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EFFECT OF SOME ANTAGONISTIC BACTERIA IN REDUCING OF PEANUT DAMPING - OFF, ROOT AND POD ROT INCIDENCE CAUSED BY *Rhizoctonia solani*

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ABSTRACT

Seventeen bacterial isolates from the soil, rhizosphere, geocarposphere, peanut roots and pegs beside three supplied bioagents (*Bacillus subtilis*, *Pseudomonas putida* and *Pseudomonas fluorescens*) used to study their effect on four isolates of *Rhizoctonia solani*. *In vitro* only nine isolates caused moderate to strong inhibition to the four tested isolates of *R. solani*. *P. fluorescens* (Pf.5) gave the highest significant antagonistic effect against the four tested isolates of *R. solani* on PDA medium followed by *B. subtilis* (Bs.1), *P. putida* (PP) and *Bacillus* sp (S.5). In greenhouse and field experiment, 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 *Bacillus* sp (S.5). Regarding to peanut pod yield, the highest total peanut pod yield in the two seasons (2009 and 2010) was obtained by *B. subtilis* (Bs1) followed by *P. fluorescens*. The obtained data clearly showed the ability of some tested bioaegents to be near to the fungicides effect (Rezolex-T) in reducing damping-off and peanut root and pod rots diseases. In this respect, in greenhouse and field trials *P. fluorescens* (Pf 5.) was the nearest one to fungicides effect in reduction of peanut damping-off and peanut root and pod rots and exceeded the commercial biocide effect (Rhizo-N), followed by *Bacillus* sp (S.5) and *B. subtilis* (Bs1) compared to other tested bioagents.

Keywords: *Rhizoctonia solani, Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas fluorescens*, bioagent, Biological control, Peanut, Damping-off, Root rot, pod rot and fungicides efficiency

INTRODUCTION

Peanut is one of the most important leguminous field crops in Egypt as well as in many parts of the world. It is used for human consumption, oil production, food industries and animal feeding. Peanut is a unique legume since it flowers above ground and the pods (fruit) formed below the soil surface that makes all peanut parts exposed to attack by many soilborne fungi especially *R. solani*, which cause root rot and pod rot diseases causing quantitative and qualitative losses in yield (Hilal *et al.,* 1990, Mahmoud, 2004 and Mahmoud *et al.,* 2006).

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), which suppress a variety of root and vascular disease caused by soilborne pathogens (Jayashree *et al.*, 2000, Meena *et al.*, 2001, and Mahmoud **2004**).

Bacillus and *Pseudomonas* considered as important genera of these bacteria (Sailaja and Podile, 1998, Meena *et al.*, 2001 and Ibrahim, *et al.,* 2008). *B. subtilis* has been used for many years in attempts to control plant pathogens and plant growth increase. Certain strains of *B. subtilis* appear to be very effective as a biological control agent. 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 and Gabr *et al.*, 1998). *B. subtilis* was used to control *Fusarium* wilt or crown rot diseases (Nemec *et al.*, 1996 and Mosa, *et al.*, 1997).While in peanut application of *B. subtilis* has a reducing effect on crown rot caused by *Aspergillus niger*, foot rot caused by *Sclerotium rolfsii* and root cankers caused by *R. solani* (Turner and Bakman, 1991, and Podile and Prakash, 1996).

Recently, *Pseudomonas* spp. had attained much attention as biological control agents. *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 (Mosa *et al.,* 1997, Karunanithi *et al.,* 2000 and Jayashree *et al.,* 2000). In peanut, under greenhouse tests, 99% of plants were protected from *S. rolfsii* infection when inoculated with *P. fluorescens*. However, in field trial, treatment increased total pod yield by 65% and resulted in 18% greater survival of plants up to harvest. *Pseudomonas* strains showed in *vitro* antibiosis against the collar rot pathogen caused by *A. niger* and gave protection to groundnut seedlings against the disease. Treated plants gave higher yields in terms of pod number and weight than control (Sheela et al., 1998 and Dileep *et al.,* 1999). 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 antagonistic effect produced *in vitro* by HCN, salicylic acid siderophore and beta–1,3 gluconase (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. phaseolina, Fusarium* spp*. and Aspergillus* spp. and added that *B. subtilis* induced the highest pod yield of peanut. Moreover, Ibrahim *et al.,* (2008) stated that some tested bioaegents to be near to the fungicide efficiency (Rizolex-T) in reducing damping-off and peanut root rot diseases. In this respect, in greenhouse and field trials *P. fluorescens* (Pf.) and *B. subtills* (Bs1) were the nearest to fungicides efficiency in reducing damping-off and peanut root rot.

