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THE ABILITY OF SOME ANTAGONISTIC BACTERIA ON CONTROL OF PEANUT ROOT ROTS DISEASES COMPARED TO FUNGICIDES EFFICIENCY

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ABSTRACT: ***Nineteen bacterial isolates obtained from known sources, soil, rhizosphere and peanut plants were evaluated in vitro, for their antagonistic effect against the pathogens causing peanut root rots. Only eight isolates B. subtilis (Bs1) P. fluorescens (Pf 5), (N.3), (N.5), (Sh.4), (Sh.5), (S.3) and (S.5) caused moderate to strong inhibition to the four tested pathogens (R. solani, S. rolfsii, F. solani and M. phaseolina). Pseudomonase fluoressens (Pf 5) followed by Bacillus subtilis (Bs1) and Bacillus sp (S.5) caused the widest inhibition zone almost to tested pathogens. In greenhouse and field trials, the most effective isolates in reducing peanut root rots were P. fluorescens (Pf 5) followed by B. subtills (Bs1) and Bacillus sp (S.5). Regarding to peanut pod yield, the highest total peanut pod yield in the two seasons (2006 and 2007) was obtained with B. subtills (Bs1) followed by P. fluorescens and Bacillus sp (S.5). The obtained data clearly showed the ability of some tested bioaegents to be near to the fungicides efficiency (Rezolex-T) in reducing damping-off and peanut root rot diseases. In this respect, in greenhouse trial P. fluorescens (Pf 5) was the nearest one to fungicides efficiency in reducing peanut pre-emergence damping-off (87.5 %), while B. subtills (Bs1) gave 100 % of fungicides efficiency in reducing peanut post-emergence damping-off. P. fluorescens (Pf.), B. subtills (Bs1) and Bacillus sp (S.5) were the nearest to fungicides efficiency in reducing peanut root rot (83.33 %). While in field trials P. fluorescens (Pf 5.) was the nearest one to fungicides efficiency in reducing peanut pre- and post-emergence damping-off and peanut root rot followed by Bacillus sp (S.5) and B. subtills (Bs1) compared to other tested bioagents during two seasons 2006 and 2007.***

Key words: ***Peanut, root rots, biological control, bioagents, Pseudomonase fluoressens, Bacillus subtilis and fungicides efficiency.***

INTRODUCTION

Peanut, (*Arachis hypogaea* L.) is one of the most export and locally consumed crops in Egypt. Root rots diseases are among the most destructive diseases attacking peanut in Egypt. It cause serious quantitative and qualitative losses in peanut yield, therefore growing peanuts in these soil becomes unprofitable (Al-Ahmer *et al.,* 1989 and Hussin, 2005).

Due to the environment need to more stringent regulations and the use of chemicals to control the plant diseases has always been an expensive remedy and may also reduce populations of beneficial microorganisms in soil, thus biological control has become more attractive (Cook. 1993). Plant growth–promoting rhizobacteria (PGPR) suppress a variety of root and vascular disease caused by soilborne pathogens (Jayashree *et al.*, 2000, Karunanithi *et al.,* 2000, Meena *et al.*, 2001, and Mahmoud 2004). *Bacillus* and *Pseudomonas* are considering the important genera of these bacteria (Sailaja and Podile, 1998, Karunanithi *et al.,* 2000, Meena *et al.*, 2001 and Mahmoud 2004). Certain strains of *Bacillus subtilis* are effective as biological control agents. Application of *B. subtilis* under greenhouse and field conditions, reduced damping–off and root rot diseases caused by *R. solani, Pythium* spp.*, Phytohpthora capsici, M. phaspolina* and *F oxysporum* (Asaka and Shoda, 1996, Mahmoud 2004, Hussin, 2005 and Mahmoud *et al.,* 2006). In peanut application of *Bacillus subtilis* has a reducing effect on crown rot caused by *Aspergillus niger*, foot rot caused by *Sclerotium rolfsii* and root rots caused by *Rhizoctiona solani* (Turner and Backman, 1991, Podile and Prakash, 1996, Hussin, 2005 and Mahmoud *et al.,* 2006).

***Pseudomonas fluorescens* is considered as an important group of the antagonistic bacteria where it was effective against several soilborne pathogens in field and greenhouse trails (Jayashree *et al.*, 2000 and Karunanithi *et al.,* 2000). In peanut, *Pseudomonas* strains showed *in vitro* antibiosis against the collar rot pathogen caused by *Aspergillus niger* and gave protection to groundnut seedlings against the disease (Sheela *et al.*, 1998). Seed treatment or soil application of powder formulation of *P. fluorescens* strain (Pf 1) effectively reduced peanut root rot compared to other strains and showed the maximum of antagonism effect produced *in vitro* by HCN, salicylic acid siderophore and beta–1,3 glucanase (Meena *et al.*, 2001, Shanmugam *et al.*, 2002 & 2003). Mahmoud (2004) found that, in greenhouse and field trials *P. fluorescens* (Pf 5) and *B. subtilis* significantly reduced incidence of all types of pod rots caused by *R. solani S. rolfsii, M. phaspolina, Fusarium* spp. and *Aspergillus* spp. and added that *B. subtilis* induced the highest pod yield of peanut. While, Hussin, (2005) and Mahmoud *et al.,* (2006) found that, in greenhouse trials the most effective isolates in reducing peanut damping-off, wilt and peanut root rot were *P. fluorescens* (Pf 5) followed by *B. subtills* (BS1)**

This work was carried out to study the effect of some bacterial isolates in reducing peanut root rots diseases under greenhouse and field conditions.

