Induction of Defense Compounds by Seed Treatment with Plant Growth-Promoting Rhizobacteria *Bacillus* sp. against Tomato Fusarium Wilt Pathogen

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Abstract: Plant growth–promoting rhizobacteria (PGPR) *Bacillus* sp. strain TB281 was evaluated for plant growth promotion and biological control of tomato Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. In the bacterized plants, there were significant growth increase in shoot length and fresh weight compared to untreated control. Seed bacterization using this *Bacillus* strain also decreased the number of diseased plants compared to non-bacterized controls. The present results also show that bacterized tomato plants contained higher levels of defense-related enzymatic activities in terms of peroxidase (PER), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) as well as endogenous phenolic compounds than non-bacterized ones. Maximum levels of defense enzymatic activities and endogenous phenolic compounds were found in *Bacillus*-treated plants growing in *F. oxysporum*-infested soil compared with their respective controls. The present data suggest that earlier and higher levels of enzymatic activities involved in phenylpropanoid metabolism and phenolic compounds were occurred in tomato plants treated with *Bacillus* TB281 in response to attack by *F. oxysporum*. The plant-pathogen interactions have also triggered the activities of defense enzymes and total soluble phenolics during the late primary events of the host-pathogen interaction, but during the secondary events the activities were declined sharply. Elevated levels of phenolics and defense related-enzymatic activities by *Bacillus* TB281 in tomato plants might have collectively contributed to induced resistance in tomato plants against tomato Fusarium wilt pathogen.

Keyword*: Bacillus* sp.; Biological control; Induced systemic resistance; Mechanism of action; rhizobacteria

INTRODUCTION

Induction of plant defense genes by prior application of inducing agents is called induced resistance (Hammerschmidt and Kuć, 1995). The defense gene products include peroxidase (PER), polyphenol oxidase (PPO) that catalyze the formation of lignin and phenylalanine ammonia-lyase (PAL) which is involved in the phytoalexins and phenolic synthesis. Other defense enzymes include pathogenesis-related proteins (PRs) such as ß-1,3-glucanases (PR-2 family) and chitinases (PR-3 family) which degrade the fungal cell wall and cause lysis of fungal cell. Chitin and glucan oligomers released during degradation of fungal cell wall act as elicitors that elicit various defense mechanisms in plants (Frindlender *et al.*, 1993). Induction of defense proteins makes the plant resistant to pathogen invasion (Beaudoin-Eagan and Thorpe, 1985; Bashan *et al.*, 1985; Rasmussen, 1991; Van Loon, 1997).

Certain Bacillus spp. are non-pathogenic rhizobacteria. Several isolates of *Bacillus* have been shown to suppress phytopathogens through rhizosphere colonization, antibiosis and iron chelation by siderophore production (Broadbent *et al.*, 1977; Van Peer *et al.*, 1991; Asaka and Shoda, 1996; Podile *et al.*, 1995; Kloepper, 1996; Podile and Prakash 1996; Dileep Kumar, 1999; Silva *et al.*, 2004; Jetiyannon, 2007). Certain *Bacilli* spp. are also found to promote plant growth by production of plant growth-promoting substances (Sailaja *et al.*, 1998; Guo *et al.*, 2004; Jetiyanon, 2007) and thus termed plant growth promoting rhizobacteria (PGPR). PGPR are known to induce resistance against fungal, bacterial, viral diseases and insect pests (Wei *et al.*, 1996; Zehnder *et al.*, 1997; Maurhofer *et al.*, 1998; Chen *et al.*, 2000). In addition to plant growth-promotion and direct antifungal activity elicited by PGPR, the activation of plant defense gene products by prior application with PGPR against a challenging pathogen is considered to be a novel strategy in plant protection. Induced resistance by *Pseudomonas fluorescens* is associated with accumulation of PR proteins (Maurhofer *et al.*, 1994; Viswanathan and Samiyappan, 1999) and synthesis of phenolics (Chen *et al.*, 2000; M'Piga *et al.*, 1997).

Fusarium wilt of tomato initiated by *F. oxysporum* is a serious soil-borne disease in tomato plants. Earlier studies indicated that seed treatment with *P. fluorescens* isolate 63-28 prevented the entry of Fusarium wilt pathogen in the vascular tissue by strengthening cell wall structures and accumulation of phenolic substances and chitinases (M'Piga *et al.*, 1997). However, there is a little information available on plant-mediated defense reactions induced by *Bacillus* spp. in plants against pathogen invasion.

The objectives of the present study were: (1) to determine the efficacy of *Bacillus* sp. strain TB281 for its effect on plant growth promotion and its ability to control *F. oxysporum* and (2) to elucidate the mode of *Bacillus* action in the biocontrol of the fusarium wilt pathogen by doing biochemical analysis in terms of activities of peroxidase (PER), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) and levels of phenolic compounds in tomato plants after infection with *F. oxysporum* f. sp. *lycopersici*.

