J. Agric. Sci. Mansoura Univ., 31 (6): 3525 - 3536, 2006

EFFICIENCY OF SOME ANTAGONISTIC BACTERIA TO REDUCE INCIDENCE OF DAMPING-OFF, WILT AND PEANUT ROOT ROT

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ABSTRACT

Seventeen bacterial isolates from known sources and peanut plants were tested, *in vitro*, for their antagonistic effect against the pathogens causing damping-off, wilt and peanut root rot. Only five isolates *B. subtilis* (BS) *P. fluorescens* (Pf 5), (Sp1), (Sp2) and (Ss2) caused moderate to strong inhibition on mycelium growth 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 (Sp2) caused the widest 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).

INTRODUCTION

Peanut, (*Arachis hypogaea* L.) is one of the most export and locally consumed crops in Egypt. Damping – off, wilt and root rot disease are among the most destructive diseases attacking peanut in Egypt. They cause serious quantitative and qualitative losses in peanut yield, therefore growing peanuts in these soil becomes unprofitable (Yehia *et al.*, 1979, Al-Ahmer *et al.*, 1989 and Hassan and Frederick, 1995).

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, Meena *et al.*, 2001, and Mahmoud 2004). *Bacillus* and *Pseudomonas* are considering the important genera of these bacteria (Sailaja and Podile, 1998, Meena *et al.*, 2001 and Mahmoud 2004). Certain strains of *Bacillus 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., *Phytohpthora capsici, M. phaspolina* and *F oxysporum* (Nemec *et al.*, 1996; Asaka and Shoda, 1996, Mosa, *et al.*, 1997and Gabr *et al.*, 1998). 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 cankers caused by *Rhizoctiona solani* (Turner and Bakman, 1991, Podile and Prakash, 1996 and Ray and Mukherjee, 1997).

Recently, *Pseudomonas* spp. 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). Generally, it seemed to be able to suppress more than one disease *e.g. P. fluorescens* was found to be the effective biocontrol agent against various soilborne diseases caused by *F. oxysporum, R. solani, P .ultimum, M. phaseolina* and others (Stutz *et al.*, 1986, Karunanithi *et al.*, 2000; Jayashree *et al.*, 2000 and Meena *et al.*, 2001).In peanut, under greenhouse tests, 99% of peanut plants were protected form *S. rolfsii* infection when inoculated with *P. fluorescens.* (Patil *et al.*, 1998). *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 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 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.

This work was carried out to study the effect of some bacterial isolates in reducing of damping-off wilt and peanut root rot diseases.

MATERIAL AND METHODS

1. Isolation and purification of the causal organism (s):

Peanut plants showing symptoms of root rot disease were collected from different locations namely Beni-Suef, Giza, Ismailia and Nobaria. The infected roots were washed thoroughly with tap water, cut into small pieces (1 cm.) each surface disinfested with sodium hypochlorite 2 % for two min., re- washed several times with sterilized distilled water, dried between folds of sterilized filter paper, and were placed onto potato dextrose agar plates (PDA) supplemented with streptomycin-sulfate (100 µg/ml). Petri dishes were incubated at 28°C for five days. The growing fungi were purified using the hyphal-tip and single spore techniques (Brown, 1924 and Hawker, 1960)

2. Identification of causal organism (s):

Identification of the isolated fungi was carried out based on taxonomic criteria for these fungi as described by Barentt and Hunter (1977) for the genera of imperfect fungi, Ellis (1976) for *Macrophomina phaseolina*, Booth (1977) for *Fusarium* spp. Maren and Johan (1988) for *Aspergillus* spp. and Sneh *et al.,* (1992) for *Rhizoctonia solani.*

3. Preparation of fungal inoculum:

Inocula of isolates of *F. solani*, *M. phaseolina*, *R. 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.

4. 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.

5. Disease assessment

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

$$\text{\% damping - off} = \frac{\text{pre- emergence + post emergence}}{\text{No. of planted seeds}} \times 100$$

The wilted plants and root rotted plants were recorded after 45 days and during harvesting, healthy survival plants were recorded using the following formula:

% Healthy survival plants =

100 - (% damping-off + % wilted plants + % root rot plants)

6. Greenhouse experiments:

The experiments were carried out at Agriculture Research Center, Giza. Peanut seeds, cv. Giza 5, were used for sowing in 50 cm-diameter pots containing soil previously infested with mixture of *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, wilt, root rot and survival plants at 15, 45 days and during the harvesting time as previously mentioned.

