Env. Biodiv. Soil Security **Vol. 2,** pp.39 - 49 (2018)

Suppression of *Rhizoctonia solani* Damping-off in Soybean (*Glycine max* L.) by Plant Growth Promoting Rhizobacteria Strains

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A STUDY was evaluated individual and combination strains of Plant Growth Promoting Rhizobacteria (PGPR) for its antagonist activity and suppression of *Rhizoctonia solani* AG2-3 causal agent of damping-off disease in soybean plant. Strains of *Bradyrhizobium japonicum* 110, *Bacillus megaterium* var. *phosphaticum* B6, *Methylobacterium aminovorans* ML3*, M. rhodinum* ML12, and *Trichoderma viride* 1433 species were used in the present study. I*n vitro,* all tested strains showed a notable ability to inhibit mycelial growth of *R. solani* on different growth media. While, *T.viride* and *M. rhodinum* showed the highest rate of antagonism against *R. solani*. *In vivo*, in generally, PGPR treatments notably decreased damping-off and increased healthy plants, as compared to the control (infested soil). As compared to the un-inoculated NPK fertilized control, higher growth parameters for shoot and root dry weight (g plant^{-1}), number of nodules and dry weight of nodules (mg plant^{-1}), chlorophyll content and NPK % of shoot and root, were recorded for the T_{11} (inoculation with *B. japonicum* 110 + *M. aminovorans* ML3 + *B. megaterium* var. *phosphaticum* B6 + *T. viride*) and T_{12} (inoculation with *B. japonicum* 110 + *M. rhodinum* ML12 + *B. megaterium* var. *phosphaticum* B6 + *T. viride*) treatments. Seeds yield of soybean plants attained higher values with all tested treatments. The obtained results of PGPR bacterial effects on damping-off disease and growth parameters of soybean recommend their use as an alternative tool rather than chemical fungicides. Such biocontrol approach should be included in the integrated management programs.

Keywords: Soybean; PGPR; Bacteria; Fungi; Damping-off

Introduction

Soybean (*Glycine max* L.) has recognized worldwide as an important source of protein (40-45%), which cheaper than the animal sources of protein such as meat, fish, milk, egg etc. **(**Kaul and Das, 1986). In the soil ecosystem, pathogenic and non-pathogenic microorganisms are in competition with each other (Sikora and Reimann, 2004). All soil has an antagonistic potential against specific pathogens to prevent or reduce the spread of a pathogen, parasite or deleterious agents (Sikora, 1992). Seedling damping – off and root rot diseases of soybean are considered as limiting factors affecting plant growth and yield (Omar, 1986). Economically, damping-off disease is one of the most important diseases all over the world. The disease complex is caused by different pathogens such as *Pythium, Phytophthora, Rhizoctonia* and *Fusarium.* These pathogens are difficult to control for their persistence in the soil and wide host range. On the other hand, it is better to use some chemicals in controlling these diseases but, it considers expensive and not environmental friendly. So, many researchers have used bacterial biological control as a means of protection against soil-borne diseases (Elad et al. 1986, Ashour and Afify, 2000). However, more attention has been given to Plant Growth Promoting Rhizobacteria (PGPR), as the most important alternative to chemicals to help eco-friendly biological control of soil-borne pathogens (Arora et al. 2001).

The potential of PGPR to protect plant roots from soil-borne pathogens was demonstrated in several research works (Attia et al. 2011, Huang et

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DOI: 10.21608/jenvbs.2018.3231.1024

al. 2015, Keshavarz-Tohid et al. 2017, Sadeghi et al. 2017, Lu et al. 2017). They found that inoculation of soybean with *Bradyrhizobium japonicum, Bacillus cereus* and *Trichoderma viride* strains significantly reduced damping-off and root rot diseases caused by *R. solani* and also enhanced nodulation status in the roots as well as increasing plant growth. There are several hypotheses about the mechanisms by which rhizobacteria improve growth dynamics such as production of the auxine indole acetic acid (Patten and Glick, 2002), and increased availability of nutrients in the rhizosphere by means of solubilization of unavailable forms of nutrients and/or production of metabolites such as siderophores, antibiotics, lytic enzyme, hydrogen cyanide that decrease the growth of phytopathogens and other deleterious microorganisms or through competition for nutrient and space can significantly improve plant health and promote growth by increasing of seedling emergence and yield (Glick, 1995). Therefore, the objectives of the present study are to evaluate some strains of PGPR singly or in combination for growth promotion of soybean plants and their ability to control soybean damping-off under laboratory and greenhouse conditions.

