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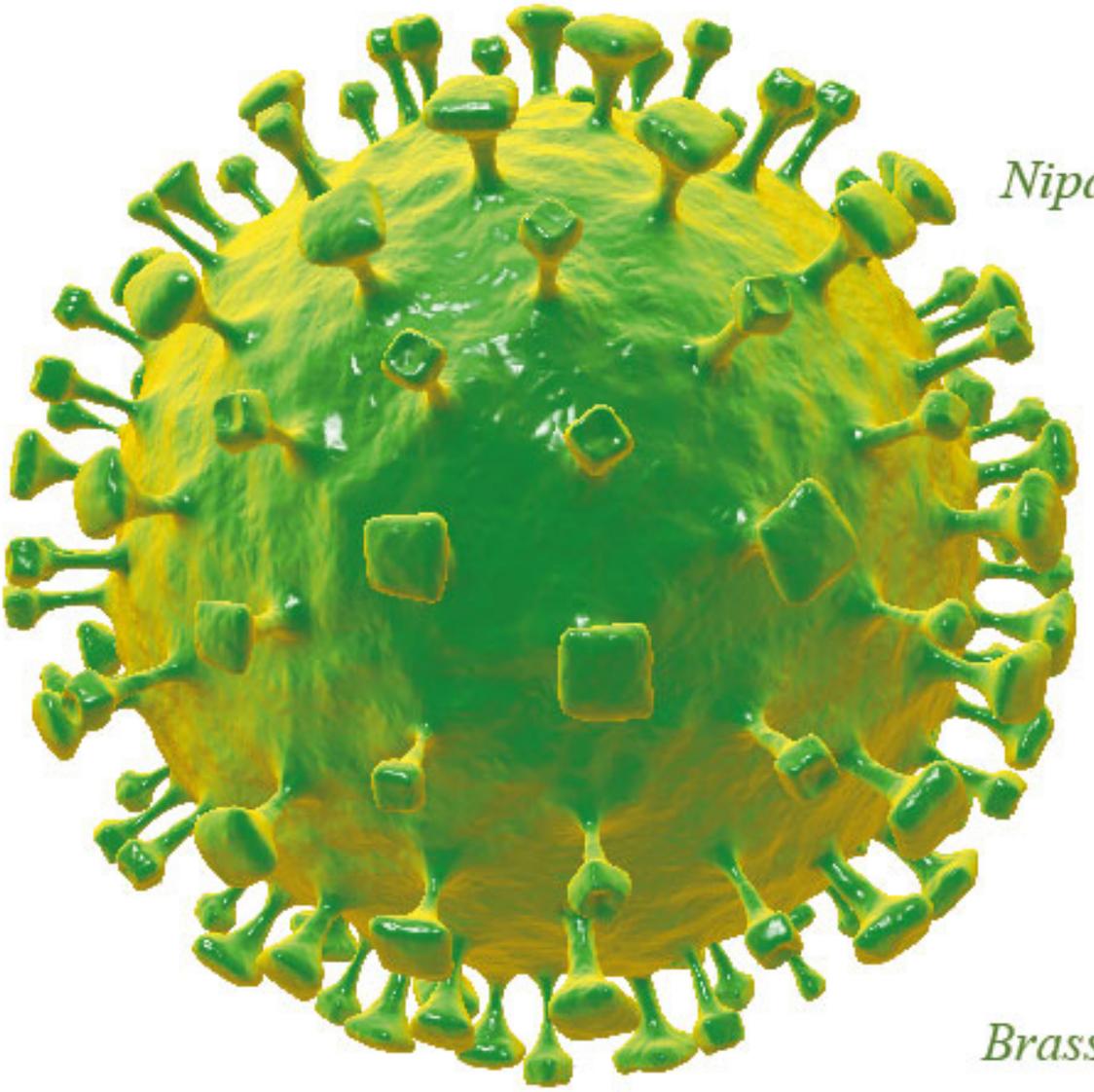

Nipah virus

Brassica napus

Bovine follicle stimulating hormone (FSH)

Sonohysterography (Gynecologic ultrasonography)

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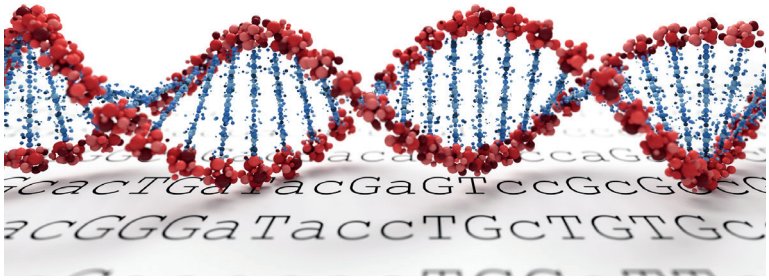

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EDITORIAL

Ultrashort peptides: minimum number in amino acid residues, maximum number in bioapplications.

Ming Ni

DOI. 10.21931/RB/2019.04.01.1

Ultra-short peptides are an intrigue new type of biomaterials for various bioapplications. Over the decades, peptides have evolved as biomaterials due to the ease of synthesis, ease of scaling up, feasibility to be functionalized/modified, and recognition by biological system[1]. Peptide-based hydrogels are widely used as scaffolds for encapsulation and delivery of drugs, genes and cells. They are made of natural amino acids. Thus they are non-immunogenic. They contain carboxylic and amine groups. Thus they can be easily functionalized with bioactive moieties. However, for peptides with more than 16 amino acid residues, they are expensive due to the high synthesis cost. As such, ultra-short peptides with only 2 to 7 amino acid residues stand out due to their easy and cost-effective synthesis, facile assembly in aqueous solution, biocompatibility and mechanical stability. These ultrashort peptides can form hydrogels without the addition of enzymes or other types of cross-linkers.

The discovery of these ultrashort peptides dates back to 2011. Dr. Charlotte Hauser (at that time she and I were both working at Institute of Bioengineering and Nanotechnology in Singapore) found a novel class of ultrashort linear peptide with 2 to 7 natural aliphatic amino acids[2]. These peptides are amphiphilic, consisting of an aliphatic amino acid tail of decreasing hydrophobicity and a hydrophilic head. These peptides self-assemble most likely via parallel–antiparallel a-helical pair formation and subsequent stacking into fibers that condense to b-turn fibrils. Fibrils further aggregate and form nanofibrous scaffolds – macroscopic hydrogels. Under scanning electron microscope, we can clearly observe nanofibrous structure at nanoscale, and honeycomb structure at microscale for this type of peptide material. The nanofibrous scaffold is capable of entrapping more than 99% water, gelation even at 1 mg/mL. These ultrashort peptides are thermally stable. They were tested with two commonly used sterilization methods in lab, UV radiation and autoclave. From NMR spectra and DSC curves, these peptides showed no different from untreated samples. Then these peptides were examined with three types of mammalian cells, including human mesenchymal stem cells, porcine nucleus pulpous cells and rabbit pigment epithelial cells. Cell culture results showed that these peptides were noncytotoxic and nongenotoxic[3]. With the preliminary data of biocompatibility for these ultrashort peptides, researchers started pursuing different bioapplications as follow:

1. Ultrashort peptides as cell culture scaffold
2. Ultrashort peptides as 3D bioprinting inks
3. Ultrashort peptides as drug delivery vehicles
4. Ultrashort peptides as bioimaging probes

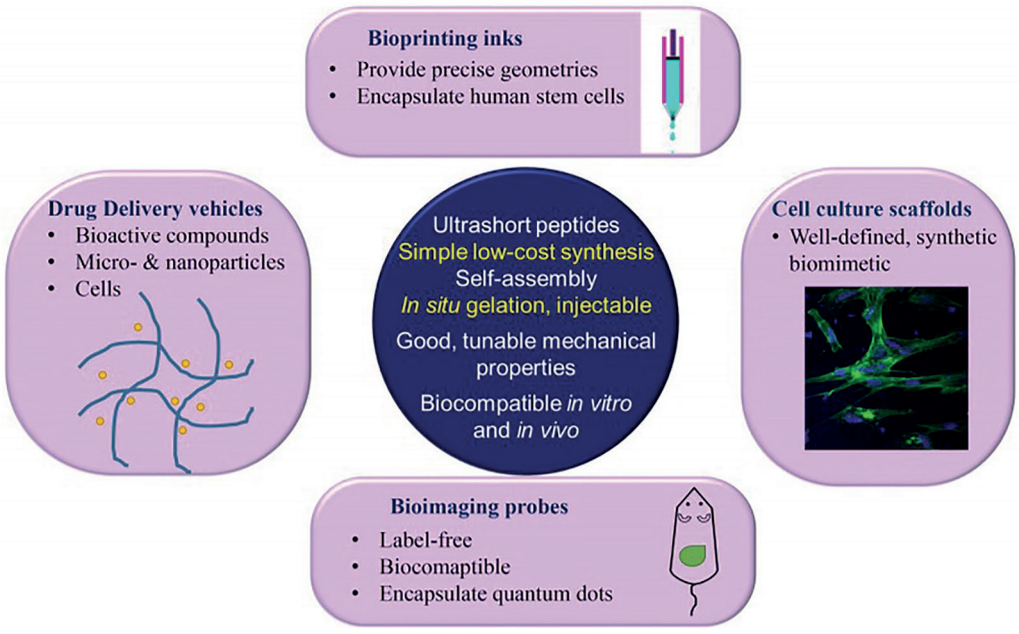

Figure 1. Bioapplications of ultra-short peptides as cell culture scaffolds, drug delivery vehicles, bioprinting inks and bioimaging probes.

1. Ultrashort peptides as cell culture scaffold
   Ultrashort peptides mimic extracellular matrix (ECM). ECM proteins, such as fibronectin and laminin, are commonly applied as surface coatings to improve cell adhesion. Amino acid sequences of RGD and YIGSR are derived from fibronectin and laminin. Instead of immobilizing the whole protein, researchers prefer to immobilize short peptides on the cell culture substrates as it is easy to control these peptides' orientation on culture substrate at a lower cost.
2. Ultrashort peptides as 3D bioprinting inks
   3D bioprinting enable us to create scaffolds with precise geometries. In 2015, Loo et al. [4] reported using ultrashort peptide hydrogels as bioink for 3D bioprinting. These peptide hydrogels undergo instantaneous gelation under physiological conditions, resulting in peptide hydrogel scaffolds. Furthermore, the hydrogel scaffolds support long-term 3D cultures of encapsulated human stem cells, as well as primary cells. The cells were further differentiated and grew into organotypic structures (skin and small intestine), which could be a useful platform for drug screening and diagnostics.
3. Ultrashort peptides as drug delivery vehicles
   Drug delivery basically faces two hurdles: targeting both in vitro and in vivo. Ultrashort peptides are a perfect choice in this regard. Reithofer et al. [5] applied "click chemistry" to conjugate anti-cancer drug to ultrashort peptides. These chemically modified ultrashort peptides can form hydrogel with pristine peptides. It was used to treat breast cancer locally in an injectable form. Significant tumor growth inhibition was observed in a mice model.
4. Ultrashort peptides as bioimaging probes
   Bioimaging refers to any imaging technique used in life sciences. It uses light, fluorescence, electrons, X-rays, ultra-sound, magnetic resonance as sources for imaging[6]. Quantum dots (QDs) are commonly use as fluorescent probes due to their superior brightness, and long fluorescent lifetimes[7]. However, QDs are highly toxic due to due to their content of heavy metal elements. Biocomaptible ultrashort peptides can be used to encapsulate QDs, providing a protective layer. Second harmonic generation (SHG) is a label-free imaging technique, offering many advantages over conventional fluorescent imaging technique[7]. Aromatic ultrashort peptides, such as FF and FFF, and aliphatic ultrashort peptides, such as IVD, are SHG-active, which make them suitable as bioimaging probes [6].

In this editorial article, we discussed ultrashort peptides (2 to 7 amino acids) and their bioapplications. They can form various types of nanostructures and possess interesting electrical, mechanical and optical properties. These ultrashort peptides can be applied as bioimaging probes, bioprinting ink, cell culture scaffolds, and drug delivery vehicles.

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LETTER TO EDITOR / CARTA AL EDITOR

Empowerment of women in Science: Myth or Reality

Hortensia M. Rodríguez Cabrera

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Recent history shows that nothing has been given to women. Every step taken in the way of the tireless task of achieving than what is fair has been synonymous of strikes, mobilization and daring, in addition to countless repressive demonstrations against the pioneers in the development of labor and social policies for the workers. Latest studies show that while political equality could be achieved in just over a century, projections for women economic empowerment, from salaries to financial control over assets, are two centuries. [1] However, what about women in STEM (Science, Technology, Engineering, and Mathematics)? Taking into account that it is precisely in this area where future jobs and sustainable growth appear to be found, the search for solutions to the gender gap in STEM is more relevant. Although in recent years the participation of women in the scientific-technological professional environment has increased, there is still an under-representation. We have been the great ones forgotten or omitted, but women have also had, we have, and we will have much to say in terms of science.

The Institute for Statistics of UNESCO [2] provided data on the positions that women occupy in science, through research carried out worldwide. The statistics concluded that, at the global level, only 28% of scientific research positions are occupied by women. Although the number of women who enroll in the university to study careers such as Natural Sciences, Engineering, Technology, Social Sciences, Humanities, and Agricultural Sciences is increasing, it is still pending in the world of science -as well as in the field of corporations- that women constitute the highest positions from researcher to project leaders.[3] Despite the advances in certain senses, at a global level, there are still enough inequalities. An example of this is that, although 20% of the engineering graduates are women, they only represent 11% of the active engineers in the job market. Of more significant impact, ten years after graduating, only 3 out of 100 continue to work in fields related to STEM. [4]

A report prepared by the UNESCO (Education sector) in 2017, for girls and women around the world, advocates for their rights to quality education, full life, and a better future, but also delves into the factors that affect this gender gap. In particular, the factors include issues such as stereotypes, family beliefs, or the sociocultural context. Likewise, and contrary to what is defended, the document discards the influence of biological factors. [5]

Moreover, numerous studies report that women in the STEM fields publish less, are paid less for their research and do not progress to the level of men in their careers. However, there are still very few data at the international level that show the real scope of these differences. Faced with this reality, it is clear that it is necessary to generate and promote measures that contribute to minimize/eliminate the gender gap in STEM.

The fact that one of the Sustainable Development Goals is "Gender Equality" shows us the importance of addressing gender gaps in any field and, especially, in younger generations, and above all, with girls. In this sense, I believe that positive discrimination will not be the path that takes us to occupy the place that belongs to us by right in science, and not to which we have been relegated/made invisible in so many years of patriarchal society. Any initiative or measures to reduce gender inequality will inevitably go through education with a gender perspective to the whole society, but especially to our children.

Educational systems and schools play a central role in determining girls' interest in STEM subjects, and in providing equal opportunities to access and benefit from quality STEM education. Besides, projects such as TeachHer, [6] which places the focus on the development of a STEM faculty capable of working and promoting these disciplines from a gender perspective, allowed to provided teachers with STEM training that allows the development of skills characteristic of these disciplines, working them in an equal, attractive and empowering way. Another positive example is Women In Science (WiSci), [7] a STEM summer camp for high school girls from around the world. The initiative focuses on empowering adolescents to pursue careers in the STEM field, fostering intercultural learning and the development of ideas that promote global social well-being.

Despite the remarkable advances made in recent decades, education is not universally available, and gender inequalities are widespread, often to the detriment of girls. Complex and interrelated cultural and socioeconomic factors affect not only the opportunities for girls to attend school, but also the quality of education they will receive, the studies they will be able to follow and, ultimately, their careers and life trajectories. One of the biggest concerns worldwide is the low participation and performance of girls in science, technology, engineering, and mathematics (STEM) disciplines. Women must grow up, especially in the scientific world, assuming without fear of failure those roles until now mostly reserved for men. We must become principal investigators, heads of research groups, directors and why not, ministers of science and education or presidents. I advocate making more aggressive policies than those that have been done so far so that all these women who are formed in STEM occupy the highest positions and receive enough

El empoderamiento de las mujeres en la ciencia: mito o realidad

La historia reciente muestra que nada se le ha regalado a las mujeres. Cada paso dado en la incansable tarea de lograr lo que es justo ha sido sinónimo de huelga, movilización y audacia, además de innumerables manifestaciones represivas contra los pioneros en el desarrollo de políticas laborales y sociales para los trabajadores. Los estudios más recientes revelan que si bien la igualdad política se puede lograr en poco más de un siglo, las proyecciones para el empoderamiento económico de las mujeres, desde los salarios hasta el control financiero sobre los activos, serán dos siglos. [1] Sin embargo, ¿Qué pasa con las mujeres en STEM (Ciencia, Tecnología, Ingeniería y Matemáticas por sus siglas en inglés)? Teniendo en cuenta que es precisamente en esta área donde se encuentran los empleos futuros y el crecimiento sostenible, la búsqueda de soluciones a la brecha de género en STEM cobra relevancia. Aunque en los últimos años ha aumentado la participación de

las mujeres en el entorno profesional científico-tecnológico, todavía están subrepresentadas. Hemos sido las grandes olvidadas u omitidas, pero las mujeres también tuvimos, tenemos, y tendremos mucho que decir en términos de ciencia.

El Instituto de Estadísticas de la UNESCO [2] proporcionó datos sobre las posiciones que ocupan las mujeres en la ciencia, a través de investigaciones realizadas en todo el mundo. Las estadísticas concluyeron que, a nivel mundial, solo el 28% de los puestos de investigación científica están ocupados por mujeres. Aunque el número de féminas que se matriculan en la universidad para estudiar carreras como Ciencias Naturales, Ingeniería, Tecnología, Ciencias Sociales, Humanidades y Ciencias Agrícolas está aumentando, todavía está pendiente en el mundo de la ciencia, así como en el campo de las corporaciones, que las mujeres asciendan a las posiciones más altas desde investigadoras a líderes de proyectos. [3] A pesar de los avances, a nivel global todavía hay desigualdades. Un ejemplo de esto es que, aunque el 20% de los graduados en ingeniería son mujeres, solo representan el 11% de los ingenieros activos en el mercado laboral. De mayor impacto resulta que diez años después de graduarse, solo 3 de cada 100 continúan trabajando en campos relacionados con STEM. [4]

Un informe preparado por la UNESCO (sector de la educación) en 2017, para niñas y mujeres de todo el mundo, aboga por sus derechos a una educación de calidad, a una vida plena y a un futuro mejor, pero también profundiza en los factores que afectan esta brecha de género. En particular, los factores incluyen temas como los estereotipos, las creencias familiares o el contexto sociocultural. Asimismo, y en contra de lo que se defiende, el documento descarta la influencia de factores biológicos. [5]

Además, numerosos estudios informan que las mujeres en los campos de STEM publican menos, les pagan menos por sus investigaciones y no progresan al nivel de hombres en sus carreras. No obstante, todavía hay muy pocos datos a nivel internacional que muestran el alcance real de estas diferencias. Frente a esta realidad, es clara la necesidad de generar y promover medidas que contribuyan a minimizar / eliminar la brecha de género en STEM.

El hecho de que uno de los Objetivos de Desarrollo Sostenible sea "Igualdad de Género" reafirma la importancia de abordar las brechas de género en cualquier campo, especialmente en las generaciones más jóvenes y, sobre todo, con las niñas. En este sentido, creo que la discriminación positiva no será el camino que nos lleve a ocupar el lugar que nos pertenece por derecho en la ciencia, y no al que hemos sido relegadas / invisibilizadas en tantos años de sociedad patriarcal. Cualquier iniciativa o medida para reducir la desigualdad de género pasará inevitablemente por la educación con una perspectiva de género de toda la sociedad, pero muy especialmente a nuestros niños.

En este sentido, los sistemas educativos y las escuelas desempeñan un papel central en la determinación del interés de las niñas en las materias STEM y en brindar igualdad de oportunidades para acceder y beneficiarse de una educación STEM de calidad. Además, proyectos como TeachHer, [6] que se centran en el desarrollo de profesionales STEM capaces de trabajar y promover estas disciplinas desde una perspectiva de género, permitieron a los docentes capacitarse en STEM y permitirles el desarrollo de habilidades propias en estas disciplinas. Inculcándoles el trabajo de forma igualitaria, atractiva y empoderadora. Otro ejemplo positivo es Women in Science (WiSci), [7] un campamento de verano STEM para niñas de secundaria de todo el mundo. La iniciativa se centra en capacitar a los adolescentes para que sigan carreras en este campo, fomentando el aprendizaje intercultural y el desarrollo de ideas que promuevan el bienestar social global.

A pesar de los notables avances logrados en las últimas décadas, la educación no está disponible de manera universal, y las desigualdades de género están generalizadas, a menudo en detrimento de las niñas. Los factores culturales y socioeconómicos complejos e interrelacionados afectan no solo las oportunidades para que las niñas asistan a la escuela, sino también a la calidad de la educación que recibirán, los estudios que podrán seguir y, en última instancia, sus carreras y trayectorias de vida. Una de las mayores preocupaciones a nivel mundial es la baja participación y el rendimiento de las niñas en las disciplinas de ciencia, tecnología, ingeniería y matemáticas (STEM). Las mujeres deben crecer, especialmente en el mundo científico, asumiendo sin temor a fallar esos roles hasta ahora mayormente reservados para los hombres. Debemos convertirnos en investigadoras principales, jefas de grupos de investigación, directoras y, por qué no, en ministras de ciencia y educación o presidentes. Abogo por hacer políticas más agresivas que las que se han hecho hasta ahora para que todas estas mujeres que se forman en STEM ocupen los puestos más altos y reciban suficiente dinero para investigar.

money to investigate.

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RESEARCHS / INVESTIGACIÓN

Response of Rapes (*Brassica napus L.*) to Nano- Iron Fertilization under semi-arid Region conditions

Lamiaa A. Mutlag[1], Nagam A. Meshaimsh[1], Hasan H. Mahdi[1], Raghad S. Mouhamad[1*], Hasan H. Khamat[1], Obeyed H. Jasaam[1], Saham M. Bajy[1]

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Abstract: The field experiment was conducted in the season of 2017-2018 at the Agricultural Research Department-AL-Azafrinih/Baghdad-Iraq. This study aimed to determine the effect of adding the Foliar Fe fertilization (Nano-Fe) and ground fertilization of (N, P) in the growth and production yield of rapes (*Brassica napus L.)*. The experiment included three levels of foliar fertilization (Nano-Fe) that is (0, 5, 10 Kg. ha^{-1}) and one level of ground fertilization (N 100 Kg. ha^{-1}, P 250 Kg. ha^{-1}). The experiment designed according to Randomized Complete Block Design (R.C.B.D) with three replications each parameter. The studied traits were nutrients content (macro-micro) in seeds and soil, the percentage of protein, oil, and carbohydrate. All data were analyzed and used statistically. Results showed a significant difference between Nano-Fe fertilization and nutrient content (N, Mn, K, Ca, Mg, S, and Cu), pH in soil at the level of 10 kg. ha^{-1}, compared to no-fertilization. Moreover, the significant difference between Nano-Fe fertilization, yield (1425.6 kg. ha^{-1}), the percentage of oil (52.16%), protein content in seeds (46.5%), carbohydrate (26.8%), Nutrient content N and P in seeds at the level of 10 kg. ha^{-1}, compared to no-fertilization and the nutrient content (Ca, Mg, Fe) in seeds at levelof5 kg. ha^{-1}, compared to no-fertilization. Current results suggested that Nano-Fe fertilization at the level of 10 kg. ha^{-1} can be adopted as the best level for rapes (*Brassica napus L.*) cultivation under semi-arid conditions.

Keywords: *Nano-iron fertilizer; yield; nutrients; protein.*

Introduction

Brassica napus L. classifies to the Brassicaceae family and has become one of the important oilseed crops in the agricultural systems of semi-arid regions where water deficit and high temperatures restrict growth and yield during the reproductive growth in Iraq. Canola seeds contain ≥49%oil contents. Oil in rapeseed is often determined by fertilization of the plants [7]. For winter type canola growth, 50 - 100 kg. ha^{-1}fertilizer containing nitrogen with potassium and about 20 - 40 kg. ha^{-1}calcium with phosphorus should be applied before or during planting [8]. In severe winters conditions, a substantial proportion of the canola leaves are killed by frost and is lost from the plant [12]. The decrease in yield oil content may be due to a deficiency of Fe that controls the metabolic transformations in the plants, so the quality of grain is determined by oil and protein seed content [26].

According to the limitations of soil usage of micro-nutrients consolidation and residual effects, the foliar spraying or leaf feeding is one of the effective ways to resolve plants food requirement to micronutrients [25]. High phosphorus in the soil, high pH, high lime, high soil moisture, are the causes of Fe deficiency in the soil [21]. If adequate and absorbable amounts of Fe are not available for the plant chlorophyll production in leaf decreases and the leaves become pale. It should be noted that not only Fe deficiency results in yellowish leaf, but also in some cases deficiency of nitrogen and some other nutrients, some pests, diseases, and low light lead to pale leaf [22]. Nano fertilizers are the most important function of nanotechnology in the production phase of agriculture.

Application of Nano fertilizers instead of conventional fertilizers, nutrients are provided to plants gradually. The nanotechnology increases the application efficiency of fertilizers, reduces soil pollution and environmental risks of chemical fertilizers [11]. Nanomaterials are much smaller and lighter; they interact better in the environment and may be a solution to the problem of iron nutrition in saline and lime soils. Iron Nano oxide is smaller than the conventional iron oxides and forms more complexes and makes the Fe more available to plants [10] Fe deficiency is a widespread nutritional problem in plants growing mainly in high pH and calcareous soils. Foliar application of Fe compounds with the technology of Nano may be a solution to the problem [1]. Iraq with arid and semi-arid climates always is faced with water shortages. Therefore, this study aimed to investigate the effect of Fe- foliar of Nano-iron on yield and yield components, soil minerals of *B. napus.*

Materials and methods

Field experiments were carried out at the Agricultural Research Department-AL-Azafrinih/Baghdad-Iraq in the season of 2017-2018. This study aimed to determine the effect of adding the Foliar Fe fertilization (Nano-Fe) and ground fertilization of (N, P) on the growth and production yield of rapes (Brassica napus L.). The soil was plowed, rotivated and leveled. The experiment divided to 3 blocks designed according to Randomized Complete Block Design (R.C.B.D) with three replications each parameter. Blocks were divided into 6 plots with an area of (1 x 2) m^2, and the plots were separated by 0.5 m in width from all sides to avoid the effect of fertilization. Soil properties of the application areas shown in Table 1.

Treatments included the application of N and P in 100, 250 kg. ha^{-1} respectively. Another plot in each block has not been fertilized. Nano-Fe was added as Foliar fertilizer with le-

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vels of 0, 5, 10 kg. ha^{-1}. Rap was planted in rows aside plot with distance of 30 cm between each rows. Soil was irrigated after depletion of 40% of F.C. using gravimetric method.

Plants were harvested after maturity; soil samples were collected at the depth of 0-30 cm and ground. Samples of a plant were oven dried at 65 °C. The powder was digested using according to (Page, 1982). The contents of the mineral elements for Ca, Na, Mg, Mn, K, Zn, Cu, and Fein soil determined using the DTPA method and analyzed by Atomic Absorption Spectroscopy (AAS). Electrical conductivity values were determined by EC meter in 1: 1soil water mixture (WTW, Page, 1982). pH was determined by a glass electrode pH meter calibrated with a standard solution in a 1: 1 soil-water mixture (Mclean, 1982). Available phosphorus (P) determined by the ascorbic acid method of 882 nm in a spectrophotometer (Olsen 1982). N percentage determined by the Kjeldahl method. Organic matter (OM) determined by Wakley - Black method (Nelson and Sommers, 1982). Soil texture determined by reading the hydrometer in water mixture for 40 seconds and 2 hours (Gee and Bauder, 1986). The contents of the mineral elements for Ca, Na, Mg, Mn, K, Zn, Cu and Fe in the plant determined using the digestion method and analyzed by Atomic Absorption Spectroscopy (AAS). The oil ratio, ash, carbohydrate, fiber and Humidity determined by the method of the Association of Official Analytical Chemists (AOAC) (5). Total protein content was determined by the Kjeldahl method (a nitrogen conversion factor of 5.95), methods described by Page (1982). Statistical analysis was done and Means have compared at Least significant differences (L.S.D). SAS and EXCEL software have been used.

pH	EC	soil texture%			OM%	N%	P	K
	ds/m^{-1}	sand	silt	clay			mg/kg	mg/kg
6.9	6	19.73	56.14	24.13	1.31	0.18	25.56	580

Table 1. Soil properties of the area under study.

Results and discussion

Table 2 showed significant increase of N content in the seeds, which were 31.6 - 33.7 mg kg^{-1}, and decrease in P content which were 7.5 - 6.1mg kg^{-1}atlevel (10 kg. ha^{-1}) Nano-Fe fertilization, compared to no-fertilization. For level (5 kg. ha^{-1}) Nano-Fe fertilization, shows significant increase in the Ca and Mg content in the seeds which were 7.3 - 8.1 mg kg^{-1}, 3.2- 3.5mg kg^{-1}respectively, and the decrease in Fe content which were 94.1- 85.2mg kg^{-1} content, compared to no-fertilization. Increased nutrient content in seeds can be explained, because of addition Foliar Fe fertilization (Nano-Fe) and ground fertilizers (N, P). Iron is a critical element in cell metabolism, and it is contributory in photosynthesis, respiration, enzymes activity, chlorophyll production. [15] Nitrogen is an essential nutrient for the synthesis of fat, which requires both N and carbon during seed development [14]. On the other hand, nitrogen plays the most critical role in building the protein structure [6]. P is an integral component of several essential compounds in plant cells, including the sugar-phosphates contributory in respiration, photosynthesis and the phospholipids of plant membranes, the nucleotides used in plant energy metabolism and in molecules of DNA and RNA [23]. Calcium (Ca) is essential in cell nucleus matrix. It activates enzymes, particularly those that are membrane-bound. It is thought that Ca is vital in the formation of cell membranes and lipid structures [18].

Values marked with the same letter no significant difference at $p \leq 0.05$

The Data of Table 3 showed significant increasing of N, K, Ca, Mg, S, Mn and Cu content in the soil which were 0.14-0.16mg kg^{-1}, 125–158mg kg^{-1}, 1200.3 - 1375.2 mg kg^{-1}, 1260–1358mg kg^{-1}, 340–420mg kg^{-1}, 3.1- 3.5 mg kg^{-1}, 52.1- 55.2mg kg^{-1} , 0.86- 1.3mg kg^{-1}respectivelyand decrease in pH from 6.91 to6.36, at level(10kg.ha^{-1}) Nano-Fe fertilization, compared to no-fertilization. For level (5 kg. ha^{-1}) Nano-Fe fertilization, showed significant increasing of K, Ca, Mg, Fe, Cu content in the soil which were 128- 135mg kg^{-1}, 1257- 1397mg kg^{-1}, 320–399mg kg^{-1}, 0.97 - 1.2 mg kg^{-1}, 0.88 - 1.17 mg kg^{-1}respectively, and decrease in pHfrom6.90to6.71, compared to no-fertilization. The increase in the concentration of nutrients in the soil can be explained, because of decrease the pH 6.91 - 6.36, when an addition of fertilizers and the soil texture of the application areas was silt clay loam. Management of plant nutrition is important to prevent reactions in reducing nutrient availability in the soil. Soil pH regulates the capacity of soils to store and

Parameter	Foliar Fe fertilization				
	0 kg. ha^{-1}		5 kg. ha^{-1}		10 kg.
	ground fertilizer (N 100 kg. ha^{-1}, P 250 kg. ha^{-1})				
	No-Fer (kg. ha^{-1})	Fer. (kg. ha^{-1})	No-Fer. (kg. ha^{-1})	Fer (kg. ha^{-1})	No-Fer. (kg. ha^{-1})
macro-micro nutrients of seeds (mg kg^{-1})					
N	24.1^{a}	27.1^{a}	33.4^{b}	33.9^{b}	31.6^{c}
K	74.9^{a}	69.1^{b}	84.5^{c}	80.2^{c}	85.2^{c}
Na	2.2^{a}	2.4^{a}	2.7^{b}	1.7^{c}	1.8^{c}
Ca	4.8^{a}	5.8^{b}	7.3^{c}	8.1^{d}	7.9^{d}
Mg	2.9^{a}	3.00^{a}	3.2^{b}	3.5^{c}	3.6^{c}
P	5.3^{a}	5.6^{a}	5.8^{b}	6.3^{b}	7.5^{d}
S	2.8	2.8	2.9	3	3.1^{a}
Fe	74.9^{a}	79.8^{a}	94.1^{b}	85.2^{c}	89.1^{d}
Mn	31.6	30.7	59.2	58.1	52.1
Cu	2.7	2.8	3	2.3	2.4

Table 2: The effect of foliar Nano- Fe fertilization and ground fertilizer (N 100 kg. ha^{-1}, P 250 kg. ha^{-1}) on the nutrient content of seed of winter rape in AL-Azafrinih station (2017-2018)

supply nutrients, and thus contributes substantially to controlling productivity [4].

