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Soil application of *Bacillus pumilus* and *Bacillus subtilis* for suppression of *Macrophomina phaseolina* and *Rhizoctonia solani* and yield enhancement in peanut

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Abstract : *Macrophomina phaseolina* and *Rhizoctonia solani* were isolated from the root of peanut plants collected from field with typical symptoms of root rot in Beheira governorate, Egypt. The two isolated fungi were able to attack peanut plants (cv. Giza 4) causing damping-off and root rot diseases in the pathogenicity test. Thirty rhizobacteria isolates (Rb) were isolated from the rhizosphere of healthy peanut plants. The inhibition effect of these isolates to the growth of *M. phaseolina* and *R. solani* was in the range of 11.1- 88.9%. The effective isolates of Rb_{14}, Rb_{18} and Rb_{28}, which showed a strong antagonistic effect (reached to 88.9) in dual culture against the growth of *M. phaseolina* and *R. solani*, were selected and have been identified according the morphological, cultural and biochemical characters as *Bacillus pumilus* (Rb_{14}), *Bacillus subtilis* (Rb_{18}) and *Bacillus subtilis* (Rb_{28}). Control of peanut damping-off and root rot by soil application with these rhizobacteria isolates in addition to two isolates of *B. pumilus* (Bp) and *B. subtilis* (Bs) obtained from Plant Pathology Dept., National Research Centre, was attempted in pots and in field trials. In pots experiment, soil application with Rb_{14}, Rb_{18}, Rb_{28}, Bp and Bs, decreased the incidence of damping-off and root rot, increased the number of survived peanut plants in *M. phaseolina* and/or *R. solani* -infested soil in comparison with the control. These treatments also increased the average length of roots and shoots; average number of branches/plant; average number of leaves/plant; average plant fresh and dry weight of the survived peanut plants compared with control. In field experiments, results reveal that soil application with Rb_{14}, Rb_{18}, Rb_{28}, Bp and Bs, significantly reduced the incidence of damping- off and root rot of peanut. At harvest, these treatments improved peanut growth (average dry weight of peanut plant) and yield components, *viz*. average number of pods per plant, average weight of pod per plant and average weight of 100 seeds. The levels of protection provided by the tested rhizobacteria isolates (Rb) represent practical potential for field control of damping-off and root rot and yield enhancement in peanut.
Key word: Peanut, damping-off and root rot, rhizobacteria isolates, growth promotion, yield enhancement.

Introduction

Peanut (*Arachis hypogaea* L.) is one of the most important leguminous and oil crops in Egypt as well as in many parts of the world. It comes after cotton, rice and onion in our export crops[1]. Damping-off and root rot

diseases caused by *Macrophomina phaseolina* and *Rhizoctonia solani*, are among the most destructive diseases, which attack peanut plants causing quantitative and qualitative losses of yield[2-6]**.** Biological control of plant diseases has attracted much attention in the past few decades as an alternative strategy for the chemical control, due to serious environmental and human health problems resulting from the application of chemical pesticides[7]. Beneficial free-living soil bacteria isolated from the rhizosphere, which have been shown to improve plant health or increase yield, are usually referred to as plant growth-promoting rhizobacteria[8] or by one group of workers in China as yield increasing bacteria (YIB) [9] and include a number of different bacteria such as *Bacillus* spp.[10]**.** The mechanisms of beneficial rhizobacteria are it causes enhancement to the plant growth, yields of many crops by possible explanations and by their antagonism against phytopathogenic microorganisms[10]**.** [11]found that *Bacillus* strains of GB-017 and GB-0356 inhibited the growth of *Botrytis cineria*, *Fusarium* sp., *Pythium* sp. and *R. solani***.** [12] reported that, from seventeen bacterial isolates obtained from known sources and peanut plants, *Pseudomonas fluorescens* (Pf_5), followed by *Bacillus subtilis* (Bs_1) and *Bacillus* sp. (Sp_2) caused inhibition effect against *R. solani*, *Sclerotinia rolfsii*, *Fusarium solani* and *M. phaseolina*, the causal pathogens of peanut root rot, *in vitro* and in greenhouse experiment. [13] reported that *B. subtilis* IX 007 inhibited the growth of *M. phaseolina* by 75%. Kumar *et al*. (2012) reported that strain *Bacillus* sp. BPR7 strongly inhibited the growth of *M. phaseolina*, *Fusarium oxysporum*, *F. solani*, *Sclerotinia sclerotiorum*, *R. solani* and *Colletotricum* sp. *in vitro* and greenhouse condition. [14] showed that *Bacillus* sp. BIN inhibited the mycelial growth of *M. phaseolina* in dual culture test by 63.3 and increased the growth of peanut in both pasteurized and non-pasteurized soil. [15]reported that *Bacillus* sp.(JDB 14) isolated from soybean rhizophere, showing antagonistic activity against *R. solani, F. oxysporum, S. rolfsii, Colletotrichum truncatum, M. phseolina* and *Alternaria alternate*. [16]reported that *B. subtili*s showed maximum inhibition by 52.2% against the growth of *M. phaseolina*. The aim of the present work is to study the efficacy of rhizobacteria isolates isolated from rhizospheric soil for controlling peanut damping-off and root rot under greenhouse and field conditions.

