



## Improving resistance of tomato plants against Fusarium root rot disease using biocontrol agents

Hofny H. H. A., Mohamed A. A., El-Fawy M. M.\*

Plant Pathology Branch, Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University (Assiut Branch), Assiut, Egypt

### Abstract

Fusarium root rot disease of tomato caused by *Fusarium solani* is one of the most important tomato diseases and causes severe problems in all production regions. The aim of the current study was to evaluate the efficacy of some *Trichoderma* isolates and *Pseudomonas fluorescense* to control this disease *in vitro* and *in vivo*. In laboratory experiments, the effect of four *Trichoderma* isolates, i.e. *T. harzianum* (T<sub>1</sub>), *T. harzianum* (T<sub>2</sub>), *T. reesei* and *T. asperellum* (T<sub>34</sub>) and one isolate *P. fluorescense*. Data indicated that all the tested isolates of biocontrol agents were able to inhibit the mycelial growth of the pathogen with variation in their antagonistic capability. In general, *T. asperellum* (T<sub>34</sub>) exhibited the highest antagonistic effect toward the pathogen, followed by *T. harzianum* (T<sub>2</sub>). Under greenhouse conditions, application of bio control agents as a soil treatment led to decrease in disease severity of tomato root rot disease compared to the control treatment. Data indicated that *T. reesei* treatment exhibited the highest reduction in the disease severity of root rot compared to other treatments in both seasons. Moreover, application of these treatments significantly enhanced plant height (cm), fresh and dry weight (gm). Laboratory estimates showed that the tested bio control agents increased catalase (m/g fresh weight), peroxidase and polyphenol oxidase (m/g f. w) in treated tomato plants compared to infected and healthy control. Furthermore, treated tomato plants contained a high level of total phenol contents (TPC) compared to untreated plants.

**Keywords:** tomato, Fusarium root rot, biological control, *Trichoderma* sp., *Pseudomonas fluorescense*.

\*Corresponding author: El-Fawy M. M.,  
E-mail address: [mansour\\_mazen@hotmail.com](mailto:mansour_mazen@hotmail.com)

## 1. Introduction

The tomato (*Solanum lycopersicum* L.) is a dicot plant, belonging to the Solanaceae family which comprises more than 3000 species; among these, the tomato is one of the most cultivated vegetables in the world (Srinivas *et al.*, 2019). Tomatoes are known as a source of vitamins and pro-vitamins (vitamin C, pro-vitamin A,  $\beta$  carotene, folate), minerals such as potassium, and secondary metabolites such as lycopene, flavonoids, phytosterols and polyphenols (Beecher, 1998, Luthria *et al.*, 2006). Thus, 100 g of fresh tomato provides over 46%, 8% and 3.4% of the daily requirements of vitamin A (being 900 UE), vitamin C (being 82.5 mg) and potassium (being 3500 mg), respectively (Canene-Adams *et al.*, 2005; Gebhardt and Thomas, 2002). Egypt considers one of the top most tomato growing countries and ranks as the fifth largest producer country in the world accounting for more than 4.0% of the global tomato production. According to the latest statistical statement (FAOSTAT, 2019); the area cultivated with tomatoes in Egypt is around 450,000 acres with a production of 7,297,108 tons. The soil borne pathogens can affect tomato and the most important of these are the soil borne fungal pathogens including *Fusarium* spp., *Rhizoctonia solani* and *Sclerotium rolfsii* (Abdel-Monaim, 2012; El-Fawy *et al.*, 2021; El-Mohamedy *et al.*, 2014; Saad 2006). Both *R. solani* and *F. solani* produced damping-off disease causing major economic losses in many crops. *F. solani* and *R. solani* are the most important soilborne fungal pathogens causing the symptoms of damping off and

root rot diseases to wide range of vegetable and crop plants including tomato (Abu-Taleb *et al.*, 2011; Dawar *et al.*, 2007). *Trichoderma* spp. are commercially marketed as biofungicide, bio-fertilizer and soil amendments. The use of *Trichoderma* fungus in agriculture can provide numerous advantages, (1) colonization of the root and rhizosphere of plant, (2) control of plant pathogens by different mechanisms such as parasitism, antibiosis and induce systemic resistance, (3) improvement of plant health by promoting plant growth, and (4) stimulation of root growth (Harman *et al.*, 2004). Biological control had attracted the interest because of increasing regulation and restriction of pesticides or unsuccessful control attempts by other means. Biological control for soil-borne pathogens by antagonistic microorganisms is potential especially for soil-borne diseases because these pathogens are difficult to be controlled with specific fungicides (Moussa *et al.*, 2006; 2007). *Trichoderma* species are soil-borne fungi that are commonly found in soil organic or inorganic matter (Harman, 2017; Kumar *et al.*, 2014; Schuster and Schmoll, 2010). There are *Trichoderma*-based biocontrol products worldwide in different forms. Unlike the action of most pesticide chemicals, *Trichoderma* protects against various diseases, and enhances plant growth, improving yield and quality of many crops. Also the use of *Trichoderma* does not cause adverse effects to human health and the environment (Agriculture Solutions, n.d.). *Trichoderma* spp. are a rich source of secondary metabolites that involves the biosynthesis of degrading

