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# Biological Control and Induction of Systemic Resistance Against Cucumber *Fusarium* Wilt by Plant Growth Promoting Rhizo-organisms

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## ABSTRACT

Cucumber *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *cucumerinum* (FOC), is one of the most important cucumber diseases in Egypt. Effects of five plant growth promoting rhizo-organisms (PGPRs) namely; *Pseudomonas fluorescens*, *Bacillus subtilis*, *Rhizobium* sp., *Trichoderma harzianum* and *T. viride* on the linear growth of FOC, controlling *Fusarium* wilt incidence and inducing systemic resistance were investigated under laboratory and glasshouse conditions, using Al-Zaeem cucumber cultivar as a susceptible cultivar and Hayel as a resistant one. Results indicated that *P. fluorescens* led to the greatest reduction in FOC mycelial growth (87.04%), followed by *T. viride* and *T. harzianum* at (83.33 and 80.74%), respectively. The tested PGPRs significantly reduced wilt disease incidence in cucumber plants as compared with control treatment (soil infected only with FOC) in both susceptible and resistant cultivars. The greatest percentages of healthy survived plants and disease reduction were observed at *P. fluorescens, T. viride* and *T. harzianum* treatments. The tested PGPRs also increased plant height, root length, leaves number and fresh weight in both studied cultivars as compared with control treatment. Moreover, phenol content, peroxidase (PO) and polyphenol oxidase (PPO) activities in PGPR treated plants were significantly higher than those in control plants either in susceptible or resistant cultivars. Also, phenol contents and the activities of oxidative enzymes were higher in cucumber plants grown in FOC-infected soil than in those grown in the controls, irrespective of PGPR treatments.

**Key words:** *Fusarium* wilt, Biological control, Rhizo-organisms, Phenol, Peroxidase, Polyphenol oxidase.

## INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important vegetable crops not only in Egypt, but also in many other countries worldwide. Cucumber plants are subject to infect by several plant pathogens (Abass, 2010 and Kanika and Raina, 2013). Among plant diseases, cucumber *Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *cucumerinum* (FOC), was reported to inflect severe damage and economic losses in plant stand, fruits quantity and quality (Pu *et al.*, 2011).

In the previous few decades, a great attention has been given to the biological control of soil-borne pathogens in vegetable crops in general and cucumber crop in particular. Great interest has recently been paid to the use of plant growth promoting rhizo-organisms (PGPRs) as an environment friendly control method (Hamed, 1999 and Cao *et al.*, 2012). These PGPRs are mainly bacterial and fungal species that have been used successfully against *Fusarium* wilt worldwide (Cao *et al.*, 2012 and Gul *et al.*, 2013).

Use of PGPRs for inducing systemic resistance against *Fusarium oxysporum* has been previously studied (Liu *et al.*, 2010). PGPRs may include various microorganisms such as *Pseudomonas* spp., *Bacillus* spp., *Rhizobium* spp. and *Trichoderma* spp. The action of induced resistance includes accumulation of phenolic compounds as well as elevation in the activities of its related oxidative enzymes such as peroxidase (PO) and polyphenol oxidase (PPO), which are important biochemical parameters for disease resistance (Pradeep and Jambhale, 2002 and Das *et al.*, 2003).

Bearing these views in mind, this study aimed to investigate the role of five PGPR species in inhibiting the mycelial growth of FOC under laboratory conditions and in controlling *Fusarium* wilt in cucumber plants under greenhouse conditions. In addition, the effects of PGPRs on phenol content and on activity of oxidative enzymes (peroxidase and polyphenol oxidase) were also studied.

## MATERIALS AND METHODS

### Sources of PGPRs and FOC

Five PGPRS species (*Pseudomonas fluorescens*, *Bacillus subtilis*, *Rhizobium* sp., *Trichoderma harzianum* and *T. viride*) were obtained from the stock cultures, kept in the Laboratory of Soil Microbiology, Department of Soil and Water, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. For FOC, this isolate was isolated from wilted cucumber plants grown in greenhouses at Ismailia Governorate. It was identified according to Booth (1971) as confirmed to be FOC using differential varieties test.