Materials and methods

Peanut cultivar

Peanut (*Arachis hypogaea* L.) seeds (Giza 4 cv.) cultivated in this study were obtained from Department of Legume Crop Research, Field Crop Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.

Isolation, purification and identification of peanut root rot pathogens

Roots of the diseased peanut plants were collected from peanut field growing in El-Tahrir district, Beheira governorate, Egypt during 2014 growing season. Root samples were firstly washed with tap water to remove adhering soil particles. Small parts of infected roots were surface disinfected using sodium hypochlorite solution (3%) for 3 minutes, and then washed with distilled sterilized water for several times. Disinfected root pieces were dried using folds of sterilized filter paper and transferred into Petri-plates containing potato dextrose agar medium (PDA) supplemented with streptomycin sulfate (0.035 g L^{-1}) and incubated at 25±2°C for 5 days. The emerge fungi were purified using hyphal tip technique and identified according to[17,18].

Pathogenicity test

The two isolated fungi were tested for determine their pathogenic ability toward peanut plants (cv. Giza 4). The experiment was conducted at the greenhouse of Pest Rearing Department, Central Agricultural Pesticides Laboratory, Agricultural Research Centre, Giza, Egypt.

Preparation of the pathogens inocula

The inocula of both *M. phaseolina* and *R. solani* was separately prepared using corn: sand: water (2:2:1 v/v) medium [19]**.** The ingredients were mixed, bottled and autoclaved for 30 min at 121°C. The sterilized medium was inoculated with the test pathogen individually using fungal disc (1cm-diameter) obtained from the periphery of 7 days-old-culture. The inoculated media were incubated at 28 ± 2°C for 15 days and then the resulting inocula was used for artificially soil infestation.

Pathogenicity procedures

Plastic pots, 30 cm diameter were sterilized by immersing it in formalin formulation for 30 minutes and then air dried for 3 days. Sterilized sandy loam soil were infested individually with each of *M. phaseolina* and *R. solani* inocula at the rate of 3% soil weight (w: w) and mixed thoroughly to ensure equal distribution of fungal inoculum, then filled in the plastic pots (5 kg for each pot). Sterilized sandy loam soil infested with sterilized non-inoculated corn medium involved in the control pots. The pots were watered and left for one week to distribute the inoculated pathogen. Six pots were used as replicates for each treatment as well as the control. Seeds of peanut (cv. Giza 4) were surface sterilized in 1% sodium hypochlorite solution for 3 min., followed by three successive rinses in sterilized distilled water. The excess water was removed by air drying. Five seeds were sown per pot. The percentages of pre- and post- emergence damping-off were calculated after 15 and 45 days from sowing, respectively while percentages of plants having root- rot symptoms and survived healthy plants were estimated after uprooting (60 days from sowing). The vegetative growth parameters *i.e.*, average root length (cm), average shoot length, average number of branches /plant, average number of leaves /plant, average plant fresh weight (g) and average plant dry weight (g), of the survived healthy peanut plants were also estimated.

Isolation of rhizobacteria isolates

The common of rhizobacteria isolates were isolated from rhizospheric soil of healthy peanut plants in order to be used as natural biocontrol agents. The rhizosphere soil samples of peanut plant had been collected during the 2014 growing season from peanut fields growing in El-Tahrir district, Beheira governorate, Egypt. The samples rhizospheric soil were placed in polyethylene bags, closed tightly, and stored in a refrigerator at 4°C until needed. Isolation of rhizobacteria was performed using a soil dilution plating technique**.** Bacterial isolates were primary selected according to cultural characters as the method described by [20, 21].

