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Potential for Improving Healthy and Productivity of Soybean By Plant Growth Promoting Rhizobacteria

[1]Heba S. Shehata, [2]Eman R. Hamed and [3]Mona E. Eleiwa

[1]Soil, Water and Environment Research Institute, Agricultural Research Center, Egypt.
[2]Chemistry of Natural and Microbial Products Dept., National Research Centre, Cairo, Egypt.
[3]Botany Dept., Faculty of Science, Cairo Univ., Egypt.

Abstract: A pot and field experiment were conducted to evaluation some rhizobacteria namely *Pseudomonas fluorescens*, and *Bacillus subtilis*. The pot experiment was executed to evaluate probable suppressive effect of rhizobacteria as bioagents against *Macrophomina phaseolina*, *Rhizoctonia solani* and *Sclerotium rolfsii* under artifically infested soil. Results showed that co-inoculation of soybean with rhizobacteria led to a significant decrease in pre- and post-emergence damping-off caused by all pathogens under investigation. In addition to enhance the nodulation status under uninfested or infested soil. Field experiments were carried out in Etay-El Baroaud to evaluate the promotive and suppressive disease effects of rhizobacteria on nodulation, plant growth and yield of soybean. Results showed that the inoculation with rhizobacteria led to a significant increase in the nodulation status, shoot dry matter and N-content after 30, 60, 90 days of planting. Moreover, the co-inoculation of *Bacillus subtillis* with *Bradyrhizobium* sp. Salient superiority in suppressive disease. The obtained results explained that the synergy between rhizobacteria (*Bacillus subtilis, Pseudomonas fluorescens*) and *Bradyrhizobium* sp. considered the efficient manner to save the protection against the phytopathogenes and promote the nodulation and symbiotic nitrogen fixation leading to a high quality yield of soybean.

Key words: *Bradyrhizobium* sp., rhizobacteria, *Bacillus subtilis, Pseudomonas fluorescens, PGPR, Damping-off,* soybean.

INTRODUCTION

Fungicides are used in control plant diseases caused by fungi, but such control is not always effective and intensive use of fungicides increases environmental pollution, health hazards and sometimes induces phytotoxicity (Polavarapu, 2000). To reduce the deleterious effect of fungicides, using the efficient alternative method of control such as rhizobzcteria and organic materials. Thus, saving of coast and decreasing environmental hazards associated with intensive fungicides use have become the valuable affair that might add essential benefits to biological control. Biological control of soil borne plant diseases by application of specific microorganisms to seed or planting materials has been investigated by several researchers (Dileep Kumar, 1999; Amer and El-Desouky, 2000; Luze, 2001 and Hassanein *et al*., 2006).

Plant growth-promoting bacteria (PGPR) may facilitate plant growth either indirectly or directly (Glick, 1995). The ability of plant growth-promoting bacteria to act as biocontrol agents against phytopathogens and thus indirectly stimulate plant growth may result from any one of a variety of mechanisms including antibiotic production, depletion of iron from the rhizosphere, induced systemic resistance, production of fungal cell wall lysing enzymes, and competition for binding sites on the root (Glick, 1995; Glick *et al*., 2007a,b). There are several ways in which plant growth promoting bacteria can directly facilitate plant growth. They may fix atmospheric nitrogen and supply it to plants usually a minor component of the benefit that the bacterium provides to the plant; synthesize siderophores which can sequester iron from the soil and provide it to plant cells which can take up the bacterial siderophore–iron complex; synthesize phytohormones such as auxins, cytokinins and gibberelins, which can act to enhance various stages of plant growth; solubilize minerals such as phosphorus, making them more readily available for plant growth; and synthesize the enzyme 1-aminocyclopropane carboxylate (ACC) deaminase, which can lower plant ethylene levels (Glick, 1995; Glick *et al*., 2007a,b). A bacterium may directly affect plant growth and development using any one or more of these mechanisms. Since many plant growth-promoting bacteria possess several of these traits, a bacterium may utilize different traits at various times during the life cycle of the plant. Typically, plant growth-promoting bacteria have little or no measurable effect on plant growth when the plants are cultivated under optimal and stress free conditions.

The exact mechanisms by which plant growth-promoting bacteria (PGPR) promote plant growth are not fully understood. Soil–plant-microbe interactions are complex and there are many ways in which the outcome can influence the plant health and productivity [(Dastager *et al.*, 2010) and (Saharan and Nehra, 2011)]. The

Corresponding Author: Eman R. Hamed, Chemistry of Natural and Microbial Products Dept., National Research Centre, Cairo, Egypt.
Email: erhamed@yahoo.com

interaction may be harmful, beneficial and neutral to the plants. However, our focus should be to exploit the beneficial interaction of plants and microbes. The objectives of this work were to evaluate the potency of some rhizobacteria to suppress some pathogenic fungi and promote plant growth under greenhouse and field conditions.

MATERIALS AND METHODS

Preperation of Inoculum:

Causal Organisms:

Source of fungal pathogens *Macrophomina phaseolina*, *Rhizoctonia solani* and *Sclerotium rolfsii* the causal agents of soybean damping off were isolated from soybean roots showing the diseases symptoms. The fungi in pure culture were identified after pathogenicity test according to keys given by Gilman 1957. Barnet and Hunter 1972 and Nelson *et al*. 1983 in National Research Centre, Chemistry of Natural and Microbial Products Dept. [(Microbial Culture Collection Unit (MCCU)].