7. Biological control:

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

7.1. 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.

7.2. 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.

7.3. 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).

7.4. Preparation of bacterial inoculum:

Bacterial suspensions (1×10^8 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.

7.5. 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 fungi, 15 days before planting.

7.6. Evaluation of antagonists under greenhouse conditions:

Pots experiment were carried out during season 2003 for studying the effect of selected nine antagonistic bacteria isolates, for controlling root rot incidence of peanut. Seeds, cv. Giza 5, were sown in pathogen infested soils as shown before at the rate of 10 seeds / pot; the antagonistic bacteria were applied as soil treatment. Damping-off, root rot incidence, and wilt were recorded.

8. 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

1. Bacterial isolates:

Fifteen bacterial isolates (Table 1) were isolated from the geocarposphere, rhizosphere, peanut peg and soil 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 season 2003.

Isolate code	Source	location
N.g	Geocarposphere	Nobaria
N.r 1	Rhizosphere	Nobaria
N.r 2	Rhizosphere	Nobaria
N.s 1	Soil	Nobaria
N.s 2	Soil	Nobaria
Sh.g	Geocarposphere	Sharkia
Sh.r 1	Rhizosphere	Sharkia
Sh.r 2	Rhizosphere	Sharkia
Sh.s	Soil	Sharkia
S.p1	Peg	Ismailia
S.p2	Peg	Ismailia
S.r 1	Rhizosphere	Ismailia
S.r 2	Rhizosphere	Ismailia
S.s 1	Soil	Ismailia
S.s 2	Soil	Ismailia

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

Fifteen bacterial isolates, in addition to two supplied bioagents, were evaluated *in vitro* for their antgagoistic effect against *R. salani, F. solani, M. phaseolina* and *S. rolfsii* on PDA medium (Table 2). Only five isolates *B. subtilis* (BS) *P.fluorescens* (Pf5), (Sp1), (Sp2) and (Ss2) caused moderate to strong inhibition to the four tested pathogens. While, (Nr1) and (Ss1) were moderately effective on *R. solani M. phaseolina* and (Shr2) isolate only had the same efficiency in inhibiting *S. rolfsii.*

Pseudomonase fluoressens (Pf5) followed by *Bacillus subtilis* (BS1) and *Bacillus* sp (Sp2) caused the widest inhibition zone almost to tested pathogens. Meanwhile most of other bacterial isolates had little effect in their inhibition of tested pathogens growth (Table 3).

Table (2): Screening of various bacterial isolates to determine their Antagonistic effect against different fungal pathogens associated with damping-off, wilt, 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.g	-	-	-	-
Bacillus sp N.r 1	+	-	+	-
Bacillus sp N.r 2	-	-	-	-
Bacillus sp N.s 1	-	-	-	-
Bacillus sp N.s 2	-	-	-	-
Bacillus sp Sh.g	-	-	-	-
Bacillus sp Sh.r 1	-	-	-	-
Bacillus sp Sh.r 2	-	-	-	+
Bacillus sp Sh.s	-	-	-	-
Bacillus sp S.p 1	+	+	+	+
Bacillus sp S.p 2	++	+	++	+
Bacillus sp S.r 1	-	-	-	+
Bacillus sp S.r 2	-	-	-	-
Bacillus sp S.s 1	+	-	+	-
Bacillus sp S.s 2	+	+	+	+

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 fungal pathogens associated with damping-off, wilt, and root rot of peanut.

Bacterial isolates	Inhibition zone [z]			
	Rhizoctonia solani	*Fusarium solani*	*Macrophomina phaseolina*	*Sclerotiom rolfsii*
B. subtilis (Bs 1)	23	22	14	11
P. fluorescens (Pf 5)	26	24	21	20
Bacillus sp (N.r 1)	12	0	11	0
Bacillus sp (Sh.r 2)	0	0	0	10
Bacillus sp (S.p 1)	14	16	9	9
Bacillus sp (S.p 2)	20	14	20	11
Bacillus sp (S.r 1)	0	0	0	10
Bacillus sp (S.s 1)	11	0	10	0
Bacillus sp (S.s 2)	13	11	14	15

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

3. Evaluation of antagonistic bacteria in the greenhouse:

Nine selected bacterial isolates were evaluated as soil drench treatment at sowing time. Results in Table (4) showed that, the most effective isolates in reducing peanut damping-off were *P. fluorescens* (Pf5) and *B. subtills* (BS1). While *Pseudomonas fluorescens* (Pf5) gave

the highest effect in reducing peanut wilt and *Bacillus* sp (Sp2) caused the highest effect in reducing peanut root rot.