### Effect of PGPRs on the linear growth of FOC

Petri dishes (9 cm diameter), each contained PDA medium, were used to study the effect of the tested PGPRs on the mycelial growth of FOC. Five mm diameter disc of a seven days old fungal

growth of FOC was placed, 2cm from the edge of the plate. On the opposite side of the Petri plate, a five mm disc of the tested PGPR or a streak of the tested bacterium was placed. In control treatment, Petri dishes were inoculated only with FOC. Three replicates for each treatment were used. All Petri dishes were incubated at 28±2°C. When FOC almost covered the medium surface in control treatment, the linear growth of FOC in different PGPR treatments was measured and percentage of reduction was calculated according to the following formula:

$$A = \frac{B - C}{B} \times 100$$

Where: A = Percentage of growth reduction, B = Mean diameter of the pathogenic fungus in the control treatment and C = Mean diameter of FOC in different treatments.

**Effect of PGPRs on the incidence of cucumber *Fusarium* wilt under glasshouse conditions**

This experiment was conducted to evaluate the efficacy of the tested PGPRs for controlling cucumber wilt caused by FOC, using Al-Zaeem and Hayel cucumber cultivars. The former is a susceptible cultivar and the latter is a resistant one. Inoculum of each of *T. viride*, *T. harzianum* and/ or FOC was grown on sterilized sorghum grains medium and incubated at 28°C for 15 days in the glasshouse of Biological Control Center, Faculty of Agriculture, Suez Canal University, Ismailia. The inoculum of FOC and the tested PGPRs were prepared by mixing one volume of each PGPR with the tested pathogen individually at the rate of (1:1 v/v). Prepared inoculum was added to each pot (20 cm diameter) at the rate of 2% (w/w) and then mixed thoroughly and watered (EL-Sharkawy, 2010). In all cases, unsterilized soils were used for all treatments. In case of the antagonistic bacteria (*B. subtilis* and *Rhizobium* sp. and *P. fluorescens*), they were separately grown in potato nutrient broth for 15 days at 30±2°C in rotary shaking incubator. Inoculum of each bacterial culture was applied at the rate of 10 ml/pot ($10^9$ CFU/ ml) before sowing (Zian, 2011). FOC- infested soil without the addition of any PGPR was used as control treatment. Five pots were used for each treatment with five cucumber seeds/pot. The pots were watered at regular intervals or when needed with equal amounts of water. Data were recorded in terms of percentages of pre- and post – emergence damping-off, survived plants (healthy and infected) and disease severity. The scale proposed by Ishikawa *et al.* (2005) was used as grades from 0 – 4 according to the percentage of internal browning through stem and root: 0 = healthy, 1= 0 - 25 % browning, 2= >25-50 % browning, 3= > 50-75 % browning, and 4= >75-100 % browning. Plant growth parameters, *e.g.* plant height, root length, number of leaves and total fresh weight were recorded 90 days post sowing.

**Effect of PGPR on phenolic content of cucumber plants**

Samples of five g of fresh leaves of susceptible cucumber variety (Al-Zaeem) and resistant variety (Hayel) were taken from each PGPR treated plants well as untreated control 45 days post planting. The leaf samples were chopped into small pieces, then stored immediately in 95% ethanol in brown bottles and kept in the dark at room temperature (25-28 °C) for one month. After that, these tissues became colorless. The ethanolic extracts were completely evaporated and the remnant dry films of the extracts were quantitatively transferred into 5ml of 50% isopropanol and stored in glass vials at -20°C until the determination of the phenol content (El-Toony, 1992).

