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IMPACT OF INOCULATION WITH EFFECTIVE STRAINS OF PLANT GROWTH-PROMOTING RHIZOBACTERIA ON FUSARIUM WILT OF PEPPER

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ABSTRACT

In greenhouse experiment, three strains of effective plant growth-promoting rhizobacteria (PGPR), *Pseudomonas fluorescens*, *Paenibacillus polymyxa* and *Azospirillum lipoferum* were evaluated for biological control of Fusarium wilt of pepper caused by *Fusarium oxysporum* f. sp. *lycopersici*. Visual inspections of root colonization by water-agar tubes technique indicated that, all the tested bacteria were able to colonize pepper root systems, whereas in separated greenhouse experiment, inoculation of bacteria by the over head-soil method caused higher colonization of the tested PGPR than root-soaking technique either in the rhizosphere or in the root material. Significant decrease of dehydrogenase and phosphatase activity was observed when soil was infested with *F. oxysporum*. Maximum values of dehydrogenase activity were obtained by using over head-soil technique with triple inoculation while, dual inoculation with *P. polymyxa* and *Az. lipoferum* gave the maximum records of phosphatase activity. The percentage of disease incidence caused by *F. oxysporum* of plants after treatment with PGPR as biocontrol agents were lower than those infected with pathogen only. Moreover, *Ps. fluorescens* or *P. polymyxa* singularly or in mixture were the best strains for controlling of Fusarium wilt of pepper since they recorded the lowest percentage of disease incidence and the maximum percentage of biocontrol efficacy. Also, inoculation of the antagonistic bacteria by over head-soil method gave lower percentage disease incidence and higher biocontrol efficacy than root-soaking technique. Data also indicated that peroxidase and polyphenol oxidase increased in plants those infected with the pathogen singularly than healthy plants those no infestation. Also, remarkable increase was observed in peroxidase and polyphenol oxidase in the plants inoculated with *Ps. fluorescens* either individually or in combination with the other PGPR. Data also demonstrated that, soil infestation with *F. oxysporum* significantly decreased root size, root dry weight, plant height and shoot dry weight of pepper as compared with control (plants not infested with *F. oxysporum*). Growth characters were significantly increased in the infested plants those inoculated with PGPR individually or the mixture of them, especially *Az. lipoferum* and *P. polymyxa*.

Key words: biocontrol, *Pseudomonas fluorescens*, *Paenibacillus polymyxa*, *Azospirillum lipoferum*, soil enzymes, Fusarium wilt, disease incidence.

INTRODUCTION

Nowadays, certain changes in agricultural practices, such as intensive tillage, and use of synthetic fertilizers and pesticides, have led to a decline in soil structure and an increase in soil-borne plant diseases (Bailey and Lazarovits, 2003). The soil-borne pathogens can rapidly colonize even in pasteurized soil, invade crops through seeds, roots, and stalks, and make considerable losses (Wheeler and Rush, 2001). These pathogenic fungi, such as *F. oxysporum*, had wide host plants and caused many difficulties in controlling the plant disease.

Biocontrol appears to be a reliable alternative to chemical fungicides, which have

raised serious concerns of food contamination, environmental pollution and botanic phytotoxicity (Fernando *et al.*, 2005). Since then, people have made considerable efforts to find different biocontrol methods for soil-borne diseases, including the application of plant extracts (Bowers and Locke, 2004), antagonistic microorganisms (Lemessa and Zeller, 2007), fungicides from antagonistic strains of fungi and bacteria (Minuto *et al.*, 2006) and disease-resistance varieties (Dong *et al.*, 2007).

Plant growth-promoting rhizobacteria (PGPR) are a group of root-colonizing bacteria in the rhizosphere of many plant species that exert beneficial effects on plants (James and Olivares, 1998). During the past several decades, many researchers have studied various PGPR strains for their capacity to increase the plant growth and yield, and to control the plant pathogens under greenhouse and field conditions (Kloepper *et al.*, 2004). To date, many mechanisms have been proposed for growth promotion by PGPR strains. First, enhancement of plant growth by PGPR was elucidated by the production of compounds that mimic plant hormones or other plant stimulants (Lucy *et al.*, 2004). Second, mechanisms for growth promotion that decrease microbial populations of pathogenic or deleterious microorganisms through antibiosis have been proposed (Kloepper *et al.*, 2004). These mechanisms have been referred to as both direct and indirect mechanisms. In addition to these mechanisms, PGPR strains must effectively colonize roots in order to provide consistent results under greenhouse or field conditions. From several studies, it has been confirmed that *Ps. fluorescens*, *P. polymyxa* and *Azospirillum sp.* can be used as plant growth promoters and also, they have antagonistic and biologically controlled a diversity of soil borne pathogens. (Diby *et al.*, 2005, Haggag, 2007; Felici *et al.*, 2008 and Ahmadzadeh and Tehrani, 2009).

