Egypt. J. Phytopathol., Vol. **40**, No. 2, pp. 79-93 (2012)

Evaluation of some Bacterial Formulations Used for Biocontrol of Pea Root-Rot Disease

Amal M. Omer* and Abeer M. El-Hadidy**

* *Microbiol. Unit, Soil Fertilization and Microbiol. Dept., Desert Res. Centre, Cairo, Egypt.*

** *Plant Pathol. Unit, Plant Protect. Dept., Desert Res. Centre, Cairo, Egypt.*

Five fungal isolates, *i.e*. *Pythium* sp., *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani*, were evaluated for their pathogenicity to pea plants. Pathogenicity test proved that *R. solani* followed by *S. rolfsii* and *F. solan*i were the most virulent pathogens which caused the highest incidence of root-rot and damping-off of pea plants. Antagonistic effect of certain effective bioagent, isolated from pea rhizosphere and identified as *Brevibacillus brevis* revealed the presence of clear antagonistic action on root-rot pathogens. The highest mean inhibition values were 94.2 and 91.3% against *F. solani* and *R. solani*, respectively.

Three dry based bioformulations, *i.e.* cellulose-clay, talc-glucose and talc-yeast, in which *B. brevis* incorporated, were used. The viability of the dry bioformulations over 6 months at room temperature was assessed, and this indicated the long shelf life of the three formulas which ranged from 68.3 to 52.4% of viable cells after 180 days of storage. The potentiality of these formulations and free spore suspension of the bioagent in controlling root-rot incidence, under greenhouse and field conditions, were evaluated. Data indicated that root-rot severity reached the highest values when mixture of pathogenic fungi was applied. All tested formulations significantly reduced pre and post emergence damping-off of seedlings and root-rot incidence. Dry bioformulations of *B. brevis*, especially cellulose-clay, proved to be more effective than its free spore suspension in soil infested with pathogens. The efficacy of all tested dry bioformulations in controlling disease was reflected on the plant growth causing significant increase in all growth parameters measured. These data indicate that dry bioformulations of *B. brevis* can be used as a promising tool for seed treatment as biocontrol agents for controlling damping-off and root-rot of pea plant.

Keywords: Bioformulations, biocontrol, *Brevibacillus brevis*, damping-off, *Fusarium solani*, *Macrophomina phaseolina*, *Pythium* sp., *Rhizoctonia solani*, root-rot and *Sclerotium rolfsii*.

Pea (*Pisum sativum* L.), of the family Papilionoideae, is an important pulse legume and high nutritive cool season vegetable crop in Egypt. Seed and soil-borne diseases are considered as important factors which restrict pea production and resulting in serious economic losses. These diseases significantly decrease both yield

and seed quality. Soil borne fungal diseases are the major limiting factors in pea production throughout the world. However, the major fungal disease problem of legume crops in Egypt is root-rot/wilt disease complex (Salem *et al*., 1990 and El-Awadi *et al*., 1990).

Damping-off, root-rot and wilt diseases are destructive diseases attacking legume crops world-wide, causing serious yield losses. These diseases are caused by several fungi, *i.e. Rhizoctonia solani, Fusarium oxysporum, F. solani* and *Sclerotium rolfsii* (Hassanein *et al*., 1996 and Abou-Zeid *et al.,* 1997). Control of these pathogens is difficult because of their persistence in the soil and wide host range. Although some chemicals fungicides are effective in controlling this disease, the excessive use of these chemical in agriculture has led to several problems including environmental pollution, development of pathogen resistance to fungicide and adverse affect on human health. Because of these problems, there is need to identify alternative methods for plant protection, which are less dependent on chemicals and are more environmentally friendly. The most important alternative method is the use of natural resources as biological control agents. Biological control is an alternative to the management of diseases caused by soil-borne microorganisms (Zavaleta, 2000). A number of rhizobacteria that were shown to act as effective biocontrol agents by suppressing a variety of economically important phytopathogens often promote overall plant vigour and yield, either when applied to crop seed or incorporated into the soil (Bashan, 1998; Brown and Surgeoner, 1991, Kloepper *et al.,* 1989 and Turner & Backman, 1991). These beneficial bacteria are known as plant growth promoting rhizobacteria or PGPR (Kloepper *et al*., 1989).

One of the most important strains used as both biocontrol and plant growth promoting rhizobacteria is *Brevibacillus brevis* (Sangita and Shah, 2000 and Sunita *et al*., 2010). *Bacillus* spp. have shown promising results for the biological control of various plant pathogens as well as growth promoters of various crops (Weller, 1988 and Podile and Laxmi, 1998). *Brevibacillus brevis* is a potential plant growth promoting and biological control agent for reducing the impact of *F. oxysporum* f.sp. *lycopersici* on tomato (Sunita, 2010). Some species from the *Bacillus* genus are particularly effective due to their capacity to form, or produce spores that survive and remain metabolically active under harsh environmental conditions (Rodgers, 1989). *Bacillus* spp. are nonpathogenic, easy to cultivate and metabolite secretors. These characteristics make them appropriate for the formulation of stable and viable biological products that could be used for soil-borne disease management (Kloepper, 1997).