Phenolic contents of cucumber plants were calorimetrically determined according to Snell and Snell (1953). Total phenols were determined by adding 10 drops of concentrated HCL to 0.1 ml of the sample, heated rapidly to the boiling point and then placed in boiling water bath for 10 min. After coloring, 0.1 ml of the reagent (Folin-Ciocalteu) and 5 ml of 20% NaCo3 were added. This mixture was diluted to 10 ml with distilled water and then the total phenols were determined using a spectrophotometer at 520 nm. As for free phenols, they were determined by adding 0.1ml of the reagent (Folin-Ciocalteu) and 3 ml of 20% sodium carbonate solution to 0.1 ml of the sample and then diluted to 10 ml with distilled water. Readings were performed after 30 min using a spectrophotometer at 520 nm after 30 min.

**Effect of PGPR on peroxidase and polyphenol oxidase activity**

Two grams of leaf samples of each of the tested cultivars were taken from each PGPR treatment as well as control, 45 days post planting. Samples were mashed and grounded well in 2ml of 0.1 M phosphate buffer (pH 6.50). Each sample was packed in Falcon tube (ca 10ml), completed to 10ml with phosphate buffer and centrifuged at 4000 rpm for 20 minutes. The supernatant was collected in another clean Falcon tube and stored at -20°C until used to determine the enzyme activities. Peroxidase activity (PO) was measured by adding 0.1ml of enzyme extract to 4ml of guaiacol solution. The guaiacol solution consisted of 3ml of 0.1M potassium phosphate (pH 6.50), 0.5ml of 2% guaiacol and 0.5ml of 0.3% $H_2O_2$ (Allam and Hollis, 1972). Enzyme activity was determined at 470 nm and was expressed as the change in absorbance at 425nm for 1min. In case of PPO assay, the methodology proposed by Matta and Dimond (1963) was used. The reaction mixture contained 0.1ml enzyme extract, 1.0ml of 0.2 M potassium phosphate buffer (pH 7.0) and 1.0ml $10^{-3}$ M catechol and completed with distilled water to 6.0ml. The reaction

mixture was incubated for 30 minutes at 30°C. PPO activity was expressed as the change in the absorbance each 0.5 min for 5 minutes at 430 nm.

**Statistical analysis**

Obtained data were analyzed using one-way ANOVA (SAS Institute, 2003). The proportional data were transformed to arcsine number before analyses. In case of significant F-values, Least Significant Difference (LSD) was used for comparison between means at the probability level of 0.05.

## RESULTS AND DISCUSSION

**Effect of PGPRs on the linear growth of FOC**

As shown in fig (1), the tested PGPRs differed significantly in reducing the mycelial growth of FOC as compared to the control treatment. *P. fluorescens* was the most effective one in reducing FOC mycelial growth (87.04%), followed by *T. viride* (83.33%), whereas *Rhizobium* sp. was the least effective one with reduction percentage of 67.78%. These findings are in harmony with those reported earlier by Kanika and Raina, (2013). These results could be attributed to the excretion of toxic or inhibitory metabolic substances or the competition with FOC for the limited nutrient resources (Sankar and Jeyarajan, 1996 and Chen *et al.*, 2012).

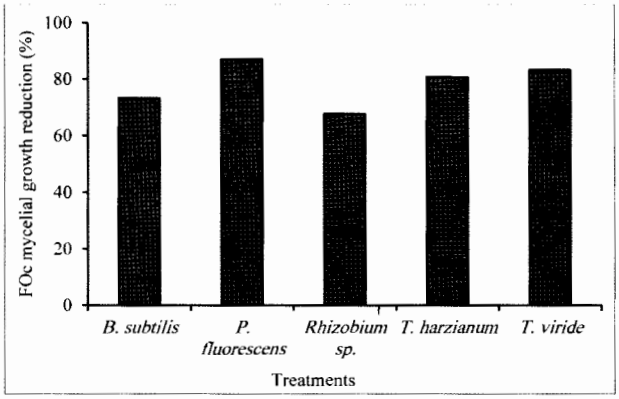


Fig. (1): Mycelial growth reduction (%) of FOC in response to PGPRs *in vitro*

FOC= *F. oxysporum* f. sp. *cucumerinum*.

