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Article

Identification of Diagnosis Fungi that Cause Potato Root Rot

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ABSTRACT

Results of collecting samples from different regions of Anbar Governorate (Al-Amiriyah, Al-Khalidya, Fallujah, Heet and Ramadi) showed that potato root rot disease is widespread in all regions collected. The results of isolation and phenotypic and molecular diagnosis using the polymerase chain reaction (PCR) technique indicated showed the presence of fungus *Rhizoctonia* spp., and Fungus *Fusarium* spp. Accompanying potato root rot disease and the pathogenicity test using radish seeds on water Agar (W.R.) culture media, all tested isolates achieved a significant reduction in radish seed plants compared with control treatment uncontaminated by any of the isolates of fungi, which recorded infection rate 0%.

Keywords: Potato Root Rot, Diagnosis, Fungi, *Rhizoctonia solani* , *Fusarium solani*.

INTRODUCTION

Potato, *Solanum tuberosum* L., is considered a strategic vegetable crop and comes in fourth place after wheat, corn and rice. It is from the family *Solanaceae*, of strategic importance in most countries of the world in general and Iraq in particular, as it is considered one of the easiest crops to manage when compared to other vegetable crops as well as Most of its varieties, especially the early ones, complete their life cycle less than 100 days [1]. Due to the ease of management of this crop and its high productivity and short life cycle, its cultivation has expanded significantly [2]. As a result of the expansion in the cultivation of this crop, many problems appeared, foremost of which are fungal causes, especially root rot pathogens, leg ulcers and black crust, which cause economic losses. It increases farmers' production [3], [4] . Root rot disease is one of the most essential plant diseases worldwide that affects many crops [5]. Root rot disease of potato plants caused by the fungi *Rhizoctonia solani* and *Fusarium solani* is among the most common diseases due to its highly destructive effects that may reach chronic effects in every potato growing area. Each of them can have an economic impact on the season and does not cause Economic losses are only on an annual basis and therefore considered a problem for producers who often suffer economic losses from this disease. Mold was first described as a potato tuber disease in Ireland in 1913, has been known to occur in North America since then, and has become a significant soil-borne disease. Statistics show that this species is becoming increasingly common, which poses a significant threat to potato production, especially in warm regions [6]. Root rot symptoms represent a

significant threat because the damage begins underground, where the first symptoms cannot be distinguished—the appearance of pathological symptoms on infected plants [7], [8]. Because of the importance of the potato crop and the danger of root rot disease, the study aimed to isolate and characterize the phenotypic and molecular diagnosis of the fungi accompanying the samples infected with root rot disease.

MATERIALS AND METHODS

Sample collection

Samples were collected from potato plants that showed symptoms of disease (yellow, plant wilt, plant death, burn edges of leaves) [9] from some fields of Anbar Governorate (Al-Khalidiya, Al-Amiriya, Sadat Al-Fallujah, Nuaimiya, Heet and Saqlawiyah) for the agricultural season Two thousand twenty-one for the spring loop, and the affected plant parts were placed in sterile polyethylene bags, with the place and date of sample collection recorded, and the samples were kept in the refrigerator at a temperature of 4 C° until the isolation process**.**

Isolation of fungi associated with potato root rot

Parts of roots that showed symptoms of rot were taken and washed with water to get rid of dust and cut into small pieces with a length of 0.5-1 cm. and superficially sterilized with sodium hypochlorite solution Nacl (chlorine 6% active substance) for 3 minutes after which the plant pieces were washed with distilled water Sterilized 3 times to get rid of the effects of the sterile material, dried with sterile filter paper and transferred by sterile forceps, and 4 plant pieces were planted in each Petri dish with a diameter of 9 cm containing the sterilized culture medium (PDA) Potato Dextrose Agar with an Autoclaved at 121 °C and a pressure of 5.1 bar/cm^3 for 20 minutes and the antibiotic (Amoxicillin) was added to it at a concentration of 200 mg. L^{-1}, after sterilizing medium. The dishes were incubated at 25 ± 2 °C for 3 days, after which a microscopic examination was conducted to investigate the presence of pathogenic fungi**.**

Phenotypic diagnosis

Fungi isolates were diagnosed after the growth of fungal colonies on the PDA medium was prepared, as microscopic slides were examined using a light microscope, and depending on the cultural characteristics and using the taxonomic key specific to the species, the fungi were morphologically diagnosed [10].

Molecular diagnosis

DNA was isolated and extracted in the Scientific Progress Laboratory / Baghdad - Al-Harithiya according to the ABIOpure protocol. Prepared by the Korean company Macrogen ITS13 -5'- TCCGTAGGTGAACCTGCGG, ITS43 - 5'-TCCTCCGCTTATTGATATGC**.** The Korean company Macrogen prepared the primers in lyophilized form in nuclease-free water to give a final concentration of 100 µl.$µl^{-1}$. A working solution of these primers was prepared by adding 10 µl of the starting primer (stored at -20 °C) to 90 µl of distilled water to obtain an effective starting solution of 10 µl. mol-1 After PCR, gel electrophoresis to confirm the presence of amplification using polymerase chain reaction (PCR) was fully approved on the parameters of the extracted DNA. After completing the DNA amplification and migration steps on Agarose Gel, the samples were sent to the Korean Macrogen Company to obtain the sequence of nitrogenous bases (Sequencing). Several studies have relied on this technique in the diagnosis of fungi, as [11] showed that the PCR technique is used to diagnose *R. solani* fungi and relied in its diagnosis on the transcribed spacer internal region between (ITS4-ITS1) to distinguish between types of fungi. specialized. [12] also used PCR technique to diagnose species of the genus *Rhizoctonia* such as *R. solani*, isolated from different families of plants.