Materials and methods

Microbial strains and growth conditions

Bradyrhizobium japonicum strain 110, *Bacillus megaterium* var. *phosphaticum* (B6), from Bacteriology Laboratory, Sakha Agricultural Research Station, Agriculture Research Center, Egypt as a biofertilizer products were used in this study. *Methylobacterium aminovorans* (ML3) and *Methylobacterium rhodinum* (ML12) were obtained from Alaa El-Dein Omara, Researcher of Bacteriology Laboratory, Sakha Agricultural Research Station, Agriculture Research Center, Egypt. These strains were isolated in previous study as a PGPR from soil which data published in J. Agric. Sci. Mansoura University, 2(4), 2013. Pure cultures were routinely maintained on Yeast Extract Mannitol Agar (YEMA) (Vincent, 1970), Nutrient Agar (NA) (Atlas, 1997) and Ammonium Mineral Salt Agar (AMSA) (Wittenbury et al. 1970), respectively.

Trichoderma viride 1433 (Tv-1433) and *Rhizoctonia solani* AG2-3 provided by Department of Microbiology, Faculty of Agriculture, Mansoura University and Department of Plant Pathology, Agriculture Research Center, respectively, were also used in this study, Pure cultures of these strains were routinely maintained on Potato Dextrose Agar (PDA) medium (Okon et al. 1977**)** and all chemicals used are obtained from Merck-Co., Germany.

Seeds used

Seeds of soybean (*Glycine max* C.V. Giza 111), were kindly supplied by Department of Leguminous Crops, Sakha Agricultural Research Station, Egypt.

Laboratory experiment (in vitro):

The antagonistic activity against *R. solani* was evaluated by a dual culture technique (Sadfi et al. 2001). Briefly, each strain was streaked on to PDA, AMSA, NA, and YEMA media, respectively, in one side of the plate while disc 5 mm in diameter of *R. solani* strain was taken from 7 day-old culture and placed at the other side of the antagonist with triplicates. Plates were incubated at 30°C for one week. Reduction percent of *R. solani* after 5 days was calculated by the following formula of (Whipps, 1987).

Reduction % = R1 – R2/ R1 x 100, where: R1 = growth in control plates (without antagonism), and R2 = growth in the presence of the bioagent

Pot experiment (in vivo):

The pot experiment aimed to investigate the effect of inoculation with *Bradyrhizobium japonicum* St.110, *Bacillus megaterium* var. *phosphaticum* B6, *Trichoderma viride*, *Methylobacterium aminovorans* ML3 and *Methylobacterium rhodinum* ML12 either alone or in combination on soil infested and non-infested with the pathogen *R. solani*. Pot experiment was conducted in loam soil in texture having the following characteristics: pH, 7.91; EC, 0.186 dS m^{-1}; organic matter (%), 1.12; particle size distribution sand, silt and clay (%), 47.10, 35.60 and 17.30, respectively; soluble cations Ca^{+2}, Mg^{+2}, Na^{+} and K^{+} (meq L^{-1}), 0.86, 0.49, 0.50 and 0.12, respectively; soluble anions CO_3^{--}, HCO_3, Cl^{-} and So_4^{--} (meq L^{-1}), 0.0, 1.0, 0.66 and 0.31, respectively; available N (Kg mg^{-1}), 6.44; available P (Kg mg^{-1}), 5.80; available K (Kg mg^{-1}), 351.1. Also, total count of bacteria, 150 x 10^6 CFU; total count of fungi, 75 x 10^4 CFU and total count of actinomycetes, 45 x 10^5 CFU. (Allen, 1959). The experiment was carried out as 2 x 12 x 7 complete randomized block designed, i.e. 2 treatments (infested and non-infested soil) and 12 inoculation treatments with 7 replicates for each treatment. Peatmoss- based inoculum contained *R. solani* (1 X 10^6 spores ml^{-1}) was mixed with

the soil of the infested treatments (6 Kg soil pot^{-1}) at the rate of 5 g Kg^{-1} of soil. This soil was put in 30 cm diameter and 35 cm depth pots. The soil moisture maintained at 60% of water holding capacity. Soybean seeds were surface sterilized, then ten seeds were sown in each pot. At the same time, all plants were inoculated with the biofertilizer inoculants $1X10^9$ CFU at the rate of 10 ml pot^{-1} (Attia et al. 2011). The mineral fertilizers comprised urea (46.5% N), calcium super-phosphate (15% P_2O_5), and potassium sulphate (48% K_2O). The experiment involved the following 12 treatments with and without *R. solani* infestation as shown in Table 1.

Growth parameters

Sixty day old, plants were taken to determine shoot and root lengths (cm $plant^{-1}$), shoot and root dry weight (g $plant^{-1}$)**,** number of nodules and dry weight of nodules (mg $plant^{-1}$)**,** chlorophyll content, nitrogen, phosphorus and potassium (%) of shoot and root.

