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A survey, Pathogenicity assay, and Molecular Identification of the Pathogenic Fungi Associated with Pistachio in Anbar Province, Iraq

Khalid W. Tarad[1], Hamood M. Saleh[1] & Tavga S. Rashid[2*]

[1]Department of Plant Protection, College of Agriculture, University of Anbar, Iraq
[2]Department of Plant Protection, College of Agricultural Engineering Sciences, Salahaddin University, Erbil, Iraq

[*]**Corresponding author email**: **T.S.R.**: tavga.rashid@su.edu.krd; **H.M.S.**: ag.hamood.saleh@uoanbar.edu.iq; **K.W.T.**: andkhalid@yahoo.com

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Abstract: This study was carried out in 6 different locations in the Anbar Province, between January and February 2021. The locations with higher pistachio cultivation, such as Kabisa, Al-Haditha, Al-Baghdadi, Anah, Al-Qaim, and Rawa were selected to study the prevalence of fungal diseases affecting pistachio crops. Twenty-six distinct fungal isolates were identified, and each isolate was examined for the pathogenicity. Although the level of pathogenicity of each isolate varied from high to moderate, they all caused lesions, damaged vascular tissue, signs of root rot and seed rot in pistachio seedlings. The fungal strains were identified depending on ITS marker. Twelve of the isolates belonged to *Fusarium solani*, 10 of *F. falciforme* isolates, 2 of *F. oxysporum* isolates, while 1 isolate of *F. vanettenii* and *Xylaria* sp. The most frequently-occurring species were *F. falciforme* and *F. solani* in the Anbar Province. Out of all the studied isolates, Al-Qaim and Al-Baghdadi were the highest pathogenic isolates. The pathogenic effects of *F. vanettenii* and *Xylaria* sp. on pistachio plants as well as the existence of *F. solani* and *F. falciforme* in Iraq were all recorded in this study for the first time.

Keywords: Disease severity, *Fusarium* sp, ITS, *Pistacia vera*, *Xylaria* sp.

Introduction

The *Pistacia vera* L., is originated the East Mediterranean Sea and the East of Central Asia (Al-Saghir & Porter, 2012). It is commonly grown in Iran, Iraq, Turkey, other Mediterranean countries, and the USA (Hormaza & Wünsch, 2007).

Pistachio crops are susceptible to a number of diseases, some of which can be extremely dangerous. For instance, *Macrophomina phaseolina* cause root rots, *Phytophthora* spp. cause foot rots (Chitzanidis, 1994), while late blights caused by different pathogenic species such as *Alternaria alternata, A. arborescens, A. tenuissima*, and *A. infectoria* (Pryor & Michailides, 2002). Many *Phytophthora* species, such as *P. taxon, P. niederhauserii*, and *P. cinnamomi* were reported to cause pistachio decline. A few other fungal species, such *as Fusarium solani* and *Macrophomina phaseolina*, were isolated from the declining

pistachio plants and rootstocks. *Cytospora* species were believed to be the most virulent fungal pathogen that could threaten pistachio cultivation in California (Eskalen *et al*., 2001). *F. proliferatum* and *Cytospora* sp. were isolated from the Californian pistachio crops (Nouri *et al*., 2020). Additionally, *Colletotrichum karstii* and *Schizophyllum commune* were isolated from the pistachio crops in California (Nouri *et al*., 2019). According to report by Nouri *et al*. (2020) *M. phaseolina* is a disease that damages pistachio crops by causing crown rot in California.

According to our knowledge, there were no recorded studies investigated the fungal diseases on pistachio in Iraq. Thus, the objective of this study was to determine the pathogenic fungi on pistachio plants in different pistachio-growing regions of the Anbar Province.

Materials & Methods

Survey and Sample collection

The survey was carried out in the Anbar Province. Six areas with higher pistachio cultivation were chosen (Kabisa, Al-Baghdadi – Haditha, Hina, Rawa and Al-Qaim). The specimens were collected from the beginning, in the middle, and at the end of the fields, randomly. The following symptoms were noticed in the pistachio samples: plant death, dryness, yellowing of leaves, dark discoloration in wooden vessels, partial or total wilting, and root rot. Pistachio plant stems and roots were cut into small pieces, placed in polyethylene bags. Ten samples from each line were randomly selected, packed in polythene bags, and delivered to the plant protection department's lab at the University of Anbar in order to isolate the fungi. According to the shown symptoms on the plants (dryness, wilting, and yellowing), the infection percentage was determined using the following equation:

$$\% \text{ Infection} = \left(\frac{\text{number of infected plants}}{\text{total number of the examined plants}}\right) \text{x } 100$$

Fungi isolation

The stems and roots were washed with water then sliced into smaller pieces (0.5 cm), and surface-sterilized for 3 mins using 1% sodium hypochlorite (NaOCl). The samples were then rinsed thrice with sterilized water to remove NaOCl and dried on sterile filter paper, placed on Potato Dextrose Agar (PDA) plates, and incubated for 3-6 days at 28°C. Following growth, fungal colonies were purified using the hyphal tip technique by transferring the individual hyphae to PDA plates using a sterilized needle and incubated until the development of pure fungal cultures.

Morphological identification

All the isolates were sub-cultured for seven days on PDA at 25°C. Pigment production and colony characteristics were noted. Slides were prepared for each isolate, the conidia, hyphae, conidial head, conidiophores, spores, etc. were observed microscopically at high magnification for morphological identification using AXIO A2 Imager Upright Microscope (Germany). They were divided into 26 isolates according to the location and cultural characteristics.

Molecular identification

DNA extraction and molecular identification: Based on a pathogenicity test, 26 fungal isolates were grown on PDA for 72 h at 30°C. The complete genomic DNA from each isolate was extracted using a commercial genomic DNA purification kit (Wizard DNA Genomic Purification System (Promega, USA). Agarose gel electrophoresis was used in combination with NanoDrop ND-100 equipment (Thermo

Fisher, USA) to examine the DNA's quantity and quality.

