Morphological and molecular Identification of the causal agent of Fusarium wilt disease on Okra and Control it using some *Trichoderma* B io-agents

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Abstract

The study included a field survey to determine the prevalence of fusarium wilt disease on okra in different regions of Najaf, Diwaniyah, and Babylon. It was found that the infection rate in the areas under study was between 18 and 73%. Results of isolation and phenotypic characteristics of wilt-infected okra plants recorded six fungal isolates belonging to *Fusarium* spp. Also, three isolates of *Trichoderma* spp. were isolated from the soil surrounding the roots of intact okra plants. The efficiency of biological fungus isolates was studied in addition to a fourth isolate which previously identified as *Trichoderma longibrachiatum* against the pathogenic fungus. The isolated fungi varied in some phenotypic characteristics regarding color variation and growth rate. The results of testing the pathogenicity of the pathogenic *Fusarium* spp. isolates on okra in a pot experiment showed that isolate F2 (Isolate Abbasia) was the most pathogenic, used in subsequent studies. The results of the molecular diagnosis using Polymerase chain reaction (PCR) showed that the F2 isolate belongs to the fungus *Fusarium solani*, which was registered in the Gen Bank under the number OQ729824 Isolate T3 of the biological fungus *Trichoderma* spp., belonging to the fungus *Trichoderma harzianum*, was registered with the accession number OQ729823. The isolate T3 *T. harzianum* showed high antibody efficiency against *F. solani* on P.D.A culture media with an inhibition rate of 82.8% in the test of fungal isolates' filters. Biologically, isolate T3 gave the highest inhibition in the radial growth rate of *F. solani* compared to other isolates T1, T2, and T4 *Trichoderma* spp. In the plastic pot experiment, the isolate of the biologically resistant fungus *T. harzianum* T3 led to the highest growth indicators of okra plants compared to other biological isolates and worked to protect okra plants from fusarium wilt disease, as no symptoms of the disease appeared on okra plants in the treatment of this isolate compared to that. By treating the pathogenic fungus *F. solani*, the infection intensity reached 60%.

Keywords: *Trichoderma* spp.**,** fusarium wilt**,** biocontrol**,** okra

Introduction

Okra, *Abelmoschus esculentus* L, belongs to the Malvaceae family, and it is a widespread crop in different regions of the world, as the annual global output of okra is about 6,000,000 tons annually (10). It is considered one of the important green crops that are grown in hot and warm regions in Asian and African countries (14). Many areas of okra cultivation suffer from low crop productivity due to infection with all pests and pathogens, the most important of which is *Fusarium* spp. The causative agent of fusarium wilt. Fusarium wilt diseases are among the destructive diseases that affect different plant hosts, which appear most often in the form of rapid or gradual wilting of the leaves and branches of affected plants, ending with the death of the entire plant, causing a reduction in the number of plants and loss of production (12). For controlling these diseases, many methods have been used, the most important of which is chemical control, as it gives quick and guaranteed results. However, the excessive and indiscriminate use of pesticides and non-compliance with the recommended instructions led to negative effects on human health and the environment in general (24 and 29). Therefore, the researchers resorted to using soil revival to control the pathogens, foremost of which are species belonging to the fungus *Trichoderma* spp. It is a widespread soil fungus (13). The fungus species *Trichderma* spp. Effective bio-agents for fungal disease control that are safe, environmentally friendly and widely used against fungal pathogens including *Fusarium* spp. (19). This study was selected due to the importance of *Fusarium* spp. and the significant losses it causes on okra yield. Therefore, the study aimed to isolate the fungus that causes fusarium wilt disease, diagnose it phenotypically and molecularly, and use the biological control agent *Trichoderma* spp. to control fusarium wilt disease on okra.

Materials and methods

The study included a field survey of Fusarium wilt caused by the pathogenic fungus *Fusarium* spp. The obtained isolates of *Fusarium* spp. The biological fungus *Trichoderma* spp. phenotypically and molecularly. The pathogenicity of the pathogenic fungus *Fusarium* spp. isolates was also tested, and the antigenic ability of the *Trichoderma* spp. isolates was tested on P.D.A culture medium. In addition to testing the effect of *Trichoderma* spp. filtrate on the growth of the pathogenic fungus *Fusarium* spp. on PDA culture medium.

Field survey

The field survey covered 10 sites/fields in three governorates: Najaf (Al-Hira, Al-Manathira, Al-Abbasiya and Bahr Al-Najaf), Diwaniya (Ghammas, Shafia and Al-Shamiyya) and Babel (Markaz, Al-Kifl and Al-Nil). Samples were collected randomly from each site, and the infected okra plants were observed in each field through morphological and anatomical wilting symptoms after making a longitudinal section of the stem of the infected plants. The infection percentage was calculated. Also, samples of healthy okra plants were collected with the soil surrounding the roots for the purpose of isolating the fungi. The isolation of wilt-causing fungi and biotrophic fungi in

plants and for each sample was carried out on the next day of sampling.

Isolation, purification and identification of wilt-causing fungi on okra plants

Infected parts were cut into small pieces, sterilized with 1% sodium hypochlorite (NaOCl) solution, and 4 pieces were transferred to a sterile petri dish containing sterile P.D.A culture medium. The plates were incubated at 25 ± 2°C for 2-4 days, after which the fungi were purified by taking a small piece from the edge of the colony and placed in the middle of a plate containing the same culture medium (P.D.A). The plates were incubated for a period of 5-7 days in the laboratory of plant pathology / College of Agriculture / University of Kufa, and the isolated fungi were diagnosed outwardly "to the level of sex through the nature of the growth of the colonies and the types, shapes and colors of the spores that they form and depending" on the diagnostic characteristics mentioned by Lesli and Summerel (18).

