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IDENTIFICATION OF THE MAIN PATHOGENIC FUNGUS OF ROOT ROT OF DATE PALM OFFSHOOTS (*PHOENIX DICTYLIFERA* L.), AND THE ANTAGONISTIC EFFECT OF *TRICHODERMA HARZIANUM IN VITRO*

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A B S T R A C T

Root rot is a significant underground disease in date palms (*Phoenix dactylifera* L.) caused by pathogenic soil-borne fungus *Fusarium oxysporum*. Polymerase chain reaction (PCR) amplification was performed, followed by analysis of the sequences of nitrogen bases of the products using the program BLAST (Basic Local Alignment Search Tool). The results revealed that most pathogenic isolates were belonging to *F. oxysporum, F. proliferatum* S1*, F. proliferatum* S2*,* and *F. fujikuroi*. The selected isolates of *Fusarium* spp. were sequenced. The result of a pathogenicity assay shows that all the assayed isolated fungi were pathogenic to date palm plantlets at different percentages, and the main causative pathogenic fungus was *F. oxysporum* with disease severity (DS) of 96% and disease incidence (DI) of 96.6%. An attempt was made to find the most appropriate bioagents that can protect date palm offshoots (*P. dactylfera* L.) from certain soil-borne fungal diseases. The antifungal efficacy of *Trichoderma harzianum* was tested *in vitro*, where it inhibited mycelial radial growth of *F. oxysporum* by 87% on PDA medium. The results showed there was no reduction in mycelial growth of *T. harzianum* at all the different concentrations of NaCl tested under *in vitro* growth conditions compared with control concentrations (0 dSm).

Keywords: Date palm, root rot disease, *Fusarium oxysporum, Trichoderma harzianum*..

INTRODUCTION

Date palm trees (*Phoenix dactylifera* L.) are susceptible to a variety of fungal pathogens that cause significant reductions in the number of trees and their production. Several soil-borne fungi attack date palms and have been recorded as causal pathogens of root rot, wilt, and declining diseases, such as *Fusarium oxysporum, Fusarium solani, Fusarium moniliforume, Fusarium semitectatium, Thialoviopsis paradoxa,* and *Phomopsis phoenopsis* (Djerbi,1983; Baraka *et al*., 2011). A good approach to controlling plant diseases requires a detailed understanding of the pathogens. The genus *Fusarium* is a global saprophyte soil fungus and includes several virulent phytopathogens (Dean *et al.,* 2012). Strains of *Fusarium* spp. may become optional abiotrophic parasites under certain environmental conditions, triggering decay of the cortex, root rot, wilting, and eventually premature death (Coleman, 2016).

Date palm trees are afflicted with a variety of soil-borne pathogenic fungi that pose a threat to adult trees and offshoots, causing significant damage and production losses around the globe (El-Morsi *et al.,* 2015; Maitlo *et al.,* 2013). The pathogenic fungi *Fusarium oxysporum, F. solani, F. moniliform,* and *Rhizoctonia solani* have been isolated from young offshoots and adults of the date palm (*Phoenix dactylifera* L.) in a variety of locations (Alwahshi *et al.,* 2019; Arafat *et al.,* 2012; Baraka *et al.,* 2011). Despite the significant economic losses caused by *F. oxysporum* (Schlecht. emend. Synder & Hansen), the only way to combat the disease and reduce the significant economic losses is through disease prevention using systemic chemical fungicides and prophylactic

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treatments, both of which are limited (Chandel and Deepika, 2010). *F. oxysporum* is a fungus that lives in the soil and has various invasion mechanisms. It attacks plants by inflicting natural sores on their roots, colonizes their vessels, and eventually kills the plant (Medeiros *et al.,* 2012).

In conditions conducive to disease, such measures quickly become ineffective to regulating phytopathogens and pose serious environmental and health concerns. Recently, focus has been drawn to sustainable, healthy and environmentally friendly alternatives. Biological control agents are living microorganisms that are used to prevent the development of plant pathogens (Pal and Gardener, 2006). However, few extensive scientific studies have been carried out on the successful protection of date palm trees against *Fusarium* spp. Several fungal biocontrol agents have been described and are available for use as beneficial organisms, such as *Trichoderma* spp, *Aspergillus* spp*, Gliocladium* spp*, Candida* spp and *Coniothyrium* spp. (Naher *et al.,* 2014).