PGPR= Plant growth promoting rhizo-microorganism

**Effects of PGPRs on Fusarium wilt incidence**

Data presented in table (1) show that application of the tested PGPRs significantly reduced wilt incidence in cucumber plants as compared with control treatment (soil infected only with FOC) either in the susceptible or the resistant cultivar. *P. fluorescens* was the most effective tested PGPR in reducing wilt incidence in the susceptible cultivar (Al-Zaeem), showed the highest percentage of healthy plants and disease reduction being (86.62 and 80.45%), respectively with subsequent lowest severity percentage of (9%). On the other hand, *Rhizobium* sp. was the least effective PGPR in controlling wilt incidence at respective percentages (66.36, 60 and 17.53%). Also, all PGPR treatments caused significant decrease in pre- and post-emergence damping off as compared to control treatment either in the susceptible or resistant cultivars. Meanwhile, the effectiveness of *T. harzianum*, *T. viride* and *B. subtilis* in controlling wilt incidence was moderate but differed significantly with the control treatment. In case of the resistant cultivar Hayel, the values of all recorded parameters were generally lower than those recorded at the susceptible one, except the percentage of healthy plants and disease reduction. The same trend of effectiveness of PGPRs against *Fusarium* wilt was also ranked in descending order as *P. fluorescens*, *T. viride*, *T. harzianum*, *B. subtilis* and *Rhizobium* sp. (Table 1). These results are in agreement with those reported earlier (Yang *et al.*, 2008; Chen *et al.*, 2010; Mohamed, 2011; Cao, 2012 and Gul *et al.*, 2013) who concluded that biological control using PGPRs was a successful control method to control cucumber *Fusarium* wilt caused by FOC.

**Effects of PGPRs on plant morphological characters**

Data in table (2) show that the tested PGPRs had significant effects on the morphological characters (*i.e.*, root length, whole plant length, leaves number and fresh weight) either for the susceptible cucumber variety (Al-Zaeem c.v.) or the resistant one (Hayel c.v.) grown in FOC-infested soil. In the susceptible cultivar, *P. fluorescens* treatment led to the longest plant height (87.30 cm) and root length (23.10 cm) with subsequent greatest number of leaves (7.60 leaves/ plant) and total plant fresh weight of (16.37 g). However, *Rhizobium* sp. gave the lowest morphological data as respective values were of 69.40 cm, 17.50 cm, 6.20 leaves/ plant and 11.73 g/ plant. Pertaining to the resistant cucumber variety, *P. fluorescens* also was the best treatment with respective values of 106.70 cm, 27.90 cm, 12.20 leaves and 20.97 g, respectively. It was observed that there were significant differences among all PGPR treatments as compared with control. These findings are in harmony with those reported earlier by Yang *et al.* (2008) who found that *T. harzianum* had an obvious growth-promoting effect on vegetable crops and could significantly improve plant height, length of root and yield. Also, application of *B. subtilis* B579 at $10^8$ CFU/ml reduced disease incidence by 73.60% and promote seedling growth (Chen *et al.*, 2010).

The mode of action of the tested PGPRs differed from one to another. *Trichoderma* spp. produce extracellular lytic enzymes and volatile substances (Elad *et al.*, 1982). Also, it has been reported that *Trichoderma* spp. may excrete toxic or inhibitory