MATERIALS AND METHODS

1. Isolation of causal organisms:

Fungal isolates being used throughout this study were previously isolated by the authors from diseased peanut plants or pods, and their pathogenic capabilities were also proved (Mahmoud, 2004)

2. Preparation of fungal inoculum:

Inocula of isolates of *Fusarium solani*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *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 isolated fungi. The inoculated media were incubated at 28°C for 15 days and were then used for soil infestation.

3. Soil Infestation:

Inoculum of each isolate of *F. solani, M. phaseolina, R. solani* and *S. rolfsii* was mixed thoroughly with the 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

(A) Disease assessment was measured as percentages of pre- and post-emergence damping-off after 15 and 30 days from sowing, respectively. Percentages of pre- and post-emergence damping-off were calculated using the following formula:

$$\text{\% Pre- emergence} = \frac{\text{Number of non germinated seeds}}{\text{Number of sown seeds}} \times 100$$

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

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

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

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

(C) Percentages of treatment efficacy in reducing the diseases infection were calculated as follows:

$$\text{\% Treatment efficiency} = \frac{\text{Control - Treatment}}{\text{Control}} \times 100$$

% Bioagents efficiency to fungicides efficacy = $\frac{\text{Bioagents efficiency}}{\text{Fungicides efficiency}} \text{X } 100$

-In all cases, the percentage of diseases incidence was related to the percentage of viable seeds

5. Source of antagonistic bacteria

This study was conducted to investigate the efficiency of some antagonistic bacteria to reduce incidence of damping – off and root rot.

5.1. Source of known antagonistic bacteria:

Two known isolates of *Pseudomonas fluorescens* (Pf 5) (Howell and Stipanovic, 1979) and *Bacillus subtilis* (Bs1) (El-Hadidy, 2003) were obtained from Culture Collection of Department of plant Pathology, Faculty of Agriculure, Ain Shams University.

5.2. Isolation of antagonist's bacteria from peanut:

Bacterial isolates were isolated from the soil and different samples of peanut plants according to Mickler *et al.,* (1995). Samples of roots, pegs and pods were collected from different fields at Ismailia, Nobaria and Sharkya districts, peanut organs with adhering soil were placed in plastic bags and transferred to the laboratory. Adhering soil was carefully brushed off from each organ. Ten grams of soil or peanut samples were suspended in 90 ml sterile water, shaken for 30 min., and serial dilutions to 10^6 were prepared. Dilutions from each sample were planted on nutrient agar media (NA) and King's B media (KB) (King *et al.,* 1954). Plates were incubated at 27°C for 2- 4 days then individual colonies were picked up, purified and stored at 4°C on the appropriate medium.

5.3. Evaluation of antagonists, *in vitro*:

All bacterial isolates were tested by streaking the bacteria in the center of culture plate containing PDA medium, and then incubated for 48 hours at 25°C. Plates were then inculated with each studied pathogen by placing two 5 mm disks, from three-day-old culture of the pathogenic fungus, 3 cm. apart from both sides of bacterial growth. Plates were incubated at 25 °C, for 4 days and fungal colony diameter in the presence or absences of bacteria were measured. The inhibition zone between bacteria and the pathogen was measured as described by Maurhofer *et al.,* (1995).

5.4. Preparation of bacterial inoculum:

Bacterial suspensions (1 x 10^8 cfu / ml) were prepared by dilution plate assay as described by Callan *et al.,* (1990). Bacterial cells from agar cultures of each isolate were inoculated into nutrient broth (NB) and centrifuged at

3000 rpm for 5 min., the supernatant was discarded, and the precipitate was re-suspended in 100 ml sterilized distilled water. The suspension was centrifuged again for 5 min. and the precipitate was finally suspended in sterilized distilled water. Bacterial concentrations were determined according to its turbidity using spectphotometer.

5.5. Methods of application:

Bacterial isolates were applied as seed treatment, Two ml bacterial suspension in 0.1 % methyl cellulose were mixed thoroughly with 10 seeds in a small Petri dish. The seeds were air dried for 30 min in a laminar flow cabinet and were planted directly seeds treated with methyl cellulose were used as control. While fungicide Rhizolex-T 50% (Tolclofos-methyl 20% + thiram 30%) were applied as seed treatment at the rate of 3g/kg seed and the commercial biocides Rhizo-N (Bacillus subtillis 3 x 106 c.f.u/ml) at the rate 5g/kg seed.