Table 4 shows significant increase in the seed yield from 1298.6 to 1425. 6kg.ha^{-1}at level (10 kg. ha^{-1}) Nano-Fe fertilization, compared to no-fertilization. This may be due to increasing the number of capsules in the plant and the number of seeds in capsules. Recent research has shown that a small number of nutrients, especially Zn, Fe, and Mn applied by foliar spraying increases the yield of crops [19] significantly. Keikha et al.[9] found in studied the effect of Fe foliar application at the beginning of the flowering stage on canola and observed that the treatment increased grain yield from 4136 to 4557 kg. ha^{-1}. The status of seed yield is the most important field scale, giving the final evaluation of agricultural operations to produce new recommendations on production.

Values marked with the same letter no significant difference at p ≤ 0.05

The results showed a significant increase in the percentage of oil 52.16% at level (10 kg. ha^{-1}) Nano-Fe Fertilization, compared to no-fertilization (Table 4). This may be due to increase in leaves area and surface area of capsules that were involved in the process of carbon representation and then increase the accumulation of dry matter and this has reflected positively on the percentage of oil in the seeds. The quality of rapeseed grain is determined by its oil and protein content [17]. Bahrani, A., [2] showed oil percent and oil yield, NPK fertilizer and foliar application of Fe, Zn and Mn treatments were significant in oil percent.

Chemical components, including protein, are essential traits to be taken into account in the study of rapes (*Brassica napus L.)*. In results show increase percentage of seed protein 46.5 %at level (10 kg. ha^{-1}) Nano-Fe fertilization, compared to no-fertilization (Table 4). This may be due to the inverse relationship between the percentage of oil and protein, increasing the percentage of oil in the seeds leads to decrease the percentage of protein, and conversely, this is similar with (24).

The other results: percentage of carbohydrate, Ash, Fiber and Humidity which were 26.8%, 5.56%,7.49 %, 9.2%, respectively found at level Nano-Fe fertilization (10 kg. ha^{-1}), compared to no-fertilization (Table 4). In addition to oil production, rapeseed leaves and stems provide high-quality feed because of their low fiber content and high protein content and can be ground in animal feeds [3].

Values marked with the same letter no significant difference at p ≤ 0.05

Table 4: The effect of foliar Fe fertilization and ground fertilizer (N 100

Peak	Title (vg/mL)	260/280 ratio
1	10^5	0.601
2	10^8	1.334

kg.ha^{-1}, P 250 kg.ha^{-1}) on the Organic composition of the seed of winter rape in AL-Azafrinih station (2017-2018)

Conclusions

Brassica napus L. (canola) responded positively with increasing level Foliar Fe fertilization (Nano-Fe10 kg. ha^{-1}) and ground fertilizers (N 100 kg. ha^{-1}, P 250 kg. ha^{-1}). Crop growth and yield significantly affected by higher amounts of applied fertilizers. Seed quality aspects, i.e. protein content and oil content represented the variable response to each increment in Foliar Fe fertilization (Nano-Fe10 kg. ha^{-1}).

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Parameter	Foliar Fe fertilization				
	0 kg. ha^{-1}		5 kg. ha^{-1}		10 kg.
	groundfertilizer (N 100 kg. ha^{-1}, P 250 kg. ha^{-1})				
	No-Fer (kg. ha^{-1})	Fer. (kg. ha^{-1})	No-Fer. (kg. ha^{-1})	Fer (kg. ha^{-1})	No-Fer. (kg. ha^{-1})
macro-micro nutrients of soils (mg kg^{-1})					
pH	7.1^a	7.3^a	6.90^b	6.71^c	6.91^b
Ec(ds/m^{-1})	5.4^a	5.5^a	5.7^a	6.01^b	6^b
N	0.1^a	0.11^a	0.11^a	0.12^a	0.14^c
K	108^a	114^a	128^b	135^g	125^b
Na	1457.3	1390^b	1300.7^c	1317.3^c	1200.3^d
Ca	1780^a	1889^a	1257^b	1397^c	1260^b
Mg	229.2^a	240.2^b	320^c	399^d	340^c
P	25.5	30.01	25.4	24.95	20.22
S	2.8^a	2.8^a	2.9^a	3^b	3.1^b
Fe	0.44^a	0.68^b	0.97^c	1.2^d	1.3^d
Mn	31.6^a	30.7^a	59.2^b	58.1^b	52.1^c
Cu	0.70^a	0.78^a	0.88^b	1.17^c	0.86^b

Table 3: The effect of foliar Nano-Fe fertilization and ground fertilizer (N 100 kg. ha^{-1}, P 250 kg. ha^{-1}) on the nutrient content of soil of winter rape in AL-Azafrinih station (2017-2018)

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RESEARCHS / INVESTIGACIÓN

AAV Purification by anion-exchange chromatography

Purificación de AAV mediante cromatografía de intercambio aniónico.

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Abstract: We describe a new optimized, scalable and reproducible method based on anion exchange chromatography to obtain high titers of rAAV vectors without empty capsids contamination. The method takes advantage of Q-sepharose matriz to development a scalable procedure. After the virus harvest from supernatant and lysate cells, virus crude was subjected to anion exchange chromatography with Q-sepharose column. Three different protocols were tested, and the elution peaks were evaluated through qPCR rAAV titration and 260/280 nm ratio determination in order to identified empty capsid-containing fractions. A 150 mM NH4Ac wash step fallowing by 1 M NaCl elution step generate a a high titer eluted fraction of rAAV with 1.334 260/280 nm ratio. The described method makes rAAV vector purification an easily adapted for a large scale GMP production format to produce empty capsid free rAAV for clinics.

Keywords: rAAV purification, anio-exchange, empty capsid

Resumen: Este trabajo describe un nuevo método optimizado, escalable y reproducible basado en la cromatografía de intercambio aniónico para obtener títulos elevados de vectores rAAV sin contaminación de cápsides vacías. El método aprovecha la matriz Q-sepharose para desarrollar un procedimiento escalable. Después de la recolección del virus del sobrenadante de cultivo y del lisado, el crudo viral se sometió a cromatografía de intercambio aniónico con una columna de Q-sefarosa. Se probaron tres protocolos diferentes, y los picos de elución se evaluaron mediante qPCR rAAV y la determinación de la relación de 260/280 nm para identificar las fracciónes que contienen cápsides vacías. Un paso de lavado con NH4Ac 150 mM seguido de un paso de elución con NaCl 1 M genera una fracción eluida de alto título de rAAV con una relación 260/280 nm de 1.334. El método descrito hace que la purificación del vector de rAAV se adapte fácilmente a un formato de producción de GMP a gran escala para producir rAAV libre de cápside vacío para clínicas.

Palabras clave: Purificación de rAAV, intercambio aniónico, capsidas vacias

Introduction

Recombinant Adeno-associated virus (rAAV) are simple virus with linear single-stranded DNA genome. rAAV have proved significant potential as DNA-delivery vectors. Their main advantages include a wide tropism, poor immunogenicity and the ability to maintain the transgene expression for long periods [1].

Since the introduction of Baculovirus expression vectors to produced high titers of rAAV, the problem of scalable production seems to be solved [2]. However, manufacture methods that enable to obtain rAAV vectors with optimal purity, potency, and consistency are needed. Some of the available rAVV purification methods include cesium chloride gradient ultracentrifugation, iodixanol gradient ultracentrifugation [3], heparin-based affinity chromatography purification(4) and ion-exchange chromatography [3]. However, none of the above mentioned methods could solved one of the key drawbacks of the scalable rAAV purification process: contamination with empty capsids.

This work reports an optimized, scalable and reproducible method based on anion exchange chromatography to obtain high titers of rAAV vectors without empty capsids contamination.

Materials and methods

rAAV production and virus harvest

rAAV were obtained with AAV-DJ Helper Free Expression Systems from Cell Biolabs (USA). This system allows the obtaining of rAAV without the use of helper virus. For recombinant virus production, pAAV-DJ and pHelper were used as helper vectors and pAAV-GFP as genome vector. The method to produce rAAV-GFP was done following manufacturer's instructions. Briefly, 100-mm-diameter culture dishes were seeded with HEK-AAV cells at 70 % confluence, which were co-transfected with pAAV-GFP, pAAV-DJ and pHelper (1:1:1) with 25 kDa branched polyethylenimine.

Three days after transfection the cells were harvested by scrapping and were lysed with Tris-Cl buffer containing 150 mM NaCl and 2mM $MgCl_2$. Virus-containing supernatant were precipitated adding 1/5 volume of PEG 40%/2.5M NaCl, incubated 1 hour in ice and spun down at 3000 g for 30 min. Both virus fractions were joined and subjected to three freeze-thaw cycles and treated with benzonaze nuclease and RNAse. The final spin down was done at 3000 g during 30 min and the virus-containing supernatant were collected.

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Virus titration

The vector genome copies number (VG) was determined by qPCR using Brilliant III Ultra-Fast SYBRgreen qPCR Master Mix (Agilent). The viral DNA was extracted from 1, 10 and 20 µl of purified virus and was treated with 0.5 U DNase I (NEB) to digest any contaminating unpackaged DNA, followed by an additional treatmentwith 10 µg of proteinase K (Thermofisher Scientific) to assist in breaking capsids and releasing viral DNA. qPCR was run in an AriaMX (Agilent technologies) with primers annealing at AAV ITRs: forward primer 5´-GGA ACC CCT AGT GATGGA GTT-3´ and reverse primer 5´-CGG CCT CAG TGA GCG A-3. The program used was: 95ºC 10 min, then cycled 40 times at 95ºC for 15 seg, 60ºC for 30 seg and 72ºC for 30 seg. To generate the standard curve, a pAAV-GFP plasmid was used in serial dilutions from 1×10^9 to 1×10^2 genome copies (vg) and performed in triplicate. A no-template negative control was also performed in triplicate [1].

Anion Exchange Chromatography

Thirty milliliters of Q Sepharose Fast Flow (GE Healthcare) were loaded on XK16 column (GE Healthcare) and equilibrated with 5 column volume (CV) of equilibrium buffer (EB) (Tris-HCl 20 mM, NH_4Ac 100mM). Eight milliliters containing $8x10^{12}$ vg were subjected to buffer interchange through Sephadex G25 molecular exclusion column and adjusted to 30 mL of final volume with EB. Virus sample was loading into the Q-Sepharose column at 8 mL/min. Virus sample was recirculated 3 times and then washed with 5 CV of EB.

In order to discard empty capsids and elute AAV particles, 3 different elution protocols were tested.

i: 120 mM to 500 mM NH4Ac linear gradient (5CV), 1 M NaCl (5CV).

ii: 150 mM NH4Ac (5CV), 500 mM to 1 M NH4Ac linear gradient (5CV), 1 M NaCl (5CV).

iii: 150 mM NH4Ac (5CV), 1M NaCl (5CV).

The elution profiles were checked for UV absorbance at 280 nm and all peaks were collected for further analysis.

SDS-PAGE

The electrophoresis was performed according to Laemmli [5] using a 12 % resolving polyacrylamide gel under reducing conditions with Coomassie blue staining. AccuRuler RGB Plus Prestained Protein Ladder was obtained from MaestroGen Inc.

rAAV/Empty capsid ratio determination.

In order to determinate rAAV/Empty capsid ratio, elution peaks from Q-sepharose chromatography were subjected to 260/280 nm in a SPECTROstar^{Nano} from BMG LABTECH. Before absorbance measurement, samples were treated with SDS 20% and heated at 75°C for 10 min [6].

Results

Due to its well-known advantageous features in scalable production processes and proven capacity to bind AAV (6), the Q-Sepharose fast flow was chosen for the rAAV purification process.

Peaks 1 and 2 were obtained from the NH4Ac 120 to 500 mM linear gradient (fig.1). The 260/280-absorbance ratio showed that peak 1 corresponds to the empty capsids-containing fraction (Table1). Little increment in 260/280-ratio of peak 2 suggest that this 120 to 500 mM linear gradient can not resolve empty capsids and rAAV. On the other hand, NaCl 1M elution step resulted in a high titer fraction of rAAV.

Since empty capsid-containing fraction start to elute near to NH4Ac 120 mM, a wash step could be a suitable condition prior to rAAV elution. Taking into account previous findings, a 500 mM to 1 M NH4Ac linear gradient was tested followed by a NH4Ac 150mM wash step, in order to find out the best NH4Ac condition to elute rAAV. Finally, a high salt elution step (NaCl 1M) was tested, similar to the elution protocol i.

Peak	Title (vg/mL)	260/280 ratio
1	10^5	0.601
2	10^8	1.334

Table 1: Fraction analysis of rAAV Q-sepharose purification with elution protocol i.

A 150 mM NH4Ac wash step produced a first peak (peak1) (fig. 2) corresponding to the empty capsid-containing fraction. A 260/280 nm ratio analysis of this fraction confirmed that this condition is appropriated to wash out empty capsids (Table 2).

The application of a 500 mM to 1 M NH4Ac linear gradient generated three peaks at different NH4Ac molarities (fig2) and with different viral titers (Table 2). Once more, the fraction corresponding to NaCl 1M elution step (peak 5) resulted in a fraction with high rAAV titer.

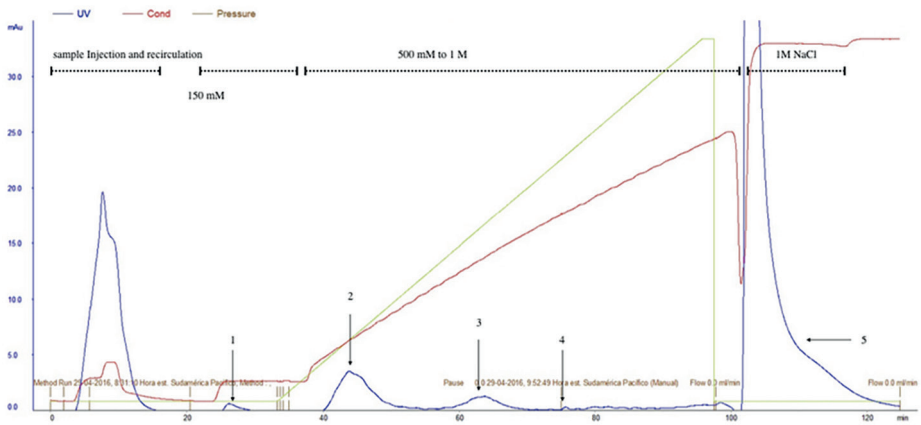

Figure 1. rAAV purification by anion chromatography interchange (Q-sepharose). Elution protocol i. 8x1012 vg were loaded into Q-sepharose XK-16 column and recirculated 3 times (8 mL/min). Empty capsids (1) and rAAV(2) were eluted by addition of a 120 to 500 mM NH4Ac linear gradient. 3: rAAV eluted with NaCl 1M solution.

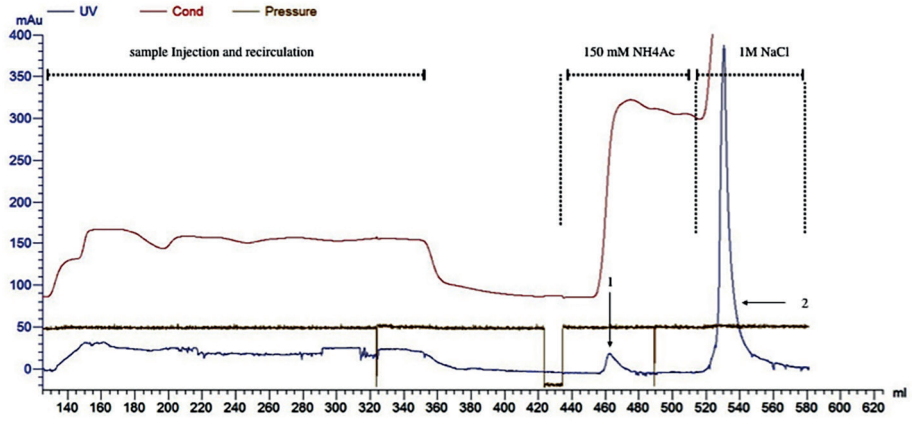

Figure 2. rAAV purification by anion chromatography interchange (Q-sepharose). Elution protocol ii. 8x1012 vg were loaded into Q-sepharose XK-16 column and recirculated 3 times (8 mL/min). 1: empty capsids were eluted by addition of 5 CV of NH4Ac 150 mM solution. 2,3,4: rAAV were eluted adding a 500 mM to 1M NH4Ac linear gradient. 5: rAAV eluted adding NaCl 1M solution.

Peak	Title (vg/mL)	260/280 ratio
1	10^4	0.582
2	10^7	1.276
3	10^5	1.350
4	10^2	1.269
5	10^8	1.383

Table 2: Fraction analysis of rAAV Q-sepharose purification with elution protocol ii.

The 500 mM to 1 M NH4Ac linear gradient did not result in a homogeneous high viral titer fraction. Moreover, the high salt step condition generated a high titer rAAV fraction with elevated 260/280 ratio. Hence, a single high salt elution step could be sufficient to obtain a homogeneous rAAV fraction with high virus titer.

Figure 3 shows a chromatogram of the rAAV purification process, using two purification steps: a 150 mM NH4Ac wash step to eliminate empty capsids and a single high salt elution step with NaCl 1M.

Like the previous elution protocols, NH4Ac 150 mM wash step resulted in a single peak (peak 1) enriched with empty capsids (Table 3). The fraction eluted with NaCl 1M was enriched with a high titer of rAAV. The SDS-PAGE analysis confirmed a rAAV-purified sample compared with the no treated sample obtained from the virus harvest step.

Peak	Title (vg/mL)	260/280 ratio
1	10^5	0.638
2	10^5	0.973
3	10^8	1.334

Table 3: Fraction analysis of rAAV Q-sepharose purification with elution protocol iii.

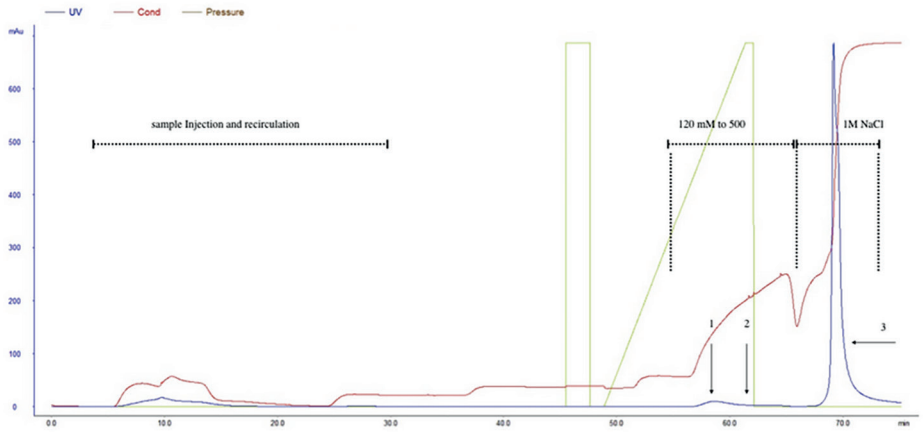

Figure 3. rAAV purification by anion chromatography interchange (Q-sepharose). Elution protocol iii. 8x1012 vg were loaded into Q-sepharose XK-16 column and recirculated 3 times (8 mL/min). 1: empty capsids were eluted by addition of 5 CV of NH4Ac 150 mM solution. 2: rAAV were eluted adding 5CV of NaCl 1M solution.

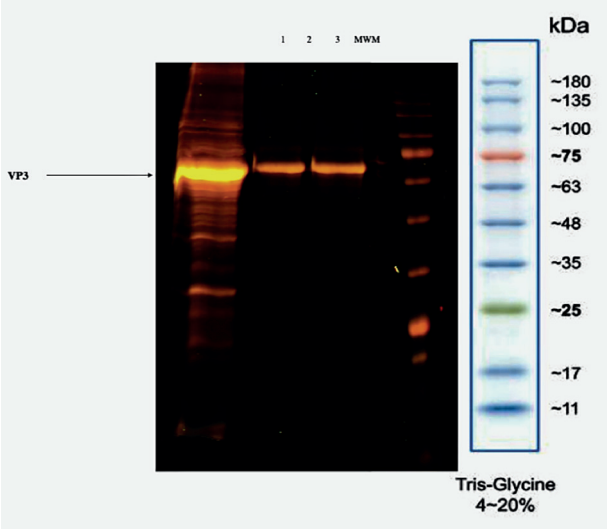

Figure 4. rAAV Q-sepharose purification. 12 % SDS-PAGE of eluted fractions with elution protocol iii. Lane 1: Injected rAAV sample. Lane 2: NH4Ac 150 mM wash step. Lane 3: NaCl 1 M elution step. MWM: AccuRuler RGB Plus Prestained Protein Ladder

Discussion

Methods like Cesium chloride gradient ultracentrifugation and iodixanol gradient ultracentrifugation can resolve empty capsids and rAAV [3]. However, these processes are time consuming, require expensive equipment and are not based on a scalable methodology. On the other hand, affinity based chromatography procedures like heparin-based affinity chromatography cannot solve contamination with empty capsids [4]. Some authors have reported the use of both, anion and cation exchange chromatography to solve this problem [7, 8].

This work describes the development of a scalable purification method to remove empty capsids from hybrid capsid rAAVs. These hybrid rAAVs have a wilder tropism than other non-hybrids rAAVs.

Guang Qu et al. reported that the use of a NH4Ac 100 to 200 mM linear gradient as wash step and NH4Ac 200 to 300 mM linear gradient to rAAV2 elution, in a Poros HP50 column, resulted in a high yield of rAAV2 [7]. Similar results were also obtained with the application of a NH4Ac 180 mM in a Q-sepharose column [8]. Although similar conditions were tested in this work, we could not be able to obtained similar results. Since hybrid rAAV carry eight different wild-type capsid viruses, the ionic strength could vary. Indeed, the application of NH4Ac linear gradient did not generate a homogeneous rAAV preparation.

In conclusion, a scalable method has been developed that allow the separation of empty capsids from hybrid rAAV with good purity yield. This method can be applied to other AVV serotypes.

Acknowledgment

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RESEARCHS / INVESTIGACIÓN

Propagación clonal *in vitro* de *Paulownia elongata x Fortunei*

In *vitro clonal* propagation of *Paulownia elongata x Fortunei*

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Abstract: The hybrids of *Paulownia elongata X fortunei* are considered as an interesting alternative for programs of production of biomass, raw material, the production of pulp and its use in processes of recovery of soils exploited by mining. Sexual propagation presents high genetic segregation, which is why the use of in vitro reproduction techniques is recommended. We used internodes and apices that after being disinfected with ethanol (70%) and sodium hypochlorite (1%) for ten minutes were established in the MS culture medium and different concentrations of BAP were evaluated (0, 0.5, 1.0, 1.5 and 3.0 mgL^{-1}) in combination with AIB (0, 0.1 and 1.0 mgL-1) and for the rooting were evaluated concentrations of AIB (0. 0.5, 1 and 3.0 mgL^{-1}). The best proliferation and shoot length results were obtained with concentrations of 1.5 mgL-1 of BAP with 0.1 mgL-1 of AIB with 4 shoots / explant and a length of 4 cm. The rooting was achieved between 85% and 82% with the use of 1 and 3 mgL-1 of AIB respectively.

Keywords, micropropagation, *in vitro* proliferation, *in vitro* rooting.

Resumen: Los híbridos de *Paulownia elongata X fortunei* se consideran como una interesante alternativa para programas de producción de biomasa, materia prima la producción de pulpa y su utilización en procesos de recuperación de suelos explotados por minería. La propagación sexual presenta alta segregación genética por lo cual se recomienda la utilización de técnicas de reproducción in vitro. Se emplearon entrenudos y ápices los cuales después de ser desinfectados con etanol (70%) e hipoclorito de sodio (1%) durante diez minutos se establecieron en el medio de cultivo MS y se evaluaron diferentes concentraciones de BAP (0, 0.5, 1.0, 1.5 y 3.0 mgL^{-1}) en combinación con AIB (0, 0.1 y 1.0 mgL^{-1}) y para el enraizamiento se evaluaron concentraciones de AIB (0. 0.5, 1 y 3.0 mgL^{-1}). Los mejores resultados de proliferación y longitud de los brotes se obtuvieron con concentraciones de 1.5 mgL^{-1}de BAP con 0.1 mgL^{-1} de AIB con 4 brotes/explante y una longitud de 4 cm. El enraizamiento se logró entre el 85% y 82% con la utilización de 1 y 3 mgL^{-1} de AIB respectivamente.

Palabras clave: micropropagación, proliferación *in vitro,* enraizamiento *in vitro.*

Introducción

Paulownia es un género maderable de la familia *Scrophulariaceae* nativo de China y que ha sido adaptado en diversas partes del mundo tales como América y Europa. Se caracteriza por ser un árbol deciduo de rápido crecimiento, con grandes hojas distribuidas en pares opuestos en el tallo. Se utiliza para programas de reforestación en suelos pobres en nutrientes y debido a la amplia distribución de su sistema radical se puede emplear para la fitorremediación de suelos contaminados por actividades industriales[1,2]. La madera se utiliza para la producción de pulpa para la fabricación de papel y para enchapes. *Paulownia tomentosa* ha tenido mayor atención respecto a otras especies del género debido a que las primeras plantaciones se establecieron en Norteamérica. Sin embargo, investigaciones con *P. elongata* en América y Europa y *P. fortunei* en Australia han mostrado que sus características de crecimiento y manejo son mejores[3]. De igual manera, se han desarrollado híbridos de *P. elongata X fortunei* que han mostrado ser promisorios.

La reproducción de *P. elongata, P. fortunei* y sus híbridos se puede realizar a partir de semilla; sin embargo, se presenta alta variabilidad genética y largos periodos de juvenilidad. Los métodos de propagación vegetativa son preferidos como es el caso de estacas que se enraízan con la utilización de reguladores de crecimiento, aunque la respuesta es inespecífica y no se producen altos porcentajes de enraizamiento[4].

Por esta razón, la utilización de técnicas de reproducción in vitro de *Paulownia sp* han tomado especial importancia para suplir material para la industria forestal[5,6]. Las técnicas de propagación in vitro permiten la obtención de materiales libres de plagas y enfermedades, mayor homogeneidad fisiológica para la producción de biomasa y aprovechamiento forestal. Se tienen investigaciones de reproducción a partir de organogénesis directa e indirecta y mediante embriogénesis somática[2,3,7,8,9].

La regeneración *in vitro* es influenciada por factores como el tipo de explantes, medios de cultivo, reguladores de crecimiento y condiciones ambientales[10,11].

El objetivo del presente estudio fue el de establecer una metodología para la propagación clonal in vitro de un hibrido de *Paulownia elongata X fortunei*

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Materiales y métodos

Área de estudio

Fuente de explantes

Fueron empleadas yemas apicales y axilares de 2 centímetros de longitud, se realizó un lavado de estas yemas con yodo (2mL/L) durante 2 minutos. En cámara de flujo se desinfectaron con etanol 70% durante 2 minutos en hipoclorito de sodio al 1% durante 10 minutos posteriormente, se realizó un triple lavado con agua destilada estéril. En el caso de las yemas axilares fueron tomados entrenudos con yemas de aprox. 0.3 cm de longitud, respecto a la yema apical se eliminaron los primordios foliares y se dejó una porción aproximada de 0.5 cm. Se realizó el establecimiento en el medio de cultivo MS[12] y se le adicionó las vitaminas MS, sacarosa (3%) y agar – agar (6.5 gL^{-1}).

Fase de proliferación

Se evaluaron diferentes concentraciones de BAP (0, 0.5, 1.0, 1.5 y 3.0 mgL^{-1} en combinación con AIB (0, 0.1 y 1 mgL^{-1}). En la tabla 1 se muestran los tratamientos realizados. Se utilizó un experimento factorial 5 x 3 con diseño completamente al azar. Cada tratamiento estuvo compuesto por 5 repeticiones. Los datos se analizaron mediante ANOVA y se utilizó el análisis de contrastes de *Tukey* para determinar diferencia entre las medias de los tratamientos.

Tratamientos	BAP (mgL^{-1})	AIB (mgL-1)
1	0.0	0.0
2	0.0	0.1
3	0.0	1.0
4	0.5	0.0
5	0.5	0.1
6	0.5	1.0
7	1.0	0.0
8	1.0	0.1
9	1.0	1.0
10	1.5	0.0
11	1.5	0.1
12	1.5	1.0
13	3.0	0.0
14	3.0	0.1
15	3.0	1.0

Tabla 1. Evaluación del efecto de la bencilaminopurina (BAP) y el ácido Indolbutírico (AIB) en la fase de proliferación in vitro de *P. elongata x P. fortunei.*

Fase de enraizamiento. Se utilizó el medio MS y se evaluó el efecto del ácido indolbutírico (0, 0.5, 1 y 3 mgL^{-1}). Se utilizó un diseño completamente al azar con 30 repeticiones por tratamiento.

Para el análisis de la información se utilizó el paquete estadístico RWizard 3.1. En todos los casos se verificaron los supuestos de normalidad de Lilliefors (Kolmogorov-Smirnov), Shapiro-Wilk y de homogeneidad de varianza de Levene's.

Resultados y discusión

Fase de proliferación

De acuerdo con los análisis estadísticos se encontró interacción entre las concentraciones de BAP y AIB respecto al número de brotes / explante. En la figura 1 se observa como cuando se utilizó AIB (0.1 mgL^{-1}) con las concentraciones de BAP hasta 1.5 mgL^{-1} se incrementó el número de brotes, obteniendo su máximo valor en esta concentración. Con las concentraciones de AIB (0 y 1 mgL^{-1}) el máximo valor se alcanzó con BAP (1 mgL^{-1}). En todos los casos concentraciones de BAP (3 mgL^{-1}) causó disminución en el número de brotes.

Al realizar la comparación de las medias de los tratamientos (figura 2) se encontraron diferencias significativas entre estos respecto al número de brotes por explante. El tratamiento donde se utilizó BAP (1.5 mgL^{-1}) más AIB (0.1 mgL^{-1}) mostró un promedio de 4 brotes / explante con una periodicidad de subcultivos de cada 21 días. Estos resultados difieren con los obtenidos por[4], con *P. tomentosa* con un promedio de 7.4 brotes por explante, lo cual se puede explicar por la especie que se evaluó. Es conocido el efecto fisiológico de las citoquininas en la estimulación de división y elongación celular y la activación de la síntesis de ARN, la estimulación de síntesis de proteínas y actividad enzimática como ha sido revisado por[13]. La combinación de BAP con AIB contribuye a mejorar la proliferación posiblemente debido a un equilibrio entre citoquininas / auxinas.

Respecto a la longitud de los brotes en la figura 3 se observó que en todos los casos la mayor longitud de los brotes se alcanzó con BAP (1.5 mgL^{-1}), mientras que la concentración de BAP (1.5 mgL^{-1}) disminuyó la longitud de los brotes. Al realizar la comparación de medias de tratamientos se encontró que cuando se utilizó BAP (0.5, 1.0, 1.5 y 3.0 mgL^{-1}) con AIB (0.1 y 1.0 mgL^{-1}) se tuvo la mejor respuesta en cuanto a la longitud de los brotes (figura 4).