This work was carried out to study the effect of some bacterial isolates in reducing damping-off, peanut root and pod rots diseases which caused by *R.solani* diseases.

MATERIALS AND METHODS

Isolation and identification of causal organisms:

The fungal isolates, which were used throughout this study were previously isolated by the authors from diseased peanut roots and the

identification was carried out based on their hyphal characteristics according to Sneh *et al.,* (1991) and confirmed with anastomosis test for AG_4 as described by Carling, 1996.

Preparation of fungal inoculum:

Inocula of isolates of *R. solani* was 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 (120^{o}c). The sterilized medium was inoculated using agar discs, obtained from the periphery of 5-day-old colony of each of the tested isolates. The inoculated media were incubated at 28°C for 10 days and were then used for soil infestation.

Soil Infestation:

Inoculum of each isolate of *R solani* mixed thoroughly with the soil surface of each pot, at the rate of 2% w/w, and covered with a thin layer of sterilized soil. The infested pots irrigated and kept for 7 days before sowing.

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 =

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

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

% 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:

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

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

Plants in individual pots/plots were dug and inverted based on an optimum maturity index. Pods were threshed, air-dried for three days, weighted and then examined for pod rot incidence.

$$\text{\% lose of yield} = \frac{\text{No. of infection pods x 100}}{\text{Total pods}}$$

Source of known antagonistic bacteria:

Two known isolates of *P. fluorescens* (Pf5) (Howell and Stipanovic, 1979) *P. putida* and *B. subtilis* (Bs1) (El-Hadidy, 2003) were obtained from Culture Collection of Department of plant Pathology, Faculty of Agriculure, Ain Shams University.

Isolation of antagonistic bacteria from peanut:

Bacterial isolates were isolated from soil and different samples of peanut plants, according to Mickler *et al.,* (1995). Samples of roots, pegs and

pods collected from different fields at Ismailia, Nobaria and Sharkia districts, peanut organs with adhering soil placed in plastic bags and transferred to the laboratory. Adhering soil carefully brushed off from each organ. Ten grams of soil or adhering soil 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 media (NA) and King's B media (KB) (King *et al.,* 1954). Peanut organ samples also cutting to small pieces (1 cm) thin sterilized and transferred on nutrient agar media (NA) and King's B media (KB). Plates incubated at 27°C for 2- 4 days then individual colonies picked up, purified and stored at 4°C on the appropriate medium.

Evaluation of antagonists, *in vitro*:

All bacterial isolates were tested by streaking the bacteria in the center of culture plate containing PDA medium, 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 of the tested isolates, 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 Maurchofer *et al.,* (1995).

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

9. Methods of application:

Bacterial isolates were applied as soil treatment, by adding 100 ml of bacterial suspensions (10^8 cfu / ml) for each pot, which previously infested with the pathogenic isolates, 15 days before planting.

10. Evaluation of antagonists under greenhouse conditions:

Pots experiments carried out during season 2009 for studying the effect of selected nine antagonistic bacteria isolates, for controlling damping off, root, and pod rot incidence of peanut. The experiment carried out at Agricultural Research Center, Giza. Peanut seeds, Giza 6 cv., used for sowing in 50 cm-diameter pots containing soil previously infested with *R. solani* (2% w/w). Ten seeds sown per each pot, five replicate pots used for experiment. Disease assessment recorded as percentage of damping- off, root rot, pod rot and survival plants at 15, 45 days and during the harvesting time as previously mentioned.

Bacterial isolates applied as seed dressing at sowing and as soil treatment after 40 days from sowing. (10^8 cfu / ml).

Evaluation of antagonists in the field:

A field experiment established at Ismailia Experimental Station, Agriculture Research Center (ARC), during seasons 2009 and 2010 to study the effect of effect of five antagonistic bacterial isolates, for controlling

damping-off, root and pod rot incidence of peanut. The selected fields known to have natural infestation with root and pod rot pathogens. The soil type was sandy loam (77% sand, 11% silt and 12% clay; pH 7.98). The antagonistic bacteria applied as seed dressing at sowing and the antagonistic bacteria applied as soil treatment after 40 days from sowing as previously mentioned. Fungicide Rhizolex-T 50% (Tolclofos-methyl 20% + thiram 30%) 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 applied as recommended. Seeds were sown on the first week of May with 10 cm spacing between plants. The experimental unit area was 10.5 m^2 (1/400 fed.).The experiment arranged in completely randomized block design with four replicates. Disease assessment recorded as previously mentioned.