The objectives of the present research were to evaluate *Ps. fluorescens*, *P. polymyxa* and *Az. lipoferum*, the well-known as PGPR and biocontrol agents (Abou-Aly, 2008) for suppression of pepper Fusarium wilt. In addition, the impact of these bio-inoculants on seedling growth of pepper was conducted.

MATERIALS AND METHODS

Microorganisms

Pseudomonas fluorescens, *Paenibacillus polymyxa* and *Azospirillum lipoferum* were previously screened and evaluated to their efficiency as PGPR and biocontrol agents. *Ps. fluorescens* exhibited more than two or three biological control trails; *Az. lipoferum* can play a great role as plant growth-promoting bacteria, while *P. polymyxa* was the most strain that showed strong PGPR qualities and pathogen inhibition (Abou-Aly, 2008). *Ps. fluorescens* and *P. polymyxa* were grown on Nutrient broth at 30° C for 72 h, while *Az. lipoferum* was grown on Semi-solid malate medium (Hegazi *et al.*, 1979) at 30° C for 72 h. Cells of the three bacteria were harvested by centrifugation (7000 rpm, 10 min), washed twice, and then resuspended in sterile water to a density of 10^8 cfu/ml.

Fusarium oxysporum f. sp. *lycopersici* was isolated from roots of a diseased pepper, and maintained on potato dextrose agar PDA. Fungal culture was identified in Plant Pathology Branch, Fac. of Agric., Benha University. Fungus inoculum were prepared on potato dextrose broth medium and incubated at 28°C for 7 days, the spores density was adjusted to contain ~ 10^6 spore/ml.

Root colonization bioassay

Pepper seeds (*Capsicum annuum* L. cv. Baladi) were surface sterilized in 50% ethanol (30 s) followed by 2% NaClO (3 min), washed three times in sterile water, soaked in 5 ml of a suspension of the tested rhizobacteria for 24 h and then transferred to sterile 0.6% water-agar tubes. Periodic visual inspections were performed daily in order to detect bacterial growth around arising roots. In case of doubt or difficulty of observation, it proved useful to remove the whole seedling from the agar gel for visual inspection. Assays were carried twice with three replicates per culture (Silva *et al.*, 2003).

Colonization capacity of the tested bacteria

Colonization capacity of *Ps. fluorescens*, *P. polymyxa* and *Az. lipoferum* was tested in separated greenhouse experiment according to Ryu *et al.* (2006). Pepper seedlings were surface sterilized by 3% NaClO (3 min) and were planted in sterilized soil after inoculation with each of the tested bacterial suspension using seedling soaking or over head-soil techniques (as mentioned below). Ten days after transplanting, three adhering rhizosphere soil from each treatment were carefully collected. One gram of the rhizospher samples was soaked in 9 ml of sterile saline with shaking at 200 rpm for 30 min. For determination of the numbers of endophytic bacteria (as described by Timmusk *et al.*, 2005), the soil was removed from the roots. Individual roots were blended in 10 mM $MgSO_4.7H_2O$ for about 3 min. The plant roots were surface sterilized in a surface sterilization solution (1% sodium hypochlorite, 0.2% Tween 20 in phosphate-buffered saline) and then followed by four washes with sterilized water. Roots were then macerated using a mortar. Serial dilutions of the cell suspension of the rhizosphere or macerated roots were plated on Nutrient agar supplemented with chloramphenical (10 mg/L) and rifampicin (50 mg/L). The number of colonies was counted after incubation at 30° C for 2 days.

