounting to 0.18323, but there were positive and negative indirect effects for the rest of the traits. They were highly and positively affecting the grain yield through the characteristic of the number of grains/panicle, which amounted to 0.8468 and negative through most of the studied traits, and the weight characteristic was achieved. One thousand tablets (gm) have a low total effect of 0.096.

Biological yield (kg/ha)

The biological yield trait achieved a high positive indirect effect through plant height with a grain yield of 0.45999 and a high negative indirect impact through the number of days from planting to physiological maturity -0.75296, as well as positive and negative effects through the rest of the traits. The biological yield trait achieved a high direct result of 0.88934. As for the value of the total impact, that is, the genetic correlation coefficient, it was few and negative, as it

amounted to -0.191. Biological yield has also been identified as a significant direct contributor towards grain yield exerted a high positive immediate effect (0.8481) by Mervat MA[18].

Harvest Index (%)

The direct effect of the harvest index was high, reaching 0.83833. There was a positive and high indirect effect on grain yield through the characteristic of the number of days from planting to physiological maturity, which amounted to 0.77963. An indirect, negative and high effect through the characteristics of plant height and biological yield, which were -0.42101 and -0.42992, respectively, which was reflected in achieving a high total positive effect of 0.761 and this is consistent with what (19,20) stated that the trait of harvest index achieved a direct and high total effect with grain yield. It can be concluded from the preceding that the harvest index is an effective selection criterion to improve Rice grain yield because it achieved the highest overall positive effect, i.e., the highest positive genetic correlation. It also achieved a high direct positive effect on grain yield. The residual effect determines how best the causal factors account for the variability of the resultant factor, the grain yield kg. ha.$^{-1}$. In the present study, the residual effect was shallow at the genotypic level (Table 3), indicating that the characters selected in this study contributed to the yield.

Discussion

Path coefficient analysis provides a view into interrelationships by separating the correlation coefficients into direct and indirect effects of characters. In crops, path analysis has been used by plant breeders to assist in identifying traits that are useful as selection criteria to improve crop yield. The rice breeders used the path coefficient analysis to estimate the desirable features in the selection to enhance the grain yield. The correlation among traits under study indicated that the short flowering period would provide more time to increase the full-grain number per panicle. This will be improved by reducing sterility. An increase in panicle number per m^2 will improve the final grain yield, which was panicle number per m^2 and grain number per panicle and harvest index correlated positively to yield traits. These results agree with the results of (21-28). Current knowledge about trait relationships helps in the appropriate selection process due to the increased share of crop improvement[29]. Breeding programs aim to increase rice production by using more genetic types and applying effective selection methods to increase yield through yield traits. Identifying the relationship between creation and yield traits via correlation analysis is an essential step[30], but dividing the influence of features into direct and indirect effects by path analysis is more critical for them a selection of yield traits[31,32]. The importance of path analysis is partitioning the correlation coefficient into its components; the first component is the path coefficient, which measures the direct effect of a predictor variable upon its response variable. The second component is the indirect effect of a predictor variable on the response variable through other predictor variables.

Conclusions

Path analysis revealed that harvest indexes are the most critical component characters that could be used as selection indices for further improvement in grain yield under any climatic condition. Hence natural selection of these traits can be made in rice breeding programs. Progress in these traits will result in simultaneous improvement in grain yield.

Author Contributions

For research articles with one author, M.A.Al-anbari. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by Al-Mashkhab Rise Research Station (AMRRS).

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Path coefficient analyses evaluate each trait's direct and indirect contribution to yield could be estimated by picking up appropriate features for indirect selection.

Acknowledgments

Authors are thankful to the Al Mushkab Rice Research Station (AMRR), Najaf, Iraq, for providing all the necessary support for conducting research and making this research study feasible.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ARTICLE / INVESTIGACIÓN

The effect of adding compound fertilizer of NPK and Humic acid on the availability of NPK soil and cabbage yield

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DOI. 10.21931/RB/2022.07.04.40

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Abstract: A field experiment was conducted in Jdeidet Al-Shatt in Diyala Governorate during the fall season of 2021. According to modern American classification, silt loam soil is classified at a level lower than Typic Torrifluvent. To study the effect of NPK and humic acid addition on soil NPK availability and total cabbage yield according to RCBD randomized complete block design with three replications. The balanced 20:20:20 NPK compound fertilizer was added at three levels, 0,150,300 kg ha^{-1} and humic acid at three levels, 0, 15 and 30 kg ha^{-1}. The fertilizers were added to the soil in two batches, the first during the planting process and the second 43 days after the date of the first batch. The concentrations of elements were measured in two stages, and the total yield of cabbage. The results of the study showed the superiority of the compound fertilizer NPK at the level of 300 kg ha^{-1} of NPK elements and in the first stage 46days (before the emergence of the head), where the concentration of NPK elements reached 36.59 mg kg^{-1}, 35.55 mg kg^{-1}, 224.90 mg kg^{-1}, respectively. While the superiority of the humic acid at the level of 30 kg ha^{-1} to 30.44 mg kg^{-1}, 25.13 mg kg^{-1}, and 209.39 mg kg^{-1}, respectively. As for the compound fertilizer at the second stage106 days (when the head is fully mature), the concentration of NPK elements and cabbage yield reached 27.17 mg kg^{-1}, 36.92 mg kg^{-1}, 208.69 mg kg^{-1}, 68.23kg Mg ha^{-1}, while the humic acid was 22.86 mg kg^{-1}, 27.91 mg kg^{-1}, 197.11 mg kg^{-1}, 57.53 Mg ha^{-1}, respectively.

Key words: NPK compound fertilizer, Humic acid, NPK readiness, cabbage.

Introduction

Most Iraqi soils are considered calcareous because of their high content of carbonate minerals such as calcium carbonate. These minerals reduce soil fertility by raising pH, exposing the nutrients added to the soil to sedimentation, loss and stabilization processes, and high temperatures and low rainfall, one of the challenges facing soils in dry and semi-arid areas, including Iraqi soils. This causes a decrease in the soil's organic material content[1,2]. Adding fertilizers to the ground dramatically increases the availability of crop nutrients and improves the soil ecosystem that contributes mainly or is secondary to 95 percent of global food production. Proper fertilizers can increase agricultural productivity by avoiding deforestation and reducing the need to use additional lands for agriculture. Chemical fertilizers are an indispensable resource for increasing agricultural production, which leads to increasing yields by up to 50 percent and improving farmers' livelihoods[3,4]. Nitrogen is the essential component of all living organisms and one of the primary nutrients that limit life on our planet. Nitrogen is exposed to various loss processes, including fixation in the bodies of microorganisms or volatilization. Phosphorus is an essential nutrient for crop growth; it is a non-renewable resource in global food security; Phosphorus is poorly soluble in soil and has weak plant uptake efficiency, Phosphorus availability in soil is affected by temperature, dryness and pH, and maximum phosphorus uptake from the soil is at pH 7-6.5. This will affects the availability of Phosphorus in the soil, especially in calcareous soils[5]. Potassium is the third phytonutrient that is not available to plants. Intensive cultivation, runoff and soil erosion cause a deficiency of Potassium in the soil[6]. Humic acid (HA) is a multifunctional natural polymer compound in most terrestrial and aquatic environments. Humic acid effectively influences the absorption of elements from the soil, as it helps to increase the readiness and transfer of significant components, including Nitrogen, phosphorous and potassium[7,8]. Humic acid is a sustainable and environmentally friendly fertilizer ready directly when added to the soil. It does not need time to decompose, free of seeds, weeds, bushes and pathogens compared to traditional organic fertilizer. Since Humic acid can dissolve in the bases, adding it to the calcareous soil, including most the Iraqi soil, leads to a lowering of the soil pH and thus drives an increase in the readiness of the elements in the ground and its absorption by the plant, Humic acid is a supplement to chemical fertilizers. It helps in reduce agricultural production input costs. To increase the amount of farm production and to meet the world's food requirements, resulting from the increased use of chemical fertilizers increased, but the high prices and fear of losing them because they cause pollution. Therefore, it was necessary to find alternative ways to reduce its loss from the soil. The use of humic acid with chemical fertilizer, according to the fertilizer's recommendation, increases the soil's elements when added as a mixture or feed in the ground. This makes it ready for absorption by the plant for a long time, which reduces the loss of added elements from the soil[9,10]. Because of the importance of cabbage cultivation in Iraq, as it is one of the main winter crops Based on the preceding, the study aimed to find out

Citation: Turki M I, Bader B R. The effect of adding compound fertilizer of NPK and Humic acid on the availability of NPK soil and cabbage yield. Revis Bionat a 2022;7(4) 40. http://dx.doi.org/10.21931/RB/2022.07.04.40
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

the effect of adding NPK and Humic acid fertilizers and the interaction between them on the availability of NPK soil and total yield of cabbage.

Materials and methods

A field experiment was conducted in one of the fields of Jdeidet Al-Shatt territory of Al-Khalis district in Diyala governorate, located 30 km from the center of Baquba in Diyala Governorate and located at longitude 44°25'33.2868'E and at latitude 33°37'29.172'N during the autumn season 2021 on silty loam soil. The study aimed to examine the effect of adding NPK compound fertilizer and humic acid on soil availability. Total cabbage yield was evaluated by adding a balanced 20:20:20 NPK compound fertilizer at three different levels. These were: 0 no addition, 150 kg h^{-1} half of the fertilizer recommendation, 300 kg h^{-1} full of the fertilizer recommendation 11 whose symbol is C1, C2, C3, respectively. The second factor is humic acid with three levels, which are 0 no addition, 0, 15 kg ha^{-1}, and 30 kg ha^{-1}; its symbol is H1, H2, and H3, respectively. A sample was taken randomly from different locations and mixed to be a composite sample representative of the field soil at a depth of 0-30 cm. It was dried and ground with a wooden hammer and passed through a sieve with a hole diameter of 2 mm to perform the physical and chemical analyzes, whose results are shown in Table No 1.

Soil preparations were carried out for cultivation, including plowing, smoothing and leveling, and it was divided into three sectors. Each sector contains nine parameters, the dimensions of the experimental unit are 3 * 2.5 m^2, and each experimental unit includes four lines. The number of plants in the experimental unit is 24 plants. The planting was done on terraces. The width of the terrace was 50 cm, seedlings were planted on one side, and the distance between one plant and another was 40 cm. The distance between an experimental unit and another 100 cm, and the distance between one sector and another 100 cm. The seedlings were planted in the field on 9/24/2021. The ground addition of NPK fertilizer and humic acid was applied in two stages, the first when planting and the second on 6/11/2021 before the stage of the emergence of the head after 43 days, by making an incision in the soil around the plant. Measurements were taken from the ground in two stages, the first stage before the emergence of the head and the second on the 7/1/2022 stage after the maturity of the head. The analyzes were carried out in the Laboratory of Soil and Water Resources Sciences, College of Agriculture, Diyala University, and the following characteristics were studied:

Nitrogen availability concentrations in soil mg kg^{-1}

Estimation was performed by extracting the soil with potassium chloride (2N), adding magnesium oxide and converting Nitrogen from nitrate to ammonium by adding Devarde alloy as a reducing agent. Estimation was carried out using the Microelectronic apparatus[12].

Phosphorous availability concentration in soil mg kg^{-1}

It is estimated using sodium bicarbonate (0.5M) at pH 8.5. Where Ammonium Molybdate and ascorbic acid are added until the blue color develops. A spectrophotometer is used at wavelength 882[13,14].

Potassium availability concentration in soil mg kg^{-1}

The determination of ammonium acetate was carried out by using a flame photometer[14].

The total yield of the plant Mg ha^{-1}

The total plant yield was calculated using the following equation:

The total plant yield was computed using the next equation:= Weight of the head without the outer leaves x the number of plants per hectare / 1000.

Results

Nitrogen availability in the soil (mg kg^{-1})

Table 2 shows that the addition of NPK compound fertilizer and humic acid in the average concentrations of nitrogen availability in the soil, There are significant differences as the C2 and C3 two transactions were significantly superior, which two reached the highest average of 22.61,36.59mg kg^{-1} on respectively compared to C1 treatment, which amounted to 16.65 mg kg^{-1}, with an increase of 35.79% and 119.75%. As for the effect of adding humic acid, the H2 and H3 two transactions were significantly superior, which two reached the highest average of 24.31, and 30.44 mg kg^{-1}, respectively, were very special to the H1 treatment, which amounted to 21.10 mg kg^{-1}, with an

Adjective	The value	Unit
Electrical conductivity EC (1:1)	2.6	Ds m^{-1}
Soil pH (1:1)	7.7	
Organic matter	8.08	g kg^{-1}
Available nutrients		
Nitrogen	30.00	mg kg^{-1}
phosphorous	12.87	
potassium	307.01	
Calcium Carbonate	242.06	
Bulk density	1.36	Mg m^{-3}
Soil Separators		
clay	27.68	%
silt	51.68	
sand	20.64	
Field capacity	27	%
soil texture	Silty loam	

Table 1. Soil characteristics study before planting.

increase of 15.21% and 44.26%, respectively. As for the interaction effect, the C3H3 treatment, which amounted to 48.83 mg kg^{-1}, showed the highest and lowest values for the C1H1 treatment, which amounted to 14.13 mg kg^{-1}, and an increase of 245.57% percent.

Phosphorous availability in the soil (mg kg^{-1})

Table 3 shows that the addition of NPK compound fertilizer and humic acid had a significant effect on the average concentrations of phosphorous availability in the soil, as the C2 and C3 two transactions were significantly superior, which two reached the highest average of 15.79, 35.55mg kg^{-1}, respectively compared to treatment C1 which amounted to 11.95 mg kg^{-1}, with an increased rate 32.13% and 197.48%. As for the effect of adding humic acid, the average H3 treatment, which amounted to 25.13 mg kg^{-1}, was significantly superior to the H1 treatment, which amounted to 17.05 mg kg^{-1}, with an increase of 47.39%. As for the effect of the interaction, the C3H3 treatment, which amounted to 42.08 mg kg^{-1}, showed the highest value and the lowest value, when C1H1 treatment, which amounted to 11.79 mg kg^{-1}, and an increase of 256.91%.

Potassium availability in the soil (mg kg^{-1})

Table 4 shows that the addition of NPK compound fertilizer and humic acid had a significant effect on the average concentrations of potassium availability in the soil, as the C2 and C3 two transactions were significantly superior, which two reached the highest average of 198.01, 224.90 mg kg^{-1}, respectively compared to treatment C1 which amounted to 181.69 mg kg^{-1}, with an increased rate 8.98% and 23.78%. As for the effect of adding humic acid, the average H3 treatment, which amounted to 209.39 mg kg^{-1}, was significantly superior to the H1 treatment, which amounted to 194.83 mg kg^{-1}, with an increase of 7.47%. As for the effect of the interaction, the C3H3 treatment, which amounted to 232.70 mg kg^{-1}, showed the highest value and the lowest value when the C1H1 treatment amounted to 174.00 mg kg^{-1} and an increase of 33.73%.

Nitrogen availability in the soil (mg kg^{-1})

Table 5 shows that the addition of NPK compound fertilizer and humic acid had a great effect on the average concentrations of Nitrogen availability in the soil, as the C2 and C3 two transactions were significantly superior, which two reached the highest average of 20.79,27.17 mg kg^{-1} compared to C1 treatment, which amounted to 14.16 mg kg^{-1}, respectively with an increase of 46.82 and 91.87 %, As for the effect of adding humic acid, as the H2 and H3 treatment two transactions were significantly superior which two reached the highest average of 20.69,22.86 mg kg^{-1}, was significantly superior to H2 and H1 treatment, which amounted to 18.58mg kg^{-1}, respectively with an increase of 11.35 and 23.03%, As for the interaction effect, C3H3 treatment which

	C1	C2	C3	
H1	14.13 f	20.83 de	28.33 c	21.10 C
H2	17.50 ef	22.83 d	32.60 b	24.31 B
H3	18.34 e	24.16 d	48.83 a	30.44 A
	16.65 C	22.61 B	36.59 A	

*The symbols in the table indicate: C = NPK compound fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H= humic acid where H1= 0 without addition, H2 = 15 kg ha^{-1}, H3= 30kg ha^{-1} According to Duncan's polynomial test, means with different letters differ significantly from each other at a 0.05% probability level.

Table 2. Effect of adding NPK and Humic acid fertilizers and the interaction between them on nitrogen concentrations in soil in the pre-emergence of the head stage (mg kg^{-1}).

	C1	C2	C3	
H1	11.79 c	12.81 c	26.55 b	17.05 B
H2	11.81 c	13.52 c	38.02 a	21.12 AB
H3	12.25 c	21.06 bc	42.08 a	25.13 A
	11.95 B	15.79 B	35.55 A	

*The symbols in the table indicate: C = NPK compound fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H= humic acid where H1= 0 without addition, H2 = 15 kg ha^{-1} ,H3= 30kg ha^{-1}. Means with different letters differ significantly from each other according to Duncan's polynomial test at 0.05%probabilitylevel.

Table 3. Effect of adding NPK compound fertilizer and humic acid and their interaction on phosphorous concentrations in the soil in the pre-emergence of the head stage (mg kg^{-1}).

	C1	C2	C3	
H1	174.00 f	193.13 d	217.36 b	194.83 B
H2	181.24 ef	195.24 cd	224.65 ab	200.38 B
H3	189.81 de	205.66 c	232.70 a	209.39 A
	181.69 C	198.01 B	224.90 A	

*The symbols in the table indicate: C = NPK compound fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H= humic acid where H1= 0 without addition, H2 = 15 kg ha^{-1} ,H3= 30kg ha^{-1} Means with different letters differ significantly from each other according to Duncan's polynomial test at 0.05%probabilitylevel.

Table 4. Effect of adding NPK and humic acid fertilizer and the interaction between them on potassium concentrations in the soil in the pre-emergence of the head stage (mg kg^{-1})

	C1	C2	C3	
H1	11.63 g	19.23 de	24.87 bc	18.58 C
H2	15.00 fg	20.40 d	26.66 ab	20.69 B
H3	15.84 ef	22.73 cd	30.00 a	22.86 A
	14.16 C	20.79 B	27.17 A	

*The symbols in the table indicate: C = NPK compound fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H= humic acid where H1= 0 without addition, H2 = 15 kg ha^{-1}, H3= 30kg ha^{-1} According to Duncan's polynomial test at 0.05% probability level, Means with different letters differ significantly from each other.

Table 5. Effect of adding NPK and humic acid fertilizer and the interaction between them on nitrogen concentrations in the soil at the stage of full maturity of the head (mg kg^{-1}).

amounted to 30.00mg kg^{-1} showed the highest value and the lowest value when C1H1 treatment which amounted to 11.63mg kg^{-1}, and an increased rate of 157.95%.

Phosphorous availability in the soil (mg kg^{-1})

Table 6 shows that the addition of NPK compound fertilizer and humic acid had a significant effect on the average concentrations of Phosphorous availability in the soil, as the C2 and C3 two transactions were significantly superior, which two reached the highest average of 21.58,36.92 mg kg^{-1} compared to C1 treatment which reached 12.49 mg kg^{-1}, respectively with an increased rate of 72.77% and 195.59%, As for the effect of adding humic acid, as the H2 and H3 two transactions were significantly superior which two reached the highest average of 23.69,27.91 mg kg-1 was significantly superior to H1 treatment which amounted to 19.40mg kg^{-1}, respectively with an increase of 22.11 % and 43.86%, As for the interaction effect, C3H3 treatment, which amounted to 45.49 mg kg^{-1} showed the highest value and the lowest value when C1H1 treatment, which amounted to 11.50 mg kg^{-1}, and an increase of 295.56%.

Potassium availability in the soil (mg kg^{-1})

Table 7 shows that the addition of NPK compound fertilizer and humic acid had a significant effect on the average Potassium availability in the soil, as the C2 and C3 two transactions were s significantly superior, which two reached the highest average of 189.81, and 208.69 mg kg^{-1} compared C1 treatment, which amounted to 169.90 mg kg^{-1}, respectively, with an increase of 11.71 and 22.83%, As for the effect of adding humic acid,as the H2 and H3 two transactions were s significantly superior which two reached the highest average of189.48,197.11mg kg^{-1} was significantly superior to H1 treatment which amounted to 181.81 mg kg^{-1}, respectively, with an increase of 4.21% and 8.41%, As for the interaction effect, the C3H3 treatment which amounted to 220.26 mg kg^{-1}, showed the highest value and the lowest value for C1H1 treatment which amounted to 162.74 mg kg^{-1}, and an increase of 35.34%.

The total yield of the plant Mg ha^{-1}.

Table 8 shows that the addition of the NPK compound fertilizer and humic acid had a significant effect on the average total yield of the plant, as the C2 and C3 two transactions were significantly superior, which two reached the highest average of 57.88, 68.23 Mg ha^{-1} compared to the treatment C1 which amounted to 33.77 Mg ha^{-1}, respectively with an increased rate 71.39 and 102.04%, As for the effect of adding humic acid, as the H2 and H3, two transactions were significantly superior, which two reached the high est average of 54.29,57.53 Mg ha^{-1}, showed a significant superiority over H1 treatment, which amounted to 48.06 Mg ha^{-1}, respectively, with an increase of 12.96 and 19.70%, As for the effect of the interaction, the C3H3 treatment which amounted to 70.61Mg ha^{-1} showed a value and the lowest value for C1H1 treatment which amounted to 26.11 Mg ha^{-1}, and an increase of 170.43%.

	C1	C2	C3	
H1	11.50 g	17.56 ef	29.13 c	19.40 C
H2	12.55 fg	22.40 de	36.13 b	23.69 B
H3	13.44 fg	24.79 cd	45.49 a	27.91 A
	12.49 C	21.58 B	36.92 A	

*The symbols in the table indicate: C = NPK compound fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H= humic acid where H1= 0 without addition, H2 = 15 kg ha^{-1}, H3= 30kg ha^{-1} According to Duncan's polynomial test at 0.05% probability level, Means with different letters differ significantly from each other.

Table 6. Effect of adding NPK compound fertilizer and humic acid and the interaction between them on phosphorous concentrations in the soil at the stage of full maturity of the head (mg kg^{-1}).

	C1	C2	C3	
H1	162.74 e	187.00 bc	195.67 b	181.81 C
H2	168.64 de	189.67 b	210.13 a	189.48 B
H3	178.33 cd	192.75 b	220.26 a	197.11 A
	169.90 C	189.81 B	208.69 A	

The symbols in the table indicate C = NPK compound fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H= humic acid where H1= 0 without addition, H2 = 15 kg ha^{-1}, H3= 30kg ha^{-1} According to Duncan's polynomial test at 0.05% probability level. Means with different letters differ significantly from each other.

Table 7. Effect of adding NPK and humic acid fertilizer and the interaction between them on potassium concentrations in the soil in the stage of full maturity of the head (mg kg^{-1}).

	C1	C2	C3	
H1	26.11 i	52.69 f	65.38 c	48.06 C
H2	35.52 h	58.66 e	68.68 b	54.29 B
H3	39.68 g	62.30 d	70.61 a	57.53 A
	33.77 C	57.88 B	68.23 A	

*The symbols in the table indicate: C = NPK compound fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H= humic acid where H1= 0 without addition, H2 = 15 kg ha^{-1}, H3= 30kg ha^{-1} According to Duncan's polynomial test at 0.05% probability level, Means with different letters differ significantly from each other.

Table 8. Effect of adding NPK and humic acid fertilizer and the interaction between them on the concentrations of the total yield of the plant(Mg ha^{-1}).

Discussion

The results in tables 2,3,4,5 and 6,7,8 show that the compound fertilizer NPK and humic acid had a role in all the characteristics mentioned in the above tables, as the use of compound fertilizer NPK at the level of 300 kg ha^{-1}. This led to an increase in the availability of Nitrogen, phosphorous and Potassium ready in the soil. This may be attributed to adding the compound fertilizer, which leads to the rapid release of the elements and an increase in their readiness [15-21].

The results of Tables 2, 3, 4,5,6,7,8 the superiority of humic acid significantly in the above-studied traits. Humic

acid may be a source of nutrients. It may also be attributed to the role of humic acid in holding water in the soil, which helps to maintain soil moisture and thus encourages the increase of root branches and thus helps in the absorption of nutrients it may be attributed to the role of humic acid, which helps to release the nutrients associated with minerals and salts available in the soil, Humic acid works to reduce the degree of soil reaction, which leads to reducing the processes of loss and sedimentation. As it attracts nutrients and helps dissolve them slowly and continuously, this positively reflects on increasing soil productivity and plant yield[7,22-25].

The combined application of the compound fertilizer NPK and humic acid led to a more significant increase in the availability of the elements compared to their effect[26-29]. And its combination with organic fertilizers leads to a rise in soil productivity beyond what can be achieved with a single use.

Conclusions

Adding NPK compound fertilizer 300 kg ha^{-1} led to an increase in the efficiency of the elements in the soil, its ability to supply the plant with what it needs, and an increase in the residual effect.

The addition of humic acid 30 kg ha^{-1} led to an increase in the concentrations of Nitrogen, Phosphorous, and Potassium and the total yield of the plant, thus meeting the plant's needs for these nutrients.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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ARTICLE / INVESTIGACIÓN

Effect of nano-zinc and nano-boron spraying on cotton growth and yield

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DOI. 10.21931/RB/2022.07.04.41

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Abstract: A field experiment was carried out in one of the fields of Karbala Governorate - Iraq, during the summer agricultural season of 2021 to know the effect of spraying with nano-zinc and nano-boron on some characteristics of cotton yield. The experiment was applied in three replications according to the randomized complete block design (RCBD) with two factors; the first factor included four levels of Nano-zinc (0, 100, 200, 300) mg L^{-1}. The second factor included four levels of Nano-boron (0, 90, 180, 270) mg L^{-1}. The concentration of 300 mg L- a of Nano-zinc caused a significant increase in the leaf area number of monopodial, number of sympodial branching, total boll number per plant, number of open bolls per plant, boll weight, number of seeds per boll, Seed index and Fibre index, as it gave averages of leaf area 1861 cm2, 8.50 branches $Plant^{-1}$. 4.45 branches $plant^{-1}$, 28.48 bolls $plant^{-1}$, 15.09 bolls $plant^{-1}$, 10.82 g, 6.85 g, respectively. The results also showed that Nano-boron caused a significant increase in the studied traits, where the concentration is 90 mg L^{-1}, which caused a significant increase in plant height, leaf area number of monopodial, number of sympodial branching, total boll number per plant, number of open bolls per plant, boll weight, number of seeds per boll, Seed index and Fibre index. It gave averages of leaf area 153.91 cm, 1908 cm^2, 8.56 branches $plant^{-1}$, 27.98 boll $plant^{-1}$, 15.07 boll $plant^{-1}$, 5.37 g, 10.77 g, and 6.60 g, respectively. It is concluded that adding zinc and boron, singly or together, to the vegetative system improved cotton growth characteristics and yield.

Key words: Nano fertilizer, foliar, zinc, boron, cotton.

Introduction

Cotton is one of the essential economic crops because it contains fiber and oil. It also can persist and survive in different environments, as some cotton can adapt to such environments and produce high-quality fibers[1]. Still, the deterioration in the productivity of the cultivated varieties has caused. It is due to the lack of fertilization with micro-nutrients, which leads to an imbalance in the nutritional balance or thus affects the growth of the plant[2]. To preserve the environment from the risk of pollution, Nano-fertilizers were used as well to reduce time and effort. Foliar feeding with nutrients is essential in changing cotton's growth and physiological characteristics through fertilization strategies. Foliar spray fertilization leads to improving the efficiency of fertilizer use and reducing the environmental pollution. It has also been proven that fertilization with foliar feeding of mixtures of micronutrients during the flower or nut development stages is adequate when optimal use of nutrients. With cotton, thus reducing the rate of walnut shedding and increasing the yield[3], indicated that foliar feeding helps to reach the nutrients to the places of their representation[4]. Indicated that it is preferable to use foliar feeding when there is a problem in the soil, such as its ability to sediment and fix nutrients and high salinity as it reduces. The readiness and absorption of these elements[5].indicated that the application of nanotechnology in agriculture leads to improving the environment and increasing crop quality and production of crops[6]. Nano fertilizers are characterized by their small size and large surface area, which leads to an increase in yield. Zinc and boron are considered nutrients. The essential microelements for plants and animals and zinc have a vital role in the plant's metabolic system, as this zinc activates the participation in the formation of proteins, fats, carbohydrates, DNA and enzymes[7]. Zinc also plays a significant role in controlling the production and toxicity of free radicals that can be caused damage to membrane lipids[8]. Zinc deficiency leads to a decrease in vegetative and fruitful growth[9]. Using zinc by spraying on leaves leads to Increasing the number of flowers, number of seeds per boll, and cotton yield[10,11]. Boron contributes to the transfer of sugar particles and sources of filling, leading to improved fruits and increasing vegetative growth and seed formation. Indicated that spraying with boron at concentrations (0.0.56.1.12) kg/ha led to a significant increase in bolls, boll weight and cotton yield[12]. It was also found[13] when using seven treatments of zinc and boron with the following concentrations (0 + 0, 0.75 + 0, 0 +1, 0.75 + 1, 1.50 + 0, 0 + 2, 1.50 + 2) added by spraying on cotton, that 0.75 kg/ha of zinc with 1.00 $kg.ha^{-1}$ caused a significant increase in the number of bolls, cotton yield and boll weight[13]. (14) showed a significant increase in cotton yield due to spraying zinc and boron. (15) when studying the effect of three concentrations of boron on the growth and yield of cotton indicated that boron caused a significant increase in the number of bolls and cotton yield. This study examines how spraying with zinc and nano-boron affects cotton growth and yield characteristics.

Citation: Fadil, NM; Alfarttoosi, H.A.Kh. Effect of nano-zinc and nano-boron spraying on cotton growth and yield. Revis Bionat a 2022;7(4) 41. http://dx.doi.org/10.21931/RB/2022.07.04.41

Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

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Materials and methods

A field experiment was carried out in the holy city of Karbala during the summer of 2021 to find out the effect of spraying with different concentrations of Nano-zinc and Nano-boron on the growth characteristics and yield of cotton. The experiment was applied with three replications according to a randomized complete block design (RCBD) with two factors; the first factor included four levels of Nano-zinc (0, 100, 200, 300) mg L^{-1} and four treatments of Nano-boron (0, 90, 180, and 270) mg L^{-1}. The fertilizer was randomly sprayed on the vegetative system of the plant, as the Nano-zinc used is from the chelating fertilizers that contain zinc in it 12%, and the Nano-boron used is from the chelating fertilizers that contain boron (9%). Nano zinc and Nano boron were sprayed when the plant reached the flowering stage by 50% after the absence of the sun.

Plants were randomly selected from each experimental unit. The following characteristics were measured: Plant height(cm), leaf area (cm^2), number of monopodial (branches $plant^{-1}$), number of sympodial branching (branches $plant^{-1}$), total boll number per plant (boll $plant^{-1}$), number of open bolls per plant (boll $plant^{-1}$), boll weight (g), number of seeds per boll (seed $boll^{-1}$), Seed cotton yield (Ton h^{-1}) , Seed index and Fibre index.

Results and discussion

Table 1 shows that there were significant differences between the treatments of nano-zinc, where the concentration of 300 mg L^{-1} gave the highest average in leaf area, number of), number of monopodial (branches $plant^{-1}$), number of sympodial branching (branches $plant^{-1}$), with averages amounting to 1861 cm^2, 8.50 branch. $Plant^{-1}$,4.45 branch $Plant^{-1}$, superior to the non-spray treatment. While the concentration of 100 mg.L^{-1} gave the high est average plant height of 154.81 cm. The reason is that zinc is essential in forming enzymes as it is a functional and structural cofactor and biomass production and is also required in producing chlorophyll and regulating enzymes[16]. At the same time, the deficiency of zinc in cotton leads to the small size of the leaf, its wrapping and its appearance it[17]. The results of Table 1 also showed that Nano-boron caused a significant increase in the studied traits; the concentration 90 mg L^{-1} gave the highest average in the plant trait: height, leaf area, number of sympodial branching with standards of leaf area 153.91 cm, 1908 cm^2, 8.56 branch $Plant^{-1}$ superior to no-spray. The boron deficiency affects the growth and yield of cotton as a result of reducing the transfer of material represented to the stalk through vascular bundles, which stops growth or causes abnormal development of the cotton reproductive parts of cotton[18]. And when spraying the solution (Zn + B) together on the vegetative treatment of cotton plants led to a significant increase between treatments, the concentration of 300 mg L^{-1} of Nano-Zn with 90 mg L^{-1} of Nano-boron gave the highest average in leaf area. The number of sympodial branches, which provided an averages leaf area of 2228 cm^2, 9.33 branch $Plant^{-1}$, respectively, is superior to no spraying, and this is because zinc and boron cause an improvement in the absorption site and its transfer of plant nutrients to plant parts and an increase in photosynthesis, which in turn causes an increase in growth characteristics, and this is consistent with what was stated[19].

Table 2 shows that there were significant differences between the treatments of Nano-Zn in the number of total bolls, the number of open bolls and the number of seeds per bolls, the concentration of 300 mg.L^{-1} caused a significant increase in the number of total bolls, the number of open bolls, as it gave averages of 28.48 bolls $plant^{-1}$, 15.09 bolls $Plant^{-1}$, respectively, superior to the non-spray treatment that offered the lowest standards. This trait increase may be due to the role of zinc in the production of IAA auxin, which prevents the dropping of buds, flowers and bolls and the rise in the number of branches[17]. This is in agreement with (16). It was also noted that the Nano boron caused a significant increase in these characteristics, as the concentration of 90 mg L^{-1} of Nano boron gave the highest rate in the total number of bolls, and the number of open bolls amounted to 27.98 bolls $plant^{-1}$,15.07 bolls $plant^{-1}$, respectively. It is superior to the non-spray treatment that gave the lowest averages, and the reason for this increase is that boron controls the growth of meristematic cells, so when it is lacking, no change occurs at standard rates. The transfer of sugars and the increase in the rate of photosynthesis enhances the growth of flowers and seeds, which increases the number of bolls[12], and this is consistent with what was reached[15]. The results also showed that spraying zinc and boron together caused a significant increase in the studied traits, the concentration (300 +90) mg L^{-1} of Nano-zinc and Nano-boron caused a significant increase in the total number of bolls and the number of open bolls, as it gave averages of 31.20 bolls $plant^{-1}$, 17.03 bolls $Plant^{-1}$, respectively, superior to the no-spray treatment that gave the lowest standards.

Table 3 showed that Nano-zinc led to a significant increase in bolls weight, seed index and Fibre index. The concentration of 300 mg L^{-1} of Nano-zinc caused a substantial increase in seed index and Fiber index, as it gave averages of 10.82 g and 6.85 g, respectively, superior to the non-spray treatment that offered the lowest standards. The reason is due to the role of zinc in cell formation and seed development, as zinc deficiency causes a decrease in the number of bolls and thus their small size[21-23]. The treatments of Nano-boron also caused a significant increase in bolls weight, seed index and Fibre index, the concentration of 90 mg L^{-1} gave the highest averages of 5.37 g, 10.77 g, and 6.60 g, respectively, superior to The non-spray treatment that offered the lowest standards. The reason is due to the effective role of boron in the vital processes of the cotton plant and its role as well in the transfer of sugars from the leaves to the fruits, as it is necessary to fill the seeds and improve the quality of the bolls[24] and this is consistent with (25). The spraying of Nano-zinc and Nano-boron together gave an increase in all traits, and spraying with 300 mg L^{-1} + 90 mg L^{-1} of Nano-zinc and Nano-boron caused a significant rise in bolls weight, seed index and Fibre index, as it gave averages amounting to 5.54 g, 11.52 g, 7.20 g, respectively, and this is consistent with what was stated[25].

Conclusions

It is concluded from this study that spraying zinc and boron caused a significant increase in all growth characteristics and cotton yield, so we suggest spraying a concentration of 300 mg.L^{-1} of nano-zinc and a concentration of 90 mg.L^{-1} of nano-boron to increase the yield and quality of cotton.

Zinc nano concentrations		Plant height (cm)	Leaf area(cm^2)	Sympodial	monopodial
0		147.86	1510	7.92	4.08
100 Mg.L^{-1}		154.81	1692	8.13	4.30
200Mg.L^{-1}		150.88	1510	8.38	4.22
300Mg.L^{-1}		151.64	1861	8.50	4.45
L SD		5.303	330.8	0.455	0.1451
Boron nano concentrations					
0		147.68	1443	7.87	4.20
90 Mg.L^{-1}		153.91	1908	8.56	4.25
Mg.L^{-1}180		151.62	1640	8.14	4.39
270Mg.L^{-1}		151.98	1506	8.23	4.20
LSD		5.303	330,8	0.455	0.1451
Zinc nano concentrations	**Boron nano concentrations**				
Control (0)	Control (0)	141.07	1309	7.10	3.87
	90Mg.L^{-1}	150.73	1742	8.57	4.13
	180Mg.L^{-1}	150.10	1602	7.70	4.27
	270Mg.L^{-1}	149.53	1385	7.80	4.03
100Mg.L^{-1}	Control (0)	153.57	1628	7.80	4.27
	90Mg.L^{-1}	156.57	2118	7.93	4.20
	180Mg.L^{-1}	155.63	1548	8.20	4.33
	270Mg.L^{-1}	153.47	1475	8.60	4.40
200Mg.L^{1}	Control (0)	145.97	1408	8.27	4.20
	90Mg.L^{-1}	152.63	1544	8.40	4.27
	180Mg.L^{-1}	154.03	1665	8.47	4.30
	270Mg.L^{-1}	150.90	1422	8.40	4.10
300.Mg.L^{-1}	Control (0)	150.13	1426	8.33	4.47
	90Mg.L^{-1}	155.70	2228	9.33	4.40
	180Mg.L^{-1}	146.73	1745	8.20	4.66
	270Mg.L^{-1}	154.00	2043	8.13	4.27
LSD		10.606	661.6	0.911	0.2902

Table 1. Effect of nano zinc–boron spray on the same growth traits of cotton.

Author Contributions

Conceptualization, Fadil, NM; Alfarttoosi, H.A.Kh..; methodology, Alfarttoosi H.A.Kh, formal analysis, Alfarttoosi, H.A.Kh.; resources Fadil, NM; data curation, Fadil, NM; writing—original draft preparation, Fadil, NM writing—review and editing Fadil, NM; supervision Alfarttoosi, H.A.Kh.; project administration, Alfarttoosi, H.A.Kh.

Funding

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

The study did not report any data.

Acknowledgments

Department of Field Crops Agriculture Collage University of Kerbala.

Conflicts of Interest

The authors declare no conflict of interest.

Zinc nano concentrations		Number of boll. Plant	Number of open bolls Plant^{-1}	Number of seeds in the boll	Seed cotton yield Tan.ha^{-1}
0		25.88	14.02	28.41	3.99
100 Mg.L^{-1}		26.52	14.08	30.82	4.21
200Mg.L^{-1}		27.45	14.04	32.7	4.17
300Mg.L^{-1}		28.48	15.09	30.17	4.43
L SD		1.780	0.941	3.471	0.36
Boron nano concentrations					
0		26.19	13.91	29.26	4.02
90 Mg.L^{-1}		27.98	15.07	31.50	4.47
Mg.L^{-1}180		27	13.98	29.35	4.06
270Mg.L^{-1}		27.17	14.28	32.98	4.17
LSD		1.780	0.941	3.471	0.36
Zinc nano concentrations	**Boron nano concentrations**				
Control (0)	Control (0)	24.27	13.33	21.57	3.55
	90Mg.L^{-1}	26.60	15.00	33.73	4.28
	180Mg.L^{-1}	26.60	13.77	28.47	3.96
	270Mg.L^{-1}	26.07	13.97	29.87	4.16
100Mg.L^{-1}	Control (0)	25.03	13.93	32.33	4.34
	90Mg.L^{-1}	26.73	14.30	28.33	4.47
	180Mg.L^{-1}	27.07	14.43	30.93	4.17
	270Mg.L^{-1}	27.27	13.67	31.67	3.86
200Mg.L^{1}	Control (0)	27.27	14.40	31.00	4.29
	90Mg.L^{-1}	27.40	13.93	32.80	4.04
	180Mg.L^{-1}	27.27	13.60	31.07	4.12
	270Mg.L^{-1}	27.87	14.23	35.93	4.23
300.Mg.L^{-1}	Control (0)	28.20	13.97	32.13	3.89
	90Mg.L^{-1}	31.20	17.03	31.13	5.09
	180Mg.L^{-1}	27.07	14.13	26.93	3.97
	270Mg.L^{-1}	27.47	15.23	34.47	4.43
LSD		3.560	1.882	6.943	0.72

Table 2. Effect of Nano zinc – boron spray on the same productivity traits of cotton.

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Zinc nano concentrations		boll Weight (g)	Seed index(g)	Fibre index(g)
0		4.69	9.83	6.06
100 Mg.L^{-1}		5.10	10.35	6.36
200Mg.L^{-1}		4.93	10.15	6.16
300Mg.L^{-1}		4.93	10.82	6.85
L SD		0.43	0.951	0.674
Boron nano concentrations				
0		4.84	9.67	6.15
90 Mg.L^{-1}		5.37	10.77	6.60
Mg.L^{-1}180		4.89	10.30	6.41
270Mg.L^{-1}		4.98	10.24	6.28
LSD		0.43	0.951	NS
Zinc nano concentrations	**Boron nano concentrations**			
Control (0)	Control (0)	4.02	9.46	5.53
	90Mg.L^{-1}	5.24	10.50	6.11
	180Mg.L^{-1}	4.70	9.47	6.98
	270Mg.L^{-1}	4.82	9.87	5.64
100Mg.L^{-1}	Control (0)	5.30	9.51	6.38
	90Mg.L^{-1}	5.25	10.67	6.63
	180Mg.L^{-1}	4.66	10.88	5.77
	270Mg.L^{-1}	5.17	10.34	6.66
200Mg.L^{1}	Control (0)	4.82	10.04	6.12
	90Mg.L^{-1}	5.45	10.39	6.47
	180Mg.L^{-1}	4.97	10.12	6.26
	270Mg.L^{-1}	4.50	10.04	5.80
300.Mg.L^{-1}	Control (0)	5.22	9.67	6.55
	90Mg.L^{-1}	5.54	11.52	7.20
	180Mg.L^{-1}	5.23	10.74	6.63
	270Mg.L^{-1}	5.43	10.69	7.02
LSD		0.86	1.902	1.349

Table 3. Effect of nano zinc–boron spray on the same productivity traits of cotton.

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ARTICLE / INVESTIGACIÓN

Detection of *LAP1* and *LAP2* genes from *Trichophyton rubrum*

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DOI. 10.21931/RB/2022.07.04.42

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Abstract: Samples of hair, nails and skin were collected, representing people of different ages and races. The number of isolated people gathered between October 2021 and February 2022 from Al-Hussein Teaching Hospital and a private clinic under the supervision of doctors(118) species was examined. Dermatophytes were found in 80 of them; among the 80 positive *Trichophyton rubrum* species, 30 were produced, which represents less than half of the dermatophytes for each of the 80 positive species (14 cutaneous, nine hair and seven nail isolates)the study's findings included hair hole testing, which came back negative, and urea degradation testing. The results were either negative or inconsistent across the isolates; the growth test in the PDA was positive, the virulence factors that enable the fungus to penetrate host tissues were studied during leucine aminopeptidase (*LAP1*) and (*LAP2*), it was observed that there were no significant differences in gene isolates of *T. rubrum*.

Key words: *LAP1*, *LAP2*, genus *Trichophyton rubrum*.

Introduction

Dermatophytes are responsible for various illnesses, including ringworm and ringworm-like infections (Tinea); dermatophytoses is the correct word for a fungus that damages corneal tissues and produces superficial skin diseases such as skin, hair, and nails. It has three principal species: *Trichophyton*, *Microsporum*, and *Epidermatophyton*. *Trichophyton spp.* is the most frequent Dermatophytoses fungus, with (25) species, the most virulent, and one of the most common diseases in the world, affecting 10- 20% of the global population[1].

Many enzymatic components, such as hydrolysis enzymes, are secreted by Dermatophytes, and the higher the ability to create these enzymes, the more ferocity and high ferocity of the attacking fungi, which may cause skin ulcers known as krion, one of the most important enzymes involved in the breakdown of keratin in the host's skin[2].

The element mercury is characterized by its high toxicity, especially when inhaled, as it causes scratches and ulcers in the respiratory tract and is mainly produced from industrial processes. Leucine aminopeptidase (LAP1) and (LAP2) are enzymes that help the fungus survive by providing food and preparing the skin for colonization; the presence and production of these enzymes have been investigated genetically in a variety of organisms, including animal cells and some species. Bacteria such as E.coli have been found in the types of genes Trichophyton and Microsporum, but it has not been investigated in depth in sex T.rubrum, even though it is an important marker of the fungus's high virulence[3].

Materials and methods

Diagnostic kits

The diagnostic kits used in this work are from the manufacturer (Presto™ Mini gDNA Yeast Kit) and Geneaid/ Germany.

Primers

The following primers were used in this work to detect proteins: LAP1(F)TGTCTACAACAACGTCCCCG.and(R) CGTCACCGTCGTAGATC)TGG. The product size is 569 bp, while the *LAp2* primer has a length of 487 bp. and the (F)TTGAGTTCCACTGGTACGCC (R) CGACAATGAGCTTAGCGTGC.

Culture media

The following is a list of the various types of media used. The culture media used to isolate and identify fungal isolates were sterilized in an autoclave at 121°C for roughly 15 minutes under 15 psi pressure, and glass was sterilized in an electric oven at 160°C for 90 minutes, per the manufacturer's recommendations[2].

Using the SDA (Sabouraud's Dextrose Agar) medium: The dermatophytes isolates were cultured in this medium agar, which was made following the manufacturer's instructions (Liofilchem, Italy) by combining 65 g of media with 1000 ml of distilled water, correcting the pH, and sterilizing everything in an autoclave.

Citation: Sadeq H S, Al-Oebady M A H. Detection of LAP1 and LAP2 genes from Trichophyton rubrum. Revis Bionat a 2022;7(4) 42. http://dx.doi.org/10.21931/RB/2022.07.04.42

Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

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Test medium for urease 1000mL of DW is mixed with the urea agar base. The solution was sterilized in an autoclave, and its pH was set to 7. cooling to 50 °C. A sterile test tube was then filled with the mixture, which had been gently mixed with 50mL of sterilized 40% urea solution. The combination was then allowed to solidify in a slant position. The majority of *T. mentagrophytes* strains caused the medium to become red from yellow. The color was unaffected by *T. rubrum*.

Trichophyton Agar No.1 medium was used to differentiate between various fungal isolates. This medium was created following the manufacturer's instructions (Oxoide UK), which included dissolving 59.4 grams in 1000 ml of distilled water, adjusting the pH to 6.8, and sterilizing the medium at 121 °C for 15 min at a pressure of 15.

Medium for Potato Dextrose Agar (PDA) This medium was created following the manufacturer's instructions (Liofilchem, Italy), which called for combining 34 g of PDA with 1000 ml of sterilized water. This medium assessed *T. rubrum's* capacity to produce a red stain.

Collection of Specimens

A total of (118) samples were collected from patients who visited the medical clinic between October 2021 and February 2022. Skin and its derivatives were used at random in the study, as well as direct diagnosis and transplantation on dishes, using a sterilized sharp blade, samples of skin were scraped from the margin of the infected area, the wounded hair was cut for examination, and the nails were extracted from a sharp blade with pieces of odd shape and color.

Sample transplantation: Samples were transplanted directly into the SDA medium, which contained anti-chloramphenicol and cycloheximide, T. rubrum was identified by planting samples on Sabouraud Dextrose Agar(SDA) and Potato Dextrose Agar (PDA) medium[4-6].

Diagnostic tests for Trichophyton rubrum

Hair perforation test: Take baby hair and lay it on a glass slide with a drop of Lacto phenol, then study it under a microscope to see how the fungus attacks the hair if the test is positive[7].

Urease test: Depending on the method, this test was used to determine the ability of fungi to make urea enzyme[8].

Growing on *Trichophyton agar* No.1: *Trichophyton agar* No. 1 is utilized. Agar 1 is a vitamin-free, casein-based control that contains extra histidine. Small, agar-free isolates are moved from SDA plates and cultured at 26oC for 2–3 weeks[9,10].

Growth on the medium of the PDA: This medium is used to distinguish *T. mentagrophytes* from *T. rubrum*, which produces red dye on the back of the plate. PDA medium is inoculated with the colony at 10-14 days and incubated in the dark at a temperature of 28°C for 2-4 weeks. Rubrum11.

Diagnosis method using PCR assay / by method[12]

Hair perforation test: Take baby hair and lay it on a glass slide with a drop of Lacto phenol, then study it under a microscope to see how the fungus attacks the hair if the test is positive[7].

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Growth on the medium of the PDA: This medium is used to distinguish *T. mentagrophytes* from *T. rubrum*, which produces red dye on the back of the plate. PDA medium is inoculated with the colony at 10-14 days and incubated in the dark at a temperature of 28°C for 2-4 weeks. Rubrum[11].

Results

There were 97 positive samples. Fungal infections were found in 80 of the 97 samples. T.rubrum 30 produced positive pieces, accounting for less than half of the cutaneous skin fungus, with 14 isolates from the skin, seven from the nails, and nine from the hair. The following methods were used to identify fungus:

The colony's shape

Some colonies were fast-growing, while others were slow-growing; the colony's diameter was around 7 cm in 14 days; the bottom surface seemed red or purple; and many forms of isolates, including fluffy, disintegrated, velvet, and granular isolates. It has a large code production[2].

Micronutrients

Fungi develop little round cones along the hypha in grape clusters, as well as massive conidial with long, smooth, long-stemmed, thin-walled, and helical walls. There is an issue with diagnosing Trichophyton spp genetically, which is called Trichophyton species complex, which is similar to the behavior of species in this species, and the different genotypes may be highly similar in appearance[13].

Microscopy revealed a significant number of little conidia that were either sitting or gripping a small bump on the fungal strings in a reciprocal fashion (Teardrop-shaped) or peg-shaped; the huge conidia were quite small and came in a variety of shapes, including pencil-shaped and cigar-shaped conidia, as shown in the following figure (2) , this result agrees with (2,4,14).

Physiological tests of the fungus *T.rubrum*

The hair hole produces a relatively negative result because wall chains developed around the hair blade but not inside it; the urea test showed a negative or mixed effect among the isolates. When growing on the PDA medium, T.rubrum produced a red or pink dye when growing on this medium[15].

The urea test

Is a method of determining how much urea when the current findings were compared to those previously reported in the literature, there was a good amount of inconsistency. Negative urease test results were obtained in *T. rubrum*, whereas positive urease test results were found in the other species; according to the researchers, *T. rubrum* had positive urease test results, but *M. gypseum*, *M. canis*, and *T. schoenleinii* had negative urease test results[8], ,the urease test results for *T. mentagrophytes* were positive.

The hair follicle

Some dermatophytes produce specialized perforating organs that allow them to penetrate and infect the hair shaft in vitro, while others attack the hair with simple peripheral erosions; as we discovered in this study, the hair perforation

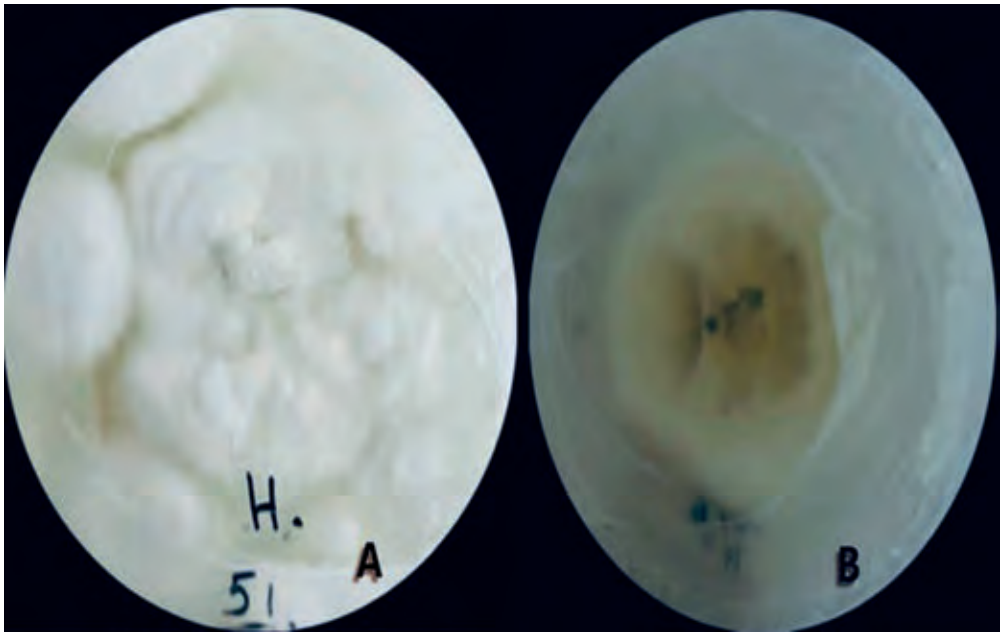


Figure 1. Phenotypic characteristics of T. rubrum and the colony growing on the medium of SDACC, (a) The external appearance from the front (b) The external appearance from the back, temperature 28 ° C, incubation period ten days.

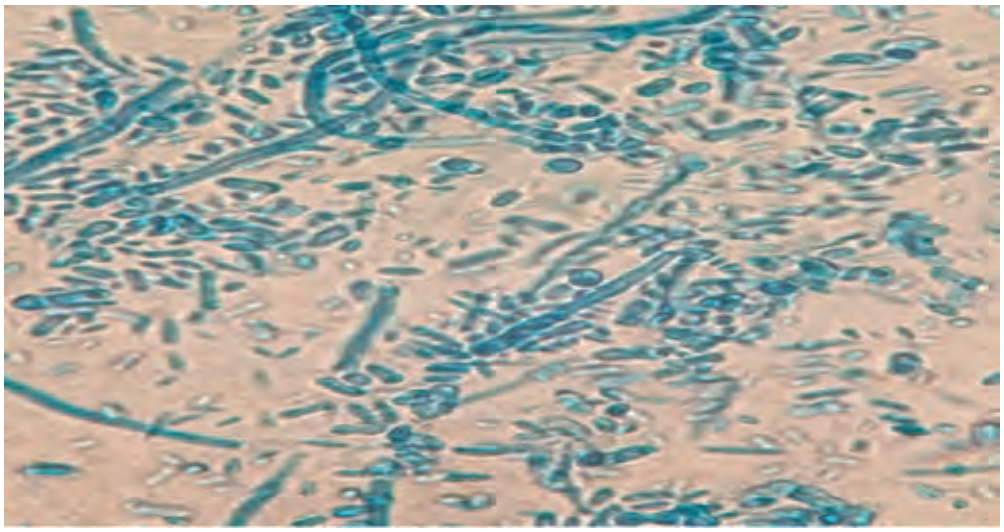


Figure 2. Macroconidia & microconidia of Trichophyton rubrum at 40X with Lacto phenol cotton blue stain.

isolated dermato-phytes	PDA media is used to produce red pigment	Test the hair hole	Production of Urease test
T. rubrum	+	-	-
T.mentogrophytes	-	(Ectothrix)+	+
T. verrcosum	-	(Ectothrix)+	-
M.canis	-	(Ectothrix)+	+
T. schoenleinii	-	(Ectothrix)+	+

Table 1. Shows the results of fungal physiological tests.

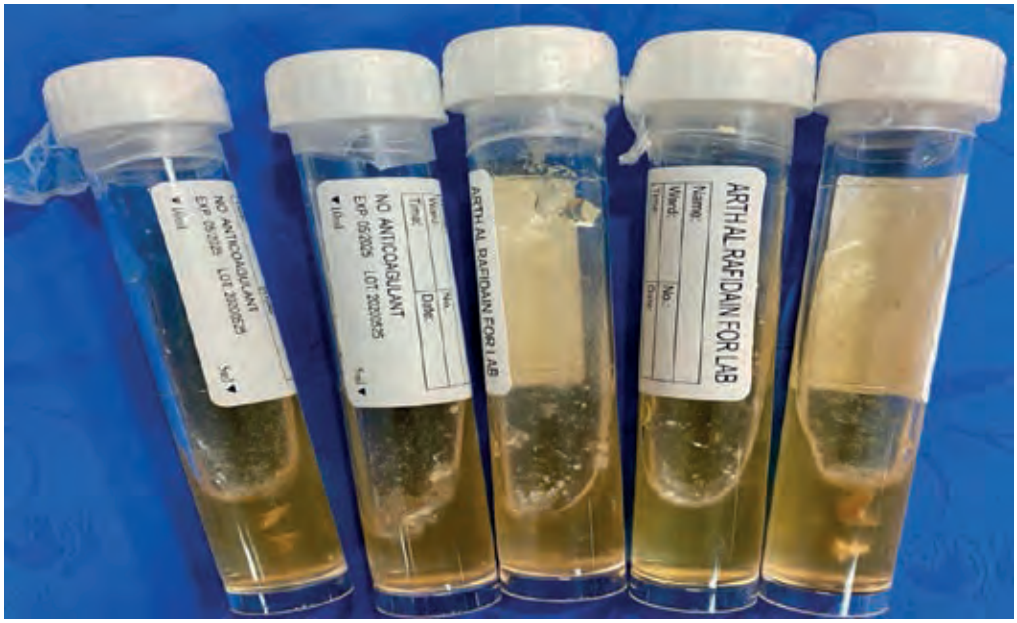


Figure 3. Urea decomposition test. (a) Non-degradation of urea to T. rubrum, the temperature is 28 □, and the incubation period is seven days.

test in vitro can distinguish *T. rubrum* isolates with no perforating organs from atypical *T. mentagrophytes* separates that produce perforating organs in 8–15 days, and it can also be used to distinguish *T. schoenleini*[2].

The prevalence of skin fungal infections by place of residence

According to the current study, the incidence of disease with skin fungi was high among patients from rural areas (60 %), whereas the rate of infection in patients from urban areas was low (40 %), as shown in Figure (5), the findings backed up to (16), who found that people in rural areas are more affected than city dwellers and that this could be attributed to a lack of hygiene, tinea capitis and tinea corporis have the highest incidence[17].

Dermatophytes Infection and gender: It has been studied at various ages, from 5 months to 70 years. In the Al-Hussein teaching hospital, it was discovered that males and female dermatophyte infections were converging. Male disorders comprised 61 samples and (51.69%) of the total infection, while female diseases comprised 57 cases and(48.31%) of the infection. These ratios revealed significant differences in condition between genera, as shown in Figure 6.

This conclusion was supported by the findings of other researchers, including those from Iraq[18] and South Africa[19]. They discovered that males are more prone to dermatophyte infection than females, in contrast to prior research conducted in water[20].

This could be because of the small size of the study sample and the reliance on patients referred to Al-Hussein Teaching Hospital, which had a lot of visitors, or it could be because of the dependence on patients referred to Al-Hussein Teaching Hospital and some clinics, both of which had a lot of visitors. Males are more numerous than females because most girls prefer to visit outpatient clinics.

Dermatophytes Infection associated with Age: The age ranges that were most affected by dermatophytes infection

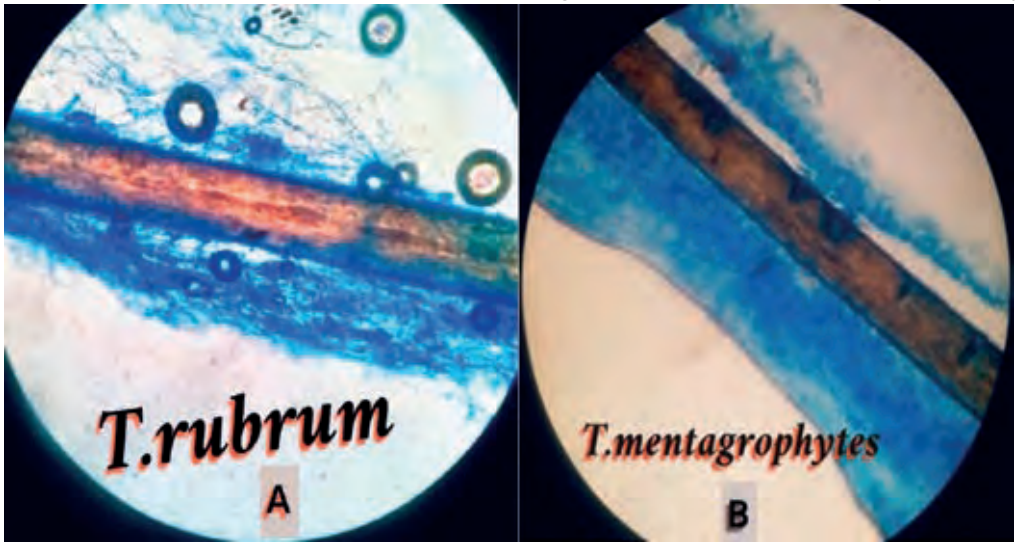
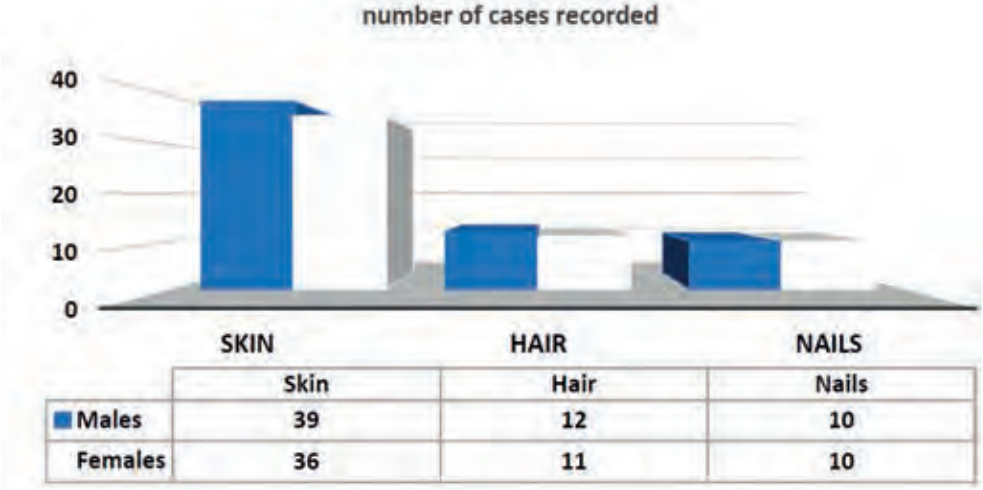


Figure 4. A hair penetration test, (a) No hair penetration in T.rubrum when cultured on SDA medium and at (25 □) for an incubation period of (21) days, (b) Hair penetration in T.mentagrophtes when cultured on SDA medium and at (25 □) for a period of (21) incubation day.

Figure 5. Infection with dermatophytes is distributed according to where the affected people live. *X2 value = 145.664, Significant differences on P≤0.05.



	Skin	Hair	Nails
Males	39	12	10
Females	36	11	10

Figure 6. Shows how the number of scales on men's and women's skin, hair, and nails varies. * Not Significant differences on P≤0.05. Error bars represent standard deviation on a significant level of 0.05.

ranged from 5 months to 10 years at (26.66 %), followed by age 11 to 20 years at (18.66 %), age 21 to 30 years at (16.66 %), age 31 to 40 years at (12 %)t, age 41 to 50 years at (8.33 %)t, age 51 to 60 years at a ratio of (7.03 %), and age 61 to 70 years at (6.66%).

The findings of this study were consistent with those of numerous previous studies; however, because sample sizes varied, there were a few modest and significant discrepancies at certain times[16].

According to this, dermatophyte infections were more common, and the age group most susceptible to this virus was 5 to 10 years old at a ratio of (80.9 %), which was consistent with the findings of other research. This result was also roughly constant with (21). Patients between the ages of 9 and 6 had the highest infection rate. While tinea is more common in children due to a low ratio of fatty acids that prevents fungus growth, it is uncommon in adults due to increased sebaceous gland activity and sebum's antifungal saturated fatty acid content as people age[22].

Diagnosis of *T.rubrum* by Polymerase Chain Reaction (PCR)

The gels were examined under UV light, and one band appeared in all wells at the same level, indicating that primers were bound to their complementary sequence in the DNA template; gel electrophoresis is a technique used to measure the quantity and size of DNA fragments produced after PCR has been completed, the current study's findings are similar to the expected length of about 500 base pairs, the results for the multiplication of a specific primer for dermatophytes *T.rubrum*, and those of a Korean study[23], with a few PCR reaction parameters adjusted that the phenotypic data were similar. The results showed a similarity in the amplified band's molecular weight, which was estimated based on the molecular weights of the band diagnosis was matched by all thirty isolates examined, which is consistent with the findings of (24).

T.rubrum was identified molecularly by performing a polymerase chain reaction (PCR) with primers for this (ITS1) and (ITS4), which yielded a 508-base-pair amplification result. This study is not an epidemiological survey but a diagnostic and pathogenic investigation focused on virulence factors, which helped guarantee that the fungus was morphologically accurate. In contrast, isolates from other species were ignored and genetically checked later[25].

Genes Virulence factors

Proteolytic enzymes, which are present in some environmental isolates obtained from soil and organic matter but less frequently, are crucial for the examination of skin components and appendages, especially structural proteins like keratin; their presence is not limited to fungi that attack the stratum corneum of the skin, however; it has been found that some environmental isolates obtained from soil and organic matter contain proteolytic enzymes[2,26].

The temperature and pH of the culture medium, the components of the culture medium, which include sources of carbon, nitrogen, and salts, the duration of incubation, as well as the aeration and fermentation method used, whether it is a surface or submerged fermentation, are just a few of the variables that affect the growth of microorganisms and the production of protease enzymes[2].

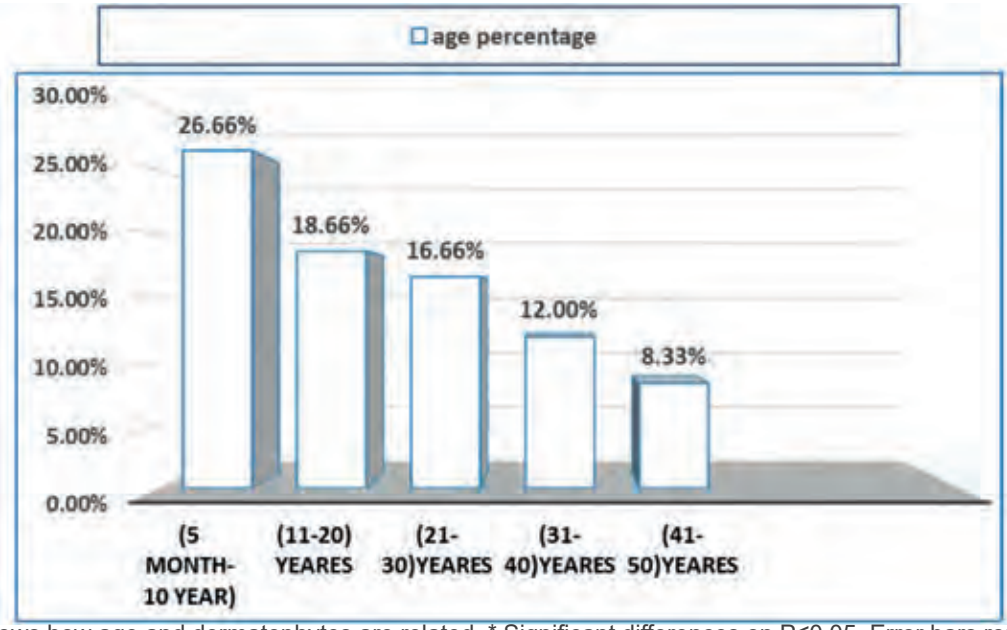


Figure 7. Shows how age and dermatophytes are related. * Significant differences on P≤0.05. Error bars represent standard deviation on a significant level of 0.05.

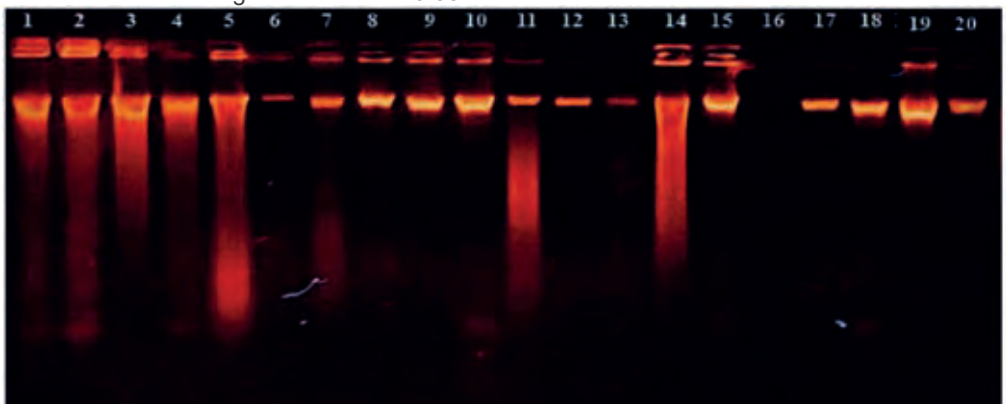


Figure 8. Agarose 1%, 40min. At 110V, stained with Ethidium Bromide agarose gel electrophoresis appearance that displays DNA that was extracted from (human), and for the diagnosis of Trichophyton rubrum, where M: Marker (1500-100bp), represents 1-20 positive fungal isolates for a 508bp assay.

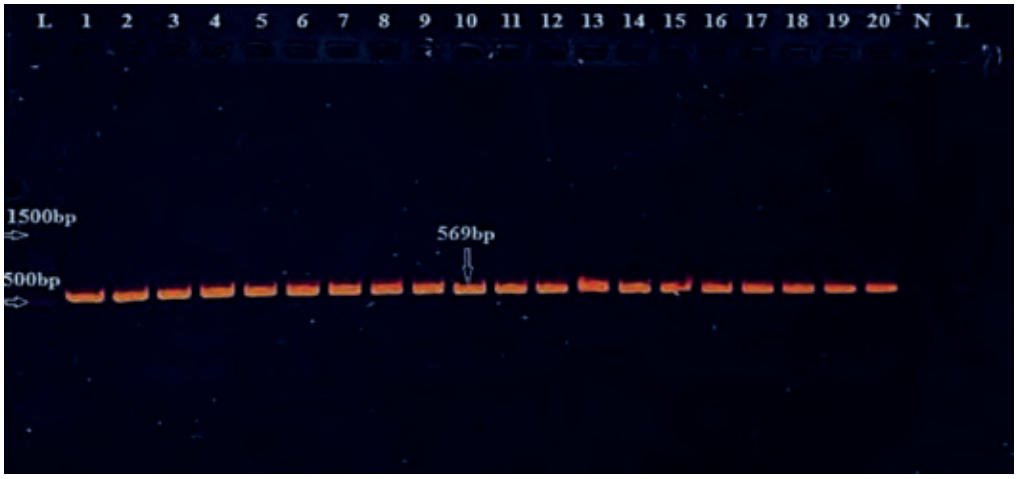


Figure 9. Gel electrophoresis for PCR product of (LAP1 primer) shows 569bp Primer TM at (59), (Agarose 2%, 15min. at 110 volts then lowered to 75 volts for 60min.). They were visualized under UV light after staining with ethidium bromide. Lane L: DNA ladder (100-1500bp).

Discussion

All ages, particularly children, are affected by fungal infections, which are a major global health issue. As a result, numerous studies on various epidemiological, economic, control, and therapeutic aspects of this infection have been carried out. About 20–25% of the world's population is thought to have skin mycoses, making dermatophytosis one of the most prevalent human fungal illnesses[27]. The term "dermatophytes" refers to a class of closely related fungi that comprises members of the genera Epidermophyton, Microsporum, and Trichophyton, each of which has several identified species. These fungi are keratinophilic, meaning they attack the skin, hair, and nails of humans and animals[28].

Most dermatophyte strains previously had a relatively narrow geographic distribution, making them one of the few fungi that cause infectious diseases. On the other hand, dermatophytosis has recently become one of the most prevalent infectious disorders affecting people worldwide and is spread globally. As many other disorders resemble the

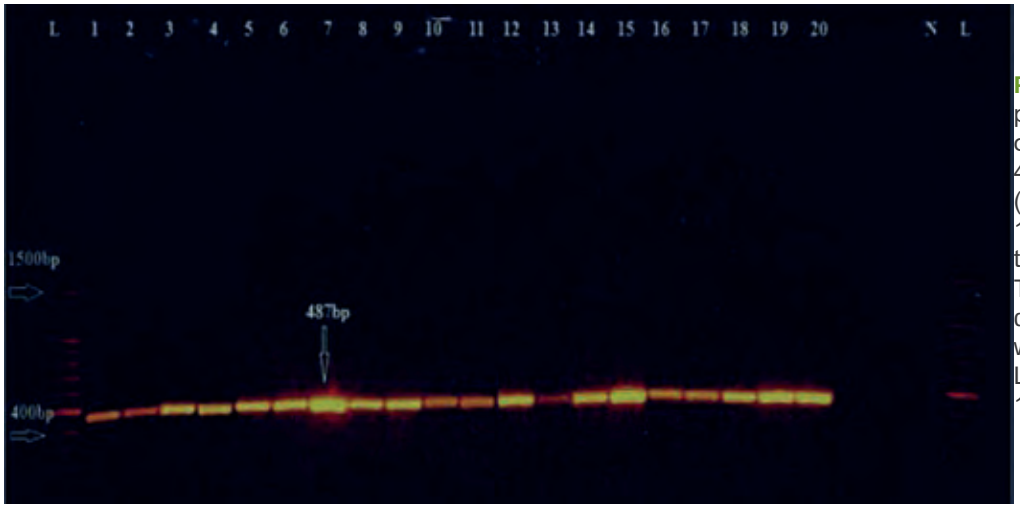


Figure 10. Gel electrophoresis for PCR product of (LAP2 primer) shows 487 bp Primer TM at (57), (Agarose 2%, 15min. at 110 volts then lowered to 75 volts for 60min.). They were visualized under UV light after staining with ethidium bromide. Lane L: DNA ladder (100-1500bp).

Type of gene	Number and percentage
LAP1	30(37.5%)
LAP2	30(37.5%)
X2	11.34
P>= 0.05	

Table 2. Molecular diagnosis of T.rubrum isolates and virulence factors.

clinical presentation, it is challenging to identify dermatophytosis based solely on clinical signs. Seborrheic dermatitis, atopic dermatitis, contact dermatitis, psoriasis, candidal intertrigo, erythrasma, eczema, etc., are among the dermatophytoses that can be diagnosed as a differential diagnosis[29].

Additionally, immunocompromised patients, such as those with AIDS, diabetes mellitus, organ transplantation, corticosteroid use, and antineoplastic agent use, are more challenging to diagnose for dermatophytosis.In order to apply the proper treatment and preventative measures, reliable laboratory methods must be available for the quick and accurate identification of the dermatophytes involved. The conventional approaches to finding fungi have limitations, such as the low specificity of KOH microscopy and the low sensitivity and lengthy nature of fungus culture. The results of culture isolation are further compromised because additional dermatophyte isolates from patients receiving antifungal treatment typically do not exhibit characteristic morphology on culture. This study's few negative results from direct microscopic examination and culture could be related to several factors, including the small amount of collected sample, which might not have been enough to produce a positive result. Because many people with dermatophytoses utilized random drugs without seeing a doctor, the adverse effects of culturing specimens may be explained. Instead of causing them to expand, this led to a shift in the dermatophytes' vitality, perhaps due to improper sample storage before culture. Storage in containers that retain moisture causes saprophytic fungus to develop, contaminating the original sample and yielding unfavorable results. The hyphae of non-dermatophytic fungi, such as molds, which frequently only appear as temporary contaminants and are not the trustworthy causative agent of the disease, are very difficult to distinguish from those of dermatophyte hyphae[30].

Tinea cruris was the third most common kind of dermatophytosis in our study, Adults in the (31-50) year age range were impacted, and more men than women experienced it. According to Rothman, 1985, ringworm of the groin is primarily a postpubertal disease of men and is most likely caused by the pubertal development of a sex-specific apocrine gland, whose secretion contributes to infection susceptibility. According to data, urban areas had a 40% infection rate, while rural areas had a 60% infection rate. However, given the predominance of persons who live in urban settings, their findings differ from those of others. This can be explained by the fact that many rural places have poor health, financial difficulties, and congestion of residents[31].

The initial PCR technique is DNA fingerprinting. It has been demonstrated that this primer is an effective tool for the molecular identification of dermatophytes. In this study, we successfully classified T.rubrum isolates as dermatophyte species[32].

Conclusions

The most common species that induced dermatophytoses were *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Microsporum canis*. The *T.rubrun* fungus outnumbers other species that cause skin infections, indicating that it is very pathogenic. Anthropophilic disorders were more prevalent than other categories, and animal interaction and poor hygiene in rural areas were the main causes of dermatophytes infection; the fungus *T. rubrum* carries the genes *LAP* and *LAP2*, which produce proteolytic enzymes that aid in adhesion, invasion, and suppression of the host response, making the fungus more likely to colonize and penetrate tissues.

Funding

Self-funding.

Conflicts of Interest

There is no conflict.

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ARTICLE / INVESTIGACIÓN

The relationship between the infestation of Whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) and the chlorophyll content in different Eggplant varieties

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DOI. 10.21931/RB/2022.07.04.43

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Abstract: The sweet potato whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), is one of the most important pests in vegetables. Although the host plant *Solanum melongena*, is considered the most preferred, the whiteflies have preferences for particular varieties of eggplant. In this study, we evaluated three eggplant varieties in choice feeding tests. We found that the varieties of Zomorrod and Barcelona were the most preferred with the highest numbers of whitefly nymphs, which were 312 and 300 per leaf, respectively, on the last week of May. In comparison, the nymph average was 171.8 per leaf on the last week of May on the kareema. Chlorophyll concentration has been negatively affected by the infestation of *B. tabaci*. The investigation of the chlorophyll content ratio between the uninfested and infested eggplant leaves showed a decrease in chlorophyll content at 13.95 and 6.60 11 mg/L of the Zumorrod variety for the uninfested and infested leaves, respectively, with 52.67% chlorophyll reduction. At the same time, the mean chlorophyll content for uninfested and infested eggplant leaves of the Barcelona variety was 12.74 and 4.95 mg/L, respectively, with 61.14% chlorophyll reduction. At the same time, the Kareema variety was recorded at 17.49 and 4.95 mg/L for the uninfested and infested leaves, respectively, with 72.39% chlorophyll reduction. Furthermore, feeding by *B. tabaci* reduced leaf photosynthesis in eggplant by restricting gas exchange through stomata and reducing chlorophyll's content and photosynthetic capacity.

Key words: Population density, piercing pest, *Bemisia tabaci*, *Solanum melongena*, host preference, plant pigments.

Introduction

Eggplant *Solanum melongena* L. is an important vegetable crop cultivated for its fruits. It is also known as brinjal, aubergine, garden egg, or guinea squash in countries[1]. Although India and Afghanistan are considered the original homeland of eggplant plants, eggplant cultivation is currently widespread in all regions of the world[2]. Several varieties of eggplant fruits show a wide range of shapes and colors, ranging from long elongated to oval to ovoid-shaped fruits, displaying multiple colors spanning from shades of purple, yellow, and green to white. Among the various factors that may cause the eggplant's low productivity is the damage caused by many pests, such as different species of aphids, mites, moths, and the whitefly *Bemisia tabaci* (Gennadius)[3].

The whitefly *B. tabaci* (Gennadius) (Hemiptera, Aleyrodidae) is a species complex with a worldwide distribution, considered a severe pest on several plant families, including Solanaceae[4]. Throughout the whitefly life cycle, it feeds on the leaves, causing direct and indirect damage to the host plant by piercing leaves, sucking sap, and producing honeydew (on which sooty mold develops). It also affects growth, photosynthesis, and chemical and phonological processes[5,6], in addition to transmitting a wide range of virus diseases such as Tomato yellow leaf curl virus (TYLCV), tomato chlorosis virus (ToCV), sweet potato chlorotic stunt virus (SPCSV), mild cowpea mottle virus (CpMMV), melon yellowing-associated virus (MYaV), lettuce infectious yellows virus (LIYV), tobacco mosaic virus (TMV), and tomato mosaic virus (TMV)[7]. The sweet potato whitefly *B. tabaci* is well known that the whitefly infestation on vegetables causes a reduction in chlorophyll content and other pigmentations in the infested plants[8]. Whitefly nymphs usually cause chlorosis in infested leaves[9]. The result of the decrease in the concentration of chlorophyll negatively affects the photosynthesis process and leaf gas exchange[8].

The control of *B. tabaci* is a challenge because of the several generations annually developing and its ability to acquire resistance to insecticides quickly[10]. Over the years, many studies on the plant host preferences of *B. tabaci* and host suitability among different plant varieties have been carried out[5,11-14]. Furthermore, several studies focused on the effect of host plant growth by whitefly infestation, such as the age and population size of whitefly infestation and the ability to reproduce it[15]. However, the physiological and biochemical changes caused by *B. tabaci* in the infected plant hosts were not seen. This study aimed to assess the impact of *B. tabaci* on the chlorophyll concentration of some eggplant varieties.

Materials and methods

Prepared greenhouse and growth conditions

The study was conducted at the University of Baghdad, Agricultural Engineering Sciences, located at the Al-Jadriyah Campus. Different varieties of hybrid eggplants Barce-

Citation: Raed, A.; Ahmed, Q.; Arif, M. The Relationship between the Infestation of Whitefly Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) and the Chlorophyll Content in Different Eggplant Varieties. Revis Bionat a 2022;7(4) 43. http://dx.doi.org/10.21931/RB/2022.07.04.43

Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022
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lona F1 (Fito Semillas company, Barcelona, Spain), Zomorrod F1 (Enza Zaden company, Netherlands), and Kareema F1 (Vilmorin Seed Generation Company, Paris, France) were obtained from the local market. Seeds were sown in a planting tray filled with potting mix under controlled conditions and kept free from pest and disease infestations. The seedlings of eggplants were transferred to the greenhouse (9 × 13 m^2) till the seedlings reached 2-4 leaves. Eggplants were irrigated and fertilized with NPK fertilizers. The greenhouse was divided into five plots with 10 planting rows. Each plot was cultivated with one variety of eggplant and was divided into 5 plots containing 10 eggplant plant seedlings, in a total of 50 plants per variety.

Population Density Calculation

Five leaves were taken from each eggplant variety and replicated each week randomly during April and May to calculate the nymph number of whitefly *B. tabaci* on both surfaces of the leaves for the three varieties of eggplants mentioned above. The samples of eggplant leaves were placed into zip lock bags with initial details such as the name of the eggplant variety, collection date, and the replicate number. The zip bags were transferred to the laboratory, examined using a stereomicroscope and needle on the same day of sample collection, and counted the nymph number.

Chlorophyll content

Based on Goodwin's method (16), the concentration of chlorophyll in the uninfested and infested leaves of three varieties of eggplants (Zomorrod, Kareema, and Barcelona) was measured by using a spectrophotometer; uninfested leaves were taken from plants regularly sprayed by chemical pesticides to prevent pest infestation. Uninfested and infested leaves extraction were prepared with three replicates. Five leaves were taken from uninfested and infested leaves from each variety for each replicates separately, leaves drying under laboratory conditions, and mortar and pestle were used for crushing the leaves. A quantity of 0.2 g of leaf powder was taken from each treatment, placed in a glass vial, added a volume of 20 ml of acetone / water (80:20) solvent, and left for 24 hours. The extract was placed on a spectrophotometer port to measure chlorophyll content for each replicate from each variety. To calculate the absorbance of the grape leaf extract sample, the wavelengths 663 nm and 645 nm were set up in the spectrophotometer to estimate the amount of chlorophyll in each instance by the concentration of (mg/mL) according to the following equations:

Chlorophyll a (mg/mL) = 12.7 D (663nm) – 2.69 D (645nm)

Chlorophyll b (mg/mL) = 22.9 D (645nm) – 4.68 D (663nm)

Total chlorophyll (mg/mL) = 20.2 D (645nm) +8.02 D (663nm)

Where D represents the spectrophotometer reading, 20.2 and 8.02 mg/g referred to the absorbance of chlorophyll a and b at 645 nm and 663 nm, respectively.

% reduction of chlorophyll was calculated by the equation below as described by Farina *et al.*[23]:

$$\%\text{Reduction} = \frac{\text{Uninfested eggplants value} - \text{infested eggplants value}}{\text{Uninfested eggplants value}} \times 100$$

Statistical analysis

All field tests were conducted using the Randomized Completely Block Design (RCBD) utilizing the statistical analysis program SPSS version 26 to analyze variance (ANOVA) on the data. The chlorophyll content of uninfested and infested eggplant leaves with whiteflies from each tested variety was compared using the least significant difference (LSD) under the probability level of 0.05.

Results

The population of whitefly *B. tabaci* nymphs in the three varieties of eggplant: Kareema, Barcelona, and Zumorrod, from the beginning of April to the end of May, are reported in Figure 1. It is shown that three peaks of the whitefly *B. tabaci* population began to gradually increase to reach their first peak, and the average of whitefly nymphs was 45.4, 52 and 40 nymphs per leaf in the last week of April for the varieties of eggplant Barcelona, Zumorrod, and Kareema, respectively. While, the average population of whitefly nymphs was recorded to reach the second peak of whitefly nymphs density of 274.2, 381.6, and 148.4 nymphs per leaf in the second week of May for the three eggplant varieties Barcelona, Zumorrod, and Kareema, respectively. However, the average population density of whitefly *B. tabaci* nymphs was recorded at 300, 312 and 171.8 nymphs per leaf on the last week of May for Barcelona, Zumorrod, and Kareema, respectively. Statistically, there were significant differences between the three eggplant varieties in the severity of whitefly *B. tabaci* between these three varieties, the highest infestation of the whitefly in the nymph instar was on Zumorrod, followed by Barcelona, and then Kareema was the least.

The population density of the whitefly nymph on the two eggplant varieties, Barcelona and Zumorrod, was more sensitive to whitefly infestation than Kareema. The infestation of whitefly on different eggplant varieties may return to eggplant morphological characteristics.

The current study demonstrated that the whitefly, *B. tabaci*, feeding on host eggplant decreased the concentration of plant pigments, especially chlorophyll a, chlorophyll b, and total chlorophyll. Plant sap, which contains plant pigments like chlorophyll, can cause reduced in the plant colors of the sap when directly sucked. There was a significant difference in the content of both chlorophyll a and b between uninfested and infested eggplant leaves for all varieties. The range of both chlorophylls a and b was 9.75 and 4.19 mg/L for uninfested and 3.16 and 3.43 mg/L for infested leaves of Zumorrod variety, respectively. While in the Barcelona variety, the chlorophyll was recorded for both chlorophyll a and b at 8.68 and 4.07 mg/L for uninfested leaves and 2.91 and 2.03 mg/L for infested leaves with whitefly, respectively. Moreover, the variety of Kareema was 12.53 and 5.41 mg/L for the content of both chlorophylls a and b in the uninfested leaves, respectively. On the other hand, the concentration of chlorophylls a and b were 3.08 and 2.07 mg/L, respectively, in the infested leaves of Kareema (Table 1 and Figure 2).

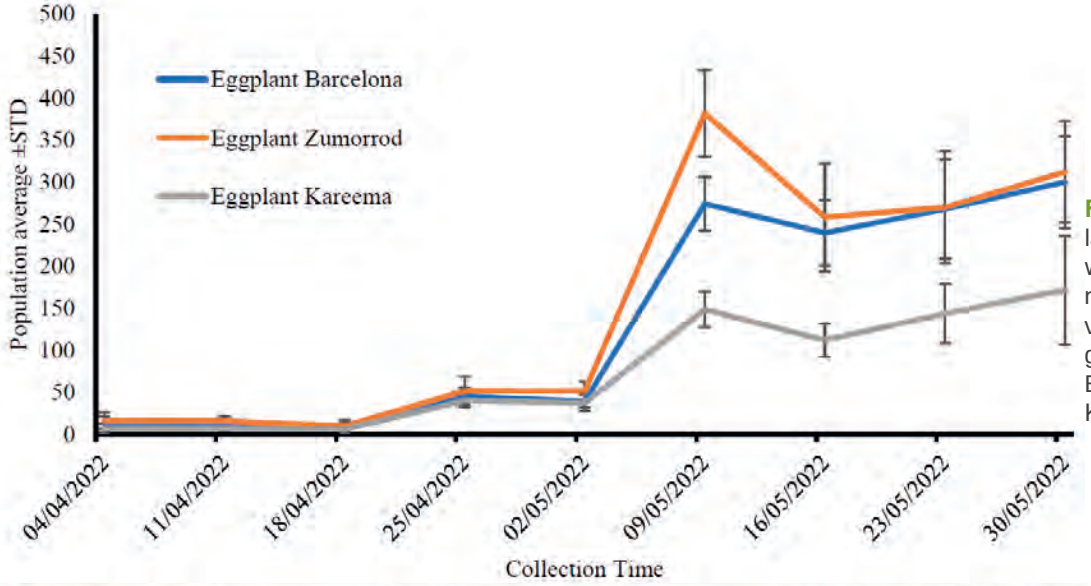

Figure 1. Population density of whitefly *B. tabaci* nymphs on three varieties of eggplant (Zumorrod, Barcelona, and Kareema).

Eggplant varieties	N [7]	Content (Mean ± STD [8] mg/L)			95% CI [9] of the Mean total chlorophyll	
		Chlorophyll a	Chlorophyll b	Total Chlorophyll	Lower Bound	Upper Bound
Zu-H [1,*]	3	9.75±0.80	4.19±0.25	13.95±1.04	11.36	16.53
Zu-I [2]	3	3.16±0.95	3.43±0.27	6.60±1.18	3.65	9.55
Ba-H [3,*]	3	8.67±2.85	4.07±1.40	12.74±4.19	2.33	23.15
Ba-I [4]	3	2.91±0.87	2.03±0.31	4.95±1.17	2.03	7.87
Ka-H [5,*]	3	12.53±0.89	5.41±0.42	17.49±0.66	16.29	19.59
Ka-I [6]	3	3.08±1.23	2.07±0.42	4.95±1.35	1.58	8.31

[1] Uninfested Zumorrod (Zu-H); [2] Infested Zumorrod (Zu-I); [3] Uninfested Barcelona (Ba-H); [4] Infested Barcelona (Ba-I); [5] Uninfested Kareema (Ka-H); [6] Infested Kareema (Ka-I); [7] Number of replicates (N); * referred to significant at the level of 0.05; [8] STD referred to the standard deviation; [9] CI referred to 95% confidence interval for the mean of total chlorophyll.

Table 1. The comparison between different uninfested and infested varieties of eggplants (Zumorrod, Barcelona, and Kareema) with whitefly *Bemisia tabaci* in chlorophyll content.

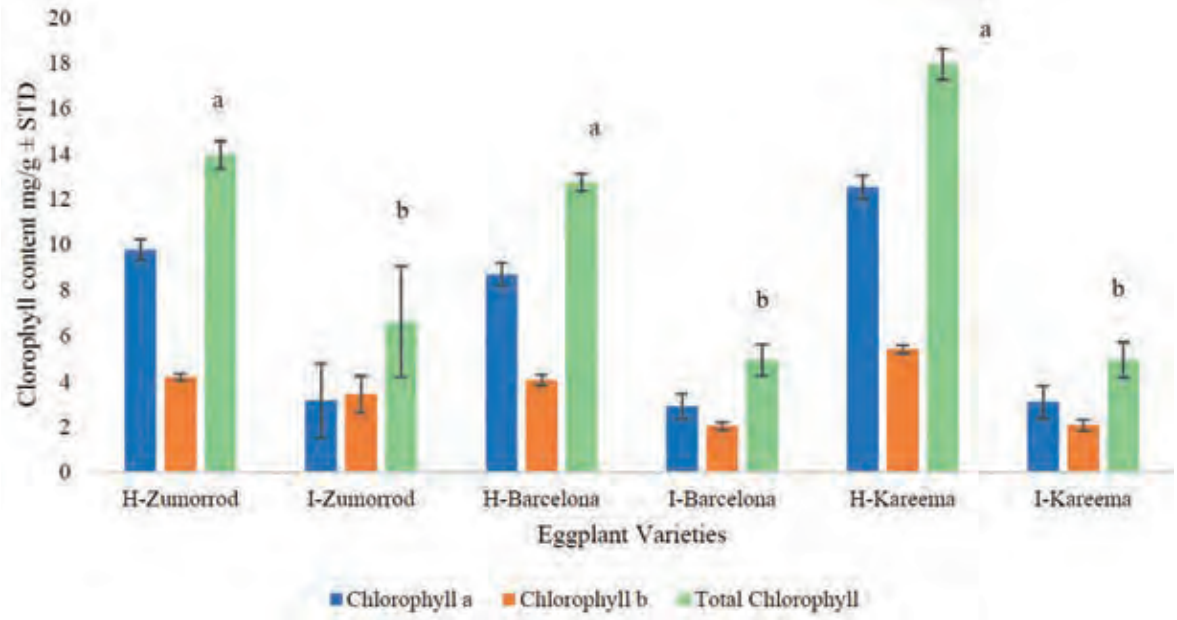

Figure 2. The concentration of chlorophyll content (mg/L) ± STD in various uninfested and infested eggplant varieties (Zumorrod, Barcelona, and Kareema) with whitefly *Bemisia tabaci*. Different litters mean significant differences at the level 0.05 for total chlorophyll.

The data on total chlorophyll content in different varieties of eggplant showed significant differences in the mean content of total chlorophyll between uninfested and infested leaves of different varieties of eggplant with whitefly *B. tabaci*. Table 1 also demonstrates that the mean content of total chlorophyll was more significant than infested leaves as shown in Zumorrod variety 13.95 and 6.60 mg/L for uninfested and infested leaves, respectively. At the same time, the total chlorophyll of the eggplant Barcelona variety was 12.74 and 4.95 mg/L in uninfested and infested eggplant, respectively. In addition, the array of Kareema was read at 17.49 mg/L of total chlorophyll in uninfested leaves compared with 4.95 mg/L in infested eggplant leaves Figure 2.

The values of chlorophyll a, b, and total chlorophyll decreased under the feeding stress of *B. tabaci*. The following means of chlorophyll content (Table 2) indicated that the reduction of chlorophyll content had significantly less chlorophyll a, b, and total chlorophyll in infested than uninfested eggplant leaves, as represented in Zumorrod variety chlorophyll a was 67.57%, chlorophyll b 18.12% and total chlorophyll 52.67%. The results showed that the reduction percentage of chlorophyll a, b and total chlorophyll in the Barcelona variety was 66.37, 50.12, and 61.14%, respectively. Similarly, the chlorophyll content values were 75.39, 61.66, and 72.39% in the Kareema variety. The whitefly *B. tabaci* infestation on different varieties of eggplant significantly reduces chlorophyll content compared with uninfested eggplant leaves (Figure 3).

Discussion

The preferences of whiteflies for several eggplant varieties (Zumorrod, Barcelona, and Kareema) were evaluated in this study. The investigation was conducted every week during the study time. The density of the whitefly nymph population on the two eggplant varieties, Barcelona and Zumorrod, was more susceptible to whitefly infestation than Kareema. A similar result was observed by (17) Proved that the two eggplant varieties, H149 and JSZ, were more preferred for feeding and egg-laying by whiteflies than other studied varieties, and adult whiteflies lived longer on these varieties. On the other hand, infection with *B. tabaci* reduces percentages in various growth parameters of eggplant, such as leaf area, fresh leaf weight, dry leaf weight, chlorophyll content, and rate of Photosynthesis[18]. This is consistent with our results, and significant differences were reported between uninfested leaves and the infested leaves of the three varieties (Zumorrod, Barcelona, and Kareema). According to our study findings, feeding by whitefly *B. tabaci* decreased the number of plant pigments such as chlorophyll a, b, and total chlorophyll. The reduced levels of chlorophyll may be because of sucking on plant sap containing plant pigments. Due to the lowering level of chlorophyll content as a result of *B.tabaci* feeding compatible with (19,20) who mentioned that the concentration of chlorophyll changes in the plant during its growth or as a result of external pressure, such as infestation with sup-sucking insects that feed on plants sap[21,23].

Eggplant varieties	% Reduction of chlorophyll content (mean±STD)			95% CI [1] of the Mean total chlorophyll	
	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Lower Bound	Upper Bound
Zumorrod	67.57±7.02	18.12±6.31	52.67±6.76	23.12	81.24
Barcelona	66.37±10.79	50.12±10.25	61.14±10.66	12.21	103.79
Kareema	75.39±5.25	61.66±4.25	72.39.3.99	55.37	89.61

[1] CI referred to a 95% confidence interval for the mean reduction percentage of total chlorophyll

Table 2. The percentage of chlorophyll reduction in different varieties of eggplant (Zumorrod, Barcelona, and Kareema) caused by whitefly *Bemisia tabaci*.

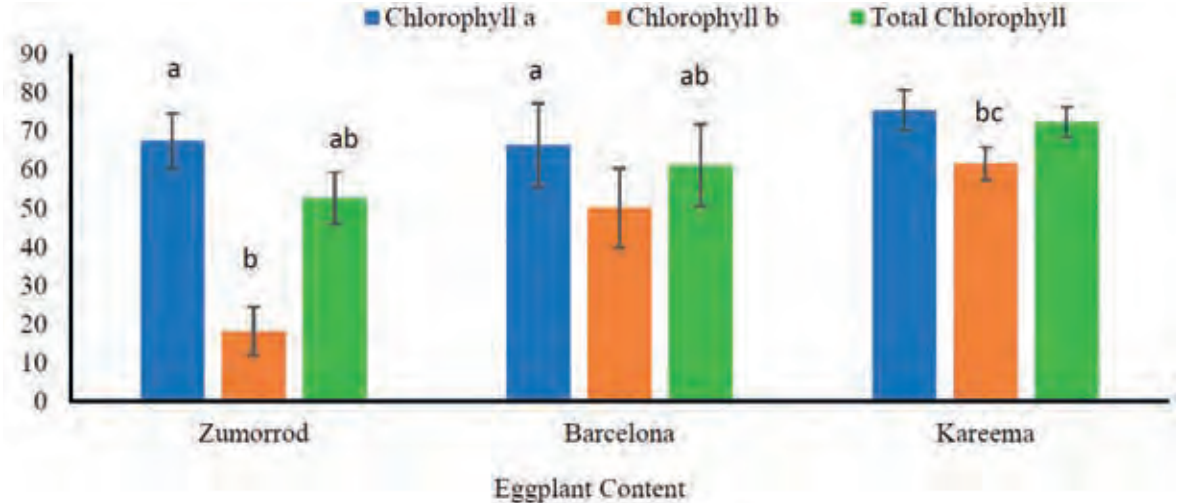

Figure 3. The percentage of chlorophyll reduction caused by the infestation of whitefly *B. tabaci*. Different litters mean there were significant differences at the level 0.05 for chlorophylls a, b and total chlorophyll.

Moreover, the feeding of *B. tabaci* caused a decrease of 9.7% and 65.9% in both chlorophyll content and photosynthesis rate, respectively, in eggplant leaves. Aside from reducing the photosynthesis rate, Shannag[22] pointed out that the infestation of *B. tabaci* also reduces the rate of water content by 32% more than uninfested plants. Because of the loss of chlorophyll, the injury may reduce photosynthesis, reducing the quantity and quality of eggplants. Chlorophyll can be reduced by sucking pests' infestations, and the chlorophyll concentration can change during the growth and development of the eggplant contents. Additionally, we found that uninfested eggplant plant chlorophyll content values were higher than those of infested plants. In eggplant plants, feeding activity by both nymphs and adults of whitefly *B. tabaci* reduces the amount of chlorophyll a, b, and total chlorophyll in the leaves. It has a detrimental effect on the rate of transpiration and photosynthesis.

Conclusions

From these results, we conclude that *B. tabaci* varies in the severity of infection between the three variants, the highest condition of the whitefly was in Zumorrod. Barcelona, and the least was Kareema. This injury accompanies by a significant reduction in chlorophyll concentration in the infected leaves compared with uninfested leaves. Furthermore, a significant decrease in chlorophyll concentration in the infected leaves compared with uninfested leaves; a reduction in leaf photosynthesis leads to lower production.

Author Contributions

Conceptualization, Q.A.; methodology, A.R., Q.A. and M.A.; validation, A.R., Q.A. and M.A.; formal analysis, Q.A.; investigation, A.R. and Q.A.; resources, Q.A.; data curation, A.R., Q.A. and M.A.; writing—original draft preparation, A.R. and Q.A.; writing—review and editing, A.R., Q.A. and M.A.; visualization, A.R., Q.A. and M.A.; supervision, Q.A.; project administration, Q.A. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Acknowledgments

We thank the University of Baghdad for its research support.

Conflicts of Interest

The authors declare no conflict of interest.