mechanisms such as antibiotics (= antibiosis), mycotoxins (= myco parasitism) and phytotoxins (= cell wall degradation) (Mukherjee *et al.*, 2012). The production of secondary metabolites is a significant mechanism for biocontrol by *Trichoderma*. Induced resistance of plants towards pathogen infection can be developed by treating plants with a variety of abiotic and biotic inducers (Walters *et al.*, 2005). This work aimed to evaluate the efficacy of *Trichoderma* isolates and *Pseudomonas fluorescense* as a soil treatment for controlling *Fusarium* root rot disease of tomato and their effect on some growth parameters of tomato plants.

## 2. Materials and methods

### 2.1 Isolation and identification of pathogenic fungus

*Fusarium solani* isolate was isolated from naturally infected tomato roots, which showed the typical symptoms of root rot diseases. Roots were cut into small pieces sections, surface sterilized by soaking into ethanol 70% for three minutes and briefly dried between sterile filter paper. The surface sterilized roots segments were subsequently mounted on to potato dextrose agar medium (PDA), amended with 150 mg/liter streptomycin and 150 mg liter chloramphenicol to inhibit bacterial growth. Emerging mycelium from the root sections, which had the morphological characteristics of the genus fungi were isolated and further propagated on PDA medium. Each

fungus was purified using the single spore colony technique (Choi *et al.*, 1999). The purified isolates were reared on PDA plates and incubated at 27°C in the dark for two weeks. The formed spores were reared on PDA plates and incubated at 27°C in the dark for two weeks. The formed spores were extracted and used for inoculation. For long-term storage, several small of agar plugs (7 mm in diameter) were disked using a sterilized cork borer and transferred into micro tubes prior to 5°C. Fungal isolate were identified by using the morphological features of mycelia and spores as described by Booth (1977) and Domsch *et al.* (1980).

### 2.2 Pathogenicity test of *F. solani* isolate

The isolate of *F. solani* was grown on sand maize medium. Sand and ground maize seeds were mixed in the ratio of 2:1 (w/w) moistened to 40% moisture content. After preparation, 200g of the medium was filled into 500 ml Erlenmeyer conical flask and autoclaved for one hour. One disc of *F. solani* was inoculated into sand maize medium and incubated at room temperature for 14 days. Plastic pots (30 cm in diam) containing sterilized clay-sand soil, sterilization of plastic bags and soil was carried out by using 5% formaldehyde solution. The content of bottles was thoroughly mixed in plastic container and used as a source of inoculum. Inoculum of pathogenic isolate was added at a rate of 1% to the plastic bags soil (w/w), one

week before sowing, mixed well with the soil and then thoroughly irrigated. Three tomato seedlings were planted into each infested pot, and the culture practice was carried out as usual, after 90 days from planting the degree of root rot disease was estimated. Disease severity of root rot plant growth parameters were recorded after 90 days from sowing date. The arbitrary (0-5) disease index scale as described by (Grunwald et al, 2003) was adopted. Where: 0= No visible symptoms, 1= slight hypocotyl lesions, 2= lesions coalescing around epicotyls and hypocotyls, 3= lesions starting to spread into the root system with root tips starting to be infected, 4= epicotyl, hypocotyls and root system almost completely infected and only slight amount of white tissue was left, and 5= completely infected root. Disease severity of root rot was recorded using the following formula:

$$\text{Disease severity (\%)} = \frac{\sum [(n \times V) / 5 \times N]}{5 \times N} \times 100$$

Where, n= number of plants in each infection category, V= numerical values of infection categories, N= total number of plants examined and 5= constant, the highest numerical value.

### 2.3 Sources of the biocontrol agents

*Trichoderma* spp. were collected from the rhizosphere of healthy tomato plants using dilution plate techniques and purified by the single spore method. The isolated fungi were identified on the basis

of their morphological characters (Rifai, 1969). *Trichoderma reesei* (AUMC No. 5829) was obtained from Microbial Resources Mycological Research Center, Assiut University, Assiut, Egypt. *P. fluorescence* (AUMC No B-151) was obtained from Microbial Resources Mycological Research Center, Assiut University, Assiut, Egypt.

### 2.4 In vitro, the antagonistic effects of *T. harzianum* and *P. fluorescence* against *F. solani*

The antagonistic effect of *T. harzianum* and *P. fluorescence* against *F. solani* was investigated in Petri dishes containing Potato Dextrose Agar (PDA) medium. Each plate was inoculated on both sides with 6-mm discs from 7-day-old cultures of individual antagonistic fungal isolates. Also, one disc from 7-day-old cultures of *C. beticola* was placed in the center of the same plate. Four plates were used for each treatment as replicates. Inoculated plates with the pathogen only were used as a control. The inoculated plates were incubated at 25°C. The antagonistic abilities of biocontrol agents' isolates were recorded when the growth of the pathogen isolate completely covered the surface of control plates. The percentage of growth inhibition was calculated according to the equation of El-Fawy et al. (2018):

$$R = (C - B/C) \times 100$$

Where R = % of growth inhibition, C =

growth in the control, and B = growth in the treatment.