Experimental design

Pepper seedlings at the age of 30 days were treated with bacterial strains in one of the following two methods. In the root-soaking, the seedling roots were soaked by dipping the root system in mixture of sucrose solution (4%) as an adhesive for inocula, and cell suspensions of the bacterial strains individually or mixtures of them for about 30 min before the seedlings were transplanted into plastic pots (containing clay loam soil, 5kg/pot). In the over head-soil technique, 50 ml suspension of each strain or the mixtures of them were poured into each pot immediately before transplanting. Control treatment was treated with the same volume of suspensions without inoculation. The soil was infested with the suspension of fungal spores 5 days before transplanting or 2 days after transplanting. The treatments were distributed in greenhouse using randomized complete block design. Six replicates of each treatment were used. After forty-five days from transplanting, each seedling was assessed for Fusarium wilt severity. The disease severity was recorded on a 0-4 scale with zero representing no infection and four denoting plants completely infected. The 0-4 scale of the disease severity was classified as follows: 0-no infection, 1-slight infection (about 25% of the leaves became yellow), 2-moderate infection (two or three leaves became yellow, 50% of the leaves became wilting), 3-extensive infection (the all plant leaves became yellow, 75% of the leaves became wilting, and growth was inhibited) and 4-complete infection (the whole plant leaves became yellow, 100% of leaves became wilting, and the plant was died). The percentage of disease incidence and biocontrol efficacy were determined using the formula as described by Song *et al.* (2004):

$$\text{Disease incidence (\%)} = \frac{\Sigma \text{ scal x number of plants infectd}}{\text{highest scale x total number of plants}} \times 100$$

$$\text{Biocontrol efficacy (\%)} = \frac{\text{disease inciedence of the control - disease incidence of bacterial treatment}}{\text{disease incidence of the control}} \times 100$$

Dehydrogenase activity

After forty-five days from the transplanting, dehydrogenase activity was estimated as described by Glathe' and Thalmann (1970), data was calculated and presented as µg 2,3,5-triphenyl formazan (TPF). gm^{-1} dry soil . day^{-1}.

Phosphatase activity

Phosphatase activity was determined by the method of Drobrikova (1961); means of data were calculated as µg inorganic phosphorus released per gm .dry $soil^{-1}$. 24 $hrs.^{-1}$ on dry basis.

Peroxidase and polyphenol oxidase activity

Peroxidase was determined according to the methods of Allam and Hollis (1972), data was calculated as absorbance (nm). g^{-1} fresh weight/15 min. while polyphenol oxidase activity was determined according to the method described by Matta and Dimond

(1963), data was calculated as absorbance (nm). g^{-1} fresh weight/30 min.

Growth characters

Plant height (cm), shoot dry weight (g), root size (cm^3) and root dry weight (g/plant) of the pepper were determined in each treatment after 60 days from the cultivation.

Statistical analysis was carried out according to Snedecor and Cochran (1989) by using the least significant differences test (L.S.D).

RESULTS AND DISCUSSION

Colonization capacity

Determination of the colonization capacity of pepper roots by *Ps. fluorescens*, *P. polymyxa* and *Az. lipoferum* applied with different inoculation methods is shown in Table (1). Visual inspections of root colonization by water-agar tubes technique indicated that, all the tested bacteria were able to colonize pepper root systems whereas the control never showed any colonization. Surface sterilization of seeds probably inactivates most of the microbial population associated with them, leaving ecological niches in the seed available for the PGPR to establish. These findings are in harmony with those obtained by Silva *et al.* (2003) who found that, the water-agar gel usually does not itself support growth of most prokaryotes unless root exudates exert positive chemotaxis, providing nutrients for bacterial growth. Consequently, rhizobacteria which are able to grow under the bioassay conditions are probable to grow on root exudates in association with a certain level of specificity. Weert and Bloemberg (2006) reported that the bioassay provides a good indication of the ability of a rhizobacterium to establish itself in the rhizoplane of the target plant. The good biocontrol agents were also good root colonizers.

Table (1): Root colonization abilities of the tested bacterial strains on seeds and seedlings of pepper.