The strains of the genus *Trichoderma* spp. are adaptable, expert root colonizers, global in nature, and quickly grow in culture. Also produce a large number of green conidia and chlamydospores, which are utilized for disease management in organic agriculture. *Trichoderma* spp. have all been employed with biological controls, biofertilizers, enzyme sources, protein makers, and prevention of plant disease infections (Harman, 2000; Puyan, 2016). *Trichoderma* strains improve the growth of root and shoots system area, as well as increase the dry and shoot weight (Yedidia *et al.,* 2003). The application of the fungus *Trichoderma* in plants can prevent infection through many mechanisms. These include induced plant resistance, competition for resources and space, antibiosis, and Mycoparasitism (Dix and Webster, 1995; Maloy, 1993).

The primary aim of this study was to isolate and identify the fungal pathogens that cause root rot infections on date palm offshoots(*Phoenix dactylifera* L.), as well as *in vitro* testing of antagonism with the fungus *T. harzianum*.

MATERRIALS AND METHODS

Isolation of the associated fungi: Date palm offshoot (root) samples were collected from several nurseries in Basra Governorate, Iraq. The samples were displaying signs and symptoms of yellowing and wilting, were washed under tap water and then cut into segments (1 cm), which were sterilized by dipping in a 1% sodium hypochloride solution for 2 minutes, followed by washing with sterile distilled water several times. The segments were dried on filter paper under growth chamber conditions and transferred separately to Petri dishes containing sterilized potato dextrose agar (PDA) medium. The Petri dishes were incubated at 25°C for 5–7 days, and then the colonies of fungal growth were isolated and purified using mycelial-growth, hyphal-apex, or single-spore methods. The fungi were classified according to morphological characteristics described by Booth (1985) and Sneh *et al.* (1991), and the appearance and frequency percentage of each fungus were calculated according to the following:

$$\% \text{ Fungi appearance } = \frac{\text{number of times each fungus appears}}{\text{total number of samples}} \times 100$$

$$\% \text{ Fungal Frequency } = \frac{\text{Total number of isolates of each fungus}}{\text{Total number of isolates}} \times 100$$

The fungal isolates obtained were kept on PDA slants and maintained at 5°C in refrigerator for future analysis.

DNA extraction, PCR amplification, and DNA sequencing: The partial ITS region of each *Fusarium* isolate's DNA was extracted and amplified using polymerase chain reaction (PCR) and the universal primer pair ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) or EF1T (5′ -ATG GGT AAG GAA GAC AAG AC -3′) and EF2T (5′- GGA GGT ACC AGT GAT CAT GTT-3′) respectively (White *et al.,* 1990). PCR amplification was performed using Taq DNA polymerase (Roche, Cat. No.11146173001) with a final volume of 20 µL. The PCR reaction mixture contained 2 µ1 of 10X PCR buffer, 1 µL of each primer (10 pmol), 2 µL of dNTPs (2 mM), 3 µL of DNA template (30 ng/µL), and 1 unit of Taq polymerase. Each sample volume was adjusted to 20 µL by adding nuclease-free water. Primary denaturation at 94°C was performed for 1 minute, followed by 35 cycles of end denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, initial extension for 1 minute, and final extension at 72°C for 5 minutes. PCR-amplified products were electrophoretically separated under UV illumination on 1% agarose gel for 140 min at 80 V and 400 mA, stained with ethidium bromide, and imaged using a gel documentation method (Vilber Lourmat, Taiwan). For DNA sequencing, the PCR-amplified products were gel purified using a Favorprep PCR purification kit (Cat. No. FAGCK 001, Favorgen, Taiwan) and submitted to the

Macrogen DNA sequencing service in Korea along with the primer pair (ITS1 and ITS4). The forward and reverse primers were used to directly sequence PCR results in both directions. Using the Basic Local Alignment Search Tool (BLAST), the acquired nucleotide sequences were aligned and compared to sequences from other isolates that had already been published in the National Centre for Biotechnology Information (NCBI). The nucleotide sequences were aligned several times, and MEGA 6 software was used to create phylogenetic tree using the neighbour-joining technique (Tamura *et al.,* 2013).