Isolation, purification and identification of biocontrol fungi used in the study

Four isolates of the biological control fungus *Trichoderma* spp. were used in this study. Three of them, T1, T2 and T3, were isolated from the soil surrounding the roots, while the fourth isolate, T4, *T. longibrachiatum*, previously identified, was obtained from the Graduate Studies Laboratory in the College of Agriculture / University of Kufa. The three isolates were isolated and purified using a soil dilution method, as 1 mL was transferred to a Petri dish with the addition of 20 ml of P.D.A. culture medium. Then the dishes were incubated at 25 ± 2°C for 2-3 days, and purification was carried out as previously. Fungi were diagnosed at the genus level by the nature of growth and color of the colonies depending on the taxonomic key (16).

Pathogenicity test of wilt-causing fungi on okra plants in plastic pots

The experiment was carried out using soil previously cultivated with okra crop, which was sterilized by autoclave twice (8). The inoculum of each fungus, isolated and loaded on millet seeds, was added at a rate of 1g/150g of sterilized soil and placed in plastic pots (1 kg/pot) (15) for 3 replicates for each treatment and watered with caution. The soil of the control treatment without contamination with fungi. After three days, all the pots were planted with local okra seeds (10 seeds/pot) surface sterilized with sodium hypochlorite (NaOCl) solution at a concentration of 1%, and the pots were placed under the conditions natural and watered as often as needed. The same experiment was carried out with the same steps without soil sterilization. The percentage of germination of seeds and dead seedlings was calculated after 10 days of sowing. After 20 days of cultivation, the plants were thinned to three plants per pot. The average measurements were taken that included shoot length, fresh and dry shoot weight, and the severity of infection was calculated according to the pathological index consisting of four degrees, where 0 = healthy plants, 1 = appearance of pallor and yellowing on the plant, 2 = temporary wilting of plants, 3 = permanent wilting (1). The percentage of infection severity was also calculated by Mckinney (20).

Molecular identification

The molecular diagnosis of the fungi isolated in this study was carried out using the polymerase chain reaction (PCR) technique (Table1).

Table 1. PCR primers used for diagnosing fungi under study

Primer	Sequence 5 ⟶ 3		PCR product size
ITS1	F	TCC GTA GGT GAA CCT GCG G	550-600 bp
ITS4	R	TCC TCC GCT TAT TGA TAT GC	

DNA sequence analysis of fungi isolated in this study

For diagnosing the isolated fungi, the PCR products of the fungal isolates with primers ITS1 and ITS4 were sent to Macrogen (South Korea) to determine the nucleotide sequence of the duplicated DNA products in both forward and reverse directions. All nitrogenous bases sequences were analyzed by BLAST program (Basic Local Alignment Search Tool) and the results were compared with globally characterized data for the same fungus at the National Center for Biotechnology Information (NCBI). Phylogenetic trees analysis was performed using MEGAX software (17).

Antagonism of *Trichoderma* spp. to the pathogenic *F. solani* F2 on P.D.A

The antagonistic ability of the four isolates of *Trichoderma* spp., against the pathogenic fungus *F. solani* F2 (Abbasia Isolate), was tested using the dual culture technique with 3 replications for each treatment, compared to the growth of the pathogenic fungus alone as a control treatment. All plates were incubated at 25±2°C until the growth of the control pathogenic fungus reached the edge of the plate, where the percentage of inhibition was calculated (27).

Effect of *Trichoderma* spp. and *F. solani* filtrate on *F. solani* growth on P.D.A. medium. A sterile Potato Sucrose Broth (P.S.B) medium was prepared, distributed in 350 ml glass flasks (250 ml/flask), each flask was inoculated with four discs of 0.5 cm in diameter (P.D.A) for an isolate of the fungus *Trichoderma* spp. The four *F. solani* isolates or isolated individually. The flasks were incubated at 25±2°C for 28 days with a shaker at 150 cycles/min to ensure the distribution of the fungi. Then, the fungi were filtered using sterile filter paper, passing the filtrate through a 0.22mm Millipore. 5mL of each filtrate was added. With 15 mL of culture medium (P.D.A) in Petri dishes with 3 replicates of the filter of each fungus, only culture medium (P.D.A) as control treatment. The dishes were inoculated with a disc of 0.5 cm of *F. solani*, incubated at 25 ± 2°C for 9 days, after which the radial growth rate was calculated (30 and 28). Effect of *Trichoderma* spp. isolates on *F. solani* (Abbassia isolate) and growth indicators of okra in plastic pots

The experiment was carried out in sterile and non-sterile soil by double contamination in plastic pots (1 kg/pot). Inoculate each pot with *Trichoderma* spp. With the isolation of the pathogenic fungus *F. solani* F2 loaded on millet seeds by 1g/150g sterilized soil with 3 replications, 3 replications of *F. solani* positive control only and 3 replications of *F. solani* soil only as negative control. Pots were watered, left for 3 days, then planted with 3 local okra seeds (superficially sterilized). After germination, seedlings were thinned to one plant where pots were distributed randomly under ambient conditions with irrigation as needed. 30 days after inoculation, plant growth indicators including shoot length, and shoot fresh and dry weight were calculated, as well as the percentage of infection severity was calculated as previously mentioned.

Experimental design and statistical analysis

The laboratory experiments were implemented according to the completely randomized design (C.R.D) while for the field experiments, the Randomized Complete Block Design (R.C.B.D) was used. The data were analyzed using the GenStat statistical analysis computing program and the Excel program in displaying results graphs. Averages between treatments were compared using the least significant difference L.S.D under the 5% probability level (3).