5.6. Evaluation of antagonists under greenhouse conditions:

Pots experiment were carried out during season 2005 for studying the effect of selected nine antagonistic bacteria isolates, for controlling root rot incidence of peanut. Seeds, cv. Giza 6, were sown in pathogen infested soils as shown before at the rate of 10 seeds / pot; the antagonistic bacteria were applied as seed treatment. Damping-off, root rot incidence were recorded.

6. Greenhouse experiments:

The experiments were carried out at Agriculture Research Center, Giza. Peanut seeds, cv. Giza 6, were used for sowing in 50 cm-diameter pots containing soil previously infested with *R. solani*, *F. solani*, *S. rolfsii* and *M. Phaseolina* (2% w/w). Ten seeds were sown per each pot. Experiment were used for each replicated for five times. Disease assessment was recorded as percentage of damping- off, root rot and survival plants as previously mentioned.

7. Evaluation of antagonists in the field:

A field experiment was established at Ismailia Experimental Station, Agriculture Research Center (ARC), during seasons 2006 and 2007 to study the effect of sex antagonistic bacterial isolates, for controlling damping-off and peanut root rot incidence. The selected fields were known to have natural infestation with pod rot pathogens. The soil type was sandy loam (77% sand, 11% silt and 12% clay; pH 7.98). The antagonistic bacteria were applied at sowing as seed treatment (approximately 50 ml of bacterial suspensions in 0.1 % methyl cellulose were mixed thoroughly with 100 seeds), while fungicide Rhizolex-T 50% (Tolclofos-methyl 20% + thiram 30%) were applied as seed treatment at the rate of 3g/kg seed and the commercial

biocides Rhizo-N (Bacillus subtillis 3 x 106 c.f.u/ml) at the rate 5g/kg seed. Cultural practices and fertilization for the peanut crop were applied as recommended. The experiment was arranged in completely randomized block design with four replicates. Disease assessment was recorded as percentage of damping - off, root rot and survival plants as previously mentioned, pod yield were determined at the end of each season.

8. Statistical analysis:

The data were statistically analyzed by analysis of variance (ANOVA) using Statistical Analysis System (SAS Institute, inc, 1996). Means were separated by Duncan's Multiple Range Test at $P \leq 0.05$ levels.

RESULTS

1. Bacterial isolates:

Seventeen bacterial isolates (Table1) were isolated from the soil, rhizosphere, geocarposphere, peanut roots and pegs obtained from different fields in three locations in Egypt. All bacterial isolates are related to the genes *Bacillus* and refer to *Bacillus sp.*

Table (1): List of bacterial isolates (*Bacillus* sp) obtained from peanut samples and soil from different locations, during seasons 2003 and 2004.

No.	Isolate code	Source	location
1	N.1	Soil	Nobaria
2	N.2	Soil	Nobaria
3	N 3	Rhizosphere	Nobaria
4	N.4	Rhizosphere	Nobaria
5	N.5	Root	Nobaria
6	N.6	Geocarposphere	Nobaria
7	Sh.1	Soil	Sharkia
8	Sh.2	Rhizosphere	Sharkia
9	Sh.3	Rhizosphere	Sharkia
10	Sh.4	Geocarposphere	Sharkia
11	Sh.5	Root	Sharkia
12	S.1	Soil	Ismailia
13	S.2	Soil	Ismailia
14	S.3	Rhizosphere	Ismailia
15	S.4	Rhizosphere	Ismailia
16	S.5	Peg	Ismailia
17	S.6	Peg	Ismailia

2. Screening of bacterial antagonists, *in vitro*:

Seventeen bacterial isolates, in addition to two supplied bioagents, were evaluated *in vitro* for their antagonistic effect against *R. solani, F. solani, M. phaseolina* and *S. rolfsii* on PDA medium (Table 2). Only eight isolates *B. subtilis* (BS) *P. fluorescens* (Pf 5), (N.3), (N.5), (Sh.4), (Sh.5), (S.3) and (S.5) caused moderate to strong inhibition to the four tested pathogens. While, both of (N.2) and (S.1) were moderately effective on *R. solani* and *M. phaseolina*. Only (N.6) had the same efficiency in inhibiting *M. phaseolina*.

Table (2): Screening of various bacterial isolates to determine their Antagonistic effect against different fungal pathogens associated with damping-off, and root rot of peanut.

Bacterial isolates	Inhibition zone [z)]			
	Rhizoctonia solani	*Fusarium solani*	*Macrophomina phaseolina*	*Sclerotiom rolfsii*
B. subtilis (Bs. 1)	++	++	++	+
P. fluorescens (Pf. 5)	++	++	++	++
Bacillus sp N.1	-	-	-	-
Bacillus sp N.2	+	-	+	-
Bacillus sp N.3	+	+	+	+
Bacillus sp N.4	-	-	-	-
Bacillus sp N.5	++	+	+	+
Bacillus sp N.6	-	-	+	-
Bacillus sp Sh.1	-	-	-	-
Bacillus sp Sh.2	-	-	-	-
Bacillus sp Sh.3	-	-	-	+
Bacillus sp Sh.4	+	+	+	+
Bacillus sp Sh.5	++	+	+	+
Bacillus sp S.1	+	-	+	-
Bacillus sp S.2	-	-	-	-
Bacillus sp S.3	+	+	+	+
Bacillus sp S.4	-	-	-	-
Bacillus sp S.5	++	++	++	+
Bacillus sp S.6	-	-	-	-

z) Inhibition of pathogens is expressed as the distance (mm) between pathogen mycelium and bacteria on potato dextrose agar (PDA), inhibition zone < 15 mm (+), inhibition zone ≥ 15 (++) while (-) no inhibition zone.