Treatments	Colonization in water-agar tube	Root-soaking		Over head-soil	
		CFU/g rhizosphere	CFU/g root	CFU/g rhizosphere	CFU/g root
Without PGPR	-	2.4×10^1	-	6.3×10^1	-
Ps. fluorescens	+	6.3×10^3	3.4×10^2	8.4×10^6	7.2×10^2
P. polymyxa	+	5.1×10^4	2.8×10^2	2.7×10^6	5.8×10^2
Az. lipoferum	+	2.5×10^5	4.1×10^2	1.5×10^6	2.6×10^2

(+), colonization; (-), no colonization

Data in Table (1) also indicated that, inoculation by the over head-soil method caused higher colonization of all PGPR than the root-soaking technique either in rhizosphere or in root material. These findings are in harmony with Xue *et al.* (2009). Also, *Ps. fluorescens* is able to colonize the rhizosphere and roots of pepper seedling but not as *P. polymyxa* and *Az. lipoferum*. *P. polymyxa* was the potent strain as colonizer bacteria in the two inoculation methods. These data are similar to those found by Timmusk *et al.* (2005) who reported that *P. polymyxa* is able to colonize the roots and forms biofilms around the roots. Weert and Bloemberg (2006) mentioned that, when bacterial cells adhere and proliferate on a surface they can form a colony and subsequently a biofilm, in which they are encased in an extracellular polysaccharide matrix. This process of colonization consists of the following steps **(i)** transport of microbes to the surface, **(ii)** initial attachment, **(iii)** formation of microcolonies and **(iv)** biofilm maturation.

Enzyme activity in rhizosphere

Data in Table (2) show that dehydrogenase and phosphatase activities were

affected by inoculation with *Ps. fluorescens*, *P. polymyxa* and *Az. lipoferum* in pepper rhizosphere infested with *F. oxysporum* f. sp. *lycopersici*. Soil infestation with *F. oxysporum* significantly decreased the activity of dehydrogenase and phosphatase. Moreover, individual inoculation with PGPR in soil infested by pathogenic fungi led to higher enzyme activity than the control soil that infested with pathogen singularly. Dual inoculation of pepper transplants showed higher values of enzyme activity than individual inoculation. This result could be attributed to the synergistic effect occurred in case of dual inoculation. Maximum values of dehydrogenase were obtained by inoculation with mixture of all bacteria while, dual inoculation with *P. polymyxa* and *Az. lipoferum* gave the maximum records of phosphatase activity. Similar trend was obtained by Khalifa (2005) and Zaghloul *et al.* (2008) who found that mixture inoculation with PGPR and biocontrol agent significantly increased dehydrogenase and phosphatase activity in the presence of *F. oxysporum*.

Soil that infested with fungal spores 5 days before transplanting led to decrease in both enzymes activity with all treatment than the soil that infested with the fungi after bacterial inoculation. Also, over head-soil method recorded higher values of enzymes activity than root-soaking.

Table (2): Effect of inoculation with PGPR on soil enzymes activity after 45 days from transplanting in the presence of *Fusarium oxysporum*.

Strains	*F. oxysporum*	Dehydrogenase (µg TPF/g dry soil/24 h)				Phosphatase (µg inorganic P/g soil/24 h)			
		Root-soaking		Over head-soil		Root-soaking		Over head-soil	
		A	B	A	B	A	B	A	B
Without PGPR	-	22.4	19.5	21.8	23.8	31.5	36.2	28.9	33.4
Without PGPR	+	15.7	12.4	16.8	20.7	27.3	28.9	19.1	29.7
Ps. fluorescens	+	28.4	25.2	23.6	34.6	29.5	53.1	29.8	51.8
P. polymyxa	+	28.9	37.1	29.5	42.9	38.2	58.3	31.2	55.7
Az. lipoferum	+	33.6	39.4	43.1	56.3	37.5	52.6	34.7	47.1
Ps. + P.	+	37.8	44.5	47.9	46.8	45.6	61.3	39.5	58.9
Ps. + Az.	+	34.8	39.6	52.0	58.7	50.2	51.3	47.1	62.5
Az. + P.	+	36.5	47.3	51.2	63.6	49.7	62.8	58.2	72.4
Ps. + P. + Az.	+	45.7	58.7	59.5	74.5	48.4	59.3	52.4	65.7
LSD at 5%		7.13	6.42	6.74	6.14	3.81	5.54	5.72	6.85

A: Soil was infested with fungal spores 5 days before transplanting.
B: Soil was infested with fungal spores 2 days after transplanting.

