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Performance of some Antagonistic Bacteria in Minimizing Occurrence of Peanut Damping-off, Root- and Pod-Rot Diseases

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Seventeen bacterial isolates from soil, rhizosphere, geocarposphere, peanut roots and pegs beside three bioagents (*Bacillus subtilis*, *Pseudomonas putida* and *P. fluorescens*) were used to study their antagonistic effects on the casual pathogens of peanut damping-off, root- and pod-rots (*Fusarium solani*, *F. moniliforme*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotium rolfsii*). *In vitro* evaluation revealed that only nine isolates caused moderate to strong inhibition to the tested pathogens. *P. fluorescens* (Pf.5) show ever, gave the highest significant antagonistic effect against the five pathogens on PDA followed by *B. subtilis* (Bs.1), *P. putida* (PP) and *Brevibacterium casei* (S.5). In greenhouse and field experiments, the most effective isolates in reducing peanut damping-off, root- and pod-rot diseases were *P. fluorescens* (Pf.5) followed by *B. subtilis* (Bs1) and *B. casei* (S.5). Also the highest total peanut pod yield in the two seasons (2012 and 2013) was obtained by *B. subtilis* (Bs1) followed by *P. fluorescens*. The results confirmed the ability of some bioaegents to be near to the action of fungicide (Rizolex-T) in reducing peanut damping-off, root- and pod-rot diseases. In this respect, in greenhouse and field trials *P. fluorescens* (Pf 5.) effect was the nearest one to fungicide effect in minimizing of peanut damping-off, root- and pod- rots followed by *B. casei* (S.5) and *B. subtilis* (Bs1).

Keywords: *Bacillus amyloliquefaciens*, *B. subtilis*, bioagent, *Brevibacterium casei*, fungicides, *Pseudomonas fluorescens* and *P. putida*.

Peanut (*Arachis hypogaea* L.) is one of the most export and locally consumed crops in Egypt. Pod- and root-rot diseases are among the most destructive diseases attacking peanut in Egypt. They cause serious quantitative and qualitative losses in peanut yield, therefore growing peanuts in infested soil becomes unprofitable (Hussin, 2005 and Mahmoud *et al.*, 2006a).

Due to the environment need to more regulations and the weaknesses of chemical control, the biological control has become more attractive (Cook, 1993). Cook and Baker (1983) defined biological control as the reduction of the amount of inocula or disease-producing activity of a pathogen accomplished by or through one or more organisms other than humans. Bacteria, especially plant growth-promoting rhizobacteria (PGPR), can suppress a variety of root and vascular disease caused by soilborne pathogens (Meena *et al.*, 2001 and Mishra *et al.*, 2013). *Bacillus* and *Pseudomonas* were considered as important genera of these bacteria (Meena *et al.*, 2001; Ibrahim *et al.*, 2008 and Mishra *et al.*, 2013). Application of *B. subtilis* under

greenhouse and field conditions, reduced damping-off and root-rot diseases caused by *R. solani, Pythium* spp., *Phytophthora capsici, Macrophomina phaseolina* and *Fusarium oxysporum* (Nemec *et al.*, 1996; Gabr *et al.*, 1998 and Hussin, 2011). *B. subtilis* was used to control Fusarium wilt or crown rot diseases (Nemec *et al.*, 1996 and Mosa *et al.*, 1997). While, application of *B. subtilis* reduced crown rot caused by *Aspergillus niger* and root-rot caused by *R. solani* (Podile and Prakash, 1996 and Hussin, 2011).

Pseudomonas fluorescens is considered as an important antagonistic bacterium where it was effective against several soilborne pathogens in field and greenhouse (Mosa *et al.*, 1997; Karunanithi *et al*., 2000 and Jayashree *et al*., 2000). In peanut, under greenhouse tests, 99% of plants were protected from *Sclerotium rolfsii* infection when inoculated with *P. fluorescens* and in field trial, this treatment increased total pod yield by 65% and resulted in 18% greater survival of plants up to harvest (Sheela *et al*., 1998). Seed treatment or soil application of *P. fluorescens* strain (Pf 1) effectively reduced peanut root-rot diseases and showed the maximum of antagonistic effect produced in vitro by HCN, salicylic acid siderophore and beta–1,3 glucanase (Meena *et al*., 2001; Shanmugam *et al*., 2002 & 2003; Ibrahim *et al*., 2008 and Hussin, 2011).