Results and Discussion

Field survey

The results showed (Fig. 1) the prevalence of fusarium wilt disease on okra plants in the surveyed fields. The percentage of infection ranged from -18 to 73%. The highest infection was recorded in Ghama's fields, while no infection was recorded in the fields of Al-Hira, Al-Shafia, Al-Shamiya, and Hilla Center. The spread of the disease in the areas where the infection appeared may be due to the repeated cultivation of the okra crop annually or the cultivation of other crops belonging to the same hibiscus family in the same fields. This led to the accumulation of fungal pollen, which remains in the soil for long periods, in addition to the appropriate weather conditions. The low infection rates in some fields, the reason may be due to the cultivation of the okra plant for the first time in the surveyed fields (11 and 15).

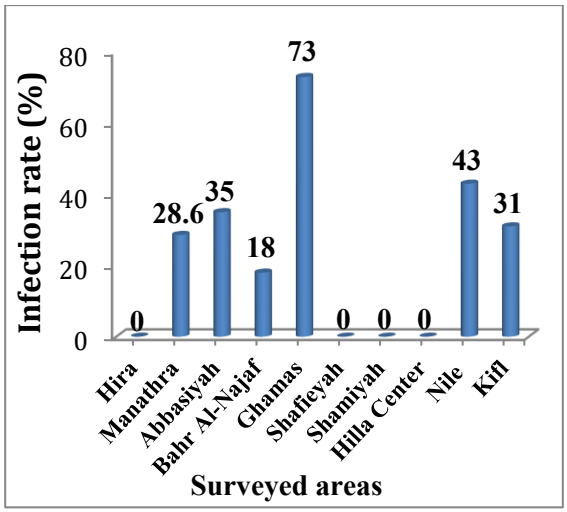


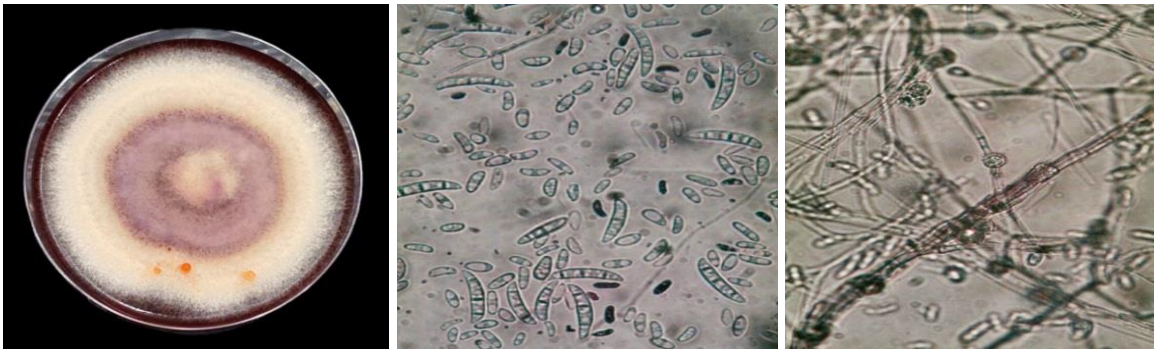
Figure 1. The rate of infection with fusarium wilt disease on okra plants in the surveyed fields in the provinces of Najaf, Diwaniyah, and Babylon

Isolation, purification and identification of wilt-causing fungi on infected okra plants

The results of isolation, purification, and microscopic examination showed that there were six fungal isolates that were initially diagnosed based on some phenotypic

characteristics (18). it was also observed that the isolates produced pigments that spread in the growth medium. All the isolates belonged to the genus *Fusarium* spp., which is distinguished by having septate hyphae and conidia, including macroconidia, microconidia, and chlamydospores.

It can be noted from the results (Table 2) that the isolate of the pathogenic fungus F4 (Ghammas isolate) led to the highest reduction in seed germination, as it recorded a rate of 68.3% compared to the rate of the control treatment that led to 91.7%, while no differences were recorded for the soil type in Reducing the germination percentage of okra seeds. Generally, the two fungus isolates F3



A B C

Figure (2) Characters of the *Fusarium* fungal colony Abbasia isolate F2. A) *F. solani* fungal colony, B) A microscopic picture of the macroconidia and Microconidia formed by the fungus, C) Chlamydospores

Phenotypic and microscopic diagnosis of the bio-control fungi used in the study

The results of isolation and purification from the soil surrounding the roots of healthy okra plants showed that three biological isolates were obtained, and they were diagnosed at the level of sex depending on phenotypic and microscopic characteristics through the nature of colony growth and their colors and according to the taxonomic key mentioned by Jaklitsch and Voglmayr (16), where the results showed that all Fungi belong to the genus *Trichoderma* spp.

Pathogenicity of *Fusarium* spp. isolates

(Bahr al-Najaf isolate) in sterile soil and F4 (Ghammas isolate) in non-sterile soil were the strongest in reducing the percentage of seed germination to 66.7% with a significant difference from some interactions. As for the effect of the fungus on Seedling death, the results showed that the fungus isolate F2 (Abbasiya isolate) led to the highest percentage of seedling death 22.2% with a significant difference from the control treatment in which no seedling death was recorded. No significant effect of soil sterilization was observed on the percentage of dead seedlings. But the highest percentage of seedling death was recorded in the treatment of the fungus F2 isolate in the non-sterilized soil and

recorded a 25% significant difference from the rest of the interactions.