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ARTICLE / INVESTIGACIÓN

Study of genetic analysis of individual hybrids derived by the half-diallel cross of some *Lycopersicon esculantum* mill

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DOI. 10.21931/RB/2022.07.04.44

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Abstract: The study was conducted for the first season of 2020 in the field of the College of Agriculture \ Diyala University which the seeds of the first generation F1 hybrids were produced for six strains that were selected according to the genetic distance among them and resulted in 15 hybrids by applying for the half diallel crossing program. In the second year, the seeds of individual hybrids were planted with their parents and a hybrid Commercial common boob cat for comparison within a field experiment to evaluate the field performance of these produced hybrids according to the design of complete randomized sectors and with three replications. The differences were tested according to the LSD test. The result showed that the highest hybrid strength compared with the highest parents for the trait of plant height 43.34 cm, number of fruits 48.20%, plant yield 77.27%, TSS ratio 41.83%, fruit content of vitamin C 33% and sugar content 25.86% for the above traits, respectively. As for the common commercial hybrid, Boob cat was 64.20%, 121.23%, 43.92%, 55%, 109.36% and 46.65%, respectively. As for the union ability, the father gave c.c. Orang (5) had the highest general combined ability for plant height which amounted to 3.972. For the number of fruits, plant yield and TSS, Father Rose (1) showed the highest general union ability, which amounted to 4.136, 0.358 and 0.095, respectively, while the vitamin C and sugars ratio were recorded by the fathers (5) Red P.t (2) and Amish Pa.(4). The highest general federating ability amounted to 1.937 and 0.975 respectively while the highest union ability for plant height in 1×5 hybrids reached 33.375 and for TSS and sugars the hybrid showed1×3The highest ability of a particular union was 1.60 and 1.77, respectively. Regarding the characteristics of fruit number and yield of a plant, the 1×2 hybrid was superior in a proportion of 16.71 and 1.11 for vitamin C, and the 3×4 hybrid was superior in a ratio of 8.06. As for the percentage of heritability was high compared to the strict sense, indicating the importance of non-host gene action of genes in the inheritance and manifestation of the trait. The mean of the degree of dominance was higher than one for all the mentioned traits, which evidences the participation of super dominance of genes in the manifestation of the trait.

Key words: Half-diallel, boob cat, genetic distance, *Lycopersicon esculantum*.

Introduction

The tomato crop (*Lycopersicon esculantum* mill) family Solanaceae is one of the world's most essential and widespread vegetable crops because of its excellent nutritional value. The tomato contains calcium, iron, and vitamins A, B2, B6 and C. It also contains proteins, carbohydrates, amino acids and some pigments such as carotene. Lycopene and some phenolic compounds[1]. It grows in various environments, from dry regions to wet areas, as well as in different soils according to its original habitat. And genetic and even at the plant molecular level[2]. Because of the low production per unit area in the world and Iraq in particular, it has become imperative for plant breeders to search for means through which production can be increased and improved in quantity and quality, Which forms the basis of any crossbreeding program that aims to develop hybrid varieties as the genetic action of any trait is related to the amount of genetic variation and the ability to combine between the parental strains, and this is one of the necessary steps in developing and improving crops including the tomato crop. By testing them, the best genetically heterogeneous strains or varieties to obtain the phenomenon of hybrid vigor to produce hybrids that outperform the widely cultivated varieties in one or more traits[3-5].

Cross-crossing is one of the most efficient breeding methods for selecting hybrids produced in the early stages or in later generations in breeding programs based on knowledge of the type of gene action that controls the inheritance of the trait[6].

This mating design has been standard in many plant species regardless of whether self-pollinating or cross-pollinating, as it satisfies the breeder's desire to obtain distinctive hybrids by identifying the best parents familiar with each other. There are three mating designs in breeding studies: the plant is the diallel, partial dialed, and line x tester.

The phenomenon of heterosis is one of the most critical agricultural phenomena that contributed to the development of agrarian production quantitatively and qualitatively. The phenomenon of hybrid vigor was first diagnosed by (7,8). The first scientists who noticed the strength of the hybrid in tomatoes were Hedrick, and Booth[9] was represented by an increase in yield and the number of fruits and then expanded to include yield components and qualitative characteristics[10], and between Al-Shammari and Hamdi[11] Showed that the genetic divergence between the parents is a helpful indicator of the performance of the crosses. Heterozygosity is more influential than homozygosity, and many studies have confir-

Citation: Al-Shammari A M A, Al- Obaiday A R H. Study of genetic analysis of individual hybrids derived by the half-diallel cross of some Lycopersicon esculantum mill. Revis Bionat a 2022;7(4) 44. http://dx.doi.org/10.21931/RB/2022.07.04.44
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

med a positive association between the genetic divergence of parents and the high performance of the crosses[11-14].

To derive individual hybrids of tomato by cross-crossing and evaluate them in comparison with one of the hybrids approved in the environmental conditions of the region and the possibility of adopting it locally and estimating the genetic parameters and the strength of the hybrid and then choosing the best hybrids produced in quantity and quality that are well cultivated under the environmental conditions of the region and the possibility of adopting it locally.

Materials and methods

First season: production of individual hybrids (F1)

The seeds of these strains were planted inside cork dishes in one of the nurseries located within the Khan Bani Saad district of Baquba Center on 13/1/2020. After the seeds germinated and the plants had reached the seedling stage, they were transferred and planted in the open field at the research station of the Department of Horticulture and Landscaping for the College of Agriculture, Diyala University within the first season on 10/3/2020, All crop service work was carried out including watering, irrigation, fertilizing and removing the grasses as needed for producing unique hybrids (F1).

During this season, half dialele was conducted among these six strains according to the first method and the fixed model of Griffing[15] methods for the production of the first generation F1 hybrids, as shown in (Table 2) and resulted in 15 hybrids. The color of the flowers is cream. The bag was sacked after castration. The rest of the flowers were removed from the plant to ensure no cross-pollination.

Studied traits

The measurements were taken from a random sample of five plants in the experimental unit, and for each replicate, the average was taken. The studied traits included the following:

Plant height (cm): It was measured at the end of the growing season from the surface of the soil to the top of the plant for five plants within the experimental unit, and the average was taken.

The number of fruits per plant (fruit plant^{-1}).

The number of fruits in the experimental unit was calculated cumulatively from the beginning of the harvest until the end of the growing season and divided by the number of plants in the experimental unit.

Plant yield (kg plant^{-1})

The cumulative yield was recorded from the beginning of harvest to the last pound for each experimental unit and then divided by the number of plants in one experimental unit.

The percentage of total dissolved solids (TSS %) was calculated using a Hand Refractometer.

The content of fruits of vitamin C (mg 100 g^{-1}): It was estimated by smearing a specific volume of tomato juice with 2.6 Dichlorophenol indophenol dye and based on the mg unit of vitamin C per 100 g of fruit juice[16].

Estimation of total sugars in juice (%)

It was estimated by taking 1 ml of diluted juice and adding to it 1 ml of 5% phenol solution (5 g of phenol in 100 ml of distilled water) and 5 ml of concentrated sulfuric acid with continuous shaking; then the light absorption was read by a spectrophotometer at a wavelength of 550 nm And to calculate the sugar content of fruits by dropping the readings on the standard curve prepared using glucose.

Statistical analysis

The standard statistical analysis was carried out according to the design of the entire random sectors with three replications, and data were entered (parents, single crosses, triple crosses and common commercial Bobcat crosses), and the averages were compared using the least significant difference (LSD) at the probability level of 5% and in the case of substantial differences between the treatments for the studied traits. The analysis is continued using the statistical method of this mating method by splitting the averages of the squares of the transactions into their components after collecting data for all the studied traits and populating using the Excel program; then, the data were statistically analyzed using the SAS program, and the data were compared according to the Tukey test at the probability level of 0.05, as the test was conducted regardless of the significance of F[17].

Hybrid vigor of individual hybrids was estimated based on.

units	characteristics	Valuable soil		units	characteristics	Valuable soil	
g kg^{-1}	6.8	Organic matter		ds	6.5	Electrical $EC_{1:1}$conductivity	
	230	Caco3		--	7.55	pH1:1	
	250	Sand	Soil	mg kg^{-1}	53.0	N	Ready-made
	450	silt	Separ		39.0	P	Elements
	300	Clay	ators		240.0	K	
%	25	Weight moisture field capacity		Mg cm-3	1.36	Bulk density for depth 0.3-3m	
				Silty loam	Soil texture		

Table 1. Some physical and chemical properties of field soil.

Breed number	Breed name	its source
1	Rose	Genetic Resource Center at the University of California, Davis, USA
2	Red P.t	
3	Nepal	
4	Amish Pa	
5	C. C. Orange	
6	T. 115	

Table 2. Breeds used in crossbreeding and their source.

Parents	Rose	Red P.t	Nepal	Amish Pa	c.c. Orang	T. 115
Rose	1	X	X	X	X	X
Red P..t		2	X	X	X	X
Nepal			3	X	X	X
Amish Pa.				4	X	X
c.c. Orang					5	X
T. 115						6

Table 3. Individual crosses are derived from pure strains according to the second Griffing method (1956).

Results and discussion

Firstly: The mean deviation of the first generation $\bar{F}1(i)$ from the best parents BP according to the following equation:

The results of Table 4 show the strength of the hybrid based on the best parents and the standard hybrid for the

$$H_{BP} = \bar{F}_{1(i)} - BP$$

Secondly. The mean deviation of the first generation $\bar{F}1(i)$ from the ordinary standard hybrid Boobcat according to the following equation

$$H_{CP} = \bar{F}_{1(i)} - C\bar{P}$$

$C\bar{P}$ = Commercial Hybrid

The degree of inheritance in the narrow and broad sense was estimated according to the following equation

$$h^2_{bs} = \frac{\sigma^2_G}{\sigma^2_P}$$

$$h^2_{ns} = \frac{\sigma^2_A}{\sigma^2_P}$$

Since:

h2bs: degree of inheritance broadly

h2ns: the degree of heritability in its narrow (limited) sense

σ2 A: the portion of the genetic variance due to the additional verb

σ2 G: genetic variance

σ2 P: phenotypic variance

The dominance degree rate was estimated from the following equations:

$$a = \sqrt{\frac{2\,\lceil^2 D}{\lceil^2 A}} = \sqrt{\frac{8\,\lceil^2 Sca}{4\,\lceil^2 gca}} = \sqrt{\frac{2\,\lceil^2 Sca}{\lceil^2 gca}}$$

From the values of a, we infer the following:

If a is zero, this indicates that there is no sovereignty

If it is zero > a > one, this shows a partial dominance

If a is equal to one, this suggests that there is complete dominance

If a < 1, this indicates that there is a super-dominance

studied traits. Most hybrids showed positive and positive hybrid vigor in the desired direction for the plant height trait. The highest was the hybrid 2×6 which scored 43.34 compared with the best parents. Compared with the commercial hybrid, the hybrid 1×5 showed the highest desirable hybrid vigor of 64.20% for the number of fruits. The hybrid 3×6 showed the highest rate of 48.20% compared with the highest parent, while the hybrid 1×2 recorded the highest hybrid strength compared with the standard hybrid, which amounted to 121.23%. It reached 77.27%, while the hybrid 1×2 recorded the highest significant and desirable hybrid strength compared with the standard hybrid, which amounted to 43.92%. As for TSS, the hybrid 1×3 showed positive and significant hybrid strength compared with the best parents, with a percentage of 41.83% compared to the commercial hybrid. The above hybrid had the highest hybrid strength of 55%. The highest hybrid strength distinguished the 2×5 hybrid compared to the best parents for vitamin C, which amounted to 33%. The hybrid 1×2 showed the highest significant and positive hybrid strength of 109.36% compared with the standard hybrid. As for sugars, the hybrid 1×3 was distinguished by the best hybrid strength compared to the highest hybrid, which amounted to 25.86%, while the hybrid 4×5 recorded the highest hybrid strength compared with the commercial hybrid 46.65%. The difference of hybrids in the above traits is mainly due to the variation of their genetic composition, which in turn affects the physiological ability and its efficiency in converting the products of photosynthesis in favor of the growth and elongation of stem cells and then the increase in plant height and number of branches which is reflected on other vegetative growth indicators. In other words, the trait Vegetativeness is mainly determined by genetic factors and shares with them the response of hybrids to the influence of environmental factors affecting their growth, including agricultural service operations[5,14,18,19].

As shown in Table (5) the results of the statistical genetic analysis and the splitting of the mean squares of the genotypes into components that belong to the general and specific abilities of the union according to the second method of the first model (Fixed Model) proposed by Griffing (1956).

The analysis of variance of the genotypes data (parents + individual hybrids + common commercial Bobcat hybrid) for all traits shows that the mean of squares was significant for all the traits that were under study. The general ability to combine was highly significant for most traits except for TSS, which did not reach the significance limit as the results presented in Table 5 showed a highly effective effect of the mean squares of genotypes and their interactions for all studied traits and identifying the nature of the work of the genes that control the inheritance of the studied traits. These results are consistent with what was found by a group of researchers including[14,20-22].

adjectives	plant height		number of fruits		plant yield		TSS		VC		sugars	
genetics	BPH	SCH	BPH	SCH	BPH	SCH	BPH	SCH	SCH	BPH	SCH	BPH
2×1	2.20	8.17	51	121.23	38	43.92	13.77	35.71	109.36	16.90	23.50	11.38
3×1	4.73	29.18	-3	42	3	7.94	41.83	55.00	77.02	-1.15	39.55	25.86
4×1	27.57	35	-3.92	40.70	23	28.29	1.34	7.86	42.49	-20.43	36.92	4.48
5×1	39.73	64.20	-34.44	-3.98	-9.52	-5.71	26.95	27.86	89.18	5.63	22.27	10.28
6×1	0	5.83	-22.35	13.71	6.42	10.92	-3.68	12.14	79.21	0	27.80	-1
3×2	-4.73	17.50	-7.37	-11	-31.5	-32.01	-7.78	10.00	103.04	22	25.78	14.96
4×2	23.34	23.34	-0.68	28.31	9	10.42	1.19	20.71	37.14	-12.69	44.72	10.43
5×2	1.98	19.84	35.34	38.93	27	26.05	-2.39	16.43	108.92	33	29.11	22.96
6×2	43.34	46.69	-19.52	-10.61	3.75	2.98	-1.19	17.86	99.64	18.98	9.65	-15
4×3	-4.73	17.50	-14.72	10.17	-17.48	-16.87	10.45	20.71	102.80	21.93	32.01	0.73
5×3	-14.19	5.83	4.74	7.524	24.16	-8.19	2.61	12.14	20.60	-27.48	22.27	11.75
6×3	14.19	40.85	48.20	64.60	77.27	35.48	-1.84	14.29	23.02	-26.67	40.51	8.82
5×4	7.94	26.84	-21.91	0.88	10.83	11.66	25.50	33.57	51.00	6.97	46.65	11.91
6×4	-2.28	0	-10.27	15.92	-2.95	-2.23	1.22	17.86	86.26	11	31.04	0
6×5	-1.98	15.17	-16.73	-7.52	66.55	27.30	0	16.43	81.40	8.11	35.95	5.29

Table 4. Shows the strength of the hybrid (%) based on the deviation of the first generation from the highest parent and the commercial hybrid (Bobcat).

SOV	replication	genotype	parents	crosses	P V c	gca	aca	error	Total
df	2	20	5	14	1	5	15	40	82
plant height	**213.06	**711.17	**356.88	**666.28	**311.11	**88.85	**286.46	0.39	28889.1
number of fruits	85.2**	139.6**	65.3**	167.6**	118.9**	52.2**	44.6**	1.2	5804.58
plant yield	17**	2.19**	1.07**	2.07**	9.53**	0.58**	0.78**	1.14	120.0055
TSS	**12.35	1.02**	0.41**	0.99**	4.40**	0.06	0.43**	0.16	72.752
Vitamin C	94.2**	75.7**	35.6**	86.5**	125.1**	20.5**	26.7**	0.03	3219.37
sugars	**2.78	**6.48	**6.06	**3.79	**46.27	**2.48	**2.05	0.22	273.857

Table 5. Results of the analysis of variance for the genotypes (parents + individual hybrids + common commercial Bobcat hybrid), fathers and individual crosses for the studied traits.

Parents	Rose	Red P.t	Nepal	Amish Pa.	c.c. Orang	T.115	*SE*
plant height	-1.777*	3.972*	-2.902*	2.972*	-4.027*	1.763*	0.117
number of fruits	-1.418*	-3.363*	-0.163*	-0.568*	1.377*	4.136*	0.207
plant yield	0.023*	-0.126*	0.051*	-0.445*	0.137*	0.358*	1.99
TSS	-0.070*	-0.1*	-0.058*	0.041*	0.091*	0.095*	0.075
Vitamin C	0.490*	-1.775*	-1.729*	-0.499*	1.937*	1.575*	0.033
sugars	0.259*	-0.357*	0.975*	-0.086*	-0.602*	-0.188*	0.087

Table 6. Estimating the effect of the general ability of the parents to combine on the indicators of vegetative growth, flowering and yield of tomato plants.

Table (6) shows the effect of the general ability to combine for all the traits under study that were estimated and their results are shown in the above table in which it is noted that Father 1 showed a general desirable and significant ability to combine for the characteristic of plant height, number of fruits, yield of one plant, TSS and vitamin C,. As for Father 2. It showed a desirable and moral effect of the general ability for the characteristics of the number of fruits, plant yield, TSS and vitamin C to combine, while Father 3 showed a broad ability of positive and significant for the trait of plant height and TSS to combine and father 4 showed a general effect on the combination significantly and in the desired direction for the trait of the yield of the plant only and the percentage of sugars. Father 5 was characterized by a general ability of significance and the desired direction for the characteristic of plant height only to combine. At the same time, father 6 showed a generally desirable and important combining ability for vitamin C traits.

Table (7) shows the effect of the special ability on the union of individual hybrids for all the studied traits, and it is noted that the plants shown by the parents due to the variation in the general ability to combine have an effect on the behavior of the resulting hybrids through the crossing tip between them. The height of the plant 9 crosses showed the power to destroy, especially for the significant and positive union. The highest reached 33.37 for the hybrid 1×5. For the number of fruits, 4 of the hybrids achieved a positive and significant unique ability to destroy the difference in the special ability to combine, reached a maximum of 16.71 for the hybrid 1×2, and 9 genotypes for the trait of the plant yield showed a special ability to combine positive and significant in the direction.

The desired one was the best of the hybrid 3×6 scoring 1.73 while 4 crosses were distinguished for TSS and showed positive and desirable values that hare special ability to combine. The maximum was the hybrid 1×3, scoring 1.60.

As for the content of the fruits of vitamin C, 10 crosses showed the highest positive and significant special to combine ability in the desirable direction. The highest hybrid was 3×4, which scored 8.06. For the percentage of sugars, 9 combinations were distinguished. They gave positive, significant and desirable values for the unique ability to combine, which reached the highest rate in the 1×3 hybrid, which scored 1.77.

Table (8) shows the components of the phenotypic variance for the traits studied, and we note that the environmental conflict differed from zero for all the traits studied. In contrast, the additional genetic variance differed from zero for the characteristics under study except for the TSS percentage while the sovereign genetic variance differed from zero.

For all studied traits, the values of dominance genetic variance were greater than those of additional genetic variance. The table above shows that the importance of gene variance is greater than the environmental variance values for all the studied traits except for TSS. Accordingly, these traits can be genetically improved. These results agree with what was found by (14,23,24) in their studies on tomato plants. Shown in the table (8) are the values of heritability, the rate of the degree of dominance and the expected genetic improvement of the traits studied; it is noted that the importance of heritability in the broad sense was high and close in all the traits studied, while the values of heritability in the narrow mind for all the traits studied for TSS were low.

Conclusions

The low values heritability in the narrow sense has appeared in the characteristics of plant height. TSS this decrease is due to the decline in the value of additional genetic variance and the increase in the value of the dominant genetic variance. As for the average degree of dominance, it was more significant than one for all the traits under study, which indicates the presence of super-dominance at some genetic sites that control the inheritance of all the traits studied. These results are consistent with what was found[25].

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adjectives	plant height	number of fruits	plant yield	TSS	VC	sugars
genetics	-6.625*	16.71*	1.11*	0.65*	3.45*	0.45*
2×1	4.375*	0.75*	0.24*	1.60*	0.32*	1.77*
3×1	15.25*	0.05	0.57*	-0.49*	-4.39*	0.40*
4×1	33.375*	-6.84*	-0.35*	0.48*	3.69*	0.07
5×1	-10.875*	-4.79*	-9.09*	-0.28*	-0.28*	0.08
6×1	0.166*	0.18	-1.10*	-0.49*	4.44*	0.61*
3×2	11.041*	0.01	0.11*	0.10	-5.68*	0.71*
4×2	1.166*	5.61*	0.88*	-0.04	6.73*	1.26*
5×2	29.916*	-4.86*	-0.16*	-0.01	2.86*	-1.57*
6×2	-0.958*	-2.14*	-0.40*	0.15	8.06*	-0.25*
4×3	-17.833*	0.45	0.08*	-0.19	-6.04*	-0.03
5×3	17.916*	11.41*	1.73*	-0.12	-7.89*	1.43*
6×3	6.041*	-1.44*	0.38*	0.90*	0.42*	1.68*
5×4	-11.208*	0.00	-0.28*	0.13	4.23*	-0.70*
6×4	-5.083*	-2.09*	1.04*	0.11	3.43*	1.18*
6×5	0.322	0.57	0.05	0.20	0.09	0.24

Table 7. Estimates of the effects of special ability on the union of individual crosses for all studied traits.

Variations Adjectives	$\sigma^2 E$	$\sigma^2 A$	$\sigma^2 D$	$h^2_{n.s}$	$h^2_{b.s}$	ā	GA	GA %
plant height	0.13* ± 0.03	7.393* ± 4	95.443* ± 32.75	0.071	0.998	5.081	1.282	1.260
number of fruits	0.41* ± 0.08	2.073* ± 1.12	14.736* ± 5.10	0.120	0.976	3.770	0.879	3.229
plant yield	0.004* ± 0.0008	0.050* ± 0.02	0.252* ± 0.08	0.164	0.986	3.159	0.160	3.859
TSS	0.05* ± 0.01	0.000 ± 0.002	0.126* ± 0.04	0.014	0.718	17.435	0.003	0.063
Vitamin C	0.01* ± 0.002	1.715* ± 0.91	8.926* ± 3.06	0.161	0.999	3.226	0.924	3.178
sugars	0.07* ± 0.01	0.200* ± 0.01	0.66* ± 0.23	0.215	0.924	2.563	0.366	2.540

Table 8. Estimates of the components of phenotypic variance, standard error, heritability, mean degree of dominance, and expected genetic improvement for the studied traits.

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ARTICLE / INVESTIGACIÓN

Response of some rice cultivars (*Oryza sativa* L.) to spraying with a natural honey solution and effect on growth and yield characteristics

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Abstract: The field experiment for the current study was carried out at the rice research station in Najaf governorate, affiliated with the Iraqi Ministry of Agriculture. To know the response of three cultivars of rice to spraying with the natural honey solution at different. The factorial experiment was carried out using a randomized complete block design with three replications. The results showed the significant effect of the various study factors with their interactions on the characteristics of the present study, as the honey solution spray treatment at 6 g. l-1 was superior to cultivar Ambar 33 in plant height and number of panicle branches (same as cultivar Mashkhab 1 with the same concentration). The number of grains in panicle, panicle length, 1000 grain weight and fertility percentage (same as cultivar Jasmine with the same concentration) achieved 67.7%, 88.3%, 104.5%, 52.7%, 21.3% and 148% increase for the traits, respectively compared to control treatment. As for the result, the spray treatment of cultivar Jasmine with a concentration of 6 g.l-1 with the natural honey solution was superior in giving the highest grain yield with an increase of 45.6%.

Key words: Rice cultivars, spraying, honey, growth, yield.

Introduction

Rice (*Oryza sativa* L.) is one of the most important crops (Poaceae) that is entered as a staple food in most countries of the world, especially in developing countries, as more than half of the world's population feeds mainly on this crop, and 20 species belonging to the genus (oryza) have been identified. However, the cultivated species among these species is (sativa) and is cultivated in more than 100 countries in the world, and its cultivation extends between latitudes 50 north and 40 south[1]. The global production of this crop reached 762.8 million tons in 2018, for a cultivated area of 165.5 million hectares, with a productivity rate of 4.6 tons. hectares^{-1}, and in the Arab world, production reached 5.6 million tons for a cultivated area of 524 thousand hectares, with a productivity rate of 3.7 tons. ha^{-1}. While in Iraq, rice production for the same year amounted to 182 thousand tons for a cultivated area of this crop of 54 thousand hectares, with a production rate of 3.4 (tons. ha^{-1})[2]. If local productivity is compared with global or Arab productivity, Iraq's productivity rate is considered low for this crop. This decline may be due to many reasons, including primitive agricultural methods and a lack of interest in crop service operations through the introduction of modern technologies in agriculture, fertilization, pest control and others. Also, it is one of the negative ways that local farmers use by alternating rice with wheat, which is reflected in a decrease in soil fertility and, thus, a reduction in the yield[3]. The quantity required to meet the global need for rice was estimated from the beginning of the second millennium, so it was necessary to increase the production rate of this crop by 50% until 2025[4].

Hence, attention must be paid to this crop, as wide varieties were developed, their characteristics were studied under different environmental conditions, and the best field practices were determined for them with one goal, which is to reach the maximum possible productivity because the productive capacity of any variety, whatever it is, depends on the operations of serving the crop[5].

One of the goals that man has sought in recent times is the optimal use of natural resources, the use of raw materials of a pure environmental nature, and the avoidance of various environmental pollutants that enter into production, especially agricultural, and man has used honey since ancient times in various fields in addition to nutrition, as it was used in treatments for sick cases. Different as well as in the food industry. Bees produce honey from the nectar of plants and the secretions of nectar-sucking insects (bees). From a nutritional point of view, natural honey is a source of the essential natural macro and micronutrients, which consists of a saturated solution of sugars, mainly fructose and glucose, as well as a wide range of secondary components[6,7]. It was found that fructose had the highest amount among the sugars contained in honey, followed by glucose and sucrose, in addition to many vitamins, minerals, proteins, enzymes, flavonoids and phenolic acids[8].

In view of the importance of the rice plant and its role in securing food for most countries of the world, as mentioned above, and to know the effect of the honey solution on some growth characteristics and the impact of some varieties of this plant, this study was conducted.

Citation: Jabbar A F; Alawadi H F. Ali, EAHM.Response of some rice cultivars (Oryza sativa L.) to spraying with a natural honey solution and effect on growth and yield characteristics. Revis Bionat a 2022;7(4) 45. http://dx.doi.org/10.21931/RB/2022.07.04.45
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

Materials and methods

The current study was carried out at the rice research station in Najaf governorate affiliated with the Iraqi Ministry of Agriculture to know the response of some rice varieties to spraying using different concentrations of honey solution and their effect on some growth characteristics and yield. The experimental land was prepared by performing soil service operations such as plowing, smoothing and dividing before planting. Samples were taken from the field soil with a depth of 0-30 cm, and some physical and chemical properties were measured, as shown in Table (1). Twelve experimental units with dimensions of 3 x 2 m^2 for one experimental unit and a distance between blocks 1.5m, and the seeds of the varieties (Amber 33, Jasmine and Mashkhab 2) were planted inside the experimental units in lines by the wet method, the process of weed control and the crop service operations were completed as fertilization, irrigation, etc., according to the approved recommendations. The plants were sprayed with different concentrations of honey solution (0, 2, 4 and 6) g.l-1 by five sprays (the first spray after 50 days of planting and between one spray and another for two weeks), as eucalyptus honey was used, which proved some of the measured qualities of this honey in Table (2). The treatments were sprayed using a 20-liter dorsal sprinkler that was calibrated before applying the treatments, taking into account that there was no overlap between the treatments when spraying using a cardboard (carton), and the used sprayer was washed well after each use.

The characteristics were studied

After completing the experiment, the following characteristics were studied:

Plant height (cm)

According to the size of the plant, when the maturity was complete from the surface of the soil to the end of the panicle as the average of a sample of (10) plants.

Number of branches carrying panicle (branch per m^2)

The average number of branches carrying the panicle per square meter was calculated for each treatment.

Number of grains in a panicle (grains per panicle)

The number of grains in the panicle was calculated as the average of a sample of (10) plants at harvest.

Panicle length (cm)

The panicle length was measured from the beginning of the panicle (at the last node of the branch) to the end of the panicle and it was calculated as an average of a sample of (10) plants.

Weigh 1000 grain (g)

1000 grains of each sample were counted for each of the different treatments and were weighed by a sensitive scale after maturation and were calculated as an average of the sample consisting of (10) plants.

Grains yield ($t.ha^{-1}$)

The yield was calculated after harvesting of a square meter of each experimental unit, the weight of the grains per meter was taken then it was converted to tons per hectare[9].

Fertility percentage (%)

The fertility ratio was calculated by the following equation[10]:

After recording all the data, the results were compiled

$$\text{fertility percentage} = \frac{\text{full seeds no.}}{\textit{total seeds no.}} \times 100$$

and analyzed using the statistical program Genstat, and the averages were compared using the least significant difference (LSD) test at a probability level of 0.05.

Results and discussion

Effect of cultivars on the growth and yield of rice plants

The results in Table (3) show that the difference of cultivars had a significant effect on the growth characteristics and yield that were measured in this study, as the Amber 33 variety was superior in giving the highest average of plant height, number of grains in the panicle, the length of the panicle and the weight of 1000 grain, which amounted to

Measured adjective		The value	Measuring unit
EC 1:1		7.6	$dS.m^{-1}$
pH 1:1		2.4	----
N		23	$Mg.kg^{-1}$ soil
P		12.3	
K		45.1	
O M		11.3	$g.kg^{-1}$
Soil Separators	sand	140	$gm\ kg^{-1}$ soil
	silt	560	
	mud	300	
Tissue class		clay mixture	----

Table 1. Analysis of the physical and chemical properties of field soils.

Measuring adjective	Value	Measuring unit
pH _ _	4.22	-------
Hydroxymethyl	5.67	g.100g $^{-1}$
Total Sugar	63.51	g.100g $^{-1}$
Hydroxy methyl furfural	16.05	Mg.kg $^{-1}$
Proline	192.65	Mg.kg $^{-1}$
Protein	0.108	Mg.kg $^{-1}$
Invert Sugar	31.29	g.100g $^{-1}$

Table 2. Some measured characteristics of the honey used for the experiment (eucalyptus honey).

Cultivars	Plant height cm	Number of branches carrying panicle (branch per m^2)	Number of grains in a panicle (grains per panicle)	Panicle length (cm)	Weigh 1000 grains (g)	Grains yield (t.ha^{-1})	Fertility percentage (%)
Amber33	118.47	286.83	479.66	52.66	20.05	4.158	11.35
Jasmine	76.33	357.33	410.41	36.41	18.72	4.624	12.17
Mashkhab1	68.66	318.83	401.25	37.66	18.33	2.944	8.21
LSD 0.05	2.47	18.19	19.85	1.46	0.25	0.128	1.41

Table 3. Effect of cultivars on growth characteristics and yield of rice.

118.47 cm, 479.66 grain .panicle^{-1} and 52.66 cm and 20.05 g for the traits preceding the succession, so the percentage increase was 72.5%, 19.5%, 39.8%, and 9.4% for the traits respectively compared to the Mashkab[1] cultivar, followed by the cultivar Jasmine, which recorded the highest average number of branches bearing panicle, the percentage of fertilization and grain yield, which amounted to 357.33 Branch per m^2 and 12.17 % and 4.624 t.ha^{-1} respectively, achieving an increase in the average of the traits by 24.6%, 48.2% and 57.1% for the above traits respectively. While the cultivar Miskhab1 recorded the lowest average for most of the traits under study compared to the rest of the cultivars. The variation in the response of the studied traits may be due to the genetic factor of each variety, which is reflected in the plant's behavior towards standards and growth factors, as one of the most important factors that determine the behavior of living organisms is the genetic factor in which the response to different growth factors varies. Several studies have confirmed the cultivar factor. It had a significant effect on the plant response and its reflection in the most important traits, such as yield. (11) indicated that the cultivars significantly affected the studied traits and varied in the various characteristics. Anbar 33 cultivar gave the highest values for plant height compared to the rest of the cultivars in the study. (12) also mentioned that there is a significant effect of cultivars that was reflected in the most critical studied characteristics, including plant height, fertility rate, dry weight, number of panicles, number of grains in panicle, grain weight and grain yield, and attributed this to the influence of the genetic factor on the behavior of the plant and its response to various growth factors.

Effect of spraying with different levels of honey solution on growth characteristics and yield of rice

The results in Table (4) show that there is a significant effect of the levels of spraying with natural honey solution, as the spraying treatment with concentration (6 g.l^{-1}) achieved the highest averages for all growth traits and the result in this study, it reached 96.66 cm, 415.66 branches per m^2, 545.11 grains per panicle, 46.56 cm, 20.32 g and 4.335 t.ha^{-1} 14.53% for plant height, number of branches carrying panicle, number of grains in panicle, panicle length, the weight of 1000 grain, grains yield and fertility percentage respectively, achieving an increase of 25.4%, 84.6%, 79.3%, 24.7%, 14.0%, 24.9% and 119.2% for the traits respectively, compared to the control treatment, which recorded the lowest averages for all the traits under study. This was followed by a concentrated spray treatment (4 g.l^{-1}). The reason for the positive effect and the increase in the average traits treated with the natural honey solution is a result of that it contains nutrients and materials important in increasing growth, as honey contains sugars, vitamins, minerals, enzymes and organic acids[13], as the presence of such materials may increase the growth rate. The activity and effectiveness of the plant in the representation of raw materials and the manufacture of its food are reflected in the increase in growth and yield. (14) indicated that honey could be used as a nutritious bio-fertilizer alone or accompanied by another fertilizer to give good results.

Effect of interaction between cultivars and honey solution on growth characteristics and yield of rice

The results in Table (5) show that there is a clear significant effect of the interaction combinations between rice cul-

levels spraying ($g.l^{-1}$)	Plant height (cm)	Number of branches carrying panicle (branch per m^2)	Number of grains in a panicle (grains per panicle)	Panicle length (cm)	Weigh 1000 grains (g)	Grains yield (t.ha-1)	Fertility percentage (%)
0	77.11	225.22	304.00	37.33	17.83	3.471	6.63
2	85.22	300.55	406.33	41.11	18.72	3.811	9.79
4	92.22	342.55	466.33	44.00	19.27	4.017	11.34
6	96.66	415.66	545.11	46.56	20.32	4.335	14.53
LSD 0.05	2.86	21.01	22.92	1.69	0.29	0.148	1.62

Table 4. Effect of levels of honey solution on growth characteristics and yield of rice.

Fertility percentage (%)	Grains yield ($t.ha^{-1}$)	Weigh 1000 grain (g)	Panicle length (cm)	Number of grains in a panicle (grains per panicle)	Number of branches carrying panicle (branch per m^2)	Plant height (cm)	treatments	
							Level spraying $g.l^{-1}$	Cultivars
5.86	3.925	18.86	48.66	302.00	140.00	102.66	0	Amber33
10.35	4.087	19.52	51.33	458.33	253.33	116.33	2	Amber33
12.74	4.217	20.21	53.66	536.67	330.00	124.33	4	Amber33
16.44	4.402	21.62	57.00	621.67	424.00	129.33	6	Amber33
9.05	4.143	17.50	32.00	345.00	329.00	67.00	0	Jasmine
11.35	4.518	18.40	35.00	377.67	345.00	73.00	2	Jasmine
12.65	4.780	18.89	38.00	440.67	363.33	80.33	4	Jasmine
15.61	5.054	20.08	40.67	478.33	392.00	85.00	6	Jasmine
4.98	2.345	17.13	31.33	265.00	206.67	61.66	0	Mashkhab1
7.67	2.827	18.24	37.00	383.00	303.33	66.33	2	Mashkhab1
8.64	3.053	18.70	40.33	421.67	334.33	71.00	4	Mashkhab1
11.55	3.551	19.26	42.00	535.33	431.00	75.66	6	Mashkhab1
3.08	0.261	0.55	2.64	37.29	33.24	4.53	LSD 0.05	

Table 5. The effect of the interaction between levels of honey solution and cultivars on growth characteristics and yield of rice.

tivars and the different levels of spraying with a natural honey solution, as the variety Amber 33 achieved the highest values of averages in most of the traits under study, except for the grain yield in which the combinations of Jasmine cultivar excelled. The treatment of spraying honey solution with a concentration of 6 $g.l^{-1}$ on the Ambar 33 variety gave the highest averages for plant height, the number of branches carrying the panicle (equally with the Mashkhab 1 cultivar with the same concentration), the number of grains in the panicle, the length of the panicle, the weight of 1000 grain, and Fertility percentage (equally with the Jasmine cultivar with the same concentration). They were 129.33 cm, 424 branches per m^2, 621.67 grains per panicle, 57 cm, 21.62 g and 16.44% for the traits, respectively. Thus, achieving an increase of 67.7%, 88.3%, 104.5%, 52.7%, 21.3% and 148% for the traits, respectively, compared to the control treatments (average of cultivars with a concentration of $0g.l^{-1}$ of honey solution). As for the yield, the treatment of spraying the cultivar Jasmine with a concentration of 6 $g.l^{-1}$ with an explanation of natural honey was superior by giving the

highest average grain yield, which amounted to 5.054 t.ha^{-1}, with an increase of 45.6%. Whereas the lowest values for the averages of the studied traits were recorded when spraying with distilled water only on the cultivar Mashkhab1 in all traits except for the number of branches carrying panicles. The mean decreased to the lowest level for all characteristics.

The percentage of decrease was for plant height, number of grains in the panicle, panicle length, weight of 1000 grain, grains yield and fertility percentage were 20%, 12.8%, 16.1%, 3.9%, 32.4% and 24.9% for the traits, respectively, compared to the control treatments. The reason for the distinctiveness of the treatments of the mixtures of spraying the honey solution with a concentration of 6 g.l^{-1} on the plants of the cultivar Anbar in most of the studied traits is the result of the cultivar responding positively to the spray with this concentration as a result of what the solution contains of nutrients, since honey contains important basic and secondary nutrients in The vital processes within the plant, thus increasing the representation and production of food for the plant, increasing the division and expansion of cells, and this is what was observed through the data of the experiment.

Conclusions

Genetic traits play a major role in responding to the different growth factors of all organisms, especially plants, so specialists have been breeding plants to produce varieties that have the ability to give the highest production in conditions that are not ideal for growth through the use of methods of selection and hybridization or the introduction of new varieties with distinctive characteristics, as well as the use of Natural substances are added to the plant in order to raise the efficiency of representation in the plant and thus be an integrative process between the genetic and environmental factor of a plant, and through the data of this study we can conclude that the Amber variety was distinguished by giving the highest averages for most of the traits under study, while the Jasmine variety was distinguished by giving the highest yield when spraying. The plant was treated with a honey solution with a concentration of 6 g.l^{-1}. In contrast, the cultivar Mashkhab 1 recorded the lowest averages for the studied traits. Based on the foregoing, we can recommend the use of honey solution at a concentration of 6 g.l^{-1} on rice plants of the two cultivars Amber and Jasmine at a rate of five sprays during the growing season. We also recommend the introduction of other varieties and crops in such experiments in which the honey solution was used alone or as a supplement as a fertilizer for the crop.

Funding

This research received no external funding.

Conflicts of Interest

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ARTICLE / INVESTIGACIÓN

Effect of chemical fertilizer and humic acid on cabbage leaves' N, P, K and S concentrations (*Brassica oleracea* var. capitata L.)

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DOI. 10.21931/RB/2022.07.04.46

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Abstract: A field study was carried out in one of the fields of Jdeidet Al-Shatt district is, located 30 km from the center of Baquba in Diyala governorate, during the autumnal season 2021-2022 on silty loam soil classified to a level under the Typic Torrifluvent according to the modern American classification to know the effect of adding chemical fertilizer and humic acid on the availability of nitrogen, phosphorus and potassium concentrations for cabbage leaves, according to of randomized complete block design (RCBD) by using three replicates. The first factor was the chemical compound fertilizer NPK(20:20:20) added at three different levels 0, 150 kg ha^{-1}, 300 kg ha^{-1}, while the second factor was humic acid at three levels 0, 15 kg ha^{-1}, 30 kg ha^{-1}. Fertilizers were added to the soil by making an incision around the plant and were added in two stages, the first when planting and the second 43 days after the date of the first batch. The results of the study showed that adding chemical fertilizer at a level of 300 kg ha^{-1} led to significant differences in the concentrations of nitrogen, phosphorous, potassium and sulfur elements in the inner leaves, where the concentrations of elements reached 2.55%,0.34%, 2.95%,1.36% respectively, the outer leaves. In contrast, the concentrations of nitrogen, phosphorous and potassium reached 4.00 %, 0.34%, and 2.67%, respectively, While the superiority of the humic acid at the level of 30 kg ha^{-1} to 2.33%, 0.32%, 2.77%,1.47% in the inner leaves, respectively, while in the outer leaves 3.80 %,0.31 %,2.49%.

Key words: Chemical fertilizer, humic acid, concentrations of N,P, K and S, cabbage.

Introduction

The cabbage crop is one of the most important winter vegetables in Iraq. It is a vegetable of great economic importance all over the world. It has high nutritional properties that are low in fat and protein and a high content of vitamins, fiber and minerals[1]. What distinguishes it from other vegetables is that it contains glucosinolate compounds, which are sulfur-rich compounds that protect against cancer[2]. Nitrogen, phosphorous and potassium are essential elements for the plant of cabbage, as this nutrient faces many challenges in the soils of dry and semi-arid regions of the world, including Iraqi soils due to high temperatures and low rainfall, which causes a decrease in its content of organic material and a high degree of interaction Soil resulting from the presence of calcium carbonate, which causes a decrease in soil fertility as a result of exposure of the added elements to the soil to the processes of loss and stabilization[3]. Nitrogen is an important and crucial element in plant growth and development. Its deficiency leads to a decrease in plant productivity by reducing the content or activity of enzymes involved in the process of carbon metabolism, leaf area and the longevity of green leaves, As well as its deficiency leading to plant aging[4]. Phosphorous is one of the main elements in regulating the reactions of the carbonic metabolism process and an essential source of energy and regulation of the breathing process. It is also included in the composition of the cytoplasm, the nucleus, nuclear proteins and lipid derivatives, as well as some enzymes. It is essential in cell division; it helps roots to early growth and spread in the soil. It also helps in early flowering, seed production and fruit ripening[5]. Potassium is one of the essential nutrients for plants, and it is required in large quantities for the growth and reproduction of plants. Potassium is in second place after nitrogen; its deficiency leads to a severe change in various physiological processes[6]. To increase the amount of agricultural production to meet the world's food requirements, it is necessary to find alternative and cheap ways at the same time working to reduce the loss of elements from the soil, as the use of humic acid (HA) is a major component of humic substances, which were found in different sources such as soil, humus, peat, lignite, and coal, and that the molecular structure of humic acid is the reason for the activity of these compounds due to the large number of functional groups present in each loop[7,8]. It effectively influences nutrients from the soil, as it increases the availability or transfer of significant elements, including nitrogen, phosphorous and potassium; it was considered a supplement to chemical fertilizers and reduces the costs of production inputs of agricultural crops. It doesn't take time to decompose free from seeds, weeds, bushes and pathogens compared to conventional organic fertilizers[9-11]. The use of humic acid with chemical fertilizer, according to the fertilizer's recommendation, works to hold the elements in the soil when added as a mixture or feed in the soil. This makes it ready for absorption by the plant for a long time in the soil. This leads to an increase in the availability of elements in the soil[12]. Because of the importance of cultivating the cabbage plants in Iraq, the study aimed to: Know the effect of adding NPK and humic acid fertilizers and the interaction between them on nitrogen, phosphorus and potassium concentrations for cabbage leaves.

Citation: Turki M I, Bader B R. Effect of chemical fertilizer and humic acid on cabbage leaves' N, P, K and S concentrations (Brassica oleracea var. capitata L.). Revis Bionat a 2022;7(4) 46. http://dx.doi.org/10.21931/RB/2022.07.04.46
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

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Materials and methods

A field study was carried out in one of the agricultural fields in Jdeidet Al-Shatt sub-district of Al-Khalis district, located 30 km from the center of Baquba in Diyala gover during the autumnal season 2021-2022 norate on silty loam soil classified to a level under the Typic Torrifluvent according to the modern American classification. It is located at 44°25'33.2868" east longitude and 33°37'29.172" north latitude to study the effect of adding chemical fertilizer NPK and humic acid on the nitrogen concentration, phosphorus and potassium in cabbage leaves. It can be conducted by adding the balanced compound chemical fertilizer NPK(20:20:20) at three different levels, which are 0 without addition, 150 kg ha^{-1}, and 300 kg ha^{-1} according to the Fertilization Recommendation[13]. It is also symbolized by C1, C2, and C3 in sequence, and the second factor is humic acid in three levels, 0(without adding), 15 kg ha^{-1} and 30 kg ha^{-1}. A sample was taken randomly from different places at a depth of 30 cm. It was mixed to become a composite sample representative of the study soil. The soil was dried and ground with a wooden hammer and passed through a sieve with a holes diameter of 2 mm to conduct some physical and chemical analyses, whose results are shown in Table 1.

Soil preparation processes for cultivation include plowing and softening the soil. It has been divided into three sectors; each sector contains 9 factors, the dimensions of the experimental unit are 3 * 2.5m2, and the experimental unit contains 4 lines. The number of plants in the experimental unit was 24, and the number of plants in each line was 6 plants. The planting was also done on the terraces; the width of the balcony was 50 cm. The seedlings were planted on one side of the terrace. The distance between one plant and another was 40 cm, between one experimental unit and another was 100 cm, and between one sector and another was 100 cm. The seedlings were also planted in the field on 9/24/2021, and the ground addition of the chemical fertilizer NPK and humic acid was conducted in two stages. The first is during the planting process, and the second is in the stage before the appearance of the head, which is after 43 days. This can be accomplished by making an incision in the soil around the plant. Measurements were also taken from plant samples in two stages, the first stage before the emergence of the head and the second stage of completion of the maturity of the head. The plant samples were prepared for elemental determination in the soil science and water resources laboratories College of Agriculture, University of Diyala.

Measuring the concentration of nitrogen in leaves (%)

Nitrogen was estimated by adding sodium hydroxide to the digested vegetable sample and extracted by using the Micro Kjeldahl apparatus[14].

Measuring the concentration of phosphorous in leaves (%)

It was estimated by adding ammonium molybdate and ascorbic acid. As well as it was measured at a wavelength of 882 nm by using a spectrophotometer[15].

Measurement of potassium concentration in leaves (%)

It was estimated by the flame apparatus[14].

Measurement of sulfur concentrations in leaves (%)

It was estimated by the turbidity method by adding barium chloride and measured at a wavelength of 420 nm using a spectrophotometer[16].

Results

The concentration of nitrogen in the Inner leaves (%)

Table 3 shows significant differences between adding chemical fertilizers and humic acid effect on the means of nitrogen element concentrations in the inner leaves. The C2 and C3 treatments were significantly superior, with the highest norm reaching 2.03 and 2.55% compared to the C1 treatment, which amounted to 1.57%, with an increased rate of 29.29% and 62.42%, respectively. It was the effect of adding humic acid, the mean of H2 and H3 treatments which amounted to 2.06% and 2.33%, respectively, showed significant superiority over the H1 treatment, which amounted to 1.76%, with an increase of 17.04% and 32.38%,

Soil Properties	values	Unit
Electrical conductivity EC (1:1)	2.6	dSm^{-1}
Soil pH (1:1)	7.7	
Calcium Carbonate	242.06	g kg^{-1}
Organic matter	8.08	
Available nutrients		
available nitrogen	30.00	mg kg^{-1}
available phosphorous	12.87	
available potassium	307.01	
Bulk density	1.36	Mg m^{-3}
Soil particles		
clay	27.68	%
silt	51.68	
sand	20.64	
soil texture	Silty loam	
Field capacity	27	%

Table 1. Soil properties study before planting

Adjective	the value
Humic acid	53%
Fulvic acid	12%
K2O	17%

Table 2. Guaranteed contents of organic fertilizer (humistar).

	C1	C2	C3	
H1	1.09 g	1.97 def	2.23 bc	1.76 C
H2	1.75 f	2.01 cde	2.43 b	2.06 B
H3	1.88 ef	2.11 cd	3.01 a	2.33 A
	1.57 C	2.03 B	2.55 A	

Table 3. Effect of adding chemical fertilizer, humic acid and the interaction between them on nitrogen concentrations in the inner leaves at the stage of full maturity of the head (%).
* The symbols in the table indicate: C = chemical fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H = humic acid where H1 = 0 without addition, H2 = 15 kg ha^{-1},H3= 30 kg ha^{-1}. Means with similar letters are not significantly different from each other according to Duncan's polynomial test at 0.05% probability level.

respectively. As for the effect of the interaction, the C3H3 treatment showed that it was significantly superior, which amounted to 3.01%, the maximum value and the minimum value when the C1H1 control treatment, which amounted to 1.09%, and an increased rate of 176.14%.

The concentration of phosphorous in the Inner leaves (%)

Table 4 shows that the addition of chemical fertilizer and humic acid in the average phosphorous element concentrations in the inner leaves was significantly superior to the C2 and C3 treatment, which reached the highest average of 0.28 and 0.34% compared to the C1 treatment, which amounted to 0.25%, respectively with an increase of 12% and 36%.

Results in table 4 show the effect of adding humic acid on phosphorus concentrations in the inner leaves; the treatment H3 reached 0.32% and showed a significant superiority to the H1 treatment, which amounted to 0.27%, with an increase of 18.51%. As well as the effect of the interaction,

	C1	C2	C3	
H1	0.23 d	0.27 cd	0.30 bc	0.27 B
H2	0.26 cd	0.27 cd	0.33 b	0.28 B
H3	0.27 cd	0.29 bc	0.39 a	0.32 A
	0.25 C	0.28 B	0.34 A	

Table 4. Effect of adding chemical fertilizers and humic acid and the interaction between them on phosphorous concentrations in the inner leaves at the stage of full maturity of the head (%).
* The symbols in the table indicate: C = chemical fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H = humic acid where H1 = 0 without addition, H2 = 15 kg ha^{-1}, H3= 30 kg ha^{-1}. Means with similar letters are not significantly different from each other according to Duncan's polynomial test at 0.05% probability level.

it gave the C3H3 treatment was significantly superior, which amounted to 0.39%, with the maximum value and the minimum in the treatment C1H1 control, which amounted to 0.23% and an increase of 69.56%.

The concentration of potassium in the inner leaves (%)

Table 5 shows significant differences in the addition of chemical fertilizer and humic acid on potassium concentrations in the inner leaves, as the C2 and C3 treatments were significantly superior, which reached the highest mean of 2.67 and 2.95%, respectively, compared with the C1 treatment, which amounted to 2.39%, with an increase of 11.71% and 23 .43%. Results in table 5 show the effect of adding humic acid on potassium concentrations in the inner leaves; the treatment H3 reached 2.77% and showed a significant superiority over the H1 treatment, which amounted to 2.59%, with an increase of 6.94%. As for the effect of the interaction, the C3H3 treatment showed that it was

	C1	C2	C3	
H1	2.31 e	2.62 cd	2.86 abc	2.59 B
H2	2.33 e	2.66 cd	2.93 ab	2.64 B
H3	2.54 de	2.74 bcd	3.05 a	2.77 A
	2.39 C	2.67 B	2.95 A	

Table 5. Effect of adding chemical fertilizer and humic acid and the interaction between them on potassium concentrations in the inner leaves at the stage of full maturity of the head (%).
* The symbols in the table indicate: C = chemical fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H = humic acid where H1 = 0 without addition, H2 = 15 kg ha^{-1},H3= 30 kg ha^{-1}. Means with similar letters are not significantly different from each other according to Duncan's polynomial test at 0.05% probability level.

significantly superior, which amounted to 3.05%, the maximum value and the minimum when the C1H1 control treatment, which amounted to 2.31%, and an increased rate of 32.03%.

The concentration of sulfur in the Inner leaves (%)

Table 6 shows significant differences in the addition of chemical fertilizer and humic acid on concentrations of sulfur in the inner leaves; there were no significant differences in the treatments of the chemical fertilizer, humic acid, the average H3 treatment, which amounted to 1.47%, showed a significant superiority over the H1 treatment, which amounted to 0.59%, with an increase of 149.15%,As for the effect of the interaction, the treatment of it gave C3H3 showed significant superiority, which amounted to 1.85%, the maximum value and the minimum with value for treatment C1H1 control which amounted to 0.56% and an increase of 230. 35%.

The concentration of nitrogen in the outer leaves (%)

Table 7 shows The effect of adding chemical fertilizer and humic acid on the means concentrations of nitrogen in the outer leaves. The C2 and C3 treatments were significantly superior, which reached the highest average of 3.71 and 4.00%, respectively, compared to the C1 treatment, which amounted to 3.44%, with an increase of 7.84% and 16.27 %, respectively. The effect of adding humic acid treatment was 3.63%, and the average H3 treatment amounted to 3.80%, with an increase of 4.68%. As for the effect of the interaction, the C3H3 treatment showed that it was significantly superior, which amounted to 4.12%, the maximum value and the minimum value when the C1H1 control treatment, which amounted to 3.34%, and an increased rate of 23.35%.

The concentration of phosphorous in the outer leaves (%)

Table 8 shows the effect of adding chemical fertilizers and humic acid phosphorous concentrations in the outer leaves, as the C2 and C3 treatments were significantly superior, which reached the highest mean of 0.28 and 0.34% compared to the C1 treatment, which amounted to 0.25%, respectively, with an increase of 12% and 36% respectively. Results in table 8 show the effect of adding humic acid on concentrations of phosphorous in the outer leaves; the treatment H3 reached to 0.31%, showed a significant superiority over the H1 treatment, which amounted to 0.27%, with an increase of 14.81%, As for the effect of the interaction, it gave the C3H3 treatment showed that it was significantly superior, which amounted to 0.37%, the maximum value and the minimum value when the C1H1 control treatment, which amounted to 0.23%, and an increased rate of 60.86%.

The concentration of potassium in outer leaves (%)

Table 9 shows the effect of adding chemical fertilizers and humic acid concentrations of potassium in the outer leaves, as the C2 and C3 treatments were significantly superior, which reached a higher mean of 2.35 and 2.67% compared to the C1 treatment, which amounted to 2.06%, respectively, with an increase of 14.07 % 29.61%. Concerning the addition of humic acid, the average H3 treatment, which amounted to 2.49%, showed a significant superiority to the H1 treatment, which amounted to 2.24%, with an increase of 11.16%. As for the effect of the interaction, the treatment of it gave C3H3 showed that it was significantly superior, which amounted to 2.82%,%, the maximum value and the minimum with value when the treatment C1H1 control, which amounted to 1.92%, and an increased rate of 46.87%.

Discussion

It is evident from tables (3,4,6,7,8,9) that there were significant differences between the treatments of the compound chemical fertilizer NPK. The reason may be due to the role of fertilizer and increasing the leaves' concentrations of nutrients N, P, and K. This increases the activity of

	C1	C2	C3	
H1	0.56 c	0.58 c	0.63 bc	0.59 B
H2	1.20 abc	1.26 ab	1.59 a	1.35 A
H3	1.26 ab	1.30 a	1.85 a	1.47 A
	1.01 A	1.04 A	1.36 A	

Table 6. Effect of adding chemical fertilizer and humic acid and the interaction between them on sulfur concentrations in the inner leaves at the stage of full maturity of the head (%).
* The symbols in the table indicate: C = chemical fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H = humic acid where H1 = 0 without addition, H2 = 15 kg ha^{-1}, H3= 30 kg ha^{-1}. Means with similar letters are not significantly different from each other according to Duncan's polynomial test at 0.05% probability level.

	C1	C2	C3	
H1	3.34 e	3.66 d	3.90 bc	3.63 B
H2	3.44 e	3.71 cd	3.99 ab	3.71 AB
H3	3.55 de	3.74 cd	4.12 a	3.80 A
	3.44 C	3.71 B	4.00 A	

Table 7. Effect of adding chemical fertilizers and humic acid and the interaction between them on the nitrogen concentrations of the outer leaves after the head maturity (%).
* The symbols in the table indicate: C = chemical fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H = humic acid where H1 = 0 without addition, H2 = 15 kg ha^{-1},H3= 30 kg ha^{-1}. Means with similar letters are not significantly different from each other according to Duncan's polynomial test at 0.05% probability level.

	C1	C2	C3	
H1	0.23 f	0.27 de	0.32 bc	0.27 B
H2	0.26 ef	0.29 cde	0.33 b	0.29 AB
H3	0.27 def	0.30 bcd	0.37 a	0.31 A
	0.25 C	0.28 B	0.34 A	

Table 8. Effect of adding chemical fertilizers and humic acid and the interaction between them on phosphorous concentrations in the outer leaves at the stage of full maturity of the head (%).
* The symbols in the table indicate: C = chemical fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H = humic acid where H1 = 0 without addition, H2 = 15 kg ha^{-1},H3= 30 kg ha^{-1}. Means with similar letters are not significantly different from each other according to Duncan's polynomial test at 0.05% probability level.

	C1	C2	C3	
H1	1.92 g	2.29 de	2.53 bc	2.24 B
H2	2.07 fg	2.33 cde	2.65 ab	2.35 B
H3	2.21 ef	2.44 cd	2.82 a	2.49 A
	2.06 C	2.35 B	2.67 A	

Table 9. Effect of adding chemical fertilizer and humic acid and the interaction between them on potassium concentrations in the outer leaves at the stage of full maturity of the head (%).
* The symbols in the table indicate: C = chemical fertilizer where C1 = 0 without addition, C2 = 150 kg ha^{-1}, C3 = 300 kg ha^{-1} and H = humic acid where H1 = 0 without addition, H2 = 15 kg ha^{-1},H3= 30 kg ha^{-1}. Means with similar letters are not significantly different from each other according to Duncan's polynomial test at 0.05% probability level.

physiological processes in the plant, including building proteins, carbohydrates and the process of carbon metabolism, which causes rapid growth, which reflects positively on the plant, and this is what had reached[17]. They explained that the nitrogen, phosphorous and potassium concentrations range between 2.77 -3.17%, 0.27-0.20% and 3.97-2.80%, respectively. These concentrations fall within their standard limit. As for the moral increase achieved by humic acid, it may be attributed to the role of acid in increasing the plant's content of nutrients and plant hormones such as auxins and cytokinins, which work to increase the division of meristematic cells, which leads to an increase in the size of the plant and stimulates enzymatic reactions and humic acid works to improve the permeability of the membranes. Thus, it increases the uptake of elements, including N, P, K, Mg, and Ca, which become available and ready for uptake in the plant's root system[18,19]. As for Table 6, there was a significant difference between the treatments of humic acid in the percentage of sulfur in the leaves. It may help to release the nutrients associated with minerals and salts in the soil. As well as it was found that 95% of the sources of sulfur are linked in the soil organically, as humic acid improves the biological properties of the soil as the soil microorganisms work to convert organic sulfur into mineral sulfur as $SO4^{-2}$ is ready for plant uptake[9,20-22].

Conclusions

The addition of the chemical fertilizer 300 kg ha^{-1} led to an increase in the concentrations of nutrients (N, P, and K) in the inner and outer leaves of cabbage. The addition of humic acid 30 kg ha^{-1} led to increased nitrogen, phosphorous, potassium and sulfur concentrations in the leaves.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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ARTICLE / INVESTIGACIÓN

Epigenetic effects of selenium and vitamin E supplementation in broiler breeder diets on the performance of their progeny

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DOI. 10.21931/RB/2022.07.04.47

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Abstract: Nutritional supplements have been commonly used in the poultry industry last few years. The study aimed to investigate the epigenetic effects of adding vitamin E and organic selenium to the diet of broiler breeders Ross-308 on their progeny meat production performance. The treatments included the control group fed with a standard diet without supplementation (T1), T2 using a standard diet supplemented with 500 mg of vitamin E / kg, T3 using a standard diet supplemented with 0.5 mg of organic selenium (Availa powder) /kg, and T4 using a standard diet supplemented with a mixture of vitamin E and organic selenium in proportions 500 and 0.5 mg/kg respectively. The eggs were collected from each treatment to obtain the progeny reared for 35 periods, and measurements were recorded for meat production and carcass traits. The results showed that the treatments had significant epigenetic effects on body weight at hatching. Hence, T2 had a significantly heavier body weight than T1, while no significant differences were observed between T3 and T4. The result of T2 recorded extremely high feed intake compared with T3. On the other hand, T3 and T4 recorded a hefty weight of breast parts compared with T1 and T2. In conclusion, organic selenium supplementation led to a significant increase in breast weight and a decrease in thigh part weight compared to the control group. In contrast, vitamin E supplementation led to an increase in chick weight at hatch, a reduction of total mortality and an improvement in feed conversion ratio compared to the control group. This refers to the epigenetic effects of organic selenium and vitamin E on progeny traits when added to the breeder diet.

Key words: Epigenetics, broiler, selenium supplementation, meat production.

Introduction

The poultry industry made significant contributions toward world food security by satisfying people's demand for animal protein[1]. Poultry breeding and production is characterized by a short production cycle and invested capital cycle[2]. It is an important issue to obtain high output and high-quality meat at low cost, which needs a good formula for the chicken diet that affects feed consumption, weight gain, and food conversion efficiency[3].

Nutrition is one of the environmental factors that must supply basic requirements for growth, development and survival, and it represents an essential factor that can directly change the phenotypic performance of living organisms, as well as the epigenetic effects in the subsequent generation through the transmission of nutritional influences on genes expression across generations[4,5]. The epigenetic effects resulting from nutrition focused on determining the necessary nutrients in feeding broiler breeders, which pass these elements to the hatching eggs or directly pass the epigenetic marks from the broiler breeder[6].

Epigenetic effects refer to changes and phenotypes that appear on an organism without a difference in the nucleotide sequence through the processes of DNA methylation and modifications in histones. Some of these changes are inherited across generations[7]. The mechanisms are carried out in small pieces of DNA by enzymes and protein complexes, which represent the language of epigenetic effects in response to external factors, and are phenotypically reflected on the cell and then the organism[8]. Epigenetic mechanisms can regulate gene expression through chemical modifications of DNA bases and changes in chromosomal structure[9], including methylation[10]. and modulation of histones[11,12].

Many studies used vitamin E supplementation in the diet of broiler breeders (using levels 100, 200 and 400 mg/kg of diet). They recorded significant increases in the body weight of their progeny at using 200 and 400 mg / kg of diet[13]. In contrast, other studies used different levels of vitamin E supplementation (0, 150, 250 and 350 mg/kg of diet) and reported no significant differences in feed consumption and feed conversion efficiency in their progeny generation[14,15] indicated an improvement in weight gain when selenium was added (0.4 mg/kg) to the diet of broiler at 1-6 weeks of age, (16) indicated no significant differences in weight gain when adding sodium selenite to broiler diets at a 1 mg/kg rate at 15-27 days of age. There are many global hybrids used widely in Iraq to produce broilers for local consumption, and sometimes the broiler industry imports chicks or eggs for hatchery, besides there are many enterprises that reared broiler breeders to produce hatching eggs, and there are many studies aimed to improve their performance in the Iraqi environment[17-20].

Most feed staff used nutritional supplementations to the diet of broiler breeders such as vitamins, minerals, and amino acids to improve broiler breeder flock performance. It did

Citation: Hassan K, Ali. F. Epigenetic effects of selenium and vitamin E supplementation in broiler breeder diets on the performance of their progeny. Revis Bionatura 2022;7(4) 47. http://dx.doi.org/10.21931/RB/2022.07.04.47.
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

not give attention to the effect of these supplementations on the performance of their progeny[21]. Hence, the recent study aimed to determine the effect of vitamin E, selenium and a mixture of vitamin E and organic selenium supplementation on the diets of Ross broilers breeders and their impact on the performance of their progeny.

Materials and methods

The experiment was conducted in the poultry house of the animal farm in the Department of Animal Production at the College of Agriculture, University of Diyala. Experiment units include 60 hens and 10 cocks of Ross 308 broiler breeders flock at 41 weeks of age, and the flock is housed in floor pens with dimensions of 2 x 1.5 m. According to the breeding company guide, the hens were fed on a diet containing 15% crude protein and metabolic energy 2775 kilocalories/kg feed and in a restricted system weighing 175 g / hen. The flock was divided into four groups, each group for treatment with three replicates (each replicate with five hens).

The treatments included the control group fed with a standard diet without additives (T1), T2 using a standard diet supplemented with 500 mg of vitamin E / kg, T3 using a standard diet supplemented with 0.5 mg of organic selenium (Availa powder) /kg, and T4 using a standard diet supplemented with a mixture of vitamin E and organic selenium in proportions 500 and 0.5 mg/kg respectively. The available powder contains organic selenium and selenomethionine hydroxy analog at a rate of 0.1%.

The treatments were introduced to the groups for three weeks as an adaptation period before hatching eggs were collected for each treatment to obtain the progeny chicks were fed on a standard broiler diet without supplementation, with a starter diet content of crude protein of 22%. Metabolic energy of 2900 kilocalories/kg during the first 21 days, then replaced with diet content crude protein 20% and metabolic energy 3111 kilocalories/kg until the end of the experiment at the age of 35 days.

Statistical analysis

Experimental data were analyzed according to a Completely Randomized Design, and the significant differences among means were detected using Duncan's Multiple Range Test at the probability of 0.05[22]. The linear model:

$$Yij = \mu + \tau_i + \varepsilon_{ij}$$

Where:

Y_{ij}= observation

μ=overall mean.

τ_i=treatment effect (i=1,2,3,4)

ε_{ij}= The experimental error of observation normally distributed with a mean equal to zero and variance equal to σ2e.

Results

Table 1 shows the significant effects of supplementation treatments on body weight at hatching; hence T2 therapy outperformed the T1 treatment for body weight at hatching, while no significant differences were observed between T3 and T4. There are no significant differences observed among treatments in all following weeks of rearing for body weight; these results may be indicated to active epigenetic effects of vitamin E supplementation to hens diet on the embryonic development only, but not extended to the period after hatching.

Table 2 shows significant differences among treatments in weekly feed consumption; hence T2 outperformed T3 in the first and second week of age, while no significant differences were observed among other therapies. The results appeared no significant differences among averages of treatments in feed consumption during the subsequent weeks of rearing.

Table 3 shows significant differences among treatments for the food conversion rate in the second week. Hence T2 and T4 outperformed the control group, which indicates that epigenetic effects resulting from vitamin E supplementation alone (T2) or in a mixture with selenium (T4) to the diet of broiler breeder hens in the feed conversion rate during the second week while no significant differences observed with the T3, and no significant differences observed among treatments during the following weeks of the rearing period.

Table 4 shows significant differences among treatments for total mortality; hence T2 outperformed T1 and T4 and did not differ significantly from T3. On the other hand, T3 exceeded T1. The results indicate that all of the supplementation treatments outperformed the chicks' viability compared to the control group, which may be a result of epigenetics due to supplementation treatments of vitamin E and selenium in the breeder's diet, which were positively reflected in the progeny viability and reduced mortality.

Table 5 represents that there are significant differences among treatments; hence T1 outperformed T4 in the aspect of dressing percentage T3, and T4, which used selenium supplementation, recorded significantly high breast weight percentage compared with T1 and T2; the differences may be due to epigenetics effect of selenium supplementation in the breeder hens that affect the progeny broiler performance. On the other hand, T1 and T2 outperformed T3 and T4 in the percentage of thigh weight.

Discussion

The results agreed with (13) who used different levels of vitamin E (100, 200, and 400 mg/kg of diet in the broiler breeders Ross-308and the results pointed to significant superiority of 400 mg supplementation compared to 100 mg during 1-42 days of age. The results did not agree with (13), who used different levels of vitamin E (100, 200 and 400 mg/kg diet) to the broiler breeders Ross-308 diet at 75 weeks of age. They did not record significant differences in their chick's body weight among groups at the hatching.

The results did not agree with (23) when 0.2 mg/kg of hydroxy selenomethionine was added to broiler breeders' diets. In the progeny flock, no significant differences were observed in the weekly feed consumption from 1 to 41 days.

The results agreed with (13) who used vitamin E supplementation in different levels (100, 200 and 400 mg/kg of diet to the diet of broiler breeder hens and recorded significant differences in the progeny groups at level 100 mg of vitamin E / kg of diet supplementation compared to levels 200 and 400 mg of vitamin E/kg of diet during the period 1-21 days of age, and no significant differences were observed in the subsequent weeks of the experiment.

The results agreed with (24) when Selenomethionine (Availia) was used in the diets of Ross 308 broilers at 36

Age	T1	T2	T3	T4
One day	47.21 ± 0.17 b	50.08 ± 1.06 a	47.97 ± 0.40 ab	48.26 ± 0.50 ab
Week1	153.96 ± 4.33	159.53 ± 1.84	156.77 ± 6.43	155.43 ± 4.69
Week2	478.70 ± 14.90	466.72 ± 4.65	446.72 ± 11.97	447.40 ± 6.84
Week3	954.66 ± 33.47	940.66 ± 7.40	875.15 ± 22.88	925.04 ± 40.13
Week4	1619.08 ± 66.63	1532.34 ± 11.61	1489.48 ± 18.35	1492.35 ± 33.27
Week5	2218.84 ± 69.15	2000.72 ± 145.33	2008.79 ± 92.99	2117.24 ± 36.94

Table 1. Means ± standard error of body weight (g) in the broiler progeny that results from supplementation treatments in the diet of the broiler breeders Ross 308 during the rearing period (5 weeks).
T1: standard diet without addition (control): T2 add vitamin E: T3 add selenium: T4 add vitamin E and selenium mixture. According to Duncan's multiple ranges test, means with different letters differ significantly at a probability level of 0.05.

Age	T1	T2	T3	T4
Week1	173.22 ± 5.19 ab	161.46 ± 1.78 a	128.34 ± 6.68 b	152.01 ± 13.39 ab
Week2	372.11 ± 11.17 ab	393.64 ± 16.22 a	344.07 ± 9.12 b	365.93 ± 12.07 ab
Week3	722.69 ± 22.01	743.29 ± 17.38	663.18 ± 8.76	675.00 ± 50.00
Week4	1175.65 ± 78.54	1175.65 ± 120.90	1010.86 ± 91.01	1092.3 ± 58.99
Week5	1092.12 ± 69.34	1025.00 ± 34.90	983.42 ± 45.91	1064.69 ± 31.95

Table 2. Means ± standard error of feed intake (g/ week) in the broiler progeny that results from supplementation treatments in the diet of the broiler breeders Ross 308 during the rearing period (5 weeks).
T1: standard diet without addition (control): T2: add vitamin E: T3 add selenium: T4 add vitamin E and selenium mixture. Means with different letters refer to significant differences at P≤0.05.

Age	T1	T2	T3	T4
Week1	1.29 ± 0.07	1.48 ± 0.02	1.18 ± 0.01	1.43 ± 0.17
Week2	1.14 ± 0.01 b	1.28 ± 0.05 a	1.19 ± 0.01 ab	1.26 ± 0.03 a
Week3	1.52 ± 0.04	1.57 ± 0.02	1.57 ± 0.13	1.42 ± 0.04
Week4	1.79 ± 0.20	1.95 ± 0.21	1.65 ± 0.16	1.94 ± 0.18
Week5	1.82 ± 0.11	2.79 ± 1.03	1.95 ± 0.20	1.71 ± 0.09

Table 3. Means ± standard error of feed conversion rate in the broiler progeny that result from supplementation treatments in the diet of the broiler breeders Ross 308 during the rearing period (5 weeks).
T1: standard diet without addition (control): T2 add vitamin E: T3 add selenium: T4 add vitamin E and selenium mixture. Means with different letters refer to significant differences at P≤0.05.

days of age at 0.2 mg/kg feed; hence they recorded significant differences between supplementation treatments and control in feed consumption. The results did not agree with 13, which added vitamin E at different levels (100, 200, 400 mg / kg of diet) to the broiler breeders Ross-308 used. The progeny appeared to have no significant differences in the weekly feed consumption during 1 - 42 days.

The epigenetic effects of vitamin E did not stabilize until the total rearing period. The results did not agree with (23), who used 0.2 mg/kg of hydroxyl selenomethionine supplementation in the diets of broiler breeders. No significant differences were recorded in the feed conversion rate of their progeny during the rearing period from 1 to 41 days.

The results did not agree with (13) in a study that used vitamin E supplementation in different levels (100, 200 and 400 mg/kg diet) to the broiler breeder diet, and the results did not record significant differences among groups with an aspect of mortality during 1 – 42 days rearing period. And

Trait	T1	T2	T3	T4
Mortality	8.54 ± 1.89 C	0.00 ± 0.00 a	1.93 ± 0.98 ab	4.92 ± 0.90 b
Male sex ratio	47.05 ± 1.51 a	22.08 ± 4.80 b	46.32 ± 3.39 a	29.77 ± 2.99 b

Table 4. Means ± standard error of the total mortality (%) and male sex ratio in the progeny flock result from supplementation treatments in the diet of the broiler breeders Ross 308.
T1: standard diet without addition (control): T2 adding vitamin E: T3 adding selenium: T4 adding a mixture of vitamin E and selenium. Means with different letters refer to significant differences at P≤0.05.

Traits	T1	T2	T3	T4
Live body weight (g.)	2307.75± 68.68	2455.25 ± 107.98	2209.00 ± 42.66	2502.25 ± 190.65
Carcass weight (g.)	1811.50± 68.35	1746.00± 103.94	1724.25 ± 38.01	1739.00± 175.01
Dressing (%)	78.49 ± 3.63 a	71.11 ± 6.30 ab	78.06 ± 1.70 ab	69.50 ± 9.23 b
Breast weight (%)	35.96 ± 1.26 b	36.41 ± 0.90 b	44.09 ± 0.34 a	42.39 ± 0.47 a
Thigh weight (%)	30.23 ± 1.38 a	28.59 ± 2.08 a	23.26 ± 0.50 b	24.70 ± 1.45 b
Heart weight (%)	0.78 ± 0.04	0.74 ± 0.04	0.81± 0.04	0.75 ± 0.05
Liver weight (%)	3.24 ± 0.09	3.20 ± 0.13	3.12 ± 0.11	3.50 ± 0.13
Gizzard weight (%)	1.35 ± 0.06	1.40 ± 0.11	1.64 ± 0.12	1.60 ± 0.13

Table 5. Means of squares ± standard error of dressing and carcass parts (%) in the progeny of groups of broiler breeders had nutritional supplementation treatments in the diet.
T1: standard diet without addition (control): T2 adding vitamin E: T3 adding selenium: T4 adding a mixture of vitamin E and selenium. Means with different letters refer to significant differences at P≤0.05

the results did not agree with (24) when selenomethionine (Availia) was supplemented to Ross 308 broiler diets at 36 days of age at 0.2 mg/kg. The results differed with (25) who used supplementation of vitamin E and selenium at levels of 200 and 0.3 mg/kg of diet, respectively, to the diet of quail at the age of 1-2 days, as it was noted that there were no significant differences in the total mortality. Shows that there are substantial differences among treatments in respect of sex ratio; hence T2 and T4 appeared to have a significantly low percentage of males in the hatching batch compared with T1 and T3, which indicates that vitamin E supplementation to the diet of breeder hens may have an epigenetic effect in determining the sex of progeny chicks.

The results agreed with (26) who used organic selenium at level 0.3 mg/kg in a broiler diet and recorded a significant increase in breast and thigh weight. The results did not agree with (14), who reported no significant differences in carcass and breast and thigh weight.

Conclusions

We can conclude from the results of the current study that the organic selenium supplementation led to a significant increase in the weight of the breast and a decrease in the importance of the thigh parts of the carcass compared with the control group. In contrast, the vitamin E supplementation led to an increase in the weight of chicks at hatching, a decrease in total mortality and an improvement in the feed conversion rate compared with the control group. These results indicate the presence of epigenetic effects of organic selenium and vitamin E in the progeny traits when added to the broiler breeder diet, which can be exploited to improve the performance of the progeny resulting from the breeding flock.

Funding

This research received no external funding.

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ARTICLE / INVESTIGACIÓN

Epigenetic effects on broiler exposure to magnetic field on progeny meat production traits

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DOI. 10.21931/RB/2022.07.04.48

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Abstract: This experiment was conducted to determine the effect of the exposure of Ross 308 broiler breeders to a magnetic field on the meat production traits of progeny. The experimental flock consisted of 60 hens and ten cocks of Ross 308 broiler breeders at 36 weeks of age, divided randomly into four groups; each group applied for treatment with three replicates. The treatments were control (T1), storage of semen in an 803 gauss magnetic field for 24 h (T2), storage of fertilized eggs in a magnetic field of 250 gausses for 72 h before entering the incubator (T3), and exposing individual cages to 250 gausses of magnetic field for 8 Weeks (T4). The progeny result from the broiler breeders groups was recorded for body weight and feed intake, compared with the control (T1). The results showed no significant differences among progeny groups in body weight, weekly weight gain and weekly feed intake during the rearing period.

Key words: Epigenetic, magnetic broiler breeders, broiler progeny performance.

Introduction

Quantitative traits are affected by genetic and environmental factors, but each trait's relative importance differs from one trait to another and can be estimated by heritability[1]. Environmental factors refer to all non-genetic factors, including ambient conditions, management, etc., and many genetics studies pointed out that the ecological effects did not transmit to the next generations[2]. Some of these factors depend on lifestyle and behavior[3]. The current knowledge improved evidence that environmental conditions can be affected gene expression without changing the DNA sequence. These changes can be heritable, which refers to epigenetic phenomena, recognized as a critical mechanism for regulating gene expression through DNA methylation and histone modification[4]. They also control the degree of gene activity, i.e., which genes are activated and inactivated[5].

Magnetic fields represent one of the critical environmental factors due to the increasing use of electrical devices, power lines, electromagnets and static magnetic in our lifestyle[6]. Some epidemiological studies have suggested a weak relationship between magnetic fields and some types of cancer in humans. The Swedish National Institute of Working Life conducted one of the most significant studies investigating various industries and occupations. The results indicated an association with chronic lymphocytic leukemia and an increased risk of brain cancer for males exposed to an average magnetic field of more than 0. 2 microteslas[7, 8]. It further reported a significant decrease in blood glucose, total protein, cholesterol and triglycerides in a magnetic group of quail compared to the control group, which may affect product performance.

In poultry production, most economic traits are quantitative traits, so the genetic and environmental factors are considered during the selection procedure for the desired traits. Epigenetics controls how the animal's genetic makeup is used; hence, epigenetics is driven by environmental cues that activate or deactivate various mechanisms that control gene expression during transcription, post-transcription, and translation levels[9]. Animal adaptation to the changes in ecological conditions occurs by adjusting their developmental growth, metabolism, and behavior to promote survival and reproduction[10,11]. The recent study aimed to investigate the epigenetic effects of the exposure of broiler breeders to magnetic fields on their progeny for meat production traits.

Materials and methods

This study was conducted in the poultry field belonging to the Department of Animal Production - College of Agriculture - University of Diyala, in the governorate of Diyala, Iraq, and the experiment aimed to determine the epigenetic effects of the magnetic field exposed to the broiler breeders Ross 308 on the productive and physiological traits of their progeny. The broiler progeny results from broiler breeder groups treated with the following conditions: (T1) the control, (T2) the stored semen exposure to the magnetic field of 803 gausses for 24 hr., (T3) the fertilized eggs exposure to a magnetic field of 250 gausses for 72 hr., (T4) the individual cages exposure to a magnetic field of 250 gausses during for 8 weeks. The resulting progeny chicks were reared for three replicates for 6 weeks, and Table 1 appears the chemical analysis of the diet used in the study.

The lengths of the chicks were measured using a ruler inserted from the beak to the end of the middle finger in the legs of chicks, and weekly feed intake= feed intake by day ×7.

Citation: Hassan K, Ebrahim A. Epigenetic effects on broiler exposure to magnetic field on progeny meat production traits. Revis Bionatura 2022;7(4) 48. http://dx.doi.org/10.21931/RB/2022.07.04.48
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

$$\text{Uniformity} = \frac{\text{Number of birds whose weight is within 10\% above or below the standard weight}}{\text{Total number of birds}}$$

$$\text{Dressing percentage.} = \frac{\text{carcass weight}}{\text{live body weight}} \times 100$$

chemical analysis of the diet	Parent		Progeny
	Male	female	
Protein	13.33	13.99	22
Energy	2788.8	2772.84	2900
Calcium	2.73	0.76	9.10
Phosphorous	0.32	0.47	4.60
Methionine	0.31	0.3	
Lysine	0.71	0.65	14.7
Fats	-	-	27
Fiber	-	-	26
methionine + cysteine	0.57	0.56	11.30
Ash	-	-	64.45

Table 1. Chemical analysis of the diet of the parent and progeny.

The statistical analysis procedure was performed using a general linear model with Complete Randomize Design CRD. The significant differences among the treatment means were detected using Duncan's multiple ranges test[12] at 0.05 probability.

Results and discussion

The epigenetic effects on progeny performance.

Table 2 shows no significant differences among groups in the length and weight of the chick at one day old. The results did not agree with (13) who recorded heaver chicks in the treated magnetic group compared with the control, hence found 46.40 and 42.90 g. for male and female chicks, respectively, while the control group was 43.80 and 41.10 g. respectively. The results agreed with (14), who found that exposing the fertile eggs to a magnetic field did not affect the chick's weight and length. This difference may be attributed to differences in magnetic field intensity, exposure period, and magnetic procedure in the current study compared to the previous studies mentioned above.

Epigenetic effects of the magnetic field in body weight of progeny

Table 3 shows no significant differences in the chick's body weight resulting from magnetically treated groups of broiler breeders and control groups. The results did not agree with (15) who reported a significant increase in body weight when the fertile eggs were exposed to a magnetic field of 1800 gauss for 30 and 90 minutes. The authors explained the differences due to the effect of the magnetic field on the thyroid gland. The results agree with (16) in body weight and feed conversion ratio, which used magnetic drinking water in the experiment. And in the same direction[17].

The results did not agree with (14), who found significant differences in weight gain among the experimental treatments. The results did not agree with (13), who used magnetic drinking water to broiler with an intensity of 500, 1000, and 2000 gauss and recorded body weights of 3490 and 2870 g at the age of 49 days for males and females in 2000 gauss treatment. Compared to the control, 2980 and 2607 g for males and females, respectively.

Epigenetic effects of a magnetic field in feed intake

Table 4 shows that were no significant differences in the feed intake in the progeny that resulted from magnetically treated groups of broiler breeders and control group. (18) reported that exposure of fertile eggs of broiler breeders with a magnetic field intensity of 18 gausses for 60 and 75 minutes reduces body weight gain and broiler feed intake

Traits	Control	Magnetization of stored semen	Magnetic fertile eggs	Magnetic of individual cages	P-Value
Chick length (cm)	17.57±0.03	17.30±0.10	17.32±0.10	17.38±0.13	0.467
Chick/egg weight (%)	72.68±1.12	70.36±0.21	74.03±0.87	74.93±1.68	0.081

Table 2. Means ± standard error of chick length, Chick/egg weight resulting from magnetic groups of boiler breeder Ross 308.

Age	Control	Magnetization of stored semen	Magnetic fertile eggs	Magnetic of individual cages	P- value
One day	45.91±0.25	44.86±0.10	46.34±0.40	45.37±1.48	0.547
Week1	144.65±3.49	141.10±1.47	147.72±6.68	149.22±4.52	0.604
Week2	379.54±7.65	376.32±5.11	376.46±8.48	375.54±8.00	0.981
Week3	823.63±216	828.66±26.46	817.28±34.45	824.04±19.17	0.992
Week4	137.83±37.48	1420.79±2.61	1467.51±40.55	1394.88±30.71	0.394
Week5	2138.63±22.28	2173.66±12.42	2144±52.43	2060.96±54.74	0.300
Week6	2790.50±40.18	2882.03±45.9	2904±53.17	2756.41±54.105	0.390

Table 3. Means ± standard error of body weight (gm) for Ross 308 broilers resulting from magnetic field treatments in the broiler breeders.

Age	Control	Magnetization of stored semen	Magnetic fertile eggs	Magnetic of individual cages	P- value
Week1	134.67±4.39	133.32±6.77	148.66±14.10	146.43±5.86	0.511
Week2	31.12±7.70	312.21±7.87	325.60±6.03	329.12±15.65	0.511
Week3	592.30±6.89	607.14±25.34	607.61±18.117	630.33±18.60	0.567
Week4	897.01±24.68	927.39±4.89	986.61±54.60	896.15±14.20	0.210
Week5	1119.53±23.64	1099.80±5.21	1105.56±26.02	1044.07±35.37	0.234
Week6	1260.86±27.50	14992.81±21.89	1285±7.85	1267.19±35.37	0.437

Table 4. Means ± standard error of the feed intake (gm) for progeny groups result from magnetic treatments in broiler breeders.

of progeny during the experimental period of 39 days. The results agree with (13), which indicated that there were no significant differences in the feed intake in the progeny resulting from fertile eggs' exposure to the magnetic field.

Epigenetic effects of the magnetic field in uniformity of body weight of progeny

Table 5 shows a significant decrease in the body weight uniformity of chicks resulting from magnetic of cages treatment (81.55%) compared to the control (90.32%) at one day old, and the difference disappeared during the rearing period. This situation may refer to the epigenetic effects of the magnetic field during embryonic development but not include the rearing period.

Conclusions

No significant epigenetic effects were detected due to the broiler breeder's magnetic field exposure and its relation to the progeny performance for meat production traits. There is evidence that the exposure of rearing cages to a magnetic field can enhance embryonic development, which reflects on the body weight of chicks at hatching

Funding

This research received no external funding.

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Age	Control	Magnetization of stored semen	Magnetic fertile eggs	Magnetic of individual cages	P- value
One day	90.32±1.62a	78.65±2.75ab	88.88±1.43a	81.55±1.40b	0.047
Week1	60.06±3.02	66.87±2.58	69.15±3.10	68.46±4.05	0.252
Week2	68.33±4.07	74.90±3.52	74.04±1.49	77.95±5.45	0.418
Week3	72.85±2.15	70.44±5.48	76.30±7.69	74.56±4.83	0.884
Week4	72.28±1.31	62.41±2.73	59.69±6.65	65.00±6.31	0.672
Week5	69.24±8.95	63.60±3.92	58.43±8.24	70.77±7.70	0.650
Week6	65.70±5.23	63.36±3.34	63.81±8.59	58.81±5.33	0.864

According to Duncan's multiple ranges test, means with different letters refer to significant differences from each other at P≤ 0.05

Table 5. Means ± standard error of the uniformity (%) for body weight for progeny groups result from magnetic treatments in broiler breeders.

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ARTICLE / INVESTIGACIÓN

Detection of polymorphism in growth differentiation factor 9 gene (GDF9) Exon1 and its association with litter size in local Iraqi goats

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DOI. 10.21931/RB/2022.07.04.49

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Abstract: Litter size is one of the most important economic traits in goats. Growth Differential Factor 9 (GDF9) Gene is suggested as a functional candidate for fertility, fecundity and twining rate. This study was performed to identify SNPs in exon1 of the GDF9 gene and their association with fertility traits in local Iraqi goats. The exon 1 of GDF9 was sequenced, and the SNP (Single nucleotide polymorphism) was determined in 36 local Iraqi goats. Then the association analyses between polymorphic locals of GDF9 and litter size were performed using the chi-square analysis procedure. The result shows that mutation (C 1902 G) was significantly associated with litter size in local Iraqi goats and the location of C1902 G mutation with three genotypes CC, CG and GG. A significant effect was found for birth weight, weaning weight and twin ratio, where the homozygous dominant genotype outperformed CC. A significant impact was seen for fertility and mortality, where CC topped CG and GG in fertility percentage and superiority of CG over CC and GG in mortality rate. The results preliminarily demonstrated that GDF9 was a critical gene affecting the fecundity of local Iraqi goats and that (C 1902 G) could be a potential genetic marker.

Key words: GDF9 gene, litter size, polymorphism, Iraqi goats.

Introduction

The interest in improving the production of farm animals, including goats, is very important to increase economic performance. The breeder follows breeding programs to increase the productive capacity of the animal by raising the frequency of good alleles in the herds and making a selection for them, including ovulation rate and size of births in the abdomen can be controlled[1]. One is inherited by several genes with few effects, and sometimes by one gene with significant effects, these genes are called fertility genes[2], as these genes in the DNA responsible for the size of the abdomen as well as fertility traits are essential in goats and have value High economic[3] and that the number of births in one ateras is considered a profit factor for educators, as the number of births in one womb depends in particular on the rate of ovulation and there are genes that have an effect on the ovulation rate where GDF9 is a growth factor and a member of the beta-type transforming growth family and it has a crucial role In the reproduction of sheep and goats through the development of the ovarian follicle and the rate of ovulation[4,5]. And not only in females, it was found to have an influential role in semen quality in male goats[6], New molecular genetics techniques enable researchers to use genes of high economic importance found in specific strains to be passed on to other strains[7], in general, the genetic makeup is the one that controls the body structure of the sheep. An organism, through the action of genes, means that the genes in the nucleotide sequence that are loaded on chromosomes in the cell nucleus are the main rulers of the animal's performance[8]. The mutation can cause a change in the part or the entire gene that controls traits, and a phenotypic variation occurs in the trait as a result[9]. The mutation can occur at the level of the gene, causing one or more nitrogenous bases, or appear at the chromosomal level, so they are called chromosomal mutations, which cause a change in chromosome number or structure[10].

Materials and methods

This study was conducted in the animal field of the Department of Animal Production at the College of the Agriculture / University of Diyala on a group of 36 local female goats, who were placed in 60% open and 40% closed pens, and they were fed green and dry fodder during pregnancy and green fodder after birth. The study verified the presence of some genetic markers that affect some reproductive traits (litter size). The DNA extraction process was carried out according to the company's steps attached to the extraction kit (Kit) of the Taiwanese company FAVORGEN. The efficiency of the extraction process was revealed by accessing the complete DNA by migrating the samples through an agarose gel. Primers were prepared for the purpose of gene phenotypic polymorphism, molecular detection and mutagenesis of the GDF9 gene.

The reproductive and productive performance of the experimental animals, which amounted to 36 goats, was calculated based on (ACAD,1996) according to the following rates:

Citation: Ali, O.; Al- Azzawi,S.; Detection of polymorphism in growth differentiation factor 9 gene (GDF9) Exon1 and its association with litter size in local Iraqi goats.Revis Bionatura 2022;7(4) 49. http://dx.doi.org/10.21931/RB/2022.07.04.49
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

$$\text{Fertility percentage} = \frac{\text{Number of mother goats}}{\text{Number of goats exposed to males}} \times 100$$

$$\text{Fertility rate at birth} = \frac{\text{Number of births produced}}{\text{Number of goats exposed to males}} \times 100$$

$$\text{Fertility rate at weaning} = \frac{\text{Number of weaned}}{\text{Number of goats exposed to males}} \times 100$$

$$\text{Childbearing rate at birth} = \frac{\text{Number of births produced}}{\text{Number of mother goats}} \times 100$$

$$\text{Newborn weight of each goat at weaning} = \frac{\text{Total weights of weaned newborns}}{\text{Number of mother goats}}$$

The significance of the percentages of these traits was tested using chi-square.

Primer sequence	Primer Name	Amplicon size (pb)	Name gene	Annealing Temp C
GAAGACTGGTATGGGGAAATG F -	Exon 1	462	GDF9	61
CCAATCTGCTCCTACACACCT R -	Exon 1			
Based on gene sequences available in the gene bank database Gene bank Sequence number				

Table 1. Primers sequence used in the study.

Results

The figure(1) shows the migration process of the PCR product of 36 female domestic goats.

Figure (2) shows the location of mutation 1902 of the first expression region, the result of using PCR, as it shows the variation from guanine to cytosine.

Table (2) shows a significant effect on the fertility rate, where the wild genotype CC is the highest significance, followed by the hybrid CG, then the recessive GG, which is less significant. The fertility rate was 44%, 8%, and 2%, respectively, and a highly effective effect on the fertility rate at birth for the structures. The three genotypes CC, CG, and GG, where the percentage reached 72, 11, and 5, respectively, where the dominant genotype is the highest significance, and the fertility rate at weaning found highly significant differences for the three genotypes CC, CG, and GG. The percentages were 63%, 8%, and 5%, respectively, where the dominant genotype was the highest significance. There were highly significant differences for the genotypes CC, CG, and GG in the proportion of twins, reaching 50%, 5%, and 5%. Also, the dominant genotype was the most signifi-

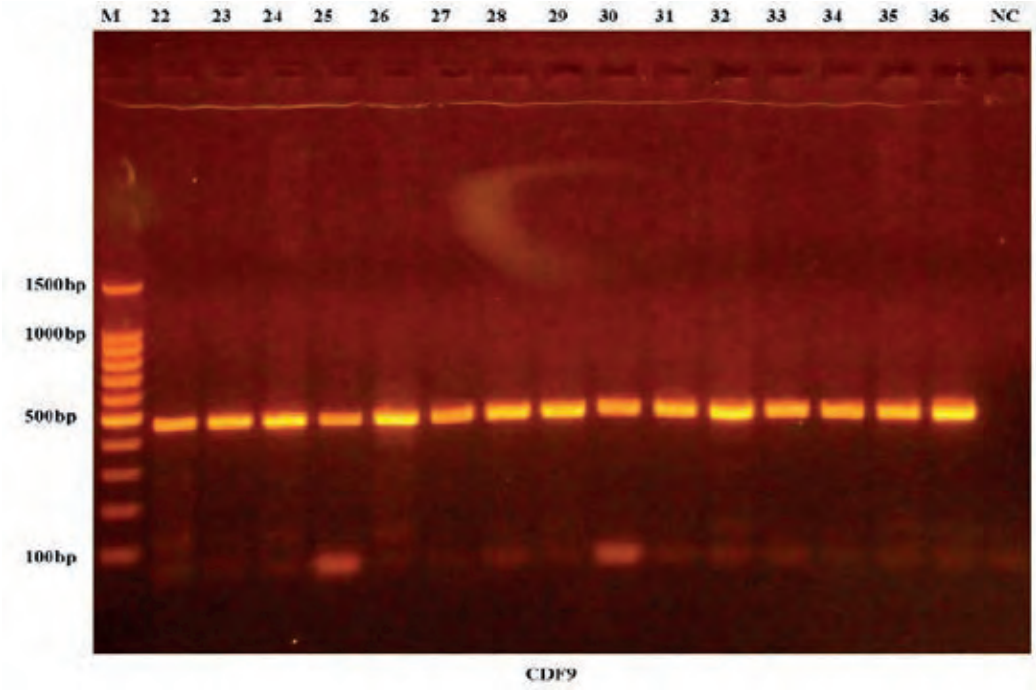

Figure 1. The appearance of the results of the polymerase chain reaction (PCR) through electrophoresis on a 1.5% agarose gel at a voltage of 100 volts and a current of 50 amperes. The standard DNA size is 100–1500 bp (21–36), representing the DNA bundles of the GDF9 gene, which appear to be 462 base pairs in length.

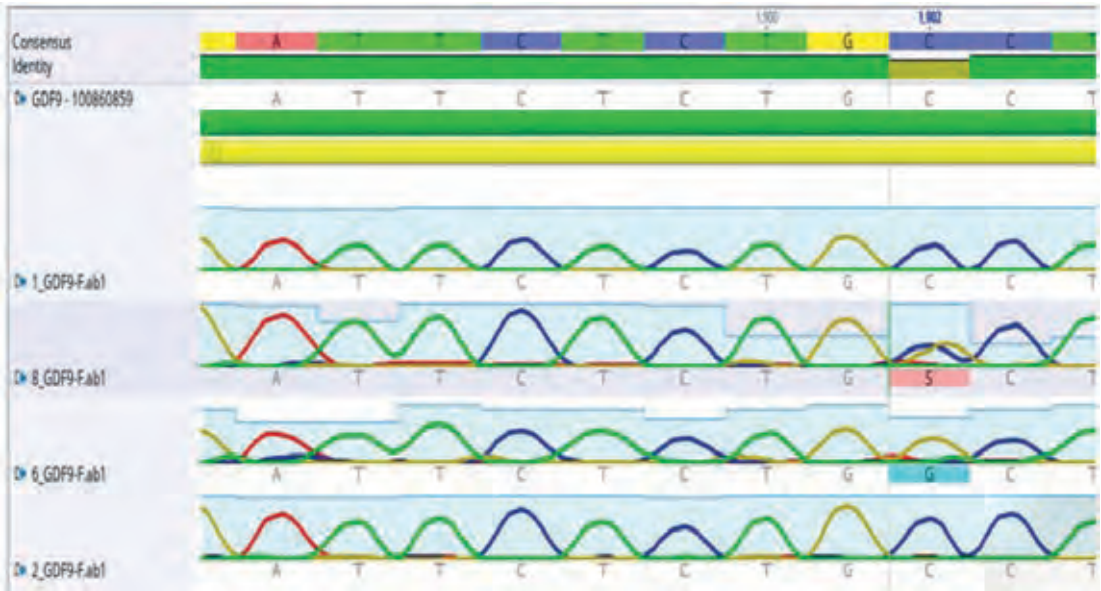

Figure 2. The location of the mutation 1902 of the first expression region, the result of using PCR, as it shows the variation from guanine to cytosine.

Adjective	Genotype			Total	Significant leve
	CC	CG	GG		
Number of goats exposed to males	28	7	1	36	NS
Number of mothers	16	3	1	20	NS
Fertility rate%	44 a	8 b	2c	54	*
number of births	26	5	1	32	NS
Fertility rate at birth%	72a	11b	5c	88	**
Fertility rate at weaning%	63a	8b	5c	76	**
Number of twin births	10	1	1	12	NS
Twins ratio%	50a	5b	5c	60	**
Weight per goat at weaning	12.6	1.7	1	15.1	NS
Peril ratio%	11b	20a	0c	31	*

Table 2. Reproductive Performance of mutant C 1902 G in goats.

cant. Table (2) shows a substantial effect of The fatalities for the genotypes CC, CG, and GG, which amounted to 11%, 20%, and 0%, respectively, where CG is the highest significance, and there was no significant effect on other reproductive traits. The GDF9 gene has an important impact on fertility and fertility, which is evident by determining the significance of the reproductive characteristics of goats. As it appears in the table, there is a significant effect On the fertility rate, fecundity at birth, fertility at weaning and the percentage of twins.

Discussion

In goats, there were no extensive studies of the GDF9 gene[12] has been mentioned through his study on the GDF9 gene in two breeds of goats, each line showing one heterogeneity site of three genotypes where the heterogeneity site of the first breed was found in which structures are AA, AG and recessive GG. The hybrid structure had the highest significant correlation with the size of the abdomen. In contrast, the site of heterogeneity for the second strain had three structures CC, CA, and AA, where a significant correlation was found with the number of births in one litter, where CC, CA had the highest correlation compared to the recessive AA, and in another study on sheep, where (13) reported a significant effect of GDF9 gene on fertility. Also, (14) mentioned, through his study on Chinese goats, that the GDF9 gene in the second exon has two sites of heterogeneity, and in each of the two sites, a significant effect was found on belly size. Different studies have suggested that GDF9 polymorphisms are associated with animal reproductive performance. A nonsense mutation (c.1111G>A) in GDF9 causing a Val→Met substitution was significantly associated with litter size in ewes[15]. The FecGE allele of a novel GDF9 polymorphism significantly increased ovulation rate and prolificacy in ewes[16]. In addition, GDF9 polymorphisms have also shown significant correlations with high prolificacy in goats[17], the number of transferable embryos and ova in cows[18], and sperm quality traits in bulls[19]. However, most

studies implicating the effects of GDF9 on reproductive performance have been conducted in mammals, and there are limited data regarding its role in the reproductive system of poultry. Numerous studies have demonstrated the importance of GDF9 in animal reproductive biology and its involvement as a candidate gene for reproductive performance[20]. However, most of these studies on the association between GDF9 polymorphisms and reproductive features have been on pigs, sheep, and goats. Twelve polymorphisms were discovered in the swine GDF9 gene[21], including one 314-bp indel and three SNPs in coding areas. Through direct sequencing of the DNA from Cambridge and Belclare sheep, (20) discovered eight SNPs, three of which were nonsense mutations and four of which were G>A mutations, in the GDF9 coding area. Four SNPs—C183A, C719T, A959C, and G1189A—were found by Dong *et al.*[22] in the goat GDF9 coding region. Investigators sequenced the whole chicken GDF9 gene's coding region and found 15 SNPs, including three in the promoter region, one in each of exons 1 and 2, and nine in the three ′ UTR. Nine of these SNPs were previously unreported in goats; two were missense mutations that caused the amino acids Ile and IleVal to be substituted in the coding sequence. These findings significantly increase our knowledge of the GDF9 polymorphisms underpinning the goat reproductive systems. This is most likely a result of the breed-specific effect or because this locus' products were linked to those of other mutations[23,24]. Therefore, it would be essential to find these six SNPs in additional goat breeds or big herds and carry out additional functional gain or loss trials to study the influencing mechanism further and provide a more precise justification for this notion. The observation that reproductive traits are complex quantitative features incorporating QTL, QTN, and interactions by the fecundity gene is also significant, as the prior studies tended to concentrate on a single SNP site[25]. Only Wang *et al.* (2019) discovered a strong correlation between the Q320P and V397I mutations in goats, which calls for more research into additional goat breeds worldwide[23].

Conclusions

Concluding that the GDF9 gene can be considered one of the important genes that affect the fertility of the local Iraqi goats, as through the technique of reading the nucleotide sequence, the location of the mutation 1902 was identified, which could be a potential genetic marker or as a marker, and that the genotype CC in this mutation is better than the two genotypes CG, GG by reproductive traits, where CC (the dominant wild genotype in Iraq) is significantly higher. Therefore, it was diagnosed that there was a highly significant effect of this gene on the characteristic of the number of litters at birth, the number of litters at weaning, and the number of twin births.