Inocula of the fungi, *Rhizoctonia solani, Macrohpomina phasiolina* and *sclerotium rolfsii* were prepared by growing on potato dextrose agar (PDA) medium (Difco , 1984) for 7 days incubation and two disks (5 mm Diameter) added to autoclaved sorghum gains-sand mixture (3/1, w/w) in 500 ml bottle for each fungus and inocubated at 25 °C for two weeks.

Bacterial Strains and Growth Conditions:

Bacterial strains were obtained from Water and Environment Research Institute, Agricultural Research Center, Egypt. *Bradyrhizobium* sp., *Pseudomonas fluorescens*, and *Bacillus subtilis* were used in pot and field experiments. *Bradyrhizobium* sp. was grown on a yeast extract manitol broth medium (Atlas, 1995) inoculated at 30°C. *Pseudomonas fluorescens*, and *Bacillus subtilis* were grown on a Kings medium B (liquid without agar) (Atlas, 1995). Each bacterial culture was incubated at 28 °C for 3 days on a rotary shaker until early log phase ($5x10^9$ CFU/ml).

Preparation of Bioagent Inoculants:

Vermiculite supplemented with 10% irish peat was packed in polyethylene bags (300 g carrier bag), then sealed and sterilized by gamma irradiation (5.0×10^6 rads). Bacterial culture was injected into sterilized carrier to satisfy 60% of the maximal water holding capacity.

Pot Experiment:

Pot experiment was executed in the greenhouse at Agricultural Research Centre, Giza, Egypt.

This experiment was carried out using pots 25 cm in diameter. Each pot was then sterilized with 5% formalin solution and left to dry before use. Soil was autoclaved at 121°C for 1h. Pots were filled with 10 kg soil for each pot. Soil was infested with 2% of pathogen *Rhizoctonia solani*, *Macrohpomina phasiolina* and *sclerotium*. Pots were watered daily for one week before planting to enhance the fungal growth. Seeds were treated with 16% Arabic gum solution as adhesive agent and thoroughly mixed with the previously prepared vermiculite carrier containing either with *Bradyrhizobium* sp. only, *Bradyrhizobium* sp. + *Pseudomonas fluorescens*, *Bradyrhizobium* sp. +*Bacillus subtilis* or in combination at the rate of 300 or 600g/ 50kg seeds. The planted non-infested soil was served as control. The treatments were arranged in complete randomized design with three replicates. These treatments were as follows:-

1- Control (untreated seeds non-infested soil)
2- Control (untreated seeds infested soil)
3- Seeds inoculated with *Bradyrhizobium* sp. +*Bacillus subtilis* at the rate of 300g/ 50kg seeds.
4- Seeds inoculated with *Bradyrhizobium* sp. +*Bacillus subtilis* at the rate of 600g/ 50kg seeds.
5- Seeds inoculated with *Bradyrhizobium* sp. + *Pseudomonas fluorescens* at the rate of 300g/ 50kg seeds.
6- Seeds inoculated with *Bradyrhizobium* sp. + *Pseudomonas fluorescens* at the rate of 600g/ 50kg seeds.

The percentage of pre- and post-emergence damping off was recorded after 15 and 45 days of soybean sowing. While, the nodulation status, shoot dry weight and n-content were evaluated after 60 days of planting. The pod yield was monitored after harvesting.

Field Experiments:

The field experiment was carried out at Etay-El Baroaud to study the effect of *Bradyrhizobium* sp. Inoculation combined with plant growth promoting and bioprotecting rhizobacteria (PGPR) on nodulation, growth, yield and yield components of soybean. The main physical and chemical properties of soil are shown in Table (1).

All treatments were applied with 20 kg N/fed. Soybean seeds were inoculated with gamma irradiated vermiculite based inoculum at a rate of 300, 600 g/50 kg seeds using 16% Arabic gum solution as a sticking agent.

Table 1: Some physical and chemical properties of the soil used in field experiments.

Soil property	Value
C. sand (%)	5.90
F. sand (%)	31.30
Silt (%)	14.50
Clay (%)	48.30
Texture grade	clay
ECe (dSlm)	9.81
Soluble cations and anions (meq/L):	
Ca^{++}	5.63
Mg^{++}	16.6
Na^{+}	88.0
K^{+}	0.32
$HCO3^{-}$	5.24
Cl^{-}	43.32
$SO4^{=}$	60.53

All treatments received the recommended dose from superphosphate (15.5% P_2O_5) at a rate of 200 kg/fed. and sulfate (48% K_2O) at a rate of 50 kg/fed. ammonium sulfate (20.5% N) was used as nitrogen fertilizer.

Methods for analysis:

- Soil were determined according to Piper (1950).
- Total nitrogen n shoots and seeds was assayed according to Bremmer (1965).
- Total phenols content were determined by the Folin-Ciocalten method described by Meda *et al.* (2005).
- Acetylene reducing activity of root was measured using the method of Hardy *et al*. (1973).
- All data of plant parameters were statistically analyzed according to Snedcor and Cochran (1980).