***In vitro* screening of rhizobacteria isolates for their antagonistic activity**

In vitro inhibition of *M. phaseolina* and *R. solani* growth by the thirty bacterial isolates obtained from peanut rhizosphere were tested using the dual culture technique [22]**.** Petri plate containing PDA medium were inoculated (by streaking) on one side with one loopful obtained from a 48-hours-old culture of the test bacterium. The opposite side was inoculated with a disc of the test pathogen and the plates were incubated at 28 ± 2°C. Plates inoculated with a disc of the test pathogen only served as control. Four replicate plates were made for each test bacterium as well as the control. Colony radius of the test pathogen was recorded when the control plates reached full growth. The percent growth inhibition (PGI %) was calculated using the following formula suggested by [23]: PGI% = C – T / C × 100. Whereas; PGI percentage = Mycelial growth reduction (%) of the pathogen, C = Radial growth of the pathogen in control plates (cm) and T = Radial growth of the pathogen in dual culture plate (cm). The percent growth inhibition (PGI%) was categorized on a growth inhibition category (GIC) scale from 0 to 4 according to [24] as follows: (0) no growth inhibition; (1) growth inhibition of 1-25%; (2) growth inhibition of 26-50%; (3) growth inhibition of 51-75% and (4) growth inhibition 76-100%.

Identification of the effective rhizobacteria

The effective rhizobacterial isolates *viz*. Rb_{14}, Rb_{18} and Rb_{28}, against the growth of *M. phaseolina* and *R. solani in vitro* test, were identified to the level of species according to the morphological, cultural and biochemical characters according the method described by [25] as *Bacillus pumilus* (Rb_{14}), *Bacillus subtilis* (Rb_{18}) and *Bacillus subtilis* (Rb_{28})**,** respectively.

Effect of rhizobacteria isolates on peanut damping-off and root rot, in experiments conducted in pots and in the field:

Rhizobacterial inoculum preparation

The effective rhizobacterial isolates, *viz. Bacillus pumilus* (Rb_{14}), *B. subtilis* (Rb_{18}) and *B. subtilis* (Rb_{28}) in addition to the isolates obtained from Plant Pathol. Dept. National Research Centre (NRC), *viz. B. pumilus* (Bp) and *B. subtilis* (Bs), that were isolated from cucumber rhizosphere in pervious study were used in experiments conducted in pots and in the field. *Bacillus pumilus* (Bp) and *B. subtilis* (Bs) isolates were found to be effective in controlling cucumber root rot diseases caused by *Fusarium solani*, *Pythium ultimum*,

Rhizoctonia solani and *Sclerotium rolfsii*. Each rhizobacterial isolate was separately grown in conical flasks (250 ml) containing 100 ml of nutrient glucose (2%) broth [NGB] medium, [Beef extract, 3 g ; Peptone, 5g; Glucose, 20 g in 1000 ml distilled water and pH 7.2 ± 0.2], and then separately incubated on a shaker incubator (125 rpm) at 28 ± 2°C for 48 .The bacterial suspension of each tested rhizobacteria was adjusted to 10^{7-9} colony forming unit (CFU) /ml [26].

Pot experiment

A pot experiment was conducted in 2015 to evaluate the performance of rhizobacteria isolates, *viz. B. pumilus* (Rb_{14}), *B. pumilus* (Bp), *B. subtilis* (Rb_{18}), *B. subtilis* (Rb_{28}) and *B. subtilis* (Bs) as a bio-control agent against peanut damping-off and root rot. The experiment was conducted using a randomized block design with six replicates for each treatment. Plastic pots (30 cm diameter) filled with 5 kg sandy loam soil infested with each of *M. phaseolina* and *R. solani* as mentioned before in pathogenicity test. After one week of soil infestation, each pot was inoculated with 300 ml of bacteria suspension [27]. Pots infested with the pathogen only served as control I, while others treated with nutrient glucose broth medium only served as control II. Then, the inoculated pots were watered and then left to one week. Five of peanut seeds (cv. Giza 4) were sown in each pot. Percentages of pre and post- emergence damping-off as well as root rot incidence were recorded after 15, 45 and 60 days of sowing, respectively. The survival healthy plants and vegetative growth parameters were estimated as mentioned before.

Field experiment

As in the pots experiment, the same peanut cultivar and five rhizobacteria isolates were studied in field experiment. The experiment were conducted during 2015 growing season in a clay loam soil, naturally infected with *M. phaseolina* and *R. solani* under a spraying irrigation system by overhead sprinklers at Nubariya region, Beheira governorate, Egypt. The experiments were conducted using a randomized block design. The field experiment was consisted of 21 plots each (3 x $2m^2$) in area, each plot composed of 3 rows with 10 holes per row. Each row was 3 m in length, 20 cm in height and 40 cm in width. Rhizobacteria isolates were applied as soil treatment before sowing at the rate of 100 ml of bacterial suspension per hole. Holes treated with nutrient glucose broth medium only and others treated with distilled water only were used as control I and II, respectively. After application, surface disinfected peanut seeds (cv. Giza 4) were sown at the first week of May of growing season in all treatments at the rate of two seeds ⁄ hole. Three plots were used as replicates for each treatment as well as the controls. Irrigation, recommended fertilizer levels and agronomical practices were used as usual in the reclaimed sandy soils without chemicals. The percentages of pre- and post- emergence damping-off were calculated after 15 and 45 days from sowing, respectively while percentages of plants having root- rot symptoms and survived healthy plants were estimated up to harvest. Harvest times were determined 120 days after sowing. The plants were dug by hand inverted and dried in the field for a week then pods were harvested by hand. At harvest, fifteen air-dried plants from the inner rows from each replication were selected to determine the average dry weight of plant growth above the ground aerial (gram/$plant^{-1}$), average number of $pods/plant^{-1}$, average weight of $pods/plant^{-1}$ and average weight (grams) of 100-Kernel.