Formulation of microbial cells has long been established for applications in the agricultural systems (Meyer, 2003). The aims of formulating viable cells are to ensure that adequate cell viability is sustained to increase the efficacy of the cells and to facilitate the delivery and handling processes (Filho *et al*., 2001). This can be achieved by producing granular formulations, powder or dust formulations, microcapsules, or oil-emulsion formulations (Brar *et al*., 2006). For biocontrol purposes, formulated microbial cells is most often applied using wet (liquid) formulations by spraying inoculum suspensions on targeted sites, or using dry (solid) formulations where granules or dust are sprayed instead (Brar *et al*., 2006).

The potential *P. fluorescens* is formulated using different organic and inorganic carriers either through solid or liquid fermentation technologies. Thus, for field application of *P. fluorescens* towards the management of the bacterial root-rot and wilt disease of pea, development of commercial formulations with suitable carriers that support survival of the bacteria for a considerable length of time is necessary (Nakkeeran *et al*., 2005).

The objectives of this study were to evaluate dry *Brevibacillus brevis* based bioformulations as potential biocontrol agents against pea root-rot and wilt disease and as plant growth promoting rhizobacteria under green house and field condition.

Materials and Methods

Isolation and identification of causal organisms:

Pea plants showing symptoms of damping-off, root-rot and wilt infection were collected in plastic bags from different growing areas at Ismailia governorate. Roots and crowns of plants were washed thoroughly with tap water. Small pieces (2-5mm) were cut from each sample and sterilized with sodium hypochlorite 1% for 2 min and dried between sterilized filter paper and placed on potato dextrose agar plats (PDA) supplemented with streptomycin-sulfate (µ/ml). Petri dishes were incubated at 25ºC for 48-72 hours. Single spores or hyphal tips taken from developed colonies and transferred onto (PDA). The fungal isolates were identified in Plant Pathology institute, Agriculture research centre, Cairo, Egypt, according to Barnett and Hunter (1987) for the genera of imperfect fungi, Booth (1977) for *Fusarium* species, Sneh *et al*. (1992) for *Rhizoctonia solani* and Plaats-Niterink (1981) for *Pythium* species and evaluated for their pathogenicity to pea plants.

Pathogenicity test:

The experiments were carried out in artificiality infested sandy clay soil at the green house of Plant Protection Dept., Desert Res. Centre (DRC). Inocula of isolated fungi *i.e. Pythium ultimum*, *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium solani* f.sp. *pisi. w*ere prepared on PDA medium and incorporated to soil. Five pots were used for each treatment to study the effect of tested fungi on the incidence of pre and post emergence damping-off, incidence of root-rot and survived plants were calculated at 15, 30 and 45 days after planting, respectively.

Isolation and identification of biocontrol agent from pea rhizosphere:

The bacterial bioagents were isolated from the rhizosphere of different pea plants collected from Ismailia governorate on Nutrient medium. On the basis of colony morphology, many colonies were randomly selected and further purified by streaking. Each strain was evaluated as potential antagonist against the previous pathogens by the paper disc plate method of Pleczer and Chan (1971). Among 57 isolates, the isolate recorded the maximum inhibition of mycelial growth was identified as *Brevibacillus brevis* by partial 16S rRNA gene sequence analysis according to Berg *et al.* (2002) in Medical University of Graz, Austria. Bacterial 16S rRNA gene sequences were amplified by PCR using the eubacterial primer pair 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-TAC GGY TAC CTT

GTT ACG ACT T-3') (Lane, 1991). The PCR was performed by using a total volume of 20 µl containing 1× Taq&Go (MP Biomedicals, Eschwege, Germany), 1.5 mM $MgCl_2$, 0.2 mM of each primer and 1 µl of template DNA (95°C, 5 min; 30 cycles of 95°C, 30 s; 57°C, 30 s; 72°C, 90 s; and elongation at 72°C, 5 min). PCR product was sequenced with the Applied Biosystems 3130l Genetic Analyser sequencer, Data Collection v3.0, Sequencing Analysis v5.2 (Foster City, USA) at the sequencing core facility ZMF, Medical University of Graz, Austria. Obtained sequences were aligned with reference RNA sequences from NCBI (National Centre for Biotechnology Information) database.

In vitro antifungal activity:

The inhibition of mycelium growth of *R. solani*, *Pythium* spp., *S. rolfsii*, *M. phaseolina* and *F. solani* by *B. brevis* strain were tested on PDA medium. One ml of bacterial suspension (10^8cfu/ml) was streaked on PDA media plates and a 6mm agar disc of each fungal isolate from fresh PDA culture was placed at the other marginal side and incubated at 25 ± 2°C for seven days. The radial growth of the developed fungal colony towards and away from the bacterial colony was measured. The percentage of growth inhibition was calculated using the following calculation:

$$\text{Inhibition (\%)} = [(R - r)/R \times 100]$$

Whereas: R is the maximum radius of the fungal colony away from the bacterial colony and r is the radius of the fungal colony opposite the bacterial colony.

Preparation of Brevibacillus brevis spore yield:

Brevibacillus brevis were grown on a modified nutrient medium supplemented with a mixture of $MnSO_4$, $CaCl_2$, $ZnSO_4$ and KCl at a concentration of 500 ppm for 3 days on an orbital shaker at 150 rpm at 30°C till the maximum spore yield was produced (Omer, 2010), these were harvested and subsequently washed by repeated centrifugation at 5,000 × g for 20 min at 4°C/resuspension in sterile distilled water. Finally, the spore pellet was re-suspended in sterile distilled water and used as active material in different formulations. The final spore titer was $\geq 10^8$ cfu/ml.