Seeds yield were recorded at harvesting time as (g $plant^{-1}$).

Growth inhibition

Growth inhibition was measured on a scale from 0 to 3 (Korsten et al. 1995), where 0 = no growth inhibition, 1 = 1 to 25% growth inhibition, 2 = 26 to 50% growth inhibition and 3 = 51 to 75% growth inhibition

Disease assessment

According to Arafa (1985), pathogenicity was tested as follows:

a- Percentage of pre-emergence damping off was determined after 15 days:

Pre-emergence (%) = No. of ungerminated seeds / No. of sown seeds × 100

b- Percentage of post- emergence damping off was determined after 30 days:

Post- emergence (%) = No. of died seedlings / No. of survival plants × 100

c- Percentage of diseased plants was determined after 60 days:

Diseased plants (%) = No. of infested plants with root-rot / No. of survival plants × 100

Statistical analysis

All obtained data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the randomized complete block designed to experiment using the L.S.D. method according to (Steel and Torrie, 1980).

TABLE 1. Treatments used for pot experiment.

Treatment	Description
T_1	Control, (Without inoculation).
T_2	Inoculation with *B. japonicum* (St.110).
T_3	Inoculation with *B. megatherium* var. *phosphaticum* (B6).
T_4	Inoculation with *T. viride*.
T_5	Inoculation with *M. aminovorans* (ML3).
T_6	Inoculation with *M. rhodinum* (ML12).
T_7	Inoculation with *B. japonicum* (St.110) + *B. megatherium* var. *phosphaticum* (B6).
T_8	Inoculation with *B. japonicum* (St.110) + *T. viride*.
T_9	Inoculation with *B. japonicum* (St.110) + *M. aminovorans* (ML3).
T_{10}	Inoculation with *B. japonicum* (St.110) + *M. rhodinum* (ML12).
T_{11}	Inoculation with *B. japonicum* (St.110) + *M. aminovorans* (ML3) + *B. megatherium* var. *phosphaticum* (B6) + *T. viride*.
T_{12}	Inoculation with *B. japonicum* (St.110) + *M. rhodinum* (ML12) + *B. megatherium* var. *phosphaticum* (B6) + *T. viride*.

Results

3.1. Laboratory experiment (in vitro):

After 5 days of incubation at 30°C, all microorganisms under study showed differences in their ability to inhibit mycelial growth of *R. solani* (Table 2 and Figure 1). The maximum mycelial growth inhibition percentage was recorded in *T. viride* followed by *M. rhodinum* (74.50 and 72.61%), while the minimum mycelial growth inhibition percentage was recorded for *B. megaterium* (54.33 %) on PDA medium respectively. But on YEMA medium the results showed that *B. japonicum* attained 61.41 %. Also, *T. viride* and *B. megaterium* gave 72.60 % and 59.90 % on NA medium. On the other hand, *M. rhodinum* and *M. aminovorans* were recorded 69.30 % and 66.80 % on AMS medium.

Pot experiment (in vivo)

Data of Table 3 and Figure 2 showed that all treatments decreased damping off % and increased healthy plants compared with the control treatment (T1). In the infested soil, the inoculation with the different bio-inoculants revealed different decreases in damping-off plants. The lowest pre-emergence damping-off plants (15 days old) were recorded for the treatments T_{10}, T_{11} and T_{12} (10%) compared to un-inoculated control (50%). Under non-infested soil with *R. solani,* the highest percentage of pre-emergence damping off was 30% in T_1 treatment compared with the lowest percentage (0.00%) for T_{10}, T_{11} and T_{12} treatments. Also, the post-emergence percentages damping-off plants (30 days old) showed notable decreases due to

TABLE 2. Percent of mycelial growth inhibition rates of *R. solani* on different media by microorganisms under study at 5 days after inoculation.

Organism	Mycelial growth inhibition (%)							
Media	PDA	GI	AMS	GI	YEMA	GI	NA	GI
B. japonicum	56.16	3	-	-	61.41	3	-	-
B. megaterium	54.33	3	-	-	-	-	59.90	3
T. viride	74.50	3	-	-	-	-	72.60	3
M. aminovorans	71.20	3	66.80	3	-	-	-	-
M. rhodinum	72.61	3	69.30	3	-	-	-	-

No test. PDA: Potato dextrose agar, AMS: Ammonium mineral salts, NA: Nutrient agar, YEMA: Yeast extract mannitol agar, GI: Growth inhibition.