This work was carried out to study the effect of some bacterial isolates in reducing of peanut damping-off, root- and pod-rot diseases under greenhouse and field conditions.

Materials and Methods

1. Isolation and identification of the causal fungal organisms:

The fungal isolates, which used throughout this study, were previously isolated by the author from diseased peanut roots and pods and their pathogenic capabilities were determined (Mahmoud *et al*., 2006a).

2. Preparation of fungal inoculum:

Inocula of *Fusarium solani, F. moniliforme, Macrophomina phaseolina, Rhizoctonia solani* and *Sclerotium rolfsii* were prepared using sorghum - coarse sand - water (2:1:2 v/v) medium. The ingredients were mixed, bottled and autoclaved for 2 hours at 1.5 air pressure. The sterilized medium was inoculated using agar discs, obtained from the periphery of 5-day-old colony of each of the tested fungi. The inoculated bottles were incubated at 28°C for 15 days.

3. Soil Infestation:

Inoculum of *F. solani, F. moniliforme, M. phaseolina, R. solani* or *S. rolfsii* was mixed thoroughly with soil surface of each pot, at the rate of 2% (w/w) and was covered with a thin layer of sterilized soil. The infested pots were irrigated and kept for 7 days before sowing.

4. Disease assessment:

Disease assessment was recorded as percentage of damping-off (pre- and post-emergence) after 15 days and 45 from sowing using the following formula:

$$\text{Pre-emergence (\%)} = \frac{\text{Number of non emerged seedlings}}{\text{Number of sown seeds}} \times 100$$

$$\text{Post-emergence (\%)} = \frac{\text{Number of dead emerged seedlings}}{\text{Number of sown seeds}} \times 100$$

Damping-off (%)= Pre-emergence (%) + Post-emergence (%)

Percentages of infected plants by root-rot and survived healthy plants were estimated after uprooting (120 days from sowing) as follows:

$$\text{Root-rot (\%)} = \frac{\text{Number of plants with root-rot}}{\text{Number of sown seeds}} \times 100$$

$$\text{Healthy plants (\%)} = \frac{\text{Number of survived healthy plants}}{\text{Number of sown seeds}} \times 100$$

Plants in individual pots/plots were dug and inverted based on an optimum maturity index. Pods were threshed, air-dried for ten days, weighted and then examined for pod-rot incidence. Percentage of pod-rot was recorded. Four categories for apparent symptoms of pod-rots beside the healthy pods were adopted according to Satour *et al.* (1978): a) Rhizoctonia rot, pods with dry brown lesion; b) Fusarium rot, pods with pink discoloration and c) complex pod-rot with general breakdown resulting from many fungi and all of types were calculates as follows:

$$\text{Pod-rot categories (\%)} = \frac{\text{Number of rotted pod category}}{\text{Number of total pods}} \times 100$$

5. Source of known antagonistic bacteria:

Two known isolates of *P. fluorescens*; Pf5 (Howell and Stipanovic, 1979) *P. putida*; PP and *B. subtilis*; Bs1 (El-Hadidy, 2003) were obtained from Culture Collection of Dept. of Plant Pathol., Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

6. Identification of unknown antagonistic bacteria:

The unknown bacterial isolates which used in field trials were identified by using Biolog technique (Carbon and amino acid utilization profile of microorganisms) Biolog TM micro-plates (Biolog, Inc., 3938 Trust way, Hayward, CA94545, USA) at the Unit of Identification of Microorganisms Plant Pathol. Res. Inst., Agric. Res. Centre (ARC), Giza, Egypt.