Bioformulation of the Brevibacillus brevis:

The spores of *B. brevis* was formulated into three different dry formulations: cellulose-clay (1:1), talc-glucose (1:1) and talc-yeast (1:1). The enrichment materials (glucose and yeast) were incorporated at 0.25% concentration. For each formula, 1% carboximethylcellulose (CMC) as binder, traces of sodium benzoate as stabilizer and 15% CaCO3 as buffer were incorporated. The inert carriers, enrichment and additive materials were mixed and sterilized by autoclaving. Twenty ml of spore suspension were added into them, mixed well under aseptic conditions, and then the mixtures were air dried in a laminar flow chamber for 48 hours. After drying, a 1-g sample was removed for initial population counts. Powder formulations were then placed in plastic Petri plates, sealed with par film, stored at room temperature as described by (Omer, 2010).

Population dynamics of Brevibacillus brevis in the different bioformulations:

The population dynamics of the bioagent *B. brevis* formulations was determined at different days after storage. Viable population of *B. brevis* in the three powder formulations was determined at 15, 30, 60, 90 and 120 days at room temperature ($25^{o}C\pm2$).

Evaluation of Brevibacillus brevis bioformulations in controlling damping-off and root-rot and promoting growth of pea plants:

A. Greenhouse experiment:

Pea seeds, at a rate of 10 seeds/ pot, were sown in 30cm pots filled with soil infested with *P. ultimum*, *S. rolfsii*, *R. solani* or *F. solani*. Bacterial formulations (cellulose-clay, talc-glucose and talc-yeast) were mixed with pea seeds treated with powder formulations at the rate of 1% (powder formulation: seeds) to give a bacterial population of $\geq 10^{7}$ cfu/seed of formulation. For the free-spore suspension treatment, seeds were moistened in CMC solution (1%) before application of inoculum to get a thin, uniform coating of inoculum on seeds. Inoculated seeds were dried in shade before sowing (Samasegaran *et al*., 1982). Uninfested soil and soil infested with tested fungi without bacterial bioformulation were served as controls. Pre and post emergence damping-off, percentage of root-rot incidence and survived plants were calculated at 15, 30 and 90 days after planting, respectively. Disease severity of root-rot and any discoloration of internal tissue were recorded. Severity of browning of internal tissue was recorded and conducted with scale proposed by Haware and Nene (1980) based on 0 – 4 scale according to percentage of foliage yellowing or necroses (0 = 0%, 1 = 1-33%, 2= 34-66 %, 67-100%, 4=dead plant). Plant growth parameters (Plant height, fresh weight, dry weight, number of pods and weight of 100 seeds/plant were recorded three months after planting for all treatments.

B. Field experiment:

An experiment was conducted in a complete randomized design with three replicates in naturally infested field at Ismailia government for controlling pea root-rot and damping-off disease. A standard plot size of 3 x $3m^2$ with 5 rows was maintained for all treatments. For each plot, 200 seeds were sown. Seeds of pea were treated with the bacterial formulations as previously mentioned in pot experiment. Untreated seeds were served as control. Soil in all treatments was amended with recommended dose of super phosphate (15.5% P_2O_5) at a rate of 250 kg/fed, ammonium nitrate (33.3% N) at a rate of 300 kg/fed and K-sulphate (48% K_2O) at a rate of 200 kg/fed. Plant growth parameters (Plant height, fresh weight, dry weight number of pods and weight of 100 seeds pea plants) were recorded three months after planting for all treatments.

Statistical analysis:

Data were subjected to statistical analysis, whenever needed, using the method described by Snedecor and Cochran (1990). The least significant difference (L.S.D) was used to differentiate means according to Waller and Duncan (1969).

Results and Discussion

1- Fungal pathogens of pea root-rot disease:

Pythium sp. and *Rhizoctonia solani* were isolated from pea plant infected with damping-off, while *Sclerotium rolfsii*, *Macrophomina phaseolina*, *R. solani* and *Fusarium solani* were isolated from rooted-rot pea plants on PDA plates. Results in Table (1) show that all the tested fungi were pathogenic to pea plants. They recorded pre and post emergence damping-off to pea seedlings with significant differences among them. *M. phaseolina* and *F. solani* recorded the lowest pre and post emergence damping-off of seedlings, while the highest ones were recorded with *P. ultimum.* The highest root-rot incidence of pea plants was recorded with *R. solani* followed by *S. rolfsii* and *F. solani*, two months after sowing.

Table 1. Effect of some soil born fungi on the incidence of pre and post emergence damping-off and survival of pea plants

Fungal isolate	Damping-off (%)		Root-rot incidence (%)	Survival (%)
	Pre-emergence	Post-emergence		
P. ultimum	32.3a	29.6a	33.6b	38.1c
M. phaseolina	6.7c	10.1b	26.8c	83.3a
S. rolfsii	13.5b	17.8b	38.7b	68.7b
R. solani	26.7a	23.5a	66.6a	49.8c
F. solani	8.6c	11.6b	38.6b	79.5b

2- In vitro assay of antagonism:

The inhibitory effect of *Brevibacillus brevis* against linear growth of fungi was *in vitro* evaluated as shown in Fig. (1). Marked inhibition in the linear growth of the tested fungi was occurred. The highest mean inhibition values, 94.2 and 91.3, were obtained against *F. solani and R. solani*, respectively. *Macrophomina phaseolina* showed more resistance to *B. brevis* than other fungi.