Statistical analysis:-

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

RESULTS

Bacterial isolates:

Seventeen bacterial isolates (Table 1) 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 genus *Bacillus* and refer to *Bacillus* sp

Screening of bacterial antagonists, *in vitro*:

Seventeen bacterial isolates, in addition to three supplied bioagents (*B. subtilis*, *P.putida* and *P. fluorescens*), were evaluated *in vitro* for their antagonistic effect against four isolates of *R. solani* which represent four locations of Egypt on PDA medium (Table 2). Only nine isolates caused moderate to strong inhibition to the four tested isolates.

P. fluoressens (Pf.5) gave the high significant antagonistic effect against the four tested isolates of *R. salani* on PDA medium followed by *B. subtilis* (Bs.1), *P. putida* (PP) and *Bacillus* sp (S.5) (Table 3). Meanwhile *Bacillus* sp (N.5), *Bacillus* sp (Sh.5) and *Bacillus* sp (S3) gave moderate effect in their inhibition of tested pathogens growth. While, both of *Bacillus* sp (N.3) and *Bacillus* sp (Sh.4) had little effect.

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

Isolate code	Source	Location
N.1	Soil	Nobaria
N.2	Soil	Nobaria
N 3	Rhizosphere	Nobaria
N.4	Rhizosphere	Nobaria
N.5	Root	Nobaria
N.6	Geocarposphere	Nobaria
Sh.1	Soil	Sharkia
Sh.2	Rhizosphere	Sharkia
Sh.3	Rhizosphere	Sharkia
Sh.4	Geocarposphere	Sharkia
Sh.5	Root	Sharkia
S.1	Soil	Ismailia
S.2	Soil	Ismailia
S.3	Rhizosphere	Ismailia
S.4	Rhizosphere	Ismailia
S.5	Peg	Ismailia
S.6	Peg	Ismailia

Table (2): Screening of various bacterial isolates to determine their antagonistic effect against different *R. solani* isolates.

Bacterial isolates	Inhibition zone [z)]			
	***R solani* (B1)**	***R. solani* (Sh4)**	***R. solani* (N6)**	***R. solani* (Is3)**
B. subtilis (Bs. 1)	++	++	++	++
P.putida (PP)	++	++	++	++
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 < 20 mm (+), inhibition zone ≥ 20 (++) while (-) no inhibition zone.

Table (3): Antagonistic effect of various bacterial isolates against different *R. solani* isolates.

Bacterial isolates	Inhibition zone [z)]			
	***R solani* (B1)**	***R. solani* (Sh4)**	***R. solani* (N6)**	***R. solani* (Is3)**
P. fluorescens (Pf. 5)	26	36	33	29
P.Putida (PP)	22	31	28	25
B. subtilis (Bs.1)	24	33	31	27
Bacillus sp N.3	11	16	15	12
Bacillus sp N.5	16	23	21	18
Bacillus sp Sh.4	10	14	13	11
Bacillus sp Sh.5	18	25	23	20
Bacillus sp S.3	14	20	18	16
Bacillus sp S.5	19	27	25	21

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

Evaluation of antagonistic bacteria under greenhouse conditions:

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

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

Results in Table (4) show 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 *Pseudomonas putida* (PP) while, *P. fluorescens* (Pf.5) was the most effective isolate in reducing peanut post-emergence. Moreover, both of *P. fluorescens* (Pf.5), *B. subtilis* (Bs.1), *Bacillus* sp. (N.3), (Sh.5) 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 and exceeded the commercial biocide (Rhizo-N) in their effect on root rot diseases, followed by *Bacillus* sp (S.5) and *B. subtills* (Bs1) compared to other tested bioagents (Table 4).

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

Six selected bacterial isolates beside standard consisting of Rhizo-N (biocides) and Rizolex-T (fungicide) were evaluated under field conditions during two successive seasons 2009 and 2010. Data in Table (5) indicate that, all tested bioagents have significant effect in reducing of damping-off and peanut root rot compared with the control during the two successive seasons,. *P. fluorescens* (Pf.5) followed by both of Bs.1, PP and S.5 were the most effective isolates in reducing peanut damping-off. While, *P. fluorescens* (Pf.5) and *B. subtills* (BS1) gave the highest effect in reducing peanut root rot compared with other tested bioagents during two seasons 2009 and 2010. *Bacillus* sp.(Sh.5) isolate gave the lowest effect in reducing damping-off and peanut root rot diseases compared with other tested bioagents during two growing seasons.

