Egyptian Journal of Biological Pest Control, 21(2), 2011, 227-232

Evaluation of some Plant Growth Promoting Rhizobacteria in Bioprotecting Lupine from Infection by *Fusarium solani*

Maha A. Hewedy[*]; A. F. Abdel-Wahab[**]; Mehrashan T. El Mokadem[*] and Soad Y. El-Sayed[**]

[*]Botany Dept., Women College for Arts, Science and Education, Ain Shams Univ., Cairo, Egypt.
[**]Department of Agricultural Microbiology, Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt

(Received: October 26, 2011and Accepted: November 27, 2011)

ABSTRACT

Serratia sp., *Paenibacillus polymyxa* and *Pseudomonas fluorescens* were tested for their ability to inhibit growth of the fungi causing lupine diseases *in vitro* and in *vivo*. All tested rhizobacteria exhibited a positive reaction for cyanide, protease, but as for chitinase test only *P. fluorescens* gave negative reaction. All tested bacteria showed antagonistic effect against *Fusarium solani*. *Serratia* sp. appeared superiority for the most PGP-related properties. A pot experiment was conducted to evaluate the co-inoculation of *Bradyrhizobium* sp. with the tested bacteria in the incidence of *F. solani*. The co-inoculation of rhizobacteria with *Bradyrhizobium* recorded an increase in all parameters compared to sole inoculation of *Bradyrhizobium*. The yield of lupine plants, represented by straw dry weight and seed yield, was increased significantly due to the co-inoculation with PGPRs and *Bradyrhizobium*, especially in case of *Serratia* sp. in the presence of *F. solani*. Nitrogen, phosphorus content and crude protein of seeds and straw were increased when the PGPRs were co-inoculated with *Bradyrhizobium* over than those inoculated with *Bradyrhizobium* alone.

Key words: Biological control, *Fusarium*, PGPR bacteria, Lupine.

INTRODUCTION

A promising strategy for replacement or reducing the injurious impacts of pesticides is biocontrol technology, used individually or as a component of integrated pest management (IPM). Biocontrol preparations of both fungi and bacteria have been applied to seeds, seedlings and planting media in several ways to reduce infection by plant diseases with various degrees of success. Biological control may exert through several mechanisms such as; competition for nutrients, competition for sites on roots or production of metabolites. Another promising mechanism for biocontrol of pathogenic fungi is induced resistance (Mao *et al.*, 1997).

White lupine (*Lupinus termis*) is one of the oldest agricultural crops widely used in the world, not only as a protein source in fodder production but also for soil improvement (Maknickiene, 2001 and Abdel-Monaim *et al.*, 2010).

Grain legumes are subject to numerous pathoge attacking both the roots and the aerial parts of the plant. Soil-borne fungal diseases are the most important factors among all, limiting the seed yield of legume crops in many countries worldwide (Shaban and El-Bramawy, 2011).

The present investigation aimed to evaluate the effectiveness of certain Rhizobacteria against *Fusarium solani in vitro* and the suppression of damping-off disease of lupine in pot experiments. Production of cyanide, chitinase and protease was investigated as potent fungicidal compounds. The effect of Rhizobacterial treatments on seed yield, pods number, protein content and P-concentration in lupine seeds and straw was also investigated.

MATERIALS AND METHODS

Microbial organisms and inocula preparations

A) Rhizobia and rhizobacteria: *Bradyrhizobium lupinus*, *Paenibacillus polymyxa*, *Pseudomonas fluorescens* and *Serratia* sp. were kindly obtained from Department of Microbiology, Soils, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Giza, Egypt.

B) Pathogenic fungi: *Fusarium solani*, used for lupine infection experiment was kindly obtained from Plant Pathology Research Institute, ARC, Giza, Egypt.

C) Seeds: Lupine (*Lupinus termis* L. cv. Giza-1) seeds were kindly obtained from Leg. Crops Dept., Field Crops Res. Inst., ARC, Giza, Egypt.