Pathogenicity essay of fungi isolates: The capabilities of pathogenesis of isolated fungi were examined under the conditions of greenhouse at the Date Palm Research Centre of Basra University in the season of 2019-2020. Plastic pots (30 cm in diameter) were sterilized with formalin solution (5%) for 10 minutes. Soil was sterilized with formalin solution (5%) for 7 days and then dried for 2 weeks until the formaldehyde dissipated. The sterilized pots were filled with sterilized soil (5 kg/pot). An autoclaved millet grain medium was used to grow the tested fungi in 500-mL glasses. The inoculation was performed with discs (0.5 cm in diameter) taken from 7-day-old colonies of each examined fungal isolate, and all glasses were incubated for 15 days at 27±1°C with shaking every 48 hours. At 5% of the soil weight, the sterilized soil was separately inoculated with fungi carried on millet grains. Before planting, the pots were watered three times weekly to ensure that the fungus inoculum was evenly distributed in the soil. In each pot, three date palm plantlets (10 months old) were grown, and five pots were used as replicates. Four pots filled with non-infested soil used as controls. Three months after pot planting, the incidence and severity percentages of disease were recorded. The pathogen was re-isolated from plants that showed symptoms of disease and contrasted with the initial culture used. Foliage signs were recorded and assessed on a scale of 0–4 according to the percentage of vegetation affected: 0 = healthy, 1 = less than 25% diseased foliage (symptoms are mild); 2 = 26–50% diseased foliage (symptoms are moderate); 3 = 51–75% diseased foliage (severe symptoms); 4 = over 76% diseased foliage (transplants are dead or near to death). The format of Liu *et al.* (1995) was used to calculate the disease severity (DS) as DS = d/ (dmax n) × 100, where d is the disease ratio of each transplant, dmax is the maximum disease rating, and n is the total number of assayed transplants in each replication.

***In vitro* antagonistic assay:** An experiment was performed to examine the antagonistic relationship between the pathogenic fungus *F. oxysporum* and the biological agent *T. harzianum. F. oxysporum* was identified using molecular methods focused on sequencing amplification products of the ITS regions by PCR with accession number MK751702.1. The relationship was examined using the technique of dual culture described by Garrett (1956) with a few modifications. The bio agent fungus *T. harzianum* was isolated and purified by BioHealth WSG Administration and Distribution (Humintech GmbH, Heerdter LandstraBe189/D*D40549Dusseldorf/GermanyE-Mail: info@humintech.com; Internet: www.humintech.com). Discs (0.5 cm in diameter) from mycelial plugs were cut using a cork borer from active culture plates of both *F. oxysporum* and *T. harzianum* and placed on opposite sides in a Petri dish containing sterilized PDA at a distance of 3 cm from each other. The Petri dishes were incubated at 27°C. In control, a disc of *F. oxysporum* (0.5 cm) was placed in a dish containing sterile PDA. The mycelial growth was recorded after 48, 72, and 96 hours, and the inhibition percentage was measured. The experiment was done in five replicates, and the inhibition percentage of the diameter of the growing colony was calculated as:

$$\% \text{ Inhibition } = \frac{(C - T)}{C} \times 100$$

where C is the diameter growth of the control colony, and T is the diameter growth of the pathogen colony with antagonism in dual culture(Garret,1956).

Tolerance assay of *Trichoderma harzainum* in PDA with various concentrations of NaCl: *T. harzainum* was cultured at 25 °C for 5 days in PDA. Mycelium discs (0.5 cm) were obtained from the colony edge of the PDA plates and inoculated into new PDA, amended with various concentrations of NaCl (0, 10, 15, 18, 21, 24, 27, and 30 dSm-1). The plates were incubated at 25 °C. The diameters of the colonies were measured daily during culturing. Each concentration (treatment) contains five replicates. The salt tolerance ability of *T. harzainum* isolates was evaluated as the percentage inhibition of mycelial growth(IMG) calculated by:

$$\% \text{ IMG } = \frac{(C - N)}{C} \times 100$$

where N = radial mycelial growth on PDA with NaCl, and C = radial mycelial growth on NaCl-free medium (0 dSm^{-1}).