### 2.5 Production of *Trichoderma* isolates inoculum

*Trichoderma* spp. were grown in 250 ml Erlenmeyer conical flasks containing Potato Dextrose Broth at  $28\pm 1^\circ\text{C}$  for 8 days. Liquid cultures of *Trichoderma* spp. were homogenized in a blender ( $5\times 10^6$  conidia/ml) and used for soil irrigation of tomato seedlings twice from time to time 21 days.

### 2.6 Production of *P. fluorescence* inoculum

To prepare the inoculum of *P. fluorescence*, a bacterial strain was grown on NA medium plates and transferred to 50ml flask containing 25ml nutrient broth (NB) medium. Flasks were incubated at  $27^\circ\text{C}$  in an orbital shaker (125 rpm) for 72 hours. Subsequently, the bacterial culture suspension was transferred to 20ml sterilized tubes that were centrifuged at 11,000 rpm for 5 minutes at  $4^\circ\text{C}$ . Pellet was discarded and supernatant was transferred to new sterilized glass tubes and colony forming units (CFU) in the supernatant was adjusted ( $4\times 10^8$  CFU/ml) by spectrophotometer at OD 660 nm.

### 2.7 Effects of *T. harzianum* and *P. fluorescence* on *F. solani* root rot disease of tomato under greenhouse conditions

Sterilized pots that containing sterilized clay-sand soil (1% w/w), were infested

by *F. solani* isolate inocula were prepared by growing fungi on barely grain medium as described before, mixed with the soil and irrigated well. After one week, three disinfected tomato seedlings (Super Jakal cv.) were transferred into each pot. Three replicates were used for each treatment. Irrigation was done twice (one and three weeks after transplanting) with spore suspensions of biocontrol agents at concentrations  $5\times 10^6$  conidia/ml. Before application, the solutions were diluted to the required concentrations using sterile distilled water as described by Shalaby and El-Nady (2008). The Foset fungicide at concentrations 2 gm/Liter of water was used as comparison (positive control), inoculated with the pathogens and irrigated pots with water only used as control. After 90 days from planting the disease index was recorded as a vascular browning and foliar yellowing percent as mentioned before. Some growth parameters including plant height (cm), fresh and dry weight (gm) were also estimated.

### 2.8 Determination of phenolic compounds

Phenolic compounds were colourimetrically determined using the phosphotungstic-phosphomolybdic acid, Folin and ciocalteu phenol reagents according to Snell and Snell (1953). Total phenols were determined as follows; ten drops of concentrated HCl were added to the sample (0.1 ml), heated rapidly to boiling point and placed

in a boiling water bath for 10 minutes. After cooling, 1.0 ml of the reagent and 5 ml of 20% Na<sub>2</sub>CO<sub>3</sub> were added. The mixture was diluted to 10 ml and determination was carried out at 520 nm after 30 minutes.

### 2.9 Determination of polyphenoloxidase (PPO) activity

The polyphenoloxidase activity was determined according to the method described by Matta and Dimond (1963). Polyphenoloxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/min.

### 2.10 Determination of peroxidase (PO) activity

Peroxidase activity was estimated according to Allam and Hollis (1972). In this method, the oxidation of pyrogallol was calculated and converted to pyrogalline in the presence of H<sub>2</sub>O<sub>2</sub> at a wavelength of 425 nm. The peroxidase enzyme activity was differentiated as the change in absorption at 425 nm /min<sup>-1</sup> g<sup>-1</sup> on a fresh weight using a spectrophotometer.

### 2.11 Determination of Catalase (CAT) activity

Catalase enzyme activity was determined according to the procedure of Aebi (1984). A total reaction mixture of 3 ml, consisting 2400 µl phosphate buffer (50 mM), 100 µl of the enzyme extract and 500 µl H<sub>2</sub>O<sub>2</sub> (10 mM) was used to

measure enzyme activity. The reaction mixture absorption was recorded at 240 nm with a spectrophotometer twice with an interval of 2 min and the enzyme activity was calculated using an extinction coefficient of 0.28 mM<sup>-1</sup> cm<sup>-1</sup>.

### 2.12 Statistical analysis

The obtained data were subjected to statistical analysis using MSTAT-C program version 2.10 (1991). Least significant difference (L.S.D., p = 0.05) for comparison between means of treatments was used as mentioned by Gomez and Gomez (1984).

## 3. Results

### 3.1 Pathogenicity test of *F. solani* isolate

The result of this experiment showed that the isolate of pathogenic fungus were able to infect super Jakal tomato cultivar (79.90%) and produced typical symptoms of Fusarium root rot disease under greenhouse conditions.