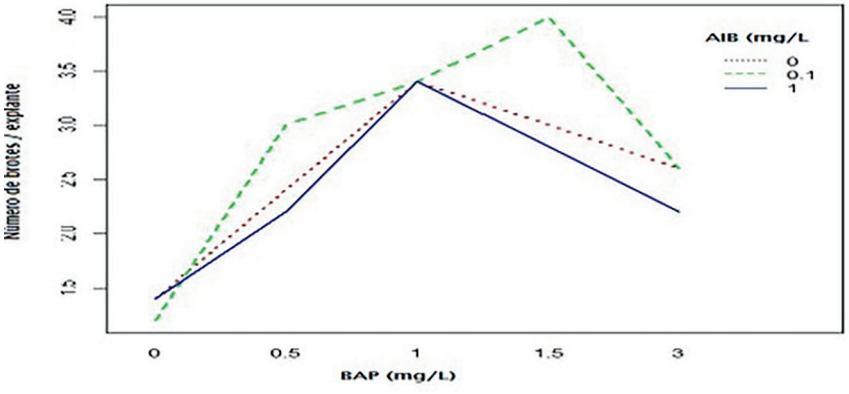

Figura 1. Interacción entre los factores correspondientes a diferentes concentraciones de BAP y AIB sobre el número de brotes in vitro en *Paulownia elongata X fortunei*

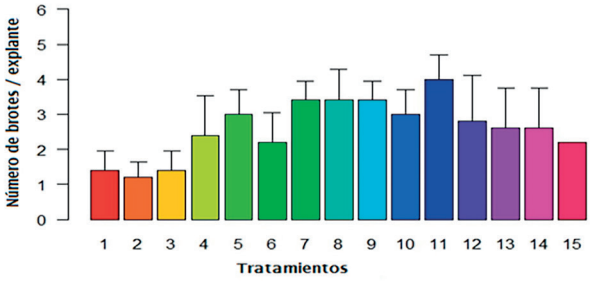

Figura 2. Evaluación del efecto del BAP y AIB sobre el número de brotes de *P. elongata x P. fortunei*

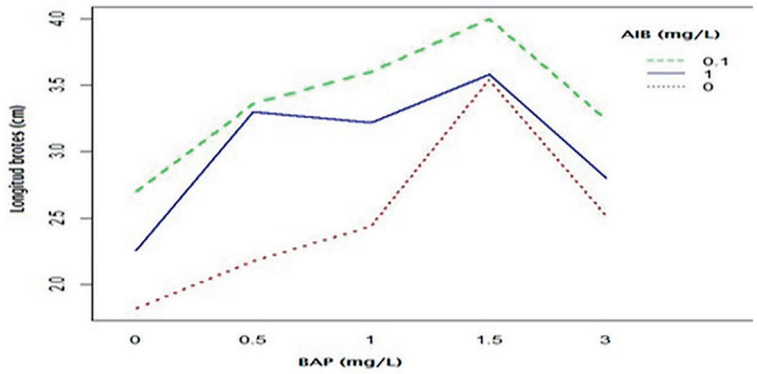

Figura 3. Interacción entre los factores correspondientes a diferentes concentraciones de BAP y AIB sobre la longitud de los brotes in vitro en *Paulownia elongata X fortunei*

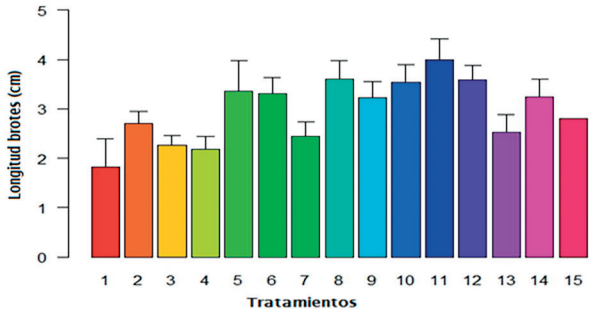

Figura 4. Evaluación del efecto del BAP y AIB sobre la longitud de los brotes de *P. elongata x P. fortunei.*

Fase de enraizamiento

Después de cuatro semanas se evaluó el efecto de cuatro concentraciones de AIB sobre el porcentaje de enraizamiento y número de raíces por explante. Respecto al porcentaje de enraizamiento (figura 5) las medias de los tratamientos presentaron diferencias significativas. De acuerdo con el test de Tukey se encontró que las concentraciones de 1 y 3 mgL^{-1} presentaron los mayores porcentajes de enraizamiento del 85% y 82% respectivamente.

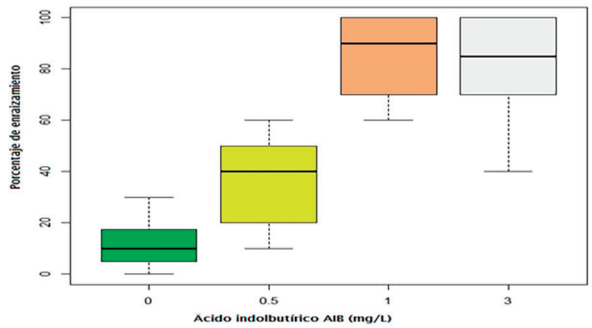

Figura 5. Efecto de diferentes concentraciones de AIB sobre el porcentaje de enraizamiento in vitro de brotes de *P. elongata x P. fortunei.*

Con relación al número de raíces los tratamientos donde se utilizaron concentraciones de 1 y 3 mgL^{-1} tuvieron la mejor respuesta con 3.4 y 3 raíces por brote respectivamente (figura 6). Estos resultados coinciden con los encontrados por[7], quienes encontraron porcentajes de enraizamiento del 66.6% y 3.57 raíces/brote en *P. kawakamii.* De igual manera[4] en *P. tomentosa* encontraron que el empleo de IBA (0.5 mgL^{-1}) tuvo la mejor respuesta de enraizamiento mayor del 95%.

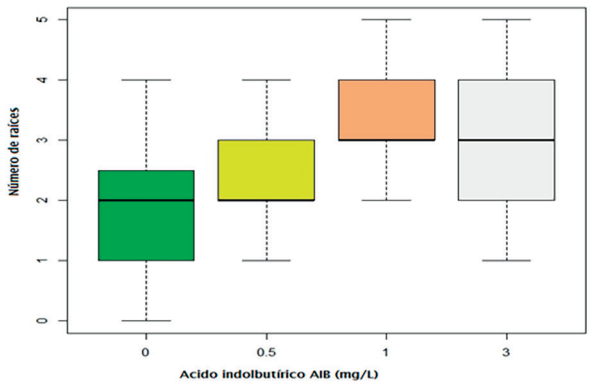

Figura 6. Efecto de diferentes concentraciones de AIB sobre el número de raíces in vitro de *P. elongata x P. fortunei*

En la figura 7 se muestran los estados de desarrollo de *P. elongata x P. fortunei* propagados in vitro. La primera fase de establecimiento se logró con la siembra de entrenudos los cuales se desinfectaron con etanol (70%) durante dos minutos e hipoclorito de sodio (1%) durante diez minutos. La proliferación se desarrolló en el medio de cultivo MS suplementado con BAP (1.5 mgL^{-1}) más AIB (0.2 mgL^{-1}), donde después de 60 días se estabilizó en la producción media de 4 brotes / explante cada cuatro semanas. El enraizamiento se desarrolló con AIB (1 mgL^{-1}) con un porcentaje del 85% a los 30 días.

Conclusiones y recomendaciones

la tecnología de cultivo in vitro permite mejorar el acceso a una gran cantidad de plantas a partir de cantidades mínimas de material vegetal, y su desarrollo productivo representa una oportunidad de crecimiento y diversificación a nivel del sector forestal de nuestro país; asimismo, estos cultivos podrían usarse para predecir las respuestas de las plantas a los contaminantes ambientales, y así reducir el costo de los experimentos de plantas convencionales.

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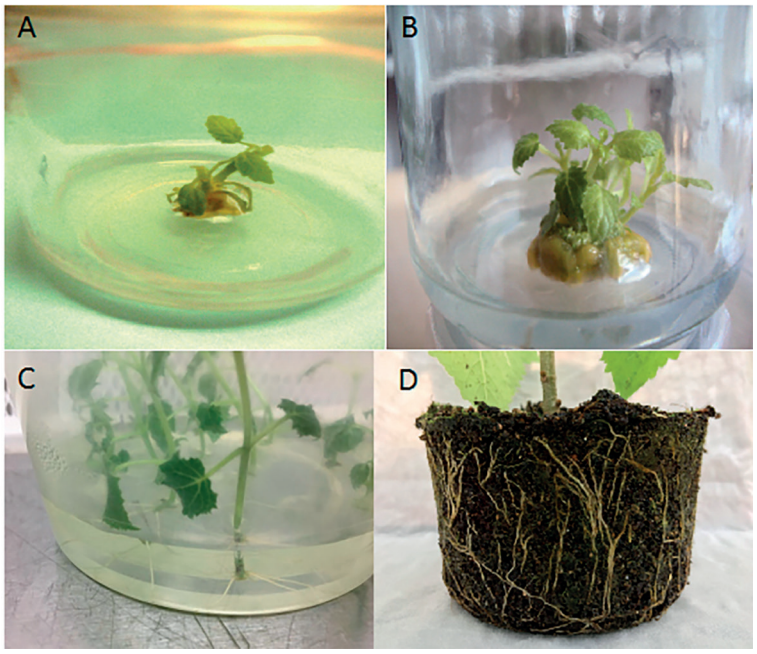

Figura 7. Diferentes fases de desarrollo de P. *Paulownia elongata X fortunei* propagado in vitro. A. Fase de establecimiento. B. Fase de proliferación. C. Fase de enraizamiento. D. Aclimatización.

RESEARCHS / INVESTIGACIÓN

Morphotaxonomy and Distribution of Marine Green Algal Flora in Kalegauk Island

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Abstract: The marine green algae were collected from Apor Seik, Pashyu Chaung, Chaytoryar Pagoda, Alè Seik, Auk Sei, and Kyunn Pyet or Cavendish Island along the Kalegauk Island from September 2016 to January 2017. In the study areas, salinity range and temperature regimes seawater were 26-27 ‰ and 29° C to 31° C, respectively. Mainly the present study had been made to know the diversity and distribution of marine green algae along the Kalegauk Island. Marine green algae were identified based on their internal and external morphological characters. The total 14 species could be recorded in Kalegauk Island. Of these species, almost all species were first new records for Kalegauk Island.Alson, it was concluded that all species would be the most diverse and abundant in Apor seik. Moreover, the present study could provide evaluating the impacts of marine green algae resources caused by infrastructures in the future.

Keywords: Morphological characters, Kalegauk, New record, and green algae.

Introduction

Marine benthic green algae are usually found in the intertidal zone and in shallow waters where there are plenty of nutrients and sunlight. Green algae usually appear in green because they contain the same ratio of chlorophyll a and b as that of higher plants. Hence, it is generally believed that green algae are closely related to terrestrial higher plants in the theory of biological evolution[1]

Kalegauk Island is the island in Ye township, Mon state, Myanmar. It is located in the northern part of the Andaman Sea, 8.25 km from the coast of Mon. The island has a long shape with a length of over 10 km and a width of 1.6 km in itsmost extensivet are, and there is a small Cavendish island lies 0.5 km off thesouthernn point of Kalegauk Island. It is mainly composed of four villages, viz., Apor Seik Village, Auk Seik Village, Alè Seik Village and Pashyu Chaung VillageAlsoon, Chaytoryar Pagoda is also one of the most famous places in Kalegauk IslandMangrove forests rather than rocky shores generally cover the coastal areas of the Kalegauk Islandes. The coastlines of Kalegauk Island dominantly comprise ocovereder by mud, silt and clay and many capes or promontories on these areas. In the study areas, salinity range and temperature regimes seawater were 26-27 ‰ and 29° C to 31° C, respectivelyNowadaysay Kalegauk Island has been declared as the island to be constructed deep sea port.

Therefore, thprimaryin objective of the present study is to record the diversity and distribution of marine benthic green algae from Kalegauk Island with their morphological characters. As a result, this study will become primaryic informational providing data for further observing on the marine green algae of Kalegauk Island.

Materials and methods

Marine green algae were collected in the forms of drift and live specimens growing in the high tide line, intertidal and shallow subtidal areas from Apor Seik, Pashyu Chaung, Chaytoryar Pagoda, Alè Seik, Auk Seik and Kyunn Pyet from September 2016 to January 2017. The site location, topography, associated flora and fauna and other related parameters of Kalegauk Island were recorded. In the field, all the adhering materials such as sand particles and other debris as well as epiphytes were removed from the samples with the help of painting brush before preservation. The seaweed samples preserved 4% formaldehyde with seawater. All the bags and containers were labeled withe th date, time of collection, locality and transport to the laboratory of Marine Science Department for further analysis.

In the laboratory, color and morphological differences between different genus, species and taxonomic characters firstly studied and then the collected seaweeds had been identified wian th emphasis on the external and internal morphologies of vegetative and reproductive features. For internal details studies of the thallcross-sectionion (c.s) were obtained freehandand with shaving blades, then stained in Aniline Blue (0.5 g water soluble aniline blue in 100 ml distilled water and 5 ml conc. Acetic acid) and mounted in glycerine. Vegetative and reproductive structures of the plants were studied under the Olympus compound microscope and Kaneko Yushima dissecting microscope. Microscopic measurements were recorded in micrometer (µm) using the ocular meter. This stuly followed the classification system of Guiry and Guiry[2].

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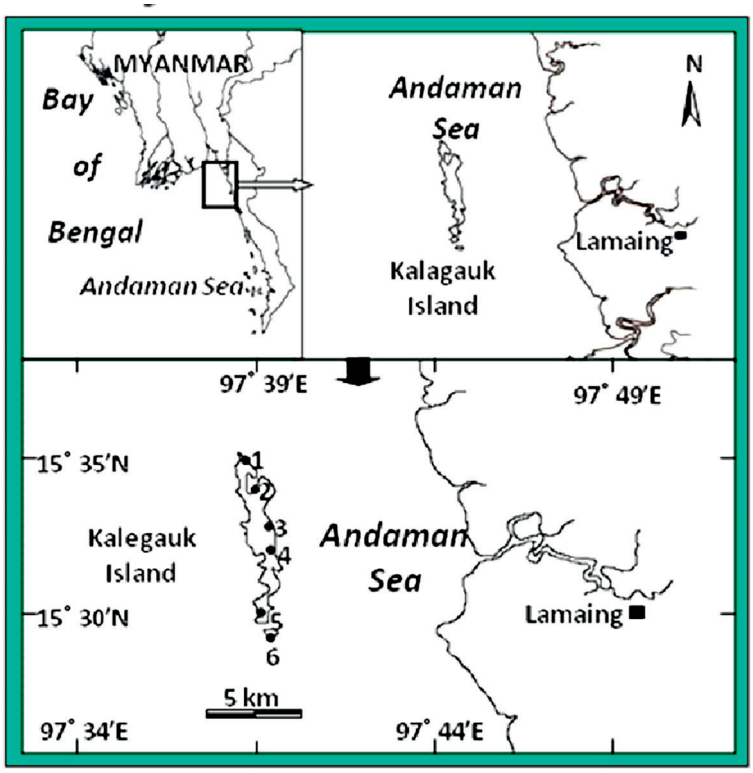


Fig 1. Map showing the specimens collection sites of the marine benthic algae of Kalegauk Island in the northern part of the Andaman Sea. 1. Apor Seik. 2. Pashyu Chaung. 3. Chaytoryar Pagoda. 4. Alè Seik. 5. Auk Seik. 6. Kyunn Pyet or Cavendish Island.

Results

Descriptions of marbenthicntic green algae (Chlorophyta) in Kalegauk Island

Key to the species of *Ulva* from Kalegauk Island

1a. Thalli tubular, the blade tapering to the base *Ulva compressa*

1b. Thalli unbranched, the blade linear to broad *U. linza*

Ulva compressa Linnaeus (figs. 2-6)

Ulva compressa Linnaeus 1753: 163[3]. Taxonomic synonyms: *Enteromorpha compressa* (Linnaeus) J. Agardh: Martens 1871: 465[4]; Anand 1940: 11, fig.1A[5]; Dawson 1944: 203[6]; Kylin 1949: 22-23[7]; Yamada 1950: 173[8]; Durairatnam 1961:18, pl.I, fig.7[9]; Soe-Htun 1998: 104[10]; Soe Pa Pa Kyaw *et al.* 2009: 44-45, fig.5[11]; Soe-Htun *et al.* 2009: 270[12]; Jha *et al.* 2009: 8, fig. A-C[13]; Pham *et al.* 2011: 52[14]; Guiry and Guiry 2018[2].

Description. Thalli tubular, gregarious, more or less compressed, dilated towards the apex, tapering to the base, to 1-3 cm tall, greenish yeolor. Profusely branched but the branches ooccasionallyally branched, branches narrowed to base, similar to centralmfrontrond, often with hollow margins. In surface view cells irregularly arranged, polygonal, 12-16 µm long and 12-20 µm wide. In transverseerse section cells irregularly rounded, 16-20 µm long and 12-16 µm wide. In the upper portion, cells 8-12 µm long and 4-8 µm wide.

Distribution. Apor Seik, Pashyu Chaung, Chaytoryar Pagoda, Alè Seik, Auk Seik, Kyunn Pyet.

Ulva linza Linnaeus (figs. 7-9)

Ulva linza Linnaeus 1753: 163[3]. Taxonomic synonyms: *Enteromorpha linza* (Linnaeus) J. Agardh: Kylin 1949: 19-20[7]; Womersley 1956: 353[1]; Arasaki 1964: 8, fig. 18A-B[15]; Taylor 1967: 63[16]; Soe Pa Pa Kyaw *et al.* 2009: 45-46, fig.6[11]; Jha *et al.* 2009: 11, fig. A-C[13]; Soe-Htun *et al.* 2009: 27A[12], Guiry and Guiry 2018[2].

Description. T arehalli unbranched, silky, often gregarious, up to 2-5 cm tall, yellowish green in color. The blade linear to broad, usually with crisped or ruffled margins and at times spirally twisted, blade alentirelyetely compressed apart from a narrow marginal cavity with the two layers separated by mucilage and moderately adherThe marginargin of blades frequehallowsallow because of separation of the two cell layer. In surface view, cells arranged irregularly, 8-16 µm in diametercross-sectionction cit is usually elongatedgated, two rows, 8-16 µm long and 6-15 µm wide. In the upper portion, cells 8-12 µm and 8-1full wide.

Distribution. Apor Seik, Pashyu Chaung, Chaytoryar Pagoda, Alè Seik, Auk Seik, Kyunn Pyet.

Key to the species of *Cladophora* from Kalegauk Island

1a. Branches profuse, in curved near the tips and common pseudodichotomous branches *Cladophora vagabunda*

1b. Branches sparse, not in curved near the tips and rare pseudodichotomous branches *Cladophora sp.*

Cladophora vagabunda (Linnaeus) Hoek (figs. 10-11)

Cladophora vagabunda (Linnaeus): Anand 1940: 28-29, figs. 12A-B[5]; Womersley 1956: 358[1]; Durairatnam 1961: 21-22, pl II, fig.1[9]; Guiry and Guiry 2018[2].

Description. Thalli of uniseriate branched filaments with apical and intercalary growth, up to 3.5 cm tall, light green in color, composed of pseudodichotomously brancentralg main axis ending in densely branched fasciculate terminal branch systems. In main branches, cells cylindrical, 875-1000 µm long and 75-100 µm widsecondaryundary branches, cells 375-625 µm and 50-75 µm wide. Branchlets pectinately arranged and the tip are cells tapering.

Distribution. - Apor Seik and Alè Seik.

Cladophora sp. (figs. 12-14)

Description. Thalli, uniseriate branched filaments with apical and intercalary growth, green to duln color, entathick, bushy, slender-celled filaments contorted, repeatedly dichotomous branch, grow in rock pools in the intertidal zone. Main axis stout, alternately branched, 180-150 µm long and 50-100 µm wide. Branches are sparse and rare pseudodichotomous branches. In lateral branches, the tip cells tapering, 100-110 µm long and 40-70 µm wide.

Distribution. Apor Seik and Alè Seik.

Chaetomorpha spiralis Okamura (figs. 15-16)

Chaetomorpha spiralis Okamura 1903: 131-132[17]; Arasaki 1964:10: fig.25[15]; Abbott and Hollenberg 1976: 101-103[18]; Kyaw Soe and Kyi Win 1977: 56, figs.73A1-2[19]; Guiry and Guiry 2018[2].

Description. Plants uniseriate, unbranched, erect, gregarious, dark green in color, rigid, much coiled and contorted when young, later loosened and entangled among other algae, short, slender, and attached to the substra atum by discoidal holdfast. Discoidal holdfasts are 300-400 µm long and 30-150 µm wide. Cells are moniliform to nearly cylindrical, 225-625 µm long and 155-250 µm wide. The length of the cells usually about 2-3 times the diameter.

Distribution. Apor Seik, Pashyu Chaung, Alè Seik and Auk Seik.

Chaetomorpha aerea (Dillwyn) Kützing (figs. 17-21)

Chaetomorpha aerea (Dillwyn) Kützing**:** Dawson 1944: 208[6]; Kylin 1949: 48, fig. 50[7]; Womersley 1956: 355[1]; Durairatnam1961: 20, pl. 1, fig.10[9]; Taylor 1967: 72[16]; Kyaw Soe and Kyi Win 1977: 55, fig. 74A1-2[19]; Guiry and Guiry 2018[2].

Description. - Plants are erect, uniseriate, unbranched, rigid, gregarious, dark green in color, slender toward the base and attached by a basal cell which is below. Cells swell one times after two or three cells, little contracted at the septa, 50-200 µm long and 50-80 µm thick. A basal cell is longer than other cells.

Distribution. Apor Seik and Pashyu Chaung.

Chaetomorpha gracilis Kützing (figs. 22-25)

Chaetomorpha gracilis Kützing**:** Durairatnam 1961: 19[9]; Kyaw Soe and Kyi Win 1977: 56, fig. 74A[19]; Phang 2006: 118[20]; Soe-Htun *et al.* 2009: 271[12]; Soe Pa Pa Kyaw *et al.* 2009: 52, fig. 17[11]; Pham *et al.* 2011: 13[14], Guiry and Guiry 2018[2].

Description. - Plants uniseriate, unbranched, erect, cylindrical, green to dark green in color, entangled among other algae, grow in tufts, attached to the substratum by irregularly ramified rhizoids, gregarious. Cells little contracted at the septa, 600-750 µm long and 200-225 µm wide. The length of the cells usually about 2-4 times than the diameter.

Distribution. Apor Seik, Pashyu Chaung, Alè Seik and Auk Seik.

Chaetomorpha linum (Müller) Kützing (figs. 26-28)

Chaetomorpha linum (O.F. Müller) Kützing**:** Kylin 1949: 49[7]; Yamada 1950: 177[8]; Dawson 1956: 78, fig.100[6]; Womersley 1956: 357[1]; Soe-Htun *et al.* 2009: 270[12]; Soe Pa Pa Kyaw *et al.* 2009: 51, fig.16[11]; Guiry and Guiry 2018[2].

Description. Plants uniseriate, unbranched, erect, gregarious, grass-green to yellowish-green in color, loosely entangled, stiff. Filaments are often very long, tapering toward the base and curved toward the back at the top. Cells slightly contracted at the septa, subclavate, 50-100 µm long and 50-90 µm wide. The length of the cells usually 1-2 times the diameter.

Distribution. Apor Seik, Pashyu Chaung, Alè Seik and Auk Seik.

Rhizoclonium curvatum Chapman (figs. 29-34)

Rhizoclonium curvatum V.J Chapman 1949: 496[22]; Womersley 1984: 170, figs. 53E-F[21]; Guiry and Guiry 2018[2].

Description. Plants forming mats, dark green in color, entangled with each other, commonly bent and laterally attached with branchlets. Branchlets 250-300 µm long and 20-30 µm wide, septate, erect and segmented. Cells are stout, 30-40 µm long and 20-30 µm wide.

Distribution. Apor Seik, Pashyu Chaung and Alè Seik.

Rhizoclonium riparium (Roth) Harvey (figs. 35-37)

Rhizoclonium riparium (Roth) Harvey: Kylin 1949: 50[7]; Womersley 1956: 361[1]; Durairatnam 1961:19[9]; Taylor 1967: 76[16]; Kyaw Soe and Kyi Win 1977: 58, fig.79,pl.1-2[19]; Soe Pa Pa Kyaw *et al.* 2009: 53, fig.18-19[11]; Soe-Htun *et al.* 2009: 271[12]; Guiry and Guiry 2018[2].

Description. Plants small, along unbranched filaments, dark green to yellowish-green in color, straight to irregularly curved, sometimes twisted and entangled in thin mats or strands. Cells are cylindrical, 12-28 µm long and 8-12 µm wide. Cells are usually 2-3 times longer than broad. Rhizoids are irregular in position and rare or nonseptate.

Distribution. Apor Seik, Pashyu Chaung, Alè Seik and Kyunn Pyet.

Rhizoclonium grande Boergesen (figs. 38-41)

Rhizoclonium grande Boergesen**:** Yoshida, Nakajima and Nakata 1985: 60; 1990: 272[23]; Abbott and Huisma 2004: 82, fig. 25B[24], Soe-Htun *et al.* 2009: 271[12]; Guiry and Guiry 2018[2].

Description. Plants in dsmallmats or short stands, dyellow-green yellow green in color, entangled with each other, stiff and irregularly curved. Rhizoidal branches many, long, twisted, septate or nonseptate and tapering toward the tip. Cells elongated, 50-120 µm long and 40-60 µm wide.

Distribution. Apor Seik, Pashyu Chaung, Alè Seik, and Kyunn Pyet.

Rhizoclonium africanum Kützing (figs. 42-45)

Rhizoclonium africanum Kützing: Lawson and Price 1968: 329[25]; Abbott and Huisman 2004: 82, fig. 25A[24]; Soe-Htun *et al.* 2009: 271[12]; Guiry and Guiry 2018[2].

Description. Plants filamentous, tending to be stiff or coarse and pale to dark green in color. Cells are 140-160 µm

long and 35-40 µm wide, 2-4 times longer than wide. Rhizoidal branches few to many, short and septate.

Distribution. Apor Seik, Pashyu Chaung, and Alè Seik.

Cladophoropsis membranaceae (Hofman Bang ex C. Agardh) Boergesen (figs. 46-48)

Cladophoropsis membranaceae (Hofman Bang ex C. Agardh) Boergesen: Anand 1940: 47-48, figs.25-26[5]; Egerod 1952; 356, fig.3[26], Dawson 1956: 80, fig.103[6]; Taylor 1967: 117, pl.2, fig.1, pl.3, fig.2[16]; Soe-Htun *et al.* 2009: 271[12], Guiry and Guiry 2018[2].

Description. Plants forming tuft or clump of branched filaments, gloomy, light green in color, up to 4.5 cm long or broad. Most of the filaments declined, twisted and contorted forming entangled masses. Branches are sparse, usually secund, irregular, and without septa. In main axis, segments 300-800µm long and 100-120 µm wide. Branches are 300-600 µm long and 80-100 µm wide.

Distribution. Apor Seik, Pashyu Chaung, Chaytoryar Pagoda, Alè Seik, Auk Seik, Kyunn Pyet.

Dichotomosiphon sp. (figs. 49-52)

Description. Plants small, composed of siphonous tubes with constriction at points of ditrichotomous branching, pale green in color and not segmented. Branches network in shape, rounded at the tip, 80-40 µm long and 12-20 µm wide.

Distribution. Apor Seik and Chaytoryar Pagoda.

Discussion

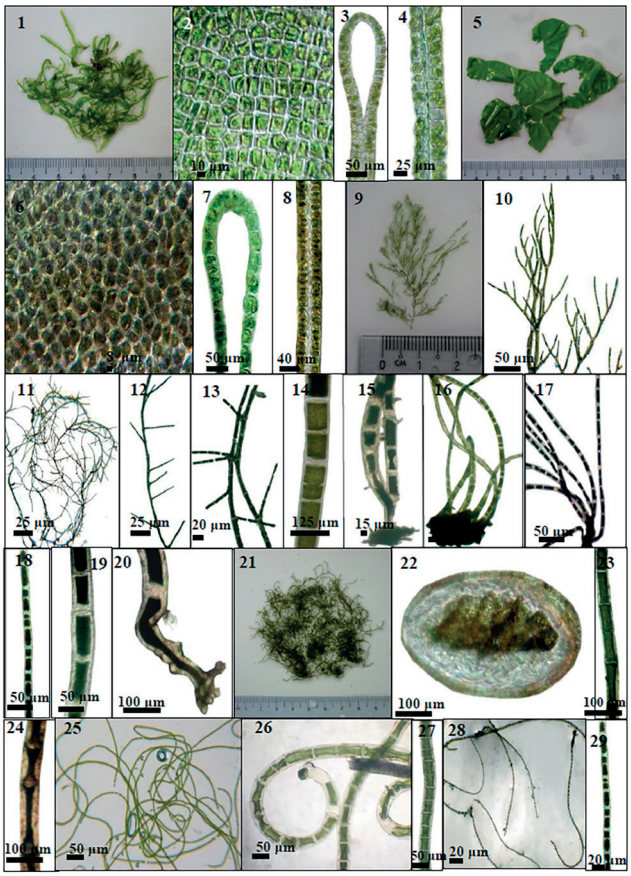
Tin Aung Myint.[27] was firstly observed the eeds around the Kalegauk Island in 1971. After that, some marine algae of Kalegauk Island had been described as a small part of their algal flora study in Myanmar by Kyaw Soe and Kyi Win in 1977[19]. Since 1977, there was no record and study about the marine algae in Kalegauk Island. Compared with the present study in which the total 12 species of green algal flora could be recorded, almost all species were new records for the seaweed resources of Kalegauk Island with the exception of *Rhizoclonium riparium*and *Dichotomosiphon sp.* which had been described in the previous studies. Therefore, it could be possible that the present study was the first specific record for the marine green algal flora of Kalegauk Island.

Compared with the previous studies of green algal flora in Myanmar, *Rhizoclonia um curvatum* was new record for Myanmar in the present study according to the references available. However, *Cladophora* sp. and *Dichotomosiphon sp.* could not be identified up to the species level due to lack of specimens and references.

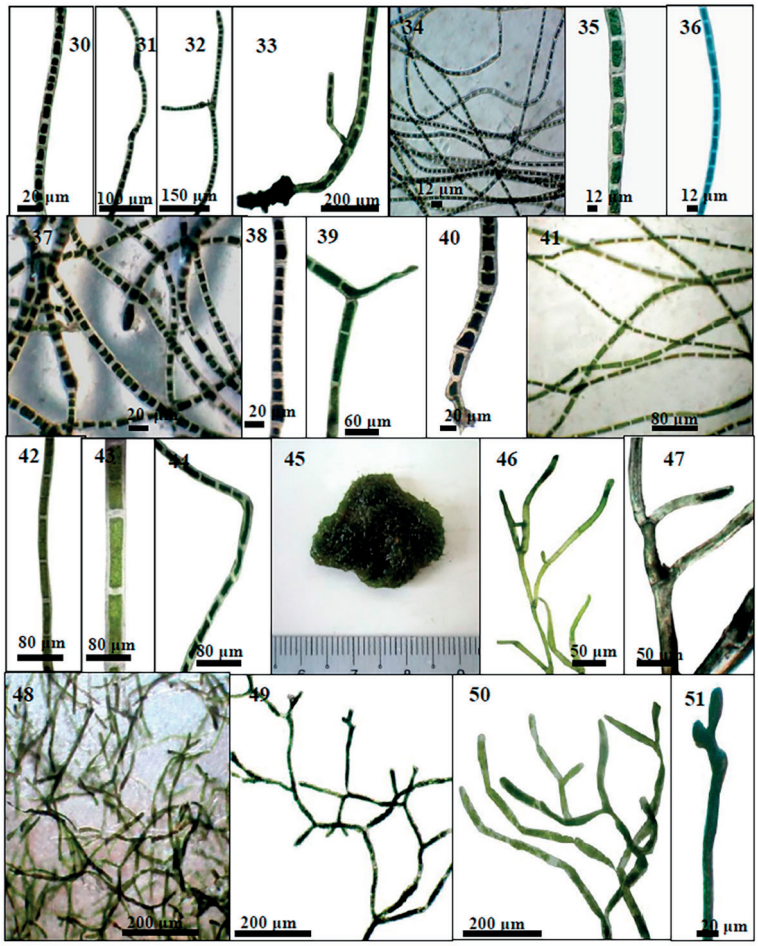
Chaetomorpha spiralis, C. aerea and *Dichotomosiphon sp.* had not been recorded in distribution and potential utilization of marine algae of Myanmar by Soe Htun *et al.* 2009[12]. However, these species were reported in seaweed for utilization by Kyaw soe and Kyi Win 1977[19]. It is well-known that Kalegauk Island can be one of the islands without depleting its algae resources compared to the other areas of Myanmar.

