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Fusarium Wilt on Pepper, Disease Incidence and Control Potential using Trichoderma Longibrachiatum

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Abstract

Fusarium wilt is one of the important and major diseases that affect pepper crops in different parts of the world. The study aimed to determine the presence of wilt fungi associated with pepper crops in different areas of central Iraq and to evaluate the response of different pepper varieties to infection with the possibility of reducing infection using the biological agent Trichoderma longibrachiatum. The results of a survey of 10 areas showed the presence of Fusarium spp. on pepper in six areas covered by the survey. The fungal isolates were purified and multiplied and their pathogenicity was tested, where two isolates with the highest pathogenicity and an isolate with medium pathogenicity were selected for field tests. The pathogenic isolates were diagnosed microscopically and morphologically, and the diagnosis was confirmed molecularly, which was found to be due to the fungus Fusarium solani and registered under the accession number (PP95237) in (NCBI). The antagonism of the biological fungus Trichoderma longibrachiatum registered under the accession number MZ021580 against the isolated fungi was tested. The results showed the highest percentage of antagonism against the isolate (F3), while the antagonism ranged from (82.35) to (83.88). The pathogenicity of the pepper plant was tested for the three hybrids Carisma, Gennext and Golden Land, as the isolates F3 and F5 of the fungus Fusarium spp. gave the highest percentage of seed rot and seedling death. To evaluate the effectiveness of the biological control agents and varieties used to control Fusarium wilt disease on pepper, the results showed that T. longibrachiatum led to a reduction in the disease severity. T. longibrachiatum was superior in the field in increasing plant height, leaf area, dry weight of vegetative and root groups, fruit weight, total yield regardless the pepper variety compared to pathogen infected and in the absence of biological factor. Golden Land was superior in the field in most of the study indicators, especially in the presence of the biological fungus T. longibrachiatum.

Keywords*: biological control, F. solani, Capsicum, yield quality*

Introduction

Pepper (*Capsicum annum* L.) like other important vegetable crops is infected with many pathogens, especially fungi such as Alternaria alternata (Fajardo-Rebollar et al., 2021), Fusarium oxysporum, Pythium capsici (Biri and Gomathinayagam, 2021) and Phytophthora spp. (Arora et al., 2021). Fusarium solani is one of the main causes of wilting and root rot of pepper, leading to significant economic losses and a decrease in production (El-Kazzaz et al., 2022). It is also considered a seed-transmitted fungus (Iwuagwu et al., 2022). It is a fungus endemic to the soil and infects a wide host range of plants (Nikitin et al., 2023). The main symptoms it causes are premature death of plants, as it causes blockage and imbalance in the work of the bundles. Vascular, leaf drop, color changes, leaf curling, damage to reproductive structures, premature maturation, irregular root rot and stem necrosis (Rivera-Jiménez et al., 2018), farmers have used chemical control for decades to control plant diseases. Although chemical pesticides are effective in controlling plant pathogens, their frequent and excessive use has led to the emergence of resistance traits in them (Wang and Ji, 2021), and pollution of water, air, soil and food and alteration of the plant biosphere characteristics, which has led to harmful effects on humans, animals and plants (Andrade-Hoyos et al., 2019). Therefore, plant pathologists have begun to find other means of control such as biological control, which is an effective and environmentally feasible method for the development of sustainable agriculture (Pérez-Torres et al., 2018). The fungus Trichoderma spp. It is one of the most important bio-fungi in controlling plant pathogenic fungi. The reason for its use and success is that it has many mechanisms such as parasitism, antagonism, competition for food and space (Nawrocka et al., 2018), production of volatile compounds (Hernández-Melchor et al., 2019), and enhancing plant growth. As well as the use of disease-resistant varieties and their cultivation in areas infested with plant pathogens (Launio et al., 2020). The study aimed to determine the presence of wilt fungi

associated with pepper crops in different areas of central Iraq and to evaluate the response of different pepper varieties to infection with the possibility of reducing infection using the biological agent Trichoderma longibrachiatum

Materials and Methods

Field survey

The field survey was carried out during the summer season of 2023)) from the beginning of June to the end of September, the survey covered 10 sites/fields in three governorates: Najaf (Al-Haidariya, Fadak Farm, Horticulture and Forestry Project, and Bahr Al-Najaf), Karbala (Tawrij and Al-Jadwal Al-Gharbi) fields, and Babylon (Al-Musayyab and Al-Kifl) fields. Samples were collected randomly from each site, noting the symptoms of wilting and anatomical symptoms in a longitudinal section of the stem of infected plants. Samples were also collected with the soil surrounding the roots for the purpose of isolating biological fungi, if any. The samples were brought to the laboratory and the fungi causing wilt were isolated from the plants, and the number of infected plants (infection rates) were calculated based on the infection symptoms (Al-Ghanimi, 2023):