Statistical analysis

All experiments were designed Complete Randomized Design and data analyzed by using least squares analysis of variance (ANOVA), Least Significant Difference (L. S. D.) test was used at the 1% level of significance [28].

Results

Isolation, purification and identification of peanut root rot pathogens

The pathogenic effect of both *M. phaseolina* and *R. solani* toward peanut plants, under artificial soil infestation are given in Table (1). Results obvious that the percentages of pre- and post-emergence damping off were 30.0 and 28.6% in case of *M. phaseolina*, while the percentages were 40.0 and 43.9% in case of *R. solani*, compared to 3.3 and 5.0%, in case of control, respectively. The percentage of root rot disease incidence was 52.8% with *M. phaseolina*, while the percentage was 41.7% with *R. solani*, compared to 3.3% in the control plants. Results showed that the percentages of survival peanut plants were 18.6 and 14.4% with *M. phaseolina*

and *R. solani*, compared to 91.7% in the control, respectively (Table, 1). *M. phaseolina* and *R. solani* reduced the tested vegetative growth parameters VGP, *i.e.* length of root and shoot, number of branches and leaves per plants as well as fresh and dry weight of infected plants. Significant differences were recorded among treatments, except between *M. phaseolina* and the control for leaves number and between *M. phaseolina* and *R. solani* for dry plant weight. *R. solani* significantly reduced the tested VGP, than *M. phaseolina* as well as the control (Table, 2).

Table 1. Pathogenicity test of *Macrophomina phaseolina* and *Rhizoctonia solani* on peanut plants.

Fungal pathogen	Damping-off (%)		Root rot incidence (%)	Survival plants (%)
	Pre-emergence (%)	Post-emergence (%)		
Macrophomina phaseolina	30.0a*	28.6a	52.8a	18.6b
Rhizoctonia solani	40.0a	43.9a	41.7a	14.4b
Control	3.3b	5.0b	3.3b	91.7a

*Means in each column followed by the same letter are not significantly different according to LSD test (P = 0.05)

Table 2. Effect of *Macrophomina phaseolina* and *Rhizoctonia solani* on growth parameters of peanut plants grown under artificial soil infestation.

Fungal pathogen	Average vegetative growth parameters					
	Root length (cm)	Shoot length (cm)	Branches No. / plant	Leaves No./plant	Plant weight (g)	
					Fresh	Dry
Macrophomina phaseolina	13.0b*	17.0b	2.8b	18.7a	4.0b	1.2b
Rhizoctonia solani	11.0c	15.0c	2.5c	15.0b	3.5c	1.3b
Control	17.0a	23.2a	3.5a	19.5a	5.9a	1.8a

*Means in each column followed by the same letter are not significantly different according to LSD test (P = 0.05)

In vitro* screening of rhizobacteria isolates for antagonism against *M. phaseolina* and *R. solani

The antagonistic effect of the thirty rhizobacteria isolates (Rb) against the growth of *R. solani* and *M. phaseolina in vitro* test are listed in Table (3). Results cleared that most of the tested Rb isolates inhibited the growth of the two pathogens by the ranges of 11.1 to 88.9%. According to growth inhibition category scale, about 26.7 and 23.3% of tested bacterial isolates had no inhibition effect against *M. phaseolina* and *R. solani*, respectively. Antagonistic results showed that isolates of Rb_3, Rb_5, Rb_8, Rb_9, Rb_{10}, Rb_{12}, Rb_{13}, Rb_{15}, Rb_{20} and Rb_{22} (about 33.3% of tested isolates) inhibited the growth of *M. phaseolina* by 1-25%, while in case of *R. solani* the same inhibition effect was obtained by isolates of Rb_3, Rb_8, Rb_{15} and Rb_{22} (about 13.3% of tested isolates). The isolates that inhibited fungal growth by 26-50% were Rb_5, Rb_9, Rb_{23}, Rb_{26} and Rb_{29} (about 16.7% of tested isolates) in case of *R. solani* by 26 -50%, while isolate Rb_6 only (about 3.3% of tested isolates) in case of *M. phaseolina*. Isolates of Rb_{16}, Rb_{17}, Rb_{19} and Rb_{24} (about 13.3% of tested isolates) inhibited the growth by 51-75% in case of *M. phaseolina*, while isolates of Rb_6, Rb_{10}, Rb_{12}, Rb_{17}, Rb_{27} and Rb_{30} in case of *R. solani*. Results also cleared that isolates of Rb_{14}, Rb_{18}, Rb_{26}, Rb_{27}, Rb_{28}, Rb_{29} and Rb_{30} (about 23.4% of tested isolates) in case of *M. phaseolina* and isolates of Rb_{13}, Rb_{14}, Rb_{16}, Rb_{18}, Rb_{20}, Rb_{24} and Rb_{28} (about 26.7% of tested isolates) in case of *R. solani* inhibited the growth by $\geq$ 75% (Table, 3).