The F2 isolate was the most aggressive in reducing the growth indicators of the plants under study with a significant difference from the uninoculated control treatment. The rates of plant height and fresh weight did not differ between the two types of sterilized and non-sterilized soils, while soil sterilization decreased the mean dry weights of okra plants with a significant difference from the non-sterilized soil. The average weights were 0.08 and 0.09 gm, respectively. On the other hand, the highest infection severity of 68.2% was recorded in the F2 fungus treatment, with a significant difference from the rate of the control treatment. The infection intensity increased to 77.6%, especially with F2 isolate in sterile soil, with a significant difference from non-sterile soil. The cause of seed rot, seedling death, and reduced growth indicators in plants infected with the pathogenic fungus *Fusarium* spp. results from the effect of the fungal mycelium of the pathogen that penetrates the plant tissue, turning the tissue brown. This is because these fungi secrete enzymes and toxins that affect and break down plant roots, which reduces the absorption of nutrients or kills seed embryos preventing them from germinating (22 and 25). The difference in the severity of infection with fungal causes may be due to the quantity and quality of substances, toxins, or enzymes produced by these fungi, and these substances disrupt the defense mechanisms in the plant (21). Stępień and Chelkowski (26) explained that the reason for the variation and difference in the pathogenicity of fungal isolates of *Fusarium* spp. may be due to genetic differences between isolates. This difference is generally in the ability to produce some substances such as lysis enzymes and toxins, which help to penetrate the plant host cells and cause infection. Based on the findings of the results of this experiment, the fungus F2 isolate (Abbasiya isolate), the most pathogenic, was chosen for the subsequent experiments.

Molecular identification of *Trichoderma* sp. and *Fusarium* sp. isolates

The results of DNA extraction from *Fusarium* sp. (F2) and *Trichoderma* sp. (T3) and subjection to the polymerase chain reaction (PCR) using the forward (ITS1) and reverse (ITS4) primers showed the possibility of DNA amplification (PCR-amplified product) with a size of about 550-600 base pairs (bp).

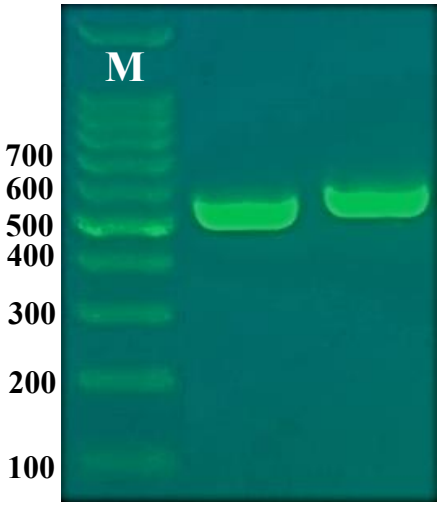


Figure 3. Amplified PCR products from fungal isolates (*Fusarium* sp. F2 and Trichoderma sp. T3) isolated from okra plants. M: ladder of the nucleic acid (Molecular-weight size marker) with the number of nitrogenous

base pairs (bp), the sizes of each of them fixed on the left

Nucleotide sequence analysis of the amplified DNA product of the fungi isolated in this study, using the BLAST program for comparison with the data available in the National Center for Biotechnology Information (NCBI), showed that isolate (F2) belongs to the fungus *Fusarium solani*, and isolate (T3) belongs to *Trichoderma harzianum*. A 100% similarity was found in the nitrogen base sequences of the genetic region (ITS1 and ITS4) of the fungi isolated in this study and other isolates of the same fungi previously registered at the National Center for Biotechnology Information (NCBI). The isolates were registered in the Gen Bank database under registration numbers. for each isolate, as shown in Table (3).

Table 2. Pathogenicity of *Fusarium* spp. isolates on okra plants in plastic pots

Measurements		Treatment								
	Soil	Control	F1	F2	F3	F4	F5	F6	Soil type effect	L.S.D. ($P \leq 0.05$)
Seed germination (%)	Sterile	96.7	70.0	70.0	66.7	70.0	76.7	73.3	74.7	Soil 6.97
	Unsterile	86.7	70.0	70.0	73.3	66.7	70.0	70.0	72.3	Fungi 13.04
Average		91.7	70.0	70.0	70.0	68.3	73.3	71.6		Interaction 18.44
Seedlings death (%)	Sterile	0.0	0.0	19.4	0.0	14.2	12.1	0.0	6.5	Soil 6.24
	Unsterile	0.0	4.7	25.0	11.3	9.5	4.7	4.7	8.5	Fungi 11.68
Average		0.0	2.3	22.2	5.6	11.8	8.4	2.3		Interaction 16.52
Plant height (cm)	Sterile	8.63	5.83	4.23	6.40	5.76	5.80	5.83	6.06	Soil 0.551
	Unsterile	8.30	6.13	5.50	6.93	6.30	6.77	6.23	6.59	Fungi 1.030
Average		8.46	5.98	4.86	6.66	6.03	6.28	6.03		Interaction 1.457
Shoot FW (g)	Sterile	1.30	0.66	0.56	0.83	0.66	0.83	0.76	0.80	Soil 0.0904
	Unsterile	1.26	0.76	0.66	0.83	0.70	1.03	0.93	0.88	Fungi 0.1691
Average		1.28	0.71	0.61	0.83	0.68	0.93	0.84		Interaction 0.2392
Shoot DW (g)	Sterile	0.13	0.07	0.06	0.09	0.07	0.09	0.09	0.08	Soil 0.00980
	Unsterile	0.12	0.08	0.07	0.10	0.08	0.11	0.10	0.09	Fungi 0.01833
Average		0.12	0.07	0.06	0.09	0.07	0.10	0.09		Interaction 0.02592

Infection severity (%)	Sterile	0.0	34.1	77.6	66.2	65.0	56.4	51.9	50.1	Soil 3.79
	Unsterile	0.0	56.8	58.9	19.4	26.0	36.5	33.3	32.9	Fungi 7.08
Average		0.0	45.4	68.2	42.8	45.5	46.4	42.6		Interaction 10.02

*Values are means of 3 replications

Table 3. The fungal isolates diagnosed in this study and recorded in the Gen Bank database with their serial codes

	Fungus	Serial code
1	*Fusarium solani*	OQ729824
2	*Trichoderma harzianum*	OQ729823

The results of the phylogenetic analysis of the nitrogenous bases sequence revealed the genetic relationship between the strains of the species identified in this study with a group of global fungal isolates of the same genus and species.