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ARTICLE / INVESTIGACIÓN

Detection of heat shock protein 70 genes (HSP70) polymorphism and its relationship with some productive and reproductive traits in local Iraqi goat

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DOI. 10.21931/RB/2022.07.04.50

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Abstract: The study was conducted on 30 local goats in the Diyala / Al-Khalis governorate belonging to goat breeders. The experimental samples' laboratory work was completed in the College of Agriculture - the University of Diyala and the Office of Scientific Progress - Al-Harithiya - Baghdad from 11/13/2021 to 6/7/ 2022. This study is to demonstrate the relationship between the genetic phenotypes of the heat shock gene (HSP70)in the productive and reproductive to the reproductive and effective performance of local Iraqi female goats. The percentage of genotypes for the C1653T mutation site in the goat sample was 53%, 37% and 10% for the CC, CT and TT genotypes, respectively, and the allelic frequency were 0.71 and 0.29 for the C and T alleles, respectively. The chi-square value was not significant between the genotypes. The genetics indicates that the population is in a state of (Hardy Weinberg) equilibrium ($p < 0.05$), which suggests that there is no selection pressure in favor of a specific genetic structure for this genetic locus. This mutation had a high effect ($P<0.01$) on the mortality rate, where the CT genotype was 27% superior to the two genotypes CC, which was 13%, and then the TT genotype had no mortality. As for the traits of milk production (daily and total), it was observed through this study that there was a balance for this mutation in this direction so that there is resistance to heat stress, which negatively affects milk production as the animal is in a state of harmony between resistance to heat stress and milk production. As for growth traits, the C1653T mutation had no significant effect on growth traits. There were no significant differences between the birth weight and weight gain genotypes. Still, concerning the importance of weaning, it was noted that the CC and TT genotypes were superior to the CT genotype (11.92 and 11.92). 11.70 and 10.17) kg, respectively.

Key words: Hsp70, Milk, litter size.Polymorphism, Iraqi goats.

Introduction

Goats are considered among the animals that were not raised in most Arab countries, despite the exploitation of this animal in many Asian and African countries for its production of twins. The poor later began to improve these animals, especially since there are breeds that have a broad scope for genetic improvement, which has a role in filling part of the deficit that results from the lack of milk and meat and its high prices[2,6]. (5) indicated that heat stress is among the various stresses that animals are exposed. Goats are considered one of the most capable animals in dealing with heat stress without affecting their health or product performance. She suffers from heat stress outside her comfort zone, and heat stress is defined as the imbalance the body produces from metabolic heat inside the body, and it dissipates to the surrounding areas. Heat stress also significantly affects some physiological, behavioral and hematological parameters of goats[1]. The importance of selection for animals that are more resistant and more adaptable to these climatic changes and different stress conditions due to their high genetic ability to protect their cells, which works to reduce the effects of heat stress. When exposed to various stress conditions[4], molecular genetics technology enables researchers to use the economically important genes present in certain Breeds and transfer them to other strains[7].

Materials and methods

This study was conducted on a sample of 30 local goats in the governorate Diyala / Al-Khalis belonging to a goat breeder. The laboratory work of the experimental samples was conducted in the laboratory of the College of Agriculture - the University of Diyala and the Office of Scientific Progress - Al-Harithiya - Baghdad for the period from 11/13/2021 to 6/7/ 2022; this study is to demonstrate the relationship of the genetic phenotypes of the heat shock gene HSP70 to the reproductive and productive performance of local Iraqi female goats. The reproductive performance of experimental animals was calculated based on[3] the following rates.

Measuring milk production The daily and total milk production of the experimental animals (30 goats) was calculated by hand milking each goat. The newborns were isolated from their dams at night and for 12 hours from eight in the evening until seven in the morning to milk the goats. The milking process began after the 15th day after birth, where they were. On this day, the first circuit lasted for two months. For each month, the milk was measured twice. A total of 4 measurements according to the following equation:

TMY= M1*2*T+ M2*2*T + M3 *2*T + M4*2*T

TMY= total milk production

M= Milk measurement (M1 the first measurement, M2 the second measurement, M3 the third measurement, M4 the fourth measurement).

T= The period between the two milkings.

Citation: Waheb O R, AL-Azzawi S H. Detection of heat shock protein 70 genes (HSP70) polymorphism and its relationship with some productive and reproductive traits in local Iraqi goat. Revis Bionatura 2022;7(4) 50. http://dx.doi.org/10.21931/RB/2022.07.04.50
Received: 25 August 2022 / **Accepted:** 12 October 2022 / **Published:** 15 November 2022

$$\text{Fertility percentage} = \frac{\text{No. of doe kids}}{\text{No. of doe exposed to male}} \times 100$$

$$\text{Fertility rate at birth} = \frac{\text{Number of births No.of kids born}}{\text{No. of doe exposed to male}} \times 100$$

$$\text{Prolificacy} = \frac{\text{No.kids born}}{\text{No. doe kids}} \times 100$$

$$\text{Prolificacy} = \frac{\text{No.kids weaned}}{\text{No. doe kids}} \times 100$$

$$\text{Loss Percentage} = \frac{\text{Number of dead births}}{\text{No. Kids in flock}} \times 100$$

$$\text{Twining rate\%} = \frac{\text{No. of kids born twins}}{\text{No. of doe kids}} \times 10$$

$$\text{* Total kids weight at weaning} = \frac{\text{Total weights of weaned newborns}}{\text{No. of doe kids}} \times 100$$

The dam's weight at birth was taken using a scale of 150 kg. The weight of the newborns was also measured at birth and at weaning three months after the date of birth using a scale of 150 kg, and then the weight gain before weaning was calculated through the difference between the weight at weaning and the weight at birth according to the following equation:

Weight gain = weaning weight - birthweight.

Blood samples were drawn from the jugular vein, and a 5 ml medical syringe was used after cleaning and sterilizing the area with 70% ethyl alcohol. After the blood was drawn, it was placed in dedicated tubes containing an anticoagulant substance (EDTA). Then these tubes were placed in a freeze at a temperature of 4-C to preserve them until the DNA extraction process was performed. And then, the initiators of the heat shock gene(HSP70), the third piece, were determined. This primer was designed by the Scientific Advancement Company (ASCO) located in Baghdad / Al-Harithiya, and this initiator consists of 903 nitrogen bases.

The data on reproductive performance traits, milk production and growth traits were analyzed using the general linear model (GLM) within the statistical program (SAS) (2012) according to the following mathematical model:

Yim= μ+Gi+eim

Yim= observation value of m

μ= overall mean

Gi= effect of genotypes

eim= The random error which is normally distributed with an average of zero and a variation of e□2

	Start	Stop	Length bp	Tm	GC%
Forward. GACCTCAACAAGAGCATCAA	1432	1452	20	60	**45**
Reverse. GATCCCAACAGTCTCCATAAC (Anti Sense)	2314	2335	21	60	**47.6**

Table 1. Initiator data of Exon 3 of HSP70. gene.

Reaction materials		Quantity
Master mix		**(µl) 12.5**
Primers	Forward	**(µl) 1**
	Revers	**(µl) 1**
Nuclease Free Water		**(µl) 7**
(DNA)genetic material		**(µl) 3**
Total volume		**25**
Aliquot per single rxn		**3 ɜ Master mix per from tube microliter 22 (µl)Template from**

Table 2. Materials used in the polymerase chain reaction (PCR) for the third segment of the HSP70. Gene.

The theChi-square-x2 test was used to compare the percentages of the genotype distribution of the studied gene mutation from the third segment of the HSP70 gene. The significant differences between the means were compared using Duncan's test (1955). The gene frequency for the studied region was calculated according to the following law.

A repeat of the first allele

As for the frequency of the second allele, it was according to the equation

qB= 1- PA

$$PA = \frac{2X \text{ No. of Homozygotus} + 1 \text{ x No of Heterozygotus}}{2X \text{ Total number of samples}}$$

Results

The extraction process was carried out successfully for the studied goat sample, consisting of 30 goats, using the diagnostic kit mentioned in the working materials and methods. 100 volts 50 mA for 60 minutes, and then the limited piece of HSP70, which had a size of 903 bp, was determined and amplified as shown in Figure (1) (2) (3) using PCR technology.

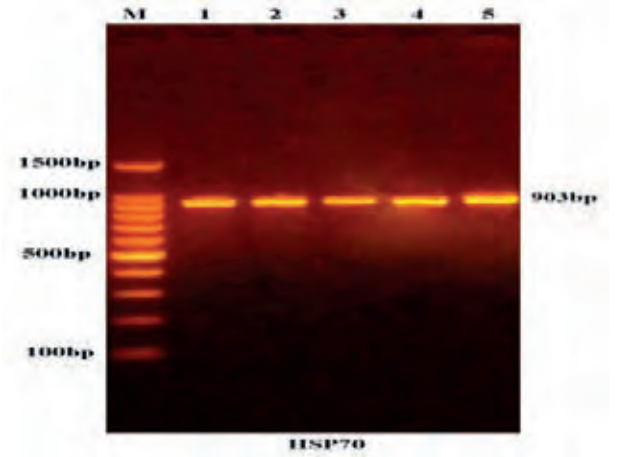

Figure 1. Detection of the product of PCR by electrophoresis on agarose gel, where M represents the standard DNA size of 100-1500bp (1-5) represents the DNA bands of HSP70 gene, which appears in size 903bp for 30 samples of female goat.

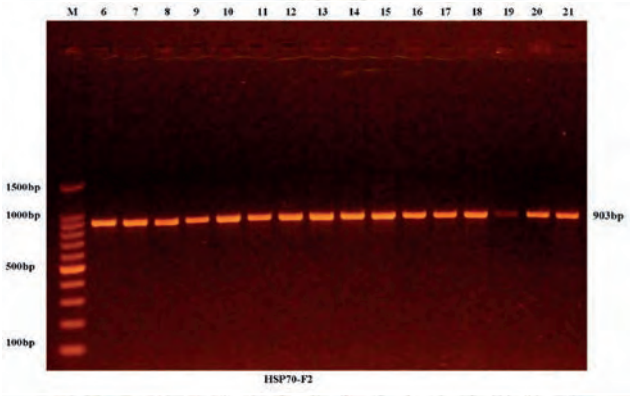

Figure 2. Results of the amplification of HSP70 specific region of Bos taurus samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 6-21 resemble 903bp PCR products.

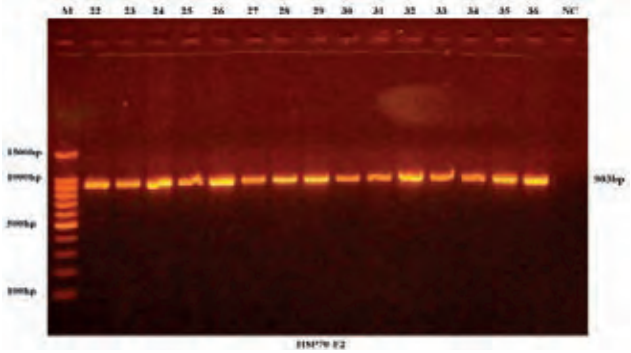

Figure 3. Results of the amplification of HSP70 specific region of Bos taurus samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 22-36 resemble 903bp PCR products.

The site of heterogeneity of the SNP in the third segment of the HSP70 . gene

The site (C1653T) contained three genotypes: CC (Wild), CT (Heterozygous) and recessive TT (Mutant), as the nitrogenous base changed from C to T.

Number, percentage and allelic frequency of the genotypes in the third segment of the heat shock gene (HSP70).

The percentage of genotypes for the C1653T mutation site in the goat sample was 53%, 37%, and 10% for the CC, CT and TT genotypes, respectively, and the allelic frequency was 0.71 and 0.29 for the C and T alleles, respectively. Hybrid CT and low percentages of TT genotype and that the value of chi-square did not have a significant difference between the genotypes, which indicates that the population was in a state of equilibrium (Hardy Weinberg) ($P < 0.05$), which suggests that there is no selection pressure in favor of a specific genotype for this locus.

Relationship of the C1653T mutation in the third studied segment of the HSP70 gene on the reproductive performance of goats.

Table (4) for the third mutation C1653T showed a significant effect ($P < 0.01$) in the percentage of deaths and that this percentage resulted from dividing the number of

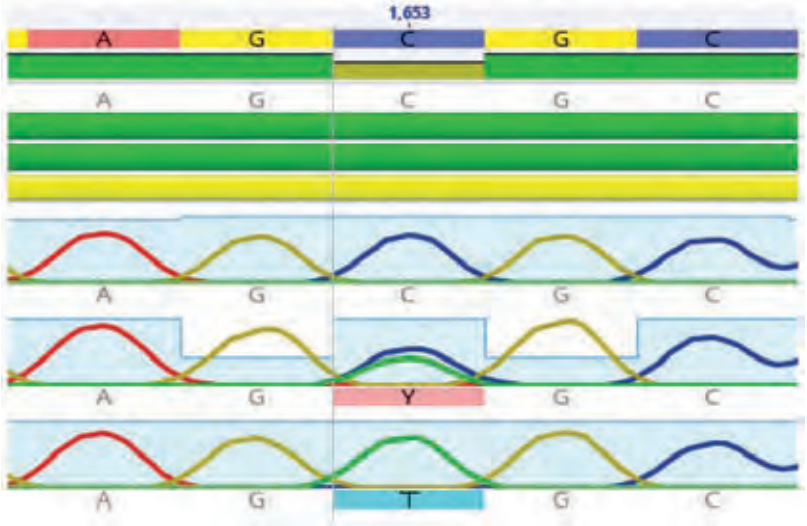

Figure 4. Analysis of the 1653 SNP locus of the HSP70 gene using Sanger sequencing. A single C peak is indicative of a homozygous C allele. A single T peak indicates a homozygous T allele, and a C peak and a T peak indicate a heterozygous T/C allele.

Adjective	genotype			total	significant level
	CC	CT	TT		
C1653T					
The number of goats exposed to male	16	11	3	30	NS
Number of mother goats	9	6	3	18	NS
Fertility percentage (%)	30	20	10	60	NS
number of births	15	11	4	30	NS
Fertility rate at birth (%)	50	36	13	99	NS
Fertility rate at weaning (%)	46	30	13	89	*
Number of twin births	6	5	1	12	NS
Twins (%)	33	27	5	65	NS
Loss Percentage (%)	13	27	0	40	**
Newborn weight per goat at weaning	8.45	5.06	2.81	16.32	NS

Table 3. The number and percentage of the HSP70 gene for the C1653T mutation site in the studied sample of female goats.

dead births by the number of births generated × 100, where the CT genotype outperformed by 27% on each of the two genotypes CC, where It was 13%, then TT had no fatalities. As for the other percentages, there are no significant differences.

Newborn weight per goat at weaning

Relationship of the C1653T mutation in the third studied segment of the HSP70 gene on daily and total milk production.

The present study showed that no-significantly differences to C1653T in daily and total milk production and lactation period traits (Table 6). The site of mutation was a balance for this mutation concerning the product characteristics (milk production) and resistance to heat stress, so this mutation worked in this direction so that there is resistance to heat stress. This negatively affected milk production, as the animal was in a state of balance between resistance to heat stress and milk production. Accordingly, according to the mutation that occurred, we did not notice any significant differences in milk production for different groups of goats.

The relationship of the studied mutation C1653T in the third studied segment of the HSP70 gene in growth traits

The results of the statistical analysis in Table (7) showed that there was no significant effect of the C1653T mutation on growth characteristics. There were no significant differences between the genotypes of birth weight and weight gain, but with regard to weight at weaning, it was noted that the CC and TT genotypes were superior to the structure. The CT genotypes were (11.92, 11.70, and 10.17) kg, respectively.

Discussion

It was shown from Table (4) that there were significant differences ($p > 0.01$) in the percentage of mortality. It was noted that the TT genotype was superior, as there were no deaths for the two genotypes, CC and CT. As for the fertility rate at weaning, it was noted that there were significant differences ($p > 0.05$), and it was pointed out that the genotype was superior CC on the other two combinations, CT and TT, while in the rest of the proportions, there were no significant differences. Regarding milk production (daily and total), as shown in table (5), it was observed that there were no significant differences. A balance was achieved between milk production and resistance to heat stress, and this is consistent with Contreras-jodar 2018[10]. The animal is in a state of resistance to heat stress, which negatively affects milk production and its components, so there is a

Adjective	genotype			total	significant level
	CC	CT	TT		
C1653T					
The number of goats exposed to male	16	11	3	30	NS
Number of mother goats	9	6	3	18	NS
Fertility percentage (%)	30	20	10	60	NS
number of births	15	11	4	30	NS
Fertility rate at birth (%)	50	36	13	99	NS
Fertility rate at weaning (%)	46	30	13	89	*
Number of twin births	6	5	1	12	NS
Twins (%)	33	27	5	65	NS
Loss Percentage (%)	13	27	0	40	**
Newborn weight per goat at weaning	8.45	5.06	2.81	16.32	NS

Table 4. The effect of the C1653T mutation on the reproductive performance of goats.

Boom site	Genotype	Number of animals 30	mean ± standard error	
			Daily milk production (kg)	Total milk production (kg)
C1653T	CC	16	0.81±0.08	48.69±5.27
	CT	11	1.13±0.12	67.92±7.63
	TT	3	0.94±0.23	56.82±14.36
Significant level			NS	NS

Table 5. The mean ± standard deviation of the total and daily milk produced for the experimental animals.

Boom site	Genotype	Number of animals 30	Mean ± standard error		
			Birth weight BWT	Weight Weaning(WWT)	Weight gain(WG)
C1653T	CC	16	2.62±0.12	a 11.70±0.35	14.17±0.64
	CT	11	2.25±0.15	b 10.17±0.75	12.14±1.06
	TT	3	2.57±0.21	a 11.92±0.97	14.50±1.17
Significant level			NS	*	NS

Table 6. The mean ± standard deviation for (birth weight(BWT), weaning weight(WWT), and weight gain(WG).

balance for the animal in resistance to heat stress and milk production for the different groups of goats according to the mutation that occurred. As for the growth characteristics, as shown in Table (6), There were no significant differences in birth weight and weight gain. As for weaning weight, it was noted that the genotypes CC and TT were superior to the genotype CT, reaching (11.70 and 11.92), respectively, over the genotype CT, which is 10.17.

Conclusions

We find the superiority of the goats carrying the TT genotype in the C1653T mutation, as no deaths were recorded. We also see the superiority of the goats carrying the CC genotype in the fertility rate at weaning. We also find the superiority of the goats carrying the CC and TT genotypes in the weaning weight of the same mutation. We find the superiority of the genotype CC and TT concerning weaning weight, which has an economic return for the breeder.

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ARTICLE / INVESTIGACIÓN

Extraction and characterization of phenolic compounds with antioxidant and antimicrobial activity from avocado seed (*Persea americana* mill)

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DOI. 10.21931/RB/2022.07.04.51

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Abstract: The increase in the demand for Hass avocado has brought a rise in the generation of inedible waste such as peel and seed, by-products that are rich in bioactive substances. In the present study, aqueous, ethanolic, and supercritical fluid extracts were obtained from fresh seed and dry seed, which were analyzed to determine the antioxidant capacity measured through 2,2-diphenyl-2-picrylhydrazyl free radical (DPPH); 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) methods as well as the content of phenolic compounds. In addition, the antimicrobial activity of strains of food interest, such as *Listeria monocytogenes*, *Salmonella enterica* Typhimurium and *Escherichia coli* was evaluated. The ethanolic extract of fresh seed presented the highest antioxidant and antimicrobial activity. The aqueous extract of fresh seed registered a significant antioxidant capacity but an absence of antimicrobial activity. In contrast, the ethanolic extract of dry seed showed a representative antimicrobial activity on both *S. enterica* Typhimurium and *L. monocytogenes*, but low antioxidant activity. *E. coli* exhibited resistance against all the assessed extracts. The results from this work highlight the opportunity to consider the Hass avocado seed extracts as a novel alternative to replace or reduce the use of synthetic antioxidant and antimicrobial additives in food.

Key words: Waste by-product, Aqueous extract, Ethanolic extract, Supercritical extraction, Polyphenols, Free radical.

Introduction

Persea americana Miller (Lauraceae) is an evergreen tree native to Central America and cultivated in tropical and subtropical areas. Its cultivation is highly valued because it presents an edible fruit known as an avocado that can ripen even after being harvested[1]. There are several varieties of this fruit; the Hass variety is the most accepted one by consumers. It is estimated that between five and six million tons of avocados are harvested annually, which continues to grow due to increased demand[2]. Hass avocado has different organoleptic and nutritional qualities that differentiate it from other fruits, including a smooth texture and a pleasant flavor and color. It stands out for its high content of fat-soluble vitamins, phytosterols, proteins and monounsaturated fatty acids such as linoleic acid[3]. These compounds have been widely related as beneficial for health against metabolic disorders such as hypercholesterolemia, arterial hypertension, diabetes and fatty liver disease[4,5].

An edible portion of the avocado is only a part of the whole fruit. It mainly corresponds to the pulp, consumed directly or used as the main ingredient for the production of guacamole or sauce or for the oil extraction that can be used in food, cosmetics or pharmaceutical preparations; the rest of the fruit is usually discarded or little used[6,7]. Avocado residues are the peel and the seed that together represent between 30-33% of the total weight of the fruit, being the seed approximately 15 to 16%[8]. These by-products are currently considered a promising source of various bioactive compounds, among which polyphenolic combinations stand out, such as flavonoids, phenolic acids, and tannins[9,10].

Several epidemiological studies have shown that a regular intake of polyphenols, especially flavonoids, reduces the impact of chronic diseases such as diabetes, various types of cancer and cardiovascular and neurodegenerative diseases[11,12]. The ability to trap free radicals generated in the course of these diseases is the mechanism that partly explains the contribution of these substances to a reduced occurrence of these pathologies[13]. On the other hand, polyphenols are also used as natural antioxidants, helping to increase the shelf life of food and other consumer products[14]. Likewise, many reports of antibacterial and antifungal capacity for these substances[15].

Significant amounts of procyanidins A and B have been reported in Hass avocado seed[16]. Also citric acid, hydroxytyrosol glucoside, caffeoylquinic acid, tyrosol glucoside, catechin and quercetin derivatives, and vanillic acid. A higher sterol content has also been reported in the seed extracts than in the pulp, which has also shown anti-inflammatory, anticarcinogenic and increased free radical scavenging potential[17].

Based on this, the present study aimed to obtain super-

Citation: David D, Alzate A F, Rojano B, Copete-Pertuz L S, Jhony Gutierrez R E, Zapata-Vahos I C. Extraction and characterization of phenolic compounds with antioxidant and antimicrobial activity from avocado seed (Persea americana mill). Revis Bionatura 2022;7(4) 51. http://dx.doi.org/10.21931/RB/2022.07.04.51
Received: 25 December 2021 / **Accepted:** 12 June 2022 / **Published:** 15 November 2022
Publisher's Note: Bionatura stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

critical fluids extracts of Hass avocado seed, evaluating the antioxidant activities and antibacterial effects (against foodborne pathogens microorganisms present in food such as *Listeria monocytogenes*, *Salmonella enterica* Typhimurium and *Escherichia coli*).

Materials and methods

Plant material

The samples of avocado fruits (*P. americana* Mill. cv. Hass) were collected in Guarne (average temperature: 21 ºC and altitude: 2,150 m.a.s.l.) department of Antioquia, Colombia.

Once the material was pulped, the seeds were sanitized with an antibacterial solution of 0.3%v/v citrosan for 5 minutes. After the disinfection process, the material was cut into smaller pieces with a knife. Half of this material was ground and labeled FS, Fresh Seed. Another half of the seeds were dried at 50 °C for six hours and finely crushed in a cyclone-type laboratory mill (Udy Corporation, Colorado, USA); this material was labeled DS, Dry Seeds.

Extraction of bioactive compounds

Extraction with ethanol and water

Ethanolic and aqueous extracts were prepared separately using 10 g of seed powder (DS and FS) and 50 mL of each solvent. The mixtures were homogenized in ultraturrax at 9000 rpm for 5 min (IKA-Werk, Staufen, German) and centrifuged for 15 min at 5000 ×g. Subsequently, the supernatants were added in amber flasks, and 50 mL of the solvents were added to the precipitates again to homogenize and centrifuge them a second time. Finally, the two supernatants were mixed and stored at -20°C until use[18]. Table 1 shows the identification and coding of the samples according to the extraction method used.

Extraction by supercritical fluid

Extraction was carried out in a Speed SFE Applied Separations equipment (Pennsylvania, USA) with a capacity of 100 mL. CO_2 in the supercritical state was employed as extraction solvent using temperatures (40°C and 50°C) under pressures of 20 MPa and 30 MPa. In this experiment, 30 g of dried seeds in the avocado powder were taken and extracted for 40 min, and later the extract was stored in sealed test tubes at -20°C until the tests were carried out[19]. Extraction capacity supercritical fluid was expressed in percentage. Assays were performed in triplicate.

Total polyphenol content

Polyphenol quantification was performed by the *Folin-Ciocalteau* colorimetric method, with some modifications[20]. In test tubes, 50 µL of the sample, 125 µL of *Folin-Ciocalteau* reagent, 425 µL of sodium carbonate solution (7.1%), and water to complete 1000 µL were mixed. The reaction mixture was kept in the darkness for 60 min, and after this time, the absorbance was determined at 760 nm in a PG-Instruments spectrophotometer (Leicestershire, United Kingdom). A calibration curve was made using gallic acid as a standard. The results were expressed as equivalent gallic acid per 100 g sample (mg GAE/100 g).

Antioxidant capacity tests

DPPH free radical scavenging activity

The antioxidant activity of Hass avocado seeds was evaluated by the ability to trap the stable radical DPPH (2,2-diphenyl-2-picrylhydrazyl free radical), according to the methodology reported by Rojano (2011)21 with some modifications. In a test tube, 10 µL of sample and 990 µL of a DPPH solution (0.2 mM) were added. The exact amount of DPPH and 10 µL of the sample solvent were used as a reference. After 30 min of reaction, the absorbance at 517 nm was measured in a Multiskan Spectrum spectrophotometer (Thermo-Scientific, Waltham, MA, USA). The calibration curve was constructed using Trolox as a reference antioxidant, and the results were reported as equivalent µmol Trolox per 100 g of sample (µmol TE/100 g).

ABTS free radical scavenging activity

The antiradical ability of Hass avocado seeds is based on the discoloration of ABTS•+. The cationic radical ABTS•+ was generated by an oxidation reaction of ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6 ammonium sulfonate) with potassium persulfate according to the methodology described by Rojano (2011) 21. In the assay, 10 µL of sample and 990 µL of ABTS•+ solution were used; after 30 min of reaction, the change in absorbance with respect to a reference was determined at 734 nm. The reference consisted of a mixture of 990 µL of ABTS•+ radical solution and 10 µL of sample solvent. A calibration curve was constructed using Trolox as the reference antioxidant, and the results were reported as equivalent µmol Trolox per 100 g of sample (µmol TE/100 g).

Ferric reducing antioxidant power (FRAP) assay

FRAP methodology evaluates the ability of a sample to reduce the complex formed between iron and TPTZ (2,4,6-tripyridyl-s-triazine), where iron in its ferric form (Fe^{+3}) becomes ferrous iron (Fe^{+2}). This change can be measured spectrophotometrically[22]. A 50 µL portion of the sample was mixed with 900 µL of a FRAP solution (1 mL of 10 mmol/L TPTZ and 1 mL of 20 mmol/L $FeCl_3$ in 10 mL of pH 3.4 acetate buffer). The mixture was incubated for 30 min, and the absorbance was measured at 593 nm on a multiscan spectrum spectrophotometer (Thermo-Scientific). A standard curve was made using ascorbic acid as a reference. The results were expressed as equivalent mg of ascorbic acid per 100 g sample (mg AAE/100 g).

Assays for Hydrophilic and Lipophilic Antioxidant Capacity (Oxygen Radical Absorbance Capacity - ORAC)

Experiments employed Trolox as a standard and controlled temperature and pH conditions (37 °C and 7.4, respectively). The assay was determined by diluting Trolox in 75 mM phosphate buffer (pH 7.4) and water-acetone (1:1, v/v) for ORAC-H (Hydrophilic) and in 7% β-methyl cyclodextrin for ORAC-L (Lipophilic). An excitation and emission wavelength of 493 nm and 515 nm, respectively, were used. 3 mL of the following mixture were prepared: 21 µL of a 10 mM fluorescein solution, 2899 µL of phosphate buffer, 50 µL of 600 mM AAPH, and 30 µL of the sample or 500 mM Trolox; as a control, the sample solvent was used. The antioxidant effect was calculated using the differences in areas under the fluorescence intensity decay curve between the negative control and the sample, and it was compared

against the area under the Trolox curve[23]. The results were expressed as equivalent Trolox values (µmol TE/100 g of sample), according to Equation 1.

$$ORAC\ Value = \frac{(AUC_{sample} - AUC_{control})}{(AUC_{Trolox} - AUC_{control})} . f\left[\frac{Trolox}{Sample}\right] \qquad \textbf{Equation 1}$$

Where AUC is the area under the curve corresponding to the sample, control or Trolox, and f is the concentration ratio between Trolox and the sample.

Antibacterial activity

The antimicrobial capacity of the extracts was determined using the good diffusion methodology for 3 strains of foodborne pathogenic bacteria, *Escherichia coli* (ATCC 25922), *Salmonella enterica* Typhimurium (ATCC 14028) and *Listeria monocytogenes* (ATCC 19118) at the first pass, according to the procedures described by Hudzicki[24]. This methodology allows us to measure and compare the areas of inhibition of microbial growth of the extracts. The activation of the pathogenic microorganisms was carried out 24 h before the tests on the trypticase soy agar (TSA) method reported by Davidson and Parish[25]. They were seeded by the streak method and incubated at 37 °C. After activation, the colonies were inoculated in Brain Heart Infusion (BHI), then the microorganisms were standardized on a scale of 0.5 Mac Farland; later, the solution was seeded on the surface Muller Hinton agar. Besides, four equidistant wells were made in the culture medium to obtain a circular well to the bottom of the Petri dishes. Then, randomly, 100 µL of each extract covered each well, and the Petri dishes were incubated for 24 h at 37 °C. Water and ethanol were used as negative controls (C-), and ciprofloxacin antibiotic (160 mg/mL) as positive control (C+). In addition, two Petri dishes were left with only the culture medium as environmental control. The results were reported as the inhibition halo diameter around the wells measured in millimeters (mm). All assays were performed in triplicate.

Statistical analysis

The results of the antioxidant capacity were analyzed using the analysis of variance (ANOVA) followed by Tukey's multiple comparison tests at 5% level of probability. The tests were carried out with the R Studio software version 3.5.0.

Results and discussion

Different Hass avocado seed extracts were obtained from some processes such as supercritical fluid extraction, as well as percolating and mechanical maceration with water and ethanol for both fresh seeds (FS) and for seeds that were dried at 50 ºC (DS) (Table 1). In total, 8 different extracts were analyzed to determine the presence and content of total polyphenols and phenolic acids; a complete antioxidant characterization was also carried out, and finally, the antibacterial capacity was presented on three of the main pathogenic bacteria. The results were promising and allowed further progress in using this by-product as a source of bioactive substances.

Extraction of bioactive substances

After performing the supercritical fluid extraction of the dried Hass avocado seeds (DS) under different pressure and temperature conditions, the capacity in the extract production was measured (Table 2). The highest extraction capacity (2.01%) was reached at 50 °C and 30 MPa pressure, followed by 50 °C and 20 MPa (1.63%), which indicates that a higher temperature substantially improves the extractive processes of this seed when this extraction methodology is used.

The extracts obtained presented lipophilic characteristics and, according to Daiuto et al. (2014)[26]. Hass avocado seed gives 3.32% lipids. However, these values may vary depending on the height and soil where the avocado is grown; this would explain why supercritical fluid extraction using CO_2 as a solvent is very efficient in extracting lipid compounds. The extraction capacity results achieved in this research are comparable with those reported by Polania (2014)[27], who obtained extractions capacities of 0.5% at 10 MPa and 60 °C, using ethyl acetate and 3% ethanol as cosolvent and capabilities of 3.6% with 6% methanol at 15 MPa and 50 °C.

Total polyphenol content

The Hass avocado seed extracts presented significant values of total polyphenols; the results are shown in Table 3. The extraction of fresh seeds (FS) with ethanol was the one that presented the best results (10.65 mg GAE/g), followed by aqueous extraction for FS (8.28 mg GAE/g); whereas the seeds dried (DS) had a polyphenol content much lower than FS. These results indicate that the drying of the sources, despite being at a relatively low temperature (50 ºC), caused significant degradation of this compound. Similar results have been reported by Segovia-Gomez *et al.* (2014)[28], who evaluated a process to optimize the extraction of polyphenols with different proportions of ethanol. The phenolic compounds in the seed extracts are of great importance since they are part of a group of secondary metabolites considered natural antioxidants with potential benefits for human health: anticancer activity, anti-inflammatory activity 17, and inhibition of gastric ulcer formation[29].

Description	Name
Aqueous extract of fresh seed	FS-H_2O
Ethanolic extract of fresh seed	FS-EtOH
Aqueous extract of dry seed	DS-H_2O
Ethanolic extract of dry seed	DS-EtOH
Supercritical fluid 40 °C and 20 MPa	SFC1
Supercritical fluid 40 °C and 30 MPa	SFC2
Supercritical fluid 50 °C and 20 MPa	SFC3
Supercritical fluid 50 °C and 30 MPa	SFC4

Table 1. Identification and coding of samples obtained by different extraction methods for Hass avocado seeds.

Pressure (MPa)	Temp. (°C)	Extraction Capacity (%)
30	50	2.01 ± 0.27
30	40	1.43 ± 0.12
20	40	1.27 ± 0.23
20	50	1.63 ± 0.18

Table 2. Percentage of extraction capacity by supercritical fluid of dried Hass avocado seeds.

Sample	Total polyphenols (mg GAE/100g)	DPPH (µmol TE/100g)	ABTS (µmol TE/100g)	FRAP (mg AAE/100g)
FS-EtOH	1064.95 ± 32.33^a	14.30 ± 1.37^a	25.98 ± 3.88^a	898.30 ± 65.25^a
FS-H_2O	828.37 ± 102.9^b	10.66 ± 1.48^b	17.56 ± 2.30^b	616.23 ± 23.03^b
DS-H_2O	433.56 ± 21.64^c	3.00 ± 0.68^c	8.69 ± 0.98^c	265.49 ± 14.4^c
DS-EtOH	177.49 ± 23.89^d	0.73 ± 0.06^d	7.61 ± 1.59^c	63.46 ± 8.49^d

The same letters per column mean no significant differences between extracts.

Table 3. Antioxidant capacity and content of antioxidant metabolites of aqueous and ethanolic extracts of Hass avocado seeds.

Antioxidant capacity

Considering that oxidation reactions are complex and that the bioactive substances present in Hass avocado seeds can exert their antioxidant action by different mechanisms, different antioxidant tests, based on the transfer of an electron (DPPH, FRAP and ABTS) and transfer of hydrogen atoms (ORAC), were carried out in this research to characterize the antioxidant potential of this by-product[30]. The antioxidant capacity of the FS and DS extracts in water, and ethanol is presented in Table 3. The highest antioxidant activity was obtained for the ethanolic extract of fresh seed (FS-EtOH), reaching the highest values in the different antioxidant tests, followed by fresh seed extract with water (FS-H_2O). On the other hand, the dry seed extracts in water (DS-H_2O) and ethanol (DS-EtOH) showed lower values, demonstrating a low antioxidant capacity. From this, it is inferred that the high antioxidant capacity presented by the FS extracts is directly related to the higher content of total polyphenols presented by these samples and that the heat treatment showed a significant decrease in the antioxidant capacity.

The results attained by FRAP showed that Hass avocado seed extracts present reducing substances that contribute to the total antioxidant capacity, especially FS-H2O and FS-EtOH, which have the highest values. Other authors reported similar behaviors of reducing power in ethanolic extracts from avocado seeds with values among 0.28 - 0.73 mg/mL $FeSO_4$[31].

The response of the DPPH free radical scavenging capacity was superior for FS compared to DS in water and ethanol. Some studies that have characterized the avocado found that the Hass variety contains greater antioxidant capacity than other avocado varieties, such as Fuerte. Wang and others (2010) evaluated the parts of the Hass avocado, finding 189.8 µmol TE/g FW in the peel and 164.6 µmol TE/g FW in the seed[6].

In ABTS assays, a similar behavior to DPPH and FRAP was evidenced, finding that the FS-EtOH sample presented the highest trapping of the cationic radical ABTS•+, followed by FS-H2O. In another investigation, values of 300 µmol TE/g DW were reported for avocado seeds[30], taller than those found in this work. Some authors have reported the presence of procyanidins, catechins, epicatechins, caffeoylquinic acid, vanillic acid, flavonoids, phenylpropanoids and tannins, among others, in by-products of avocado[32,33]; compounds that contribute to the stabilization of DPPH and ABTS free radicals. Thus, the Hass avocado seed extracts presented a high reducing power and a remarkable antioxidant capacity by the methodologies used in this research.

Regarding the ability to trap oxygen free radicals (ORAC), this methodology was used in its two variants; the hydrophilic variant (ORAC-H) was performed on the extracts of FS and DS obtained in water and ethanol, and the lipophilic variant (ORAC-L) was used for the seed extracts which were attained by supercritical fluid. Results are summarized in Table 4.

The aqueous extracts had a greater capacity to trap the hydroxyl radical (52.23 µmol TE/ g sample and 51.47 µmol TE/ g sample for DS-H2O and FS-H2O, respectively) than the ethanolic extracts (10.62 µmol TE/ g sample and 14.75 µmol TE/ g sample for DS-EtOH and FS-EtOH, respectively), showing statistically significant differences. Regarding the lipophilic samples, no statistically significant differences were found, ORAC-L values were around 30 µmol TE/ g sample. The potential of these extracts to trap radicals is very important due to the harmful effect of free radicals in food and various biological systems[34].

Wang *et al.* (2010)[6] reported ORAC activity in various avocado varieties, finding 428.8 µmol equivalents of Trolox TE/g of fresh seed for the Hass variety. The authors

ORAC-H (µmol TE/100 g sample)		ORAC-L (µmol TE/100 g sample)	
DS-H_2O	5222.7 ± 799.6[a]	**SFC1**	2716.5 ± 262.2[a]
FS-H_2O	5146.6 ± 722.9[a]	**SFC2**	3029.7 ± 219.8[a]
DS-EtOH	1061.8 ± 75.5[b]	**SFC3**	2905.9 ± 238.0[a]
FS-EtOH	1474.9 ± 135.4[c]	**SFC4**	2946.8 ± 291.6[a]

The same letters per column mean that there are no significant differences.

Table 4. ORAC values of extracts obtained by supercritical fluid, water and ethanol from fresh and dried Hass avocado seeds.

found, for 7 types studied, that in the avocado seed, there is a remarkable antioxidant activity measured by DPPH and ORAC, in addition to the content of phenols and procyanidin, which were above the results indicated for the avocado skin and pulp. Another investigation reported ORAC activity of 310 µmol Trolox/g, dry weight[30]. Soong and Barlow (2004)[35] reported that the content of secondary metabolites and the antioxidant capacity are higher in the seed than in the Hass avocado pulp.

Antibacterial capacity

Figure 1 illustrates the inhibition halos for *S. Typhimurium* (ATCC 14028), *L. monocytogenes* (ATCC 19118) and *E. coli* (ATCC 25922) facing the extracts evaluated. Significant differences (p-value <0.05) with respect to the positive control (ciprofloxacin, 160 mg/mL) were found. The FS-EtOH and DS-EtOH extracts presented a greater growth inhibition of *L. monocytogenes* and *S. Typhimurium* than the others. Ethanolic extracts were obtained from fresh and dry seeds, reaching an inhibition range similar to the positive control, with diameters of 38.16 mm and 26.94 mm for *L. monocytogenes* and 26.17 mm and 19.90 mm. for *S. Typhimurium*, respectively (Figure 1). Some studies attribute this activity to compounds such as phytosterols, triterpenes, fatty acids, furoic acids, flavonoids, polyphenols, and proanthocyanidins[36]. Raymond and Dykes (2010)[37] reported higher antimicrobial activity of ethanolic extracts than aqueous ones in Gram-positive and Gram-negative bacteria except for *E. coli*, with minimum inhibitory concentrations of 104.2 µg/mL for *Salmonella enteritidis* and 416.7 µg/mL for *L. monocytogenes*.

The FSC2 and FSC3 extracts showed growth inhibition of *L. monocytogenes*, whereas the other extracts evaluated did not show an antimicrobial effect. *E. coli* presented resistance against all assessed extracts, and both positive and negative (water and ethanol) control behaved according to expectations (Figure 1). The results obtained for *E. coli* agree with results previously reported by Hennessey (2019)[38], who found resistance from *Staphylococcus aureus* subsp. ATCC 29213 and *E. coli* against Lorena variety avocado seed extracts extracted with solutions of sodium hydroxide, ethanol, and water; while Romaní *et al.* (2017)[39] found that the ethyl acetate fraction of the seeds of *Persea americana* Mill, Hass variety, presented phenolic compounds with antibacterial activity at a concentration of 10% facing the *E. coli* strain with a minimum inhibitory concentration of 0.625 mg/mL. Rodríguez *et al.* (2011)[40] evaluated the antimicrobial activity of the seed, skin and pulp of two varie-

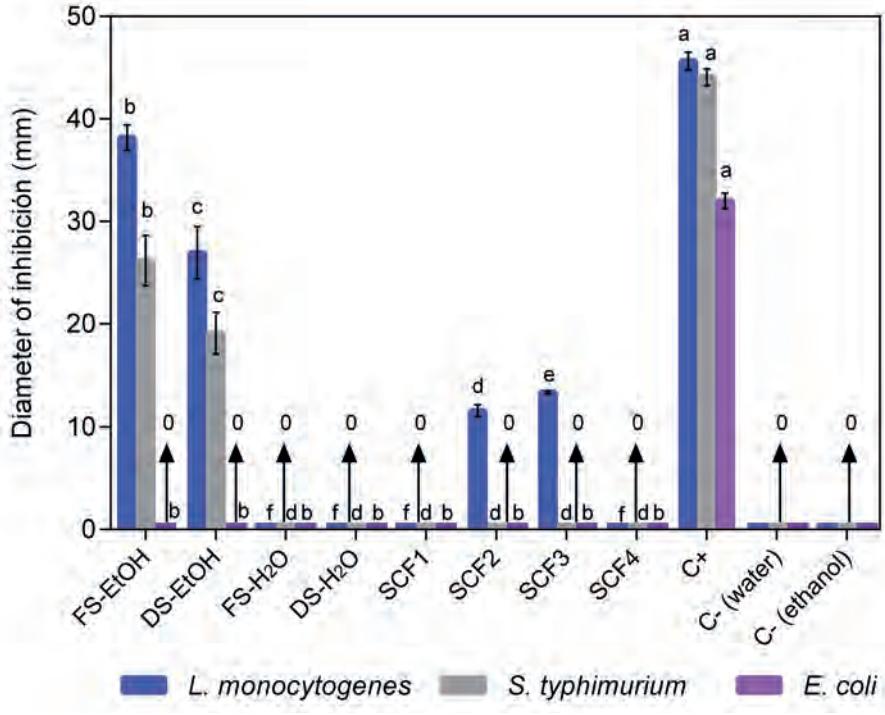

Figure 1. Antibacterial activity of FS-EtOH, DS-EtOH, FS-H2O, DS-H2O, SFC1, SFC2, SFC3, SFC4 avocado seed extracts, C+ and C- on L. monocytogenes, S. typhimurium and E. coli. The same letters per column mean that there are no significant differences.

ties of avocado on *E. coli* CECT 4267. The authors found antimicrobial activity for the Fuerte variety and resistance facing the Hass variety extract, which suggests dependence of the antimicrobial response to the plant variety and the type of solvent used in the extraction processes.

Conclusions

The seeds generated as by-products of avocado industrialization are an interesting source of extracts with essential concentrations of polyphenols and antimicrobial potential. In this work, different extracts were obtained in various solvents, and the best results of antioxidant and antimicrobial capacity were for the ethanolic extract of fresh seed (FS-EtOH), being very effective in the growth inhibition of *S. Typhimurium* and *L. monocytogenes* microorganisms. The aqueous extract of fresh seed (FS-H_2O) also had a great antioxidant capacity, although it did not show any inhibitory effect on the bacteria evaluated. The dry seed ethanolic extract (DS-EtOH) showed significant antimicrobial activity on *S. typhimurium* and *L. monocytogenes*, but low antioxidant activity. With these results, natural Hass avocado seed extracts can be considered a good alternative in the food industry to replace or reduce the use of antioxidant additives and synthetic antimicrobial agents.

Acknowledgements

The authors would like to thank Servicio Nacional de Aprendizaje -SENA (Tecnoparque nodo Rionegro) and the Universidad Católica de Oriente (UCO) for the financial support of this research.

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REVIEW / ARTÍCULO DE REVISIÓN

Efecto de los hongos formadores de micorriza arbuscular (HFMA) en la producción de aceites esenciales en romero (*Rosmarinus officinalis* L.)

Effect of arbuscular mycorrhizal fungi (AMF) on essential oil production in rosemary (*Rosmarinus officinalis* L.)

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DOI. 10.21931/RB/2022.07.04.52

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Resumen: Se realizó una campaña experimental en invernadero con el fin de evaluar el efecto de los hongos formadores de micorriza arbuscular (HFMA) en la producción de aceites esenciales en romero (*Rosmarinus officinalis* L.). Para el efecto se implementó un diseño experimental completamente al azar, con tres tratamientos compuestos por suelo más inóculo multiespórico (*Glomus* spp, *Acaulospora* spp, *Entrophosfora* spp, *Scutellospora* spp.), suelo más inóculo monoespórico (*Entrophosfora colombiana*), y el tratamiento control sin inocular, todos estos con diez repeticiones por tratamiento. El suelo utilizado para todas las unidades experimentales fue esterilizado y ajustado a 0,02 mg L^{-1} de P (fósforo), concentración óptima para la condición micorrizal. Las variables respuesta fueron: contenido de P foliar, la masa seca aérea, la colonización micorrizal, y el rendimiento en aceites esenciales. Los resultados de masa seca aérea indican incrementos significativos de los tratamientos inoculados comparados con el tratamiento control. El P foliar no presento diferencias entre tratamientos. La colonización micorrizal promedio de los tratamientos inoculados fue del 73%. En cuanto a los aceites esenciales producidos por las plantas de romero, los resultados fueron inversos a los de la masa, donde se evidencio mayor rendimiento en el tratamiento control. De acuerdo con los datos, el uso de HFMA en la producción de romero, favorece el desarrollo de la planta, de otro lado, el rendimiento en aceites esenciales podría ser compensado por la mayor biomasa producida, lo que permitiría a los productores incrementar la cantidad de aceite extraído.

Palabras clave: Aceites esenciales, hongos micorrízicos arbusculares, *Rosmarinus officinalis*.

Abstract: An experimental greenhouse campaign was conducted to evaluate the effect of arbuscular mycorrhizal fungi (AMF) on the production of essential oils in rosemary (*Rosmarinus officinalis* L.). For effect, a completely randomized experimental design was implemented, with three treatments composed of soil plus multisporic inoculum (*Glomus* spp, *Acaulospora* spp, *Entrophosfora* spp, *Scutellospora* spp.), soil plus monosporic inoculum (*Entrophosfora colombiana*), and the control treatment without inoculation, all of these with ten replicates per treatment. The soil used for all experimental units was sterilized and adjusted to 0.02 mg L^{-1} of P (phosphorus), the optimum concentration for the mycorrhizal condition. The response variables were: foliar P content, aerial dry mass, mycorrhizal colonization, and essential oil yield. The results of aerial dry mass indicate significant increases in the inoculated treatments compared to the control treatment. Leaf P showed no differences between treatments. The average mycorrhizal colonization of the inoculated treatments was 73%. As for the essential oils produced by the rosemary plants, the results were the inverse of those of the mass, where a higher yield was found in the control treatment. According to the data, the use of HFMA in the production of rosemary favors the development of the plant; on the other hand, the yield of essential oils could be compensated by the higher biomass produced, which would allow producers to increase the amount of oil extracted.

Key words: Essential oils, arbuscular mycorrhizal fungi, *Rosmarinus officinalis*.

Introducción

El Romero (*Rosmarinus officinalis*) es una planta aromática de origen mediterráneo, que pertenece a la familia Lamiaceae, sus hojas presentan altas concentraciones de aceites esenciales con propiedades anti microbiales[1,2], se ha utilizado para incrementar la circulación de la sangre, y es efectiva contra reumatismo y migraña[3]. Debido a su importancia y demanda internacional, el cultivo de romero se ha incrementado en todo el mundo. En Colombia, una de las regiones con mayor potencial para el cultivo de esta planta es el altiplano del oriente antioqueño (AOA). Los suelos del AOA donde se produce romero, generalmente tienen características de Andisoles, suelos desarrollados a partir de materiales provenientes de erupciones volcánicas (ceniza, pómez, lava, etc.) y/o de materiales volcaniclásticos en los que la fracción coloidal está dominada por minerales de bajo rango de ordenamiento o por complejos Al-humus[4].

Citation: Sierra-Escobar J A, Arteaga M M, Quiroz C. Efecto de los hongos formadores de micorriza arbuscular (HFMA) en la producción de aceites esenciales en romero (Rosmarinus officinalis L.). Revis Bionatura 2022;7(4) 52. http://dx.doi.org/10.21931/RB/2022.07.04.52
Received: 25 January 2022 / **Accepted:** 22 October 2022 / **Published:** 15 November 2022

Los materiales de estos suelos le dan unas características únicas y distintivas, llamadas propiedades ándicas, las cuales se manifiestan en una baja densidad aparente, una alta carga variable y una alta capacidad de retención de fosfatos y de humedad[5]. En general, los Andisoles del AOA se han desarrollado bajo régimen de humedad údico caracterizados por presentar, en la capa arable, pH fuertemente ácido, alto contenido de materia orgánica, colores muy oscuros, alta carga variable, bajos contenidos de bases intercambiables y de fósforo disponible, además de desbalances entre las bases y contenidos de Al intercambiable[6]. El fósforo, elemento que es esencial en la fotosíntesis, la respiración celular y en el metabolismos energético de la planta[7] se hace menos disponible cuando el suelo es de origen Ándica, es por esto que las plantas tienen que desarrollar mecanismos especializados para la toma de este elemento, uno de estos mecanismos, es la necesidad de realizar simbiosis benéficas con microorganismos del suelo como los hongos formadores de micorrizas, los cuales juegan un gran papel al hacer rápida la disponibilidad de este elemento y de otros para el buen funcionamiento fisiológico de las plantas[8].

La palabra micorriza, se deriva del griego *myco*, que significa hongo, y *rhiza*, que hace referencia a las raíces de las plantas. Se podría decir que es una simbiosis mutualista formada entre los órganos de absorción sanos (raíces y rizomas), se cree que entre 86% al 94% de las plantas del mundo poseen simbiosis con algún tipo de micorriza[9], ya sean terrestres, epífitas o acuáticas[10]. Existes varios tipos de micorriza, los principales son: micorrizas arbusculares, ericoides, orquidioides, y ectomicorrizas. Las micorrizas arbusculares, son el tipo más cosmopolita, el cual se encuentra en aproximadamente el 74% de las plantas con flor del mundo[9]. Se caracteriza por la integración estructural y metabólica entre ambos simbiontes que se manifiesta en la nutrición, sanidad, productividad y adaptabilidad de las plantas a las condiciones ambientales[11]. Se encuentran en la literatura numerosos artículos que demuestran la efectividad de los hongos formadores de micorriza arbuscular (HFMA) para mejorar el rendimiento y productividad de las plantas[12-14]. Estas ventajas que dan a las plantas los HFMA en el rendimiento vegetal, se están utilizando con mayor frecuencia por parte de los cultivadores de diversos cultivos agrícolas. Incluso, algunos productores de romero del AOA utilizan con frecuencia inóculos comerciales de HFMA, con el fin de aumentar la productividad. El efecto positivo de HFMA en romero ya ha sido probado en estudios llevados a cabo por investigadores de Egipto e Irán, dando como resultado incrementos significativos en el crecimiento de las plántulas evaluadas, es de aclarar que ambos estudios se realizaron en condiciones de suelo diferentes[15,16]. Sería interesante conocer si en las condiciones del AOA, la aplicación en el suelo de HFMA incrementaría la biomasa y productividad del romero. Y si esto favorece también su concentración en aceites esenciales. Por tal motivo, el presente estudio pretende conocer la respuesta de romero a la inoculación de hongos formadores de micorriza arbuscular (HFMA) en el rendimiento en biomasa, y la concentración de aceites esenciales.

Materiales y métodos

Localización

El experimento se realizó en uno de los invernaderos de la Universidad Católica de Oriente (6° 9´ 15.2" N, 75° 22´ 10.4" W y altitud de 2112 m) ubicado en la cordillera central de los Andes (Rionegro, Colombia). El sitio presenta, una temperatura promedio de 17°C y una precipitación anual de 2200 mm con régimen bimodal dos periodos de lluvia y dos secos. Además, el invernadero se encuentra en la zona de vida bosque húmedo montano bajo[17].

Suelo

Se utilizó un suelo orgánico (Andisol), el cual tenía características similares al suelo de las áreas cultivadas de romero del AOA. Éste suelo fue usado como materia prima para todos los análisis y ensayos posteriores. Los 499 kg de suelo que se utilizaron, se desinfectaron en caldera, donde se vaporizaron a 90 psi y a 90oC durante dos horas, esto con el fin de eliminar los microorganismos patógenos, semillas y propágulos vegetales indeseados.

Análisis físico-químico del suelo

Submuestras de suelo fueron utilizadas para realizar varios análisis como la isoterma de adsorción de P, que fue elaborada con base en la metodología propuesta por Fox & Kamprath[18], los resultados de este procedimiento sirvieron para ajustar el suelo a 0,02 mg L^{-1} de P (concentración óptima para la condición micorrizal). Además, se realizó un análisis de suelos de fertilidad en el laboratorio de suelos de la Universidad Nacional Sede Medellín (tabla 1). Aquello sirvió para ajustar los niveles de Mg y S que presentaban deficiencias, para lo cual se aplicó 12,31 g $MgSO_4$ por kilogramo de suelo. Los métodos analíticos utilizados para el análisis de suelos fueron: textura (Bouyucos), pH (agua, 1:1, v:v), materia orgánica % (Walkley y Black), Calcio, Magnesio y Potasio cmolc kg^{-1} (Acetato de Amonio 1M, pH 7), Fósforo (Bray II); Hierro, Manganeso, Cobre y Zinc mg kg^{-1} (Olsen – EDTA); Boro no detectable mg kg^{-1} (Agua Caliente); Nitrato mg kg^{-1} (Sulfato de Aluminio 0.025M) y Amonio mg kg-1 (KCl 1M).

Inóculos y análisis biológico

Para los tratamientos se trabajaron dos inóculos micorrizales, uno monoespórico (*Entrophosfora colombiana*) y otro multiespórico compuesto por especies de los géneros *Glomus* spp, *Acaulospora* spp, *Entrophosfora* spp, y *Scutellospora* spp.

Para garantizar la efectividad de los inóculos de HFMA y homogenizar su aplicación en los diferentes tratamientos,

Textura				pH	C.E	M.O	Al	Ca	Mg	K	Na	CICE	P	S	Fe	Mn	Cu	Zn	B
A%	L%	Ar%	Clase		dSm-1	%	Cmolc Kg^{-1}						mg kg^{-1}						
58	32	10	FA	5,6	5.6	14,5	-	7,2	0,7	0,49	0,04	8,4	32	9	38	76	1	5	0.23

Tabla 1. Análisis fisicoquímico del suelo utilizado para el experimento.

se realizaron conteos de esporas y se utilizó la técnica del número más probable de propágulos micorrizales infectivos (NMP) propuesta por Porter[19] para hongos micorrizales. El conteo de esporas se realizó para conocer el número de esporas por gramo de cada inóculo. Para el efecto cada muestra se transfirió a un vaso de precipitación con 300 mL de agua corriente y 0,15 g de pirofosfato de sodio. Se agito y se dejó decantar pasando el sobrenadante por una batería de tamices (250, 106 y 53 µm). Se centrifugó con una solución de sacarosa al 50% durante 5 minutos; el sedimento fue recogido del tamiz en un embudo cubierto con papel filtro. Finalmente se realizó el conteo en estereoscopio separando los morfotipos más representativos. Por otro lado, se determinó NMP. Para esto se utilizó un sustrato compuesto por una mezcla esterilizada compuesta por suelo (50%) y arena (50%), que se llevó a una concentración de P soluble de 0,02 mg L^{-1} por medio de una isoterma (tal como se explicó anteriormente). La especie indicadora fue *Leucaena leucocephala*, ampliamente utilizada para este fin[20]. Para el montaje, se emplearon diluciones de 10^{-1} a 10^{-5} cada una con 5 repeticiones por dilución. Para un total de 25 unidades experimentales por muestra de inóculo. Cada unidad experimental estaba compuesta por 20 g de inóculo y de 180g de sustrato esterilizado. Una vez germinadas las semillas fueron colocadas en los potes con su respectiva dilución, aplicándoles semanalmente la solución nutritiva Hoagland libre de P hasta el final del experimento. Posteriormente, las plántulas de *L. leucocephala* se cosecharon a los 65 días y se les realizó tinción de raíces de acuerdo con lo propuesto por Kormanik *et al.*[21]. La observación de la raíces se realizó en el estereoscopio con la técnica de presencia-ausencia y los resultados se analizaron con la tabla de Cochran[22]. De acuerdo con los resultados del NMP se aplicaron 13,4 g/kg de suelo de *A. colombiana*, y 12,69 g/kg de suelo de inoculo multiespórico.

Material vegetal

Se escogió el genotipo R2 de romero producido por la Unidad de Biotecnología de la Universidad Católica de Oriente y se multiplicaron vegetativamente por la técnica de enraizamiento de mini esquejes, de los cuales se tomó material vegetal entre 3 y 5 cm. Este material fue propagado en bandejas de germinación con un sustrato compuesto por turba más cascarilla de arroz quemada en relación 2:1 esterilizado, utilizando para el efecto la metodología propuesta por Castro *et al.*[23].

Diseño experimental

El diseño experimental fue completamente al azar, el cual estaba compuesto por tres tratamientos: el tratamiento 1 (*A. colombiana*), tratamiento 2 (inoculo multiespórico) y el tratamiento 3 (control sin inocular) (Tabla 2). Cada tratamiento tuvo 10 réplicas. La unidad experimental correspondió a una plántula de romero contenida en una caneca plástica con capacidad de 20 litros de agua, la cual fue llenada con 12 kg de suelo más su respectivo tratamiento. El total de unidades experimentales fueron 30.

La cosecha del experimento tuvo dos períodos de tiempo: el primero para realizar la evaluación del fósforo foliar, la masa aérea seca y el porcentaje de colonización (cosecha a los 119 días después de la siembra) en la cual se cosecharon 15 unidades experimentales, cinco por tratamiento. Y el segundo periodo tuvo la finalidad de producir mayor masa (100 g por unidad experimental como mínimo) requeridos para la extracción del aceite, y la masa seca aérea (nuevamente), la última cosecha ocurrió a los 280 días después de la siembra y con las 15 unidades experimentales restantes.

Análisis estadístico

Para el análisis estadístico se realizaron las pruebas de normalidad de Kolmogorov-Smirnov y Shapiro-Wilk. Además, los datos fueron sometidos a análisis de varianza y a la prueba de rangos múltiples de Duncan, para lo cual se empleó un nivel de significancia P £ 0,05. Se utilizó para estos análisis el paquete estadístico Statgraphics Centurión XVII y Rwizard 1,0.

Variables respuesta

Contenido de P foliar. Se estimó a través de la concentración de P en el disco de hoja al momento de la cosecha[24], en donde se analizó el contenido de P en las hojas jóvenes completamente desarrolladas 20. Para tal fin, una porción circular del tejido foliar se removió con un perforador (6 mm de diámetro). El contenido de P se expresó en términos de µg por disco de hoja. El P se determinó por el método del azul de molibdato[25], luego de reducir los discos de hoja a cenizas en la mufla a 500°C por 3 h. Este método de muestreo no destructivo fue originalmente propuesto por Aziz y Habte[26].

Masa seca aérea

Para determinar la masa seca aérea, cada plántula se cortó en el cuello de la raíz, se secó el material vegetal en horno a 60 °C por 72-96 horas (hasta obtener un peso constante) y finalmente se pesó cada unidad experimental. Este procedimiento se realizó en dos momentos, a los 119 y a los 280 días después de la siembra.

Colonización micorrizal

Para la colonización micorrizal, se tomaron submuestras de 0,6 a 1 g de raíces finas de cada unidad experimental, se lavaron y se cortaron aproximadamente a 1 cm de longitud, las cuales se sumergieron en una solución de KOH al 10% para su aclaración, durante 24 horas, después se acidificaron en una solución de HCl (10%)[27], y se tiñeron con fucsina ácida al 0,15 % en ácido láctico con base en la metodología propuesta por Kormanik *et al.*[21]. Para la cuantificación de la colonización, se extienden 20 raicillas en un portaobjetos, cada raicilla se leyó en tres puntos

Tratamiento	Fuente de inóculo
1	*Entrophosfora colombiana*
2	*Glomus* spp. *Acaulospora* spp. *Entrophosfora* spp. *Scutellospora* spp.
3	Control (sin inocular)

Tabla 2. Nomenclatura para los tratamientos.

(arriba, abajo y en el medio), se observaron al microscopio en 10X y 40X, y se marcaron las estructuras micorrícicas observadas (esporas, hifas y arbúsculos) que equivalen a los campos colonizados, y los campos observados son la totalidad de campos, independientemente de que estén colonizados o no. El porcentaje de colonización se calculó con la fórmula[28].

$$\% \text{ colonización micorrizal} = \frac{\text{No. de campos colonizados}}{\text{No. total de campos observados}} * 100$$

Rendimiento en aceite esencial

A las 15 plantas restantes se les extrajo el aceite por medio de la técnica de hidrodestilación con trampa de Clevenger. El tiempo estimado para la extracción fue de 2 horas y 56 minutos. Una vez obtenido el aceite fue secado con sulfato de sodio anhidro. El rendimiento en la extracción se expresó en ml de aceite esencial/100 g de materia seca.

Resultados

La concentración de P foliar no presentó diferencias estadísticamente significativas entre los tratamientos, aunque el tratamiento control (sin inoculación) presento un valor ligeramente superior de 5,0 mg de P por disco (Tabla 3).

No se presentaron diferencias significativas (LSD, P £ 0.05) entre los promedios comparados.

En cuanto a masa seca aérea, en la primera cosecha (119 días después de la siembra) se presentaron diferencias significativas entre todos los tratamientos, con valores superiores en MSA a favor de los tratamientos inoculados, con valores de 42,5 y 34,5 para *E. colombiana* y Comercial respectivamente. En la segunda cosecha (280 días después de la siembra), se continuo con la misma tendencia, en donde los inóculos exhibieron diferencias significativas (Figura 1). Estos resultados evidencian un efecto positivo de los HFMA en romero, siendo el tratamiento 1 (inóculo monosporico con *E. colombiana*), el que presentó mejor efectividad.

La colonización micorrizal fue similar en los tratamientos 1 y 2, con valores de 77 y 69% respectivamente. Mientras qué, el tratamiento 3 (control) presentó colonización del 4,7%, muy por debajo de los demás tratamientos. La colonización en el tratamiento control se debe, posiblemente, al tamaño de los potes utilizados (que facilitan la diseminación de esporas) y al ambiente confinado del invernadero (Tabla 4).

El rendimiento en aceite esencial evaluado mediante la técnica de destilación por arrastre de vapor con trampa Clevenger, tuvo diferencias estadísticamente significativas entre los tratamientos T3 (control) yT1 (*E. colombiana*) con valores de 2,17 y 1,775 ml 100g-1 respectivamente. De acuerdo con los datos, no hubo efecto indirecto de la inoculación de HFMA en romero en cuanto al rendimiento en la extracción de aceite (Tabla 5).

Tratamiento	P foliar (µg/disco	Desviación estándar
T1 (*E. colombiana*)	3.66	0,926283
T2 (Comercial)	3,98	0,752994
T3 (Control)	5,0	2,00375

Tabla 3. Contenido de P en los discos de hoja de romero en función de la concentración de P en la solución del suelo y la inoculación micorrizal.

Tratamiento	Colonización micorrizal (%)	Desviación estándar
T1 (*E. colombiana*)	77	8,52485
T2 (Comercial)	69	17,1864
T3 (Control)	4,7	5,68133

Tabla 4. Porcentaje de colonización micorrizal.

Tratamientos	Rendimiento en aceite (ml $100g^{-1}$)	Desviación estándar
T1 (*E. colombiana*)	1,775 b	0.236291
T2 (Comercial)	1,98 ab	0.216795
T3 (Control)	2,17 a	0.198997

Columnas con letras disimiles indican diferencias significativas (Duncan, P ≤ 0.05)

Tabla 5. Rendimiento en la extracción de aceite.

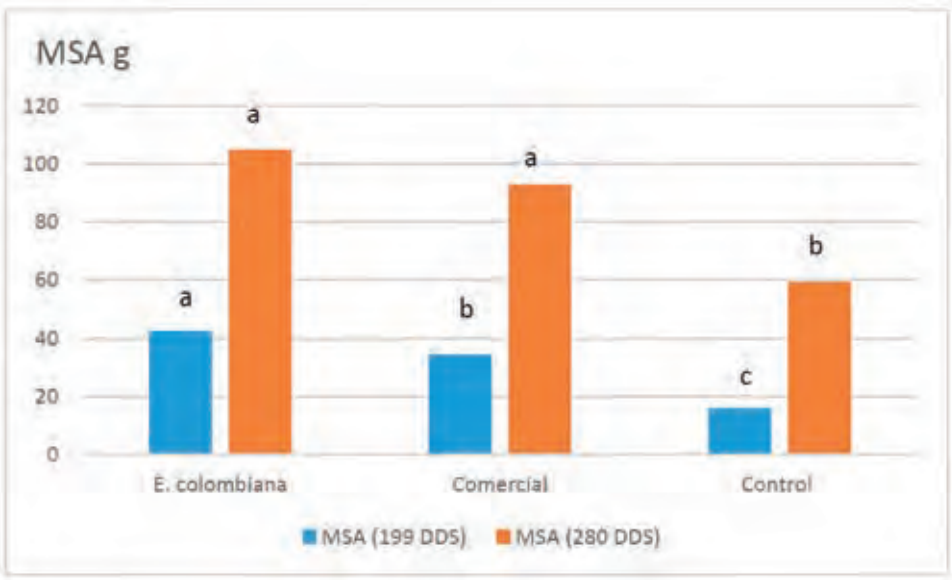

Figura 1. Masa seca aérea (MSA) de los diferentes tratamientos aplicados a romero en función de la inoculación micorrizal, y en dos momentos de cosecha. Columnas con letras disimiles indican diferencias significativas (Duncan, P £ 0,05).

Discusión

Los resultados indican que romero (*Rosmarinus officinalis*) respondió bien a la inoculación de HFMA, lo cual mejoró significativamente la masa seca aérea. La respuesta positiva, en cuanto a masa, de plantas de romero inoculadas ha sido ampliamente reportada, aunque en condiciones diferentes. Por ejemplo, Sanchez-Blanco[29] evaluaron *R. officinalis* en condiciones de estrés hídrico y observaron que las plantas micorrizadas tuvieron incrementos significativos en masa seca aérea. En otro estudio en Irán, conducido por Bahonar *et al.*[15] y en condiciones salinas, *R. officinalis* respondió bien a la inoculación micorrizal, con incrementos significativos en masa. Lo mismo ocurrió en suelos calcáreos de Egipto, en donde Shehata *et al.*[16] evaluaron diferentes microorganismos (HFMA, *Bacillus megatherium*, *Azospirillum brasilense*) y sus mezclas y encontraron respuesta positiva a la inoculación en cuanto a la masa, sobre todo cuando las mezclas incluían HFMA. La anterior información confirma que *R. officinalis* puede adaptarse bien a diferentes condiciones de suelo, pero para hacerlo requiere de los HFMA.

Al igual que la masa, la colonización micorrizal en romero ha sido ampliamente estudiada, los porcentajes de colonización micorrizal varían dependiendo del mico simbionte, pero en general *R. officinalis* responde bien a la mayoría de ellos. Valores entre 20 y 70% de colonización han sido reportados[30]. Lo que está en concordancia con los resultados aquí obtenidos (Tabla 5.).

La relación de los HFMA con metabolitos secundarios se encuentra estudiada, y se ha reportado que dependiendo de la especie de planta se favorecen los HFMA. Por ejemplo en *Valeriana officinalis*., *Salvia officinalis*., *Trifolium pratense*.y *Origanum vulgare* los HFMA mejoraron su productividad, incluyendo la acumulación de metabolitos secundarios, aunque es de resaltar que este comportamiento depende de la especie de planta[31]. Además, se ha encontrado que los flavonoides, estimulan el crecimiento de los hongos micorrizales[32]. Otros autores han observado en raíces de plantas micorrizadas, cambios en la biosíntesis de metabolitos secundarios[33], los cuales van desde la acumulación de flavonoides[34] triterpenoides[35], ciclohexanona y apocarotenoides[36], fitoalexinas[37] y compuestos fenólicos[38], es decir que los HFMA tienen un efecto fisiológico en los metabolitos secundarios producidos por las plantas. En este sentido, los cambios en las raíces colonizadas de *R. officinalis* pueden afectar la producción de aceites esenciales de forma diferencial, por ejemplo, en el presente estudio no hubo respuesta positiva en el rendimiento de aceites esenciales y su concentración, resultados similares encontró Bagheri *et al.*[39], en donde evidenciaron incrementos significativos en la masa seca cuando se inocularon las plantas de romero con *Glomus intraradices* y *G. mosseae*, cosa que no ocurrió con los aceites esenciales. De otro lado, Bahonar *et al.*[15] observaron incrementos en masa y aceites esenciales cuando inocularon plántulas de romero en condiciones salinas. Igual tendencia presento Shehata *et al.*[16] en suelos calcáreos utilizando la mezcla de HFMA, *Bacillus megatherium* y *Azospirillum brasilense*. La anterior información sugiere que los mecanismos que estimulan el incremento en la producción de aceites esenciales en las plantas de *R. officinalis* no son claros, y pueden ser diferenciales, ya que sus efectos cambian dependiendo de las condiciones del suelo y del mico simbionte utilizado.

Conclusiones

Las plántulas micorrizadas exhibieron diferencias significativas en cuanto a biomasa en los dos períodos evaluados, es decir a los 199 y 280 días después de la siembra. Sin embargo, el mayor rendimiento en aceites esenciales lo presento el tratamiento control (sin inocular), lo que sugiere que los HFMA disminuyen la concentración en los tejidos de romero de estos aceites. Es de aclarar, que, si se tiene en cuenta la cantidad total de biomasa de los tratamientos inoculados, el aceite producido para el cultivador sería mayor, por lo cual sería recomendable la micorrización de las plántulas para incrementar la producción de romero.

Agradecimientos

Agradecemos el apoyo económico de la Dirección de Investigación y Desarrollo de la Universidad Católica de Oriente.

También agradecemos al grupo de Estudios Florísticos y a los programas de Ingeniería Ambiental y Agronomía de la misma Universidad, que siempre estuvieron prestos a orientarnos en todo momento.

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ARTICLE / INVESTIGACIÓN

Computational discovery of novel anthelmintic natural compounds from *Agave Brittoniana* trel. Spp. *Brachypus*

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DOI. 10.21931/RB/2022.07.04.53

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Abstract: Helminth infections are a medical problem in the world nowadays. This report used bond-based 2D quadratic indices, a bond-level QuBiLs-MAS molecular descriptor family, and Linear Discriminant Analysis (LDA) to obtain a quantitative linear model that discriminates between anthelmintic and non-anthelmintic drug-like organic-compounds. The model obtained correctly classified 87.46% and 81.82% of the training and external data sets, respectively. The developed model was used in a virtual screening to predict the biological activity of all chemicals (19) previously obtained and chemically characterized by some authors of this report from *Agave brittoniana* Trel. spp. *Brachypus*. The model identified several metabolites (12) as possible anthelmintics, and a group of 5 novel natural products was tested in an in vitro assay against *Fasciola hepatica* (100% effectivity at 500 µg/mL). Finally, the two best hits were evaluated *in vivo* in bald/c mice and the same helminth parasite using a 25 mg/kg dose. Compound 8 (Karatavinoside A) showed an efficacy of 92.2% in vivo. It is important to remark that this natural compound exhibits similar-to-superior activity as triclabendazole, the best human fasciolicide available in the market against *Fasciola hepatica*, resulting in a novel lead scaffold with anti-helminthic activity.

Key words: TOMOCOMD-CARDD Software, QuBiLs-MAS, nonstochastic and stochastic bond-based quadratic indices, LDA-based QSAR model, Computational Screening, Anthelmintic Agent, *Agave brittoniana* Trel. spp. *Brachypus*, Fasciola hepatica.

Introduction

Helminths remain among the most common chronic infections, with more than one-third of the world's population infected at any time[1]. Currently, the high cost and toxicity of anthelmintics as well as the emergence of resistant strains of pathogenic helminths, have stimulated the desire to search for additional chemotherapeutic agents allowing a more efficient control of these parasites[2-4]. A practical solution to this problem is to develop effective drugs from less expensive and more available raw materials[5]. Natural products (NP) can be one of these materials for various reasons: 1) They inspired most of the active ingredients in medicines, 2) NP exhibit enormous structural diversity, 3) NP are the result of centuries of evolutionary pressure to create biologically active molecules, 4) the structural similarity of protein targets across many species, and so on. 5) It is extensively known that NP share more similar than synthetic compounds to the 'chemical space' of drug molecules[6-18]. Unfortunately, only a small proportion of that diversity has been extensively explored for its pharmacological potential so far[19-21].

Until now, the search for new anti-helminthic compounds from natural origin has generally been based on traditional trial-and-error methods[5,22]. Unfortunately, these methods are highly inefficient and expensive[9,23]. For this reason, new technologies have emerged to replace these old "hand-crafted" approaches for synthesis and testing new chemical entities[12,24-26]. Virtual screening is an example of these modern approaches. Specifically, Quantitative Structure-Activity Relationships (QSAR) predictive models have been extensively used to filter large databases of compounds to identify new bioactive chemicals[27-34]. Compared to other areas of pharmaceutical research; however, the screening of NPs has suffered from a lack of data in an appropriate format. Such information can significantly impact virtual screening, where new natural agents would be identified as potential therapeutic anthelmintics.

Citation: González-Castañeda Y, Marrero-PonceY, Guerra J O, Echevarría-Díaz Y, Pérez N, Pérez-Giménez F, Simonet A M, Macías F A, Nogueiras C M, Olazabal E, Serrano H. Computational discovery of novel anthelmintic natural compounds from Agave Brittoniana trel. Spp. Brachypus. Revis Bionatura 2022;7(4) 53. http://dx.doi.org/10.21931/RB/2022.07.04.53
Received: 25 January 2022 / **Accepted:** 22 October 2022 / **Published:** 15 November 2022
Publisher's Note: Bionatura stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

On the other hand, some authors of this report used an in-house computational approach to discover new anthelmintic synthetic compounds with rather good results[35-37]. A similar approach has been used to find new tyrosinase inhibitors from natural origin[38,39]. However, no scientific report about discovering NPs with an anti-helminthic activity using an analogous computational strategy has been published.

This report presents the creation/validation of the QSAR model able to identify potential anthelmintic compounds. Next, we used this model in the virtual screening of NPs previously obtained and chemically characterized from *Agave brittoniana* Trel. spp. *Brachypus*. Finally, the identification/selection of the most promising anti-helminthic NPs for *in vitro* and *in vivo* experimental evaluation and the results of these evaluations are presented.

Materials and methods

Experimental Section

Materials

Compounds 1-5 were derived from previous studies made with *Agave brittoniana* Trel. spp. *Brachypus*[63]. The rest of the chemicals were obtained using a similar approach described by the same research team[63]. The extraction and purification of all compounds with a purity higher than 99% were carried out employing previously described methods.63 To obtain the initial dissolutions, each product dissolved in water at a concentration of 10 mg/mL (1%). The insoluble products were first dissolved in dimetylsulphoxide (DMSO) so that the concentrations of this product in the final solution did not exceed 1%. The necessary dilutions of each product to make possible the biological evaluation was obtained starting from the initial solutions. In addition, a solution of TCB was utilized as reference drug.

Animals

Healthy balb/c mice of both sexes (body weight: 0.018±0.001 Kg) and food were purchased from the National Center for Laboratory Animal Production (CENPALAB, Havana, Cuba). Quarantine, labeling, climatization and good maintenance conditions of animals were strictly obeyed.

General Experimental Procedures

To measure the chemical effectiveness against *F. hepatica*, an experimental technique reported in the literature was selected for biological material processing and *F. hepatica* egg extraction[66]. Mitterpak *et al*.'s technique for the host (*Lymnaea cubensis*) invasion was carried out[67]. Afterwards, we followed the steps reported by Olazábal *et al.*[68] to obtain the metacercariae. Metacercariae were conserved in the cold until the *in vivo* experiment[66].

Biological Experiments

The anthelmintic activity of the compounds was evaluated, first, against *F. hepatica* in an *in vitro* assay using an earlier described procedure and second, against metacercariae of the same pathogen in an *in vivo* experiment, applying another well-established procedure.

Several treatment groups with ten mice per group were created. One group (infected control group) was treated with Miglyol 810N (administration vehicle). The second group was neither infested nor treated. The remaining groups were treated with new chemicals. All mice received the new compounds through an oral route. Mouse invasion with metacercariae of *F. hepatica*, 2 weeks old, 14 days before drug administration, was carried out by Corba *et al.*'s method[69]. The effectiveness was evaluated based on the following:

1) determination of the E% index. This is a quantitative indicator of effectiveness introduced by Steward[70] and defined as E% = [(XC–XT)/XC] × 100[71]. Here, E% is the percentage of effectiveness, XC is the average amount of *Fasciola* in the control group, and XT is the average amount of *Fasciola* in the treated group. Effectiveness was measured based on the elimination or not of *F. hepatica*, in its juvenile stage, as shown by laboratory diagnostics, using the helminthological necropsy on day 7 after the inoculated treatment[69].

2) Determination of the hepatic index[72], by mean of the formula A = (B/C) × 100. In this case, A = hepatic index, B = liver weight and C = body weight.

3) Degrees of lesions of the liver[69].

4) Spleen relative weight[73].

5) Intensity of invasion making use of the formula I = A/B, where A = total amount of parasites, B = total amount of positives.

6) Extension of invasion by use of the formula %E.I = [T(t)/T(a)] × 100, where %E.I is the percent of invasion extensity, T(t) = number of total positives, and T(a) = total of infected animals[74].

7) Gain of weight (final weight) (initial weight).

From these different effectiveness indexes[72-74], the E% index was selected.

Computational method

In the present report, we used a defined mathematical algorithm, which is characterized in this case by bond-based QuBiLs-MAS (acronym for Quadratic, Bilinear and N-Linear mapS based on graph–theoretic electronic-density Matrices and Atomic weightingS) MDs family (bond-level nonstochastic quadratic indices) to encode the chemical information in numbers[50-52]. The CARDD extension of the TOMOCOMD approach has been previously successfully used to discover new bioactive molecular entities[35,36,38,44-49].The general principles of these indices and the main steps for the application of the QuBiLS-MAS[50] software (http://tomocomd.com/software/qubils-mas) in QSAR/QSPR for drug design have been described in detail elsewhere[35,36,38,44-49].

To find the classification function that discriminates between active and inactive compounds, we select the LDA because it is one of the most broadly used and straightforward techniques to obtain QSAR equations[35,36,48,49,75-85]. It was carried out with the STATISTICA software[53]. Forward-stepwise and best subset search procedures were fixed as the strategy for variable selection. The best model was selected considering the principle of parsimony (Occam's razor). The considered tolerance parameter was the default value for minimum acceptable tolerance, which is 0.01. The quality of the model was determined by examining Wilks' λ parameter (*U* statistic), the square Mahalanobis distance (D^2), the Fisher ratio (*F*), and the corresponding *p* level [*p*(*F*)] as well as the percentage of good classification (accuracy) in the training and test sets (see Schemes 1 and 2). The classification of cases was performed by means of the posterior classification probabilities where one compound can then be classified as active if Δ*P*% > 0, being Δ*P*% = [*P*(Active) - *P*(Inactive)] >100, or as inactive otherwise. *P*(Active)

and *P*(Inactive) are the probabilities with which the equation classifies a compound as active or inactive, respectively. On the other hand, the probability density approach implemented in the Ambit Disclosure software was used to evaluate the applicability domain of the model developed[60].

Results and discussion

In silico study and virtual screening

Developing and validating linear QSAR models

To obtain a mathematical relationship between chemical structures and biological activity, the chemical information contained in many compounds must be statistically processed. Therefore, we build a data set containing 212[40-43] and 305[40,41] inactive compounds from the literature. It was build including 517 (active + inactive) compounds and was randomly divided into two subgroups: a set of 352 compounds (138 active and 214 inactive) that was used as the training set for developing the classification model and a second set of 165 compounds (74 active and 91 inactive) that was used as a test set for testing the predictive power of the model developed (see figure 1).

Each structure was parameterized by using one TOMOCOMD-CARDD[35,36,38,44-49] molecular descriptor (MDs) family, named bond-based nonstochastic 2D quadratic indices (QuBiLs-MAS Software)[50-52] (see the experimental section for more details). Linear discriminant analysis (LDA), implemented on the STATISTICA software, was used as the statistical technique for model building[53]. The best classification model obtained is given below, together with the LDA-statistical parameters:

$$Class = -2.2558 + 0.00001\,{}^{M}q_{5L}{}^{H}(\bar{x}) - 0.00024\,{}^{M}q_{3}{}^{H}(\bar{x}) + 0.06528\,{}^{P}q_{1}(\bar{x}) - 0.00280\,{}^{M}q_{1L}(\bar{x}) - 0.00360\,{}^{P}q_{3}{}^{H}(\bar{x}) + 0.00447\,{}^{M}q_{3}{}^{H}(\bar{x}) \quad (1)$$

$$N=352 \quad \lambda=0.443 \quad D^2=5.261 \quad F(6,344)=72.196 \quad p<0.0001$$

where, N is the number of compounds, λ is the Wilks' statistic, D^2 is the squared Mahalanobis distance and *F* is the Fisher ratio.

The Wilks' parameter is equal to the proportion of the total variance in the discriminant scores not explained by differences among the groups. Smaller values of Wilks' lambda indicate the greater discriminatory ability of the function. Its statistic parameter can take values in the range of 0 (perfect discrimination) to 1 (no discrimination)[54]. That is, Wilks' lambda is a direct measure of the proportion of variance in the combination of dependent variables unaccounted for by the independent variable (the grouping variable). Suppose a large proportion of the variance is accounted for by the independent variable. In that case, it suggests an effect from the grouping variable and that the groups (active and inactive) have different mean values. The Mahalanobis distance is a statistical technique that can be used to measure how distant a point is from the centre of a multivariate normal distribution, and its parameter indicates the separation between the respective groups[55]. It shows whether the model has an appropriate discriminatory power for differentiating between the two respective groups. The classification of cases was carried out by means of the posterior classification probabilities. Using the Mahalanobis distances to do the classification, we can now derive probabilities. The probability that a case belongs to a particular class is basically proportional to the Mahalanobis distance from that group centroid. In summary, the posterior probability is the probability, based on our knowledge of the values of other variables, that the respective case belongs to a particular group.

This equation can correctly classify 87.46% (307/352) of the compounds in the training set and showed values of the Matthews correlation coefficients of 0.74 on it. More important, the model achieves a balanced classification accuracy in each group.

The results of the most relevant statistical parameters for this model are presented in Table 1, and the classification of compounds in the training set using Eq. 1 is presented in Table 2.

Once a model is trained, its validation is another crucial aspect in this kind of analysis which can be performed by internal and external validation techniques (see Scheme 2)[56,57]. Here, a leave-many-out (LMO) cross-validation technique was carried out where groups of 176, 117, 70, 35, and 17 compounds of the training data (352 chemicals) were taken like cancellation groups and at each step. Then, the newly trained model was used to predict the left-out compounds. The results of this analysis are shown in Table 3,

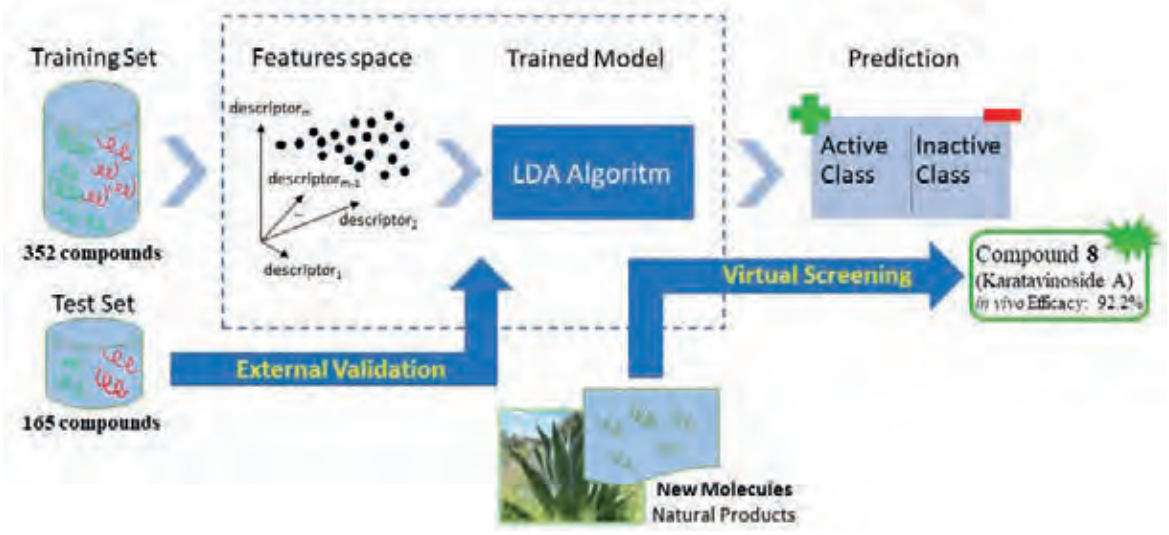

Figure 1. Schematic representation of the process used to design training and test sets.

Matthews Corr. Coefficient	Accuracy 'Q_{Total}'	Sensitivity 'True Positive rate' (%)	Specificity 'True Negative rate' (%)	False positive rate 'false alarm rate'(%)
Training Set				
0.74	87.46	87.59	87.38	**13**
Test Set				
0.64	81.82	83.78	80.22	**20**

Table 1. Prediction Performances and Statistical Parameters for QSAR Models in the Training and Test Sets.

and the model's parameters and predictions are rather stable when a perturbation is applied to the training set. This proofs that our model is robust.

In addition, to check the possibility of random correlations, the Y-randomization test (Y-scrambling) was performed by calculating the quality of the model randomly modifying the sequence of the response vector y (binary response: active or inactive) of the 5%, 10%, 20%, 30% y 40% of the compounds in the training set and recalculating the statistical parameters of the obtained models[57]. The final conclusions of this test are present in Figure 2, indicating that the achieved level of random correlation is significantly lower than the original regression, leading to the conclusion that the models are not random.

A more strict performance evaluation of a model is provided by an external validation where the model predictively is a challenge by compounds (external test set) that were not used in the model training (see Figure 3)[57]. Therefore, the equation obtained was evaluated in the test set (external prediction), showing accuracies of 81.82 % (135/165) and values of the Matthews correlation coefficients of 0.64. In addition to the external validation, the results of the statistical parameters described in Table 1 show that our model is not only robust but also predictive; therefore, it can be used in ligand-based virtual screening. The classification of both compounds in the external prediction set are depicted in Table 2.

Finally, to define the applicability domain[57] of Eq 1, a city-block distance-based approach[58,59] implemented in the Ambit program[60] was used. The model's applicability domain was defined from the training set, and all compounds belonging to the external test series were inside it.

In silico identification of active compounds from natural products

Taking into consideration that NPs have inspired most of the active ingredients in medicines[10], in the last years a number of recent investigation was carried out to discover new active compounds from the natural origin using computational strategy[61]. In our research, the developed model (Eq. 1) was used to filter an extensive database of NPs. All details of this database and other active (anthelmintics) NPs discovered by using our approach will be shown in the following reports.

Here, we only present the discovery of novel anthelmintic compounds from *Agave brittoniana* Trel. spp. *Brachypus*: a plant that grows like one of two endemic subspecies (ssp. *Brachypus* and ssp. *Spirituana*) of *Agave brittoniana* Trel. in the central region of Cuba[62]. A group of nineteen compounds composed by 12 steroidal saponins, 6 steroidal sapogenins and 1 phytosterol (see Figure 4) that have been previously obtained and chemically characterized from this subspecies of *Agave* was evaluated *in silico* using the Eq. 1. These compounds were: agabrittonosides A–D[63], agabrittonosides E–K, karatavioside A[64], Diosgenin, Chlorogenin, Hecogenin, Tigogenin, Rockogenin, and β-Sitosterol.

As result of this virtual screening, twelve compounds were identified by the model as potential anti-helminthic hits (see Table 4).

However, it is generally acknowledged that QSARs are valid only within the same domain for which they were developed. Even if the models are developed on the same chemicals, the DA for new chemicals can differ from model to model, depending on the specific MDs. One of the present reports aims is to develop a model for predicting the anthelmintic activity of NP at the early stages of the drug discovery and development pipelines. Therefore, the chemicals selected in this study were only evaluated in vitro after plotting them into the model's previously obtained AD. In this analysis, all compounds were inside the DA of the model, ensuring excellent reliability for the prediction of this kind of lead used in the virtual screening. Moreover, all new leaders fall within the model's DA, so the predictions are reliable.

Experimental corroboration

In vitro assay

Compounds were limited in availability; therefore, not all compounds were experimentally tested. Only three of the compounds detected *in silico* as potential anti-helminthic hits (Karataviosido A, Agabrittonósido A, Agabrittonósido B) and a mixture of Agabrittonósidos D and Agabrittonósidos E could be tested *in vitro* against F. hepatica at 5×10^{-1}, 5×10^{-2}, 5×10^{-3}, 5×10^{-4}, 5×10^{-5} and 5×10^{-6} mg/mL. Triclabendazole (TCB) was included in this experiment as a reference drug because it is the one of choice in treating human fasciliasis[65]. Besides, Yucagenin (predicted as inactive) was also included in determining the influence of the glycoside moiety in the anti-helminthic activity. The biological *in vitro* evaluation results can also be seen in Table 4.

The experimental results agreed with the virtual screening predictions. As predicted, Yucagenin is not active at any test concentrations. However, its glycoside derivative (8, 9) had a bioactivity profile as TCB. This first saponin (8) has a glycoside rest joined to the C-3 atom identical to compound 9, its structural difference in the opening of the ring F and the glycosidation in the C-26 atom. The responsible for the little activity of 10, can be this structural modification or the increase of polarity of this zone. The mixture of compounds 12 and 13 presented *in vitro* activity higher than that observed for TCB. Compounds 12 and 13 are very similar structurally; both have the diosgenin-like central scaffold, but in compound 13 one xylose unit in 12 is substituted by a rhamnose group. In addition, 12 have a hydroxyl moiety in C-2, which is the only difference from 9. The combination of these subtle changes notably increases the activity of 12 and 13 concerning 9.

name	ΔP%[a]	name	ΔP%[a]
Training active group			
Tetrachloromethane	99.96	Benacyl	64.94
Hexachloroethane	99.99	Imcarbofos	-11.47
Antimony sodium thioglycollate	-12.40	Etibendazole	58.88
Dichlorvos	95.20	Fluranteel	99.92
1-Chlorobutane	-84.87	Pararosaniline embonate	88.20
Dimetrizadole	-52.17	Amphotalide	81.43
Disoferol	-57.14	Praziquantel	67.48
Lindane	19.06	Diuredosan	58.21
Mindazole	8.40	153C51	74.88
Fospirate	87.49	Trichlorophen	99.75
Oltipraz	96.40	Febantel	61.19
Certuna	-37.26	Lucanthone	81.00
Butonate	99.95	Miracil A	56.87
Antienite	73.81	Diamphenetide	48.22
Sodium antimony dimethylcysteine tartrate	53.94	Alazanine triclofenate	98.23
Wormin	35.99	Antelmycin	-42.25
Nitrodam	83.60	Diospyrol	96.63
Lobendazole	-58.33	Becantone	91.94
Bromothymol	81.12	Desaspidin	97.44
Iodothymol	70.57	Methylrosanilidium chloride	-5.71
Famophos	12.06	Bunamide	84.38
Thiacetaisamide	4.78	Bidimazium oidide	94.02
Antazonite	76.66	Pyruinium chloride	74.74
Vincofos	98.97	Desoine	34.28
Niclofolan	98.21	Thymoloverm	97.10
Bromofenofos	80.53	Teroxalene	96.95
Sinid	99.82	Pretamazium iodide	97.82
Phenotiazine	79.08	Hedaquinium chloride	97.71
Nitazoxanide	75.50	Dryocrassin	99.97
Bendamizole	38.85	Netobimin	4.02
Tioxidazole	56.19	Phenithionate	91.48
Phoxim	70.22	Artesunate	73.02
Albendazole	20.75	Abamectin B	99.45
Anthiolimine	67.17	Alantolactone	48.31
Carbantel	40.67	Antimony potasium tartrate	90.38
Cetovex	-33.89	Arecoline	-70.97
4-Hexylresorcinol	25.63	Aspidin	96.52
Crufomate	86.81	Benomyl	-6.42
Butynorate	-13.36	1,4-Bistrichloromethylbencene	100.00
Hexachlorophenimonophosphate	97.18	Carbacol	36.93
Niclosamide	96.88	Triclabendazole sulphoxide	95.99
Nitroscanate	86.36	Epripantel	53.65
G-572	76.60	Acid filix (c)	99.91
Resurantel	87.70	Oentian violet	-32.44
Phenzidole	45.11	Hectolin	98.35
Ontianil potasium	77.13	Hidroxyquinoline	7.47
Atractyl	57.00	Urea stibamine	-81.83
Amidantel	1.49	alfa-Kosin	95.91
Mirasan	-4.31	Mandelic acid isoamyl ester	72.81
Triclabendazole	96.56	Mibbemycin Oxime A3	96.35
Nocodazole	19.80	Moxidectin	98.94
Styrylpyridiniumchloride	69.52	Naftalofos	85.30
Haloxon	87.78	Naphthalene	13.12
Coumafos	95.29	Nicotine	-61.44
Coralop	93.40	Nitroxymil	53.93

Table 2. Results of the Classification of Compounds in the Training and Test Set using QSAR Models.

name	ΔP%[a]	name	ΔP%[a]
Evultin	74.23	Paraherquanude	51.83
Oxamniquine	28.86	Thymol	35.82
Brotianide	98.98	alfa-Santonin	42.82
Afesal	98.55	Arsamilate	-88.07
Furodazole	78.42	Quinacrine	79.37
Oxfendazole	57.98	Kainic acid	-63.64
Dribendazole	53.95	Hexachlorophene	99.65
Butamisole	80.95	Tetrachloro-difluoroethane	99.70
Spirazine	70.66	Diamphenethide Deacetylated (metabolite)	43.69
Lubisan	17.98	Doramectin	99.57
S72014	86.50	Dymanthine	-24.86
Flubendazole	75.13	Bromoxanide	99.91
Mebendazole	54.79	Tetrachloroethane	-17.65
Egresin	58.67	Paramomycin	-12.13
Training inactive group			
3-Episiostatin B	-66.80	2-etoxybenzamide	-95.05
Thiacetazone	-63.38	2-isopropil-4-pentenoyl urea	-93.63
ZDPFA	-3.61	Carbetimer	-65.19
Foscarnet	-98.33	Etimidin	-82.76
Ethoxene	-82.33	Leucenol	-89.40
Arildone	87.74	Azatepa	-54.26
Fanciclovir	-27.92	Asperlin	-53.90
Valerium Paul Thibault	-63.43	Pipobroman	-99.98
Ferriscorbone magnesienne	-53.98	Glucin	-98.56
Aponal	-51.73	Isomalt	-86.18
Carbavin	-51.30	S-2346	-96.34
Zonisamide	-35.28	DL 204 IT	-97.47
Atrolactamide	-38.70	Equilenin	-96.11
Beclamide	-53.84	Ethyllysergamide	-99.39
Tetrantoin	-19.04	Trenbdone	-96.14
Tiletamine	13.30	Methallenestril	-94.65
Ferro-Drops	-79.75	Estradiol	-97.10
Cobalti glutamas	-72.22	Metexaminum	-82.55
Fructosum Ferricum	-80.55	Solution A40	-94.81
Ferromaltose	-74.93	Proxamine	-92.92
Erythrophyll	99.96	Clonazoline	-94.53
Butanolum	-74.30	Tymazoline	-91.30
A-Peest	-71.84	Isopropylmethoxamine	-96.06
Iprazochrome	-35.89	Angiotensinamide	-99.79
Esculamine	35.44	Terlipressin	-97.99
Morfafen	95.81	Isomylamine	-88.00
Acetazolamide	-57.16	Dimebamate	-78.19
Trometamol	-82.01	DEP	-85.12
Butazolamidum	-32.90	Silamprobamate	-89.54
Merbiurelidin	-95.50	Chlorphenesin carbamate	-81.89
Chlorothiazide	-3.72	Guaifenesin	-93.80
Disulfamide	-40.20	Murexine	-99.52
Trichlormethiazide	83.08	Nafomine	-97.56
WR-2823	-97.28	AHR-2666	-82.70
Peucedanin	87.50	Pifexole	-83.22
Batilol	21.98	Tybamate	-85.86
Glipentide	61.98	Carmecolina cloruro	-99.27
Glisindamide	76.76	Eseridine	-97.46
Glimepiride	79.06	Distigmine bromide	-99.46
PIDH	-11.49	D-935	-95.24
Carbutamide	-23.95	Ergocristine	-96.60
Ag-307	-65.39	Etilefrine pivalate	-74.11

Table 2. Results of the Classification of Compounds in the Training and Test Set using QSAR Models.

name	ΔP%[a]	name	ΔP%[a]
Diazoxide	48.58	Pivenfrine	-72.63
Alarmine	-67.86	Etafedrine	-98.00
MK-534	86.29	Dimetofrine	-97.44
Oxonazine	-78.29	Methoxyphenamine	-96.85
Guanisoquine sulfate	18.67	Clorprenaline	-85.85
Stilonium iodide	29.75	Phenylethanolamine	-94.85
Pancuronium bromide	-70.89	Norfefrine	-93.92
Dimecolonium iodide	-97.46	Oxidopamine	-90.52
Chlorisondamine chloride	-62.91	Pacamine	-90.78
Mecamylamine	-77.27	Metaraminol	-94.36
Methylene chloride	-91.59	Synephrine	-96.92
Vinyl ether	-72.01	Corbadrine	-93.16
Neothyl	-83.80	Phenamazoline	-97.60
Novasil	-39.87	Pholedrine sulfate	-95.71
Acidum isobutiacilicum	-14.08	Etilefrine	-97.13
Iodothiouracil	-39.43	Para-Aminoephedrine	-97.11
Basthioryl	-94.03	AMT	-96.63
Pyrglutargine	-68.78	Aphidicolin	-93.27
Acustasin	-76.09	Pipratecol	-98.77
Gluronsan	-59.23	Ftorin	-78.82
Dicumarol	96.78	Alprostadil	-92.58
Chloracyzine	81.07	Prostaglandin F1alpha	-95.10
Aminoethylnitrate	-58.55	Azaclorzine	-71.68
Nitrodimethylin	-69.40	Aceperone	-97.98
Propatyl nitrate	72.94	Prenylamine	-99.48
Carpronium chloride	-96.05	Ericolol	-50.93
6,9-Didesmethylartemisinin	28.85	Ancarolol	-86.07
Strychnobrasiline	-51.99	Cicloprolol	-94.58
Cilional	-42.36	Pafenolol	-97.54
7,7 Difluoro-*ß*-arteether	59.45	Nafetolol	-87.75
Mezepine	25.77	Sulfinalol	-98.59
Tandamine	17.54	ROM-203	-99.01
Perafensine	50.44	Spirendolol	-84.25
Doxepin	16.96	Flusoxolol	-95.37
Ketimipramine	10.33	Dobutamine	-96.87
Tisocromide	93.66	Carbazeran	-97.71
Cotriptyline	42.34	Bufalin	-97.30
Diminazone	38.39	Niludipine	-81.15
Lauroguadine	-31.96	Locundieside	-95.58
Glycobiazol	74.00	Covallatoxal	-96.67
Clioquinol	61.50	Peruvoside	-96.94
Noscapine	-97.31	Olitoriside	-94.64
Tilidine	-99.19	Deltamethrin	100.00
Bextrometorfano	-98.11	Phenothrin	-88.12
Levallorphan	-98.61	KC-8973	-90.43
Fenyltoloxamine	-99.27	Lipothiamine pyrophosphate	-99.45
Clorfenoxamine	-98.03	Xanthine	-94.25
Medrylamine	-99.58	Methioninol	-97.15
Homoclorciclicine	-97.98	Silidianin	-95.38
Pimetixene	-96.08	Bietamiverine	-98.35
Borimamide	-98.97	Cimetropium Bromide	-95.13
Nigrifactin	-94.21	Diponium Bromide	-97.74
Octastine	-94.55	Feclomine	-99.66
Acidum etidronicum	-99.57	Flavoxate	-95.16
Nibet	-99.04	Flopropione	-86.14
Refortan	-26.69	Pipoxolan	-97.17
Clorotepine	-88.43	Fludalanine	-87.26

Table 2. Results of the Classification of Compounds in the Training and Test Set using QSAR Models.

name	ΔP%[a]	name	ΔP%[a]
Docloxytepin succinate	-87.52	Cryptargol	-93.20
Diclorpromazine	-79.47	Mepartricin A	-99.86
5-fluorocitosicine	-83.27	Protoanemonin	-76.14
5-nitro-2-firfurilmetil eter	-84.77	Contramine B	-97.95
4(2-aminoetil)imidazole	-94.04	F-8	-95.26
2 hidroxibenzamine	-93.21	Nitrofurylather	-84.77
3-hidroxyacetanilide	-93.99	Protoxyl	-99.98
***O*-acetilsalicylamide**	-90.46	Bismuth Cevitamate	-73.39
Test active group			
Tetrachloroethylene	98.42	Artemethei	9.05
Metrifonate	99.96	Santoperonin	99.88
Stibomen	46.92	Agrimonolide	80.21
26354R-P	13.02	Dibutyltin dilarate	100.00
VUFB-7904	-43.77	Uredofos	58.90
Bitoscanate	33.74	Salantel	97.29
SQ18506	-64.91	Miracil	85.40
Carbendazim	-44.87	Hycanthone	72.64
Tiabendazole	43.77	Miracil C	6.53
F30066	25.19	Dithiazanine iodide	98.37
Ascaridole	14.50	Dicroden	70.73
Eucalyptol	-16.31	RO2-9009	-49.19
R8231	85.91	Zilantel	99.77
Nitramisole	69.53	Bisbendazole	99.80
Levamisole	22.03	Chuanliansu	-86.51
Pyrantel	24.06	Stilbaziumiodide	90.80
Clorsulon	85.46	Coib	65.81
Feniodiumchloride	100.00	Luxabendazole	80.84
Stibofen	3.76	Abamectin A	99.31
Sodium stibocaptate	93.88	Amorcazine	91.74
Oxibendazole	-16.25	Aspidinol	37.83
Morantel	41.11	Bethionol	99.64
Oxyclozanide	99.38	Embelin	77.42
Nitroclofene	98.39	Acid filix (a)	99.94
Amoscanate	76.60	Acid filix (b)	99.93
Dichlorophen	94.80	Glycarsamide	-93.83
Ciclobendazole	-2.50	Dichlorophenersine	66.43
Parbendazole	-4.81	beta-Kosin	95.95
SRC-4402	94.38	Mibbemycin Oxime A4	96.09
Cambendazole	79.05	Moxidentin	99.47
Thiophanate	-57.67	2-Naphthol	18.87
Clioxanide	69.61	Albendazole sulphoxide	-2.36
Fenbendazole	81.78	Rafoxanide	98.65
Santolactone	42.82	Tenium Closylate	52.10
Ticarbodine	91.51	Dibromomsalan	97.71
Meclorazepan	92.18	Milbemycin D	98.09
RP12869	32.95	Tribromsalam	97.87
2-Azamizoribine	-47.22	Buparvaquone	94.66
2-fluoroNpcA	-14.23	Quinapyramine	-13.66
Futhan	72.13	Metadone	-99.57
Ribavirin	-64.08	Dextromoramide	-99.56
Test inactive group			
Aciclovir	-81.10	Antazoline	-98.95
RS-21592	-74.34	Histamithizine	-98.22
Zalcitabine	-46.89	Tironamine	-94.24
Triclofos	97.57	Clofibrique Acid	-34.00
Chlorobutanol	99.44	Nicofibrate	-47.60

Table 2. Results of the Classification of Compounds in the Training and Test Set using QSAR Models.

name	ΔP%[a]	name	ΔP%[a]
Bromisoval	-94.72	Flutizenal	8.37
Baldrianol	-66.64	Trimetilsulfonium hidroxide	-98.91
Ethchlorvynol	-51.93	1-fenilsemicarbazide	-98.35
Trimethadione	-19.96	*p*-Etoxyanilinometane sulfonic acid	-99.20
Phenacemide	-54.72	Pyrimazid	-95.70
UK-17022	71.11	Improsulphan	-99.42
Cinromide	51.40	Phenetylurea	-97.84
Zebromal	-99.43	Diethylstilbestrol	-97.20
Phenythilone	17.72	Norclostebol	-85.07
Iron aspartate	-75.26	Mephentermine	-92.42
Orotonsan Fe	-65.18	Mtrafazoline	-97.26
Ferrogluconat	-83.98	Chlorzoxazone	-61.01
Ferrotrenine	-77.68	Luvatren	-86.42
Cupriaseptol	-60.00	Furtrethonium iodide	-99.02
Besunide	53.38	Benzyllephedrine	-98.80
Pytamine	-8.01	Isoprenaline	-93.75
Lemidosul	48.68	Dopamine	-91.79
Propazolamide	-42.63	Octodrine	-74.53
Sulclamide	-17.71	Isometheptene	-88.25
Propamin soviet	-80.83	Metanephrine	-97.20
Calcii mesoxalas	-58.45	Bufeniode	-99.29
Mebetide	-18.70	Buphenine	-97.90
Olmidine	-37.91	Nimedipine	-95.34
Ganglefene	66.58	Odiphalin	-99.01
Gaplegin	-98.87	Acebutolol	-93.61
Trepirium	-98.48	Indopanolol	-95.92
Enflurane	23.59	Pirepolol	-98.34
Subcutin	-9.76	Colforsin	-63.68
Thiamazole	-88.68	Frutisin	-100.00
Orotric	-64.99	PR-H 286 BS	-97.99
Esorben	-78.28	Nicoxamat	-94.78
Efloxate	91.34	Aminopromazine	-99.13
Nitronal	0.11	Emepronium	-99.77
Berberine	67.88	Hymecromone	-82.19
Aecachinium	46.06	Taurultam	-97.54
Elanzepine	66.48	Fluoramphenicole	-85.13
Almoxatone mesilate	85.13		

Table 2. Results of the Classification of Compounds in the Training and Test Set using QSAR Models.