Table 3. Antagonistic activity of rhizobacteria isolates against *Macrophomina phaseolina* and *Rhizoctonia solani in vitro*.

Rhizobacterial isolate	Average linear growth (cm) and growth reduction (%)					
	Macrophomina phaseolina			*Rhizoctonia solani*		
	Growth (cm)	Reduction (%)	GICS[1]	Growth (cm)	Reduction (%)	GICS
Rb1	9.0a*	0.0	**0.0**	9.0a	0.0	**0.0**
Rb2	9.0a	0.0	**0.0**	9.0a	0.0	**0.0**
Rb3	7.5d	16.7	**1.0**	7.5c	16.7	**1.0**
Rb4	9.0a	0.0	**0.0**	9.0a	0.0	**0.0**
Rb5	8.0b	11.1	**1.0**	6.5e	27.8	**2.0**
Rb6	6.0i	33.3	**2.0**	2.5l	72.2	**3.0**
Rb7	9.0a	0.0	**0.0**	9.0a	0.0	**0.0**
Rb8	7.8bc	13.3	**1.0**	7.8bc	12.2	**1.0**
Rb9	7.1e	21.1	**1.0**	6.6e	26.7	**2.0**
Rb10	7.6cd	15.6	**1.0**	4.0h	55.6	**3.0**
Rb11	9.0a	0.0	**0.0**	9.0a	0.0	**0.0**
Rb12	6.8fg	24.4	**1.0**	3.5i	61.1	**3.0**
Rb13	7.6cd	15.6	**1.0**	2.0n	77.8	**4.0**
Rb14	1.0n	88.9	**4.0**	1.0t	88.9	**4.0**
Rb15	7.5d	16.7	**1.0**	7.0d	22.2	**1.0**
Rb16	2.9l	67.8	**3.0**	1.5s	83.3	**4.0**
Rb17	6.3h	30.0	**3.0**	3.5i	61.1	**3.0**
Rb18	1.0n	88.9	**4.0**	1.0t	88.9	**4.0**
Rb19	3.5k	61.0	**3.o**	1.0t	88.9	**4.0**
Rb20	6.7g	25.6	**1.0**	1.0t	88.9	**4.0**
Rb21	9.0a	0.0	**0.0**	9.0a	0.0	**0.0**
Rb22	8.0b	11.1	**1.0**	8.0b	11.1	**1.0**
Rb23	9.0a	0.0	**0.0**	4.8f	46.7	**2.0**
Rb24	3.5k	61.1	**3.0**	1.0t	88.9	**4.0**
Rb25	9.0a	0.0	**0.0**	9.0a	0.0	**0.0**
Rb26	1.2mn	86.7	**4.0**	4.5g	50.0	**2.0**
Rb27	1.0n	88.9	**4.0**	3.5i	61.1	**3.0**
Rb28	1.0n	88.9	**4.o**	1.0t	88.9	**4.0**
Rb29	1.4m	84.4	**4.0**	4.5g	50.0	**2.0**
Rb30	1.2mn	86.7	**4.0**	2.9k	67.5	**3.0**
Control	9.0a	-	**-**	9.0a	-	**-**

[1]GICS = Growth inhibition category scale

*Means in each column followed by the same letter are not significantly different according to LSD test (P = 0.05)

Efficiency of rhizobacteria isolates in pot and in field experiments

Pot experiment

Effects on peanut damping-off and root rot

The efficiency of the effective Rb isolates *viz. B. pumilus* (Rb_{14}), *B. pumilus* (Bp), *B. subtilis* (Rb_{18}), *B. subtilis* (Rb_{28}) and *B. subtilis* (Bs) for protecting peanut plants from *M. phaseolina* and *R. solani* infection in pots are showing in Table (4). Results showed that the isolates of Rb_{14}, Bp, Rb_{18}, Rb_{28} and Bs significantly reduced the incidence of damping off and root rot disease caused by two pathogens, compared to the control. The percentages of per-emergences damping off were in the range of 10.0 to 16.7% (caused by two pathogens),

compared to 26.7 and 36.7% in peanut plants that artificially infested with both *M. phaseolina* and *R. solani* only, respectively. Isolate of Bs highly reduced the pre-emergence of damping off % caused by *M. phaseolina*, followed by Rb_{18}, Rb_{14}, Rb_{28} and Bp, respectively. Isolate of Rb_{14} highly reduced the disease incidence caused by *R. solani*, followed by Rb_{28}, Bs, Bp and Rb_{18}, respectively (Table, 4).