Laboratory Experiments:

Effect of Antagonistic Microorganisms on Mycelial Growth:

Petri dishes containing potato dextrose agar (PDA) medium were inoculated with a disc (5 mm diam) of one of pathogenic fungi. This pathogenic fungus was inoculated at one side, whereas, the opposite side was inoculated with streak for antagonistic bacteria 6 cm apart. Plates only inoculated with pathogenic fungus were kept as control. All plates were incubated at 25˚C for 3-5 days. When mycelia growth covered the entire medium surface in control treatment, plates were then examined and linear growth was determined.

Determination of Total Phenols:

A total phenols content were determined by the Folin-Ciocalten method described by Meda *et al.* (2005).

RESULTS AND DISCUSSION

1. Effect of Rhizobacteria on Damping off, Nodulation and Growth of Soybean Plants as Affected by Artificial Infection Conditions in Greenhouse:

-Damping-off of Plants:

For damping-off of soybean (Table 2) results clearly revealed that all bacterization treatments exhibited a significant reduction in pre-and post-emergence damping-off caused by the three pathogens under investigation, as compared with the absolute control. Data also displayed that the pathogens under investigation varied in their reaction according to the bioagent used and their concentration. As a general trend, the potency of the bioagents for reducing the damping-off of soybean either in used the first rate (300g/ 50kg seeds) or the second rate (600g/ 50kg seeds). The second rate of inoculum showed more effective than the first rate.

The coinoculation of soybean with *Bradyrhizobium* sp. and *Pseudomonas fluorescens* had a salient superiority for suppressing the soybean damping-off caused by the three tested pathogens. In fact, the decrease occurred in damping-off of soybean resulted in addition of bioagents. PGPR as a bioagent may control the pathogen via antibiosis through the production antagonistic substances such as lytic agents, enzymes, volatile compounds, sidrophores and antibiotics (Stuz and Christie, 2003). In this concern, Dowling *et al*. (1996) indicated that sidrophores producing bacteria with high affinities for iron, had been found to inhibit certain phytopathogens. Pieterse *et al*. (1996) reported that PGPR can induce systemic acquired resistance that characterized by an accumulation of salicylic cid (SA) and pathogenesis -related protein. Biological control against soilborne pathogens by several rhizobacteria was reported by many investigators (Hassanein *et al*., 2006).

-Nodulation Status:

Nodulation formed on soybean roots as affected by rhizobacteria under artificial infection was recorded in Table (3). It is apparent that nodulation status was affected by the infection with the tested pathogens, particularly in case of *R. solani* in comparison with uninfested soil. Inoculation of soybean with *Bradirhizobium* only in combination with PGPRs led to a significant increase in the nodulation status as compared to control either in infested or uninfested soil. The highest values of nodular tissues accumulated due to the second rate of Bradyrhizobium and *Pseudomonas* (420, 96.67, 86.67 mg/ plant) followed by the second rate of *Bacillus* (86.67 mg/plant).

Table 2: Effect of bioprotecting rhizobacteria on damping-off of soybean plants in artificially infested soil.

Treatment	(Damping off) First rate (300g/ 50kg seeds)								
	Infested (R. solani)			Infested (S. rolfsii)			Infested (M. phasiolina)		
	Pre-%	Psot-%	Surviv-%	Pre-%	Psot-%	Surviv-%	Pre-%	Psot-%	Surviv-%
	First rate (300g/ 50kg seeds)								
Control	76.17	0	23.83	71.4	19.07	9.53	57.1	28.6	14.3
Rhizobium (Rh)	38.13	0	61.87	52.37	4.77	42.86	38.13	14.3	47.57
Rh+ Bacillus (B)	28.60	0	71.40	28.60	1.43	69.97	19.07	14.3	66.63
Rh+ Pseudomonas (P)	0	14.30	85.70	7.77	52.33	39.90	9.53	71.3	19.17
Rh+B+P	0	38.13	61.87	0	47.6	9.53	0	57.13	42.87
L.S.D. at 0.05	38.49								
	Second rate (600g/ 50kg seeds)								
Control	90.47	0	9.53	57.13	33.33	9.54	47.6	33.33	19.07
Rhizobium (Rh)	42.87	9.53	47.60	57.13	14.3	28.57	28.56	14.30	57.14
Rh+ Bacillus (B)	18.97	0	81.03	19.07	0	80.93	14.3	4.77	80.93
Rh+ Pseudomonas (P)	4.77	0	95.23	9.53	0	90.47	9.53	4.77	85.70
Rh+B+P	4.77	0	95.23	19.07	0	80.93	14.30	4.77	80.93
L.S.D. at 0.05	27.57								

The highest number of nodules/ plant were obtained in the case of the second rate of *Pseudomonas* (39 nodules/ plant) followed by the second rate of the mixture (30.33 of nodules/ plant). Indeed, the rhizobacteria may enhance nodulation process via providing more noule sites (Srinivasan *et al.* (2006) and decreasing the verulance of pathogens by producing antibiotic substances (Kloepper, 1993). Similar tendency was noticed by Dileep Kumar *et al.* (2001) and Hassanein *et al.* (2006), who reported that coinoculation of soybean, pea, fababean and chickpea under natural and artificial infection conditions.