As regards the distinguishing morphological characters, the filaments of *Rhizoclonium curvatum* were laterally attached with segmented branchlets or celled rhizoids at their curves whereas those of other *Rhizoclonium* only had rhizoidal branchlets. Likewise, Chapman 1949[22]also described this species seems fosharpight to distinct curves sepacalledby fairly celled rhizoids, forming position Y at the junction. The present ady was found the similar result. Moreover, it was strange that *Dichotomosiphon sp* was only one the species which is composed of siphonous tubes with constriction at points of ditrichotomous branching.

During the field study, *Ulva compressa, U. linza* and *Cladophoropsis membranaceae* were ubiquitous and were found everywhere in Kalegauk Island. *Cladophora vagabunda* and *Cladophora sp.* were commonly found in the mangrove forests of Apor Seik and Alè Seik. *Chaetomorpha gracilis, C. linum* and *C. spiralis* were too numerous in the rocky shore as well as mangrove forests of Apor Seik, Auk Seik, Alè Seik and Pashyu Chaung. *Chaetomorpha aerea* were found in Apor Seik and Pashyu Chaung. *Rhizoclonium riparium, R. grande, R. africanum* and *R. curvatum* were also scattered in the rocky shore and mangrove forest of Apor Seik, Alè Seik and Pashyu Chaung. *Dichotomosiphon* sp grows on silt and mud of Apor Seik and Chaytoryar Pagoda and then it was too smaquicklycoAlsoeasily. In addition, the green algal flora species were found 14 numbers of species in Apor Seik, 11 numbers of species in Pashyu Chaung, 4 numbers of species in Chaytoryar Pagoda, 12 numbers of species in Chaytoryar Pagoda, 6 numbers of species in Auk Seik and 3 numbers of species in Kyunn Pyet.



Figs. 2. Habit of Ulva compressa; 3) Surface view; 4) Cross section of older thallus margin.; 5) Cross section of the upper part of a blade; 6) Habit of Ulva linza; 7) Surface view; 8) Cross section of older thallus margin; 9) Cross section of the upper part of a blade; 10) Habit of Cladophora vagabunda; 11) Upper branch system; 12) Habit of Cladophora sp; 13-14) Branch system; 15) Middle part ofChaetomorpha spiralis; 16) Discoid Holdfast; 17) Discoid holdfast of C. aerea; 18) Habit of C. aerea; 19) Middle part; 20) Lower cells; 21) Base of filaments: 22) Habit of Chaetomorpha gracilis; 23) Cross section; 24) Uppermost cells of filaments; 25) Lower cells of filaments;; 26) Habit of Chaetomorpha linum; 27) Part of a coiled filament; 28) Middle part; 29) Habit of Rhizoclonium curvatum; 30) Cells of R. curvatum.



Figs. 3. Cells of R. curvatum; 32) Arcuate filaments of R. curvatum; 33) Apical branchlet of R. curvatum; 34) Lower branchlet of R. curvatum; 35) Habit of Rhizoclonium riparium; 36-37) Cells of R. riparium; 38) Habit of Rhizoclonium grande; 39) Cells of R. grande; and 40) Rhizoidal branchlet of R. grande; 41) Holdfast of Rhizoclonium grande; 42) Habit of R. africanum; 43-44) Cells of R. africanum; 45) Arcuate filament of R. africanum; 46) Habit of Cladophoropsis membranaceae; 47) Branch system of C. membranaceae; 48) Segment of C. membranaceae; 49) Habit of Dichotomosiphon sp; 50-51) Branch system of Dichotomosiphon sp; and 52) Oognium of Dichotomosiphon sp.

Conclusion

In relation with the present study, it can be considered that marine green algae seem to be the most diverse and abundant species in Apor Seik and there would be other unrecorded seaweed in Kalegauk Island because Kalegauk Island iAfterwardpulation. Aferwards, Kalegauk Island is necessary for the study of other marine algae and the observations of the seasonal ecologyAcknowledgments.

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RESEARCHS / INVESTIGACIÓN

Evaluation of Rapeseed (*Brassica napus L.*) maturity and productivity for accumulative temperature

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Abstract: Field experiment conducted during the winter agricultural season 2017-2018 at Al-Zafraniya Agricultural Research Station and Al-Tuwaitha Agricultural Research Station, according of Randomized Complete Block Design (RCBD) with three replications, to study maturity and productivity for accumulative temperature of rapeseed (*Brassica napus L.*). The experiment planting in Last week of September 2017 at Al-Zafraniya station and Last week of October 2017 at AL- Tuwaitha station. The results show increasing significantly in the yield (1110.11 kg. ha^{-1}), percentage of oil (47.12 %), protein content in seeds (43.1%), carbohydrate (25.7%), nutrient content of seed (K, Ca) in Al-Zafraniya location; compared to Al-Tuwaitha location, yield (352.8 kg. ha^{-1}), percentage of oil (41.1 %), protein content in seeds (41.2%), carbohydrate (26.2%), nutrient content of seed (Na, Ca, Mg, Fe) at fertilization, compared to no-fertilizer. It showed significant differences with nutrient content of soil (Na, Mg, Fe) for Al-Tuwaitha location and nutrient content of soil (K, Ca, Mg, Fe, S, Cu), pH, EC for Al-Zafraniya location at fertilization, compared to no-fertilizer. These results shows that the delay at time of cultivation leads to a decrease in seed yield, oil ratio, and percentage of protein in seeds, also high temperatures at seed filling stage leads decrease in seeds, oil and protein content.

Keywords: Productivity, Seed yield, Oil content, Rapeseed (Brassica napus L.)

Introduction

Brassica genus is native in the wild in Western Europe, the Mediterranean and temperate regions of Asia. In addition to the cultivated species, which grown worldwide, many of the wild species grow as weeds, especially in North America, South America, and Australia [1] (Cohen et al., 2000). Brassica napus L. can be cultivated under different environmental conditions; there are winter varieties grown early and late. The rapeseed effected by frost, which negatively affects the composition of flowers and pollen, the maturity of seeds and the proportion of oil in seeds. Rapeseed plants need a light period between 10-24 hours. Shading and low temperatures lead to fewer flowers, pods and seeds [2] (Taifour et al., 1990). Robertson et al., 1999 [3] found that the delay in the date of cultivation leads to fluctuation in seed yield between -10 to +4% per week delay. It is important genus in the Brassicaceae family, several species and types of Brassicas. In Iraq has a suitable climate for many oilseeds crops. This is not completely true in North with low temperatures and short growing season, where the oilseed crops usually perform poorly except sunflower and rapeseed.

Rapeseed (Brassica napus L.) is a new and promising oilseed crop for this region, its seed high oil content ranging from 45-60 percent and is rich in protein (34-40) percent [4] (Shirani and Ahmedi 1995). It mainly used in the meat packing industry as an aid to flavor, emulsification, water binding, slicing, bologna and other processed meats. Ground Rapeseed can absorb excess fat and fluid (approximately 4.5 times its own weight) and also used with seasoned hamburger, meatloaf, liver sausage, chili, various canned meat products. Under iraqi conditions, Al-Mandil and Kanah (2017)[5] reported that Brassica napus that were collected from four sites of the city of Mosul, Al-Rashidiya, Shalalat AlMosul, Mosul University, while the fourth site at the village of Abukudoor in spite of the soils were varying in the values of the elements that have been insignificant in Phenolic compounds (Hydroquinone, Resorcinol, Gallic acid, Vanillin and Salicylic acid) , Expect Gallic acid was absence from all of these extracts with the exception of sample that collected from Shalalat Al-Mosul. Which characterized with highest value for phosphorus compared to other sites. Also, in Al-Mosul that AL-Doori and Hasan (2010)[6] investigated the effects of different row spacing (30 - 60 cm) in rapeseed, they concluded that number of siliques per plant, seed weights and dry matter per plant, weight of thousand seed and oil yield increases as row spacing increased.

In AL-Sulaimani, Abdulkhaleq et al. (2018)[7] she expertise at The Qlyasan Agricultural Research Station, College of Agricultural Sciences, University of Sulaimani, using three levels of zinc fertilizer on seed yield showed positive and highly significant correlation on seed yield recorded by an average pod weigh with 0.898, while the maximum positive indirect effect on seed yield recorded by biological yield via average pod weight with 0.840.

Previous study (Hassan and El-Hakeem, 1996) revealed that nitrogen reduced oil percentage of the seed. Nitrogen increases seed and oil yields by influencing a number of growth parameters such as seeds per silique and seed weight and by producing more vigorous growth and development [8] (Sharief, 2000). Winter oilseed rape can be used as a catch crop to reduce nitrogen leaching during the autumn–winter period because of its high capacity to take up nitrate from the soil. Other work on oilseed rape grown hydroponically or in field conditions showed that NO_3 uptake increased from stem extension to the start of the flowering, whereas little NO_3 uptake was observed during Silique filling [9] (Rossato et al, 2001). Yield response of Rapeseed to increasing Nitrogen rate with different environmental variables, including weather, soil type, residual fertili-

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ty (especially nitrate), soil moisture, and cultivars [10] (Hocking, 1997).

Materials and methods

This study carried out in Iraq at the Al-Zafraniya Agricultural Research Station (Latitude 33 ° 13 20.8″; N, Longitude 44° 30 16.0″ E, 765 MASL), located 2 km south of Baghdad city and the Al-Tuwaitha Agricultural Research Station (Latitude 33° 12′ 17″; N, Longitude 44° 30′ 52″ E, 765 MASL) located 5 km south west of Baghdad city during the winter seasons of 2017-2018. The meteorological data of two stations location is shown in Table 1.

The experimental area plots were ploughed twice, harrowed and well leveled. A brief account of some physical and chemical properties of the experimental soil is given in Table (2). The experiment was carried out in (Last week of September 2017 at Al-Zafraniya station, Last week of October 2017 at AL- Tuwaitha station) according of Randomized Complete Block Design (RCBD) with three replications. Rapeseed were planting in row with spacing 30 cm in plot (1 x 2) m^2; the plots were separated by 0.5 m in width from all sides to avoid the effect of fertilization and Field fertilizer (N 100 kg. ha^{-1}, P 250 kg. ha^{-1}) added. The nutrient parameters measured during period study, and nutritive value in terms of minerals of K, Ca, Mg, Na, Zn, Mn, Cu, and Fe. One gram of tissues and soil form each replicate were dried in an oven for 24 h at 105° C (weighed and re-weighed until a constant weight was reached). The samples were allowed to cool at room temperature before the final weight was taken. The contents of the mineral elements for K, Ca, Na, Mg, Mn, Zn, Cu and Fe were determined using the standard method (aqua regain digestion method) and analyzed by Atomic Absorption Spectroscopy (AAS), method of described by Page [16].

The chemical composition of triplicate plant samples was grounded and analyzed for moisture content, carbohydrate, ash, protein (the total protein content was determined by the Kjeldahl method (a nitrogen conversion factor of 5.95), and oil as per the methods of the Association of official Analytical chemists AOAC [5]. Data were analyzed using the SAS 1999 software and means were separated using the stander error [21].

Statistical Analysis: The LSD test was done to find the significant differences between treatment means at 5% probability level. Mature plants were harvested on July 20, of 2017 for estimating seed yield, yield components and growth rate.

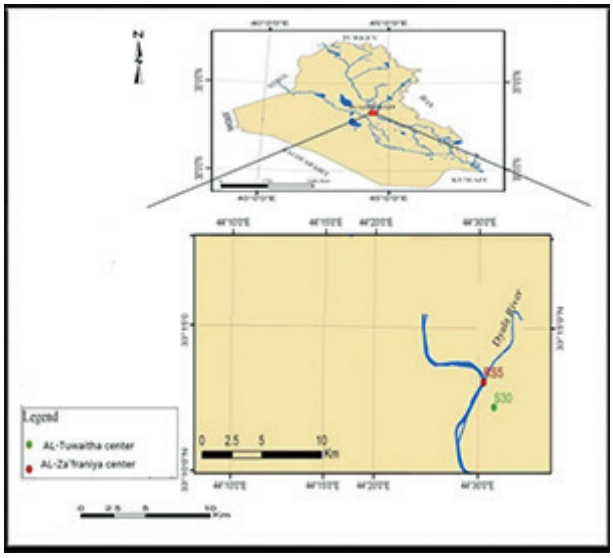

Fig 1. Map showing the location of the studied area

Month	Field fertilizer					
	Al-Zafraniyastation			Al-Tuwaithastation		
	Monthly absolute minimum temperature C	Monthly absolute maximum temperature C	Aver.temp. C	Monthly absolute minimum temperature C	Monthly absolute maximum temperature C	Aver. temp. C
Sep.2017	19.3	39	29.21	19.5	40	30
Oct.	06.2	19.3	12.74	06.8	18.9	13
Nov.	06.8	18.7	12.78	06.8	18.9	13
Dec.	01.2	13.41	07.39	01.4	14.1	07.9
Jan.2018	01.35	10.56	06.1	01.7	11	06.4
Feb.	04.44	16.87	10.55	04.9	17.1	11.1
Mar.	06.54	18.10	12.60	06.8	19.1	13.01
Apr.	11.0	25.67	12.58	11.2	26.1	13.2
May	14.89	30.12	23.2	15.02	31.02	20.1

Table 1. Growing season (29/9/2017–29/5/2018) weather summary for Al-Zafraniya station and Al-Tuwaitha station (29/10/2017–29/6/2018) location study.

Location	Coordinate	Grain size analysis %			Type of soil	CEC	O.M%	Fe_2O_3%
		Sand	Silt	Clay		meq/100gm		
Al-Zaifraniya	N 33 ° 13 ′20.8″ E 44° 30́ 16.0″	19.73	56.14	24.13	sandy silt	12.5	1.31	5.4
AI-Tuwaitha	N 33° 12′ 17″ E 44° 30′ 52″	2.53	54.27	43.20	Mud	17.2	1.27	4.8

Table 2. Physico-chemical properties of area under study

Results and discussion

Results of Table 3 were shows increasing significantly in the seed yield (898.5^{b} - 1110.11^{c} kg. ha^{-1}) in Al-Zafraniya station at fertilization, compared to no-fertilization. Al-Tuwaitha station found decrease significant differences in the seed yield (366.2^{a} - 352.8^{a} kg. ha^{-1}) at fertilization, compared to no-fertilization (Fig 2). The status of seed yield is the most important field scale, giving the final evaluation of agricultural operations to produce new recommendations on production. Through the results, we show increasing the seed yield when sowing date (29-Sept-2017) compared to the second date (29-Oct-2017). The high seed yield on the first date may be due to climatic conditions and increase the number of capsules in the plant and the number of seeds in capsules. (Rahman et al., 2000)[14] showed agriculture in September was better than agriculture in October for a high yield of seeds and oil. The delay in sowing date of rapeseeds leads to slow vegetative growth of the plant and low speed of photosynthesis due to low paper area of the plant and an increase in the fall of flowers and capsules (abortion), (Mendham., 1981)[15]. The decrease in the second date (29/10/2017) was due to high temperature (19.1^{0}C) during the flowering period (Mar. month) (Table 1). Anne et al. (1999) [16] found the high temperature from 18-26 ^{0}C during the flowering period of rapeseeds leads to decrease in the seed yield.

Chemical components, including percentage of oil, protein content in seeds, carbohydrate, are important traits to be considered in the study of rapes (Brassica napus L.). The results shows increasing significantly in the percentage of oil (47.12 %), protein content in seeds (42.1%), carbohydrate (25.7 %) in Al-Zafraniya station at fertilization (Table 3, Fig 3). As well as in Al-Tuwaitha station found decrease significant differences in percentage of oil (41.1 %), protein content in seeds (41.2%), carbohydrate (26.2 %) at fertilization (Table 3, Fig 3). It is show that the percentage of oil, protein content in seeds, increase in sowing date (29/9/2017) and decreased at the delay. The decrease in the second date (29/10/2017) was due to high temperature (19.1^{0}C) between the flowering and the beginning of the formation of corns (Mar. month) (Table 1). This is confirmed by (Pritchard et al.1999)[17] that the high temperature of more than 16 ^{0}C in the flowering stage leads to the decrease of oil in seeds between 12-15% for each high in the temperature of the atmosphere one degree Celsius. Hauska et al. (2007)[18] found the percentage of oil affected by climatic conditions.

In the present study, Field fertilizer application of (N 100 kg.ha^{-1}, P 250 kg.ha^{-1}) to winter rapeseed significantly affected the content of macronutrients and micronutrients in seeds and soil. In Al-Tuwaitha station showed increase significant differences in the K (64.9^{a} - 69.1^{b} mg kg^{-1}) and Ca (3.8^{a} - 5.8^{b} mg kg^{-1}) content of seeds, the content of other macronutrients (N, Na, P, Fe, Mg, Cu, Mn and S) in the seeds of winter rapeseed was not significantly influenced at fertilization, compared to no-fertilization (Table 4). In Al-Zafraniya station shows increasing significantly in the Na (2.7^{b} - 1.7^{c} mg kg^{-1}) , Ca (7.3^{c} - 8.1^{d} mg kg^{-1}) , Mg (3.2^{b} - 3.5^{c} mg kg^{-1}), and decreased Fe (94.1^{b} - 85.2^{c} mg kg^{-1}), content of seeds , The content of other macronutrients (N, K, P, Cu, Mn and S) in the seeds of winter rapeseed was not significantly influenced at fertilization, compared to no-fertilization (Table 4). The uptake and accumulation of micronutrients in different parts of crop plants (roots, shoots, seeds) are determined by the humus content of soil, the activity of microorganisms in the rhizosphere, soil pH, soil moisture content and fertilization.[19]

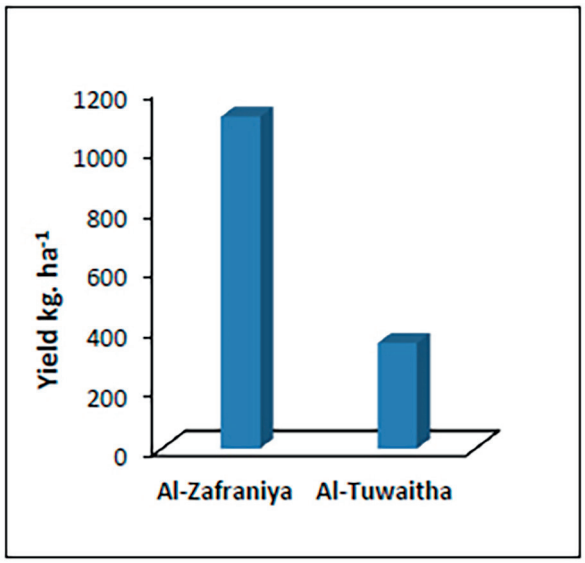

Fig 2. The yield (kg. ha^{-1}) in locations

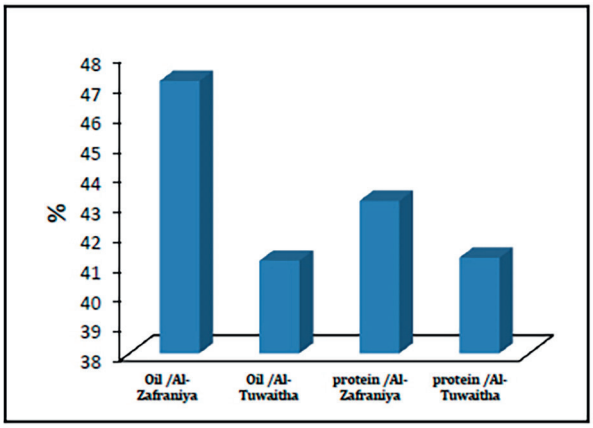

Fig 3. The percentage of oil and protein in locations

Parameter	AL- Tuwaitha station (29/10/2017–29/6/2018)		AL- Zafraniya station (29/9/2017–29/5/2018)	
	No-Fer. (kg.ha^{-1})	Fer. (kg.ha^{-1})	No-Fer. (kg.ha^{-1})	Fer. (kg.ha^{-1})
Yield kg. ha^{-1}	366.2^{a}	352.8^{a}	898.5^{b}	1110.11^{c}
HI %	22.04^{a}	24.5^{a}	27.07^{a}	28.21^{b}
Oil %	40.1^{a}	41.1^{a}	45.11^{a}	47.12^{a}
Seed protein %	40.3^{a}	41.2^{a}	42.1^{a}	43.1^{b}
Carbohydrate %	25.41^{a}	26.2^{b}	25.1^{a}	25.7^{a}
Ash %	3.94^{a}	4.36^{b}	3.5^{a}	4.1^{b}
Fiber %	5.63^{a}	5.98^{b}	5.63^{a}	6.27^{c}
Humidity %	6.3^{a}	6.52^{a}	7.05^{b}	7.21^{b}

Table 3. Effect of field fertilizer on Yield seeds and Organic composition of seed of winter rapeseed in Al-Zafraniya station and AL- Tuwaitha station (2017-2018). Values marked with the same letter do not differ significantly at $p \leq 0.05$

macro-micro nutrients (mg kg^{-1})	Al-Tuwaitha station		Al-Zafraniya station	
	No-Fer(kg.ha^{-1})	Fer. (kg.ha^{-1})	No-Fer. (kg.ha^{-1})	Fer. (kg.ha^{-1})
N	22.1^{a}	25.1^{a}	33.4^{b}	33.9^{b}
K	64.9^{a}	69.1^{b}	84.5^{c}	80.2^{c}
Na	1.2^{a}	1.4^{a}	2.7^{b}	1.7^{c}
Ca	3.8^{a}	5.8^{b}	7.3^{c}	8.1^{d}
Mg	2.1^{a}	2.5^{a}	3.2^{b}	3.5^{c}
P	4.3^{a}	4.6^{a}	5.8^{b}	6.3^{b}
S	1.8	1.8	2.9	3
Fe	64.9^{a}	66.8^{a}	94.1^{b}	85.2^{c}
Mn	34.6	35.7	59.2	58.1
Cu	3	2.8	3	2.3

Table 4. Effect of field fertilizer on the nutrient content of seed of winter rapeseed in Al-Zafraniya station and Al-Tuwaitha station (2017-2018).

Macro-micro nutrients. Values marked with the same letter do not differ significantly at $p \leq 0.05$

Data presented in Table (5) showed, in Al-Tuwaitha station found increasing significantly in the Mg (219.2 - 222.2 mg kg^{-1}), Fe (3.8 - 5.8 mg kg^{-1}) and decreased Na (1257.3 – 1190 mg kg^{-1}) content of soil. The content of other macronutrients (N, K,Ca, P, Cu,Mn and S) was not significantly influenced at fertilization, compared to no-fertilization. In Al-Zafraniya station shows increasing significantly in the EC (5.7^{a} - 6.01^{b}), K (128^{b} - 135^{g} mg kg^{-1}) , Na (1300.7^{c} - 1317.3^{c} mg kg^{-1}) , Ca (1257^{b} - 1397^{c} mg kg^{-1}), Mg (320^{c} - 399^{d} mg kg^{-1}) , S (2.9^{a} - 3^{b} mg kg^{-1}) , Fe (0.97^{c} - 1.2^{d} mg kg^{-1}), Cu (0.88^{b} - 1.17^{c} mg kg^{-1}) content of soil and decreased pH (6.55^{b} - 6.11^{c}), at fertilization, compared to no-fertilization . The results show that the soil in Al-Zafraniya station contains nutrients higher than the Al-Tuwaitha station; this may be due the fertility of Al-Zafraniya soil compared to Al-Tuwaitha soil. As well as soil texture (silt clay loam), decrease the pH (6.55^{b} - 6.11^{c}) when addition of fertilizers and different weather condition of temperature, rainfall, and humidity (Table 1). Management of plant nutrition is important to prevent acidic reactions in reducing nutrient availability in the soil. Soil pH regulates the capacity of soils to store and supply nutrients, and thus contributes substantially to controlling productivity in terrestrial ecosystems (Brady, N. C. & Weil, R. R., 2002)[20].

Values marked with the same letter do not differ significantly at $p \leq 0.05$

Conclusions

The results showed increases with Yield (1110.11 kg. ha^{-1}), percentage of oil (47.12 %), protein content in seeds (43.1%), carbohydrate (25.7%) in Al-Zafraniya location compared with Al-Tuwaitha location which gave decrease in Yield (352.8 kg. ha^{-1}), percentage of oil (41.1%), protein content in seeds (41.2%), carbohydrate (26.2%). Agriculture in 29- September was better than agriculture in 29-October for a high yield of seeds, protein and oil. The high temperature from 18-26 °C during the flowering period of rapeseeds leads to decrease in the seed yield and percentage of oil, protein in seeds.

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macro-micro nutrients (mg kg^{-1})	Al-Tuwaitha station		Al-Zafraniya station	
	No-Fer(kg.ha^{-1})	Fer. (kg.ha^{-1})	No-Fer. (kg.ha^{-1})	Fer. (kg.ha^{-1})
N	22.1^{a}	25.1^{a}	33.4^{b}	33.9^{b}
K	64.9^{a}	69.1^{b}	84.5^{c}	80.2^{c}
Na	1.2^{a}	1.4^{a}	2.7^{b}	1.7^{c}
Ca	3.8^{a}	5.8^{b}	7.3^{c}	8.1^{d}
Mg	2.1^{a}	2.5^{a}	3.2^{b}	3.5^{c}
P	4.3^{a}	4.6^{a}	5.8^{b}	6.3^{b}
S	1.8	1.8	2.9	3
Fe	64.9^{a}	66.8^{a}	94.1^{b}	85.2^{c}
Mn	34.6	35.7	59.2	58.1
Cu	3	2.8	3	2.3

Table 5. Effect field fertilizer on the nutrient content of soil of winter rapeseed in Al-Zafraniya station and Al-Tuwaitha station (2017-2018)

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RESEARCHS / INVESTIGACIÓN

Control of bacterial pathogens isolated from water using Actinomycetes extracts at Egerton University, Kenya

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Abstract: Diseases are the worst enemy to man currently. This study was aimed at isolating pathogenic bacteria from water obtained from shallow wells in Dundori Kenya. Also, the study aimed at testing the isolates for sensitivity to antibiotic metabolites previously extracted from Actinomycetes isolates from soils of Egerton University. Water samples were collected from shallow wells randomly selected from Dundori and abbreviated as A, B, C, D, and E. Bacterial pathogens were isolated from the water samples using the membrane filtration technique. The isolates were characterized using biochemical means. Antimicrobial sensitivity testing was carried out using Kirby Bauer disk diffusion method. Data analysis was carried out using the Statistical Package for Social Sciences (SPSS). Comparison of means was carried out using one way ANOVA. Shallow wells B, D and E were highly contaminated with pathogenic bacteria. Biochemical characterization of the isolates indicated that the most common isolates were *Vibrio cholera, Klebsiella pneumoniae, Proteus sp, Escherichia coli*, and *Staphylococcus aureus.* There was no significant difference between the zones of inhibition produced by the antibiotic metabolites (F=2.149 P=1340) when tested against the test isolates. There were no significant differences between the MIC›s of the antibiotic metabolites on the bacterial pathogens (F=2.01 P=0.15). Water from some shallow wells in Dundori is highly contaminated with *Klebsiella pneumoniae, Escherichia coli, Proteus sp., Vibrio cholerae* and *Staphylococcus aureus.* The pathogens can effectively be controlled using antibiotics from the Actinomycetes. There is a need to sensitize the residents of the study area on ways of preventing seepage of contaminants into the shallow wells.

Keywords: Bacteria, Egerton, Control, Pathogens, Isolation, Water.

Introduction

Water from wells is considered contaminated or unsafe for drinking when tested for toxic chemicals or pathogenic microorganisms 1. The primary source of contamination by microorganisms could be fecal waste from warm-blooded animals including humans. Different pathogenic bacteria cause enteric diseases to humans while others are non-pathogenic 2. The water could also support the growth of protozoan and viral pathogens; hence water test for each pathogen would be extremely expensive and tedious 3 . Water is considered unsafe for human consumption when it has pathogenic microorganisms. Water test for bacteriological safety depends on microbiologists' ability to detect coliform bacteria in the wells 4. *Escherichia coli* is the abundant bacteria in the test since it lives longer in water than other intestinal bacteria 5

Presence of pathogenic microorganisms in water sources may arise from contamination with fecal material, wastes from vegetation and animal dung resulting in growth of coliform bacteria 6. As a result, it becomes economically expensive for the communities to dig new wells with a low level of contamination 7. People who take contaminated water visit health facilities due to stomachache, diarrhea and flu-like symptoms. According to GOK (2014) 8 health statistics shows that pupils in Dundori have a high absenteeism rate and the cost of living in the area is rising at a very high rate.

In developing countries, contamination of water resources is indicated by the presence of enteric pathogens 9. However, rural communities use water disinfection methods such as chlorination, coagulation, sedimentation, and boiling 10. Some of these methods become impractical due to the high cost of the required equipment and low availability of chemical coagulants 11. Hundreds of people in Dundori area depend on both private and public wells which are infested with coliform bacteria. As a result, there is a need to control and improve the health safety of the water to levels that are considered safe for human consumption 12. The area is facing a challenge on how to offer more people access to clean and safe drinking water 13.

Actinomycetes have been used as potential sources of many bioactive compounds which have diverse clinical effects and essential applications in human medicine 14. Antibiotic metabolites screened from Actinomycetes isolated from soils of Egerton University may be a remedy to contamination of water with bacterial pathogens 15. The aims of this study were to isolate pathogenic bacteria from shallow wells of Dundori and carry out the sensitivity test of the isolates to antibiotics previously screened from Actinomycetes isolated from the soils of Egerton University.

Materials and methods

Study area

The study was carried out in Dundori ward with a population of 43,482. The geographical area is sloppy hence the risks of contamination of shallow wells with bacteria. According to Amata *et al.* (2014) 16, Dundori region has an average temperature of 17oC 63oF and lies at latitude 0.27 and longitude 36.1.

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The Actinomycetes antibiotic metabolites

Four antibiotic metabolites coded EU30, EU37, EU41 and EU 154 had previously been extracted from Actinomycetes isolated from soils of Egerton University were used in this study. The Actinomycetes were grown in Luria Bertani broth for 7 days. The metabolites were extracted using ethyl acetate [17].

Water sampling

Five shallow wells coded A, B, C, D, and E were randomly selected from the study area. A total of 255 water samples were collected using sterile sample bottles. The sample size was determined using the formula by Waithaka *et al.* (2015) 18.

$$n = \frac{Z^2 pqD}{d^2}$$

Where; n = sample size, p = anticipated prevalence which was 21% (0.21) in this study, q = failure which was calculated as 100-21 giving 79% (0.79), Z = is the appropriate value from the normal distribution for the desired confidence level which was 1.96 in this study, d = allowable error (0.05) and D = design effect. Based on a confidence interval of 95%, allowable error of 5% and a Z value of 1.96 the sample size was;

$$n = \frac{1.96^2(0.21 - 0.79)}{0.05^2} = 254.928 = 255$$

When collecting the samples, an empty ½ inch was left in the bottle for air space. The samples were transported to Egerton University Department of Biological Sciences Laboratories in iced containers and stored under refrigerated conditions at -4oC awaiting further processing.