Table 4. Efficacy of *Bacillus pumilus* and *Bacillus subtilis* on damping-off and root rot diseases in pot experiment.

Rhizobacteria isolate	Disease parameters			
	Damping-off (%)		Root rot incidence (%)	Survival plants (%)
	Pre-emergence (%)	Post-emergence (%)		
M. phaseolina only (Control I)	26.7abc*	27.8ab	50.0a	22.2g
M. phaseolina + *B. pumilus* Rb_{14}	16.7cd	8.9bc	8.9b	82.2cd
M. phaseolina + *B. pumilus* Rb	16.7cd	3.3c	3.3b	93.4a
M. phaseolina + *B.subtilis* Rb_{18}	13.3cd	11.7bc	10.8b	77.5f
M. phaseolina + *B. subtilis* Rb_{28}	16.7cd	8.9bc	6.7b	84.4cd
M. phaseolina + *B. subtilis* Rb	10.0cd	11.7bc	6.7b	81.6de
R. solani only (Control I)	36.7a	43.0a	38.9a	18.1h
R. solani + *B. pumilus* Rb_{14}	10.0cd	14.2bc	8.9b	76.9f
R. solani + *B. pumilus* Rb	13.3cd	8.9bc	6.7b	84.4cd
R. solani + *B.subtilis* Rb_{18}	16.7cd	8.3bc	6.7b	85.0c
R. solani + *B. subtilis* Rb_{28}	13.3cd	7.5c	3.3b	89.2b
R. solani + *B. subtilis* Rb	13.3cd	12.5bc	8.9b	78.6ef
Without treatment(control II)	3.3d	3.3c	3.3b	93.4a

*Means in each column followed by the same letter are not significantly different according to LSD test (P = 0.05)

The isolates of Rb_{14}, Bp, Rb_{18}, Rb_{28} and Bs also significantly reduced the post-emergences damping off. The incidence was in the range of 3.3 to 11.7% for *M. phaseolina*, while it was in the range of 7.5 to 14.2 % for *R. solani*, compared to disease incidence of 27.8 and 43.0% in plants infested with each pathogen only, respectively. *Bacillus pumilus* (Bp) isolate followed by Rb_{14}, Rb_{28}, Rb_{28} and Bs, highly reduced the disease incidence in case of *M. phaseolina*. On other hand, Rb_{28} isolate highly reduced *Rhizoctonia* post-emergence damping off %, followed by Rb_{18}, Bp, Bs and Rb_{14}, respectively (Table, 4).

The tested isolates significantly reduced the incidence of root rot disease, where the incidence was in the ranges of 3.3 to 10.8% in case of *M. phaseolina* and 3.3 to 8.9% in case of *R. solani*, compared to the percentages of 50.5 and 38.9% in cases of pathogen only, respectively. Both *Bacillus pumilus* (Bp) and Rb_{28} highly reduced the incidence of disease caused by *M. phaseolina* and *R. solani*, respectively. Results showed that the percentages of survival plants were in the ranges of 77.5 to 84.4 % with *M. phaseolina* and 78.6 to 89.2% with *R. solani*, compared to 22.2 and 18.1 % with pathogen only, respectively (Table, 4).

Effects on peanut vegetative growth

The Rb_{14}, Bp, Rb_{18}, Rb_{28} and Bs isolates treatments increased the vegetative growth parameters (VGP) of peanut plants, compared to the control (Table, 5). Results revealed that average length of root, average length of shoot, average number of branches/plant, average number of leaves /plant, average fresh weight /plant and average dry weight /plant, resulted from Rb isolates treatment were in the ranges of 16.5 - 29.3 cm; 27.8 - 36.0 cm; 3.5 - 4.8; 20.3 - 41.3; 8.3-21.2g and 2.9 - 6.9 g in case of *M. phaseolina*, compared to 12.9 cm, 15.2 cm, 2.9, 15, 3.5g and 1.4g in pathogen only, respectively. In case of *R. solani* infestation, the above VGP resulted from Rb isolates treatments were in the ranges of 17.8 - 23.5cm ; 24.5 - 33.8cm; 4.8 - 5.0 branch; 32.0 - 36.3 leaf; 11.7 - 15.5g and 3.1 - 4.1 g, compared to 14.2 cm, 17.3cm, 2.9 branch, 18.9 leaf, 4.0g and 1.5g with pathogen only, respectively (Table,5).