Pseudomonase fluoressens **(Pf.5) gave the high significant antagonistic effect against *R. salani, F. solani, M. phaseolina* and *S. rolfsii* on PDA medium followed by *Bacillus subtilis* (Bs.1) and *Bacillus* sp (S.5) (Table 3). Meanwhile *Bacillus* sp (N.5), *Bacillus* sp (Sh.5) and *Bacillus* sp (S3) gave moderately effect in their inhibition of tested pathogens growth. While, both of *Bacillus* sp (N.3) and *Bacillus* sp (Sh.4) had little effective.**

Table (3): Antagonistic effect of various bacterial isolates against different fungal pathogens associated with damping-off and root rot of peanut.

Bacterial isolates	Inhibition zone [y]			
	Rhizoctonia solani	*Fusarium solani*	*Macrophomina phaseolina*	*Sclerotiom rolfsii*
P. fluorescens (Pf. 5)	27 a [z]	23 a	20 a	18 a
B. subtilis (Bs.1)	25 a	21 ab	16 b	13 b
Bacillus sp N.3	10 de	11 e	8 de	8 c
Bacillus sp N.5	15 bc	15 cd	12 c	10 c
Bacillus sp Sh.4	8 e	6 f	6 e	5 d
Bacillus sp Sh.5	18 b	13 de	11 cd	10 c
Bacillus sp S.3	13 cd	11 e	12 c	8 c
Bacillus sp S.5	19 b	18 bc	19 ab	13 b

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

z) Means in each column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

3. Evaluation of antagonistic bacteria under greenhouse conditions:

Eight selected bacterial isolates beside standard consisting of Rhizo-N (biocides) and Rezolex-T (fungicides) were evaluated as seed treatment at sowing time under greenhouse conditions. Results in Table (4) showed that, all tested bioagents have significant effect in reducing damping-off and peanut root rot compared to control. In this respect the most effective isolates in reducing peanut pre-emergence damping-off was *P. fluorescens* (Pf 5) and *B. subtills* (Bs1) was the most effective isolate in reducing peanut post-emergence damping-off. Moreover *Pseudomonas fluorescens* (Pf 5) and *Bacillus* sp (S.5) gave the highest effect in reducing peanut root rot compared with other tested bioagents and without any significant with Rhizo-

N, while S.3 isolates gave the lowest effect in reducing damping-off and peanut root rot diseases compared to other tested bioagents.

Table (4): Effect of different tested bioagents on damping-off, and root rot of peanut cv. Giza 6, grown in artificially infested soil, under greenhouse conditions [x].

Isolates [y]	Disease incidence (%)			Survival (%)
	Damping-off		Root rot	
	Pre-emergence	Post-emergence		
P. fluorescens **(Pf. 5)**	6 ef	6 cd	12 de	76 b
B. subtilis **(Bs.1)**	8 de	4 d	14 cd	74 bc
Bacillus **sp N.3**	10 cd	10 b	16 bc	64 e
Bacillus **sp N.5**	8 de	8 bc	12 de	72 bc
Bacillus **sp Sh.4**	12 bc	8 bc	14 cd	66 de
Bacillus **sp Sh.5**	8 de	6 cd	16 bc	70 cd
Bacillus **sp S.3**	14 b	10 b	18 b	58 f
Bacillus **sp S.5**	8 de	6 cd	12 de	74 bc
Rhizo-N	8 de	6 cd	12 de	74 bc
Rezolex-T	4 f	4 d	10 e	82 a
Control	20 a	14 a	22 a	44 g

x) Soil in each pot was infested with a mixture of pathogenic fungi at the rate of 2% (w/w).
y) Bacterial isolates were applied as seed treatment, 10 seeds were mixed thoroughly with two ml of bacterial suspensions (10^8 cfu/ml) before sowing
z) Means in each column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05).

Data in Fig (1) showed that Pf 5, Bs1 and S.5 isolates gave the highest efficiency in reducing damping-off and peanut root rot compared to other treatments, while S.3 and N.3 isolates gave the lowest efficiency compared to other tested bioagents.

Data in Table (5) clearly showed the ability of some tested bioaegents to be near the fungicides efficiency in reducing damping-off and peanut root rot diseases. In this respect, *P. fluorescens* (Pf 5) was the nearest one to fungicides efficiency in reducing peanut pre-emergence damping-off (87.5%).