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

Bacterial isolates	Damping-off		Total	Root rots	Survival
	Pre	Post			
P. fluorescens (Pf. 5)	8	6	14	10	76
P.putida (PP)	6	10	16	12	72
B. subtilis (Bs.1)	8	8	16	10	74
Bacillus sp N.3	10	10	20	10	70
Bacillus sp N.5	8	8	16	12	72
Bacillus sp Sh.4	12	10	22	12	66
Bacillus sp Sh.5	12	10	22	10	68
Bacillus sp S.3	14	10	24	14	62
Bacillus sp S.5	8	8	16	10	74
Rhizo-N	8	6	14	12	74
Rizolex-T	6	6	12	8	80
Control	16	12	28	16	56
L.S.D. 5%:	2.14	1.88	2.52	1.92	3.51

Moreover, *P. fluorescens* (Pf 5.) was the nearest one to fungicides effect in reduction of peanut damping-off and peanut root rots and exceed the commercial biocide (Rhizo-N) in their effect on damping off and root rots diseases, followed by *Bacillus* sp (S.5) and *B. subtills* (Bs1) compared to other tested bioagents **(Table 5)**

Table (5): Effect of antagonistic bacterial isolates on peanut damping-off and root rot incidence under field conditions during two seasons 2009 and 2010.

Bacterial isolates	Season 2009			Season 2010		
	Damping-off	Root rots	Survival	Damping-off	Root rots	Survival
P. fluorescens (Pf. 5)	7.20	10.45	82.35	9.17	11.83	79.00
P.putida (PP)	9.77	13.90	76.32	10.98	15.80	73.21
B. subtilis (Bs.1)	9.71	11.65	78.64	10.92	13.34	75.74
Bacillus sp S.5	9.92	12.89	77.20	12.95	13.70	73.36
Bacillus sp N.5	14.48	15.92	69.60	15.94	18.41	65.65
Bacillus sp Sh.5	11.75	14.30	73.95	12.95	16.64	70.41
Rhizo-N	10.09	10.83	79.08	11.13	14.84	74.02
Rizolex-T	6.50	8.49	85.01	8.21	10.28	81.51
Control	15.93	18.01	66.06	17.72	20.10	62.18
L.S.D. 5%:	1.963	1.895	3.643	1.711	1.509	3.221

On peanut brown pod rot 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 brown pod rot incidence. The most effective isolates were Pf.5 followed by S.5. Bs.1 and PP. Meanwhile, isolates N.5 and Sh.5 caused moderate effect and isolates N.3, Sh.3 and S.3 caused slight effect compared to non-treated control.

On the other hand, data showed that *P. fluorescens* (Pf 5.) exceed the commercial biocide (Rhizo-N) in their effect on dry brown lesion followed

by *Bacillus* sp (S.5) and both of *B. subtills* (Bs1) and *P.Putida* (PP) compared to other tested bioagents (Table 6).

On peanut brown pod rot incidence under field conditions:

Field experiments were carried out during growing seasons 2009 and 2010 to study the effect of six antagonistic bacterial isolates for controlling brown pod rot incidence of peanut

Data in Table (7) show clearly that, all tested antagonistic bacteria significantly reduced incidence of all types of pod rot compared with the control. The most effective isolates were Pf.5 followed by S.5 in both growing seasons. Isolates Bs1, PP showed moderate effect, while N.5 and Sh.5 gave the lowest effect in reducing of pod rot incidence.

In the same time, data also showed that, *P. fluorescens* (Pf 5.) was the nearest one to fungicides effect in reduction of peanut pod rot (dry brown lesion) and exceed the commercial biocide (Rhizo-N) followed by *Bacillus* sp (S.5) compared to other tested bioagents **(Table, 7)**.

Table (6): Effect of antagonistic bacterial isolates on peanut brown dry lesion incidence under artificial conditions.

Bacterial isolates	Dry Brown Lesion	Apparent healthy
P. fluorescens (Pf. 5)	12.85	87.15
P.Putida (PP)	14.28	85.72
B. subtilis (Bs.1)	14.08	85.92
Bacillus sp N.3	22.17	77.83
Bacillus sp N.5	16.29	83.71
Bacillus sp Sh.4	21.12	78.88
Bacillus sp Sh.5	15.49	84.51
Bacillus sp S.3	23.09	76.91
Bacillus sp S.5	13.28	86.72
Rhizo-N	14.48	85.52
Rizolex-T	12.00	88.00
Control	25.57	74.43
L.S.D. 5%:	2.027	2.078

Table (7): Effect of antagonistic bacterial isolates on peanut pod rots incidence under field conditions during two seasons 2009 and 2010.