Disease assessment

Data in Table (3) show that disease incidence percentage in plants not treated with bacteria but inoculated with *F. oxysporum* alone reached from 70.8 to 83.3 %. The percentage of disease incidence caused by *F. oxysporum* of plants after treatment with PGPR as biocontrol agents was lower than those infected with pathogen only. Similar trend was observed by Khalifa (2005) and Xue *et al.* (2009). Moreover, *Ps. fluorescens* or *P. polymyxa* singularly or in mixture were the best strains for controlling Fusarium wilt followed by *Az. lipoferum* since they recorded the lowest percentage of disease incidence and the maximum percentage of biocontrol efficacy. In this respect, the two bacteria (*Ps. fluorescens* and *P. polymyxa*) were the potential strains used as the best biocontrol agents against *F. oxysporum in vitro*; these data were confirmed in previous study by Abou-Aly (2008) who found that *Ps. fluorescens* and *P. polymyxa* are more potent to produce catechol-type siderophores, chitinase, cellulase and protease and they were more efficient for inhibition of fungal growth, either by using dual culture technique or by using filtrates of their respective cultures. Results also showed

that inoculation of antagonistic bacteria by over head-soil method (as a simple inoculation method) gave lower percentage disease incidence and higher biocontrol efficacy than root-soaking technique. Soaking the seedling roots into a bacterial suspension might slightly or even severely injure the root system. These injuries may give both of the pathogen and biocontrol agents an opportunity for colonization, and the serious injury may inhibit plant growth (Xue *et al.*, 2009). This suggestion might explain whey biocontrol efficacy in the plants inoculated by root-soaking was lower than the counterparts inoculated with the over head-soil method.

Table (3): Pepper wilt assessment as affected by inoculation with PGPR after 45 days from transplanting in the presence of *Fusarium oxysporum*.

Strains	*F. oxysporum*	Disease incidence (%)				Biocontrol efficacy (%)			
		Root-soaking		Over head-soil		Root-soaking		Over head-soil	
		A	B	A	B	A	B	A	B
Without PGPR	-	4.1	0.0	0.0	0.0	95.0	100	100	100
Without PGPR	+	83.3	75.0	79.1	70.8	-	-	-	-
Ps. fluorescens	+	45.8	29.1	41.6	29.1	45.0	61.2	47.4	58.9
P. polymyxa	+	50.0	33.3	45.8	31.2	39.9	55.6	42.1	57.3
Az. lipoferum	+	58.3	41.6	54.1	33.3	30.0	44.5	31.6	52.9
Ps. + P.	+	33.3	20.8	37.5	12.5	60.0	72.2	52.5	82.3
Ps. + Az.	+	35.4	25.0	29.1	25.0	57.5	66.6	63.2	64.6
Az. + P.	+	39.5	20.8	33.3	16.6	52.5	72.2	57.9	76.5
Ps. + P. + Az.	+	27.0	22.9	25.0	14.5	67.5	69.9	68.3	79.5

A: Soil was infested with fungal spores 5 days before transplanting.
B: Soil was infested with fungal spores 2 days after transplanting.

Peroxidase and Polyphenol oxidase

Data in Table (4) indicate that peroxidase and polyphenol oxidase increased in plants those infected with the pathogen singularly than healthy plants those no infestation. Increased peroxidase and polyphenol oxidase activity upon infection might be required for an additional deposition of lignin around the lesion court induced by pathogen. Peroxidase is a key enzyme in the biosynthesis of lignin and other oxidized phenols which are highly toxic to the pathogen (Khatun *et al.*, 2009). In addition, application of PGPR singularly or in mixture led to increase of the two enzymes than infested plants with pathogen alone. These findings are in harmony with those obtained by Zaghloul *et al.* (2008). Also, remarkable increase was observed in peroxidase and polyphenol oxidase in the plants those were inoculated with *Ps. fluorescens* either individually or in combination with the other PGPR. The role of polyphenol oxidase in plant-microbe interactions appears to be complex. Thipyapong *et al.* (2007) found that PGPR could induce polyphenol oxidase activity in some plant species, such induction could presumably be beneficial to plant hosts by protecting them from attacking pathogens. Application of a biocontrol agent, *P. fluorescens*, also induced peroxidase and polyphenol oxidase activities and resulted in significantly reduced infestation of the pathogen.

Growth characters

Data in Tables (5 & 6) show the effect of inoculation with PGPR on pepper growth characters in the presence of pathogenic fungi. Results from the greenhouse pot experiment demonstrated that soil infestation with *F. oxysporum* significantly decrease root size, root dry weight, plant height and shoot dry weight of pepper as compared with control plants those not infested with the pathogen fungi. Growth characters were significantly increased in the infested plants those inoculated with PGPR individually or the mixture of them, especially *Az. lipoferum* and *P. polymyxa*.