Isolation of bacterial pathogens

One hundred milliliters water samples were filtered using membrane filters (Whatman GmbH, Germany), pore size 0.45µm, 47 mm diameter 19 . The membrane filters were aseptically placed on sterile nutrient agar in nutrient agar. Incubation was carried out at 37oC for 24h. Enumeration of bacteria was carried out with the help of a colony counter.

Biochemical characterization

Biochemical identification of the isolates was carried out using API 20E strip kit (bioMe´rieux®, Inc., France). The reagents used included API NaCl 0.85 % medium, API 20 E reagent kit, Zn reagent, oxidase, mineral oil, and API 20E Analytical Profile Index 20. The strips were prepared by the use of anincubation box (tray and lid). In this preparation, 5ml of distilled water was distributed into the honey-combed wells of the tray to create a humid atmosphere 21. Inocula of pure isolates were, emulsified in 5ml of normal saline 0.85% NaCl to achieve a homogeneous bacterial suspension. Anaerobiosis in the tests arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), H2Sproduction (H2S), Urease (URE), citrate utilization (CIT), fermentation/oxidation of glucose (GLU), mannitol (MAN), inositol (INO), sorbitol (SOR), rhaminose (RHA), melibiose (MEL) and arabinose (ARA), gelatinase (GEL), tryptophane deaminase (TDA), fermentation/oxidation of sugars sucrose (SAC) and amygdaline (AMY), Indol (IND) and acetoin (VP) production was maintained by overlaying with mineral oil. The incubation box was closed and incubated at a temperature 37°C for 24h as described by the manufacturer, and the results were determined according to API 20E catalog. The microorganisms were further identified using colony morphology, Gram staining and motility test 22 .

Antimicrobial sensitivity testing

The antimicrobial susceptibility testing was carried out by use of Kirby Bauer disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) 22. Sterile wire loop was used to pick 3 colonies of each *Klebsiella pneumoniae, Escherichia coli, Proteus Sp., Vibro cholera and Staphylococcus aureus.* Emulsification in 3 ml of sterile physiological saline was carried out. Standardization of the suspended colonies was performed by diluting the normal saline suspension until the turbidity matched 0.5 McFarland standards. A sterile cotton swab was dipped into the standardized suspension, drained, and used for inoculating 20ml of Mueller-Hinton agar in sterile Petri plates. Discs (ABTEK BIOLOGICAL LTD., UK) 8mm in diameter were aseptically dipped into each of the antibiotic metabolites. The discs were air dried and placed on the agar using sterile forceps. The plates were incubated aerobically at 37°C for 24h. The zones of inhibition were measured in millimeters .

Statistical Analysis

Data obtained was presented in the form of tables. ANOVA was used to compare the means of viable cells in well A, B, C, D and E, zones of inhibition of the bacterial pathogens by the Actinomycetes crude extracts and minimum inhibitory concentration of the crude extracts.

Results

Number of viable cells obtained from the shallow wells from Dundori

There were no viable cells isolated from well A and well C (Table 1). The number of viable cells isolated from well B varied from 25 to 46 per ml of water. However, in well D the range of viable isolated cells was 227 to 325. In well E, the viable cells ranged from 680 to 1020 per ml of water sample. There was a significant difference in the number of viable isolated cells in well A, B, C and D (F=154.265 P=0.0009).

S. No	Well				
	A	B	C	D	E
1	0	40	0	289	970
2	0	25	0	300	680
3	0	32	0	322	795
4	0	46	0	227	1020
5	0	30	0	325	1000

Table 1. Number of viable cells (CFU/ml) isolated from well A, B, C, D, and E

Biochemical characteristics of the isolates

All the isolates apart from *Vibrio cholarae* were positive for catalase test. Also, the isolates were positive for the indole test except for *Klebsiella pneumoniae* and *Proteus sp* (Table 2). All the isolates were positive for Voges proskauer test except *Escherichia coli* and *Proteus sp.* However, the isolates were positive for citrate utelization except *Escherichia coli* and *Vibrio cholerae.*

On the other hand, all the isolates tested negative for methyl red except *Proteus sp.* and *Vibrio cholerae.* All the isolates tested negative for coagulase test except *Staphylococcus aureus*. Also, the isolates tested negative for oxidase test except for *Staphylococcus aureus*.

Zones inhibition of the isolates by the Actinomycetes metabolites

The zones of inhibition of *K. pneumoniae* varied from 21±0.2mm in EU 154 to 23±0.3mm in EU 30 (Table 3). In *E. coli,* the zones of inhibition ranged between 18±0.3mm in EU 37 to 23±0.2mm in EU 41. Besides, the zones of inhibition in *Proteus sp.* ranged between19±0.3mm in PAN 154 to 23±0.2mm in EU 30. However, in *V. cholera* the zones of inhibition varied from 18±0.2mm in EU 154 to 25±0.3mm in EU41. In *S. aureus,* the zone of inhibition ranged between 22±0.3mm in EU 154 to 27±0.2 in EU 41. There was no significant difference between the zones of inhibition produced by the antibiotic metabolites (F=2.149 P=1340) when tested against the test isolates. The zones of inhibition were indicated by clearing around the disk having the antibiotic metabolites (Figure 1).

Minimum inhibitory concentration (MIC) of the antibiotic metabolites

The minimum inhibitory concentration (MIC) in *K. pneumoniae* varied between 0.23±0.03mg/ ml in EU 41 to 0.25±0.01mg/ml in EU 30 (Table 4). In *Escherichia coli,* the MIC varied from 0.19±0.01mg/ml in EU 37 to 0.24±0.02mg/ml in EU 30. However, in *Proteus sp.* the MIC ranged between 0.18±0.01mg/ml in EU 41 and EU 154 to 0.25±0.02mg/ml EU 30. In *V. cholera,* the MIC varied from 0.17±0.02mg/ml in EU 41 to 0.25±0.02mg in EU 41. In addition, in *S. aureus,* the MIC varied from 0.23±0.01mg/ml in EU 41 to 0.25±0.01mg/ml in EU 30. There was no significant differences between the MIC's of the antibiotic metabolites on the bacterial pathogens (F=2.01 P=0.15).

Indole	M. Red	V. Proskauer	C.utilization	Coagulase	Catalase	Oxidase	Pathogen
-	-	+	+	-	+	-	*K.pneumoniae*
+	-	-	-	-	+	-	*E. coli*
-	+	-	+	-	+	-	*Proteus sp.*
+	+	+	-	-	-	+	*V. cholerae*
+	-	+	+	+	+	+	*S. aureus*

M. Red; methyl Red, V. Proskauer; Voges Proskauer, C. utilization; citrate utilization, K.pneumoniae; Klebsiella pneumoniae, E. *coli*; *Escherichia coli*, *V. cholera*; *Vibrio cholerae, S.aureus; Staphylococcus aureus.*

Table 2. Biochemical tests of the isolates

Pathogen	Zone of inhibition (mm)			
	EU 30	EU 37	EU 41	EU 154
K.pneumoniae	23±0.3	22±0.1	21±0.3	21±0.2
E. coli	22±0.2	18±0.3	23±0.2	20±0.3
Proteus sp.	23±0.2	20±0.2	20±0.2	19±0.3
V. cholera	21±0.3	22±0.3	25±0.3	18±0.2
S. aureus	25±0.2	25±0.1	27±0.2	22±0.3

K. pneumoniae; *Klebsiella pneumoniae*, *E. coli*; *Escherichia coli*, *V. cholera*; *Vibrio cholerae, S.aureus; Staphylococcus aureus.*

Table 3. Zones of inhibition of the isolated bacteria by metabolites from the selected Actinomycetes.

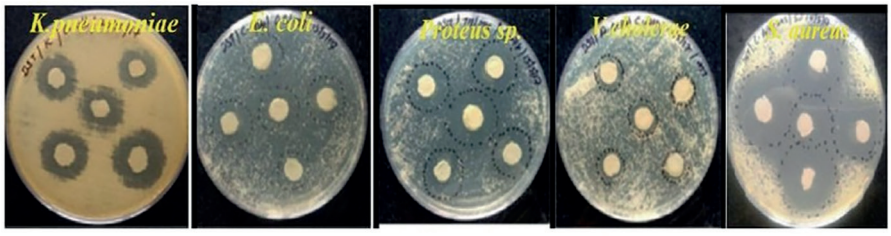

Figure 1. Zones of inhibition of the isolated bacteria by metabolites from the selected Actinomycetes.

Pathogen	Minimum inhibitory concentration (mg/ml)			
	EU 30	EU 37	EU 41	EU 154
K.pneumoniae	0.25±0.01	0.24±0.02	0.23±0.03	0.24±0.02
E. coli	0.24±0.02	0.19±0.01	0.23±0.01	0.22±0.03
Proteus sp.	0.25±0.02	0.22±0.02	0.18±0.01	0.18±0.01
V. cholera	0.23±0.03	0.24±0.02	0.25±0.02	0.17±0.02
S. aureus	0.25±0.01	0.23±0.03	0.23±0.01	0.23±0.02

K. pneumoniae; *Klebsiella pneumoniae*, *E. coli*; *Escherichia coli*, *V. cholera*; *Vibrio cholerae*, *S.aureus; Staphylococcus aureus.*

Table 4. Minimum inhibitory concentration of the isolated bacteria by metabolites from the selected Actinomycetes.

Discussion

Shallow wells A and C had no viable cells (Table 1). However, viable cells were isolated from wells B, D and E. The lack of viable cells from wells A and C could be attributed to lack of seepage of wastewater in the area 18. The isolation of viable cells from wells B, D and E concurred with a study carried out in Pakistan 23 . The similarity in results between the two studies may be attributed to the proximity of the wells to pit latrines 24 .

The results on biochemical characterization of the isolates obtained in this study are typical of the isolates (Table 2). According to Joao and Cabra (2014) 25, biochemical characteristics of bacterial isolates are an indispensable tool in their identification. The results of the current study concur with those of a previous study by Poornima (2018) 26. The possible reason for the similarity in the results could be the isolation of the same bacteria 27. Florica *et al.* (2015) asserted that the same strains of bacteria react the same way biochemically.

The zones of inhibition of the bacterial pathogens by the Actinomycetes crude extracts are presented in Table 3. These results differed with a previous study obtained by Waithaka *et al.* (2017b) 15. Sukhvir *et al.* (2016) 28 explained that the type of antibiotics produced by Actinomycetes determines the zones of inhibition shown by the tested pathogens. Besides, the evolutionary characteristics of the test pathogens also determine the size of inhibition 29.

However, the minimum inhibitory concentrations presented by the metabolites in the current study partially agreed with a previous study by Rajeswari *et al.* (2015) 30. This may be attributed to the Actinomycetes utilizing the same mechanisms of inhibiting the growth of the test pathogens 31. In addition, the concentration of the antibiotics may have led to the observed results 32 .

Conclusions

Water from some shallow wells in Dundori is highly contaminated with *K. pneumoniae*, *E. coli, Proteussp*., *V. cholerae* and *S. aureus.* The pathogens can effectively be controlled using antibiotics from the Actinomycetes.

Recommendations

There is a need to sensitize the residents of the study area on ways of preventing seepage of contaminants into the shallow wells. Further studies aimed at determining the mechanisms of antimicrobial control of actinomycete need to be carried out.

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Conflict of interest

No conflict of interest declared.

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RESEARCHS / INVESTIGACIÓN

Mycorrhizal symbiotic effectiveness as a tool for decision making in restauration of the tropical dry forest

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Abstract: A greenhouse experiment was designed to determine the mycorrhizal symbiotic effectiveness in native mycorrhizal fungi population associated with different soil coverage in the Cesar department. The experimental design was completely randomized with nine treatments and six variations per treatment, 54 experimental units in all. Treatments consisted of combination of soils which contained a substrate from different mulches or soil coal mining (soil-coverage): natural forest (NF), transition soil (TS), a horizon (AH), mining waste (MW), palm (PM), pastures (PT), undisturbed soil (US), with its respective controls, positive *Glomus mosseae* (GM) and negative sterile substrate without inoculum (WI). The variables studied were foliar P content was monitored as a function of time; at harvest, shoot dry weight, shoot P content, and mycorrhizal colonization represented the time increments. The higher contents of P foliar obtained in the sampling period were for the positive control (GM) showing significant differences between soil-coverage, except for ST and US assessed on the sampling day 74. Shoot dry weight had a significant difference in GM, NF, TS, AH, PM and US treatments compared to the remaining three. Treatments with the most weight were US and GM (positive control). Mining waste (MW), PT and WI (negative control) had the lowest values in mass. As expected, shoot P content in the GM samples was higher and had significant differences compared to the other treatments. Soil-coverage closest to the positive control were NF, US, and TS. All assessed treatments showed mycorrhizal colonization except the negative control (WI). Three soil-coverages PM, PT, and US were similar to the positive control, with colonization percentages of 29, 24 and 48 respectively. In conclusion, this kind of research suggests that symbiotic effectiveness experiments are an excellent tool for the selection of native arbuscular mycorrhizal fungi. Besides, and as evidenced, soil-coverage NIT was statistically similar to the positive control (GM), which makes it a candidate for mass crude inoculum production for restoration purposes.

Keywords: arbuscular mycorrhiza, *Glomus mosseae*, mycorrhizal effectiveness.

Resumen: Se realizó un experimento para determinar la efectividad simbiótica micorrizal en diferentes suelos-cobertura del departamento del Cesar. Para el efecto se utilizó un diseño experimental completamente al azar con nueve tratamientos y seis repeticiones por tratamiento, para un total de 54 unidades experimentales. Los tratamientos consistieron en la combinación de muestras de suelos con un sustrato de crecimiento, procedentes de diferentes coberturas vegetales o suelos de minería de carbón (suelos-cobertura): bosque natural (NF), suelo de transición (TS), horizonte A (AH), residuos mineros (MW), palma (PM), pastos (PT), suelo no intervenido (US), con sus respectivos controles, positivo *Glomus mosseae* (GM) y negativo sustrato sin inocular (WI). Como variables respuesta se emplearon el contenido de P foliar, el P total, la masa seca aérea (MSA) y la colonización micorrizal. Los mayores contenidos de P foliar obtenidos en el periodo de muestreo fueron para el control positivo (GM) presentando diferencias significativas entre los suelos-cobertura, con excepción de TS y US evaluados en el día 74 del muestreo. En la masa seca aérea existieron diferencias significativas de los tratamientos GM, NF, TS, AH, PM y US comparados con los tres restantes. Los tratamientos con mayor masa fueron US y GM (control positivo). Por el contrario MW, PT y WI (control negativo) tuvieron los valores más bajos en cuanto a masa. Tal como se esperaba, en cuanto al P total absorbido, GM fue superior y tuvo diferencias significativas comparadas con los demás tratamientos, los suelo-cobertura más cercanos al control positivo fueron NF, US y TS. Todos los tratamientos evaluados exhibieron colonización micorrizal con excepción del control negativo (SI), se resaltan tres suelos cobertura PM, PT y US que se asemejan al control positivo, con porcentajes de colonización de 29, 24 y 48 respectivamente. En conclusión, esta clase de investigaciones sugieren que los experimentos de efectividad simbiótica son una excelente herramienta para la selección de inóculos de hongos micorricico arbusculares nativos. Además, y Tal como se evidencia en los presentes resultados, el suelo-cobertura US fue estadísticamente similar al control positivo (GM), lo que lo hace candidato para la producción en masa de inóculos crudos para fines de restauración.

Palabras clave: efectividad micorrizal, micorriza arbuscular, *Glomus mosseae.*

Introduction

The tropical dry forest (td-F) is an intermediate form between the tropical savanna and the tropical rainforest[1]. These tropical ecosystems are the most biologically diverse in the world[2] and belong to a vegetative formation between 0 and 1000 m.a.s.l, with temperatures above 24 ºC and with two or three times of drought per year[3, 4, 5, 6, 7]. Nearly 42% of the tropical and subtropical habitats belongs to td-F[8]. South America represents 22% of the total forest area[7]. Miles et al.[9] estimated that more than half of the td-F remaining in the world (54,2%) is located in South America. In Colombia, the td-F is the second largest ecosystem in existence occupying 24.97 % of the country[10]. Over the last century, the biodiversity in these ecosystems has been

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deteriorating because of human activity, replacement of forest by agricultural fields, cattle, and mining. Such is the advance of urbanization and industrialization processes[11,12].

According to Janzen[3] the td-F is considered one of the most fragile ecosystems, due to the slow regeneration capacity and the persistent deforestation threats. These are both natural and anthropic issues. Moreover, the dry conditions which they are subjected, seedling recruitment and the growth rates are lower than the rainforest[13,14]. The td-F has become one of the most threatened ecosystems in the world[3,11].

The td-F in the Cesar department is not unaffected by the changes caused by different anthropic activities to the soil. Soils in these areas have different uses with negative consequences. Everyday activities such as farming, raising cattle, growing oil palm crops, corn crops, as well as cotton production represents 44% of national productions in its best times. Cotton and rice production were essential for the Cesar department's development in 1976, using 52% of the agricultural land[15]. As of late, coal open pit mining has been the primary source of income for the area.

Coal mining started in the 1980s and was already an underway in the departments of Cesar, La Guajira, and Córdoba. Mining presented a significant dynamic growth in the regions and economic sustainability. According to Sánchez et al[16], between 1988-2003, Colombian coal production grew 39%, reaching 47 million tons in 2003. Production grew to 96%, achieving an extraction of 19 million tons in the same year. Therefore, the department of Cesar yielded 40% of the Colombian coal in 2003[15]. Coal mining is one of the largest industries in the department of Cesar and is one of the most significant sources of environmental deterioration. The mining exploitation system involves sinkholes up to 400 m deep, making dramatic changes in vegetation and soil. Once the coal has been extracted, the soil layers are mixed and piled up in mounds up to 400 m high called dumps. The high demand of mineral resources generates disturbances in the ecosystems. It also changes the natural hydrology, reliefs, soil, biological communities, land uses which in turn change human population activities[17]. It also makes temperature increase and acidity, reduces the soil humidity, organic matter and nutrient content. All these factors make it difficult to recover the vegetal coverage[18, 19].

Open pit mining has a wide range of environmental impacts that are sometimes irreversible in the environment, unlike other productive processes, this takes place in a finite period of time[20]. The mine operation and closure plans, which in Colombia are established in laws 685 of 2001, provide guidelines for the mining owner to establish rehabilitation activity and restore vegetal restoration of the affected areas by the mining extraction processes. Moreover, the law 99 of 1993 made a change in the execution of mining projects in Colombia, establishing mechanisms and technical instruments for any activity that may cause severe deterioration to renewable natural resources or the environment or introduces considerable or notorious modifications to the landscape[21].

Vegetation is one of the areas most affected by mining. Evidence that plants show effects is in their growth due to the low availability of nutrients present in soils, especially degraded soils. Plant size becomes limited, or they mature in poor form. In order to reverse this effect, plants perform beneficial symbiosis with microorganisms found in the soil making mutualistic associations. This association is established between the plants and specific groups of soil fungi that are found mainly in the rhizosphere. One of the most common mutualistic relationships is mycorrhizal symbiosis. The word mycorrhiza has a Greek origin and means a symbiotic association between a fungus (mycos) and the root (rhizo) of plants. The existence of several types of mycorrhiza, the endomycorrhiza or arbuscular mycorrhizal fungi (AMF), is the largest group forming associations with the 73% of the plant species of the world, mainly tropical[22]. AMF allows plants to improve their nutritional development and overcome stress phenomena in the colonization of terrestrial ecosystems. This is due to the roots ability for symbiosis establishment[23]. Extraradical hyphae can explore larger volumes of soil and reach sites where the root is not able to penetrate[24, 25, 26], increasing the nutrient uptake, mainly from P[27, 28, 29, 30]. This P uptake in the soil by the AMF and the subsequent translocation and transfer to the host plant allows it to obtain a level of equivalence or higher than that obtained by non-associated plants with AMF [31,32,33]. Regardless of the nutritional role, the AMF colonization contributes significantly to improve soil structure, increasing the plant's resistance to biotic and abiotic stress and favors the establishment of interactions with other beneficial microorganisms[27, 34, 35, 36, 37, 38]. Techniques have been developed to assess the AMF and learn the state of these fungi in the soil. One of these is the mycorrhizal symbiotic effectiveness technique that was initially developed by Habte and collaborators[39] of the University of Hawaii. This is based on the theory which is defined as the ability of soil or an inoculum to successfully perform mycorrhizal symbiosis, which is reflected in the infectivity and effectiveness of the infective propagules of the sample. Subsequently, the technique was adjusted by Habte and Osorio[40]. It is clear that in this technique no defined range measures the effectiveness, it is measured indirectly with the statistical analysis that is presented in the response variables such as leaf P, air dry mass and percentage of mycorrhizal colonization. This technique requires reference controls (positive and negative) used in the experimental design. We expect to determine mycorrhizal symbiotic effectiveness as a tool for decision making in the restoration of the tropical dry forest. We think that with the knowledge of microorganism from the soil, especially AMF, we could start the restoration process adequately. Therefore, we should choose the appropriate native inoculum.

Materials and methods

Study area. The department of Cesar is located in the northwest area of Colombia, limited to the north by the Guajira, to the east by the northern of Santander and Venezuela, to the south by Santander and the west by Bolivar and Magdalena. It has an average temperature between 28 and 30 °C and an average annual rainfall of 1940 mm, a bimodal regime with two wet periods between April and June in which 31% of annual rainfall occurs and between August and November with 53% of the annual rainfall [41]. It is located in a tropical dry forest life zone[8]. Here, we worked with several soils or materials, as described in Table 1, comparing areas of the department of Cesar, which were called soil-coverage to facilitate the understanding of the results. Samples were taken from soil-coverage areas of the Calenturita mine at different points, proportional to the production stage (sterile material, horizon A, not used). Also, samples of the most representative soils of the department were also used from: natural forest, stubble, palm, and grass.

Experiment area. The symbiotic effectiveness experiment was carried out in the greenhouse of the Las Mercedes farm, owned by the Universidad Católica de Oriente (6 ° 1'32.3 "N, 75 ° 10' 4.5" W, the altitude of 1116 m.a.s.l) Cocorna (Antioquia Colombia). It has an average temperature of 24 °C and an average annual rainfall of 4,200 mm; the site is located in a predominately humid forest life zone[8].

Soil-coverage	Predominant plants	location	Altitude (m)	Municipality	Description
Natural forest NF	*Pseudobombax septenatum, Bursera simaruba, Pterocarpus acapulcensis*	10° 43.408'N 073° 16.132'W	370	Valledupar	Soil from the Los Besotes natural reserve, classified as entisoles and eceptisols. They are moderately deep soils, moderately fine and moderately thick textures, well-drained, strongly acid and neutral reaction, low and high fertility [42]
Transition soil (low stubble) ST	*Byrsonima crassifolia, Curatella americana, Xylopia aromatica,*	09° 32.683'N 073° 22.388'W	108	La Jagua de Ibirico	Soil from an area of stubble in early succession, composed of isolated trees and shrubs, or grouped into islands of vegetation but without making a canopy. These soils are classified as Entisols, Inceptisols, Vertisols, Alfisols and Mollisols, deep and superficial soils, well drained, fine and moderately coarse textures, light and moderate, strongly acid and very high base saturation [42]
A Horizon (mine) AH	*Machaerium microphyllum, Senegalia macbridei, Cuatella americana, Desmanthus virgatus*	09° 41.124'N 073° 27.389'W	115	La Jagua de Ibirico	Soil coming from the superficial layer, which is separated and piled up before the coal extraction.
Sterile material (mining waste) MW		09° 41.844'N 073° 27.127'W	126	La Jagua de Ibirico	Waste soil from the mining production (MW), a mixture of different edaphic materials that are piled up in the dumps.
Undisturbed soil (mine) US	*Muntigia calabura Byrsonima crassifolia, Xylopia aromatica, Mimosa pigra, Attalea butyracea*	09° 40.641'N 073° 27.992'W	56	La Jagua de Ibirico	Soil from a forest area with the recent removal of vegetation coverage, with soil and subsoil without alteration within the mine.
African palm soil PM	*Elaeis guineensis Jacq*	09° 41.948'N 073° 15.368'W	113	Becerril	Soil from African palm crops used in oil production. These soils are classified as Entisols, Inceptisols, Vertisols, Alfisols and Mollisols, deep and superficial soils, well drained, fine and moderately coarse textures, light, and moderate, strongly acid and very high base saturation.
Pasture soils PT	*Brachiaria sp.*	09° 41.943'N 073°15.358'W	118	Becerril	Soil used in livestock activities in this region.

Table 1. coordinates of the different soils or materials (soil-coverage) from the department of Cesar.

Growth substrate. The growth substrate, was a mixture between soil and sand in a 6-4 proportion (v / v) respectively, as suggested by Habte and Osorio[40]. The soil sample corresponded to an A horizon of Andisol (supplied by a commercial company). Sand 68%, Loam 24%, Clay 8%, texture Frank-Sandy (Boyucos), pH 5.0, organic matter 3.4% (by ignition); Calcium, Magnesium and Potassium, 0.180, 0.06 and 0.05 cmolckg^{-1} (ammonium acetate1M, pH 7), Al 0.4 cmolckg-1 (1M KCl); Phosphorus 105 mg kg^{-1} (Bray ll), Sulfur 24 mg kg-1 (Calcium Phosphate 0.008M), Iron, Manganese, Copper and Zinc 210, 5, 8 and 6 mg kg^{-1} (modified Olsen); Boron 0.1 mg kg^{-1} (hot water); Nitrate 49.1 mg kg^{-1} (Aluminum Sulfate 0.025M); Cationic Exchange Capacity 17.07 cmolckg-1, electrical conductivity C.E 0.1 dS / m.cmolckg^{-1}, electrical conductivity C.E 0.1 dS /m.

We adjusted the pH of the growth substrate to 6.0 with 2g of CaCO3 per kg of soil. We used dolomite lime (57% CaCO3 and 38% MgCO3) in order to improve the concentration of Mg in the substrate. For lime concentrations determination, we made an incubation curve of $CaCO_3$according to the Uchida & Hue[43] methods. The growth substrate was disinfected by vaporization, and a week later it was sterilized in an autoclave at 120 °C and 0.1 MPa, for an hour. In order to establish the substrate solution phosphorus concentration of 0.02 mg / L, the optimal level for mycorrhizal activity[44]. An isotherm of P sorption (Figure 1), was based on the methods proposed by Fox & Kamprath[45].

Sources of inoculum (soil-coverage). The growth substrate was transferred to 54 pots (11.5x15 cm) with a mass of 800 g / pot. 350 g was directly added to each pot, and the remaining 450 g of the substrate was inoculated and mixed uniformly with 20 g of each of the sources of inoculum (soil-cover), previously mentioned. This with the purpose of facilitating contact of the root with each inoculum. Likewise, we used a crude inoculum of *Glomus mosseae* (GM) as a positive control. This mycorrhizal inoculum was purchased from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) and later multiplied in sorghum by a commercial company. It contained spores, fragments of infected roots and hyphae of the fungus suspended in a solid matrix composed of soil and sand. As a negative control, we used the same growth substrate, that is, without inoculation (WI), which received 20 g of sterile substrate. The negative control was the same growth

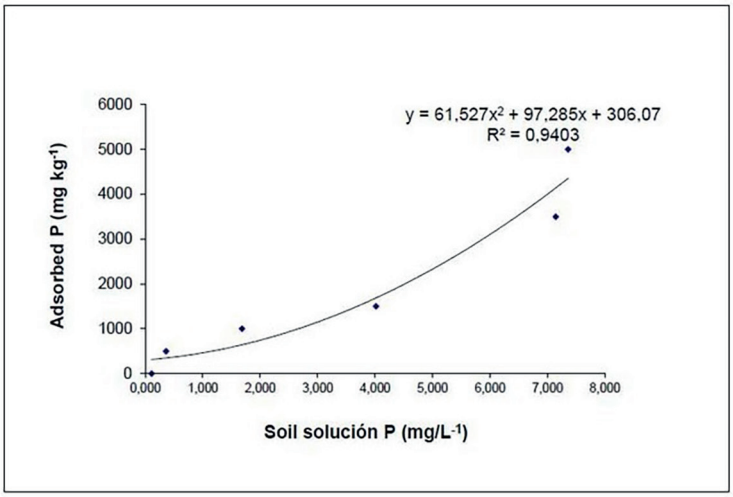

Figure 1: isotherm of P sorption of the growth substrate

substrate without inoculation (WI), which received 20 g of sterile substrate. For each of the soils or materials (soil-coverage), we performed soil analysis, in order to record its properties, and facilitate the final analysis (Table 2).

Indicator plant. *Leucaena leucocephala* var. K11 was used as a trap species or indicator plant because it is highly dependent on the mycorrhizal condition and has rapid growth[40]. Certified *L. leucocephala* seeds were introduced to decrease the experimental variability. Seeds were scarified with H_2SO_4 for 20 min, washed 6 times with deionized water[44] and placed in a humid chamber with filter paper for germination. Once pregerminated, one seed was placed per pot or experimental unit in the different treatments (soil-cover). The second week after planting, each experimental unit received 10 cm^3 of the P-free Hogland nutrient solution in the following doses (mg L-1): N 50, K 132, Mg 106, S 204, Zn 10, Cu 5, B 0.8 and Mo 0.5. Plants were stored under natural light for growth and were periodically watered to maintain them between 50 and 60% of the maximum water retention capacity.

Experimental design. A completely randomized experimental design with nine treatments and six repetitions per treatment, for a total of 54 experimental units was analyzed.

Response variables

AMF spore extraction was used a sub-sample of each soil-coverage. Each subsample was transferred to a beaker with 300ml of running water and 0.15g of sodium pyrophosphate. It was stirred and left to rest for 5 minutes; the supernatant was passed through a battery of sieves (250, 106 and 53 µm), and centrifuged with a sugar solution at 70% for 5 min. Later the sedimented product of the centrifugation was collected on filter paper[40]. We are concluding with the spores count in the stereoscope. Leaf P content was measured through frequent monitoring (every 21 days) as a function of time in the youngest mature leaf following using the non-destructive sampling method Habte et al. [46]. P determination was carried out using the molybdate blue method[47], after reducing the leaf samples to ash in a muffle at 500 °C for 3 hours[48]. Shoot dry weight (SDW); plants were harvested at 95 days after the experiment planting. SDW determination was made after drying the plant material at 60 °C for 72 hours[40]. Mycorrhizal colonization, this was determined by the method of plates[49] from the number of positive fields (presence of arbuscular, vesicles and hyphae) inside the root, for this, the finest roots were rinsed with KOH at 10%[50] and then stained with a 0.15% acid fuchsin in lactic acid[51]. The total P content absorbed (TPC) was estimated through the concentration of P in the fourth pineal at the time of harvest[46], as described above.

Analysis of results. We made and verified the statistical analyzes and the assumptions, to verify that the standardized residuals are normally distributed with zero mean and variance one, the standardized residuals were plotted with the independent variable. Data were subjected to Duncan's multiple range test and Fisher's LSD test, which a level of significance of P £ 0.05. We used the statistical packages R wizard and Statgraphics. Obtained data from the aerial dry mass, was necessary to perform a natural logarithmic transformation (Ln) to meet the conditions of normality and homogeneity of variance (ANOVA).

soil-coverage	pH	M.O.	Al	Ca	Mg	K	C.E.C	P-Bray II	Fe
		%	----------cmolckg^{-1}----------					mg/kg	
BN	6.5	3.2	0	4.6	2.2	0.75	7.57	1	42
TR	4.6	1.2	1.2	6.9	0.1	0.38	8.54	7	441
MM	6.5	2.9	0	50	15	0.55	65.55	4	42
MA	5.8	2.1	0	50	12.8	0.41	63.17	2	46
NIT	5.6	1.7	0	50	5.7	0.61	56.34	2	61

Table 2. Soil or material analysis (soils-coverage) of the department of Cesar

Results

The soil-coverage with the most significant amount of spores were GM, TS, PM, and US with values of 11500, 3360, 2070, and 1060 respectively. The number of spores in the sample of soil from the palm crop (PM) is striking, which did not show up in the other results. As will be seen later (Table 3).