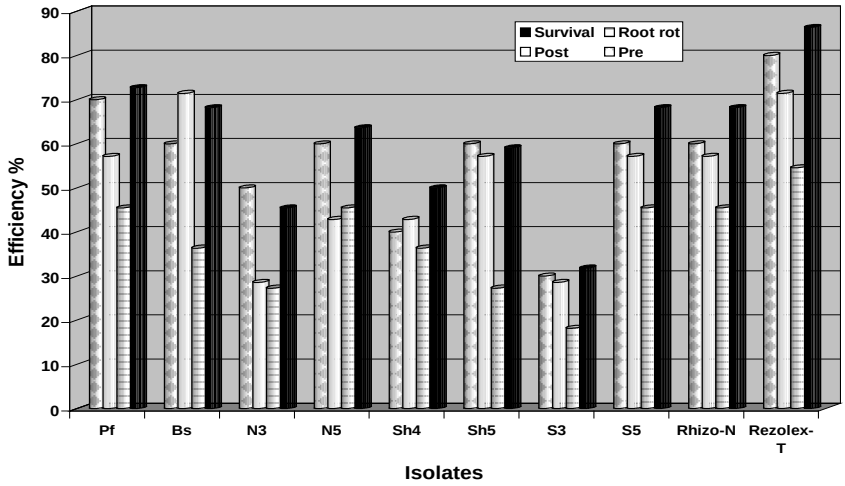

Fig (1): Efficiency of different tested bioagents for controlling damping-off and root rot of peanut cv. Giza 6, grown under greenhouse conditions in artificially infested soil.

B. subtills **(Bs1) gave the same as fungicides efficiency in reducing peanut post-emergence damping-off.** ***P. fluorescens*** **(Pf.),** ***B. subtills*** **(Bs1) and** ***Bacillus*** **sp (S.5) were the nearest to fungicides efficiency in reducing of peanut root rot (83.33 %).**

Table (5): The percentage efficiency of different tested bioagents to fungicides efficacy on damping-off, and root rot of peanut cv. Giza 6, grown in artificially infested soil, under greenhouse conditions [x)].

Isolates [y)]	% Biological efficiency to fungicides efficacy			
	Damping-off		Root rot	Survival
	Pre-emergence	Post-emergence		
P. fluorescens **(Pf.5)**	87.50	80.00	83.33	84.21
B. subtilis **(Bs.1)**	75.00	100.00	66.66	78.95
Bacillus **sp N.3**	62.50	40.00	50.00	52.63
Bacillus **sp N.5**	75.00	60.00	83.33	73.69
Bacillus **sp Sh.4**	50.00	60.00	66.66	57.90
Bacillus **sp Sh.5**	75.00	80.00	50.00	68.42
Bacillus **sp S.3**	37.50	40.00	33.33	36.84
Bacillus **sp S.5**	75.00	80.00	83.33	78.95
Rhizo-N	75.00	80.00	83.33	78.95
Rezolex-T	100.00	100.00	100.00	100.00

z) % Bioagent efficiency to fungicides efficacy = (% Biological efficiency/ fungicides efficacy) x 100

4. Evaluation of antagonistic bacteria under field conditions during two successive seasons 2006 and 2007:

Five selected bacterial isolates beside standard consisting of Rhizo-N (biocides) and Rezolex-T (fungicides) were evaluated as seed treatment at sowing time under field conditions during two successive seasons 2006 and 2007. Data in Table (6) indicated that, all tested bioagents have significant effect in reducing of damping-off and peanut root rot compared with control during the two successive seasons,. *P. fluorescens* followed by *Bacillus* sp (S5) were the most effective isolates in reducing peanut pre- and post-emergence damping-off. While *P. fluorescens* (Pf5), *Bacillus subtills* (BS1) and *Bacillus* sp (S5) gave the highest effect in reducing peanut root rot compared to other tested bioagents and without any significant with Rhizo-N during the two seasons 2006 and 2007. *Bacillus* (Sh.5) isolate gave the lowest effect in reducing damping-off and peanut root rot diseases compared to other tested bioagents in the two grown seasons.

Table (6): Effect of different tested bioagents on damping-off, and root rot of peanut cv. Giza 6, grown under field conditions during the two seasons 2006 and 2007 [x)].

Isolates [x)]	Disease incidence (%)			Survival (%)	Yield Ton/fed
	Damping-off		Root rot		
Season 2006 [y)]	Pre-emergence	Post-emergence			
P. fluorescens (Pf.5)	5.61 ef	4.20 dc	4.26 de	85.93 b	1.139 c
B. subtilis (Bs.1)	8.61 bcd	6.16 c	5.18 cd	80.05 c	1.155 b
Bacillus sp S.5	6.90 cde	5.91 c	5.40 cde	81.79 bc	1.131 cd
Bacillus sp N.5	9.55 bc	9.36 b	6.53 c	74.56 d	1.126 cd
Bacillus sp Sh.5	11.24 ab	9.40 b	8.95 b	70.41 d	1.118 d
Rhizo-N	7.35 de	6.15 c	4.75 cd	81.75 bc	1.160 b
Rezolex-T	3.00 f	3.01 e	3.06 e	90.93 a	1.179 a
Control	13.31 a	12.09 a	13.25 a	61.35 a	0.980 e
Season 2007					
P. fluorescens (Pf.5)	3.32 de	2.21 c	4.82 d	89.65 ab	1.141 cd
B. subtilis (Bs.1)	6.45 cd	4.21 bc	5.61 c	83.73 c	1.160 bc
Bacillus sp S.5	4.18 bc	4.08 bc	6.11 cd	85.63 c	1.133 d
Bacillus sp N.5	6.11 bc	5.00 a	9.94 b	78.95 d	1.122 d
Bacillus sp Sh.5	8.31 ab	7.42 a	10.59 b	73.68 e	1.056 e
Rhizo-N	5.20 cde	3.15 bc	6.82 cd	84.83 bc	1.165 ab
Rezolex-T	2.05 e	2.15 c	2.49 e	93.31 a	1.185 a
Control	10.50 a	9.00 a	15.00 a	65.50 c	0.999 f