Tabe 3: Response of nodulation status to rhizobacteria as affected by artificial infection under greenhouse conditions.

Treatment	No. of nodules/ plant				Dry weight of nodules (mg/plant)			
	Infested (R. solani)	Infested (S. rolfsii)	Infested (M. phasiolina)	Uninfested	Infested (R. solani)	Infested (S. rolfsii)	Infested (M. phasiolina)	Uninfested
Control	1	2.67	2	4.67	1	2	2	60
Rhyzobium (Rh) 1	13.67	4.67	4	15.33	56.67	6.67	19.33	96.69
Rhyzobium (Rh) 2	16.67	7.33	8		110	90	20	
Rh+ Bacillus (B) 1	18.67	9.33	8	13	40	56.67	66.67	150
Rh+ Bacillus (B) 2	21	11.67	11.67		186.67	56.67	86.67	
Rh+ Pseudomonas (P) 1	13.67	7.33	8.67	16.67	186.67	36.67	66.67	190
Rh+ Pseudomonas (P) 2	39	8	12.67		420	96.67	86.67	
Rh+B+P 1	16	9.67	12.67	18	28.67	26.67	66.67	176.67
Rh+B+P 2	30.33	12.67	12.67		80	60	70	
L.S.D. at 0.05	6.05				68.89			

1-First rate (300g/ 50kg seeds)
2-Second rate (600g/ 50kg seeds)

-Shoot Dry Matter and N-Content:

Data in Table (4) revaled that the shoot dry matter and N-content exerted a valuable reduction due to infestation with pathogens, particularly *R. solani*. However, the coinoculation with different rhizobacteria tended to significantly increase the plant dry matter and N-content in both infested and uninfested soil.

The coinoculation with the second rate of *Bacillus* exerted salient superiority for increasing the shoot dry matter and N-content in the infested and uninfested soil. In fact, the shoot dry matter and N-content are considered as a good indication to plant growth and performance of N-fixation particularly under suffering conditions such as diseases incidence. Hence, it is possible to deduce that exploitation of sufficient bioagents such as rhizobacteria may restrict the plant damping-off caused by such used pathogens via enhancement of plant vigour and performance of biological nitrogen fixation. Similar findings were observed by Dileep Kumar *et al.* (2001).

Tabe 4: Effect of rhizobacteria on shoot dry matter and N-content of soybean plants influenced by artificial infection under greenhouse conditions.

Treatment	Shoot dry weight (g/ plant)				Shoot N-content (mg/plant)			
	Infested (*R. solani*)	Infested (*S. rolfsii*)	Infested (*M. phasiolina*)	Uninfested	Infested (*R. solani*)	Infested (*S. rolfsii)*	Infested (*M. phasiolina*)	Uninfested
Control	0.95	1.35	1.2	1.3	15.4	23.67	25.37	140.43
Rhyzobium (Rh) 1	5.9	7.35	7.2	6.9	178.37	194	160.17	210.3
Rhyzobium (Rh) 2	6.35	12.4	7.33	7.54	213	261.2	318.2	
Rh+ Bacillus (B) 1	5.97	8.07	5.07	6.35	167.07	225.67	170.07	296.7
Rh+ Bacillus (B) 2	5.75	12.57	10.35	9.67	233.2	476.6	309.33	
Rh+ Pseudomonas (P) 1	4.12	7.45	4.07	4.12	123.7	124.73	174.87	202.1
Rh+ Pseudomonas (P) 2	6.37	9.67	7.05	7.35	190.3	191.87	271.33	
Rh+B+P 1	5.27	7.25	6.4	6.85	152.4	185.5	134.93	289.73
Rh+B+P 2	4.48	6.85	7.75	6.37	215.63	199.7	203.23	
L.S.D. at 0.05	0.44				175.46			

1-First rate (300g/ 50kg seeds)
2-Second rate (600g/ 50kg seeds)

-Dry Weight of Root and Biological Yield:

Data in Table (5) revaled that the coinoculation of soybean with the first rate of *Bradyrhizobium* sp. and *Pseudomonas fluorescens* had a salient superiority for increasing the soybean dry weight (1.8 g/plant), while the second rate of *Bradyrhizobium* sp. and *Bacillus subtilis* was the most effective for the biological yield (25.67 g/pot).

Tabe 5: Effect of rhizobacteria on dry weight of root and biological yield of soybean plants influenced by artificial infection under greenhouse conditions.