To identify the selected fungal isolates, the ITS region was amplified using the ITS1 forward primer and ITS4 reverse primer (White *et al*., 1990). PCR amplification was carried out in 25 µL reaction volume, where the reaction mixture contained 10×PCR buffer (200 mM Tris-HCl, 500 mM KCl), 0.2 mM of every dNTP, 1 unit of Taq DNA polymerase, 1.5 mM MgCl2, 0.5 M primer, and template DNA (100 ng). The PCR amplification was carried out in thermal cycler machine (XP, TC-XP – G, BIOR TECHNOLOGY CO., LTD) using the following protocol: initial denaturation at 95°C for 8 min; 30 cycles of denaturation at 95°C for 30s; annealing at 55°C for 20 s; elongation for 60 s at 72°C; and final extension step for 5 min at 72°C in (XP Thermal Cycler, TC-XP-G, BIOR TECHNOLOGY CO., LTD).

Agarose gel preparation and electrophoresis

The agarose gel (1%) was prepared by dissolving 1g of agarose in 1× Tris-Borate-EDTA buffer (TBE) by heating it in the microwave. The mixture was mixed with FluroSafe dye (Fisher Scientific USA) and cooled to 60°C. The mixture was carefully poured into a casting tray. The samples were loaded in agarose gel wells then electrophoresed at 100 V for 45 min with the observation and photo caught under ultra violet (UV) illumination in Dark Hood DH-40, BIOTECH FISCHER).

Sequencing

For sequencing, the PCR products were purified in two directions using the EXOSAP-IT PCR Product Clean-Up Reagent (Thermo Fisher Scientific, USA). The SeqStudio Genetic Analyzer was used to sequence the purified PCR products, using the same forward and reverse primers (Hall, 2011). The sequences that were generated were further assessed using ChromasPro software.

To categorize and assess the homologous sequences in the bacterial and fungal isolates utilized in this work with those listed in the GenBank database, the BLAST (Basic Local Alignment Search Tool) search software from NCBI (http://blast. ncbi.nlm.nih.gov) was employed.

Phylogenetic Analysis

MEGA 6.1 was used to perform a phylogenetic analysis utilizing the ITS sequences of the selected fungal strains and control sequences (Neighbour-joining algorithm with 1000 bootstrap replications). Based on the overall character differences, neighbor-joining trees were built, and bootstrap values from 1,000 replications were determined (Huang *et al*., 2012).

Pathogenicity test

A pathogenicity test was performed on 26 fungal isolates. to conduct the experiments in the Dept. of Plant Protection, College of Agriculture, University of Anbar. A nursery (Western nursery for rare crops) in Anah provided the Ashouri cultivar seeds. In February 2021, sterilized soil sand-peat moss (1:1) were used in 2 kg plastic pots.

The soil was inoculated with fungal isolates (one fungal plate/2 kg soil) (Christensen *et al*., 1988). Every pot was irrigated, wrapped using polyethylene bags, and left for 3 days. After soaking the seeds in water for 24 h, 2 seeds were then placed in every pot at a depth of 1 cm. A pathogen-free (control) was also prepared. The

percentage of germination was observed after seed germination.

The final colony characteristics were examined to confirm that the isolated fungal species were identical to the species used for pathogenicity analysis, demonstrating the validity of Koch's hypotheses. The experiments were repeated twice, using two plants in a planting pot for each replicate.

After eight weeks of cultivation, disease severity was calculated based on the scale (Table 1) by Latha *et al.* (2009) and according to the following equation:

$$\%\ \textbf{Disease Severity} = \frac{\text{Class frequency} \times \text{score of rating class}}{\text{total number of plants)} \times \text{(maximal disease index)}} \times 100$$

Table (1): Disease severity alternate rating scale.

Score chart	Grade	Disease severity Range (%)
0	Healthy	0
1	Initial	1-10
2	Low	11-25
3	Medium	26-50
4	High	51-75
5	Very high	76-100

Score chart 0–6 scale (0 = healthy; 1 = 1–20%; 2 = 20–40%; 3 = 40–60%; 4 = 60-80% and 5 = 80–100% of the plant area infected).

Statistical analyses

A Completely Randomised Design (CRD) with 4 replications was used for all experiments. The Analysis of Variance (ANOVA) was carried out utilizing SAS software ver. 9.2. The statistical differences between the various fungal isolates were determined using the Duncan test at the level of probability of 0.05 (Cary, NC: SAS Institute Inc. 2011.

Results

Scanning study

The results of the scanning study across six Anbar Province regions (Kabisa, Al-Baghdadi, Haditha, Anah, Rawa, and Al-Qaim) are presented in table (2), the lowest infection rate was shown in Haditha (16.4%), while Anah reported the highest infection rate (76%).

Table (2): The percentage of infection in different regions of Anbar Province for pistachio fields.

	LOCATION	% INFECTION
1	Kabisa	70
2	Al-Baghdadi	22
3	Haditha	16.4
4	Anah	76
5	Rawa	66.6
6	Qaim	47

Sample collection and isolation of fungal strains

Each area yielded a different variety of fungal strains. The two areas with the most isolates, Al-Baghdadi and Haditha, each contributed seven isolates (Figs. 1 and 3) and Rawa (6 isolates) were next (Fig. 4). In addition, Al-Qaim yielded 3 isolates (Fig. 3) and 4 isolates from Anah (Fig. 6); only one isolate from Kabisa (Fig. 2) was chosen. From Al Baghdadi two isolates were *F. solani*; four isolates were *F. falciforme*; and one isolate was *F. vanettenii*. Isolates form Haditha, two were *F. falciforme* and four were *F. solani*. Al Rawa isolates two were *F. oxysporum*, one was *F. solani*; and three were *F. falciforme*. Al Qaim two isolates were *F. solani*; one was *Xylaria sp.* while isolates from Al Anah, one was *F. falciforme*; the other isolate was *F. solani*. Only one isolate was isolated from Al Kabisa which was *F. solani*.