MATERIALS AND METHODS

Source of the bacterial isolate and inoculum preparation

Bacillus sp. strain TB281 was previously isolated from the rhizoplane of healthy tomato plants grown in the Farm of Faculty of Agriculture, Suez Canal University. This strain showed an antagonistic activity

against several phytopathogens *in vitro* and thus it was considered as a biocontrol agent for a number of phytopathogens, including *Fusarium oxysporum* f. sp. *lycopersici* (Youssef and Abdl-Wahid, 2005). *Bacillus* TB281 cultures were stored at –20 °C in Nutrient Broth (NB) medium (beef extract 3 g, peptone 6 g, and sucrose 10 g in 1.0 L distilled water) (Fang, 1998) amended with 20% glycerol. The inoculum was prepared by streaking bacteria onto Nutrient agar (NA) plates and incubated at 30 °C for 3 days and scraping bacterial cells off plates in sterile distilled water (SDW) to yield 10^9 colony forming units (CFU) per ml.

Fungal isolate and inoculum preparation.

Tomato plants showing vascular wilt symptoms were collected from different locations at Ismailia Governorate during the growing seasons of 2005 and 2006. Plant samples were thoroughly washed under running tap water, surface sterilized in 2% sodium hypochlorite solution for 3 min and rinsed three times in sterilized distilled water (SDW). Small pieces were planted on potato dextrose agar (PDA) medium. The isolated fungus was identified as *Fusarium oxysporum* f. sp. *lycopersici* on the basis of the mycological characteristics described by Domsch *et al.* (1980) and Nelson *et al.* (1983).

Fungal inoculum was prepared using sterilized sorghum medium inoculated with 4 mm discs taken from a 7-day-old *F. oxysporum* culture. The inoculated media were incubated at 27 °C for 15 days. Soil were infested with the fungal inoculum at the rate of 3% (w/w) and watered every other day for one week. Soil provided with the same amount of fungal free medium was used as control.

Pathogenicity test

F. oxysporum f. sp. *lycopersici* was examined for its pathogenicity according to the modified method described by Haglund (1989).

Seed bacterization with *Bacillus* TB281

A modified method of Weller and Cook (1983) was applied. Tomato seeds were surface sterilized by dipping in 2.4% sodium hypochlorite solution for 2 min, rinsed in SDW and then air-dried. Bacillus culture grown on potato dextrose agar (PDA) for 48 h at 28 °C was scraped with a rod and suspended in 10 ml of 1% carboxymethylcellulose (CMC). 1 g of surface sterilized seeds was placed into the bacterial suspension in CMC for 1 h and air-dried for 2 to 3 h in sterilized Petri plates. Seeds treated with 1% CMC only were served as control. The dried seeds were sampled and colony-forming units (CFU) were determined on PDA. The bacterial suspension was adjusted to give 1.2×10^7 CFU/ seed.

Effect of seed bacterization on growth and control of Fusarium wilt of tomato

This experiment was carried out in four treatments in a randomized block design. The first treatment contained non--bacterized seeds grown in non-infested soil (no rhizobacteria + no pathogen). The second one contained non-bacterized seeds (seeds treated with only 1% CMC) sown in sandy soil infested with *F. oxysporum* (pathogen alone). The third treatment contained bacterized seeds sown in sandy soil infested with *F. oxysporum* as described above (rhizobacteria + pathogen), whereas the fourth one contained bacterized seeds sown in soil uninfested with the pathogen (rhizobacteria alone). Seeds were sown in pots (15 cm diameter; 30 cm height) filled with sterilized sandy soil at 5 seeds per pot. Tomato seedlings were properly fertilized through the irrigation water with a solution of N, P and K at 50, 14 and 41 mg l^{-1}, respectively. The effect of seed bacterization on growth promotion in terms of shoot length, and fresh weight was calculated on the 28th day. Data are the mean values (± SD) of three independent experiments, each with three replicates, 10 pots each.

Effect of seed bacterization on quantification of disease severity and induction of defense compounds

At 28 days after seed planting, the plants were harvested and the number of plants showing wilt symptoms, *i.e.* stunting of shoot, dropping of leaves and root discoloration, was counted.

To understand the mechanism of the disease suppression in terms of physiological and biochemical aspects of defense compounds resulted from the interaction between the *Bacillus* sp. strain TB281 and the phytopathogen *F. oxysporum*, both non-bacterized and bacterized tomato seedlings (treatments 1, no rhizobacteria + no pathogen & 4, rhizobacteria alone) were carried out, as described above. After 15 days, half of the treatments 1 & 4 was challenge inoculated with *F. oxysporum* to give rise treatments 2 (pathogen alone) and 3 (rhizobacteria + pathogen), respectively. Seeds were sown in pots (15 cm diameter; 30 cm height) filled with sterilized sandy soil at 5 seeds per pot. The experiment was performed in a randomized block design and the environmental conditions are identical to the experiment described above.