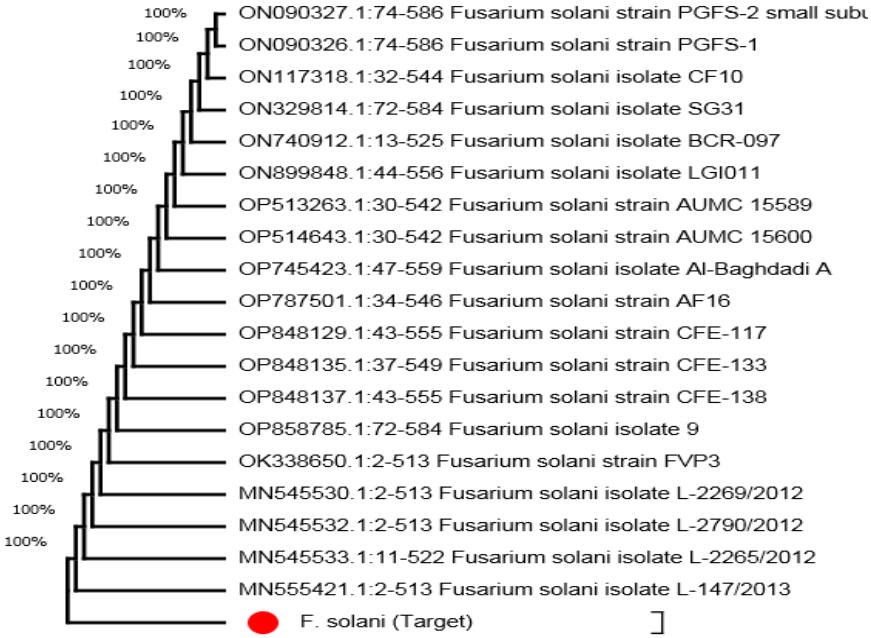


Figure 4. The Neighbor-Joining tree shows the genetic relationship between the *Fusarium solani* isolate (target) in this study and other isolates previously registered at the National Center for Biotechnology Information (NCBI)

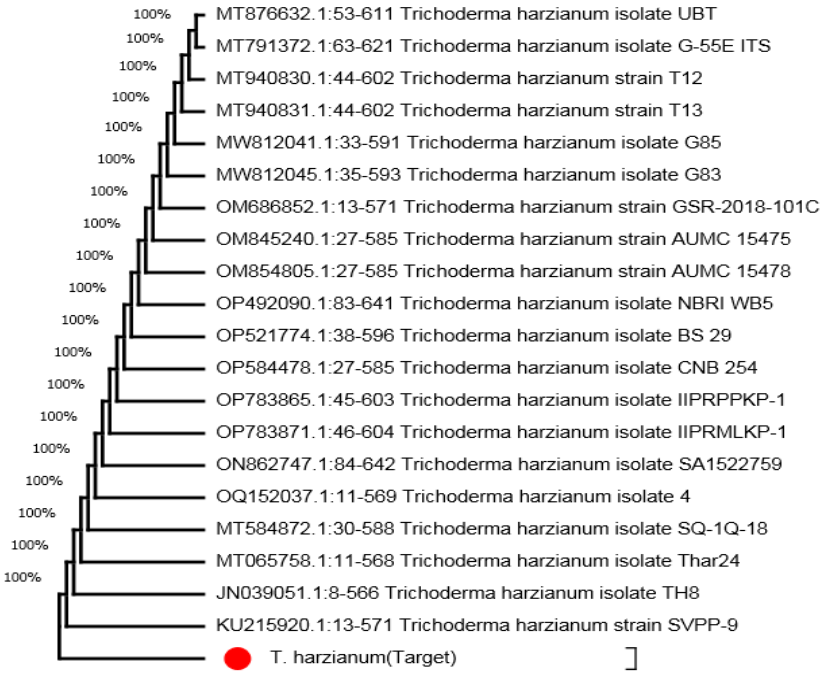


Figure 5. The Neighbor-Joining tree shows the genetic relationship between *T. harzianum* isolates in this study and other isolates previously registered at the National Center for Biotechnology Information (NCBI).

Antagonistic of *Trichoderma* spp. to the pathogenic *Fusarium solani* on P.D.A

The results showed (Fig. 6) that the *T. harzianum* T3 isolate led to an 82.8% inhibition in the growth of the pathogenic fungus with a significant difference compared to the rest of the isolates. *Trichoderma* spp., including the species used in this study, has high efficiency in resisting many fungi. Pathogen-like fungi *F. oxysporum* f.sp. *lycopersici*, *F. solani* and *R. solani* (2). Zewain (31) showed that *T. harzianum* possesses an antigenic property that makes it an antifungal agent. It is biologically active against many plant pathogenic fungi, it parasitizes directly on the hyphae of other fungi by wrapping around the filaments and analyzing its of the competing fungus and limits its reproduction.

Effect of infiltrates of *Trichoderma* spp. isolates. On the growth of *F. solani* on P.D.A

The results showed in Figure (7) that the fungus isolate T3 was significantly superior to the rest of the isolates in inhibiting the radial growth of the pathogenic fungus at a rate of 2.96cm. Many studies indicated the efficiency of the fungus filter *Trichoderma* spp. in inhibiting the growth of many plant pathogens (9). Castillo et al (7) showed that the metabolites of *Trichoderma* fungus produced in liquid culture media inhibited the radial growth of *Sclerotinia sclerotiorum* by 100%. The same study also showed that different strains and species of *Trichoderma* fungus often

produce different compounds. This is evidence that each fungal species differs from the rest of the species belonging to the same fungus through secondary metabolites.