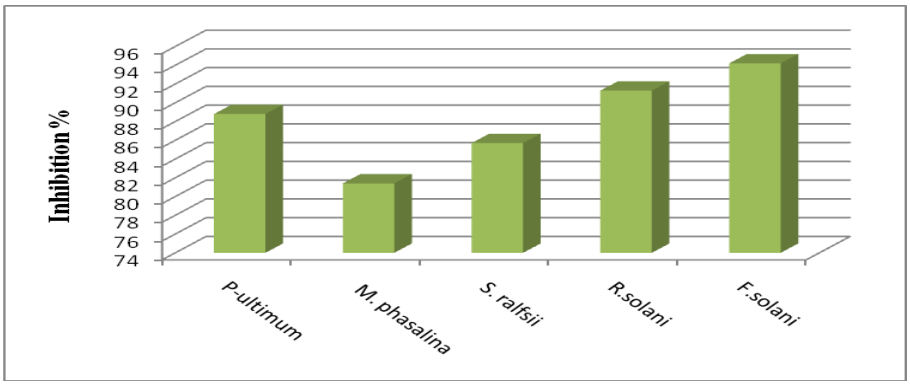

Fig. 1. *In vitro* antagonistic effect of *Brevibacillus brevis* on the root-rot and damping-off fungi.

3- Survival of bioagent in different formulations:

Data illustrated in Fig. (2) show that in all formulations, bacterial populations were declined steadily over time. The bacteria survived even up to 180 days of storage with different percentage although the population declined from 60 days of formulation. The number of viable cells detected in T-yeast carrier was slightly higher than that in T-glucose and cellulose-clay after six months of storage. Only 68.3 and 60% of viable cells were detected in T-yeast and T-glucose, while almost 52.4 viable cells were detected in cellulose-clay after six months. Greater loss of viable cells stored at 30°C could be explained by the desiccation process that occurred as a result of prolong exposure to high temperature (Cigdem and Merih, 2005). A higher PGPR (strain B4) population was found to be maintained in talc/peat based formulation using CMC or Arabic gum as adhesive (Suslow and Schroth, 1982). Glucose and yeast were the most efficient ones in preserving bacterial populations at different formulations. Also, the formula composed of cellulose-clay was effective formulation in survival of bacterial populations. This is may be due to the advantages of both clay and cellulose as carriers (Omer, 2010). Bacterial existence in talc-based formulation is one of the most functional products useful in economic purposes, especially in industrial and half-industrial scale of bacterial productions (Sadi and Ahmadzadeh, 2012).

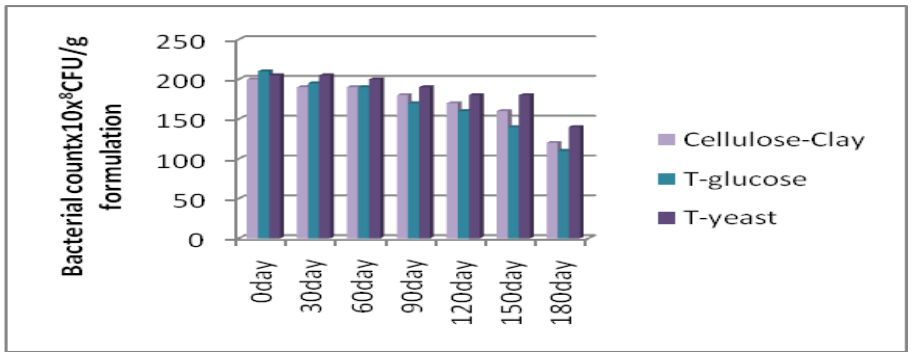

Fig 2. Viable population of *Brevibacillus brevis* in 3 different powder formulations stored at room temperature (25±2°C).

4- Biocontrol efficacy of Brevibacillus brevis:

A. Under greenhouse condition:

In this experiment, the efficiency of three formulations and free spore suspension of *B. brevis* in controlling damping-off and root-rot diseases caused by *P. ultimum, S. rolfsii, R. solani* and *F. solani* and their mixture were evaluated. Data presented in Table (2) show that all bacterial formulations decreased damping-off, root-rot incidence and disease severity compared with the controls. Data indicated that soil infested with pathogenic fungi used as control show the highest percentage of pre and post emergence damping-off compared with non-infested soil. While pea plants infested with *P. ultimum* or mixture of pathogenic fungi showed the highest pre and post emergence damping-off compared to other pathogenic fungi, plants infested with mixture or *R. solani* recorded the highest root-rot incidence.

Table 2. Effect of bacterial application on damping-off, root-rot incidence and survival of pea plants grown in soil infested with pathogenic fungi