Isolation, purification and diagnosis of fungi causing wilt on pepper plants

The pathogenic fungi were isolated from the plants with symptoms of wilt infection. The stem area close to the soil surface and the root area were separated from the rest of the plant parts at a height of 5 cm above the crown area. The infected plant parts were washed with running water, cut into 0.5-1 cm pieces and sterilized with a solution of sodium hypochlorite (NaOCl) at a concentration of 1% for 3 minutes, then washed with distilled water and dried on sterile filter paper. 4 pieces were transferred to sterile Petri dishes with a diameter of 9 cm containing 15-20 ml of sterile P.D.A. culture medium with 3 plates for each sample and the plates were placed in the incubator at 25±2°C for 2-4 days. The fungi were then purified by planting a small piece of the hyphal tip in the center of a P.D.A. plate, incubated at 25±2°C for 5-7 days (Pathak, 1974). Fungi were identified to the genus level by the growth pattern, colony color, and spore types, shapes, and colors, and based on diagnostic features (Summerell and Leslie (2011).

Molecular identification of fungal isolates isolated in this study

DNA was extracted from fungal isolates using the kit (Cat. No: FAPGK100) provided by Favorgen (Taiwan, China). The purity of the extracted DNA was measured (Williams et al. (1997). For the purpose of identifying the fungal isolates, the PCR products amplified from the fungal isolates by polymerase chain reaction (PCR) with primers ITS1 and ITS4 were sent to Macrogen (South Korea) for the purpose of determining the nucleotide sequence. All nitrogenous base sequences were analyzed using BLAST (Basic Local Alignment Search Tool) and compared with the data available at the National Center for Biotechnology Information, NCBI)) belonging to the same fungus and identified globally. Based on the nitrogenous base sequences of the identified isolates, the phylogenetic tree was drawn using MEGA-X software (Kumar et al., 2016).

Pathogenicity of fungal isolates on pepper seeds in pot experiment

This experiment was carried out using sterile agricultural soil inoculated with fungal isolates pre-loaded on millet seeds at a rate of 1.5% (1.5 g/100 g soil). The inoculum was mixed in plastic bags to homogenize the inoculum with the soil, then placed in 1 kg plastic pots, after which the soil was moistened with water, covered and left for 48 hours. Then, pepper seeds were planted five seeds/pot and watered carefully whenever needed. After 10 days after germination, the percentage of rotten seeds and dead seedlings was calculated (Al-Ghanimi, 2023).

Antagonism of *T. longibrachiatum* to *Fusarium solani* on P.D.A medium

The inhibitory ability of *T. longibrachiatum* was tested against three *F. solani* isolates (F3), F5 and F6) using Dual Culture Technique on P.D.A medium with 3 replicates for each treatment, 3 replicates for F. solani in the absence of *T. longibrachiatum* and vice versa. All plates were incubated at 25±2°C until the growth of the biocontrol fungus reached the edge of the plate, after which the percentage of efficiency of the biocontrol fungus in inhibiting the growth of F. solani was calculated (Swami and Alane, 2013).

Field experiment

Pepper seeds were planted in nursery dishes containing 50 cells and two seeds were planted in each cell, the dishes contained peat moss only on 10/1/2024 and after two months of planting, the seedlings were transferred to the field experiment soil. The experimental land was prepared in the winter season on 11/3/2024 where the soil was treated with

the three *F. solani* isolates of the fungus and the biological *T. longiprachiatum* at a rate of 10 g of millet seeds loaded with the fungus to 1 kg of soil. The soil was lightly irrigated whenever necessary to activate the fungal inoculum. After two days, two pepper seedlings (previously planted) were transplanted to each unit with three replicates distributed as Randomized Complete Block Design (RCBD).

The studied indicators

At the end of the experiment, the vegetative growth indicators included plant height (cm), leaf area (cm^2 $leaf^{-1}$), shoot and root dry weight (g. $plant^{-1}$) were calculated. The production and yield indicators also included fruit weight (g. $fruit^{-1}$), plant yield (g. $plant^{-1}$) and total yield (ton ha^{-1}) were also measured.