Culture media

Different media were used for cultivation and maintenance of certain microbial organisms as follow:

a) Yeast extract mannitol (Vincent, 1970) for of rhizobia.
b) Nutrient agar medium (Dowson, 1957) for *Paenibacillus polymyxa*.
c) Peptone glycerol medium (Grimont and Grimont, 1984) for *Serratia* sp.
d) Kings-agar B medium (Alef, 1995) for *Pseudomonas fluorescens*.

e) Potato dextrose agar (PDA) (ATTC, 1992) for the pathogenic fungus, *F. solani*.
f) Luria-Bertani (L.B) agar medium (Bric *et al.*, 1991) amended with 4.4 gm of glycine. This media was used for the detection of hydrogen cyanide (HCN) production.
g) Chitinolytic activity agar medium (Strzelezyk *et al.*, 1990). This media was used for the detection of bacteria antagonistic the pathogenic fungus.
e) Protease production: Protease was detected on minimal agar medium according to the method described by Dunne *et al.* (1997).

Soil used

Sandy soil was collected from 20 cm surface layer of Ismailia Experimental and Research Station, Ismailia Governorate, ARC, Egypt.

Assay of rhizobacteria activities *in vitro*

1- Detection of hydrogen cyanide: was carried out as described by Bakker and Schippers (1987).
2- Detection of chitinase and protease enzyme production: was carried out according to the method described by Strzelezyk *et al.* (1990).
3- Fungal inhibition test: was carried out according to the method described by Alvarez *et al.* (1995).

Evaluation of rhizobacteria as bio-protecting agent *in vivo*

A pot experiment was conducted in a greenhouse at SWERI, ARC, Giza, Egypt during the winter season of 2006/07 to evaluate the ability of some rhizobacteria namely; *P. polymyxa, Serratia* sp. and *P. fluorescens* for their ability to promote and bio-protect lupine plants grown in sandy soil. Earthenware pots (35 cm diameter) were filled by 10 kg non-sterilized soil, then fertilized by superphosphate, at the rate of 2 g/pot (200kg/ fed) and manured by 25g compost (2.5 ton/fed). After 15 days of sowing date, all these pots were fertilized by ammonium sulphate at the rate of one g/pot (100 kg/fed) and potassium sulphate at the rate of 0.5 g/fed (50 kg/fed). It was prepared for sowing lupine (cv. Giza-1) and the following treatments were practiced:

1- Seeds inoculated with *Bradyrhizobium lupinus* only (Br) as control in case of lupine.
2- Seeds inoculated with Br + *P. polymyxa* (Bp).
3- Seeds inoculated with Br + *Serratia sp* (S).
4- Seeds inoculated with Br + *P. fluorescens* (Ps).
5- Seeds inoculated with Br + Bp + S + Ps.

At lupine experiment, the treatments were executed in the absence and presence of artificial inoculation by *F. solani*. Soil infestation was done via sterilization of infested pots, with 5% formalin solution and left to dry before use. Potted soil was infested with the inoculum of *F. solani* in irrigation water and left for seven days to provide suitable moisture for fungal growth.

Seed inoculation with rhizobia or/ and rhizobacteria was manipulated via mixing each inoculant with seeds at the rate of 10 g inoculants/ 1 kg seeds, with the Arabic gum as adhesive material. Each treatment of both experiments comprised of six replicates to satisfy duration of both sampling and harvesting and it was arranged in randomized complete block design.

Percentage of pre- and post- emergence damping-off was recorded after 45 days of planting in the infested soil only. The nodulation status, growth vigor and plant content of N and P, as well as the yield and its component of each plant were evaluated at 60 and 120 days, post planting, respectively.

Analytical methodology

Plant materials

1-Nitrogen and Phosphorus contents were measured using Micro-Kjeldahl method according to Page and Von-Tigerstrom (1982).
2- Crude protein contents of seed and straw were calculated by multiplying the percentage of N by 6.25 (Page and Von-Tigerstrom, 1982).