Table (4): Effect of some antagonistic bacteria on damping-off, wilt, and root rot of peanut cv. Giza 5, grown in artificially infested soil, under greenhouse conditions [x].

Isolate code [y]	Disease incidence (%)			Survival (%)
	Damping-off	Wilt	Root rot	
Bacillus sp (S.r1)	24 a [z]	16 ab	24 ab	36 ef
Bacillus sp (Sh.r2)	20 c	16 ab	26 a	38 ed
Bacillus sp (S.s1)	24 a	16 ab	20 bc	40 d
Bacillus sp (S.s2)	22 ab	10 d	22 bc	46 c
Bacillus sp (S.p1)	18 cd	12 cd	10 d	60 b
Bacillus sp (S.p2)	16 c	10 ed	8 d	66 a
P. fluorescens (Pf 5)	12 e	8 e	10 d	70 a
B. subtilis (Bs 1)	10 e	10 ed	14 dc	66 a
Bacillus sp (N.r1)	22 ab	12 cd	24 ab	42 d
Control	24 a	18 a	26 a	32 f

x) Soil in each pot was infested with a mixture of pathogenuic fungi at the rate of 2% (w/w).

y) Soil was treated with 100 ml per pots 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 Pf5, Bs1 and Sp2 isolates gave the highest efficiency in reducing damping-off, wilt and peanut root rot compared to other treatments, while Sr1 and Shr2 isolates gave the lowest efficiency compared to other treatments.

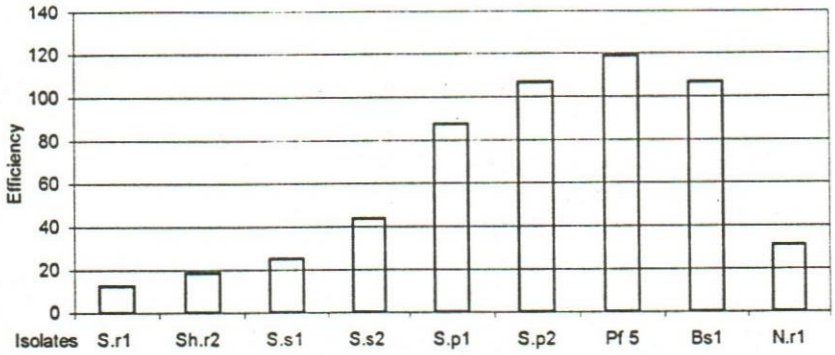

Fig (1): Efficiency of different bioagents for controlling damping-off, wilt, and root rot of peanut cv. Giza 5 under greenhouse conditions in artificially infested soil.

DISCUSSION

The results demonstrate the antagonistic effect of some rhizobacterial isolates, which obtained from soil, rhizosphere and peg of peanut and two stander isolates from *Bacillus subtilis* and *Pseudomonase fluorescence*.

From 17 tested isolates only five isolates *Bacillus subtilis* (BS) *P. fluorescens* (Pf5), *Bacillus* sp. (Sp1), *Bacillus* sp. (Sp2) and *Bacillus* sp. (Ss2) caused moderate to strong inhibition to the four tested pathogens. While *Bacillus* sp. (Nr1) and *Bacillus* sp. (Ss1) were moderately effective on *R. solani, M. phaseolina* and *Bacillus* sp. (Shr2) isolate only had the same efficiency in inhibiting *S. rolfsii*. *Pseudomonas fluoressens* (Pf5) followed by *Bacillus subtilis* (BS1) and *Bacillus* sp. (Sp2) caused the widest inhibition zone almost to tested pathogens. Meanwhile most of other bacterial isolates had little effect in their inhibition of tested pathogens growth. This is in agreement with Lazzaretti *et al.,* (1994); Asaka and Shoda, (1996), Ashour and Afify, (1999) and Mahmoud (2004), 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 *Pseudomonase fluorescens* was found to be the effective bio-control agent against various soil-borne diseases caused by *F. oxysporum, R. solani, P. ultimum, M. phaseolina* and others (Karunanithi *et al.*, 2000; Jayashree *et al.,* 2000, Meena *et al.*, 2001 and Mahmoud, 2004).