Table 5. Efficacy of *Bacillus pumilus* and *Bacillus subtilis* on peanut vegetative growth parameters in pot experiment.

Treatment	Vegetative growth parameters (VGP)					
	Root length (cm)	Shoot length (cm)	Branches No. / plant	Leaves No./plant	Plant weight (g)	
					Fresh	Dry
M. phaseolina only (control I)	14.2h	17.7h	2.8d	18.9e	4.0gh	1.5h
M. phaseolina + *B. pumilus* Rb_{14}	16.5g	27.8e	3.5cd	20.3de	8.3f	6.9a
M. phaseolina+ *B. pumilus* Rb	25.8b	31.8c	4.3abc	27.8cd	10.5e	2.9f
M. phaseolina + *B.subtilis* Rb_{18}	28.3a	33.8b	5.3a	36.8ab	20.3a	4.7c
M. phaseolina + *B. subtilis* Rb_{28}	29.3a	28.5e	4.8ab	34.5abc	13.6bcd	4.5c
M.phaseolina + *B. subtilis* Rb	26.0b	36.0a	4.0bcd	41.3a	21.2a	5.7b
R. solani only (Control I)	12.9i	15.2i	2.8d	15.0e	3.5 h	1.4h
R. solani + *B. pumilus* Rb_{14}	20.5e	25.3f	5.0ab	36.0abc	11.7de	3.1ef
R. solani + *B. pumilus* Rb	21.5e	33.8b	4.8abc	34.0abc	13.1cd	3.2e
R. solani + *B.subtilis* Rb_{18}	17.8f	29.0e	4.8ab	34.8abc	13.7bc	4.1d
R. solani + *B. subtilis* Rb_{28}	23.5cd	24.5f	5.0ab	36.3ab	13.1cd	3.3e
R. solani + *B. subtilis* Rb	23.3d	30.8d	5.0ab	32.0bc	15.5b	3.9d
Without treatment (Control II)	17.0fg	23.3g	3.5cd	19.5de	5.9g	1.8g

*Means in each column followed by the same letter are not significantly different according to LSD test (P = 0.05).

Field experiment

Effects on peanut damping-off and root rot

Results of field experiment revealed that rhizobacteria Rb isolates treatments significantly reduced the percentage of damping off and root rot and increased the survival peanut plants, compared to the control (Table, 6). The percentages of root rot in rhizobacteria Rb isolates treated plots were in the range of 5.9 - 12.7%, compared to the percentage of 26.2% in the control. Results also revealed that the survival plants in the plots treated with rhizobacteria Rb isolates were in the range of 78.6 - 80.0%, compared to 51.5% in the control (Table, 6).

Table 6. Efficacy of *Bacillus pumilus* and *Bacillus subtilis* on damping-off and root rot in field experiment.

Rhizobacteria isolate	Disease parameters			
	Damping-off (%)		Root rot incidence %	Survival plants %
	Pre-emergence %	Post-emergence %		
Bacillus pumilus Rb_{14}	19.1b*	9.7b	6.3b	84.0a
Bacillus pumilus Rb	15.0b	6.9b	12.7b	80.4ab
Bacillus subtilis Rb_{18}	14.2b	11.6bc	9.8b	78.6ab
Bacillus subtilis Rb_{28}	16.7b	14.1ab	5.9b	80.0ab
Bacillus subtilis Rb	15.0b	10.5b	9.9b	79.6ab
Medium only (control I)	40.8a	20.0a	12.9ab	67.1b
Without treatment (control II)	41.7a	21.3a	26.2a	51.5c

*Means in each column followed by the same letter are not significantly different according to LSD test (P = 0.05).

Effects on growth and yield parameters

The tested rhizobacteria Rb isolates increased the yield component at harvest in field experiments compared to the un-treated control (Table, 7). The dry weight of plant growth above the ground aerial (gram/plant^{-1}) was in the range of 75.6 to 101.8g compared to 66.2g in the un-treated control. The number of

pods/plant^{-1} was in the range of 30 to 34 leaves compared to 27 leaves in the un-treated control. The average weight of pods/plant^{-1} was in the range of 74.0 to 83.7g compared to 63.0 g in the un-treated control. The weight of 100-Kernel (grams) was in the range of 92.4 to 104.0g compared to 80.1g in un-treated control (Table, 7).