7. Isolation of antagonistic bacteria:

Bacterial isolates were isolated from soil and different samples of peanut plants according to Mickler *et al.* (1995). Samples of roots, pegs and pods with adhering soil were collected from different fields of Ismailiya, Nubariya and Sharkiya. Adhering soils were carefully brushed off from each organ. Ten grams of soil or

adhering soil were suspended in 90 ml sterile water, shaken for 30 min., and serial dilutions to 10^6 were prepared. Dilutions from each sample transferred on nutrient agar medium (NA) and King's B medium (KB) (King *et al.,* 1954). Peanut organ samples were cut to small pieces (1 cm), then sterilized and transferred on nutrient agar media (NA) and King's B media (KB). Plates were incubated at 27°C for 2-4 days then individual colonies was picked up, purified and stored at 4°C on nutrient agar medium.

8. In vitro evaluation of antagonists:

All bacterial isolates were tested by streaking the bacteria in the centre of culture plate containing PDA medium, and then incubated for 48 hours at 25°C. Plates were inoculated with the pathogen by placing two 5-mm-disks, from five days old culture, 3 cm. apart from both sides of bacterial growth. Plates were incubated at 25°C for 5 days and fungal colony diameter in the presence or the absence of bacteria was measured. The inhibition zone between bacteria and the pathogen was measured as described by Maurhofer *et al.* (1995).

9. Preparation of bacterial inoculum:

Bacterial suspensions ($1x10^6$ cfu / ml) were prepared by dilution plate assay as described by Callan *et al.* (1990).

10. Methods of application:

The antagonistic bacteria (10^6 cfu/ml) were applied as seed treatment before sowing by suspension in 0.1 % gum Arabic were mixed thoroughly with peanut seeds and re-applied as foliar spray after 40 days from sowing. While fungicide Rizolex-T 50% (Tolclofos-methyl 20% + thiram 30%) were applied as seed treatment at the rate of 3g/kg seed and re-applied as a soil treatment after 40 days at the rate of 3 kg/fed.

11. Evaluation of antagonists under greenhouse conditions:

Pot experiments were carried out during 2011 season in order to study the effect of selected nine antagonistic bacteria isolates in controlling damping-off, root- and pod-rots incidence (%). The experiment was carried out at Agric. Res. Centre, Giza. Peanut seeds, (Giza 6 cv.) were used for sowing in 50 cm-diameter pots containing sterilized soil previously infested with mix of pathogens (2% w/w). Ten seeds were sown per each pot, five replicate (pots) were used for each treatment. Disease assessment was recorded as percentage of damping-off, root- and pod-rots and survival plants at 15, 45 days after planting and during the harvesting time as previously mentioned.

The bacterial isolates applied as seed dressing at sowing and as foliar spray after 40 days from sowing (at pegging time of peanut) and the fungicide Rizolex-T 50% was applied as mentioned before.

12. Evaluation of antagonists in the field:

The field experiments were performed at Nubariya, during 2012 and 2013 seasons to study the effect of five antagonistic bacterial isolates in controlling damping-off, root- and pod-rots incidence (%). The selected fields were known to have natural infestation with root- and pod-rot pathogens. The antagonistic bacteria were applied as seed dressing at sowing and were foliar spray after 40 days at

pegging time of peanut and the fungicide Rizolex-T 50% was applied also as previously mentioned. Cultural practices and fertilization for the peanut crop were applied as recommended. Seeds were sown on the first week of May with 10 cm spacing between halls. The experimental unit area was 10.5 m^2 (1/400 fed.).The treatments were arranged in completely randomized block design with four replicates. Disease assessment was recorded as mentioned before.

12. Statistical analysis:

The data were statistically analysed by analysis of variance (ANOVA) using the Statistical Analysis System (Anonymous, 1996). Means were separated by least significant difference (LSD) test at $P \leq 0.05$ levels.

Results

1. Bacterial isolates:

Seventeen bacterial isolates (Table 1) were collected from the soil, rhizosphere, geocarposphere, peanut roots and pegs obtained from different fields in three locations in Egypt.