### 3.2 Effects of some biocontrol agents on the growth of *F. solani* in vitro

The ability of *Trichoderma* isolates and *Pseud fluorescense* isolates to inhibit the mycelial growth of *F. solani* in dual culture was determined on PDA medium. Data presented in Table (1) indicated that all the tested isolates of biocontrol agents were able to inhibit the mycelial growth of the pathogen with variation in their

antagonistic capability. In general, *T. asperellum* (T34) exhibited the highest antagonistic effect toward the pathogen, followed by *T. harzianum* (T2). While *P. fluorescens* was the least in their

antagonistic effect. It was clear from Figure (6) that the mycelium of *Trichoderma* isolates was grown rapid over the mycelium of the pathogen and prevented its development.

Table (1): Effect of some biocontrol agents on the growth of *F. solani* *in vitro*.

Bioagents	Mycelial growth inhibition (%)
<i>T. harzianum</i> (T1)	67.9
<i>T. harzianum</i> (T2)	80.41
<i>T. reesei</i>	72.21
<i>T. asperellum</i> (T34)	80.6
<i>P. fluorescens</i>	58.6
Control	0
LSD 5%	6.40

### 3.3 Effect of soil treatment with biocontrol agents on Fusarium root rot of tomato under greenhouse conditions

Data presented in Table (2) indicate that application of biocontrol agents as a soil treatment at concentration 50 ml/pot significantly decreased Fusarium root rot disease of tomato compared to untreated plants. Among the biocontrol agents, *T. reesei* was more effective in reducing the disease severity more than the other treatments in both seasons (35.50 and 31.11%) followed by Foset fungicide (19.90 and 26.60 %). While the *T. asperellum* (T34) gave the lowest effect in controlling the disease (46.60 and 40.00 %) during 2019 and 2020 growing seasons, respectively. There were significant differences among the treatments.

### 3.4 Effect of soil treatments with biocontrol agents on some plant growth

#### characters of tomato under greenhouse conditions

Data presented in Tables (3) indicated that all the biocontrol agents improved plant growth characters of tomato plants (Super Jakal cv.) in both seasons (2019 and 2020) compared to untreated plants. The highest plant length was achieved in soil amended with *T. reesei* (43.16 and 44.00 cm) in both seasons 2019 and 2020, respectively, followed by *T. harzianum* (T5). The application of bio agents to infested soil with *F. solani* caused the best increase of plant length, fresh and dry weight. Treated infested soil with Foset 80 fungicide showed the lowest plant length and stem diameter compared to the treatments. Data also showed that all tested biocontrol agents were effective in reducing dead plants and increased survival plants as well as improved plant growth characters compared to untreated plants.

Table (2): Effect of soil treatment with biocontrol agents on Fusarium root rot of tomato under greenhouse conditions.

Treatments	Disease severity (%)		Mean
	Season 2019	Season 2020	
<i>T. harzianum</i> (T5)	33.33	37.70	35.50
<i>T. harzianum</i> (T8)	38.80	40.00	38.80
<i>T. reesei</i>	35.50	31.11	22.20
<i>T. asperellum</i> (T34)	46.60	40.00	43.30
<i>P. fluorescens</i>	37.30	42.60	39.85
Foset 80	19.90	26.60	23.25
control	88.84	91.90	89.96
L.S.D 5%	3.2	3.56	-

Table (3): Effect of application of biocontrol agents on growth characters of tomato under field conditions.

Treatments	Plant height (cm)		Mean	Fresh weight (gm)		Mean	Dry weight (gm)		Mean
	Season 2019	Season 2020		Season 2019	Season 2020		Season 2019	Season 2020	
<i>T. harzianum</i> (T5)	43.30	42.30	42.80	249.80	252.70	251.25	65.51	52.46	58.98
<i>T. harzianum</i> (T8)	41.00	36.50	38.75	231.96	231.60	231.78	46.61	42.27	44.44
<i>T. reesei</i>	43.16	44.00	43.58	229.93	225.06	227.49	53.09	49.71	51.40
<i>T. asperellum</i> (T34)	37.10	38.00	37.55	222.40	232.76	227.58	35.49	37.62	36.55
<i>P. fluorescens</i>	40.83	39.83	40.33	188.20	234.10	211.15	47.05	46.35	46.70
Foset 80	40.10	39.10	39.60	192.40	198.06	195.23	25.55	28.80	27.17
Control	21.80	26.30	24.20	90.60	103.40	97.00	15.05	9.80	12.42
L.S.D 5%	1.40	1.73	-	11.70	15.11	-	5.51	3.43	.

### 3.5 Effect of treatments with biocontrol agents on content of the total phenolic compounds in tomato plants

Data presented in Figure (1) indicate that all tomato plants treated with these biocontrol agents as soil treatments showed the highest contents of total phenolic compounds compared to the untreated plants (healthy and diseased). The highest amounts of total phenolic compounds were found in the treated plants by *T. harzianum* (T5) (87.31 mg/g fresh weight) followed by *T. reesei* (87.25 mg/g fresh weight). Generally, data also indicate that the different treatments with all biocontrol agents enhanced the accumulation of total

phenols compounds in the treated tomato plants compared to the healthy and diseased plants.