Sampling, extraction and determination of enzymatic activities

Leaf samples of treated and untreated tomato seedlings were collected at specified times after inoculation with the pathogen. Samples were frozen immediately in liquid nitrogen and stored at -20 °C. Samples was crushed in liquid nitrogen using a prechilled mortar and pestle and then homogenized with different buffers containing 1% polyvinylpolypyrolidone (PVPP; ratio: 1 fresh weight: 2 buffer, w/v) to assay different enzymes: 0.2 M sodium phosphate buffer (pH 6.4) for peroxidase (PER) and polyphenol oxidase (PPO) and 0.05 M sodium borate buffer (pH 8.8, containing 5 mM β-mercaptoethanol) for phenylalanine ammonia-lyase(PAL) The samples were homogenized and centrifuged at 20,000 g for 60 min at 4 °C. The supernatants were used as the crude enzyme source to assay enzymatic activities. Protein concentration was determined by the method of Bradford (1976), using bovine serum albumin as a standard.

Activity of PER was assayed according to a modified method of Hammerschimidt *et al.* (1982). Briefly, the assay mixture in a total volume of 3 ml

contained 10 mM potassium phosphate buffer, pH 7.5 at 25 °C, 2 mM H_2O_2 and 9 mM guaiacol as the substrate. After dilution of 5 µl of crude enzyme extract, the increase in absorbance was measured spectrophotometrically at 470 nm at intervals of 30 seconds up to 2 min. Activities of PER were expressed as enzyme units min^{-1} mg^{-1} protein.

Activity of PPO was determined according to a modified method of Canal *et al.* (1988). The reaction mixtures in a total volume of 3 ml contained 0.5 ml of crude enzyme extract and 2.5 ml of 500 mM pyrogallol as substrate in 0.02 M potassium phosphate buffer (pH 0.7). The increase in absorbance was measured spectrophotometrically at 420 nm over 2 min at the steepest increase at 25 °C. One unit of enzymatic activities is defined as the amount of enzyme giving, under the assay conditions, a change in absorbance of 0.001 per min. PPO activities were expressed as enzyme units min^{-1} g^{-1} fresh weight.

Activity of PAL was assayed by measuring the amount of trans-cinnamic acid formed at 290 nm as described by Beaudoin-Eagan and Thorpe (1985). The reaction mixtures consisted of 100 µl of plant extract and 900 µl of 6 µM L-phenylalanine in 500 mM Tris-HCl buffer, pH 8.5. After 60 min at 37 °C, the reaction mixture was stopped by the addition of 0.05 ml N HCl and measured spectrophotometrically at 290 nm. PAL activities were expressed as µmoles trans-cinnamic acid min^{-1} mg^{-1} protein.

Extraction and determination of phenolic content

Total phenolic compounds were determined using a modified method described by Singleton and Rossi (1965). Samples (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged at 10, 000 g for 15 min and the supernatant was saved. The residue was re-extracted twice with 80% ethanol and supernatants were pooled, and evaporated to dryness at room temperature. Residue was dissolved in 5 ml of distilled water. One-hundred µl of this extract was diluted to 3 ml with water and 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min, 2 ml of sodium carbonate (20%) were added and the contents were mixed thoroughly. The color was developed and absorbance was measured spectrophotometrically at 650 nm after 60 min. The standard curve was prepared using catechol. The absorbance was converted to the phenolic content in terms of catechol equivalent. The results were expressed as µg of phenolics g^{-1} fresh weight. Data are the mean values (± SD) of three independent experiments.

RESULTS

Effect of seed bacterization on tomato growth

Seed bacterization increased the shoot length in *F. oxysporum*-infested soil in relative to the control plants (Table 1). The growth of plants was stunted in *F. oxysporum*-infected soil as compared to the treatment "no rhizobacteria + no pathogen". Seeds inoculated with PGPR *Bacillus* TB281 and grown in *F. oxysporum*-infested soil attained greater height than those grown from non-bacterized seeds in the same soil. Thus, the PGPR used for seed bacterization, enabled the plants to withstand the *F. oxysporum*-infested soil and to attain better growth (shoot length) than those grown from by non-bacterized seeds. Plants produced from bacterized seeds attained significantly greater fresh weight than those grown from nonbacterized seeds in *F. oxysporum*-infested soil (Table 1).