Table (1): Effect of some PGPRs on *Fusarium* wilt incidence and severity of two cucumber cultivars grown in FOC-infected soil under glasshouse conditions

| PGPR treatment | Susceptible variety (Al-Zaeem) | | | | | | Resistant variety (Hayel) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Damping-off (%) | | Survival plants (%) | | Wilt severity (%) | Disease reduction (%) | Damping-off (%) | | Survival plants (%) | | Wilt severity (%) | Disease reduction (%) |
| | Pre-emergence | Post-emergence | Infected | Healthy | | | Pre-emergence | Post-emergence | Infected | Healthy | | |
| Control | 26.00a | 20.86a | 30.09a | 23.05c | 46.66a | -- | 12.00a | 7.50a | 23.48a | 57.02c | 25.57a | -- |
| *B. subtilis* | 4.80b | 2.23b | 15.19b | 77.78ab | 16.17bc | 64.17bc | 1.56b | 2.00ab | 12.15b | 84.29b | 10.17b | 60.23a |
| *P. fluorescens* | 1.21b | 2.00b | 10.17b | 86.62a | 9.00c | 80.45a | 1.58b | 0.00b | 1.980c | 96.26a | 2.00b | 92.00a |
| *Rhizobium* sp. | 6.00b | 6.66b | 20.98ab | 66.36b | 17.53b | 60.00c | 3.12b | 4.00ab | 13.15b | 79.73b | 10.33b | 62.87a |
| *T. harzianum* | 2.20b | 4.44b | 12.39b | 80.97a | 11.38bc | 74.73abc | 1.56b | 2.66ab | 9.41bc | 86.37ab | 5.76b | 77.47a |
| *T. viride* | 2.80b | 2.45b | 12.69b | 82.06a | 10.55bc | 76.65ab | 1.56b | 2.00ab | 5.05bc | 91.39b | 4.78b | 82.18a |
| LSD 0.05 | 5.53 | 8.66 | 11.12 | 12.61 | 7.90 | 15.65 | 4.97 | 6.31 | 8.46 | 11.17 | 10.13 | 43.98 |

Table (2): Effect of some PGPRs on morphological characteristics of two cucumber cultivars grown in FOC-infected soil under glasshouse conditions

| PGPR treatment | Susceptible variety | | | | Resistant variety | | | |
|---|---|---|---|---|---|---|---|---|
| | Plant height (cm) | Root length (cm) | Leaves number | Fresh weight (g) | Plant height (cm) | Root length (cm) | Leaves number | Fresh weight (g) |
| Control | 30.15d | 8.80d | 4.00c | 6.50c | 45.65c | 13.45c | 6.30d | 8.82c |
| *B. subtilis* | 79.90b | 20.40abc | 6.70ab | 11.78b | 91.30b | 25.70ab | 8.20c | 14.76b |
| *P. fluorescens* | 87.30a | 23.10a | 7.60a | 16.37a | 106.70a | 27.90a | 12.20a | 20.97a |
| *Rhizobium* sp. | 69.40c | 17.50c | 6.20b | 11.73b | 91.60b | 23.90b | 8.10c | 14.63b |
| *T. harzianum* | 80.90ab | 19.50bc | 6.20b | 11.73b | 91.60b | 25.60ab | 9.40bc | 15.60b |
| *T. viride* | 85.90ab | 22.50ab | 7.00ab | 12.59b | 96.85ab | 26.8a8b | 10.70b | 16.82b |
| LSD 0.05 | 6.44 | 3.12 | 1.05 | 2.17 | 11.06 | 3.89 | 1.43 | 3.07 |

Table (3): Phenol content (mg/ 100 g fresh weight) of two cucumber cultivars grown in soil infested with FOC and PGPRs under glasshouse conditions