STATISTICAL ANALYSIS

Analysis of variance was conducted using MSTAT-C software version 2.10 (1991). The least significant

difference (LSD) was estimated at $p \leq 0.05$.

RESULTS

Percentage of appearance and frequency of the isolated fungi: Most of fungal isolates were identified based on descriptions of colony morphology, conidiophores, and conidia(Figure 1).The data in Table (1) show that the pathogenic fungus *F. oxysporum* had the highest percentage of appearance and frequency, reaching 100% and 41.46%, respectively. In contrast, the lowest percentages of appearance and frequency of isolation were recorded for *Thielaviopsis paradoxa* (39 and 7.3%, respectively).

Table 1. Appearance and percentage of frequency of isolation fungi from the roots of date palm offshoots located in Basra Governorate.

Isolated Fungal Pathogens	Fungi Isolates		
	No. of samples	% appearance	% Frequency
F. oxysporum	120	100	41.463
F. proliferatum S1	100	83.3	24.391
F. proliferatum S2	85	71	14.634
R. fujikuroi	80	66.6	12.195
T. paradoxa	46	39	7.317
No. of total samples	120	-	100

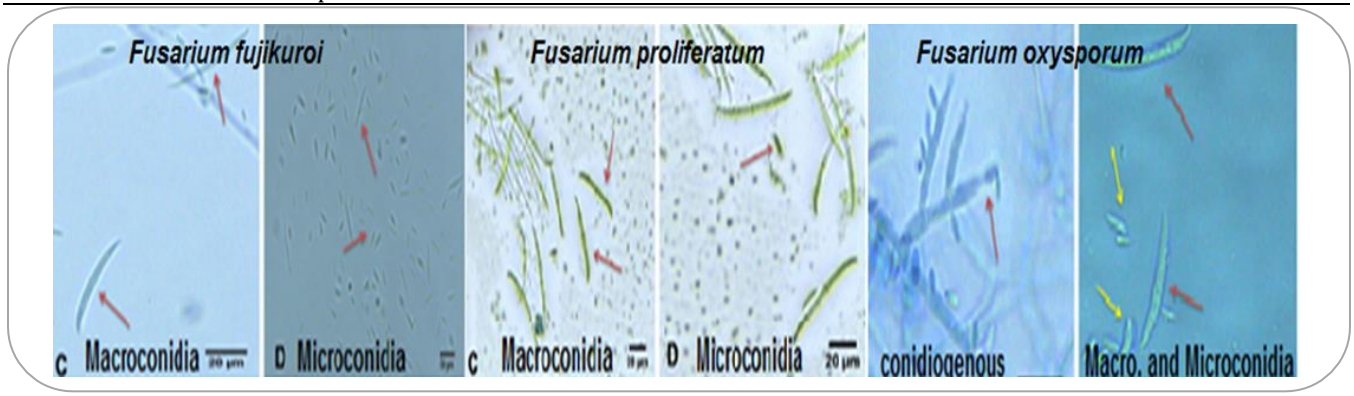

Figure 1. The descriptions of colony morphology, conidiophores, and conidia of the most isolated pathogenic fungi(*Fusarium oxysporum, F. proleferatum, F. fujikuroi*)

Pathogenicity assays: The results of isolation experiments from the infected samples of date palm offshoots showed that the fungi *F. oxysporum, F. proliferatum S1, F. proliferatum S2, F. fujikuroi, and T. paradoxa* were the main causal pathogens in date palm offshoots growing in the Basra Governorate. These infected offshoots showed exemplary symptoms of root rot and wilt diseases. Data in Table (2) showed that all the isolates of assayed fungi were pathogenic to inoculated plantlets. Isolates of pathogenic fungi slightly differed in their capability of establishing disease and the appearance of disease symptoms. The pathogenic fungus *F. oxysporum* had the highest disease rating and disease severity (DS) of the percentage of root rot of date palms (4–96%), followed by *F. proliferatum* S1 (3–37%) and *F. proliferatum* S2(3–31%). Similarly, *F. oxysporum* caused a high incidence of root rot symptoms on date palm plants (66.66%). However, *T. paradoxa* showed the lowest disease severity of 8.33% for root rot diseases and had a disease rating scale of 2.