# compounds out in each step	Q%[a]	λ	D^2	*F*	Q%[b]
LMO *for the model obtained* **[Eq. (1)]**					
17	85.43	0.52	3.80	45.15	82.42
35	85.17	0.53	3.72	45.91	82.97
70	85.11	0.53	3.83	40.94	83.52
117	85.96	0.51	3.97	36.76	80.22
176	81.82	0.56	3.55	22.34	80.22
average	**84.70**	**0.53**	**3.77**	**38.22**	**81.87**
SD	**1.64**	**0.02**	**0.15**	**9.60**	**1.55**

[a,b] Global accuracy from both models in training and test sets (group out), respectively.

Table 3. Results of the Leave-Many-Out (LMO) Cross-Validation Analysis.

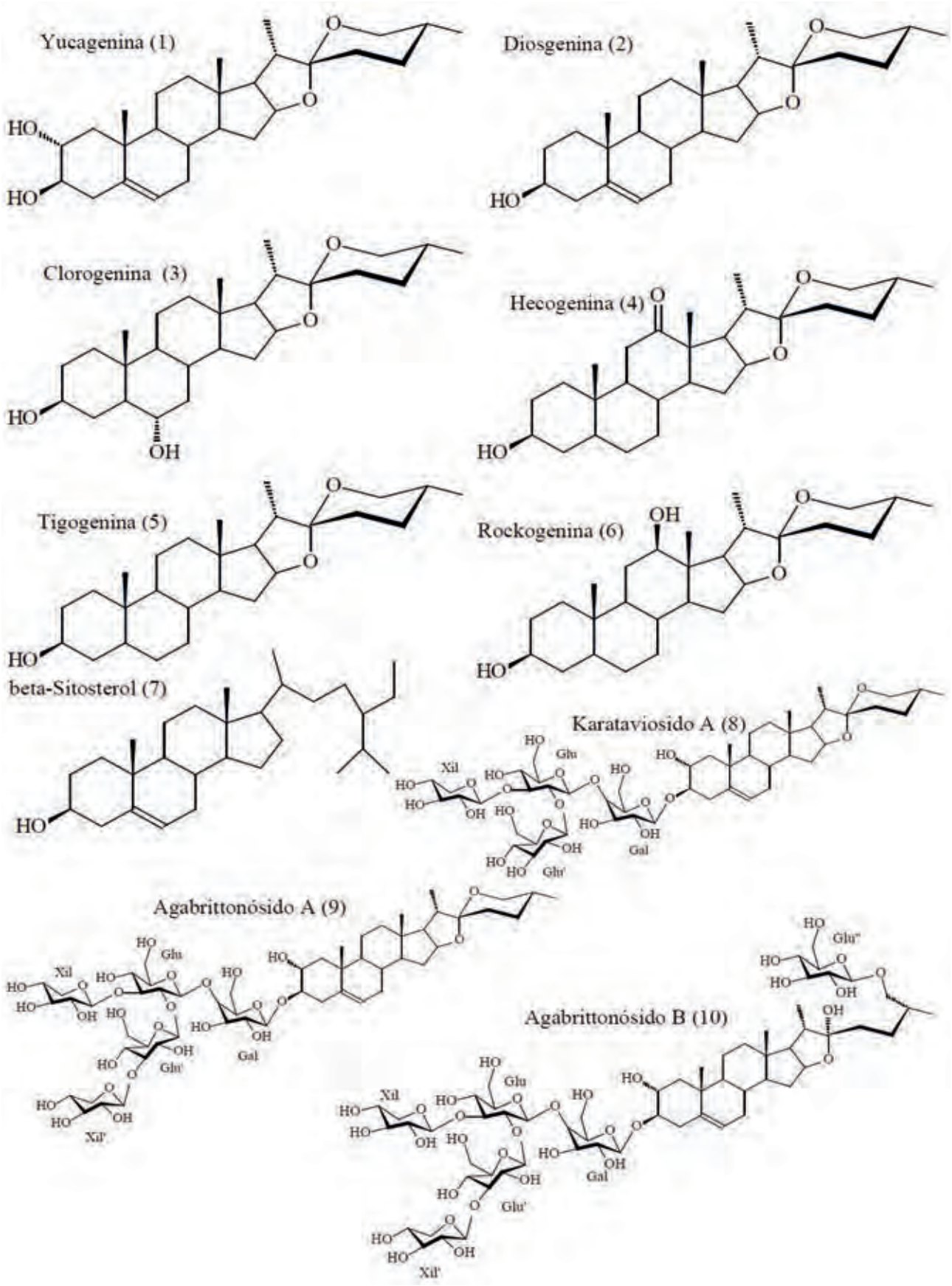

Figure 2. Chemical Structures of Compounds Evaluating in the in silico Experiment from Agave brittoniana Trel. spp. Brachypus.

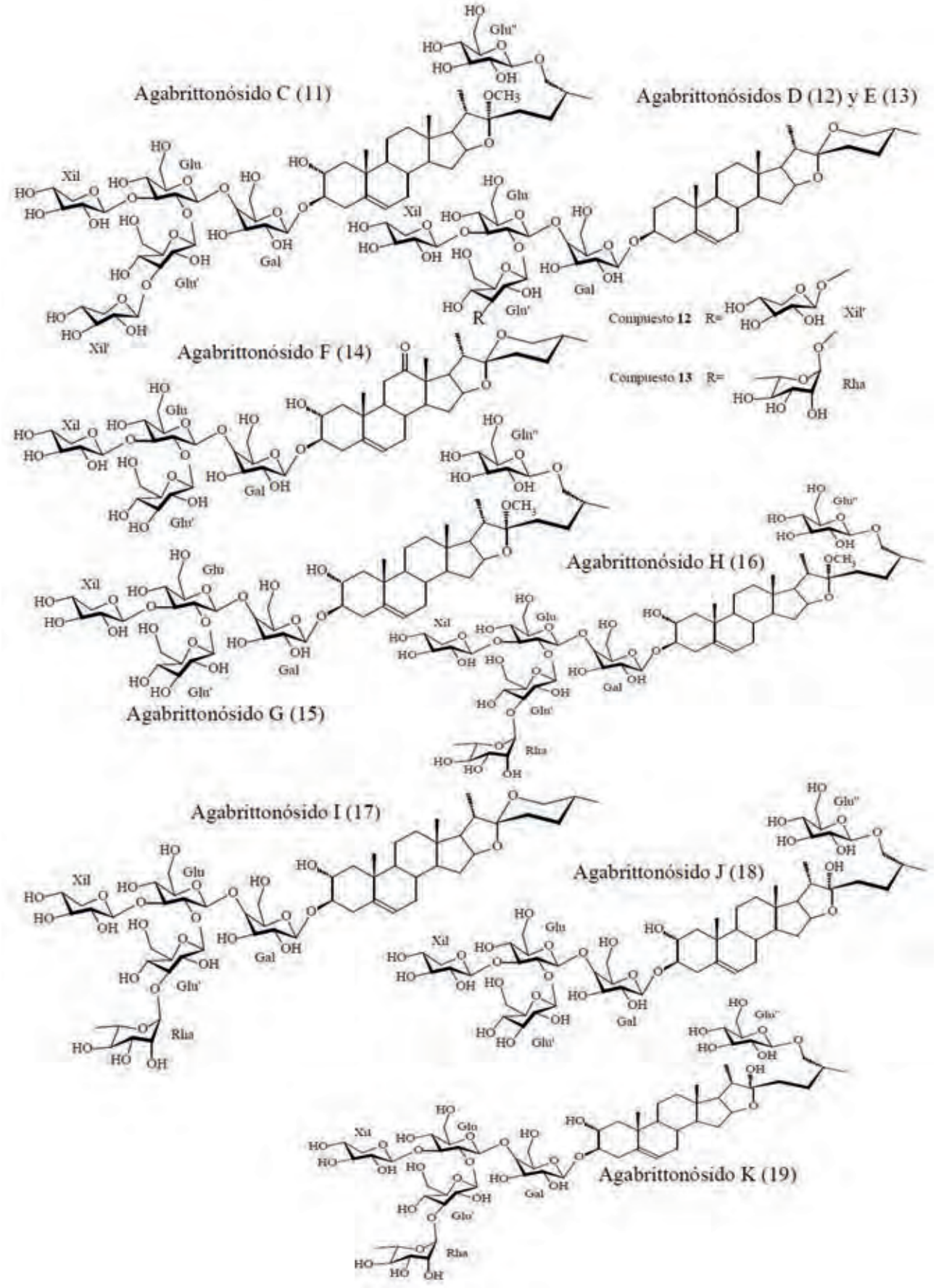

Figure 2. Chemical Structures of Compounds Evaluating in the in silico Experiment from Agave brittoniana Trel. spp. Brachypus.

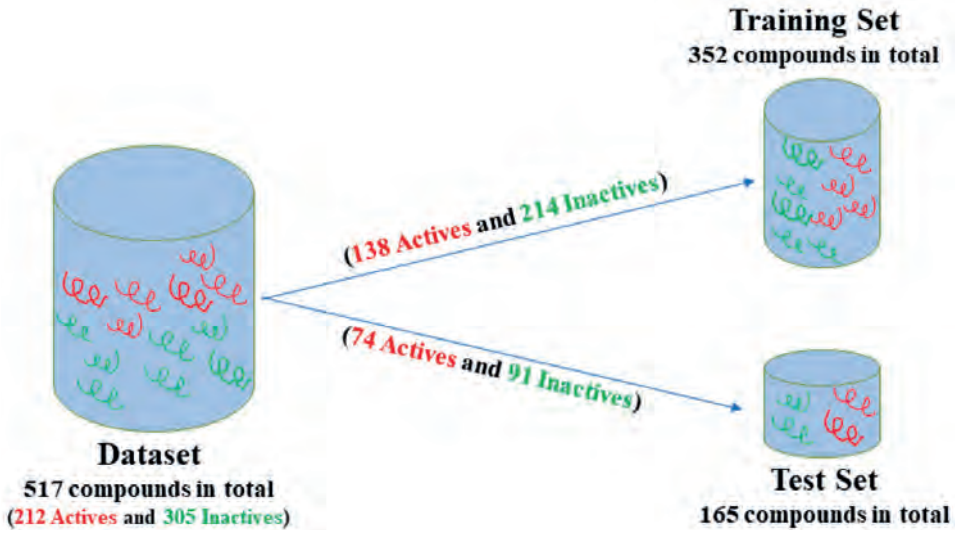

Figure 3. General overview of the computational procedure.

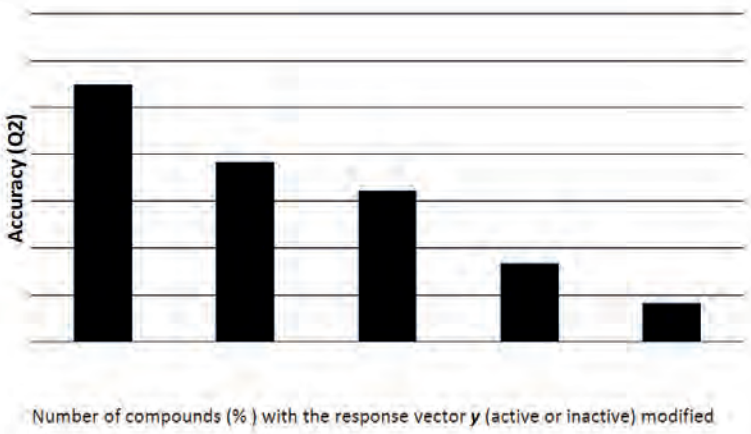

Figure 4. Behavior of the Percentage of Good Classification in the Y-scrambling Analysis.

	In silico	*In vitro**						*In vivo***
Compounds	ΔP % (Ec.1)	5.10^{-1}	5.10^{-2}	5.10^{-3}	5.10^{-4}	5.10^{-5}	5.10^{-6}	Efficacy (%)
Yucagenin (1)	-51.28	0	0	0	0	0	0	-
Diosgenin (2)	-40.23	-	-	-	-	-	-	-
Clorogenin (3)	-71.14	-	-	-	-	-	-	-
Hecogenin (4)	-50.95	-	-	-	-	-	-	-
Tigogenin (5)	-59.48	-	-	-	-	-	-	-
Rockogenin (6)	-81.05	-	-	-	-	-	-	-
β-Sitosterol (7)	-42.83	-	-	-	-	-	-	-
Karatavinoside A (8)	56.69	100	100	100	100	0	0	92.2
Agabrittonoside A (9)	99.30	100	100	100	100	0	0	52.9
Agabrittonoside B (10)	61.41	100	0	0	0	0	0	-
Agabrittonoside C (11)	71.16	-	-	-	-	-	-	-
Agabrittonoside D (12)	77.75	100	100	100	100	100	100	-
Agabrittonoside E (13)	82.35							
Agabrittonoside F (14)	64.46	-	-	-	-	-	-	-
Agabrittonoside G (15)	56.10	-	-	-	-	-	-	-
Agabrittonoside H (16)	76.95	-	-	-	-	-	-	-
Agabrittonoside I (17)	77.31	-	-	-	-	-	-	-
Agabrittonoside J (18)	42.95	-	-	-	-	-	-	-
Agabrittonoside K (19)	68.80	-	-	-	-	-	-	-
Triclabendazole (TCB)	96.56	100	100	100	100	0	0	92.2

*Concentration used (in mg/mL). **Compounds **8** and **9** as well as TCB were tested at 10 mg/Kg of weight of balb/c mice.

Table 4. Results of the in silico Classification and Percentages of Anthelmintic Activity of the Selected Compounds from Agave brittoniana Trel spp. Brachypus in vitro and in vivo Assayed.

In vivo assay

An *in vivo* experiment using Bald/c mice-like biological models was conducted to obtain more profound conclusions about the pharmacological activity of *in vitro* hits. In this case, we only include in this experiment the two more active and pure substances (8 and 9) at doses of 3 mg/Kg. Table 4 shows the results of this study, where compound 8 was more active (92.16 % of efficacy) than 9 (52.94 %). The *in vivo* efficacy of compound 8 was identical to that of the control TCB. It is important to emphasize that this experiment was performed with a reduced dose (3 mg/kg). For instance, the TCB (the best human fasciolicide on the market[65]) is only wholly effective at 10 mg/kg. In addition, the few injuries in the livers and low inflammation of the spleens observed during the postmortem examination are qualitative criteria that positively appraise the effect of the tested compounds.

Conclusions

Today virtual screening has become an essential tool in drug discovery protocols. Here, bond-level quadratic indices (QuBiLs-MAS software, http://tomocomd.com/software/qubils-mas) and LDA were used to obtain a QSAR model that discriminates anthelmintic from inactive ones. Virtual screening of several metabolites from *Agave brittoniana* Trel. spp. *Brachypus* was carried out to discover new lead scaffold anthelmintics, and experimental corroboration showed that Karatavinoside A (8) exhibits similar-to-superior activity as triclabendazole (fasciolicide reference drug), with 100% *in vitro* effectivity (at 500 µg/mL) against Fasciola hepatica and 92.2% *in vivo* efficacy (25 mg/kg). This natural compound has been identified as a promising starting point for the rational optimization/design of new chemical derivatives with more potent anthelmintic activity.

Program availability

The QuBiLS-MAS software (portable standalone) and the respective user manual are freely available online at http://tomocomd.com/software/qubils-mas50

Acknowledgments

One of the present authors (M-P. Y) thanks the program '*Estades Temporals per a Investigators Convidats*' for a fellowship to work at Valencia University (2020). Y.M.-P. and Noel Pérez acknowledge the support from Collaboration Grant 2019–2020 (Project ID16897) and Med Grant 2019-2020 (Project ID16911).

Competing interests

The authors declare no conflict of interest.

Author Contributions Statement

YG-C, FP-G, JOG, YE-D, NP and YM-P proposed the computational applications, QSAR modeling and performed the statistical analysis as well as prepared the manuscript. AMS, JOG, FAM, and CMN worked in the chemical methods and prepared the manuscript. EO and HS worked in the Parasitology tests. YG-C, FP-G, YE-D, NP and YMP worked in the QSAR modeling and performed the statistical analysis. All authors read and approved the final manuscript.

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REVIEW / ARTÍCULO DE REVISIÓN

Current situation of snakebites envenomation in the Neotropics: Biotechnology, a versatile tool in the production of antivenoms

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DOI. 10.21931/RB/2022.07.04.54

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Abstract: Snakebite envenomation is a neglected tropical disease that affects millions of people around the world with a great impact on health and the economy. Unfortunately, public health programs do not include this kind of disease as a priority in their social programs. Cases of snakebite envenomations in the Neotropics are inaccurate due to inadequate disease management from medical records to the choice of treatments. Victims of snakebite envenomation are primarily found in impoverished agricultural areas where remote conditions limit the availability of antivenom. Antivenom serum is the only Food and Drug Administration-approved treatment used up to date. However, it has several disadvantages in terms of safety and effectiveness. This review provides a comprehensive insight dealing with the current epidemiological status of snakebites in the Neotropics and technologies employed in antivenom production. Also, modern biotechnological tools such as transcriptomic, proteomic, immunogenic, high-density peptide microarray and epitope mapping are highlighted for producing new-generation antivenom sera. These results allow us to propose strategic solutions in the Public Health Sector for managing this disease.

Key words: Antivenom, biotechnology, neglected tropical disease, omics, recombinant antibody.

Introduction

Neglected diseases occur in tropical and subtropical climates, specifically in rural areas where access to clean water, sanitary conditions, and medical care are limited[1]. They are caused by various pathogens such as viruses, fungi, bacteria, parasites and toxins, causing health, economic and social consequences. The term neglected is because these diseases are absent in most public health programs[2] affecting many people generating disability[3] and unemployment[4]. In addition, these diseases cause significant effects on the economy of developing countries due to the high cost of the treatments[5,6].

The World Health Organization WHO, in its NTDs portfolio, included 17 snake-caused diseases. Snakebite diseases were previously not on the NTD list, but since 2017, public health strategies have been planned for prevention, control and treatment[7]. Snakebites envenomation is found in Latin America, Africa, Asia and Oceania, poor rural tropics populations[8-11]. These countries have an absence of public health policies[12] pointed to snakebites diseases, so; they have no access to health services[1,13] and have a shortage of both medical supplies and trained human medical equipment.

More than 4 000 snake species worldwide, but only 250 are known of medical relevance[2]. Regions with the most incredible diversity of venomous snakes include Latin America and Asia[12]. Snakebite should be considered an NTD priority because it involves a wide snake diversity of species and, thus, a variety of toxins[14]. Snakes causing most ophidian accidents belong to the Viperidae and Elapidae families and the genera *Bothrops* and *Micrurus*[5,15,16]. Several risk factors, such as climate[17] and ecology[18], predispose to increased ophidian accidents[19]. Both rainy seasons and snake abundance5 cause a higher snakebites incidence[5,20,21].

Generally, global data for snakebites are not accurate, showing variability mainly due to scarce and not representative epidemiological studies[5,19,21]. Most hospital reports[22] and surveys[12] do not report important data such as incidence, mortality, and physical and psychological consequences suffered by patients[23]. According to Pach *et al.*[21], five million snakebites are reported annually worldwide, two to three million results in poisonings and 80 000 to 130 000 people die from these diseases. In Latin America and the Caribbean, hospital reports indicate approximately 70 000 cases of snakebites per year, which may be underestimated[24,25].

As a mega-diverse country, Ecuador has 40 poisonous snake species[22], of which 17 are responsible for 99% of poisoning cases. The most significant number of patients are found in the Amazon region, followed by the coastal area and the Andean region[26], which correlates with one of the studies of the geographic pattern of poisonous snakes[22]. Among the toxic snake families, the most representative is *Viperidae* and *Elapidae*[23,26].

Poisons are a set of proteins, peptides and enzymes that cause toxic effects[20] in the pathology of snakebite envenomation. The toxic profiles of each venom vary according to the geographic location and snake taxonomy[27,28], generating a wide range of local and systemic pathologies, inclu-

Citation: Romo, E.; Torres, M.; Martin-Solano, S. Current situation of snakebites envenomation in the Neotropics: Biotechnology, a versatile tool in the production of antivenoms. Revis Bionatura 2022;7(4) 54. http://dx.doi.org/10.21931/RB/2022.07.04.54
Received: 25 May 2022 / **Accepted:** 22 October 2022 / **Published:** 15 November 2022

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ding blindness[29,30], necrosis[31,32], paralysis[33], respiratory[34,35], renal[36,37] and cardiac insufficiency[38]. The only specific treatment currently available is antivenom (Table 1) or antisera since its development in 1894[39,40]. The traditional method for producing antivenoms is based on animal hyperimmunization with non-lethal venom doses with the following collection of large amounts of plasma[11,41].

Almost all countries in the world have limited antivenom availability[1,13,42,43] due to low interest in drug research and development, costs, safety, efficacy, and inefficient antivenom distribution[5,13,21,23]. However, several investigations are carried out with different types of antibodies[44,45], using pharmacological molecules[46,47] and innovative DNA immunization strategies[48,49] to inhibit or reduce toxic effects.

This review aims to update the real situation of snakebites in the Neotropics from the epidemiological point of view and also to expose current biotechnological tools that could be implemented in the future to solve the drawbacks in the production and availability of antivenoms.

Snakebite in the Neotropics: Ecological and Environmental Aspects

Ecosystem alteration, agriculture and environmental conditions modify the ecological patterns in the geographic distribution of the different snake species[59]. The highest incidence rate is concentrated in rural areas; urban spaces report snakebite cases[60,61].

Venomous and non-venomous snakebites are the result of the interaction between humans and various snake species found in specific habitats such as jungles[62], forests[63,64], arid[65,66] and urban areas[67,68]. Snakes, humans and the environment are the three components of this ecological interaction where one part can influence the other one while also affecting the third one. A circular dynamic characterizes this interaction, so it is necessary to know and relate these components. According to Guedes *et al.*[69], 659 snake species were recorded in the Neotropics, where species richness and phylogenetic diversity are mainly concentrated in the Amazon region of Brazil, the Andean region of Colombia, Ecuador and Peru, and some Central America regions. On the other hand, few species are recorded in the Caribbean. In the Neotropics, the most abundant species are *L. muta*, *Micrurus frontalis*, *B. jararaca*, and *Bothrops erythromelas*[69]. These snakes are of medical and clinical importance in the Neotropics and belong to the *Colubridae*, *Elapidae* and *Viperidae* families[70].

Agriculture causes geographic and ecological change in ecosystems, as forests are replaced by cultivated croplands[71]. Humans have been engaged in agricultural activities such as planting crops and raising animals since the Paleolithic era (12 000 to 5 000 years BP), which continued over the millennia to the present day[72-74]. Many people do this job and find themselves vulnerable to venomous snakebites[75-77]. According to Suazo-Ortuño *et al.*[78] research on agricultural conversion, snakes are not susceptible to changes in their habitat; the species diversity in agricultural areas is not diminished, and snakebites risk is maintained. Several epidemiological studies determined a higher incidence in tropical rural areas where farming and grazing activities are carried out under unfavorable conditions[8,14,19].

The most significant number of cases are recorded in rainy seasons associated with natural phenomena such as floods, hurricanes and cyclones[79]. In the Neotropics, the El Niño phenomenon causes heavy rains that increase the incidence of snakebites[80]. Snakes are ectoderm animals; temperature increases cause snakes to migrate to more temperate zones that are inhabited by humans[81]. Climate change then causes a geographic redistribution of snake species due to alterations in environmental temperature[82-84].

Continent	Snake species	Treatment
Australia[50,51]	*Pseudonaja* spp. *Oxyuranus* spp. *Notechis scutatus* *Tropidechis carinatus* *Austrelaps* spp. *Hoplocephalus* spp.	Monovalent antivenom Polyvalente antivenom
Asia[52]	*Naja* spp. *Bungarus* spp. *Daboia* spp. *Echis* spp. *Trimeresurus* spp.	Monovalent antivenom Polyvalente antivenom
United States[53] **and Canada**[54]	*Crotalus* spp. *Agkistrodon* spp. *Sistrurus* spp. *Micrurus* spp.	Polyvalente antivenom
Latin America[11,55]	*Bothrops* spp. *Crotalus* spp. *Lachesis* spp. *Micrurus* spp.	Polyvalente antivenom
Africa[56,57]	*Echis* spp. *Bitis* spp. *Naja* spp. *Dendroaspis* spp.	Polyvalente antivenom Monovalent antivenom
Europe[58]	*Vipera berus* *V. aspis* *V. ammodytes*	Monovalent antivenom

Table 1. Treatment for snakebite envenomation around the world.

Clinical findings, Social, and Economic Impact of Snakebite Envenomation

Venomous snakes can cause several local and systemic pathologies such as hemorrhage[85,86], necrosis[87,88] and renal failure[89,90]. Complications result in physical and psychological injuries[91,92] in the short and long term, which can have an economic impact, including death.

Short-term sequelae occur immediately after the snakebite but can be controlled in a reasonable time. Among them, local hemorrhage[93], anemia[94], edema[95], abscesses[88] and bacterial infections[96,97] are found. If these complications are not treated promptly and adequately, they can lead to systemic complications that can result in disability[98].

Long-term sequelae appear approximately six months after snakebite envenomation and may persist for months or even years[92,99]. These sequelae are of the physical and psychological type, although the psychological effects have a late onset. For both, there is no follow-up for adequate treatment[100]. Among the physical sequelae, the most common are tissue injuries. Tissue necrotization can trigger compartment syndrome[101], which must be addressed by surgical treatment. This procedure causes loss of tissue and skeletal muscle function[3]. In some cases, amputations must be performed that generate permanent disabilities[102]. The renal dysfunction developed in patients affected by ophidian accidents can be persistent and progress to acute and chronic renal dysfunction that strictly requires dialysis treatment[103-105].

Psychological sequelae do not derive from the toxic effects of the envenomation. Still, they are the result of the traumatic process of the snakebite in which the patient suffers from the physical and economic consequences of the ophidian accident. Depression and post-traumatic disorder are the most reported effects affecting 25-45% and 43% of the patients evaluated, respectively, being the leading causes of morbidity[106,107]. Less common psychological effects are headaches, vertigo, hysteria, and delirium, but cognitive functions are not yet determined to be affected[91,99]. Long-term psychological sequelae also cause deterioration in the family and educational context. Patients present negative attitudes that prevent them from continuing their work, generating an economic and social impact[107-109]. Timely treatment would improve the life quality of snakebite victims. A first aid intervention, cognitive and behavioral psychotherapy allows for to reduction of psychological and psychiatric symptoms[92,110].

In addition to the physical and psychological sequelae, the economic consequences are aggravated by the absence of rehabilitation after the ophitic accident. This fact hinders labor insertion. Most of the time, the rehabilitation cost must be assumed by the patient itself, who usually does not have the resources to do so. The family economy in areas with a high rate of snakebites is categorized as impoverished rural areas[12,111].

Agricultural activity is the livelihood of these communities, being limited by the economic expense of the sequelae they suffer. According to these situations, conditions for a worthy life are reduced[112-114]. Finally, the economic impact affects the nuclear family and negatively affects local and national productivity[115].

Unfortunately, some case studies do not detail the patient's conditions under which they die due to snakebites[116]. This fact makes it difficult to show how high a priority this disease is. Lizarazo *et al.*[117] reported a case of a farmer who suffered a snakebite caused by the *B. asper* snake that produced a cerebral hemorrhage. Unfortunately, the antivenom administration was late; he presented a multiorgan failure and died. Hospital reports are vital in managing this neglected disease, so standardized processes should be implemented in public health centers.

Global and Regional Snakebites Burden

Global and regional snakebite envenomation burden has not been accurately determined because of scarce information and studies estimating the incidence and mortality of SBE[118]. Existing data are based on hospitals' epidemiological reports that do not provide evidence of the true SBE burden[24]. Collecting information on snakebites and envenomations is difficult because most victims live in rural and remote areas with limited access to health services[55]. In addition, people in rural communities prefer to treat themselves with traditional methods and do not go to hospitals[1].

In 1954 an estimated 500 000 poisonings were estimated[119], and in 1998 the estimate increased to 5 million snakebites per year[1,120]. According to Gutiérrez *et al.*[121], the snakebite burden has an estimated 2.5 million bites per year, concentrated in South Asia, sub-Saharan Africa and Latin America. Through a regional comparison, it has been possible to determine the global level and the regions most affected by snakebite envenomation. The annual envenomation cases vary from region to region: Europe, where non-venomous snakes mainly cause snakebites, reported 8000 cases; North America 5 000 to 10 000 patients; the Middle East 15 000 to 40 000, Africa 43 000 to 1 000 000, Asia 121 000 to 2 000 000, Australia 10 000, Oceania 10 000 to 500 000 and Latin America 60 000 to 300 000[1,120-122] respectively.

Snakebite occurs in different geographical environments, whose social, economic and ecological factors may be similar, allowing the development of social and technological strategies to cope with this disease in the context of public health. Gutiérrez *et al.*[123] evaluated the snakebite envenomation situation in Costa Rica, Nigeria and Sri Lanka, the most affected regions worldwide. The annual SBE burden is similar in Nigeria, with 43 000 reported cases, and in Sri Lanka, 40 000 reported cases, while in Costa Rica, case reports were much lower, reporting just only 500 cases.

The scientific community in Neotropical countries has conducted several epidemiological studies. The results may overlap with other existing ones, and some countries do not publish any information[24]. In the Neotropics, the countries with the most scientific publications on snakebite envenomation are Costa Rica, Colombia, Ecuador, Argentina and French Guiana[1].

The lack of data reliability and accuracy is due to deficient information systems in the different countries and because victims from rural areas prefer to use traditional treatment methods based on medicinal plants[124,125]. In Latin America, Chippaux[5] reported around 60 000 cases between 2014 and 2016 of snakebites per year and just 370 deaths. In another study, Kasturiratne *et al.*[1] made epidemiological estimates of SBE obtaining 115 000 cases of snakebites and 2 000 deaths from 1985 to 2007.

Variations in epidemiological indicators such as burden, incidence, prevalence and mortality are due to the influence of environmental and anthropogenic factors. Also, the period and geographical area of epidemiological evaluation and the El Niño current have different effects according to geographical location[126]. The countries with the highest incidence of around 100 000 cases of SBE per inhabitant

in the Neotropics, according to the official health reports of each country, are Panama with 55.8, French Guiana with 21.1, Venezuela with 18.9, Costa Rica with 15.0 and Brazil with 13.4[5]. Values of SBE burden vary among scientific publications and may be over or underestimated. The annual incidence of snakebites worldwide is about 6.2 per 100,000 inhabitants, while mortality is 0.04 per 100,000 inhabitants[5].

Antivenom Production: Current Status in the Neotropics

Antivenom production in the Neotropics dates back to the beginning of the 20th century at the Butantan Institute in Brazil in 1901, considered one of the pioneering laboratories in the region[118,127] together with the Clodomiro Picado Institute in Costa Rica, founded in 1970[128]. In 2014, countries such as Mexico, Costa Rica, Venezuela, Colombia, Ecuador, Peru, Bolivia, Brazil and Argentina were antivenom producers with laboratories in Public Institutions[129,130]. Brazil, Mexico and Costa Rica were able to satisfy the antivenom demand at the national level and even cover the regional and global market[118,131-134].

In other cases, where antivenom needs are not supplied, it must be imported from other countries in the region and even from other continents, as in the case of Martinique and Saint Lucia, Caribbean islands, which import antivenom from France and the United States, respectively[118,135]. In the case of Ecuador, antivenom production was local at the former antivenom producer "Instituto de Higiene y Medicina Tropical, Leopoldo Izquieta Perez", which operated up to 2012 and was closed due to deficiencies in the production processes[13]. At the beginning of 2022, the National Institute of Public Health Research INSPI signed a cooperative agreement with the Regional Amazonic University IKIAM to implement a research project to optimize the experimental production of effective antiophidic sera in Ecuador. This project promotes a change in public health perception and in the snakebite envenomation victims' lives.

In addition to the importance of producing antivenoms to reduce the SBE burden, they should be validated through pre-clinical and clinical trials to determine their effectiveness against various venoms. This becomes in an essential task due to the diversity of snake species in the Neotropics. Several clinical studies have been conducted with antivenoms from the region to treat endemic snake envenomation136 and have successfully reduced the envenomation signs developed in the victims[137-139]. The results of these assays indicate the existence of cross-reactivity[106,130,133,140,141]among antivenoms and toxins affecting patients, while others cannot neutralize heterologous venoms[142]. Antivenoms produced commercially in the Neotropics at a laboratory scale are mostly derived from equine serum and neutralize venoms of the genus: *Bothrops*[143-145], *Crotalus*[146-148], *Micrurus*[149-151], *Lachesis*[140,152].

Currently, there are only three antivenoms approved by the Food and Drug Administration FDA for exclusive use in the United States: Antivenin® Wyeth[153], CroFab®[154] and Anavip®[155]. In the Neotropics, only the antivenom Antivipmyn®, manufactured by Bioclon Laboratories of Mexico, was recognized by the FDA as an orphan drug[156]. These orphan drugs are intended to treat diseases affecting a small number of people, less than 200,000[157]. However, the FDA-approved drugs derived from snake venoms such as Captopril and Batroxobin[158], obtained from the venom of *B. jararaca*, and the latter from *B. moojeni* and *B. atrox*[32,159]. These drugs have proved biomedical applications.

The efforts of antivenom research and production are evident in the published scientific literature; however, there are drawbacks related to the heterogeneity in the used production technologies, the quality and innovation of the pharmaceutical products obtained and the production volumes[129,160,161]. These variables cannot be analyzed due to the absence of updated and reliable epidemiological information that would allow having a base-line and thus supply the antivenom requirements. In addition, antivenom production in Latin America during 2020 was reduced as a consequence of the COVID-19 pandemic caused by SARS-CoV-2. Medical supplies and research were mainly focused on developing therapies and diagnostic kits to cope with COVID-19 health emergencies[162].

Snakebite in Ecuador

Epidemiological studies in Ecuador are limited[62,163]. No data has existed in the country's health system records in the last three years. From 2014 to 2018, the Public Health Ministry of Ecuador, through the Epidemiological Gazette, recorded 7 714 cases of snakebites in the country, with an average incidence of 9.37 / 100 000 inhabitants and an annual incidence of 9.0, 11.3, 10.4, 8.6 and 7.6 respectively[164]. In the Chippaux[5] study, incidence and mortality rates of 9.5 and 0.057 cases per 100,000 inhabitants were estimated in 2014-2015. In another study, Ecuador reported 9.8 cases per 100,000 inhabitants that resulted in 0.06 deaths per 100,000 inhabitants each year in 1998-2007[80]. The annual incidence of snakebites in Ecuador does not have significant variations even though the Spatiotemporal analysis differs, 11.5 /100 000 inhabitants[80],7.7-11/100 000 inhabitants[165] and 9.5 / 100 000 inhabitants[5].

Morona Santiago and Manabí are the provinces that register the highest number of snakebite cases. However, it is observed that in both the coastal and Amazonic regions were found a more significant number of cases[164]. Snakebites are distributed geographically in the Coastal region (56-58%), Andean region (5-33%) and Amazonic region (11-37%)[80]. Studies conducted in indigenous communities indicate that the highest incidence of snakebites occurs in the Amazon region[142,166-168], associated with the distribution of snake species in the country[22,26]. Among the species causing snakebites in the country is the genus: *Bothrops, Bothriopsis, Lachesis* y *Micrurus*[79] and the species: *B. atrox, B. asper, L. muta, B. bilineata* y *Bothrops lojanus*[26,168]. Information obtained from available epidemiological studies indicates that those mainly affected are agricultural workers in rural areas[80]. Heavy rains such as the El Niño Phenomenon in January to June increase the incidence of snakebite cases throughout the country[80].

Biotechnological Approach to Snakebite Therapy

Drawbacks of available Antivenoms

Antivenom therapy is the most widely used therapy to treat several pathologies, including snakebite envenomation, by neutralizing the venom proteins[169]. Traditional antivenom production has several drawbacks affecting their safety and efficacy, such as the venom complexity, adverse reactions and production costs. Venom composition varies by snake diversity[69], geographic distribution[170,171], ontogenetic[172] variations and snake growth stage[173]. Toxins that make up snake venom have a great molecular and biological diversity, including protein and peptide content; non-protein

components such as carbohydrates, lipids, amino acids, nucleosides, amines and metal ions[174]. The main molecules of medical importance are grouped into dominant and secondary proteins: phospholipases, metalloproteinases, serine proteinases, three-finger toxins, hyaluronidases, and myotoxins, among others (Table 2)[175]. These factors limit the antivenom's neutralization spectrum, although several studies in the Neotropics indicate a good neutralization capacity of heterologous venoms, as mentioned in the previous sections.

Antivenoms are obtained from animal immunization, such as horses[184,185], donkeys[186], and sheep[187,188], hence the name heterologous antivenoms[189,190]. Antivenoms currently present several drawbacks, as 59% of patients treated with this therapy develop adverse events and side effects[191]. Early anaphylactic reactions cause headaches, vomiting, fever, and urticaria[192,193] and may or may not be an IgE-mediated response[194]. Antivenom can be composed of complete IgG, antibody fractions (Fab or (Fab')2) and other serum proteins of animal origin that can also cause adverse reactions. In addition, many antibodies do not neutralize the target antigen[195]. Different types of antibodies are involved in developing late anaphylactic adverse reactions, such as human anti-horse antibodies named heterophile. These antibodies form an immune complex deposited in the target tissues causing inflammation known as serum sickness[196,197].

Purification and enzymatic digestion techniques are being employed to improve the quality and safety of the drug product while reducing the effects of adverse reactions[198]. Antivenom formulations with antibody fragments maintain neutralizing capacity and minimize adverse effects[137,199]. This antibody-based production technique currently employed has several disadvantages due to the costs of animal maintenance and antibody purification techniques. These facts limit the reproducibility of this technology[14,189].

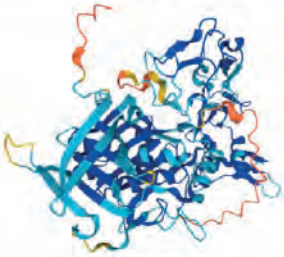
Toxins	Physiologic effects	3D-Structure
Phospholipases (PLA2s)[176,177]	Neurotoxic and myotoxic effects Severe necrosis Paralysis due to blockage	 UniprotKB: P24605 Myotoxin II *Bothrops asper*
Snake Venom Metalloproteinases (SVMPs)[178,179]	Systemic lethal hemorrhage Edema Hyperalgesia Inflammatory pain	 UniprotKB: P30431 HF2-proteinase *Bothrops jararaca*
Snake Venom Serine Proteinases (SVSPs)[180,181]	Alteration of hemostasis Edema Hyperalgesia External and internal bleeding	 UniprotKB: Q9PTU8 Bothrops protease A (BPA) *Bothrops jararaca*
Three-Finger Toxins (3FTXs)[182,183]	Neurotoxicity Hemotoxicity Flaccid paralysis Necrosis	 UniprotKB: C0HJR1 Micrurotoxin 1 *Micrurus mipartitus*

Table 2. Main snake venom toxins, physiological effects and its 3D-structure.

Based on this background, we expose the use of biotechnological tools with an innovative approach to improve the neutralization of venoms, increasing their efficacy and improving production yields. It is essential to consider biotechnology as a solution to the shortage of antivenoms in Latin America and the world since production costs can be reduced, and the pharmaceutical market in the production of antivenoms will be empowered.

Recombinant Antibody Technology

The first recombinant antibodies were developed by Georges Kohler and Cesar Milstein in 1975 using the hybridoma technology[200]. Recombinant antibody technology since then has become a potential therapy for snakebite envenomation. They are more effective because of their neutralizing capacity and reduced side effects such as anaphylactic reactions compared to animal serum-based antivenoms[198]. Using this technology, several antibody formats have been obtained in chimeric and humanized versions, whose therapeutic use is approved by the FDA in treating several diseases[201,202].

The hybridoma technology, nevertheless, has several disadvantages. The main one is related to the development of human anti-mouse antibodies that cause allergic reactions and decrease the lifetime of the therapeutic antibody[203]; however, this is still the most widely used technique for antibody production. Production of antibodies involves an immunological process in which the antigen undergoes proteolytic degradation, so the derived antibodies will not distinguish the antigen in its initial form[204]. Therefore, researchers work to find other technologies that solve these drawbacks and do not activate the complement system.

Antibodies for therapeutic purposes like IgG have a longer life and are more permeable[205]. Recombinant antibodies have various formats in the structure; they are assembled according to different combinations of heavy and light chains[206]. The single-chain variable fragment (scFv) and fragment antigen-binding[137] are the most commonly used formats because of their high affinity to the antigen, structural stability and shorter generation[207].

Currently, several studies demonstrate the efficacy and therapeutic potential in neutralizing various snake venoms using recombinant antibodies of multiple formats, e.g., camelids nanobodies against the poison of *B. atrox*[208] and *C. durissus terrificus*[209]; scFv against the venom of *Bothrops pauloensis*[210] and *L. muta*[211]. Recombinant antibodies, such as scFv and nanobodies, are FDA approved for treating the diseases, except for the treatment of snakebite envenomation[212]. In addition, the FDA and European Medicines Agency EMA has not approved the production of recombinant antibodies in *E. coli*[189].

The isolation of therapeutically effective antibodies has presented low yields due to the high purification costs using traditional methods[213-216]. Smith[217], in 1985 developed the phage display technique (PDT) that is independent of an immune system, making this technique the most selected since it does not generate immunogenicity in patients[218]. The PDT, as shown in Figure 1, consists of an *in vitro* phenotypic selection of antibodies expressed with the fusion proteins of M13 filamentous phages. At the same time, a genotypic selection is needed because the various genes encoding the antibodies of interest are found inside the phages[219,220]. The antibody selection and enrichment process are performed by affinity to the molecules of interest, in this case, the venom toxins[218,221]. This technology's advantages focus on controlling the process conditions such as antigen selection, immobilization, design of the antibody libraries, and binding and washing needs. In addition, it is a faster and low-cost technique compared to hybridomas production[204]. It is good to stand out that antibody production against snake venom toxins has only reached the laboratory scale; research is still ongoing for their optimization and further scaling up.

The next stage of affinity antibody selection is heterologous expression. There are several expression systems ranging from bacteria, yeast, insects, and plants to mammals, each with advantages and disadvantages[222]. Bacteria such as *Escherichia coli*[223,224] and *Bacillus subtilis*[225,226] have been employed as factories to produce heterologous proteins for therapeutic purposes because their genome is characterized and genetic manipulation is simple; they have rapid growth and bioprocesses have enabled large-scale production at low cost[167,227]. Castro *et al.*[228] produced a recombinant antibody, scFv, that neutralizes the BaP1 metalloprotein from *Bothrops asper* snake venom by expressing the antibody in a bacterial system using *Escherichia coli* as host (Figure 2). After protein extraction and purification, the yield was 280 ug of scFV per liter of bacterial culture. The drawbacks of these systems are due to the absence of post-translational modifications and a poor excretion system, as the stability of the proteins depends on the oxidative environment where it is secreted. In addition, inclusion bodies can be formed, hindering antibody purification[229-231]. Strategies to optimize antibody production in *E. coli* are listed in Table 3.

The most commonly used yeasts for these purposes are *Pichia pastoris*[240-242] and *Saccharomyces cerevisiae*[243,244]. Both are easy to grow, perform post-translational modifications such us disulfide-bonded and protein glycosylation, have a high growth rate and protein secretion levels are very high. Contrary to bacterial expression systems, protein secretion in yeasts constitutes a great productive advantage since the secreted proteins are harvested relatively quickly from the culture medium, so downstream processes are cheaper[242,245]. *Pichia pastoris* is also the most widely used yeast strain due to its ease of industrial scaling. It reduces costs and minimizes equipment used for implementing pilot or industrial bioreactors[246]. Yeast expression systems are used to produce recombinant antibodies and proteins with inhibitory action against venom toxins (Figure 2). The antimyotoxic protein DM64, which acts against phospholipases A2 of *Bothrops asper* venom[247], was successfully produced by a recombinant *Pichia pastoris*.

Mammalian cells are commonly used to produce biopharmaceuticals, antibodies and active protein[248]. Antibody production in mammalian cell systems is mainly selected by its ability to carry out post-translational modifications that maintain antibody stability so a correct protein function. However, expensive culture media due to nutrient requirements and high contamination rates limit this technology. In addition, yields are low and the slow production time increases costs[236,249].

Laustsen *et al.*[251] and Jenkins and Laustsen[250] estimated the cost of large-scale production of antibodies in the Chinese Hamster Ovary (CHO) cell expression systems using a fed-batch fermentation strategy. The production cost ranged from 20 to 250 USD for these pharmaceutical products. On the other hand, plasma-derived antivenom production is around a thousand dollars. Currently, no studies are estimating the cost of new screening and expression technologies applied to large-scale antivenom production.

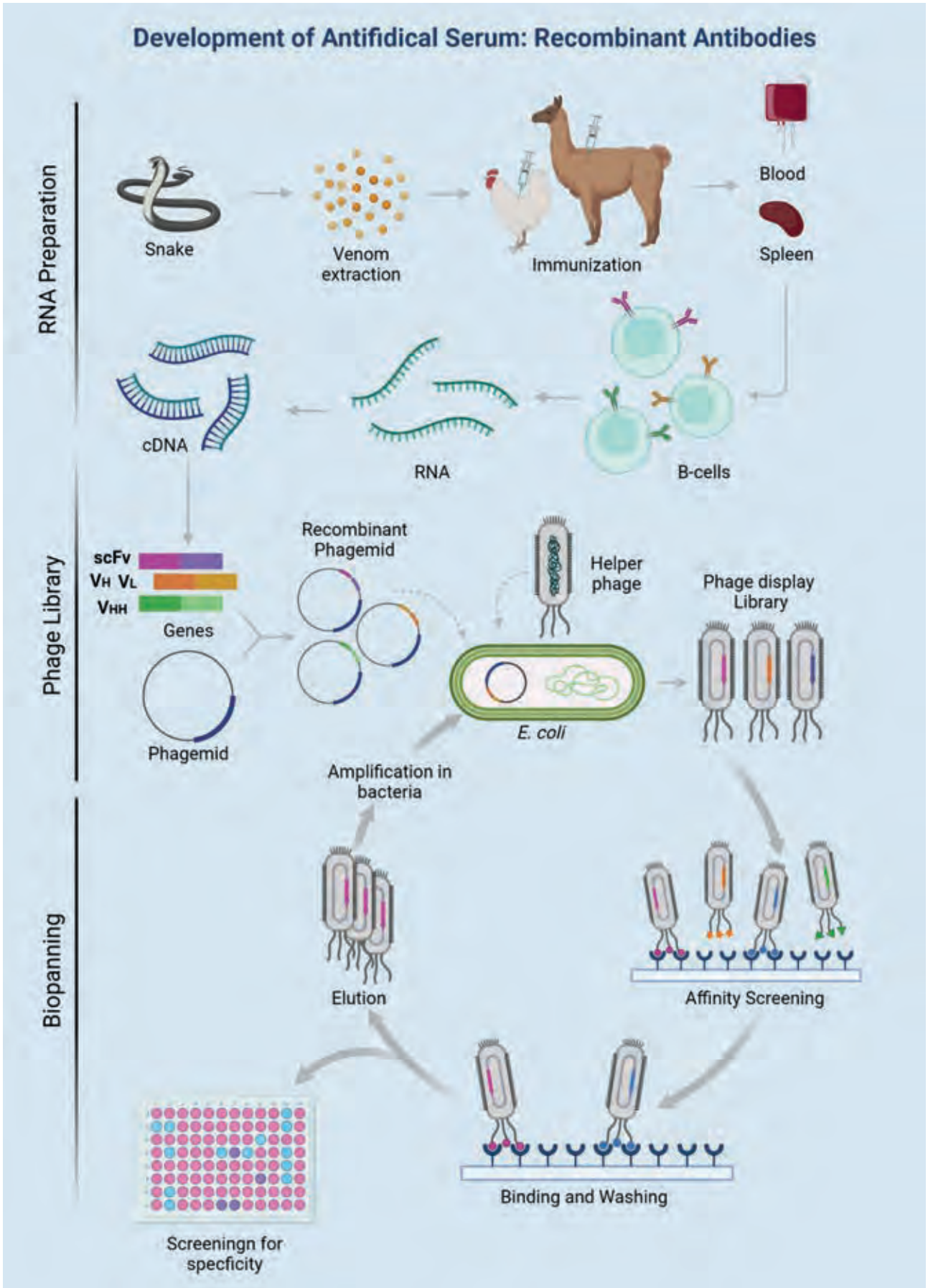


Figure 1. Phage display for selection of antibodies against snake antivenom. Image created using BioRender (https://biorender.com/).

Expression system	Advantages	Challenges
Periplasmic Expression[232-234]	• Correct protein folding by chaperones that catalyze the formation of disulfide bonds.	• Signal sequences are required, which affect production yields because they are unpredictable. • Low membrane transport performance. • Limited volume of periplasmic space.
Co-Chaperone Expression[235-237]	• Cytoplasmic expression of disulfide-rich proteins • It requires the expression of other chaperones that provide oxidative equivalents to generate de *novo* disulfide bonds.	• Low yields.
Inclusion Bodies[238,239]	• Protect against the potential toxicity of the expressed protein. • High performance and potential purities.	• Complex protein purification protocol. • Misfolded and inactive proteins.

Table 3. Strategies for the expression of disulfide-rich proteins in *E. coli*.

An alternative system to those described above includes insect cells advantageously as production hosts. This expression system can use chaperones for correct protein folding and own key metabolic pathways to carry out post-translational modifications, such as acetylation or glycosylation[251,252]. The system works with the baculovirus expression vector[253]. Insect cells are used as hosts to a greater extent for toxins production used as immunogens and for different *in vitro* toxicity assays. This technology is more complex but with high throughput and reproducibility at low costs[254].

Finally, we have plant-based expression systems. To date, several types of toxin antibodies have been produced experimentally[255-258]. Plants are considered biofactories because of the amount of biomass they generate, allowing large-scale production. They are low-cost and not susceptible to contamination. Nevertheless, even though the initial steps of N-glycosylation and N-glycan processing are highly conserved between plants, mammals and yeast, N-glycosylation patterns differ between them[259].

Antibody expression titers in plants are low, so approaches for expression improvement have pointed to expression cassette design, plant and tissue selection and plant material extraction techniques[260]. There are few studies of antibody expression in plants (Figure 2). One reported the extraction and purification from *Nicotiana tabacum* leaves of scFv against *B. pauloensis* venom[210].

Although different techniques currently produce monoclonal antibodies, other biotechnological alternatives can be employed to enable regional and global scale of antivenoms production.

Omics for the production of Antivenoms

Omics enable innovation in the health sector to broaden the understanding of physiological processes of pathologies involving various molecules such as nucleic acids and proteins[261] so, facilitating effective diagnosis and treatment[262]. Omics tools such as proteomics and transcriptomics are a fundamental axis in the design and production of antivenoms (Figure 3) as they are part of the preclinical evaluation and improvement of antivenom efficacy[170,263].

These technologies must comply with the good manufacturing practices detailed in the WHO Guidelines for the industrial production of antivenoms[43,264] to ensure the quality and safety of the pharmaceutical product[265]. Proteomic and transcriptomic guide researchers to understand the biochemical and toxicological variations in venoms to antivenoms[265] response. Omics tools should be included in antivenom production processes to validate the safety and quality of the bioproduct[266].

Transcriptomics

Transcriptomics studies genome-encoded RNA transcripts such as mRNA, rRNA, tRNA, miRNA, and non-coding RNA[267]. The mRNA is required for protein synthesis, and its abundance indicates the presence of a target gene. The transcriptome is subject to change due to time, environmental and physiological conditions[268]. Transcriptomics gives information on RNA diversity, transcriptional units, splicing mechanisms, post-transcriptional modifications and information of gene expression, regulation and signaling[267]. The transcriptomics workflow is depicted in Figure 3.

Transcriptomic studies of venoms and venom glands of some snakes from the Neotropics were carried out by Rodrigues *et al.*[269]. He compared the transcriptomic profiles of the venom and venom gland of *Bothrops pauloensis*, finding qualitative variations and low concordance with the proteomic profiles. Ontogenetic changes affect venom composition; in young species of *B. jararaca* there is a greater diversity of toxin precursors and elevated amounts of metalloproteinases compared to adult species[173]. The analysis of the ontogenetic factor is fundamental in the production of antivenom since the efficacy in neutralizing envenomations caused by juvenile species may be limited. Freitas-de-Sousa *et al.*[270] evaluated the environmental effect in captive and wild species of de *B. atrox*, the composition of the venoms does not present significant quantitative differences, thus, supporting the use of venoms from captive species for the production of the antivenom.

The use of transcriptomics as a tool for toxin discovery

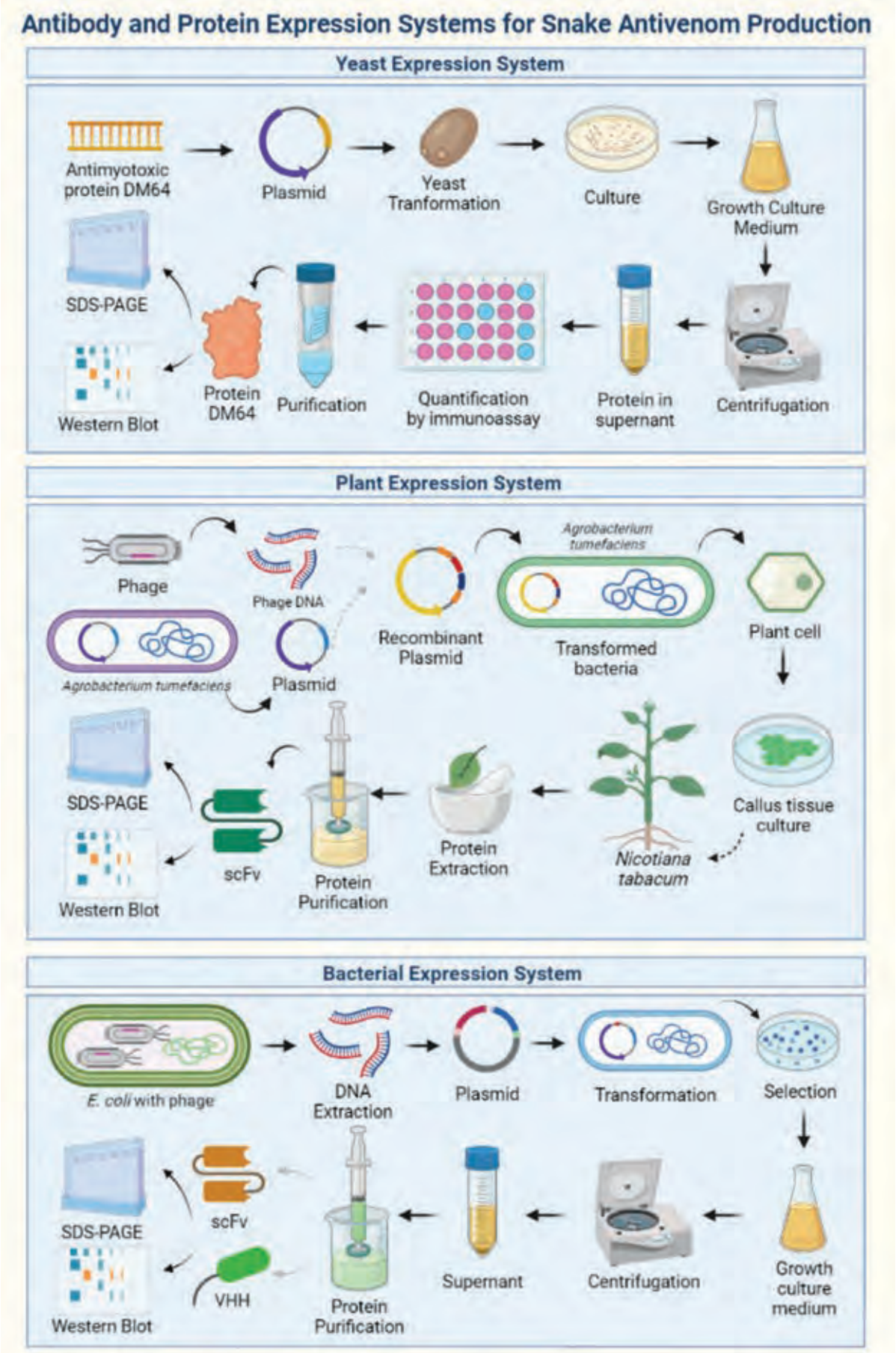


Figure 2. Protein and recombinant antibody expression systems for the production of snake antivenoms. Image created using BioRender (https://biorender.com/).

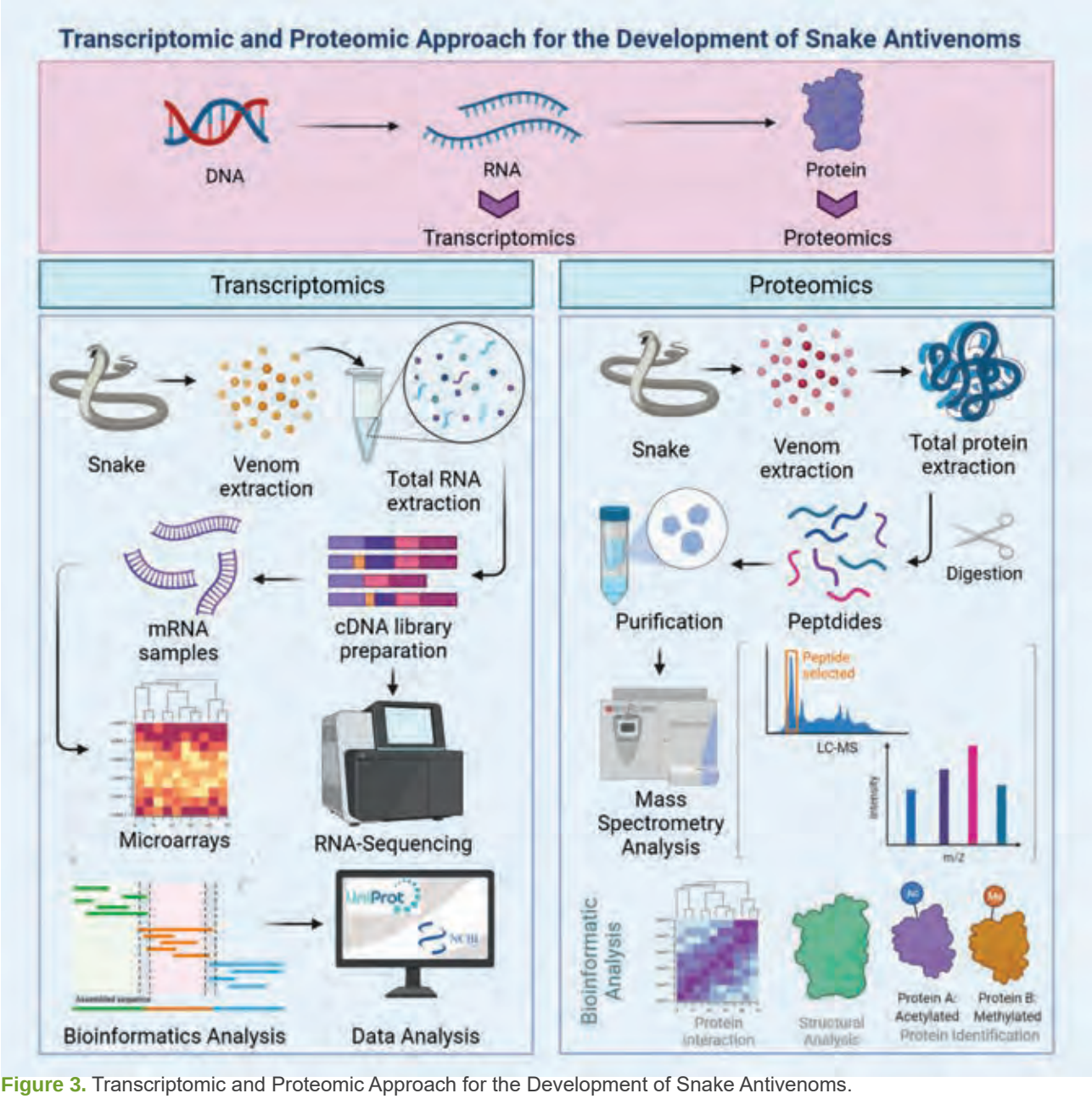


Figure 3. Transcriptomic and Proteomic Approach for the Development of Snake Antivenoms.

has displayed good results. In the venom gland of *Bothrops moojeni*, new toxins have been discovered, and amino acid sequences of unreported toxins have been obtained. These findings promise to know the function of new toxins and to design effective and neutralizing antivenoms[271]. Transcriptomics is used to know the complexity and composition of snake venoms and to evaluate toxins' immunogenicity at the molecular level, specificity and affinity for epitopes. The study of phospholipase A2 and three-finger toxin from *Micurus nigrocinctus* venom presented low immunogenicity[272].

Proteomics

Proteins are expressed in cells and perform cellular processes related to biological functions. Proteomics studies the entire set of proteins in a cell or organism[273]. It is characterized by being dynamic and influenced by time, space, environment and cellular modifications such as post-translational modifications[274,275].

Proteomics has several approaches to obtaining information about protein structure and functionality, including cellular expression, modifications, interactions and signaling[275]. The study of proteomics is essential if we consider proteins as gene products since proteins determine the phenotype. Genomics is static; the expression level of a gene will not always correlate with protein levels[276] due to post-transcriptional and post-translational modifications. The study of snake venom proteins is also known as venomics. Both venomics and peptidomics allow understanding of the biological processes in envenomation, development of new therapeutics and potential pharmacological applications of snake venom toxins[277]. Proteomics follows two main experimental approaches for its study: gel-based and mass spectrometry. The mass spectrometry approach is divided into two modalities: Bottom-up or Shotgun proteomics, where proteins undergo enzymatic digestion, and top-down analysis employs intact protein[278]. The proteomics workflow is depicted in Figure 3.

Venom proteomics studies showed variations in the

composition and functionality of toxins by several factors. A phylogeographic approach with proteomic tools has determined the venom phenotypes of snake species belonging to the Micrurus; the geographic distribution of venomous snakes and evolutionary mechanisms are very influential factors[279]. There are interspecific, intraspecific, qualitative and quantitative variabilities of snake venoms under different environmental conditions. Oliveira *et al.*[165] evaluated the proteomic profiles of 22 individuals of the *C. durissus terrificus*, new venom components were found with various enzymatic activities that cause other immunological and biochemical effects affecting antivenom production. Snake species, such as *B. atrox* and *B. jararaca*, develop a response to adapting environments that can produce several venoms-protein isoforms at the molecular level with different biological activities, complexity and enzymatic activity, which limits antivenom efficacy[280,281].

Immunogenomics

Immunogenomics is an essential tool for antivenoms development since epitopes mapping antibodies capable of recognizing these antigenic sites can be designed. This fact increases the neutralization capacity of antivenoms[282]. This tool, also known as antivenomics, allows identifying the recognition of certain immunogens by antibodies, a key factor in the clinical efficacy of antivenom in snakebite envenomation[283].

The antivenoms produced at Instituto Butantan and Instituto Clodomiro Picado present efficient neutralization of the venom of *B. atrox* and *Bothrops erythromelas* species in the northern region of South America and Brazil, respectively[170,284]. The commercial antivenom Antivipmyn Tri produced in Mexico by Instituto Bioclón exhibits immunoreactivity of *C. durissus cumanensis* venom in Colombia[285].

Toxins used to determine antibody responsiveness are also produced without the need for host cells. Protein synthesis is performed with the necessary components: dNTPs, amino acids, ATP, GTP, biological machinery that includes ribosomes, tRNA, RNA polymerases, initiation and elongation factors and a motor-like plasmids DNA carrying the correct information for transcription, translation and accurate folding *in vitro*. The main drawback of cell-free production is the low yield of proteins and their poor stability[286,287].

Bioinformatics, the best omics ally

The results obtained from these omics tools are massive, so computational tools are needed to facilitate data analysis[288,289]. Bioinformatics, through the use of algorithms and computational strategies, develops methods to analyze biological data, which include: data organization and curation, processing, annotation, statistical analysis, prediction and simulation[290]. The most commonly employed bioinformatics analyses are listed in Table 4.

Scientific evidence for decision-making in Public Health

The vast amount of information found in the published scientific literature on snakebite epidemiology, strategies and other associated issues can create confusion among decision-makers in governmental entities in the region and hinder the formulation of public health policies. Based on this systematic review, we propose actions that governments should implement according to their country's needs.

Start with creating a single national registry system to obtain a database on the epidemiology of snake bites. The information obtained will make it possible to know which groups are most affected. With this base information, strategic programs for prevention, control, monitoring, planning and research can be developed.

Follow-up programs for victims of venomous snakebites can reduce long-term sequelae. Prevention strategies should include educational programs and the provision of protective equipment in rural areas whose main activity is agriculture. Efforts should be made to strengthen the medical room for rapid action in snakebite emergencies.

Last but not least, governments should provide resources for the characterization of clinically meaningful snake venoms and promote research to create effective and low-cost diagnostic and therapeutic tools.

Conclusions

Snakebite disease is considered a neglected tropical disease due to its global, regional and national burden, as well as its social and economic impact on society. Although there are several prevention strategies and tools for treating this disease, a One Health approach is required because several actors are involved in its dynamics. Ecological, political, technological and medical aspects should be considered to allow us to manage and administer the correct registration of snakebite cases from public policies. It is essential to ensure the development of preventive programs and effective treatments for snakebite envenomation using current biotechnological tools for vulnerable populations. Preventive programs will improve the economic and social situation of the most affected regions today. The most modern biotechnological tools have been applied experimentally, but only on a laboratory scale, and the support of governmental entities is a crucial factor in enhancing the future industrial production and snakebite antivenoms scaled up.

Funding

Authors declare no such funding related to this article.

Acknowledgments

Not applicable.

Conflicts of Interest

Authors do not claim any conflict of interest.

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Technology	Application	Supplementary techniques	Bioinformatics Analysis	Target Venom
Phage Display	Development of Antibodies, selection of scFv[210,211] and delection of VHHs [208,209].	Library construction Bio panning Sequencing ELISA Western Blot Colony PCR MALDI-TOF2	Multiple sequence alignment	*Bothrops atrox* *Bothrops pauloensis* *Crotalus durissus terrificus* *Lachesis muta*
Recombinant antibodies	Development of Antibodies, selection of scFv[210] and selection of VHHs[208,209].	Cloning PCR SDS-PAGE Western Blot Antibody expression and purification	N/D*	*B. atrox* *B. pauloensis* *C. d. terrificus*
Plant transformation	Production of recombinant antibodies[210]	Plasmid construction PCR Sequencing	N/D*	*B. pauloensis*
Proteomics	Venom phylogeny[279,291]. Identification of venom toxins[149,270,280,292]. Venom variability[165,170,284].	Protein, peptide and amino acid sequencing Liquid Chromatography: FPLC, RP-FPLC, RP-HPLC SDS-PAGE Mass spectrometric analysis: ESI , nESI-MS/MS, MALDI-ToF, MALDI-TOF-TOF, LC-MS/MS	Peptide sequence analysis	*B. atrox* *Bothrops jararaca* *Bothrops erythromelas* *Crotalus durissus collilineatus* *Micrurus ruatanus* *Ophryacus sphenophrys* *Porthidium lansbergii*
Peptidomics	Composition of snake venom[293].	Enzyme digestion LC-MS/MS	Peptide alignment	*B. atrox*
Transcriptomic	Identification of venom toxins[271]. Characterization of toxins[173,270]. Molecular basis of venom composition differences[173,269]. Antibody responses to toxins at the molecular level[272].	cDNA library construction PCR Sequencing Generation of ESTs High-throughput sequencing Next-generation sequencing	Assembly of contigs, sequence alignment, Dendogram (Neighbor-joining method), Protein modelling	*B. atrox* *Bothrops moojeni* *B. pauloensis* *B. jararaca* *Micrurus nigrocinctus*
Antivenomics	Efficacy of antivenoms[170]. Immunoreactivity[284,285].	Immunodepletion RP-HPLC SDS-PAGE Immunoaffinity assay	N/D*	*B. atrox* *B. erythromelas* *Crotalus durissus cumanesis*
High-density peptide microarray	Epitope mapping[294].	Library of peptides *in silico* Photolithographic synthesis Protein homology model	Multiple sequence alignment	*B. asper* *Crotalus simus* *Lachesis stenophrys*

Table 4. Current biotechnological technologies for producing snake antivenoms in the Neotropics use bioinformatics tools.

Bioinformatics	Identification of epitopes[295] Phylogeny-based comparative analysis[291]	DNA extraction PCR Sanger Sequencing Protein sequence analysis Prediction of secondary protein structures Molecular modeling Peptides synthesis	Multiple sequence alignment Phylogenetic reconstruction (Bayesian inference)	*L. stenophrys* *P. lansbergii*
B-cell epitope mapping	Identification of peptides capable of inducing neutralizing antibodies[296-298] Synthetic antigens for immunization protocols[298-300]	SPOT synthesis technique: peptides and genes Fmoc solid-phase synthesis MALDI-TOF ELISA Homology modelling	Sequence alignment	*B. atrox* *B. jararaca* *C. durissus* *Micrurus corallinus* *Micrurus frontalis*
Liposome encapsulation	Encapsulation of synthetic peptides for immunization[298,300]	N/D	N/D*	*B. atrox* *C. durissus*
Heterologous protein expression	Development of antitoxic protein as antivenom therapy[301]	Plasmid construction Transformant screening Protein expression Immunoassay Western Blot	N/D*	*B. asper*
Cell culture	*In vitro* alternative assay for antivenom pre-clinical evaluation[247]	Cell viability assay Neutralization assay Phase-contrast microscopy	N/D*	*B. jararaca*

*NO DATA

Table 4. Current biotechnological technologies for producing snake antivenoms in the Neotropics use bioinformatics tools.

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Evaluation of new genetic structures under the dimensions from *Cicer arietinum*

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Abstract: Twenty-one chickpea genotypes were tested to evaluate genetic variation in some agronomic traits, heritability and genetic advance. The experiment was conducted in field crops during the 2018 -2019 growing season. A Randomized Complete Block Design (CRD) with three replications was used; data were collected for days to 50% flowering, plant height, number of primary branches per plant, number of secondary branches per plant, the height of the first pod from the ground, number of pods per plant, number of seeds per pod, biological yield per plant, seed yield per plant, harvest index and 100-seed weight. Analysis of variance showed a highly significant mean square difference for all traits except the number of pods per plant, harvest index and 100-seed weight. Genotypes (7,18) were more distinctive than the other genotypes in most characteristics. The high value of the genotypic coefficient of variation was found for biological yield per plant (29. 772), seed yield per plant (24.757) and the number of primary branches (24.849). High heritability was recorded for the first pod height from the ground (67.8) and plant height (60.8). High expected genetic advance as a percentage of the mean was estimated for biological yield per plant (41.144), seed yield per plant (39.61), and the number of primary branches per plant (38.382).

Key words: Genetic variability, Heritability, genetic advance, Chickpea.

Introduction

Chickpea (*Cicer arientinum* L.) is considered the third among pulses, and the world pulses production is 12%[1]. Chickpea ranks as an essential source of protein for the rural poor who cannot buy animal products. In Iraq, chickpeas' productivity is unstable due to cultivars with a narrow genetic base, which exposes them to biotic stresses in fencing production. Genetic diversity is needed in crop breeding programs to improve the productivity of cultivars by using the introduced germplasm one method. Information on genetic parameters in new germplasm is needed to improve genetic diversity in breeding and breeding programs[2]. High variations in days to maturity, the number of plant pods and seed yield in chickpea[3], plant height and the number of plant branches[4], are decisive factors in deciding which traits still show high variability value by phenotypic and genotypic value and coefficient of variation, giving the idea about the amount of variability in the population, (genetic status). In addition to genetic variation, heritability is an important parameter in the selection of specific traits, high heritability value in a broad sense was found in 100-seed weight, and the number of seed plant^{-1} in chickpea by (4), seed yield and number of branches plant F1[5]. Estimated high broad-sense heritability for (6), biological yield and its related traits in soybean, days to 50% flowering in chickpea by (1),(7) found high broad-sense heritability for the number of branches and biological yield along with genetic advance in chickpea with the same traits[8]. Genetic variability, heritability and genetic advance in chickpeas were studied, and a low genetic coefficient of variation for days to 50% flowering and plant height indicated common environmental effects on these traits. Heritability effects are essential in expressing the reliability of phenotypic characters with high heritability, which is influential in selecting such characters and desiring future chickpea breeding programs. The present study aims to determine the genetic variability, heritability and genetic advance in 21 genotypes of chickpeas.

Materials and methods

The experiment was carried out in the field crops Department. College of Agricultural Engineering Science, Duhok University, Iraq. In the growing season, March 2018 using, twenty genotypic and one check (local cultivars) were used in this study (Table 1). The source of genotypic seed was obtained from International Center for dry Agriculture ICARDA; all the genotypes seeds were sown in randomized complete block design R C B D with three replication in three-row 4 m long 50cm between and 20cm within the row all Agricultural practices were performed recommended for chickpea productions Five plants selected round only for each phenotype to recording data of the following traits:-number of days to 50%vflowering, number of secondary branches per plant height of fresh pod.cm., number of pods per plant, number of seed per pods, Biological yield gm, grain yield per plant gm, selection index and 100 seed weight gm.

Citation: AliAgwan I M M, Altaweel M S, Ali Askander H S. Evaluation of new genetic structures under the dimensions from Cicer arietinum. Revis Bionatura 2022;7(4) 55. http://dx.doi.org/10.21931/RB/2022.07.04.55
Received: 27 March 2022 / **Accepted:** 22 October 2022 / **Published:** 15 November 2022

Data were subjected to analysis of variance to define the phenotypic and genotypic, and environmental variance and coefficient of variation according to the formula suggested by (9),

Environmental variance O'²e=MSE

GA=KO'Ph²

Where:-

K=selection intensity under 10%=1.76

O'p=standard variance of the phenotype

h=heritability in a broad sense

GA=genetic advance.

GAM=GAIX̄*100

Where:-

GAM=genetic advanced percent of the mean

x̄=mean of population.

Genetic advance is considered high when it is more than 30% and 10-30%medium less than 10% low[12].

The study was carried out to evaluate the performance of 21 new inputs derived from the International Center for Dry Agriculture ICARD shown in Table 1.

GA=KOPh²
Where:-
K=selection intensity under 10%=1.76
Op=standard variance of the phenotype
h=heritability in a broad sense
GA=genetic advance.
GAM=GAIX̄*100
Where:-
GAM=genetic advanced percent of the mean
x̄=mean of population.

NO.	Genotypes	Pedigree
1	FLIP07-180C	X03TH-29/(S99858XFLIP97-26)XS00432
2	FLIP07-193C	X02TH 61/S99520XL.Mt-1
3	FLIP09-63C	X05TH7/X04TH-126XFLIP01-18
4	FLIP09-88C	X05TH64/X04TH-202XFLIP00-17
5	FLIP09-97C	X05TH64/X04TH-202XFLIP00-18
6	FLIP09-113C	X05TH64/X04TH-202XFLIP00-19
7	FLIP09-114C	X05TH64/X04TH-202XFLIP00-20
8	FLIP09-122C	X05TH64/X04TH-202XFLIP00-21
9	FLIP09-220C	X05TH64/X04TH-202XFLIP00-22
10	FLIP09-221C	X05TH64/X04TH-202XFLIP00-23
11	FLIP09-222C	X05TH64/X04TH-202XFLIP00-24
12	FLIP09-223C	X05TH64/X04TH-202XFLIP00-25
13	FLIP09-224C	X05TH64/X04TH-202XFLIP00-26
14	FLIP09-225C	X05TH64/X04TH-202XFLIP00-27
15	FLIP09-226C	X05TH64/X04TH-202XFLIP00-28
16	FLIP09-227C	X05TH64/X04TH-202XFLIP00-29
17	FLIP09-228C	X05TH64/X04TH-202XFLIP00-30
18	FLIP09-230C	X05TH64/X04TH-202XFLIP00-31
19	FLIP09-231C	X05TH64/X04TH-202XFLIP00-32
20	FLIP09-232C	X05TH64/X04TH-202XFLIP00-33
21	Duhok variety	Local check variety

Table 1. Numbers, Names and Pedigrees genotypes of chickpea.

The land was plowed with the plowing disc plow, and the soil was mowed with disc harrows the twenty. On 23-12-2014, under the demographic conditions in the fields of one of the farmers of the region of the province of Nineveh in the experiment design of the random segments full RCBD three replicates where the experimental unit consists of two lines length of 4 m for the line and 50 cm between the line and another and (20 cm) between Joura and other, The fertilizer was added Luria (N% 46) at an average rate of (120 kg / e) in the first two steps after germination and the second at the beginning of the stage of holding flowers, and the amount of precipitation during the growing season. At the end of the growing season, the studies were carried out on five plants randomly selected from the experimental unit. The yield was estimated by harvesting the experimental unit plants and studying the number of days for 50% flowering, plant height (cm), total plant count, Qurna length (Kg/ha), dry seed yield (kg/ha), harvest index (%), the weight of 100 seeds (g). The data were analyzed statistically according to the design of the entire random sectors (RCBD. Using the SAS (2004) program, genetic, phenotypic, and environmental variations were estimated according to the way they were explained[11].

$$\sigma^2_g = (M.S.t - M.s.e)/r$$

$$\sigma^2_e = M.S.e \quad \sigma^2_p = \sigma^2_g$$

The standard error of phenotypic variation was estimated (12) according to the equation:

$$SE(\sigma^2 G) = \sqrt{\frac{1}{r^2}\left[\frac{2(msg)^2}{k+2} + \frac{2(mse)^2}{k+2}\right]}$$

$$SE(\sigma^2 E) = \sqrt{\frac{2(mse)^2}{k+2}} \qquad SE(\sigma^2 p) = \sqrt{\frac{2(\sigma^2 p)^2}{N}}$$

Note that k = degrees of freedom for each source (genetic structures or experimental error), r = number of replicates, where N = total degrees of freedom of genetic structure and experimental error and calculation of the values of phenotypic differences (PCV) and genotype (9), depending on the ranges used by(12), which is less than 10%, low, 10-30% medium, and more than 30% high.

$$Phenotypic Coefficient of Variation (P.C.V.) = \frac{\sqrt{\sigma^2_P}}{\bar{X}} \times 100)$$

And to find inheritance values broadly in how they have been explained[9]. The scales described above were based on (1999). The inheritance values in the broad sense are less than 40%, 40-60% medium, and 60% or higher.

$$H^2 = \frac{\sigma_G{}^2}{\sigma_P{}^2}$$

The predicted genetic improvement was estimated when 5% of the plants were selected[11,12] suggested the expected genetic improvement as a percentage of the mean: less than 10%, 10-30% medium, and more than 30% high.

The physical and genetic correlations between the pairs of studied traits were found as explained by using the Excel program and tested its significance in how it was described[10].

$$Msp_{(cov.)} = Msg_{(cov.)} + Mse_{(cov.)} \qquad rP = \frac{Msp_{(cov.)}}{\sqrt{Msp_{(1)} \times Msp_{(2)}}}$$

$$\sigma_{G\times Y} = \frac{Msg_{(cov.)} - Mse_{(cov.)}}{r} \qquad rG = \frac{\sigma_{G\times Y}}{\sqrt{Msg_{(1)} \times Msg_{(2)}}}$$

Results

Analysis of variance

Analysis of variance (Table 2) for all genotypes studied traits showed highly significant differences in the mean square for all studied traits except the number of pods per plant, harvest index and 100seed weight; this indicates the high genetic variability in genotypes can be used in chickpea breeding program. Similar results were obtained by (13–15).

Mean performance :

S.O.V	d.f	Days to 50% flowering	Plant height (cm)	No. of primary branches per plant	No. of second branches per plant	First height pod (cm)	No. pods per plant	No. seed per pod	Biology yield of Per plant	Grain yield of Per plant	Harvest index %	100 seed weight g.
Replication	2	5.762	1.714	0.008	87.246	1.440	2407.284	0.146	953.581	1184.736	3314.336	5.168
Genotype	20	** 197.071	** 48.537	** 1.024	** 230.100	** 45.276	n.s. 614.419	** 0.205	** 2213.293	** 470.206	n.s. 655.777	n.s. 174.356
Error	40	76.662	8.575	0.211	42.430	6.197	474.265	0.052	640.596	88.550	311.030	86.546

Table 2. Mean square of variance analysis for studied traits in chickpeas *And **indicating significance at levels 0.05 and 0.01, respectively.

Characters Genotype	Days to 50% flowering	Plant height (cm)	No. of primary branches per plant	No. of second branches per plant	First height pod (cm)	No. pods per plant	No. seed per pod	Biology yield of Per plant (gm/plant)	Grain yield of per plant (gm/plant)	Harvest index (%)	100 seed weight (g)
1	77.000	26.700	1.700	49.600	26.700	34.300	1.200	100.140	30.052	29.905	24.700
2	84.000	28.200	1.700	48.867	28.200	33.600	1.500	100.140	19.333	29.905	20.355
3	76.000	22.900	2.500	36.733	22.900	22.300	1.500	162.600	55.810	34.167	21.314
4	80.000	21.000	2.500	27.467	21.000	28.300	1.500	88.350	39.857	56.109	21.040
5	76.000	29.800	2.200	46.200	29.800	29.100	1.500	140.388	42.253	29.880	11.955
6	95.000	23.200	2.000	32.933	23.200	30.800	1.100	102.750	46.075	51.663	29.793
7	65.000	30.700	3.000	65.800	30.700	45.400	1.750	135.900	70.355	54.151	24.094
8	90.000	29.300	3.500	21.200	32.900	38.400	1.100	94.820	26.001	27.295	20.501
9	67.000	26.900	2.200	57.133	26.900	36.600	1.300	154.180	63.830	42.430	20.958
10	70.000	29.600	1.800	30.667	29.600	33.400	1.500	107.400	42.018	40.487	22.446
11	80.000	25.200	2.900	62.733	25.200	41.700	1.500	128.080	56.570	44.240	21.880
12	86.000	24.100	1.500	28.800	24.100	24.000	1.500	88.950	58.490	77.377	21.510
13	72.000	22.300	1.700	53.267	23.300	37.300	1.200	119.910	42.135	40.134	21.020
14	72.000	25.300	1.500	24.600	25.300	18.800	1.700	136.820	50.320	36.990	21.376
15	81.000	20.200	2.300	59.267	20.200	39.200	1.500	111.640	45.140	42.194	20.635
16	78.000	28.100	1.700	40.733	28.100	45.400	1.500	146.700	52.757	36.016	20.800
17	85.000	32.600	2.300	47.600	29.100	32.200	1.300	77.250	50.640	74.175	42.090
18	64.000	25.600	2.600	60.133	26.500	32.700	1.100	131.740	37.123	27.831	46.920
19	70.000	24.200	1.700	32.467	29.200	25.100	1.200	149.900	42.100	27.869	20.115
20	72.000	24.800	1.300	35.533	24.800	23.400	1.600	166.240	53.338	32.198	23.655
21	75.000	15.300	1.400	20.333	16.800	10.667	0.600	130.500	32.535	26.316	19.935
Average	**76.905**	**25.524**	**2.095**	**42.003**	**25.929**	**31.556**	**1.364**	**76.905**	**45.559**	**76.905**	**23.671**
L.S.D. (0.05)	**14.870**	**4.973**	**0.780**	**11.063**	**4.228**	**36.985**	**0.387**	**42.984**	**15.981**	**29.952**	**15.799**
			1.062	**15.057**	**5.754**	**50.339**	**0.527**	**58.504**	**21.751**	**40.766**	**21.504**

Table 3. Mean performance of twenty–one genotypes of chickpea for studied traits.

Showed the mean performance of Twentyone's –genotypes for studied traits, the result discovered many genotypes earlier than the check variety for days 50% flowering genotypes (6 and 8) were the latest with (95,90)days respectively while the genotypes18were the earliest with (64) days. For plant height which is one of the desirable traits in chickpeas which decreases lodging effect and improves ultimate seed yield, the genotype with modest plant height and high yield traits could be essential to use for genetic improvement of chickpea varieties; the result showed the tallest plant among the twenty–one genotype, found in genotypes (17,18) with value (32,60) and (30.70) cm. respectively, while the shortest plants with (15.30) cm for genotypes (21). Regarding the number of primary branches per plant, genotype (8) had the highest number, with (3.50) branches and the lowest number for genotype (20), with (1.30) branches; for the number of secondary branches, per plant, genotype (7,11) recorded the highest number with (65.8 and 62.73) respectively with the lowest number of genotype (8) with (21.20). The same table showed that genotype (8), with (32.90) cm was the highest of the first pod from the ground, while genotype (21) was the shortest for the same trait, for the number of pods per plant genotypes (7 and 16) with (45.4) had the highest number and lowest with (10.66) for genotypes (21). Regarding the number of seed per pod, genotypes (7 and 14) gives a maximum number of (1.75 and 1.700) respectively, and the minimum number of seed per pod is recorded by genotypes (8,18) with (1.100). For

Characters	VG	VE	VP	GCV	PCV	H²%	GA	GA as a percent of mean
Days to flowering 50%	** 40.136	** 76.662	** 116.798	8.238	14.053	39.4	7.650	9.948
Plant height (cm)	** 13.321	n.s. 8.575	** 21.896	14.299	18.333	60.8	5.864	22.976
No. of primary branches per plant	n.s. 0.271	n.s. 0.211	** 0.482	24.849	33.139	56.20	0.804	38.382
No. of second branches per plant	** 62.557	** 42.430	** 104.987	18.830	24.394	59.60	12.577	29.943
First height pod (cm)	** 13.026	n.s. 6.197	** 19.223	13.920	16.909	67.80	6.120	23.604
No. pods per plant	** 46.718	** 474.265	** 520.983	21.660	72.332	9.0	4.216	13.362
No. seed per pod	n.s. 0.051	n.s. 0.052	n.s. 0.103	16.557	23.529	49.50	0.327	24.000
Biology yield of plant (gm)	** 524.232	** 640.596	** 1164.828	29.772	44.379	45.0	31.642	41.144
Seed yield per plant (gm)	** 127.219	** 88.550	** 215.769	24.757	32.242	59.0	17.841	39.161
Harvest index %	** 114.916	** 311.030	** 425.946	13.939	26.836	27.0	11.470	14.915
100 seed weight (gm)	** 29.270	** 86.546	** 115.816	22.856	45.464	25.30	5.603	23.669

Table 4. Genetic parameters of studied traits in chickpeas.

biological yield per plant, genotypes (20 and 2) with values (166.240 and 162.600g) give the highest value (166.240 and 162.600g) provide the highest value of biological yield per plant and lowest for genotype (1x) with (xx.25g) the same table showed that genotype (x^{-}) had the highest value with (70.355)for grain yield per plant and lowest for genotype (2) genotype (2) with (19.330g), for harvest index genotypes (12) record the high value (77.377) and low value for genotype (21) with (26.316). Genotype (18) records the high value of 100 seed weight with (46.92)g and the lowest for genotype (5) with (11.955); we conclude from the previous results that genotype (7) was more distinctive than other genotypes in number of secondary branches per plant, number of pod per plant, number of seed per pod and grain yield per plant, followed by genotype (18) was earliest with day 50% flowering and 100 seed weight (g). In contrast, genotype (8)was superior in the number of primary branches per plant and the highest first pod from the ground; the results were consistent with other researchers[1,15–17].

Illustrated some of the genetic parameters for studied traits, it is clear that the genetic variation was highly significant for all traits except the number of primary branches per plant and number of seeds per pod; the high phenotypic variance as compared to genotypic variance explains the role of environment in the expression of the trait such value provides information of the extent of variability, it is clear the highest value of the phenotypic coefficient of variation were record in the number of pod per plant with (x^{-}2.332)100 seed weight (45.464)g .and biological yield per plant (44.379) g., while the higher genetic coefficient, of variation, were found in natural product per plant (29.772)g.,seed yield per plant (24.757) g and the number of primary branches per plant (24.849). Similar results were observed by (18)[1,19,20]. From the same table, the heritability estimate value was high for the height of the first pod from the ground (67.8) and plant height (60.8). At the same time, it was medium for the number of secondary branches per plant (59.6), Number of seeds per pod (49.5), biological yield per plant (45) and grain yield per plant (59) and low for other traits. The results were in agreement with other researchers(13,21). genetic advance as present of mean at 10% selection intensity was high for biological yield (41.144),seed yield per plant (39.161)and number primary branches per plant (38.382). At the same time, it was low for the number of days 50%flowering (9.948)and medium for other traits, from the present study showed that the high-value heritability followed by the medium of expected genetic advance as percent value for plant height and high the first pod from the ground these two traits could be improved easily more than the other traits in the present study, a similar result was found by (5,13,15,22,23).

Discussion

In Colombia, approximately 176,000 cultivated hectares benefit 52,000 families in 422 municipalities of 30 departments,

Monte Carlo Simulation Analysis (MCS)

After structuring costs, the most influential cost component was direct labor, representing 53% of the total cost. The cost of culture media was 12% of the total, IMC represented 5%, and operating expenses, including administrative expenses and infrastructure, were 30% (Figure 2).

Conclusions

Many genotypic structures of chickpeas are estimated in this study many characters. The mean square has a highly significant difference for all traits except the number of pods per plant, harvest index and 100 seed weight. The genotypic coefficient variation has a high value in biological yield, seed yield per plant and number of primary branches. Heritability has a high value in the first pod and plant high. The expected genetic advance has a high value as percent in many characteristics such as biological and seed yield number of primary branches in the plant.

Funding

Self-funding

Acknowledgments

In this section, we acknowledge any person who supports us in completing this project.

Conflicts of Interest

There is no conflict

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ARTICLE / INVESTIGACIÓN

Evaluation of the effects of mobile phone electromagnetic radiation on some physiological parameters and histological structure in some laboratory male mice organs

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DOI. 10.21931/RB/2022.07.04.56

Abstract: Recently, the researcher has shown great interest in Electromagnetic radiation released from different devices such as TV, microwaves, medical apparatus, and satellites because of its effect on animals' growth and health. Exposure to "EMR" from mobiles phone can cause adverse effects on different cell functions. This study aimed to evaluate the effects of these radiations on histological and some blood parameters. The present study used 20 mice divided into two groups, the first one contains five animals as control, and the second experiment group contains 15 animals. EMR exposed from mobile for 12 h\day for one month. Histological examination of lungs, hearts and spleen showed a dramatic effect in these organs, such as necrosis, congestion, infiltrations, edema, splitting of muscle bundles and degenerations. This study shows that radiation from mobile phones contributes to histological changes in various visceral organs. Blood parameters showed a significant increase in platelets, bleeding and clotting time compared to the control group. The effect of EMR (Electromagnetic Radiation) on histology related to free radicals, increased lipid peroxidation in the cell membrane, and change in electrolyte concentration. An increase in platelets, bleeding and clotting time can also affect the rise in body temperature, ions and stimulations of stem cell divisions.

Key words: Electromagnetic radiations, mice, physiology, histology, mobile phone.

Introduction

Mobile phones have become an essential instrument of communication. It is also a form of entertainment and free time, particularly for kids and people[1]. Exposure to electromagnetic waves (EMW) from mobile phones and much other equipment like microwave cookers, electric motors, stations of electricity and MRI systems equipment may have adverse effects on cell function such as chromosomal aberrations, damage to the tissues, neurological degeneration, migraines and headache in children, low birth weights and heart diseases[2]. Some research on magnetic fields and cancer found the different conditions in reproduction and neurobehavioral related to electromagnetic radiation (EMR), such as mobile phones[3]. Free radical formation in other tissues caused by cell phones was reported[4]. Everyone is exposed to two types of electromagnetic fields: the first one, from power lines and electronic appliances the second, electromagnetic waves from wireless devices such as cell phones, cordless phones, cellular antennae and towers[5]. The role of electromagnetic field theory in biology and medicine was an excellent introduction to electromagnetics in these sciences[6]. International Agency for Research on Cancer (IARC) and World Health Organization (WHO) conclude that the waves released from mobile phones are considered carcinogenic to humans, causing headaches, hearing loss and changes in brain activity[7,8]. Bioelectromagnetics fields interact with living systems which depends on the wave's shape, frequency and exposure time[9]. In 1775, an Austrian scientist, Frans Mesmer, declared the presence of electricity and magnetism in the human body. He had been criticized for his announcement[10]. in 1953, professor Iwada Yasuda found new bone formation in the rabbit femur when the current in the (μA) range was applied for three weeks, which means Electrical stimulation of bone marrow to form osteoblasts and osteocytes in osteoid tissue, that means without the mechanism of cell proliferation and differentiation, capable for production of new bone tissue[11]. Blood is a living mobile tissue that moves around the body via blood vessels to carry nutrients and oxygen[12]. Platelets are small, non-nucleated components of the blood that play an essential role in hemostasis by forming an initial plug that helps to stop acute bleeding from damage blood vessels and provides the physiological surface for activation of coagulation factors[13]. Platelet function affect by many environmental and behavioral factors such as body temperature, exposure to allergens, air pollution and nutrition[14]. The aim of present study to investigation effects possibility of electromagnetic waves (EMW) for mobile phone on the histological structure of some organs and effect on blood parameters.

Materials and methods

Experimental and study design

Twenty mature male mice weighed 25-30g and aged 12-14 weeks were used; animals were put in cage food and water were provided daily. The mice were divided into two groups; group 1 included five mice used as control. Group

Citation: Yousif Al-Fatlawi A C. Evaluation of the effects of mobile phone electromagnetic radiation on some physiological parameters and histological structure in some laboratory male mice organs. Revis Bionatura 2022;7(4) 56. http://dx.doi.org/10.21931/RB/2022.07.04.56
Received: 25 January 2022 / **Accepted:** 22 October 2022 / **Published:** 15 November 2022

2 had fifteen mice exposed to EMR (Electromagnetic Radiation) from a mobile Nokia 2690 GSM frequency band (850-1900 MHZ) with dimensions of (107.5 × 45.5 × 13.8) mm and weighing (80.7) g, a mobile connected to a phone network. The distance between animals and the device is about 15 cm. Mice are exposed for (12 hours\ day) when mobile in a standby state; the total period of exposure is one month.

Histological process Technique

At the end of the experiments, samples of organs collections for histological study after animals were sacrificed under anesthesia. Hearts, lungs and spleen organs are fixed immediately by putting in formalin 10% for 24 hours. Tissues were dehydrated in ethanol, embedded in paraffin wax, tissues were sectioned at 5µm and stained with hematoxylin and eosin[15].

Blood collection

The mechanism of mammalian blood coagulation was designed to reduce blood loss due to injury and to keep blood fluid in the organism's blood vessels. Cardiac puncture blood collection is a widespread method for collecting blood from mice; by these processes, only small blood volumes can be obtained[16].

Blood assay

Platelets are critical mediators of hemostatic blood clot formation, and many methods to assess platelet function; one of these is Clotting time, which was measured by (17), while bleeding time is measured by (18).

Statistical analysis

The results analysis with the software SPSS, version 24, at level (p≤0.0001) by using a t-test for compared all treatment data with control mice.

Results

The result showed that the examination of a light microscope for the paraffin section of cardiac muscles of mice when exposed to electromagnetic radiation, many histological disorders such as congestions, dilated blood vessels, disruption of few fibers, degeneration of cardiomyocytes, disarrangement of cardiac muscle tissue and splitting of muscle bundles (fig. 1,2). Examination of the lung tissue of mice showed increased infiltrations of inflammatory cells, edema for interstitial space thickness of alveolar walls and congestions (fig. 3,4). Tissue sections of the spleen showed congestion, necrosis in the white pulp, and an increase in red pulp (fig. 5). Blood parameters results showed a highly significant increase (p≤0.0001) in clotting time and bleeding time exposed to 12/ hrs of electromagnetic radiation from mobile phone (fig. 6) compared to control. Also, platelet counts showed a significant increase (p≤0.0001) as compared to the control group (fig. 7).

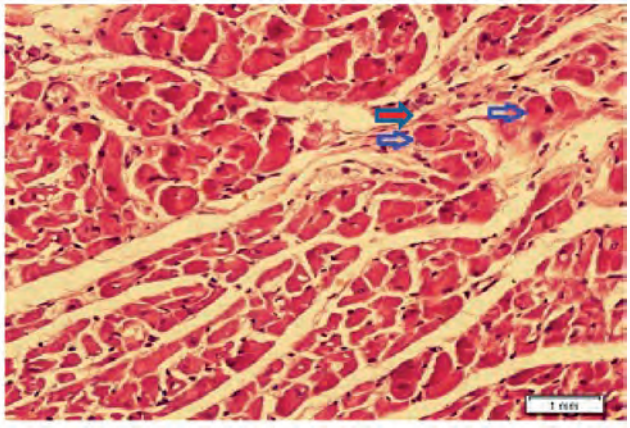

Figure 1. Shows heart tissue with degeneration of cardiomyocytes (blue arrow), disarrangement of cardiac muscle tissue and splitting of muscle bundles (red arrow) (H&E stain x400).