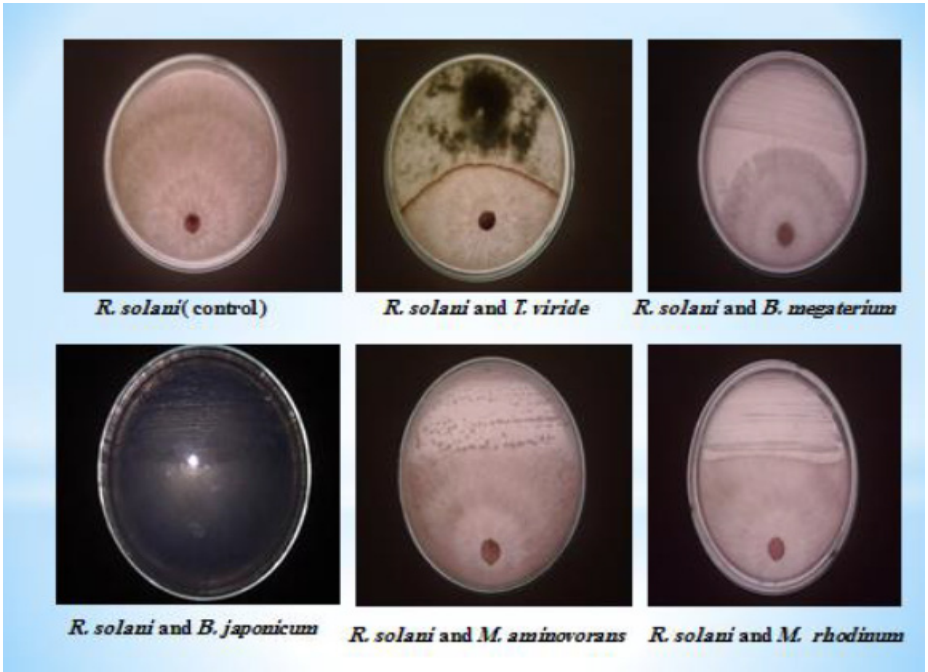

Fig. 1. *In vitro* inhibition of *R. solani* by studied microorganisms.

TABLE 3. Damping-off % and diseased plants % of soybean plants under different bio-inoculants affected by infested and non-infested with *R. solani*.

Treatment	Pre-emergence (%)		Post-emergence (%)		Diseased plants (%)	
	Non.	In.	Non.	In.	Non.	In.
T_1	30.00 ^{c}	50.00 ^{d}	16.66 ^{c}	66.66 ^{c}	33.33 ^{b}	100 ^{c}
T_2	10.00 ^{b}	30.00 ^{c}	12.50 ^{b}	33.33 ^{b}	0.00 ^{a}	33.33 ^{b}
T_3	10.00 ^{b}	30.00 ^{c}	12.50 ^{b}	33.33 ^{b}	0.00 ^{a}	33.33 ^{b}
T_4	10.00 ^{b}	30.00 ^{c}	12.50 ^{b}	33.33 ^{b}	0.00 ^{a}	33.33 ^{b}
T_5	10.00 ^{b}	30.00 ^{c}	12.50 ^{b}	33.33 ^{b}	0.00 ^{a}	33.33 ^{b}
T_6	10.00 ^{b}	30.00 ^{c}	12.50 ^{b}	33.33 ^{b}	0.00 ^{a}	33.33 ^{b}
T_7	10.00 ^{b}	30.00 ^{c}	12.50 ^{b}	33.33 ^{b}	0.00 ^{a}	0.00 ^{a}
T_8	10.00 ^{b}	30.00 ^{c}	12.50 ^{b}	33.33 ^{b}	0.00 ^{a}	0.00 ^{a}
T_9	10.00 ^{b}	20.00 ^{b}	12.50 ^{b}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
T_{10}	0.00 ^{a}	10.00 ^{a}	11.11 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
T_{11}	0.00 ^{a}	10.00 ^{a}	11.11 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
T_{12}	0.00 ^{a}	10.00 ^{a}	11.11 ^{a}	0.00 ^{a}	0.00 ^{a}	0.00 ^{a}
LSD (5%)	**10.4**		**1.325**		**0.070**	

T1: Control, T2: inoculation with *B. japonicum* T3: inoculation with *B. megaterium* T4 : inoculation with *T. viride* T5: inoculation with *M. aminovorans* T6: inoculation with *M. rhodinum* T7: inoculation with *B. japonicum* + *B. megaterium* T8: inoculation with *B. japonicum*+ *T. viride* T9: inoculation with *B. japonicum*+ *M. aminovorans* T10: inoculation with *B. japonicum*+ *M. rhodinum* T11: inoculation with *B. japonicum*+ *M. aminovorans* +*B. megaterium*+ *T. viride* T12: inoculation with *B. japonicum*+ *M. rhodinum* + *B. megaterium*+ *T. viride*. Non: Non-infested, In: Infested.