The morphological symptoms seen in Figures 1 and 2 are typically highly obvious on date palm offshoots.

Table 2. Pathogenicity assay of fungal pathogens on date palm plantlets under greenhouse conditions after 90 days post-inoculation. (D = disease degree; DSI = disease severity index; DI = disease incidence).

Pathogens	Disease severity and disease incidence of root rot disease				
	D	DSI	No. of tested plants	No. of infected plants	DI%
F. oxysporum	4	96	12	12	100
F. proliferatum S1	3	37.5	12	6	50
F. proliferatum S2	3	31.15	12	5	41.66
F. fujikuroi	2	12.5	12	3	25
T. paradoxa	2	8.33	12	2	16.66
Control	-	-	12	0	0

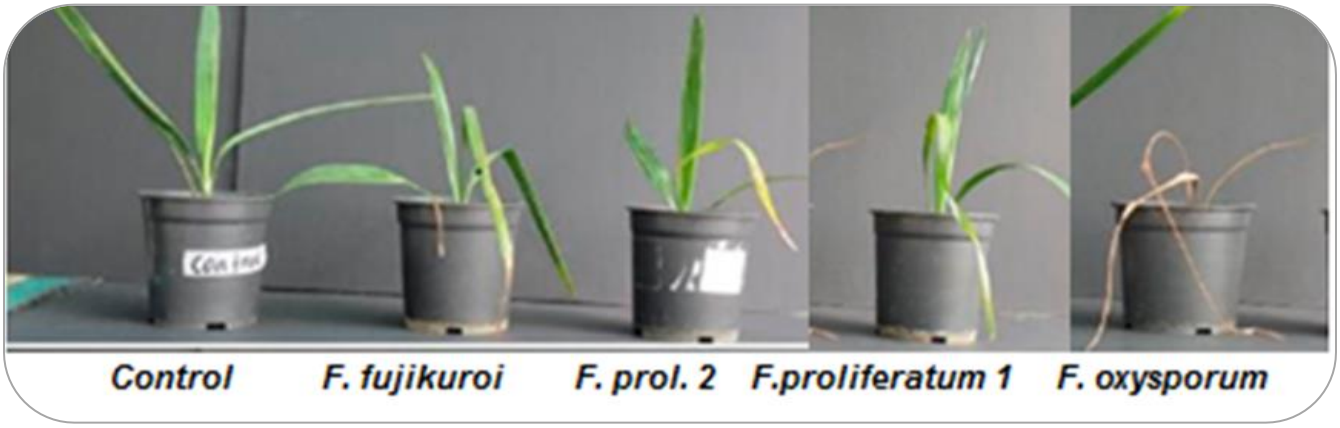

Figure 2. Typical symptoms of the root rot disease on date palm offshoots treated with pathogenic fungi isolates and control.

Figure 3. Typical symptoms of the root rot disease on the roots of date palm offshoots.

Molecular identification of the fungal pathogen: The results showed that the identified isolates belong to fungi *F. oxysporum, F. proliferatum* S1, *F. proliferatum* S2, and *F. fujikuroi*. The sequences of nitrogen bases obtained from the identified fungal isolates were compared with those available at the NCBI. The identified sequences of these fungi have been deposited in the Gen Bank database (NCBI) with PCR amplification and nucleotide sequencing of the ITS region of fungal isolates: MK751702.1 for *F. oxysporum*; LT970774.1 for *F. proliferatum* S1; KX582247.1 for *F. proliferatum* S2; and KY29366.1 for *F. fujikuroi* (run ends: 20/4/2019).Figure (2)describes the DNA products amplified by polymerase chain reaction(PCR)from Fusarium isolates (*F. oxysporum* (10), *F. proliferatum* S1(1), *F. proliferatum* S2 (2), and *F. fujikuroi* (13).