Results and Discussion

Field Survey

The results of the field survey (Figure 1) showed the spread of root rot and wilt disease on pepper plants in most of the fields covered by the survey, with an infection rate of 15-40%. The highest infection rate was recorded in the fields of Al-Kifl, Babylon Governorate (40%), while Al-Hussainiya in Karbala recorded a rate of 15%. The spread of the disease in these areas is attributed to the repeated cultivation of pepper crops or the cultivation of other crops belonging to the Solanaceae family in the same fields. This led to the accumulation of pathogenic fungal inoculum that remains in the soil, in addition to the suitability of environmental conditions for the growth of the causative fungus, especially temperatures (Al-Ghanimi, 2023; Al-Badrani, 2023).

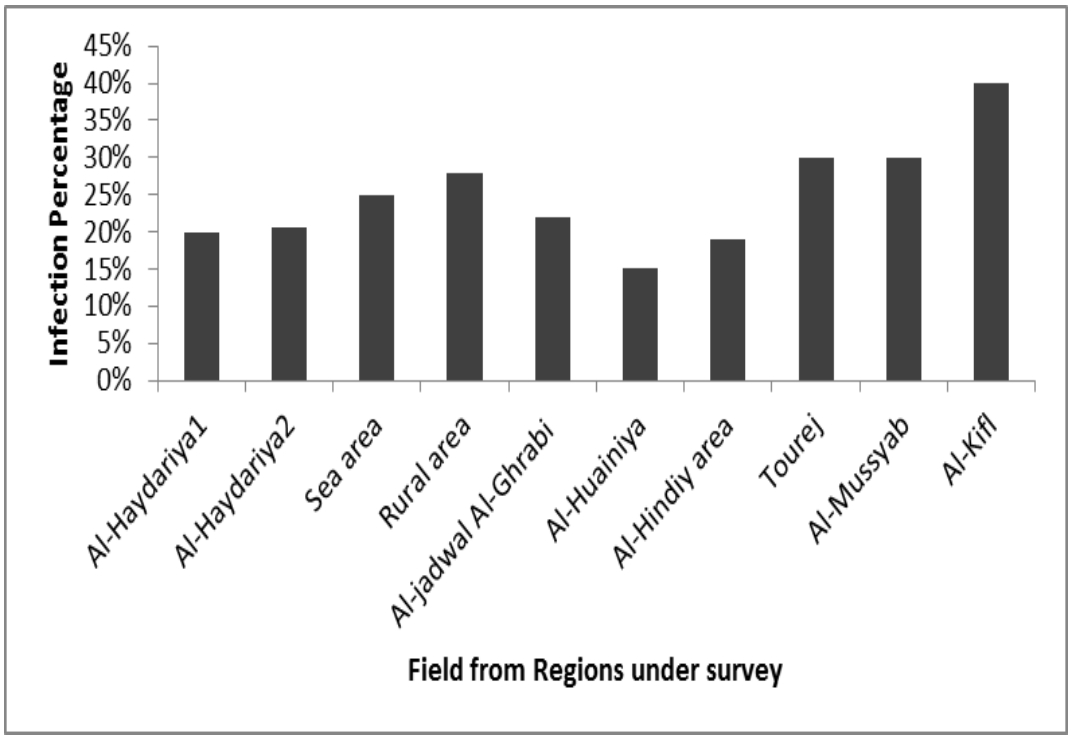

Figure1. The percentage of infection with wilt and root rot fungi in 10 areas from three governorates (Najaf, Karbala and Babylon) in central Iraq included in the study.

Isolation, purification and identification of fungi causing pepper wilt

The results of isolation, purification and microscopic examination of the PDA culture medium of the infected pepper plant parts showed the presence of six fungal isolates *Fusarium* spp. (Figure 2). They were initially identified based on some morphological characteristics (Leslie and Summerell, 2006). White to cream colonies were observed with scattered and divided fungal hyphae and long, slightly oblique, broad and relatively strong conidia with almost disc-shaped ends divided by 5-7 septa (Macroconidia), and other spores were smaller, spindle-shaped or oval-shaped and rounded at both ends (Microconidia), in addition to recording the Chlamydospores, that were large, spherical and with a thick, dark-colored wall (Bissett, 1984).