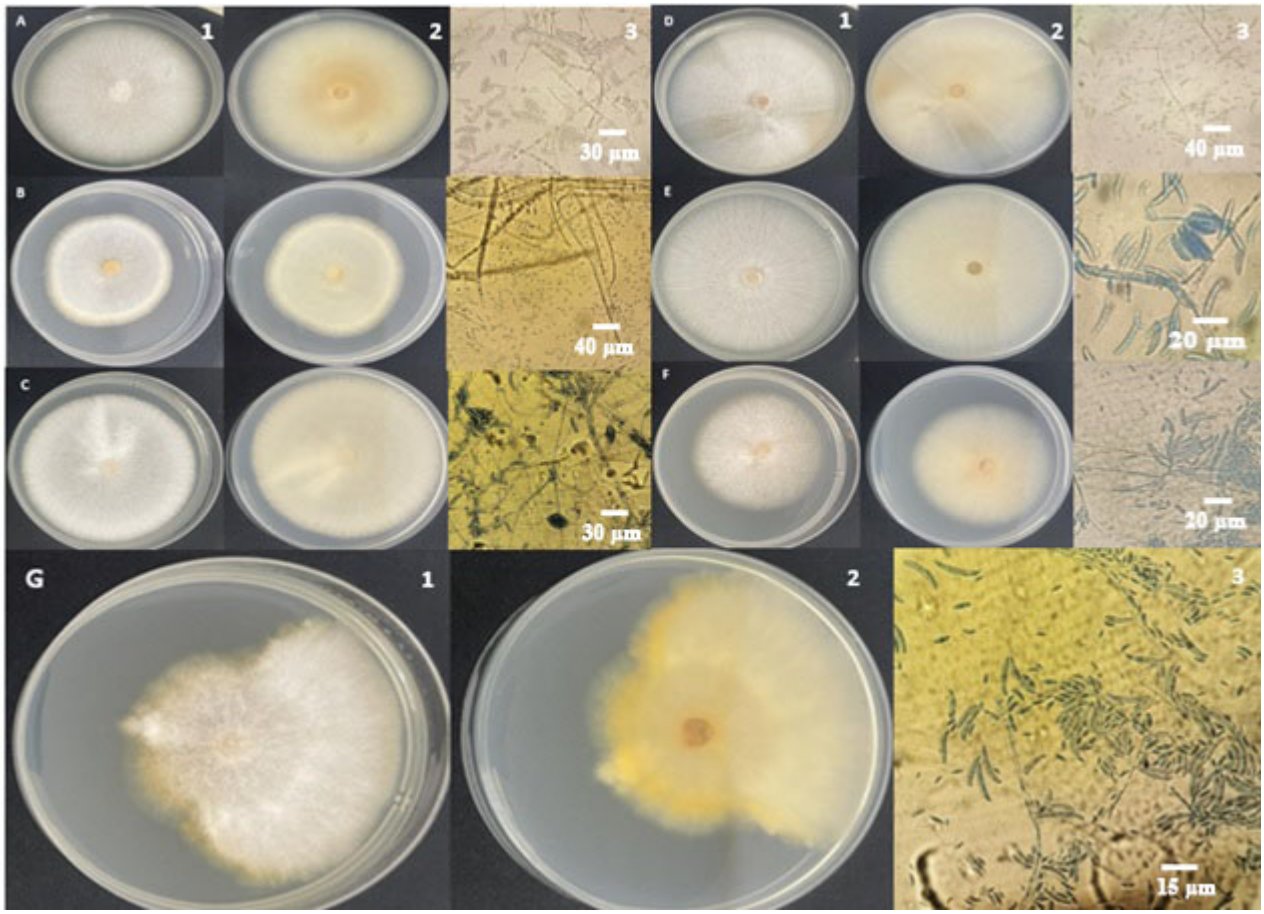

Fig. (1): The fungal isolates of: Al Baghdadi A and B were *F. solani*; C, D, E and F were *F. falciforme*; G was *F. vanettenii.*

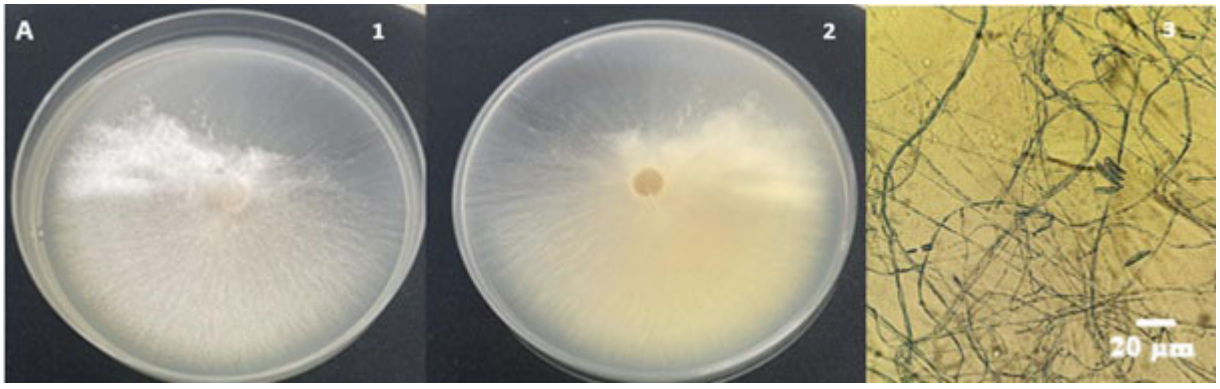

Fig (2): The fungal isolates of A Kabisa was *F. solani*.