x) Bacterial isolates were applied as seed treatment, 10 seeds were mixed thoroughly with two ml of bacterial suspensions (10^8 cfu/ml) before sowing

y) Each season was analyzed separately.

z) Means in each column with the same letter are not significantly different according to Duncan's Multiple Range Test (P = 0.05)

Concerning the effect of tested bioagents on peanut yield during the two seasons 2006 and 2007, the data presented in Table (6) demonstrated that, all tested bioagents caused significant increase in total pod yield compared to the control during the two growing seasons. The highest total peanut pod yield in the two seasons obtained with *B. subtills* (Bs1) and without any significant with Rhizo-N followed by *P. fluorescens* and *Bacillus* sp (S5). While *Bacillus* (Sh.5) gave the lowest total peanut pod yield in the two successive seasons compared to the other bioagents.

Data in Fig (2) showed that Pf 5, Bs1 and S5 isolates gave the highest efficiency in reducing damping-off and peanut root rot compared to other bioagents, while N5 and Sh5 isolates gave the lowest efficiency compared to other tested bioagents during the two successive seasons.

Data in Table (7) proved that, some tested bioaegents gave efficiency in reducing damping-off and peanut root rot diseases near to the fungicides efficiency. In this respect, *P. fluorescens* (Pf 5.) was the nearest one to the fungicides efficiency in reducing peanut pre- and post-emergence damping-off and peanut root rot followed by *Bacillus* sp (S.5) and *B. subtills* (BS1) compared to other tested bioagents during the two seasons 2006 and 2007.