Statistical analyses

Obtained data were subjected to Analysis of Variance (ANOVA). L.S.D. test was used to compare the treatment means according to the procedures outlined by Snedecor and Cochran (1980) using MSTAT computer program software program (MSTAT Ver., 1.42).

RESULTS AND DISCUSSION

Characteristics of some rhizobacteria *in vitro*

Cyanide production

According to the amounts of HCN released in rhizosphere by microorganisms, this mechanism may be benefited the plants via suppressing the phytopathogens or may become deleterious to plants at high amounts. Therefore, the ability of the tested rhizobacteria to produce cyanide was done by qualitative screening (Fig. 1). The visual inspection of the plates showed that each of the rhizobacteria assayed had a cyanogenic potential as a result of changing the color of indicator paper. *Serratia* sp. and *P. fluorescens*, respectively turned the color into reddish and brown, as indication to their potential to produce cyanide. On the other hand, *P. polymyxa*, and *B. lupinus* were considered a moderate cyanogens (showed yellow to light brown color). Ability of rhizobacteria to produce HCN in reasonable quantities may be useful to imply such rhizobacteria a suppressive bioagents against soil borne phytopathogens. From such point of view, some rhizobacteria, i.e. *P. fluorescens* and *P. polymyxa* exhibited a cyanogensis activity and

secreted suitable HCN quantities. Accordingly, they may belong to the biological control agents (Antoun *et al.*, 1998).

Assessment of chitinolytic and protolytic activities

Serratia sp. and *P. polymyxa* exhibited a chitinolytic activity, which is reflected by forming clear zones (Fig. 2). These results are in accordance with those obtained by Hashimoto *et al.* (2000) and El-Tahlawy (2006) who detected the activity of chitinase enzyme mediated by *P. lichenifoinis; B. circulans; P. polymyxa* or *Serratia* sp. when grown in the presence of colloidal chitin. Used strains of *P. fluorescens* and *B. lupinus* failed to exhibit chitinolytic activity.

Serratia sp. and *P. polymyxa* exhibited a strong proteolytic activity. On the other hand, *P. fluorescens* was moderate for exerting the protolytic activity, while *B. lupinus* was the lowest in such mode of action (Fig. 3).

Hyphal cell wall of the pathogenic fungi consists mainly of chitin and protein (Sivan and Chet, 1989). Hence, the destructive parasitizing of lyses of the pathogen by extracellular detractive enzymes such as; chitinase and glucanase which are considered an effective mechanism implicated in biological control against soil borne pathogenic fungi (Friedlender *et al.*, 1993).

Exploitation of rhizobacteria as bioagents under *in vitro* and *in vivo* conditions

1- *In vitro* assessment

Antagonistic activity of the studied rhizobacteria was tested *in vitro* by assaying its ability to inhibit the mycelia growth of *F. solani* on PDA media (Fig. 4).

It is clear that all the three tested rhizobacteria were able to restrict the mycelia growth of the investigated fungi. This finding may be considered as a primary indication for using such tested rhizobacteria as bioagents against the challenged phytopathogen under *in vivo* conditions.

Similar results were obtained by Montealegre *et al.* (2003) and Hassanein *et al.* (2006). There are various mechanisms that can mediated by rhizobacteria to suppress the mycelial growth of such soil borne pathogens comprised a variety of antifungal factors such as hydrolytic enzymes (Bangera and Thomashow, 1999). In this concern, Dunne *et al.* (1997) demonstrated that the exposure of selected phytopathogenic fungi to lytic enzymes such as chitinase, protease or glucanase can result in the degradation of the structural matrix of fungal cell walls.