### 3.6 Effect of treatments on activities of polyphenoloxidase, peroxidase and catalase enzymes

Data presented in Figure (2) showed that treated tomato plants with the tested biocontrol agent significant variation ( $P \leq 0.05$ ) in polyphenoloxidase (PPO), peroxidase (POD) and catalase (CAT) enzymes activity among the treatments compared to untreated control plants. Data also showed that *T. harzianum* (T5) recorded significantly higher level of POD and PPO enzymes activity (21.50



and 3.32 mg/g fresh weight) over all other treatments followed by *T. reesei*. While *T. asperellum* (T34) also recorded significantly higher level of poly phenol oxidase enzyme activity. Moreover, healthy and untreated control plants recorded lowest level of all determined enzymes activity.

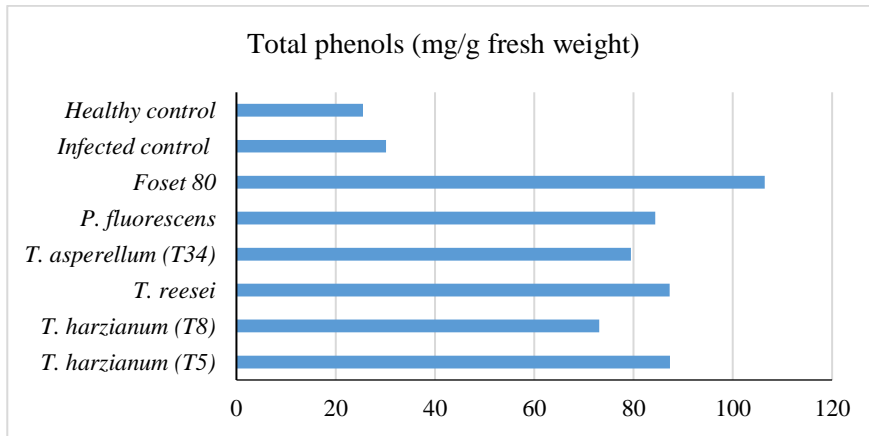


Figure (1): Effect of treatments on accumulation of total phenol content in tomato plants under artificially inoculation with *F. solani* in greenhouse conditions.

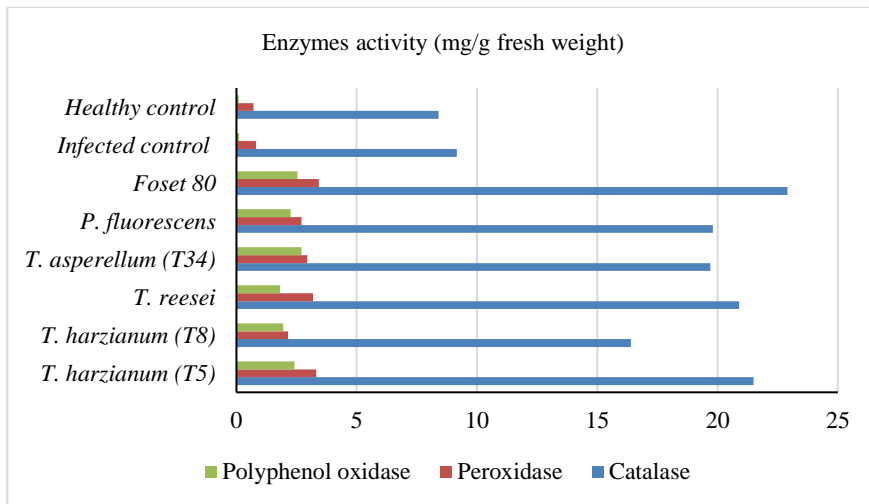


Figure (2): Effect of treatments on activities of polyphenol oxidase, peroxidase and catalase enzymes in tomato plants under artificially inoculation with *F. solani* in greenhouse conditions.

#### 4. Discussion

Biological control is one of the best alternative ways to use pesticides to control plant diseases in many crops, especially in clean farming. In the current study showed that all the tested biocontrol agents were able to inhibit the mycelial growth of the pathogen with variation in their antagonistic capability. The results reported herein indicated that all tested isolates of *Trichoderma* were able to inhibit the mycelial growth of the causal pathogen with different degrees. The highest inhibition of mycelial growth was caused by *T. asperellum* (T34). These results are in agreement with those obtained by Elad *et al.* (1983) and Tsrer *et al.* (2001). This can be explained in the light of results recorded by Abd El-Moity (1981), who stated that *T. harzianum* works through different mechanisms, *i.e.* production of gliotoxin, mycoparasitism and growing very fast and act as barrier between susceptible plant tissues and virulent pathogens. Mycoparasitism by *T. harzianum* is a complex process, involving recognition of the host, attachment to the mycelium, coiling round the hyphae, partial degradation of the cell wall and penetration of the host mycelium (Elad *et al.*, 1983; Benhamou and Chet, 1993 and Pal, 2005). Scanning electron microscopic observation of parasitism of *T. harzianum* and *T. hamatum* on *R. solani* revealed that the hyphae of *Trichoderma* coil around the host. *Trichoderma harzianum* attached to host mycelium by forming hooks and