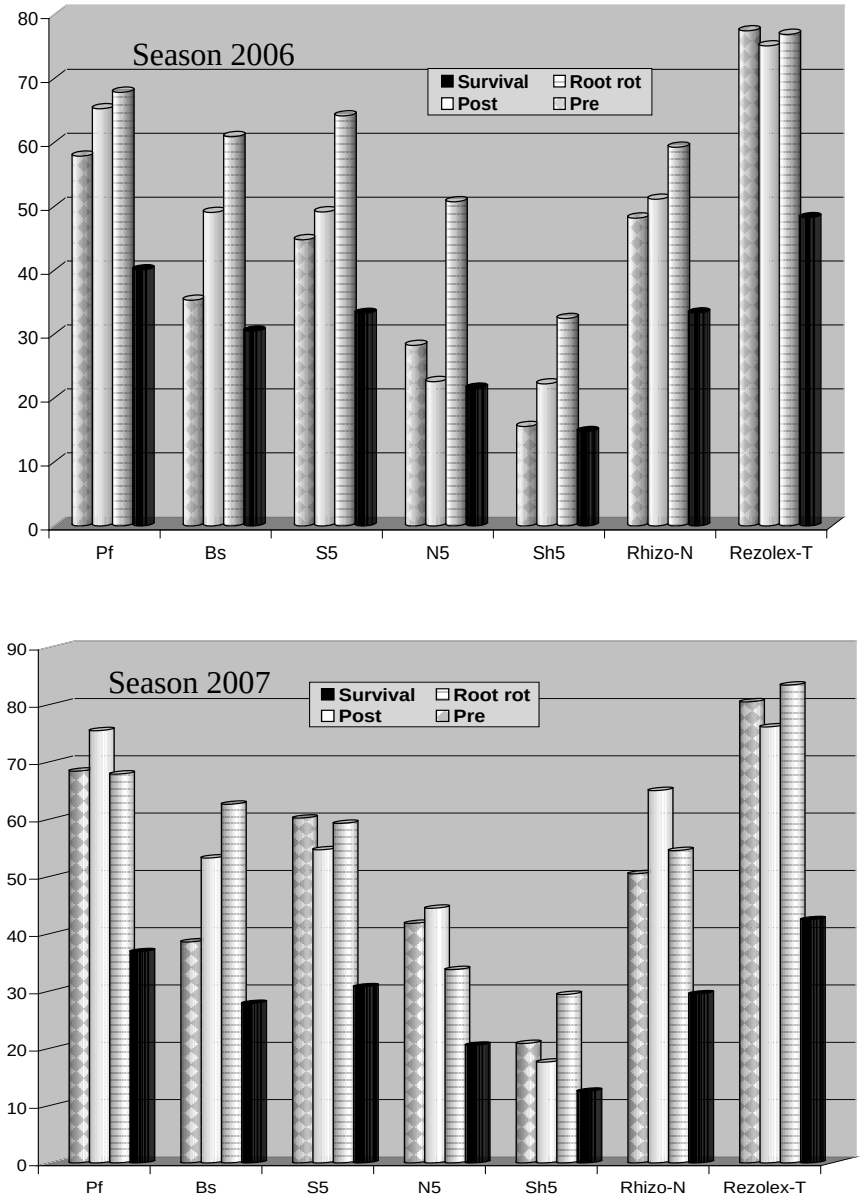

Fig (2): Efficiency of different tested bioagents for controlling damping-off and root rot of peanut cv. Giza 6, grown under field conditions during the two seasons 2006 and 2007

Table (7): The percentage efficiency of different tested bioagents to fungicides efficacy on damping-off, and root rot of peanut cv. Giza 6, grown under field conditions during two season 2006 and 2007 [x].

Isolates [y]	% Biological efficiency to fungicides efficacy			
	Damping-off		Root rot	Survival
Season 2006	Pre-	Post-		
P. fluorescens	74.69	86.90	88.22	83.09
B. subtilis (Bs.1)	45.59	65.31	79.19	63.21
Bacillus sp S.5	57.81	65.42	83.41	68.96
Bacillus sp N.5	36.47	30.07	65.94	44.65
Bacillus sp Sh.5	20.08	29.59	42.20	30.61
Rhizo-N	62.17	68.06	77.03	69.09
Rezolex-T	100.00	100.00	100.00	100.00
Season 2007				
P. fluorescens	84.97	99.13	81.37	83.83
B. subtilis (Bs.1)	47.93	69.93	75.06	63.28
Bacillus sp S.5	74.79	71.83	71.06	69.88
Bacillus sp N.5	51.95	58.40	40.45	46.69
Bacillus sp Sh.5	25.92	23.11	35.25	28.41
Rhizo-N	62.72	85.40	65.39	67.10
Rezolex-T	100.00	100.00	100.00	100.00

z) % Biological efficiency to fungicides efficacy = (% Biological efficiency/ fungicides efficacy) x 100

DISCUSSION

The results of this study demonstrate the antagonistic effect of some rhizobacterial isolates, which obtained from soil, roots, geocarposphere, and peg of peanut and two stander isolates of *B. subtilis* and *P. fluorescence*. Of 19 tested isolates, only eight isolates caused moderate to strong inhibition of mycelial growth to the four tested pathogens (*R. solani, F. solani, M. phaseolina* and *S. rolfsii*). *P. fluorescens* (Pf 5) followed by *B. subtilis* (Bs1) and *Bacillus* sp. (S5) isolate were the best antagonistic bacteria for limiting growth of the tested pathogens as they caused the widest inhibition zone. This is in agreement with Asaka and Shoda, (1996), Ashour and Afify, (1999), Mahmoud (2004), Hussin, (2005) and Mahmoud *et al.,* (2006) who stated that, certain strains of *Bacillus* appear to be most effective as a biological control agent, by inhibition the mycelial growth of plant pathogenic fungi. While *P.*

fluorescens **was the effective bio-control agent against various soil-borne diseases caused by** ***F. oxysporum, R. solani, P. ultimum, M. phaseolina*** **and others (Jayashree** ***et al.,*** **2000, Karunanithi** ***et al.,*** **2000, Meena** ***et al.*****, 2001, Hussin, 2005 and Mahmoud** ***et al.,*** **2006).**

In greenhouse experiment, the same 8 isolates were tested beside standard consisting of Rhizo-N (biocide) and Rezolex-T (fungicide). ***P. fluorescens*** **(Pf 5),** ***B. subtilis*** **(Bs1) and** ***Bacillus*** **sp. (S5) isolates gave the highest efficiency in reducing damping-off and peanut root rot compared to other treatments. Data were obtained from field experiments supported greenhouse results and showed extent efficiency of** ***P. fluorescens*** **(Pf 5),** ***B. subtilis*** **(Bs1) and** ***Bacillus*** **sp. (S5) isolates in reducing damping-off and peanut root rot diseases. Data also clearly proved the ability of some tested bioaegents to nearest to the fungicides efficiency in reducing of damping-off and peanut root rot diseases. This is in agreement with Podile and Prakash, (1996), Sheela** ***et al.*****, (1998), Mahmoud, (2004), Hussin, (2005) and Mahmoud** ***et al.,*** **(2006).**