Treatment	dry weight of root (g/ plant)				Biological yield (g/ pot)			
	Infested (R. solani)	Infested (S. rolfsii)	Infested (M. phasiolina)	Uninfested	Infested (R. solani)	Infested (S. rolfsii)	Infested (M. phasiolina)	Uninfested
Control	0.23	0.19	0.32	0.32	9.4	6.93	8.17	9.8
Rhyzobium (Rh) 1	0.55	0.31	0.62	0.57	11.83	18.67	15.35	18.1
Rhyzobium (Rh) 2	0.67	0.62	0.57		15.6	20.6	13.37	
Rh+ Bacillus (B) 1	0.46	0.35	0.43	0.57	12.93	14.15	14.85	13.8
Rh+ Bacillus (B) 2	0.71	0.42	0.91		23.27	25.67	21.7	
Rh+ Pseudomonas (P) 1	1.8	0.48	0.46	0.83	12.07	19.1	17.27	15.43
Rh+ Pseudomonas (P) 2	1.55	0.81	0.59		13.93	20.37	19.77	
Rh+B+P 1	0.55	0.38	0.43	0.59	9.07	17.4	16.6	13.77
Rh+B+P 2	1.33	0.68	0.51		18.8	20.9	1.8	
L.S.D. at 0.05	0.19				3.13			

1- First rate (300g/ 50kg seeds)
2- Second rate (600g/ 50kg seeds)

II- Effect of Rhizobacteria on Soybean Plants Under Field Conditions:

For damping-off of soybean under field conditions, (Table 6) results clearly revealed that all bacterization treatments exhibited a significant reduction in pre-and post-emergence. the second rate of *Bradyrhizobium* sp. and *Pseudomonas fluorescens* was the most active dose for suppressing damping-off.

Tabe 6: Effect of bioprotecting rhizobacteria on damping-off of soybean plants under field conditions.

Treatment	Damping off		
	Infested (R. solani)		
	Pre-%	Psot-%	Surviv-%
Control	22.0	12.3	65.7
Pesticide	10.2	5.5	84.3
Bradyrhizobium sp. (Rh) 1	18.5	11.8	69.7
Bradyrhizobium sp. (Rh) 2	18.5	12.6	68.9
Rh+ Bacillus (B) 1	20.0	16.7	63.7
Rh+ Bacillus (B) 2	20.0	6.0	74.0
Rh+ Pseudomonas (P) 1	14.0	12.0	74.3
Rh+ Pseudomonas (P) 2	4.0	2.0	94.9
Rh+B+P 1	23.3	10.8	65.9
Rh+B+P 2	18.9	4.4	76.7
L.S.D. at 0.05	27.57		

1- First rate (300g/ 50kg seeds)
2- Second rate (600g/ 50kg seeds)

-Shoot Dry Matter and N-content:

Data in Table (7) showed that coinoculation in soybean with *Bradirhizobium* and rhizobacteria led to a significant increases of the shoot dry weight and N-content as compared to control or sole rhizobial inoculation after 45 and 75 days of sowing. However, the highest values of shoot dry weight and N-content were attended in case of the second rate of mixture. These results emphasis the exchangeable promotive effect of rhizobacteria on

nitrogen fixation performed by soybean plants. Many investigators observed the enhancement of plant growth and biological nitrogen fixation by legumes due to rhizobacteria (Zhang *et al.*, 1996; Dashti *et al*., 1997 and Li and Alexander, 1999).

Tabe 7: Effect of rhizobacteria on shoot dry weight of shoot and N-content of soybean plants under field conditions.

Treatment	dry weight of shoot (g/ plant)	N-content (mg/plant)
Control	2.90	390.97
Pesticide	2.49	268.77
Rhizobium (Rh)1	4.45	459.63
Rh+ Bacillus (B)1	4.66	667.4
Rh+ Pseudomonas (P)1	5.19	823.97
Rh+B+P 1	6.49	752.07
Rh+ Bacillus (B)2	6.01	784.8
Rh+ Pseudomonas (P)2	6.26	764.67
Rh+B+P 2	6.52	834.57
Rh+ Bacillus (B)3	4.56	503.7
Rh+ Pseudomonas (P) 3	5.37	549.6
Rh+B+P 3	4.88	668.9
L.S.D. at 0.05	1.84	231.76

1- First rate (300g/ 50kg seeds)
2- Second rate (600g/ 50kg seeds)
3- Third rate (900g/ 50kg seeds)

-Root Dry Weight and Yield:

Root dry weight and yield developed after 45 days of soybean sowing as affected by rhizobacteria coinoculation in presented in Table (8). Inoculation of soybean with rhizobacteria exerted a considerable improvement productivity of soybean indicated by significant increases in root dry weight and yield in the inoculated treatment in comparison to the uninoculated ones, the second rate of *Bradyrhizobium* sp. and *Pseudomonas fluorescens* had a salient superiority for increasing the soybean yield. These results according to Hassanein *et al*., 2003 and 2006. In conclusion, the strain concentration showed an important role on the biological efficacy and preservation of biocontrol agents. Some studies showed the higher the strain concentration, But some other reports detected there was no positive trend on the strain concentration to the biological efficacy It is possible that too much foreign biocontrol agents in the micro-ecological environment in soil creates unsuitable condition for indigenous bacteria and host plant, which might make it easier for the infection of pathogens. There are several mechanisms for different biocontrol agents, and the ultimate aim of these biocontrol bacteria is to regulate ecological balance for controlling pathogen and promoting host plant growth, which also needs to cooperate with the indigenous bacteria in soil and plant. Therefore, the strain concentration of biocontrol agents is very important in practical application (Zhan, *et al*., 2011).

Tabe 8: Effect of rhizobacteria (PGPR) on root dry weight of shoot and yield of soybean plants under field conditions.