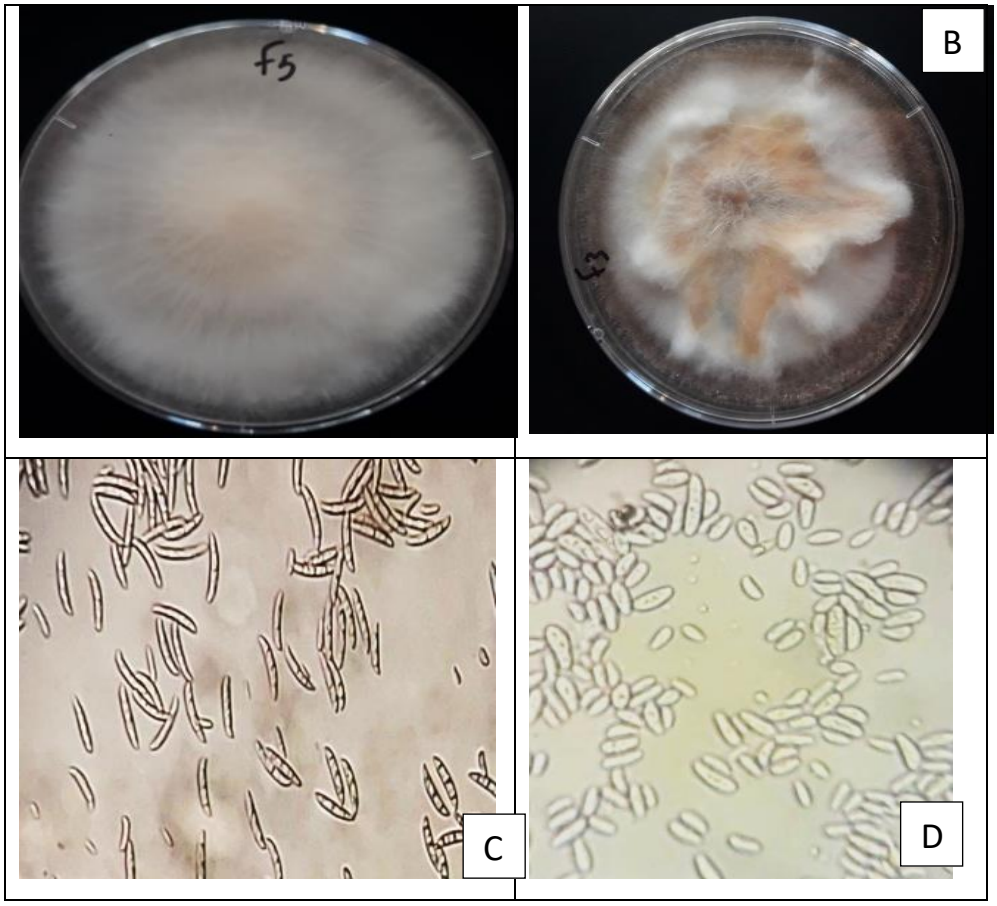

Figure2. Growth of fungal colonies on P.D.A. medium of *F. solani* isolated from pepper plants (A) and (B), macroconidia (C) and microconidia (D) under the light microscope

Molecular diagnosis of *F. solani* isolates under study

Polymerase Chain Reaction (PCR) technique was used for molecular diagnosis of two isolates of the highly pathogenic Fusarium spp. isolated from pepper plants, and an isolate with moderate pathogenicity. Bands of 550bp sequences of nitrogenous bases were obtained, Figure (4-3). Molecular diagnosis proved that the three isolates are identical and belong to the fungus *Fusarium solani*, as one of them (F3) was registered in the National Center for Biotechnology Information (NCBI) as a new isolate under accession number PP952373.

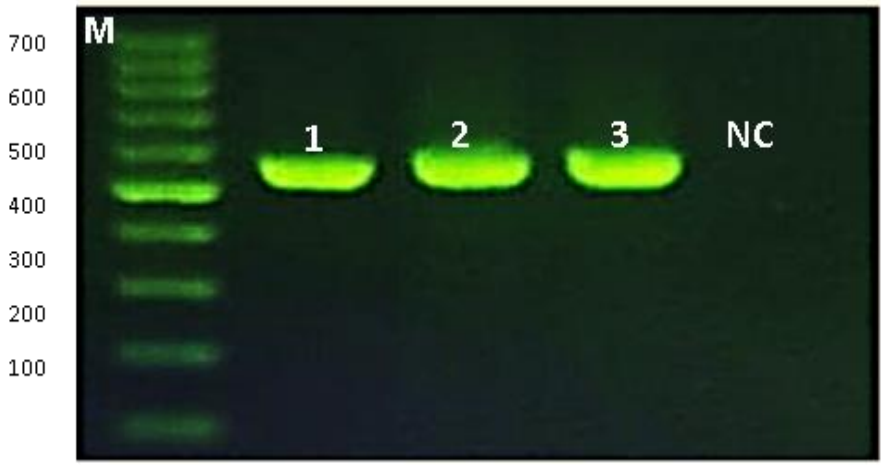

Figure 3. 550bp-PCR products amplified by PCR from *F. solani* isolates (F3, F5, and F6) isolated in this study from pepper plants. NC: Negative control; It was performed by PCR mixture containing all reaction components except the DNA of the fungal mycilium. M: Molecular-weight size marker by number of nitrogenous base pairs (bp)