Treatment *		Damping-off (%)		Survival plants (%)	Root-rot incidence (%)	Disease severity (%)
		Pre-emergence	Post-emergence			
Uninfested plant		0.0	0.0	100.0	0.0	0.0
Infested plants with *P. ultimum*	Control	8.7a	20.5ab	70.8bc	54.3b	3.6a
	Formula 1	2.5c	2.5d	95.3a	18.4d	1.0c
	Formula 2	2.6c	2.5d	94.9a	19.3d	0.9c
	Formula 3	3.7b	6.7bc	89.6ab	31.2c	1.7b
	FSS	4.3b	8.9bc	86.8b	39.3bc	1.9b
Infested plants with *S. rolfsii*	Control	3.9b	13.3b	82.8b	38.3bc	3.1ab
	Formula 1	1.3d	7.4bc	91.3b	21cd	1.8b
	Formula 2	1.6d	3.9cd	94.5a	19.4d	1.4c
	Formula 3	3.2c	4.2cd	92.6ab	26.2cd	2.3b
	FSS	2.3c	6.3c	91.4ab	30.1c	2.5ab
Infested plants with *R. solani*	Control	5.5b	20.5ab	74bc	63.4a	3.5a
	Formula 1	1.7d	13.6b	84.7b	42.6bc	1.1c
	Formula 2	2.5c	13.7b	83.8b	39.7bc	1.3c
	Formula 3	3.3bc	16.8b	79.9bc	48.4b	2.4b
	FSS	2.6c	17.2b	80.2b	50.7b	2.6ab
Infested plants with *F. solani*	Control	4.2b	9.6bc	86.2b	48.3b	2.9ab
	Formula 1	1.4d	3.9cd	94.7a	21.2cd	0.8c
	Formula 2	2.1c	7.4bc	90.5ab	25.2cd	1.2c
	Formula 3	2.5c	6.8bc	90.7ab	34.3c	1.7b
	FSS	3.3c	6.9bc	89.8ab	39.5bc	2.3b
Infested plants with mixture	Control	6.7ab	30.3a	63c	66.6a	4a
	Formula 1	2c	8.2bc	89.8ab	27.5c	1.6b
	Formula 2	2.3c	9.2bc	88.5ab	28.2c	1.5b
	Formula 3	3.4bc	13.3b	83.3b	42.3bc	2.8ab
	FSS	4.2b	19.4ab	76.4bc	54.2b	3.2ab

* Control : No bacterial inoculation.
Formula 1: Cellulose-clay based formulation.
Formula 2: Talc-glucose based formulation.
Formula 3: Talc-yeast based formulation.
FSS : Free spore suspension.

Generally, *B. brevis* application significantly reduced the root-rot infection at pre and post emergence damping-off, root-rot incidence and disease severity caused by all tested fungi. The maximum reduction in disease severity was recorded with *F. solani* (72.4%) and *P. ultimum* (72.2%) while the minimum one was recorded with *S. rolfsii* (54.8%) where mean that *F. solani* and *P. ultimum* were more sensitive to *B. brevis* than other tested fungi. Development of symptoms on glasshouse-raised tomato plants was markedly reduced in co-inoculations of *F. oxysporum* with *B. brevis*, compared with inoculations with the pathogen alone (Sunita *et al.*, 2010). *Brevibacillus brevis* has significant potential as a gramicidin-producing biocontrol agent against *Phytophthora* spp., *Pythium* spp., *Rhizoctonia solani*, *Colletotrichum acutatum* and *F. oxysporum* (Sangita and Shah, 2000).

Bioformulations of *B. brevis* proved to be more effective than its free spore suspension in soil treated with pathogens. Cellulose-clay and talc-glucose based formulations recorded the highest reduction of pre and post emergence damping-off for all tested fungi except for *F. solani* where cellulose-clay based formulation showed the maximum reduction followed by other formulations and free spore suspension. No significant variations detected between cellulose-clay and talc-glucose based formulations in reducing all the traits studied. Data also indicated that all tested formulations significantly reduced root-rot disease. The most effective formula were cellulose-clay and talc-glucose based formulations which reduced root-rot incidence by 18.4 and 19.3% compared with talc-yeast based formulation and free spore suspension. Root-rot severity reached the highest rate (4.0) when mixture of fungi was applied to the soil. The severity was reduced to 0.9, 1.4 and 1.5 in plants treated with talc-glucose based formulation and infested with *P. ultimum*, *S. rolfsii* or fungal mixture, respectively, and reduced to 1.1 and 0.8 in plants treated with cellulose-clay based formulation and infested with *R. solani* or *F. solani*, respectively, comparing with control.

Data presented in Table (3) revealed that seed inoculation with *B. brevis* bioformulations caused a significant increase for all parameters measured over the controls, and this is compatible with that talc-based formulations prepared with bacteria had the longest stability and biocontrol activity which had significant effect on sunflower growth compare to control (Sadi and Ahmadzadeh, 2012). Data showed that the lowest values of plant height, plant fresh and dry weight number of pods and seed weight were observed with the controls. The greatest values for all traits were recorded with plants inoculated with dry formulation compared with control. No significant variation among dry bioformulations was recorded. For plant height, the highest remarkable increase was recorded with dry bioformulations followed by free spore suspension application relative to control. The formulations when applied in seed, root and soil were more effective in reducing disease severity possibly due to the all round placement of the antagonist viz. on the seed, from which the antagonist migrated to the elongating roots (Burr *et al.*, 1978).

B. Under field condition:

The effect of different formula on the incidence and severity of root-rot disease of pea under field condition is shown in Fig. (3); the incidence of diseased pea plants (6-8%) recorded from plants grown in pots treated with formula 1 followed by formula 2 (12.4%) and formula 3 (21.3%) compared with the untreated control (67.3%). Data in Table (5) also illustrate significant differences among control and treatments. Cellulose-clay based formulation recorded the lowest degree of incidence (0-1) compared to control which revealed the highest value (3-6). These mean that cellulose-clay based formulation is the most effective treatment followed by the other two bio formulation. *B. brevis* ATCC 8185 synthesizes two kinds of antibiotic peptides, cyclopeptidetyrocidine and linear gramicidin (Marlow and Linderman, 1996). *B. brevis* may have potential as a biocontrol agent against fusarial wilt in pigeon pea by producing extra-cellular antagonistic substance which induced swelling of the pathogen's hyphal tips, and cells were bulbous and swollen with shrunken and granulated cytoplasm. Also, antagonistic substance inhibited conidial germination and act as a fungicide to pathogen mycelia (Sangita and Shah, 2000).

