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Induction of Systemic Resistance and Growth Promotion by Selected Strains of Rhizobacteria against Lupine Fusarium Wilt

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Pots and field experiments were conducted in Ismailia, Agric. Res. Station, to evaluate the impact of some strains of plant growth promoting rhizobacteria (PGPR) (*Azotobacter chroococcum*, *Azospirillum brasilense*, phosphate solubilising bacteria (*Bacillus megaterium* var. *phosphaticum*), potassium solubilising bacteria (*B. cereus*, *B. polymyxa* and *Pseudomonas fluorescens*) for their efficacy against *Fusarium oxysporum* on lupine plants in pots, all tested PGPR significantly decreased wilted plants and increased healthy plants compared with the control treatment. *P. fluorescens* was the most effective in controlling wilt disease and increasing the levels of total, free and conjugated phenol in lupine followed by *B. polymyxa* and *B. megaterium*. While, *A. brasilense* was the least one. In the field trial during 2008/2009 and 2009/2010 growing seasons, results indicated that, *P. fluorescens* treated plots reduced wilt disease incidence of lupine plants resulting in a significant increase of lupine plant growth and seed yield compared to the control treatment.

Keywords: *Azospirillum brasilense*, *Azotobacter chroococcum*, *Bacillus cereus*, *B. megaterium* var. *phosphaticum*, *B. polymyxa* and *Pseudomonas fluorescens*, lupine wilt and total phenols.

White lupine (*Lupinus albus* L.) belongs to the leguminous family which has been cultivated in Egypt for human and animal nutrition, also for medical and industrial purposes. It can be considered as a friendly crop to the environment related to its efficient nitrogen fixation system, in addition to its improvement to the traditional cereal rotation and protein supply in low input farming systems (Hamblin *et al.*, 1993 and Julier *et al.*, 1994). Lupine seeds contain alkaloids, protein, oil, cholesterol, lecithin, salts (phosphorus and potassium) and carbohydrates (Ibrahim *et al.*, 1990). Soil-borne fungal diseases are among the most important factors limiting the yield production of lupine, resulting in serious economic losses. Several soil-pathogens including *Fusarium oxysporum* attack the roots and stem base of lupine plants (El-Barougy and El-Sayed, 2003 and Zian, 2005). Some chemicals are effective in controlling these diseases but, these chemicals are expensive and not environmental friendly. Therefore, alternative control methods are needed for managing these pathogens. Many researchers have used bacterial biological control as a mean of protection against soil-borne diseases as an alternative control method to fungicides (Zavaleta, 2000). From the plant growth promoting rhizobacteria,

Bacillus has shown promising results for the biological control of various plant pathogens as well as growth promoters of some crops (Weller, 1988 and Podile and Laxmi, 1998). Beneficial bacteria can be a significant component in the management of the soil environment so as to achieve attainable crop yield (Cook, 2002). These beneficial bacteria live in the rhizosphere, the region around the root, which is rich in nutrients due to the exudation of 0.40 of plant nutrients from the roots (Nelson, 2004). By benefiting from the nutrients secreted by plant roots within the rhizosphere, the bacteria influence the plants in a direct or indirect way. One influence may be stimulation of plant growth (Bloemberg and Lugtenberg, 2001). Bacteria inhabiting the rhizosphere and positively influencing plant growth are referred to as plant growth promoting rhizobacteria (PGPR) (Kloepper *et al.*, 1986). Significant yield increases have been achieved in crops such as maize, rice, potato, wheat and canola after inoculation with PGPR (Bertrand *et al.*, 2001 and Thakuria *et al.*, 2004) which resulted in increased interest in PGPRs (Asghar *et al.*, 2004). There are several hypotheses about the mechanisms by which rhizobacteria enhance growth. One direct mechanism is production of the auxin indole acetic acid (IAA) (Patten and Glick, 2002). Another direct mechanism may be increased availability of nutrients in the rhizosphere by means of solubilisation of unavailable forms of nutrients and/or production of siderophores (Glick, 1995 and Rodriguez and Fraga, 1999). Free living diazotrophic bacteria such as *Azosprillum* also are involved in promoting the growth of many tropical grasses, by fixing nitrogen as symbiotically and transferring it to the plant (Saubidet *et al.*, 2002). Biological control of plant pathogens, through the production of antibiotics, or through competition for nutrient and space can significantly improve plant health and promote growth by increasing of seedling emergence, vigour and yield. Antibiotics produced by *Pseudomonas* species (Thomashow and Weller, 1988); *Bacillus cereus* (Silo-Suh *et al.*, 1994) play an important role in the biological control of plant diseases. Seed and soil treatments with biocontrol agents *Bacillus megaterium, B. subtilis* and *Pseudomonas fluorescens* significantly reduced chickpea Fusarium wilt disease intensity of chickpea (Landa, 2004).