Discussion

Excessive exposure to (EMF) due to increased technologies becomes dangerous because of its effect on different organisms' biological systems and health. This research aimed to investigate histological disorders of some organs. The present study's result agrees with (19) who found damage in the heart tissue of mice exposed to the mobile phone (MP); this can be attributed to the use closely the heart can absorb EMR emitting from MP. In consonant with the present study, (20) found many disorders in the lung, such as infiltration of inflammatory cells, blood vessel obstruction and interalveolar septal disturbance. (21) found exposure to EMR emitted from mobile can cause enlarged in the white pulps of the spleen and dilatation of its sinusoids; the degree of these changes increased with the duration of EMR exposure. Also, (22) found atrophy in seminiferous tubules and vacuolation in hepatocyte cells of guinea pigs exposed to EMW. The present study agreed with (23) when they discovered that many lesion in the tissue of brain rat exposed to EMW includes degeneration and edema, in lung found hemorrhage, emphysema and alveolar congestion. Atrophy with vacuolations in the hepatocyte, also necrosis in the pancreas. These changes can be attributed to many mechanisms: EMW with high energy causes increased local temperature leading to break down protein bound and denaturation[24]. The second mechanisms where wave contact together to formed free radical production and antioxidant consumption which leads insufficient defense system, these free radicals attack lipids, proteins and nucleic acid, when causes genetic mutation leading to the breaking of DNA strands and cell death[25,26]. Also a 900 MHz EMF application adversely influenced the learning behavior of female pups

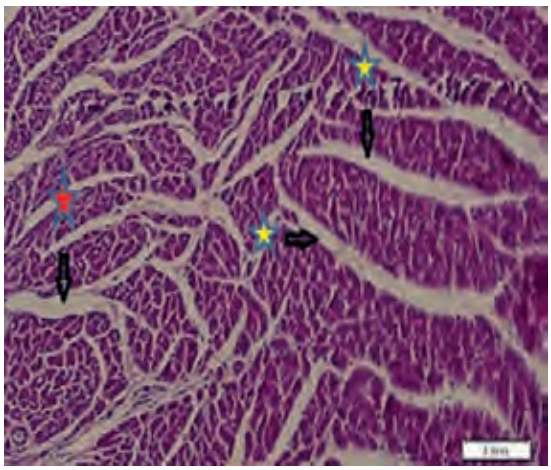

Figure 2. Shows heart tissue with marked degeneration of cardiomyocytes (yellow star) and dilated interstitial spaces (red star) (H&E stain x200).

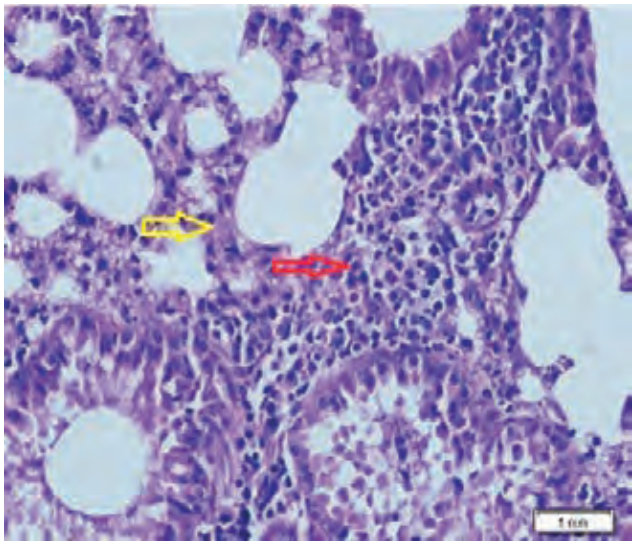

Figure 3. Showed lung tissue with marked lymphocyte infiltration (red arrow), edematous interstitial spaces and thickened alveolar walls (yellow arrow) (H&Ex400).

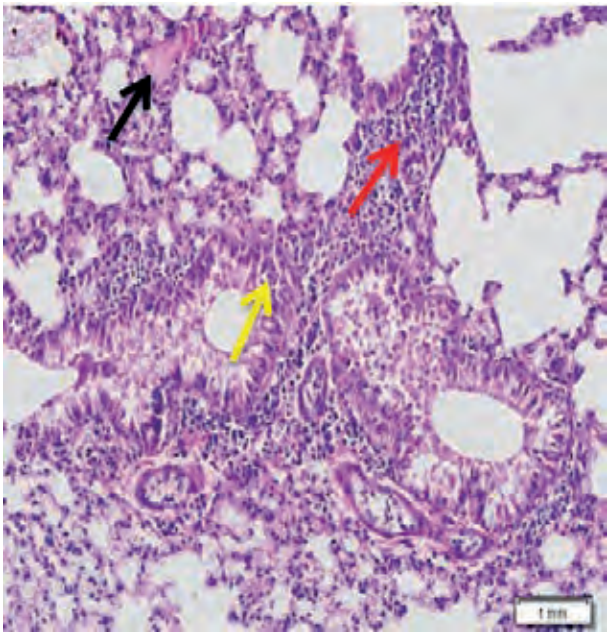

Figure 4. Shows lung tissue with edematous interstitial spaces and thickened alveolar walls (yellow arrow), marked lymphocytic infiltrate (red arrow) and congestion (black arrow) (H&Ex200).

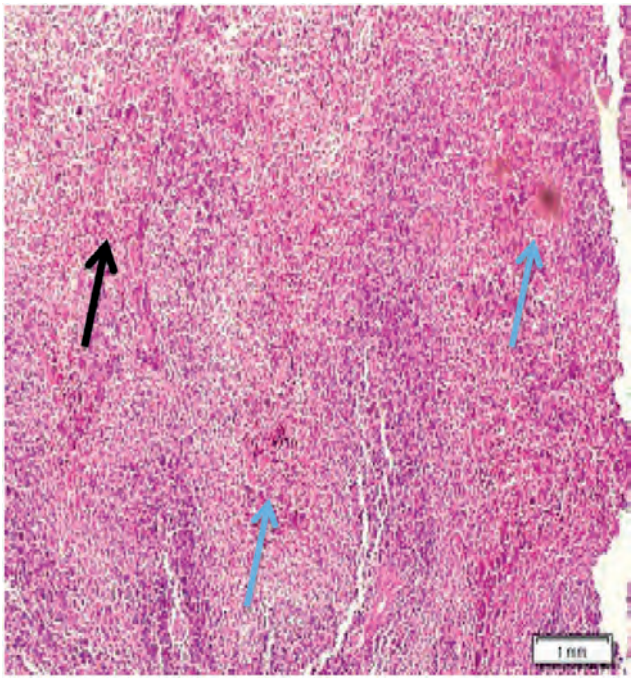

Figure 5. Shows spleen tissue with congestion(blue arrow) and a mild increase in red pulp area necrosis in white pulp (black arrow) (H&Ex100).

in the prenatal period and also resulted in histopathological changes occurring in the hippocampus[27]. Histological changes can be due to the formation of free radicals through exposure to EMF, which in turn targets membrane lipids and changes their nature by breaking protein bonds[22]. A study on workers, welders and computer operation exposed to EMW found increased RBC, MCV and platelets due to critical change in the erythropoiesis system[5]. The present study, in agreement with (28), saw an increase in RBC, MCV and platelets. At the same time, a decrease in WBC, Hb and lymphocytes cell may be related to the effect of EMW on shortening cell cycles and increasing the synthesis of DNA. Another study found an increase in RBC and platelets correlated to the impact of EMW that causes stimulated division of stem cells in the bone marrow and increases immature reticulocyte cells[29]. Results were found to increase bleeding and clotting time, and these deal with (12) who attributed these increase to the following reasons the first one, The increase in body temperature reduces blood viscosity, which eventually contributes to an increase in blood clotting time. The second, enzymatic chains or hormones experimentally affected the strength of the electromagnetic field can created around the outside of the cell wall and pull the ions to the opposite directions[30].

Conclusions

The effects of EMF on living tissues have been proven beyond a shadow of a doubt, and the level of damage is

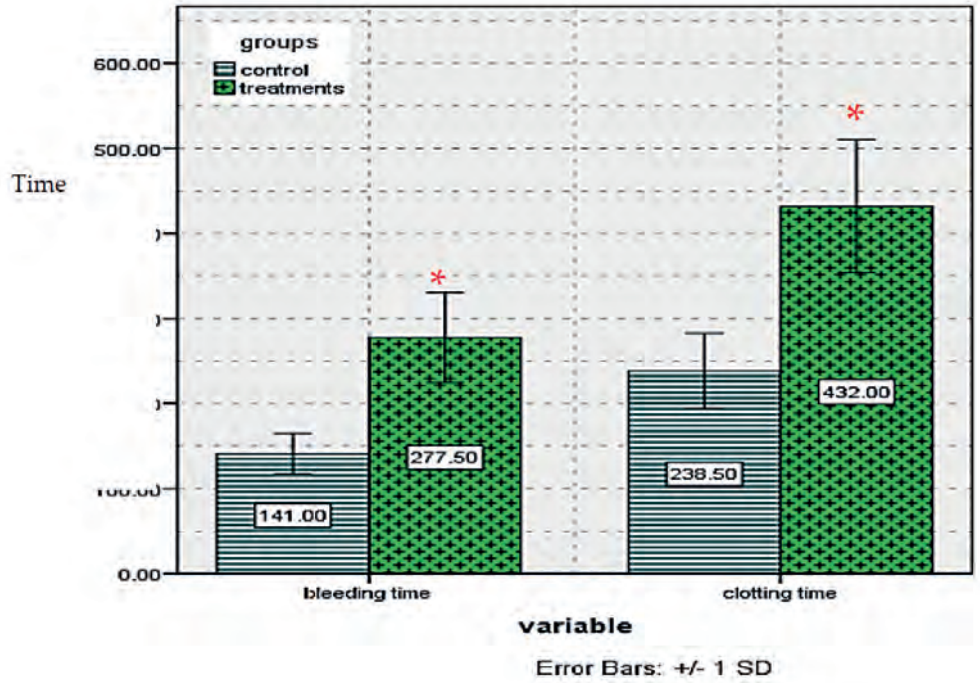

Figure 6. Showed bleeding, clotting time of treatment and control male mice after exposure to electromagnetic waves from mobile phone.

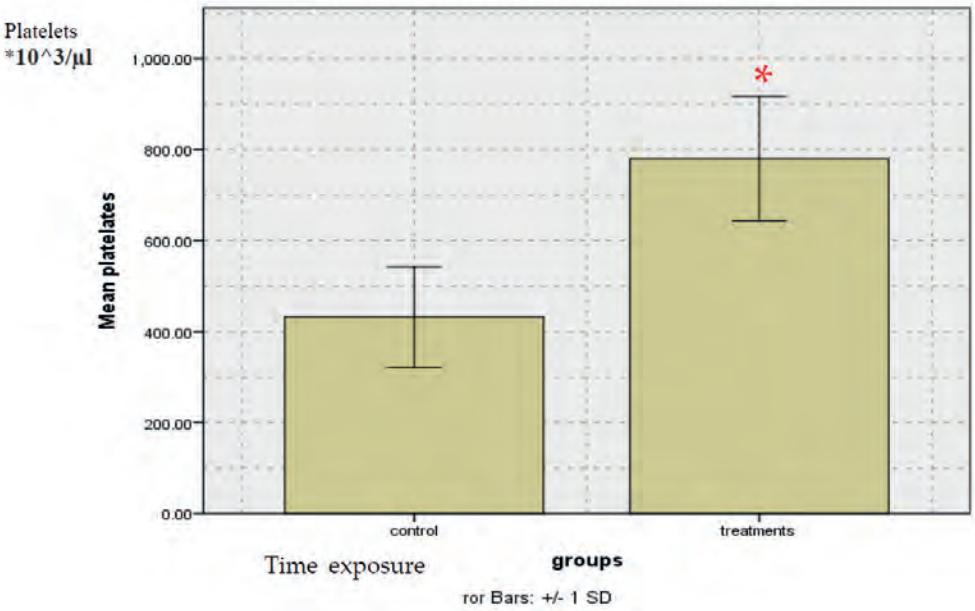

Figure 7. Showed platelets counts of treatment and control male mice after exposure to electromagnetic waves from mobile phone*= significant differences (p≤0.0001).

directly proportional to the magnetic field strength, time of exposure, and kind of tissue exposed.

Acknowledgments

We thank the Iraqi ministry of higher education and the University of Kerbala for the facilities needed to carry out this study. The authors personally funded this work.

Ethical approval

The research related to human use has complied with all the relevant national regulations and institutional policies and, following the tenets of the Helsinki Declaration, has been approved by the author's institutional review board or equivalent committee. Project no. 1025 was approved on January 20th, 2019.

Conflicts of Interest

The authors declare they have no conflict of interest.

Informed consent statement

Informed consent has been obtained from all individuals included in this study.

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ARTICLE / INVESTIGACIÓN

Efficacy of zinc oxide nanoparticles and *Bifidobacterium bifidum* Extraction on anaerobic bacteria isolated from patients with diarrhea

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Abstract: Infectious acute diarrhea may be prevented with probiotics; because they make up the majority of the colonic flora in breastfed newborns and are likely to contribute to the lower incidence of diarrhea in this population, *Bifidobacteria* are particularly appealing as probiotics agents. The present study was designed to identify anaerobic bacteria, especially *C. difficile* the main reason for dysentery associated with antibiotics. Detect the ability of each ZnoNPs and *B. bifidum* to inhibit bacterial growth. During the period from March to October 2019, (100) children and adults who came to Salah al-deen hospital in Tikrit city participated in the study under the supervision of a physician. All samples were transported using a carry Blair if late one or two hours after collection and culturing. The collected models were also cultured on Xylose Lysine Deoxycholate agar, Salmonella Shigella agar, Eosin methylene blue agar, and MacConkey agar. For initiated diagnoses of the Enterobacteriaceae, Blood agar is used to detect beta-hemolytic isolates, recover enteric bacteria other than Enterobacteriaceae, and evaluate the results of oxidase tests. To diagnose bacterium kinds, biochemical reactions and motility tests were used. Impact of ZnoNPs, and *B. bifidum* antibiotic *in vitro*. The results of 100 dysentery feces samples were obtained into (60%) samples for males and (40%) for females. Eighty-two positively impacted anaerobically on growth media like Clostridium complicate agar and MacConkey agar (18%) other than bacteria. In contrast, negative samples revealed 10 (55.56 percent) samples for males and 8 (44.44 percent) samples for females. The same stool samples were taken and cultured on Clostridium difficile agar and MacConkey agar under anaerobic and ideal incubation conditions. 15% and 67% of isolates appeared on MacConkey agar of the total number of samples, while 18% showed negative growth. Finally, Zn NPs showed their ability to inhibit Clostridium complicated segregate lean on the condensation 5 mg/ml, and it caused the inhibitory effect on Clostridium to complicate by 10-22 of the diameter of inhibition. The Inhibition Zone Dimeter ranged from 8 to 25 mm for isolates when condensation was utilized at 2.5 mg/ml. According to the findings, the widths of the inhibitory zones for isolates of *C. difficile* containing *B. bifidum* supernatant mg/ml ranged from 9 to 24 mm.

Key words: Zinc oxide nanoparticles, Probiotic, *Bifidobacterium bifidum*, *Clostridium difficile*.

Introduction

Diarrhea is a confusing matter in older people, especially in people with general weakness due to other problems in the body, such as stool or fluid electrolyte disturbances. A complication associated with the use of antibiotics is *Clostridium difficile* infection (CDI)[1]. Intestinal flora dominates. Using broad-spectrum antimicrobials may destroy the patient's normal flora and encourage the dissemination of *C. difficile* toxins. Thus, antimicrobial therapy is critical to the development of CDI[1]. In the last year, many studies using Nanotechnology as antimicrobial activity and zinc oxide nanoparticles as an antibacterial factor appeared in biologists' medicine, chemists and physicists[2]. Scientists have searched for many years for antibacterial agents for *Helicobacter pylori*, for examples[3,4]. A significant reduction in apoptosis was demonstrated in infants with viscera[5]. Changes to the body's defensive[6,7]. Measures to reduce inflammation reduced visceral-induced morbidity, as well[8,9]. Hence, this study identified the types of aerobic bacteria that cause diarrhea. Detect the ability of each ZnoNPs and *B. bifidum* to inhibit bacterial growth. The current investigation aimed to identify anaerobic bacteria, particularly *C. difficile*, the primary cause of diarrhea linked with antibiotic use. Find out whether ZnoNPs and *B. bifidum* can both stop bacterial growth.

Materials and methods

Sample collection

Samples were taken from patients of different age groups in Salah al-Din Hospital in Tikrit governorate from March 2019 to October 2019; the study included 100 samples, and vectors were used for transplanting them on the following media[10]. For initial isolation of Enterobacteriaceae, such as MacConkey agar; XLD agar, SS agar, and EMB agar; and blood agar to disclose beta-hemolytic isolates recover enteric bacteria other than Enterobacteriaceae, as well as oxidase test performance[10]. Aerobic and anaerobic isolates were incubated in a jar at 37 C° for 24 hours, and all biochemical tests were performed to detect Gram-positive and negative species, as well as a motility test[11].

Citation: Atiyea Q M, Younus R W, Abdulkareem H S, Hamad A M. Efficacy of zinc oxide nanoparticles and Bifidobacterium bifidum Extraction on anaerobic bacteria isolated from patients with diarrhea. Revis Bionatura 2022;7(4) 57. http://dx.doi.org/10.21931/RB/2022.07.04.57
Received: 25 January 2022 / **Accepted:** 22 October 2022 / **Published:** 15 November 2022
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ZnO NPs and *Bifidobacterium bifidium* were shown to have a minimal inhibitory concentration

ZnO NPs and *Bifidobacterium bifidius* were studied according to the agar Dilution method. The concentrations of ZnO in the supernatant of *Bifidobacterium bifidum* varied from 1-2 mg/ml. Muller Hinton agar center in glass bottles (20 ml each), sterilized by inventory and returned to a temperature of 4°C. Different amounts of ZnO and *Bifidobacterium bifidus* were added, the media was fed correctly, and then the media was placed on sterile plates and maintained at 4 C° until use. The infection was caused by bacteria examined and compared to a set turbidity standard solution after 18-24 hours. The micropipette of five µl was made from the micropipette, then the dishes with various dosages of ZnO and *Bifidobacterium bifidus* were extracted, and the dishes were allowed for a while before drying. Dishes were incubated upside down at 37°C for 24 hours. After incubation, determine the minimum inhibitory concentration (MIC), the lowest concentration of the chemical utilized that did not result in noticeable bacterial growth or the development of only a small number of bacterial colonies[12].

In vitro assessment of the efficacy of ZnoNPs and *Bifidobacterium bifidus* as microbial antibiotics

Fifteen isolates were selected from different stool samples and tested for the effectiveness of ZnONPs on them, as reported in NCCLS (2004), which included: Preparation of bacterial suspension from samples treated with the treatment (*Bifidibactrium bifidum* and ZnO). It was compared with the tubes containing McFarland standard (0.5)[13] to obtain a dilution of 1.5x 10^8 colony forming units (CFU/ml) and then taken from it (0.1 ml) and planted on (Muller Hinton agar) and distributed on the surface of the medium and left for 15 minutes. Then, 100 µl of each treatment were added to ZnO NPs and *Bifidobacterium bifidum* at a concentration of 2 mg/ml, and the plates were incubated at 37 °C for 24 hours. Next, the inhibition zone was evaluated[14].

Statistical Analysis

The SPSS[15], IBM version 20, program was used to perform the analysis on the data[18]. Statistical significance is assumed when the p-values are less than 0.05.

Results

Sample distribution

Table (1) shows the growth of anaerobic species from stool samples in both gender; 100 stool samples were collected and distributed among 60 males and 40 females. Anaerobic samples showed positive growth of gram stain on each of *Clostridium difficile* agar and MacConkey agar medium. At the same time, 18% of the samples showed a non-bacterial increase, distributed among (55.56%) samples for males and 8 (44.44%) for females.

Bacterial Isolation

Under anaerobic conditions at 37°C, a hundred patient samples were grown on *Clostridium difficile* agar and MacConkey agar. It causes bacterial isolates to emerge on 15 % and 67 % of the total samples on MacConkey agar, respectively, whereas 18 % showed no growth on any of the utilized mediums.

Diagnoses of *Clostridium difficile*

The bacterial isolates appear flat and have a ground-glass appearance, which is apparent on the *Clostridium difficile* agar and TCCFA medium. According to the results of bacterial transplanting, the bacteria manifested as circular, convex colonies with distinct borders and a gray tint. The bacteria were positive bacilli according to the results of the pigmentation gram stain. The results of biochemical identification, such as catalase and oxidase, were found to be negative, whereas gelatin liquefaction was determined to be positive (gelatin liquefaction +ve). In addition, the fermentative of glucose, fructose, and mannose changed color, indicating positive findings, and the enzymatic reactions were negative for casein hydrolysis. Still, positive results for lecithinase and lipase were detected for the esculin hydrolysis test, as shown in table (2).

Diagnoses of Enterobacteriaceae species

The shape and diameter indicate bacterial isolates grown anaerobically on MacConkey agar for 18-24 h (Figure. 2) to the following Enterobacteriaceae. The diagnosis was based on microscopic examination using Gram stain. The appearance of Gram-negative species, which are given to these species as references to Enterobacteriaceae. The production of the capsule and characteristics of the colony, such as mucus and metallic luster, all these parameters were used to determine the bacterial genus. The hemolysis, catalase, oxidase, urease production, and IMViC test were performed, according to (11). The study showed that 100 samples of cultured stool and 67 samples gave a positive culture with enteric isolates bacteria that caused diarrhea in patients. The triple sugar iron agar test uses sugar and iron to diagnose different types of intestinal bacteria. Containing three fermentable sugars, the isolates were diagnosed by the appearance of the characterization results. *Escherichia coli* was a response to Triple Sugar Iron agar. While *Pseudomonas* and *Shigella Sonni* can't ferment it, the results are Alk/Alk, Alk/Alk, with no gas and no H_2S generation. *K.pneumoniea* was also found to alter medium to Acid/Acid, produce gas, although it was variable (d) to produce H_2S. While *Serriatia marcessens* appeared to be Alk/Acid, produced gas, and was unable to create H_2S. These bacteria exploited the egg yolk suspension in the medium to produce lecithinase and lipase, which were also employed in diagnostics.

Culture results	Male		Female		Total	
	No.	%	No.	%	No.	%
-ve culture (No growth)	10	55.56	8	44.44	18	18
+ve culture	50	60.97	32	26.82	82	82
Total	60	60.00	40	40.00	100	100

Table 1. The growth of anaerobic species from stool samples in both gender.

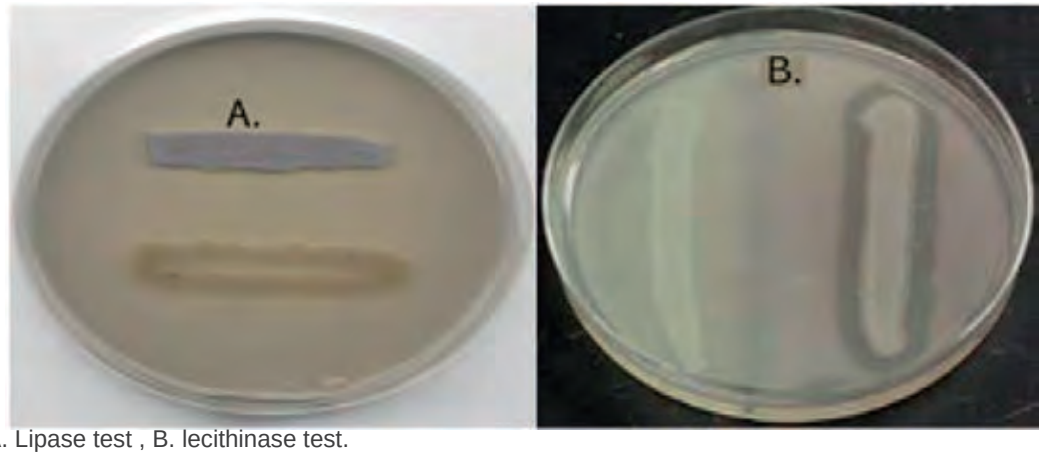

Figure 1. A. Lipase test , B. lecithinase test.

Gram stain	Motility	Catalase	Lecithinase	Lipase	Caseinase	Sugar	Carbohydrates Fermentation		
							Glucose	Fructose	Maltose
100% (+)	100% (+)	100% (-)	100% (+)	100% (-)	100% (-)	100% (-)	100% (+)	100% (+)	100% (+)

Table 2. Biochemical tests for Clostridium difficile.

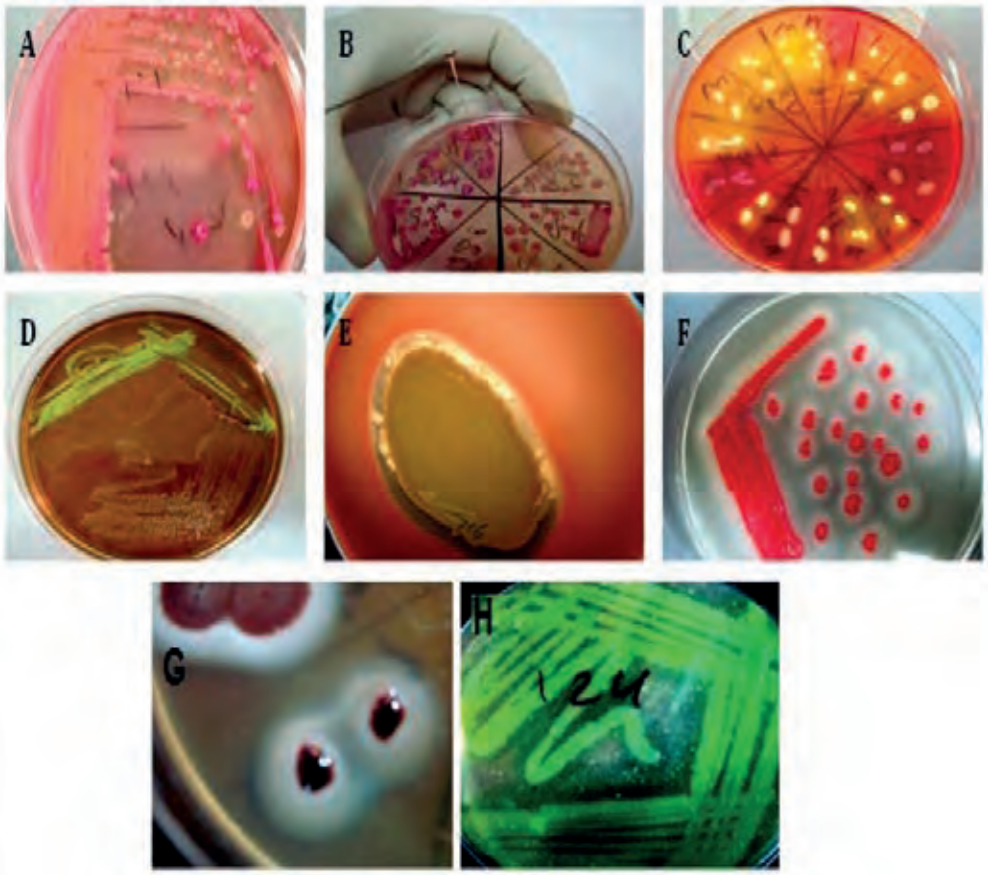

Figure 2. Primary, selective and differential media are used for bacterial isolation and identification.
A-Pink colonies represent lactose fermenter, while pale colonies represent non-lactose fermenter colonies on differential and selective Mac Conkey agar media. B- Mucoid characters of Klebsiella spp. on Mac Conkey agar media. C- Organisms such as E. coli and Klebsiella-Enterobacter species may utilize more than one carbohydrate and produce bright yellow colonies, species that use non of the carbohydrate produce translucent colonies. Most species of Salmonella have red colonies, most with black centers from H2S gas and Citrobacter colonies are yellow with black centers. D-Typical strong lactose fermenter, notably E. coli, produce colonies that are green-black with a metallic sheen on Eosin methylene blue agar (EMB). E-The clear zone around the swarming colony represents beta-hemolysis on blood agar. F-The opacity around the squeaking culture represents lipase producer Serratia marsessence on Sierra media. G-The opacity around the colony represents lecithinase producer bacteria on Egg yolk agar media. H- The clear zone on Skim milk agar around the streaking culture represents protease-producing bacteria.

C. defficile isolated from patients with diarrhea was inhibited by ZnoNPs

Table (3) and figure 3 demonstrate the inhibitory effects of zinc oxide particles (ZnO NPs) on *C. difficile* isolates from individuals with diarrhea (5). The metronidazole antibiotic was chosen for its distinct mode of action against *C.difficile* isolated from diarrhea patients, and the outcomes of the ZnO NPs inhibition investigation were contrasted with those of this drug. Additionally, the diameter of the inhibitory zone served as the basis for the measurement (IZD). The results demonstrated that, depending on the concentration, ZnO NPs could inhibit *C.difficile* isolates, with a concentration of 5 mg/ml inhibiting *C.difficile* isolates with diameters ranging from 10 to 22 mm. For the isolates with a concentration of 2.5 mg/ml, the IZD appeared between 8 and 25 mm. Furthermore, only three isolates with an IZD of 5,10,10 mm seemed susceptible to ZnONPs at a concentration of 1.25. While the 0.625 mg/ml concentration of ZnONPs was ineffective in suppressing *C.difficile* isolates.

The *C. defficile* isolate from diarrhea patients that *Bifidobacterium bifidum* supernatant inhibits

The effect of *Bifidobacterium bifidum* supernatant on the growth of *C. difficille* isolates from diarrhea patients is shown in Table (4). The inhibition zone diameters IZD of *B. bifidum* supernatant were observed to be between 9 and 24 mm against *C.difficile*.

C. difficile isolates	ZnONPs concentration (mg/ml) 5.0	2.5	1.25	0.625
S1	20 mm	25mm	-mm	-mm
S2	12	-	-	-
S3	10	8	-	-
S4	18	16	-	-
S5	16	12	-	-
S6	22	17	10	-
S7	14	12	-	-
S8	17	13	-	-
S9	19	15	-	-
S10	12	9	-	-
S11	21	18	-	-
S12	16	11	-	-
S13	18	15	7	-
S14	19	17	5	-
S15	15	11	-	-

(-) = mean non-inhibit. The means were referred to 3 replicates.

Table 3. Inhibition zone diameter in mm of ZnONPs against C.difficile isolates.

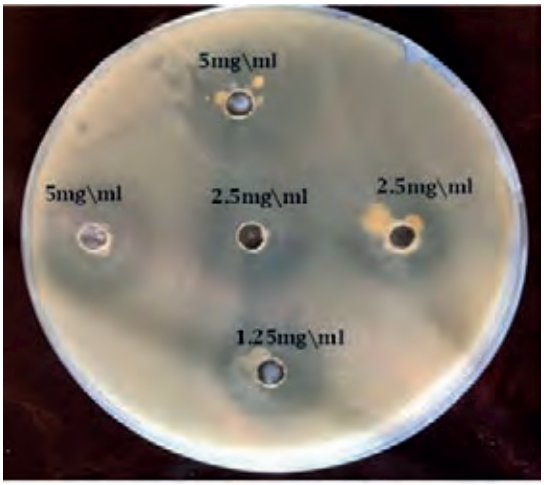

Figure 3. Zno NPs gradient concentration inhibition growth bacteria C. difficile.

C. difficle isolates	Inhibition zone diameter (mm)
S1	22
S2	18
S3	21
S4	24
S5	18
S6	21
S7	19
S8	15
S9	11
S10	13
S11	9
S12	17
S13	22
S14	24
S15	16

Table 4. The size of the B.bifidus supernatant's inhibitory zone against C.difficle isolates.

Discussion

There are several reasons, including the growth of aerobic bacteria or diarrhea resulting from antibiotics. Despite the tremendous development in diagnostic techniques, many of the causes leading to gastroenteritis have not been explained to this day[16]. The other samples from diarrhea patients were positive for bacterial growth in 82 % of cases. Males had 50 positive samples (60.%), while females had 32 (39.02 %), and the male-to-female ratio was 1.2: 1. These findings were confirmed by (17), who discovered that males had 62.26 % diarrhea while females had 37.74 %. It was also agreed with (17), who discovered that 62 percent of males and 38 % of girls in hospitals had diarrhea. *Clostridium difficile* isolation from diarrhea is extremely rare, owing to the need for a persistent anaerobic environment to promote optimum development. Because it prevents the growth of normal fecal flora, they used the taurocholate-cefoxitin-cycloserine-fructose agar (TCCFA), which is selective and differential for *C. difficile*[18]. By having both cefoxitin, which more broadly prevents the growth of Gram-negative and -positive bacteria, and *c. difficile* and the majority of enterococci strains, it can act as a bacteriostat for Gram-negative bacteria.

However, 67 % of the bacterial isolates appeared on MacConkey agar media and appeared in various sizes and shapes, indicating that they belonged to the Enterobacteriaceae family of bacteria. There are methods for creating and counting spore stocks in vitro for various downstream applications, including microscopy. Since fructose fermentation lowers pH and changes the medium's color from red/orange to yellow, the pH indicator neutral red can be added[18]. The fermentative results of sugars such as glucose, fructose, and mannose led to changed color and were considered positive findings; these results, which show on the parameters above, were conformity for the isolates as *C.diffcille*[19]. The

breakdown of the lecithin in the egg yolk causes an opaque precipitate to develop surrounding the colonies. The Lipase enzyme hydrolyzes the lipids in the egg yolk, giving the colony's surface an iridescent shine[12]. Except for *P.flourscence*, all isolates appear to be negative for this test. The gelatin hydrolysis test was also done, and all isolates were determined to be negative, except for *Serratia marscence*, which was found to be positive[20].

Interest in probiotics' potential to control RV diarrhea has grown over the past decades. There is evidence that certain Bifidobacterium strains can treat gastroenteritis in this situation[21,22]. However, *B. bifidum* and other *Bifidobacterium* strains have effectively reduced viral clearance in babies, (23) even in mice[24]. *In vitro* studies have shown that *Bifidobacteria* and *Lactobacillus* can inhibit RV infection, for example, by interfering in the adhesion step. On the other hand, the reduction of viral shedding exerted by *Lactobacillus* is consistent with other studies, which found that L. rhamnosus GG could reduce RV elimination in gnotobiotic piglets infected with human RV and in children[25]. There was no previous study, at least locally, that demonstrated the ability of ZnONPs to inhibit *C.difficile*. Still, the ability of ZnONPs to inhibit *C.difficile* isolated from diarrhea patients is visible, confirming the ability of these nanoparticles were shown to penetrate the cell walls of bacterial isolates, destroying cellular activity and causing cell death. The antibacterial activities of Zn NPs were investigated by determining the lowest inhibitory concentration against *E.coli* bacteria, which was corroborated by (26). Diffusion in the pits was used to monitor the antibacterial nanoparticle's quantitative assessment, and it was shown that the area of inhibition mostly relied on the concentration and agreement with results by the (14). It showed that bacterial inhibition increased as ZnO NP concentration was raised. The results were also in agreement with (27). The MIC was measured using a concentration of ZnONPs of 1.25 mg/ml, and it was discovered that the MIC against *C.difficile* isolates was 1.25 mg/ml. While this was discovered, the MBC for the identical bacterial isolates was 2.5 mg/ml. The capacity of ZnO NPs to disrupt the bacterial membrane, resulting in cytosolic component leakage and bacterial cell death, is one of the mechanisms of bacterial inhibition. The findings were in line with those of (28); in dosages of 1.2-1.6 mg/ml, ZnO NPs were shown to be more effective at inhibiting *V.cholerae* and Enterotoxigenic *Escherichia coli* (ETEC)[29] discovered that Zn NPs had anticoccidial and antioxidant activities in the jejunum after infection with *Eimeria papillata*. Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus epidermis*) bacteria were shown to have different antibacterial effects from nanoparticles, with Gram-negative bacteria having stronger antimicrobial activity than Gram-positive bacteria[30]. The findings of The inhibitory activity of *Bifidobacterium bifidum* supernatant against *C. defficile* isolated from diarrhea patients concurred with (31) who showed that probiotics reduce the risk by 42% compared to advanced diarrhea associated with antibiotics. Thus (32) who used a probiotic *B. bifidusm* to treat mice infected with *H. pylori* and who used probiotics in the treatment of *Clostridium difficile* infection agreed that probiotics were beneficial for both adults and children, reducing the risk of diarrhea associated with *Clostridium difficile* by 59.5 and 59.5. 65.9%, respectively. Several variables contribute to the effectiveness of *Bifidobacterium* species. Major Probiotic mechanisms of action include competitive exclusion of pathogenic microorganisms, production of anti-microorganism substances, and immune system modulation. They also include epithelial barrier enhancement, increased adhesion to the intestinal mucosa, and concurrent inhibition of pathogen adhesion[33,34].

In the previous study, there has been some debate about the possibility of using a wide variety of chemicals to stop the growth of hazardous microbes. One example of this would be the direct effect that Ag and TiO2 nanoparticles had on dangerous bacteria in the earlier study number[35]. This would be an illustration of this. The testing of these nanoparticles included the utilization of *P. mirabilis* and *P. vulgaris*, examples of bacteria that can be dangerous to humans. On the other hand, several studies have shown that treating harmful bacteria with therapy that involves physical forces, such as audible noises and magnetic fields, can help diminish the resistance of *S. aureus* to infection[36]. Several different researchers carried out these studies. A wide range of researchers from a variety of institutions carried out these investigations. Due to the researchers' efforts, this discovery was made.

Conclusions

When *C. difficile* was grown in isolation from diarrhea, our findings led us to the conclusion that zinc oxide nanoparticles and probiotics both have a good effect in preventing the growth of the pathogen. This assumption was proven beyond a reasonable doubt by the use of an approach known as good diffusion in the pits. It was discovered in the lab that increasing the concentration leads to a bigger increase in the total quantity of inhibition that can be seen on the plate. This was one of the discoveries made. Both the bacteria that are responsible for diarrhea and the bacteria that are isolated from diarrhea are candidates for therapy with a variety of enhancers and nanomaterial, according to the research group that conducted the study.

Acknowledgments

We extend our thanks, appreciation and gratitude to everyone who helped write, prepare and publish this research paper and proofread it.

Conflicts of Interest

No conflict.

Funding

Self by authors.

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ARTICLE / INVESTIGACIÓN

MiR-144 as a novel biomarker in breast cancer diagnosis and treatment

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Abstract: Exosomes naturally carry the biomolecules in the body; they perform this task efficiently without compromising the immune system and by breaking through all the biological barriers, so they can be the best choice for designing and introducing a drug and gene transfer system. Extraction of the exosomes from the cell culture medium was performed by precipitation with an Exoquick kit solution. Nanoparticle specificity analysis was performed using scanning electron microscopy and dynamic light scattering. Trizol reagent (Invitrogen) was used for RNA extraction. Single-strand cDNA synthesis was performed from the miRNA and RT-PCR. Data were analyzed using a threshold cycle comparative method and cell cycle analysis using flow cytometry. Exosomes containing miR-144 can dramatically decrease the expression level of crucial TGF-β pathway genes, SMAD4 and TGF-βR2, in breast cancer cells. Botulinum toxin A inhibits cancer cell growth by inhibiting the TGF-β pathway. The simultaneous combination of engineered exosomes containing miR-144 and bacterial botulinum toxin A has increased effects on inhibiting the TGF-β signaling pathway. It causes cell cycle arrest in breast cancer cells. The present study's findings showed that overexpression of miR-144 in breast tumor cells results in the packaging of miRNA in exosomes derived from these cells. As a result, the exosomal platform for nucleic acid transfer to the cell appears to be an effective transducer for gene transfer to the cell. It could be used as a suitable adjunct to cancer therapeutic studies.

Key words: Breast cancer, botulinum toxin A, exosome, miRNA, biomarker.

Introduction

Breast cancer is the second most common malignancy in the world. Breast cancer is the most common cause of death in women less than 45 years of age. Statistics show that cancer is rare in the age group of 20 to 24, but it is more prevalent in women aged 34 to 39. Modifiable and non-modifiable risk factors in breast cancer predictor models of body mass index, BRCA1 and BRCA2 mutations, Parity Li Fraumeni syndrome, high alcohol intake, lifestyle, radiation exposure in utero, breastfeeding, and smoking have been noted. Studies in the molecular, histopathological, genetic, and genomic domains have shown that young women with breast cancer have an increased incidence of more invasive subtypes with worse overall prognosis, increased genetic susceptibility, differential tumor genes, and genomic-specific signatures[1].

The miRNAs are a group of 25 nucleotide-long RNAs. They are small non-coding RNAs that approximately 18 normally regulate gene expression at the post-transcriptional stage by binding to the 3'UTR portion of a target mRNA. Studies have shown that alterations in miRNA expression occur in a range of cancers. MiRNAs also control cancer-related processes such as proliferation, apoptosis, migration, and invasion. Recent studies have also shown that miRNAs play a key role in stem cell differentiation. The role of miRNAs as new predictive and prognostic markers has been considered in several studies and has been prominent as potential therapeutic targets[2]. MiR-548c-5p, miR-181d, miR-487b, miR-206, miR-195, miR-30d, miR-149, miR-183, miR-182 and miR-320, miR-10a, miR-130, miR-127-3p, miR-143, miR-10b, miR-125b, and miR-195 are used as molecular signatures early in the onset of breast cancer[3].

In this study, we aimed to evaluate the regulation pattern of MiR-144 in response to exosome therapy of breast cancer cells, as well as evaluate the breast cancer cells micro-environment.

Materials and methods

Cell culture

The cell line of MDA-MB-231 (invasive breast cancer cell line) was bought from the Pasteur Institute, Iran. The cells were cultured in a DMEM medium containing 11% fetal calf serum (FBS), 1mM L-glutamine, 100 unit/ ml penicillin, and 100µg /ml streptomycin in wet incubation; they were grown with 5% CO_2 at 37 °C.

Cell passage of adherent cells was performed by evacuating the cell culture medium, washing twice with PBS, adding 2ml of trypsin solution, and incubating at 37 °C for 3 to 5 minutes. After transferring to Falcon, the cell suspension was centrifuged at 1500rpm for 5min. The resulting precipitate was dissolved in 1 ml of a complete medium. The resulting suspension was divided between 2 to 3 new flasks. Cell culture flasks were incubated and maintained at 37 °C with 5% carbon dioxide.

Citation: Simin P T, Marandi S J, Ardakani R B . MiR-144 as a novel biomarker in breast cancer diagnosis and treatment. Revis Bionatura 2022;7(4) 58. http://dx.doi.org/10.21931/RB/2022.07.04.58
Received: 2 May 2022 / **Accepted:** 12 June 2022 / **Published:** 15 November 2022

Exosome Isolation from Cell Culture Using Sedimentation with ExoQuick Kit

The culture medium or cell supernatant collected at different passages was used to isolate the exosomes based on the manufacturer's guidelines[4].

Scanning electron microscopy (SEM)

A small volume of the exosome was purified and washed with glutaraldehyde 2.5% and fixed with PBS. The sample was then dialyzed with ethanol and dried on a glass surface with a thin layer of gold. The size and morphology of the exosomes were assessed by scanning electron microscopy (Digital SEM, KYKY-EM3200, China).

Dynamic light scattering (DLS)

The volume of 40µL of extracted exosomes that had been dissolved in PBS reached 300µL by PBS. Then the solution was sonicated. Exosome size was measured by the Zetasizer Nano ZS (Malvern Instruments, UK) apparatus.

Overexpression of microRNA

The tube containing the lyophilized microRNA precursor sequence was briefly centrifuged to collect all material at the end of the tube. The precursor sequence was dissolved in 331µl of nuclease-free water according to the manufacturer's proposed protocol, increasing this amount to give a 11µM solution. The tube was kept at room temperature for a few minutes, and then the contents of the tube were mixed with gentle pipetting. The resulting suspension was stored at -20 °C.

Total RNA Extraction

In the present study, the Trizol (Invitrogen) reactor was used for RNA extraction. The manufacturer's guideline was followed to isolate RNA from the cell or exosome: they were first centrifuged in a Falcon tube and then added to the pellet containing 1ml of Trizol reagent and incubated at room temperature for 5min. 200µl chloroform per 1ml Trizol was added to the mixture. The micro-tube centrifugation was performed at 12000g at 4 °C for 15min. Isopropanol was added to the volume of the transferred fluid and incubated on ice for 20 minutes. The supernatant was discarded, and 1 ml of 75% ethanol was added to the precipitate. The microtube was vortexed and continued until the sediment was separated from the bottom of the microtube. The sample was centrifuged at 7500g for 8 minutes at 4 °C[5].

Statistical analysis

The data were presented as the mean and standard deviation (SD) of two or three independent experiments and the t-test was used for statistical analysis of data changes. P values of 1 were considered statistically significantly less than 0.05. data were analyzed using SPSS 18.0 and PRISM.

Results

Breast tumor cells for transfection and isolation of engineered exosomes

After breast tumor cells were cultured, the cells were transfected with the miR-144 precursor sequence as described. The supernatant of transfected cells and the control cell group was collected at different passages and used for exosome isolation. The morphology of the MDA-MB-231 breast tumor cell lines by contrast phase microscopy after transfection of the miRNA precursor sequence is shown in Figure 1.

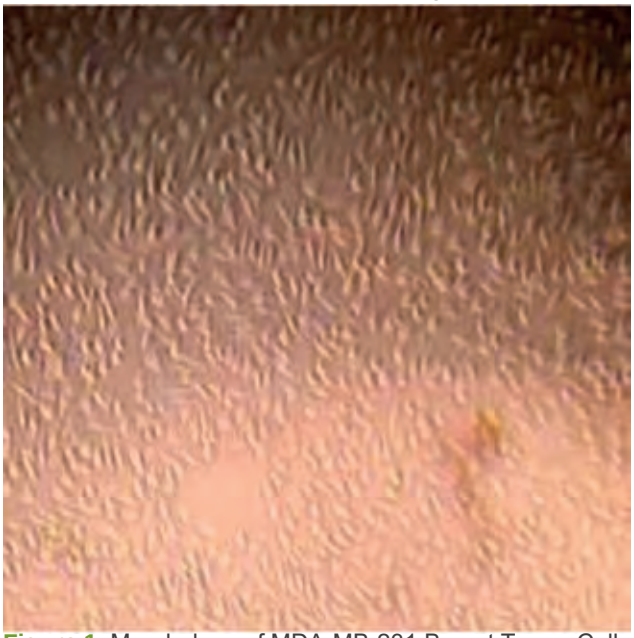

Figure 1. Morphology of MDA-MB-231 Breast Tumor Cells by Contrast Phase microscopy.

Melting curve analysis

In this study, melting curve analysis was used to confirm the accuracy of the amplified fragment and to ensure the absence of nonspecific product, primer-dimer, and contamination. By examining the generated peaks, it can be concluded that the peaks formed at low temperatures are directly related to the amount of nonspecific products formed at the end of the PCR process. As shown in Figure 2, the presence of only one peak for each gene at its specific melting temperature indicates the specificity of the replication product. This ensures no such thing as nonspecific replication, primer dimers, or contamination.

Regulation pattern of miR-144

Following the transfection of breast cancer cells with miR-144 precursor sequence, Real-time PCR results in two transfected, and untransfected cell lines (control group) showed that miR-144 expression in transfected cells was compared. There was a significant increase in the control group. As shown below, after 24h of transfection with the precursor sequence, miR-144 expression levels were significantly increased in transfected cells compared to basal miRNA expression levels in the control cell group ($p < 0.001$) evidence of the adequacy of the strategy chosen to increase miR-144 expression. Figure 3 shows the expression of miR-144 from real-time PCR.

Size and Morphology of Exosomes Isolated from Breast Cancer Cells by Scanning Electron Microscopy

SEM assessed the size and morphology of exosomal extracellular vesicles. The results showed that the isolated exosomes had less than 150 nm spherical appearance. Figure 4 shows the image taken by this microscope.

Evaluation of relative amounts of miR-144 in engineered exosomes compared to control exosomes

Real-time PCR was used to demonstrate the production of engineered exosomes containing miR-144. As shown,

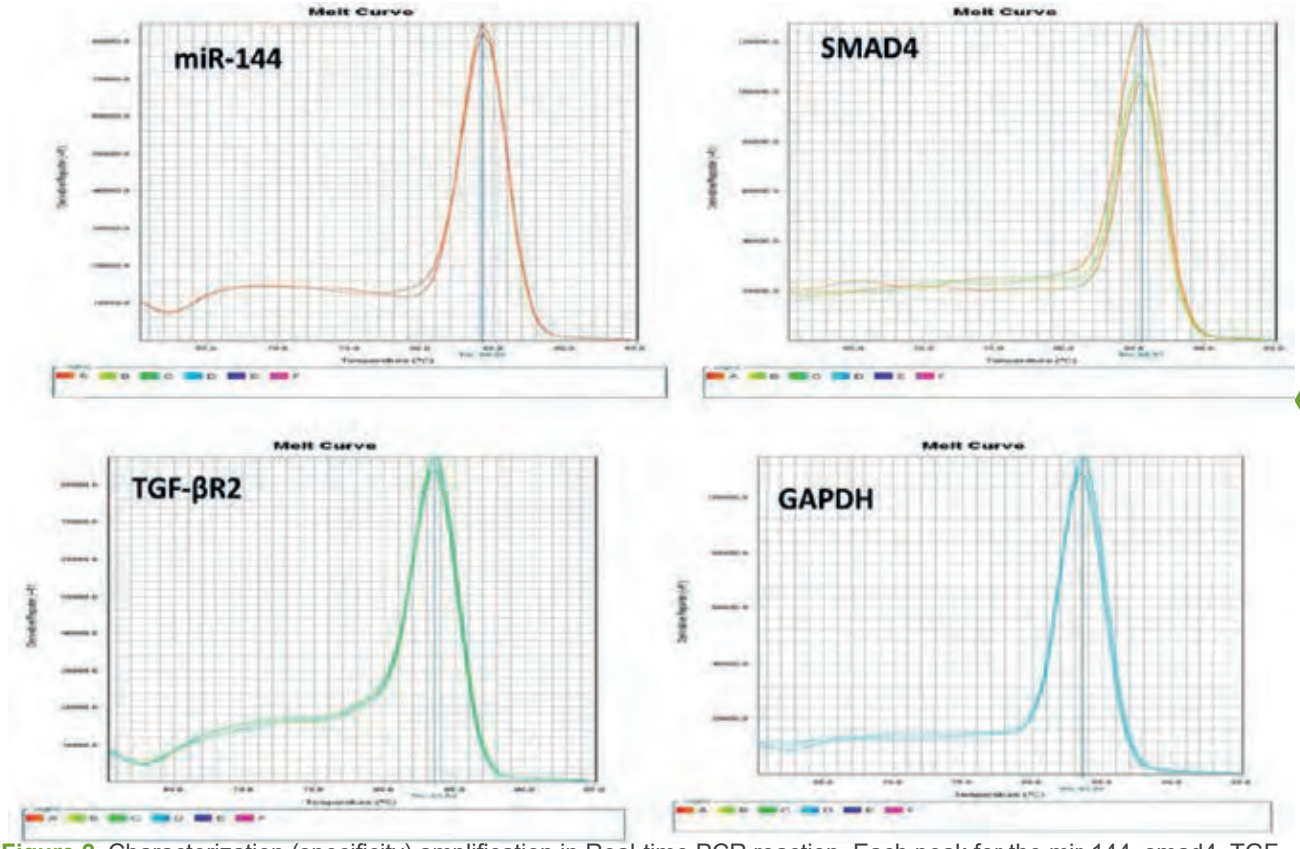

Figure 2. Characterization (specificity) amplification in Real-time PCR reaction. Each peak for the mir-144, smad4, TGF-bR2 and GAPDH genes represents the melting temperature of a PCR product.

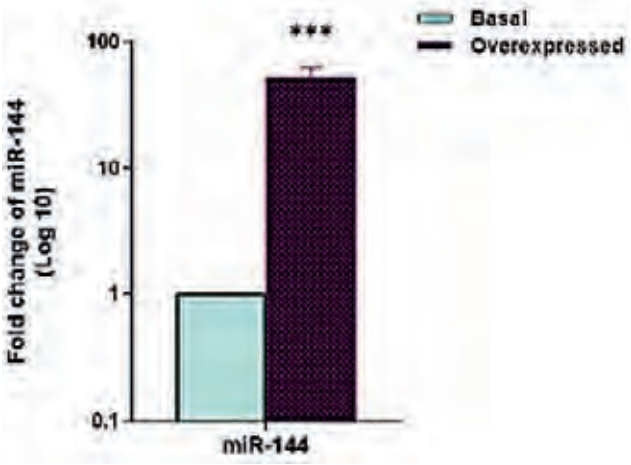

Figure 3. Expression of miR-144 from Real-time PCR. Significant increase in miR-144 expression levels in cells transfected with the miR-144 precursor sequence compared to its basal expression levels in the control cell group.

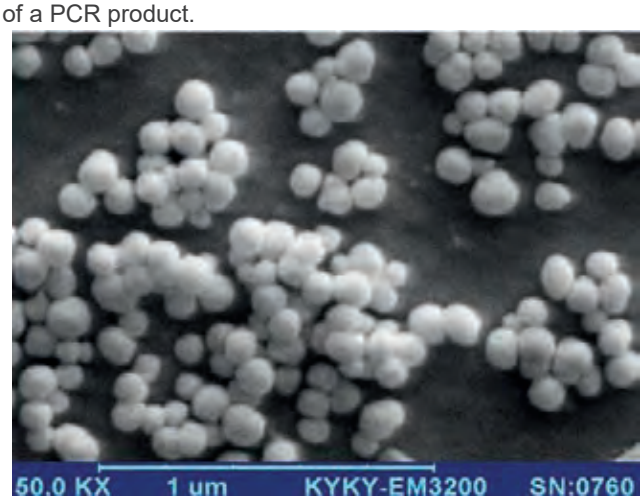

Figure 4. Investigation of exosomal extracellular vesicles isolated by SEM.

engineered exosomes (Exo-miR-144) showed significantly higher expression of miR-144 than control exosomes ($p < 0.001$). Figure 5 shows the expression levels of miR-144 in the two engineered and control exosomes.

Assessment of SMAD4 gene transcript expression in breast cancer cells following treatment with the bacterial toxin, engineered exo-miR-144 exosomes, and combination treatment of toxin A and exo-miR-144

To identify the effect of engineered exosomes containing miR-144 (Exo-miR-144) on breast cancer cells, the cells were treated with the engineered exosomes derived from both transfected and Non-transfected breast cancer cells (compared). Real-time PCR results as shown in figure 6 a decrease in Exo-miR-144 at 24 and 48 hours after treatment with SMAD4 gene expression. The results also showed that bacterial toxin A-induced reduced the expression of the SMAD4 gene in breast cancer cells. More importantly, the combination treatment of bacterial toxin A and Exo-miR-144 was engineered to create a time-dependent synergism to reduce SMAD4 gene expression.

Assessment of TGF-βR2 gene transcript expression in breast cancer cells following treatment with bacterial toxin A engineered exo-miR-144 exosomes and combination treatment of toxin A and exo-miR-144

To identify the effect of engineered exosomes containing miR-144 (Exo-miR-144) on breast cancer cells, these

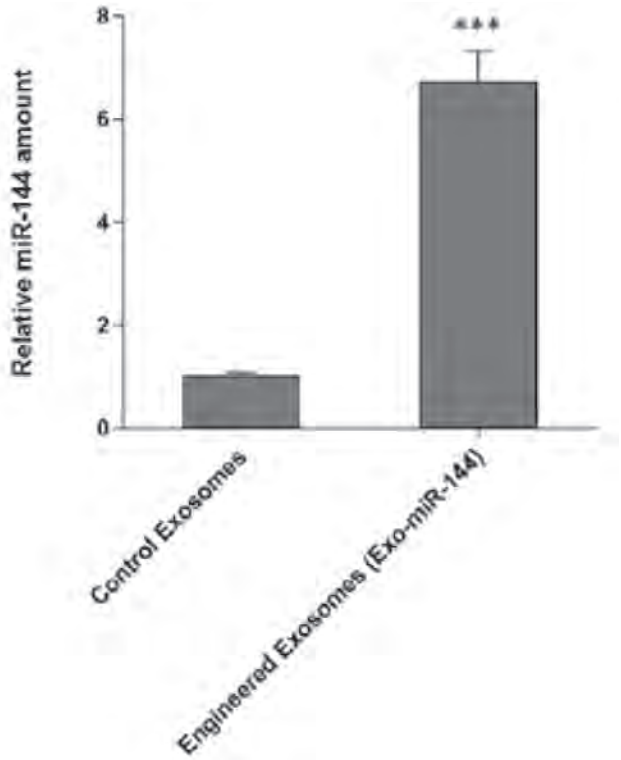

Figure 5. Determination of expression levels of miR-144 in the two engineered exosomes and control showed significant levels of miR-144 in the exo-miR-144 engineered exosomes. Exosomes are derived from cells transfected with the miR-144 precursor sequence.

cells were treated with these engineered exosomes and with cells treated with the control exosomal group), exosomes derived Non-transfected breast cancer cells (compared). Real-time PCR results, as shown in Fig. 7 showed that at 24 and 48 h after TGF-βR2 gene expression was decreased in the Exo-miR-144 treated group. The results also showed that bacterial toxin A-induced a decrease in the expression of the SMAD4 gene in breast cancer cells. But more importantly, the combination treatment of bacterial toxin A and Exo-miR-144 was engineered to produce a time-dependent synergism to reduce TGF-βR2 gene expression.

Evaluation of cell cycle progression in breast cancer cells following combination treatment of bacterial toxin and engineered exosomes (Exo-miR-144)

A strong synergy was observed in the reduction of expression of SMAD4 and TGF-βR2 genes following the combination treatment of bacterial toxin A and engineered exosomes (Exo-miR-144) in MDA-MB-231 breast cancer cells. As can be seen, the simultaneous treatment of bacterial toxin A and engineered exosomes (Exo-miR-144) increased the cell population in the Sub-G1 phase from 1.92% to 35.54%. This combination treatment is remarkable for stopping the cell cycle (Figure 8).

Discussion

Some bacterial toxins' role in inhibiting cancer cell proliferation has been elucidated. The most well-known function of Botulinum toxin is its effects on cell integrity and cytoskeleton. In 2013, Bandala and colleagues demonstrated the effect of Botulinum toxin on the proliferation and apoptosis of T47D cancer cells. They determined that Botulinum treatment may be a standard treatment for breast cancer. However, the molecular pathways induced by Botulinum that affect its cytotoxic activity have not been well identified[6].

Delivery of efficient and functional miRNA mimics and/or antagonists to tumor cells is a major challenge in miRNA-based cancer therapy. The current standard approach for gene-based and RNAi-based treatments uses viral or non-viral vector systems. Methods that directly utilize viral vectors lead to efficient gene transfer, although in some cases, they also have inefficiencies[7]. Cationic liposomes are composed of two positively charged lipid layers. They can form with negative DNA, which has a negative charge, through the simple mixing of lipid and complex DNA so that the resulting complex (lipoplex) has a generally positive control. The lipoplex is readily attached to the cell and transfected efficiently to a large extent[8]. Currently, numerous clinical trials are underway using cationic liposomes to deliver genes. Liposomes for delivery of chemotherapeutic agents such as Doxorubicin have also been previously available for chemotherapy of breast cancer[9]. An essential drawback of using cationic liposomes is that they lack specificity for the tumor and have relatively low transfection efficiency compared to viral vectors. However, the tumor specificity of lipoplexes can be dramatically enhanced by carrying a ligand identified by the cell surface receptor. Endocytosis mediated by the cellular entry pathway receptor is highly efficient in eukaryotic cells. Ligand placement on the lipoplex facilitates the entry of DNA into cells by the initial binding of the ligand to the cell surface receptor and subsequent entry of the lipoplex into the cell. Upon entry into the cell, DNA exits the endocytosis pathway to express it in the cell nucleus[10]. To efficiently deliver DNA to the cell, tumor-specific nanoparticle-specific lipopolysaccharides have been developed with ligand-targeting and self-assembling capabilities for cancer gene therapy[11]. Exosomes are one of these biological nanostructures that efficiently transfer macromolecules and nucleic acids between cells. These natural nanofluids in the human body transfer proteins, types of RNAs, and in some cases, DNA from DNA. Cell by cell is responsible[12].

In 2013, Katakowski et al. used exosomes secreted by mesenchymal stem cells to load miR-146b, showing that miRNA treatment using exosomes can effectively inhibit tumor growth. In addition to siRNAs and miRNAs, mRNAs can also be transported by exosomes as a product[13]. Exosomes can also load other types of medicine. For example, in 2015, Zhang *et al.* found that curcumin, loaded in murine lymph node cell exosomes, could successfully transfer to brain tissue and improve the apoptosis of microglial cells in the brain. The results of their work show that this strategy may provide a new non-invasive and therapeutic approach to treating inflammatory brain diseases. There are also other studies on the effect of the genetic content of exosomes in cancer treatment, all of which confirm the efficacy of exosomes in gene transfer[14].

Conclusions

As shown in the results section, exosomes secreted from tumor cells did not affect the expression levels of TGF-B pathway genes in breast cancer cells. However, when miR-144 was overexpressed in tumor cells, this overexpression caused miR-144 to be packaged in tumor-derived exosomes. Interestingly, exosomes derived from these engineered cells that contained significant amounts of miR-

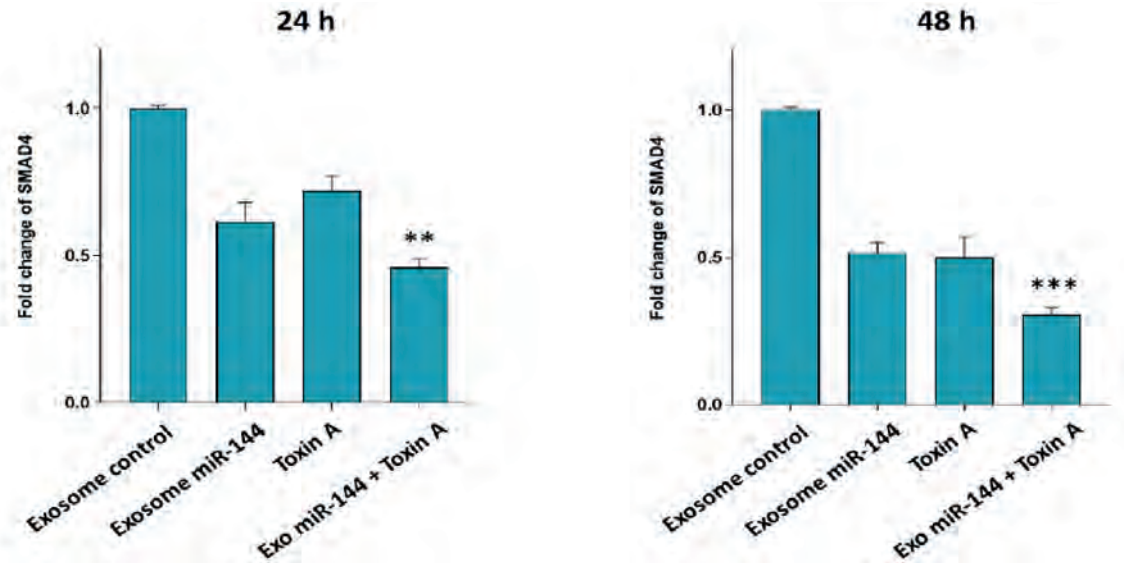

Figure 6. Assessment of SMAD4 Transcript Expression in Breast Cancer Cells Following Treatment with Exosomal Control, Bacterial Toxin A, Exo-miR-144 Engineered Exosomes and Toxin A + Combined Treatment Exo-miR-144. Treatment of engineered exosomes containing miR-144 resulted in a significant decrease in the expression level of the SMAD4 gene. In this regard, the synergistic effect of the bacterial toxin A + Exo-miR-144 significantly reduced the expression of SMAD4 transcripts in a time-dependent manner. The results were normalized to GAPDH reference gene expression.

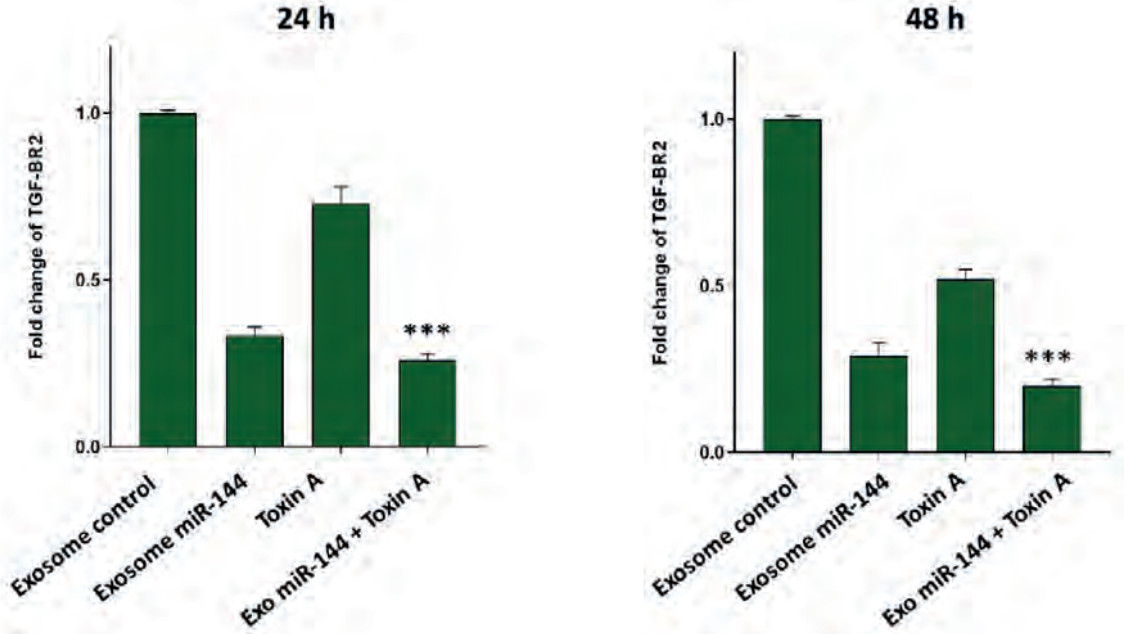

Figure 7. Evaluation of TGF-βR2 Transcript Expression in Breast Cancer Cells Following Treatment with Exosomal Control, Bacterial Toxin A, Exo-miR-144 Engineered Exosomes and Toxin A + Combined Treatment Exo-miR-144. Treatment of engineered exosomes containing miR-144 resulted in a significant decrease in the expression level of the TGF-βR2 gene. The synergistic effect of Exo-miR-144 + bacterial A toxin was important in this regard, which significantly reduced the expression of TGF-βR2 transcripts in a time-dependent manner. The results were normalized to GAPDH reference gene expression.

144 reduced the expression of the SMAD4 and TGF-BR2 genes. Also, bacterial toxin A's inhibitory effects on these genes' expression were observed in a time-dependent manner. What is essential is that the exosomal combination of miR-144 and Botulinum toxin A has a synergistic effect on the expression of the SMAD4 and TGF-B genes, which may underline the importance of this therapeutic strategy.

Conflicts of Interest

The authors declare no conflict of interest.

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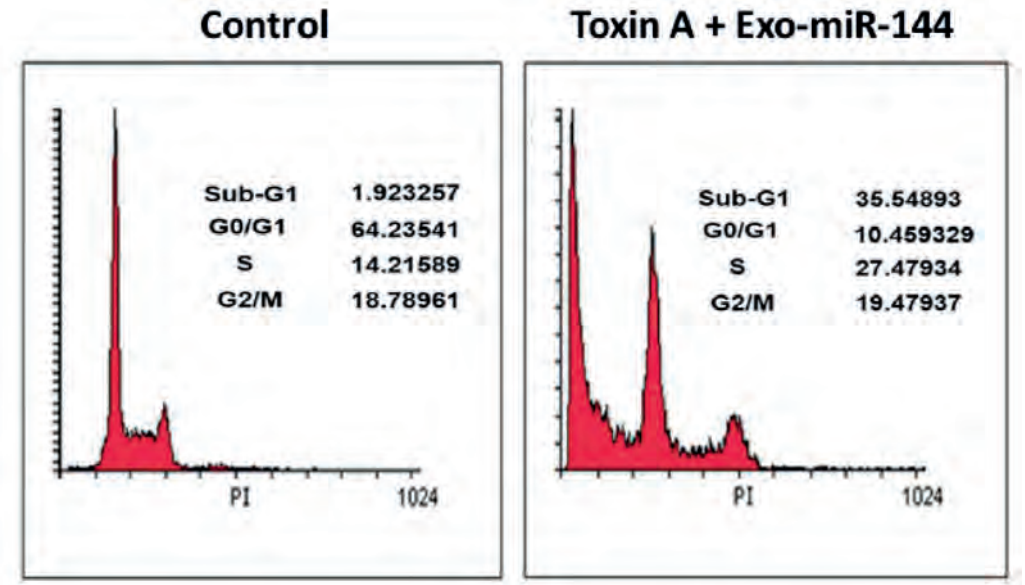

Figure 8. Cell cycle distribution in the breast cancer cell line treated with bacterial toxin A and engineered exosomes (Exo-miR-144). As shown, the suppression of TGF-β pathway gene expression increased the amount of subG1 phase cells.

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ARTICLE / INVESTIGACIÓN

Incidence of symptomatic aerobic vaginitis among some Iraqi women in Baghdad city

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DOI. 10.21931/RB/2022.07.04.59

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Abstract: Aerobic vaginitis (AV) is a newly adopted type of vaginal infection caused by aerobic bacteria; it was defined by Donders in 2002 and diagnosed according to Donder's criteria. AV is associated with increased vaginal pH, decreased vaginal microbiota and overgrowth of facultative anaerobic or aerobic bacteria, including Gram-positive cocci and Gram-negative bacilli. Because knowledge of the aerobic bacterial types causing AV is very important and only limited studies are available in Iraq about this type of vaginal infection, this study aimed to report the prevalence of AV in symptomatic women in Baghdad City, investigate the aerobic bacterial types associated and to evaluate the most critical symptoms and risk factors associated with AV. One hundred fifteen high vaginal swabs (HVSs) and 115 vaginal swabs (VSs) were collected from women of age 18-50 years attending some hospitals and private clinics in Baghdad City under the supervision of a competent gynecologist. Vaginal swabs were prepared for direct wet mount preparation to test under the microscope and Gram staining for AV identification according to Donders Criteria. High vaginal swabs were cultured on different culture media; the primary diagnosis of obtained colonies was based on phenotypic characteristics, conventional biochemical tests and Gram staining. The diagnosis was confirmed using Gram-positive (GP) and Gram-negative (GN) identification cards of the VITEK 2 System. Statistical analysis was carried out using (SPSS v 20) and p-value ≤ 0.05 at 95% CI was considered statistically significant. Out of the 115 swabs, only 89 (77.3%) swabs showed bacterial growth. Ninety-five bacterial isolates were obtained, including (65.2%) Gram-positive and (34.7%) Gram-negative bacteria. G+ve bacterial isolates included: (46.3%) *Staphylococcus* spp., (6.3%) *Kocuria* spp., (6.3%) *Enterococcus* spp., (5.2%) *Micrococcus luteus* and (1.0%) of *Streptococcus agalactiae*. G-ve bacterial isolates included (15.7%) *Escherichia coli*, (11.2%) *Klebsiella pneumoniae*, (3.2%) *Pseudomonas aeruginosa* (3.2%) *Pseudomonas aeruginosa*, (2.1%) *Acinetobacter baumannii* and (2.1%) *Proteus mirabilis*. More than half (58.4%) of patients showed severe AV. Mixed bacterial infections were reported in 6 (6.7%) cases only. Vaginal pH ranged between (5.5- 6.5). The most frequent(100%) testified symptoms were abnormal vaginal discharge and itching, or irritation, the less frequent symptoms (51.6%) was vaginal dyspareunia, and the most significant symptoms in associated with different bacterial types and age groups were foul smelling and burning (p= 0.001, 0.008, 0.0001). Among the risk factors, age was significantly associated with most bacterial types obtained with p values (0.05 and 0.02). *Staphylococcus* spp. and *E.coli* were the predominant bacterial types in AV patients in the current study, and the lower rate of bacteria was *Streptococcus agalactiae* (1.0%). A high prevalence of AV was reported; therefore, regular screening and proper diagnosis of AV using microscopic examinations, culturing of swabs and determining vaginal pH using specific vaginal pH test strips should be stimulated to develop AV management.

Key words: High vaginal swabs, Vaginal swabs, Aerobic vaginitis, aerobic G+ve and G-ve bacteria, Risk factors, Iraq.

Introduction

In 2002 the term 'aerobic vaginitis (AV) was coined for the first time to describe an essential condition of vaginal dysbiosis besides the existing entity, bacterial vaginosis (BV). BV is a rather well designated over the last 100 years and has become well-known as a condition that is very prevalent among women all over the world. AV is a type of vaginal infection caused by aerobic bacteria and is characterized by abnormal (dysbiotic) vaginal microflora containing aerobic, enteric bacteria, inconstant levels of vaginal inflammation and inadequate epithelial maturation[1]. The standard lower genital or reproductive tract of the female is inhabited by several different bacteria that live in balanced populations; the dominant bacterial species is Lactobacillus, which can prevent facultative and obligate anaerobes outnumbering in vaginal microbiota, maintaining healthy microbial homeostasis[2]. When lactobacilli decrease in number, it facilitates the growth of potentially pathogenic bacteria[3]. AV is a form of dysbiosis in the ecology of aerobic microorganisms that belong to the vaginal microflora. These microorganisms include *Staphylococcus aureus*, *S.epidermidis*, *Enterococcus faecalis*, and *Streptococcus agalactiae*. *Escherichia coli*, *Pseudomonas* spp, *Acinetobacter* spp and *Klebsiella pneumoniae*[4,5]. Patients with AV present with red vaginal wall, itching, purulent discharge and vaginal epithelial cell lysis[6]. AV is associated with miscarriage, placental histological inflammation, preterm delivery, premature rupture of membranes and major cervical cytological abnormalities[7-10]. The prevalence of AV is reported to be 5-27.6% in various

Citation: Raheem Z K, Said L A. Incidence of symptomatic aerobic vaginitis among some Iraqi women in Baghdad city. Revis Bionatura 2022;7(4) 59. http://dx.doi.org/10.21931/RB/2022.07.04.59
Received: 2 May 2022 / **Accepted:** 12 June 2022 / **Published:** 15 November 2022

areas[11-13],[15]. *S. aureus*, *S.epidermidis*, *E.faecalis*, *Streptococcus* spp and *E.coli* have been found to colonize in significant numbers in AV patients[1,10,13]. Not all AV cases are symptomatic or can be treated by antibiotics targeting the causative bacteria, so the study on AV bacterial causatives is critical. The data about AV prevalence, pathogens and risk factors are limited in Iraq. Therefore, the present study aimed to report the prevalence of AV in symptomatic women in Baghdad City, evaluate the correlation of symptoms, and risk factors with AV, and investigate the aerobic bacterial types associated with AV.

Materials and methods

Ethics and patient permission for data collection

Firstly, what the procedure will include was clarified gently for each patient using patient-friendly language to obtain their verbal consent to participate in this study and secondly, to answer the questions that are included in the questionnaire form: age, is she currently married or not, is she pregnant, contraceptive type, have previous or repeated vaginal smears or not.

Also, the clinical symptoms, including the presence of abnormal vaginal discharge, vaginal itching and burning, foul smelling and vaginal dyspareunia, were collected. The present study was approved by the Research Committee of the College of Science/ Biology department/at Mustansiriyah University.

Specimens Collection

From January to June 2021, 115 high vaginal swabs and 115 vaginal swabs were collected from married women suffering from at least one of the clinical symptoms included in the symptoms form; patients were asked if they had taken antibiotics or intra-vaginal medication during the preceding two weeks. All VSs were prepared for direct wet mount preparation by microscope and Gram staining to identify aerobic vaginosis (AV) according to Donders Criteria; all HVSs were placed in Carey-Blair transport media and transported to the laboratory throughout three hrs., then cultured on different culture media.

Vaginal swabs, macroscopic examination

Vaginal swabs were observed in terms of consistency, odor, color, presence of mucus, blood and pH. The pH of the women's vaginae was determined using specific vaginal pH test strips (NATURE LAND/China); women's vaginae pH > 4.5 were considered abnormal, as mentioned by (7).

Vaginal swabs, wet mount preparation and Donders scoring

One drop of each vaginal swab suspension with normal saline was placed on a slide and covered with a coverslip to study the criteria employed for diagnosing AV in infected women as described by Donders[1]. The diagnosis of AV is based on five microscopic criteria, each of which can be absent 0 points, moderate 1 point or severe 2 points. The sum of the points establishes the composite AV score, with a maximum score of 10. AV score <3, no signs of AV; (AV) 3 or 4, light AV; 5 or 6, moderate AV; >6, severe AV.

Identification of bacterial isolates

For primary diagnosis, all obtained swabs were cultured on sterile prepared plates of MacConkey agar, blood agar and mannitol salt agar by gently streaking and incubated at 37°C aerobically for 24-48 hrs[16]. The colonies obtained were read out as standard laboratory protocols, growth was identified based on morphological characteristics Gram stain, motility and conventional biochemical tests including catalase and oxidase[17]. Gram-positive (GP) and Gram-negative (GN) colorimetric identification cards of VITEK 2 Compact Automated System and (BioMérieux/ France) were used to confirm the identification of all obtained isolates.

Data Analysis

Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 20. Chi-square values were calculated at a 5% ($p \leq 0.05$) significance level for studying the significance levels between the different factors included in this study.

Results

Characteristics and symptoms of study participate

As shown in (Table 2), the most frequently (100%) reported symptoms were the presence of abnormal vaginal discharge and itching or irritation in a total of (89) women that showed positive culture, and the less frequent 46 (51.6%) was vaginal dyspareunia.

According to Table 2, most (93.2%) of patients in the present study were currently married, and only a few (34.8%) of patients were pregnant; this indicates that the prevalence of AV in non-pregnants was higher than in pregnant women in the present study.

AV Severity percentages among study participates

Wet mount preparation shows the epithelial cells, WBCs, Para basal cells, different bacteria and PMNs in the vaginal swabs under study, which indicate that the patient is suffering from AV infection. AV severity was determined for all patients with positive culture results according to the Donders criteria and scoring. The present study showed that 58.4%, 30.3%, and 11.2% of patients had severe, moderate and light AV, respectively, as shown in (Figure 1).

AV score	LBG	Leukocytes no.	Proportion of leukocytes	Background flora	Proportion of parabasal epitheliocytes
0	I	≤ 10/hpf	Non or sporadic	Notable or cytolysis	Non or <10%
1	II	> 10/hpf ≤ 10 epithelial	≤50% of leukocytes	Small coliform bacilli	≤10%
2	III	>10 epithelial cell	>50% of leukocyte	Cocci or chain	>10%

Table 1. Donders Criteria for the microscopic diagnosis of AV using high power 400 x· of microscope[1].

Characteristics	No.	%	Characteristics	No.	%
Currently married	83	93.2%	Abnormal vaginal discharge	89	100%
pregnant	31	34.8%	Itching or irritation	89	100%
Use contraceptive	30	33.7%	Foul-smelling	84	94.3%
Intrauterine device (IUD)	14	15.7%	Burning	80	89.8%
Oral contraception	8	8.9%	Vaginal dyspareunia	46	51.6%
Male condom	7	7.8%	Have previous vaginal smears	22	24.7%
Contraceptive implant	1	1.1%	Total	89 women	

Table 2. Numbers and percentages of patient's data and symptoms.

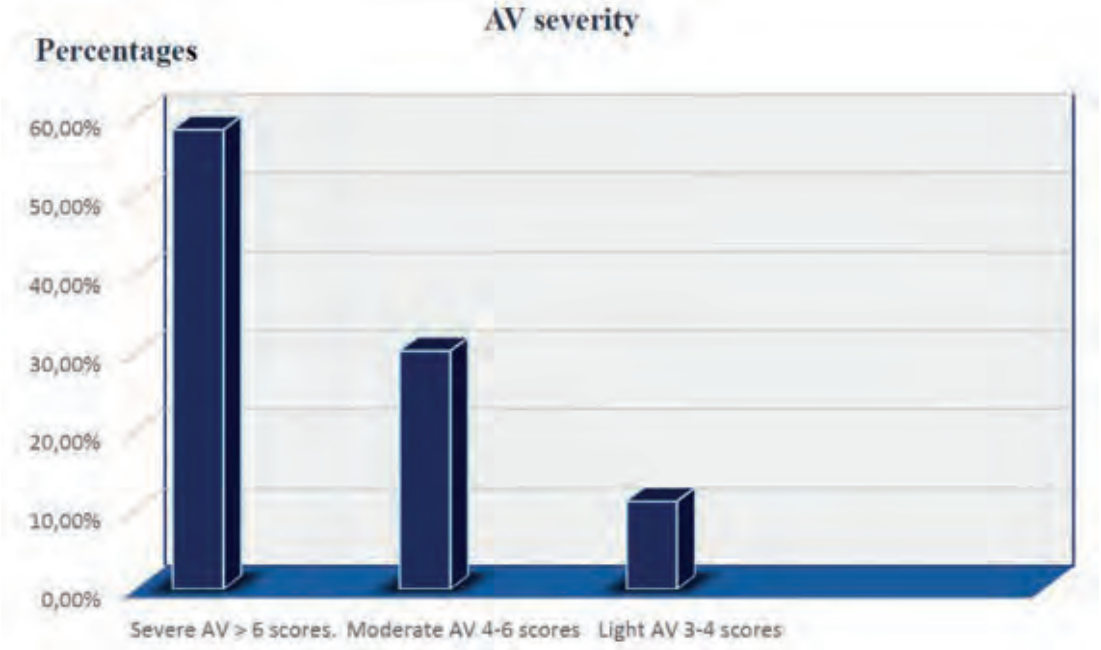

Figure 1. Aerobic vaginitis severity percentages among study participate.

Vaginal pH Test

Different color changes were obtained using the commercial-specific vaginal pH test strips; pH was determined according to the color change in strips after being touched with vaginal discharge and compared with the pH ranges colors on the manufacturer's box. Vaginal pH values and their percentages were obtained, including; pH 5, 5.5, pH 6 and 6.5 in 2%, 10%, 42% and 45% of infected women, respectively.

Identification of bacterial isolates using GP and GN Cards of VITEK 2 Compact Automated System

GP and GN identification cards of VITEK 2 system identified different G+ve and G-ve bacterial isolates with varying values of confidence when 54 isolates (56.8%) showed confidence values 99-96% (excellent identification), 29 isolates (30.5%) showed confidence values 93-95% (perfect identification), 12 isolates (12.6%) showed confidence value (89-92%) good identification value. It is worth mentioning that only 7 isolates were unidentified with both cards and were excluded from this study.

Numbers and percentages of obtained bacterial types

Bacterial growth was observed in 89 (77.3%) of 115 swab samples. Ninety-five bacterial isolates were obtained, including 62 (65.2 %) Gram-positive and 33 (34.7 %) Gram-negative bacteria, as shown in (Table 4). Mixed bacterial infections were noted in 6 (6.7%) cases only. Gram-positive bacteria belong to 5 genera and 13 species, while Gram-negative bacteria belong to 5 genera and 5 species.

Distribution of AV patients according to age

AV patients were distributed into four age groups: ≤ 20 years in 13 (14.60) cases, (21-30) years in 41 (46.06%) cases, (31-40) years in 24 (26.96) cases and (41-50) years in only 11 (12.35%) case as shown in (Figure 2). It is worth mentioning that most (46. 0%) of the patients belonged to

Gram-positive bacteria					
Type of isolates	**No.**	**%**	**Type of isolates**	**No.**	**%**
Staphylococcus aureus	10	10.5	*Micrococcus luteus*	5	5.2
Staphylococcus haemolyticus	11	11.5	*Enterococcus faecalis*	5	5.2
Staphylococcus hominis	11	11.5	*Kocuria kristinae*	4	4.2
Staphylococcus epidermidis	7	7.3	*Kocuria rosea*	2	2.1
Staphylococcus warneri	1	1.0	*Streptococcus agalactiae*	1	1.0
Staphylococcus saprophyticus	2	2.1	*Enterococcus faecium*	1	1.0
Staphylococcus auricularis	2	2.1	**Total**	62	65.2%
Gram-negative bacteria					
Type of isolates	**No.**	**%**	**Type of isolates**	**No.**	**%**
Escherichia coli	15	15.7	*Acinetobacter baumannii*	2	2.1
Klebsiella pneumoniae	11	11.5	*Proteus mirabilis*	2	2.1
Pseudomonas aeruginosa	3	3.2	Total	33	34.7%
Mixed Bacteria Infection					
Types of isolates	**No.**	**%**	**Types of isolates**	**No.**	**%**
S.hominis, S.haemolyticus	2	2.2	*P.mirabilis, P.aeruginosa*	1	1.1
E.coli, S.aureus	1	1.1	*S.hominis, K.kristinae*	1	1.1
K.pneumoniae, K.kristinae	1	1.1	Total	6	6.7%

Table 3. Numbers and percentages of identified bacterial types in HVSs.

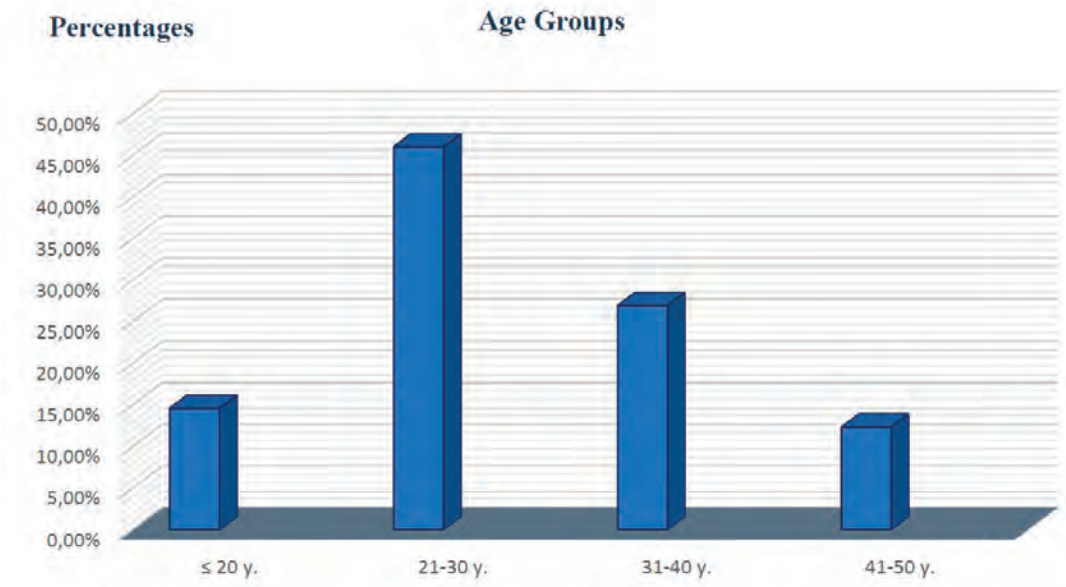

Figure 2. The column chart clarifies the percentage of patients' age groups.

the age group (21-30) years, while only (12.3%) of the patients belonged to the age group (41-50) years.

The mean±SE (standard error) and the probability *P* value of patients' age according to the pathogenic bacterial type were determined as shown in (Table 4); the diversity of AV bacteria according to the age group was significantly different, and the results indicate that the age (p= 0.02, CI 95%) was considered a significant factor in women infected with *Micrococcus* spp and *Enterococcus* spp, also in women infected with *Staphylococcus* spp and *P.aeruginosa* (*p*= 0.05, CI 95%). While in women infected with *Kocuria* spp., *E.coli* and *K.pneumoniae* (*P* value =NS), the age was considered statistically non-significant.

Statistical Analysis

Statistical analysis revealed that few factors were non-significant regardless of bacterial type, other factors were significant with different bacterial types, and some

Type of isolates	Age mean±SE	*P* value	Type of isolates	Age mean±SE	*P value*
***Staphylococcus* spp.**	29.49±3.5	0.05	*E.coli*	31.1±4.2	NS
***Kocuria* spp.**	34.6±4.3	NS	*K.pneumoniae*	31.8±6.5	NS
***Micrococcus* spp.**	26.6±3.7	0.02	*P.aeruginosa*	29±3.4	0.05
***Enterococcus* spp.**	26±2.8	0.02	CI 95%		

SE= standard error, P value= probability value, C.I= confidence interval, NS= non-significant

Table 4. Patients' ages mean±SE and p-value according to causative age.

Bacterial types and Numbers.	Abnormal vaginal discharge	Foul-smelling	Itching or irritation	Burning	Vaginal dyspareunia	PH Mean±SE
No growth 26	25	19	22	8	0	25
***Staphylococcus* spp. 42**	42	42	42	42	23	6.3±0.2
Relative risk	1.04	1.36	1.18	32.5	29.511	---
CI 95%	0.9-1.1	1.08-1.7	1.1-31.9	1.8-57.8	1.8-47.6	----
P value	0.3	0.008	0.04	0.0001	0.01	NS
***Kocuria*. Spp. 6**	6	6	6	6	5	6.5±0.1
Relative risk	1.04	1.36	1.13	32.5	0.8	---
CI 95%	0.9-1.1	1.08-1.7	0.4-1.3	1.8-57.8	0.5-1.1	----
P value	0.3	0.008	0.08	0.0001	0.3	NS
***Micrococcus* Spp. 5**	5	5	5	4	4	6.3±0.2
Relative risk	1.12	1.14	1.7	32.5	34..6	---
CI 95%	0.9-1.3	0..9-1.6	1.6-6.6	1.8-57.8	2.1-51.6	----
P value	0.0001	0.001	0.001	0.0001	0.01	NS
***Enterococcus* Spp. 6**	6	6	6	6	6	6.5±0.2
Relative risk	1.04	1.36	1.13	32.5	50.1	---
CI 95%	0.9-1.1	1.08-1.7	0.4-1.3	1.8-57.8	3.1-71.7	----
P value	0.3	0.008	0.08	0.0001	0.005	NS
***Escherichia coli* 15**	15	15	15	11	3	6.1±0.3
Relative risk	1.1	1.3	1.3	0.6	1.18	---
CI 95%	0.9-1.2	1.1-1.7	1.1-1.7	0.2-2.6	0.5-1.9	----
P value	0.3	0.008	0.008	0.001	0.4	NS
***Klebsiella pneumoniae* 11**	11	11	11	8	3	6±0.2
Relative risk	1.1	1.3	1.3	0.6	0.8	---
CI 95%	0.9-1.2	1.1-1.7	1.1-1.7	0.2-2.6	0.2-2.7	----
P value	0.3	0.008	0.008	0.001	0.8	NS
***Pseudomonas aeruginosa* 3**	3	3	3	3	2	6.5±0.2
Relative risk	1.1	1.3	1.3	0.6	3.6	---
CI 95%	0.9-1.2	1.1-1.7	1.1-1.7	0.2-2.6	0.8-5.9	----
P value	0.3	0.008	0.008	0.001	0.01	NS

Table 5. The statistical analysis compares relative risk and the P value of bacterial types in the study according to the patient's symptoms.

aspects were substantial only with some bacterial types. As shown in (Table 6), the infection with *Staphylococcus* spp was significantly associated with the following clinical symptoms: foul-smelling (*p*=o.oo8), itching or irritation (*p*= 0.04), burning (*p*=0.0001) and vaginal dyspareunia (*p*=0.01) while pH was significantly non-significant (*p*=NS), on the other hand, colonization with *Staphylococcus* spp. were associated considerably with currently married patients (*p*=0.01) and age (*p*=0.05) when the age mean ±SE = 29.49±3.5. Another finding was that the infection with *Kocuria* spp was significantly associated with clinical symptoms, including foul smelling (p=0.008) and burning (p=0.0001). On the other hand, colonization with *Kocuria* spp. was significantly non-significant with all the patient data in (Table 5) despite that, the patients suffer from many symptoms. Colonization with *Micrococcus luteus* was significantly associated with all AV symptoms, including; abnormal vaginal discharge (*p*= 0.0001), foul smelling (*p*=0.001), itching or irritation (*p*=0.001), burning (*p*=0.0001) and vaginal dyspareunia (*p*=0.01). Also, it was significantly associated with age (*p*=0.02) and if the patient has previous vaginal smears (*p*=0.01). According to the statistical analysis, *Enterococcus* spp. was associated considerably with AV symptoms, including foul smelling (*p*=0.008), burning (*p*=0.0001) and vaginal dyspareunia (*p*=0.005). Also, it was significantly associated with patients' age (*p*=0.02). Another finding, infection with *E.coli* and *K.pneumoniae* were significantly associated with foul smell (*p*=0.008), itching or irritation (*p*=0.008) and burning (*p*=0.001). On the other hand, *E.coli* was significantly associated with pregnancy (*p*=0.04), while *K.pneumoniae* was associated considerably with contraceptive use (*p*=0.03). Statistical analysis revealed that colonization with *P.aeruginosa* was significantly associated with foul smelling (*p*=0.008), itching or irritation (*p*=0.008) and burning (*p*=0.001), and vaginal dyspareunia (*p*=0.01), while according to patients data, *P.aeruginosa* were associated

Bacterial types and Numbers	Age Mean ±SE	Is the patient currently married	Is the patient pregnant	Is she use contraception ?	Is she have previous vaginal mears?
No growth Control 26	38.35±2.8	20	3	5	5
***Staphylococcus* spp. 42**	29.49±3.5	42	14	17	8
Relative risk	---	1.3	2.9	2.1	0.9
CI 95%	----	1.1-1.6	0.9-1.09	0.3-0.9	0.3-2.5
P value	0.05	0.01	0.06	0.09	0.8
***Kocuria* spp. 6**	34.6± 4.3	5	0	2	2
Relative risk	---	1.08	0.55	1.7	1.7
CI 95%	----	0.7-1.6	0.05-9.4	0.4-6.8	0.3-2.5
P value	NS	0.7	0.6	0.4	0.8
***Micrococcus* spp. 5**	26.6± 3.7	5	1	1	0
Relative risk	---	1.08	1.4	0.86	28.1
CI 95%	----	0.7-1.6	0.12-11.5	0.1-6	1.4-4.7
P value	0.02*	0.7	0.7	0.8	0.01
***Enterococcus* spp. 6**	26±2.8	6	0	1	4
Relative risk	---	1.08	0.55	1.7	1.7
CI 95%	----	0.7-1.6	0.05-9.4	0.4-6.8	0.3-2.5
P value	0.02	0.7	0.6	0.4	0.8
***Escherichia coli* 15**	31.1± 4.2	13	6	3	7
Relative risk	---	1.1	3.6	1.04	2.4
CI 95%	----	0.8-1.5	1.1-1.8	0.2-3.7	0.9-6.3
P value	NS	0.4	0.04	0.9	0.06
***Klebsiella. spp* 11**	31.8±6.5	10	3	6	2
Relative risk	---	1.5	2.3	2.8	0.9
CI 95%	----	0.9-2.4	0.9-8.5	1.1-7.3	0.2-4.4
P value	NS	0.08	0.2	0.03	0.9
***Pseudomonas aeruginosa.* 3**	29±3.4	3	1	1	0
Relative risk	---	2.3	2.8	1.7	0.6
CI 95%	----	1.6-3.4	0.2-10.3	0.2-8.6	.004-1.1
P value	0.05	0.001	0.2	0.5	0.7

Table 6. Statistical analysis of relative risk and the P value of bacterial types in the study according to patient's data.

only with age (p=0.05) and married patients (p=0.001).

The relative risk (RR) or risk ratio is the ratio of the probability of an outcome in an exposed group to the possibility of a development in an unexposed group. Together with risk difference and odds ratio, relative risk measures the association between exposure and the outcome.

Discussion

The present study results showed that *Staphylococcus haemolyticus*, *Staphylococcus aureus* and *Escherichia coli* were the most prevalent bacterial types among AV-infected women. A local study conducted by Jabuk[18], in Hilla City/ Iraq reported that 64% of women using an intrauterine device (IUD) were infected with aerobic bacteria, including; *E.coli, S.aureus, K.pneumoniae* and *Proteus mirabilis* and the most frequent symptom was the presence of abnormal vaginal discharge and the less frequent (18%) symptom was vaginal dyspareunia. Their results agree with the present study results when 100% of patients have abnormal vaginal discharge. In this investigation, abnormal vaginal discharge, foul smelling, itching, burning and vaginal dyspareunia were found to be statistically significant in most patients infected with different obtained bacterial types when p value < 0.05 as mentioned in statistical analysis (Table 5). Another study by Yalew *et al.*[19] reported that the prevalence of AV in pregnant women was 8.1%. The present study agreed with Mulu *et al.*[20] when they found that the prevalence of vaginal infection was higher in nonpregnant women than in pregnant women. According to the patient's data, the most frequent factor was marriage (93.2%), and the less was if the patient had previously repeated smears (24.7%), as shown in (Table 2). AV is a form of vaginitis affecting millions of women worldwide that are distinguished from BV; many studies have found that the incidence of AV was approximately 11.77% included, 4.34% in nonpregnant women and 13.08% in pregnant women[21]. Another study by Wang *et al.*[14] found that the AV rate is 15.40% in women who presented with AV symptoms in Southwestern China. Although several researchers have found that AV infection may be companied by other forms of vaginitis BV and candidiasis and present different clinical manifestations, the precise mechanism remains unclear[9,22]. Medium and severe AV in mixed AV infection was more frequent than in simple AV infection in terms of AV score[23].

As reported in the present study, the pH level of the vaginae of AV-infected women was higher than tha5.5-6. It was recognized that the pH level was higher than 4.5 in AV patients because vaginal microflora and lactobacilli levels were lower in comparison[24]. In most patients with AV, the vaginal pH was high > 4.5[12]. The vaginal pH range (3.8-4.5) indicates healthy women's vaginae. Research conducted by Kaambo *et al.*[25] agrees with pH test results obtained in this study, when they reported that the vaginal pH in AV-infected women was> 4.5, typically 6.

The most prevalent Gram-positive bacteria were 11(11.5%) *S.haemolyticus* and 10 (10.5%) *S.aureus*, while the most prevalent Gram-negative bacteria was *E.coli* 15 (15.7%); this result was in agreement with previous studies conducted in Iraq when Al-Suadi[26], found that the most frequent bacterial type was *S.aureus* in 12 (27.9%) and *E.coli* in 11 (25.5%) isolated from AV infected women, similar results were obtained by Mohammed *et al.*[27] when found that the most prevalent bacterial types in AV infected women were *S.aureus* and *E.coli*, and similar results were obtained in a study conducted in a foreign country by Wang *et al.*[14]. While the different result was obtained in a study conducted in Iraq by Shaker[28], when the most frequent bacterial species isolated from HVSs were *K.pneumoniae*, *Streptococcus* spp. and *E.coli*. As shown in (Table 4), mixed infections were noted in 6 (6.7%) cases; similar results were obtained by Mohammed *et al.*[27] when their AV mixed infection was found in 4 (9.3%) cases only.

As shown in (Figure 2), most (46.06%) of AV-infected women belonged to the age group (21-30) years; this finding is in agreement with a study conducted by Wang *et al.*[14] when they found that the AV was usually isolated from the sexually active patients age group of 20-30 years, followed by those in the age group of 30-40 years. The highest prevalence in the age groups (21-30) and (31-40) may perhaps be due to these being the highest reproductively active and high sexual exposure groups. A study conducted by Zhang *et al.*[29] reported that simple AV patients were aged > 50 years, while mixed AV patients were aged 30-40 years and also found that age was a significant factor in AV infections (p=0.003); this result is in agreement with the results of the present study when age is a significant factor in associated with most AV bacterial types.

The differences in bacterial colonization prevalence, types and risk factors associated with AV may be due to several details, including differences in geographical settings, ethnicity, sample size and many other factors[30]. AV, without a correct diagnosis, may be diagnosed incorrectly as BV, leading to more severe complications such as Desquamative inflammatory vaginitis, which increases the risk of preterm delivery, chorioamnionitis and many other risk factors[31]. In a recent study conducted by Kim *et al.*[32], they reported that vaginal colonization with *S.aureus* and *E.coli* was significantly associated with many pathogens isolated from neonates infected with initial-onset sepsis. The above-reported results (Table 6) showed that AV symptoms were significantly associated with different bacterial types. At the same time, according to the patient data, age was the most significant risk factor associated with most bacterial types, followed by married (p=0.01), especially when the causative pathogen was *Staphylococcus* spp.

Conclusions

As concluded, there is a high prevalence of AV among women seeking gynecological care in Baghdad City; AV is a common cause of abnormal vaginal discharge, foul smelling, itching, burning and high pH levels, and the patient may present with all or some of these signs and symptoms. The most prevalent bacterial type in AV patients was *Staphylococcus* spp. colonization of different genera and species of Gram-positive and Gram-negative bacteria were significantly associated with two or four tested factors and symptoms except with *Kocuria* spp. Only limited studies on AV have been performed in Iraq. So, similar studies must be carried out to improve Iraqi women's health status. On the other hand, healthcare professionals should consider enhancing the education of patients to improve their knowledge of vaginal health and prevent the risk factors of AV.