The positive control (GM) was statistically superior in leaf P content in time in comparison with the other treatments (soils-coverage), only on day 74 the TS and US treatments had no statistical differences with GM. Conversely, the negative control showed, in general, the lowest concentration of leaf P content, followed by PT and MW. It is noteworthy that the three soils with the least anthropic intervention (US, NF, and TS) showed a similar tendency in terms of leaf P content in the first two samples (days 31 and 54) with very similar values to the negative control (WI). Apparently, from day 60, all these treatments had significant increases of P until the end of the experiment, presenting significant differences compared to WI (Figure 2).

Soil- coverage	Spores per 100g of soil
Glomus mosseae (GM)	11500
Natural forest (NF)	140
Transition soil (TS)	3360
Mining waste (MW)	40
A horizon (AH)	70
Undisturbed soil (US)	1060
Palm (PM)	2070
Pastures (PT)	SN

Table 3. AMF spore extraction in different soil-coverage. Without data (WD)

The total P content absorbed (TPC) at the end of the harvest, the GM positive control had significant differences compared to the other assessed soil-coverage treatments.

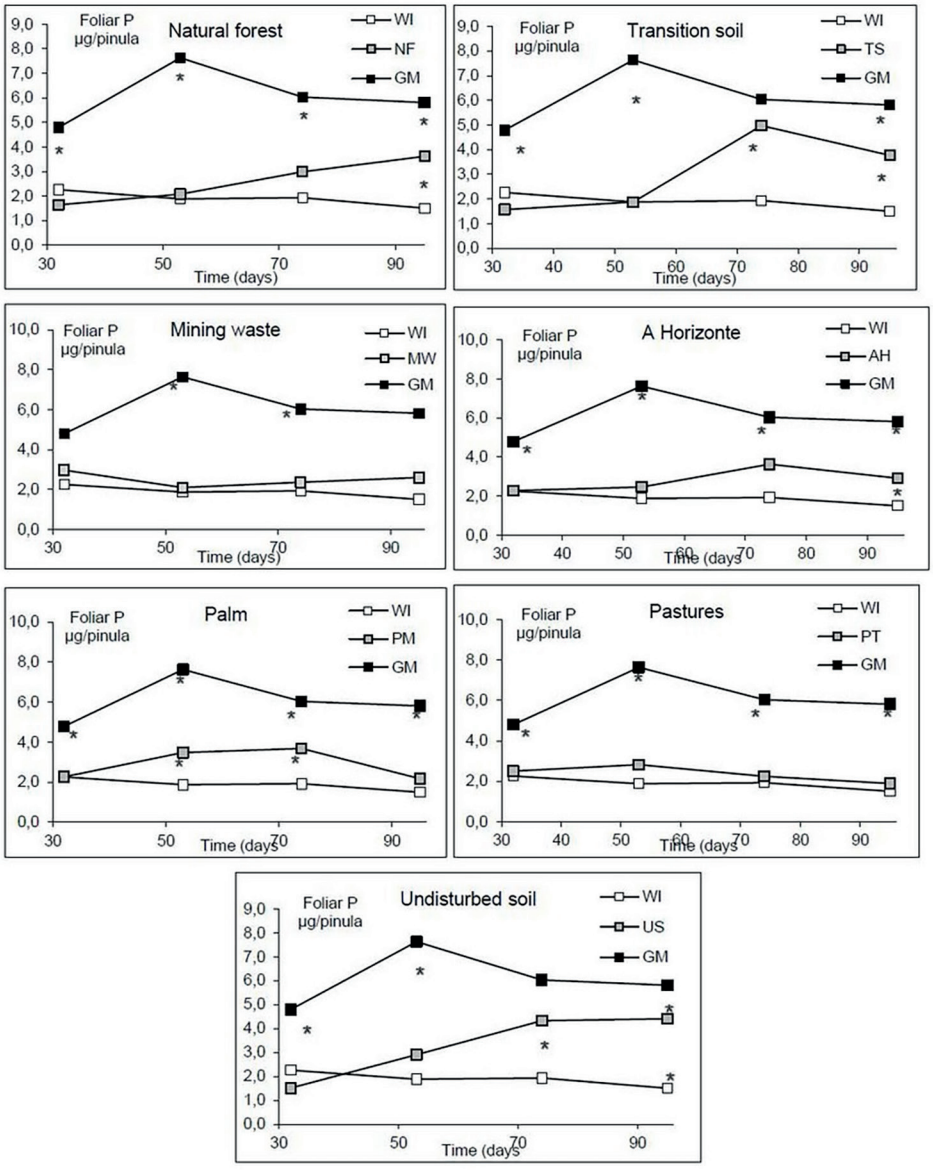

Figure 2. Leaf P content evaluated over time according to the inoculation with different sources of inoculum (soil-coverage). Asterisk indicates significant differences.

In addition, we found that treatments or soil-coverage NF, TS and US had a similar behavior to the GM positive control in the contents of total P. As for the WI treatment (negative control), it obtained the lowest contents of total P content absorbed, followed by the soil-coverage MW, PM, and PT with similar results (Figure 3).

The treatment with the shoot dry weight (SDW) was US, which even exceeded the positive control (GM), although not with significant differences. Overall, the remaining soil-coverage presented two tendencies, one similar to the positive control (GM), grouped in TS, NF, AH, and PM, where the soil-coverage is again found from soils not much intervened and another similar to the negative control (WI) next to the MW and PT treatments (Figure 4).

Mycorrhizal colonization was present in all the evaluated treatments, with the exception of the negative control WI, as expected. In addition, three soils-coverage PT, PM and WI showed similarity to the GM positive control; these coverage-soils obtained colonization percentages of 24, 29 and 48 respectively (Table 4).

Soil-coverage	AMF colonization (%)
WI	0
NF	13
TS	4
MW	3
AH	15
PM	29
PT	24
GM	34
US	48

Table 4. Percentage of mycorrhizal colonization present in the Leucaena root in the different inoculum sources (soil-coverage).

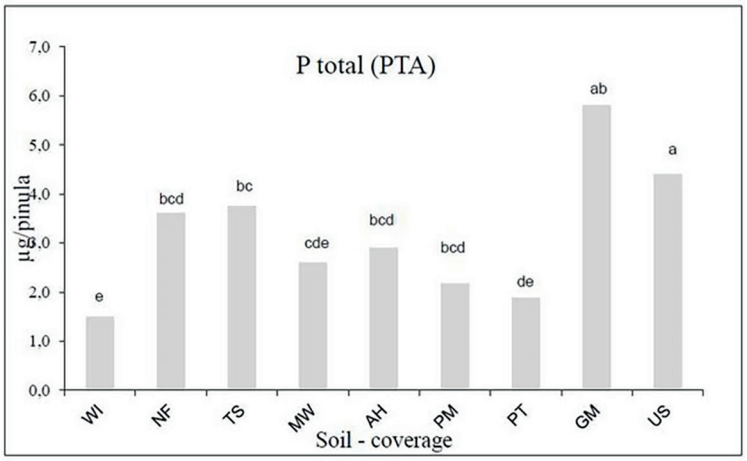

Figure 3. Total P (PTA) in the Leucaena pineal at the end of the harvest in the different sources of inoculating (soils-coverage). Columns with different lowercase letters indicate significant differences (Duncan, P £ 0.05).

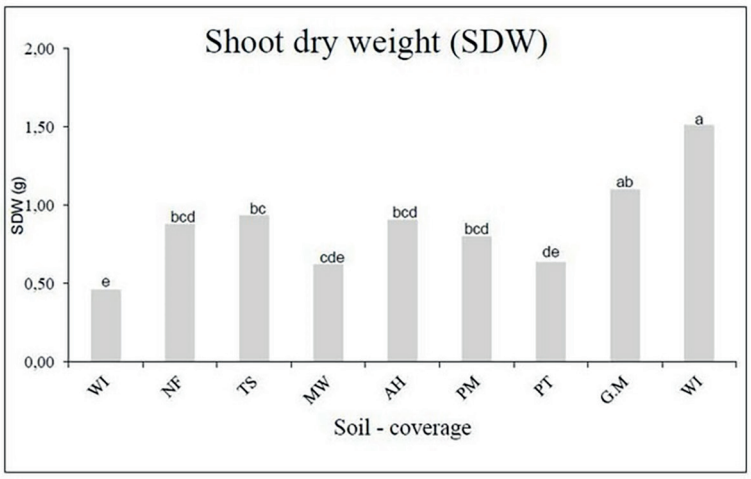

Figure 4. Shoot dry weight (SDW) of Leucaena at the end of the harvest in the different sources of inoculate (soils-coverage). Columns with different letters indicate significant differences (Duncan, P £ 0.05).

Discussion

Results indicate that the AMF of the different soil-coverage has very variable dynamics[52] since in general two clearly defined trends were presented, two sets of data were grouped, the first (US, NF, and TS) associated to the positive control and the other remaining group associated with the negative control. These results are possibly due to the lack of infective mycorrhizal propagation absent in the ground coverage of the second group. This behavior has been reported by Osorio et al.[53]. Although with opposite tendencies, which found that forests, stubbles and forest plantations of high areas (low montane forests) had a similar behavior to the negative control. On the contrary crops and pastures evaluated by these research-

ers, had similar tendencies to the positive control (*Glomus aggregatum).*

From the ecological perspective Stürmer & Siqueira[52] found that the conversion of primary forest to different land uses (secondary forest, pastures, agroforestry systems and crops) apparently does not reduce the diversity of AMF in the Brazilian Amazon, this could explain in part , because soil-coverage US, NF and TS, showed similarity with the positive control in terms of SDW and leaf P. On the other hand, Lovera & Cuenca[54] found a contrary effect, when they compared a natural savanna versus a disturbed savanna in Venezuela, where a significant decrease in soil spore diversity was observed in the disturbed savanna.

Previously mentioned, it is noteworthy that the undisturbed soil-coverage (US) from the area of the Calenturita mine, presented values very similar to the positive control. This soil was not disturbed at the time of the samples. Therefore, it is assumed that the diversity of AMF and the number of spores in the sample were not affected.

Glomus mosseae inoculation increased significantly in the *Leucaena* plants, the leaf P contents, and the SDW plant growth, with respect to the plants that were inoculated with the soil-coverage. Similar behavior was showed by Jaramillo et al[55]. They used *Glomus aggregatum* as a positive control in the evaluation of several soils from oil palm, secondary forest, and soil degraded by alluvial mining. It is noteworthy that in the study by Jaramillo et al[55], the soils from the palm and the alluvial mining showed low mycorrhizal symbiotic effectiveness, with values very similar to those presented here (Figure 3 and 4).

The low symbiotic effectiveness of the soil-coverage of African palm (PM) and pasture (PT) is possibly due to the management of these soils, which includes the use of pesticides and tillage. Both of which negatively affect the diversity of AMF and its number of effective propagules. There are several documents reported by literature confirming these effects[54, 56, 57, 58]. Likewise and as expected, the mixture of soils and materials from the coal mining operation negatively affected the soil-coverage sterile material (MW) and A horizon (AH) resulting in low symbiotic effectiveness. The negative consequences of mining on microorganisms have been well documented by different authors[59]. Finally, with the obtained results here, some soil coverage (US, NF, and TS) can be selected that have high symbiotic effectiveness, and that can be used (after multiplying the crude inoculum) in restoration and reforestation processes. Studies carried out by Souza et al[60] suggest that the same degraded areas, such as those subjected to mining activities, may contain efficient AMF populations, contributing positively to rehabilitation.

Conclusions

Assessing treatments, we found that in general there are significant differences between two groups of data, first was evidenced that the soil-coverage US, NF, and TS had similar results to the positive control (GN), but MW, PM, PT and AH had behaviors similar to the negative control.

According to the data found, the soil-coverage with the best mycorrhizal symbiotic effectiveness were Undisturbed Soil, (US), Natural Forest (NF) and transition soil (TS). They have a high potential to be used in fundamental processes of ecological restoration.

Acknowledgments

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CASE REPORTS / REPORTE DE CASO

Signo de Leser Trèlat como forma de presentación de cáncer de pulmón. A propósito de un caso.

Sign of Leser Trèlat as a form of presentation of lung cancer. About a case.

Adrian Isacc Nieto Jiménez

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Resumen: Se presenta el caso de un paciente de 65 años valorado en el hospital Provincial Docente Arnaldo Miliàn Castro en Santa Clara por presentar manchas color café a nivel de la espalda de rápida aparición en alrededor de 2 meses, que antes no estaban, acompañadas de prurito molesto en ocasiones. Fue tratado por dermatología anteriormente por este motivo con diagnóstico de queratosis seborreica. Llamó la atención el comienzo brusco de esta entidad por lo que se planteó un posible signo de Leser Trèlat y se indicaron varios estudios complementarios y radiológicos. Se realiza biopsia cutánea de dos de las lesiones en la espalda y se concluyen como queratosis seborreicas, se realiza Rx de tórax que arroja imagen nodular sospecha de neoplasia de pulmón que se confirma con biopsia. Este signo cutáneo constituye una dermatosis paraneoplàsica poco frecuente y diagnosticada en nuestro medio. Se asocia a neoplasias del tracto digestivo y respiratorio respectivamente.

Palabras clave: signo de Leser Trèlat, neoplasia, queratosis seborreicas.

Abstract: We present the case of a 65-year-old patient who was evaluated at the Provincial Teaching Hospital Arnaldo Miliàn Castro in Santa Clara, due to coffee-colored spots on the back that appeared rapidly in about two months, which were not there before, accompanied by annoying pruritus. Sometimes. He was treated by dermatology previously for this reason with a diagnosis of seborrheic keratosis. The sudden onset of this entity attracted attention, so a possible sign of Leser Trèlat was raised, and several complementary and radiological studies were indicated. A skin biopsy was performed on two of the lesions on the back, and they were concluded as seborrheic keratoses. A chest X-ray was performed that showed a nodular image of a suspected lung neoplasm confirmed by biopsy. This cutaneous sign is a rare paraneoplastic dermatosis diagnosed in our environment. It is associated with neoplasms of the digestive and respiratory tract respectively.

Keywords: Leser Trèlat sign, neoplasm.

Introducción

Diversas dermatosis paraneoplásicas, como la tromboflebitis migratoria superfi cial (signo de Trousseau), la acantosis nigricans (AN), la acantosis palmaris, la hipertricosis lanuginosa adquirida, la papilomatosis cutánea florida y el signo de Leser-Trélat (LT) han sido descritas en asociación con distintas neoplasias internas, especialmente del tracto gastrointestinal [1] . Una de las entidades más controversiales es el signo de LT, el cual fue descrito como la súbita aparición e incremento en tamaño y número de múltiples queratosis seborreicas en asociación con una neoplasia interna [2, 3,4.] La mayoría de los casos que han sido reportados son adenocarcinomas (especialmente estómago, colon y mamas), en menor número se han reportado casos asociados a neoplasias de pulmón, linfoma/leucemia, carcinoma de células escamosas y malignidades hematológicas. [5]

El signo de Leser-Trélat es una dermatosis paraneoplásica sumamente infrecuente, caracterizada por la súbita aparición e incremento en tamaño y número de múltiples queratosis seborreicas en asociación a una neoplasia interna. [1]

En la literatura médica hay solo cerca de 100 artículos de reporte de casos con pacientes que presentan los dos elementos del signo: el cutáneo y el oncológico, pero también hay casos publicados en los que se describen las lesiones de queratosis seborreica eruptiva junto con otras condiciones no neoplásicas, inclusive en pacientes sin otro hallazgo, lo cual ha sido llamado pseudosigno de Leser Trélat. [6]

El signo de Leser-Trélat se fundamenta en la aparición súbita de lesiones de queratosis seborreica antes del diagnóstico de cáncer, concomitantemente o luego del hallazgo del mismo; sin embargo, no existen criterios universales en cuanto al número de lesiones y tiempo de evolución de las mismas: Fink et al (55) proponen la aparición de 20 o más lesiones en un lapso menor o igual a seis meses, y Lindelöff et [7] al definen el intervalo de tiempo en 3-18 meses. Algunos autores [8,9] describen el signo de LeserTrélat semejando a un árbol de navidad o tipo salpicadura; la mayoría de las lesiones comprometen primordialmente el tronco en 18.9%, la espalda en 15.8%, el pecho en 11.7%, las extremidades en 10.8%, la cara en 8.6%, el cuello en 8.1%, y el abdomen en 5.1%. [10]

El prurito puede ser una característica prominente en 26-51% de los casos. [6]

Las edades más frecuentemente observadas suelen corresponder a pacientes añosos (promedio de presentación es de 61 años). [11]

La tasa de incidencia en Europa es desconocida hasta ahora; en Estados Unidos se han reportado 48 casos verdaderos de este síndrome[12.] En Ecuador se registró una tasa de 0.8 en el sexo femenino con respecto a un 0.9 del sexo femenino en

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el año 2017, lo que indica que hay no hay distinción de sexo. En Cuba se registran 3 casos hasta el momento y ninguno en Villa Clara.[13]

Esta entidad ha suscitado una gran atención debido al incremento de la incidencia de las neoplasias malignas a las cuales se atribuye como el cáncer de pulmón en este caso. En la actualidad sigue sin existir un tratamiento efectivo para este tumor cuando se ha diseminado. La detección temprana y el tratamiento de la causa subyacente continúan siendo los pilares en su manejo.[10]

Caso clinico

Paciente masculino de 65 años de edad que acudió de manera espontánea al hospital provincial docente Arnaldo Miliàn Castro en abril 2018 a consulta de dermatología por manchas color café, múltiples a nivel de la espalda de aparición brusca, las cuales se diseminaron en un período de 2 meses. Se acompañaba de prurito moderado y molesto. Ya había sido valorado anteriormente por un especialista en dermatología quien realizó el diagnóstico de queratosis seborreicas atribuidas al envejecimiento de la piel, sin nada más a señalar, pero el paciente al notar el incremento de las lesiones acude nuevamente a consulta por otra opinión.

Al interrogatorio, refirió como antecedente hipertensión aretrial sin tratamiento médico y fumador de larga data de evolución, desde los 24 años, fuma 1 cajetilla de cigarrillos diaria. Además, refiere tos con expectoración amarillenta y dolor en la espalda de 15 días de evolución y fiebre en las tardes hasta 38 grados que cede con antipiréticos sin especificar cuáles.

A la exploración física se observó dermatosis en el tronco constituido por lesiones "maculopapulosas", negroparduzcas, con aspecto verruciformes, que abarcaban toda la espalda de 2 meses de evolución intensamente pruriginosas. (Figura 1).

Al examen físico del sistema respiratorio se apreció a la auscultación estertores crepitantes bibasales y disminución de la expansibilidad torácica en ambos campos pulmonares.

Se procedió a realizar dermatoscopìa y biopsia cutánea de las lesiones para confirmar el diagnóstico de queratosis seborreicas.

La dermatoscopìa y el estudio histológico confirmaron el diagnóstico planteado.

Se realizaron los siguientes complementarios:

Leucograma completo y eritro sedimentaciòn, esta última en 75.

Perfil hepático y renal normales.

Perfil lipídico y glicemia normales.

LDH normal.

Antígeno prostático sin dentro de límites normales.

Parcial de orina negativo.

BAAR en esputo sin alteraciones.

Rx de tórax que informa áreas radiopacas en hemitórax derecho con posibles cavitaciones. (Figura 2)

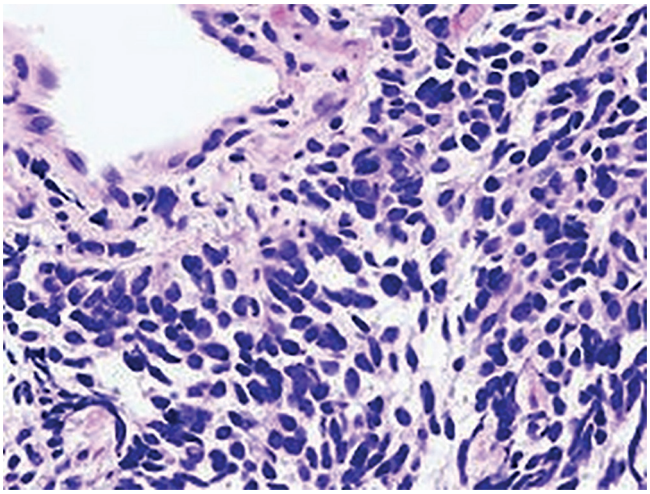

Figura 2. Imágenes con radioopacidades en hemitórax derecho sugestivo de cavitaciones.

Se procede a interconsulta con Neumología quien indica tratamiento con penicilina cristalina (bulbo de 1 millón de unidades) a razón de 16 millones de unidades diarias por vía endovenosa durante 7 días con mejoría del cuadro respiratorio. Además, se indica un TAC de pulmón que arroja un nódulo sugestivo de cáncer de pulmón que se confirmó con la biopsia del mismo a forma de carcinoma de células pequeñas. (Figura 3)

El paciente fue referido a oncología para su posterior tratamiento médico.

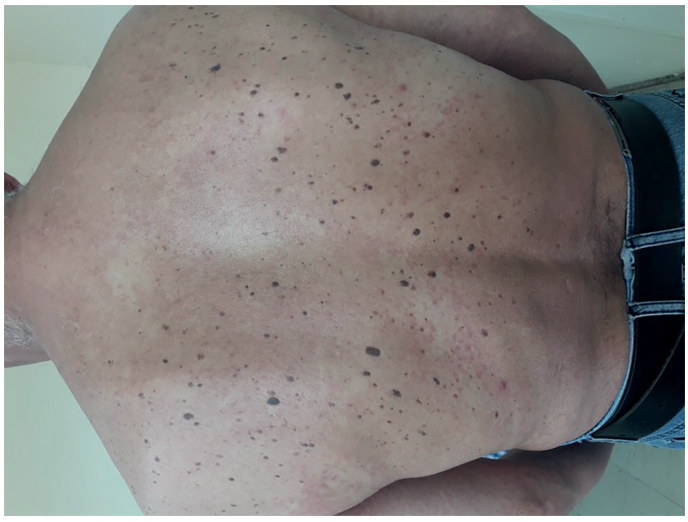

Figura 1. Se observan las queratosis seborreicas diseminadas en la espalda. A la palpación de superficie verruciforme.

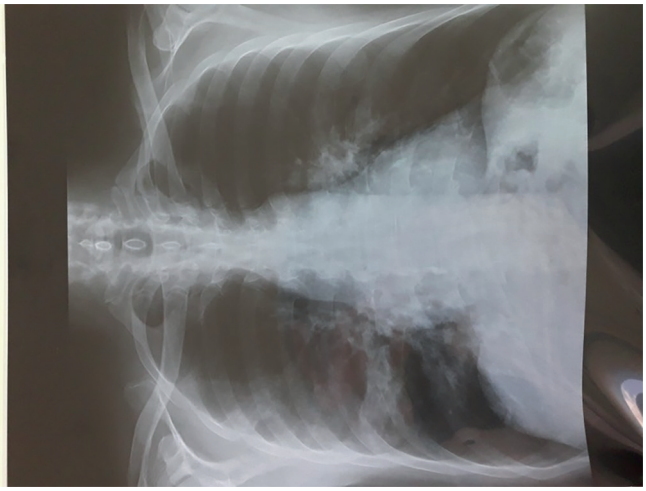

Figura 3. Histopatología de la muestra tomada del tumor de pulmón. Carcinoma de células pequeñas.

Comentario

El signo de Leser Trèlat fue descrito y acreditado de forma separada por Edmund Leser y Ulysse Trélat, ambos, cirujanos europeos, de ahí a que lleve sus nombres. Sin embargo, aparentemente estuvieron observando angiomas rubí en pacientes con cáncer y no queratosis seborreicas; por lo que este signo de sería un nombre erróneo. La primera asociación entre queratosis seborreica y una neoplasia interna fue hecha por Hollander en 1906. [7]

El 20% de pacientes con este signo presenta acantosis nigricans concomitantemente o luego de un tiempo, y un 43% presenta prurito asociado. [7,8]

La localización más frecuente suele ser la espalda, [7] coincidiendo con nuestro caso. En el 2014, Schwartz [1] resumió el tipo y localización de las neoplasias en 86 pacientes con el signo de Leser Trèlat; 51 pacientes presentaron un adenocarcinoma, siendo la localización más frecuente el estómago, luego las mamas, el colon/recto y uno de células pequeñas en pulmón.

Sin embargo, cerca del 20% de pacientes reportados en otras series presentaron un linfoma o leucemia. Sólo existe un reporte de adenocarcinoma de pulmón asociado al signo de LT en la literatura, [3] siendo el nuestro el segundo de este tipo. La etiología es desconocida en la actualidad. [2]

En nuestra paciente, el simultáneo y súbito desarrollo de múltiples queratosis seborreicas, su monomorfismo y poco grosor, y su proximidad apoyarían una teoría de hiperplasia más que de neoplasia en su histogénesis; muy similar a lo reportado por Heaphy y cols. [3]

Las manifestaciones clínicas cutáneas de este signo lo comprenden las queratosis seborreicas ya descritas indistinguibles de las que se observan cómo proceso natural de envejecimiento, con bordes bien definidos y con coloración típica color café obscura sobre todo en la cara y tronco. [9]

El diagnóstico positivo se realiza comprobando la existencia de las queratosis seborreicas por histología, de aparición reciente que concomiten con una neoplasia maligna y desaparezcan de forma simultánea. [8]

Dentro de los exámenes clínicos apropiados, se ha recomendado bioquímica sanguínea completa, sangre oculta en heces, antígeno carcino embrionario, examen de orina completo, mamografía y examen completo de cérvix en mujeres, antígeno prostático en hombres, radiografía de tórax, y considerar la posibilidad de tomografía de abdomen y pelvis. [14]

Dentro de las posibilidades terapéuticas, el mejor acercamiento es el tratamiento de la neoplasia asociada; el uso de retinoides tópicos es una opción para las queratosis seborreicas. El curso del signo de Leser Tèlat suele ser paralelo a la malignidad subyacente; salvo en un paciente con dos malignidades primaria[15]. Muchas de las neoplasias asociadas a este signo se comportan de manera agresiva, por lo que el pronóstico no es bueno; el promedio de supervivencia es alrededor de 10.6 meses. [14,15]

La persistencia de las queratosis seborreicas puede significar un fracaso en el tratamiento o una reactivación de la neoplasia.[13]

Creemos que nuestro paciente y el peso acumulativo de varios reportes de este signo asociado a una malignidad, muestran la necesidad de evaluar de manera integral a dichos pacientes.

Conclusiones

El Signo de Lesar Trèlat constituye una variante infrecuente de dermatosis paraneoplàsica a tener en cuenta ante queratosis seborreicas eruptivas.

Recomendaciones

Es importante la evaluación clínica y multidisciplinaria ante la sospecha de este signo para brindarle al paciente un mejor tratamiento y calidad de vida.

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REVIEW / ARTÍCULO DE REVISIÓN

Unravelling the endometrium: a pictorial review of saline infusion sonohysterography in the evaluation of abnormal uterine bleeding.

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Abstract: This article describes the diagnosis of causes of abnormal uterine bleeding with experience of the biggest medical institute in Kashmir, India. We work in a low resource setting where unavailabity of hysteroscopy made us acknowledge the accuracy and efficacy of saline infusion sonohysterography in diagnosis of patients with AUB thus helping guide their management.

Introduction

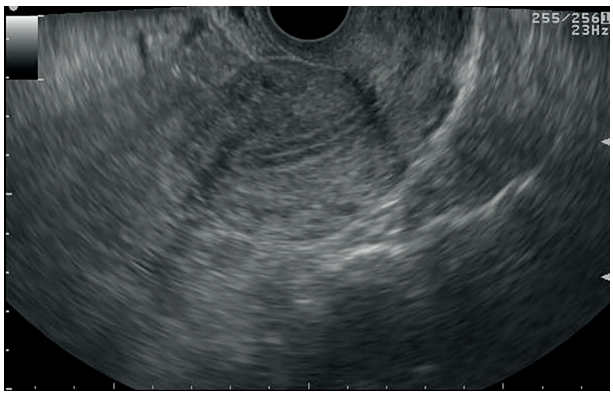
Abnormal uterine bleeding (AUB) is defined as an alteration in the volume, pattern, and duration of menstrual blood flow and is the most common reason for gynecologic referrals(1). In India, the prevalence of AUB is reported to be around 17.9%(2). Women with abnormal uterine bleeding have a considerably lower quality of life than the average population with a multitude of symptoms. (3)

The PALM-COEIN Classification System for causes of Abnormal Uterine Bleeding

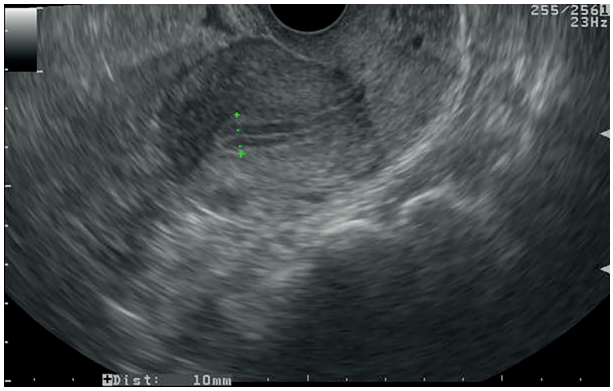
- P Polyps
- A Adenomyosis
- L Leiomyoma
- M Malignancy
- C Coagulopathy
- O Ovulatory disorders
- E Endometrial
- I Iatrogenic
- N Not classified(4)

In about 25% of patients, abnormal uterine bleeding is the result of a well defined organic abnormality(5)

For the initial assessment of possible gynecologic abnormalities, pelvic ultrasonography is most often used. (6) The introduction of saline infusion sonohysterography (SIS) has been a significant advance in the evaluation of the endometrial cavity. SIS provides an unparalleled, clear, enhanced view of the endo myometrial complex that cannot be obtained with transvaginal sonography (TVS) alone. (7)Sonohysterography is a technique in which the endometrial cavity is distended with saline, allows evaluation of the single layer of the endometrial lining and enables the radiologist to reliably distinguish focal from diffuse endometrial pathologic conditions.(8) SIS was first described in 1981 by Nannini et al.(9)



a.



b

Figure 1. a. Visualization of endometrium as a trilaminar echo on TVS. b. Placement of electronic calipers for measurement of endometrial thickness on TVS

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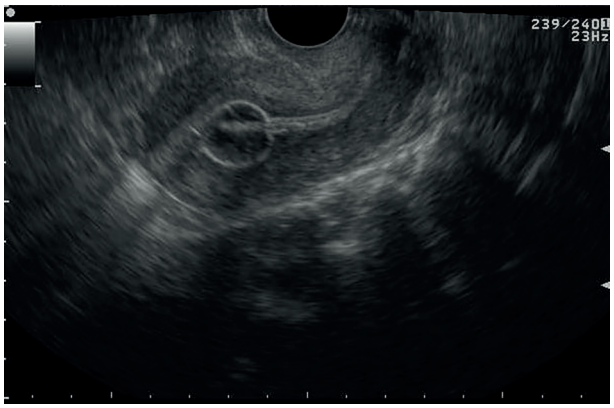
Technique of saline infusion sonohysterography

Performance of SIS after induced or natural menses minimizes confusion because at that time functions should be entirely shed, leaving only the basalis layer. (10) In premenopausal women, the best time to perform SIS is in the proliferative phase (first half) of the menstrual cycle. In postmenopausal women taking HRT, the SIS should be scheduled approximately six days after the last progestin pill when the endometrium is thinnest. (11) The baseline ultrasound study (transabdominal and transvaginal) is performed first.(10) A speculum is used to allow visualization of the cervix. After cleansing the external os, the cervical canal is catheterized using aseptic technique, and normal saline is instilled slowly using manual injection under real-time sonographic imaging into the endometrial cavity. (12) The saline distends the cavity, pushing the opposed walls of the endometrium apart. The anechoic fluid is then juxtaposed against the echogenic endometrium, giving exquisite detail of the uterine lining. (13)



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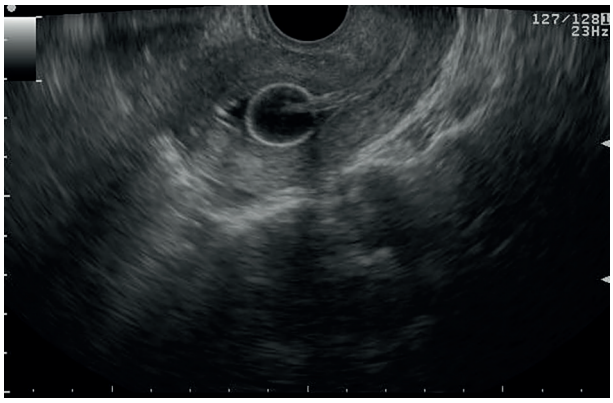

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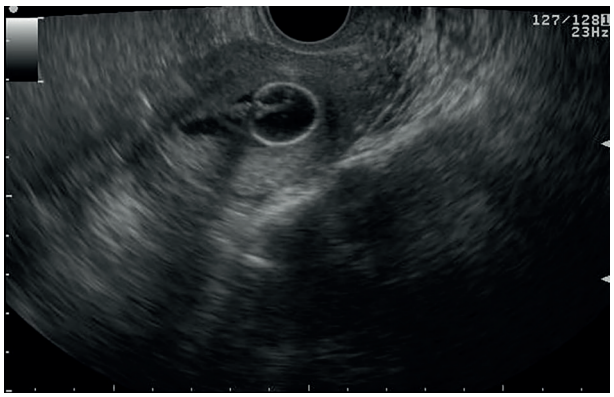

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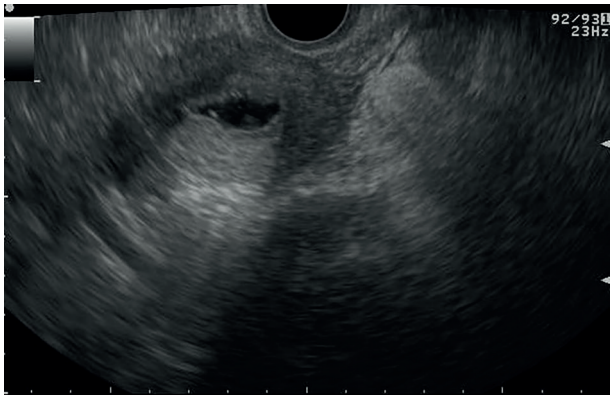

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Figure 2 Saline infusion sonohysterography. a. TVS image revealing catheter in situ with bulb inflated with saline. b. Saline infusion is started and begins filling the endometrial cavity. c. d. Endometrial layers separated by saline

Evaluation of endometrium

Endometrial thickness should be measured on a sagittal (long-axis) image of the uterus, and the measurement should be performed on the thickest portion of the endometrium, excluding the hypoechoic inner myometrium. It is a "double-thickness" measurement from basalis to basalis.(11) If the fluid is seen within the endometrial cavity, the individual wall thicknesses of the 2 sides of the endometrium are summed, excluding the intervening fluid.(13) This central quality assurance reading provides a consistent evaluation of endometrial thickness on US image.(14)

The normal endometrium

Normal endometrium should be uniform in thickness, homogeneous in echotexture, and not displaced by any submucosal, myometrial abnormality.(8) A thin endometrium of 5 mm or less had a high negative predictive value.(13) In premenopausal patients, a thickness greater than 8mm during proliferative phase or more excellent than 16mm during the secretory phase is considered abnormal. (15)

P - Endometrial Polyps

Endometrial polyps are a common gynecologic condition associated with symptoms of AUB. They account for 39% and 21% to 28% of pre- and postmenopausal women, respectively(1).