Table 3. Effect of bacterial application on growth parameters of pea plants grown in soil infested with four pathogenic fungi

Treatment *		Plant height (cm)	Plants fresh weight (g)	Plants dry weight (g)	Pods No./plants	Weight of 10 seeds
Uninfested plant		48.1a	12.2a	4.0a	28.5a	15.4a
Infested plants with *P. ultimum*	Control	36.4b	5.8c	1.4c	12.3c	4.2c
	Formula 1	47.2a	9.2a	3.9a	23.6ab	11.5ab
	Formula 2	43.5a	8.9a	3.6a	25.4a	10.3ab
	Formula 3	40.9ab	8.4ab	3.2ab	21.0ab	9.8b
	Broth	41.6ab	8.7a	3.2ab	19.9ab	9.4b
Infested plants with *S. rolfsii*	Control	39.0ab	5.3c	1.6c	13.5bc	5.4c
	Formula 1	46.3a	8.6a	3.8a	21.3ab	9.8b
	Formula 2	45.5a	9.2a	4.2a	23.2ab	10.6ab
	Formula 3	45.2a	8.3ab	3.5a	18.1b	9.5b
	Broth	41.7ab	8.1ab	3.8a	17.6b	9.5b
Infested plants with *R. solani*	Control	38.3ab	4.6c	1.1c	11.8c	4.4c
	Formula 1	45.7a	7.7ab	3.4ab	19.4ab	10.5ab
	Formula 2	44.9a	7.5ab	3.1ab	18.9b	11.2ab
	Formula 3	43.6a	6.8b	2.6ab	18.5b	9.7b
	Broth	42.3ab	6.7b	2.4b	17.8b	8.9b
Infested plants with *F. solani*	Control	37.8ab	5.3c	1.2c	13.6bc	6.2c
	Formula 1	46.7a	7.9ab	3.6a	27.4a	12.3a
	Formula 2	46.2a	7.6ab	3.8a	25.6a	10.8ab
	Formula 3	43.8a	6.7b	2.6ab	26.3a	9.6b
	Broth	43.5a	6.5b	2.4b	24.2ab	9.7b
Infested plants with mixture	Control	35.7b	4.3c	0.89c	11.4c	3.8c
	Formula 1	44.5a	6.5b	2.9ab	18.2b	10.4ab
	Formula 2	45.7a	6.6b	2.6ab	18.6b	9.7b
	Formula 3	43.5a	5.9bc	2.9ab	16.4b	9.2b
	Broth	39.9ab	5.5bc	2.5ab	15.2bc	9.0b

* As described in footnote of Table (2).

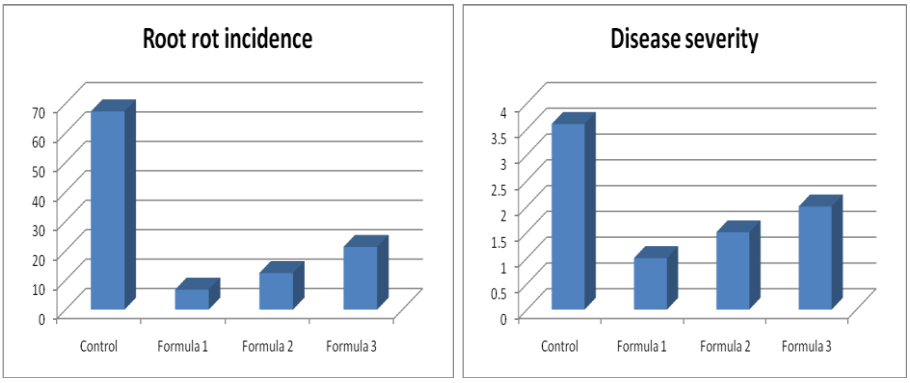

Fig 3. Effect of bacterial formulations on root-rot incidence and disease severity of pea plants under field condition.

Data in Table (4) indicate clearly that all bioformulations of *B. brevis* have a positive effect on growth of pea plants. This result is in agreement with that reported by Vivas *et al*. (2005) who demonstrated that *B. brevis* isolated from soil increased plant growth and improved nodule production due to its ability to produce Indole acetic acid. However, cellulose-clay based formulation showed maximum increase for all traits studied, no significant differences were observed between other two formulas. It is clear that the efficacy of all tested dry bioformulations in disease control was reflected on the plants and causing increase in all growth parameters measured. Cellulose-clay based formulation recorded the heights significance value for seed yield of bean (Omer, 2010).

Table 4. Effect of different bioformulations of *B. brevis* on growth parameters of pea plants grown under field condition

Treatment *	Plant height (cm)	Plant fresh weight (g)	Pods No./plant	Weight of 10 seeds
Control	49.6c	3.1c	20.4c	18.5c
Formula 1	78.5a	7.2a	41.7a	33.8a
Formula 2	70.5b	5.8b	35.2b	21.6b
Formula 3	68.3b	5.3b	31.5b	20.5b

* As described in footnote of Table (2).