Data (Table 1) also indicate a distinct decrease of diseased plants in the infested soil as a result of seed bacterization. *Bacillus* TB281 caused a decrease in the number of wilted plants. It is evident that percentage of decrease in disease syndrome over the phytopathogen alone was 57.8 % (Table 1) The increase in shoot length and fresh weight as well as disease suppression were statistically significant (Table 1). Thus, the present data suggested that bacterization of tomato seeds with a PGPR *Bacillus* TB281 increased the shoot length and fresh weight in soil infested with *F. oxysporum*. Seed bacterization also resulted in a significant reduction in tomato wilt caused by the same pathogen.

Table (1): Effect of seed bacterization on the growth promotion and suppression of Fusarium wilt of tomato plants caused by *F. oxysporum* 28 days after planting.

Treatments		Shoot length	Fresh weight	Disease severity	Decrease
Rhizobacteria	pathogen	(mm)	(mg)	(%)	(%) *
-	-	67.3 (± 2.4)	376 (± 17)	0.0	
-	+	60.7 (± 2.8)	273 (± 13)	50.5 (± 3.0)	
+	+	68.3 (± 3.5)	369 (± 21)	21.3 (± 1.3)	57.8
+	-	72 (± 3.9)	403 (± 25)	0.0	

* Value represents % decrease in disease severity over the phytopathogen alone

Effect of seed bacterization on induction of defense compounds

Activity of PER was increased in plants grown from *Bacillus*-treated seeds sown in soil infested with *F. oxysporum*. The maximum PER activity was obtained on the 4th day after challenge inoculation and PER activity was maintained at higher levels throughout the experimental period. Plants inoculated with *F. oxysporum* alone had comparatively less PER activity. PER activity in *Bacillus*-treated plants alone remained unchanged during the experimental period but compared to control, the activity was higher (Figure 1). A similar pattern of increased activity of PPO was observed in bacterized tomato plants challenged with the pathogen and the activity reached maximum on the 5th day after challenge inoculation (Figure 2).

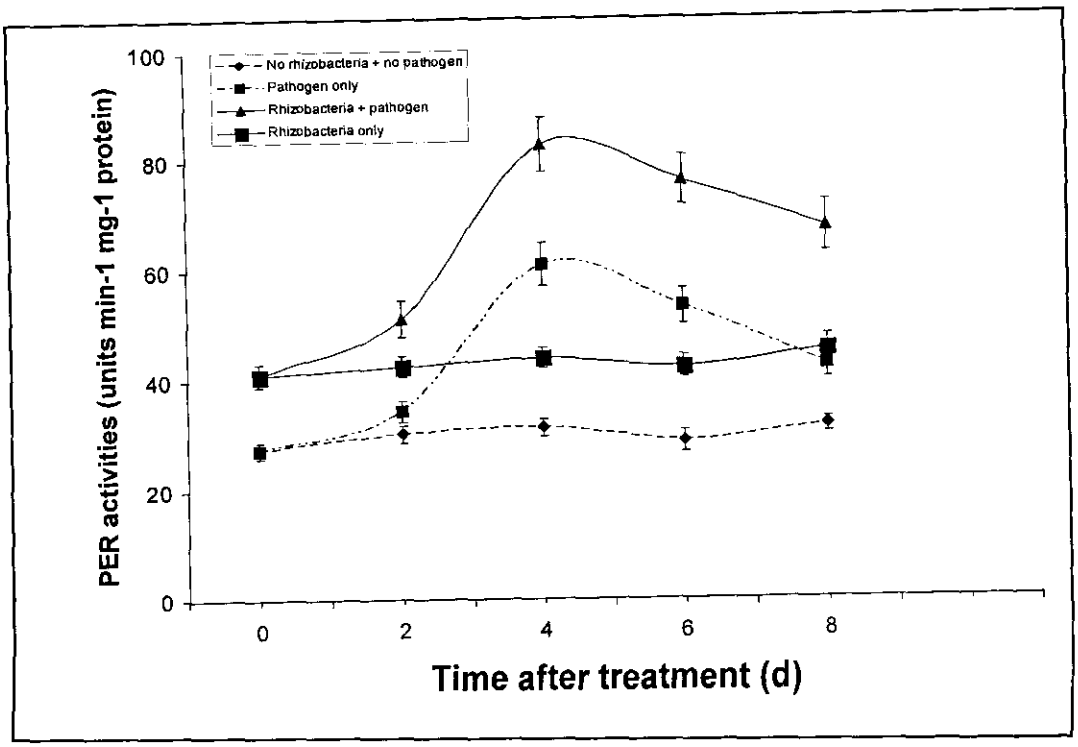

Fig. (1): Effect of tomato seed bacterization on the activity of PER during the early stages of the interaction between PGPR *Bacillus* and *F. oxysporum*.