Acknowledgments

The authors would like to thank Al-Mustansiriyah University/ College of Science/ Department of Biology. Special

thanks to all study participants for their cooperation and understanding of this work's importance. Many thanks to the medical staff in Al -Elwea Maternity Hospital and many private gynecology clinics and labs in Baghdad City for presenting the facilities, services and essential support to achieve this work.

Conflicts of Interest

This study's authors declare that they have no conflict of interest.

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ARTICLE / INVESTIGACIÓN

The association of serum visfatin in women with polycystic ovary syndrome: A case-control study

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DOI. 10.21931/RB/2022.07.04.60

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Abstract: Polycystic ovarian syndrome (PCOS) is the most common endocrinopathy among women of reproductive age. Visfatin is an adipokine secreted by fat tissue and macrophages involved in regulating glucose homeostasis, adipose tissue inflammation, chronic systemic inflammation, cardiovascular disease and endothelial dysfunction. The study sample (100 patients) includes 50 PCOS women and 50 control matched for age and body mass index (BMI). The women with PCOS were divided into obese or overweighed according to BMI ≥ 25 Kg/m^2 and non-obese BMI ≤ 25 Kg/m^2. The control group was also divided into obese and non-obese. The results showed that serum visfatin was significantly increased in obese women with POCS compared to obese control (5.61 ± 1.27 ng/mL vs. 0.48 ±0.28 ng/mL) and in non-obese women with polycystic ovarian syndrome compared to non-obese control (5.22± 1.36 ng/mL vs. 0.33± 0.26 ng/mL). These findings might suggest that visfatin could play a role in pathogenesis and the long-term consequences of PCOS.

Key words: Visfatin, polycystic ovarian syndrome, body mass index, obese women.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine, a metabolic disorder affecting 5% to 10% of women in the reproductive age[1]. It has significant and diverse clinical implications, including reproductive, infertility, hyperandrogenism, hirsutism, metabolic; insulin resistance, impaired glucose tolerance, diabetes mellitus type[2], adverse cardiovascular risk profiles and psychological features; increased anxiety, depression and worsened quality of life 2. Its management should focus on support, education, addressing psychological factors and strongly emphasizing healthy lifestyle with targeted medical therapy, monitoring and control of long-term metabolic complications[3].

The adipose tissue (AT) secretes many bioactive factors, called adipokines, that act locally within the fatty tissue and affects distant organs. These are tumor necrosis factor (TNF-α), interleukins (IL), angiotensinogen, plasminogen activator inhibitor-1 (PAI-1), leptin, visfatin, resistin, and apelin[4,5].

Visfatin is highly in various tissues and cell types, including adipocytes, lymphocytes, bone marrow, liver, muscle, trophoblast, and fetal membranes[6]. Although there are conflicting data on the relationship between visfatin and obesity, a recent meta-analysis revealed that plasma visfatin is significantly increased in subjects diagnosed with overweight/obesity, DM type 2, metabolic syndrome, and cardiovascular diseases (CVD)[7]. Many studies demonstrated that visfatin displayed proinflammatory properties and modulated immune functions; as a result, rising visfatin levels correlate with the degree of endothelial dysfunction, and it is expressed higher in the atherosclerotic lesions of symptomatic patients than in the lesions of asymptomatic patients, further emphasizing the role of this adipokine in plaque destabilization and acute cardiovascular events[8,9]. Likewise, elevated visfatin levels in PCOS may also signal heightened cardiovascular risk in particular women with this syndrome, particularly in those with insulin resistance[10].

This study aims to determine the serum levels of visfatin in women with PCOS and compare them with healthy control.

Materials and methods

This case-control study was done at Fertility Center, Al-Sadder Medical City and Al-Zahra'a Teaching Hospital for maternity and pediatrics, Najaf, Iraq, from March 1, 2019, to December 1, 2019. In this study, one hundred women were included, sub-divided into 50 patients with PCOS and 50 cases as control (were healthy matched for ages and BMI with the regular menstrual cycle). The women with PCOS were further divided into obese BMI≥ 25 Kg/m^2 and non-obese BMI ≤25 Kg/m^2. The control group was divided into obese and non-obese. The diagnosis of PCOS depends on the European Society of Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE / ASRM) in Rotterdam, Netherlands, in Oct 2003[11].

Inclusion criteria (two of three)

1) Oligo ovulation and anovulation.
2) Clinical manifestations of androgen excess and/or hyperandrogenism.
3) Polycystic ovaries changes: ≥ 12 follicles (2-9 mm in diameter) in one side or both sides of the ovaries and/or ovarian volume ≥ 10 mL.

Citation: Al- Ghazali B S, Mohammed A A, Fahad A M. The association of serum visfatin in women with polycystic ovary syndrome: A case-control study. Revis Bionatura 2022;7(4) 60. http://dx.doi.org/10.21931/RB/2022.07.04.60
Received: 22 April 2022 / **Accepted:** 18 June 2022 / **Published:** 15 November 2022

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Exclusion criteria

1) Diabetes, cardiovascular disease, hypertension, infections, hypothyroidism, hyperprolactinemia, or other serious medical problems.

2) Patients use anti-inflammatory drugs (within the previous three months), or drugs are known to affect carbohydrate and lipid metabolism, oral contraceptives, glucocorticoids, ovulation induction agents, antidiabetic and antiobesity drugs, estrogenic, or antiandrogenic or antihypertensive medication.

3) Other causes of hyperandrogenism like Cushing's syndrome, congenital adrenal hyperplasia adrenal and pituitary tumors.

After explaining the whole procedure, patients information were documented in detail, including age and BMI. A physical examination and baseline assessment were done for the patients on day 2 or 3 of the menstrual cycle. BMI was calculated as body weight in kilograms divided by height in squared meters (Kg/m^2). A transvaginal ultrasound scan was performed for all participants by real-time ultrasound device using a vaginal probe 7.5 MHZ. The morphology of polycystic ovaries was considered if there were 12 or more follicles of 2-9 mm in diameter in each ovary and/or an enlarged ovary (ovarian volume >10 cm^3)[12]. The scan is also used to measure the endometrial thickness and check the surrounding structures. Blood samples were taken through a standard venipuncture of the antecubital vein to withdraw about 5ml at the first 2-3 days of the menstrual cycle and centrifuged to collect serum. Part of the serum was used to measure hormones, including estradiol, FSH, LH and prolactin. Serum visfatin was determined by ELISA quantitative in vitro diagnostic measurement. The kit name was (cloud-clone corporation 1304 Langham Greek organization suit 226 Houston Tx 77084, USA).

Statistical analysis was performed using SPSS 20.0 statistical software. Measurement data were expressed as mean ± standard deviation, and one-way ANOVA (analysis of variance) was used to compare different measurements (numerical data). The p-value <0.05 is statistically significant.

Results

In this study, the mean age for PCOS women was 26 years, and for control, women were 28 years. BMI for PCOS women was 25.7 Kg/m^2, and for management, women were 26.1 Kg/m^2. Regarding hormonal assay for PCOS women and control women, respectively, were shown in table 1.

Regarding visfatin level in PCOS women was 5.41 ng/mL, while in control women was 0.40 ng/mL. In Obese, PCOS was 5.61 ng/mL, while in non-obese, PCOS was 5.22 ng/mL, as shown in Table 2.

Discussion

Visfatin is considered to have many actions, such as

Variables	PCOS women mean ± SD	Control women mean ± SD	P-value
Age (years)	26 ± 3.63	28 ± 3.99	0.941
BMI (Kg/m^2)	25.7 ± 7.10	26.1 ± 5.74	0.237
E2 (mLu/mL)	50.4 ± 10.14	36.5 ± 7.22	0.0001[1]
LH (mLu/mL)	4.6 ± 1.87	3.1 ± 1.34	0.0001[1]
FSH (mLu/mL)	4 ± 2.01	4.2 ± 1.74	0.596
LH/FSH ratio	1.62 ± 0.33	0.7 ± 0.36	0.0001[1]
Prolactin (ng/mL)	19.9 ± 7.99	17.5 ± 5.6	0.0869

[1] Significant.

Table 1. Hormonal, BMI and age differences between PCOS women and control women.

	PCOS women		Control women		P-value
	Obese	Non-obese	Obese	Non-obese	
	mean ±SD				
Visfatin ng/mL	5.41±1.28		0.40± 0.17		0.00001[1]
	5.61±1.27	5.22± 0.36	5.22±1.36	0.33±0.26	0.0001[2]
					0.001[3]
					0.001[4]
					0.0001[5]

[1] Significant between PCOS women and the control group.
[2] Significant between obese PCOS women and obese control women.
[3] Significant between non-obese PCOS women and non-obese control individuals.
[4] Significant between obese and non-obese PCOS women.
[5] Significant between obese and non-obese control group.

Table 2. Visfatin level in Obese PCOS, non- obese PCOS women and control women.

insulin-like activity under physiological conditions, lowering blood glucose levels, an ability to stimulate proinflammatory activity by enhancing TNF-α and IL-6 secretion, a direct contributor to vascular inflammation and endothelial dysfunction that are considered key features of atherosclerosis diseases linked to metabolic syndrome[13-15].

The present study showed that serum visfatin levels were significantly higher in the PCOS group than in the control group (P=0.0001). There was a significant difference between obese PCOS women and non-obese PCOS (P=0.001) concerning their visfatin levels. It was significantly increased in obese women with PCOS compared to obese control (P=0.0001) and in non-obese women with PCOS compared to non-obese control (P=0.0001).

Dıkmen et al. found that serum visfatin levels were similar in normal-weight PCOS and control group, and the level of visfatin observed in obese and overweight patients with PCOS was higher than that found in control women with equal BMI; in addition, obese women with PCOS also had significantly higher levels than normal weight women with PCOS[16].

El-said et al. found that visfatin levels are increased in women with PCOS compared to healthy controls. There was a positive correlation between visfatin and free androgen index in PCOS patients[17]. While Kandasamy *et al.*, and Jongwutiwes *et al.*, found that visfatin level of visfatin was significantly increased in women with PCOS than in healthy control subjects, and also it was positively correlated with insulin resistance and BMI, so the result indicates that South Indian women with PCOS exhibit higher levels and elevated insulin resistance, which suggest that visfatin could be a potential biomarker for PCOS[18,19].

On the other hand, Kowalska et al. found that the PCOS group had lower insulin sensitivity and higher serum visfatin than the control group, and a decrease in insulin sensitivity was present in both the lean and obese PCOS subjects. In contrast, the increase in visfatin level was observed only in lean PCOS subjects. In addition, serum visfatin was negatively correlated with insulin sensitivity. This may indicate that it is associated with insulin resistance and markers of hyperandrogenism in lean PCOS patients[20].

Another study done by Al Dallow Yamam et al. reported that plasma visfatin levels were significantly increased in normal weight and obese women with PCOS compared to control. It had a positive linear correlation with BMI, fasting insulin and HOMA-IR. These findings suggest that it may be related to obesity and insulin resistance[21].

Güdücü et al. reported that visfatin levels were higher in normal-weight PCOS compared to obese PCOS, but it did not reach statistical significance. It correlated negatively with fasting blood glucose, total cholesterol (TC), low-density lipoprotein (LDL) and lipoprotein-a levels in PCOS patients. It had no correlation with homeostasis model assessment-insulin resistance and fasting insulin levels, but the negative correlation between plasma visfatin levels and lipoprotein-a, fasting plasma glucose, TC and LDL levels may indicate a role for it in cardiovascular disease independent of insulin resistance[22]. Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted.

Conclusions

This study concludes that plasma visfatin level was increased in polycystic ovary syndrome women and in obese women more than non-obese. These findings might suggest that visfatin could play a role in pathogenesis and the long-term consequences of PCOS.

Author Contribution

Conceptualization, B.S.A., A.A.M. and A.M.F.; methodology, B.S.A., and A.A.M.; software, B.S.A.; validation, B.S.A., A.A.M. and A.M.F.; formal analysis, A.A.M. and A.M.F.; investigation, A.A.M. and A.M.F.; resources, A.A.M. and A.M.F.; data curation, A.A.M. and A.M.F.; writing—original draft preparation, A.A.M. and A.M.F.; writing—review and editing, A.A.M. and A.M.F.; visualization, A.A.M. and A.M.F.; supervision, B.S.A.; project administration, B.S.A.; funding acquisition, B.S.A.. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of College of Medicine, Kufa University (protocol code 2021.2298734 and 04/02/2019).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Written informed consent has been obtained from the patient(s) to publish this paper.

Conflicts of Interest

The authors declare no conflict of interest.

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ARTICLE / INVESTIGACIÓN

Antibiogram of Eucalyptus and Sesame seed oil against clinical isolates of *Pseudomonas aeruginosa*

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DOI. 10.21931/RB/2022.07.04.61

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Abstract: Because of the frequent use of antibiotics, which leads to the occurrence of resistance by pathogenic bacteria, and for the benefit of oils and the ease of their availability with minor side effects, thus, the study aimed to investigate the anti-microbial activity of Sesame seed oil and Eucalyptus against *P. aeruginosa* bacteria. Ten clinical isolates of *Pseudomonas aeruginosa* were previously isolated from wounds and have multi-drug resistance to common antibiotics. The results of Sesame oil against the bacterial isolates showed no antibacterial activity not only in the agar well diffusion method but also in the disc diffusion method. At the same time, the findings showed that eucalyptus oil had an antibacterial activity with concentrations (20%,40%, 60%, 80%, and 100%) against these isolates. Further, the antibiotics susceptibility test results showed that bacterial isolates were resistant to Amikacin, sensitive to each of cefotaxime, chloramphenicol and levofloxacin, and the effect of Gentamicin differed against the tested bacteria. Our finding concludes that eucalyptus oil may be used as an alternative drug in the treatment as an external ointment for wound infection by *Pseudomonas aeruginosa*.

Key words: Antibiotic-resistant, disc diffusion method, pathogenic bacteria, the wounds, plant essential oils.

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen that causes disease and death in patients with cystic fibrosis and those with impaired immune systems. *P. aeruginosa* is a Gram-negative aerobic bacterium. It can thrive in temperatures ranging from 4 to 42 degrees Celsius and survives with low nutrition levels[1]. These properties enable it to cling to and live on medical equipment and other hospital surfaces, making infections more likely in immunocompromised individuals[2]. Patients with cystic fibrosis are at a higher risk of morbidity and mortality due to the chronic infections that *P. aeruginosa* can produce. These infections can lead to lung damage, deficiency, pneumonia, and urinary tract infections. *P. aeruginosa* can also cause bacteremia[3,4]. *P. aeruginosa* infections are notoriously difficult to cure because the bacteria possess a high level of innate resistance and can develop resistance to a wide range of medications. Metallo-b-lactamases (MBL), altered penicillin protein binders (PBP), porin mutations, plasmid enzymatic modification, DNA-gyrase mutation, and active expulsion pumps are all examples of broad-spectrum b-lactamases. These are just some of the many mechanisms that *P. aeruginosa* uses to evade antibiotics[5]. Carbapenemic drugs (imipenem and meropenem) are broad-spectrum antibiotics used to treat *P. aeruginosa* nosocomial infections. The formation of metalloenzymes, a lack of porin permeability and a rise in the expression of active expulsion pumps are all linked to Carbapenemic resistance[6]. The development of MBL is linked to carbapenem-resistant *P. aeruginosa*, which can, except for aztreonam, hydrolyze all b-lactam antibiotics[5,7]. Because of the active substances found in various components, plants have drawn researchers from all over the world as a source of treatment. For decades, plant oils and extracts have been used for several purposes. In today's world, antibiotic resistance in microorganisms is a grave concern[8]. As a result, herbal remedies derived from plants are considered safe alternatives to synthetic drugs. The antibacterial activity of extracts and plant oils, in particular, paved the way for various applications, including food preservation, medicines, and cosmetics. Therapies and alternative medicine plant oils are aromatic oily fluids obtained through steam distillation from different plant parts like flowers, shoots, seeds, grasses, stems, leaves, forests, fruits, and stocks. Compared to antibiotics, these oils have been shown to be highly effective antibacterial agents. These have anti-microbial activities against viruses, bacteria, and fungi, including antibacterial, antifungal, anticancer, antiviral, and anti-oxidant characteristics[9]. The genus of *Eucalyptus* is an aromatic and medicinal plant that is widely distributed around the world. It has therapeutic properties because it produces bioactive chemicals interacting with other species in the environment, preventing bacterial and fungal growth[10,11]. Candidates for creating novel anti-microbial medications include compounds that can suppress infections while causing little harm to host cells. Today, plant-based treatments are regaining favor because the efficacy of antibiotics, which are widely regarded as almost universal solutions for infectious diseases, is waning. This is due to the widespread use of these chemical agents and their prescription on a large scale, which is sometimes inappropriate, resulting in bacterial strain adaptability and selection strains that are multiresistant and pose a public health risk[12]. *Eucalyptus* is a native Australian genus in the Myrtaceae family, with approximately 900 varieties and subspecies. *Eucalyptus* contains bioactive chemicals. These chemicals have been shown to

Citation: Khalaf R J, Hussein A R. Antibiogram of Eucalyptus and Sesame seed oil against clinical isolates of Pseudomonas aeruginosa. Revis Bionatura 2022;7(4) 61. http://dx.doi.org/10.21931/RB/2022.07.04.61
Received: 22 April 2022 / **Accepted:** 18 June 2022 / **Published:** 15 November 2022

have antibacterial, antifungal, anti-inflammatory, analgesic, and anti-oxidant properties[10,13]. Sesame, also known as *Sesamum indicum* L., is a crop that has been farmed for a very long time and has antifungal characteristics. It is believed that it originated in the continent of Africa. The anti-oxidant and health-promoting effects of sesame oil, along with a rise in the rate of fatty acid oxidation in the mitochondria and peroxisomes of the liver, can be attributed to sesame oil[14]. Sesame oil consumption appears to increase plasma gamma-tocopherol and vitamin E activity, which are thought to aid in cancer and heart disease prevention., as well as antibacterial activity[15]. The widespread use of antibiotics causes pathogenic bacteria to develop resistance, and because of the convenience with which oils may be obtained with the fewest side effects. Thus, the study aimed to examine if sesame seed oil and *Eucalyptus* oil could limit the growth of *P. aeruginosa*.

Materials and methods

Bacterial isolates

All isolates of *P. aeruginosa* previously isolated from patients from the deep skin surface of the wound using sterile swabs, then cultured directly on blood agar and MacConkey agar aerobically overnight at 37 c for bacteria and identified by Vitek 2 system, kept in refrigerator till beginning the work.

Extraction of volatile oil from *Eucalyptus* and fixed oil of Sesame

Leaves of Eucalyptus that were collected from the University of Baghdad, Aljadiryaa campus, after cleaning and rinsing leaves were dried and ground finely and placed in the Clevenger apparatus to extract the volatile oil using hydro distillation method by applying a ratio 1:5 between plant and distilled water[16]. Meanwhile, the sesame seed is ground to a paste and heated to 80-90 c for 15 min. Then add enough boiling water to suspend the ground seed on stirring for 15 min. The upper oil layer separated after cooling and dried by heating[17]. Sesame seed oil was obtained from the local market, but its origin is Iran; after we sell it, kept at 4^{o}C until use.

Antibacterial activity of extracted oil

A- Agar well diffusion method

In the first step, activation of bacterial isolates in (BHI) broth and incubate the tubes at 37°C for 18-24hr. Making dilute the bacterial broth in normal saline compared with Mcfarland 0.5 tube (1x10^{8} CFU/ml). Then, culturing on Mueller Hinton agar (MHA) plates were prepared sterilely. Inoculation (MHA) plates with the diluted tested bacteria by the Swabbing method. We added 200µl from each sesame oil concentration (100000µg/ml,50000µg/ml,25000µg/ml), while *Eucalyptus oil* concentrations (20%, 40%, 60%, 80%,100%) were put in three wells and one well full by (DMSO) used as control(since we dissolve the oil with it) and incubated the plates at 37°C for (18-24hr). Finally, detected inhibition zone for anti-microbial activity.

B- Agar disc diffusion method

Using the activation of bacterial isolates previously, then diluted the bacterial broth and cultured on (MHA) as mentioned above. Adding 0.2ml of each sesame seed oil concentration (5000 µg/ml,10000 µg/m,25000 µg/ml,50000 µg/ml) was used to the submerged disc that we synthesized it using sterile filter papers. In contrast, the control disc was soaked with DMSO and put on the surface of a cultured (MHA) plate aseptically and incubated at 37°C for (18-24hr) to detect the inhibition zone as a clear zone around the discs and measured in mm using a ruler.

Antibiotic susceptibility test for *P. aeruginosa*

The Kirby-Baurer method was used to test anti-microbial sensitivity. The antibiotics that were used in this study were Amikacin, Azithromycin, cefotaxime, chloramphenicol, Gentamicin, and levofloxacin. The isolated colonies were collected using a sterile loop and emulsified in 3-4 ml of sterile physiological saline. A sterile brush that had been dampened in the bacterial suspension was then used to make streaks across the surface of the Mueller Hinton agar. The anti-microbial disc was diced using antiseptic forceps, and then it was regularly distributed on the inoculated plate. After incubating the plate aerobically at 37°C for 24 hours, the zone of growth inhibition around each disc was measured in millimeters, and the results were reported as sensitive, intermediate, or resistant to a specific anti-microbial agent by comparison with standard inhibition zones as mentioned in the clinical laboratories standards institute[6].

Statistical Analysis

The SPSS, IBM version 20, program was used to perform the analysis on the data[18]. Statistical significance is assumed when the p-values are less than 0.05.

Results

Antibiotics sensitivity test for *Pseudomonas aeruginosa*

The antibiotic sensitivity test results showed that the isolates of *P. aeruginosa* were resistant to Amikacin with a diameter of inhibition zone 14 mm and 12 mm, respectively. Azithromycin differs in its effect against the two test isolates, 8mm and 19mm, respectively. The two isolates of *P. aeruginosa* were sensitive to each of the cefotaxime, chloramphenicol, and levofloxacin antibiotics, while the effect of Gentamicin differed against the two tested isolates. Isolates 1 to 6 were sensitive to Gentamicin with a diameter of inhibition zone 16mm, while isolates 7 to 10 were resistant to the same antibiotics with a diameter of inhibition zone 12 mm (figure 1).

Antibacterial activity of sesame seed oil against *P. aeruginosa*

The results of the antibacterial activity of sesame seed oil against *P. aeruginosa* by the two methods are shown in Figures 2 and 3. The bacterium *P. aeruginosa* offers high resistance to the concentrations used in both agar well methods and disc diffusion of sesame seed oil, which is unable to inhibit their growth.

Antibacterial activity of *Eucalyptus* oil against *P. aeruginosa*

Figure 4 shows the different concentrations of *Eucalyptus* oil used; the lowest inhibition zone was found at 20% concentration with a measurement of 10 mm, while the biggest inhibition zone was measured at 18 mm and increased to 23 mm at 80% and 100% concentrations, respectively. DMSO was used as a control which give a negative effect against *Pseudomonas aeruginosa* as shown in figure 5.

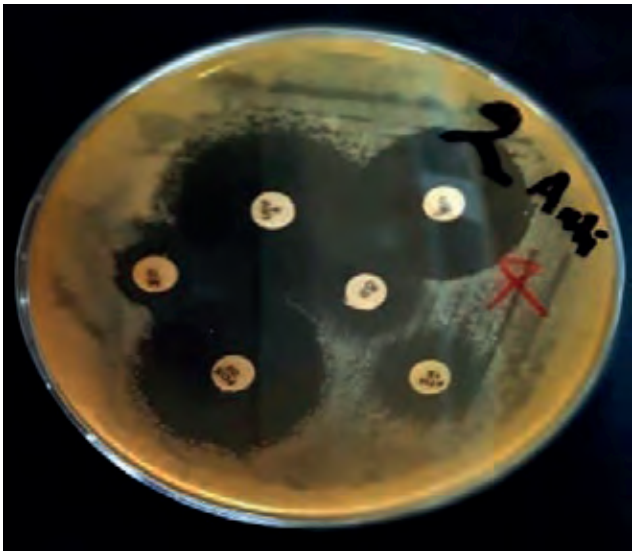

Figure 1. Antibiotics susceptibility test for P. aeruginosa.

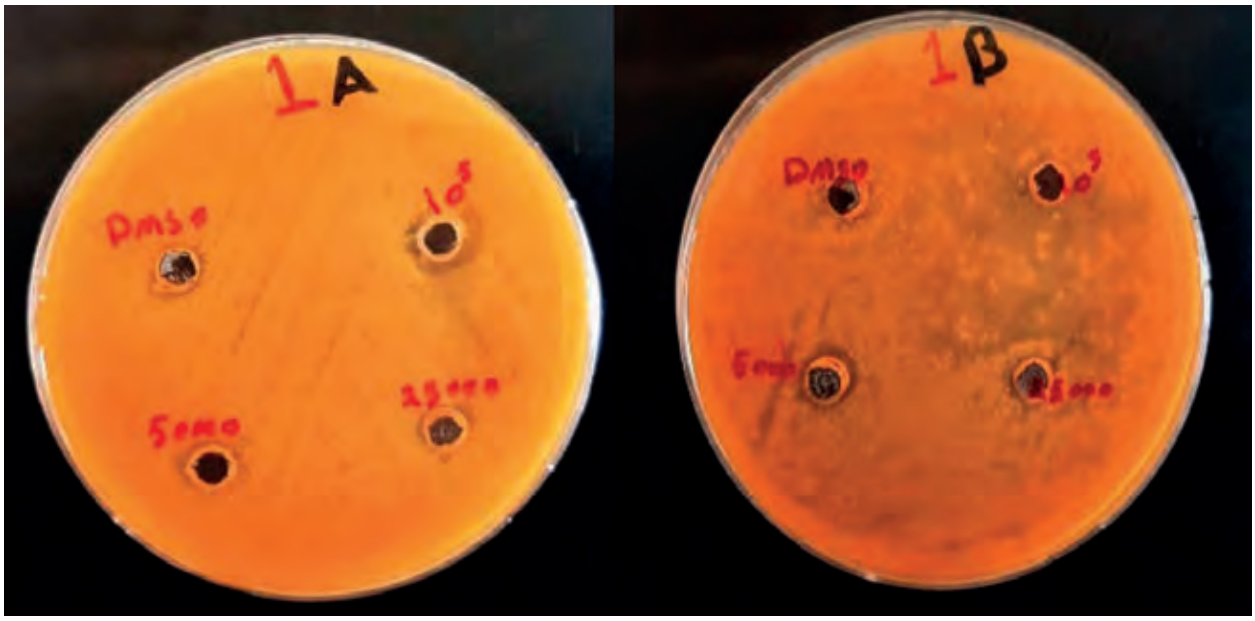

Figure 2. Resistance pattern of P. aeruginosa against sesame oil in agar well diffusion method.

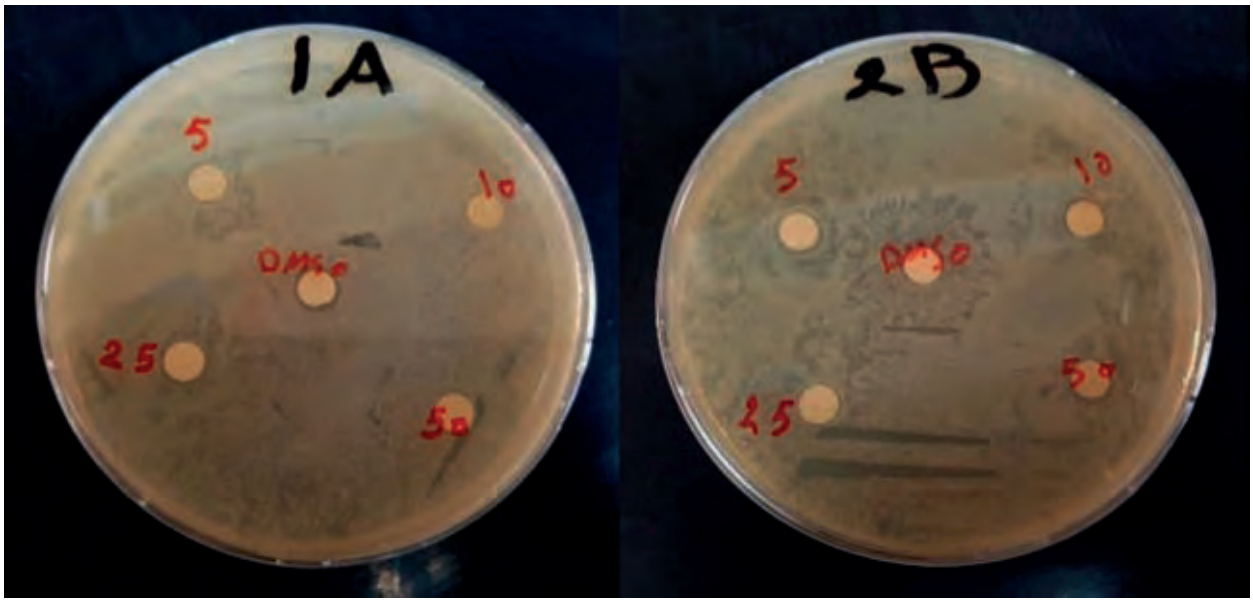

Figure 3. The effect of sesame oil on P. aeruginosa in the agar disc diffusion method.

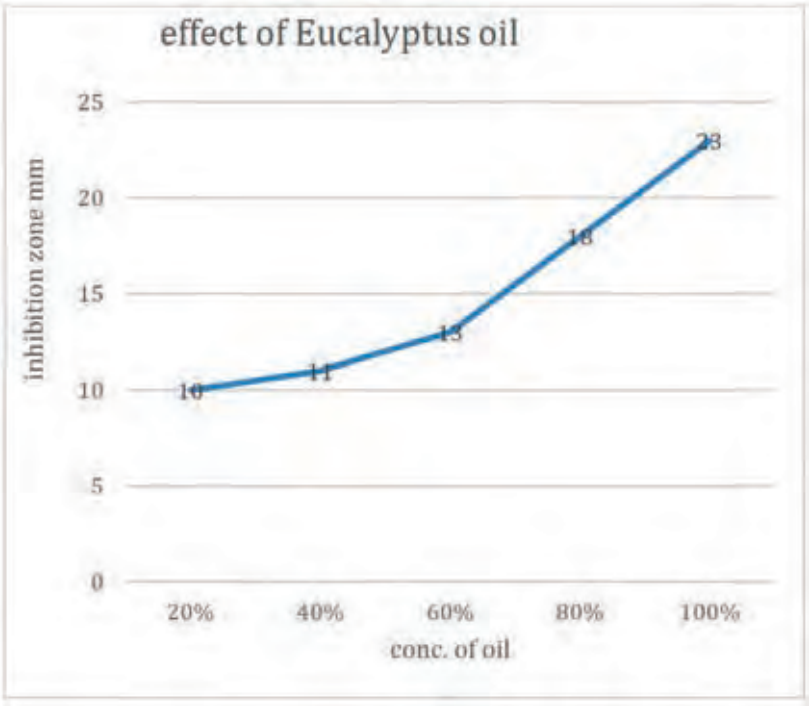

Figure 4. The anti-microbial activity of Eucalyptus oil on P. aeruginosa.

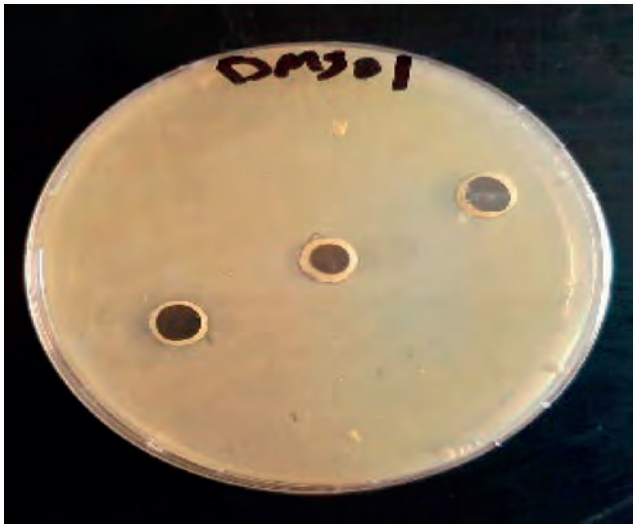

Figure 5. The negative effect of DMSO as a Control against P. aeruginosa.

Discussion

Some references showed that Azithromycin was not approved for the treatment of infection caused by *P. aeruginosa*, and there are no published breakpoints for this species. Still, other references showed that the direct effect of Azithromycin was on the outer membrane of *P. aeruginosa*, which might contribute to its bactericidal activity against this organism. *P. aeruginosa* is not only naturally resistant to a wide variety of anti-microbials but also possesses an extraordinary capacity for developing resistance to commonly used anti-microbials through the selection of mutations in chromosomal genes or by horizontally acquiring resistant determinants. *P. aeruginosa* is resistant to anti-microbials not only because it is naturally resistant to them, but also because it has an extraordinary capacity to develop resistance to commonly used anti-microbial Amikacin and colistin were shown to be the most effective antibiotics against blood. Respiratory *P. aeruginosa* isolates, according to certain investigations[6,19]. Our findings were in contrast to those of earlier research. Given that two of our country's isolates showed resistance to Amikacin, we suspect this disparity may be attributable to inappropriate antibiotic prescribing practices. By attaching to the 30S subunit of the ribosome, the aminoglycoside antibiotics gentamicin and Amikacin can disrupt the process of protein synthesis. The low permeability of *P. aeruginosa's* cell wall, particularly its outer membrane, is generally thought to be the cause of the bacteria's intrinsic resistance to all antibiotics. Our results of sesame oil antibacterial activity have differed from another researcher's since[20] showed that sesame oil exhibited strong anti-microbial effects against the tested bacteria with inhibition zone ranging from 15 to 25 mm. This could be because the organism used for testing was pseudomonas aeruginosa, which is highly resistant to many antibiotics and anti-microbial agents. Additionally, the sesame oil that we used came from the market, and it's possible that it lost some of its activity because of improper storage because it was imported from Iran. Methanol extracts of different parts of Sesame Indicum l. (root, seed, and leaves) had different levels of anti-oxidant and anti-microbial activity. This was assayed by agar disc diffusion and agar well diffusion method against five bacterial species. Also, these results disa-

greed with our results, which may be due to the use of imported sesame oil from other countries rather than sesame oil derived from local products[21]. A previous study performed by Yepola and Adeniyi[22] showed that phytochemical screening of leaf extracts of *Eucalyptus tannins*, saponins, and cardiac glycosides was discovered in camaldulensis after testing the plant. Also, this demonstrated that the methanol extract, dichloromethane fraction, and methanol residue represent a broad spectrum of activity; the methanol extracts demonstrated greater activity against *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus subtilis* (15-16 mm) than they did against *Klebsiella ssp.*, *Yersinia enterocolitica*, and *Pseudomonas aeruginosa* (14 mm). The findings of our research were consistent with those of a previous study. The essential oils extracted from *Eucalyptus globulus* leaves have a density lower than that of water, a hue similar to a pale yellow, and an aromatic scent that is spicy. The antibacterial activity of essential oil displayed considerable against different strains of Staphylococcus aureus that were tested. However, the antibacterial activity of essential oil remained lower than that of aqueous extract, which showed the highest antibacterial activity. Bowras and his coworker showed this. Our findings regarding the antibacterial activity of eucalyptus oil against ten clinical isolates of *Pseudomonas aeruginosa* coincide with the literature in that a higher oil concentration had a more potent effect on *Pseudomonas aeruginosa* than a lower concentration of the oil did[1]. On the other hand, Damjanovic – Vrantica et al. reported that the anti-microbial activity test demonstrated that the essential oil of E. globulus possesses a rather potent anti-microbial activity, particularly against *Streptococcus pyogenes*, *E. coli*, *Candida albicans*, *Staphylococcus aureus*, *Acinetobacter baumanni*, and *Klebsiella pneumoniae*, with the exception of *Pseudomonas aeruginosa* & *Salmonella infantis*[23]. *Pseudomonas* spp. are recognized to have the ability to metabolize a wide range of organic chemicals, and because of this property, it is utilized extensively in bioremediation. This may be the reason for their high-level resistance. Chao's team[24] demonstrated this. This bacterium may be simplifying and metabolizing the chemicals in the oils that are inhibitory to many of the other bacteria. This would be beneficial to the overall bacterial population. The primary bioactive compounds in Eucalyptus were -pinene, p-cymene, limonene, and –terpinene[25]. According to the researchers' findings, the essential oils of rosemary and *Eucalyptus* have the highest levels of 1,8-cineole of any other essential oils. Another study investigated the efficacy of various essential oils against multidrug-resistant strains of *P. aeruginosa*. The essential oils investigated included *Cinnamomum zylanicum* (Dalchini oil), *Eucalyptus globulus* (Nilgri oil), *Eugenia caryophyllata* (Clove oil), *Ocimum sanctum* (Tulsi oil), and *Allium sativum* (Garlic oil). Compared to other oils, the inhibitory action of *Cinnamomum zylanicum* oil against multidrug-resistant bacteria was shown to be the most potent, followed by the inhibitory action of *Eucalyptus* globulus oil (Nilgri oil).

Conclusions

Pseudomonas aeruginosa is an opportunistic pathogen. Infections by *P. aeruginosa* are challenging to treat since these bacteria can acquire resistance to different antibiotics. Our findings conclude that eucalyptus oil may be used in the treatment as an external ointment for wound infection by *Pseudomonas aeruginosa* as an alternative or along with antibiotics

Acknowledgments

Thanks going to all who support us.

Conflicts of Interest

No conflict.

Funding

Self by authors.

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ARTICLE / INVESTIGACIÓN

Polyene macrolide antibiotic nanoemulsion: a proposal for the treatment of cutaneous leishmaniasis

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DOI. 10.21931/RB/2022.07.04.62

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Abstract: Leishmaniasis is a neglected tropical disease that requires timely and inexpensive treatment. For this purpose, a nanoemulsion with a polyene macrolide antibiotic, or amphotericin B (NE-AmB), was developed. This study quantified the amount of drug permeated and retained in intact and lacerated human skin, simulating cutaneous leishmaniasis (CL) processes. Toxicity in macrophage and keratinocyte cell lines, activity against promastigotes and amastigotes of *Leishmania tropica*, *in vivo* irritant activity, and histological evidence was evaluated. Results. The amount of drug retained in intact and damaged skin was 750.18 ± 5.43 and 567.97 ± 8.64 µg/g/cm^2, respectively. There was no permeation. No apparent toxic effect was observed in HaCaT cell lines. The IC50 of NE-AmB found for promastigotes and amastigotes was 0.26 ± 0.09 and 0.37 ± 0.05 µg/mL, respectively. NE without AmB did show antiparasitic activity. The formulation showed lower IC50 values on both parasite stages than the AmB solution. There was no skin irritation, and histology showed skin improvement with treatment. We suggest that this NE-AmB may be a candidate for *in vivo* studies in CL patients.

Key words: Leishmaniasis, Amphotericin B, *ex vivo* permeation studies, *in vitro* cytotoxicity, *in vitro* leishmanicidal activity, Draize test, histology.

Introduction

Leishmaniasis is a parasitic disease caused by protozoa of the genus *Leishmania*. It is estimated that about one million new cases and more than 20,000 deaths occur annually due to this parasitosis[1]. In European, Asian, and African countries, this disease is transmitted by biting female sandflies (vectors) belonging to the genus *Phlebotomus*. However, in the American region, it is caused by *Lutzomyia*, specifically *Psychodidae*[2]. *Leishmania* has two primary cell morphologies: promastigotes in the vector and amastigotes in the mammalian host. Its life cycle begins with promastigotes inoculated into the host, which are phagocytosed by macrophages. Once inside the macrophages, they lose their flagella and become amastigotes that multiply by binary fission. The infected macrophages then "explode" and release their amastigotes to infect other macrophages, thus reproducing the cycle[3].

There are three types of leishmaniasis: cutaneous (CL), mucocutaneous (MCL), and visceral (VL), with CL being the most common form of the disease. Depending on the infecting species, this disease can cause anything from skin ulcers, which may heal independently, to deep wounds that can spread to other body parts[4]. Antimonials, such as sodium stibogluconate (Pentostam®) or meglumine antimoniate (Glucantime®), are the first choice for treating all leishmaniasis. However, their use presents problems such as invasive administration (intravenous, intramuscular, and intralesional) and adverse effects: musculoskeletal pain, renal failure, and hepatic and cardiac toxicity. Other therapeutic options include intramuscular pentamidine, paromomycin ointment alone, or imiquimod (in combination with paromomycin). Azithromycin, miltefosine, antifungal drugs (all oral administration), and, in particular, itraconazole and fluconazole could be considered an alternative in complex lesions or with possible mucosal involvement. However, despite the existence of a vast therapeutic arsenal, the increase in resistance to antimonials, their high toxicity, high cost, and prolonged treatment regimens, especially in immunocompromised patients, has generated that drugs such as amphotericin B (AmB) have become the most appropriate treatment alternative for LC[5].

AmB is a polyene macrolide antifungal agent isolated from *Streptomyces nodosus* and collected in 1955 from the Orinoco River in Venezuela. AmB was rapidly introduced into clinical medicine and received Food and Drug Administration (FDA) approval in 1958, even without elucidating its chemical structure. This molecule binds explicitly to ergosterol in the parasite cell membrane, establishing aggregated transmembrane pores, and causing membrane depolarization[6].

AmB deoxycholate (AmB-Deox) has been used during the last few years due to the increasing number of immunosuppressed patients. However, it has been associated with a high rate of side effects, especially renal toxicity. For this

Citation: Sosa L, Espinoza L C, Fuentes J M, Siwady J A, Rivas F R, Díaz M R. Polyene macrolide antibiotic nanoemulsion: a proposal for the treatment of cutaneous leishmaniasis. Revis Bionatura 2022;7(4) 62. http://dx.doi.org/10.21931/RB/2022.07.04.62
Received: 22 September 2022 / **Accepted:** 18 October 2022 / **Published:** 15 November 2022

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reason, other formulations have been developed: a lipid formulation (liposomal, AmBisome®, Gilead Sciences, Foster City, CA, USA), a lipid complex (Abelcet®, Sigma Tau Pharmaceuticals, Pomezia, Italy), and a colloidal suspension (Amphocil®, Penn Pharmaceuticals, Ltd, Tredegar, UK), which share the same spectrum of action but differ in efficacy and toxicity, in addition to being administered invasively and having a high cost, making them difficult to acquire in developing countries[7].

Given the above, topical application of AmB could be an alternative for treating LC. Topical administration has several advantages: it is a simple technique, quite comfortable, is not painful for the patient and allows self-administration, direct application to the site of infection, and could prevent systemic effects caused by the drug, ensuring its local effect and retention of a sufficient amount on the skin[8]. To date, there is no marketed drug containing AmB for topical administration, so there is a need to develop new formulations of AmB against leishmaniasis which are more effective, safe, and affordable.

Taking into account the above, in the previously published work by Sosa et al., a nanoemulsion with Amphotericin B (NE-AmB) was developed to treat LC. For this purpose, castor oil as the oil phase, Transcutol® P as the aqueous phase, and an emulsifier system composed of a combination of labrasol®/plurol oleic acid® were used as excipients. In the present study, permeation and retention analysis evaluated the amount of AmB retained and permeated in healthy and damaged human skin. In addition, the cytotoxic effect of NE-AmB was studied in three cell lines: HaCaT, RAW 264.7, and J774A.1, and the leishmanicidal activity against both parasite stages (promastigotes and amastigotes) of *Leishmania tropica*. Finally, a Draize test was performed on New Zealand rabbits, and histological analysis of rabbit skin was done to test dermal irritation and its effect at the microscopic level.

Materials and methods

Materials

The AmB used for the study was obtained from Acofarma (Barcelona, Spain). Dimethyl sulfoxide (DMSO), methanol, castor oil, and acetonitrile were obtained from Sigma-Aldrich (Darmstadt, Germany). Transcutol®P, Labrasol®, and Plurol Oleico® were kindly provided by Gattefossé (Barcelona, Spain). The water used in all experiments was obtained from a Milli-Q® Plus system (Millipore Co., Burlington, MA, USA). All chemicals and reagents were of analytical grade.

Parasite strains and cultures

Leishmania tropica species was isolated from a patient with LC in Barcelona, Spain (MHOM/ES/2010/BCN-809). Promastigote growth curves were performed by counting the number of parasites daily for six days to determine their kinetics and establish the logarithmic growth phases. The promastigotes obtained were seeded at 26 °C in a Shneider culture medium at pH 7.0, supplemented with heat-inactivated 20% fetal bovine serum, 25 µg/mL gentamicin solution (Sigma, St. Louis, MO, USA), and 1% penicillin (100 IU/mL) with streptomycin solution (100 mg/mL) (Sigma, St. Louis, MO, USA).

Preparation of NE-AmB

First, a selection of solvents was tested to determine the solubility of AmB. DMSO was selected as the best solubilizing component of AmB. The oily component, aqueous component, and emulsifying system were chosen. Subsequently, a ternary phase diagram was performed to find the best combination that was a single phase and had the smallest droplet size and the lowest polydispersion. The results of this formulation have been reported in a previous study[9].

Ex vivo permeation studies

Franz diffusion cells were used to perform the permeation assays, placing the studied sample on human skin from a donor undergoing plastic surgery at the Barcelona-SCIAS Hospital. The patient gave prior written informed consent by the provisions of the Barcelona Hospital Ethics Committee (ethical experimental, protocol reference number: BEC/001/16, Barcelona, Spain). Human skin was brought to the laboratory, cut with a Zimmer® dermatome (Ohio, USA) in 400 µm thick samples, and the integrity of the skin was verified by measuring transepidermal water loss (TEWL) parameters.

The stratum corneum of healthy skin was partially removed by applying an adhesive tape seven times (thus simulating damaged skin in leishmaniasis processes). Transcutol®P was used as the receptor médium, and a temperature of 32 ± 0.5 °C was maintained with constant agitation. A total of 300 µL of NE-AmB was deposited in the compartment with the donor skin and placed towards the stratum corneum (Figure 1).

To extract the drug retained in the skin, the pieces of skin that had contact with the drug were cut, weighed, and washed with a 0.05% sodium lauryl sulfate solution and then with distilled water. Extraction was performed by puncturing the skin pieces 70 times with a sterile needle, and after the skin pieces were weighed, 1 mL of DMSO was added and placed in an ultrasonic bath for 30 minutes and cold. The amount of AmB from the permeation samples and that extracted from the skin pieces were determined using the High-Performance Liquid Chromatography (HPLC) method, previously validated and described by Sosa et al.

AmB was determined using HPLC, with a Waters® 515 chromatograph, a 717 Plus autosampler, and a 2487 dual absorbance detector (Waters®, Milford, MA, USA). The assay was performed with a Kromasil® Eternity C18 (250 mm x 4.6 mm x 5 µm, Teknokroma, Barcelona, Spain). The mobile phase was a mixture of acetonitrile, acetic acid, and water (52:4.3:43.7 v/v/v) and was pumped through the C18 column at a flow rate of 0.5 mL/min. A 10 µL per sample was injected, and finally, the fluid was analyzed at 406 nm. All measurements were performed at room temperature and under isocratic elution conditions. Calibration curves were prepared with freshly prepared AmB stock solutions in a concentration range of [0.39 to 200] µg/mL. The analytical method was accurate, with a coefficient of variation between 0.02% and 8.79%, relative percentage error between -1.16% and 3.46%, and linear within the concentration range used [0.39-200] µg/mL, with a p-value corresponding to ANOVA applied to the mean values of 0.05.[9]

In vitro cytotoxicity test

Three cell lines were used to establish the cytotoxic effect of NE-AmB: two macrophage cell lines, RAW 264.7 and J774A.1, and one keratinocyte cell line (HaCat) (Eppel-

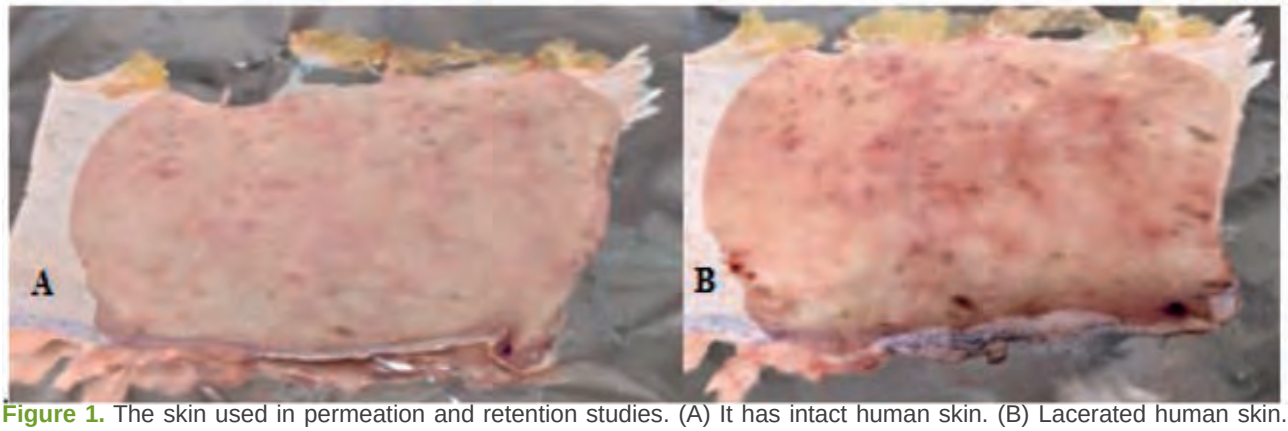

Figure 1. The skin used in permeation and retention studies. (A) It has intact human skin. (B) Lacerated human skin. Source: own elaboration.

heim, Germany). A suspension of 5.0 x 10^4 cells/mL of each cell line was seeded in 96-well plates (Costar 3596, Corning Incorporated, NY, USA) and incubated at 37 °C, 5% CO_2 atmosphere and in RPMI-1640 complete medium supplemented with 20% fetal bovine serum, thermoinactivated, and 1% penicillin (100 U/mL)-streptomycin (100 mg/mL) solution for 24 h. After that, serial double dilutions of the NE-AmB, NE without AmB, and AmB solution were added, and incubation conditions were maintained for another 24 h. Finally, 10% WST-1 reagent (Roche Diagnostics GmbH) was added to all Wells, and these were incubated for four h under the same conditions of temperature and CO_2 atmosphere. Cultures were included as positive control and negative control. Absorbance was read at 450 nm (Multiskan EX, ThermoElectron Corporation, Shanghai, China). Different concentrations of AmB were assayed, from 150 µg/mL to 0.14 µg/mL. The following equation was used to determine the % cell viability:

$$Viability\ \% = \frac{OD\ Positive\ control - OD\ Cells\ in\ the\ presence\ of\ experimental\ dilution}{OD\ Positive\ control} x100 \quad \text{(equation 1)}$$

***In vitro* leishmanicidal activity in promastigotes**

Promastigote cultures were performed as explained in section 2.2. To evaluate the antimicrobial activity of NE-AmB, serial double dilutions of NE-AmB, drug-free NE and AmB solution were made using Schneider culture medium and plated in a 96-well plate (Costar 3596, Corning Incorporated, NY, USA). To the dilutions, a suspension of 1 x 10^6 promastigotes/mL (in log phase) was added and incubated at 26 °C for 48 h. Briefly, the samples were lysed, and alkalinization carried out the enzymatic reaction with p-nitrophenyl phosphate. The optical density was read at 405 nm (Multiskan EX, ThermoElectron Corporation, Shanghai, China). Cultures were included as positive control and negative control. The IC50 (the concentration inhibiting 50% of parasite growth) was calculated by variable transformation analysis and linear regression using Excel version 2019, and experiments were performed in triplicate. Different concentrations of AmB in the range of [150-0.14] µg/mL were tested.

***In vitro* leishmanicidal activity on amastigotes**

RAW 264.7 cell line was used to study the activity of NE-AmB, drug-free NE, and AmB solution against amastigotes. A concentration of 5 × 10^4 cells/mL was seeded on a LabTek 8-well chamber slide system (Nunc, Rochester, NY, USA) and incubated for 24 h at 37 °C in a 5% CO_2 atmosphere. *Leishmania tropica* promastigotes were added to the cells in a 1:10 ratio (macrophages: parasites) and incubated for 24 h under the same conditions. After removal (washing with sterile 0.1 M PBS) of free promastigotes, fresh RPMI-1640 was added as culture médium. Then NE-AmB, drug-free NE, and AmB solution were added, making double dilutions. Plates were incubated for 48 h at 37 °C in a 5% CO_2 atmosphere. Cultures were included as the positive and negative control. Slides were fixed and stained with Giemsa stain and counted to assess the number of amastigotes in 300 macrophages to determine the percentage of infected cells (in triplicate). The IC50 was expressed as the percentage of growth inhibition concerning untreated controls.

Draize test

Adult male New Zealand albino rabbits weighing 1.9 and 2.1 kg (San Bernardo farm, Spain) were used. The dorsal area of the trunk was shaved with an electric shaver 24 h before the start of the test, checking that the chosen animals had completely healthy skin. Four 2-cm-long scarifications were made with a lancet, 2 cm apart. The backs of the rabbits were divided into four zones with the help of adhesive tape. In rabbits number 1, 2, and 3, 0.5 mL of NE without active substance was applied on the upper left side, 0.5 mL of the NE-AmB was applied on the lower left side, and no formulation was applied on the upper right and lower right sides, taking them as a control. After 48 h of applying the different formulations, the substances under study were removed to score from 0 to 4 (from lowest to highest) in each area, both the formation of edema and irritation on the exposed skin. Scoring was repeated at 72 h. The mean value of the individual primary dermal irritation index was determined for each rabbit by summing the edema and erythema scores at 48 h and 72 h and dividing the result by 4. The PDI is the average value of the individual indices for each rabbit. According to the obtained PDI value, the products are classified as "non-irritant" (PDI<0.5), "mildly irritant" (0.5 <PDI<2), "moderately irritant" (2 <PDI<5), and "severely irritant" (PDI > 5). Three animals were used for each formulation. Ethical approval for the handling of experimental animals was obtained from the Institutional Animal Ethics Committee (AICE) of the University of Barcelona.

Histological analysis of rabbit skin

Rabbits were anesthetized and sacrificed with sodium pentobarbital. For histological analysis, skin samples from the back of the rabbits were cut and placed for 24 h on plastic disks immersed in 4% buffered formaldehyde at room temperature. After fixation, all samples were embedded in kerosene blocks cut into 5 µm sections and mounted on microscope slides. Subsequently, the samples were stained with hematoxylin and eosin and finally viewed under a light microscope (Olympus BX41 and Olympus XC50 camera) at 100X magnification to evaluate tissue structure.

Results

Preparation of NE-AmB

The physicochemical characterization of NE-AmB has been published, presenting a low viscosity (12.20 ± 0.02 mPa.S) and a Newtonian flow (r=1). It remained chemically stable for at least three months, and the pH remained between 5 and 6. Accelerated stability studies using Turbiscan®Lab showed that this formulation would remain stable (physically) for at least six months. The 55:05:40 composition was finally chosen, i.e., 55% Labrasol®/Plurol Oleic® (5:1), 5% castor oil, and 40% Transcutol®P. NE-AmB presented a droplet size of 112.90 ± 10.15 and a polydispersity index of 0.22 ± 0.02 (Figure 2)[9].

Ex vivo permeation studies

The amount of AmB retained in both healthy and damaged skin is shown in Table 1. No AmB was found in the receptor medium of the Franz cell, demonstrating that the drug could not permeate through either healthy or damaged skin. Furthermore, 567.97 µg/g/cm^2 was found to be retained in damaged skin and 750.18 µg/g/cm^2 in intact skin.

Cytotoxicity assay

Regarding the HaCaT cell line, these are human keratinocytes, so it is essential to determine toxicity in these cells since NE-AmB would be placed directly on the skin. No cytotoxic effect of this formulation or its excipient was observed. We only observed toxicity with the AmB solution at concentrations of 150 µg/mL (Figure 3A).

On the other hand, the cellular toxicity of AmB, NE-AmB, and NE solutions without drug was studied in two macrophage lines, considering that AmB could be absorbed into the bloodstream when the LC is ulcerated. In these cases, it is essential to determine the concentration at which the drug to be tested would be toxic, although, in the permeation studies on damaged skin, we did not find AmB in the receptor medium. The AmB solution showed imminent toxicity at 150 and 75 µg/mL concentrations in the RAW 264.7 and J771A.1 cell lines, respectively. In contrast, at concentrations of 37.5 µg/mL, cell viability above 80% was shown (Figure 4B). NE alone shows toxicity only in the J774A. One cell line (Figure 4B) and NE-AmB showed simi-

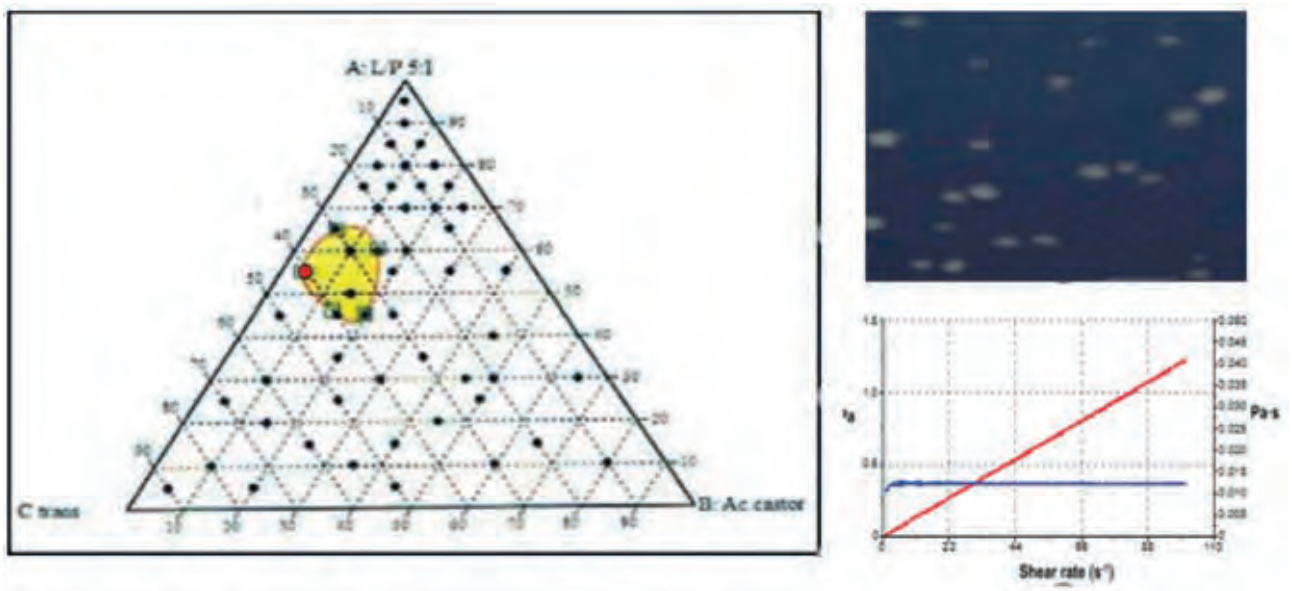

Figure 2. (A) Ternary phase diagram where the dots are the tested mixtures and the nanoemulsion zones in yellow. The red dot indicates the composition selected for the preparation of the NE-AmB. (B) The image taken by Transmission Electron Microscopy (TEM) can also be seen. (C) The rheological behavior of the NE-AmB.

Human skin	Essay	
	Permeation(µg/cm^2)	Retention (µg/g/cm^2)
Intact skin	Not quantified	750.18 ± 5.43
Damage skin	Not quantified	567.97 ± 8.64

Table 1. AmB Amount permeated and retained in human skin.

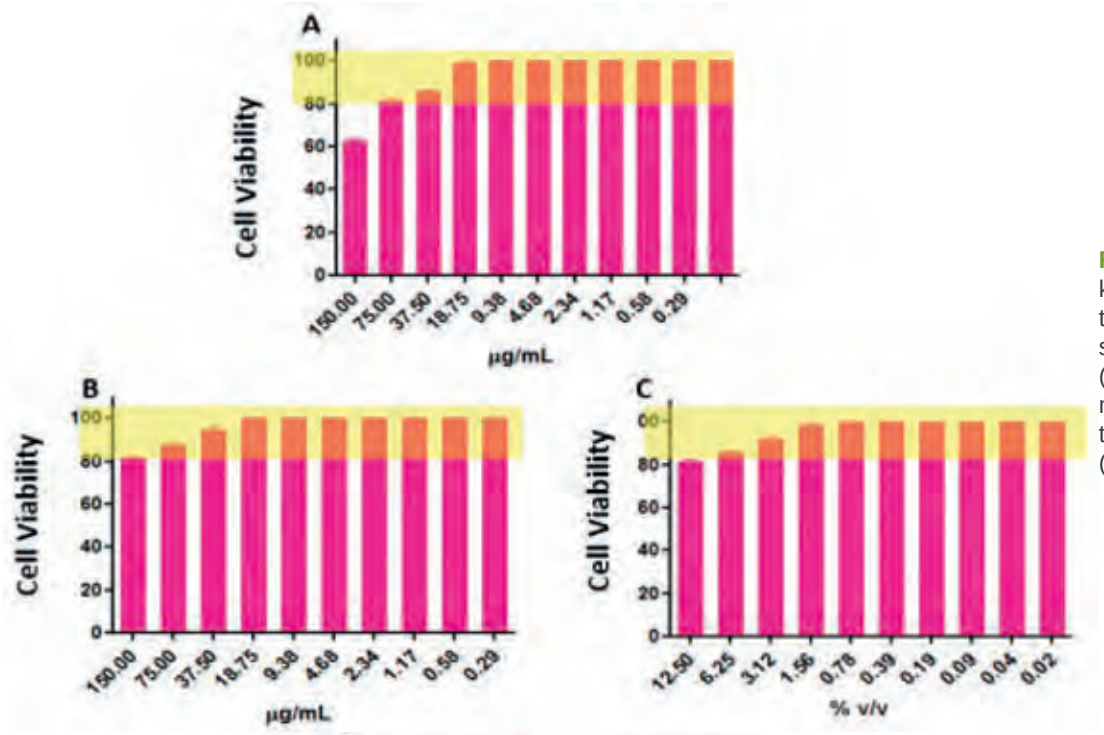

Figure 3. HaCaT keratinocytes cell toxicity. (A) AmB solution (µg/mL) (B) NE-AmB (µg/mL) (C) NE without the drug (%v/v).

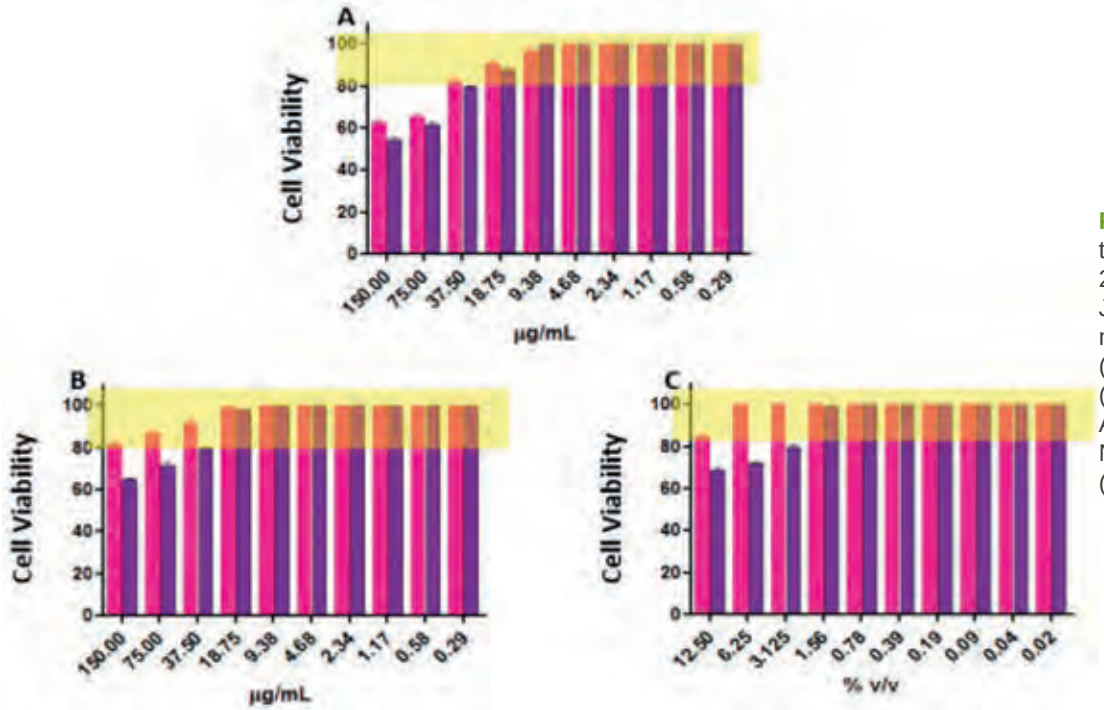

Figure 4. Cell toxicity in RAW 264.7 (pink) and J774A.1 (purple) macrophages (A) AmB solution (µg/mL) (B) NE-AmB (µg/mL) (C) NE without AmB (%v/v).

lar behavior as the excipient (Figure 4C). NE-AmB and NE observe no cell toxicity without the drug in the RAW 264.7 cell line (Figure 4B and 4C).

In vitro leishmanicidal activity

Table 2 summarizes the activity on promastigotes and amastigotes. The IC50 of NE-AmB for promastigotes and amastigotes was 0.26 ± 0.09 and 0.73 ± 0.02 µg/mL, respectively. The IC50 of the AmB solution on promastigotes and amastigotes was 0.73 ± 0.08 and 1.79 ± 0.02 µg/mL, respectively. The drug-free NE showed activity against both parasite stages: 0.16 ± 0.03% (promastigotes) and 0.39 ± 0.02% (amastigotes). Likewise, Figure 5 shows the infected and untreated macrophages (5A) and the cultures treated with NE-AmB (5B). Table 2. IC50 values in promastigotes and amastigotes of the genus *Leishmania tropica*.

Draize test

48 h after the application of NE-AmB and NE without the drug on the back of the rabbits, no discomfort was observed on the part of the animal throughout the test. No apparent changes (to the naked eye) were detected in the skin of the rabbits, and no edema, erythema, or irritation phenomena were observed (Figure 6).

Histological analysis of rabbit skin

Regarding histological evaluation, micrographs revealed that provoked scarification or control without AmB (sca-

rifications only) showed histological alterations and the presence of nonspecific inflammatory cells in the skin (Figure 7B). Topical application of NE without AmB (Figure 7C) caused some improvement, but inflammatory cells were still observed. However, NE-AmB (Figure 7D) markedly repaired this alteration, resulting in a less pronounced inflammatory process than the control. Figure 7A shows skin without scarification.

Discussion

Leishmaniasis is a parasitic infection of high medical, social and economic importance. The absence of effective vaccines and the limitations of current treatment's search for effective therapies is a real need, especially in developing countries, where there is little accessibility to antimony

Formulation	IC50 (µg/mL)	
	Promastigote	Amastigote
AmB solution	0.73 ± 0.08	1.79 ± 0.02
NE-AmB	0.26 ± 0.09	0.73 ± 0.02
Formulation	%v/v	
NE	0.16 ± 0.03	0.39 ± 0.02

Table 2. In vitro leishmanicidal activity.

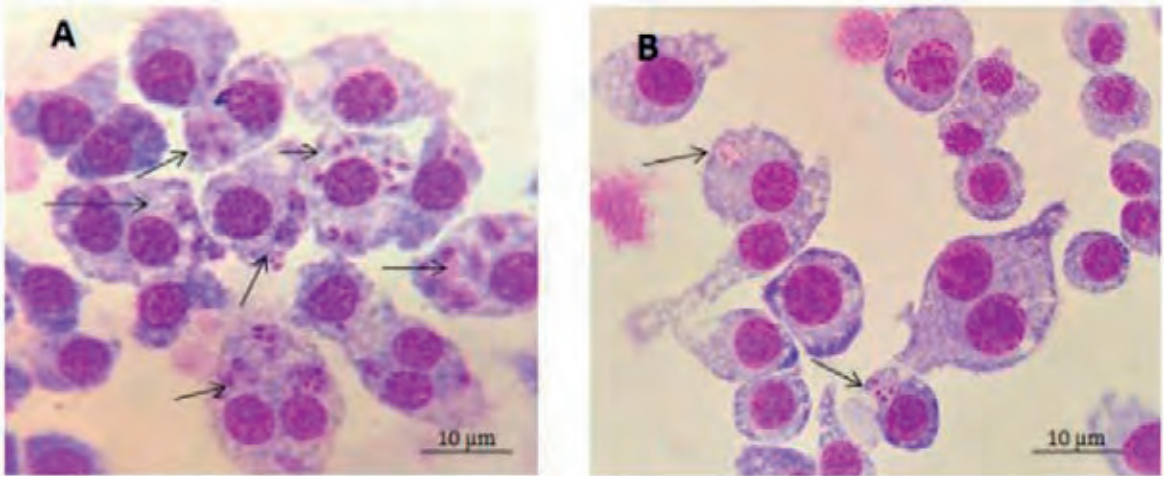

Figure 5. Effect of NE-AmB on infection of RAW 264.7 macrophages with Leishmania tropica. (A) Infected and untreated control cultures (B) NE-AmB-treated cultures. Arrows indicate the amastigotes.

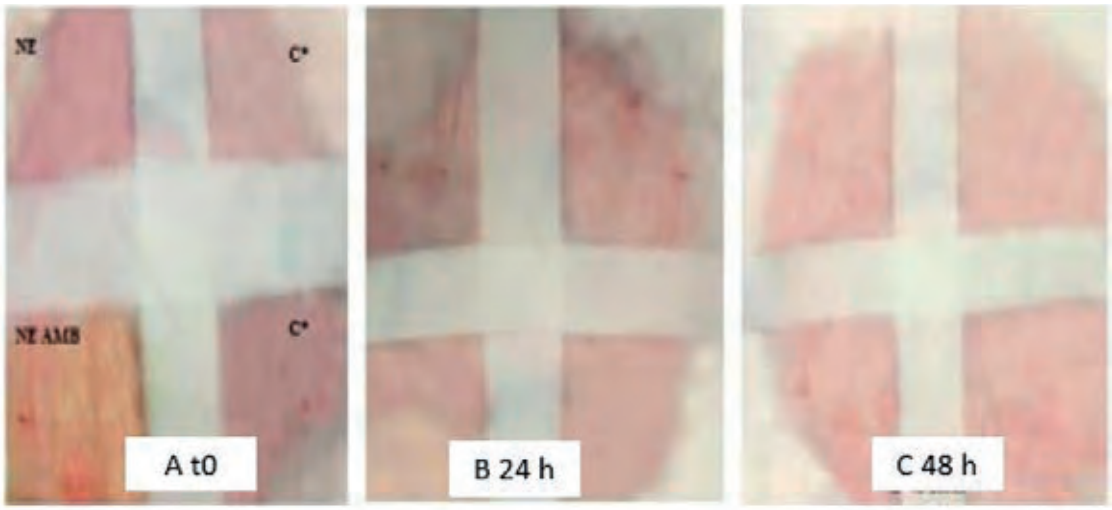

Figure 6. Draize test with emulsified systems at t0, 24 h, and 48 h. (A) NE = nanoemulsion without AmB. (B) NE AmB = nanoemulsion with AmB. (C) Cº = control (scarification skin without drug).

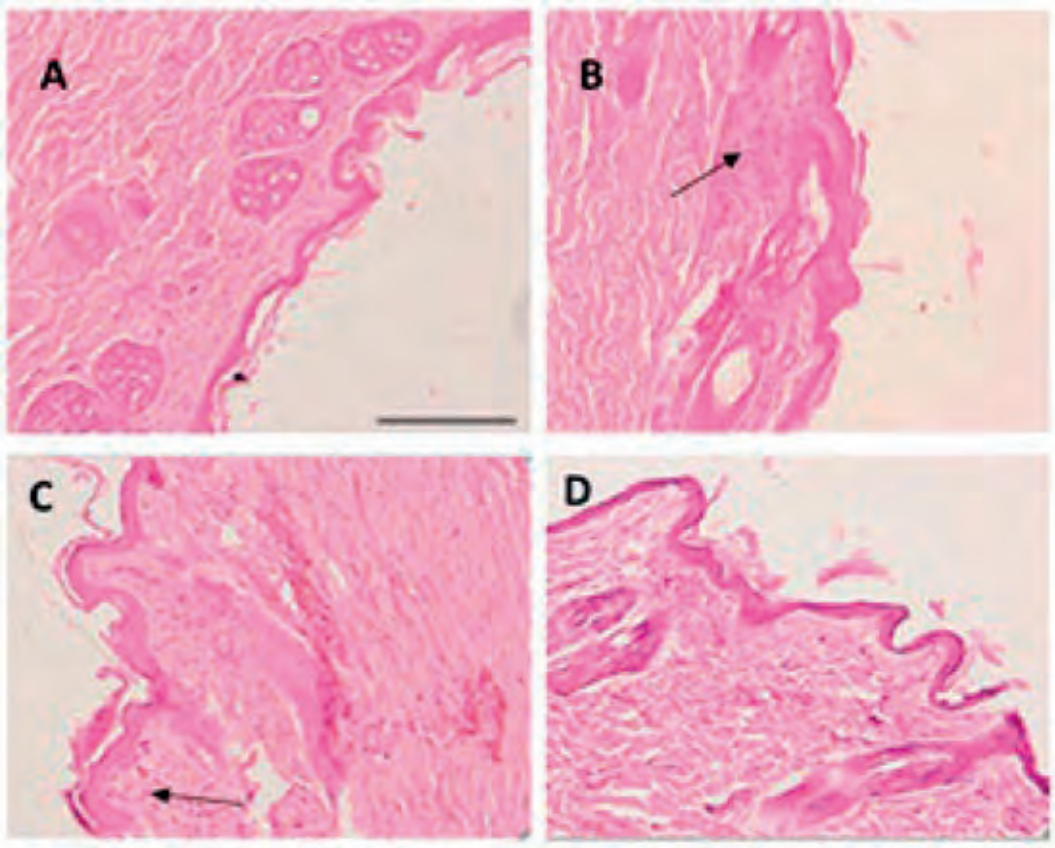

Figure 7. (A) Skin without scarification. (B) Skin with scarification and without drugs. (C) NE without AmB. (D) NE-AmB. The arrow indicates the inflammatory process—bar scale of 200 µm.

salt treatments, considered the first line[10]. AmB is considered an option for antimony salts treatment[11]. To this end, we propose an alternative, an easily prepared and applied NE-AmB for LC treatment.

Ex vivo permeation studies provide valuable information to predict the *in vivo* behavior of the formulation. Human skin used for testing has been lacerated to simulate skin in leishmaniasis processes. In this investigation, in the permeation tests, AmB was not quantified in the receptor compartment of the Franz cell, indicating that there was probably no absorption through the skin. Previous studies have shown that AmB does not tend to be absorbed[9,12-14]. This can be explained because AmB has a high molecular weight (926 Da), high hydrophobicity, and liposolubility, which would limit the passage of the drug through the dermis due to the aqueous structure of the dermis. Moreover, it can penetrate the skin without reaching the systemic circulation, which is confirmed by a large amount of drug retained in the tissue, which is indicative that the formulation, having a more local effect on the target area without side effects, is useful in the topical treatment of ulcers present in the LC. Other studies show that AmB can be absorbed in small amounts. However, the absorption tests in these studies were performed on rat skin, which is more permeable than human skin. The quantities found in the receptor medium were minimal, below the detection limit of the methodical used[15,16].

As we can see in Table 1, there was less AmB retained in the lacerated skin. This could be explained by the fact that AmB, which is more lipophilic, is more contained in the stratum corneum in the epidermis. In addition to being a high molecular weight drug, it takes longer to be absorbed by the dermis[17]. In a previous study by Berenguer *et al.* (2020), in which they prepared an AmB gel, they also found a lower amount of the drug in the lacerated skin. However, it is essential to note that in leishmaniasis processes, nodules and papules may contain epidermis, so AmB would be retained in both layers of the skin, exerting an eminently local effect[13].

Cellular toxicity studies are essential as they indicate that a formulation can be administered into the body. The AmB solution showed similar toxicity in both macrophage cell lines. However, NE-AmB and NE alone showed more significant toxicity in J774A.One than in Raw 264.7 cell lines. The latter cell line is known to exhibit multiple drug resistance properties caused by the presence of P-glycoprotein in the structure of these cells[18].

Regarding toxicity in keratinocytes, it was observed that only AmB solution presented toxicity at a concentration of 150 and 75 µg/mL. This could be because AmB is dissolved in DMSO, which is the cause of toxicity in this solvent. This is evidence that we can place NE-AmB on the skin without presenting any toxic effect. On the other hand, *Leishmania tropica* was used because it is one of the species that causes LC. It is characterized by causing relapsing leishmaniasis and is usually more resistant to drugs. The IC50 values of NE-AmB found in this study, both in amastigotes and promastigotes, were less than 1.00 µg/mL, lower than those found for the AmB solution, so at the *in vitro* level, it would be considered more effective. This could be because NE alone produced an effect against the parasite. However, being non-toxic, it is regarded as a suitable vehicle.

Previous research has demonstrated the efficacy of various AmB-based formulations in a range of IC50 values between 0.2-1.62 µg/mL, similar to those found in this study (Table 2). However, the values vary because they depend

on the infecting species[19]. In addition, the level of AmB retained in the injured skin (567.97 ± 8.64 µg/g/cm^2) and the intact skin (750.18 ± 5.43 µg/g/cm^2) was higher than the AmB IC50 values found in both parasite stages, amastigotes (0.73 ± 0.02 µg/mL) and promastigotes (0.26 ± 0.09 µg/mL). Therefore, after topical application of the formulation, the drug found in the dermis is phagocytosed by infected macrophages that hydrolyze the molecule through acidic lysosomal enzymes, releasing AmB where they live. Then Leishmania parasites multiply[20].

Finally, in the Draize test, no signs of irritation, itching, or edema were evident in the animal. Histological studies showed that NE alone caused a decrease in inflammation, but not entirely. Nevertheless, NE-AmB generated a considerable improvement in the inflammatory process of the rabbit skin compared to the positive control (or only with bedsores). This would indicate that NE-AmB could be applied directly to the skin. This can be observed in a previous study, where the obtained values of transepidermal water loss and stratum corneum hydration (SCH) evidenced no problems directly applying this formulation on human skin[9,12].

Conclusions

The low IC50 values found in this study for both parasite stages show that NE-AmB is promising for treating LC. The amounts of drug retained in the skin (intact or damaged) would be sufficient to eliminate the parasite from the site of infection, in this case, the skin. No drug was quantified in the Franz cell receptor compartment, showing no permeation or that amounts that might permeate are undetectable. This indicates that AmB will probably not be absorbed into the bloodstream. Both the excipient and the formulation with the drug did not show cytotoxicity on keratinocytes, so we can assume that its application on human skin is safe.

Furthermore, the formulation was non-irritant and did not produce edema or allergy during the Draize test. The NE-AmB showed microscopic improvements in scarified skin, which would benefit the level of skin leishmaniasis. The low viscosity of NE would allow its easy spray application, avoiding direct contact with the ulcer and preventing concomitant infections. Tests on infected animals are recommended to continue the study and ensure the efficacy of NE-AmB at the *in vivo* level.

Funding

The name of IN2UB, grant number [2017.3.IN2UB.2] funded this research.

Conflicts of Interest

The authors declare no conflict of interest..

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ARTICLE / INVESTIGACIÓN

The Effect of Chicken *Gallus gallus* (Domestics) feathers on the sorption properties of polyurethane foam

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DOI. 10.21931/RB/2022.07.04.63

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Abstract: A comparative absorption capability analysis was conducted using adapted polyurethane foam as crude oil Sorbents. The used Crude oil has been brought from the west of the Qurna city oil field with A.P.I. equals 22.2- 27. API measures how heavy or light a petroleum liquid is compared to water; crude oil's sorption and absorption ratio amounts are investigated. The findings demonstrate that the absorption ratio of fluff feather to wing feather is very distinct. The fluff feather absorbed much more crude oil than the wings. Moreover, much crude oil absorption causes the three types of feathers to plunge into crude oil. Owing to the disparity of the capillary structures of pure and modified polyurethane and the particular arrangement of the feathers, the absorption of modified polyurethane foam is beyond pure foam. The absorption ratio is saturated at (240-270) % (where the modified foam releases some additional volume of crude oil rather than the saturation ratio). Because of the cross-link density inside the modified foam, the last results were clarified. Also, we analyzed the effect of 10 holes on the absorption ratio in which the absorption is less than the unpinned ratio.

Key words: Chicken feather, Qurna, West Qurna oil field, Iraqi crude oil, polyurethane, capillary structure.

Introduction

If oil leaks happen, we must then scrub the leaked oil to prevent humans and significant environmental problems. Human health and environmental protection are at threat[1]. The ecological threats of spilled crude oil are not only related to water and sea life but also to the future of human beings. It is of great concern to use sorbents to minimize the chances of oil contamination and to consider the use of sorbents as one of the most economical and effective ways of cleaning up hydrocarbon pollution on the shoreline[2,3]. Nature is the main source of many sorbents, and the industry created many modern sorbents[4-6]. The mechanism of both natural and synthetic sorbents depends on inverting absorbed oil from the liquid phase to semi soil phase. In such a way, preventing spilled oil from controlling more water surfaces is easier and faster[7-10]. The ultra-light polyurethane foams are one of significant oil absorbers. They can trap oil from oil-water mixtures several times its weight since that absorption capacity is associated with different ratios of bubbles spread within the foam matrix where more bubbles mean more oil absorption ability[11-13]. In natural conditions, the cross-linking density of polyurethane foam is considered to be one of the major factors that affect the absorption ability to both water or crude oil where the amount of taking water or oil by foams is highly related to that density since it affects the movement of molecules or jamming the way of absorbed molecules through the inside of polymer matrix[14]. In such a way, Polymer with such properties has taken much attention due to their wide area, different size formation, ease of handling, and transfer from one place to another. It is well known that sorbents can be either natural organic (eat moss, feathers), natural inorganic [clay, sand]", or synthetic [polyethylene, nylon[15-17]. Owing to their low density, low water absorption, and excellent physical and chemical resistance, synthetic polymers such as polypropylene are said to be suitable materials for marine oil-spill recovery[18-20]. The main objective of this research is to compare the oil spill removal capacity of the small, medium, and large feathers and then to use the three different sizes of feathers to modify the foam. We based on the combination of natural and synthetic sorbents feather to adjust synthetic sorbents polyurethane foam and analyze the modified foam as spill oil sorbent in this paper.

Materials and methods

In this study, the used feather is obtained from the chicken (*Gallus gallus* domestics) bird. The used feathers were retrieved from the chicken farm site and washed multiple times to remove dust or filth. Two kinds of feather fluff and wings feathers were used. Each type of feather was distributed into the reaction container and mixed with reaction chemicals, as shown in figure (1). Di-isocyanates (part B) and polyols (part A) are reacted to produce polyurethane foam. A 1:1 (50:50) mixture ratio was used, which indicates that part A was equivalent to part B. The synthesis of Gallus gallus domestics-filled polyurethane foam was accomplished by combining 200 grams of part A and (200 g) of part B in a container, then adding (15 g) of *Gallus Gallus* domestics feathers directly to the mixing container.

The manual mixing of the used feathers and the reacting chemicals takes (3-4) minutes. At the end of the mixing

Citation: Abdullah N A, Hamdi H A, Shaban A D. The Effect of Chicken Gallus gallus (Domestics) feathers on the sorption properties of polyurethane foam. Revis Bionatura 2022;7(4) 63. http://dx.doi.org/10.21931/RB/2022.07.04.63
Received: 22 September 2022 / **Accepted:** 18 October 2022 / **Published:** 15 November 2022

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phase, the mixture would rise, forming the upper layer, before being broken into random shape parts and left for one day. The crude oil brought from the Qurna West oil field has an A.P.I. of 22.2-27. For the absorption tests[21], crude oil was poured into a (3 liters) exposed glass container with a diameter of 14.8 cm. In each experiment, a container was filled with (2 liters) of water and 300ml of oil. The amount of fat in the beaker was selected so there would be plenty of oil left during the absorption test. The dry weights of pure polyurethane foam parts are measured. Each piece was carefully mounted on the oil surface. After a specific time (As seen in the diagrams below), the samples were regularly taken out of the test jar. The wet surfaces of immersed foam are dried between tissue papers and measured directly to the nearest (+0.2 g). The samples are immediately returned to the specimen jar, and the tests are carried out. The steps of the method were repeated under the same conditions, but this time with modified polyurethane. On a weight basis, the sorbent's oil sorption was measured. Oil absorption capacity is calculated by the formula [g/g] = $[S_t–S_0]$ /S_0 that according to (22). Where S_0 denotes the original dry weight of a sorbent and St indicates the importance of the sample after immersion. Figure 1 shows the modified polyurethane foam added with feathers.

Results

To use the modified polyurethane foam as a crude oil absorbent, we measured the absorption capacity of the feathers of chicken (Gallus domestics). Three different sizes of feathers were used (big, medium, and small). Figures 2, 3, and 4 show the changes made to the absorption ratio related to immersion time in crude oil. Figure (5) depicts the difference in the absorption ratio of modified polyurethane due to natural oil immersion time. The effect of pinholes and the obtained result in such a case are shown in figure (6). Figure (7) shows the shape of the modified foam after the immersion in crude oil and how the absorption of that oil blacked the inside matrix of that foam.

Discussion

It is evident from the last figures (2, 3, and 4) that the immersion time is limited to one minute. After that, all three feather sizes diving into the crude oil, indicating the heavy amount of crude oil absorbed by the feather. A second noticeable thing from the last figure is that the absorption capacity is more prominent with a small feather than that with a

Figure 1. Shows the modified polyurethane foam added with feathers.

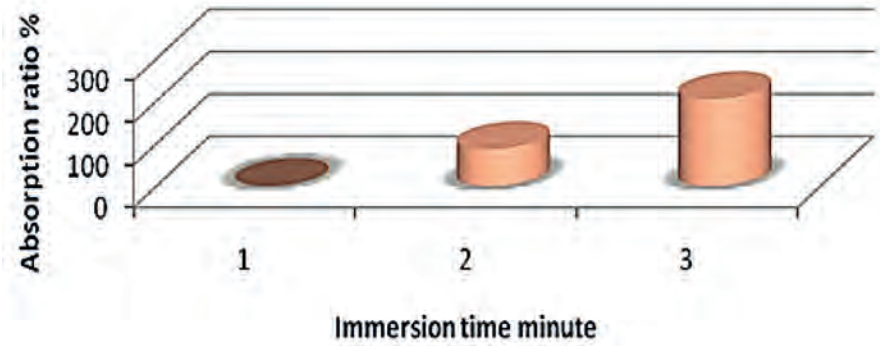

Figure 2. Absorption ratio of the medium feather as a function of immersion time.

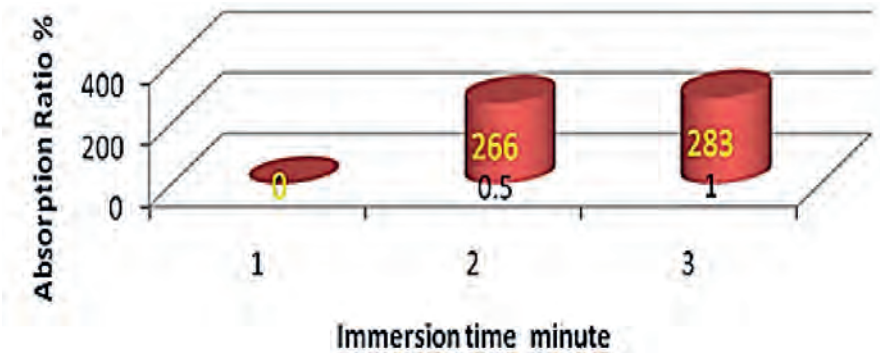

Figure 3. Absorption ratio of the big-size feather as a function of immersion time in crude oil.

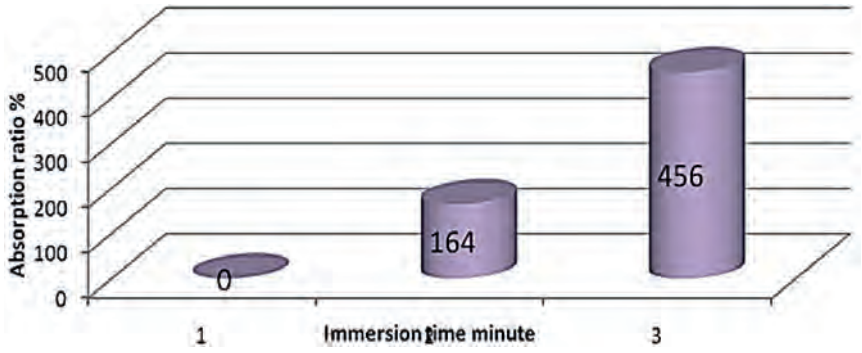

Figure 4. Absorption ratio of small feather as a function of immersion time.

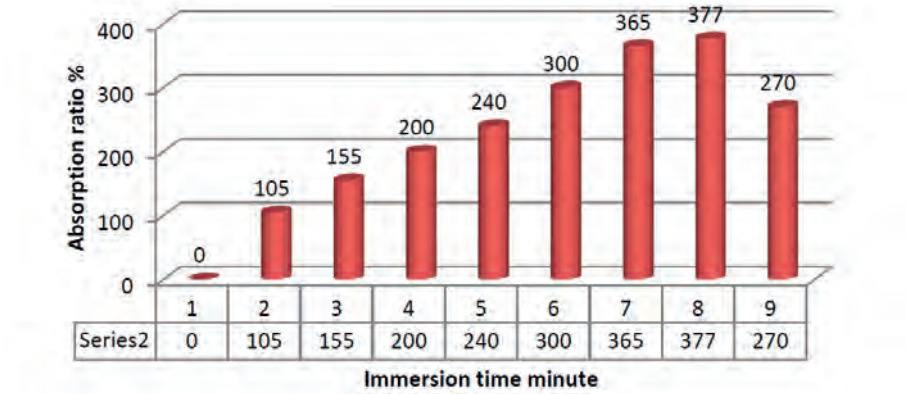

Figure 5. Absorption ratio of modified polyurethane foam as a function of immersion time.

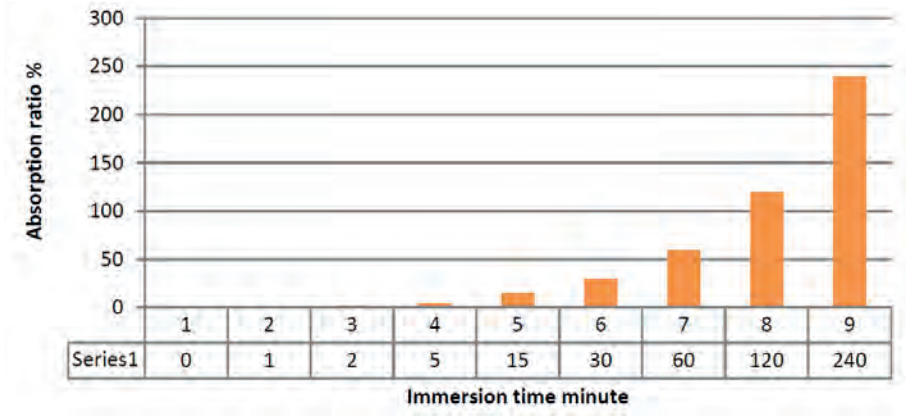

Figure 6. The effect of pinholes on absorption ratio of modified polyurethane.

Figure 7. The shape of modified polyurethane foam after immersion in crude oil.

medium and large size feather. The previous result is due to the unique structure of feather-like barbs linking density per feather[23,24]. Figure 5 indicates that the time range starts with one minute to 240 minutes. The saturation absorption ratio is restricted to 240 %, which is more than pure polyurethane foam. We must note that we used big feathers since they had the highest absorption ratio, as shown in the last three photographs—[105 %].

Moreover, in another sample of modified foam, the reached saturation ratio is 270%. The difference between these two ratios is surely related to the geometrical distribution of feathers into the foam matrix. This saturation and limitation of the absorption capacity are also due to the heavy cross-linking density of polyurethane foam chains. This cross-linking density prevents further oil molecules from taking into the foam since no more space between chains is available in that foam. Thus we see the absorption ratio is increasing with time, especially at 120 minutes, but it decreases at 240 minutes. The last decrease in absorption ratio may explain in terms of no space to control molecules of oil into it due to the high cross-linking density. Thus for the previous result, we moved to study the effect of pinholes on the absorption ratio. The reason for choosing pinholes is to make a new way for oil molecules to cross their way up into the polyurethane foam matrix. We decided 10 holes per ($25cm^2$) distributed through the foam matrix. The diameter of each pinhole was (1mm). Figure 6 shows less absorption ratio is obtained, but no absorption saturation is reached. Even though they managed new ways into the matrix of foam, the lower absorption ratio still has much space in that foam.

More tests are needed to determine the appropriate ratio between the two components, A and B, of polyurethane. Compared with other natural additives added to polyurethane foam, the absorption ratio obtained in this study is less than that obtained by other researchers[25-28]. Aside from the lower absorption potential, natural organic sorbents include hay, feather, straw, peat moss, and other carbon-based materials[29-32]. Natural crude oil sorbents have the unfavorable characteristics of being gritty, difficult to use under windy weather and having no oil absorbency. Furthermore, specific natural organic sorbents accumulate oil and water, allowing the sorbents to sink. In such a manner, the modified foam seems to solve the problematic use under windy conditions, but the small absorption capacity is still less than the aim of this study[33]. It is vital to note that the immersion time ranges from one minute to three minutes, with a two-minute break in between.

Conclusions

The absorption action is related to the kind and size of the used feathers. The fluff feathers are becoming more suited for the absorption of crude oil and the modification of polyurethane foam as an absorbent. The absorption capacity is larger with a small feather than with a medium and large size feather. Furthermore, the absorption ratio of modified polyurethane is improved due to capillary structure improvements made to the polyurethane foam, which is attributed to adding a chicken feather. Modified polyurethane has significant potential as an oil sorbent, especially within the first hour of an oil leak or spill. The 120-minute duration is long enough to achieve a saturation absorption ratio. Pinholes have the opposite effect on the absorption properties of modified polyurethane foam.

Acknowledgments

Thanks going for all who support us.

Conflicts of Interest

No conflict.

Funding

Self by authors.

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ARTICLE / INVESTIGACIÓN

Phenotypic and genotypic investigation of three weeds residues allelopathic effect on the growth of three hybrid wheat cultivars

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DOI. 10.21931/RB/2022.07.04.64

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Abstract: The present study was carried out to determine the allelopathic effect of extraction of three herbs (*Silybum marianum* L. and *Malva parviflora* L. and *Loliuim rigidum* L.) on three wheat cultivars (Rashid, Abo Ghrib and IPA99) on germination, growth and ear formation by classical research methods (laboratory and greenhouse experiments and molecular detection of GA3-oxase2 (TaGA3ox2-1) gene expression and its crucial role in wheat growth and Ta14S gene expression as a gene responsible for ear development. The study analyzed the influence of weed residues on the germination and growth of three wheat cultivars. According to the mean effect of aqueous weed extract on the cultivars, the laboratory experiments revealed a significant difference in all the characteristics studied.

Key words: Weeds, hybrid wheat cultivars, allelopathy, RT-PCR.

Introduction

Wheat (Triticum aestivum) is a grass that belongs to the Poaceae family and is a widely cultivated agronomic crop for grains used in household consumption and the creation of a multitude of processed materials, which was labeled as a profitable agricultural venture; the crop has a long and illustrious history in human culture and civilization. It is essential to the world economy and food security[1]. Weeds can produce many allelochemicals; they have a range of effects on other plants, including reducing or activating the germination and growth of receiving plants[2]. Allelopathy from specific weed species impacts crop development; allelochemicals from allelopathic weed products can harm budding crop seedlings by disrupting root and shoot growth[3]. Allelochemicals secreted from *Chenopodium murale* L. root hairs were found to be responsible for cell cycle disruption and oxidative damage in wheat[4]. Allelochemicals are a type of secondary metabolite that isn't necessary for metabolism; they have been linked to decreased seed germination and seedling growth; their inhibition is complicated, involving interactions between various chemical classes such as phenolic chemicals, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids[3].

Allelopathic water extracts from weed species, such as *Malva parviflora* L., were also found to limit barley growth and photosynthetic activity[5]. While found that adding aqueous extract vegetative sections of *Malva rotundifolia* L, *Silybum marianum*, and *Sonchus oleracens* to wheat seedlings reduced plumule and extreme length and weight[6]. Allelopathic weeds have been reported in enormous numbers; they harm crop plants from emergence through maturity, causing significant economic losses[7]. A varied range of weeds in wheat-growing fields is one of the critical limiting factors in wheat output[8]. Compared to wheat infested with weeds (*A. fatua* and *S. Marianum*), the results showed that wheat grown in weed-free circumstances had the highest biomass and leaf area index during both growing seasons. To reduce crop-weed conflict and increase crop output, control of certain weeds, namely *A. fatua* and *S. marianum*, is particularly desirable[9]. Many kinds of research were achieved overall the world. In a seed bioassay,(10) utilized aqueous extracts of different extracts of four common weeds (*A. fatua*, *Phalaris minor*, *Melilotus alba*, and *Chenopodium album*) and found allelopathic effects on wheat at levels of 6,8,10, and 12g/100ml. The allelopathic effect of weeds (*Avena fatua*, *Melilotus officinalis*, and *Polypogon hissaricus*) on germination, growth, dry biomass and chlorophyll concentration of three wheat cultivars was discovered by (11).

Gibberellins (GAs) are plant hormones that control many growths and developmental processes in plants[12]. They are especially critical in stem elongation regulation[13,14]. As a result, it's reasonable to look for a link between GAs and plant height heterosis. In previous research, GA levels have been linked to the robust plant development seen in hybrid F1 plants. There are three lines of evidence that support this relationship. In maize, hybrids have greater GA levels than parental inbreds[15], while an interspecific hybrid between Liriodendron chinense and *L. tulipifera* has higher GA levels than parental inbreds[15]. There are three phases in the production of GA in higher plants. As a molecular biomarker, we chose the GA20ox gene, which is involved in the third stage of GA biosynthesis and is linked to a critical role in the corrosion precursor of bioactive GAs to GA1. The bioactive GA is a factor in to increase in hybrid shoot cylinders than in inbred shoot cylinders[16]. Many experiments, on the other hand, focus on molecular mechanisms of developmental regulation that have the potential to accelerate wheat improvement. Even though 14-3-3 proteins are increasingly being linked to developmental control[15]. In the formation of wheat seeds, little is known about such genes. Because hexaploid There are three types of wheat chromosomes. We focused

Citation: Al-Doree E K, Al-Sffar R S, Jasim I R. Phenotypic and genotypic investigation of three weeds residues allelopathic effect on the growth of three hybrid wheat cultivars. Revis Bionatura 2022;7(4) 64. http://dx.doi.org/10.21931/RB/2022.07.04.64
Received: 22 September 2022 / **Accepted:** 18 October 2022 / **Published:** 15 November 2022

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on the relevance of the Ta14S gene in wheat cultivars under study to better understand a regulatory function in wheat seed development[17].In many scientific domains, molecular research utilizing quantitative polymerase chain reaction (qPCR) is regarded as a gold standard technology for confirming traditional results. The activation of relevant genes for numerous metabolic pathways is detected by screening at the molecular level of allelopathic action, which leads to increased plant germination, growth, seedling, flower, and fruit formation[18].

One of the most severe issues in agricultural productivity is weeds. They have an impact on crops through direct resource competition as well as allelopathic consequences. Allelochemicals can be found in almost every part of a plant, including the leaves, flowers, fruits, roots, rhizomes, seeds, and pollen.

Materials and methods

In 2021,the research was carried out at Mosul University's Biology Department College of Science. Three weeds(-*Silybum marianum* L., *Lolium rigidum* L., and *Malva sylvestris* L.) were collected and transported to the laboratory for allelopathic testing after harvesting. Wheat seeds were obtained from the sources testing and confirmation center in Ninava governorate/Iraq.

Laboratory assay

Preparation of aqueous extracts

Weeds were mixed and stored for 24 hours at room temperature in distilled water (5gm per 100ml). After that, the solutions were filtered twice: once through a double layer of muslin gauze and then again through Whatman No.1 filter paper. These water extracts include 5% (w/v) water. The effect of water extracts at concentrations 5 %(w/v) was evaluated in a Petri dish bioassay. Ten wheat cultivars (Rashid, Abu Graib and IPA99) seeds were placed in sterilized Petri dishes lined with twice-folded filter paper. In each Petri dish, 8 ml of (5%,w/v) of three weed extracts was added, while distilled water was used in control. There were three frequencies in all of the treated samples. The dishes were incubated at 22 °C(±2) for 7 days in a Completely Randomized (CR)Design. The percent of seeds germination was calculated in the following equation[19].

$$\text{Germination percent} = \frac{\text{Number of germination seeds}}{\text{Number of cultimative seeds}} \text{ x } 100$$

Seedling (cm), plumule and radical length (cm), in addition to plumule and radical fresh weight (mg), were measured at the end of this study.

Preparation of weed residues

In 2021, a pot experiment was carried out at the Department of Biology's greenhouse. The effect of incubated dry weed residues(5%,w/w)during one week in pots with soil was determined, 10 seeds were placed in Plastics pots with a diameter of 20cm for each of the wheat cultivars (Rashid, Abo Ghrib, and IPA-99) at 0.5cm depth from the surface of the soil, then irrigated with water and placed in a greenhouse at a temperature of 22 (±2)°C, 10 wheat seeds were sowed in the soil without residues as a control. After 60 days of seedling, the germination percent was assessed using a complete randomized block design, with each pot having three frequencies[20].