Conclusion

Cellulose-clay based formulation of *B. brevis* as biocontrol agent was found to be highly effective bioformulation with respect to long shelf life and suppression of fungal root-rot disease when applied to pea seed. Therefore, application of biological agents as seed treatment could be used as an effective and non-hazard applicable technique for controlling soil borne plant pathogens in addition to avoid environmental pollution due to decrease the usage of chemical fungicides.

References

Abou-Zeid, N.M.; El-Morsy, G.A.; Hassanein, A.M. and Arafa, M.K. 1997. Identification of Fusarium wilt caused by *Fusarium oxysporum* and pathogen variability in faba bean, lentil and chick-pea crops in Egypt. *J. Agric. Res.*, **75**(3): 529-541.

Omer, Amal M. 2010. Bioformulations of *Bacillus* spores for using as biofertilizer. *Life Sci. J.*, **7**(4): 124-131.

Barnett, H.L. and Hunter, B.B. 1987. *Illustrated Genera of Imperfect Fungi*. Burgess Publishing Company, Minneapolis, 241p.

Bashan, Y. 1998. *Azospirillum* plant growth promoting strains are nonpathogenic on tomato, pepper, cotton, and wheat. *Can. J. Microbiol*., **44**: 168-174.

Brown, A.E. and Surgeoner, R. 1991. Enhancement of plant growth by *Zygorrhyn chumoelleri. Ann. Appl. Biol*., **118**: 39-46.

Brar, S.K.; Verma, M.; Tyagi, R.D. and Valero, J.R. 2006. Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides. *Process Biochem*., **41**: 323-342.

Booth, C. 1977. *Laboratory Guide to the Identification of the Fusarium species*. Commonwealth Mycological Institute, Surrey, England. 58p.

Burr, T.J.; Schroth, M.N. and Suslow, T. 1978. Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *Pseudomonas putida. Phytopathology*, **68**: 1377-1383.

Cigdem, K. and Merih, K. 2005. Effect of formulation on the viability of biocontrol agent, *Trichoderma harzianum* conidia. *African J. Biotechnol.*, **85**: 483-486

El-Awadi, F.A.; Smail, I.A.I. and Nadia, E.A. 1990. Evaluation of lupine entries and fungicides against root-rot and wilt diseases of lupine, Proc. 8th Congress of the Egyptian Phytopathol. Soc., Cairo, pp. 393-395.

Filho, A.B.; Alves, A.B.; August, N.T.; Pereira, R.M. and Alves, L.F.A. 2001. Stability and persistence of two formulations containing Anti Carsiagemmatalis Nuclear Polyhedrovirus (AgMNPV). *J. Neotrop. Entomol.,* **30**: 411-416.

Haware, M.B. and Nene, Y.I. 1980. Sources of resistance to wilt and root-rot of chickpea. *INT Chickpea Newsletter*, **3**: 11-13

Hassanein, A.M.; El-Morsy, G.A. and Abou-Zeid, N.M. 1996. Biological control of damping-off, root-rot and wilt diseases of lentil. Proceedings du Symposium Regional sur les Maladies des cereals et des Legumineuses, pp.369-374.

Kloepper, J.W. 1997. Current status and future trends in biocontrol research and development. The U.S. International Symposium on Clean Agriculture. Sapporo: OECD, pp. 49-52.

KIoepper, J.W.; Lifshitz, R. and Zablotowicz, R.M. 1989. Free-living bacterial inocula for enhancing crop productivity. *Trends Biotechnol*., **7**: 39-44.

Lane, D.J. 1991. 16S/23S rRNA sequencing. Pages: 115-175. In: *Nucleic Acid Techniques in Bacterial Systematics*. Stackebrandt, E. and Goodfellow, M. (eds.). Chichester, John Wiley & Sons.

Meyer, S.L.F. 2003. United States department of agriculture-agricultural research service research programs on microbes for management of plant-parasitic nematodes. *J. Pest Manag. Sci*., **59**: 665-670.

Marlow, J.L. and Linderman, R.G. 1996. Antibiosis of gramicidin from *Bacillus brevis* on fungal pathogens and bacterial antagonists. *Phytopathology*, **86**: 54.

Nakkeeran, S.; Fernando, D.W.G. and Siddiqui, A.Z. 2005. Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. Pages: 257-296. In: *PGPR: Biocontrol and Biofertilization*. Siddiqui, Z.A. (ed.). Springer, Dordrecht, The Netherlands.

Plaats-Niterink, V.A.J. 1981. Monograph of the Genus *Pythium*. Studies in Mycology No. 21 Central bureau for Schimmelculture Barhn, The Netherlands, 242pp.

Pleczer, M.J. and Chan, E.C. 1971. *Laboratory Exercise in Microbiology*. McGraw Hill Book Co., Berlin, 356pp.

Podile, A.R. and Laxmi V.D.V. 1998. Seed bacterization with *Bacillus subtilis* AF1 increases phenylalanine aminonialyase and reduces the incidence of Fusarial wilt in pigeon pea. *J. Phytopathol*., **146**: 255-259.

Rodgers, P.B. 1989. Potential of biological control organisms as a source of antifungal compounds for agrochemical and pharmaceutical product development. *Pest Sci*., **27**: 155-164.

Sadi, M.S. and Ahmadzadeh, M. 2012. Effect of pH on stability, sunflower growth promotion and biocontrol potential of a talc-based formulation of *'Pseudomonas fluorescens'* UTPF61. *Australian J. Crop Sci.*, **6**(3): 112:118.