Pathogenicity tests of isolated fungi on radish seeds

The pathogenicity of seven isolates of *R.solani* spp. (Al-Amiriya - Rs1, Al-Amiriya 2 - Rs2, Al-Khalidiya - Rs3, Sadat Al-fallujah - Rs4, Al-Nuaimiya - Rs5, Heet - Rs6 and Al-Saqlawiyah- Rs7) And seven isolates of *Fusarium* spp. (Al-Amiriya - Fu1, Al-Amiriya 2 - Fu2, Al-Khalidiya - Fu3, Al-Khalidiya - Fu4, Al-Nuaimiya - Fu5, Heet - Fu6Furthermore, Al-Saqlawiyah - Fu7) was tested. In the Department of Plant Protection at the College of Agriculture / University of Anbar, the test was carried out according to the method of [13] according to a Randomized completely design (CRD) sterile nutrient medium (Water Ager) was prepared and distributed in Petri dishes with a diameter of 9 cm containing15-20 mm. From the more significant culture medium and water (W.A.) (20 g acre, liter of distilled water), sterilized by an autoclaved 121 °C and a pressure of 5.1 bar/cm3 for 20 minutes, added to the antibiotic Amoxicillin at a concentration of 200 mg/L^{-1}, the dishes were inoculated by taking a 5 mm diameter disc using A sterile cork piercing was placed in the middle of the dish. 3 dishes were used for each isolate of the tested fungi, and 3 were used as a comparison treatment (without adding a tablet from the mushroom colony). Then the dishes were transferred to the incubator and left at a temperature of 25 ± 2 for three days, after which the sterilized radish seeds were sown superficially with a solution of Sodium hypo chlori; 100%, 10 seeds were used for each plate. The seeds were sown in a circular motion around the fungal perennial disc so that the seeds were separated by 1 cm from the edge of the plate. Then, the plates were incubated for 14 days, after which the percentage of infection was calculated for each isolate according to the following equation:

$$\text{Germination \%} = \frac{\text{Number of Infected Plants}}{\text{Total Number of Plants}} \text{ x } 100.$$

RESULTS AND DISCUSSION

The results of sampling. in Fig. (1) and Fishow g. (1) Potato root rot disease is widespread in most potato growing areas in the world, and that is consistent with findings found by [14, 15].

Figure 1: Potato plants infected with root rot disease.

No.	Sample Code	Location	Collection Date 2021
1	A1	Al-Amiriya	**25 /10**
2	A2	Al-Amiriya2	**27 /10**

3	A3	Al-Khalidiya	**3 /11**
4	A4	Sadat Al-Fallujah	**5 /11**
5	A5	Al-Nuaimiya	**11 /11**
6	A6	Heet	**12 /11**
7	A7	Al-Saqlawiyah	**14 /12**

Table 1: Areas from which samples were collected from Potato root rot disease.

Phenotypic diagnosis

The results of the phenotypic diagnosis shown in Table (2) showed the presence of the fungus *R.solani* spp. and *Fusarium* spp., which were associated with potato root rot disease, which is the main cause of the disease, and this is consistent with what was indicated by [16, 17].

No.	Sample Code	Fungi	Location	Collection Date 2021
1	A1	*Rhizoctoina spp.* and *Fusarium spp.*	Al-Amiriya	**25 /10**
2	A2	*Rhizoctoina spp.* and *Fusarium spp.*	Al-Amiriya 2	**27 /10**
3	A3	*Rhizoctoina spp.* and *Fusarium spp.*	Al-Khalidiya	**3 /11**
4	A4	*Rhizoctoina spp.* and *Fusarium spp.*	Sadat Al-Fallujah	**5 /11**
5	A5	*Rhizoctoina spp..*and *Fusarium spp.*	Al-Nuaimiya	**11 /11**
6	A6	*Rhizoctoina spp* and *Fusarium spp.*	Heet	**12 /11**
7	A7	*Rhizoctoina spp.* and *Fusarium spp.*	Al-Saqlawiyah	**14 /12**

Table 2: The most important fungi related to potato root rot were isolated and diagnosed phenotypically to the genus level.

Molecular Diagnostics

Table (3) shows the results of the molecular diagnosis that the fungi that cause potato root rot disease are of the genus *Rhizoctonia* spp. and *Fusarium* spp., according to the arrangement of nitrogenous bases in the single DNA strand. These results are consistent with what was mentioned by [16, 18], and 19, and these fungi are the leading cause of potato root rot disease.