Table (4): Peroxidase and polyphenol oxidase (absorbance/g fresh leaves) as affected by inoculation with PGPR after 45 days from transplanting in the presence of *F. oxysporum*.

Strains	*F. oxysporum*	Peroxidase				Polyphenol oxidase			
		Root-soaking		Over head- soil		Root-soaking		Over head- soil	
		A	B	A	B	A	B	A	B
Without PGPR	-	1.12	1.64	1.26	1.32	0.15	0.11	0.11	0.13
Without PGPR	+	2.58	2.61	2.53	2.36	0.18	0.15	0.21	0.17
Ps. fluorescens	+	3.48	3.95	4.13	4.42	0.34	0.25	0.43	0.38
P. polymyxa	+	4.06	4.27	3.93	3.78	0.28	0.25	0.34	0.31
Az. lipoferum	+	2.96	3.31	3.12	3.17	0.22	0.26	0.30	0.27
Ps. + P.	+	4.39	4.86	5.24	5.61	0.32	0.36	0.41	0.46
Ps. + Az.	+	4.51	4.97	4.18	5.28	0.26	0.38	0.38	0.48
Az. + P.	+	3.72	4.16	4.02	4.95	0.26	0.42	0.46	0.49
Ps. + P. + Az.	+	4.69	5.83	4.80	5.45	0.29	0.37	0.43	0.38
LSD at 5%		1.05	0.87	0.74	1.12	0.05	0.08	0.04	0.09

A: Soil was infested with fungal spores 5 days before transplanting.
B: Soil was infested with fungal spores 2 days after transplanting.

Table (5): Root characters as affected by inoculation with PGPR after 60 days from transplanting in the presence of *Fusarium oxysporum*.

Strains	*F. oxysporum*	Root size				Root dry weight (g)			
		Root-soaking		Over head- soil		Root-soaking		Over head- soil	
		A	B	A	B	A	B	A	B
Without PGPR	-	8.7	8.4	8.3	9.1	0.43	0.45	0.39	0.41
Without PGPR	+	6.3	7.5	7.1	7.3	0.32	0.39	0.31	0.33
Ps. fluorescens	+	8.1	10.3	9.8	9.2	0.46	0.43	0.40	0.48
P. polymyxa	+	10.5	11.6	9.7	9.8	0.43	0.48	0.46	0.57
Az. lipoferum	+	12.7	11.5	9.6	10.8	0.53	0.53	0.48	0.54
Ps. + P.	+	9.6	11.5	11.4	12.7	0.51	0.58	0.51	0.55
Ps. + Az.	+	10.4	12.9	10.7	12.6	0.51	0.60	0.53	0.63
Az. + P.	+	11.3	13.8	11.9	14.2	0.57	0.57	0.58	0.68
Ps. + P. + Az.	+	11.0	14.9	11.8	13.5	0.54	0.61	0.51	0.62
LSD at 5%		1.3	1.2	1.2	1.5	0.06	0.05	0.06	0.06

A: Soil was infested with fungal spores 5 days before transplanting.
B: Soil was infested with fungal spores 2 days after transplanting.

The beneficial effect of these bacteria as PGPR was previously confirmed by Abou-Aly (2008) who demonstrated that *Az. lipoferum* and *P. polymyxa* were potent for phosphate solubilization and increased the nitrogen in free-nitrogen medium; also they were able to produce IAA eithr with or without tryptophan as a precursor. These beneficial traits may be enhanced and stimulated the growth characters of pepper. Russo *et al.* (2008) reported that the presence of *A. brasilense* increased root system, root hair biomass production and apical activity so; it had a positive effect on rooting. Aside from the promotion of plant growth, *Az. lipoferum* protects plants against pathogen attacks. This study indicates that *Az. lipoferum* could be employed as a tool in plant biotechnology. On the other hand, although *A. lipoferum* lacks the capacity to produce significant amount of catechol-type siderophore or lytic enzymes (Abou-Aly, 2008), *P. polymyxa* was potent for

production of these traits which make this bacteria as a typical biocontrol agent and as an enhancer for the plant fitness.

In general, using the effective strains of *Ps. fluorescens*, *P. polymyxa* and *Az. lipoferum* not only as plant growth-promoting rhizobacteria but also to control soil-borne fungal pathogen may be a promising ecological alternative to chemical treatments in order to develop a sustainable agricultural management to either replace or reduce agrochemical abuse in.