Treatment	Root dry weight (g/ plant)	yield (ton/feddan)
Control	0.74	16.96
Pesticide	0.92	12.61
Rhizobium (Rh)1	1	19.08
Rh+ Bacillus (B)1	1.07	22.23
Rh+ Pseudomonas (P)1	1.06	24.1
Rh+B+P1	0.98	16.96
Rh+ Bacillus (B)2	0.9	26.16
Rh+ Pseudomonas (P)2	1.32	29.61
Rh+B+P 2	0.87	16.79
Rh+ Bacillus (B)3	1.23	16.79
Rh+ Pseudomonas (P) 3	1.17	18.32
Rh+B+P 3	1.01	12.2
L.S.D. at 0.05	0.203	8.42

1- First rate (300g/ 50kg seeds)
2- Second rate (600g/ 50kg seeds)
3- Third rate (900g/ 50kg seeds)

Effect if Antagonistic Microorganisms on Mycelial Growth:

Results, Table (9) and Figure (1) show that pathogens showed a different level of sensitivity towards the PGPR isolates. *Pseudomonas fluorescens* was high efficiency in antagonism. *Pseudomonas fluorescens* more severely interacted against plant pathogenic than *Bacillus subtilis.*

Table 9: Effect of antagonistic microorganisms on mycelial growth:

Bacterial strains	Pathogenic fungi		
	R. solani	*S. rolfsii*	*M. phasiolina*
Bacillus subtilis	22	12	8
Pseudomonas fluorescens	27	14	10
Bradyrhizobium sp.	12	8	5

Numbers express the diameter (mm) of inhibition zone.

Fig. 1: Photograph showed inhibition growth of *Rhizoctonia solani* by PGPR.
(A): PGPR, *Rhizoctonia solani* (B): *Rhizoctonia solani* (Control).

Total Phenols Content:

Figure (2) show that application with the combined PGPR under study caused increase of phenols content in tissues of soybean plant comparable with the untreated control.

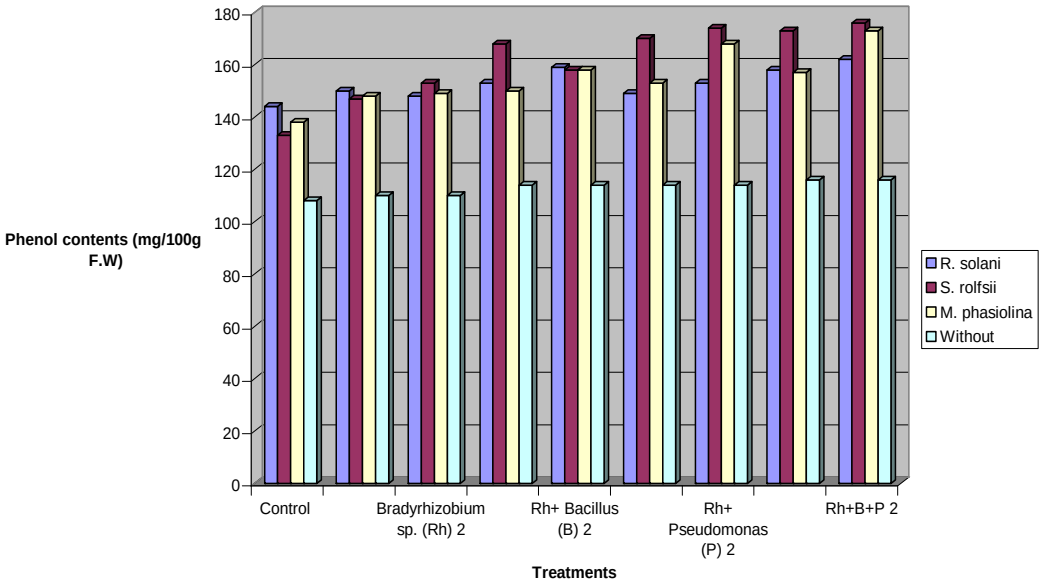

Fig. 2: Effect of PGPR on phenol contents in soybean at 50 days of planting, greenhouse.

Acetylene Reducing Activity of Root:

Figure (3) show Acetylene reducing activity. Infection with pathogenic fungi significantly inhibited ethylene production. Unexpectedly, the situation compeletely changed in *Bradyrhizobium*- bioagent- pathogen and bioagent-inoculated plants simultaneously infected by fungal preparation exhibited significantly higher acetylene reducing activity than fungi-untreated corresponding. Inoculation with *Bradyrhizobium* alone slightly enhanced the ethylene production treated with *Bacillus* and *Pseudomonas* of combined the enzymatic activities significantly increased by up to 6%. This indicates the necessity of Rhizobium inoculatiob to express a potential N_2-fixing ability. *Bradyrhizobium*- seed soaking inoculation with PGPR, whatever it is seems the proper procedure to secure a positive diazotroph -biocontrol agent interaction.