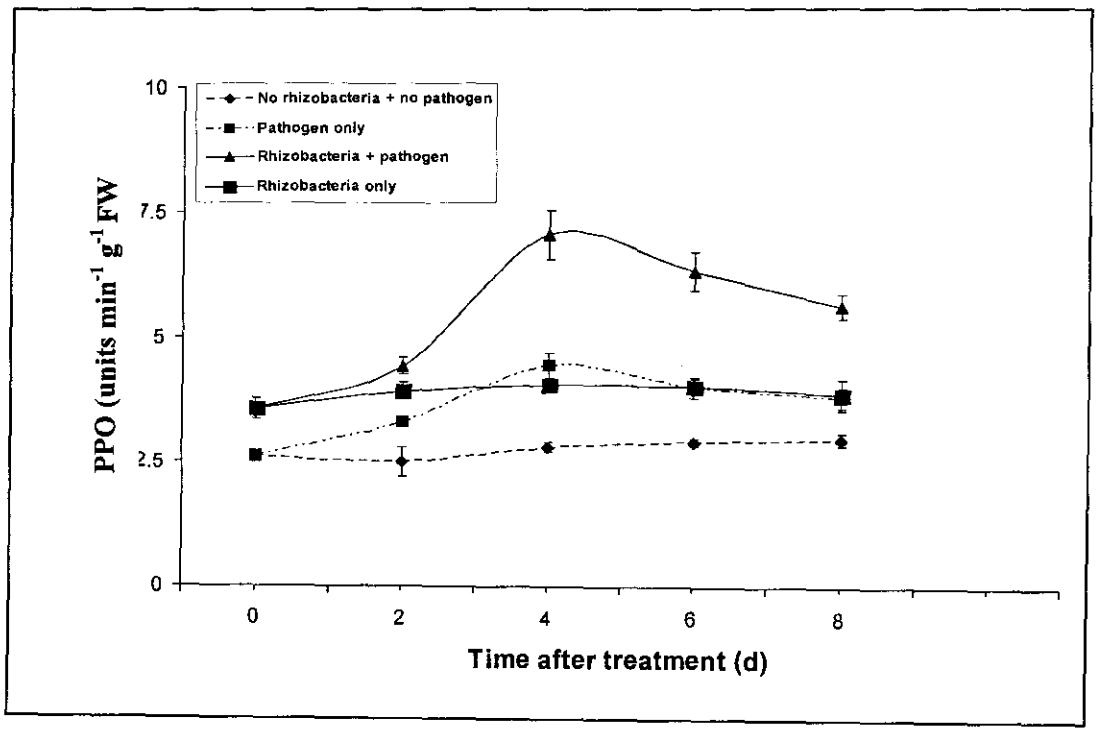

Fig. (2): Effect of tomato seed bacterization on the activity of PPO during the early stages of the interaction between PGPR *Bacillus* and *F. oxysporum*.