Bacterial isolates	Season 2009			Season 2010		
	Dry brown lesion	Other Rots	Apparent healthy	Dry brown lesion	Other rots	Apparent healthy
P. fluorescens (Pf. 5)	6.72	10.69	82.59	8.69	9.85	81.46
P.Putida (PP)	8.15	13.81	78.04	9.19	13.00	77.81
B. subtilis (Bs.1)	8.95	12.40	78.65	9.33	12.22	78.45
Bacillus sp S.5	6.76	12.5	80.74	9.13	11.00	79.87
Bacillus sp N.5	11.22	14.32	74.46	13.77	15.17	71.06
Bacillus sp Sh.5	9.09	14.36	76.55	14.11	15.60	70.29
Rhizo-N	7.35	13.25	79.40	10.22	11.00	78.78
Rizolex-T	6.11	9.81	84.08	8.32	9.78	81.90
Control	14.01	16.39	69.60	15.18	16.21	68.61
L.S.D. 5%:	0.611	0.858	1.384	0.771	1.029	1.409

On peanut yield and loss of yield under field conditions:

Concerning the effect of tested bioagents on peanut yield and yield loss during the two seasons 2009 and 2010. The data presented in Table (8) demonstrate that, all tested bioagents caused significant increase in total pod yield and reduction of yield loss compared with the control during the two growing seasons. The highest peanut pod yield in the two seasons obtained with *B. subtills* (Bs1) followed by *P. fluorescens* and *P.putida* (PP). While *Bacillus* (Sh.5) gave the lowest peanut pod yield and the lowest effect on reducing yield loss in the two successive seasons 2009 and 2010 compared with the other bioagents.

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

Bacterial isolates	Season 2009		Season 2010	
	Yield (Ton)	Loss of yield (%)	Yield (Ton)	Loss of yield (%)
P. fluorescens (Pf. 5)	1.126	22.4	1.095	25.5
P.putida (PP)	1.115	23.5	1.083	26.7
B. subtilis (Bs.1)	1.135	21.5	1.101	24.9
Bacillus sp S.5	1.108	24.2	1.077	27.3
Bacillus sp N.5	1.098	25.2	1.072	27.8
Bacillus sp Sh.5	1.065	28.5	1.033	31.7
Rhizo-N	1.141	20.9	1.107	24.3
Rizolex-T	1.212	13.8	1.150	20.0
Control	0.977	30.3	0.930	39.0
L.S.D. 5%:	0.087		0.096	

DISCUSSION

The results demonstrate the antagonistic effect of some bacterial isolates, which were obtained from soil, rhizosphere and peg of peanut and three stande isolates from *B. subtilis*, *P. putida* and *P. fluorescence*. All tested bioagents have significant effect in reducing damping-off root and peanut pod rot diseases compared to control. In this respect the most effective isolates were *P. fluorescens, B. subtilis* and *Bacillus* sp S.5. This is in agreement with Lazzaretti *et al.,* (1994); Ashour and Afify, (1999), Mahmoud (2004), Mahmoud *et al.,* (2006c) and Ibrahim *et al.,* (2008) who stated that, certain strains of *Bacillus* appear to be most effective as a biological control agent, by inhibiting the mycelial growth of plant pathogenic fungi. While *P. fluorescens* was found to be the most 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, Meena *et al.*, 2001, Mahmoud, 2004, Mahmoud *et al.,* 2006 c and Ibrahim *et al.,* 2008).