The results of the genetic analysis using the Neighbor-Joining tree showed that the studied isolate aligned with most of the isolates recorded in the NCBI data with a similarity rate of up to 95% (Figure 4-4), which indicates the global spread of this fungus in various environments.

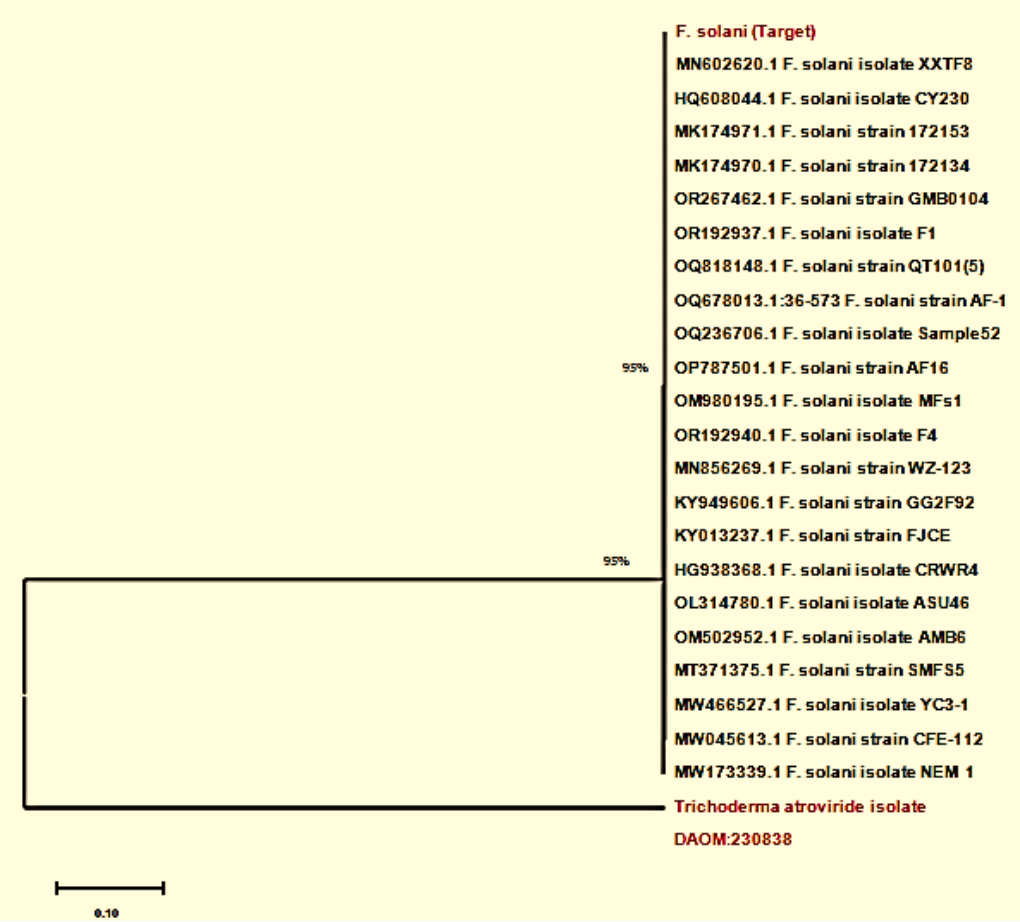

Figure 4. Neighbor-Joining tree shows the genetic relationship between the fungus isolate *F. solani*, registered under the number PP952373, isolated from pepper plants, and some isolates registered in the US NCBI for the same fungus, with the isolate *Trichoderma atroviride* used as an out-group

Pathogenicity of *F. solani* isolates on pepper seeds in plastic pots

The results of Table (4) showed that all tested fungal isolates led to a high percentage of seed rot with a significant difference from the healthy control treatment that did not record any rotten seeds and complete germination. The isolates F3 and F5 outperformed the isolate F6 in the highest percentage of seed rot regardless of the hybrids used, as the isolates F3 and F5 recorded a rot percentage of 53-86%.