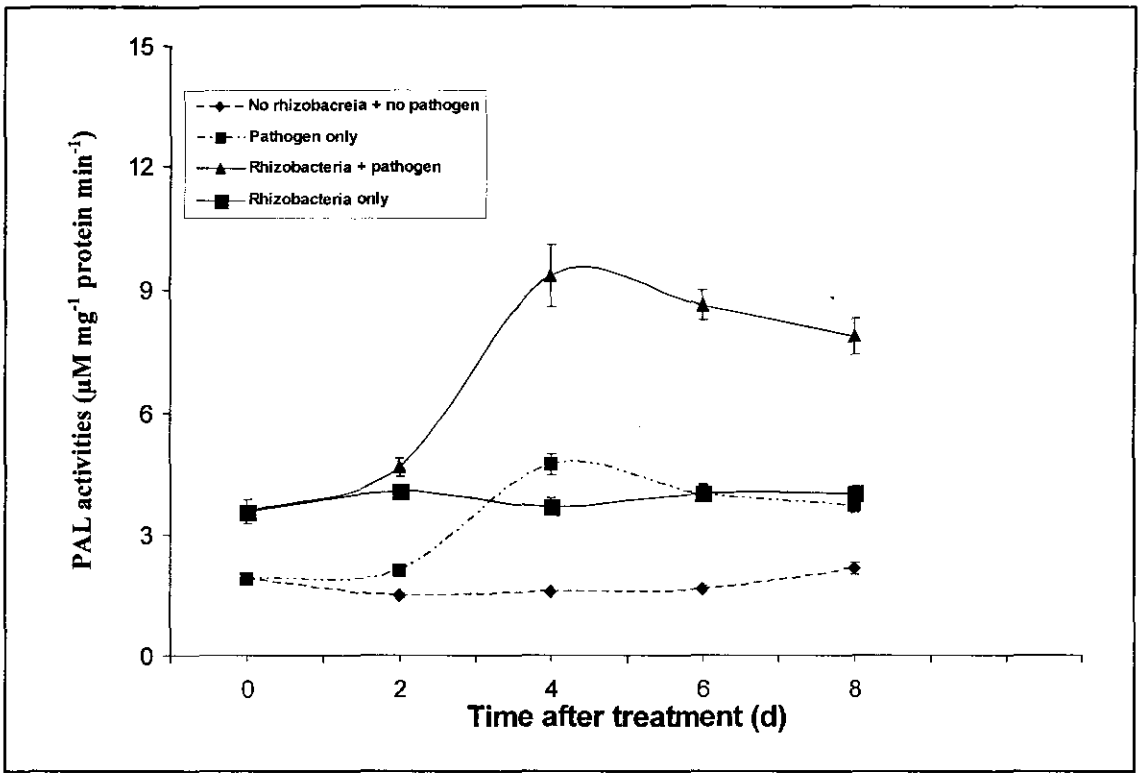

Fig. (3): Effect of tomato seed bacterization on the activity of PAL during the early stages of the interaction between PGPR *Bacillus* and *F. oxysporum*.

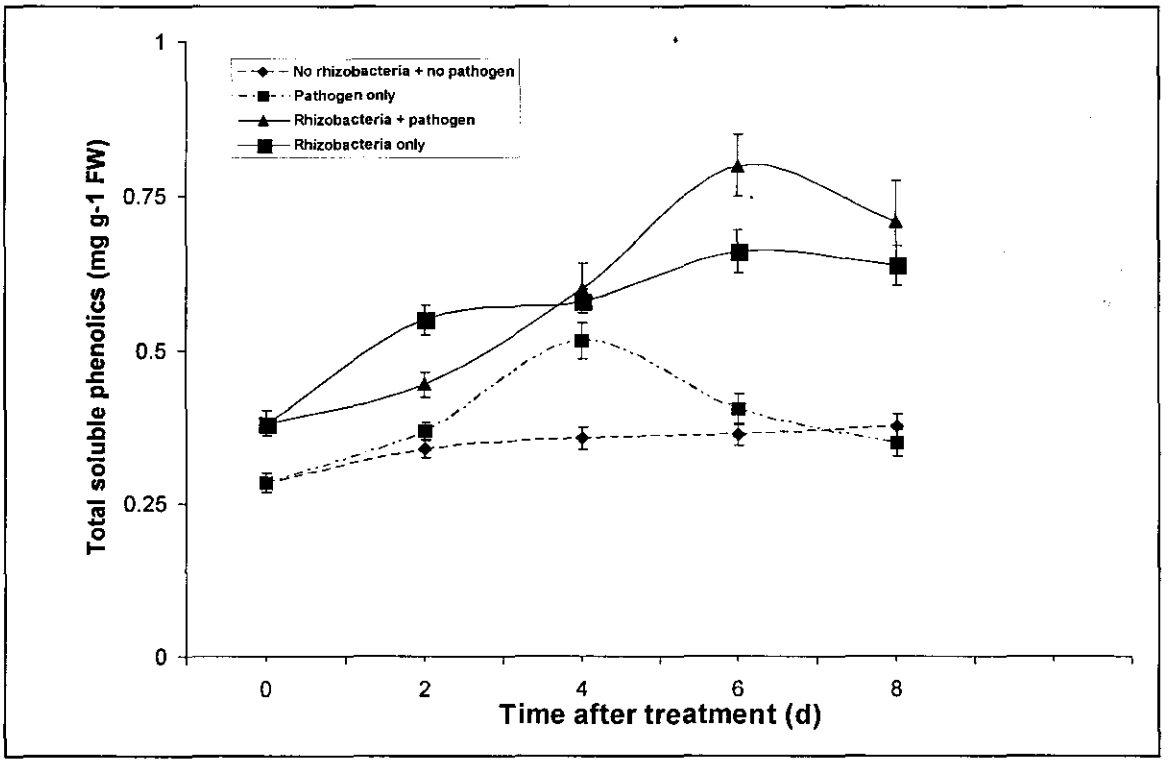

Fig. (4): Effect of tomato seed bacterization on the levels of total soluble phenolics during the early stages of the interaction between PGPR *Bacillus* and *F. oxysporum*.

Upon pathogen challenge in bacterized tomato plants, PAL activity started to increase one day after inoculation and reached maximum on the 4th day after the pathogen challenge. Similarly, tomato plants treated with the pathogen alone contained higher activity of PAL, but the induction of activity was noted for 2-4 days, thereafter declined sharply. *Bacillus*-treated plants alone also had significantly higher PAL activity compared to untreated control, but activity was less compared to bacterized plants grown in soils infested with *F. oxysporum* (Figure 3).