Table 7. Efficacy of *Bacillus pumilus* and *Bacillus subtilis* on peanut vegetative growth parameters in field experiments.

Treatment	Average of growth parameters			
	Dry weight of plant	Pod No. plant^{-1}	Pod weight plant^{-1}	Weight of 100 seeds
Bacillus pumilus Rb_{14}	79.6ab*	31bc	77.3c	93.5c
Bacillus pumilus Rb	101.8a	34a	83.7a	104.0a
Bacillus subtilis Rb_{18}	85.3ab	30c	80.8b	98.3b
Bacillus subtilis Rb_{28}	75.6ab	31bc	74.0d	92.4c
Bacillus subtilis Rb	88.8ab	33ab	82.5a	100.5b
Medium only (control I)	66.8b	27d	62.9e	80.6d
Without treatment (control II)	66.2b	27d	63.0e	80.1d

*Means in each column followed by the same letter are not significantly different according to LSD test (P = 0.05).

Discussion

Macrophomina phaseolina and *R. solani* found to be prevalent associated fungi with root rot of peanut in Beheira governorate where peanut is intensively cultivated in Egypt. Pathogenicity test proved that the obtained fungi were pathogenic and virulent for peanut plants. These findings are in harmony with reports from Egypt and other parts of the world[29, 30, 31, 32, 13,16,6]**.** Controlling such diseases mainly depends on fungicides treatments. However, fungicidal applications cause hazards to human health and increase environmental pollution. In the present study, we isolated the common bacteria found in the rhizosphere of healthy peanut plants for controlling the two tested pathogens. Thirty rhizobacteria Rb isolates were obtained and subsequently screened for their antagonistic activity against *M. phaseolina* and *R. solani*. Results of *in vitro* tests revealed that the most tested rhizobacteria Rb isolates had antagonistic effect against the growth of *M. phaseolina* and *R. solani*, especially the isolates of Rb_{14}, Rb_{18} and Rb_{28} that identified as *B. pumilus* Rb_{14}, *B. subtilis* Rb_{18} and *B. subtilis* Rb_{28}. Bio control capacity through antagonistic bacteria involves either competition [33] or bacterial metabolite production, such as siderophores, hydrogen cyanide, antibiotics or extracellular enzymes for antagonism towards plant pathogens [34, 35]**.** It has been reported that *Bacillus* spp. contains various biocontrol characteristics including secondary metabolites, the colonizing potential, and the production of competitors[36]**.** Therefore, the effective rhizobacterial isolates *viz. B. pumilus* (Rb_{14}), *B. subtilis* (Rb_{18}) and *B. subtilis* (Rb_{28}) in addition to the two rhizobacterial isolates, *viz. B. pumilus* (Bp) and *B. subtilis* (Bs) that were isolated from cucumber rhizosphere were applied in pots and in field experiments. Results in pots and in field experiments showed that the rhizobacteria Rb isolates applications highly reduced the damping-off and root rot disease incidence when compared with un-treated control. The treatments also highly increased the survival plant as well as the tested growth parameters and yield components. These results are agreement with those reported by[37]**.** *Bacillus* spp. form spores, are resistant to unfavorable conditions and can thus be adapted to the field. According to[38]**,** diverse populations of aerobic endospore-forming bacteria appear in agricultural fields; this may directly and indirectly contribute to crop productivity. Multiple *Bacillus* spp. can promote crop health in varied ways. In addition, through the work of [39, 40]**,** we know that some *Bacillus* spp. are good root colonizers and can effectively protect infection regardless of soil borne or airborne pathogens. [41] demonstrated that the number of *Bacillus* strain activities suppress pathogens or otherwise promote plant growth. Improvements in plant health and productivity are mediated through three different ecological mechanisms: (i) pathogen antagonism, (ii) host nutrition and growth promotion, and (iii) plant host defense stimulation. Rhizobacteria are ideal for use as biocontrol agents. Rhizobacteria inhabit the rhizosphere that provides the front line defense for roots against attack by pathogens. Pathogens find antagonism from rhizobacteria before and during primary root infection. Rhizobacteria are reported to provide protection against several plant pathogens. Generally, rhizobacteria traits associated with plant pathogens biocontrol include: antibiotic synthesis**,** production of low molecular weight metabolites such as hydrogen cyanide with antifungal activity, production of enzymes

including chitinase, b-1-3-glucanase, protease, and lipase. These enzymes can lyses some fungal cells [42]**.** This study led to the selection of potential biocontrol agents against peanut damping-off and root rot diseases caused by *R. solani* and *M. phaseolina*, and demonstrated that local rhizobacteria Rb isolates of *Bacillus* spp. have a prospective use as biological control agents to protect peanut plants.

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