Table 1. List of bacterial isolates obtained from peanut samples and soil from different locations

Isolate code	Source	Location
N.1	Soil	Nubariya
N.2	Soil	Nubariya
N 3	Rhizosphere	Nubariya
N.4	Rhizosphere	Nubariya
N.5	Root	Nubariya
N.6	Geocarposphere	Nubariya
Sh.1	Soil	Sharkiya
Sh.2	Rhizosphere	Sharkiya
Sh.3	Rhizosphere	Sharkiya
Sh.4	Geocarposphere	Sharkiya
Sh.5	Root	Sharkiya
S.1	Soil	Ismailiya
S.2	Soil	Ismailiya
S.3	Peg	Ismailiya
S.4	Rhizosphere	Ismailiya
S.5	Peg	Ismailiya
S.6	Root	Ismailiya

2. Screening of bacterial antagonists, in vitro:

Seventeen bacterial isolates in addition to the three known bioagents (*Bacillus subtilis*, *Pseudomonas putida* and *P. fluorescens*) were *in vitro* evaluated for their antagonistic effect against *F. solani*, *F. moniliforme*, *M. phaseolina*, *R. solani* and *S. rolfsii* on PDA medium (Table 2). Only nine isolates caused moderate to strong inhibition to the four tested isolates.

Table 2. Screening of various bacterial isolates to determine their antagonistic effect against five pathogenic fungi to peanut

Bacterial isolate	Inhibition zone*				
	Rhizoctonia solani	*Fusarium moniliforme*	*Fusarium solani*	*Macrophomina phaseolina*	*Sclerotium rolfsii*
B. subtilis (Bs.1)	++	++	+	++	++
P. putida (PP)	++	+	++	+	++
P. fluorescens	++	++	++	++	++
N.1	-	+	-	+	-
N.2	-	-	-	-	-
N.3	+	++	+	++	+
N.4	-	-	-	-	-
N.5	+	++	++	++	++
N.6	-	-	-	-	+
Sh.1	-	-	-	-	-
Sh.2	+	-	+	-	-
Sh.3	+	+	+	+	+
Sh.4	-	-	-	+	-
Sh.5	+	++	++	++	++
S.1	-	+	-	-	-
S.2	+	-	-	-	-
S.3	+	++	++	+	++
S.4	-	-	-	-	-
S.5	++	++	++	++	++
S.6	-	-	+	-	-

* Inhibition of pathogens is expressed as the distance (mm) between pathogen mycelium and bacteria on potato dextrose agar (PDA), inhibition zone < 20 mm (+), inhibition zone $\geq$ 20 (++) while (-) no inhibition zone.

Pseudomonas fluorescens (Pf.5) gave the high significant antagonistic effect against the tested pathogens on PDA medium followed by *B. subtilis* (Bs.1), *P. putida* (PP) and (S.5) (Table 3). Meanwhile, S.3, N.5, N.3, Sh.5 and Sh.4 gave moderate effect in their inhibition of tested pathogens growth. While, both of N.3 and Sh.4 had little effect.

3. Evaluation of antagonistic bacteria under greenhouse conditions:

3.1. On peanut damping-off and root-rot incidence under artificial conditions:

Nine selected bacterial isolates beside standard consisting of Rizolex-T (fungicide) were evaluated under greenhouse conditions.

Table 3. Antagonistic effect of various bacterial isolates against five pathogenic fungi to peanut

Bacterial isolate	Inhibition zone*				
	R. solani	*F. moniliforme*	*F. solani*	*M. phaseolina*	*S. rolfsii*
P. fluorescens (Pf.5)	26	36	35	29	33
P. Putida (PP)	22	31	30	25	28
B. subtilis (Bs.1)	24	33	32	27	31
N.3	11	16	16	12	15
N.5	16	23	22	18	21
Sh.3	10	14	14	11	13
Sh.5	18	25	24	20	23
S.3	14	20	19	16	18
S.5	19	27	26	21	25
L.S.D. at 5%	1.85	2.14	2.04	2.02	2.07

* Inhibition of pathogens is expressed as the distance (mm) between pathogen mycelium and bacteria on potato dextrose agar (PDA).