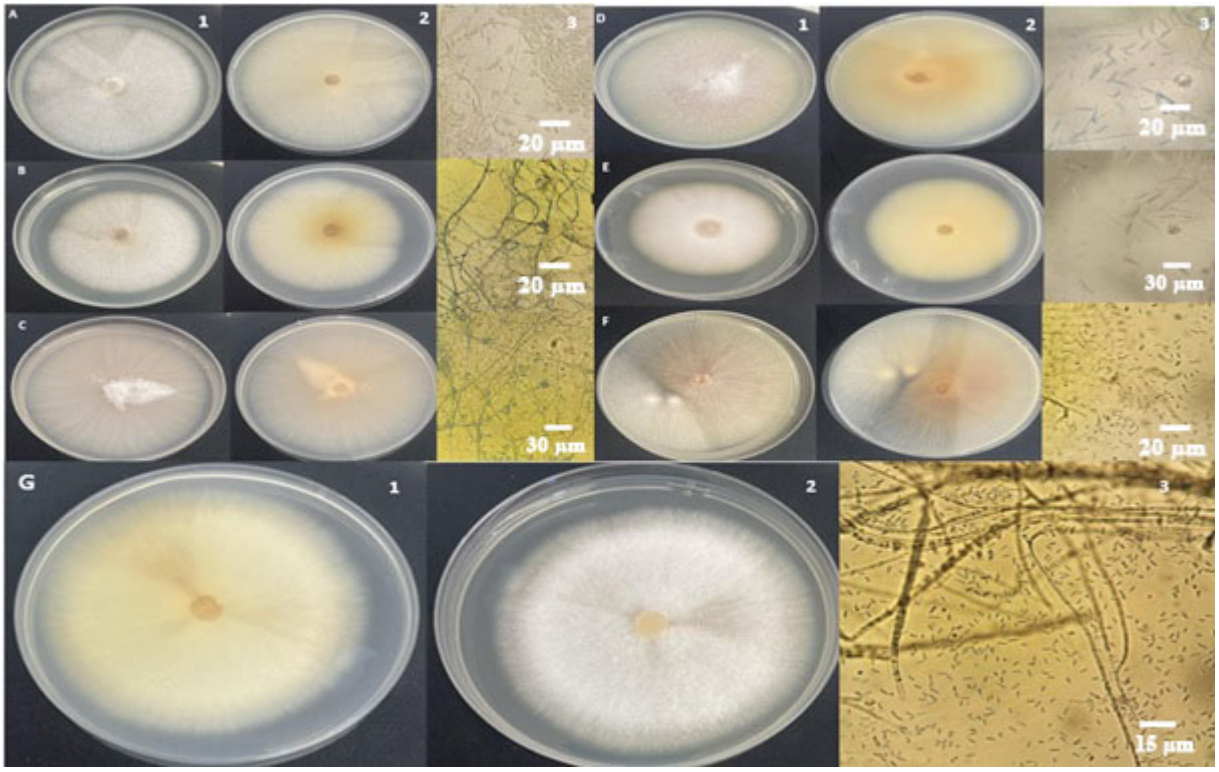

Fig. (3): The fungal isolates of: HadisaA, B and G were *F. falciforme*; C, D, E and F were *F. solani*.

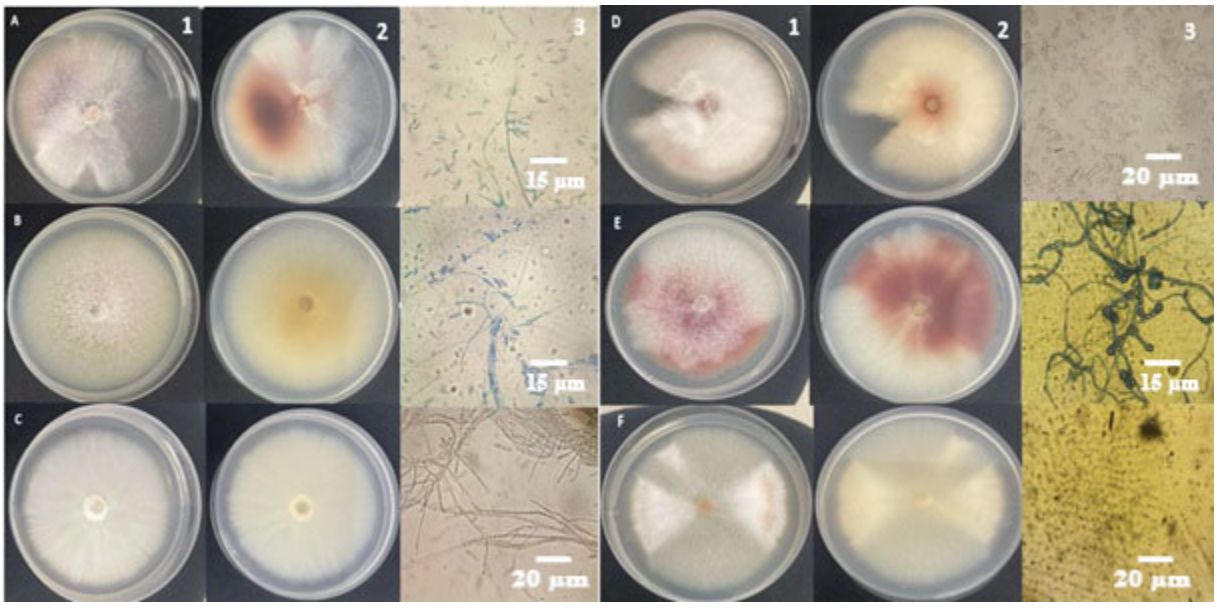

Fig (4): The fungal isolates of: Al Rawa A and E were (*F. oxysporum*), B was (*F. solani*); C, D and F were (*F. falciforme*).