Table (6): Effect of inoculation with PGPR on plant height and shoot dry weight after 60 days from transplanting in the presence of *Fusarium oxysporum*.

Strains	*F. oxysporum*	Plant height (cm)				Shoot dry weight (g/plant)			
		Root-soaking		Over head- soil		Root-soaking		Over head- soil	
		A	B	A	B	A	B	A	B
Without PGPR	-	30.5	34.3	29.5	32.6	6.5	6.9	6.4	7.0
Without PGPR	+	19.7	22.9	23.6	20.8	5.1	4.3	4.8	4.2
Ps. fluorescens	+	24.5	25.9	27.8	29.5	6.2	5.8	6.7	6.9
P. polymyxa	+	26.7	31.3	23.7	29.4	5.7	7.1	6.2	6.4
Az. lipoferum	+	29.3	34.5	28.8	29.1	6.9	6.8	6.7	6.3
Ps. + P.	+	27.1	32.6	28.6	29.7	7.2	7.7	7.3	7.4
Ps. + Az.	+	29.5	29.2	31.4	34.5	7.3	7.4	8.9	7.6
Az. + P.	+	32.0	35.7	27.4	35.8	7.5	8.4	7.5	10.5
Ps. + P. + Az.	+	30.6	32.4	35.2	33.7	6.9	7.8	7.2	8.6
LSD at 5%		6.12	4.73	3.43	5.71	0.75	2.04	0.93	1.16

A: Soil was infested with fungal spores 5 days before transplanting.
B: Soil was infested with fungal spores 2 days after transplanting.

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تأثير التلقيح بسلالات فعالة من البكتريا المشجعة لنمو النبات على الذبول الفيوزاريومى فى الفلفل

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فى تجربة أصص تم تقييم ثلاثة سلالات فعالة من البكتريا المشجعة للنمو وهى *Ps. fluorescens* و *P. polymyxa* و *Az. lipoferum* لمقاومة الذبول الفيوزاريومى فى الفلفل حيويا. وقد أظهرت تلك البكتريا قدرة على استيطان الجذور باستخدام طريقة أنابيب ماء الأجار، كما أن كل البكتريا المستخدمة أظهرت قدرة عالية على استيطان الجذور أو الريزوسفير عند تلقيحها بطريقة الإضافة فوق سطح التربة عن طريقة نقع الشتلات. بالإضافة إلى ذلك فقد انخفض النشاط الإنزيمى للتربة بإصابة الشتلات بفطر الفيوزاريوم ولكن عند تلقيح التربة بمخلوط ثلاثى من البكتريا المستخدمة أعطى أعلى نشاط لإنزيم الديهيدروجينيز ، وكذلك فإن أعلا نشاط لإنزيم الفوسفاتيز قد سجل باستخدام التلقيح المزدوج ببكتريا *P. polymyxa* و *Az. lipoferum* فى وجود الفطر الممرض.

وعلى الجانب الأخر فإن نسبة حدوث الإصابة بفطر الفيوزاريوم بعد تلقيحها بالبكتريا المشجعة للنمو قد انخفضت عن غير الملقحة بالبكتريا. وقد أظهر استخدام مخلوط ثنائى من بكتريا *P. polymyxa* و *Ps. fluorescens* كفاءة فى تقليل نسبة حدوث المرض وزيادة المقاومة للفطر الممرض. كذلك فإن تلقيح البكتريا بالإضافة فوق سطح التربة أعطى أقل نسبة إصابة بالفطر وأعلى كفاءة فى المقاومة عن طريقة نقع الشتلات. وقد زاد تركيز إنزيمات البيروكسيديز والبولى فينول أوكسيديز فى النباتات المصابة عن النباتات السليمة. وقد زاد تركيز هذين الإنزيمين مقارنة بالنباتات المصابة زيادة معنوية عند استخدام بكتريا *Ps. fluorescens* سواء منفردة أو فى مخلوط مع البكتريا المشجعة للنمو. وقد أثبتت النتائج أيضا أن إصابة التربة بفطر الفيوزاريوم قد أدى إلى انخفاض معنوى فى صفات النمو للنبات مقارنة بالنباتات غير المصابة. وقد زادت صفات النمو زيادة معنوية فى النباتات المصابة والملقحة بالبكتريا المشجعة للنمو منفردة أو مختلطة وخاصة بكتريا *P. polymyxa* و *Az. lipoferum*.