Results in Table (4) show that, all tested bioagents have significant effect in reducing damping-off and peanut root-rot compared to the control except S3and Sh3. In this respect, the most effective isolates was *P. fluorescens* (Pf.5) followed by *B. subtilis* (Bs.1) and (S.5).

Table 4. Effect of antagonistic bacterial isolates on peanut damping-off and root-rot incidence (%) under artificially infested conditions

Bacterial isolate	Damping-off		Total (%)	Root-rot (%)	Healthy survival (%)
	Pre-emergence	Post-emergence			
P. fluorescens (Pf. 5)	8	6	14	10	76
P. putida (PP)	10	10	20	12	68
B. subtilis (Bs.1)	8	8	16	14	70
N.3	10	10	20	14	66
N.5	10	10	20	16	64
Sh.3	12	12	24	14	62
Sh.5	12	10	22	14	64
S.3	14	12	26	16	58
S.5	8	8	16	12	72
Rizolex-T	6	6	12	8	80
Control *	16	14	30	20	50
L.S.D. at 5%	2.4	3.9	6.1	4.9	8.3

* Treatment without bacteria.

Moreover *P. fluorescens* (Pf.5) followed by *P. putida* (PP) and (S.5) gave the highest effect in reducing peanut root-rot compared to other tested bioagents. On the other hand, S.3 isolate gave the lowest effect in reducing damping-off and peanut root-rot diseases compared with the other tested bioagents.

Data also showed that, *P. fluorescens* (Pf 5.) was the nearest one to fungicides effect in reduction of peanut damping-off and peanut root-rots followed by (S.5) since there were no significant differences between their effect and Rizolex-T (Table 4).

3.2. On peanut damping-off and root-rot incidence (%) under field conditions:

Six selected bacterial isolates beside standard consisting fungicide (Rizolex-T) were evaluated under field conditions during two successive seasons (2012 and 2013). Data in Table (5) indicate that all tested bioagents have significant effect in reducing of damping-off and root-rot compared with the control during the two successive seasons. *Pseudomonas fluorescens* (Pf.5) followed by both of *Brevibacterium casei* (S.5) and *P. putida* (PP) were the most effective isolates in reducing peanut damping-off. Meanwhile, *P. fluorescens* (Pf.5) followed by *B. casei* (S.5) and *B. subtilis* (Bs.1) gave the highest effect in reducing peanut root-rot compared with other tested bioagents during 2012 and 2013 seasons. While, *B. amyloliquefaciens* (N.5) isolate gave the lowest effect during the two growing seasons.

The effect of *P. fluorescens* (Pf.5) was the nearest one to fungicide effect in reduction of peanut damping-off and peanut root-rot, followed by *B. casei* (S.5) and *B. subtilis* (Bs.1) compared to the other tested bioagents, although there were no significant differences between their effect and the fungicide (Table 5).

Table 5. Effect of six antagonistic bacterial isolates on peanut damping-off and root-rot incidence under field conditions, during 2012 and 2013 seasons

Bacterial isolate	Season of 2012			Season of 2013		
	Damping-off (%)	Root-Rot (%)	Survival (%)	Damping-off (%)	Root-Rot (%)	Survival (%)
P. fluorescens (Pf.5)	8.08	11.44	80.48	9.42	12.17	78.41
P. putida (PP)	10.74	15.01	74.25	11.29	16.27	72.44
B. subtilis (Bs.1)	10.89	12.96	76.15	12.32	13.10	74.58
B. casei (S.5)	10.67	12.68	76.65	11.23	12.73	76.04
B. amyloliquefaciens (N.5)	14.61	16.1	69.29	15.42	17.97	66.61
B. amyloliquefaciens (Sh.5)	11.78	14.42	73.80	12.32	16.14	71.54
Rizolex-T	6.35	8.41	85.24	7.42	9.56	83.02
Control (without bacteria)	16.11	18.25	65.64	17.26	19.72	63.02
L.S.D. at 5%	1.23	1.21	1.44	1.22	1.23	1.45