The results of the pathogenicity capacity in plastic pots (Table (4)) showed that all the fungal isolates under study gave different indicators for the percentage of seedling death similar to those recorded in the case of the percentage of seed rot, as the isolates F3 and F5 had a similar effect on all varieties sometimes and superior at other times in the hybrids Carisma and Genext where the percentage of seedling death in them was 26.67% - 46.67%, Coldland recorded the lowest percentage of seedling death and seed rot in all isolates (13.33 to 33.33)%.

The variation in the percentage of seed rot and seedling death may be due to the parasitic nature of the fungus Fusarium spp. and the abundance of fungal growth inside plant tissues. This obstructs the access of water and nutrients to different plant parts, in addition to the ability of the fungus to produce enzymes and mycotoxins that lead to the decomposition of the plant cell wall, allowing the fungus to enter stem and root tissues which prevents the seed germination and embryos growth (Al-Ghazali, 2022). The results of the current study agree with Engalycheva (2024) that the pathogenic fungus *Fusarium* spp. is one of the most important factors causing pepper wilt, seedling death, and blockage of the transport

vessels. Al-Ghanmi (2023) showed that the fungus *F. solani* was the most common species in the areas under study and causes the rot and death of pepper seedlings with an infection rate of 100%.

Table1. Pathogenicity of six *F. solani* pathogenic isolates on three pepper hybrids grown in 100 gm pots for ten days after germination under growth chamber conditions

Treatments	**Carisma**		**Gennext**		**Golden Land**	
***Fusarium* isolates**	Seed rot %	Seedlings death %	Seed rot %	Seedlings death %	Seed rot %	Seedlings death %
F3	73.33	26.67	53.33	46.67	73.33	26.67
F5	73.33	26.67	73.33	26.67	86.67	13.33
F6	46.67	20	40	40	60	33.33
LSD (P≤0.05)	Seed rot= 15.42 Seedlings death= 12.13					

Antagonism of *T. longibrachiatum* to *F. solani* on P.D.A. culture medium

The results of the antagonism test between the bio-fungus and the pathogenic fungus showed that the bio-fungus *T. longibrachiatum* inhibited the pathogenic isolates of F. solani (Table 3) where it recorded an inhibition rate of 82.00%. In general, the bio-isolate Tri recorded an inhibition ability on the pathogenic isolates F3, F5 and F6 by 83.88, 82.35 and 80%, respectively. The results of the current study are consistent with Lone et al. (2012), Subash et al. (2013) and Anjum et al. (2020) where it was found that different isolates of the bio-fungus Trichoderma spp. significantly reduced Fusarium wilt on pepper, especially using *T. longibrachiatum*. This is often due to the rapid growth of the fungus, the production of antibiotics, the metabolic rates, the antimicrobial secondary metabolites, the physiological structure and other mechanisms exhibited by *Trichoderma* spp. are major factors that affect the fungus' ability to antagonize and inhibit other fungi.

Table2. Inhibitory effect of the bio-agent *T. longibrachiatum* (Tri) against pathogenic isolates of *F. solani* in the dual culture method on PDA medium

Treatments	**Inhibition rate by**
***Fusarium solani* isolates**	***Trichoderma longibrachiatum***
F3	83.88
F5	82.35
F6	82.71
L.S.D 0.05	0.78

Field experiment

The results showed that the pathogenicity of F. solani isolates differed on different pepper varieties, as the isolate F3 showed an infection severity ranging from 50% on the hybrid Carisma to 76% on Gennext and Golden Land, while the isolate F5 showed almost equal infection severity on the three varieties, compared to the lowest infection severity for isolate F6 regardless of the variety (Figure 5). In general, the difference in infection severity between different isolates is attributed to the ability of the isolate to secrete enzymes and toxins, especially Trichothecene Mycotoxins. These toxins are considered one of the most important specialized virulence factors in addition to the pathogenic chromosomes (strain-specific chromosomes) which are considered one of the indicators of the pathogen's virulence (Rampersad, 2020).

The results indicate that the Fusarium F5 isolate had the highest effect in reducing most of the vegetative growth and yield indicators, which generally led to recording the lowest rates regardless of the pepper variety, followed by the F3 isolate with a sometimes similar effect, while the F6 isolate had the least effect in reducing the growth indicators and yield under study.