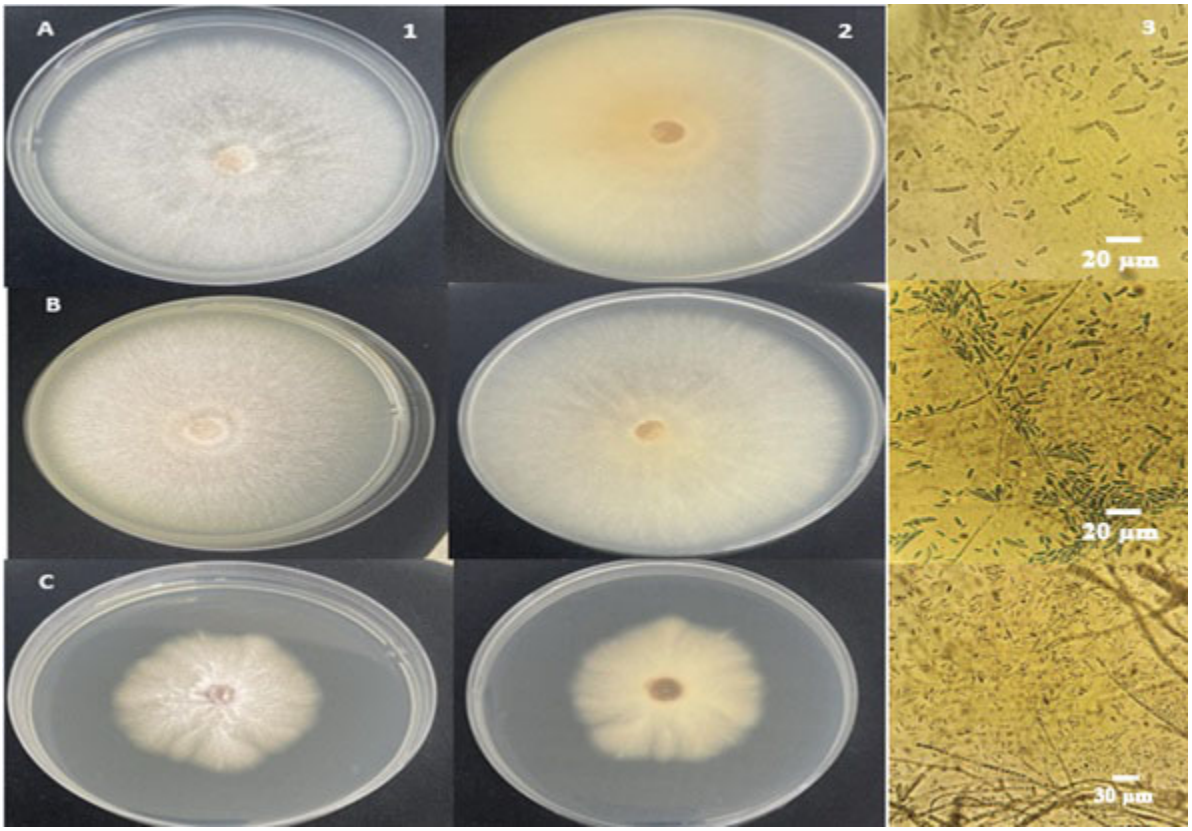

Fig. (5): The fungal isolates of: AlQaim A and B were (*F. solani*); C was *Xylaria*.

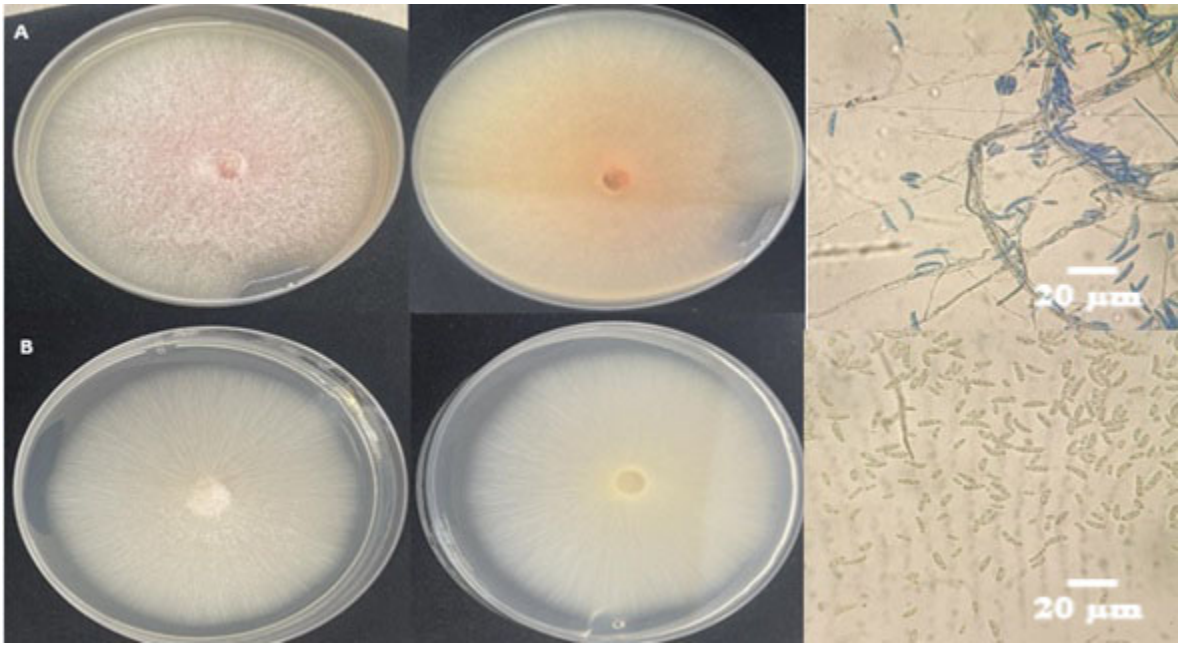

Fig (6): The fungal isolates of: Al Anah was *F. falciforme*; B was *F. solani*.

Molecular identification

The gel electrophoresis results (Fig. 7) confirmed the amplification of ITS region for all examined fungal strains.

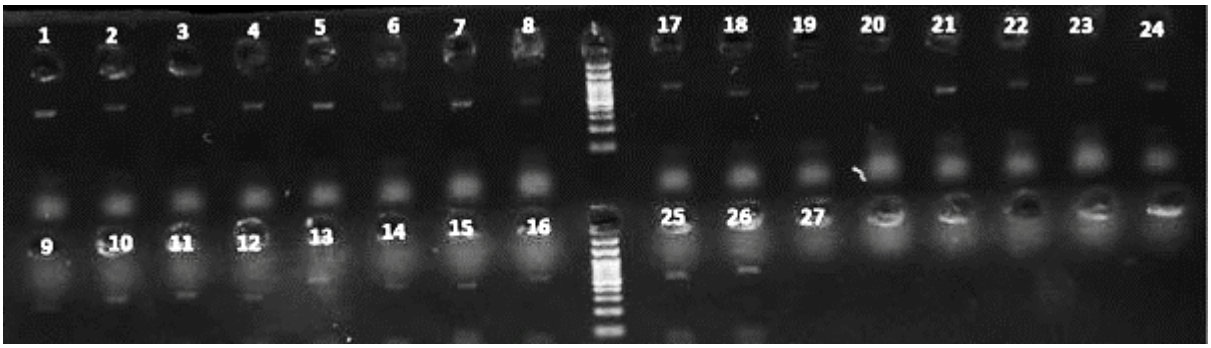

Fig (7): Gel electrophoresis profiles of ITS gene for selected fungal isolates. Well 1-26: all isolates; 27: nill, L: ladder.