| PGPR treatment | Phenol contents (mg/ 100 g fresh weight) in, | | | | | |
|---|---|---|---|---|---|---|
| | Susceptible cultivar (Al-Zaeem) | | | Resistant cultivar (Hayel) | | |
| | Free | Conjugated | Total | Free | Conjugated | Total |
| Control | 6.73c | 2.19a | 8.92c | 9.06d | 2.52a | 11.58d |
| Control+ FOC | 10.89b | 3.30a | 14.19b | 14.22c | 2.62a | 16.84c |
| *B. subtilis* | 16.52a | 2.94a | 19.46a | 16.94bc | 3.15a | 20.09bc |
| *P. fluorescens* | 19.10a | 3.09a | 22.19a | 20.97a | 3.86a | 24.83a |
| *Rhizobium* sp. | 16.83a | 3.02a | 19.85a | 18.20ab | 3.51a | 21.71ab |
| *T. harzianum* | 17.67a | 2.98a | 20.65a | 19.50ab | 3.53a | 23.03ab |
| *T. viride* | 17.50a | 3.57a | 21.07a | 21.20a | 3.28a | 24.48a |
| LSD | 2.66 | 3.58 | 4.44 | 3.09 | 2.62 | 3.63 |

Table (4): Peroxidase and polyphenol-oxidase activity of two cucumber cultivars grown in soil infested with FOC and PGPRs under glasshouse conditions

| Treatment | Peroxidase activity unit/g fresh weight/1 min | | Polyphenol-oxidase unit/g fresh weight/5 min | |
|---|---|---|---|---|
| | Susceptible Variety | Resistant Variety | Susceptible Variety | Resistant Variety |
| Control | 0.41c | 0.58c | 0.33d | 0.49e |
| Control + FOC | 0.79abc | 0.84bc | 0.59d | 0.80de |
| *B. subtilis* alone | 0.63bc | 0.95bc | 0.81cd | 1.01de |
| *P. fluorescens* alone | 0.80abc | 0.92bc | 0.96bcd | 1.149d |
| *Rhizobium* sp. alone | 0.72abc | 0.94bc | 0.86cd | 1.04 d |
| *T. harzianum* alone | 0.76abc | 1.01abc | 1.01bcd | 1.25cd |
| *T. viride* alone | 0.79abc | 1.05abc | 1.01bcd | 1.24d |
| *B. subtilis* +FOC | 1.39abc | 1.88ab | 1.81ab | 2.22ab |
| *P. fluorescens* FOC | 1.69ab | 1.89ab | 2.06a | 2.55a |
| *Rhizobium* sp. +FOC | 1.39abc | 1.51abc | 1.59abc | 1.78bc |
| *T. harzianum* +FOC | 1.81a | 2.17a | 2.14a | 2.54a |
| *T. viride* +FOC | 1.75a | 1.96ab | 2.01a | 2.36a |
| LSD | 1.113 | 1.214 | 0.92 | 0.54 |

Means in the same column followed by different alphabetical letters indicate significant differences (LSD, P> 0.05)
Each figure represents the mean of five replicates
FOC= *F. oxysporum* f. sp. *cucumerinum*. PGPR= Plant growth promoting rhizo-microorganism

metabolic substances such as gliotoxin (Li *et al.*, 2004), occupy infection courts, induce resistance, produce protease and fungal cell wall degrading enzymes (Perello *et al.*, 2003). *Pseudomonas* spp. have several mechanisms including rapid growth *in vitro*, rapid utilization of seed and root exudates, colonization and multiplication in the rhizosphere, production of wide spectrum of bioactive metabolites, its aggressive competition with other microorganisms and its adaption to environmental stresses (Weller, 2007). As for *B. subtilis*, its antagonistic activity is mainly due to dipeptide compounds namely Bacilysin and Fengymycin (Loeffler *et al.*, 1986) and to the antibiosis, lysis, and competition with pathogens for limited nutrients (Wang *et al.*, 1993). It has also been reported that *B. subtilis* provides protection for crop seedlings against the infection by *F. oxysporum* due to the production of antibiotics (bacteriocin and subtilisin), which