The objectives of the present study are therefore to evaluate some strains of PGPR for inducing resistant and growth promotion of lupine plants under greenhouse and field conditions. It is also discusses the efficiency of these strains for producing phenol compounds which associate with secondary plant metabolites to express resistant to pathogen infection.

Materials and Methods

1-Isolation and identification of the causal pathogen and pathogenicity test:

1-1- Isolation of F. oxysporum from wilted lupine plants:

Fusarium oxysporum was originally isolated on potato dextrose agar (PDA) medium from diseased lupine plants collected from different localities of Ismailia, Sharkia, Assuit, Menia and Beni-Suef governorates. Purification of the isolated fungus was carried out using hyphal tip techniques as described by Toussoun and Nelson (1968). Pure culture was identified according to their morphological characters according to Nelson *et al.* (1983) and Barnett and Hunter (1986) in Agric. Botany Dept., Fac. Agric., Suez Canal Univ.

1-2- Pathogenicity test:

Pathogenicity test of *F. oxysporum* isolates which isolated from wilted lupine plants were carried out on the susceptible cultivar (Giza 2) at Ismailia Agric. Res. Station, ARC.

A sterilized sorghum medium (100 g sorghum/bottle (1Liter) and enough water to cover the sorghum) was used for preparation of fungal inoculum. The medium was autoclaved, then inoculated with each of the isolated isolates, each alone, and incubated at 25±2°C for 15 days. Pots (30 cm in diameter) were filled with unsterilized sand clay soil .The soil was infested with the fungal inoculum at the rate of 3% (w/w) of soil weight (El-Barougy, 2008). The inoculated soils were watered and mixed thoroughly for one week to ensure even distribution of the inoculum. Lupine seeds (cv. Giza 2) were sown at the rate of five seeds/pot. A set of five replicates were used for each treatment. Five pots containing non-inoculated soil were used as a control. Percentages of early and late wilt were recorded at 30 and 90 days, respectively after sowing, while the numbers of the survived plants (healthy and infected) were recorded at 120 days after sowing. Infected survived plants were evaluated by cutting longitudinally of stem and root, healthy survived plants= no visual evidence of the disease.

Disease severity of visual wilt symptoms and any discoloration of internal tissue were recorded at one, three and four months after sowing according to the scale proposed by (Ishikawa *et al.*, 2005) with some modifications, based on 0-4 grades according to the percentage of inside browning through stem and root: 0= healthy, 1= 0-25% browning, 2= >25-50% browning, 3= >50-75% browning, and 4= >75-100% browning. The same fungi were reisolated from the diseased tested plants.

2-Bacterial inoculum, preparation and inoculation techniques:

Highly efficient strains of plant growth promoting rhizobacteria (PGPR) *Azotobacter chroococcum* (Beijerinck), *Azospirillum brasilense* (Tarrand), *Bacillus megaterium* var. *phosphaticum* (de Bary), *B. cereus* (Cohn), *B. polymyxa* (Prazmowski) and *Pseudomonas fluorescens* (Migula) were obtained from the cultural collocation of Agric. Microbiology Dept. National Research Centre.

The growth promoting rhizobacteria were independently grown in nutrient broth (Difco) for 48 hours at 30±2°C in a rotary shaking incubator. The density of each bacterial culture in the broth was counted using a haemocytometer, and the Liquid broth was adjusted to 10^9 cfu/ml suspension. In PGPB treatments, 10 ml of either tested microorganisms suspension were added to the soil in each pot just after sowing. In field inoculation, 100 ml of each microorganism, each alone mixed with some soil before adding to the plot after sowing.

3- Greenhouse experiments:

3-1- Effect of some strains of plant growth promoting rhizobacteria (PGPR) on the incidence of lupine Fusarium wilt:

In this experiment, PGPR were used to evaluate their efficiency in controlling lupine wilt disease (*F. oxysporum*) on cv. Giza 2 Sterilized sorghum grain media was infested with the pathogenic fungus (*F. oxysporum*) and incubated at 25±2°C for 15 days. Five lupine seeds per pot were sown in 30-cm pots filled with *F. oxysporum* infested unsterilized soil at the rate of 3% (w/w) per pot as previously

mentioned, seven days before planting. 10 ml of either tested microorganisms suspension were added to the soil in each pot just after sowing. Each pot received equal amounts of water. Other agricultural processes were performed according to normal practice. The treatments were as follows: 1) *Azotobacter chroococcum* 2) *Azospirillum brasilense*, 3) *B. megaterium*, 4) *B. Cereus* and 5) *Pseudomonas fluorescens*. The control treatments were soil infested with *F. oxysporum* used as control (1). Non-infested soil used as control (2). A set of five pots for each treatment was used. Percentages of early wilt recorded at 30 days from sowing. Late wilt and survived plants were recorded at 90 days from sowing while disease severity of wilt and any discoloration of internal tissue were recorded at 120 days from sowing. The wilted plants were evaluated by cutting longitudinally through each plant (stem and root), healthy plants= no visual evidence of disease.