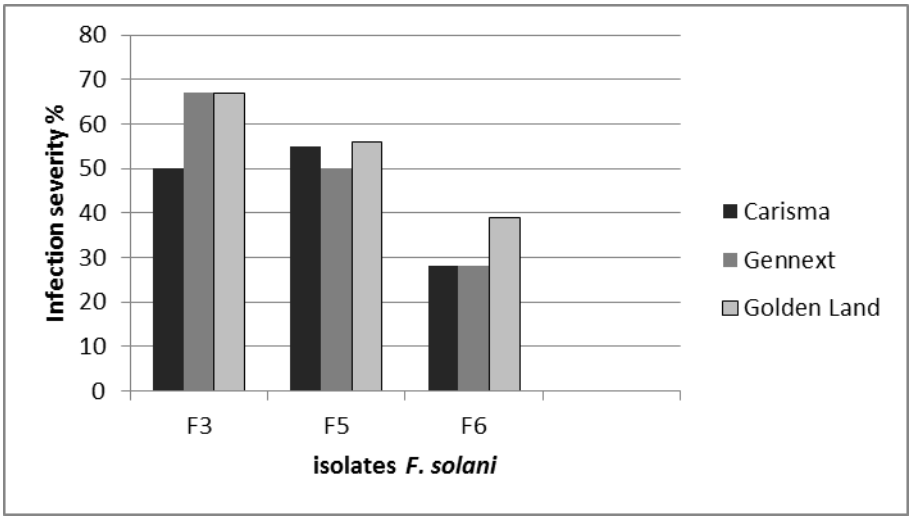

Figure5. Field infection severity of three *F. solani* isolates on Bell pepper cultivars Carisma, Gennext and Golden Land

All plants inoculated with the pathogenic fungus recorded lower values for plant height, number of leaves, and leaf area with a significant difference from the uninoculated control treatment. Regardless of the type of isolate, it was found that the appearance of the Trichoderma long fungus led to an increase in the values of the growth indicators for plant height and leaf area with a significant difference from the values recorded in the absence of the pathogenic fungus in plants inoculated with the pathogen only, Table (4).

The pathogenic fungus also led at the same time to an increase in the leaf area rate even in the presence of pathogenic fungi and recorded a rate three times higher than that recorded in the absence of the pathogenic fungus. The biotrophic fungus, especially in the presence of the pathogen on infected plants, led to a significant increase of 6-10 times in the green dry weight higher than that recorded in plants inoculated with the pathogen only, regardless of the variety. The same context in the effect was recorded in the effect of the treatments on the dry root weight, Table (4).

On the other hand, the effect of the treatments did not differ much from the previous in the case of the effect on the yield indicators for fruit weight and total yield. It was noted that the values of the yield indicators decreased significantly (by 30-40%) due to the effect of the pathogenic isolates compared to those recorded in the control treatment. At the same time, the presence of the biotrophic fungus, even in the presence of infection with the pathogenic fungus, had a

Table4. Field infection severity of three *F. solani* isolates on Bell pepper cultivars Carisma, Gennext and Golden Land

Pepper Cultivars	Treatments		Plant height	Leaf area	Shoot DW g	Root DW g	Fruit weight	Total yield
Carisma	Con	C	31.33	12.39	113.47	19.86	29.76	595.13
		T	45	26.36	136.15	20.67	54.45	1089.00
	F3	C	16.67	8.50	12.22	4.81	1.33	26.67
		T	34.67	10.58	120.66	15.12	29.73	594.67
	F5	C	13	2.32	10.57	6.99	0.00	0.00
		T	33	12.30	117.37	13.65	30.40	608.00
	F6	C	20.33	6.62	22.34	9.38	1.96	39.13
		T	37	9.08	123.50	13.36	25.18	503.53
Average			28.87	10.31	94.62	12.98	21.60	432.02
Gennext	Con	C	33.33	9.65	66.97	11.79	24.81	496.27