Studies on induction of defense mechanisms revealed that higher accumulation of phenolics was noted in bacterized tomato plants growing in *F. oxysporum*-infested soils. Levels of phenolics started to increase one day after challenge inoculation. The maximum increase was found on the 5th day after challenge inoculation. *F. oxysporum*-treated plants alone also contained higher level of phenolics, but accumulation started on the 3rd day after infection with *F. oxysporum* and declined sharply 5 days after inoculation. Moreover, the elevated levels of phenolics were less compared to bacterized plants challenged with the pathogen. There was no marked change in *Bacillus*-treated plants alone during the time-course of experimental period and the levels of phenolics remained higher compared to the untreated control (Fig. 4).

DISCUSSION

Seed bacterization of tomato by *Bacillus* TB281 had a positive effect on shoot length and fresh weight in soils infested with *F. oxysporum*. Seed bacterization also resulted in significant reduction of tomato Fusarium wilt. *Bacillus* spp. has been used to control soil-borne or seed-borne and post-harvest fungal diseases (Utkhede and Smith, 1992 & 1993; Sholberg *et al.*, 1995). Dileep Kumar (1996) reported an antibiotic-producing strain of a *Bacillus* sp. isolated from peanut rhizosphere which decreased the number of wilted chick pea plants grown in *F. oxysporum*-infested soils as a seed inoculant.

Plants have endogenous defense mechanisms that can be induced in response to attack by phytopathogens. It is well known that the defense genes are inducible genes and appropriate stimuli or signals are needed to activate them. Inducing the plant's own

defense mechanisms by prior application of a biological inducer is thought to be a novel plant protection strategy. The use of certain *Bacilli* for controlling soil-borne diseases has been studied (Weller and Cook, 1983; Paulitz and Loper, 1991). Recent studies implies that prior application of *Bacillus* strengthen host cell wall structures, via PER activity, resulting in restriction of pathogen invasion in plant tissue. In fact, PER is a key enzyme in the biosynthesis of lignin (Bruce and West, 1989). Increased activity of cell wall bound PERs has been elicited in different plant species due to pathogen infection (Goy *et al.*, 1992; Reimers *et al.*, 1992; Mohan *et al.*, 1993; Chen *et al.*, 2000). In bean, rhizosphere colonization of various bacteria induced the PER activity (Zdor and Anderson, 1992). In the present study, earlier and increased PER activity is found in *Bacillus* TB281- treated plants challenged with the pathogen. The same results were obtained by Chen *et al.* (2000) in cucumber roots treated with *P. corrugata* challenged with *P. aphanidermatum*. PPO activity was also induced by *Bacillus* TB281 against *F. oxysporum*. Chen *et al.* (2000) reported that various rhizobacteria and *P. aphanidermatum* induced the PPO activity in cucumber root tissues.

Additionally, PAL is the first enzyme in phenylpropanoid metabolism. PAL activity could be induced in plant-pathogen interactions and fungal elicitor treatment (Podile and Laxmi, 1998; Ramanathan *et al.*, 2000; Daayf *et al.*, 1997). In the present study, increased activity of PAL was noticed in *Bacillus*-treated plants challenged with the pathogen, reached maximum on the 4th day after challenge inoculation and maintained at higher level throughout the experimental period. In plants treated with the pathogen alone, the activity was declined sharply 4 days after treatment. Invasion of root plants by the pathogen might have resulted in decreased activity of PAL whereas earlier and increased activity of PAL due to *Bacillus* treatment might have prevented the fungal invasion, and thus, the activity maintained at higher levels during the experimental period. Results of the present study indicate that the enzymatic activities involved in phenylpropanoid metabolism in tomato plants have been induced by *Bacillus* TB281 in response to challenge inoculation with *Fusarium*. These results are in harmony with those obtained by Chen *et al.* (2000) who reported that high levels of PAL were induced in cucumber roots inoculated with *P. aphanidermatum* but roots treated with *P. corrugata* had initially higher levels of PAL and the levels were lower after plant treatment with *P. aphanidermatum*. Increase in mRNAs encoding for PAL synthase could be recorded in the early stage of the interaction between bean roots and various rhizobacteria (Zdor and Anderson, 1992). DeMeyer *et al.* (1999) reported that rhizosphere colonization by *P. aeruginosa* 7NSK2 activated PAL in bean roots and increased the salicylic acid levels in leaves.