Disease severity of wilt and any discoloration of internal tissue were recorded as mentioned before. Plant growth parameters (plant height, number of branches, number of pods, seed weight and root length / plant) were also recorded four months after planting.

4- Field experiment:

4-1- Effect of some strains of plant growth promoting rhizobacteria (PGPR) on lupine wilt and root rot diseases under field condition:

An experiment was conducted in a field at Ismailia Agric. Res. Station, 7 and 8 November during 2008/2009 and 2009/2010 growing seasons, respectively, in soil known to have a high inoculum density of the lupine wilt pathogen, for controlling wilt of lupine in a naturally infested field. Some bacterial plant growth promoting and bacterial biological agents tested as soil treatments, 100 ml cell suspension (El-Barougy *et al.*, 2009) (at the rate of 10^9 cfu / ml) of each microorganism was just diluted with sandy soil and added to the rows of the plot after planting. The treatments were arranged in a complete randomized block design with three replicates. The field plot was 2×3 m^2 with four rows; each row contained 10 hills on the eastern side. 100 seeds of cv. Giza 2 were sown in each plot. Percentages of early wilt and damping-off were recorded at 30 days after sowing, while infected plants (late wilted and root-rotted plants) as well as survived plants were recorded at 90 days after sowing and weight of seed yield/feddan were recorded after harvest till all plants and seeds dried.

5- Chemical analysis:

5-1- Effect of PGPR on the levels of total phenolic compounds of lupine plants under greenhouse conditions:

5-1-1- Preparation of the leaves extract:

Samples of five gm of susceptible lupine cultivar (Giza 2) and resistant mutant (Mutant 7) leave tissues were obtained from each treatments (PGPR) and controls, then cut into small portions and immediately stored in 95% ethanol in brown bottles and kept in the dark at room temperature for one month until the tissues were colourless. The ethanolic extracts were left to air current at room temperature till approximately dryness, then the extract was quantitatively transferred into 5 ml of 50% isopropanol and stored in vials at -20°C till the determination of total, free and conjugated phenols (El-Toony, 1992).

5-1-2- Determination of phenolic compounds:

Phenolic compounds were colorimetrically determined using the phosphotungstic – phosphomolybdic acid; (Folin–Ciocalteu) phenol reagent according to Snell and Snell (1953).

6-Statistical analysis:

All the data were statistically processed by the analysis of variance and by determining the significance threshold using Duncan's test (Duncan, 1955).

Results

1-Isolation and identification of the causal pathogen and pathogenicity test:

Eighteen *Fusarium oxysporum* isolates, isolated from the roots of lupine plants showing typical symptoms of wilt disease, collected from different locations of Ismailia, Sharkia, Assuit, Menia and from Beni-Suef governorates were tested for the pathogenicity on lupine cv. Giza 2. Data presented in Table (1) indicate that all the tested isolates were pathogenic and the eighteen isolates showed a significant variation in their pathogenicity in pots experiment. *F. oxysporum* isolate No.3 isolated from Assuit governorate showed to be the most virulent isolate to cause 28% early wilted plants at 30 days and 32% late wilted plants at 90 days , followed by isolates No.15, No.2 and No.6 which were isolated from Ismailia, Assuit and Menia governorates, respectively. These *F. oxysporum* isolate No.3 was selected for further investigation.