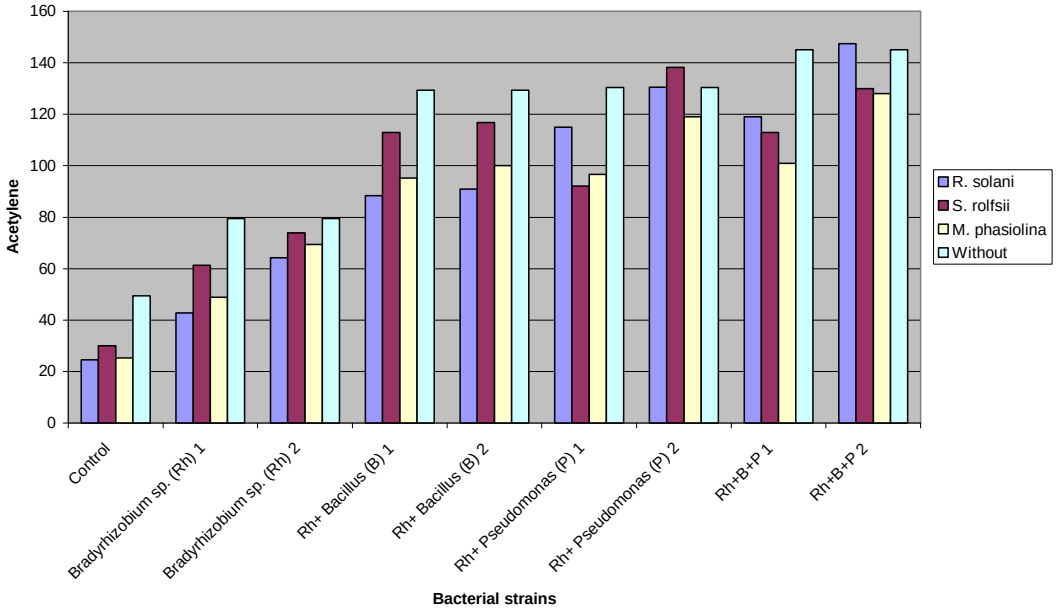

Fig. 3: Acetylene reducing of 50 days old soybean plants as affected by inoculation with PGPR and infection by pathogenic fungi.

-One Hundred-Seeds Weight and Oil Content of Soybean Seeds:

Table (10) show the 100-seeds weight and seed oil percentage of soybean plants. With respect to the effect of interaction between *Bradirhizobium* and different PGPR inoculation, there was significant effect on seed oil percentage of soybean. Cassan *et al*. (2009) found that PGPR and *Bradirhizobium japonicum* singly or in combination, showed the capacity to promote seed germination nodule formation and early development of corn and soybean seedlings. Both strains *Pseudomonas fluorescens*, and *Bacillus subtilis* were able to excrete IAA and gibberillic acid into the culture medium, at aconcentration sufficient to produce morphogical and physiological changes in young seed tissues. Because the ability of most strains of *Pseudomonas fluorescens* to colonize both the surface and root interior, including the stem and leaves of grass, (Baladoni *et al*. (1997). Endophytic bacteria reside intercellular or intracellular with the host tissues and therefore may again advantages for themselves by being protected from environmental stress and microbial competition.

Endophytes offer a wide range of benefits to plants such as promoting growth, reducing disease severity, including plant defense mechanisims (Senthilkuman *et al*., 2007), biologically fixing nitrogen increasing plant mineral uptake (Malinowski *et al*., 2000). PGPR can produce of phytohormones, such as auxins and gibberellins, which can induce plant.

REFERENCES

Amer, G.A. and Sh. M. El-Desouky, 2000. Suppression of bean damping-off caused by *Sclerotium rolfsii* using *Trichoderma and Gliocladium* species. Minufiya J. Agric. Res., 25: 921-932.

Atlas, R.M., 1995. Handbook of Media for Environmental Microbiology. Boca Raton, Florida,USA, CRC Press.

Baladoni, J., L. Caruso, V. Baladoni, S. Goinad and J. Dobereiner, 1997. Recent advances in BNK with non-legume plants. Soil Biochem., 29: 911-922.

Barnett, H.L. and B.B. Hunter, 1972. Illustrated Genera of Imperfect fungi. Burgess Publishing co. Minnesota, 3rd Ed., 241.

Bremmer, J., 1965. Inorganic forms of nitrogen. In: Methods of soil Analysis, Part2 C.F. Black pp. 1179-1237. American Society of Agronomy, Madison, Wisconsn, USA.

Cassan, F., Perrig, V. Sgroy, V. Mascriarell, O. Penna and V. Lina, 2009. *Azospirillum brazilense* Az 39 and *Bradirhizobiu japonicum* B 109 inoculation singly on in combination, promote seed germination and early seedling growth in corn (*Zea maize* L.) and soybean (*Glycin max* L.). Europian Journal of Soil Biology, 45: 28-35.

Dashti, N., F. Zhang, R. Hynes and D. Smith, 1997. Application of plant growth promoting rhizobacteria to soybean (*Glycine max*) increases protein and dry matter yield under short-season conditions. Plant and Soil, 188: 33-41.

Dastager, S.G., C.K. Deepa and P. Ashok, 2010. Characterization of plant growth-promoting rhizobacterium Exiguobacterium NII-0906 for its growth promotion of cowpea (*Vigna unguiculata*). Biologia, 65: 197-203.

Difco Manual, 1984. Dehydrated Culture Media and Reagent for Microbiology. 10thEd., Difco laboratories, Detroit Michigan, USA. 689-690.