The BLAST results showed that ITS sequences of Kabisa A isolate was 99% compatible with the *F. solani* (Acc. No. MK530101), while Al-Baghdadi A, B, and C were 99.9% compatible with the *F. solani* (Accession No. MN856440, KJ193849, and MN545531), respectively. Meanwhile, the Al-Baghdadi G strain 99% compatible with the *F. vanettenii* (Acc. No. MH855496), whereas Al-Baghdadi D, E, and F isolates were 99% compatible with *F. falciforme* (Acc. Nos. MN396537, OP482314, and OP482357), respectively; The AlQaim C isolates showed a 99% compatibility with the *Xylaria* sp. (Acc. No. ON843621), while AlQaim A and B strains were found to be 99% compatible with *F. solani* (Acc. Nos. OL851828 and MT594367), respectively. Anah A (Acc. No. OP747041) strain was similar to the *F. falciforme* sequences in GenBank (Acc. No. MN907526), while the Anah B strain showed a 99% compatibility with *F. solani* (Acc. No. MN646254). AlHaditha A, B, and G strains were 99% identical to the *F. falciforme* (Acc. No. MW045574, MN907531, and MN907496), respectively; while AlHaditha C, D, E, and F strains were 99% compatible with *F. solani* (Acc. No. MF401578, MN817707, MZ723886, and MZ723895), respectively.

AlRawa A and E isolates showed a 99% compatibility with *F. oxysporum* (Acc. No. KF751873 and OL504748), respectively; whereas AlRawa B was 99% identical to *F. solani* (Acc. No. MZ723891). The AlRawa C, D, and F isolates were 99% compatible with the *F. falciforme* (Acc. No. MN907513, MN907491, and ON394614), respectively.

The results of the phylogenetic analysis indicated that all the fungal species that were identified were clustered together and showed 95-99% similarity to other reference sequences acquired from GenBank (Fig. 8). The identified isolates ITS sequences were deposited in GenBank under Accession numbers presented in table (4).

Table (4): The identified fungal isolates with their genebank accession numbers.

No.	Isolate name	Pathogen name	Accession number
1	Kabisa A	*F. solani*	OP750997
2	Al-Baghdadi A	*F. solani*	OP745423
3	Al-Baghdadi B	*F. solani*	OP750992
4	Al-Baghdadi C	*F. solani*	OP745473
5	Al-Baghdadi D	*F. falciforme*	OP745516
6	Al-Baghdadi E	*F. falciforme*	OP750991
7	Al-Baghdadi F	*F. falciforme*	OP745648
8	Al-Baghdadi G	*F. vanettenii*	OP751507
9	Haditha A	*F. falciforme*	OP747192
10	Haditha B	*F. falciforme*	OP747191
11	Haditha C	*F. solani*	OP747295
12	Haditha D	*F. solani*	OP747296
13	Haditha E	*F. solani*	OP747305
14	Haditha F	*F. solani*	OP747413
15	Haditha G	*F. falciforme*	OP747417
16	Anah A	*F. falciforme*	OP747041
17	Anah B	*F. solani*	OP750993
18	Rawa A	*F. oxysporum*	OP747464
19	Rawa B	*F. solani*	OP747463
20	Rawa C	*F. falciforme*	OP747457
21	Rawa D	*F. falciforme*	OP747467
22	Rawa E	*F. oxysporum*	OP747468
23	Rawa F	*F. falciforme*	OP747490
24	Al-Qaim A	*F. solani*	OP745695
25	Al-Qaim B	*F. solani*	OP747675
26	Al-Qaim C	*Xylaria* sp.	OP746070

All identified fungi we obtained were clustered together 95-100% related with other reference sequences that aquired from the gene bank based on the phylogenetic analysis, *Fusarium falciforme* and *F. venetteni* (Fig. 8); *Fusarium solani* (Fig. 9); *Fusarium oxysporum* and *Xylaria* sp. (Fig. 10).

Pistachio seeds inoculated with Haditha A, D, and Rawa D isolates showed the maximal germination percentage (66.7%), followed by Al-Baghdadi A, Rawa B, and C (50%) and Kabisa A, Haditha B, C, Rawa E, and F (33.4%). The results further showed that all the seeds inoculated by Al-Baghdadi B, C, D, E, F, G; Haditha G, Anah B, and Al-Qaim A, B, C isolates did not germinate (Table 3).

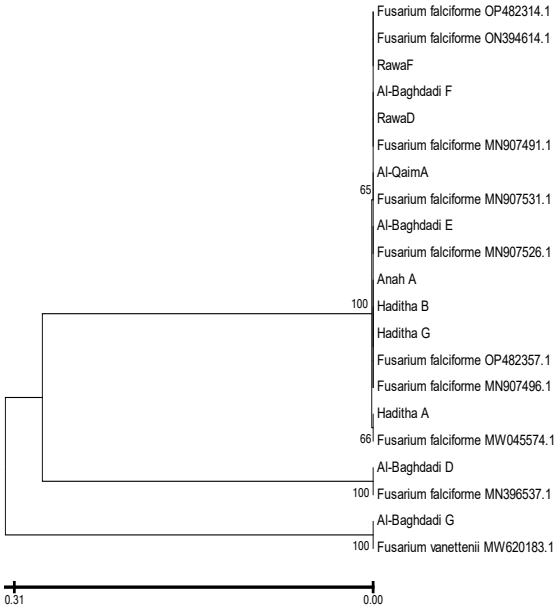

Fig (8): Phylogenetic tree that was constructed using the neighbor-joining approach, which displays the evolutionary relationships of the isolated *Fusarium falciforme* and *F. venetteni* species compared to the GenBank reference sequences.