2- Greenhouse experiments:

2-1- Effect of some strains of plant growth promoting rhizobacteria (PGPR) on the incidence of lupine Fusarium wilt:

In this experiment, plant growth promoting rhizobacteria namely *Azotobacter chroococcum Azospirillum brasilense, Pseudomonas fluorescens, Bacillus megaterium, B. cereus* and *B. polymyxa* were added singly into soil to study their effect on lupine Fusarium wilt disease development in pots. Data in Tables (2a & b) indicate that all treatments decreased percentages of wilted plants one and three months after sowing and increased healthy plants compared with the infested soil (control 1) and non-infested untreated seeds (control 2) treatments. Data indicated that soil planted with the susceptible cultivar (Giza 2) and infested with *F. oxysporum* (control 1) showed the highest disease severity score (2.8) and the lowest percentage of healthy plants (20%) compared with the lowest disease severity score (0.4). Whereas, the highest percentage of healthy plants (84%) was recorded with *P. fluorescens* treatment followed by both of *B. megaterium* and *B. polymyxa* (0.6 and 80%). The highest percentage of disease reduction over the control was obtained from *P. fluorescens* (85.7%) followed by each of *B. megaterium* and *B. polymyxa* treatments which recorded 78.5%. Data also, indicated that soil planted with Mutant 7, infested with *F. oxysporum* and treated with *P. fluorescens* or *B. polymyxa* were the most effective treatments, judged by the lowest disease severity score (0.2) and the highest increase in disease reduction (90%) compared with the control 1 (infested soil only).

Table 1. Pathogenicity of various *Fusarium oxysporum* isolates isolated from wilted lupine plants on cv. Giza 2

Tested isolate	Wilted plants (%)		Survived plants (%)		Disease * severity score
	One month after seeding	Three months after seeding	Infected plants (%)	Healthy plants (%)	
Isolate No.(1)	28	20	20	32	2.8
Isolate No.(2)	28	24	20	28	3.0
Isolate No.(3)	28	32	24	16	3.4
Isolate No.(4)	16	12	32	40	2.2
Isolate No.(5)	16	24	28	32	2.6
Isolate No.(6)	28	24	24	24	3.2
Isolate No.(7)	0.0	20	32	48	1.6
Isolate No.(8)	24	24	32	20	3.0
Isolate No.(9)	16	24	32	28	2.6
Isolate No.(10)	16	20	24	40	2.0
Isolate No.(11)	20	28	16	36	2.6
Isolate No.(12)	12	20	36	32	2.2
Isolate No.(13)	32	24	12	28	3.2
Isolate No.(14)	8.0	12	20	60	1.6
Isolate No.(15)	28	20	20	32	2.6
Isolate No.(16)	4.0	24	24	48	2.2
Isolate No.(17)	20	24	12	44	2.4
Isolate No.(18)	20	24	28	28	2.4
Control	8.0	8.0	4	80	0.8
L.S.D. at 0.05%	3.30	3.74	10.41	9.87	0.97

* Disease severity of wilt and any discoloration of tissue were recorded according to Ishikawa *et al.* (2005) with some modifications, based on 0-4 scale according to percentage of inside browning or necrosis (1= 0 > 25%, 2= >25-50 %, 3= >50-75% and 4= >75-100).

Table 2a. Effect of some strains of PGPR on the incidence of lupine Fusarium wilt under greenhouse condition

Treatment	Giza 2 (Susceptible cultivar)					
	Wilted plants (%)		Survived plants (%)		Disease severity *	
	One month after seeding	Three months after seeding	Infected survived plants (%)	Healthy survived plants (%)	Disease severity score	reduction over the control (%)
Azotobacter chroococcum	8	12	12	68	1.0	64.2
Azospirillum brasilense	8	4	12	76	0.8	71.4
Pseudomonas fluorescens	4	4	8	84	0.4	85.7
Bacillus polymyxa	4	4	12	80	0.6	78.5
Bacillus megaterium	4	4	12	80	0.6	78.5
Bacillus cereus	8	4	12	76	1.0	64.2
Infested soil only (Control 1)	20	28	32	20	2.8	-
Untreated seeds (Control 2)	8	8	12	72	1.0	-
L.S.D. at 0.05	3.08	3.31	9.90	10.39	0.69	-

* As described in footnote of Table (1)

Table 2b. Effect of some strains of PGPR on the incidence of lupine Fusarium wilt under greenhouse condition

Treatment	Mutant 7 (Resistant)					
	Wilted plants (%)		Survived plants (%)		Disease severity*	
	One month after seeding	Three months after seeding	Infected survived plants (%)	Healthy survived plants (%)	Disease severity score	Reduction over the control (%)
Azotobacter chroococcum	8	4	16	72	0.8	60.0
Azospirillum brasilense	8	4	8	80	0.6	70.0
Pseudomonas fluorescens	4	0	0	96	0.2	90.0
Bacillus polymyxa	4	0	4	92	0.2	90.0
Bacillus megaterium	4	4	8	84	0.4	80.0
Bacillus cereus	4	8	8	80	0.8	60.0
Infested soil only (Control 1)	8	8	28	56	2.0	-
Untreated seeds (Control 2)	4	4	8	84	0.8	-
L.S.D. at .05	2.14	3.21	7.01	9.82	0.68	-

*As described in footnote of Table (1).