Phenotypic characteristics investigation

Germination percent, shoot length(cm), fresh weight of shoot(gm), spike length, number of leaves per plant, spike weight(g), and weight of 100 seeds. The water content (WC) can be expressed on a dry weight (DW) or fresh weight (FW) basis, as listed in the following equation[21].

$$WC_{(DW\ basis)} = \frac{FW-DW}{DW} \text{ X } 100$$

$$WC_{(FW\ basis)} = \frac{FW-DW}{DW} \text{ X } 100$$

Molecular investigation assay

RNA isolation

RNA was extracted according to the manufacturer's instructions for the RNA kit (Geneaid, Taiwan).

Single-strand DNA synthesis

After RNA isolation, the cDNA synthesis phase was started. Amplification mixture(GeneAll,S.Korea)was composed of 10X Reaction Buffer2 µl,20X dNT P(1µl),Rtase(1µl),RNase Inhibitor(0.5µl),RNase free Water (3.5µl), RNA Sample (6µl), primers (3µl) and RNA Sample(10µl). Amplification steps included 25°C (10min),27°C (120min) and 85°C(5min).

Real-Time qPCR profile

Amplification mixture was used in this step composed of 2X MasterMix(S YBR Green)10µl, primers 2µl,DNA template2µl and RNase free distilled water 6 µl(GeneAll,S. Korea),primers that used were, wheat growth indicator,Ta-GA3ox2-1-S-5'-GTACATGGGCGTGCGCAAGAAG-3',Ta-GA3ox2-1-A-5'-GCACGCATCC ACCAGCATCATC-3 (26), spike development,Ta14S-R1-5'-CGCCT GCTAC-GCTACAAGGAC-3', Ta14S-F2- 5'-GTCAATGACCGTTG CAATGTG3', in addition to β-actin-F-5'-TTTGAAGAGT-CGGTGAAGGG-3',β-actin-R-5'-TTT CATACAGCAGGCAA-GCA-3'[17]. Amplification mixture plate was placed in Applied Biosystem 7500 fast thermocycler; an amplification program was included, initial unwinding at 95°C(5min), then 40cycles of unwinding at 95 °C(30 sec), primer hybridization at 60°C, and 57°C(30sec) respectively and extension 72°C (30sec).

Statistical analysis

Phenotypic outcomes analysis

The data were statistically evaluated by SPSS Program, and differences in treatment means were compared using the Completely Randomize Design (CRD) test at a probability level of 0.05[23].

Molecular outcomes analysis

The quantification findings were expressed in terms of the cycle threshold value calculated using the manually modified baseline. The method mentioned earlier was used to determine relative gene expressions. To detect the difference between control and treated cases, differences in the amplification values of target and reference genes were estimated as expression levels(fold change) for each sample[18,24].

Results

The current findings (table1) revealed a substantial difference in wheat germination growth characteristics after

being treated with an aqueous solution of three weed residues(5%,w/v). According to the mean effect of cultivar, the results showed the highest effect of seed germination percent in IPA-99(91.67%). After treatment with *Malva parviflora* L.aqueous extracts, the highest increase in seed germination percent (96.67%) was reported in the Rashid cultivar. According to the mean effect of weeds, the highest seed germination percent (93.33%) was noticed after treatment with *Malva parviflora* L.

Regarding the mean effect of cultivar, the outcomes revealed the highest impact of plumule length in Abo Garib (4.38cm). The treatment with *Silybum marianum* L., aqueous extracts showed the highest increase in plumule length (4.93cm) reported in the Abo Garib cultivar. The means effect of weeds showed the highest value in plumule length (4.04cm)after treatment with *Malva parviflora* L.

The mean effect of cultivar outcomes showed the highest impact of plumule weight in Rashid(0.07gm). Due to treatment with *Silybum marianum* L., aqueous extracts' highest increase in plumule weight(0.092gm) was documented in the Ras-hid cultivar. The mean effect of weeds showed the highest value in plumule weight (0.054gm)after treatment with *Silybum marianum* L. The mean effect of cultivar outcomes showed the highest impact of radical length in Abo Garib (6.19cm). Because of therapy with *Loliuim rigidum* L.,aqueous extracts, the highest reducing effect of radical length(2.97cm) was noticed in the Rashid cultivar. The mean effect of weeds showed the highest value in radical length (4.93 cm)after treatment with *Silybum marianum* L.

The mean effect of cultivar outcomes showed the highest effect of radicale weight in Rashid(0.039gm).because of treatment with *Silybum marianum* L.,aqueous extracts, the highest increasing effect of radicale length(0.045cm)was noticed in Abo Garib cultivar. The mean impact showed the highest value in weight(0.038gm)after treating with *Loliuim rigidum* L.

Figure 1. Shows that the three weed residues were affected in the seed germination percent of three wheat cultivars. The highest increasing value was linked with Abo Ghraib cultivar after cultivation in the soil incubated with *Silybum marianum* L. and *Malva parviflora* L. residues. In contrast, the highest reduction percent of IPA99 seed germination was documented after cultivation in soil with *Silybum marianum* L. and *Malva parviflora* L.

The three cultivars that were cultivated in the soils incubated with weeds residues showed variant increasing values in spike length. However, the remainder of the treatments varied in spike length with different cultivars; the highest spike length was linked with Abo Garib after treatment with *Malva parviflora* L., as represented in the histogram (figure2).

Figure (3) demonstrated a variance in the number of leaves of the three wheat cultivars cultivated in the weed-containing soil compared to the number of plants grown in the control soil(without weeds). The number of leaves of the cultivar IPA99 treated with *Loliuim rigidum* L. revealed the highest increasing value.

The present results demonstrated that the weight of 100grains was differed according to the three wheat cultivars after treated with soil weed residues. Cultivar Rashid treated with soil having *Malva parviflora* L. residues showed the most significant value of growth (figure 4).

In the soil incubated with weed residues, there were changes in the relative water content in wheat cultivars, as shown in Figure(5). The relative water content of Abo Ghraib plants grown in soils incubated with *Malva parviflora* L. residues showed the highest increasing value.

Relationship between phenotypic and genotypic results

This study showed significant positive predictive values as phenotypic results associated with specific molecular outcomes(table2). In term of wheat length, there was a significance increasing percentage in Rashid cultivar after treat-ing with *Loliuim rigidum* L. and *Malva parviflora* L.(L-Treated(T),45cm: control (c),34cm and M-treated,44cm: control,34cm resp.), these results were confirmed

Cultivars	Treatment	Seed germination (%)	Plumule length (cm)	Plumule Fresh weight (gm)	Radical Length (cm)	Radical fresh weight (gm)
Rashid	**Control**	80.00 d	4.0 ab	0.040 c	7.10 ab	0.038 cd
	Loliuim rigidum L.	90.0 bc	4.93 a	0.074 b	8.50 a	0.063 a
	Silybum marianum L	83.33 cd	2.83 de	0.092 a*	2.97 d*	0.026 fgh
	Malva parviflora L	96.67 ab*	3.80 ae	0.075 b	6.17 abc	0.03 ef
	Mean cultivars effect	**87.50 b**	**3.89 a**	**0.070 a**	**6.18 a**	**0.039 a**
Abo Gharib	**Control**	83.33 cd	3.60 b-e	0.019 f	7.53 ab	0.019 i
	Loliuim rigidum L.	83.33 cd	4.33 abc	0.040 c	5.30 bc	0.029 efg
	Silybum marianum L	86.67 cd	4.93 a*	0.041 c	6.40 abc	0.045 b*
	Malva parviflora L	83.33 cd	4.63 ab	0.041 c	5.53 bc	0.026 fgh
	Mean cultivars effect	**84.17 b**	**4.38 a**	**0.035 b**	**6.19 a**	**0.029 b**
IPA 99	**Control**	86.67 cd	3.23 de	0.030 d	5.67 bc	0.033 de
	Loliuim rigidum L.	100.0 a	2.60 e	0.029 e	4.60 cd	0.023 hi
	Silybum marianum L	80.0 d	3.37 cde	0.029 e	5.43 bc	0.024 ghi
	Malva parviflora L	100.0 a	3.70 b-e	0.039 c	6.33 abc	0.039 c
	Mean cultivar effect	**91.67 a**	**3.23 b**	**0.031 c**	**5.51 a**	**0.029 b**
Mean weeds effect	**Control**	83.33 b	3.61 a	0.029 c	6.77 a	0.030 b
	Loliuim rigidum L.	91.11 a	3.96 a	0.046 b	6.13 ab	0.038 a
	Silybum marianum L	83.33 b	3.71 a	0.054 a	4.93 b	0.031 b
	Malva parviflora L	93.33 a	4.04 a	0.052 a	6.01 ab	0.032 b

Green color, highest increasing value; red color, highest decreasing value and*, highest weed effect.

Table 1. Effect of aqueous extracts of weeds in germination and growth of three wheat cultivars.

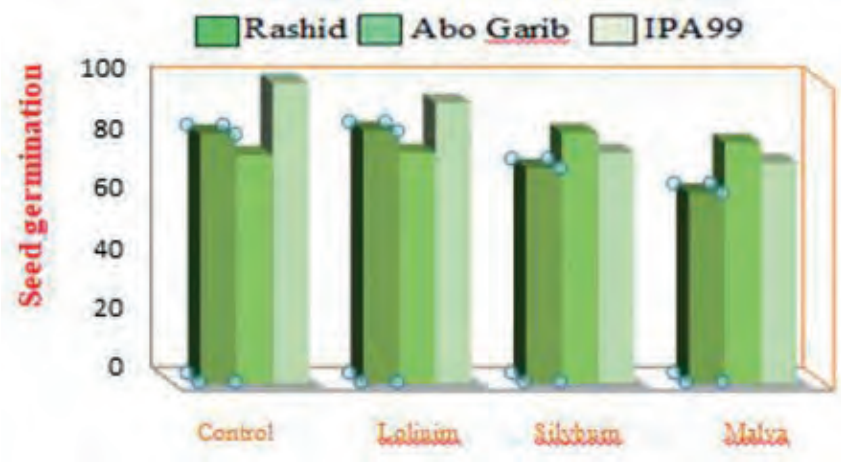

Figure 1. Histogram of seed germination of three cultivars of wheat plants treated with three weeds (*Silybum marianum* L., *Malva parviflora* L., and *Loliuim rigidum* L.).

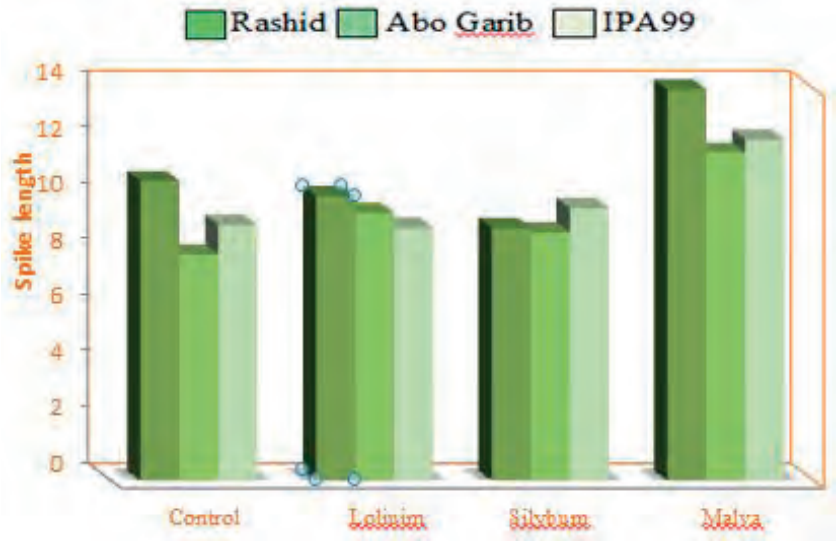

Figure 2. Histogram of spike length of three cultivars of wheat plants treated with three weeds(*Silybum marianum* L. and *Malva parviflora* L. and *Loliuim rigidum* L.).

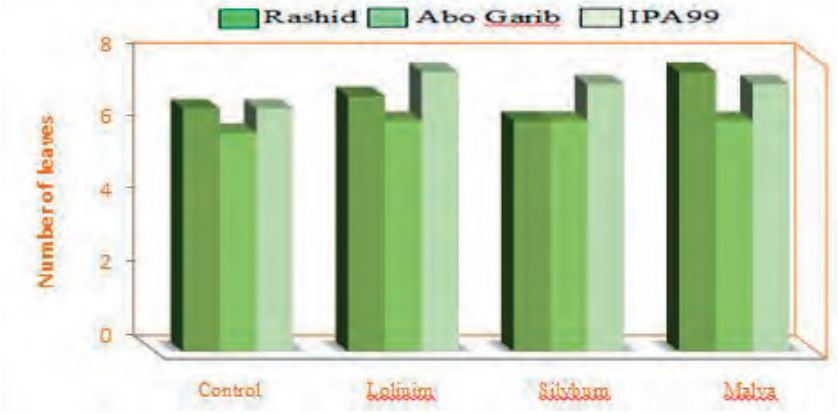

Figure 3. Histogram of the number of leaves of three cultivars of wheat plants treated with three weeds(*Silybum marianum* L. and *Malva parviflora* L. and *Loliuim rigidum* L.).

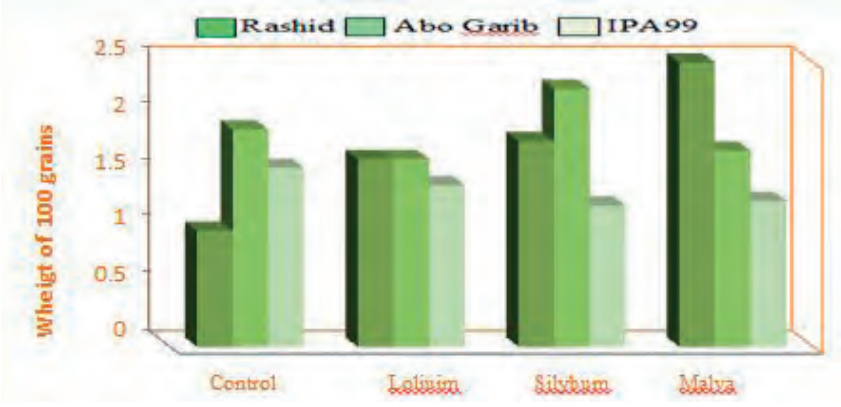

Figure 4. Histogram of the weight of 100 grains of three cultivars of wheat plants, treated with three weeds(*Silybum marianum* L. and *Malva parviflora* L. and *Loliuim rigidum* L.).

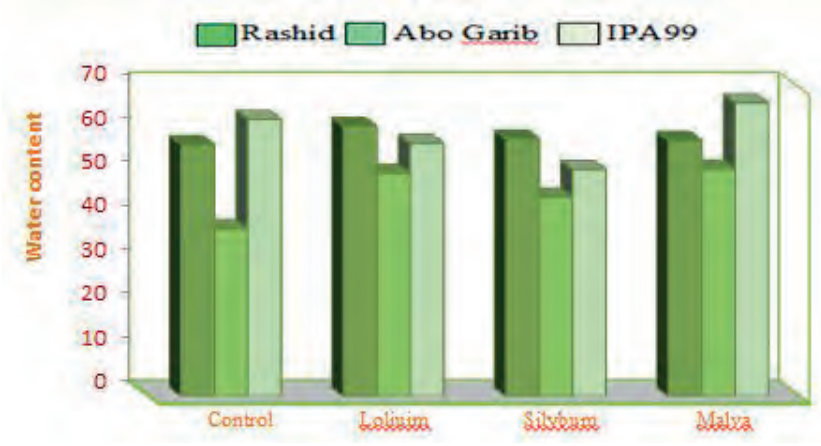

Figure 5. Histogram of the water content of 100 grains of three cultivars of wheat plants treated with three weeds(*Silybum marianum* L. and *Malva parviflora* L. and *Loliuim rigidum* L.).

with TaGA3ox2-1gene expression related with the same case(L-t,5.1043:c,0.870 67 and M-t,3.96014:c,0.87067resp.) other outcomes showed high expression with previous gene (T,7.96787:c,0.942034), that associated with phenotypic finding(T, 55:c,35) after treating of Abo Grib cultivar with *Malva parviflora* L. as well as, growth value was increased in IPA99 cultivar(T,44:c,21), which agreed with that documented genotypically (T,6.85702:c,1.01002). Spike weight showed the highest increasing value phenotypically in Rashid(t,55gm:c35, gm)after treating Abo Grib cultivar with *Malva parviflora* L., which in turn agreed with Ta14S-R1 gene expression(T,6.71807:c,1.01065).

Discussion

Weeds leave massive amounts of residue in the field, affecting the linked crops and subsequent crops in various cropping systems. Allelochemicals released by weeds impact agricultural plant germination, stand establishment, growth, yield, and physiology. By regulating enzyme activity, protein synthesis, photosynthesis, respiration, cell division, and enlargement, among other physiological functions, they produce significant reductions in crop germination and growth, resulting in a significant drop in crop production. Allelopathic weeds, in short, are a possible hazard to crop plants and can result in financial losses[25].

The present results showed a synergistic relationship between utilizing weed residues and wheat cultivars growth in which the studied parameters were increased. In turn, these outcomes agreed with the documented results of many researchers and disagreed with others. According to to (26), some plant species have a more considerable inhibitory impact when used as residues due to higher allelochemical extraction during the decomposition process. *Flaveria bidentis* L., an invasive weed, was shown to exude allelopathic phenolic chemicals, and residues from this weed hindered the growth and biomass of cotton seedlings. There was the highest decrease in root fresh (64%) and dry biomass (64%) and root/shoot ratio(64%)after being treated with *V. sativa* raw residues. The outcomes showed that the interaction of leaches and residues could lead to a synergetic effect in nature. In turn, this causes significant economic and environmental disposal of waste and organic materials accumulate-tion[27].

On the other hand, (28) used various concentrations of aqueous extracts(5,10 and 15g/l)of the three weeds, significantly reducing the percent germination of Pirsabaq cultivar. In pot culture, root and shoot length and dry seedling biomass of the three wheat varieties showed differential responses to different weeds. Aqueous extract at 15g/l of *A. fatua* increased the source, shot height, and dry weight of Pirsabaq cultivar[11].

Phenotypic investigation (Greenhouse)				**Molecular screening (RTqPCR)**				
Control	***L. rigidum***	***S. marianum***	***M. parviflora***	**Gene**	**Control**	***L. rigidum***	***S. marianum***	***M. parviflora***
1.25	1.13	0.85	3.0	Ta GA3ox2-1	1.01078	0.94783	0.95015	4.21653
0.65	2.05	0.98	0.91		0.94891	3.01892	1.40132	0.76258
1.20	1.21	0.70	2.50		1.78756	2.20189	0.90143	4.10397
34	45	35	44	Ta14S-R1	1.00912	5.01891	2.10442	3.95403
35	45	42	55		1.01065	4.88201	4.01627	6.71807
21	35	33	44		0.81223	1.77189	1.53041	5.10176

Table 2. Comparison between phenotypic results and molecular screening outcomes.

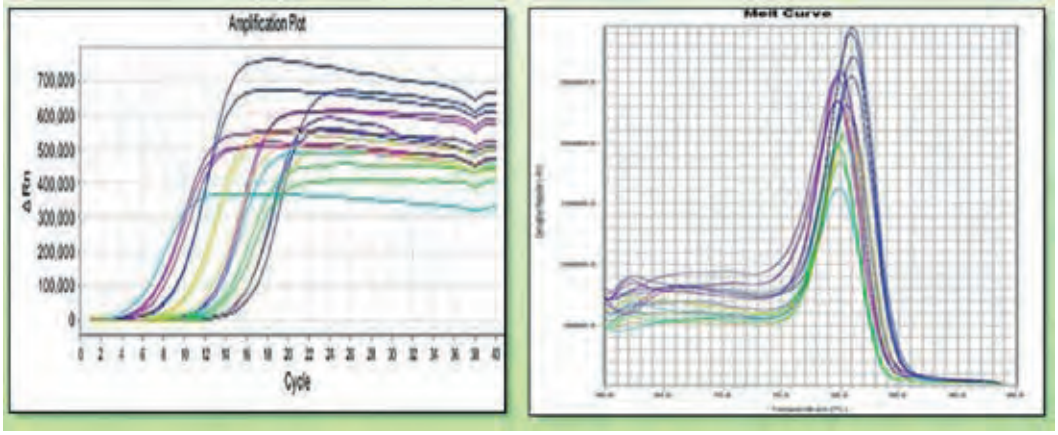

Figure 6. Shows the events of the *TaGA3ox2-1*gene amplification curve via RTqPCR, in which different values of previous gene expression were documented.

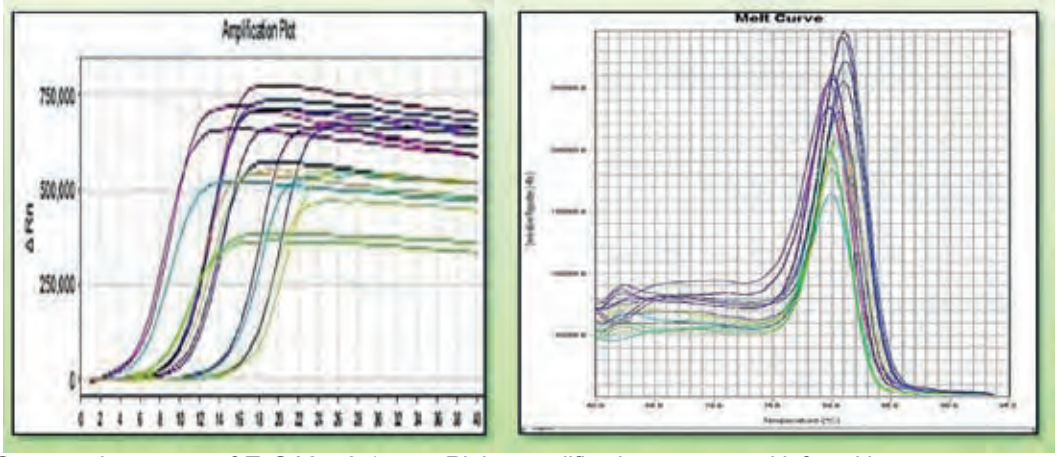

Figure 7. Comparative curves of *TaGA3ox2-1*gene. Right, amplification curves and left melting curves.

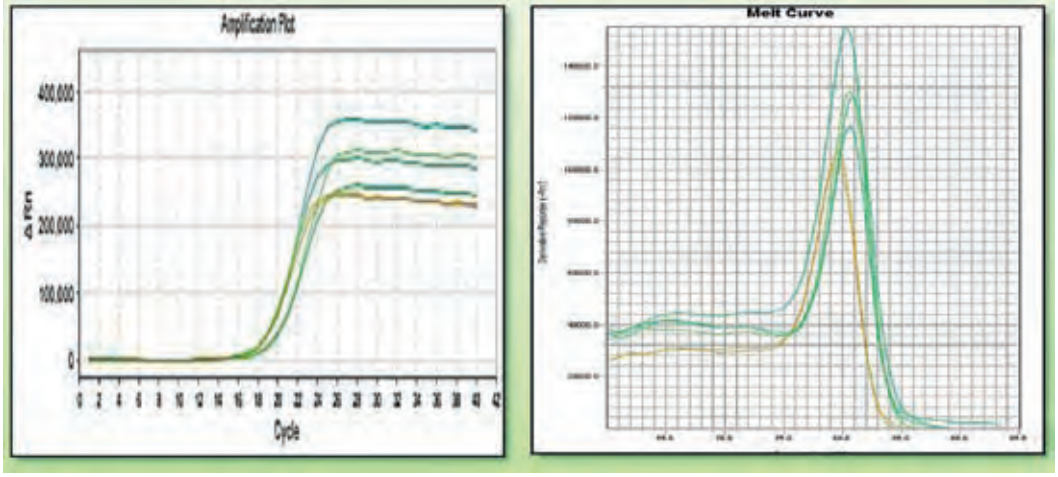

Figure 8. Showed the events of *B-actin* gene amplification curve via RTq- PCR as a reference gene.

Conclusions

In terms of molecular investigation, many studies were achieved in this field over the world[22] noticed that among the 18genes analyzed, genes encoding enzymes that promote the synthesis of bioactive gibberellin like *TaGA20ox-2, TaGA20oxD, TaGA3ox2-1, TaGA3ox2-2, TaGA3ox2-2*, these genes act as positive components in its response pathways were up-regulated in hybrid cultivars. A previous study showed that three *Ta14S* homoeologous genes have regulatory roles in seed development and germination via synthesis of 14-3-3 proteins are involved in signal transduction pathways with significant roles in late embryo development in seeds and germination, which represented a high transcription of *Ta14S-2B*[29].

Funding

Self-funding.

Acknowledgments

In this section, we acknowledge any person who supports us to complete this project.

Conflicts of Interest

There is no conflict.

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ARTICLE / INVESTIGACIÓN

Serological detection of Cytomegalovirus in blood samples from infected women in Misan province, Iraq

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DOI. 10.21931/RB/2022.07.04.65

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Abstract: Cytomegalovirus is a species of Herpesviridae can that infect humans and cause latent and severe infections. Severe human infection caused by Human Cytomegalovirus (HCMV) usually occurs in humans who suffer from immunodeficiency. This research aims to detect antibodies IgM as a primary infection and IgG as a secondary infection in women suffering from repeated abortion and determines the more ages susceptible to HCMV infection. Blood sera were taken from females of different ages, from 15-45 years, based on clinical manifestation and abortion. The sample sizes in this study were taken from 150 patients, of which 100 cases were positive for HCMV. We recorded a high percentage of CMV infection (25%) and a high abortion rate (27.5%) in patients at age 20-24 years with a high rate of IgG, and we noticed a low rate of CMV infection (10%) in the age group 35-39 years with a low rate of IgG, we also reported a high abortion rate (27.27%) related with present IgM. The percentage of abortion was (26.66%) reported in patients ages 15-19 and 40-45 years, with (a 33.33%) rate of IgM and IgG.

Key words: Human Cytomegalovirus HCMV, abortion, IgM, IgG.

Introduction

Human Cytomegalovirus (HCMV) refers to the Herpesviridae family, subfamily Betaherpesvirinae. HCMV can cause severe human diseases, especially in immune-compromised patients and children. The CMV infections in adult women cause abortion, while in children can lead to hearing loss[1]. The virus can be transmitted horizontally and vertically, by direct contact, from the mother to her fetus during pregnancy, or through breastfeeding[2–4].

Furthermore, it was reported that the virus transmits sexually three and via blood transfusion four from one infected person to another. HCMV virion is round in shape with 150 to 200 nm diameter and surrounded by a lipid bilayer envelope[5]. The viral genome composes of double-strand DNA linear with a size reaching 236 KB[6]. The genome consists of two segments (units), long unit (UL) and short unit (US), containing around 150 open reading frames (ORFs) that encode 158 proteins, 47 essential and 117 nonessential proteins[7].

The virus infects the human body by attaching to the target cell via viral glycoprotein that is prolonged from the viral envelope[5]. HCMV uses some tegument proteins to invade the immune system of humans, including intrinsic, innate, and adaptive immune systems[8,9]. The virus sheds in body fluids such as saliva, blood, milk, tears and urine and causes asymptomatic infection[8,10]. On another side, the virus causes symptomatic infections in people suffering from weak immunity or immunodeficiency[4].

Detection of IgM and IgG are used for virus identification widely. Testing antibodies in blood samples is highly sensitive and straightforward for viral diagnosis. It was reported that the IgM antibody is the first line of defense, and IgG has a significant role in long-term immunity[11]. IgM can be detected in the case of SARS coronavirus infection after 3-6 days of infection; IgG can also be seen after eight days[12,13]. Therefore, detecting antibodies is one of the effective methods for testing samples from patients suspected of virus infection.

Materials and methods

Collection of blood sera

One hundred samples were obtained from women attending to Obstetrics gynecology Out-patients department in Al-Sadder Hospital and Central Health laboratory in Misan city. Blood sera were taken from women suffering from CMV infection and abortion, fifty women were chosen as a healthy control group, and their ages ranged from 15-45 years. The sera were separated and stored at -20 °C for HCMV antibodies estimation using the Minividas technique.

Detection of Cytomegalovirus

The sera samples were tested using VIDAS (CMVG), and BioMerieux and the procedure were carried out according to the manufacturer's instructions. The serum samples were placed in a ten-pits strip. The pits were: 1: Sample well (serum) contains antigen, Empty well: 2, 3, 4, 5, Conjugate wells: 6, Wash buffer well: 7, 8, 9: Substrate wells (SPR): 10 contains anti-antibody. SPR sucks out the serum in well 1, and then the antigen interacts with the antibody in well 6. During this process, SPR sucks the washing buffer from one of the 7, 8, and 9 pits and return it to empty wells for

Citation: Alrashedi H S H, Hasson H J. Serological detection of Cytomegalovirus in blood samples from infected women in Misan province, Iraq. Revis Bionatura 2022;7(4) 65. http://dx.doi.org/10.21931/RB/2022.07.04.65
Received: 22 September 2022 / **Accepted:** 18 October 2022 / **Published:** 15 November 2022

antigen-antibody conjugation, as this process repeats several time. Next, the unreacted cognates aspirate and return to the empty well. Then, SPR interacts with fluorinated material in the last well, where the enzyme is attached to the substrate to produce a flash (phosphorescent light). Finally, the Mini VIDAS (BioMerieux) device records and analyses the results according to the phosphorescent light produced.

Statistical analysis

The collected data were analyzed using SPSS 26 software.

Results

Effect of CMV infection on fetal cases

Infection with CMV significantly impacts fetus status during and at the end of the pregnancy period in women. In this study, we noticed that the high percentage of fetal death was 70%, while the low rate was 2.4% for abnormal birth. It was recorded that 27.5% of fetuses were normal, as shown in Figure 1. This variation in fetus status is due to many factors, such as congenital CMV that affect fetus growth, high CMV viral load due to low immunity or changes in normal flora count in the uterus and vagina. Many women use contraceptive drugs. These findings agree with the study carried out by (14) as they recorded a high percentage of abortion at 21 weeks and premature fetus cases in women infected with CMV. Therefore, from the figure shown below, it is clear that the infection of CMV does not entirely affect the fetus, and some other factors may be correlated with the normality or abnormality of fetus delivery.

Relation between age groups and CMV infections

This study was based on variables to identify the relationship between age groups and women infected with CMV. The sample size was taken from 125 patients, and 100 cases were positive for CMV. We found that the high percentage of CMV infection was at age 20-24 years (25%), and the low rate of CMV infection was at age 35-39 years (10%), as shown in Figure 2. This might be because the account of normal flora in the uterus and vagina of infected women with HCMV is changed due to the estrogen hormone that affects the immune system and the uterus environment. These results are similar to the finding done in the United States by (15). The authors reported a high rate of CMV infections in women aged between 20 and 24. Therefore the age of the women has a significant role in resistance to CMV infection.

Relation between education levels and CMV infections

Infection with CMV in humans can also depend on the patient's education level factor. In this study, it was found that the high rate of CMV infection, 45%, was in primarily educated women, while the low rate of CMV infection, 15%, was in university-educated women, as shown in Figure 3. This could be because of a lack of health awareness compared to women with higher education levels who care for their health, including preventing pathogens and biological vectors. However, these findings disagreed with a report showing that CMV infection was high in secondary educated women level and low rate of CMV infection in women with primarily educated women[16].

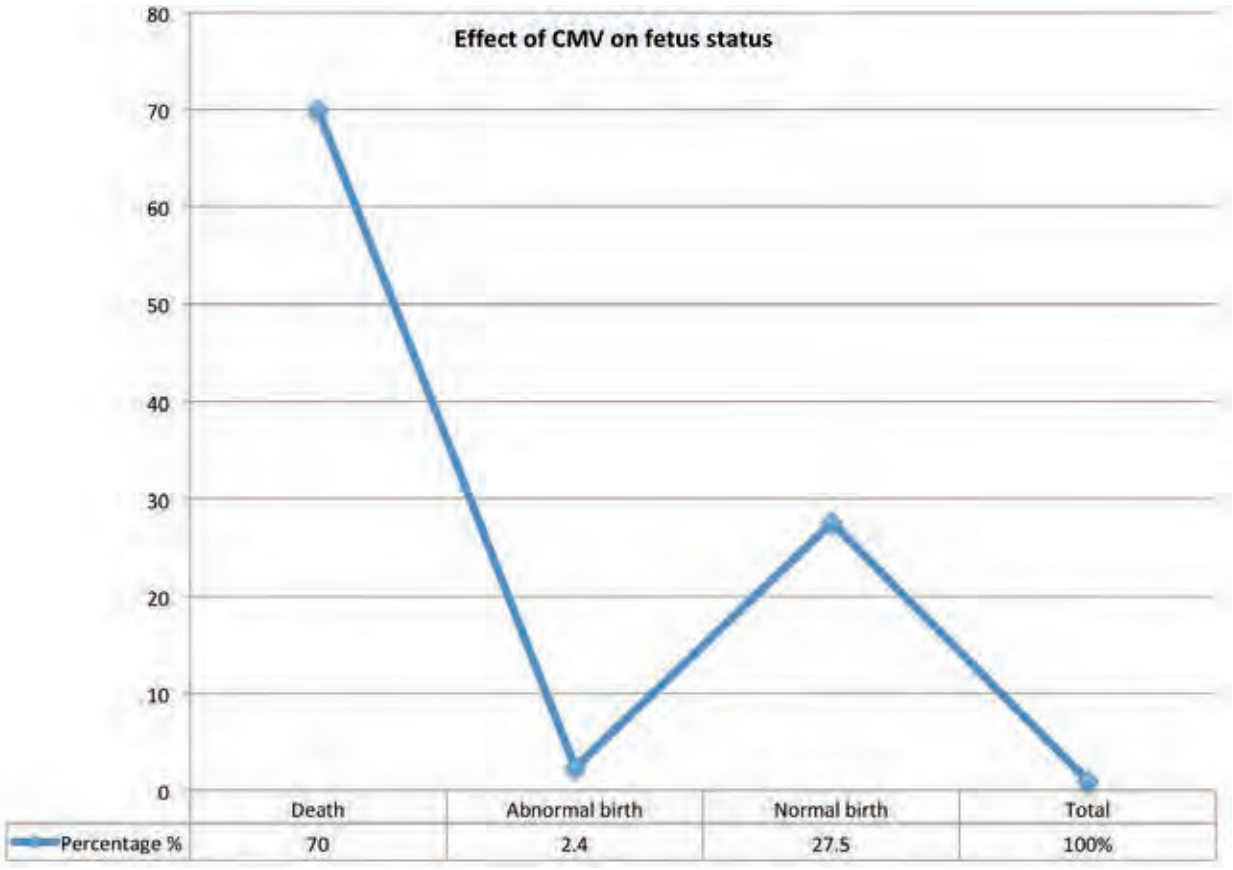
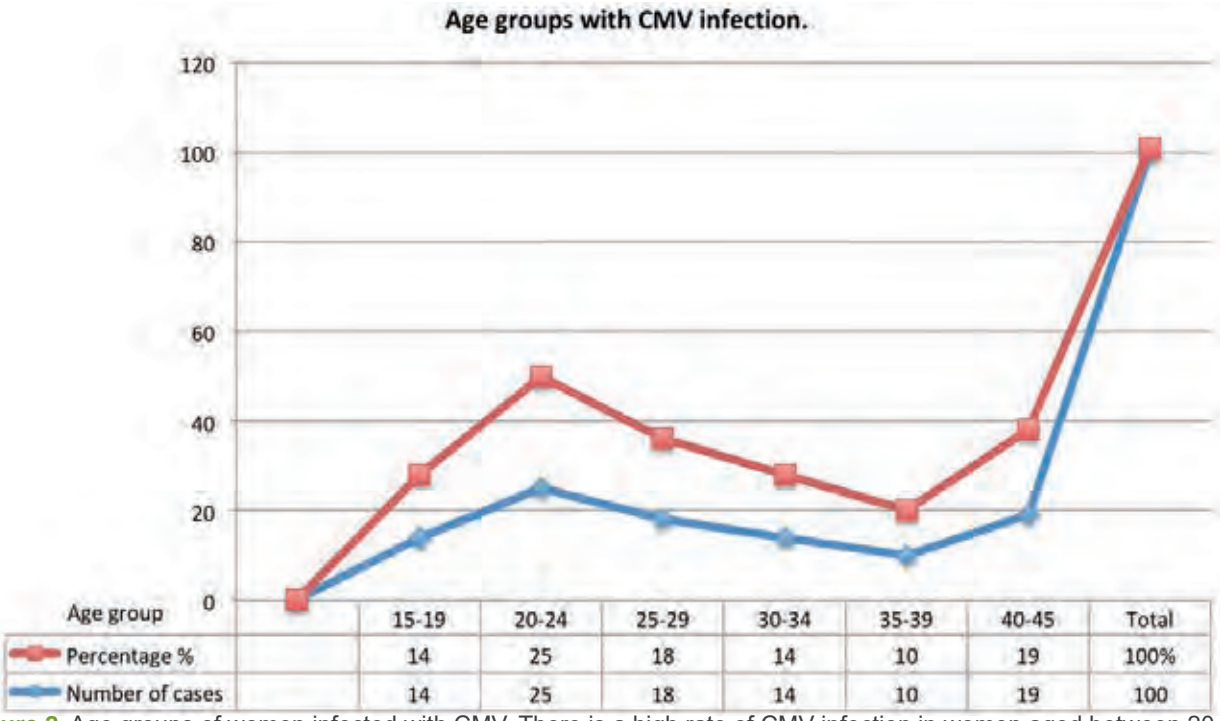
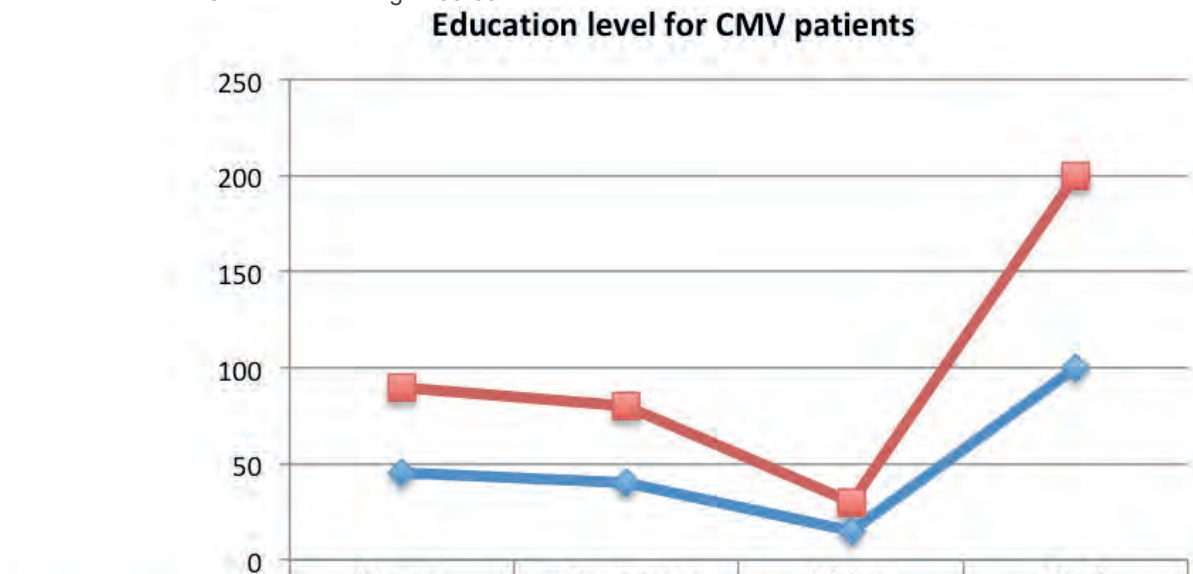


Figure 1. A percentage of cases of birth. The figure shows a high rate of fetal death cases, around a quarter percentage of normal birth, and a low rate of abnormal birth.



Age group	15-19	20-24	25-29	30-34	35-39	40-45	Total
Percentage %	14	25	18	14	10	19	100%
Number of cases	14	25	18	14	10	19	100

Figure 2. Age groups of women infected with CMV. There is a high rate of CMV infection in women aged between 20-24 years and a low rate of CMV in women aged 35-39.



	Primary	Secondary	Higher	Total
Percentage %	45	40	15	100
Number of cases	45	40	15	100

Figure 3. The effect of education level in CMV patients. There is a high percentage of CMV infection in women with a low education level compared to infected women at the university level.

Relation between Immunoglobulin (IgM, IgG) and age of infected women

The levels of immunoglobulin IgM and IgG vary during HCMV infections. In this present study, we detected Immunoglobulin IgM in women that suffer from acute infection. While IgG in women with reactivation or past infection, we also detected IgM and IgG in reactivation or recent infection. In addition, we found that the high rate of IgG was 26 (26.96 %), IgM was 1(20)%, and both IgG and IgM were 2(33.33%), as shown in Table 1. These IgG and IgM rate variations could happen because of many factors, such as changing hormones and infection in reproductive age groups. These findings agreed with other results carried out by (17), in which they reported a high level of IgG. Contrary to these results, a low IgG rate was revealed in women infected with CMV in the Kurdistan region of Iraq[18].

Immunoglobulin and age-related abortion in women

In this study, as shown in Table 2, we found that the high rate of abortion was 27.5 % in the age group 20-40 years associated with IgG 24 (26.9%); it was noticed that the low rate of abortion was 11.1% at age group 35-39 with

Age groups	IgG	Percentage %	IgM	Percentage %	IgM & IgG	Percentage%
15-19	11	12.35	1	20	2	33.33
20-24	24	26.96	1	20	0	0
25-29	16	17.97	1	20	1	16.66
30-34	13	14.60	0	0	1	16.66
35-39	9	10.11	1	20	0	0
40-45	16	17.97	1	20	2	33.33
Total	89	100	5	100	6	100

Table 1. Relation between immunoglobulin and the age group.

a lower rate of IgG 9 (10.1%). In the case of the level of IgM, it was recorded a high rate of abortion in the age group 25-29 and 35-39 years old with 1 (20%)IgM level, while there was no abortion occurred in the case of 0 IgM level at age 15-19. Moreover, the results show that the high rate of abortion was 26.66% in the age group 15-19 and 40-45 years old, corresponding to 2 (33.3%) levels of IgM and IgG that similar to results carried by (19), whereas no abortion in case of 0 IgG and IgM level at age 20-24 and 35-39 years. There is a positive correlation between IgG, IgM and abortion in women infected with CMV.

Relation between IgG, IgM and miscarriage

Miscarriage can cause by HCMV infection. In this study, we recorded a high rate of IgG 89(89%) and 90% miscarriage, while the rate of IgG and IgM were 6(6%) and miscarriage was 5.76%, as shown in Figure.4. Therefore, it is clear that there is a high rate of a miscarriage occurring in women associated with a high level of IgG. These findings are similar to the result revealed by (20). Therefore, there is a high level of IgG measured in women who suffered from miscarriage caused by HCMV.

Conclusions

Human Cytomegalovirus (HCMV) causes abortion in women. In this study, we found that the infections with CMV were different according to age groups, and level of the education of the infected women. We found 25% of CMV infection that is the highest rate in an age group ranging from 20-24 years, this is could be because of the women were in reproductive age, changing hormones, contraceptive and more susceptible to CMV. The relation between IgM is equal in all age groups as found that it was 1(20%) as a primary immune response and IgG as a secondary immune response was 24(26.9%), with age group 20-24 years and 40-45 years. Finally, the total IgM and IgG 2(33.33)% at age 15-19 and 40-45 years respectively, In addition, the percentage of abortion was 4(26.66)% at the same age groups. Therefore, CMV infection in women can lead to abortion and changing fetus status, and further investigation about HCMV in women are recommended.

Age	IgG	Abortion	IgM	Abortion	IgG & IgM	Abortion
15-19	11(12.3 %)	9.8 %	1(20 %)	9.09 %	2(33.3 %)	26.66 %
20-24	24(26.9 %)	27.5 %	1(20%)	18.18%	0	0
25-29	16(17.9%)	20.9%	1(20%)	27.27%	1(16.6%)	20%
30-34	13(14.6%)	16.2%	0	0	1(16.6%)	13.33%
35-39	9(10.1%)	11.1%	1(20%)	27.27%	0	0
40-45	16(17.9%)	14.5%	1(20%)	18,18%	2(33.3%)	26.66%
Total	100%	100%	100%	100%	100%	100%

Table 2. Immunoglobulin and age-related with abortion in women.

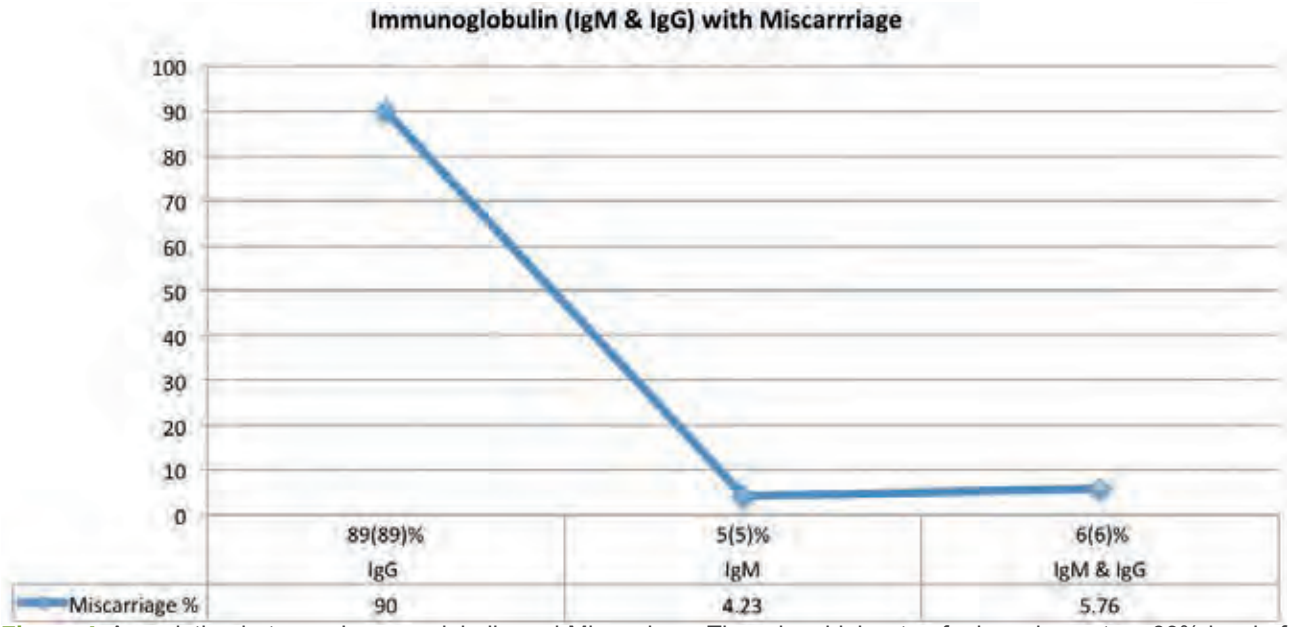


Figure 4. Association between Immunoglobulin and Miscarriage. There is a high rate of miscarriage at an 89% level of IgG, while a low rate of miscarriage at a 5% level of IgM.

Author Contributions

HSHA writing—original draft preparation, editing and project administration. HJH review and validation.

Funding

This research was self funded.

Acknowledgments

Thanks to Mr. Ali Mansi Hummod form Central Health labor tory in Misan city, Iraq, Hussein Majeed Muhammad and Noor Al-Hoda Ali Hussein for their contribution in this research.

Conflicts of Interest

The authors declare no conflict of interest.

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REVIEW / ARTÍCULO DE REVISIÓN

Predominant genetic mutations leading to or predisposing diabetes progress: A Review

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DOI. 10.21931/RB/2022.07.04.66

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Abstract: Diabetes mellitus (DM) arises following poor capacity to generate or secrete insulin or insulin resistance; hence insulin production impairment creates the illness. Individuals can control their weight, impulsivity, blood pressure, and blood lipids at the commencement of the disease. A single genetic mutation affects nearly 3% of people with diabetes. Surprisingly, beta cell function is regulated by more than 20 genes. Benefits of genetic diagnosis include improved therapy, better prediction of illness prognosis and progression, genetic counseling, and possibly prevention. Alpha HNF1 mutations in the early stages may respond to the regimen. Still, most patients need it because they control their blood glucose and will be subject to microvascular or macrovascular complications. In cases where insulin does not control sugar, using low-dose sulfonylureas would be beneficial and lower four times the glucose metabolism of metformin. These patients are susceptible to sulfonylureas and may be treated for years in case of no blood glucose attack complications. The drug will start at one-fourth of the adult dose: MODY1. It is caused by a mutation in the alpha-HNF 4 gene and is relatively uncommon. The same is true, but the threshold for renal excretion is not low, and the incidence of upward alpha-HNF 4 mutations in cases where there is a robust clinical panel for alpha HNF 1 but not confirmed by genetic sequencing should be considered. The disease is also susceptible to sulfonylureas: MODY4 with a mutation in the MODY6 gene, IPF1, with a mutation in MODY7, NeuroD1 is characterized by a carboxy sterilise mutation, which is not common: MODY2. In children and adolescents, an increment in fasting blood glucose of 100 to 150 mg/dl is not typical. The incidence of this condition is usually considered to be type 1 or 2 diabetes, but a large percentage of the above patients are heterozygote individuals, the glucokinase mutations. Specific mutations, including those rare variants in WFS1 and ABCC8 genes, insulin receptor (IR), fructose 6-phosphate aminotransferase (GFPT2), and nitric oxide synthase (eNOS), as well as mouse pancreatic β-cell lines (Min6 and SJ cells), showed that the HDAC4 variant (p. His227Arg) had been directly linked with T2DM.

Key words: Type-2 diabetes, genetic mutations, risk factors.

Introduction

Diabetes is a metabolic disorder that affects the human body[1]. The body's ability to produce or release insulin hormone is lost or becomes insulin-resistant, resulting in decreased insulin production. The primary function of insulin is to reduce blood sugar levels through various processes[2].

Diabetes can be categorized into two types. Pancreatic β-cell degeneration causes defective insulin production in type 1 diabetes. In contrast, in type 2 diabetes, a progressive resistance to insulin occurs in the body that can eventually result in pancreatic-cell degeneration and complete insulin-producing insufficiency. Genetic factors, obesity, and dementia 3 influence type 2 diabetes.

Diabetes impairs the body's ability to use and metabolize glucose and lower blood glucose levels, culminating in hyperglycemia[2,4]. Long-term sugar accumulation in the body destroys very small veins, affecting different organs such as the kidneys, eyes, and nerves[5]. Since diabetes raises the risk of cardiovascular disease, screening and early diagnosis of the condition in high-risk people can help prevent these complications[6].

Factors Affecting Type 2 Diabetes

Many factors contribute to the onset of diabetes, some of which can be controlled by individuals, such as weight, impulsivity, blood pressure, and lipids. Other cases, however, appear to be linked to the disease[7]. For instance, if someone in your family has diabetes, you are also at risk of developing it (which points to genetic influences in the disease)[7,8]. Some surveys have found that certain breeds, including blacks, Spaniards, and American Indians, are more likely to show the impact of race on the disease[9]. The risk of developing diabetes, however, rises with age. The prevalence of diabetic patients with the polycystic ovarian syndrome was shown to be higher (PCOS) in females[10-12].

Gestational Diabetes

Any elevation in blood glucose during pregnancy reaching a high level of 5-10% of the population is called gestational diabetes[1]. It has been determined that pregnancy itself can be one of the causes of diabetes. This effect is due to increased body resistance to insulin and increased insulin to compensate for this problem. Pregnancy can reveal even mild deficiencies of insulin secretion, leading

Citation: Banoon S R, Alfathi M Y, Shokouhi Mostafavi S K, Ghasemian A. Predominant genetic mutations leading to or predisposing diabetes progress: A Review. Revis Bionatura 2022;7(4) 66. http://dx.doi.org/10.21931/RB/2022.07.04.66
Received: 22 September 2022 / **Accepted:** 18 October 2022 / **Published:** 15 November 2022

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to glucose intolerance and gestational diabetes[13]. On the other hand, some patients with mildly diabetic glucose are screened during pregnancy under this group[4]. Gestational diabetes involves 3-8% of pregnancies and is also one of the risk factors for poor pregnancy outcomes. This condition can also cause type 2 diabetes. Research has outlined that almost half of these women will develop diabetes over the next 20 to 30 years[14]. Increased gestational diabetes has been reported in recent years. Several factors have contributed to this increase. These include the high prevalence of obesity among young patients and improving the survival of female children whose birth weight is at the two ends of the normal birth weight range. In adulthood, these children have impaired insulin function or the ability to secrete insulin that can predispose them to gestational diabetes[15]. Gestational diabetes can cause serious complications for the mother and the baby. These complications can be mitigated by proper diagnosis and treatment. Women with high risk for 75G glucose tolerance test (OGTT) are tested at the first post-pregnancy visit, and a re-test is implemented at 24 to 28 weeks of pregnancy. Treatment for gestational diabetes is primarily a diet and physical activity, and insulin therapy is used to control sugar in the absence of response. These women are also tested regularly after pregnancy[16].

Other types of diabetes

Genetic defects of β-cells

Diabetes is a complex and heterogeneous condition characterized by persistent hyperglycemia caused by defects in insulin secretion, insulin resistance, or a combination of the two. It has been estimated that roughly 5% of all types of diabetes are caused by these mutations. Nevertheless, accurate diagnosis is vital in treatment, prognosis, and assessment of the risk in the family[17]. The most frequent type is usually linked with an increase in low glucose levels (under 25 years of age) and is acknowledged as the most commonly diagnosed diabetes mellitus in young patients (called MODY, which is the acronym for maturity-onset diabetes of the young). This type is an autosomal recessive disorder which affects six chromosomal locations. The mutations mainly occur (50-70 percent of cases) on chromosome 12 in the hepatic transcription factor, also known as HNF-1 mutation[18].

The second one is associated with the glucokinase mutation on the 7p chromosome, leading to the production of a defective glucokinase molecule (the enzyme catalyzes the formation of glucose-6-phosphate) and stimulation of insulin secretion. Higher glucose levels are required for normal insulin secretion due to this mutation. Other gene transcription factors with sporadic mutations include HNF-4α, HNF-1β, IPF-1 and NeuroD1[19]. Genetic tests for this type are commonly used in cases where the incidence of diabetes is low and unusual symptoms associated with type 1 diabetes and 2 are observed or a strong family history of this type is recommended[20]. Maternally Inherited Diabetes and Deafness (MIDD) is caused by an alteration or mutation in mitochondrial DNA (the most frequent change is known as 3243A>G) and was first identified in the early 1990s[21]. The disease is associated with diabetes and deafness[22]. The most common form of mutation in position 3243 is in the tRNA of the leucine gene. A similar lesion is observed in MELAS (Mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes); however, diabetes is not part of the syndrome[23]. In small families, genetic disorders resulting in the inability to convert proinsulin to insulin have also been observed. This is inherited as an autosomal dominant trait. In this case, the glucose disorder is mild[24]. Neonatal diabetes mellitus is another heterogeneous group of diabetics that occurs up to 6 months of age and has an approximate incidence of 1:100,000 live births with variations within different ethnic groups. Several mutations that interrupt the process of pancreatic organogenesis, the formation of -cells, and the production of insulin cause the disorder. Abnormalities of the 6q24 region and mutations of the genes coding for the ATP-dependent potassium channel are the most common genetic causes of neonatal diabetes with normal pancreatic shape[17].

Disorders of the insulin function

Genetic disorders of insulin function include unusual cases of diabetes. Metabolic disorders caused by these mutations comprise hyperinsulinemia, mild hyperglycemia, and severe diabetes. Some patients with these defects may have nigricans acanthosis[25]. Women may exhibit male body traits and have cystic ovaries. In the past, this syndrome was considered an insulin resistance type. Leprechaunism (also known as Donohue syndrome) and Rabson-Mendenhall are two syndromes in children that contain mutations in insulin receptors resulting from severe insulin resistance[26].

Outbreaks of pancreatic bronchitis

Diabetes is one of the chronic complications of chronic pancreatitis. The difference in this type of diabetes is the destruction of the pancreatic endocrine, and therefore the glucagon secretion also mitigates; thus, in these diabetic patients, the risk of hypoglycemia (a decrease in blood sugar) is sought after treatment[27]. Apart from pancreatitis, diabetes can be a complication of any damage to the pancreas, including infections, pancreatic surgery, and pancreatic cancer[28].

Endocrine disorders

Several insulin-like hormones participate, and excessive discharge can lead to diabetes initiation. Usually, this situation is observed in patients predisposed to diabetes due to defective diabetes insulin secretion[29]. Increased growth hormone and cortisol are commonly caused by hormonal disorders that lead to diabetes, resulting in increased complications and cardiovascular mortality in these diseases owing to diabetes. These hormonal disorders have been attributed. It is estimated that 16-56% of patients have acromegaly, and a 20-50% increase in diabetes mellitus occurred in Cushing's syndrome[30].

Diabetes through drugs or chemicals

Irreversible degeneration of β-cells may occur in rare cases following the administration of mouse poison vapor or intravenous pentamidine. Some medications can also interfere with insulin function. For example, nicotinic acid and glucocorticoids are from this category. Patients taking interferon-alpha also have antibodies to pancreatic anesthetics or severe insulin deficiency in some cases[31]. Some high-performance and relatively safe drugs are associated with an increased risk of diabetes, including anti-hypertension, statins and beta-blockers[32]. Regarding statins studies have exhibited that this increase in risk is meager, and at present, this increase in trouble does not justify stopping or reducing statin use[33].

Rare types

Infections: Some infections such as measles, congenital cytomegalovirus, and coxsackie virus subtypes by 3 and 4[34].

Types of Rare immunity: Stiff man's syndrome, anti-insulin-receptor antibodies[35].

Other genetic syndromes that are associated with increased risk of diabetes include Down syndrome, Klinefelter syndrome, Turner syndrome, Wolfram Syndrome, Friedreich's ataxia (FRDA), Huntington's Disease, Laurence-Moon-Bardet-Biedl syndrome (LMBBS), Myotonic Dystrophy, Porphyria, and Prader Willi Syndrome (PWS)[36].

Informal categories

The last official categorization of diabetes was by the American Diabetes Association in 1997, and despite its problems, it is still underlined by the main authorities[37]. However, diabetes has long been characterized by a heterogeneous disease and may require a new division. Below are some suggestions for categorization:

Type one and a half diabetes

Latent Autoimmune Diabetes of Adults (LADA), a subtype of type I that has characteristics of type 2 diabetes, is supposed to be one and a half in between the two types of diabetes[38]. Those under age 50 are slimmer, have a history of autoimmune diseases, and will likely require insulin within 5 years after the onset of the disease[39].

Type 3 diabetes

Epidemiologic and scientific evidence suggests a common pathophysiology of type 2 diabetes (T2DM) and Alzheimer's disease (AD). As far as the hypothesis is concerned that AD may be "type 3 diabetes". Researchers have found that insulin resistance being the main marker as a mechanism for diabetes, also occurs in the brain. It is worth considering that, unlike insulin-dependent members, the muscle, the liver, and the fatty tissue, the brain is one of the insulin-independent organs, not requiring insulin to enter glucose into the cells. Then they disclosed that the resistance is associated with Alzheimer's disease[40]. Another study outlined that Alzheimer's can develop in the brain without hyperglycemia[41].

There is growing interest in detecting the role of insulin genes and insulin-like growth factor (IGF) and its related receptors (T2DM) in developing cognitive impairment, Alzheimer's disease and Aβ lesions in the brain. This relationship suggests that insulin and the mechanism of insulin signaling are essential for the survival of neurons. Noticeably, studies have exhibited reduced brain growth and increased tau phosphorylation in insulin-receptor-2-impaired mice. On the other hand, various studies have revealed that extractable amyloid beta (ADDL) ligands may also be responsible for this phenomenon. ADDL has similar morphology and size oligomers to prions associated with neurodegenerative diseases. ADDLs may lower insulin levels and insulin resistance in the brain of Alzheimer's patients. It is concluded that the term "type 3 diabetes" indicates that Alzheimer's disease is a type of diabetes that selectively involves the brain and has molecular and biochemical properties that overlap with type 1 and type 2 diabetes[41-43].

Signs and symptoms

In the early stages, diabetes may be asymptomatic. Many patients are accidentally diagnosed in a test or during screening. As blood glucose levels enhance, symptoms of diabetes will become more apparent. Hyper-uremia, overdrinking, overeating, and weight loss, despite high appetite, fatigue, and blurred vision, are common symptoms of diabetes. Many patients have had diabetes for several years at diagnosis and even have diabetes complications. In children with type 1 diabetes, symptoms usually appear suddenly; these patients are generally healthy, while not obese previously. In adults, these symptoms tend to be more pronounced. Ketoacidosis can be observed as a symptom of the onset of the disease in type 1 diabetes[44]. In type 2 diabetes, an individual is usually asymptomatic for several years. Symptoms are generally mild and gradually deteriorate. Eventually, the person suffers from excessive fatigue and blurred vision and may develop dehydration. In these patients, the incidence of ketoacidosis is lower due to insulin production. However, blood glucose can increase to very high levels, and the person may develop hyper-muscular shock. The results of a study exhibit that stress and depression increase the likelihood of stroke and death from cardiovascular disease in patients with diabetes more than twice[45].

Risk factors for type 2 diabetes

Some risk factors are as follows:

- Age over 40 years
- First-degree family with type 2 diabetes
- A history of pre-diabetic glucose disorders (impaired glucose tolerance, impaired fasting glucose)
- The history of gestational diabetes
- The history of the birth of a baby with macrosomia (overweight)
- The presence of complications of diabetes on the organs of the body
- There are risk factors for cardiovascular disease (such as high blood fat, high blood pressure, and obesity)
- The presence of diabetes-related diseases (polycystic ovarian syndrome, acanthosis nigricans, HIV infection, and some psychiatric disorders such as schizophrenia, depression and bipolar disorder)
- Use of diabetes medications: Corticosteroids, atypical antipsychotics, HIV / AIDS treatment[46-49].

Pre-diabetic disorders

In patients with type 2 diabetes, diabetes develops gradually over several years. Patients undergo an asymptomatic preparatory phase before reaching clear and distinct diabetes. The group prescribes pre-diabetes as some source of "increased risk of diabetes." The World Health Organization also calls them "hyperglycemia"[50,51].

In this group of patients, the criteria for diagnosis of diabetes, such as fasting plasma glucose FPG or hemoglobin A1C, or oral glucose tolerance test (OGTT), is higher than the normal values and does not go well enough to diagnose diabetes mellitus. Accordingly, in each of the three methods of diagnosis of diabetes, we are confronted with a group that has normal values to diabetic levels in the middle[52].

Regarding fasting blood glucose, the normal value is below 100 mg / dL, and the limit for diagnosis of diabetes is 126 mg / dL. Therefore, patients with fasting blood sugar of between 100 and 125 mg / dL, having dysfunction of pre-diabetics, are called "Impaired fasting glucose" (IFG).

In hemoglobin A1C, normal values are below 5.6%, and the diagnostic limit of diabetes is more significant than

6.5%. Hence, individuals with values between 5.7% and 4.6% are included in the pre-diabetic group.

In the glucose tolerance test, normal blood glucose levels are less than 140 mg / dL two hours after eating 75 milligrams of sugar, whereas, for diabetes, this value should be greater than or equal to 200 mg / dL, which is why this subgroup of patients, whose values range from 140 to 199 milligrams per deciliter, is called "impaired glucose tolerance" (IGT)[53].

Patients in these three groups do not necessarily overlap. In other words, someone may have normal fasting blood glucose but with a diabetic pre-diabetic glucose tolerance test.

The next point is that, in the clinical practice field, the use of a sugar tolerance test, although accurate, is not usually recommended for diagnosis of diabetes, nor pre-diabetic diagnosis, and is most often used for research and epidemiological research. The current criteria are fasting blood glucose or hemoglobin A1C, considered the most appropriate test for asymptomatic individuals[50].

But the most crucial issue in diabetic patients is their outcome and how to deal with this disorder. Patients with type 2 diabetes should have a pre-diabetic condition before they develop diabetes, which will put them at risk of being able to prevent diabetes by appropriate treatment. Today, there is a controversy about the drug treatment of these patients. The American Diabetes Association ADA says that if a predisposed person has high-risk criteria (body mass index BMI greater than 35, a family history of diabetes in first-degree relatives or a woman who has a history of gestational diabetes), it is better to use metformin to prevent the onset of diabetes. However, most patients do not have these criteria and must change their lifestyles. These patients must have a curriculum to lose weight and increase physical activity by regular exercise at least five times a week. These patients should also be tested annually for diabetes, while they, besides diabetes, are at a higher risk for cardiovascular disease and therefore need to be screened for cardiovascular disease[54].

Exercise and diabetes

Exercise helps people with diabetes avoid heart attacks, blindness, and nerve damage. When you eat, your blood sugar levels increase. The more blood levels increase, the more sugar the cells stick. When sugar is attached to the cell, it can no longer be separated and become a harmful substance called sorbitol, which can cause blindness, deafness, brain damage, and burning legs syndrome[55].

When sugar is delivered to your body, it can only be stored in the liver and muscle cells. The sugars have no place to go if the liver and muscle cells are saturated with carbohydrates. If the storage of muscle cells is consumed after exercise, after the meal, sugars are absorbed by the muscle, and their amount does not increase in the blood[56].

However, transcription factors seem not to be the only factors involved in differentiating α to β cells. In a study in this regard, stem cells were used to create insulin-producing β cells. However, observations suggest that these cells cannot respond to glucose extracellular stimuli. By inserting these cells into mice's bodies and maturation of these cells for five months, these cells find the ability to balance the blood sugar relative to the extracellular stimulus of glucose. Epigenetic changes play a significant role in determining the fate of endocrine cells and their full maturity. In other words, transcription factors and epigenetic modifications both play a role in deciding the fate of a cell and a specific feature of that cell[57].

DNA methylation is one of the epigenetic changes contributing to a cell's survival. Recent studies have exhibited that the role of methylation in α pancreatic cells is not limited to cell survival. In these cells, DNA methylation maintains the specific character of α cells and differentiates them from other endocrine cells. Dnmt1 is one of the enzymes involved in DNA methylation, and the researchers study simultaneously the enzyme and the Arx transcription factor to differentiate α to β cells[58].

Removing the Arx transcription factor alone causes 30% of α cells to become β-like cells after 12 weeks. However, newly created cells do not have specific markers of β-cell maturation. In contrast, with the removal of Dnmt1, none of the particular early factors of β cells occurred after 10 months. In the next step, the removal of both Arx and Dnmt1 factors took place. In the 12th week, the elimination of these two factors resulted in more than 50% of α-cells able to produce insulin, and the expression of specific factors associated with β-maturity was observed. Notably, the newly produced β cells are similar to those of the β core in terms of their physiological properties. As a result of this research, the importance of two factors, Arx and Dnmt1, was identified in maintaining the characteristics of α cells. It was also exhibited that the elimination of these two factors contributes to the specific properties of β cells[59]. Diabetes treatment can also be considered. Treatment for diabetic wounds is implemented in different ways:

Foot care: This includes moisturizing the wound environment by choosing the right ingredient, as well as keeping the edges of the wound dry. In patients with insulin-dependent diabetes mellitus, care lasts for about 3 years.

Antibiotics: Antibiotics are prescribed even when infections have not occurred (prophylaxis) to prevent infection.

Blood Glucose Control: One of the causes of diabetic wounds is high blood sugar. High blood glucose reduces immunity and delay wound healing. Blood glucose control, either as a medicine or as a diet, as well as short-term insulin administration, would improve ulcer healing.

Skin transplant: This can also treat diabetic wounds.

Surgery: Removing dead tissue around the wound site is usually implemented to cleanse and heal the wounds. Bypass surgery improves blood flow to the legs, which may help heal the wound and prevent amputation, and it is needed at the end of the amputation to stop the infection from spreading.

Hyperthermia Oxygen Therapy: Increasing the concentration of O_2 from 20% to 100% by 5 times and increasing its pressure from 1 atm to 2 atm total leads to a 10-fold increase in oxygen content. One of its effects is the formation of more blood vessels in the area mitigating blood flow and more proper flow to areas that have blocked blood. Hyperbaric oxygen therapy seems to help lower amputation.

American researchers believe that the current treatment of diabetes can help adults to live well with the aging process[60].

Types of Monogenic Diabetes and Their Care

Diabetes mellitus under the age of six months and neonatal diabetes

There is usually no diabetes mellitus in this age range. The HLA analysis also identifies more protective types against type one[61]. Neonatal diabetes begins at the beginning of the first three months of life, and insulin needs to control the sugar. Clinically, there are two subgroups for the neonatal subgroup. The transient type of neonatal diabe-

tes is characterized by an improvement of about 12 weeks, though more than 50% of them eventually relapse. This type needs lifelong treatment after diagnosis. There is a neonatal transitional diabetes mellitus and a neonatal mutation gene (ABCC8, KCNJ11 receptor sulfonylurea)[62]. For both cases, the use of sulfonylureas may be beneficial. If both parents have glucose intolerance, heterozygote or homozygote mutations are common. At the onset of neonatal diabetes, it can be challenging to detect transient or persistent diabetes. The common cause of transitional neonatal diabetes is a disorder of the gene 6 q24[63]. Parental or dystonic polysaccharides and methylation are common disorders. Diabetes mellitus begins in the first week and recovers at 12 weeks. In 50% of cases, diabetes recurs in a child[64].

Macroglossia is observed in 23% of cases. Blood glucose is between 200 and 1000 mg/ dL and is needed to control insulin requirements, but insulin needs to be reduced quickly. In the phase of patient improvement, care should be taken[65]. Treatment with sulfonylureas and metformin has not been appropriately evaluated. The second most persistent and transient diabetes infection in the first 6 months of the mutation is Kir 6.2 gene. While 10% may be temporary (although the likelihood of relapse is high), most cases of diabetes are due to this permanent gene deficiency. Most patients only have diabetes, but there may be neurological manifestations in 20% of cases. In 90% of cases, the disease is due to a new mutation. Severe cases may be associated with severe developmental delay and epilepsy, similar to West syndrome. This condition is known as the DEND syndrome (Delay, Epilepsy Neonatal Diabetes Developmental), with a complete clinical profile of insulin dependence, and 30% of them are referred to by ketoacidosis. Peptide C levels cannot be measured, and in these patients with sulfonylureas, The whole face is not treated. Still, relatively good control can be given to them without the risk of hypoglycemia[66]. A dose of 0.5 mg/kg is used to manage glibenclamide properly.

Wolcott-Rallison syndrome is characterized by diabetes, episodial dysplasia, renal dysfunction, acute liver failure, and developmental delay with recessive inheritance. This disease is associated with the mutation of E1F2AK3. Diabetes usually precedes early life. (Although there may be delayed onset), and decreasing beta cells and reducing insulin secretion by an immune mechanism[67].

There is a need for insulin for treatment. This diagnosis should be considered in diabetic patients starting at age 3 and with episodial dysplasia or acute renal failure[68].

Familial types of diabetes

MODY3 The possibility of monogenic diabetes in cases where one of the parents is diabetic (type 1 or 2) should be considered[69]. The most common form of familial diabetes mellitus is due to the mutated nuclear factor of hepatocytes, alpha-HNF 1, which are characterized by:

- Diabetes mellitus that begins in the lower ages but is not insulin-dependent (not associated with ketoacidosis, the low metabolic rate of insulin is produced appropriately). There is a secretion of the C Peptide outside the honeymoon period[70].
- There is a family history of diabetes. Diabetes begins in parents at 20-30 or even at 40 and may be treated with either insulin. They may even have one of the grandparents or grandparents[71].
- Glucose tolerance testing in the early stages of high blood sugar (usually more than 90 mg). Some patients may have normal fasting blood glucose. Still, at the second glucose test, they reach the diagnostic range of diabetes[72].

Blood glucose is often observed even though blood glucose is average because the threshold for renal excretion is low in patients.

Severe hypersensitivity to sulfonylureas causes hypoglycemia (despite poor glycemic control before the onset of the drug)[73].

Major mutations in diabetes

Mutations are genetic sequence variations that can have a wide range of effects on a person's health[86]. A single genetic mutation affects about 3% of diabetic patients. Surprisingly, more than 20 genes are involved in beta cell activity. Improved therapy, better prediction of disease prognosis and progression, genetic counseling, and possibly prevention are all benefits of genetic diagnosis[74].

Alpha HNF1 mutations in the early stages may respond to the regimen. Still, most patients need it because control of their blood glucose will be confused and subject to microvascular or macrovascular complications. In cases where insulin does not control sugar, low-dose sulfonylureas will be beneficial and lower 4 times the glucose metabolism of metformin[75] (Table 1). These patients are susceptible to sulfonylureas and may be treated for years if they have no problems with blood glucose attacks. Glycemic control with sulfonylureas will be better than insulin to avoid hypoglycemic episodes. The drug will start at one-fourth of the adult dose: MODY1[76]. It is caused by a mutation in the alpha-HNF 4 gene and is relatively uncommon. The same is true, but the threshold for renal excretion is not low, and the incidence of upward alpha-HNF 4 mutations in cases with a solid clinical panel for alpha HNF 1[77], but not confirmed by genetic sequencing, should be considered. The disease is also susceptible to sulfonylureas:

MODY4 mutation in the MODY6 gene, IPF1, with a mutation in MODY7, NeuroD1, is characterized by a carboxy sterilipase mutation, which is not typical: MODY2. The increase in fasting blood glucose in 100 to 150 mg/dl is uncommon in children and adolescents. The incidence of this condition is usually considered to be type 1 or 2 diabetes, but a large percentage of the above patients are heterozygote individuals, the glucokinase mutation mutations[78] (figures 1 and 2).

Identifying the genetic mutations underlying early-onset diabetes is critical for determining the specific diabetes subtype, allowing for proper treatment and assessment of recurrence risk in offspring. Given the disease's great genetic and clinical heterogeneity, high-throughput sequencing may provide additional diagnostic insight if Sanger sequencing is ineffective[79]. In one survey, 102 genes were re-sequenced in 30 patients who tested negative for mutations in the GCK, GCK, HNF1α, HNF4α, HNF1β, and IPF1 genes using Sanger sequencing. Undetermined mutations in the RFX6 gene were discovered in three patients, and rare variants in the WFS1 and ABCC8 genes were discovered in two of them[23]. All of the patients responded favorably to dipeptidyl peptidase-4 (DPP4) inhibitors. According to their findings, next-generation sequencing (NGS) is a susceptible method for identifying variants in new diabetes-causing genes. This path may help to understand the molecular etiology of diabetes and provide more personalized treatment for each genetic subtype[80].

Diabetes mellitus is linked to several natural mutations in the human insulin gene. Wakayama, Los Angeles, and Chicago mutant molecules were evaluated using molecular docking and molecular dynamics (MD) to investigate mechanisms of deprived binding affinity for insulin receptors

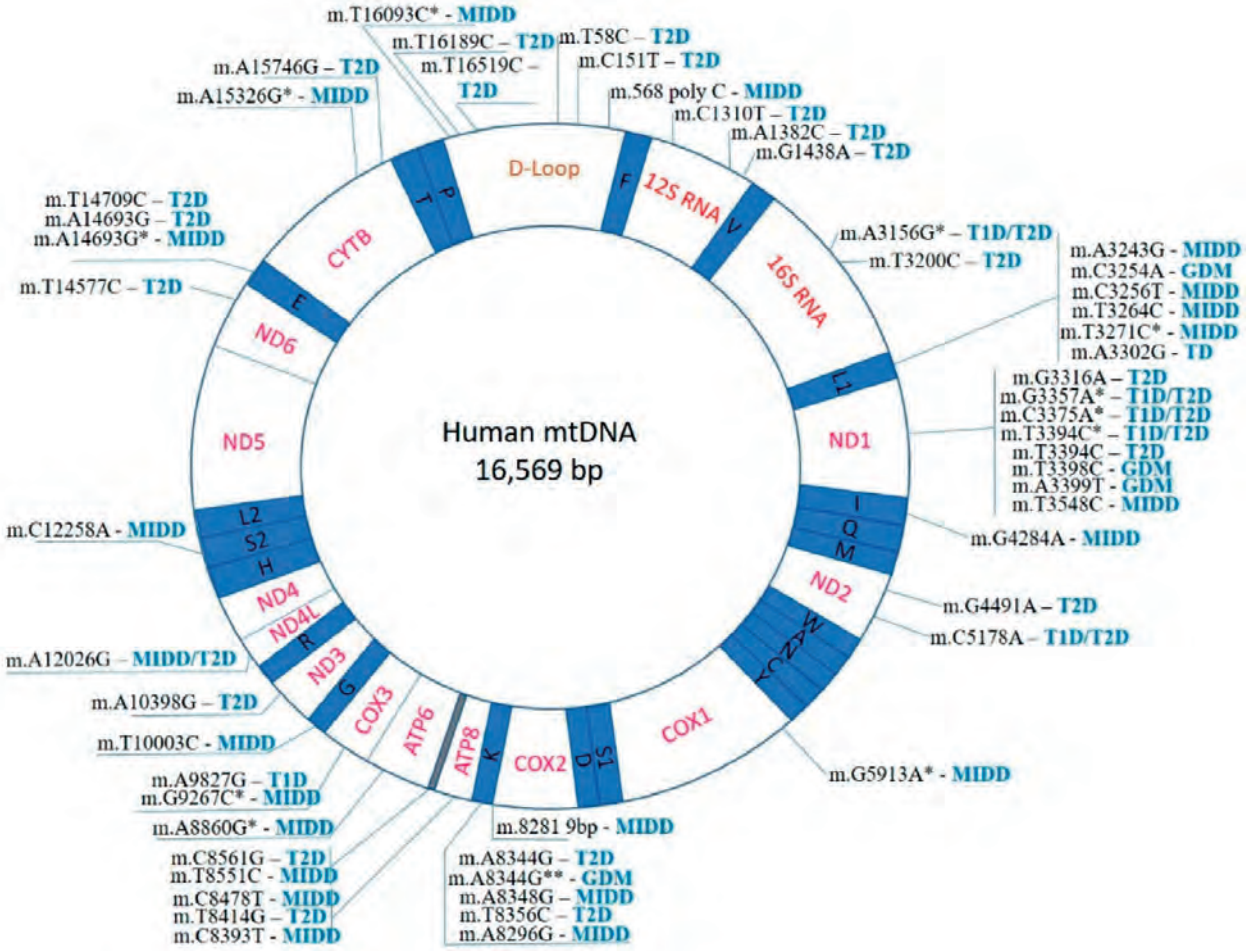

Figure 1. Mutations in the human mitochondria and diabetes (Biorender Program).

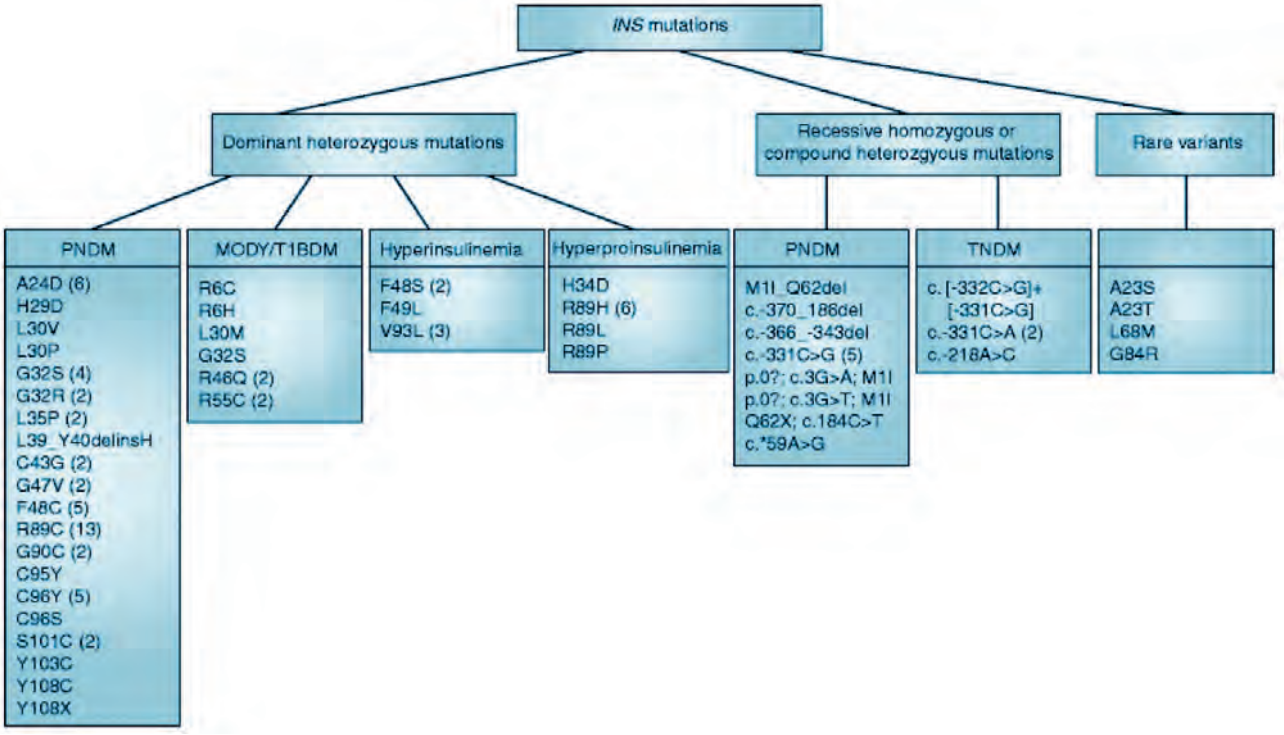

Figure 2. Pediatrics diabetes and insulin mutations (Biorender Program).

Function	Gene, Reference
C282Y gene mutation is a potential genetic marker for type 2 diabetes	Cysteine at position 282 (C282Y). [87]
A functional link between *MTNR1B* variants and the risk of developing T2D	The rs10830963 variant is located in the intron of the *MTNR1B* gene. This gene encodes the melatonin MT_2 receptor, a member of the family of G protein-coupled receptors involved in regulating circadian and seasonal rhythms. [88]
The presence of the G319S mutation in the HNF-1alpha gene in Indigenous youth with type 2 diabetes is associated with higher pancreatic lipid content	G319S mutation in the HNF-1alpha gene. [89]
Variant allele of H63D polymorphism appears to be associated with diabetic nephropathy.	Three missense mutations in HFE gene, C282Y, H63D, and S65C have been known to influence body iron status [90]
Independently of T2D, increased hepatic expression of HNF1A promoted a pro-inflammatory and pro-atherogenic serum profile mediated partly by enhanced transcription of risk genes, including PCSK9. In summary, ~1:300 individuals carry a GOF variant in HNF1A that protects carriers from diabetes but enhances hepatic secretion of metabolic disease risk factors.	Hepatocyte Nuclear Factor 1A (HNF1A) [91]

Table 1. Major mutations in the T2DM

(IR)[23]. Insulin Wakayama, is a variant in which valine at position A3 is substituted by leucine. In contrast, in insulin, Los Angeles and Chicago, phenylalanine at positions B24 and B25 are replaced by serine and leucine, respectively[81]. These mutations cause significant changes in IR binding affinity. The molecular docking was done using the ZDOCK server, and the MD study was done using AMBER 14. MD was also performed using the previously published crystal structure of IR bound to natural insulin. In detail, the binding interactions and MD trajectories explained the critical factors for deprived binding to the IR. When valine was replaced by leucine, the surface area around position A3 increased. In contrast, at positions B24 and B25, the aromatic amino acid phenylalanine was replaced by non-aromatic serine and leucine, which may be responsible for fewer binding interactions at the IR binding site, leading to complex instability. The standard mode analysis, rmsd trajectories, and fluctuation prediction in the MD simulation indicated instability of complexes with mutant insulin in the order of native insulin Chicago insulin, los Angeles insulin Wakayama molecules, which corresponds to the biological evidence of the mutant insulins' differing affinities for the IR[81,82].

Direct sequencing was used to screen the INS gene. A mixed-meal test was performed on the probands and their affected relatives. I-TASSER was used to model mutation predictions, which were then visualized using Swiss-Pdb Viewer[83]. In three generations of patients with clinically distinct diabetes, a novel heterozygous frameshift mutation p.Gln78fs in the INS gene was discovered. The single nucleotide deletion (c.233delA) is predicted to change and lengthen the amino acid sequence, resulting in aberrant proinsulin lacking native C-peptide and A-chain structures. The heterozygous mutation c.188-31G>A within the terminal intron was discovered in the second family. The mother and her daughter were misdiagnosed with type 1 diabetes when they were 6 and 2 years old, respectively. This finding contrasts with the previously reported case of a carrier of the same mutation diagnosed with permanent neonatal diabetes. We discovered a new coding frameshift mutation and an intronic mutation in the INS gene that cause childhood diabetes. INS mutations can cause a variety of phenotypes, implying that additional mechanisms are at work in the pathogenesis and clinical manifestation of diabetes[84].

The glutamine fructose 6-phosphate aminotransferase (GFPT2) gene, which is located on the long arm of chromosome 5, has recently been linked to type 2 diabetes. The TT genotype was linked to the disease in one study. Compared to the control, the nitric oxide synthase gene (eNOS) mutation in Glu 298 Asp was associated with type 2 diabetes[23]. Mendelian diabetes develops as a result of molecular mechanisms that modulate cell pathophysiology. As a result, Class IIa histone deacetylases (HDAC4, 5, 7, and 9) regulate pancreatic endocrine cell activity and glucose homeostasis in mammals. Sanger sequencing on mouse pancreatic cell lines (Min6 and SJ cells) identified an HDAC4 variant (p.His227Arg) as a disease determinant. However, two other variants, p.Asp234Asn and p.Glu374Lys were also found in non-autoimmune diabetes[85].

Conclusions

Diabetes mellitus (DM) is caused by a lack of ability to produce or secrete insulin or by insulin resistance; thus, insulin production impairment causes the disease. Individuals can control some factors that contribute to the onset of the disease, such as weight, impulsivity, blood pressure, and lipids. A single genetic mutation affects about 3% of diabetic patients. Interestingly, more than 20 genes are involved in the function of beta cells. Improved therapy, better prediction of disease prognosis and progression, genetic counseling, and possibly prevention are all benefits of gene diagnosis.

Alpha HNF1 mutations in the early stages may respond to the regimen. Still, most patients need it because they

control their blood glucose and will be subject to microvascular or macrovascular complications. In cases where insulin does not control sugar, using low-dose sulfonylureas will be beneficial and lower 4 times the glucose metabolism of metformin. These patients are susceptible to sulfonylureas and may be treated for years in case of no blood glucose attack complications. The drug will start at one-fourth of the adult dose: MODY1. It is caused by a mutation in the alpha-HNF 4 gene and is relatively uncommon. The same is true, but the threshold for renal excretion is not low. The incidence of upward alpha-HNF 4 mutations in cases with a robust clinical panel for alpha-HNF 1 but not confirmed by genetic sequencing should be considered. The disease is also susceptible to sulfonylureas: MODY4 with a mutation in the MODY6 gene, IPF1, with a mutation in MODY7, NeuroD1 is characterized by a carboxy sterilipase mutation, which is not typical: MODY2. The increase in fasting blood glucose in 100 to 150 mg/dl is uncommon in children and adolescents. The incidence of this condition is usually considered to be type 1 or 2 diabetes, but a large percentage of the above patients are heterozygote individuals, the glucokinase mutations. Other mutations associated with T2DM include rare variants in the WFS1 and ABCC8 genes, insulin receptor (IR), fructose 6-phosphate aminotransferase (GFPT2), nitric oxide synthase (eNOS), and mouse pancreatic cell lines (Min6 and SJ cells) revealed HDAC4 variant (p.His227Arg).

Conflicts of Interest

None.

Acknowledgments

The authors wrote this study.

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ARTICLE / INVESTIGACIÓN

Early appendectomy in appendicular mass

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Abstract: Appendicular mass is a well-known complication of acute appendicitis. It is conventionally treated conservatively, followed by interval appendectomy. This study aimed to determine the feasibility and safety of an early appendectomy in appendicular mass. Patients and methods: The analysis was performed at the Department of Surgery Al-Shafaa Hospital Diyala from March 2017 to December 2021. The patients with appendicular mass (n = 100) were included in this study. Patients were divided into two groups, viz. group A (n = 50) and group B (n = 50), regardless of age and gender. After preliminary investigations, appendectomy was performed in group A patients immediately. Group B patients were initially treated with the conventional procedure followed by interval appendectomy. Patient compliance, readmission and overall expenses were recorded for both groups. Results: A total of 60 (64%) males and 40 (40%) females with a mean age of 25.09 years (Range 8-44 years) are included in the study population. Post-operative wound sepsis occurred in 3 (6%) patients in group A. Treatment failure, patient compliance, readmission and overall expenses occurred in group B patients. Early appendectomy was a safe and superior option in patients with appendicular mass compared to conventional treatment.

Key words: Acute appendicitis. Appendicular Mass, Conservative management, immediate surgery.

Introduction

The most frequent cause of acute abdomen in teenagers needing surgery is acute appendicitis[1]. Patients admitted late in the acute appendicitis course showed complications such as developing an inflammatory mass in the right iliac fossa[2]. The inflamed appendix, omentum, and intestinal loops make up this swelling of inflammation. The treatment of appendicular mass is controversial; however, several management options exist for appendicular mass[2-5]. Traditionally, these patients are managed conversantly, followed by interval appendectomy after 4-6 weeks. It is believed that early appendectomy is hazardous, time-consuming and may lead to life-threatening complications such as faecal fistula[6,7]. The need for interval appendectomy has also been questioned[8,9]. The initial conventional approach claims to be a lower complication rate than the early operative approach[10]. Several studies reported that the immediate appendectomy claims to have an early recovery and complete cure during admission[11-13]. The current study aimed to compare patients treated alternatively and then had interval appendectomy to assess the feasibility and safety of immediate appendectomy in the treatment of appendicular mass in the Iraqi population.

Materials and methods

Patients and Methods

A prospective comparative study was conducted at the Department of Surgery, Al-Shafa private hospital, from March 2017 to December 2021. The patients (n=100) with appendicular mass were induced in this study. All the patients were clinically evaluated. Their blood chemistry, urine analysis, abdomen ultrasound, and plain abdomen x-ray were investigated. The patients were divided into two groups viz. Group A and group B. Treatment options were informed to each patient, and consent was taken.

Operational procedure

Group A was operated on within 24 hours of admission. Patients in group B were kept on conventional treatment comprising hospitalization with intravenous fluids and broad-spectrum antibiotics such as Cefuroxime, Metronidazole and analgesics. The mass progress and the vitals were recorded regularly to monitor the response to conventional treatment. The patients in group B were discharged after complete resolution of the acute inflammatory mass and re-admitted after 6-8 weeks for interval appendectomy.

Studied parameters

The variables studied in both groups included operative difficulties, total operating time, operative and postoperative complications, total duration of hospital stay and patient compliance.

Results

The study included 60 (60 %) males and 40 (40%) females with a mean age of 25.09 years with a range of 8-44 years. The major clinical features included tenderness in the right iliac fossa, vomiting, palpable mass in the right iliac fossa, anorexia and diarrhea. Tachycardia and fever were other vital signs observed. 85% of the patients had a leukocytosis of more than 10000/cm, while a neutrophilia of >75% was present in 90% of cases. Ultrasound of the ab-

Citation: Al-Obaidi J H. Early appendectomy in appendicular mass. Revis Bionatura 2022;7(4) 67. http://dx.doi.org/10.21931/RB/2022.07.04.67

Received: 22 September 2022 / **Accepted:** 18 October 2022 / **Published:** 15 November 2022

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domen detected a mass in the right iliac fossa in 30 (60%) patients, while the remaining 20 (40%) cases were identified at operation. A simple mass composed of an inflamed appendix. Omentum was found in 30 cases, pelvic abscess 5 cases, intussusception in one case, fish bone in one case, vermicularis in one case, gangrene of the omentum in one case and Fecolith in 4 cases. The pattern of operative findings and operative problems differed significantly in both groups, as shown in Table 1.

Immediate appendectomy needed lengthening of the incision to overcome the difficulty in dissection due to adhesions in 9 (18%) patients. The pattern of postoperative complications in both groups is shown in Table 2.

The total hospital stay in group A patients included only 3-5 day hospital admission compared to group B patients who were admitted 7-10. In group B, total patients treated conventionally, 35 (70%) were successfully operated on after 4-8 weeks. Seven patients refused interval appendectomy, and in 13 patients, we had to stop the conservative treatment and resort to operation because of the deteriorating condition of the patients. Five of these patients had perforated appendices, which led to spreading peritonitis. Eleven patients were lost to follow-up and never returned for interval appendectomy. Patients on conservative management remained hospitalized for 7-10 days during their first admission and for another 4-8 days after interval appendectomy.

Discussion

The treatment of appendicular mass is taking a turn from the traditional approach of initial conservative treatment followed by interval appendectomy to immediate appendectomy[14,15]. However, these changes are not widely accepted, and many surgeons continue to adopt the same traditional conservative approach[16]. Because it significantly shortens the overall hospital stay and prevents the need for a second readmission, early surgical intervention has long been recognized as a successful and alternative conservative approach[17]. This leads to reducing the total expenses substantially. The conventional treatment comprises hospitalization, intravenous fluids, antibiotics, analgesics and strict monitoring of the vitals and general state of the patient. In 0-20% of the cases, it proves unsuccessful, and patients need emergency operation due to spreading infection, which is comparatively more difficult[18,19]. In addition, patients may suffer a recurrence of appendicitis after being discharged from the hospital[20,21]. Many patients effuse readmission for operation once their acute problem is solved, which seems to be a significant advantage of the initial conservative approach. Another disadvantage of conservative management is the chance of misdiagnoses like intussusception and carcinoma caecum, fish bone, Fecolith and vermicularis, but in early surgery; we can deal with it and early return to work. Our study highlights the feasibility and effec-

Condition	Parameters	Type of treatment	
		Group A (50%)	Group B (50%)
Operative findings	Simple mass	21	6
	Perforated appendix	4	0
	Loculated pus collection	6	4
	Appendicular abscess	7	5
	Adhesions	9	26
	Enterobius vermicularis	1	0
	Intussusception	1	5
	readmission to the hospital	0	4
	Gangrenous in omentum	1	0

Table 1. Operative findings in both groups.

Operative problem	Group A	Group B
Bleeding	3	2
Minor trauma to the bowel	1	1
Difficult on adhesion	6	4
Time of operative	45 min	45-60 min

Table 2. Post-operative findings in both groups.

Operative problem	Group A	Group B
Wound infection	5	1
Hospital stay (day)	3 days	10 days
residual abscess	1	1

Table 3. Comparison of HbA1C with another parameter.

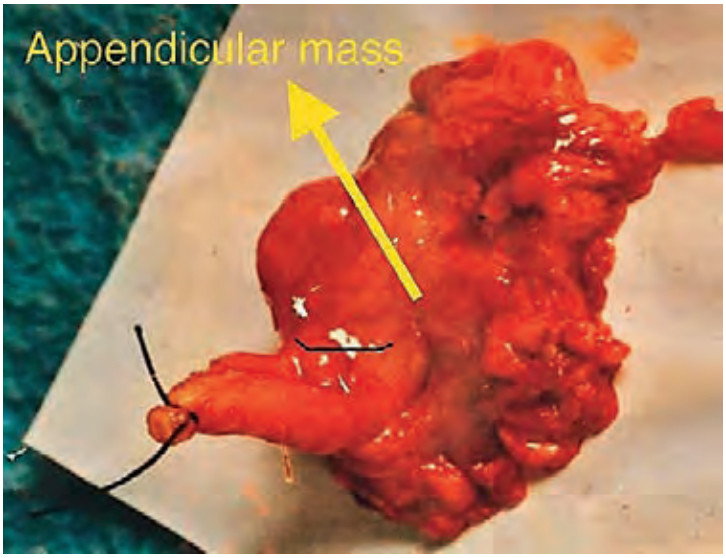

Figure 1. Appendicitis mass.

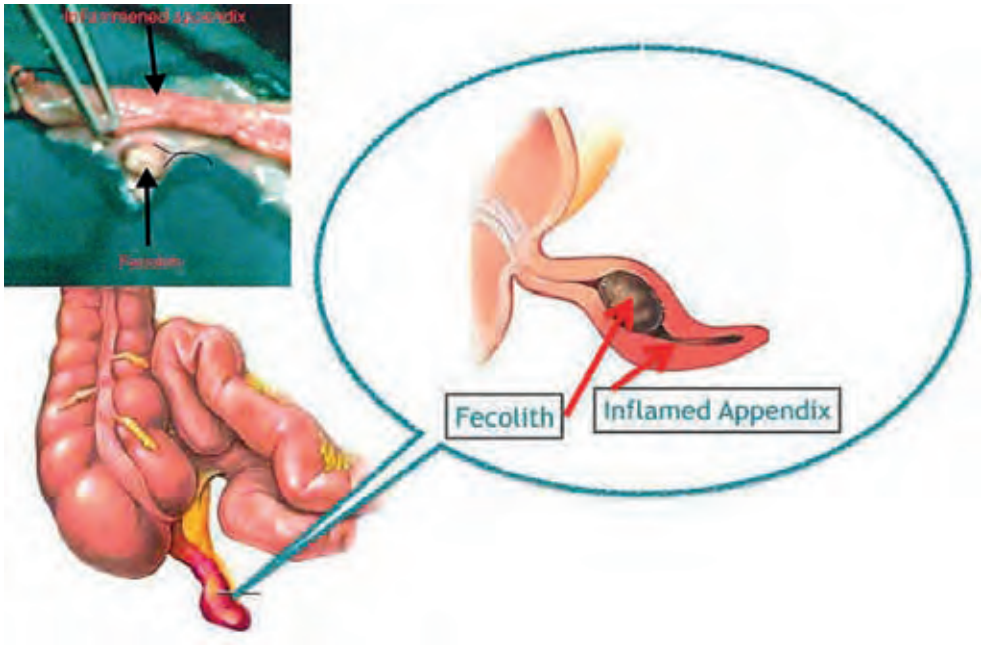

Figure 2. Appendix obstructed by Fecolith.

tiveness of early appendectomy in appendicular mass, and the results are consistent with several similar studies[22,23]. Early appendectomy is a more appropriate and effective way of managing Appendicular mass. Early appendectomy has several benefits, including a fully curative procedure, shorter hospital stays, minimum morbidity, and patient compliance. Due to an overall improvement in anesthesia, supportive care, and antibiotics, the old opinion that surgery is complex in states when the inflamed appendix is deeply embedded in the mass, and the intestinal loops are friable is more relevant now. The operative problems such as localization of appendix, adhesiolysis and bleeding are more pronounced and troublesome with interval appendectomy as shown findings of this study. Wound infection, however, remains a common postoperative complication of early appendectomy in appendicular mass, but the rate of wound infection is not so high as to preclude this early operative approach. The benefits of early appendectomy overweigh the results of interval appendectomy as evident from our results and also supported by many other studies referred to in comparison to our findings.

Conclusions

Early appendectomy on appendiceal mass is a safe and effective alternative to conventional conservative treatment followed by interval appendectomy. It reduces mortality, morbidity and hospital readmission for surgery, mainly when caused by fishbone-induced appendicitis, vermicularis, omentum gangrene, fecoliths and intussusception. This reduced this problem and patients' hospital stay and early return to work, making it safer and effectively better than conservation treatment.

Funding

This research received no external funding.

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ARTICLE / INVESTIGACIÓN

Study on the anti-microbial effect of Sinigrin against some pathogenic bacterial species

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DOI. 10.21931/RB/2022.07.04.68

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Abstract: The increasing anti-bacterial drug resistance is one of the biggest challenges facing doctors around the globe, so finding alternative treatments is one of the ideal options to overcome this problem. The cruciferous family is one of the wealthiest plants worldwide because it contains the most important secondary metabolites, glucosinolates, known for their anti-microbial properties. The present study aimed to evaluate the anti-bacterial effect of glucosinolates (Sinigrin) against eight bacterial isolates (*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Actinomyces, Proteus mirabilis* and *Streptococcus pneumoniae*). The current study investigated six concentrations of pure Sinigrin (100, 300, 500, 700, 900, and 1100 µg/ml). The sensitivity of bacterial isolates to various antibiotics was tested by VITIK 2DensiCheck equipment. The anti-bacterial activity of Sinigrin was assessed using the agar diffusion method, and the microtiter plate method measured the minimal inhibitory concentration (MIC). The highest anti-bacterial effect of Sinigrin was observed against *S. aureus*, *E. coli*, and *E. faecalis*. The anti-bacterial activity started as lower as 100 µg/ml, while a moderate effect was seen against *P. aeruginosa* and *K. pneumoniae* at a concentration lower than 700 µg/ml. On the other hand, Sinigrin was not effective against *Actinomyces*, *P. mirabilis*, and *S. pneumoniae*. It can be concluded from the present study that Sinigrin has an anti-bacterial effect on some isolates of bacteria which suggests the possibility of using Sinigrin as alternative medicine in the future.

Key words: Anti-bacterial activity, Agar well diffusion, Glucosinolates, Minimum inhibition concentration and antibiotic susceptibility, Sinigrin.

Introduction

The appearance of antibiotic-resistant microbes to multiple anti-microbial agents has become a life-threatening issue to public health. Therefore, several actions must be taken to reduce this problem, for example, by controlling antibiotic usage, developing research for enhancing the genetic mechanisms of resistance, and developing new synthetic natural anti-bacterial drugs since the ultimate goal is to offer appropriate and efficient anti-microbial drugs to thepatients[1]. Their botanical derivatives are safer in comparison with high efficacy[2].