Salem, D.S.; Omar, E.A. and Khattab, A.M. 1990. Screening for resistance to root-rot wilt disease complex in chickpea (*Cicer arictum* L.). *Agric. Res. Rev*., **69**(3): 581-595.

Samasegaran, P.; Hoben, H. and Halliday, J. 1982. The NIFTAL (Nitrogen Fixation in Tropical Agricultural Legumes) Manual for methods in Legume-Rhizobium Technology. US Agency for International Development, College of Tropical Agriculture and Resources, University of Hawaii.

Sangita, B. and Shah, A.K. 2000. Biological control of Fusarial wilt of pigeon pea by *Bacillus brevis. Canad. J. Microbiol.*, **46**(2): 125-132.

Snedecor, G.W. and Cochran, W.G. 1990. *Statistical Methods*. 8th Ed. Iowa State Univ. Press, Ames, Iowa, U.S.A.

Sunita, C.; Eunice, J.A. and Woodward, S. 2010. Biological Control of *Fusarium oxysporum* f.sp. *lycopersici* on Tomato by *Brevibacillus brevis*. *J. Phytopathol*., **158**(7-8): 470-478.

Sneh, B.; Burpee, L. and Ogoshi, A. 1992. *Identification of Rhizoctonia species*. APS Press, USA, 133p.

Suslow, T.V. and Schroth, M.N. 1982. Rhizobacteria of sugar beets: effects of seed application and root colonization on yield. *Phytopathology*, **72**: 199-206.

Turner, J.T. and Backman, P.A. 1991. Factors relating to peanut yield increases after seed treatment with *Bacillus subtilis. Plant Dis*., **75**: 347-353.

Vivas, A.; Barea, J.M. and Azcón, R. 2005. Interactive effect of *Brevibacillus brevis* and *Glomus mosseae*, both isolated from Cd contaminated soil, on plant growth, physiological mycorrhizal fungal characteristics and soil enzymatic activities in polluted soil. *Environ. Pollut.*, **134**(2): 257-266.

Waller, R.A. and Duncan, B.D. 1969. A way for the symmetric multiple comparison Problem. *Amer. Stat. Assoc. J*., **3**: 1485-1503.

Weller, D.M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Phytopathology*, **26**: 379-407.

Zavaleta, M.E. 2000. Alternativa de manejo de lasenfermedades de lasplantas. *Rev. Mex. de Fitopatol*., **17**: 201:207.

(Received 25/06/2012;
in revised form 24/07/2012)

تقييم بعض المستحضرات البكتيرية المستخدمة لمقاومة مرض عفن الجذور فى البسلة

أمل محمد عمر* ، عبير المرسى أحمد الحديدى**
* وحدة الميكروبيولوجي – مركز بحوث الصحراء.
** وحدة أمراض النبات – مركز بحوث الصحراء.

تُصاب البسلة بأمراض أعفان الجذور وموت البادرات التي تسبب خسائر شديدة فى المحصول، ونظراً لتباين المسببات المرضية المختلفة المسببة لذلك المرض وحساسيتها المختلفة للمبيدات الفطرية المستخدمة فى المكافحة وعدم جدواها أحياناً بالإضافة للتأثيرات البيئية الناشئة عن استخدامها فقد هدف هذا البحث لدراسة إمكانية المكافحة الحيوية لتلك الممرضات. اختبرت القدرة التضادية للعديد من العزلات البكتيرية المعزولة من منطقة الجذور المحيطة لنباتات البسلة على خمسة فطريات هى (*Pythium* sp., *Sclerotium rolfsii*, *Macrophomina phaseolina*) *Rhizoctonia solani* and *Fusarium solani*. تم إختيار أفضل العزلات وتعريفها على أنها *Brevibacillus brevis* . حملت البكتريا على ثلاث تراكيب جافة هى (Cellulose-clay, Talc-glucose and Talc-yeast). وقد لوحظ أن حيوية البكتيريا على هذه التراكيب استمرت لأكثر من ستة أشهر وذلك عند درجة حرارة الغرفة. تم تقييم قدرة البكتيريا المنتخبة المحملة على الثلاث تراكيب لمكافحة مرض عفن الجذور لنبات البسلة تحت ظروف الصوبة والحقل. أوضحت النتائج أن شدة الاصابة بعفن الجذور تزداد فى حالة التربة المعدية بخليط من الفطريات الممرضة وأن جميع المستحضرات البكتيرية للمكافحة المستخدمة أحدثت خفضاً معنوياً لأمراض موت البادرات قبل وبعد الظهور فوق سطح التربة وعفن الجذور. كانت أفضل النتائج عند استخدام البكتيريا (*B. brevis*) محملة على Cellulose-clay. انعكست قدرة البكتيريا المحملة على الثلاث تراكيب المختلفة فى خفض الإصابة على الزيادة فى قياسات النمو المختلفة للبسلة. حيث أدت إلى زيادة الصفات الخضرية للنبات بدرجة كبيرة تصل إلى ٥٣%.

ومن ثم توصي الدراسة على استخدام مستحضر البكتيريا *B. brevis* المحملة على أى من تلك التراكيب الجافة كأحد عوامل المكافحة الحيوية لمعاملة بذور البسلة قبل الزراعة للحد من أمراض أعفان الجذور وموت البادرات.