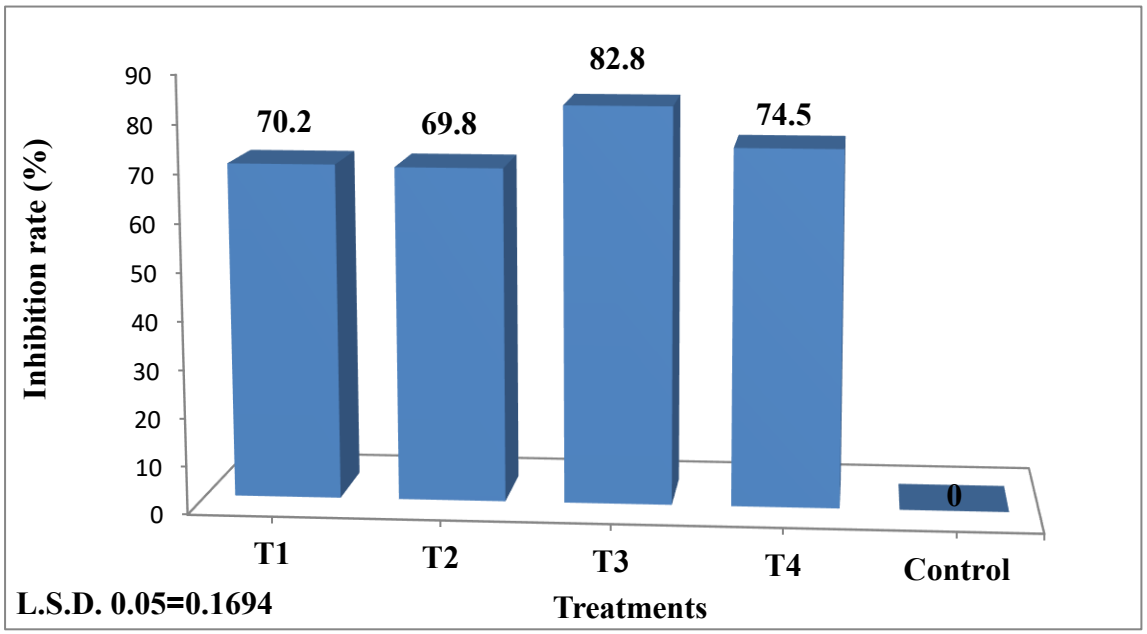


Figure 6. Effect of different *Trichoderma* isolates; T1 and T2 (*Trichoderma* spp.), T3 *T. harzianum* and T4 ، *T. longibrachiatum* on *Fusarium solani* growth on PDA culture medium

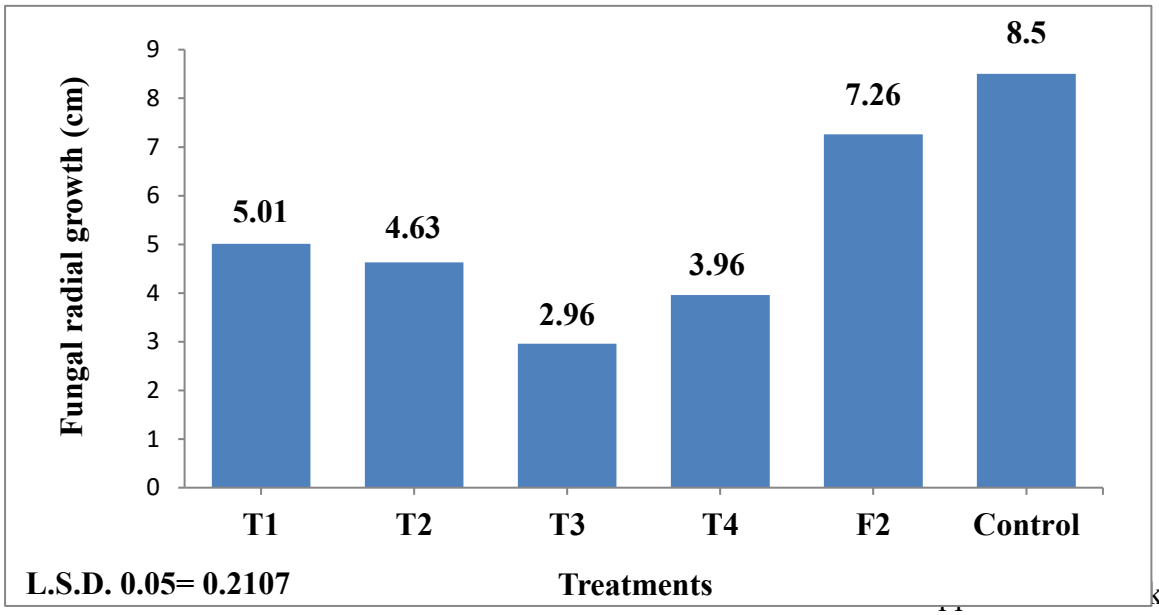


Figure 7. Effect of fungal filtrate of different *Trichoderma* isolates; T1 and T2 (*Trichoderma* spp.), T3 *T. harzianum* and T4 ، *T. longibrachiatum* on *Fusarium solani* growth on PDA culture medium

The results showed (Table 4) that isolate T3 led to the highest rate of okra growth indicators with a significant increase in plant height even in the presence of the pathogenic fungus compared to the uninfected control treatment. Growth indicators were not affected by soil sterilization or non-sterilization, while there was no significant difference in the effect of soil type on plant heights. As for the weights of okra plants, the T3 fungus isolate was superior in the treatment T3+ *F. solani* was significantly higher than the comparison treatment and the rest of the treatments in the value of fresh and dry weight. The treatment T3+*F. solani* in non-sterilized soil recorded the highest values for fresh weight 2.66g and dry weight 0.74g. As for the severity of infection, the results showed that the treatments T2+*F. solani*, T3+*F. solani* and T4+*F. solani* gave the lowest percentage of infection severity 0.0% similar to the control treatment, while *F. solani* only recorded an infection severity of 60%, T1+*F. solani* did not give complete protection from the fungus The pathogen was recorded for the severity of the infection by 20% with a significant difference compared to the treatment of the pathogenic fungus *F. solani* only, while there was no significant difference for the effect of soil type on the percentage of infection severity.