Fig. 2. Infested (Right) and Non-infested (Left) of soybean plant with *R. solani* (*In vivo*).

the different studied bio- inoculants, the most effective treatments were T_{10}, T_{11} and T_{12} which highly decreased it by percentages of 11.11,11.11 and 11.11% respectively for non-infested soil and completely prevent post-emergence % for the infested soil. Concerning the percentage of diseased plants (60 days old), the most effective treatments (0.00%) were those between T_7 to T_{12} compared to the control (T_1) under infested and non-infested soils (100 and 33.33 %).

Vegetative growth and Yield

The inoculation with *T. viride* and/or other studied inoculants revealed notable increases over un-inoculated control. The differences mostly were significant. T_{12} treatment gave the highest shoot and root dry weights under circumstances of non-infestation or infestation. It recorded 8.50 and 0.68 g plant^{-1} for non-infested and 7.86 and 0.63 g plant^{-1} for infested soil (Table 4).

TABLE 4. Shoot, root dry weight (g plant^{-1}), number of nodules, dry weight of nodules (mg plant^{-1}) chlorophyll content and seed yield (g plant^{-1}) of soybean plants infested and non-infested with *R. solani* under different bio-inoculants.

Treatment	Shoot dry weight		Root dry weight		Number of nodules		Dry weight of nodules		Chlorophyll content		Seed yield	
	Non.	In.	Non.	In.	Non.	In.	Non.	In.	Non.	In.	Non.	In.
T_1	4.25f	5.10 de	0.51 g	0.49 g	17.0 f	13.7 e	146.7 f	140.43 g	36.76 c	34.93 de	8.48 f	7.99 g
T_2	7.76 ab	7.30 ab	0.57 ef	0.54 ef	34.3 b	28.3 ab	181.9 ab	170.46 bc	40.66 ab	37.30 bc	13.25 ab	11.20 b
T_3	6.73 cd	6.36 bc	0.55 f	0.52 ef	22.0 e	19.3 d	162.0 de	151.7 ef	35.70 c	34.70 e	10.36 e	9.32 f
T_4	5.31 e	4.70 e	0.58 de	0.55 de	22.0 e	19.0 d	153.1 ef	145.2 fg	39.06 b	37.10 bc	10.92 d	9.47 ef
T_5	7.23 bcd	6.96 ab	0.63 bc	0.59 bc	26.3 de	24.3 bc	166.5 cd	159.0 de	39.26 b	37.06 bc	11.01 d	9.87 de
T_6	7.93 ab	7.66 a	0.65 ab	0.60 ab	27.7 d	25.0 bc	176.0 bc	166.46 cd	38.90 b	36.80 bc	10.18 e	9.19 f
T_7	6.36 d	5.90 cd	0.57 ef	0.54 e	25.3 de	23.0 cd	171.1 cd	160.0 de	36.76 c	33.50 e	11.12 d	9.46 ef
T_8	6.73 cd	5.80 cd	0.55 f	0.51 f	29.3 cd	27.0 abc	170.6 cd	158.8 de	36.43 c	34.6 e	11.14 d	9.84 de
T_9	7.63 abc	7.13 ab	0.62 c	0.59 bc	33.0 bc	27.7 abc	187.2 a	177.9 ab	39.50 b	37.16 bc	11.58 c	10.20 d
T_{10}	7.66 abc	7.06 ab	0.60 cd	0.57 cd	29.7 bcd	26.0 abc	176.0 bc	166.7 cd	36.76 c	36.63 cd	11.85 c	10.65 c
T_{11}	8.10 ab	7.60 a	0.67 a	0.61 ab	39.0 a	31.0 a	190.8 a	181.2 a	42.10 a	38.70 ab	13.02 b	11.46 ab
T_{12}	8.50 a	7.86 a	0.68 a	0.63 a	40.0 a	30.7 a	190.2 a	181.3 a	41.83 a	40.00 a	13.66 a	11.77 a
LSD (5%)	0.885		0.026		4.5		9.498		1.763		0.427	

T1: Control, T2: inoculation with *B. japonicum,* **T**3: inoculation with *B. megaterium*, T4 : inoculation with *T. viride,* T5: inoculation with *M. aminovorans,* T6: inoculation with *M. rhodinum*, T7: inoculation with *B. japonicum* + *B. megaterium,* T8: inoculation with *B. japonicum*+ *T. viride,* T9: inoculation with *B. japonicum*+ *M. aminovoran*s, T10: inoculation with *B. japonicum*+ *M. rhodinum,* T11: inoculation with *B. japonicum*+ *M. aminovorans* +*B. megaterium*+ *T. viride,* T12: inoculation with *B. japonicum*+ *M. rhodinum* + *B. megaterium*+ *T. viride*. Non.: Non-infested, In.: Infested.