2-2- Effect of some strains of PGPR on some growth parameters of lupine plants (Giza- 2 and Mutant 7) under greenhouse conditions:

Data presented in Table (3) reveal that the application of *Azotobacter chroococcum, Azospirillum brasilense, Bacillus megaterium* var. *phosphaticum, B. cereus, B. polymyxa* and *P. fluorescens* caused a significant increase in the values of plant height, number of branches, number of pods, seed weight and root length plants over the control treatment (infested soil without any antagonists). Data in Table (3) show that cv. Giza 2 recorded the lowest values (43.2 cm, 5.7 branches, 7.2 pods, 8.6 gm/plant and 23.3 cm/plant, respectively, as a mean of five plants) of plant height, number of branches, number of pods, seed weight and root length of lupine seedlings grown in infested soil (control 1), while the greatest values (77.4 cm, 7.9 branches, 15.4 pods, 15.7 gm/plant and 50.4 cm/plant, respectively) of plant height, number of branches, number of pods, seed weight and root length of lupine plants grown in *F. oxysporum* infested soil were recorded from *P. fluorescens* treatment compared with 56.5 cm, 6.9 branches, 9.6 pods, 11.2 gm/plant and 26.2 cm/plant, respectively, obtained from untreated lupine seeds grown in non-infested soil (control 2). Data also showed that lupine plants of Mutant 7 grown in *F. oxysporum* – infested soil treated with *P. fluorescens* treatment recorded the highest values of plant height, number of branches, number of pods, seed weight and root length (86 cm, 8.9 branches, 17 pods, 20.6 gm/plant and 54.6 cm/plant, respectively), followed by *B. polymyxa* (82 cm, 8.9 branches, 16.6 pods, 18 gm/plant and 55.5 cm/plant, respectively) and *B. megaterium* (78cm, 7.8 branches, 16 pods, 17.6 gm/plant and 50.3 cm/plant, respectively), comparing with the lowest values obtained from control 1 (61.6 cm, 6.8 branches, 10.4 pods, 15 gm/plant and 40.4 cm/plant, respectively).

Table 3. Effect of some strains of PGPR on some growth parameters of lupine plants (Giza 2 and mutant 7) under greenhouse conditions.

Treatment	Giza 2 (Susceptible cultivar)*					Mutant 7 (Resistant)				
	P.H.	N.B.	N.P.	W.S.	R.1.	P.H.	N.B.	N.P.	W.S.	R.1.
Azotobacter chroococcum	66	6.8	12.0	11.0	44.0	68.0	6.8	14.2	16.5	46.0
Azospirillum brasilense	71.8	7.3	13.8	13.8	44.7	74	7.7	15.6	16.7	49.8
Pseudomonas fluorescens	77.4	7.9	15.4	15.7	50.4	86	8.9	17	20.6	54.6
Bacillus polymyxa	74.2	7.5	14.4	14	50.2	82	8.9	16.6	18	55.5
Bacillus megaterium	73	6.8	13.2	13.5	46	78	7.8	16	17.6	50.3
Bacillus cereus	66.5	6.9	10.6	12.6	40	73	7.3	15	16.2	44.2
Infested soil (control 1)	43.2	5.7	7.2	8.6	23.3	61.6	6.8	10.4	15	40.4
Untreated seeds (control 2)	56.5	6.9	9.6	11.2	26.2	75.4	7.6	13.2	19.3	45.5
L.S.D. at 0.05	6.41	0.72	2.29	2.68	1.82	6.33	0.92	2.03	2.30	5.67

* P.H.= Plant height (cm), N.B.= Number of branches, N.P.= Number of pods/plant, W.S.= Weight of dry seeds/plant (gm), R.l.= Root length (cm),

3- Field experiment:

3-1- Effect of some strains of plant growth promoting rhizobacteria (PGPR) on wilt and root rot diseases of lupine plants under field conditions:

The effect of PGRB (*Azospirillum brasilense*, *Pseudomonas fluorescens*, *Bacillus megaterium* var. *phosphaticum* and *B. polymyxa*) on lupine wilt disease incidence under field conditions during 2008/2009 and 2009/2010 growing seasons is shown in Table (4). Data show that all the tested PGPR treatments increased the percentage of survived plants compared with the control. The highest percentage of plants survival (94 and 95.6%) was recorded during the first and second season, respectively when *P. fluorescens* treatment was used followed by 93 and 94% and 89 and 93.3% recorded from *B. polymyxa* and *B. megaterium* comparing with 71.3 and 75.6% which were obtained from control treatment. However, the differences among the treatments were not significant. Similar results were noticed with seed yield; the highest values of seed yield (2.650 and 2.800 kg/plot) were recorded during the first and second season, respectively when *P. fluorescens* was used. It is also clear from data (Table 4) that *P. fluorescens* treatment increased the percentage of survival plants compared with non-treated treatment (control) from 73.45 to 94.8 as means of the two seasons. Similar trend was noticed with yield weigh, the highest mean of yield weight (2.735 kg/plot) was recorded for *P. fluorescens* treatment compared with 0.987 kg/plot in the control treatment.