Further study in this respect showed that certain *P. fluorescens* and *B. subtilis* isolates were the most effective rhizobacteria for the suppression of damping – off, root and peanut pod rots, which showed great inhibition of hyphal growth *in vitro.* This suggested that, their biocontrol activity had been

associated with the production of certain such as enzymes, phenazines, pyrrole type antibiotics, pyo-compounds, indole derivatives peptide antibiotic, moenomycins, difficidins, bacillomycins and bacillaenes (Battu1 and Reddy2, 2009 and Awais *et al.,* 2010). The ability of antagonistic isolates to inhibit growth of the four 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) (Howell and Stipanovic 1979) against soil borne pathogens. Moreover, certain strains of *Pseudomonas* can produce several siderophores such as pyoverdine (pseudobactin), pyochelin, and salicylic acid (SA). The bacterium produced an antibiotic compounds called pyrollnitrin, HCN and lytic enzymes (Leeman *et al.,* 1996; De Meyer and Hofte, 1997; Karunanithi *et al.,* 2000, and Meena *et al.*, 2001). Meanwhile, several biocontrol agents such as *Pseudomonas* spp. showed induce resistance activity in several plants (Liu *et al*., 1997). Vanwees *et al*. (1997) elucidate the molecular mechanisms responsible for this type of defense reaction. *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, peanut plants, when 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 antagonistic effect produced *in vitro* by HCN, salicylic acid siderophore and beta–1,3 gluconase (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. Mahmoud *et al.,* (2006c) found that, *B. subtilis* (BS) *P. fluorescens* (Pf 5), (Sp1), (Sp2) and (Ss2) caused moderate to strong inhibition on the mycelium growth of the four tested pathogens (*R. solani, S. rolfsii, F. solani* and *M. phaseolina*). *P. fluoressens* (Pf 5) followed by *B. subtilis* (BS1) and *Bacillus* sp (Sp2) caused the best inhibition zone almost to tested pathogens. In greenhouse experiment, the most effective isolates in reducing peanut damping-off, wilt and peanut root rot were *P. fluorescens* (Pf 5) followed by *B. subtills* (BS1) and *Bacillus* sp (Sp2).

Moreover Ibrahim *et al.,* (2008) stated that some tested bioaegents just to be near to the fungicide efficiency (Rizolex-T) in reducing damping-off and peanut root rot diseases. In greenhouse trial *P. fluorescens* (Pf 5) was the nearest one to fungicide efficiency in reducing peanut pre-emergence damping-off while *B. subtills* (Bs1) gave 100 % effect compared with fungicides efficiency in reducing peanut post-emergence damping-off. *P. fluorescens* (Pf.), *B. subtills* (Bs1) and *Bacillus* sp (S.5) were the nearest to fungicide efficiency in reducing peanut root rot. While in field trials *P. fluorescens* (Pf 5.) was the nearest one to fungicide 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.

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تأثير بعض العزلات البكتيرية المضادة في خفض نسبة الإصابة بأعفان الجذور والثمار لفطر *Rhizoctonia solani* على نبات الفول السوداني

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تم اختبار كفاءة التضاد لسبع عشر عزلة بكتيرية معزولة من الفول السوداني ومن مصادر معروفةً وهي *Pseudomonas fluorescens Pseudomonas putida Bacillus subtilis,* وتحديد تأثيرها على عزلات الريزوكتونيا . أظهرت النتائج أن حوالي تسعه عزلات كان لها تأثير من قوي إلي متوسط على تثبيط عزلات الريزوكتونيا الأربع المختبرة. أظهرت عزلة *Pseudomonas fluorescens* (Pf5) أعلي قدرة تضادية على عزلات فطر *R. solani* المختبرة على بيئة PDA يليها *Bacillus subtilis* (BS1) و *P. putida* (PP) و *Bacillus* sp. (S.5) . في تجارب الصوبة والحقل كانت عزلة *P. fluorescens* (Pf5) أكثر العزلات تأثيراً علي خفض نسبة الإصابة بموت البادرات و أعفان جذور و ثمار الفول السوداني تلاها عزلة *Bacillus subtilis* (BS1) و *Bacillus* sp. (S.5). بالنسبة لإنتاجية المحصول من ثمار الفول السوداني سجلت المعاملة بعزلة *Bacillus subtilis* (BS1) أعلي إنتاجية تلاها عزلة *P. fluorescens* (Pf5) في كلا الموسمين 2009و2010. أظهرت النتائج المتحصل عليها قدرة بعض العزلات المختبرة علي الإقتراب من قدرة المبيد (Rizolex-T) في خفض الإصابة بموت البادرات و أعفان جذور و ثمار الفول السوداني في تجارب الصوبة والحقل و كانت العزلة *P. fluoressens* (Pf 5) هي الأقرب لقدرة المبيد في مقاومة موت البادرات، و أعفان جذور وثمار الفول السوداني بل وتفوقت علي المبيد الحيوي ,(Rhizo-N) وتلاها كلاً من *B. subtilis* (Bs1) و*Bacillus* sp. (S5) في كلا الموسمين 2009و2010.

قام بتحكيم البحث

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