3.3. On peanut pod-rots incidence (%) under artificial conditions:

Nine selected bacterial isolates were evaluated as seed treatment at sowing time and later as foliar spray at pegging time of peanut. Results in Table (6) show that all tested bacterial isolates significantly reduced incidence of pod-rot categories. The most effective isolates were Pf.5 followed by S.5 and Bs.1. Meanwhile, isolates PP, Sh.5 and N.5 caused moderate effect and isolates S.3, Sh.3 and N.3 caused slight effect compared to non-treated control. On the other hand, the action of *P. fluorescens* (Pf.5) was the nearest one to fungicide effect in reduction of peanut pod-rot diseases followed by (S.5) and *B. subtilis* (Bs.1) compared to the other tested bioagents (Table 6).

Table 6. Effect of nine antagonistic bacterial isolates on pod-rots incidence (%) under artificial infested conditions

Bacterial isolate	Dry brown lesion	Pink discoloration	General breakdown	Apparent healthy
P. fluorescens (Pf. 5)	9.00	0.75	11.86	78.39
P. putida (PP)	10.81	1.25	14.10	73.84
B. subtilis (Bs.1)	9.84	0.91	13.25	76.00
N.3	11.73	3.71	16.13	68.43
N.5	12.03	2.06	14.78	71.13
Sh.3	12.38	3.39	15.96	68.28
Sh.5	10.73	2.71	15.13	71.43
S.3	13.03	3.06	15.78	68.13
S.5	10.46	1.05	12.19	76.30
Rizolex-T	8.08	0.06	10.24	81.62
Control (without bacteria)	16.17	5.78	16.84	61.22
L.S.D. at 5%	1.03	0.24	1.27	2.54

3.4. On peanut pod-rots incidence (%) under field conditions:

Data in Table (7) show that, all tested antagonistic bacteria significantly reduced incidence of all categories of pod-rots compared with the control. The most effective isolates were Pf.5 followed by Bs1 and S.5 in both growing seasons, while N.5 and Sh.5 gave the lowest effect in this respect. In the same time, the effect of *P. fluorescens* (Pf.5) was the nearest one to fungicides effect in reduction of peanut pod-rots followed by *B. subtilis* (Bs.1) compared to other tested bioagents.

3.5. On peanut yield under field conditions:

Data presented in Table (8) demonstrate that, all tested bioagents caused significant increases in pod yield than the control pod yield. Percentage of increases however reached (9.0-16.18) and (11.8-18.39) in the first and second seasons respectively. The highest peanut pod yield in the two seasons obtained with *B. subtilis* (Bs.1), followed by *P. fluorescens* and *P. putida* (PP). While, *B. amyloliquefaciens* (Sh.5) gave the lowest peanut pod yield and the lowest effect

Table7. Effect of six antagonistic bacterial isolates on peanut pod-rots incidence (%) under field conditions during 2012 and 2013 seasons

Bacterial isolate	Season of 2012			
	Dry brown lesion	Pink discoloration	General breakdown	Apparent healthy
P. fluorescens (Pf.5)	6.89	0.35	10.42	82.84
P. putida (PP)	9.78	0.98	13.82	75.42
B. subtilis (Bs.1)	9.20	0.52	11.83	78.45
B. casei (S.5)	8.14	0.80	12.87	78.19
B. amyloliquefaciens (N.5)	11.82	1.30	14.82	72.06
B. amyloliquefaciens (Sh.5)	9.64	2.13	14.92	73.31
Rizolex-T	5.21	0.44	8.82	85.53
Control	14.70	4.29	16.88	64.13
L.S.D. at 5%	0.69	0.43	0.52	1.20
Bacterial isolate	Season of 2013			
P. fluorescens (Pf.5)	8.07	0.50	10.42	81.01
P. putida (PP)	9.30	0.26	13.79	76.65
B. subtilis (Bs.1)	9.20	0.55	11.34	78.92
B. casei (S.5)	9.26	0.25	12.49	78.01
B. amyloliquefaciens (N.5)	12.62	0.57	14.85	71.96
B. amyloliquefaciens (Sh.5)	14.83	3.29	16.37	65.52
Rizolex-T	6.86	0.43	8.77	83.94
Control	14.70	3.63	15.89	65.77
L.S.D. at 5%	0.71	0.05	0.56	1.24