Table 4. Effect of plant growth promoting rhizobacteria (PGPR) on survival plants of lupine planted in naturally infested soil under field conditions

Treatment	2008/2009 season		2009/2010 season		Mean of two seasons			
					Survived plants (%)		Weight of yield/plot (kg)	
	Survived plants (%)	Weight of yield/plot (kg)	Survived plants (%)	Weight of yield/plot (kg)	Survived plants (%)	Increasing over the control (%)	Weight of yield/plot (kg)	Increasing over the control (%)
Azospirillum brasilense	91.4	2.400	90.3	2.410	90.85	21.6	2.405	143.6
Pseudomonas fluorescens	94	2.650	95.6	2.800	94.8	29.06	2.735	177.1
Bacillus polymyxa	93	2.500	94	2.700	93.5	27.29	2.600	163.4
Bacillus megaterium	89	2.350	93.3	2.550	91.15	24.07	2.450	148.2
Control	71.3	0.900	75.6	1.075	73.45	-	0..987	-
L.S.D. 0.05%	5.81	0.32	6.22	0.23				

4- Chemical analysis:

4-1- Effect of some strains PGPR on levels of total phenolic compounds of lupine plants (Mutant 7 and cv. Giza 2) under greenhouse conditions:

Data presented in Table (5) show the effect of PGPR on total, free and conjugated phenols in leave tissues of lupine plants (Giza 2 and Mutant 7) grown in infested soil with *F. oxysporum* (control 1). Furthermore, data show that total phenolic compound content was higher in Mutant 7 lupine plants than that recorded in cv. Giza 2. Infection by *F. oxysporum* led to an increase in total and free phenols in the two tested cultivars as comparing with non- infested (healthy control 2). Furthermore in Mutant 7, the highest values in total and free phenols content (5.14 and 2.82 mg/ gfw (gram fresh weight), respectively) were recorded from *Pseudomonas fluorescens* treatment followed by *Bacillus polymyxa* (4.44 and 2.42 mg/gfw) and *B. megaterium* (4.13 and 2.20 mg/gfw) treatments, respectively. Meanwhile, the least values in total and free phenols were recorded in healthy control 2 treatment (3.56 and 1.98 mg/gfw, respectively). Concerning to cv. Giza 2, the highest records of total and free phenols were obtained from *P. fluorescens* treatment (4.26 and 2.04 mg/gfw) followed by *B. polymyxa* (4.10 and 2.24 mg/gfw) and *B. megaterium* (3.95 and 2.10 mg/gfw), respectively. Generally, in the present study, it was found that conjugated phenols were higher in Mutant 7 than in cv. Giza 2 and phenolic compounds were accumulated faster in Mutant 7 than in cv. Giza 2 as a result of infection.

Table 5. Effect of some strains of PGPR on levels of phenolic compounds of lupine plants (Mutant 7 and cv. Giza 2) under greenhouse conditions

Treatment	Cultivar	Phenolic contents (mg/g fresh weight)		
		Total phenols	Free phenols	Conjugated phenols
Azotobacter chroococcum	Mutant 7	3.77	1.88	1.89
	Giza 2	3.54	1.80	1.74
Azospirillum brasilense	Mutant 7	3.87	1.85	2.02
	Giza 2	3.68	1.75	1.93
Pseudomonas fluorescens	Mutant 7	5.14	2.82	2.32
	Giza 2	4.26	2.04	2.22
Bacillus polymyxa	Mutant 7	4.44	2.42	2.02
	Giza 2	4.10	2.24	1.86
Bacillus megaterium	Mutant 7	4.13	2.20	1.93
	Giza 2	3.95	2.10	1.85
Bacillus cereus	Mutant 7	3.65	1.98	1.67
	Giza 2	3.33	1.81	1.52
Infested soil (Control 1)	Mutant 7	3.77	2.10	1.67
	Giza 2	3.11	1.74	1.37
Healthy untreated seeds (Control 2)	Mutant 7	3.56	1.98	1.58
	Giza 2	2.95	1.62	1.33

Discussion

White lupine is suffering from infection with many diseases caused by fungi, bacteria and viruses. However, fungal diseases especially wilt disease caused by Fusarium oxysporum is considered the most serious disease of white lupine in Egypt, causing a considerable damage and loss in seed yield (Abou Zeid *et al.*, 2002).