*Trichoderma* produces appressoria at the tips of short branches (Elad *et al.*, 1983). Under greenhouse conditions, application of *Trichoderma* isolates and *P. fluorescence* for controlling Fusarium root rot disease in tomato had a beneficial effect when addition to the infested soil with the pathogen. Generally, soil treatment with bio-agent isolates was effective in decreasing Fusarium root rot symptoms on plants. In this study, *T. reesei* caused the highest reduction in disease severity of Fusarium root rot in tomato. Application of all treatments enhanced significantly all studied agronomic characters, *i.e.*, plant height (cm), fresh and dry weight (gm). *T. reesei* was the highest effect on all studied agronomic characters of faba bean plants. Similar results were obtained by Yedidia *et al.* (2001) who showed that treatment of cucumber plants in soil with *T. harzianum* (T-203) resulted in large increase in root area and cumulative root lengths, and significant increase in dry weight, shoot length and leaf area over that of the untreated control. Application of *Trichoderma* to the soil as a biocontrol agent, in the greenhouse or under field conditions, not only resulted in reduced disease severity but also enhanced plant growth (Atef, 2008). This result is consistent with those obtained by Yedidia *et al.* (2001) who showed that treatment of cucumber plants in soil with *T. harzianum* (T-203) resulted in large increase in root area and cumulative root lengths, and significant increase in dry weight, shoot length and

leaf area over that of the untreated control. Yedidia et al. (2001) suggested a direct role for *T. harzianum* in mineral uptake by the plant at a very early stage of the fungal-plant association. In addition, Harman (2000) established that *Trichoderma* spp. are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via the production or control of plant hormones. Increased growth response has been demonstrated by several other investigators (Altomare et al., 1999; Anusuya and Jayarajan, 1998). The ability of *Trichoderma* to reduce diseases caused by soil borne pathogens is well known and it is related to the antagonistic properties of *Trichoderma*, which involve parasitism and lysis of pathogenic fungi and competitions for limiting factors in the rhizosphere mainly iron and carbon (Sivan and Chet, 1986). Abou-Aly et al. (2008) showed that *B. megaterium*, *B. subtilis*, *B. coagulans* *P. fluorescens* and *Paenibacillus polymyxa* exhibited beneficial mechanisms against *Fusarium oxysporum* f. sp *lycopersici* and *F. solani*. In this study, application of bio control agents as soil treatments increased level of total phenol content and activities of peroxidase, polyphenoloxidase and catalase enzymes in tomato plants compared to untreated control plants. *T. harzianum* (T5) recorded higher level of POD and PPO enzymes activity (21.50 and 3.32 m/g fresh weight). While *T. asperellum* (T34) also recorded significantly higher level of

poly phenol oxidase enzyme activity. These results are in conformity with the results reported by El-Habbaa et al. (2016) they found that application of bio-formulations such as Rhizo-N, Plant guard and Bio-Zeid as foliar treatment increased activities of peroxidase, polyphenoloxidase and chitinase in grapevine plants compared to the control treatment. In another study, *T. viride* induced higher levels of defense enzymes such as peroxidase, polyphenol oxidase and phenyl alanine ammonia lyase in black gram during pathogenesis by *F. oxysporum* and *A. alternata* (Surekha et al., 2014). The increase in the antioxidant enzymes system such as catalase, ascorbate peroxidase and glutathione reductase was associated with resistance to Fusarium wilt in chickpea (Garcia-Limones et al., 2012). Amer et al. (2014) reported that application of the bio-control agents could play an important role in inducing partial resistance and exhibit greater potential to protect tomato plants against wilt. This study concludes that biocontrol agent treatment led to reduce Fusarium root rot disease severity and improve agronomic characters of tomato plants such as plant height, fresh and dry weight.

## References

- Abdel-Monaim, M. (2012), "Induced systemic resistance in tomato plants against Fusarium wilt diseases", *International Research Journal of Microbiology*, Vol. 3, pp. 14–23.