Table 8. Effect of antagonistic bacterial isolates on peanut yield and loss of yield under field conditions during two seasons 2009 and 2010

Bacterial isolate	Season of 2012		Season of 2013	
	Yield (Ton)	Increases (%)*	Yield (Ton)	Increases (%)
P. fluorescens (Pf.5)	1.140	15.27	1.127	17.76
P. putida (PP)	1.129	14.16	1.114	16.41
B. subtilis (Bs.1)	1.149	16.18	1.133	18.39
B. casei (S.5)	1.121	13.35	1.108	15.78
B. amyloliquefaciens (N.5)	1.111	12.34	1.103	15.26
B. amyloliquefaciens (Sh.5)	1.078	9.00	1.063	11.08
Rizolex-T	1.227	24.07	1.183	23.62
Control	0.989	--	0.957	--
L.S.D. at 5%	0.079		0.088	

* Increases related to the control.

on increase yield in the two successive seasons 2012 and 2013 compared with the other bioagents. On the other hand, differences between yield weight of Rizolex-T and this of the bacterial isolates were significant, except *B. subtilis* (Bs.1), in the first season, while they were no significant in the second season.

Discussion

The antagonistic effects of some bacterial isolates, which were obtained from soil, rhizosphere and peg of peanut and three standard isolates from *Bacillus subtilis*, *Pseudomonas putida* and *P. fluorescence* was studied. Most of all tested bioagents have significant effect in reducing damping-off, root- and peanut pod-rot diseases. In this respect the most effective isolates were *P. fluorescens*, *B. subtilis* and *Brevibacterium casei* S.5. This results are in agreement with Lazzaretti *et al.* (1994); Ashour and Afify (1999); Mahmoud *et al.* (2006b) and Ibrahim *et al.* (2008) who stated that certain strains of *Bacillus* appear to be most effective as a biological control agent through inhibiting the mycelial growth of plant pathogenic fungi. Also, *P. fluorescens* was found to be the most effective biocontrol agent against various soilborne diseases caused by *Fusarium oxysporum, Rhizoctonia solani, Pythium ultimum, Macrophomina phaseolina* and others (Jayashree *et al.,* 2000; Meena *et al.*, 2001; Mahmoud *et al.,* 2006b; Ibrahim *et al.,* 2008 and Hussin, 2011). While, *Brevibacterium* spp. consider one of the plant growth promoting bacteria (Collins, 2006 and Faisal and Hasnain, 2004 & 2006).

Many studies in this respect showed that certain *P. fluorescens* and *B. subtilis* isolates were effective rhizobacteria for suppression of damping-off, root- and pod-rots, when they recorded great inhibition to the *in vitro* hyphal growth. This suggested that their biocontrol activity had been associated with the production of certain substance such as enzymes, phenazines, pyrrole type antibiotics, pyo-compounds, indole derivatives peptide antibiotic, moenomycins, difficidins, bacillomycins and bacillaenes as reported by Battu1 and Reddy (2009) and Awais *et al.* (2010).

The ability of antagonistic isolates to inhibit growth of the five pathogens, *in vitro* and to produce certain secondary metabolites has been claimed to be important for biological control (Defago and Hass 1990 and Maurhofer *et al.,* 1995). Antibiosis is well documented for *P. fluorescens* (Pf5) against soil borne pathogens (Howell and Stipanovic, 1979). Moreover, certain strains of *Pseudomonas* can produce several siderophores such as pyoverdine (pseudobactin) pyochelin, pyrollnitrin (antibiotic), salicylic acid, HCN and lytic enzymes (Leeman *et al.,* 1996; De Meyer and Hofte, 1997; Karunanithi *et al.,* 2000 and Meena *et al.*, 2001).