Antibiotics and other anti-microbial drugs have been used extensively in treating infectious disorders for many years to combat bacterial and fungal infections. Infectious organisms have been exposed to these substances on a large scale due to the widespread use of antibiotics, their repeat prescription in healthcare facilities, and an overuse of antibiotics in animal-rearing practices in agriculture. Because of this, some organisms have developed a resistance to them, and certain bacterial strains now significantly threaten overall health. Myrosinase-activated glucosinolates produce thiocyanates, isothiocyanates, and nitrile compounds. *In vitro* studies show that these chemicals are ideal for anti-bacterial testing in treating such infections[3,4].

There are many diverse sources of natural anti-microbials, including plants, animals, bacteria, algae, and fungi. Among them, the advantages of glucosinolates and the products derived from them for human nutrition, plant defense, and as powerful anti-bacterial agents have gained recognition. Plants produce thousands of phytochemicals, many of which have nutritive value in both medicinal and health-promoting properties[5]. Sinigrin(allyl-glucosinolate or 2-propenyl-glucosinolate) is natural aliphatic glucosinolate present in cruciferous or Brassicaceae plants such as broccoli, broccoli sprouts, cabbage, mustered, Brussel sprouts, cauliflower and many others. These plants have essential components of a healthy diet, and their pic flavor profile with several physiological processes[6]. To activate, the glucosinolates molecules require conversion by the myrosinase enzyme to bioactive thiocyanate, isothiocyanate, and nitrile derivatives; therefore, glucoraphanin and Sinigrin are converted into bioactive sulphoraphanin (SFN) and allyl isothiocyanate (AITCs) with fungicidal and bactericidal properties[6]. Melrose in 2019 illustrated sinigrin uses and benefits in biomedicine[4]. Previous studies showed Sinigrin's role in modulating the immune system[7]. Therefore, the present study aims to evaluate the potential anti-microbial activity of sinigrin secondary metabolite against different bacterial isolates, which may suggest the possibility of bringing it as an alternative treatment in the future.

Citation: Hasan, A.M.; Ghafil, J.A. Study the anti-microbial effect of Sinigrin against some pathogenic bacterial species.Revis Bionatura 2022;7(4) 68. http://dx.doi.org/10.21931/RB/2022.07.04.68
Received: 22 September 2022 / **Accepted:** 18 October 2022 / **Published:** 15 November 2022

Materials and methods

Preparation of glucosinolates (sinigrin) solutions

Pure Sinigrin (extracted from the mustard plant/ Brassicaceae family) was purchased from Sigma-Aldrich. The stock solution of 3 mg/ ml of Dimethyl sulfoxide (DMSO) (99 %, v/v) (Fluka) was prepared. Different concentrations of Sinigrin were prepared from stock solution (100, 300, 500, 700, 900, and 1100 µg/mL). The dilutions were prepared by using (DMSO) (99 %, v/v) (Fluka) aseptically. The Sinigrin was dissolved in a sterile container and shaken gently at room temperature[8].

Bacterial strains

Eight isolates of pathogenic bacterial species, including *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Actinomyces, Proteus mirabilis, Streptococcus pneumoniae* (blood agar was used in growing the fastidious isolated bacteria) were isolated and identified in the Microbiology Laboratory. Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq, in the level 2 biosafety lab. Basic biochemical tests and microscopic examination were used to identify the bacterial isolates. VITEK 2 fluorescence system (ID-GNB card) was used to confirm the identification of bacterial isolates[9].

Preparation of bacterial suspension and growth medium

All bacterial isolates were stored for a long time at −80 °C in a sterile cryovial containing 30 % glycerol (v/v, glycerol/overnight bacterial growth). Before starting the experiments, a loop full of bacterial isolates was inoculated onto Mueller-Hinton Agar (MHA) and incubated at 37 oC overnight. The suspension of bacterial growth was prepared by mixing the loop full of the isolated colony with sterile normal saline. The normal saline was used to adjust the number of bacteria to be 1.5 x 10^8 c.f.u/ml by using a 0.5 McFarland tube[10].

Antibiotic susceptibility

VITEK2DensiCheck (bioMe'rieux) technology was employed to test the susceptibility of isolated bacteria to various antibiotics, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Imipenem, Gentamicin, Ampicillin, Amoxicillin/Clavulanic Acid, Ampicillin/Sulbactam, Piperacillin/Tazobactam. The CLSI 2021 manual was followed for the selected drugs[11].

The anti-bacterial effect of Sinigrin against bacterial isolates

Diffusion method

The anti-microbial effect of different concentrations of Sinigrin was evaluated by measuring their clear inhibition zone against eight bacterial isolates cultured onto Mueller-Hinton Agar plates. A hundred microliter of standard bacterial inoculum 1.5 x 10^8 of each bacterial isolate was separated onto Mueller-Hinton Agar plates. A sterile crock-poorer made several 0.45 mm diameter wells on Mueller-Hinton Agar plates. Fifty microliters of the serial concentrations (100, 300, 500, 700, 900, and 1100 µg/mL) of Sinigrin were put into the six wells, and 50 µl of DMSO was placed in the seventh well (as control). The plates were incubated overnight at 37 C. Scales were used to measure the clear inhibition zones.

Minimum inhibition concentration (MIC) of Sinigrin

The minimum inhibition concentration (MIC) of pure Sinigrin was determined using a microtiter plate. A hundred microliters of each concentration of Sinigrin (100, 300, 500, 700, 900, and 1100 µg/mL) was added into the wells of microtiter plates, 100 µl of sterile tryptic soya broth (double concentration of TSB) and 10 µl of overnight growth of bacterial isolates (1.5 x 10^8 c.f.u/ml). In control wells, 100 µl of DMSO was used instead of the sinigrin solution. Three duplicates of each trial were made. The microtiter plates were shaken gently and incubated overnight at 37 C[10].

Statistical analysis

Statistical analysis was done by using Origin 8 software. The data were expressed as means ± SE. The chi-square was used to evaluate the difference between the diameters of the test (different concentrations of Sinigrin) and the diameters of wells that were filled with DMSO (control negative). A value of $P<0.05$ was considered to be statistically significant.

Results

The anti-microbial effect of Sinigrin

The anti-bacterial effect of Sinigrin was estimated in this study against eight bacterial isolates that were isolated and diagnosed in the microbiology laboratory at the Department of Biology, College of Science, University of Baghdad. Table 1 shows that the different concentrations of Sinigrin have affected the growth of five bacterial isolates (*S. aureus, E. coli, P. aeruginosa, K. pneumoniae*, and *E. faecalis*) but not against three isolates (*Actinomyces, S. pneumoniae*, and *P. mirabilis*). It was shown that the highest anti-bacterial effect of Sinigrin was seen in the case of *S. aureus* starting from the lowest concentration of 100 µg/ml though the effect was not in a concentration-dependent manner. Similarly, in the case of *E. coli* but the highest concentrations of Sinigrin (900 µg/ml and 1100 µg/ml) were non-significant. In the cases of *P. aeruginosa* and *K. pneumoniae*, the anti-bacterial effect of Sinigrin starts at 700 µg/ml concentration upwards, and the effect was in a concentration-dependent manner. In the case of *E. faecalis*, the effect of Sinigrin appeared at low concentrations, i.e., at 100 µg/ml, 300 µg/ml, and 500 µg/ml. No anti-bacterial effect of Sinigrin was observed at high concentrations, i.e., at 700 µg/ml, 900 µg/ml, and 1100 µg/ml. Finally, the growth of *Actinomyces, S. pneumoniae*, and *P. mirabilis* isolates was not affected (Figure 1).

MICs of Sinigrin against the bacterial species

The MIC method was used to evaluate the anti-microbial effect of pure sinigrin standard solution against different bacterial isolates of *S. aureus, E. faecalis, E. coli, K. pneumoniae, P. aeruginosa, Actinomyces, P. mirabilis*, and *S. pneumoniae*. The current study showed that the MICs of Sinigrin against *S. aureus, E. coli*, and *E. faecalis* were 300µg/ml, while the MICs of Sinigrin against *K. pneumoniae* and *P. aeruginosa* were 300 and 700 µg/ml respectively. The MIC method showed no anti-bacterial effect of Sinigrin against *Actinomyces, P. mirabilis*, and *S. pneumoniae*.

No	Bacterial species	100 µg/ml	300 µg/ml	500 µg/ml	700 µg/ml	900 µg/ml	1100 µg/ml
1	*S. aureus*	15 ± 1.2	12 ± 1.2	12 ± 1.1	17 ± 1.3	14 ± 2.4	15 ± 1.1
2	*E. coli*	15 ± 1.4	14 ± 2.1	20 ± 2.2	14 ± 1.5	NS	NS
3	*P. aeruginosa*	NS	NS	NS	14 ± 1.9	15 ± 1.8	20 ± 1.7
4	*K. pneumoniae*	NS	NS	NS	14 ± 1.7	14 ± 1.5	18 ± 1.3
5	*E. faecalis*	11 ± 1.5	13 ± 2.4	15 ± 1.5	NS	NS	NS
6	*Actinomyces*	NS	NS	NS	NS	NS	NS
7	*S. pneumoniae*	NS	NS	NS	NS	NS	NS
8	*P. mirabilis*	NS	NS	NS	NS	NS	NS

NS: non-significant inhibition zone

Table 1. Anti-microbial activity of different concentrations of Sinigrin expressed as the diameter of inhibition zones in millimeters (mm) against 8 bacterial species. Each treatment was performed in triplicate.

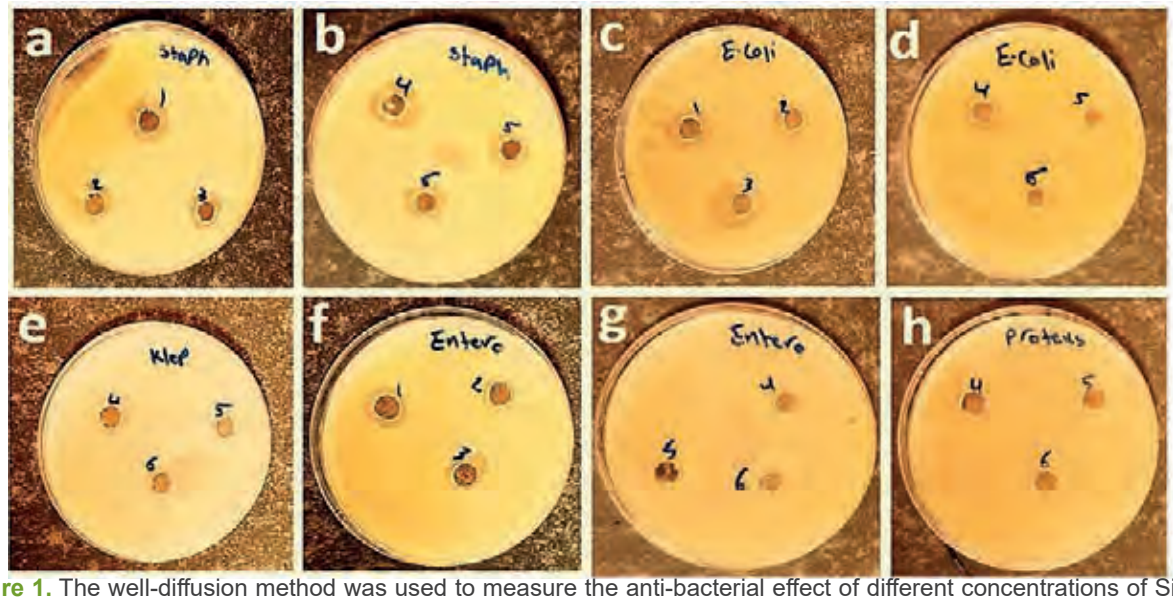

Figure 1. The well-diffusion method was used to measure the anti-bacterial effect of different concentrations of Sinigrin (well 1, 100 µg/ml; well 2, 300 µg/ml; well 3, 500µg/ml; well4, 700 µg/ml; well 5, 900 µg/ml; well 6, 1100 µg/ml) in Mueller-Hinton Agar (MHA) that was inoculated (spared) previously inoculated with a and b (*S. aureus*); c and d (*E. coli*); e (*K. pneumoniae*); f and g (*E. faecalis*); h (*P. mirabilis*).

No	Bacterial species	MICs (µg/ml)
1	*S. aureus*	300
2	*E. coli*	300
3	*P. aeruginosa*	700
4	*K. pneumoniae*	300
5	*E. faecalis*	300
6	*Actinomyces*	Not effect
7	*S. pneumoniae*	Not effect
8	*P. mirabilis*	Not effect

Table 2. Minimum inhibition concentrations (MICs) of Sinigrin against 8 bacterial species. Each treatment was performed in triplicate.

Discussion

Previous studies were focused on the treatment application of Sinigrin because of the safe and non-toxic effect of this substance as it is extracted from plant[12]. Several studies highlighted the therapeutic impact of Sinigrin; Tanaka *et al.* (1992) established the anticancer effect of Sinigrin[13], while the anti-inflammation effect was attributed by Lee and Lee (2015)[14]. As for the anti-oxidant activity, it was reported by Ippoushi *et al.* (2010). The main effect of this herbal extract was the anti-microbial effect[15]. Several studies reported the anti-bacterial effect of Sinigrin. Other investigators highlighted the anti-bacterial impact of Sinigrin against *E. coli*[16], *Bacillus subtilis*, and *Listeria monocytogenes*[17], hence the development of resistance to the conventional antibiotic by pathogenic bacteria makes it necessary to find alternative anti-microbials to eradicate them[18].

The use of different serial concentrations was done according to the manufacturer company of sinigrin phytochemicals are routinely classified as anti-microbials based on susceptibility tests that produce inhibitory concentrations in the range of 100 to 1,000 µg/mL, which is why this range was used in the present study[19,20]. In the current study, eight bacterial isolates were isolated and identified using biochemical tests and VITEK 2 DensiCheck (bioMe'rieux) technology. The diffusion method was used to check the anti-bacterial effect of different concentrations of Sinigrin. As illustrated above, the results showed that Sinigrin has an anti-bacterial effect on five isolates. Sinigrin is not usually an anti-microbial substance; when it is enzymatically hydrolyzed from allyl isothiocyanate, it exhibits potent anti-microbial activity against food spoilage and pathogenic organisms[21]. Allyl isothiocyanate molecules showed anti-microbial effects against *E. coli* O157:H7 at low pH. Based on

the results that proteins and sulphydryl compounds could suppress various isothiocyanates, it was hypothesized that the mechanism of isothiocyanates' anti-microbial activity was related to the intracellular inactivation of sulphydryl-enzymes[9,22]. It is known that the thioredoxin system has an essential role in DNA formation, which suggests that the anti-bacterial activity of allyl isothiocyanate could be related to the inhibition of DNA formation. Allyl isothiocyanate inhibits the catalysis of thioredoxin reductase and acetate kinase, which are responsible for essential metabolic reactions in bacteria. Thus, it can be proposed that allyl isothiocyanate has many targeted anti-microbial activities, as they can cause enzymatic inhibition and membrane damage[16,17]. A study by Herzallah and Holley (2015) evaluated the use of carboxymethyl cellulose (CMC) nanoparticulate on the anti-microbial activity of CMC films containing Sinigrin against *E. coli* O157:H7 on fresh beef[23]. An investigation by Lara-Lledo and their group found that pure Sinigrin after hydrolysis can interfere with the metabolism of bacteria and inhibit the monocytogenes growth[14,18].

In the novelty of the current study, different concentrations of pure Sinigrin were used to check the anti-bacterial effect of Sinigrin against other bacterial isolates, and two methods to approve the anti-bacterial effect of Sinigrin *in vitro* were used as well[13].

Conclusions

The current study has shed light on one of the most important natural compounds, glucosinolates. Glucosinolates (from the Brassicaceae family) and their hydrolysis products (such as Sinigrin) have decisive bioactive benefits, one of which is their anti-microbial properties. We have discussed Sinigrin's anti-bacterial effect (at low concentrations) against different bacterial isolates such as *S. aureus, E. coli*, and *E. faecalis*. In contrast, moderate concentrations were effective against *P. aeruginosa* and *K. pneumoniae*. Further studies on glucosinolates and their hydrolysis products are recommended to investigate their anti-microbial effect, which can be a valuable approach to enhance the therapeutic activities of such critical secondary metabolites.

Author Contributions

Alaa M. Hasan conducted the research experiments and wrote the manuscript, and Jenan A. Ghafil, conducted the research experiments, data interpretation, statistical analysis, and writing the manuscript.

Funding

Self-funding.

Institutional Review Board Statement

This work was approved by the Institutional Review Board Statement of the Department of Biology, College of Science, University of Baghdad (No. 1045).

Acknowledgments

We sincerely thank the management of the Department of Life Sciences for their support in the laboratory experiments carried out in the department's laboratories.

Conflicts of Interest

The authors declare no conflict of interest.

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ARTICLE / INVESTIGACIÓN

Assessment of lipid profile with HbA1c in type 2 diabetic Iraqi patients

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DOI. 10.21931/RB/2022.07.04.69

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Abstract: Insulin-induced hyperglycemia is the hallmark of diabetes mellitus (DM), including various metabolic disorders. Diabetic people are more likely to develop dyslipidemia, hypertension, and obesity. Type 2 diabetes (T2DM), the most common illness, is generally asymptomatic in its early stages and can go misdiagnosed for years. Diabetes screening may be beneficial in some cases since early identification and treatment can lessen the burden of diabetes and its consequences. This study aimed to find the relationship between Glycated hemoglobin (HbA1c) and lipid profile components in T2DM patients. Methods: This descriptive-analytical and cross-sectional study was performed on the control group and T2DM patients in Medical City in Baghdad between March and June 2021. A total of 90 patients with T2DM and 45 healthy control were included in this study. In the control group, healthy volunteer individuals participated. For all subjects, HbA1c, fasting blood sugar/FBS, and lipid profile (Total Cholesterol/TC, Triglyceride/TG, High-density Lipoprotein/HDL, Low-density Lipoprotein/LDL, and Very Low-density Lipoprotein/VLDL) were assessed. Among T2DM patients, 62.22% (n= 56) were male, and 37.78% (n= 34) were female. Mean ± SD levels of HbA1c, TC, TG, LDL, VLDL, HDL, and FBS were 7.33±0.56 % (168.21±9.23, 146.10±9.64, 137.23±8.32, 41.05±5.86, 43.85±6.17, and 208.81±52.1) mg/dl respectively in the T2DM group. In the control group, the Mean ± SD results of the same parameters were 4.91±0.27%, (171.20±3.57, 116.60±8.25, 105.05±2.11, 41.83±4.92, 44.04±5.54, 96.20±7.8) mg/dl respectively. Results demonstrated statistically significant differences between T2DM patients and control groups in HbA1c (p equal to 0.0025), TG (p equal to 0.015), LDL (p=0.0029), and FBS (p=0.02). Pearson correlation analysis of HbA1c with other variables showed a significant positive correlation with serum TC, TG, LDL, and FBS (r=0.573, P<0.01; r=0.655, P <0.001; r=0.498, P<0.05; r=0.691, P<0.001; respectively). At the same time, the data showed a negative connection between HbA1c and HDL (r= - 0.562, P<0.01). The findings of this study reveal that diabetic people do not have a satisfactory HbA1c level. Furthermore, HbA1c shows a significant correlation with TC, TG, LDL, and VLDL, whereas it has a significant negative correlation with HDL. The study's findings showed that HbA1c might be a useful marker for predicting dyslipidemia in T2DM patients.

Key words: T2DM, Lipid profile, HbA1c.

Introduction

Hyperglycemia, which can be induced by insulin production, insulin action, or a combination of both, is the hallmark of diabetes mellitus. Diabetic people have a high chance of developing hyperlipidemia, obesity, and hypertension. T2DM, the most common illness, is generally asymptomatic in its early stages and can go misdiagnosed for years. DM is a long-term metabolic condition brought on by a complicated interplay of genetic, environmental, and behavioral variables. Death rates from diabetes are rising for various reasons, including poor nutrition, obesity, smoking, and physical inactivity[1]. Diabetes screening may be beneficial in some cases since early identification and treatment can lessen the burden of diabetes and its consequences[2].

Moreover, it was a condition marked by hyperglycemia brought on by a deficiency in insulin action, secretion, or both (insulin resistance)[3]. Noncommunicable illnesses affect not just adults but children and adolescents throughout the world[4]. In both emerging and developed countries, people should be aware of the high rate of diabetes in their populations. In 2012, 1.9 million persons in the United States were diagnosed with diabetes, while worldwide, the prevalence of diabetes was reported to be 8.3%5. An estimated 451 million individuals between 18 and 99 years with diabetes today. According to the latest estimates, nearly half (49.7 %) of patients are undiagnosed. Diabetes implications include coronary heart disease (CHD), peripheral artery disease (PAD), stroke, and other ailments. Endothelial dysfunction will very probably become more common as diabetes rates rise[5].

The long-term glycemic control marker HbA1c is frequently utilized to reflect the typical blood glucose level in diabetics, suggesting the probability of diabetic complications. The HbA1c is currently considered an independent risk factor for cardiovascular disease (CVD) in diabetics and non-diabetics, among other well-established risk factors, in-

Citation: Al-Shaheeb S, Hashim H K, Mohammed A K, Almashhadani H A, Al Fandi A. Assessment of lipid profile with HbA1c in type 2 diabetic Iraqi patients.Revis Bionatura 2022;7(4) 69. http://dx.doi.org/10.21931/RB/2022.07.04.69
Received: 22 September 2022 / **Accepted:** 18 October 2022 / **Published:** 15 November 2022

cluding hyperlipidemia and hypertension[6]. The HbA1c is a valuable diabetes biomarker because it provides information on average blood glucose levels over the last few months[7]. Monitor glycemic management in people with diabetes by testing their HbA1c. The objective is to go down to less than 7%. Multiple variables can influence HbA1c levels, including sugar intake, exercise, and medication adherence. HbA1c might likely predict dyslipidemia and CVD in some studies[8,9].

The risk of diabetic complications was strongly associated with the leading causes of CVD in diabetics. Cardiovascular disease is one of the primary causes of mortality among diabetes people. As a result, in addition to HbA1c management, it is critical to analyze diabetes patients' blood lipid profiles and take appropriate treatment based on age and type of illness[10]. T2DM patients are four times as likely than healthy people to die from CVD[11]. Diabetic management, early screening, and prompt prevention of diabetes complications dramatically improve disease prognosis and save treatment cost[12]. Therefore, our study aimed to estimate the relationship between HbA1c and lipid profile components in T2DM patients.

Materials and methods

This study was performed on control and T2DM patients in Medical City in Baghdad between March and June 202. In the control group, healthy volunteer individuals participated. Patients with type 1 diabetes, gestational diabetes, or prednisolone-induced diabetes were not acceptable. Ninety patients with T2DM and 45 with control were included in this study. 5 ml of blood was collected from the patients by venepuncture after overnight fasting for 12 hours. Blood samples were divided into two aliquots; the first contains EDTA and is used for the HbA1c estimation. The second one was in a plain tube to collect serum and stored at -20ºC until used. Assessment of FBS and lipid profile were measured by an enzymatic colorimetric method with a commercially available kit (Spinreact, Spain). HbA1c levels measurement was performed with an available kit (Roche, Germany) using Roche Diagnostics Cobas e411 analyzer. These parameters define the DM as having HbA1C ≥ 6.5%, FBS of ≥ 126 mg/dl, and 2-hour plasma glucose levels of ≥ 200 mg/dl (11.1 mmol/l). In a study of the data, the usual descriptive statistics (Mean± Standard Deviation) for the directly measured variables were calculated. The relationships between FBS, HbA1c, and lipid profiles were established using an unpaired t-test. SPSS (Statistical Package for Social Science) version 25.0 was used to analyze the data. p> 0.05 was used as the statistical significance criterion.

Results

The age range for the patients and control groups was between 45 and 70. Among those with T2DM patients, 62.22% (n= 56) were male, 37.78% (n= 34) were female, as shown in (Table 1), and in the control group, 26.67% (n= 12) were male, and 73.33% (n= 33) were female. The mean± SD of age for the T2DM (50.10± 12.86 years) and control group (49.05± 9.66 years) showed no statistical difference (p=0.106).

Mean ±SD of TC, HbA1c, LDL, TG, VLDL, HDL, and FBS were 7.33±0.56 % (168.21±9.23, 146.10±9.64, 137.23±8.32, 41.05±5.86, 43.85±6.17, and 208.81±52.1) mg/dl respectively in the T2DM group. In the control group, the mean ± SD results of the same parameters were 4.91±0.27%, (171.20±3.57, 116.60±8.25, 105.05±2.11, 41.83±4.92, 44.04±5.54, 96.20±7.8) mg/dl respectively. Results demonstrated statistically significant differences between T2DM patients and control groups in HbA1c (p=0.0025), TG (p=0.015), LDL (p=0.0029), and FBS (p=0.02). On the other hand, the mean ± SD of TC, VLDL, and HDL in serum between study groups showed no significant difference between levels p>0.01, as shown in Table (2) and Figure (1).

Pearson correlation of HbA1c with other variables revealed a substantial positive connection with serum TC, TG, LDL, and FBS (r=0.573, P<0.01; r=0.655, P<0.001; r=0.498, <P0.05; r=0.691, P<0.001) respectively. While results demonstrated a negative correlation between HbA1c and HDL (r= -0.562, P <0.01), as shown in Table (3).

Discussion

Some lipid profile parameters showed a significant increase in T2DM groups in table (3) many contradicting findings in the literature regarding the correlation between HbA1c and lipid profile parameters. The present study revealed increasing HbA1c levels with an increase in lipid profile parameters like TC, TG, LDL, and VLDL. Moreover, the study demonstrated a negative correlation between HbA1c levels and HDL in DM patients. The positive association between HbA1c and lipid profile parameters has been found in several studies, such as research in Turkey which identified a substantial correlation between TC, LDL, TG, and HbA1c[13]. According to one study, there is a significant negative connection between HbA1c and LDL[14]. Another study stated that HbA1c had no significant correlation with lipid profile except TG[15]. HbA1c has been linked to elevated TG levels suggesting that it may predict CVD and is a risk factor in T2DM[15].

Based on the results of this investigation, FBS levels were found to be greater than the upper limit for patients with a clinical diagnosis. In T2DM, the HbA1c test is used to check blood glucose management. The HbA1c is a strong predictor of diabetes complications and the length of time a person has had diabetes[16]. HDL, LDL, TG, and TC levels are well-known risk factors for diabetes complications such as coronary heart disease and CVD. In a study by Rani *et al.* (2005), when compared to control, FBS and postprandial plasma glucose, TC, VLDL, LDL, TG, and HDL levels were higher in the survey respondents[17].

Selvin *et al.* (2005) studied the relationship between

	Diabetic Patients (n =90)	Control (n= 45)	*P value*
Female	37.78% (34)	73.33% (33)	
Male	62.22% (56)	26.67% (12)	
Age (year)	50.10 ± 12.86	49.05 ± 9.66	0.106

Table 1. Distribution of age and gender in T2DM and control group. Age represents as mean ± SD.

Biochemical Parameters	Diabetic patients (n =90)	Control (n= 45)	*P value*
HbA1c %	7.33±0.56	4.91±0.27	**0.0025****
TC (mg/dl)	168.21±9.23	171.01±3.57	0.64
TG (mg/dl)	146.10±9.64	116.60±8.25	**0.015***
LDL (mg/dl)	137.23±8.32	105.05±2.11	**0.0029****
VLDL (mg/dl)	41.05±5.86	41.83±4.92	0.86
HDL (mg/dl)	43.85±6.17	44.04±5.54	0.97
FBS (mg/dl)	208.81±52.1	96.20±7.8	**0.02***

** (P≤0.05) significant,*

*** (P≤0.01) highly significant.*

Glycated hemoglobin (HbA1c), fasting blood sugar (FBS), Total Cholesterol (TC), Triglyceride (TG), High-density Lipoprotein HDL, Low-density Lipoprotein (LDL), and Very Low-density Lipoprotein (VLDL). All the data represented as mean ± SD

Table 2. Comparison between biochemical parameters (Mean± SD) in T2DM and control groups.

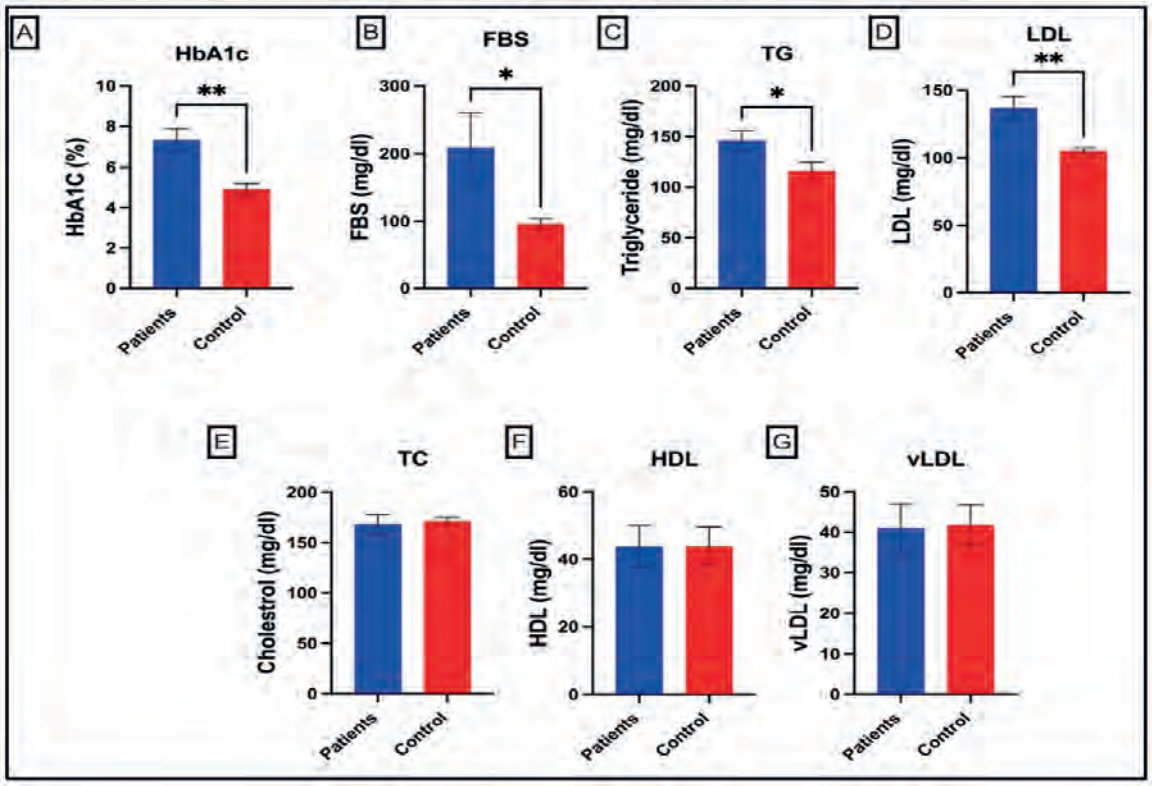

Figure 1. Comparison of biochemical parameters between T2DM and control groups; the highly significant difference between control and T2DM in HbA1c, FBS, TG, and LDL, no significant difference in cholesterol, HDL, and VLDL, independent sample t-test. All data are presented as mean ± SD.

HbA1c and LDL and HDL TC. In addition, our results agree with Selvin *et al.* (2005) study, which represents that the correlation between HbA1c and HDL was negative, but the correlation between HbA1c and LDL was positive[18]. Our results go along with a study by Patil *et al.*[16]. People with diabetes often have abnormalities in their lipids, and people with T2DM are no exception. Insulin resistance has previously been linked to the T2DM aberrant lipid profile because insulin resistance causes an increase in fatty acid release, reduces insulin-dependent muscle-free fatty acid uptake, and increases hepatic fatty acid production in the liver[19]. Diabetic individuals often have high LDL and triacylglycerol values and low HDL. The current investigation results revealed that diabetes individuals had a higher lipid profile. Our study showed a statistically strong positive correlation between HbA1c and lipid profile parameters. Our results are consistent with Wexler *et al.*, 2005 who found that HbA1c and lipid profiles (TC and LDL) have a very positive significant correlation[20]; Other researchers have shown a relationship between HbA1c and these lipid profiles in T2DM patients[15, 21].

Monte Carlo Simulation Analysis (MCS)

After structuring costs, the most influential cost com-

	HbA1c	
Parameters	**r**	***P value***
TC (mg/dl)	0.573	**0.01***
TG (mg/dl)	0.655	**0.001****
LDL (mg/dl)	0.498	**0.05***
VLDL (mg/dl)	0.647	**0.001****
HDL (mg/dl)	-0.562	**0.01***

** (P≤0.05) significant,*

*** (P≤0.01) highly significant.*

Glycated hemoglobin (HbA1c), Fasting Blood Sugar (FBS), Total Cholesterol (TC), Triglyceride (TG), High-density Lipoprotein HDL, Low-density Lipoprotein (LDL), and Very Low-density Lipoprotein (VLDL)

Table 3. Person Correlation analysis between HbA1c and FBS and lipid parameters of diabetic patients.

ponent was direct labor, representing 53% of the total cost. The cost of culture media was 12% of the total, IMC represented 5%, and operating expenses, including administrative expenses and infrastructure, were 30% (Figure 2).

Conclusions

According to the results of this study, diabetic people do not have appropriate HbA1c values. Furthermore, HbA1c showed a significant positive correlation with TC, TG, LDL, and VLDL and a significant negative correlation with HDL. In T2DM patients, HbA1c may be a valuable marker for predicting dyslipidemia and CVD.

Financial Disclosure

This research received no external funding.

Competing interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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ARTICLE / INVESTIGACIÓN

Estimation of DNA damage in the roots of *Allium cepa* exposed to heavy metals using comet assay

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DOI. 10.21931/RB/2022.07.04.70

Abstract: Higher plants were used as a bioindicator of environmental toxicity to estimate the severe problems related to the health of living organisms and the environment. *Allium cepa* plant was used to evaluate the DNA damage caused by heavy metal exposure, the roots of *A.cepa* plant. They were treated with four concentrations (5, 10, 20 and 25 ppm) for each of the metals Cadmium, zinc, copper and lead. At the same time, concentrations (50, 100, 200 and 500 ppm) were used for the preservative (sodium benzoate). The comet assay, a sensitive and suitable test for assessing DNA damage caused by chemical exposure, was used in this study. The Comet's six characteristics were measured: Head intensity, Head DNA%, Tail length, Tail intensity, Tail DNA% and Tail moment. The results showed that the metals are causing the DNA damage of meristematic cells of the roots of the *A. cepa* plant, depending on the tail length from most to least effective Cadmium> zinc > sodium benzoate > copper > lead > wastewater. I consider that it is not necessary to write down these values. The results of this study confirm that the meristematic cells of the roots of *A. cepa* are a suitable model for detecting DNA damage analyzed by the comet assay.

Key words: Toxic metals, Bio-indicator, Single cell gel electrophoresis (SCGE).

Introduction

Environmental pollution is one of the most critical problems facing the world today. Its relationship with the health of the environment and living organisms, including humans, has generated a growing global concern in this regard since the increase in human activity in recent decades has led to the emission of various types of pollutants in the environment, heavy metals are among the most dangerous of this pollutants[1].

Lawal *et al.*[2] also mentioned that heavy metals might enter the human body by consuming contaminated drinking water or crops grown in contaminated soil. Heavy metals such as lead, mercury, Cadmium, and copper are toxins that cause environmental hazards and are considered toxic. These metals are essential sources of oxidative stress in the cell and play an important role in various human pathogens, such as carcinogenesis. Heavy metal toxicity exposure leads to brain damage, mental retardation, cerebral palsy, lung cancer, gastrointestinal abnormalities, and dermatitis. It has been shown that many metals directly modify and damage DNA by forming DNA adducts that induce chromosomal breaks.

A large number of chemical compounds have been added to meals by humans for various purposes, such as flavor, texture, and shelf-life extension. A food additive must be included in the food supply. A preservative is any chemical substance or solution of a substance that is added to food under scientific regulations where it is not the main ingredient of the food. Although preservatives can preserve food for a specific period, they have adverse effects on human health, especially those antimicrobial preservatives which are toxic to living organisms and mutagenic in various test systems[3,4]. Among the types used as a food preservative is sodium benzoate, a sodium salt represented by the chemical formula C7H5O2Na, with a molecular weight of 144.1 g / mol. This odorless compound is soluble in water and ethanol, which has genotoxic and carcinogenic effects. In vivo studies have indicated the efficacy of sodium benzoate in causing anxiety and causing oxidative stress, and many toxic factors[5,6]. Higher plants are recognized as excellent genetic models for detecting environmental mutations and are frequently used in ecological monitoring studies. Allium cepa has been used to assess DNA damage among plant species. The use of *A. cepa* as a test system to detect mutations dates back to the 1940s. It has been used to this day to evaluate many chemical agents, contributing to its further application in environmental monitoring. *Allium cepa* has the advantage of being a low-cost test. It is easily handled and has advantages over other short-term tests that require prior preparation of the tested samples[7].

Comet assay or single-cell gel electrophoresis in meristematic cells of *A. cepa* roots was used to assess DNA damage to environmental pollutants. This assay was first developed by Ostling and Johansson in 1984 and was later revised by Singh in 1988. The assay is relatively low-cost, simple, fast and reliable. It gives reproducible results and can be studied independently of mitosis in addition to a small number of cells[8,9].

The study aims to examine genome-wide DNA changes induced by various heavy metals (Cadmium, zinc, copper, lead), the preservative sodium benzoate (SB) and wastewater in the roots of Allium cepa using the comet assay.

Citation: Naf'i AL E K, M I Khalil. Estimation of DNA damage in the roots of Allium cepa exposed to heavy metals using the comet assay. Revis Bionatura 2022;7(4) 70. http://dx.doi.org/10.21931/RB/2022.07.04.70
Received: 22 September 2022 / **Accepted:** 18 October 2022 / **Published:** 15 November 2022

Materials and methods

Sample preparation

Source of *Allium cepa*. *A. cepa* (2n=16) with a diameter of (1-2.5 cm) of the crystal cultivar was obtained from the local markets of Mosul city.

Treatment of onion roots with heavy metals

Standard solutions of the four metals cadmium, zinc, copper and lead were prepared for the following concentrations (5, 10, 20 and 25) ppm. Depending to the study by Abubacker *et al.*[10], as well as a sample of contaminated water was taken and prepared with two concentrations, diluted with a concentration of 50 ppm and an undiluted sample utilizing the law of mitigation, By dividing the final volume by the volume taken, one may calculate the number of dilutions. Also, standard solutions of the preservative sodium benzoate were prepared for the following concentrations (50, 100, 200 and 500) ppm Depending on the study Kostadinova *et al.*[11]. More information should be given on the contaminated water samples and how it was diluted to 50 ppm should be clarified.

The bulbs were treated with heavy metals solutions for four days, while the preservative SB was treated for three days, after that the roots were cut with a length of (0.5-1.5 cm) and then placed in Clark solution (ethanol: acetic acid 1:3) for 24 hours. After the end of the treatment period, the roots are placed in 70% ethanol and kept at a temperature of 4oC˙ until the examination is performed.

Application of the comet assay (single-cell gel electrophoresis)

In this study, safe red dye was used, and 4µL of dye was added for both types of agaroses, then the glass slides were immersed in Normal Melting Point Agarose (1%NMPA), which was prepared (1.5mM EDTA; 30mM NaOH, Ph 12.3) and the drops were left to cool at room temperature.

The roots were taken after treating them with heavy metals, preservatives and wastewater and placed in an Eppendorf tube with the addition of 500 µL of Tris Mgcl-2buffer solution consisting of (0.2MTris, pH7.5; 4mMMgcl2-6H2O; 0.5%W/V Triton X-100) and the roots were mashed well using to obtain the cell suspension, 100 µL of (Low Melting Point Agarose) 0.8% LMPA prepared from (1.5mM EDTA; 30mM NaOH, Ph 12.3) is mixed with 20µL of the cell suspension and mixed in an Eppendorf tube. The mixture is placed on top of the slide containing NMPA. While avoid bubbles, the slide cover is placed directly; then, the slides are placed on ice and left for 5 minutes. Then the slides were immersed in Lysing solution prepared from (1M NaCl;30mM NaOH;0.5% w/v SDS, Ph 12.3) for an hour. Slides are transferred to the relay tank containing the TBE solution and placed horizontally. The device was operated at a voltage of 60 V/cm so that the direction of the relay was from the negative pole to the positive pole for a period of 12 minutes. Slides are lifted from the migration basin and washed with distilled water three times. Slides were examined using a fluorescence microscope at 200X magnification[12].

Statistical analysis

Comet characteristics were analyzed using mean ± standard error. The significant levels of the different samples were also analyzed using SPSS 23 version for Windows software. $p<0.01$ and $p<0.05$ were set as statistical significance. The collected comet test photographs were examined. They were utilizing Comet Score™ software (TriTek Corp, Sumerduck, VA).

Results

Due to the ability of the comet assay to detect low levels of DNA damage in different types of cells exposed to heavy metals, the comet assay represents a powerful tool for identifying DNA damage[12]. The results obtained from the comet assay are summarized as shown in tables (1) and (2); the values of the tables do not appear in the manuscript. The head intensity of the meristematic cells of onion plant roots exposed to heavy metals was estimated at different concentrations compared to the control treatment; it was noted that the head intensity property of both Cadmium and zinc at concentrations (5, 10, 20 and 25 ppm) was less than the control treatment at the probability level $p<0.05$, except for the 25 ppm concentration of cadmium metal which was at the probability level $p<0.01$. At the same time, the results of copper showed an increase of 833663.7±0.05774 for the concentration of 25 ppm at a probability level of $p<0.05$ compared with 479198±0.54874,

Treatment	Con. c ppm	Head Intensity	Head DNA (%)	Tail Length (px)	Tail Intensity	Tail DNA (%)	Tail Moment
Control	0	479198 ±0.54874	*99.33623±0.05774	0	0	0	٨
Cd	5	*13572.41±0.05774	*26.3303±0.05774	*588.25± 0.05774	*20086.73±0.05774	*27.27752±0.05774	*8.897649±0.05774
	10	*4597.226±0.05774	*31.3399±0.05774	*96.25± 0.05774	*474.5325±0.05774	*3.308904±0.05774	*1.374614±0.05774
	20	*447585.5±0.05774	*66.2918±0.05774	*41.83333± 0.05774	*326461.3±0.05774	*33.30337±0.05774	*20.17954±0.05774
	25	**161417±0.05774	*90.51757±0.05774	*14± 0.05774	*19893.5±0.05774	*9.482431±0.05774	*1.623789±0.05774
Zn	5	211563.7±0.08819	*87.2453±0.05774	*14± 0.05774	*40414.33±0.05774	*18.48518±0.05774	*2.409278±0.05774
	10	266186.3±0.06009	*60.27805±0.05774	*23.75± 0.05774	**513451±0.05774	*39.72196±0.05774	*14.70296±0.05774
	20	*182751.7±0.05774	*37.47186±0.05774	*44.33333± 0.05774	*52915.41±0.05774	*31.21766±0.05774	*4.655569±0.05774
	25	*158752.2±0.05774	*63.27672±0.05774	*17.6± 0.05774	*128136.2±0.05774	*36.72328±0.05774	*7.152688±0.05774
Cu	5	*75654.7±0.05774	*56.12131±0.05774	*15± 0.05774	*41783.6±0.05774	*43.87869±0.05774	*9.453823±0.05774
	10	*66939.25±0.05774	*54.696±0.05774	*9± 0.05774	*34915.75±0.05774	*45.304±0.05774	*5.102031±0.05774
	20	*117469.6±0.05774	*31.38726±0.05774	*17.6± 0.05774	*117135.2±0.05774	*68.61274±0.05774	*12.03159±0.00577
	25	*833663.7±0.05774	**77.60359±0.05774	*28.9± 0.05774	*135492.8±0.05774	*22.39641±0.05774	5.689423±0.03941
Pb	5	**506943±0.54874	*77.41454±0.05774	*19.25± 0.05774	184030.8±0.08819	*22.58546±0.05774	*4.576296±0.05774
	10	*421729.5±0.05774	*82.73664±0.05774	*8.75± 0.05774	**72734±0.54874	*17.26336±0.05774	*1.747267±0.05774
	20	*527927.3±0.05774	*91.51076±0.05774	*25± 0.05774	**22114±0.54874	*8.489235±0.05774	1.963501±0.06854
	25	*422693.1±0.05774	*79.56153±0.05774	*19± 0.05774	*113352.4±0.05774	*20.43847±0.05774	*6.644985±0.05774

Table 1. Detection of DNA damage in meristematic cells of roots of *Allium cepa* exposed to heavy metals using the comet assay.

Treatment	Con. c ppm	Head Intensity	Head DNA (%)	Tail Length (px)	Tail Intensity	Tail DNA (%)	Tail Moment
W. W	undiluted	**172811±0.54874	*51.27855±0.05774	*16± 0.05774	*73658.67±0.05774	*48.72145±0.05774	5.416357±0.05774
	50	*1192037±0.05774	*92.5456±0.05774	*24.16667± 0.05774	**85238±0.54874	**7.454404±0.05774	*3.441796±0.05774
SB	50	*711886.9 ±0.57735	*73.79022±0.05774	*32.86667±0.05774	*196268.6±0.05774	*21.44343±0.05774	*12.47737±0.05774
	100	**669185±0.54874	*56.40031±0.05774	*15.69478±0.05774	**4561±0.54874	*43.57331±0.05774	*15.44112±0.05774
	200	*227712.5±0.05774	*95.90194±0.05718	*24±0.05774	*5684.5±0.05774	*4.098054±0.00663	*0.953467±0.05963
	500	404469.8±0.05821	*78.92408±0.05094	*20.44444±0.05774	124902.6±0.0596	21.07592±0.03013	*5.666019±0.06409

WW: wastewater without dilution
W.W-50ppm: Dilute wastewater
SB: preservative/sodium benzoate
* The Mean ± standard error was significant at the 0.05 level (2-tailed).
** The Mean ± standard error is significant at the 0.01 level (2-tailed).
Data without a sign (*) means that it does not contain significant differences
Tail moment=tail length x % of DNA in the tail [16]

Table 2. Detection of DNA damage in meristematic cells of *Allium cepa* roots exposed to wastewater (w.w) and preservative using the comet assay.

while the rest of the concentrations of the same metal have lower values than the control treatment. Also, an increase in lead values was observed (506943 ± 0.54874, 527927.3 ± 0.05774 for concentrations (5 ppm and 20 ppm) at the probability level of p<0.01 and p<0.05, respectively, compared with the control treatment 479198 ±0.54874. While a difference was observed in the values of contaminated water (WW), where it was noted that the diluted treatment with a value of 1192037±0.05774 was higher than the control treatment at the p<0.05 probability level, while the undiluted water sample was 172811±0.54874 less than the control treatment at the p<0.01 level. As for the preservative SB, it was observed that the comet head density increased by 0.57735 ± 711886.9 for each of the concentrations 50 ppm and 100 ppm at the probability level of p<0.01 and p<0.05, respectively.

The concentrations of metals were also compared with each other for the same characteristic, where it was found that Cadmium and lead at a concentration of (20 ppm) (527927.3 ± 0.05774, 447585.5 ± 0.05774), respectively, had a higher intensity compared with the rest of the concentrations. In comparison, the concentration of 10 ppm with an intensity of 266186.3 ± 0.06009 was higher for zinc metal. As for copper, the density was 833663.7±0.05774, higher at 25 ppm concentration. As for the wastewater, the intensity was 1192037±0.05774 higher at 50 ppm concentration, while the preservative SB showed higher intensity of 711886.9 ±0.57735 at 50 ppm concentration.

The results also showed that the percentage of DNA in the Comet's head DNA% reached the highest percentage in the control treatment, 99.33623±0.05774 compared with the heavy metal treatments; the results for this trait showed that all heavy metal treatments showed significant differences in the probability level of P<0.05 compared with the control treatment. This may be because the control treatment did not cause any breakage or damage to the DNA of its cells, compared with the heavy metal treatments that showed significant changes in the length of the Comet. The concentrations of minerals were also compared with each other for the same characteristic to find out at which of the concentrations the highest value for the percentage of DNA appeared in the head of the Comet. It was observed that Cadmium and copper, with values of 90.51757 ± 0.05774, 77.60359 ± 0.05774, were higher at the concentration of 25 ppm compared with the rest of the concentrations of both metals. In comparison, the value of zinc metal was 87.2453 ± 0.05774 higher at the concentration of 5 ppm. While lead, the value was 91.51076 ± 0.05774 higher at 20 ppm concentration compared with the other lead concentrations. The results of contaminated water showed that the percentage of DNA in the head of the Comet was more significant in the diluted sample with a value of 92.5456 ± 0.0574. In contrast, for sodium benzoate, the percentage of DNA in the head of the Comet was higher with a value of 95.90194 ± 0.0571 at a concentration of 200 ppm.

As for the characteristic Tail length (TL) measured in pixels (px), the results showed that the comet length for all heavy metal treatments contains significant differences at the probability level of p<0.05 compared with the control treatment that did not show significant differences. The concentrations were compared with each other for all samples to show how harmful heavy metals are to DNA breakage in the meristematic cells of the roots of Allium cepa. It was found that Cadmium had a higher effect at 588.25 ± 0.05774 at a concentration of 5 ppm, zinc 44.33333 ± 0.05774 at a concentration of 20 ppm, and copper at a concentration of 28.9 ± 0.05774 at a concentration of 25 ppm, lead 25±0.05774 at 20 ppm concentration, undiluted water (WW) 24.16667±0.05774 at 50 ppm concentration respectively and preservative SB 32.86667±0.05774 as the greatest DNA damage was for A.cepa roots exposed to (50 ppm) concentration

The tail intensity was also measured, showing that all minerals showed significant differences, meaning that they had a higher intensity than the control treatment, which did not show significant differences. The results showed that the comet-tail density of Cadmium and copper for all concentrations was p<0.05. As for zinc, it was found that the concentrations (5, 20 and 25 ppm) at the level of p<0.05, while the concentration of 10ppm was at the level of probability p<0.01, as for lead, the significant differences of the concentrations were (10, 20 ppm) at the level of p<0.01, while there were no significant differences at 5ppm. Wastewater showed significant differences between concentrated treatments at p<0.05 and diluted p<0.01. In comparison, the preservative showed a p<0.05 probability level for the concentrations (5 and 200 ppm), while the cells exposed to 100ppm concentration showed a p<0.01 probability level, it

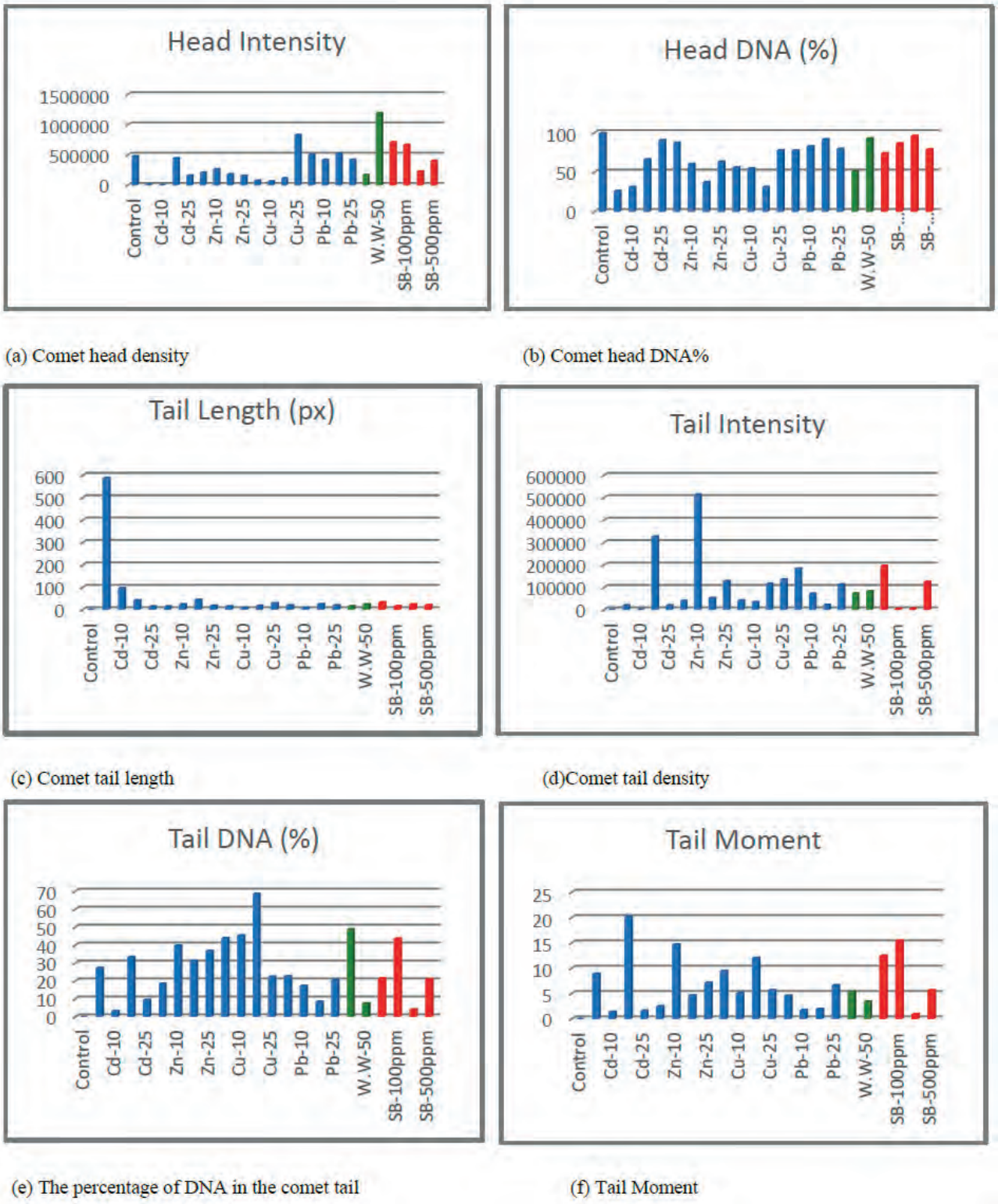

Figure 1. Shows the characteristics of the Comet of meristematic cells of the roots of Allium cepa.

was noticed that the cells exposed to the 500ppm concentration did not show significant differences.

The metal concentrations were compared with each other for the Tail intensity, where it was found that the highest intensity of Cadmium was 19893.5±0.05774 at a concentration of 20ppm, while zinc was 513451±0.05774 at a concentration of 10ppm, while the intensity of copper was 135492.8±0.05774 at a concentration of 25ppm, as for lead, the highest intensity was 184030.8±0.08819 at a concentration of 5ppm. At the same time, the wastewater was more dense Comet 83238±0.54874 at the diluted sample with a concentration of 50ppm, as for the preservative 196268.6±0.05774, it was at a concentration of 50ppm. Tail DNA% was measured, and the results showed significant differences at the probability level $p<0.05$ compared with the control treatment, which did not show significant differences, except for the 500ppm preservative concentration, which did not show significant differences. Figure (1) shows fluorescent microscopy imaging of the DNA damage caused by the effect of heavy metals on Allium cepa cells.

Tail moment (TM) also showed significant differences at the p<0.05 probability level for all treatments except for copper at 25 ppm concentration, lead at 20 ppm and contaminated water did not show significant differences. The concentrations were compared with each other for Tail Moment and Tail DNA. It was noted that the values highest for metals for each of Cadmium were at a concentration of 20 ppm, and for zinc at a concentration of 10 ppm, and copper at a concentration of 20ppm; in wastewater, the results showed that the highest values in the concentrated water sample, as for the preservative, the values for both characteristics were at a concentration of 100ppm. While lead showed a change in concentrations, the results showed that the highest value of Tail DNA % was at a concentration was 5 ppm, while the highest value of Tail moment was at a concentration of 25 ppm. Figure (1) shows the characteristics of the Comet and the variance ratio between treatments compared to the control treatment.

Discussion

The results of this study confirm that the roots of the onion plant were exposed to heavy metals (lead, zinc, copper, lead) both individually and in concentrations (25,20,10,5 ppm) and the preservative sodium benzoate at concentrations (50,200 ppm) and wastewater in both diluted treatments. And undiluted caused damage to DNA in the meristematic cells of the roots of onion plants treated with heavy metals compared to the control treatment.

The results showed that the wastewater showed the highest value of the intensity of the fluorescence of the head of the Comet, followed by the preservative and lead metal, which showed the highest value of the intensity of the fluorescence of the Comet. The study of Lyu *et al.*[13] indicated that the fluorescence intensity reflects the level of ROS accumulation. The study's results showed that the relative fluorescence intensity increased with increasing concentration and treatment time of metals. This confirms that the accumulation of reactive oxygen species (ROS) in onion roots was caused by lead stress, wastewater, and preservatives.

Ashraf *et al.*[15] reported a higher rate of DNA damage and breakage correlates with oxidative effects that occur when organisms are exposed to heavy metals, indicating the accumulation of ROS in plant tissues, which in turn causes systemic DNA damage. The study of Luo *et al.*[16] confirmed that ROS is a toxic agent and is a major source of DNA damage either by DNA strand breakage or by nucleotide removal or base mutation in nucleotides.

The results of the Tail length indicate the effect of the preservative on DNA breakdown; DNA damage to the preservative at the lowest concentration is due to inducing oxidative stress, which negatively affects physiological development, and interacts with antioxidant enzymes aimed at reducing oxidative damage[17], the result is consistent with the study of Dosay[18], which indicated the effect of sodium benzoate on DNA at low concentrations.

It was also found that Cadmium caused the most significant effect on DNA at a concentration of 5 ppm compared to the rest of the treatments used; the DNA-damaging activity of heavy metals treatments can be associated with the generation of free radicals (reactive oxygen species ROS), this leads to breaks in DNA strands and damage/binding to proteins involved in DNA replication, repair, recombination and transcription, other than oxidative stress. Cadmium may have caused the DNA-protein cross-links that generally occur with heavy metals at low concentrations; the decrease in TL for all treatments indicates that the DNA damage repair process is not affected; this may be due to cells that have adopted defensive strategies to counteract the harmful effects of reactive oxygen species caused by heavy metal[19].

DNA damage caused by exposure to heavy metals may be due to their high binding to DNA and lead to modifications in nitrogenous bases, and it may also be possible that it interferes with the DNA repair mechanism. According to previous studies, copper forms the highest degree of binding to DNA compared to other metals. Also, the decrease in TM is due to the characteristic of TL[20]. A comet test is a good tool for assessing the damage of pollutants to DNA in eukaryotic cells because it is sensitive to respond to toxic effects.

Conclusions

The current study provides valuable research information about the toxic effects of heavy metals by evaluating the DNA damage of meristematic cells of the roots of the Allium cepa plant, which is incurred by the discharge of heavy metals from natural processes or human practices into the environment. The Comet assay is a sensitive tool for assessing the potential risks associated with exposure to pollutants containing such dangerous chemicals that can cause many serious diseases.

Acknowledgments

The authors want to thank the University of Mosul for the provision of equipment, which aided in improving the quality of this work.

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REVIEW / ARTÍCULO DE REVISIÓN

Antimicrobial properties of nanoparticles in biofilms

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DOI. 10.21931/RB/2022.07.04.71

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Abstract: Biofilm is a structure in the shape of a surface adherent composed of a microbe's community and plays a crucial role in stimulating the infection. Due to the Biofilm's complex structure compared with the individual microbe, it occasionally develops recalcitrant to the host immune system, which may lead to antibiotic resistance. The National Institutes of Health has reported that more than 80% of bacterial infections are caused by biofilm formation. Removing biofilm-mediated infections is an immense challenge that should involve various strategies that may induce sensitive and effective antibiofilm therapy. In the last decade, nanoparticle NPs application has been employed as one of the strategies that have grown great stimulus to target antibiofilm treatment due to their unique properties. Nanobiotechnology holds promise for the future because it has various antimicrobial properties in biofilms and promising new drug delivery methods that stand out from conventional antibiotics. Studying the interaction between the Biofilm and the nanoparticles can deliver additional insights regarding the mechanism of biofilm regulation. This review article will define synthetic nanoparticle NPs, their medical applications, and their potential use against a broad range of microbial biofilms in the coming years. The motivation of the current review is to focus on NPs materials' properties and applications and their use as antimicrobial agents to fight resistant infections, which can locally terminate bacteria without being toxic to the surrounding tissue and share its role in improving human health in the future.

Key words: Biofilms, antimicrobial, nanoparticles, bio-nanotechnology, drug resistance.

Introduction

Nanobiotechnology syndicates biological applications with physical or chemical methods to produce nanoparticles with specific characterizations. Nanotechnology is a complicated science as it uses materials and compounds to create devices on near-atomic levels and is a new promising emerging field[1,2].

Nanoparticles have unique properties to combat conditions and diseases and have established substantial consideration in various majors such as biomedicine. Technology is in the nano range[3]. Nanoparticles come in multiple shapes, such as in a spherical form, such as a rod or plate, among other shapes. They can also be rigid or incompact and fabricated from diverse resources. Nanoparticles can be synthesized from Top-down and Bottom-up (Figure 1). In the top and bottom-up (chemical and biological) process[4]. The primary use of nanotechnology in the biomedical field is to deliver medications directly to cells or to create chemical cascades that can alter one's health and immune system to combat a range of diseases such as cancer, infectious diseases, or autoimmune diseases[5,6].

Physical-chemical properties of nanoparticles include particle size/size distribution, shape, solubility, agglomeration state/aggregation, purity and composition, surface area, surface chemistry, porosity, and other features that provide valuable information on nanoscale systems and could be melodramatically dissimilar from the particles in the range of micrometer size[8]. The nanoparticle's features, either the chemical or physical properties, should permit them to interrelate closely with bacterial biofilms and consequently provoke an antibacterial consequence that is not exclusively due to the release of metal ions which is extremely useful when used inside the body as it holds promising theories to how this technology can be used enhance one's health and amplify what is possible[9].

Human infections can be caused by a wide variety of microorganisms plus bacteria, fungi, parasites, and viruses; microorganisms of all groups are related to infections. Bacteria are the predominant constituent of microflora, and the assortment of species reflects the extensive range of endogenously resulting nutrients and the wide-ranging types of habitats to build the colonization[10]. The Biofilm could be categorized as a cumulative of microorganisms, including bacteria, in which microorganism cells adhere to each other and to a surface where they are accumulative. Though, the relationship between biofilms and the host can be disturbed in several pathways after the collapse of the microflora. Nanotechnology can interact with biofilms and microflora to improve drug delivery to these areas[11].

Citation: Ali Jabber Al-Saady M A, Aldujaili N H, Banoon S R, Al-Abboodi A. Antimicrobial properties of nanoparticles in biofilms. Revis Bionatura 2022;7(4) 71. http://dx.doi.org/10.21931/RB/2022.07.04.71
Received: 22 September 2022 / **Accepted:** 18 October 2022 / **Published:** 15 November 2022

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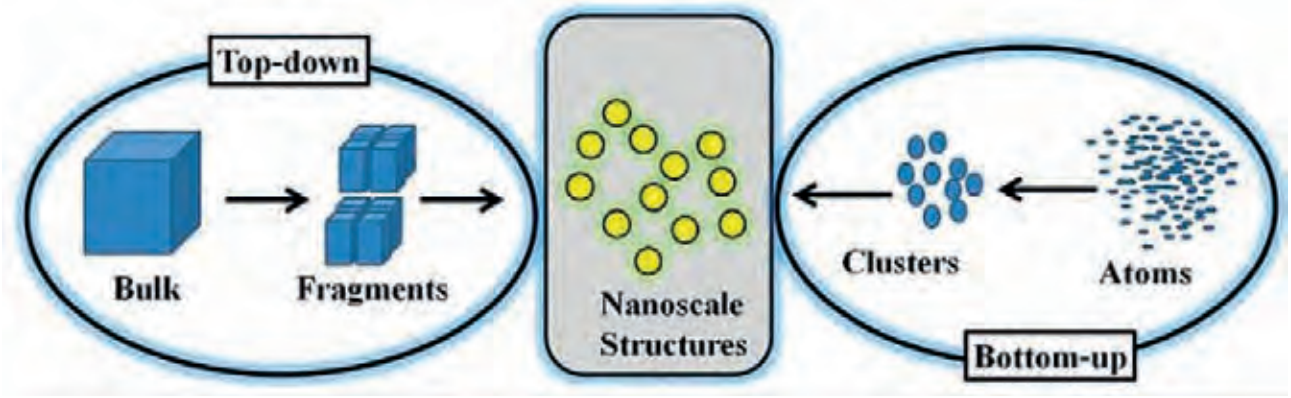

Figure 1. Illustrates the top-down and bottom-up approaches for making nanoparticles[7].

The polymicrobial phenomenon is dominant in most bacterial infections, and it is relatively infrequent to discover any that are obviously due to solitary types. The comparative influence of diverse bacterial species components in a specific infection is thus hard to control. The use of nanobiotechnology offers the possibility to control the formation of biofilms using nanoparticles as antimicrobial, antibiofilm, or with antibacterial, antiadhesive, and enhanced delivery competences (Figure 2)[12,13].

Quorum Sensing (QS)

Quorum sensing is the cell-to-cell signaling procedure that determines multi-cellular performance in microorganisms involving bacteria. Gram-negative bacteria species habitually engage minor substances such as autoinducers, signaling substances, proteins, etc. These performances in recital with a protein receptor modulate alterations in gene expression to stimulate a response to vicissitudes in the population of the cells. At the same time, in Gram-positive bacteria, oligopeptides are preferred. Increasing concentrations will result in microorganisms producing and releasing chemical signal molecules as a function of cell density[15]. Quorum Sensing has significant applications in nanotechnology because of its newfound antibacterial and antiviral properties. Due to Quorum Sensing essentially means the communication between multiple cells, nanotechnology can take advantage of this interaction and use it to help kill the Biofilm by doing things such as blocking communication, instead of taking the traditional course of broad-spectrum antibiotics[16].

Microbial biofilms and infection

Planktonic cells are isolated, free-living cells that can form the Biofilm that does not make part of the sessile cells. A biofilm is a cumulative of microorganisms with a diverse construction where cells adhere to a static surface, and biofilms are everywhere, such as on the surface of water or human teeth. Biofilms might be constructed on living and nonliving materials, which are of extensive alarm both from the environment and from a medical point of view. These

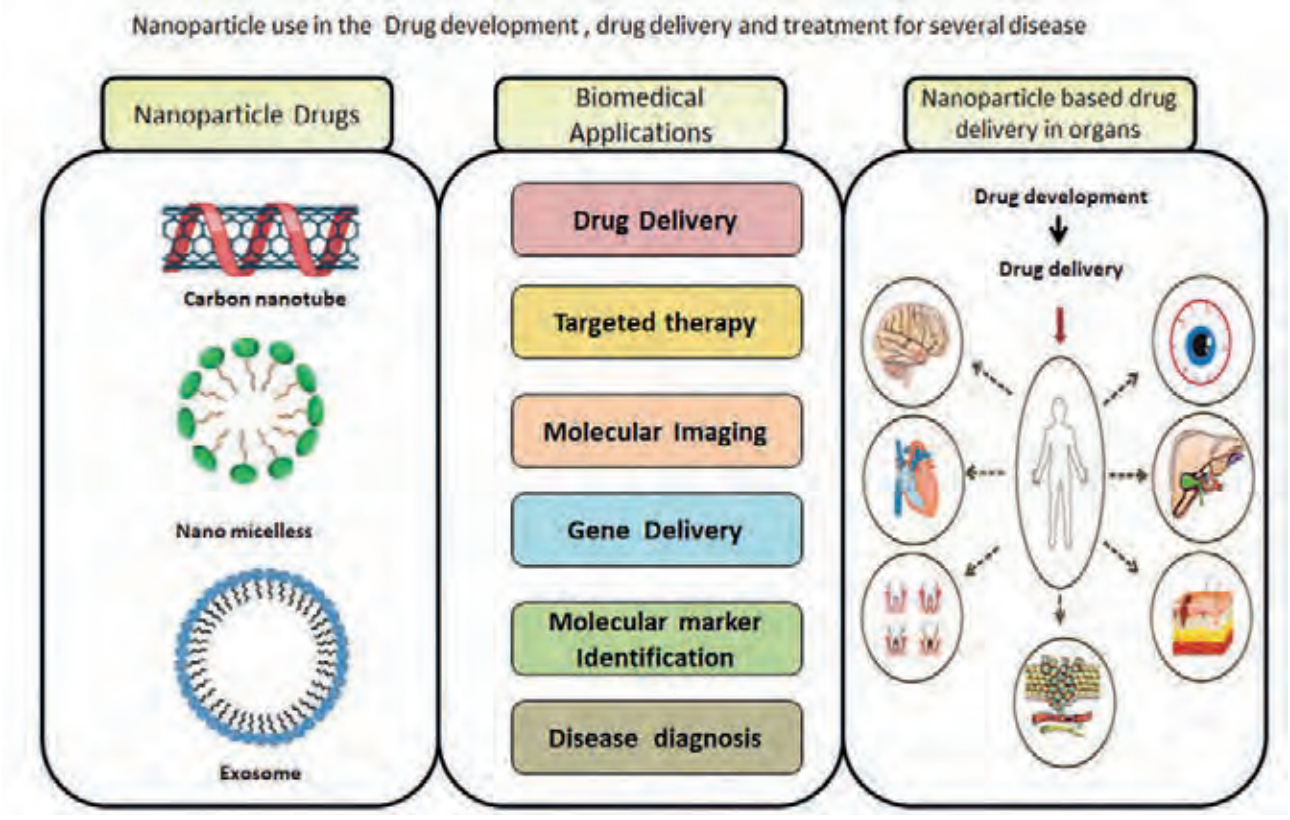

Figure 2. Nanobiotechnology offers the possibility to control many diseases[14].

cells are entrenched in the self-produced medium or environment[17].

Planktonic cells are much easier to kill with antibiotics than cells that are part of a biofilm. This is because, in a biofilm, all species in one can benefit from each other's existence. After all, different microbes can collaboratively unlock newfound capabilities and amplify them, leading to rapid and uncontrolled growth of the Biofilm, and this can lead to severe illness or even death in humans due to the corresponding bacterial infection. This is where biomedical nanotechnology is useful, as it can interfere with the Biofilm using novel approaches to remove the infection at efficiency and speeds that antibiotics cannot achieve[18,19].

The biofilm milieu

The biofilm milieu has sponge-like makings that reflect organizational integrity to the Biofilm although still permitting the movement of small substances to penetrate the Biofilm and to spread on the out-layer surface. The milieu is highly hydrated and composed of up to approximately 97% H_2O, often involving diverse types of polysaccharides along with other components such as proteins, lipids, DNA, and Ca^{+2} ions. The biofilm matrix is required for nanotechnology to pass through biofilms to enhance its drug delivery[20-23].

Factors of Microbial Biofilm

Generally, the Biofilm might be affected by many factors counting the cellular recognition for various attachment sites on a surface, contact with the planktonic cells with a sub-inhibitory concentration of antimicrobe, nutrition shortage, incidence of toxic metals, and other stress circumstances which can influence how the Biofilm grows and interacts with its environment[24]. Conditions as to whether a biofilm even forms in the first place are numerous, including temperature and nutrients, among other external growth factors. Without the right conditions, no biofilm will form. For a biofilm to keep existing, there needs to be a continued nutrient supply and a good ecosystem, so the microflora remains stable[25,26].

Microbial Advantages of a Biofilm

Biofilm advantages include many aspects that may be briefed to increase the expression of valuable genes, phenotypic vicissitudes in colony morphology, the manufacture of copious quantities of extracellular polymers that improve the admission to nutrients, achievement of antibiotic resistance genes by plasmid transmission way, and closer proximity between cells easing mutualistic or synergistic links and protection[27,28]. The biofilms usually assist bacteria in producing the virulence influences coordinately and disguising from the animal or plant's immune system[29]. The signal transduction could enhance bacterial mating among bacterial nearness available in a biofilm and, therefore, the achievement of original DNA by the transformation, which is improved and supplements the bacterial diversity[30].

The importance of Biofilm in the field of bacteriology

The reputation of biofilm development has been documented with microorganisms in one tend to vary decidedly from their planktonic counterparts in relation to behavior, construction, and physiology. These changes have consequences for the pathogenic possibility of microorganisms and their vulnerability to antimicrobials[31].

Biofilms make up a surprising amount of microbial activity, and planktonic bacteria are rarely a problem to human health. Adapting nanotechnology to biofilms to efficiently deliver drugs and disable bacteria means that the international scientific community must better understand biofilms and their relation to nanotechnology[32,33].

Biofilm stages

The development of biofilm formation is a highly complex process. Still, it is commonly recognized as containing five stages, starting with the development of a surface that the Biofilm can attach to, the crusade of microorganisms into the closeness with the surface, adhesion (in either way the reversible or irreversible of microbes adhesion to the habituated surface), development and reproduction of the organisms within the colonization of the biofilm surface, microcolony construction, and biofilm development; phenotype and genotype variations and biofilm cell detachment/dispersal[34]. It is well known that identified antibiotics can attack single bacteria and Biofilm in their early formation stages. However, it is relatively tough to destroy the late formation stages of the biofilms using traditional antibiotics, and the nanoparticles may play a vital role in terminating the multiple layers of biofilms, as shown in Figure 3.

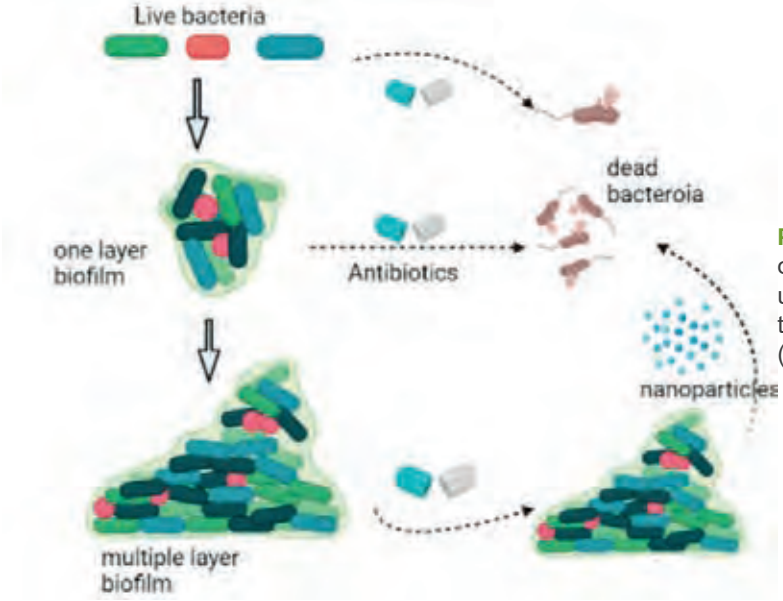

Figure 3. Biofilm growth stages, the inhibition of single bacteria cells and the one-layer Biofilm using traditional antibiotics, and the inhibition of the multiple-layer Biofilm using nanoparticles (Biorender Program).

Occurrence and examples of Biofilm

Dental plaque forms on our teeth when we neglect oral health and is an example of a biofilm; these have since been stated to be in diverse environments, for example, chronic wounds, in individuals with Cystic Fibrosis in their lungs, or plants and agricultural systems. It has been reported that 60–80% of microorganisms exist in the biofilm lifeform. Relevant biofilms are present in up to 80% of infections. Many bacterial species, such as *Bacillus sp.*, *Pseudomonas sp.*, *E. coli*, *Lactobacillus sp.*, form biofilms under the correct environmental conditions[35].

Biofilms are everywhere on Earth and can rapidly grow to substantial sizes covering entire animals and rainforests and even growing to the size of small countries. There are many different types of biofilms, however, among the most common ones are algae, fungus (mold), dental plaque, and moss[36,37].

Multispecies Biofilms

Multispecies biofilms often comprise algae, bacteria, fungi, and protozoa reliant on the colonization environment. Advantages include Interspecies interactions within biofilms resulting in increased antimicrobial tolerance, protection from foreign predators, degradation of pollutants, and indorse the spread of drug-resistance indicators and other virulence factors. Estimates of antimicrobial efficiency contrary to multispecies biofilms relying on monoculture assays may not be practicable[38].

The contaminating bacteria are adherent to some substratum or are surface-related; direct investigation of affected tissue displays bacteria living in cell clusters, or microcolonies, enclosed in an extracellular matrix. The matrix might often consist of bacterial and host contents. The infections are commonly limited to a specific site. Though dissemination might occur, it is a subordinate phenomenon. The infections are problematic or impossible to eliminate with antibiotics even though the accountable organisms are vulnerable to them in a planktonic state[11,39].

Detection methods of microbial Biofilm

Biofilm can be detected with various methods such as a tissue culture plate, the tube method, the Congo Red Agar method, Confocal Laser Scanning Microscopy (CLSM), fluorescent microscopy, bioluminescence assays, electro voltammetric detection of biofilm markers, and the Biofilm Ring Test. Biofilms come in many shapes and forms, so frequently, a human can detect a biofilm with the naked eye, such as when algae form on stagnant water or dental plaque forms on teeth[3].

Fighting Microbial Biofilms

The omnipresence and difficulty of biofilms in industrial, environmental and clinical systems present challenges for therapeutic interference. Their aptitude to perform as a breeding base for multidrug resistance and horizontal gene transfer, enabling the emergence of pathogenic strains, additional highlights the need to report their control[40]. The principal challenge is the broad-mindedness of these biofilms to most traditional or classical antibiotics. Most drugs currently used in the clinical setting have been developed and optimized to kill planktonic cells. Even when killing is accomplished, the concentration mandatory for biofilm control (minimum Biofilm inhibiting concentration, (MBIC) far exceeds that essential for control of planktonic cultures (MBIC). When the death of biofilm cells is accomplished, the capability of a subdivision of the population to survive this task, mentioned as persisted cells, means that the Biofilm can regenerate once conditions become favorable again. Therefore, new approaches are required to target the biofilm mode growth[41].

A diverse range of methods has been labeled in the literature. Antimicrobial peptides, exopolysaccharides, repurposed drugs, enzymes, chelating agents, bacteriophage therapy, quorum sensing inhibitors, and nanoparticles have all acknowledged significant considerations[42-44].

Antibiofilm activity of Nanoparticles

The administration of therapeutics into Biofilm is highly affected by the penetration of antimicrobial agents into the Biofilm. Nanotechnology deals with the design where the small size of the nanoparticles supports the procedure due to the relatively large surface area and potential group dynamic nature, these features play a crucial role in controlling biofilms through either their biocidal or antiadhesive activities[45]. The research by Watson et al. used the “Leeds *in situ* models,” which considers a tool that assists dental plaque in growing *in situ* on a detachable human enamel layer, has aided in the valuation of innovative antimicrobial agents, and is considered the extremely complex microbial composition and architecture of plaque biofilms. Using such a tool model of intact Biofilm would help gain information on the penetration of the nanoparticles on natural tooth surfaces, which may indicate that there are channels and voids in the plaque. It may occasionally spread entirely through the biomass to the underlying enamel and considerably influence the transfer of nanoparticles through biofilms[46].

Metals such as copper, silver, zinc, and gold have been employed for the last period as antimicrobial agents; they have appealed to specific attention due to their particular chemical and mechanical properties that have affected their potential roles. Many products, including toothpaste, now incorporate powdered zinc citrate or acetate to control the formation of dental plaque. Metallic nanoparticles have also been considered to improve antimicrobial efficiency[47-50].

The ability of the nanoparticles to be absorbed within the depth of the Biofilm is a key consideration in making these nanoparticles of potential effects. Therefore, the physical and chemical properties of the nanoparticles used, such as the biodegradability, biocompatibility, surface charge, and degree of hydrophobicity[51]. Nanoparticles play a significant role in causing cell death or apoptosis through different mechanisms, as shown in Figure 4.

The antimicrobial characterizations of silver and copper have established the most consideration. Both have been layered into various base materials, including PMMA and hydrogel. It has been shown that smaller silver nanoparticles are more toxic than larger particles, more so when oxidized. At the nanoscale, Ag^1 ions are known to be released (leached) from the surface. Using silver (Ag) nanoparticles (100nm), the antimicrobial action was dominated by Ag^1 ions.

In comparison, for larger particles (15 nm), the aids of Ag^1 ions and particles to the antibacterial activity are comparable. The Ag^1 ion release is proportional to the showing nano-silver surface area. Because of their small size, nanoparticles (Figure 5) may offer other advantages to the biomedical field by improved biocompatibility[52-55].

Numerous theories and descriptions have been suggested for diverse nanoparticles for their microbicidal ac-

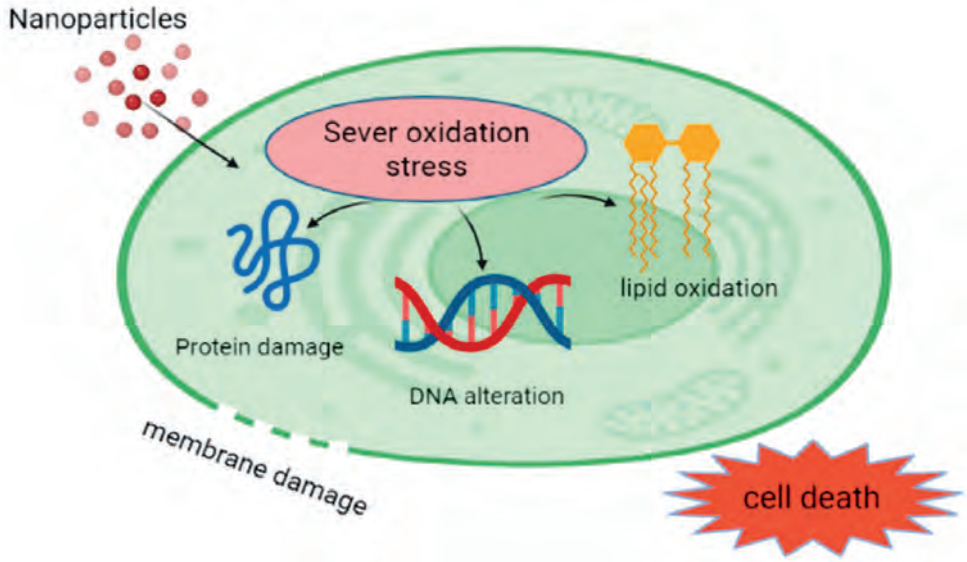

Figure 4. Nanoparticle effects on the living cells (Biorender Program).

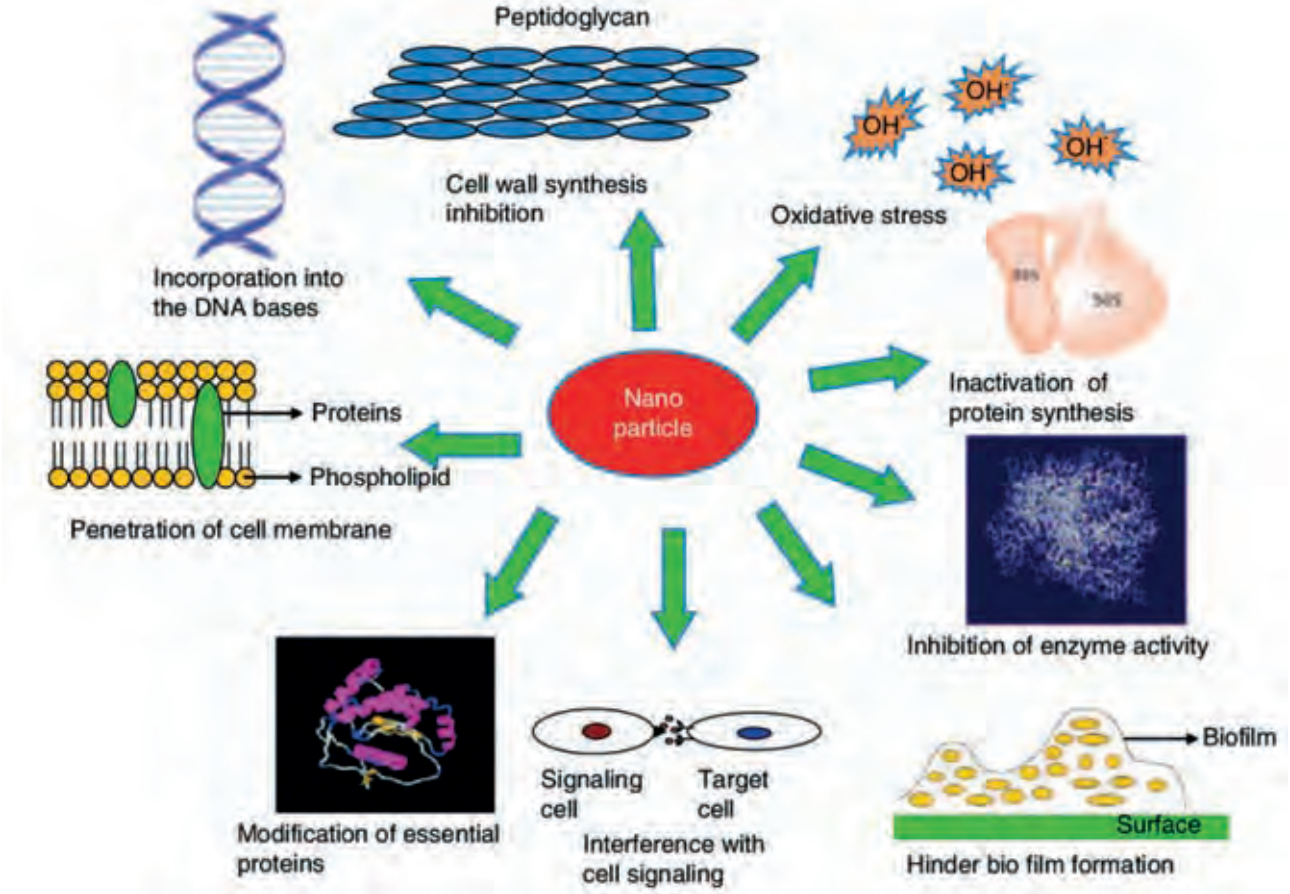

Figure 5. Mechanisms for antibacterial activity of nanoparticles[56].

tivity (Figure 2). It seems that bacteria are distant and less likely to obtain resistance to metal nanoparticles than they are to other traditional antibiotics with narrow-spectrum. This is supposed to happen because metals may act on a wide variety of bacterial boards, and frequent mutations would have to occur for the microorganisms to resist their antimicrobial activity[47]. Shape may also affect the activity of nanoparticles. The form of silver nanoparticles has been studied. Table 1 explains the most updated information about the toxicity mechanisms of different nanoparticles against biofilms.

Since NPs were shown to affect antibacterial activity in Escherichia coli, this conclusion may be drawn. Exhibiting the exception of round and rod-shaped silver nanoparticles, those with a lattice structure on the basal plane demonstrated superior biocide activity. The discrepancies appear to be explained by the number of active facets in nanoparticles of various shapes[68].

This shows that mistreatment of the toxic properties of nano particulate metals and metal oxides is feasible; in particular, those that method reactive oxygen species under UV light, such as titanium dioxide and zinc oxide, are discovered to increased use in antimicrobial formulations, with silver metal nanoparticles (5240 nm) having been reported

Nano biomaterials	Cytotoxicity mechanisms of NPs
Silver NPs [57]	-Disruption of extracellular polymeric substances network by the highly positive surface charge. -Releasing the silver ions interact with bacterial sulfhydryl groups and interfere with cell membrane integrity, respiratory chains, enzyme activities and cell proliferation.
Polycationic NPs [58] **nanocomposites** [59] **liposome** [60]	-Disruption of extracellular polymeric substances network by the highly positive surface charge. -Ions bound with DNA and interfere with electron transport, injuring bacterial enzymes and causing biofilm disruption.
ZnO NPs [61] **TiO_2 nanotube arrays** [62] **Ag NPs** [63]	-Alteration of the protein adsorptions by zinc oxide nanoparticles. The positive surface of quaternary ammonium salt disintegrates the negatively charged bacteria. -Releasing ciprofloxacin that inhibits enzymes such as DNA gyrase and topoisomerase causes bacterial disruption -Interaction of the free radicals with endogenous molecular oxygen and hydrogen peroxide damages bacteria membrane integrity and causes irreparable bacteria lysis.
Silica NPs [64] **CuO** [65]	-Releasing the ions will lead to the disintegration of the bacteria and inhibit biofilm development. Inhibition of the ergosterol synthesis by inhibiting the 14-alpha sterol demethylase produced antifungal activity. -DNA damage and oxidative stress.
Gold [66]	-Disruption of the protein conformation.
SiO_2 [67]	-Reactive oxygen species production. -Protein unfolding. -Cell membrane disruption.

Table 1. An update on the Nanoparticle Cytotoxicity mechanisms to prevent and treat the biofilms.

to inactivate most microorganisms, including HIV-1[69,70].

The large responsiveness of nano titanium dioxide and nano silicon dioxide (SiO_2) is exploited expansively for their bactericidal characterizations in filters and coatings on substrates for example, polymers, glasses, ceramics and alumina. In 2009, a novel strain of Influenza recombined in Mexico, leading to a pandemic of H1N1, a common strain of Influenza A. This led to renewed development in nanotechnology to combat such viruses and new interest in using them as antiviral medication. Substantial achievement using metal and metal oxide nanoparticles and their composite clusters against fungal and bacterial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *E. coli* has been confirmed. These have also shown the competence to inactivate viruses, including severe acute respiratory syndrome (SARS), H1N1 swine flu, and H5N1 bird flu. For example, new broad-spectrum materials (5260 nm) can reduce virus levels by anywhere from 80-2100% through direct or indirect exposure[71].

Nanoparticle arrangements, counting those based upon copper, titanium (TiO_2), (ZnO), (Al_2O_3), nickel (Ni, NiO), zirconium (ZrO_2), silicon (IV) nitride (Si_3N_4), silver (Ag), and tungsten carbide (WC) have been compared in regards to their antimicrobial potential[53,72].

Substantial activity when using Ag, TiO_2, and ZnO in the presence of UV light), SiO_2, and CuO in contradiction of bacterial pathogens, counting MRSA and *Pseudomonas aeruginosa*, have been demonstrated. MBCs were found to be in the range of 0.1-5 mg/mL

In evaluation, the conventional antibiotics are potential at concentrations 1000-fold lower. NiO, Ni, Al_2O_3, Si_3N_4, TiO_2 (in the absence of UV light), WC (tungsten carbide), and ZrO_2 lead to a lack of antimicrobial ability at the concentrations experienced. The oral pathogens *Streptococcus inter-*

medius, *P. gingivalis, F. nucleatum, P. intermedia*, and *A. actinomycete mcomitans* were also found to be vulnerable to Ag and CuO nanoparticles under anaerobic conditions with MBC values in the range 0.025-2.5 mg/mL[73-75].

Conclusions

Biofilms are structures of an accumulative of divided members of the microorganisms that may be of a single species or accumulative of a variety of microbial species communicating community-based drug resistance. Therefore, treating biofilm-mediated infections using the usual classic medicines is undoubtedly problematic. Treatment of biofilms using nanoparticle technology as antibiofilm agents is an up-and-coming method to eliminate conditions raised by microbes, including bacteria. Numerous types of NPs have been experimented with to check their activity as antibiofilm agents, and several of these NPs possess excellent anti-biofilm activity. Immobilization or impregnations of NPs are ways used to prepare biomedical surfaces. Fabrication of intelligent nanoparticles that can eradicate or treat biofilms is a step toward biofilm termination. However, many obstacles and limitations still require more research and trials. The toxic effects of some tested nanoparticles can be resolved by developing different eco-friendly methods. Despite numerous studies that have been conducted on experimenting with nanoparticles against biofilms, however, the mechanism of action is still a mystery. In the future, the potential nanoparticles that apply as antibiofilm agents will assist in improving human health by regulatory the Biofilm mediated infections.

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ARTICLE / INVESTIGACIÓN

Comparative morphoanatomical study for the same rodents species in Iraq

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DOI. 10.21931/RB/2022.07.04.72

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Abstract: Samples (4th) reviewed are deposited and stored in the Iraqi Natural History Museum (INHM), and there are 4th of them. *Sciurus anomalous* (Güldenstädt, 1785) species are preserved and mummified. It is a Caucasian squirrel (*S. anomalus*) that was medium in size, with a grayish-to-chestnut color, a golden gray back, and a golden tail. It is found in the forests of East and Southeast Asia. The variety possessed for the study was previously registered in the vertebrate literature by several authors and was stored by scientific methods in the museum. As a result of the multiplication and growth of these species, and to know the environmental changes that occurred in them, they were compared with models and samples found throughout Iraq.

Key words: Caucasian Squirrel, Morphology, *Sciurus anomalous*, Voucher Specimens, Iraq.

Introduction

The numerous correspondence in terms of shape and size for rodents and the diversity of species being the most significant order in mammals made it challenging to identify them, as it contained about 2277 species[1]. Some rodents are of identical types in terms of form and may be genetically compatible and are called sister species, and it was necessary to describe new species regularly. However, taxonomic studies are still ongoing for them. The studies examined the effects of the types that cause the destruction of crops and the transmission of diseases; therefore, a specification of each type was adopted at a certain level[2]. Taxonomy is the science of studying and documenting shape, size, and biological diversity[3]. This research has become more significant than ever due to the many biological diversity and environmental changes with human disturbances, all causing interactions that lead to the emergence of new species and vehicles of infectious diseases[4,5].

The species *Sciurus anomalusis* found in Southeast Asia and on the southern slopes of the Caucasus Basin and in the North Caucasus, Georgia, Armenia, and Azerbaijan[6-10]. It is located in most areas of Turkey[11], and it is also found in Greece; specifically, the island of Lesbos[12,13] it is also found in northern Iraq[8,14-17]. It is also distributed in Lebanon, Syria, and Jordan[8]. The study aimed to compare the current species study with the species deposited in the Iraqi Natural History Research Center and Museum (INHM), noting the differences that affect animals and the environment and the current distribution and adaptation.

Materials and methods

The four samples of male squirrel *Sciurus anomalous* (Güldenstädt, 1785) collected from the Iraqi market specialized in animals and collected from different regions of Iraq; the measurements were after the animal was anesthetized with chloroform, the measures were possessed by means of digital Vernia, ruler, electron balance and the pictures were taken with a mobile phone camera (I phone 11 pros) by the author in the laboratories of Iraq natural history research center and museum. While samples stored in the Iraqi Natural History Museum were taken of the same species, which was collected from different regions of northern Iraq, and their number was four were preserved (mummified). The specimen labels included are the common name, scientific name, and collection site. It should be mentioned that the names of the regions are based on the museum records. At present, from Iraq to compare them with those preserved in the museum in terms of the morphological measurements (T.L. = Total body length, hl = head size, W. = body width, T. =Tail, LE= length of ear, LHF = length of hind lamb, LFL = length of fore lamb) were measured in millimeters (mm) for one sample to represent all studied samples and stored in the museum.

Results

The species of squirrel (Sciurus anomalous) collected for the current study and the species deposited in Iraq at the Natural History Research Center and Museum (INHM) were a group of different regions from the north and north-east of Iraq, especially the regions (mountainous areas of Mount Horman, Safeen mountainous, the neighborhoods near Ghali Ali Beg, Soran, Mar Matti, and Sinjar in Mosul. It is also found in northeastern Iraq, such as in the Penguin Dam areas of Darbandikh and Koshak).

The voucher specimen of squirrel (Sciurus anomalous) belonging to a family of vertebrates (Sciuridae: Sciurus) was recorded in INHM according to (Figure 1).

Citation: Yousif N H. Comparative morph-anatomical study for the same rodents species in Iraq. Revis Bionatura 2022;7(4) 72. http://dx.doi.org/10.21931/RB/2022.07.04.72

Received: 22 September 2022 / **Accepted:** 18 October 2022 / **Published:** 15 November 2022

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Scientific classification

Kingdom: Animalia
Phylum: Chordata
Class: Mammalia
Order: Rodenita
Family: Sciuirdae
Genus: Sciurus
Species: *S.anamalus*[18,16,19]

The author's description, measurements, weight, and distribution of the current study were described and deposited in (INHM). It became evident that there are slight differences that the animal may have in considering the environmental conditions or adapting to the environment.

Morphology study for life Squirrel

The species collected was the color of the body sunny or golden, with the tail on the ventral side, while the dorsum was gray or dark gray. The total body length is 342 mm,(Fig.1), and the length of the trunk is 165 mm(Fig.2). The size of the tail is 157 mm, (Fig.3). The length of the hind limb is 155 mm(Fig.4). The length of the forelimb is 115 mm, (Fig.5).

The head was relatively small, comparable to the head of rodents. Its color was sunny or golden, with a head length of about 55 mm. the eye was almond, surrounded by a pale yellow halo. The size of the vibrations was (35-33 mm)(Fig. 6). The ears were comparatively long. The ear's length is 30 mm (Fig.7). Adult body mass ranges from 250–350 g.

Morphology study Mummified Squirrel

Mummified squirrel (*Sciurus anomalous*), sunny golden colored with a gray dorsum, according to the voucher specimens deposited in the Iraq Natural History Research Center and Museum (INHM). The total body length is 320 mm, (Fig.8). The size of the trunk is 152 mm (Fig.9). The length of the tail is 124 mm, (Fig.10). The length of the hind limb is 127 mm(Fig.11). The length of the forelimb is 80 mm (Fig.12). The ears were long, the length of the ear, the 19 mm (Fig.13). The head was relatively small a length of about 49 mm the vibration length (30-32mm)(Fig. 14). Adult body mass ranges from 230–400g.

Discussion

According to the survey study of geographical distribution carried out by (15,17), the family: Sciuridae, the Caucasian squirrel, *Sciurus anomalous*. Is found in 34 locations in northern Iraq, and is abundant in the mountainous areas of Mount Horman , Safeen mountainous and the areas near Ghali Ali Beg, Soran, Mar Matti, and Sinjar in Mosul. It is also found in northeastern Iraq, such as in the Penguin Dam areas Darbandikh and Koshak explained that seasonal variation might affect its color.

The current study revealed the vouchers specimens deposited with the Iraq Natural History Museum (INHM), it is clear that there are minor differences in length and measurements; that is, there are no sympatric congeners for *Sciurusanomalus*, and this is identical to what was stated[18] *S. anomalus* was distinguished in the whole study by its golden-sunny color and gray dorsum[20-22]. Observed[20,23] The tail is yellowish-brown with thick fur, consistent with current studies and voucher specimens deposited in the Iraq Natural History Research Center and Museum (INHM). Comparative measures for previous combined studies were[24-26]. Total length, 322-358 compared to the current research the entire length (320-342); tail length, 130-180 while tailing length (157-152); and the length of the ear, 23-31 compared to the size of the ear (30-19). adult body masses. Its weight varies between 250 and 410 g compared to the group of the body of the studied squirrel (250 and 350). According to research, the dorsum size of 4 samples from Iraq was 13 mm, and the maximum length of the vibrations was 38 mm[12] while the measurement of the oscillations was (35-33 mm). The eye-ring was yellow or pale orange combined with the study[22,27]. Seven samples[28] of *Sciurus anomalus* were studied. It was found that the forelimb was about (47.1 ± 0.55) and the hind limb (was 69.5 ± 0.77), compared to the samples recorded in the Natural History Research Center and Museum(INHM). The forelimb (29.0 ± 0.25) and the hindlimb (47.5± 0.56) were anterior. The current samples for the study (were 32.5 ± 0.47). The front end was 32 mm, and the hindlimb was (35.0 ± 0.45), with the claws noted as dark brown, medium in length, approximately 0.55 mm, and 0.34

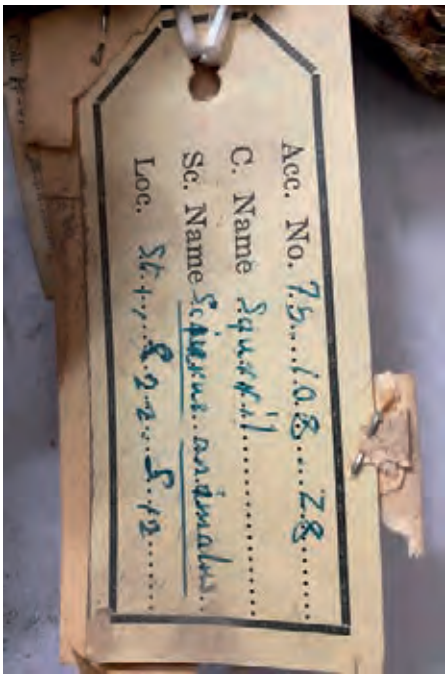

Figure 1. Shows the collection labels of the squirrel voucher specimens deposited in the Iraq Natural History Research Center and Museum (INHM).

Figure 2. Show total body length from crown to end tail.

Figure 3. Show total trunk length from neck to start tail.

Figure 4. Show tail length from coccygeal reign to end tail.

Figure 5. Hindlimb length from hip joint to end.

Figure 6. Forelimb length from shoulder joint to end.

Figure 7. Total head length & the almond eye.

Figure 8. External ear (auricle) length.

Figure 9. Total length of the mummified squirrel.

Figure 10. Trunk length of a mummified squirrel.

Figure 11. Total tail length of a mummified squirrel.

Figure 12. Hindlimb length of a mummified squirrel.

Figure 13. Forelimbs length of a mummified squirrel.

Figure 14. Head length of a mummified squirrel.

Figure 15. External ear (auricle) length of a mummified squirrel.

mm wide. [24,29]. Research continued, and the authors unanimously agreed that seasonal variations that affect crust color do not significantly vary between summer and winter. Yet, it may occupy a rusty hue in winter[30-33] observed in winter, the hair color is dark gray, the chestnut body-colored, and the head is black. Some researches study have found terminal tufts were growing in the inner part of the ear[24], and hind feet are covered with hair[5].

Conclusions

This species was previously recorded by several authors and was deposited in the collection of the Natural History Museum (INHM). It was compared with the current species, and it was confirmed that these studied species are still found throughout Iraq, the study determined the absence of any relatively significant differences between the species preserved in INHM and the current ones, and therefore the genus must be maintained from risk exposure.

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