Endometrial polyps may be visualized at transvaginal ultrasound as nonspecific endometrial thickening(16). Preservation of the endometrial-myometrial interface and echogenic appearance have been described as features of a typical polyp. (8) Some of the atypical features of polyps include cystic components, multiplicity, a broad base, and hypoechogenicity or heterogeneity that may indicate hemorrhage, infarction, or inflammation within the polyp. A small percentage of endometrial polyps may contain malignant foci or foci of endometrial hyperplasia. (17)(17)

Addition of Color Doppler to sonohysterography may help distinguish an endometrial polyp, which usually has a single feeding vessel – the pedicle artery, from an intracavitary submucosal fibroid, which usually has several vessels arising from the inner myometrium. (18)

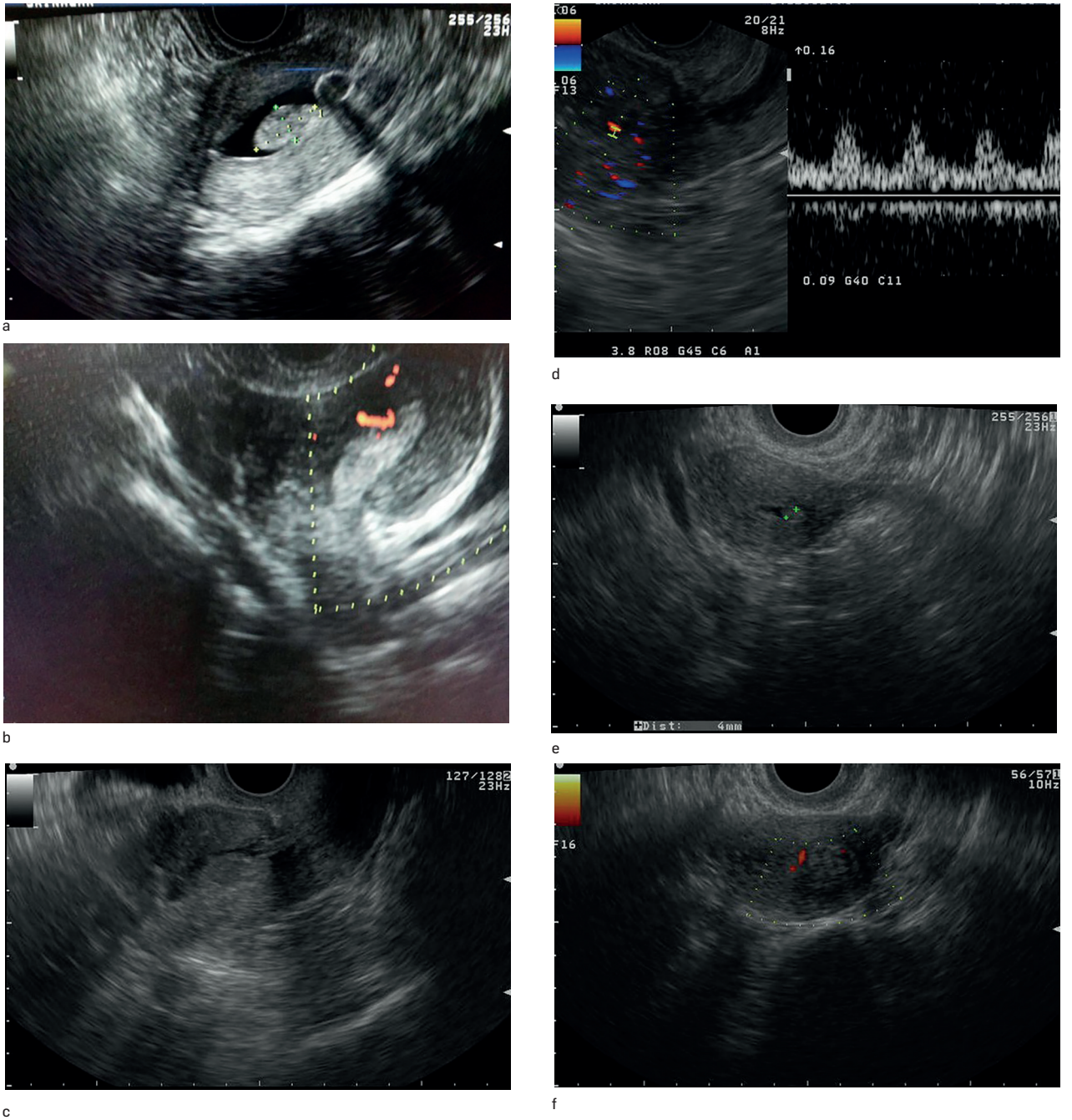


Figure 3. Endometrial Polyp. a. Saline infusion reveals broad based, echogenic lesion on posterior aspect of endometrial cavity – polyp. b. Demonstration of the pedicle artery by color doppler examination. c. Another patient with SIS revealing an echogenic polyp within the endometrial cavity. d. Arterial waveform obtained from pedicle artery of the above endometrial polyp. e. A very small endometrial polyp identified by SIS in a patient with metrorrhagia with otherwise normal imaging. f. Identification of pedicle artery by power doppler in the same patient

A - Adenomyosis

Adenomyosis is a common benign disease of the uterus characterized by ectopic endometrial glands and stroma within the myometrium associated with surrounding smooth-muscle hypertrophy(19). Approximately 70% of patients with adenomyosis have symptoms of AUB; 30% have symptoms of dysmenorrhea; and 19% present with both(1). Diffuse globular uterine enlargement is a common sonographic manifestation of adenomyosis(17).

The reported frequency of adenomyosis varies widely, ranging from 5 to 70% (20). The most common sonographic finding in adenomyosis on SIS is asymmetric myometrial thickening and heterogeneous echotexture. The second most common findings include myometrial cracks and myometrial cysts followed by an indistinct endo-myometrial junction(19).

L - Leiomyomas

Uterine fibroids are the most common benign tumor of the female genital tract(1). The PALM-COEIN system adds categorization of intramural and subserosal myomas as well as a category that includes lesions (''parasitic'') that appear to be detached from the uterus(4). Submucous myomas are most likely to cause menorrhagia(21).

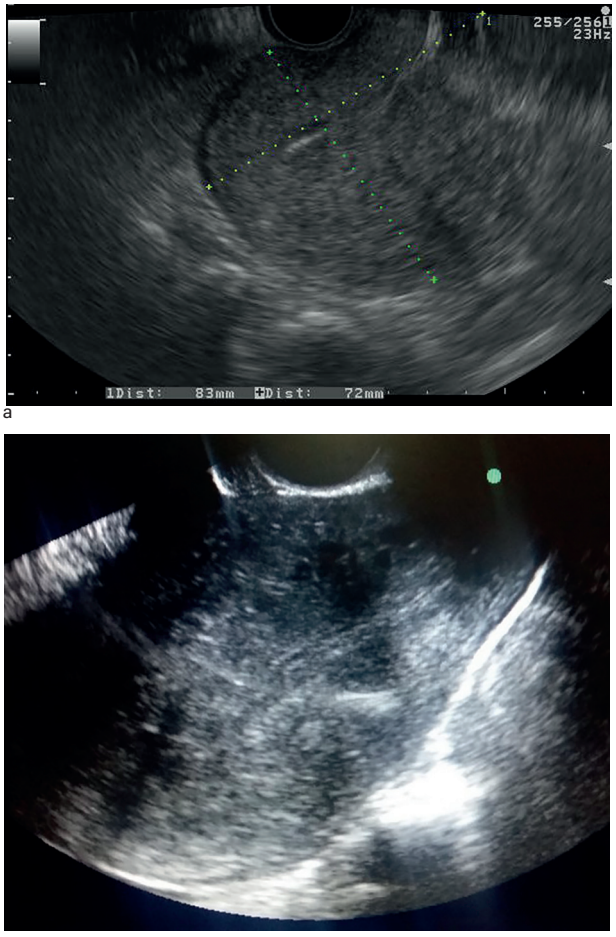


Figure 4. Adenomyosis. a. TVS images revealing enlarged, globular uterine morphology with heterogenous myometrium and thick irregular endomyometrial junction. SIS demonstrated normal endometrium will poor distensibility. b. Another patient with thick EMJ and multiple myometrial cysts with SIS showing normal endometrium with diagnosis of adenomyosis

Fibroids are commonly identified at US as hypoechoic solid masses, but they may be heterogeneous or hyperechoic, depending on the degree of degeneration and calcification. Fibroids tend not to interrupt the endometrium unless they are submucosal in location. Submucosal fibroids may distort the uterine cavity with varying degrees of intracavitary extension and are best visualized at sonohysterography(16). The addition of SIS not only shows hypoechoic, smooth rounded masses with an overlying echogenic endometrium and some degree of acoustic shadowing, but also clearly demonstrates the wall of origin, the exact localization (intramural or submucosal), and the extension into the uterine cavity (22). This feature is important because only those fibroids in which at least 50% of the mass projects into the endometrial cavity may be removed hysteroscopically.(23)

Differentiation of endometrial polyp from submucosal fibroid at sonohysterography is most effectively done by echotexture assessment and identification of an overlying echogenic endometrium. The classic submucosal fibroid is hypoechoic with shadowing and similar in texture to the myometrium with an overlying echogenic endometrium defining the subendometrial location. Because a submucosal fibroid may present almost entirely within the endometrial cavity as does a polyp, location is not a reliable distinguishing feature. (9)

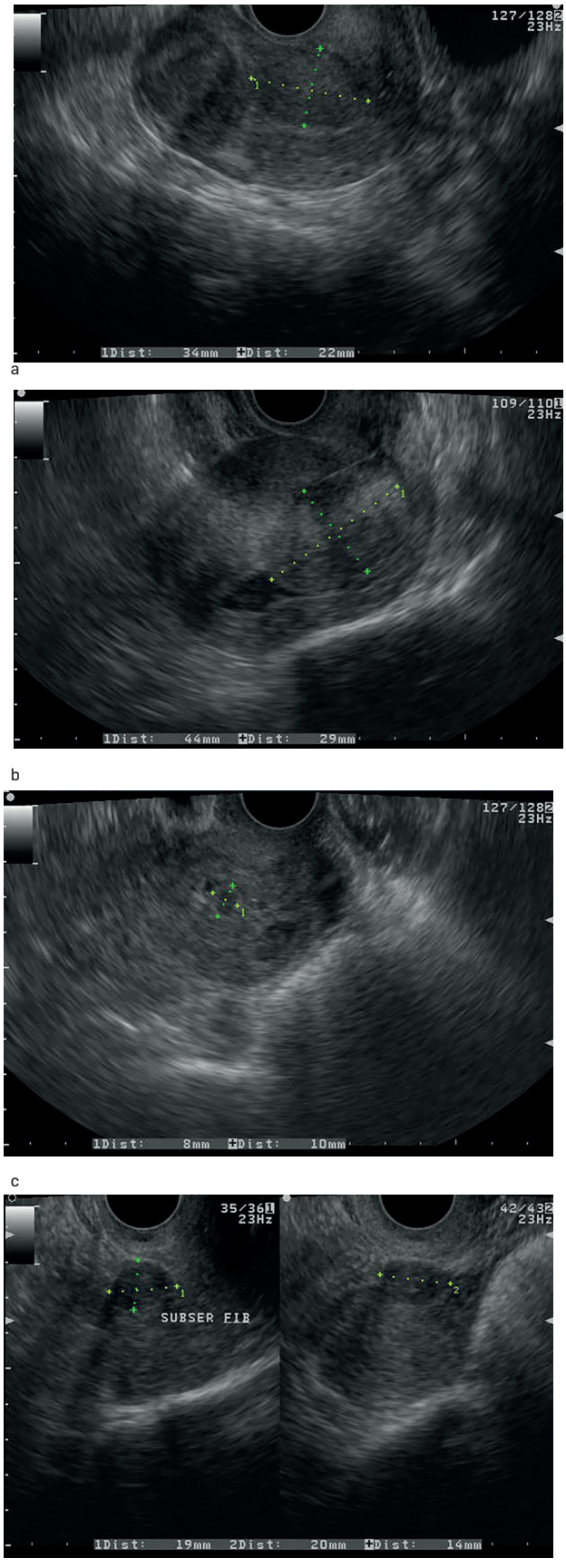


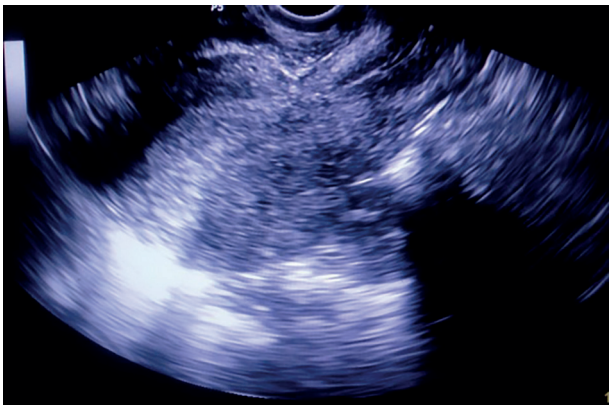
Figure 5. Leiomyomas. a. TVS images showing patient with intramural and submucous fibroids. b. A large submucous fibroid shown on SIS image with significant protrusion into the endometrial cavity. c. A small submucous fibroid identified in a patient with SIS. d. TVS images showing subserosal fibroids in a patient

E- Endometrial Hyperplasia

Endometrial hyperplasia is an abnormal proliferation of endometrial stroma and glands and represents a spectrum of endometrial changes ranging from glandular atypia to frank neoplasia. A definitive diagnosis can be made only with biopsy, and imaging cannot reliably allow differentiation between hyperplasia and carcinoma(16). It is the result of unopposed estrogens on the endometrium. It is most commonly encountered in perimenopausal women. (24)

On saline infusion sonohysterography, endometrial hyperplasia has been described as focal or diffuse thickening of the endometrium without a focal mass(25). A meta-analysis of 85 published studies that included 5892 women showed that an endometrial thickness of greater than 5 mm identified 96% of endometrial cancer. (26).Definitive criteria for distinguishing between endometrial hyperplasia and carcinoma have not been established, and both present with endometrial thickening.(25) The diagnosis of endometrial carcinoma should be suspected when the single layer of the endometrium is thicker than 8 mm, irregular, broad based, or poorly marginated or when the endometrial-myometrial interface is disrupted. However, endometrial thickness measurements often overlap in benign and malignant conditions(8).

Smaller endometrial thicknesses markedly reduce but do not exclude, the possibility of abnormalities such as polyps, hyperplasia, and cancer. (27)



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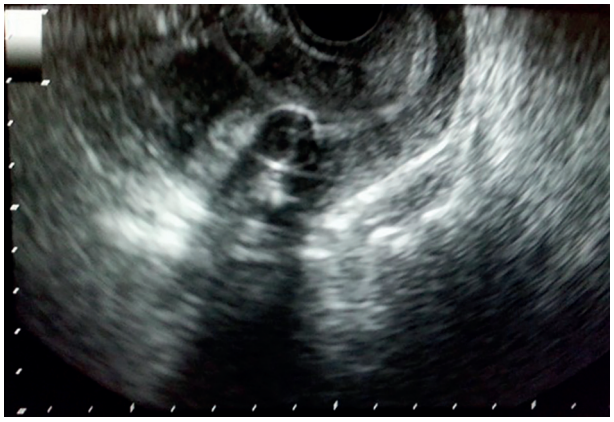

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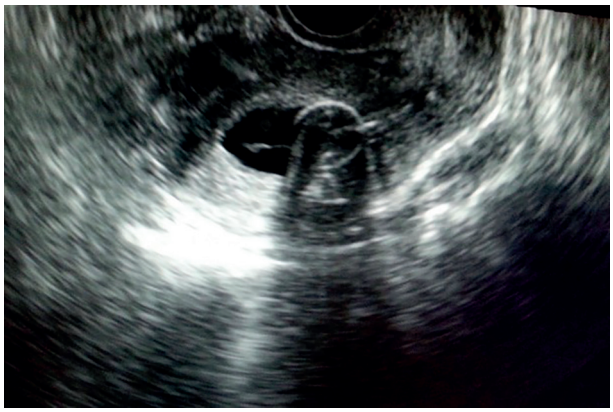

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Figure 6. Endometrial hyperplasia. a. Sagittal section TVS image of uterus demonstrating increased endometrial thickness. b. c. Endometrial cavity distended with saline revealing no intraendometrial lesion, and smooth and regular endometrial contour.

Significance

Initially, curettage was the ''gold standard'' for assessment of endometrium. First described in 1843, its performance in the hospital became the most common operation performed on women in the world. As early as the 1950s, a review of 6907 curettage procedures found the technique missed endometrial lesions in 10% of cases. Of these, 80% were polyps. (11)

Hysteroscopic evaluation for abnormal uterine bleeding is an option providing direct visualization of cavitary pathology and facilitating directed biopsy. (24) It is both sensitive and specific but is generally performed in an operating room under anesthesia, and requires specialized and highly skilled personnel, which increases the risks and costs. (28)

A saline hysterosonogram depicting focal abnormalities such as endometrial polyps or submucosal leiomyomas can guide the hysteroscopic resection of these masses. A saline hysterosonogram depicting myomas in a solely intramural location may be used to determine that an abdominal myomectomy is the optimal surgical approach for their resection. A saline hysterosonogram with specific negative findings can be extremely informative and obviate unnecessary procedures, and distinguish women who require medical therapy and investigation from those who require surgery. A normal endometrium on saline hystero sonography combined with a regular uterine appearance will direct the clinician to search for causes of vaginal bleeding other than anatomic causes, such as ovulatory versus anovulatory dysfunctional uterine bleeding or systemic disease. (25)

Safety and short-term effectiveness of hysteroscopy are now accepted for lesions limited to the uterine cavity. Submucous myomas up to 30 mm diameter and polyps can be resected endoscopically, allowing short hospital stay and quick recovery. (29) SIS on comparison outpatient diagnostic hysteroscopy is both less invasive to the patient and less expensive. (30) An approach using endometrial thickness measurement by TVS and reserving SIS for patients who have an endometrial thickness greater than 5 mm or an intracavitary abnormality visualized by TVS would be the effect to reduce the number of hysteroscopies.(31)

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REVIEW / ARTÍCULO DE REVISIÓN

FSH in bovine superovulation

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Abstract: Bovine follicle stimulating hormone (FSH) is the hormone mainly used for superovulation treatments. It is used so that several secondary follicles can reach a dominant state at the same time and thus, treated cows can release up to ten or more ovules in each zeal, decreasing the generational interval and increasing livestock production. The hormones available in the current market are obtained mostly from pituitary extracts of swine and sheep, and although they are widely used. Several negative aspects have been reported, implying high risks of contamination with pathogens, contamination with other hormones that interfere with assisted fertilization processes, significant variations between each production batch and the decreased half-life that exhibit FSH leading to excessive handling of donor cows. In this review, we detail some new approaches to overcome these problems, like slow-release FSH formulations that have been developed in order to increase the half-life of FSH and, finally the use of recombinant DNA technology to ensure a pure product.

Keywords: FSH, bovine, superovulation, recombinant.

Introduction

Follicle-stimulating hormone (FSH), produced by the anterior pituitary gland, is a glycoprotein which plays an essential role in reproductive processes1 in several vertebrates2. When the liberation from anterior pituitary occurs, FSH acts in the ovarian granulosa cells and the Sertoli cells in testis3, stimulating folliculogenesis and steroidogenesis in the ovary and spermatogenesis4. FSH, like other glycoprotein hormones, consists of two polypeptide chains (α and β subunit), non-covalently associated. The α subunit, encoded by a single gene, is identical in the amino acid sequence in all gonadotropins within a particular species5, whereas, the biological specificity of these hormones arises from the β subunit which, depending on the type of gonadotropin, is encoded by a different gene (FSHβ, LHβ or CGβ)[i]6. The α subunit of the bovine follicle-stimulating hormone has five intrachain disulfide bridges, while the β subunit, containing 12 cysteine residues7, has six disulfide bridges intrachain8.

Both subunits present post-translational modifications like glycosylation in which sugar moieties like mono- or oligosaccharides9 are transferred from donor molecules to nascent proteins by glycosyltransferases10. The glycans addition is essential for assembly, integrity, secretion and signal transduction in the α-subunit and assembly and secretion in the β-subunit. 1 Two types of glycosylations are present in FSH, N-glycosylations, and O-glycosylations. However, the predominant are of type N5, having two potential N-glycosylation sites in both alpha and beta subunits being of the Asn-Xaa-Thr type11. In bovine FSH, these N-linked oligosaccharides are located at positions N56 and N82 in the α-subunit and N7 and N24 in the β-subunit. Sialylated Asn-linked carbohydrates predominate in bovine FSH 7.

It has been elucidated that exposure of galactose residues, due to lack of glycosylation, on oligosaccharides increases the clearance from plasma 1 due to receptor-mediated endocytosis of asialoglycoproteins by hepatocytes 12. These number of exposed galactose residues is essential in elucidating the FSH half-life1. Some studies reported that FSH with fewer sialylations has a higher receptor binding activity and *in vitro* bioactivity than the sialylated FSH 13,14. However, the *in vivo* bioactivity of acidic FSH is 20-higher than the basic FSH15. Despite this, it has been shown that sialic acid plays an important role to prevent rapid clearance of FSH from circulation and it is not critical for receptor binding 7.

Oestrus cycle in cows

Different hormones regulate the estrous cycle: the gonadotropin-releasing hormone (GnRH), which is secreted by the hypothalamus, stimulates the secretion of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by Gonadotropic cells of the adenohypophysis16; in addition, by progesterone (P4), estradiol (E2) and inhibin's in the ovaries; and prostaglandin F2α (PGF) in the uterus 17. In cattle, the oestrus cycle lasts from 18 to 24 days. Two phases are present in the cycle: the luteal phase (14–18 days) and the follicular phase (4–6 days) 18,19. In the luteal phase, the corpus luteum (CL), formed from the collapsed ovulated follicle, is developed from the follicular wall and produces prevalently progesterone stimulated by LH pulses 19. During the follicular phase, final maturation and ovulation of the ovulatory follicle occur. Additionally, the progesterone levels decrease significantly due to the declining of corpus luteum function 18.

During the entire cycle, there are two or three waves of ovarian follicle growth **(**Fig. 1.), for dairy cows and beef cows, respectively, in which occur the emergence of a cohort of 5-20 follicles stimulated by the increase of FSH concentrations. Then, selection and dominance of one follicle occur due to the secretion of inhibins and estradiol (E2) by the growing cohort that decreases FSH concentrations20. This decrease in FSH leads to subordinate follicles regression 21.

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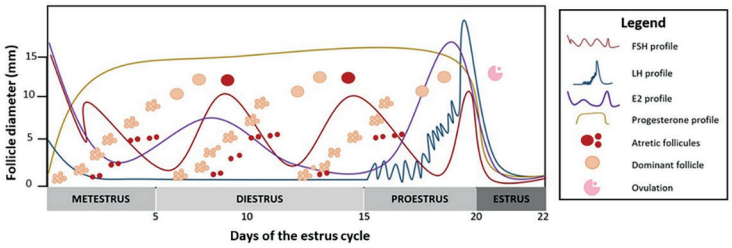

Figure 1. Schematic description of the secretion pattern of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and progesterone. Waves of ovarian follicles growth.

Knowledge of the estrous cycle is crucial when discovering new techniques to obtain an increase in livestock production.

Embryo transfer

Embryo transfer (ET) is a technique in which one or more embryos are removed from the reproductive tract of a donor female and then transferred to the lumen of the oviduct or uterus of one or more recipient females 22. It is applied to obtain a maximum number of embryos of a genetically superior animal in the shortest possible time and thus maximizes the genetic potential in a herd obtaining elite females or stallions 23. For ET, different steps must be carried out like: selection of a donor cow, superovulation, insemination, collection and evaluation of embryos, selection and preparation of recipient females, embryo transfer and finally, embryo transplant evaluation 24.

Superovulation is a hormonal treatment that induces follicular growth allowing a more significant number of oocytes to be recovered in the donor bovine female 25, being a pivotal procedure to maximize the number of viable embryos with high capacity to produce pregnancy 26. The basic principle of superovulation is to stimulate follicular development by hormonal treatment and induce ovulation of several follicles simultaneously 27. Also, by natural reproduction, a cow can breed a calf per year, reaching an average of eight to ten calves in its entire life. With an adequate hormonal regimen of superovulation, treated cows can release up to ten or more ovules in each zeal 25, decreasing the generational interval and increasing the genetic potential and production of livestock. 28

Superovulation can be achieved through the application of gonadotropin hormones29, allowing to emulate the natural reproductive processes in order to increase reproductive efficiency. The most commonly used gonadotropins in cattle reproduction include stimulating follicle hormone (FSH) and luteinizing hormone (LH), extracted from the pituitary gland of swine and sheep 29, in addition to equine chorionic gonadotropin (eCG), produced by the endometrial cups of pregnant mares 30.

Common superovulation treatment

In superovulation treatments, exogenous FSH, obtained from porcine and sheep pituitaries 31, is used to rescue secondary follicles from regression so that they could reach a dominant state too 32. The most common protocol for superovulation includes GnRH-induced ovulation of the persistent follicle and follicle wave emergence. Then, the super stimulation starts with the administration of exogenous FSH during four days with twice-daily decreasing doses 33.

As the FSH used in treatments for superovulation comes from pituitary of animals, this may be contaminated with traces of other hormones, leading to variations in oocyte quality and quantity. Several authors showed that FSH products with low LH levels improve the ovulation rate, fertilization rate and embryo quality 34,35. Kelly et al. (1997) 36 evaluated the follicular growth pattern and the performance of embryos in cattle after superovulation with two preparations of FSH differing in LH content (Serovet; FSH:LH ratio 1:1 y Vetrepharm; low LH). A higher number of ovulations was observed in cows treated with Serovet compared with Vetrepharm, this increase was mainly the result of a higher number of unfertilized ova and degenerated embryos unfit for transfer. Therefore, FSH with high purity or reduced LH content has become the method of choice for commercial use for the production of superovulatory cows.

On the other hand, it has been seen that the repeated use of exogenous hormones to induce superovulation in different species could induce a humoral immune response 37. eCG began to be used, as a superovulatory treatment in cows 31. Because of its long half-life being of 40 hours and its persistence in the circulation of up to 10 days, it is necessary a single dose for the superovulatory response 38. Its persistence in circulation is due to a large number of glycosylations sites in its structure, being the most glycosylated glycoprotein, covering 45% of its molecular weight 39. However, one study showed that treatment with eCG in bovines induced a humoral immune response at repeated doses 40. Therefore, the humoral response generated makes these treatments ineffective over time due to the production of neutralizing antibodies, thus reducing the super stimulating response.

There are several problems also associated with prolonged stimulation of the eCG including the continuous stimulation of the ovaries, follicles without ovulation, abnormal endocrine profiles and reduction of embryo quality 41. Besides, a better superovulatory response has been established after treatment with FSH compared with eCG 42. Additionally, due to the high molecular weight of eCG, a more significant percentage of ovarian cysts (40%) appeared about the treatment of FSH (8%) 43. The leading cause of economic losses and reproductive problems is attributed to the presence of these cysts, it also causes abnormal hormonal profiles and a low embryo quality 44.

The twice-daily administration of FSH, because of its short half-life being five days only 45, leads to several problems in the field including excessive handling of donor cows causing stress and decreased superovulatory response 46. Recently, there have been many alternative treatments to try to avoid these problems, for example, the application of slow-release FSH formulations 45. This could be achieved by mixing FSH with polyvinylpyrrolidone (PVP) which maintains a high concentration of FSH in blood enough to stimulate the development of multiple follicles47. However, this treatment has had variable results being unable to induce superovulation in some cases 48. Another alternative is the use of hyaluronan, a glycosaminoglycan, used in a 2% solution to dilute FSH, having the same results with the traditional two-day dose of FSH. However, it is difficult to mix FSH with hyaluronan because of its viscous state leading to problems in the field, and a more diluted solution decreases its effectiveness 49. Another alternative is

the use of aluminum hydroxide gel, a vaccine adjuvant, to induce superovulation in cattle 50. However, the use of adjuvants could cause the development of antibodies against FSH 49.