On the other hand, several biocontrol agents such as species of *Pseudomonas* showed induce resistance activity in several plants (Liu *et al*., 1997). Vanwees *et al*. (1997) elucidate the molecular mechanisms responsible for this type of defence reaction. Also, *B. subtilis* can induce resistance in peanut to rust disease by stimulation of phytoalexins production and increasing the activity of lytic enzymes (Sailaja and Podile, 1998 and Sailaja *et al.,* 1998). However, treated seed or soil with powder formulation of *P. fluorescens* strain (Pf.1) effectively reduced peanut root-

rot compared to other strains and showed the maximum of antagonistic effect produced *in vitro* by HCN, salicylic acid siderophore and beta–1,3 glucanase (Meena *et al.*, 2001 and Shanmugam *et al.*, 2002 & 2003). Mahmoud *et al.* (2006b) and Ibrahim *et al.* (2008) found that, *B. subtilis* (BS) and *P. fluorescens* (Pf 5), caused strong inhibition on the mycelium growths of the four tested pathogens (*R. solani, S. rolfsii, F. solani* and *M. phaseolina*).

The presented study confirmed the ability of some tested bioaegents to be near the fungicides efficiency in reducing damping-off, peanut root- and pod-rot diseases as well as increasing the total pod yield.

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فاعلية بعض العزلات البكتيرية المضادة فى خفض نسبة الإصابة بأمراض موت البادرات وأعفان الجذور والثمار على الفول السوداني

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تم اختبار كفاءة التضاد لسبع عشر عزلة بكتيرية معزولة من التربة وريزوسفير وجيوكربوسفير ومشاجب الفول السوداني إلي جانب ثلاث عزلات وهي *Bacillus subtilis*, *Pseudomonas fluorescens*, *P. putida* وتقدير تأثيرها على الفطريات المسببة لأعفان جذور الفول السوداني المتسبب عن الفطريات (*Fusarium solani*, *F. moniliforme*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotium rolfsii*). وقد أظهرت النتائج أن تسعه عزلات كان لها تأثير من قوي إلي متوسط على تثبيط الفطريات المختبرة. وأظهرت عزلة *Pseudomonas fluorescens* (Pf.5) أعلي قدرة تضادية على الفطريات المختبرة على بيئة PDA يليها *Bacillus subtilis* (Bs.1) و*P. putida* (PP) و*Brevibacterium casei* (S.5) وفي تجارب الصوبة والحقل كانت عزلة *P. fluorescens* (Pf.5) أكثر العزلات تأثيراً علي خفض نسبة الإصابة بموت البادرات و أعفان جذور و ثمار الفول السوداني تلاها عزلة *Brevibacterium casei* (S.5) و *Bacillus subtilis* (Bs.1). أما بالنسبة لإنتاجية المحصول من ثمار الفول السوداني سجلت المعاملة بعزلة *Bacillus subtilis* (Bs.1) أعلي إنتاجية تلاها عزلة *P. fluorescens* (Pf5) في كلا الموسمين ٢٠١٢ و٢٠١٣. كما أظهرت النتائج المتحصل عليها قدرة بعض العزلات المختبرة علي الاقتراب من قدرة المبيد (Rizolex-T) في خفض الإصابة بموت البادرات وأعفان جذور وثمار الفول السوداني في تجارب الصوبة والحقل وكانت العزلة *P. fluorescens* (Pf.5) هي الأقرب في معظم الحالات لقدرة المبيد في المقاومة وتلاها كلاً من *Brevibacterium casei* (S.5) و*B. subtilis* (Bs.1) في كلا الموسمين ٢٠١٢ و٢٠١٣.