They gave significant increases over control (T_1) reached 39.0 and 40.0 for non-infested soil and 31.0 and 30.7 nodules plant^{-1} for infested one compared to 17.0 and 13.7 nodules plant^{-1} for T_1 treatment. It is noticed that inoculant type containing rhizobia showed higher nodules number over other treatments having no rhizobia. The dry weight of nodules of soybean plants showed similar trend as number of nodules. The treatments T_{11} and T_{12} were the superior in this context under both infested or non-infested circumstances and the differences than un-inoculated control were highly significant. The increase in number and dry weight of nodules in case of inoculated treatments may be attributed to the effective specific rhizobia contained in the applied inoculum. While, the increase in number and dry weight of nodules resulted from the other inoculants which did not contain rhizobia may be due to the positive effect of the other microbial inoculants like *B. megaterium* and methylotrophic bacteria on the native rhizobia present in soil. Among the single microbe inoculants, the inoculant of rhizobia (T_2) attained the highest chlorophyll value (40.66 and 37.30) under non-infested and infested soils. The chemical fertilized control treatment have the lowest value (36.76 and 34.93), but the treatments T_{11} and T_{12} which were composite inoculants were the best in this context. They obtained 42.10 and 41.83 for non-infested soil and 38.70 and 40.0 for infested soil (Table 4). Seeds yield of soybean plants responded positively due to the application of the different inoculants (Table 4). The yields in case of non-infested soil were higher than those of the infested one in respect to all studied inoculants and control. The composite inoculants of T_{11} and T_{12} attained the highest seeds yield in both non-infested and infested soils over control (T_1) followed by the rhizobia inoculum (T_2). T_{11} and T_{12} recorded 13.02 and 11.77 for infested one, the T_1 treatment gave 8.48 and 7.99 respectively.

Mineral uptake

Nitrogen percentages of shoot and root system of soybean plants increased as a result of inoculation with the studied inoculants (Table 5). The increase in percentages of shoot N over control treatment ranged from 2.77% to 2.50%, due to $T_{12,}$ and T_7 treatments, respectively, in non-infested plants and from 2.65% to 2.41%, for $T_{12,}$ and T_3 treatments, respectively, in infested plants. The data of N% of root approximately exhibited similar trend, whereas the increase ranged from 1.61% for T_{12} to 1.42% for T_7 in respect of the non-infested plants, while in the infested plants, the percentages ranged from 1.43% for T_{12} to 1.34% for T_6. It is noted that, nitrogen percentages of shoots showed a consistent increase over those of roots. The differences over T_1 control were significant.

As shown in Table 5, the influence of the studied inoculants on the shoot - p% have a similar trend under non-infestation and infestation. Whereas, T_{12} treatment gave 0.47% and 0.36 % followed by T_{11} (0.43% and 0.35 %) then T_7 (0.42% and 0.35%) respectively, in relation to control (T_1) which exhibited 0.28% and 0.23%. The increases over control were highly significant. On the other hand, the results of root – p% have a similar trend as shoot – p% except for the high values of p – percentages of shoot compared to root – p%. Whereas, the T_{12} treatment attained p% evaluated 0.39% and 0.30 % followed by T_{11} which gave 0.36% and 0.29 % then T_7 (0.35% and 0.29 %). The differences over control which exhibited 0.21% and 0.18 % were highly significant.

Data presented in Table 5 showed that inoculation with the different inoculants variably increases potassium percentages of shoots and roots of soybean plants. In case of non-infestation with *R. solani* in shoots, the increases ranged from 0.18% caused by T_{12} treatment to 0.14% for treatments of T_4 and T_5 compared to 0.13% for control (T_1). Also, the infested plants responded positively due to inoculation with the different inoculants ranged from 0.16% for T_{12} as compared to 0.11% for control (T_1). The differences than control were highly significant. The concentrations of potassium in roots have the same trend of shoots.

Discussion

Since many years, pesticides have been used to control soil borne disease and pest of economically important crops. Also, the use of chemicals has been reduced which due to pollution of environment, especially water and food. The results indicated that several individual strains and several mixtures of PGPR may provide significant disease suppression against specific tested pathogens of soybean.

Herein, the effect of some microorganisms plant growth promoting (*Bradyrhizobium japonicum*, *Bacillus megaterium* var. *phosphaticum*, *Methylobacterium aminovorans, Methylobacterium rhodinum*, and *Trichoderma viride*) were evaluated *in vitro* and *in vivo* against *R. solani* infected soybean plants which can promote plant growth directly or indirectly. Indirect effects are related to production of

TABLE 5. Nitrogen, phosphorus and potassium (%) in shoot and root of soybean plants infested and non-infested with *R. solani* under different bio-inoculants.