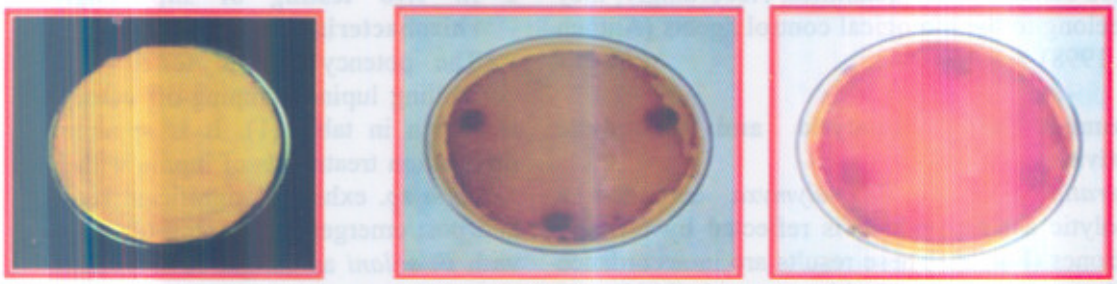
2- *In vivo* testing of suppressive ability of rhizobacteria against lupine damping-off

The potency of the tested rhizobacteria for controlling lupine damping-off caused by *F. solani* is shown in table (1). It is apparent that all co-inoculation treatments of lupine with *B. lupinus* and *Serratia* sp. exhibited significant reductions in pre- and post emergence damping-off of lupine infected with *F. solani* as compared with the sole rhizobial inoculation. In another meaning, survival of lupine was greatly pronounced when it was co-inoculated with rhizobia and rhizobacteria rather than rhizobial inoculation only. However, *P. fluorescens* and the mixture of PGPRs were nearly mimic to *Serratia* sp. for suppressing lupine damping-off, while *P. polymyxa* failed to exhibit a significant efficiency. Using of various rhizobacteria as bioagents against soil borne pathogens was reported by several investigators (Montealegre *et al.*, 2003 and Abdel–Wehab *et al.*, 2006). Rhizobacteria may suppress the soil borne fungal diseases by one or more mechanisms which act to reduce the damping-off of seedlings. These mechanisms may comprise the nutrient competition and antibiosis which may accomplish by producing of antagonistic substances such as; lytic enzymes, volatile compounds and antibiotics (Van loon and Bakkepiner, 2003).

Effect on some plant parameters

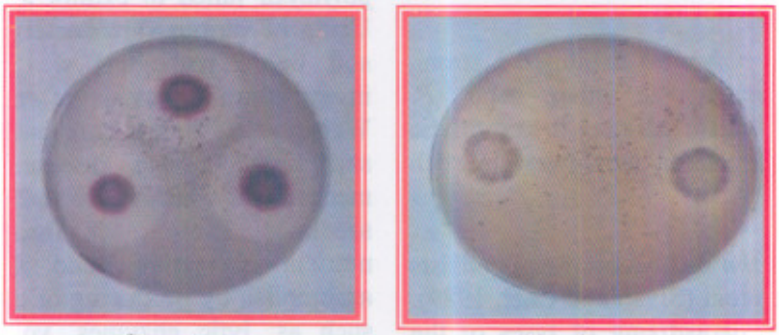
At infestation treatments, data in table (2) clearly reveal that the artificial infection of lupine with *F. solani* tended to a significant depress of the yield and its components as compared to non-infested treatment. From this result, it is possible to conclude that infection of lupine by *F. solani* resulted in reduction of the yield either its quantity or quality traits. This may be due to the plant growth and nutrients status and this depression may be leading to reduce the yield and its components. Many reports concerned with the depression of yield caused as a result of the incidence of soil borne diseases (Luz, 2001 and Abdel-Wahab *et al.*, 2006).

In respect of the interaction between *Fusarium* infection and rhizobacterial co-inoculation, data in table (3) confirmed again the independency of the two factors under investigation for affecting the measured parameters. Generally, co-inoculation of lupine plants with *B. lupinus* and any tested rhizobacteria markedly enhanced the crop productivity and its components in the presence or absence of *F. solani*. The highest values of seed yield/ pot was obtained in case of combined inocula between *B. lupinus* and *Serratia* sp. either in the absence or the presence of *F. solani* (9.67 and 8.17 g/ plant), respectively. These results clearly exerted that co-inoculation of lupine with *Bradyrhizobia* sp. and rhizobacteria resulted in a marked enhancement