In greenhouse experiment, the same isolates bio-agents gave the highest reducing effect on damping-off, wilt and root rot diseases incidence under artificial conditions. The most effective isolates in reducing peanut damping-off, wilt and peanut root rot were *Pseudomonas fluorescens* (Pf 5) followed by *Bacillus subtills* (BS1) and *Bacillus* sp (Sp2). This is in agreement with Turner and Bakman, (1991), Podile and Prakash, (1996), Patil *et al.*, (1998), Sheela *et al.*, (1998), Dileep *et al.*, (1999) and Mahmoud, (2004). Further study in that respect showed that certain *Pseudomonase fluorescens* and *Bacillus subtilis* isolates were the most effective rhizobacteria for suppression of damping – off, wilt and peanut root rot, which showed great inhibition of hyphal growth *in vitro.* This suggested that, their biocontrol activity had been associated with the production of certain secondary compounds. 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; 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 antibiotic compounds called pyrollnitrin, HCN and lytic enzymes (Meyer *et al.,* 1992; 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. have been showed induce resistance activity in several plants (Wei *et al.*, 1996; Liu *et al.*, 1997). Vanwees *et al.* (1997) elucidate the molecular mechanisms responsible for this type of defense reaction. *Bacillus 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 *Pseudomonase 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 *Pseudomonase fluorescens* (Pf 5) and *Bacillus subtilis* significantly reduced incidence of all types of pod rots caused by *R. solani S. rolfsii, M. phaspolina, Fusarium* spp. and *Aspergillus* spp.

REFERENCES

Al-Ahmer, B.A; El-Mandoh, N.E; El-Deeb, A.A and L.A. El-Ghareeb, 1989. Reaction to root and pod rot diseases of peanut *(Arachis hypogaea L.)* Annals of Agric. Sci. Moshtohor, 27: 1577-1592.

Asaka, O. and M. Shoda 1996. Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. J.Appl. Environ. Microbiol., 62: 4081-4085.

Ashour, A.Z.A. and A. H. Afify, 1999. Use of *Bacillus* strains mixture to improve biological control of cotton seedling disease. Proceeding of the Second International Conference on Fungi: Hopes and Challenges, 29 September to 1 October, 1999, Cairo, Egypt. Africa. J. Mycol. and Biotechnol., 7: 109-120.

Barentt, H.L. and B.B. Hunter 1977. Illustrated genera of imperfect fungi. Burgess Publishing Company, Minnesota, 241 pp.

Booth, C. 1977. *Fusarium* laboratory guide to the identification of the major species. C.M.I. Kew, Surrey, England, 608 pp.

Brown, N. 1924. Two mycological methods. II Amethod of isolated single strain fungi by cutting hyphal tip. Ann. Bot., 38: 402 – 406.

Callan, N. W.; D. E. Mather and J. B. Miller 1990. Biopriming seed treatment for biological control of *Pythium ultimum* pre-emergence damping off in sh 2 sweet corn. Plant Dis., 74: 368-372.

Cook, R.J. 1993. Making greater use of introduced microorganisms for biological control of plant pathogens Annu. Rev. Phytopathol., 31: 53-80.

De Meyer, G. and M. Hofte, 1997. Salicylic acid produced by the rhizobacterium *Pseudomoncs aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. Phytopathology, 87: 588-593.

Defago, G. and D. Hass 1990. Pseudomonads as antagonists of soil borne plant pathogen: modes of action and genetic analysis. pp. 249-291.In soil Biochemistry.Vol.6. (J.M. Bollag, and G. Stotzky, eds.) Marcel Dekker, New York, Basel.

Dileep, C.; B.S.D. Kumar and H.C. Dube 1999. Disease suppression and plant growth promotion of peanut by fluorescent *pseudomonad* strains, FPC 32 and FPO 4, in soil infested with collar-rot fungi, *Aspergillus niger.* Indian Phytopathol., 52: 415-416.

El-Hadidy, A.M. 2003. New approaches for controlling some soil borne fungal pathogens on pepper in reclaimed soil. Ph.D. Thesis, Fac. of Agric. Ain Shams Univ., 169 pp.

Ellis, M.B. 1976. More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.