Isolation's trials from wilted lupine plants yielded *Fusarium oxysporum* which was the most isolated fungus, conforming to other reports of El-Barougy and EL-Sayad (2003) and Zian (2005).

Pathogenicity test on cv. Giza 2 was conducted with eighteen of *F. oxysporum* isolates led to symptoms which were almost similar to those noticed under field conditions. All the *F. oxysporum* isolates were pathogenic to lupine with some variations when amended into the pot soil. The present investigation demonstrated that the isolated *F. oxysporum* isolates from naturally infected field could increase wilted plants and reduce percentages of healthy survival plants and could directly affect the yield. *F. oxysporum* isolate No. (3) showed to be high virulent, it gave the highest percentage of wilted plants, These results were in agreement with those recorded by EL-Barougy and EL-Sayad, 2003 and Zian, 2005 they found that the application of inoculum of *F. oxysporum* caused significant disease and increased losses in plants and yields of white lupine .

The use of pesticides to control soil-borne diseases and pests of economically important crops has been used in agriculture for many years. Recently, an increasing desire to reduce the use of pesticides is seen through the attempts to develop integrated pest management approaches. Biological control is high on the list of potential alternative control methods. The effects of some bacterial plant growth promoting [*Azotobacter chroococcum, Azospirillum brasilense, Bacillus cereus, B. megaterium B. polymyxa* and *Pseudomonas fluorescens*] were evaluated against *F. oxysporum* infecting lupine plants.

The obtained results revealed that percentage of healthy survival plants remaining in the pots, depending on the treatments. In the infested soil, non-treated with bacterial plant growth promoting (control 1), only about 20% for cv. Giza 2 and 56% for Mutant 7 of the plants were still alive at the end of the season. However, it has been found that sufficient control of wilt disease was obtained by using bacterial agents. All pots treated with *Azotobacter chroococcum, Azospirillum brasilense, Bacillus cereus, B. polymyxa, B. megaterium* and *Pseudomonas fluorescens* decreased wilted plants caused by *F. oxysporum* and increased healthy survival plants over the control , these findings are in agreement with those recorded by many researchers such as Whips (2001) who reported that many bacterial genera are being used and tested in bacterization including, *Azospirillum, Azotobacter, Bacillus, Bradyrhizobium,* and *Pseudomonas* to enhancement symbiotic or associative nitrogen fixation, degradation of xenobiotic compounds, plant growth promotion and biological control of plant pathogenic. In this regard, Seed and soil treatments with biocontrol agents *Bacillus megaterium, B. subtilis* and *Pseudomonas fluorescens* significantly reduced chickpea Fusarium wilt disease intensity (Landa, 2004 and Badawi *et al.,* 2007) and decreased disease incidence caused by *M. phaseolina* in soybean plants (El-Barougy *et al.,* 2009). Akhtar and Siddiqui (2008) mentioned that *Pseudomonas alcaligenes* and *Bacillus pumilus* decreased disease incidence caused by *M. phaseolina* in chickpea plants. The mechanism by which bacterial biocontrol agents affecting fungal growth may be attributed to the presence of some effective substance such as antibiotics which play an important role in the biological control of plant diseases. Many investigators confirmed these results such as Liu and Sinclair (1989 and 1990) and Handelsman *et al.* (1990).

The reduction in disease incidence reflected on plant height, number of branches, root length ,weight of dry seeds and number of pods of plants grown in infested soil with *F. oxysporum* fungus and treated with bacterial biocontrol which were greater compared with the control 1 (infested soil) and the control 2 (untreated seeds non-infested soil).These results are relatively similar to those obtained by Yuming *et al.* (2003) they reported that three *Bacillus* strains, *i.e. B. subtilis* NEB4 and NEB5 and *B. thuringiensis* NEB17 provided increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen, and grain yield of soybean plants.

Bacterial plant growth promoting (BPGP) can promote plant growth directly or indirectly. Indirect effects are related to production of metabolites, such as antibiotics, that decrease the growth of phytopathogens and other deleterious microorganisms (Raaijmakers *et al.*, 2002). Direct effects are dependent on

production of plant growth regulators. Some rhizobacterial strains promote legume nodulation and nitrogen fixation by producing flavonoid-like compounds and/or stimulating the host legume to produce more flavonoid signal molecules (Parmar and Dadarwal, 1999). Nodulation and subsequent nitrogen fixation by lupine plants are inhibited by low root zone temperatures (RZTs). Plant growth promoting bacteria can help overcome these deleterious effects. (Yuming *et al.*, 2003) reported that three *Bacillus* strains, *B. subtilis* NEB4 and NEB5 and *B. thuringiensis* NEB17 were shown to have plant growth promoting activity on pouch-grown soybean plants under greenhouse conditions at low root zone temperatures (RZTs) and under field conditions in a short growing season area. And provided increases in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen, and grain yield of soybean plants.