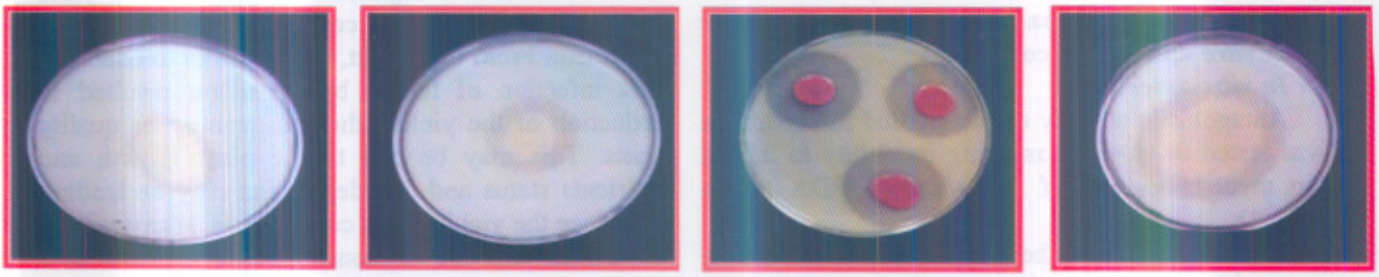
Control (filter paper without colonies) *P. fluorscens* *Serratia* sp.

Figure (1): Cyanogensis assay of some PGPRs *in vitro*.



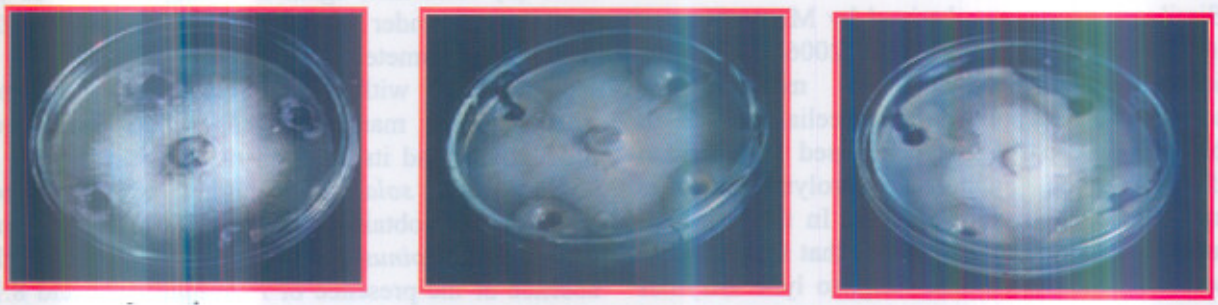
Serratia sp. *P. polymyxa*

Figure (2): Chitinase assay of some PGPRs *in vitro*.



B. lupinus *P. fluorescens* *Serratia* sp. *P. plymyxa*

Figure (3): Protease assay of some PGPRs *in vitro*.



Serratia sp. *P. fluorscens* *P. polymyxa*

Figure (4): Antagonistic effect of some PGPRs on the mycelia growth of *F. solani*.

Table (1): Suppressive potency of rhizobacteria against lupine damping-off grown in artificially infested soil

Treatments	Lupine inf. With *F. solani*		
	Pre-emergence (%)	Post-emergence (%)	Survival (%)
Control (Br)	13.33 a	26.67 a	60.00 b
Bp+Br	13.33 a	20.00 a	66.67 b
S+Br	0.00 b	13.33 b	86.67 a
Ps+Br	0.00 b	20.00 a	80.00 a
Br+Bp+S+Ps	6.67 a	13.33 b	80.00 a
L.S.D at 0.05	9.81	11.61	12.58

Table (2): Effect of pathogen on lupine yield and its components 120 days after sowing

Treatments	Seed yield (g/plants)	Straw yield (g/plant)	Pods No. (Per plant)	Pods dry weight (g/plant)
Without *F.solani*	8.38 a	19.29 a	12 a	11.07 a
With *F,solani*	7.00 b	17.71 b	10 b	8.90 b
$L.S.D._{0.05}$	0.49	0.64	0.76	0.61