Gabr, M. R.; N. A. Hussein; O. I. Saleh and M. A. Khalil 1998. Susceptibility of certain varieties and genotypes and control of wilt and root rot diseases of sesame attributed to *Fusarium oxysporum* f.sp. *sesami* and *Macrophomina phaseolina*. Egypt. J. Microbiol., 33: 403-428.

Hassan, A.M. and M.S. Frederick 1995. Peanut Health Management. The American Phytopathological Society Press., 117 pp.

Hawker, L.E. 1960. Physiological of fungi: univ. of London press, LTD warwish square, London.

Howell, C.R. and R.D. Stipanovic 1979. Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and whith an antibiotic produced by bacterium. Phytopathology, 69: 482-484

Jayashree, K.; V. Shanmugam; T. Raguchander; A. Ramanathan and R. Samiyappan 2000. Evaluation of *Pseudomonas fluorescens* (Pf-1) against blackgram and sesame root-rot disease. J. Biol. Cont., 14: 55-61.

Karunanithi, K.; M. Muthusamy, and K. Seetharaman, 2000. Pyrolnitrin production by *Pseudomonas fluorescens* effective against *Macrophomina phaseolina*. Crop Res. (Hisar), 19: 368-370 (C.F. CAB Abstracts 2000).

King, E.O.; M.K. Ward and D.E. Raney 1954. Tow sample media for the demonstration of pyocyanin and fluorescin. J. Lab., Clin Med., 44: 301-307.

Lazzaretti, E.; J. O. M. Menten, and W. Bettiol, 1994. *Bacillus subtilis* antagonistic to the principal pathogens associated with bean and wheat seeds. Fitopathologia Venezuelan, 7: 42-46 (C.F. CAB Abstracts 2000).

Leeman, M.; F.M. den Ouden; J.A. van Pelt; RP.M. Dirkx; H. Steiil; P.A.H.M. Bakker, and B. Schippers 1996. Iron availability affects induction of systemic resistance to Fusarium wilt of radish by *Pseudomonas fluorescens.* Phytopathology, 86:149-155.

Liu X.; P.E. Kolattukudy and M.X .Liu 1997. Expression of an anionic peroxidase and oxidative burst in tomato cells induced by the elicitor from *Verticillium albo-atrum.* Acta Phytophysiologica Sinica., 23: 220-226. (C.F. CAB Abstracts 2000)

Mahmoud, E.Y. 2004. Integrated control of pod rot diseases of peanut. Ph.D. Thesis, Fac. Of Agric, Ain – Shams Univ.154 pp.

Maren, A.K. and I.P. Johan 1988. A laboratory guide to the common Aspergillus species and their teleomorph. Commonwealth Scientific and Industrial Res. Org. Division of Food Processing. 116 pp.

Maurhofer, M.; C. Keel; D. Hass and G. Defago 1995. Influence of plant species on disease suppression by Pseudomonas fluorescens strain CHAO with enhanced antibiotic production. Plant Pathol., 44: 40-50.

Meena, B.; T. Marimuthu; P. Vidhyasekaran and R. Velazhahan 2001. Biological control of root rots of groundnut with antagonistic *Pseudomonas fluorescens* strains. Z. Pflanzenkr. Pflanzensch., 108: 369-381. (C.F. CAB Abstracts 2003).

Meyer, J.M.; D. Hohnadel and F. Halle 1992. Cepabactin from *Pseudomonas cepacia* a new type of siderophore. J. Gen. Microbiol., 135: 1479-1487.

Mickler, C.J.; K.L. Bowen and J.K, Kloepper 1995. Evaluation of selected geocarposphere bacteria for biological control of A. flavus in peanut plant soil, 175: 291 – 299.

Mosa, A.A.; S.T. Shehata and Soad M. Abd-Allah 1997. Biocontrol of cucumber damping-off by Fluorescent pseudomonads. Egypt. J. Appl. Sci., 12: 268-286.

Nemec, S.; L.E. Datnoff and J. Strandberg 1996. Efficacy of biocontrol agents in planting mixes to colonize plant roots and control root diseases of vegetables and citrus. Crop Protection, 15: 735-742.

Patil R.P.; K.S. Jagadeesh; P.U. Krishnaraj and J.H Kulkarni 1998. Bacterization of groundnut with *Pseudomonas fluorescens* for the control of collar rot caused by *Sclerotium rolfsii* Sacc. Karnataka J. Agric. Sci. 11: 423-425. (C.F. CAB Abstracts 2000).