- Abou-Aly, H. E. (2008), "Evaluation of some rhizobacteria as potent biological control agents *in vitro*", *Annals of Agricultural Science, Moshtohor*, Vol. 46 No. 4, pp. 81–90.
- Abu-Taleb Amira, M., Kadriya El- Deeb and Al-Otibi Fatimah, O. (2011), "Assessment of antifungal activity of *Rumex vesicarius* L. and *Ziziphus spina-christi* (L.) wild extracts against two phytopathogenic fungi", *African Journal of Microbiology Research*, Vol. 5 No. 9, pp. 1001–1011.
- Aebi, H. (1984), "Catalase *in vitro*", *Methods in enzymology*, Vol. 105, pp. 121–126.
- Allam, A. I. and Hollis, J. P. (1972), "Sulfide inhibition of oxidase in rice roots", *Phytopathology*, Vol. 62, pp. 634–639.
- Amer, M. A., El-Samra, I. A., Abou- El-Seoud, I. I., El-Abd, S. M. and Shawertamimi, N. K. (2014), "Induced systemic resistance in tomato plants against Fusarium wilt disease using biotic inducers", *Middle East Journal of Agriculture Research*, Vol. 3 No. 4, pp. 1090–1103.
- Anusuya, D. and Jayarajan, R. (1998), "Solubilization of phosphorus by *Trichoderma viride*", *Current Science*, Vol. 74, pp. 464–466.
- Atef, N. M. (2008), "*Bacillus subtilis* an *Trichoderma harzianum* as wheat inoculants for biocontrol of *Rhizoctonia solani*", *Australian Journal of Basic and Applied Sciences*, Vol. 2 No. 4, pp. 1411–1417.
- Beecher, G. R. (1998), "Nutrient content of tomatoes and tomato products", *Proceedings of the Society for Experimental Biology and Medicine*, Vol. 218, pp. 98–100.
- Benhamou, N. and Chet, I. (1993), "Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*: Ultrastructure and gold cytochemistry of the mycoparasitic process", *Phytopathology*, Vol. 83, pp. 1062–1071.
- Canene-Adams, K., Campbell, J. K., Zaripheh, S., Jeffery, E. H. and Erdman, J. W., Jr (2005), "The tomato as a functional food", *The Journal of Nutrition*, Vol. 135, pp. 1226–1230.
- Dawar, S., Younus, S. M., Tariq, M. and Zaki, M. J. (2007), "Use of *Eucalyptus* sp., in the control of root infecting fungi on mung bean and chick pea", *Pakistan Journal of Botany*, Vol. 39 No. 3, pp. 975–979.
- Elad, Y., Chet, I., Boyle, P. and Henis, Y. (1983), "Parasitism of *Trichoderma* spp. on *R. solani* and *Sclerotium rolfsii*: scanning electron microscopy and fluorescence microscopy", *Phytopathology*, Vol.

- 73, pp. 85–88.
- El-Fawy, M. M., El-Sharkawy, R. M. I. and Abo-Elyousr, K. A. M. (2018), "Evaluation of certain *Penicillium frequentans* isolates against *Cercospora* leaf spot disease of sugar beet", *Egyptian Journal of Biological Pest Control*, Vol. 28: Article number: 49.
- El-Fawy, M. M., Saeed, A. S., Abdel-Fatah, B. E. S., Abo-Elnaga, H. I. G. and Amein A. M. (2018), "Effect of certain biocides on *Fusarium* wilt disease and their effect on gene expression alterations in tomato plants", *Assiut Journal of Agricultural Sciences*, Vol. 49 No. 2, pp. 106–120.
- El-Fawy, M. M., Saeed, A. S., Abdel-Fatah, B. E. S., Abo-Elnaga, H. I. G. and Amein A. M. (2021), "Effect of soil drenching with humic acid, L-methionine and phosphoric acid on *Fusarium* wilt and induction of enzymes related to oxidative stress and defense in tomato plants", *Archives of Phytopathology and Plant Protection*, Vol. 54, pp. 1876–1895.
- El-Habbaa, G. M.; Abdou, M.M Mahdy; F. G. Mohamed and S. A. El- Shaery (2016). "Biological and chemical control of grapevine dieback disease and their effect on defense related enzymes", *International Journal of Scientific & Engineering Research*, Vol. 7 No. 3, pp. 345–351.
- El-Mohamedy, R. S., Jabnoun-Khiareddine, H. and Daami-Remadi, M. (2014), "Control of root rot diseases of tomato plants caused by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium rolfsii* using different chemical plant resistance inducers", *Tunisian Journal of Plant Protection*, Vol. 9: 45–55.
- FAOSTAT (2019), *FAO Statistical Pocketbook*, Food and Agriculture Organization of the United Nations, Rome, Italy.
- Garcia-Limones, C., Navas-Cortes, J. A., Jimenez-Diaz, R. M. and Tena, M. (2012), "Induction of antioxidant enzyme system and other oxidant stress markers associated with compatible and incompatible interactions between chickpea (*Cicer arietinum* L.) and *Fusarium oxysporum* f.sp. *ciceris*", *Physiological and Molecular Plant Pathology*, Vol. 61, pp. 325–337.
- Gebhardt Susan, E. and Thomas, R. G. (2002), *Nutritive Value of Foods*, Nutrient Data Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland, USA.
- Giovanelli, G., Lavelli, V., Peri, C. and Nobili, S. (1999), "Variation in antioxidant components of tomato during vine and post-harvest ripening", *Journal of the Science of Food and Agriculture*, Vol. 79, 1583–1588.