Table (3): Interaction effect between rhizobacteria and *Fusarium* infection on lupine yield and its components after 120 days of sowing

	Without *Fusarium*				With *Fusarium*			
Control (Br)	6.37	14.67	10	7.70	5.23	13.30	7	6.87
Bp+Br	8.80	20.67	13	11.67	7.37	18.90	10	9.33
S+Br	9.67	22.03	15	13.23	8.17	20.57	11	10.57
Ps+Br	8.53	19.80	12	11.23	7.13	18.33	9	8.70
Br+Bp+S+Ps	8.53	19.27	12	11.53	7.10	17.47	10	9.03
$L.S.D._{0.05}$	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

of the yield productivity and its components in despite of incidence of the disease. This indicates that the tested rhizobacteria participatory *Serratia* possessed the ability to restrict the infection with *F. solani*. Several mechanisms such as hydrocyanic, siderophores, lytic enzymes and induction of resistance may be implicated in suppressive traits which triggered by the tested rhizobacteria against infection with *F. solani*. Beneficial effects of PGPRs and fungal bio-protecting on the plants have been reported by many investigators (Dileep_Kumar, 2001and Abdel-Wahab *et al.*, 2006).

Luz (2001) found that rhizobacterial agents will probably be one of the most significant strategies for disease management in the third millennium. Therefore, the PGPRs tested represents prospective inoculants conjugated with *Rhizobium* for enhancing the legumes productivity, particularly with sustainable agricultural system.

In conclusion, the current study demonstrated that some Rhizobacteria, especially *Serratia* sp., can be used for biocontrol of lupine damping-off disease. Thus, these bacteria can contribute to minimize the risk and hazard of toxic fungicides. Further research concerning these Rhizobacteria will help to improve
their fungicidal activity in order to be applied to control plant diseases.

REFERENCES

Abdel-Monaim, M.F.; K.A.M. Abo-Elyousr and K. M. Morsy 2010. Effectiveness of plant extracts on suppression of damping-off and wilt diseases of lupine. Elsevier B.V, 30: 185-191.

Abdel-Wahab, A. F.; G. A. A. Mekhemar; H. S. Shehata and A. A. Hanafi 2006. Effect of plant growth bioprotecting and promoting rhizobacteria and compost on the healthy and productivity of peanut crop in sandy soil. Minoufiya J. Agric. Res., 31:1323-1348.

Alvarez, M. B.; S. Gagne and H. Anton 1995. Effect of compost on the biosphere microflora of the tomato and the incidence of plant growth promoting rhizobacteria. Appl. Environ. Microbiol., 61: 194-199

Anonymous (ATTC) 1992. Catalogue of Bacteria and Bacteriophages, 18th ed., pp. 428.

Antoun, H.; J. Chantal; N. Goussard; R. Chabot and R. Ialande 1998. Potential for *Rhizobium* and *Bradyrhizobium* species on plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.) Plant and Soil., 204: 57-67.

Bakker, A. W. and B. Schippers 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* sp. mediated plant growth stimulation. Soil Biol., Biochem., 4: 451-457

Bangera, M. G. and L. S. Thomashow 1999. Identification and characterization of a gene cluster for synthesis of the polyketide antibiotic 2, 4-diacetylphloroglucinol from *Pseudomonas fuorescens* Q2-87. J Bacteriol., 181: 3155-3163.

Bric, J. M.; R. M. Bostock and S. E. Silverstone 1991. Rapid *in situ* assay for indole acetic acid production by immobilized on a nitro cellulose membrane. App. And Environmental Microbiology., 535- 538.

Dileep-Kumar, B. S.; I. Berggren and A. M. Martensson 2001. Potential for improving pea production by co-inoculation with *Pseudomonas fluorescens* and *Rhizobium*. Plant and Soil., 229: 25-34.