Further study in that respect showed that ***Bacillus*** **and** ***Pseudomonas*** **consider the important genera of plant growth-promoting rhizobacteria (PGPR) (De Meyer and Hofte, 1997; Sailaja and Podile, 1998 and Meena** ***et al.*****, 2001). Growth-promoting rhizobacteria (PGPR) effect on soilborne pathogen by several mechanisms like, produce antibiotics to antagonize pathogenic microorganisms (Kloepper, 1991, 1993), compete for substrates that are essential for growth of the pathogens (Raupach** ***et al.,*** **1996), compete with the pathogens for infection sites (Gutteridge and Slope, 1978 and Wong and Siviour, 1979), Production of lytic enzymes (Sailaja** ***et al.,*** **1998 and Meena** ***et al.*****, 2001), produce plant-growth-promoting compounds, such as gibberellin-like substances or indolyl-3-acetic acid (Brown, 1974), induction of resistance to plant pathogens, activate plant defense resulting in systemic protection against different fungal, bacterial, and viral pathogens (Picterse** ***et al.,*** **1996; and De Meyer and Hofte, 1997) and inhibition or displacement of nonpathogenic inhibitory rhizosphere bacteria (Elliott and Lynch, 1984).**

Bacillus subtilis **can be induction of diseases resistance in peanut by stimulate of phytoalexins production and increase the activity of lytic enzymes (Sailaja and Podile, 1998 and Sailaja** ***et al.,*** **1998). However, peanut plants, when sprayed or seed treatment with** ***P. fluorescens*** **showed increase in activity of phenylalanine ammonia-lyase, phenolic content and lytic enzymes (chitinase and beta-1,3-glucanase) (Meena** ***et al.,*** **2000a,b). Moreover, genus of** ***Pseudomonas*** **can produce several siderophores such as pyoverdine (pseudobactin), pyochelin, and salicylic acid (SA). The bacterium produced an antibiotic compound called pyrollnitrin, HCN and lytic enzymes (De Meyer and Hofte, 1997, and Meena** ***et al.*****, 2001).**

Results also showed that, ***Bacillus subtilis*** **(Bs1) treatment recorded the highest total yield of peanut pods compared To other tested bioagents. This is in agreement with Turner and Backman, (1991), and Mahmoud, (2004) who**

stated that, *Bacillus subtilis* can be increased the peanut yield by improving emergence, and enhanced plant nutrition.

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قدرة بعض العزلات البكتيرية في مقاومة أمراض أعفان الجذور في الفول السوداني بالمقارنة بكفاءة المبيد علي إحداث المقاومة

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الملخص العربي

تم إختبار القدرة التضادية لتسعة عشر عزلة بكتيرية معزولة من التربة و الريزوسفير ونباتات الفول السوداني ومن مصادر معروفةً على تثبيط نمو الفطريات المسببة لأعفان الجذور في الفول السوداني معملياً و أظهرت النتائج أن ثمانية عزلات فقط *B. subtilis* (Bs1) *P. fluorescens* (Pf 5), (N.3), ((S.5),(S.3), (Sh.5), (Sh.4), (N.5) هي التي أظهرت تثبيط متفاوت ما بين متوسط إلى عالي للنمو الميسليومي لهذه المسببات المرضية(*R. solani, S. rolfsii, F. solani* , *M. phaseolina*) و أظهرت عزلة *Pseudomonase fluoressens* (Pf 5) وعزلتي *Bacillus subtilis* (Bs1) و *Bacillus* sp. (S5)أعلي قدرة تضادية لمعظم الفطريات المختبرة.

في تجارب الصوبة والحقل أوضحت الدراسة أن عزلة *P. fluoressens* (Pf 5) وعزلتي *B. subtilis* (Bs1) و*Bacillus* sp. (S5) كانت أكثر العزلات قدرة علي خفض الإصابة بأعفان الجذور في الفول السوداني. أما بالنسبة لمحصول الفول السوداني من القرون أعطي استخدام العزلة البكتيرية *B. subtilis* (Bs1) أعلي محصول تلاها استخدام كلا العزلتين *P. fluoressens* (Pf 5) و *Bacillus* sp. (S5) في كلا الموسمين ٢٠٠٦و٢٠٠٧.

أظهرت النتائج المتحصل عليها قدرة بعض العزلات المختبرة علي الإقتراب من كفاءة المبيد في خفض الإصابة بموت البادرات و أعفان الجذور في الفول السوداني ففي تجارب الصوبة كانت العزلة *P. fluoressens* (Pf 5)هي الأقرب لكفاءة المبيد في مقاومة موت البادرات قبل الإنبات (٨٧.٥% بالنسبة لكفاءة المبيد) بينما أعطت العزلة *B. subtilis* (Bs1) ١٠٠% من كفاءة المبيد في مقاومة مرض موت البادرات بعد الإنبات، أما في مقاومة أعفان الجذورفي الفول السوداني فأعطت العزلات *P. fluoressens* (Pf 5) و *B. subtilis* (Bs1) و*Bacillus* sp. (S5) أعلي كفاءة بالنسبة لكفاءة المبيد (٨٣.٣٣%). أما في تجارب الحقل فكانت العزلة *P. fluoressens* (Pf 5) هي الأقرب لكفاءة المبيد في خفض نسبة الإصابة بموت البادرات وأعفان الجذور في الفول السوداني تلاها كلاً من العزلتين *B. subtilis* (Bs1) و*Bacillus* sp. (S5) في كلا الموسمين ٢٠٠٦و٢٠٠٧.