Dileep Kumar, B., 1999. Fusarial with suppression and crop improvement through two rhizobacterial strains in chickpea growing in soil infested with *Fusarium oxysporum* f. sp. *ciceris.* Biol. Fert. Soils, 29: 87-91.

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

Dowling, D., R. Sexton, A. Fenton, I. Delany, S. Fedi, B. McHungh, M. Callanan, Y. Mcenne-Loccoz and F. Gara, 1996. Iron regulation in plant-associated *Pseudomonas fluorescence* M114: implications for biological control. In: Nakazawa, K.; Furukawa, K., Haas, D.; Silver, S. (Eds.). Molecular Biology of Pseudomonas. ASM Press. Washington DC, USA, pp: 502-511.

Glick, B., 1995. The enhancement of plant growth by free-living bacteria. Can. J. Microbiol, 41: 109-117.

Glick, B.R., Z. Cheng, J. Czarny and J. Duan, 2007b. Promotion of plant growth by ACC deaminase-containing soil bacteria. Eur. J. Plant Pathol., 119: 329-339.

Glick, B.R., B. Todorovic, J. Czarny, Z. Cheng, J. Duan and B. McConkey, 2007a. Promotion of plant growth by bacterial ACC deaminase. Crit. Rev. Plant Sci., 26: 227-242.

Gilman, J.C., 1957. A Manual of soil fungi. Cambridge univ. press, U.S.A., pp: 450.

Hardy, R., R. Burms and Holsten, 1973. Application of the acetylene- ethylene assay for measurement of nitrogen fixation. Soil Biol. Biochen., 5: 47-81.

Hassanein, A. and A. Mekhemar, 2003. Biological control of soybean damping-off caused by *Sclerotium rolfsii* using *Pseudomonas fluorescence* and *Pseudomonas putida.* Egypt J. Appl. Sci., 18: 73-86.

Hassanein, A., A. El-Garhy and 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.

Kiopper, J., 1993. Plant growth promoting rhizobacteria as biological control agents. In: metting, B. (Ed.). Soil Microbial Technologies. New York, Marcel Dekker, pp: 255-274.

Li, M. and M. Alexander, 1999. Factors affecting coinoculation with antibiotic producing bacteria to enhance rhizobial colonization and nodulation. Plant and Soil., 129: 195-201.

Luze, W., 2001. Evaluation of plant growth promoting and bioprotecting rhizobacteria on wheat crop.Fitopathol. Bras, 26: 597-600.

Malinowski, D., G. Alloush and D. Belesky, 2000. Leaf endophyte *Neotyphodum coenophialum* modifies mineral uptake in tall fescue. Plant and Soil, 277: 115-126.

Meda, A., C. Lamien, M. Romito, J. Millogo and O. Nacoulma, 2005. Determination of total phenolic, flavonoid and praline contents in Burkina fasanhoney, as well as their radical scavenging activity. Food Chemistry, 91: 571-577.

Nelson, P.E., T.A. Toussoun and W.F.O. Marasas, 1983. *Fusarium species*: An Illustrated Manual of Identification. Pennsylvania univ. Press, USA, pp: 193.

Pieterse, C., S. Van Wees, E. Hoffland, J. Van Pelt and L. Van Loon, 1996. Systemic resistance in Arabidpsis induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. Plant Cell., 8: 1225-1237.

Piper, S., 1950. Soil and Plant Analysis. 1st ed. Interscience Publishers Inc., New York, USA.

Polavarapu, S., 2000. Evaluation of phytotoxicity of diazinon and captan formulation on high bush blueberried. Horttechnology, 10: 308-315.

Saharan, B.S. and V. Nehra, 2011. Plant Growth Promoting Rhizobacteria: A Critical Review Life Sciences and Medicine Research, Volume 2011: LSMR-21.

Sendcor, G. and W. Cochran, 1980. Statistical Methods. 7th ed., Iowa State Univ. Press, Ames, USA, pp: 255-269.

Senthilkuman, M., V. Govindasany and K. Annapurna, 2007. Role of antibiosis in suppression of charcoal rot disease by soybean endophyte *Paenibacillus* sp. HKA15. Current Microbiology, 55: 25-29.

Srinivasan, M., D. Peterson and F. Holl, 2006. Influence of indole acetic acid producing *Bacillus* isolates on the nodulation of *Phaseolus vulgaris* by *Rhizobium etli* under gnobiotic conditions. Can. J. Microbiol., 42: 1006-1014.

Stuz, A. and B. Christie, 2003. Benifical microbial allelopathies in the root zone; the management of soil quality and plant disease with rhizobacteria. Soil and Tillage Res., 72: 107-123.

Zhang, F., N. Dashti, H. Hynes and D. Smith, 1996. Plant growth promoting rhizobacteria and soybean (*Glycine max* L.) nodulation and nitrogen fixation at suboptimal root zone temperatures. Ann. Bot., 77: 453-459.

Zhan, Y., Q. Liu, Y. Wang and J. Guo, 2011. Effect of different concentrations of bacterial suspensions on biocontrol efficacy and storage stability of Bacillus cereus AR156, Pantoea ananatis YT11 and Pseudomonas fluorescens ABc9. African J. of Microbiology Research, 5(23): 4019-4022.