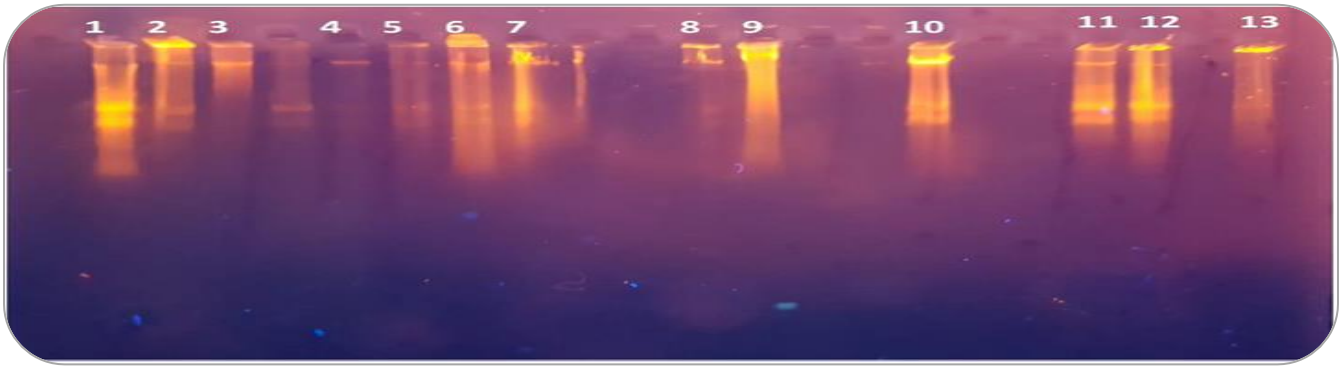

Figure 4. DNA products amplified by polymerase chain reaction (PCR) from Fusarium isolates (*Fusarium oxysporum* (10),*F. proliferatum* S1(1),*F. proliferatum* S2 (2),and *F. fujikuroi* (13).

***In vitro* antagonistic assay:** The *in vitro* experiment was carried out based on the biological evidence obtained thus far to gain the best understanding of the ability of competition and biocontrol capacity of *T. harzianum* against *F. oxysporum*. In dual-culture tests on petri dishes, *T. harzianum* quickly stopped the mycelial growth of the

fungus pathogen *F. oxysporum* and fully covered the colony of *F. oxysporum*. *T. harzianum* showed faster mycelial growth than *F. oxysporum*, and a wide area of the culture medium was colonized (Figure 5 A). A thin pre-contact zone of growth inhibition for *T. harzianum* could be observed for several hours just prior to physical contact between hyphae after 48 hours of growth. This initial partnership between *F. oxysporum* and *T. harzianum* can include both defensive and offensive fighting by both species. This shows that their interaction is possibly related to the production of inhibitory organic compounds like diffusible and non-volatile compounds. Such secondary metabolites or antibiotics can be introduced with the activity of cell metabolism correlating to development and expansion at a short distance. However, it can be fairly hypothesized that *T. harzianum* is able to detoxify them easily. Indeed, the hyphal development of *F. oxysporum* definitively stopped and reached an occupancy of 65% relative to the control (Figure 5 B). After this short 72-hour period of incubation with cross-signalling by pre-contact chemical dialog, *T. harzianum* was able to invade the inhibition zone and interfere with the colony of *F. oxysporum* between. Furthermore, after 96 hours, *T. harzianum* quickly showed intense sporulation at various points of its hyphal expansion, including the colony of *F. oxysporum* (Figure 5 C).