		T	**53**	**11.28**	**133.62**	**18.18**	**36.02**	**720.40**
	F3	C	**12.67**	**2.41**	**21.08**	**6.58**	**3.62**	**72.40**
		T	**37.67**	**12.20**	**126.32**	**14.89**	**20.82**	**416.47**
	F5	C	**23**	**1.74**	**23.33**	**8.59**	**9.57**	**191.47**
		T	**34.67**	**10.86**	**126.33**	**17.56**	**33.73**	**674.67**
	F6	C	**24.33**	**2.25**	**26.01**	**7.35**	**4.00**	**80.07**
		T	**32.67**	**16.04**	**116.64**	**11.42**	**28.02**	**560.40**
Average			**31.41**	**8.30**	**80.04**	**12.05**	**20.08**	**401.52**
	Con	C	**29.67**	**15.49**	**117.63**	**9.98**	**31.40**	**628.07**
		T	**39**	**22.74**	**117.45**	**16.07**	**65.91**	**1318.20**
	F3	C	**18.67**	**2.13**	**12.09**	**8.40**	**0.00**	**0.00**
Golden land		T	**26.67**	**29.11**	**93.48**	**14.10**	**44.81**	**896.20**
	F5	C	**16.67**	**1.66**	**12.09**	**7.77**	**4.52**	**90.40**
		T	**28.67**	**17.79**	**78.26**	**14.75**	**51.29**	**1025.80**
	F6	C	**21.67**	**3.38**	**44.06**	**8.42**	**17.34**	**347.20**
		T	**34**	**21.98**	**72.15**	**10.47**	**35.45**	**712.60**
Average			**26.87**	**14.29**	**69.03**	**11.24**	**31.34**	**627.31**
	Cont. uninfected		**38.56**	**16.32**	**114.21**	**16.09**	**40.39**	**807.84**
	F3		**24.50**	**9.88**	**81.92**	**10.65**	**16.72**	**334.40**
	F5		**24.83**	**7.78**	**61.32**	**11.55**	**21.59**	**431.72**
Average	F6		**28.33**	**9.89**	**67.45**	**10.06**	**18.66**	**373.82**
	Tri positive		**36.33**	**16.69**	**122.30**	**15.02**	**37.98**	**759.99**
	Tri negative		**21.78**	**5.24**	**40.15**	**9.16**	**10.69**	**213.90**
	Cultivar		**2.69**	**2.12**	**1.11**	**1.27**	**5.31**	**107.50**
L.S.D. ($P \leq 0.05$)	*Tri*		**2.19**	**1.73**	**0.90**	**1.03**	**4.34**	**87.80**
	F. isolates		**3.10**	**2.45**	**1.28**	**1.46**	**6.14**	**124.20**
	Interaction		**7.60**	**6.002**	**3.13**	**3.58**	**15.03**	**304.2**

significant effect on increasing the fruit weight by approximately 3 times and the total yield by 3-4 times compared to those in which the biotrophic fungus did not appear (Table 4).

The increase in growth parameters in the presence of the biological fungus *T. longiprachiatum* is often attributed to the fungus activities (Patrick et al., 2001) in promoting plant growth, especially enhancing root length, increasing the number of root hairs, and transporting more available nutrients. This also includes the release of organic acids and the synthesis of plant-stimulating compounds, such as growth hormones (indole acetic acid, cytokines, gibberellins, and zeatin) (Zhang et al., 2013). As indole acetic acid has the ability to promote root growth (Nieto-Jacobo et al., 2017). It is also noted that plant shoot and root dry weights were always higher in the presence of *T. longiprachiatum* over the control treatments uninoculated with pathogenic isolates and even those infected with the pathogenic *F. solani* isolates. The biological factor reduces the effect of the pathogen, enhances plant growth, and improves vegetative and root growth indicators to give higher fresh and dry weight (ZK, 2024). In addition, *T. longiprachiatum* ability to produce (IAA) that enhances root growth, leading to an increase in root mass and the area of colonization of beneficial microbes, thus enhancing nutrients absorption (Al-Janabi, 2022). The biological fungus may also help the plant to produce gibberellin and its importance in increasing vegetative growth, root growth development, and wood and cellulose formation (Castro-Camba et al., 2022). This eventually leads to a greater yield of fruit weight, which is also reflected in the total yield depending on the treatments (Duan et al., 2023). These results are consistent with Ji et al. (2020) on flowering Chinese cabbage that the biological fungus enhanced the absorption of nutrients and improved the quality and productivity of plants. Similarly, Bedine et al. (2022) found that the use of six strains of the probiotic fungus *T. gamsii* isolated from bean roots liberated phosphate and enhanced the bean plant's utilization of available phosphorus, thereby improving plant growth, total protein content, and chlorophyll content. Other results were observed in tomato, where the use of the probiotic fungus *T. harzianum* enhanced fruit quality, nutrient content, and total yield (Nzanza et al. (2012). This is consistent with similar

results (Duan et al. (2023) on pepper, where *Trichoderma* spp. enhanced the plant's ability to absorb nutrients, increase pepper fruit quality, and total yield.

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