It was known that there is a correlation between the degree of resistance and the phenol level in healthy plants. In the present study, it was found that, phenolic compounds content in healthy resistant mutant (Mutant 7) were higher than that found in susceptible cultivar (Giza 2). Infection by *Fusarium oxysporum* resulted in an increase in total and free phenols in the two tested cultivars compared with the healthy control 2. The highest increase in total and free phenols content was recorded in *P. fluorescens* treatment in both resistant and susceptible cultivars. The increase in phenolic compounds in the infected plants may be attributed to formation of these compounds under the stress of infection in order to decrease the determinable effect or induce resistance against the pathogen. Farkas and Kiraly (1962) indicated that phenols are oxidized to quinone or semi-quinone which plays a great role as antimicrobial substances. Moreover, Gupta *et al.* (1992) explained the importance of phenolic compounds in the host – parasite interaction is that they act as hydrogen donors/acceptors in oxidation-reduction reactions and their involvement in resistance by oxidation to quinones which are more toxic to a microorganism(s).

Effect of bacterial plant growth promoting on survival lupine plants under field condition was also studied. The obtained results showed that soil treated with *P. fluorescens* or *B. polymyxa* or *B. megaterium* or *Azospirillum brasilense* increased survival plant by 29.06, 27.29, 24.07 and 21.6%, respectively, as a means of two seasons compared with control treatment. Such increasing in survival plant was accompanied by an increase in yield weight.

In view of the apparent bacterial plant growth promoting and bacterial biocontrol agents could provide a mean for reducing the incidence of wilt disease of lupine plants in addition to avoiding the use of fungicides. Such biocontrol approach should be employed as a part of integrated pest management (IPM) system.

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تحفيز المقاومة الجهازية وتشجيع النمو فى نباتات الترمس ضد مرض الذبول الفيوزاريومى بواسطة بعض سلالات بكتريا الجذور

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يصاب الترمس بالعديد من الأمراض ويعتبر مرض الذبول المتسبب عن فطر *Fusarium oxysporum* عامل رئيسي فى تقليل إنتاجية نباتات الترمس وقد تكون المبيدات الكيمائية فعالة فى السيطرة على هذا المرض إلا أن هذه المواد مكلفة وضارة بالبيئة ولهذا يلجأ كثير من العلماء و الباحثين الى استخدام طرق مقاومة بديلة للمبيدات. لذلك أجريت تجارب الأصص والحقل فى محطة البحوث الزراعية بالإسماعيلية لتقييم بعض سلالات البكتريا المشجعة لنمو النباتات (*Azotobacter chroococcum, Azospirillum brasilense, Bacillus megaterium* var. *phosphaticum, B. cereus, B. polymyxa and Pseudomonas fluorescens*) الذى يصيب نباتات الترمس وتأثيرها على نسبة النباتات السليمة الباقية وأيضاً مقاييس النمو. وقد أوضحت نتائج تجربة الأصص ان كل سلالات البكترية المختبرة خفضت بشكل معنوى نسبة النباتات المصابة بالذبول وزادت من نسبة النباتات السليمة الباقية مقارنة بمعاملة الكنترول المنقح بالفطر . كذلك أوضحت النتائج أن سلالة بكتريا *P. fluorescens* كانت الأفضل فى الحصول على أعلى نسبة نباتات سليمة وسجلت اعلى تحفيز لأنشطة إنزيمات الشيتينيز ، البيروكسيديز و البولى فينول أوكسيديز ، كما أنها زادت من مستويات الفينولات الكلية، الحرة والمرتبطة فى كل من الصنف الحساس للإصابة والطفرة المقاومة. وقد كانت نتائج تجربة الحقل مشابهة لنتائج الصوبة حيث ان كل سلالات البكتريا المختبرة زادت من نسبة النباتات الباقية مقارنة بالكنترول وقد تلازمت زيادة نسبة النباتات الباقية مع زيادة وزن البذور الجافة. وقد تبين أن معاملة بكتريا *P. fluorescens* كانت الأكثر تأثيراً تليها معاملة *B. polymyxa* ثم *B. megaterium* ثم *A. brasilense* .