Treatment	N (%)				P (%)				K (%)			
	Shoot		Root		Shoot		Root		Shoot		Root	
	Non.	In.	Non.	In.	Non.	In.	Non.	In.	Non.	In.	Non.	In.
T_1	1.72 g	1.52 f	1.29 e	1.24 e	0.28 g	0.23 e	0.21 e	0.18 de	0.13 d	0.11 e	0.10 ab	0.08 cd
T_2	2.72 a	2.49 bc	1.57 b	1.39 bc	0.35 cde	0.25 de	0.28 cd	0.18 de	0.15 c	0.13 bcd	0.11 a	0.10 abc
T_3	2.56 de	2.41 e	1.49 c	1.35 cd	0.37 c	0.33 b	0.30 c	0.26 b	0.16 bc	0.14 abc	0.11 ab	0.10 abc
T_4	2.61 bcd	2.43 e	1.50 c	1.34 d	0.35 cde	0.27 cd	0.30 c	0.21 c	0.14 cd	0.13 de	0.10 ab	0.09 bcd
T_5	2.62 bc	2.48 bcd	1.52 c	1.35 cd	0.32 ef	0.25 de	0.28 cd	0.16 e	0.14 cd	0.13 de	0.10 ab	0.10 ab
T_6	2.57 cde	2.46 cde	1.48 c	1.34 d	0.31 f	0.26 de	0.26 d	0.17 e	0.15 c	0.13 bcd	0.11 a	0.08 d
T_7	2.50 f	2.44 de	1.42 d	1.35 cd	0.42 b	0.35 ab	0.35 b	0.29 a	0.17 ab	0.15 ab	0.12 a	0.11 a
T_8	2.53 ef	2.42 e	1.44 d	1.36 cd	0.33 def	0.28 cd	0.30 c	0.20 cd	0.15 c	0.12 de	0.09 b	0.09 bcd
T_9	2.63 b	2.53 b	1.52 c	1.37 cd	0.34 def	0.26 de	0.29 c	0.18 de	0.15 c	0.13 cde	0.11 a	0.08 d
T_{10}	2.61 bcd	2.52 b	1.51 c	1.38 cd	0.35 cd	0.29 c	0.30 c	0.22 c	0.15 c	0.12 de	0.10 ab	0.10 abc
T_{11}	2.71 a	2.60 a	1.60 ab	1.42 ab	0.43 b	0.35 ab	0.36 b	0.29 a	0.17 ab	0.15 a	0.12 a	0.10 ab
T_{12}	2.77 a	2.65 a	1.61 a	1.43 a	0.47 a	0.36 a	0.39 a	0.30 a	0.18 a	0.16 a	0.12 a	0.10 ab
LSD (5%)	0.051		0.036		0.028		0.024		0.015		0.018	

T1: Control, T2: inoculation with *B. japon*icum, T3: inoculation with *B. megaterium*, T4 : inoculation with *T. viride,* T5: inoculation with *M. aminovorans,* T6: inoculation with *M. rhodinum*, T7: inoculation with *B. japonicum* + *B. megaterium,* T8: inoculation with *B. japonicum*+ *T. viride,* T9: inoculation with *B. japonicum*+ *M. aminovorans,* T10: inoculation with *B. japonicum*+ *M. rhodinum,* T11: inoculation with *B. japonicum*+ *M. aminovorans* +*B. megaterium*+ *T. viride,* T12: inoculation with *B. japonicum*+ *M. rhodinum* + *B. megaterium*+ *T. viride*. Non.: Non-infested, In.: Infested.

metabolites, such as antibiotics, that decrease the growth of phytopathogens and other deleterious microorganisms. Direct effects are depended on production of plant growth regulators (Chin-A-Woeng et al. 2001, Raaijmkers et al. 2002).

The obtained results confirmed that the biocontrol capability of some species within the genus *Trichoderma* against a wide range of important plant pathogens (McLean et al. 2004). Strains of the *Trichoderma* species are known to produce a number of antibiotics, such as trichodermin, trichodermol A and harzianolide (Claydon et al. 1991), which are responsible for most of the inhibition of fungal phytopathogens. Also, the interaction between the antagonists as well as the pathogen and occurrence of inhibition zone on agar media could be commonly considered as a result of the production of the antibiotics and competition for nutrients and space as observed by (Ashour and Afify, 1999, El-Katatny et al. 2001). Also, Schirmbock et al. (1994), reported that antibiotics and hydrolytic enzymes are not only produced together but act synergistically in mycoparasitic antagonism. As well as *Bacillus megaterium* is considered a biocontrol agent which gave the same effect of inhibition zone on agar madia through production of antibiotics. *Bacillus megaterium* is a potential bacterial biocontrol agent against *Rhizoctonia*