The efficacy of the fungus *Trichoderma* spp. may be due to the fungus secretion of some peptides and enzymes such as the peroxidase enzyme and some low molecular weight compounds that stimulate defense mechanisms in plants.

This leads to an increase in the production of some phenolic and alcoholic compounds that inhibit pathogens. Also, "the reason may be due to the increased secretion of some proteins produced by the fungus *Trichoderma* spp. when sensitive to the presence of the pathogen and stimulate the plant to activate its defenses against the pathogen (23 and 6). The reason for the increase in the growth indicators of okra plants may be due to the fact that many species of fungi, *Trichoderma* spp., stimulate plant roots to absorb water and some nutrients such as manganese, magnesium, phosphorus, calcium, Sodium, nitrogen, and iron also increase the concentrations of many nutrients in the soil and the vegetative system of plants. These results are consistent with what was mentioned by Azarmi et al, (5) and Alshimaysawe (4).

Table 4. Effect of *Trichoderma* spp. isolates on okra growth indicators and infection severity with *F. solani* in plastic pots

Measurements		Treatment							
	Soil	Control	F.2	T1+F2	T2+F2	T3+F2	T4+F2	Soil type effect	L.S.D. ($P \leq 0.05$)
Shoot length cm	Sterile	8.83	6.93	7.77	11.87	14.07	11.90	10.22	Soil 0.521
	Unsterile	10.13	7.47	9.53	9.33	14.17	11.03	10.27	Fungi 0.902
Average		9.48	7.20	8.65	10.60	14.12	11.46		Interaction 1.276
Shoot FW (g)	Sterile	1.21	0.50	0.66	1.66	2.34	1.68	1.34	Soil 0.1596
	Unsterile	1.27	0.52	0.84	1.31	2.66	1.73	1.38	Fungi 0.2765
Average		1.24	0.51	0.75	1.48	2.50	1.70		Interaction 0.3910
Shoot DW (g)	Sterile	0.18	0.08	0.09	0.28	0.64	0.31	0.26	Soil 0.1094
	Unsterile	0.20	0.09	0.10	0.17	0.74	0.33	0.27	Fungi 0.1896
Average		0.19	0.08	0.09	0.22	0.69	0.32		Interaction 0.2681
Infection severity (%)	Sterile	0.0	66.7	26.7	0.0	0.0	0.0	15.56	Soil 4.66
	Unsterile	0.0	53.3	13.3	0.0	0.0	0.0	11.10	Fungi 8.07
Average		0.0	60.0	20.0	0.0	0.0	0.0		Interaction 11.42

Conclusion

The isolate T3 *T. harzianum* showed high antibody efficiency against *F. solani* on P.D.A culture media. In the test of fungal isolates' filters Biologically, isolate T3 gave the highest inhibition in the radial growth rate of *F. solani* compared to other isolates T1, T2, and T4 *Trichoderma* spp. In the plastic pot experiment, the isolate of the biologically resistant fungus *T. harzianum* T3 led to the highest growth indicators of okra plants compared to other biological isolates and worked to protect okra plants from fusarium wilt disease.

Conflict of interest

The authors declare no conflict of interest.

References

1- Abdul Hadi, A. K. 2017. Evaluation of the efficiency of the fungus *Penicillium corylophilum* (Dierckx) as a biocontrol agent against *Fusarium oxysporum* that causes fusarium wilt on tomato. Journal of University of Babylon, 25 (4).

2- Al-Fadhal, F. A.; A. N. AL-Abedy and Al-Janabi, M. M. 2018. Molecular identification of novel isolates of *Rhizoctonia solani* Kühn and *Fusarium* spp. (Matsushima) isolated from petunia plants (*Petunia hybrida* L.). Plant Archives, 18(1): 703-711.

3- Al-Rawi, K. M. and A. M. Khalaf-Allah. 2000. Design and analysis of agricultural experiments. Faculty of Agriculture. Second Edition. University of Al Mosul. Ministry of Higher Education and Scientific Research. The Republic of Iraq. Page, 487.

4- Alshimaysawe, U. 2018. Factors affecting biocontrol of *Rhizoctonia* diseases and growth promotion of potato by *Trichoderma* species .Ph.D. University of New England, Australia

5- Azarmi, R.; B. Hajieghrari and Giglou, A. 2011. Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake. African Journal of Biotechnology, 10(31): 5850-5855. DOI**:** 10.5897/AJB10.1600

6- Bisen, K.; C. Keswani; J. S. Patel; B. K. Sarma and Singh, H. B. 2016. *Trichoderma* spp.: efficient inducers of systemic resistance in plants. *Microbial-mediated induced systemic resistance in plants*, 185-195.

7- Castillo, F. D. H.; A. M. B. Padilla; G. G. Morales; M. C. Siller; R. R. Herrea; C. N. A. Gonzales and Reyes, F. C. 2011. In vitro antagonist action of *Trichoderma* strains against *Sclerotinia sclerotiorum* and *Sclerotium cepivorum*, Am. J. Agri. & Biol. Sci., 6(3): 410 – 417. DOI: 10.3844/ajabssp.2011.410.417

8- Dewan, M. M.; E. L. Ghisalberti; C. Rowland and Sivasithamparam, K. 1994. Reduction of symptoms of take – all of wheat and Rye–grass seedlings by the soil–borne fungus *Sordaria fenicola*. Applied Soil Ecology 1: 45-51. DOI: https://doi.org/10.1016/0929-1393(94)90022-1

9- El-Gali, Z. I. 2015. Antagonism capability in vitro of *Trichoderma harzianum* against *Alternaria alternata* on *Ceratonia siliqua*. European Journal of Pharmaceutical and Medical Research, 2(2): 20-44.