Podile, A.R. and A.P. Prakash 1996. Lyasis and biological control of *Aspergillus niger* by *Bacillus subtilis* AF1. Can. J. Microbiol., 42: 533-538.

Ray, S.K. and N. Mukherjee 1997. Studies on *in vitro* antagonism of some bacterial isolates against *Sclerotium rolfsii* Sacc. causing foot rot of groundnut and sugarbeet. J. Microbiol. Res., 35: 99-105 (C.F. CAB Abstracts 2000).

Sailaja P.R. and A.R. Podile 1998. A phytoalexin is modified to less fungitoxic substances by crown rot pathogen in groundnut (*Arachis hypogaea* L.). Indian J. Exp. Biol., 36: 631-634.

Sailaja P.R.; A.R. Podile and P. Reddauna 1998. Biocontrol strain of *Bacillus subtilis* AF1 rapidly induces lipoxygenase in groundnut *Arachis hypogea* L. compared with crown rot pathogen *Aspergillus niger*. Eur. J. Plant Pathol., 104: 125-132.

SAS Institute, Inc 1996. SAS/STAT Users Guide, Version 6, 12 th Ed. Volume 2, 846 pp. SAS Institute, Inc. Cary, North Carolina.

Shammugam. V.; N. Senthil, T. Raguchander, A. Ramanathan and R. Samiyappan 2002. Interaction of *Pseudomonas fluorescens* with Rhizobium for their effect on the management of peanut root rot. Phytoparasitica, 30: 169 – 179.

Shanmugam. V; T. Raguchander, A. Ramanthan, and S. Samiyappan 2003. Management of groundnut root rot disease caused by *Macrophomina phaseolina* with *pseudomonas fluorescens*. Annals of plant protection. Sciences; 11: 304-308.

Sheela, J.K.; S. Vaprakasam and K. Seetharaman 1998. Effect of soil application of *Pseudomonas fluorescens* using different carriers on groundnut collar rot disease incidence. Madras Agric. J., 85: 191-192 (C.F. CAB Abstracts 2000).

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

Stutz, E.; G. Defago and H. Kern 1986. Naturally occurring fluorescent pseudomonals involved in suppression of black root of tobacco. Phytopathology, 76: 181-185.

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

Vanwees, S.C.; C.M.J. Pieterse; A. Trijssenaar; T. Van; Y.A. Westende; F. Hartog and L. . Vanloon 1997. Differential induction of systemic resistance in Arabidopsis by biocontrol bacteria. Mol. Plant Microb. Interact., 10: 716-724.

Wei, G.; J.W. Kloepper and S. Tuzun 1996. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth promotinhg rizobacteria under field condition. Phytopathology, 86: 221-224

Yehia. A.H., H.M. El-Sald, A.A. Ali, and A.A. El-Deeb, 1979. Fungicidal control of damping – off and root rot diseases of peanuts in Egypt. Agricultural research Review 2: 95-102.

فاعلية بعض العزلات البكتيرية في المقاومة الحيوية لأمراض سقوط البادرات والذبول وأعفان الجذور في الفول السوداني

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تم إختبار القدرة التضادية لسبعة عشر عزلة بكتيرية معزولة من الفول السوداني ومن مصادر معروفة على تثبيط نمو الفطريات المسببة لموت البادرات و أعفان الجذور والذبول في الفول السوداني معمليا و أظهرت النتائج أن خمسة عزلات فقط (*B. subtilis* (BS1), *P. fluorescens* (Pf 5), Sp1, Sp2, Ss2) أظهرت تثبيط متفاوت ما بين متوسط إلى عالي للنمو الميسليومي لهذه المسببات المرضية (*R. solani, S. rolfsii, F. solani M. phaseolina*) أظهرت عزلة *Pseudomonase fluorescens* (Pf 5) وعزلتي *Bacillus subtilis* (BS1) و *Bacillus* sp. (Sp2) أعلي قدرة تضادية لمعظم الفطريات المختبرة. في تجارب الصوبة و أوضحت الدراسة أن عزلة *P. fluoressens* (Pf 5) وعزلتي *B. subtilis* (BS1) و *Bacillus* sp. (Sp2) كانت أكثر العزلات قدرة علي خفض الإصابة بموت البادرات و أعفان الجذور والذبول في الفول السوداني.