solani that are shown by (Zheng and Sinclair, 2000). In addition to, several other workers have noticed the beneficial effects of *Bradyrhizobium* on plant growth and reduction of diseases incidence (Hussain and Ghaffar, 1990). Since, the rhizosphere provides front line defense for roots against attack by pathogens, the *Bradyrhizobium* present in the rhizosphere are ideal for use as biocontrol agents. Reduction of fungal growth *in vitro* by some *Bradyrhizobium* and formation of inhibition zones were presumably due to the metabolites released by the bacteria into the culture medium. Also, (Khan et al. 1997) reported that *Bradyrhizobium* produces toxic metabolites which have inhibitory effect on soil-borne plant pathogens.

Genus *Methylobacterium* is well-known growth regulator producers and also having *in vitro* biocontrol ability of against the phytopathogen *Rhizoctonia solani* that showed by (Poorniammal et al. 2009). Effect of plant growth promoting on root rot and wilt disease complex of soybean plant under greenhouse conditions was also studied. Data showed that soil treated with *NFB* plus *Bacillus cereus* (BC) or *Bacillus megaterium* (BM) or *Pseudomonas fluorescens* (PF) significantly reduced diseased plants comparing with the control (Attia et al. 2011). Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance wherein plant defenses are preconditioned by prior infection or treatment that results in resistance against subsequent challenge by a pathogen or parasite (Choudhary et al. 2007). The widely recognized mechanism of biocontrol mediated by PGPR is competition for an ecological niche/substrate, production of inhibitory allelochemicals, and induction of systemic resistance (ISR) in host plants to a broad spectrum of pathogens (Hass et al. 2002).

Application of *B. japonicum* isolates significantly reduced the wilting index and increased plant growth of chickpeas. These *Bradyrhizobium* also increased nitrogen content, dry weight of nodules and dry weight of roots and shoots under infested soil with damping-off fungi (Hussain and Ghaffar, 1990). In addition, the results showed that inoculation alone or combined with *Bradyrhizobium, B megaterium* and *T. viride* gave reduced the damping-off and infested soybean plants as compared with untreated control and also increased dry weight of nodules and dry weight of roots and shoots (Mazen et al. 2008, Attia et al. 2001).

It is noticed that inoculant type which contains rhizobia showed higher nodules number over other treatments had no rhizobia. The dry weight of nodules of soybean plants showed similar trend as number of nodules. The treatments T_{11} and T_{12} were the superior in this context under both infested or non-infested circumstances and the differences than un-inoculated control were highly significant. The increase in number and dry weight of nodules in case of inoculated treatments may be attributed to the effective specific rhizobia contained in the applied inoculum. While, the increase of number and dry weight of nodules resulted from the other inoculants which did not contain rhizobia may be resulted from the positive effect of the other microbial inoculants like *B. megaterium* and methylotrophic bacteria on the native rhizobia present in soil.

Many researchers were worked on soybean plant and observed that inoculation of soybean with *Bradyrhizobium* has increased the nodulation parameters, nitrogen fixation and yield of crop (Radha 2007, Meenakshi et al. 2009). The results showed complete similarity to observations of (Madhaiyan et al. 2004) where, they observed that the *Methylobacterium* inoculation was found to increase the photosynthetic activity by enhancing the number of stomata, chlorophyll concentration and malic acid content of crops.

The increase in N uptake due to combined inoculation of two or more organisms has been documented by several workers (Deranada 2000). Similarly, (Radha et al. 2009) which worked on soybean plant and observed that inoculation of soybean with *Bradyrhizobium* and PPFMs has been reported to increase the uptake nitrogen % in shoot and root, nodulation and yield of crop as compared to control. Several biocontrol agents alone or in combination with three phosphate-solubilizing strains of *Bacillus megaterium* have been employed to control the root-rot disease complex of chickpea caused by *Meloidogyne incognita* and *Macrophomina phaseolina* and increase in P uptake (Siddiqui and Akhtar 2007). The concentrations of potassium in roots have the same trend of shoots. The increase in K uptake due to combined inoculation of two or more organisms has been documented by (Radha et al. 2009) which worked on soybean plant and observed that inoculation of soybean with *Bradyrhizobium* and PPFMs has been reported to increase the uptake of K % in shoot and root, nodulation and yield of

crop as compared to control.

Acknowledgments

We are extremely grateful to all staff members and colleagues in The Bacterial Research Laboratory, Sakha Agricultural Research Station, Agriculture Research Center, Egypt and Institute of Sciences of Food Production (ISPA-CNR), Bari, Italy.

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(*Received* 14/3/2018;
accepted 14/6/2018)