10- Elkhalifa, A. E. O.; E. Alshammari; M. Adnan; J. C. Alcantara; A. M. Awadelkareem; N. E. Eltoum; K. Mehmood; B. P. Panda and Ashraf, S. A. 2021. Okra (*Abelmoschus esculentus*) as a Potential Dietary Medicine with Nutraceutical Importance for Sustainable Health Applications. Molecules, Vol. 26, Page 696, 26(3), 696. DOI: https://doi.org/10.3390/MOLECULES26030696

11- El-Mougy, N. S.; M. D. Aly; E. I. Imbabi and Abdel-Kader, M. M. 2011. First record of Sclerotinia foliage blight disease on pepper under protected

cultivation system in Egypt. Egyptian Journal of Phytopathology, 39(2): 209-210. DOI: 10.21608/ejp.2011.230221

12- Fayyad, M. A. and M. H. Abbas. 2018. Plant diseases (basic and advanced). Shahryar House, Basra, Iraq. I 1. P: 297

13- Ferreira, F. V. and M. A. Musumeci. 2021. *Trichoderma* as biological control agent: Scope and prospects to improve efficacy. World Journal of Microbiology and Biotechnology, 37(5): 1-17.

14- Hayamanesh, S. 2018. The effect of high temperature on physiological and metabolic parameters and reproductive tissues of okra (*Abelmoschus esculentus* (L.) Moench) (Doctoral dissertation). DOI: http://hdl.handle.net/2123/18097

15- Imran, M. R. 2021. Isolation and molecular identification of the fungus *Fusarium solani,* which causes Fusarium wilt disease on okra in Najaf and Qadisiyah governorates, and its biological resistance. M.Sc. Thesis. College of Agriculture - University of Kufa, Iraq.

16- Jaklitsch, W. M. and H. Voglmayr. 2015. Biodiversity of *Trichoderma* (Hypocreaceae) in Southern Europe and Macaronesia. Studies in Mycology, 80: 1-87. DOI: https://doi.org/10.1016/j.simyco.2014.11.001

17- Kumar, S.; G. Stecher; M. Li; C. Knyaz and Tamura, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35(6): 1547. DOI: 10.1093/molbev/msy096

18- Leslie, J. F. and B. A. Summerell. 2006. The *Fusarium* manual laboratory. Blackwell Publishing Ltd, UK. pp: 388.

19- Maitlo, S. A.; N. A. Rajput; R. N. Syed; M. A. Khanzada; A. Q. Rajput and Lodhi, A. M. 2019. Microbial control of Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris*. Pakistan Journal of Botany, 51(6): 2261-2268. DOI: http://dx.doi.org/10.30848/PJB2019-6 (23)

20- McKinney, H. 1923. Influence of soil temperature and moisture on infection of wheat seedlings by Helmin. Journal of Agricultural Research, 26: 195.

21- Perincherry, L.; J. Lalak-Kańczugowska and Stępień, Ł. 2019. *Fusarium*-produced mycotoxins in plant-pathogen interactions. *Toxins*, 11(11), 664. DOI:https://doi.org/10.3390/toxins11110664

22- Roman-Aviles, B. R.; S. S. Snapp and Kelly, J. D. 2003. *Fusarium* root rot of common beans. Extension Bulletin E 2876, Michigan St. Univ. USA. Pp.2.

23- Sáenz-Mata, J. and J. F. Jiménez-Bremont. 2012. HR4 gene is induced in the Arabidopsis-*Trichoderma* atroviride beneficial interaction. International Journal of Molecular Sciences, 13(7): 9110-9128. DOI: https://doi.org/10.3390/ijms13079110

24- Scott, K.; M. Eyre; D. McDuffee and Dorrance, A. E. 2020. The efficacy of ethaboxam as a soybean seed treatment toward *Phytophthora*, *Phytopythium* and *Pythium* in Ohio. Plant Disease, 104(5): 1421-1432. DOI: https://doi.org/10.1094/PDIS-09-19-1818-RE

25- Stack, A. J.; M. A. Yaghmour; S. C. Kirkpatrick; T. R. Gordon and Bostock, R. M. 2017. First report of *Fusarium brachygibbosum* causing cankers in cold-stored, bare-root propagated almond trees in California. Plant Disease, 101(2): 390-390. DOI: https://doi.org/10.1094/PDIS-06-16-0929-PDN

26- Stępień, Ł. and J. Chełkowski. 2010. Fusarium head blight of wheat: pathogenic species and their mycotoxins. World Mycotoxin Journal, 3: 107-119. DOI: https://doi.org/10.3920/WMJ2009.1193

27- Swami, C. S. and S. K. Alane. 2013. Efficacy of some botanicals against seed-borne fungi of green gram (*Phaseolus aureus* Roxb.). Bioscience Discovery, 4(1): 107-110.

28- Tariq, M.; S. Yasmin and Hafeez, F. Y. 2010. Biological control of potato black scurf by rhizosphere associated bacteria. Brazilian Journal of Microbiology, 41: 439-451. DOI: https://doi.org/10.1590/S1517-83822010000200026

29- Wang, L. and P. Ji. 2021. Fitness and competitive or ability of field isolates of *Phytophthora capsici* resistant sensitive to fluopicolide. Plant Disease, 105(4): 873-878. DOI: https://doi.org/10.1094/PDIS-08-20-1729-RE

30- Whipps, J. M. 1987. Effect of media on growth and interactions between a range of soilborne glasshouse pathogens and antagonistic fungi. New Phytologist, 107(1): 127-142. DOI: 10.1111/j.1469-8137.1987.tb04887.

31- Zewain, Q. K. 2020. Control of white mold disease on eggplant caused by fungus *Sclerotinia sclerotiorum* by integration between soil solarization and biological control with fungus *Trichoderma harzianum*. Anbar Journal of Agricultural Sciences, 18(1): 97–108. DOI -Crossref: 10.32649/ajas