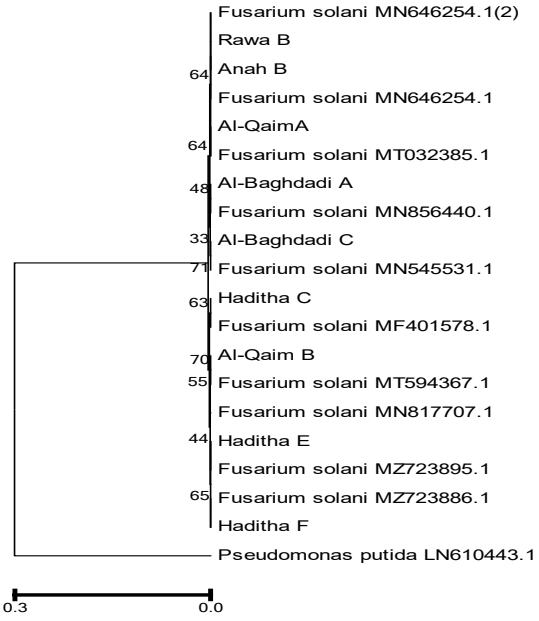

Fig. (9): Phylogenetic tree that was constructed using the neighbor-joining approach, which displays the evolutionary relationships of the isolated *Fusarium solani* species compared to the GenBank reference sequences.

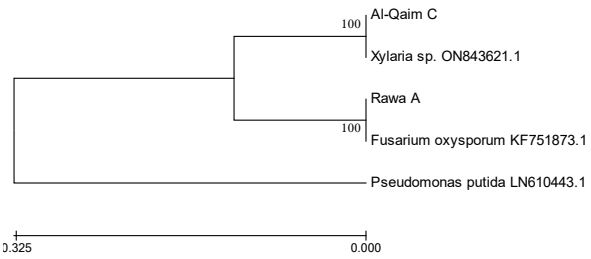

Fig (10): Phylogenetic tree that was constructed using the neighbor-joining approach, which displays the evolutionary relationships of the isolated *Fusarium oxysporum* and *Xylaria sp.* species compared to the GenBank reference sequences

Pathogenicity assay:

The results also revealed that the maximal percentage of pre-emergence seed rots was recorded after 20 days of treatment. Haditha C, D, E, Anah A, and Rawa A were the isolates that caused the pre-emergence seed rots (100%), followed by Al-Baghdadi B, C, D, E, F, G, Haditha G, and AlQaim A, B, C (83.3%). Additionally, Kabisa A, Haditha B, F, and Rawa E, F recorded 66.6%, and Al-Baghdadi A, and Rawa B, C showed 50% of pre-emergence seed rot. The pots that were treated with Haditha A, D, E, and Rawa D isolates showed the lowest percentage of developed root rot and pre-emergence seed rot (33.3%).

After two months of implantation, the germinated pistachio seedlings displayed typical disease symptoms. The initial symptoms of *Fusarium* sp. infections were represented by yellowing and wilting, while the roots turned dark brown. The un-inoculated seedlings (i.e., controls) displayed no disease symptoms. To confirm Koch's hypotheses, potential fungal pathogens were re-isolated. The colonies that had appeared after re-inoculation exhibited identical fungal morphological traits.

Table (3): The pathogenicity of the examined fungal isolates.

No.	Isolate name	% Germination	% pre-emergence seed rot	%post emergence rot
1	Kabisa A	33.4 ^{bc}	66.6 ^{c}	83.3^{b}
2	Al-Baghdadi A	50 ^{b}	50 ^{cd}	83.3 ^{b}
3	Al-Baghdadi B	0 ^{d}	100 ^{a}	-
4	Al-Baghdadi C	0 ^{d}	100 ^{a}	-
5	Al-Baghdadi D	0 ^{d}	100 ^{a}	-
6	Al-Baghdadi E	0 ^{d}	100 ^{a}	-
7	Al-Baghdadi F	0 ^{d}	100 ^{a}	-
8	Al-Baghdadi G	0 ^{d}	100 ^{a}	-
9	Haditha A	66.7 ^{a}	33.3 ^{e}	33.3 ^{e}
10	Haditha B	33.4 ^{bc}	66.6 ^{c}	66.6 ^{c}
11	Haditha C	16.7 ^{d}	83.3 ^{b}	83.3 ^{b}
12	Haditha D	66.7 ^{a}	33.3 ^{e}	66.6 ^{c}
13	Haditha E	16.7 ^{d}	83.3 ^{b}	83.3 ^{b}
14	Haditha F	33.4 ^{bc}	66.6 ^{c}	100 ^{a}
15	Haditha G	0 ^{d}	100 ^{a}	-
16	Anah A	16.7 ^{d}	83.3 ^{b}	83.3 ^{b}
17	Anah B	0 ^{d}	100 ^{a}	-
18	Rawa A	16.7 ^{d}	83.3 ^{b}	83.3 ^{b}
19	Rawa B	50 ^{b}	50 ^{cd}	50 ^{cd}
20	Rawa C	50 ^{b}	50 ^{cd}	50 ^{cd}
21	Rawa D	66.7 ^{a}	33.3 ^{e}	50 ^{cd}
22	Rawa E	33.4 ^{bc}	66.6 ^{c}	66.6 ^{c}
23	Rawa F	33.4 ^{bc}	66.6 ^{c}	66.6 ^{c}
24	Al-Qaim A	0 ^{d}	100 ^{a}	-
25	Al-Qaim B	0 ^{d}	100 ^{a}	-
26	Al-Qaim C	0 ^{d}	100 ^{a}	-

Means in each column with same letter are not statistically different at 0.05 level.