No.	Location	Code	Fungi	Accession
1	Al-Amiriya	**A1**	*Fusarium Falciforme*	**MN545530.1**
2	Al-Khalidiya	**K**	*Fusarium Oxysporum*	**OL691079.1**
3	Al-Saqlawiyah	**B**	*Rhizoctoina solani*	**LC507904.1**
4	Sadat Al-fallu-jah	**C**	*Rhizoctoina solani*	**MG654436.1**
5	Al-Nuaimiya	**D**	*Rhizoctoina solani*	**KY965394.1**
6	Heet	**E**	*Rhizoctoina solani*	**KY965394.1**
7	Al-Khalidiya	**F**	*Rhizoctoina solani*	**AJ318420.1**
8	Al-Amiriya 2	**A2**	*Fusarium Kerato-plasticum*	**KY965394.1**
9	Heet	**A3**	*Rhizoctoina solani*	**MF440630.1**

Table 3: Fungi associated with potato root rot diagnosed by PCR technique.

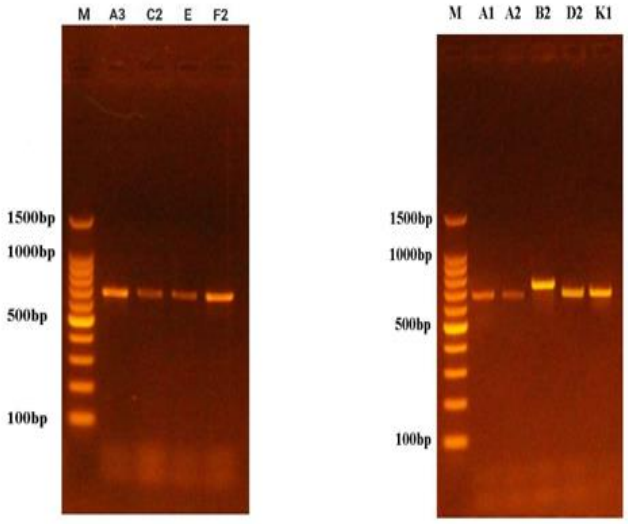

Figure 2: Results of ITS gene amplification of fungal species on Agarose Gel.

The pathogenicity test of some fungi isolates and their effect on the germination of radish seeds on the culture medium Water agar (W.A.)

Results showed in Table (4) that all tested fungi isolates tested on radish seeds were isolates of *Rhizoctonia* spp. and isolates of *Fusarium* spp. It significantly reduced the germination of radish seeds on water agar (W.A.) culture media compared to the control treatment that was not contaminated with any of the tested isolates *Rhizoctonia*, in which the infection rate was 0%. The test also showed the variation of the isolates in their effect on the germination of radish seeds, and the most influential of them was the isolate *Rhizoctonia* taken from Al-Anbar, Heet region, in which the infection rate was 90%. In contrast, *Fusarium* isolate is the strongest in the Al-Saqlawiya region, which achieved a 90% infection rate. Results agreed with that mentioned, and y [20, 21].

Location	**Isolation code *Rhizoctonia***	**Infection ratio %**	**Isolation code *Fusarium***	**Infection ratio %**
Amiriyah	*Rhizoctonia* Rs1	83.334	*Fusarium* Fu1	**83.334**
Amiriyah 2	*Rhizoctonia* Rs2	80	*Fusarium* Fu2	**86.667**
Khalidiya	*Rhizoctonia* Rs3	80	*Fusarium* Fu3	**80**
Sadat Al-Fallujah	*Rhizoctonia* Rs4	76.667	*Fusarium* Fu4	**83.334**
Nuaimiya	*Rhizoctonia* Rs5	90	*Fusarium* Fu5	**83.334**
Heet	*Rhizoctonia* Rs6	76.667	*Fusarium* Fu6	**86.667**
Saqlawiyah	*Rhizoctonia* Rs7	83.334	*Fusarium* Fu7	**90**
	Control	0.00	Control	**0.00**

	LSD 0.817		**LSD 1.236**

Table 4: Effects of different isolates of *Rhizoctonia* spp. and *Fusarium* spp. in germination of Radish seeds on water agar medium.

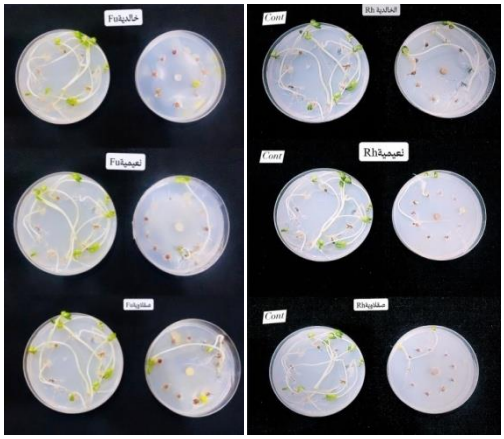

Figure 3: Fungus Rhizoctonia spp. and *Fusarium* spp. in germination of radish seeds on Water Agar media.

CONCLUSIONS

Potato root rot was observed as a disease in all sample collections. Two fungi, Rhizoctonia spp, mainly caused and *Fusarium* spp. according to the results of phenotypic and molecular diagnosis.

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