- Gomez, K. A. and Gomez, A. A. 1984. "Comparison between treatment means", In Lviley A. ed., *Statistical procedures for agricultural research*. InterScience Publication New York, USA, pp. 187. *Research*, Vol. 9 No. 53, pp. 3838–3852.
- Grunwald, N. J., Coffman, V. A. and Kraft, J. M. (2003), "Sources of partial resistant to Fusarium root rot in the Pisum core collection", *Plant Disease*, Vol. 87, 1197–1200.
- Luthria, D. L., Sudarsan, M., Krizek, D. T. and Krizek, D. T. (2006), "Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation", *Journal of Food Composition and Analysis*, Vol. 19 No. 8, pp. 771–777.
- Harman, G. E. (2000), "Myths and dogmas of biocontrol: Changes in perceptions derived from research on *Trichoderma harzianum* T-22", *Plant Disease*, Vol. 84, pp. 377–393.
- Matta, A. and Dimond, A. E. (1963), "Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems", *Phytopathology*, Vol. 53 No. 5, pp. 574.
- Harman, G. E. (2017), "*Trichoderma* spp., including *T. harzianum*, *T. viride*, *T. koningii*, *T. hamatum* and other spp. deuteromycetes, moniliales (asexual classification system), Retrieved <https://biocontrol.entomology.cornel.edu/pathogens/trichoderma.php>.
- MSTAT-C (1991), *A software program for the design, management and analysis of agronomic research experiments*. East Lansing (MI), Michigan State University, USA, pp. 400.
- Harman, G. E., Howell, C. R., Viterbo, A., Chet, I. and Lorito, M. (2004), "*Trichoderma* spp opportunistic, avirulent plant symbionts", *Nature Review Microbiology*, Vol. 2, pp. 43–56.
- Mukherjee, P. K., Horwitz, B. A. and Kenerley, C. M. (2012), "Secondary metabolism in *Trichoderma* – a genomic perspective", *Microbiology*, Vol. 158, pp. 35–45.
- Kumar, S., Thakur, M. and Rani, A. (2014), "Trichoderma: Mass production, formulation, quality control, delivery and its scope in commercialization in India for the management of plant diseases", *African Journal of Agricultural*
- Pal, R., Chakrabarti, K., Chakraborty, A. and Chowdhury, A. (2005), "Pencycuron application to soils: Degradation and effect on microbiological parameters", *Chemosphere*, Vol. 60 No. 11, pp. 1513–1522.
- Rifai, M. A. (1969), "A revision of the

- genus *Trichoderma*", *Mycological Papers*, Vol. 116, pp. 1–56.
- Saad, M. (2006), "Destruction of *Rhizoctonia solani* and *Phytophthora capsici* causing tomato root-rot by *Pseudomonas fluorescens* lytic enzymes", *Research Journal of Agriculture and Biological Sciences*, Vol. 2, pp. 274–281.
- Schuster, A. and Schmoll, M. (2010), "Biology and biotechnology of *Trichoderma*", *Applied Microbiology and Biotechnology*, Vol. 87 No. 3, pp. 787–799.
- Sivan, A. and Chet, I. (1986), "Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma*", *Journal of Phytopathologische Zeitschrift*, Vol. 116, pp. 39–47.
- Snell, F. D. and Snell, C. T. (1953), *Colorimetric methods of analysis, including some turbidimetric and nephelometric methods*, Volume III, D. Van Nostrand Co., Inc., Princeton, Jersey, Toronto, New York and London, pp. 606.
- Srinivas, C., Devi, D. N., Murthy, K. N., Mohan, C. D., Lakshmeesha, T. R., Singh, B. And Srivastava, R. K. (2019), "*Fusarium oxysporum* f. sp. *lycopersici* causal agent of vascular wilt disease of tomato: Biology to diversity—A review", *Saudi Journal of Biological Sciences*, Vol. 26 No. 7, pp. 1315–1324.
- Surekha, C. H., Neelapu, N. R. R., Siva Prasad, B. and Sankar Ganesh, P. (2014), "Induction of defense enzymes and phenolic content by *Trichoderma viride* in *vigna mungo* infested with *Fusarium oxysporum* and *Alternaria alternata*", *International Journal of Agricultural Science and Research*, Vol. 4 No. 4, pp. 31–40.
- Tsrer, L., Baraka, R. and Snehb, B. (2001), "Biological control of black scurf on potato under organic management", *Crop Protection*, Vol. 20 No. 2, pp. 145–150.
- Walters, D., Walsh, D., Newton, A. and Lyon, G. (2005), "Induced resistance for plant disease control: maximizing the efficacy of resistance elicitors", *Phytopathology*, Vol. 95, pp. 1368–1373.
- Yedidia, I., Srivastva, A. K., Kapulnik, Y. and Chet, I. (2001), "Effective of *Trichoderma harzianum* Microelement concentration and increased growth of cucumber plants", *Plant and Soil*, Vol. 235, pp. 235–242.