Discussion

All the studied fungal isolates exhibited seed rot and seedling root rot symptoms in the inoculated pistachio seedlings. All *F. solani* and *F. falciforme* strains developed seed rot and seed deaths before seed germination or developed seedling root rot following germination. The primary symptoms of the *Fusarium* infection were yellowing and wilting, or sudden drying in warm conditions. Discoloration of vascular bundles was noted when the root collars of diseased plants were cut transversely similar findings have been reported by Alakaşli & Aydin (2022). A few *Fusarium* species, particularly in nurseries, were reported to infect crops in pistachio plantations (Nouri *et al.*, 2018). The most characteristics of *Fusarium* infection was represented by root rotting and root collars. Reddish-brown or blackish patches appeared in the rotting regions. Finally, the plants started wilting and drying. Different *Fusarium* species. like *F. oxysporum*, *F. solani*, *F. equiseti*, and *F. proliferatum* species caused the discoloration and wilting of the vascular bundles in the Californian pistachio trees (Nouri *et al*., 2018).

The decayed and discolored roots of infected seedlings were reddish brown or dark to light brown in colour. Several fungal crown rot and root rot diseases exhibit similar symptoms. A few researchers (Summerell *et al*., 2001; Saremi 2005) demonstrated that *Fusarium* pathogens caused identical symptoms in various infected crops, which include cortical root decay, wilting, yellowing, root rot, and premature plant death. Similar results were presented by Triki *et al.* (2013), who published the first report where *F. solani* was responsible for root rot in pistachio plants in Tunisia. Rasheed & Naffaa (2017) reported the involvement of *F. solani* in the crown and root rot in pistachio seedlings in Syrian nurseries. Also, Tezerji *et al*. (2018) reported that *F. solani* caused crown and root rot infection in pistachio seedlings, while Crespo *et al*. (2019) demonstrated that *F. oxysporium* and *F. solani* were responsible for stem rot and discolouration in the pistachio crops.

Even though several *Fusarium* species, including *F. solani*, *F. equiseti*, *F. proliferatum*, *F. oxysporum*, *F. brachygibbosum*, *F. redolens*, and *F. chlamydosporum*, have been associated with pistachio disease (Nouri *et al* 2018; Crespo *et al*., 2019), only the *F. solani*, *F. falciforme*, *F. oxysporum*, and *F. vanettenii* strains were isolated in Iraq.

Although *F. vanettenii* led to 100% seed rot and mortality, to our knowledge there is no previous report about the effect of this pathogen on pistachio in Iraq. *F. vanettenii* was found to be responsible for tomato root rot disease in India (Debbarma MSc. *et al.,* 2021). The same is true for *Xylaria* sp., which has not yet been linked to any pistachio diseases in the past, however, it was responsible for 100% of seed rot in this study. An earlier study observed that *Xylaria* sp. caused tuber rot in glory lilies (Kousalya *et al.,* 2019), and led to Taproot decline in Soybean crops (Guyer *et al*., 2020). Furthermore, *X. necrophora*, sp. nov. also led to taproot decline in soybean plants (Garcia-Aroca *et al.,* 2021).

Conclusions

In this study, several fungal pathogens were isolated from pistachio plants in Anbar Province (Kabisa, Al-Baghdadi, Al-Haditha, Anah, Rawa and Al-Qaim). A pathogenicity analysis revealed that all the isolates were pathogenic to pistachio plants. The study revealed that one isolate of *Xylaria* sp. and four isolates of the *Fusarium* (*F. solani*, *F. falciforme*, *F. oxysporum*, and *F. vanettenii*). To our knowledge, this was the first study that reported *F. vanettenii* and *Xylaria* sp. causal agents of seed rot in pistachio crops.

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Contributions of authors

H.M.S.: Idea, design the study, revising the manuscript.

K.W.T.: Setting up the experiments, Lab and field work, data collection.

T.S.R.: Drafting the manuscript, revising the manuscript critically.

ORCID

K. T.: https://orcid.org/0000-0001-7355-7326?lang=en

T. R.: https://orcid.org/my-orcid?orcid=0000-0001-5105-1594

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المسح الحقلي، شدة الإصابة، والتشخيص الجزيئي للفطريات الممرضة لاشجار الفستق الحلبي في محافظة الأنبار، العراق

خالد وليد طراد[1] ، حمود مهيدي صالح[1] ، تافكة رشيد سليمان[2]

[1]قسم وقاية النبات، كلية الزراعة، جامعة الانبار، العراق

[2]قسم وقاية النبات، كلية علوم الهندسة الزراعية، جامعة صلاح الدين، أربيل، العراق

المستخلص: أجريت هذه الدراسة في 6 مناطق مختلفة من محافظة الانبار، للفترة بين كانون الثاني - شباط 2021. تم اختيار المواقع التي تزرع فيها نسبة عالية من الفستق الحلبي، مدينة (كبيسة، حديثة، البغدادي، عنة، القائم وراوة) لدراسة انتشار الامراض الفطرية التي تصيب اشجار الفستق الحلبي وتحديد العوامل الممرضة المسؤولة عن المرض . تم الحصول على 26 عزلة فطرية مميزة، وتم اجراء اختبارات الامراضية لكل عزلة. أوضحت النتائج ان جميع العزلات المذكورة أعلاه الى حدوث تلف في الانسجة الوعائية و تعفن الجذور والبذور في شتلات الفستق الحلبي، ومع ذلك تراوحت درجة الامراضية بين عالية ومتوسطة. تم التعرف على السلالات الفطرية بمساعدة تقنية ITS (والتي تضمنت ITS1 وITS2، 5.8S rRDNA). وكانت 12 عزلة تنتمي الى *Fusarium solani* ، 10 عزلة تنتمي الى *F. falciforme* ، 2 عزلة تنتمي الى *F. oxysporum* وعزلة واحدة لكل من *F. vanettenii* و Xylaria sp. *F. falciforme* و *F. solan* اكثر الأنواع تكراراً بالتزامن مع أشجار الفستق الحلبي في محافظة الانبار. ومن بين جميع العزلات المدروسة كانت عزلات القائم والبغدادي اكثر العزلات امراضية . وتعتبرهذه الدراسة الأولى التي حددت التأثير الممرض للفطر *F. vanettenii* و*Xylaria* sp. على نبات الفستق الحلبي وكذلك تواجد *F. solani* و *F. falciforme* في العراق.

الكلمات المفتاحية : شدة المرض، *Fusarium* sp، ITS، الفستق الحلبي , *Xylaria* sp.