**Effect of PGPRs on phenol content**

Data in table (3) show that total phenols differed significantly among all tested PGPRs and control treatments. Highest total phenols was observed in *P. fluorescens* treatment (22.19 mg/100 g fresh weight), whereas the lowest was recorded for *B. subtilis* at (19.46 mg/100g fresh weight) as compared to non-infected control (8.92 mg/100g fresh weight) and control with FOC infestation (14.19 mg/100g fresh weight). In the resistant cultivar (Hayel c.v.), the levels of phenol compounds in all tested PGPR treatments were higher than those recorded in the susceptible variety being greatest with *P. fluorescens* (24.83/100 g fresh weight) and lowest with *B. subtilis* (20.09 mg/100 g fresh weight) as compared to control without FOC infection (11.58 mg/100 g fresh weight) and control with FOC infection (16.84 mg/100 g fresh weight). These findings are in agreement with those reported earlier for cucumber (Abass, 2010 and Mohamed, 2011) and other crops (Zahra, 1990). inhibit the pathogenic fungi (Sankar and Jeyarajan, 1996).

The action of phenol system and related oxidative enzymes as well as accumulation of phytoalexins represents one of the accepted mechanisms of plant resistance (Hare, 1966). Farkas and Kiraly (1962) mentioned that phenols are oxidized to quinines or semi quinines and play a crucial role as antimicrobial substances. Accumulation of phenolics is considered a major defense mechanism in many plant species by acting as hydrogen donors/acceptors in the oxidation reaction and their involvement in resistance by the oxidation of quinines, which are more toxic to microorganisms (Gupta *et al.*, 1992). These compounds were shown to accumulate both in roots and shoots in response to various fungal infections and elicitors and their levels significantly increase in plants pre-inoculated with antagonistic microorganisms.

**Effect of different PGPRs on PO and PPO activity in cucumber plants**

As shown in table (4), the tested PGPRs increased PO and PPO activities compared to un-infected control treatment and FOC-infected control in the two cultivars, and it was higher in resistant cultivar than the susceptible one. Highest total PO activity in the susceptible variety was observed with *T. harzianum* + FOC, followed by *T. viride* + FOC and *P. fluorescens* + FOC treatments being 1.81, 1.75 and 1.69 unit/g fresh weight/min, respectively, compared with the lowest PO activity in un-infected control at 0.41 unit/g fresh weight/min. The same trend of activity was observed in the resistant cultivar. Pertaining to PPO in susceptible variety, the highest PPO activity was recorded at *T. harzianum* + FOC treatment, *P. fluorescens* + FOC and *T. viride* + FOC treatments at 2.14, 2.06 and 2.01 unit/g fresh weight/5min, respectively as compared with the un-infected control (0.33 unit/g fresh weight/5min). In the resistant variety, the highest PPO activity was recorded with *P. fluorescens* + FOC and *T. harzianum* + FOC treatments being 2.55 and 2.54 unit/g fresh weight/5min as compared with 0.49 unit/g fresh weight/5min in un-infected control. These findings are in harmony with those reported earlier (Zhuang *et al.*, 2005; Abass, 2010 and Mohamed, 2011). Enhancing the activity of PO and PPO enzymes in plant tissues in response to pathogenic infection was also reported by other earlier researchers (Das *et al.* 2003; Abo-Elyousr *et al.* 2008 and Cherif *et al.*, 2007). *Fusarium* species are known to produce toxic metabolites that play a vital role in tissue browning through their ability to oxidize phenols to quinines (Gupta *et al.*, 1992). These fungal metabolites may also activate the production of phenol-oxidizing enzymes such as PO and PPO (Ramadoss, 2002).

It could be concluded that the application of PGPRs caused -significant reduction in mycelial growth of FOC. Also, the application of all these PGPRs in general and *P. fluorescens* in particular as cucumber seed coating led to significant reduction in wilt incidence and disease reduction. Moreover, this application also increased phenol content and activities of the oxidative enzymes (peroxidase and poly phenol oxidase) that are responsible for the induced resistance in treated plants. However, it is recommended to conduct these experiments on large scale under field conditions before any ultimate conclusion could be drawn.

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