Due to these problems such as variability, immunogenic effects, and inconvenient formulations, other alternatives should be sought to produce superovulation hormones on a larger scale, which are safe for livestock and at a lower cost.

Recombinant DNA technology

The technology of recombinant DNA has allowed building a variety of hormonal analogs with different biological characteristics 51. It has been verified that the construction of recombinant FSH reduces the variation observed in the different types of FSH derived from pituitary gland 31,52. Also, with the recombinant bovine follicle-stimulating hormone, possible risks of immunogenicity are avoided, also resulting in higher purity and less variability product concerning animal extracted hormones.

Today, there are several expression systems for the production of large-scale recombinant proteins, which include expression in *E. coli* bacteria, baculovirus-mediated insect cells, yeast, and several systems in mammals 53. Expression systems using mammalian cells have a superior capacity to produce biologically active and lower cost proteins 54. In addition, these expression systems are used for the production of recombinant proteins when complex post-translational modifications are necessary for their bioactivity 55, as is the case of FSH, with several N-glycosylation sites, which increase the solubility and stability of the proteins, facilitate its adequate, its appropriate charge and the formation of disulfide bridges 31.

Several researchers have developed the recombinant bovine follicle stimulating a hormone in different expression systems such as yeast 8,56, mammalian cell lines 57,58, insect cells 59, plants 60, mammary gland of mice 61 and rabbit mammary gland 62 (Table 1). Chinese hamster ovary (CHO) cells have been chosen preferably to produce recombinant FSH at a commercial level, especially for those that require posttranslational modifications. However, the culture of mammalian cells is expensive due to the use of rich growing media and supplements, besides its slow-growing 63.

Species	Gestation (months)	Milk yield per lactation (L)	Recombinant protein per year (kg)
Rabbit	1	1–1.5	0,002
Pig	4	200–400	1,5
Sheep	5	200–400	2,5
Goat	5	600–800	4

Table 1. Expression systems of recombinant bovine FSH

Many laboratories and pharmaceutical companies have been able to produce a variety of therapeutic proteins in mammals as expression systems, including cows, pigs, sheep, goats and rabbits 64,65. These recombinant proteins are produced from body fluids of animals like milk, egg white, blood, urine and seminal plasma 66.

In recent years, researchers have focused on the mammary gland as an expression system of recombinant proteins, has several advantages. With the direct *in vivo* transduction of mammary gland, the desired protein is secreted to the milk, having relatively easy purification steps 67. Large volumes of milk could be easily collected 66, depending on the species (Table 2), so large amounts of protein could be obtained 68. The proteins obtained in this expression system have appropriate post-translational processing resulting in proper biological activity 69. Also, these proteins can be used for a long period given its low immunogenicity.70

Conclusion

The bovine FSH is of great importance in the production of cattle having a high economic implication. The aim of the application of pure FSH in superovulation protocols is to prevent the variations in oocyte quality and quantity.

Several investigators have used different expression systems to produce pure recombinant hormones, including plants, insect cells, yeasts, bacteria, and mammary gland. The development of proteins in the milk of animals could generate high quality and cost-effective products, preserving the animal wellbeing. The production of recombinant proteins has offered a safe, efficient and economical way to overcome the need for biopharmaceutical products.

Name	Cell factory	Company
Follistim®AQ	CHO cells	Organon USA. Inc
Puregon®	CHO cells	*Laboratories* NV Organon
Gonal-F®	CHO cells	Serono Laboratories
BoviPureFSH™	CHO cells	AspenBio Pharma, Inc.
EquiPureFSH™	CHO cells	AspenBio Pharma, Inc.
FSH	HEK cells	Nanocore Biotecnologia SA
bFSH	Yeast (*Hansenula polymorpha*)	8
pFSHb	Yeast (*Pichia pastoris*)	56
rbFSH	Sf21 insect cells	59
sc-bFSH	Plants (Nicotiana benthamiana)	60
rbFSH	Milk of transgenic mice	61
boFSH	Milk of transgenic rabbits	62

Table 2. Milk expression systems in different species

The developing of FSH is still a challenge in the scientific world; many expression systems could be tested for better yields. FSH variants of high purity, efficiency and stability can be used in cattle to obtain a higher number of ovules and the subsequent production of embryos of a selected cow and thus reduce the generational time and increase the genetic potential of their offspring.

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NEWS AND VIEWS / NOTICIAS Y OPINIONES

The obscure impact of Nipah virus

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Abstract: The motivation behind this introduction is to feature the known and deep impact subjected to the Nipah Virus (NiV). Principally concentrating on preventive measures and the treatment of the Nipah Virus. NiV is an individual from the family *Paramyxoviridae*, class *Henipavirus*. NiV was at first recognized in 1999, amid a flare-up of *Encephalitis* and respiratory sickness among pig ranchers and individuals with close contact with pigs in Malaysia and Singapore. Nipah infection caused a generally gentle ailment in pigs, yet almost 300 human cases with more than 100 passings were accounted for in 1999. Not just in Malaysia its spread around the world. A case-controlled survey ponder is directed to decide word related hazard factors for disease. Contact with live pigs, earmarks of being the essential hazard factor for human Nipah infection disease. Coordinate contact with life, possibly contaminated pigs ought to be limited to forestall transmission of this conceivably lethal ZOONOSIS to people. Understanding the significance and in light of specific records, it is an endeavor to assemble it to diagram the qualities and to be worried about this issue. This essential survey is gone for giving a knowledge into this fatal flare-up and to bring it into concern.

Keywords: Zoonosis, Transmission, Nipah Infection, Preventive Measures, Obscure Impact, Treatment and Review.

Introduction

Nipah infection is another paramyxovirus known to contaminate pigs, canines, people, and, perhaps, bats. By 27 April 1999, the Malaysian Ministry of Health had reports of 257 febrile encephalitis cases, including 100 passing's. The lion's share of cases had lab proof of Nipah infection contamination and most happened in the territory of Negeri Sembilan. Pigs were involved as the essential wellspring of human contamination. Most cases had guide presentation to pigs, and viral separates got from pigs and people had the same nucleotide sequences. [1]It is an outstanding issue worldwide and has influenced India too. This case-controlled diagram examines composed to pick word related hazard factors for infirmity which is caused by the Nipah Virus. Contact with live pigs are stores of being the most significant risk factor for human Nipah pollution ailment. Encourage contact with life, conceivably sullied pigs ought to be compelled to block transmission of this perhaps savage ZOONOSIS to people. Understanding the vitality and in light of specific records, it is an endeavor to gather it to outline the qualities and to be worried over this issue. Malady with Nipah contamination is connected with encephalitis (irritation of the cerebrum). After the introduction and an incubating time of 5 to 14 days, illness presents with 3-14 long stretches of fever and cerebral agony, trailed by sluggishness, bewilderment and mental confusion. These signs and signs can progress to daze like state inside 24-48 hours. A couple of patients have a respiratory infection amid the early bit of their defilements, and half of the patients giving extraordinary neurological proposals gave pneumonic insights furthermore. During the Nipah disease affliction scene in 1998-99, 265 patients were sullied with the contamination. Around 40% of those patients who entered recuperating focuses on certifiable uneasy illness passed on from the ailment. Whole deal sequelae following Nipah disease defilement have been noted, including tireless fits and personality changes. Inactive maladies with coming about reactivation of Nipah disease and death have similarly been represented months and even quite a while after introduction. The ascent of NiV into the pig people and in this way into the human masses is acknowledged to be a result of changes in normal conditions. Urbanization, deforestation, and drought realizing an insufficiency of advantages for bat masses could have compelled bats to move from their essential living spaces to rural districts. Among the components that add to the ailment ascend in Malaysia is the establishment of pig develops inside the extent of the standard host that provoked the basic introduction into the pig people. The upkeep of high densities of pigs incited the brisk dispersal of the sullying inside close-by pig masses, and the vehicle of pigs to other geographic regions for business provoked the quick spread of infirmity in pigs in southern Malaysia and Singapore. This critical examination is a way to give data into this dangerous emit and to bring it into concern. The proximity of high thickness, opening up have people empowered transmission of the disease to human.

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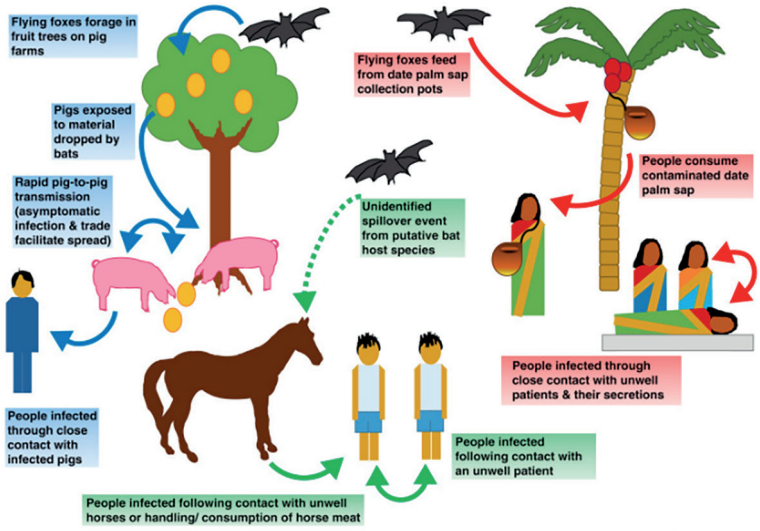

Figure 1. Nipah virus disease is an emerging infectious disease spread by secretions of infected bats. It can spread to humans through contaminated fruit, infected animals, or through close contact with infected humans. Nipah virus infection, newly emerging zoonotic infection with acute respiratory syndrome and severe encephalitis. (

Impact on India

The contamination and pollution, a creating peril, has killed in every way that matters the lion's share of its abused individuals so far in India. An unprecedented, personality was hurting disease that experts consider a possible torment threat has broken out in the region of Kerala, India, unexpectedly, defiling something like 18 people and killing 17 of them, as shown by the World Health Organization.[2] There is no sensible preventive or treatment for Nipah, an as of late rising affliction spread by bats, pigs and people who have ended up being corrupted, according to the Centers for Disease Control and Prevention and the World Health Organization. WHO has been instructed about Nipah contamination cases being represented in a family from a town in Kozhikode territory of Kerala,» he said. «Both the central and the state prosperity masters have been smart in responding to the condition and have immediately passed on gatherings and authorities to the town also to overview the situation. WHO is in close contact with the gatherings of pros passed on to the affected zones. We foresee the evaluation reports of the gatherings to explain the condition and guide advance action". [3] The present scene is suspected of having been spread by polluted natural organic product bats. [4]

Amid January and February of 2001, an episode of febrile ailment with adjusted sensorium was seen in Siliguri, West Bengal, India. Siliguri is a critical business focus with a populace of ≈500,000. It is close outskirts with China, Bangladesh, Nepal, and Sikkim. The flare-up happened among hospitalized patients, family contacts of the patients, and restorative staff of 4 doctor's facilities. Japanese encephalitis, which is endemic around there, was at first suspected. However, the age assembles influenced, and the epidemiologic highlights recommended another infection. Research facility examinations directed at the season of the flare-up neglected to distinguish an irresistible operator. Nipah infection (NiV), an as of late emanant, zoonotic paramyxovirus, was ensnared as the reason for an exceedingly deadly (case-casualty proportion 38%– 75%), febrile human encephalitis in Malaysia and Singapore in 1999 and Bangladesh amid the winters of 2001, 2003, and 2004. The regular repository of NiV is attempted to be fruit bats of the sort Pteropus. Proof of NiV disease was identified in these bats in Malaysia, Bangladesh, and Cambodia. In the Malaysian flare-up, NiV was brought into the pig populace, and the vast majority of the human cases came about because of introduction to sick pigs. In any case, a middle of the road creature has been not distinguished amid the Bangladesh episodes, which recommends that the infection was transmitted either individually or in a roundabout way from contaminated bats to people. Human-to-human transmission of NiV was additionally archived amid the episode in Faridpur, Bangladesh. Since the clinical indications of the cases in Siliguri were like those of NiV cases in Bangladesh, and because Siliguri is close influenced regions in Bangladesh, a review investigation of clinical examples was embraced to decide whether NiV was related with the Siliguri outbreak. [5] Not just in India yet additionally influenced in the neighboring nation too is influenced.

NiV has been set up as the reason for deadly, febrile encephalitis in human patients in Bangladesh amid the winters of 2001, 2003, and 2004. A NiV like the infection was recognized as the reason for the episodes in 2001 and 2003 based on serologic testing. Two flare-ups comprising of 48 instances of NiV were recognized in 2004 of every 2 adjacent areas (30 km separated) of focal Bangladesh (Rajbari and Faridpur) with a case-casualty rate of almost 75%. Due to high observation, other little groups and confined cases (n = 19) were recognized amid a similar period in seven different locales in focal and northwest Bangladesh. Even though antibodies to NV were recognized in organic product bats from the influenced regions in 2004, a halfway creature have been not distinguished, which recommends that the infection was transmitted from bats to people. Human-to-human transmission of NiV was additionally recorded amid the Faridpur episode. Which depicts the hereditary attributes of 4 NiV secludes from the episode in Bangladesh in 2004. [6] (table 1)

Sr. No.	Date	Location	Case
1.	2001 January 31–23 February.	Siliguri, India.	66 cases with a 74% mortality rate. 75% of patients were either hospital staff or had visited one of the other patients in hospital, indicating person-to-person transmission.
2.	2001 April – May.	Meherpur District, Bangladesh.	13 cases with nine fatalities (69% mortality).
3.	2003 January.	Naogaon District, Bangladesh.	12 cases with eight fatalities (67% mortality).
4.	2004 January – February.	Manikganj and Rajbari districts, Bangladesh.	42 cases with 14 fatalities (33% mortality).
5.	2004 19 February – 16 April.	Faridpur District, Bangladesh.	36 cases with 27 fatalities (75% mortality). 92% of cases involved close contact with at least one other person infected with Nipah virus. Two cases involved a single short exposure to an ill patient, including a rickshaw driver who transported a patient to hospital. In addition, at least six cases involved acute respiratory distress syndrome, which has not been reported previously for Nipah virus illness in humans. This symptom is likely to have assisted human-to-human transmission through large droplet dispersal.
6.	2005 January.	Tangail District, Bangladesh.	12 cases with 11 fatalities (92% mortality). The virus was probably contracted from drinking date palm juice contaminated by fruit bat droppings or saliva.
7.	2007 February – May.	Nadia District, India.	Up to 50 suspected cases with 3–5 fatalities. The outbreak site borders the Bangladesh district of Kushtia where eight cases of Nipah virus encephalitis with five fatalities occurred during March and April 2007. An outbreak preceded this in Thakurgaon during January and February affecting seven people with three deaths. All three outbreaks showed evidence of person-to-person transmission.
8.	2008 February – March.	Manikganj and Rajbari districts, Bangladesh.	Nine cases with eight fatalities.
9.	2010 January.	Bhangra subdistrict, Faridpur, Bangladesh.	Eight cases with seven fatalities. During March, one physician of Faridpur Medical College Hospital caring for confirmed Nipah cases died.
10.	2011 4th February.	Hatibandha, Lalmonirhat, Bangladesh.	An outbreak of Nipah Virus occurred at Hatibandha, Lalmonirhat, Bangladesh. The deaths of 21 schoolchildren due to Nipah virus infection were recorded on 4 February 2011. IEDCR confirmed the infection was due to this virus. Local schools were closed for one week to prevent the spread of the virus. People were also requested to avoid consumption of uncooked fruits and fruit products. Such foods, contaminated with urine or saliva from infected fruit bats, were the most likely source of this outbreak.
11.	2018 May.	Calicut, Kerala, India.	Deaths of seventeen people in Perambra near Calicut, Kerala, India were confirmed to be due to the virus. Treatment using antivirals such as Ribavirin was initiated.

Table 1: Date, location and number of cases Niv virus.

Disease in Humans

NiV caused genuine, rapidly powerful encephalitis that passed on a high demise rate. In light of the time break between the last introduction to pigs and subsequent start of the malady, the agonizing period stretched out from 4 days to 2 months with more than 90 % of patients giving a past loaded up with around fourteen days or less. In NiV, the rate of subclinical sickness kept running from 8 to 15 %. The bigger piece of patients showed symptoms related to the central tactile framework, yet respiratory system commitment was in the like manner seen in various patients in Singapore. Around 40 % of patients gave were ran with respiratory sickness. Over the prior decade, the beforehand dim paramyxoviruses Hendra sullying (HeV) and Nipah infection (NiV) have made in people and subdued animals in Australia and Southeast Asia. The two illnesses are overpowering, exceedingly harming, and arranged for dirtying diverse mammalian species and causing possibly destructive infirmity.[7] Because of the nonattendance of an endorsed checking operator or antiviral drugs, HeV and NiV are doled out as biosafety level (BSL) four experts and are potential bioterrorist overseers. The genomic structure of the two defilements is that of a run of the mill paramyxovirus. In any case, by constrained movement homology and immaterial immunological cross-reactivity with different paramyxoviruses, HeV and NiV have been depicted into another variety inside the family Paramyxoviridae named Henipavirus. The clinical signs were fever, cerebral torment, dazedness, and hurling. More than 50 % of the patients had a diminished dimension of mindfulness and recognizable cerebrum stem brokenness. Progressively settled patients, especially those having diabetes mellitus and those with genuine cerebrum stem commitment passed on a poorer expectation. The signs found in patients amid Siliguri erupt fever, headache, and myalgia, spewing, balanced sensorium, respiratory signs (tachypnea to extraordinary respiratory inconvenience) and programmed advancements or fits. Patients were normotensive at affirmation yet wound up hypertensive

before death. The case loss in clinical cases was around 40 % in the Malaysian scenes in Bangladesh and India, and it was on a typical 75 %.[8] The partition of another paramyxovirus, as such named Nipah disease, from cerebrospinal fluid precedents from a couple of patients demonstrated this was the etiologic authority. Starter examinations of nucleotide sequencing revealed that this contamination is solidly related to, not unclear to, Hendra disease, which caused ailment among steeds and affected three patients in Australia.[9] Nipah disease is most immovably related to Hendra contamination, which was first seen in Australia in 1994 amid a respiratory sickness scene among horses and individuals. Normal examinations of next Australian untamed life entangled natural organic product bats of the family Pteropus as the first animal supply for Hendra disease. Despite two occurrences of an extraordinary respiratory ailment, Hendra disease caused a case of deadly encephalitis. Hendra contamination transmission in all likelihood results from contact with body fluids, including pee, from corrupted animals. However, most paramyxoviruses are species specific, Hendra and Nipah diseases are fit for sullying an arrangement of vertebrates. In 10 March through 19 March 1999, 11 occurrences of febrile encephalitis or pneumonia that realized one passing occurred among pros at 1 of 2 abattoirs in Singapore. Simply outside made pigs were set up in the abattoirs; 82% started from Malaysia. Serologic or viral imprisonment inspects included Nipah disease tainting in every one of the 11 patients. [10]

Respiratory Infection

The respiratory epithelium is a basic first line of boundary and viably connected with exacerbation and host opposition against powerful diseases. In human cases of NiV ailment, NiV can be perceived in bronchiolar epithelial cells and is shed generally by nasopharyngeal and tracheal releases in the early time of the infection. Patients with symptomatic respiratory tract defilements were out and out increasingly slanted to transmit NiV. Histological changes in the lungs of NiV cases fuse necrotizing alveolitis with depleting, aspiratory edema, and want pneumonia. Multinucleated mammoth cells are now and again noted in the alveolar septum and alveolar spaces close-by necrotic zones. Intra-alveolar red hot cells are ordinary. The important deadly human occasion of HeV tainting realized outrageous respiratory disease in which the lungs had net wounds of stop up deplete and edema related with steady histological alveolitis. For the most part, histopathological changes in tracheal/bronchial epithelium were remarkable. In exploratory animal models, viral antigen is at first discernible in the bronchi and alveoli, generally concentrating on the bronchial epithelium and type II pneumocytes. We are starting late exhibited that HNV can capably corrupt epithelial cells from the lower human respiratory tract and rehash to high titers. While human-to-human transmission has been observed just in flare-ups with NiV-B, these data prescribe that both NiV and HeV have the potential for human-to-human transmission through pressurized canned items. HNV sullying of the respiratory epithelium results in the acknowledgment of red hot cytokines which result in the selection of immune cells and can progress to an Acute Respiratory Distress Syndrome (ARDS) - like affliction. Infection of the lower respiratory tract epithelium results in a combustible differential response depending upon the goals of defilement. HNV tainting of the little avionics course epithelium realized enrollment of key provocative go-betweens, for instance, IL-6, 8, IL-1α, MCP-1, G-CSF, GM-CSF, and CXCL10. Strikingly, red hot cytokine enunciation was out and out lower in trachea/bronchial epithelium. This recognition is in simultaneousness with past reports that no exacerbation is found in the bronchial epithelium of NiV cases. An impressive part of these key cytokines in HNV defilement accept a vocation in ARDS and are in like manner exceedingly imparted during illness with other unsafe respiratory contaminations, for instance, H5N1 and SARS-CoV. During the late periods of affliction, contamination replication spreads from the respiratory epithelium to the endothelium in the lungs. The malady can occur on occasion trigger a prominent vasculitis in little vessels and vessels as depicted by endothelial syncytium and divider painting rot. Broad vessels are typically not impacted. HNV would then have the capacity to enter the circulatory framework and dissipate all through the host fit as a fiddle or by limiting host leukocytes. Despite the lungs, vital target organs are the brain, spleen, and kidneys, and viremia following respiratory illness can incite multi-organ disappointment. [11]

Entry in The CNS

HNV disease of the CNS and the advancement of neurological signs are related with the disturbance of the blood-mind boundary (BBB) and articulation of TNFα and IL-1β. These expert fiery cytokines have been appeared to assume a job in expanding the porousness of the blood-cerebrum hindrance just as the enlistment of neuronal damage and demise. While the wellspring of TNF-α and IL-1β articulation in the cerebrum is as of now obscure, they can be discharged by microglia, which are additionally tainted by HNV. In any case, regardless of whether disturbance of the BBB is a direct cytopathic impact of infection replication in the microvasculature or an aberrant impact through the articulation of TNF-α and IL-1β by onlooker cells, for example, neurons and microglia stay misty. Exploratory examinations in different creature models have demonstrated right section of the CNS by HNV, through the olfactory nerve. In these models, NiV contaminates the olfactory epithelium in the nasal turbinates. NiV along these lines taints neurons stretching out through the cribriform plate into the olfactory knob, giving a next course section into the CNS. NiV then scatters to the olfactory tubercle and all through the ventral cortex. It is at present obscure whether this course is likewise naturally applicable in human diseases since the olfactory epithelial surface is generally substantial in these species contrasted with the man. [11]

Disinfection

Like distinctive paramyxoviruses, Nipah contamination is speedily inactivated by chemicals, chemicals, and various disinfectants. Routine cleaning and filtration with sodium hypochlorite or financially open disinfectants are required to propel. Sodium hypochlorite was endorsed for the filtration of pig develops in Malaysia. The effect of warmth may depend upon the substrate. Nipah contamination centers decreased yet the disease was not completely discarded in phony palm sap held at 70°C for an hour. In any case, it was completely inactivated by warming at 100°C for over 15 minutes. [12]

Current treatment procedures

Treatment is restricted to strong consideration. The medication ribavirin has been appeared to be viable against the infections in vitro, yet human examinations to date have been uncertain, and the clinical convenience of ribavirin stays unverifiable. Nipah infection contamination can be anticipated by keeping away from an introduction to wiped out pigs and bats in endemic territories and not drinking raw date palm sap.[13] However, for good countermeasures and readiness, a more extensive and increasingly far-reaching approach and venture are

earnestly required. Notwithstanding diagnostics, therapeutics, and antibodies, observation foundation must be enhanced to distinguish and check cases quickly, lead point by point contact following, research overflows, and better comprehend the environment of bats and Nipah infection disease, particularly outside of flare-up situations. As essential is the requirement for social change: nearby networks must be better upheld to guarantee disease avoidance and control measures in wellbeing offices to decrease transmission and to direct the network commitment and training required to alter conduct and lessen hazard. We should notice that Nipah infection requests an expansive, long haul procedure and pandemic arrangement. [14]

Oral administration of favipiravir completely shields from deadly Nipah infection disease in the hamster demonstrate. Favipiravir has been exhibited to diminish mortality in different exploratory models of viral hemorrhagic fever, encephalitis, or respiratory malady. To assess the in vivo viability of favipiravir against NiV-M, we used the Syrian hamster display which intently reflects most parts of human ailment, for example, far-reaching vasculitis, pneumonia, and encephalitis and has been generally acknowledged for the assessment of antiviral therapeutics and antibody hopefuls. Hamsters were tainted with a terminal portion of 104 PFU NiV-M using the intraperitoneal (i.p.) course like past examinations and treatment was started following contamination. Favipiravir was controlled twice every day utilizing the perioral (p.o.) course for 14 days, again like past investigations assessing the antiviral action of favipiravir. On test day, a stacking portion of 600 mg/kg/d was directed following disease, trailed by 300 mg/kg/d on days 1– 13.

A similar timetable with vehicle dosed control creatures as it were. All vehicle-treated NiV-tainted creatures consistently created clinical indications of sickness including hyperreflexia, ataxia, irregular breathing, and dormancy and surrendered to malady or were others consciously euthanized on days 5 or 6 PI. Creatures treated with favipiravir did not create clinical indications of sickness throughout the investigation through 42 days of post disease. Besides, weight information uncovered soak weight reduction before death or killing in vehicle-treated creatures, while favipiravir-treated creatures consistently put on load all through the length of the examination. Infection titrations from tissues were uncertain as we were just ready to recoup possible infection in one of the four vehicle-treated creatures, yet no infection was identified in the favipiravir-treated gathering (information not appeared). Constant RT-PCR for the viral P quality was directed on minds, spleens, and lungs to think about viral load between deadly creatures that were euthanized and survivors. Not surprisingly, large amounts of viral P quality articulation were recognized in each of the three tissues in the vehicle-just controls contrasted with favipiravir-treated creatures, where no popular RNA was distinguished. Two of five survivors created killing neutralizer titers (PRNT50s) of >80 and >1280, separately, while the staying three survivors had titers of <20. These outcomes show that favipiravir regulated twice day by day p.o. starting following disease is very strong in forestalling NiV-instigated grimness and mortality in the hamster demonstrate.

Association of favipiravir subcutaneously shields hamsters from destructive Nipah disease defilement. To choose the sufficiency of once to step by step subcutaneous (s.c.) association of favipiravir, for instance, starting late used in a Lassa disease (LASV) guinea pig model33, hamsters were polluted with a fatal segment of 104 PFU NiV-M through the i.p. course and treatment were begun rapidly after infection. Like the oral association think about portrayed more than, a stacking bit of 600 mg/kg/d was controlled rapidly after pollution, trailed by 300 mg/kg/d on days 1– 13. All vehicle-treated animals ended up being wiped out inside 7 DPI and demonstrated signs of loss of movement, ataxia, and sporadic unwinding. Favipiravir-treated animals made due until the completion of the examination (42 DPI) with no enhancement of clinical signs of ailment and reliably put on load over the range of the examination. Correspondingly similarly as with the past examination, attempts at disease titration from tissue were dubious, and RT-PCR recognized high stores of the viral P quality in every one of the three tissues assessed in non-treated animals, while the viral load in all survivors was not noticeable. Of the five enduring animals, three made slaughtering insusceptible reaction titers (two > 80 and one > 20), while the remaining two survivors had titers of <20. These results demonstrate that association of favipiravir s.c. At the point when step by step beginning right away after the illness is furthermore significantly satisfactory in maintaining a strategic distance from NiV-actuated frightfulness and mortality in the hamster model.[15] Virus partition is required for finish assurance; managing NiV requires a BSL4 investigate office. Led the Swine Health Information Center by the Center for Food Safety and Public Health, School of Veterinary Medicine, Iowa State University in September 2015. Quantitative consistent polymerase chain reaction (qRT-PCR) is open, as is immunohistochemistry. Immunofluorescence may be confounded by cross-reactivity with Hendra contamination, though a couple of monoclonal antibodies are as of now available for henipavirus detachment. An underhanded protein associated immunosorbent look at (ELISA), contamination balance tests using pseudotype particles, and multiplexed microsphere measures have all been delivered to perceive antibodies to NiV at the BSL2 level.[16] Another methodology which is encountering is upon Fusion Glycoprotein system.

The paramyxoviruses include a social event of major human pathogens, for instance, measles, mumps, human parainfluenza diseases, and the significantly pathogenic Nipah (NiV) and Hendra (HeV) contaminations. NiV defilements have a demise rate in individuals of up to 75%, and NiV is named a BSL4 pathogen because of its profile oragro-mental fighting potential. The suitability of segment inhibitors centered against HIV suggests that a prevalent cognizance of Paramyxovirus entry and blend will energize similarly strong antiviral therapeutics.[17]

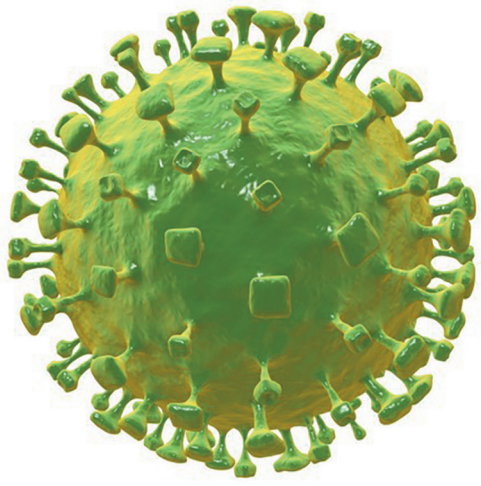

Figure 2. Nipah virus (NiV) is a member of the family Paramyxoviridae, genus Henipavirus. Henipaviruses are pleiomorphic with spherical or filamentous structures ranging from 40 to 2000 nm in size. Nucleocapsids are visible in electron microscopy with a diameter of about 18 nm. 19 (ID 117970591 © Katerynakon | Dreamstime.com)

Conclusion

For good countermeasures and readiness, a more extensive and more far-reaching approach and venture are critically required. Notwithstanding diagnostics, therapeutics, and antibodies, reconnaissance foundation must be enhanced to recognize and confirm cases quickly, direct point by point contact following, research overflows, and better comprehend the environment of bats and Nipah infection contamination, particularly outside of flare-up situations. As vital is the requirement for social change: nearby networks must be better upheld to guarantee disease anticipation and control measures in well-being offices to diminish transmission, and to lead the network commitment and instruction required to alter conduct and decrease chance. We should notice that Nipah infection requests an expansive, long haul system and pandemic arrangement. Favipiravir treatment results in diminished viral antigen and histopathological changes. With the end goal to decide the neurotic changes present in favipiravir-treated NiV-M-contaminated hamsters, we inspected cerebrum, spleen, and lung gathered from euthanized creatures amid the examination and survivors at 42 DPI utilizing H&E stains and IHC against NiV nucleoprotein. Vehicle-treated creatures showed trademark obsessive sores of NiV disease: Lungs showed perivascular penetration of provocative cells, and NiV antigens were recognized in endothelial cells, which infrequently shaped syncytia, and also in smooth muscle cells of pneumonic vessels. Gentle to direct interstitial pneumonia with alveolar edema or drain and intermittent increment in sort II pneumocytes were likewise observed. In the spleen, follicles were less unmistakable, and the red mash string showed necrotic territories scattered with mononuclear or reticular cells with NiV antigens. In cerebrums, meningitis with a penetration of neutrophils and mononuclear cells was found, and viral antigens were identified in mononuclear cells with lengthened cytoplasm in meninges and every so often in neurons in the parenchyma. Tissues of creatures which were treated with favipiravir, either p.o. or on the other hand s.c., were comparative: no remarkable discoveries of sicknesses were distinguished in minds, lungs, and spleens in the H&E areas. None of the treated hamsters showed perceptible NiV antigens in cerebrums, lungs, or spleen spleens. Lungs of treated hamsters did not demonstrate cell penetration in pneumonic veins, albeit gentle union of lung parenchyma was watched.

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