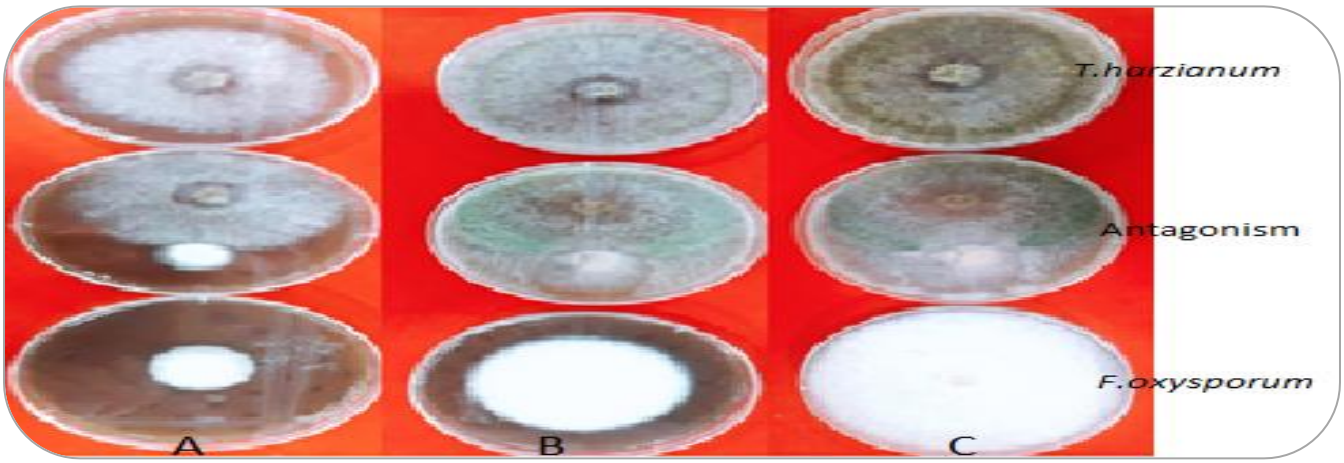

Figure 5. Antagonistic potential of *T. harzianum* isolate against *F. oxysporum* in dual culture at different periods of time on potato dextrose agar (PDA) medium. A: After 48 hours, B: After 72 hours, and C: After 96 hours of antagonism (each culture plate represents the mean of four replicates for each treatment).

Effects of various NaCl concentrations on colony growth of *T. harzianum* isolates: It is mandatory to assay the *T. harzianum* isolate's ability to tolerate different NaCl concentrations as a prerequisite before the application of this isolation in saline areas with the plants to improve their physiological properties. In Figure (6), the results obtained from the mycelial growth of *T. harzainum* are shown at different NaCl concentrations. As expected, there was no inhibition of mycelial growth at all the different NaCl concentrations tested under *in vitro* growth conditions compared with control (0 dSm).

Figure 6. The Effect of different concentrations of NaCl on the radial mycelial growth of the fungus *T.harzianum* on PDA medium at 28 C in the laboratory (Each culture plate represents the mean of four replicates for each treatment).

DISCUSSION

Percentage of appearance and frequency of isolated fungi: *F. oxysporum* is a significant and diverse phytopathogenic fungus that infects approximately 150 plant species through a variety of infection methods. *Fusarium* species can be found as parasites, endophytes, or saprophytes on plants, in soil, and in water in both agricultural and natural settings (O'Donnell *et al*., 2000). Consistent with other studies, the results of our study showed that the root rot disease of the date palm is caused by various pathogenic fungi, such as *F. oxysporium, F. ptoliteratum, F. fujikuroi, F. moniliform, T. paradoxa,* and *Rhizoctonia solani* (Abedalrad, 2019; Ahmed, 2018).

Pathogenicity assays: The pathogen variations observed may be related to date palm roots producing various exudates that cause an increase in the number of saprophytic fungi around the roots, which are affected by soil-borne fungal pathogens (Xue *et al*., 2011). The ability of the fungus pathogen *F. oxysporum* to cause disease in the plant is related to many mechanisms by which the pathogen works, such as the production of types of mycotoxins like trichothecene, fusaric acid, and enzymes, such as cell wall enzymes. A few virulence factors also degrade many metabolic compounds produced inside the host plant or pathogenic factors related to a specific feature function, such as siderophores, amino acids, and osmolites (Rana *et al*., 2017). In all Fusarium genomes, the genes that encode for the enzymes that degrade cell walls and hydrolytic enzymes are important in enhancing access to nutrients during infection (IIgen *et al*., 2008). According to our findings, the most pathogenic fungi were *F. oxysporum, F. proliferatum, F. fujikuroi, F. moniliforme,* and *T. paradoxa,* which were responsible for the incidence of root rot diseases in date palms. These findings are supported by those of Ahmed (2018), who discovered *Fusarium* spp. when isolating fungi from date palm roots. Also, El-Morsi *et al*.(2015) showed that *F. oxysporum* was the major pathogenic fungus when they measured percentage root rot/wilt severity (89.26%), followed by *F. solani* and *F. moniliforme*, which caused 82.18% and 73.26% disease severity, respectively.