Dowson, W. J. 1957. Plant Disease due Bacteria. Second ed., Cambridge, the University Press, London, pp. 231.

Dunne, C.; J. J. Crowley; Y. Moenne-Loccoz; D. N. Dowling; F. J. de-Bruijn and F. O'Gara 1997. Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular protiolytic activity. Microbiology., 143: 3921- 3931.

El-Tahlawy, A. 2006. Microbial impact productivity of some medicinal plants. M. Sc. Thesis, Fac. of agric, Ain Shams University, Egypt, 117pp.

Friedlender, M.; J. Inbar and I. Chet 1993. Biological control of soil borne plant pathogens by a b-1,3-glucanase-producing *Pseudomonas cepacia*. Soil Biol. Biochem., 25:1211–1221.

Grimont, P. A. D. and F. Grimont 1984. Bergey`s Manual of Systematic Bacteriology, Williams & Wilkins, Krieg, N.R. (ed), Vol. 1, pp. 477-483. Baltimore/London.

Hashimoto, M.; T. Ikegami; S. Seino; N. Ohuchi; H. Fukada; J. Sugiyama; M. Shirokova and T. Watanabe 2000. Expression and characterization of the chitin-binding Domain of chitinase A1 from *Bacillus circulans*. WL-12. 3045-3054.

Hassanein, A. M.; A. M. El-Garhy and A. A. Mekhemar 2006. Symbiotic nitrogen fixation process in faba bean and chickpea as affected by biological and chemical control of root-rot. J Agric. Sci. Mansoura Univ. 31: 963-980.

Luz, W. C. 2001. Evaluation of plant growth-promoting and bioprotecting rhizobacteria on wheat crop. Fitopatologia Brasileira., 26:597-600.

Maknickiene, Z. 2001. Effect of genotype on seed yield in lupine (*Lupinus leteus* L. and *Lupinus angustifolius* L.) and resistance to fungal disease (*Colletotrichtum lindemuthianuim* Br. Et Cav., *Fusarium oxysporum*), Biologija 3: 27–29.

Mao, W.; J. A. Lewis; P. K. Hebber and R. D. Lumsaen 1997. Seed treatment with a fungal or a bacterial antagonist for reducing corn damping-off caused by species of *Pythium* and *Fusarium*. Plant Dis., 81(5): 450-454.

Montealegre, J. R.; R. Reyes; L. María; P. Rodrigo; H. P. Silva and X. Besoain 2003. Selection of bioantagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. Journal of Biotechnology, 6 (2):115-127.

Page, W. J. and M. Von-Tigerstrom 1982. "Iron- and molybdenum- repressible outer membrane proteins in competent *Azotobacter vinelandi*. J. Bacteriol., 151: 237-242.

Shaban, W. I. and M. A. El-Bramawy 2011. Impact of dual inoculation with *Rhizobium* and *Trichoderma* on damping off, root rot diseases and plant growth parameters of some legumes field crops under greenhouse conditions. International Research Journal of Agricultural Science and Soil Science, 1(3): 98-108.

Sivan, A and I. Chet 1989. Determination of fungal cell wall by lytic enzymes of *Trichoderma harzianum*. J. of General Microbiol., 135: 675-682.

Snedecor, G. W. and W. G. Cochran 1980. "Statistical Methods" 7th Ed., Iowa State Univ. Press, Amr, USA, pp. 255-269.

Strzelezyk, E.; K. Gorlack and H. Rozycki 1990. Chitionlytic and protiolytic activity of Streptomycetes isolated from root free soil, rhizosphere and mycorhizsphere of pine (*Pinus sylvestrist*). Biol. Fertile Soils, 9:268-272.

Van–loon L. C. and P. M. Bakker 2003. Signaling in rhizobacteria-plant interactions. Ecological Studies, 68: 290-330.

Vincent, J. M. 1970. A manual for the Practical Study of Root Nodule Bacteria. International Biological Program Handbook, vol. 15. Blackwell Scientific Publishers, Oxford. Pp. 1-166.