The phenolic compounds may contribute to enhance the mechanical strength of host cell wall and may also inhibit the fungal growth, as phenolics are fungitoxic in nature. Seed bacterization in this study elevated level of phenolics in tomato plants. According to Benhamou *et al.* (1996), the hyphae of the pathogen surrounded by phenolic substances exhibited considerable morphological changes including cytoplasmic disorganization and loss of protoplasmic content. Higher levels of phenolics by prior application of *Bacillus* in pea plants have been reported against *P. ultimum* and *F. oxysporum* f. sp. *pisi*. Data of the present study also indicate that the higher and earlier increases of phenolics were noted in *Bacillus*-treated plants challenged with *Fusarium*. In agreement with the present results, Benhamou *et al.* (2000) reported that an endophytic bacterium, *Serratia plymuthica* induced a transient increase of phenolics in cucumber roots following infection by *P. ultimum*. The increase of

phenolic content in the treated plants with *Bacillus* indicates that seed bacterization triggered the plant to synthesize defense compounds, but greater increase of defense compounds was found when *Bacillus*-treated plants were challenged with the pathogen during the time course of study.

In conclusion, the results of the present study indicate that earlier elevated levels of enzymatic activities involved in phenylpropanoid metabolism and phenolic compounds have been found in tomato plants treated with *Bacillus* TB281 in response to invasion by *F. oxysporum*. The plant-pathogen interactions have also triggered the activities of defense enzymes during the late of primary events, but later during the secondary events of host-pathogen interaction the activities declined sharply when the pathogen colonized the root tissues. Elevated levels of defense enzymatic activities induced by *Bacillus* strain TB281 in tomato plants might have collectively contributed to induced resistance in tomato plants against *F. oxysporum*, the causal agent of Fusarium wilt. This strain can be used after testing it in a large-scale field trail to biocontrol plant diseases.

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حث المركبات الدفاعية بمعاملة البذور ببكتيريا الجذور المشجعة للنمو باسيلس ضد المسبب المرضى للذبول الفيوزاريومى في الطماطم

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اجري هذا البحث بهدف دراسة تأثير معاملة بذور الطماطم بأحد السلالات البكتيرية التي تنتمي لجنس باسيلس *Bacillus* sp. TB281 المعزولة من التربة الرملية بمزرعة كلية الزراعة- جامعة قناة السويس على: (١) تشجيع نمو البادرات ومدى مقاومتها الحيوية ضد مرض الذبول الفيوزاريومى المتسبب عن *Fusarium oxysporum* f. sp. *lycopersici* ، (٢) حث وتكوين المركبات الدفاعية المتمثلة في أنشطة انزيمات البيروكسيديز peroxidase ، البولي فينول أكسيديز polyphenol oxidase ، فينيل ألانين أمونيا ليييز phenylalanine ammonia-lyase وأيضا الفينولات الكلية الذائبة . تم زراعة البذور المعاملة بالمعلق البكتيري المحتوى على المادة اللاصقة كربو كسي ميثيل سليولوز carboxymethyl cellulose في أربعة معاملات هي كالآتي: (١) بذور غير معاملة بالبكتيريا وتم زراعتها في تربة غير معدية بالمسبب المرضى *F. oxysporum*، (٢) بذور غير معاملة بالبكتيريا وتم زراعتها في تربة معدية بالمسبب المرضى، (٣) بذور معاملة بالبكتيريا وتم زراعتها في تربة معدية بالمسبب المرضى، وأخيرا بذور معاملة بالبكتيريا وتم زراعتها في تربة غير معدية بالمسبب المرض الفيوزاريومى. وقد أسفرت تلك التجربة على أن معاملة البذور بالمعلق البكتيري أدى إلى تشجيع نمو البادرات وزيادة وزنها الطازج معنويا بالإضافة إلى زيادة قدرتها على مقاومة مرض الذبول المتسبب عن فطر الفيوزاريوم . وقد أسفرت نتائج البحث فيما يتعلق بالتحليلات البيوكيماوية على احتواء البادرات المعاملة على مستويات مبكرة وعالية من أنشطة إنزيمات البيروكسيديز، والبولي فينول اكسيديز، فينيل ألانين أمونيا ليييز الدفاعية وأيضا الفينولات الذائبة الكلية ذات العلاقة بقدرة النباتات على مقاومة المسبب المرضى للذبول بمقارنتها بالنباتات غير المعاملة وقد زادت أيضا أنشطة إنزيمات البيروكسيديز، والبولي فينول اكسيديز، فينيل ألانين أمونيا ليييز وأيضا الفينولات الذائبة الكلية في النباتات غير المعاملة والنامية في تربة معدية بنفس المسبب المرضى غير أن زيادة الأنشطة الإنزيمية ومستوى الفينولات الكلية كان مؤقتا. يستنتج من تلك الدراسة أن معاملة البذور ببكتيريا الجذور المشجعة للنمو plant growth promoting-rhizobacteria ذات تأثيرات معنوية على زيادة النمو وقدرة النباتات على مقاومة الأمراض وتعزى قدرة النباتات على المقاومة إلى ارتفاع مستويات أنشطة الإنزيمات ذات العلاقة بالمقاومة وأيضا الفينولات من خلال التأثير لبكتيريا الجذور المشجعة للنمو باسيلس.