Molecular identification of the fungal pathogen: Although the use of characteristics can lead to accurate results on occasion, many researchers do not rely on them because they require extensive experience in the subject of categorization, especially when dealing with fungus groupings that are closely related, such as *Fusarium* spp. Identification at the genome level by PCR has led to precision, sensitivity, and the ability to classify genetic variations and remove the drawbacks of traditional approaches in the identification of many species (Abedelrad, 2019; Stanis *et al*., 2016).

***In vitro* antagonistic assay:** Many mechanisms of action have been identified in the *Trichoderma* genus in relation to their ability to act as biocontrol agents against fungi. This antagonist may result in mycoparasitism, antibiosis, competition for nutrients and space, and cell-wall degrading enzymes (Kumar, 2013). Furthermore, the findings observed may be caused by different pathogens having distinct defence mechanisms against enzymes and toxic substances produced by various bioagents (Ahmed, 2013). *Trichoderma* secretes certain chemical metabolites that may be responsible for their inhibitory or antagonistic action *in vitro*, such as phenols, steroids, flavonoids, quinines, terpenoids, peptides, cytocatalasins, alkaloids, and phenylpropanoids (Mausam *et al*., 2007). In addition, through mycoparasitism, *Trichoderma* spp. mycopathogens antagonize other fungi as they weaken their cell walls by developing lytic enzymes such as chitinases, peroxidases, and glucan 1-3 B-glucosidases (Ahmed, 2013; Muthu *et al.,* 2006; Sood *et al*., 2020). The ability to develop quickly gives the antagonist a significant advantage over other fungi in terms of space competition, nutrients, and outperformance, all of which are equally significant phenomena with shared inclusion (Benitez *et al*., 2004). Our finding of antagonism inhibiting growth is consistent with the findings of Rudresh *et al*. (2005), who discovered that *T. harzianum* and *T. viride* inhibited the growth in vitro of two pathogens, *R. solani* and *F. oxysporum*, by 72.1 and 77.0%, respectively. This was achieved by producing antifungal metabolism and strong mycoparasitic activity due to completely overgrowing competing mycelia.

Effects of salinity on colony growth of *T. harzianum* isolate: Soil microbial communities have an important role in food cycling, organic matter volume, and plant productivity. According to Zahran (1997), filamentous fungi are extremely resistant to hydric stress and the increased pressure of osmotic stress and may change their physiology and appearance as a result. Killham (1994) described two microorganism adaptation methods to osmotic stress. Both result in the assembling of solutes in the cell to counterbalance the increment in osmotic pressure and the selective prevention of the amalgamated solutes (Na, Cl^-), resulting in the accumulation of the ions required for metabolism.

Current study is supported by Kumar *et al*. (2016), who examined the tolerance of 70 *Trichoderma* spp. isolates to various NaCl concentrations. The results revealed that 92.8% (65) of the isolates could withstand a NaCl concentration of 5%. While 45.7% (32) of the isolates were able to withstand 10% of the NaCl concentration when the concentration was increased further. Poosapati *et al.*, (2014), found that all the tested *Trichoderma* isolates were significantly tolerant with the highest mean colony growth at all saline concentration rates except the 1M concentrate of NaCl which had a 65-70% reduction in colony growth.

CONCLUSION

PCR was used in this analysis to diagnose *Fusarium* spp. isolates because of its high precision in the diagnosis of many species, which was useful for detecting *F. oxysporum* in infected date palm offshoots quickly, sensitively, and consistently. The pathogenicity test of the fungal isolates found that the pathogenic fungus *F. oxysporu*m had the highest disease severity (DS) and disease incidence (DI) in cases of root rot of date palm. *In vitro*, the findings of *T. harzianum* antagonism showed the greatest percentage of mycelial growth suppression of *F. oxysporum.* Nevertheless, a more comprehensive study is needed to confirm the potential of these organisms for the biological control of pathogenic fungi and regulating plant growth, which may be beneficial in the future as a biological agent.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Contribution of Authors:

Naji S. Jassim	:	Conceived the idea, planned the experiments, performed the all experiments, did computational analysis of the results, and finally prepared and wrote the manuscript.
Mahmood O. Jaafer	:	Assisted in computational analysis of the results and also helped in writing and reviewing the manuscript.