EFFICIENCY OF SOME BACTERIAL STRAINS FOR CONTROLLING LIMB ROT DISEASES OF PEANUT IN SANDY SOIL

ABSTRACT

Four biocontrol agents; *Bacillus subtilis*, *B. amyloliquefaciens*, *Pseudomonas synxantha* and *Brevibacterium otitidis* were tested individually and/or in combined mixture for suppression of *Rhizoctonia solani* on peanut. All tested bacteria moderately inhibited the growth of *R. solani*. *B. amyloliquefaciens* was the best for inhibiting growth. In a field experiment, combined mixture of the four tested bacteria completely suppressed incidence disease also combined mixtures of *B. amyloliquefaciens* and *Brevibacterium otitidis* or *Pseudomonas synxantha* and *Brevibacterium otitidis* gave same result. Also, dehydrogenase and nitrogenase activities, root nodulation and peanut biomass yield were determined. All tested strains increased plant parameters however, *B. amyloliquefaciens* and *B. subtilis* were the most effective ones.

Key words: Biological control, *Bacillus subtilis*, *B. amyloliquefaciens*, *Pseudomonas synxantha*, *Brevibacterium otitidis*, *Rhizoctonia solani*, Peanut, enzyme activity and biomass yield.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) growers must protect their crop from foliar and soilborne diseases to optimize profits and maintain high yields **(Franke *et al.*, 1999).**

One of the most common soilborne pathogen is *Rhizoctonia solani* AG-4. It induced many diseases in peanut including seed decay, pre- and post- emergence damping-off, hypocotyls and root necrosis, peg rot, limb and root rot. **Bell and Summer (1984).** Control of *Rhizoctonia* limb rot in peanut is based on an integrated management approach using crop rotation, proper fertilization, irrigation management, chemical and biological control. In response to environmental and health concerns about extended use of pesticides, there is growing interest for finding alternative control approaches for use in integrated pest management (IPM) strategies for crop diseases. Biological control of plant diseases has been suggested as alternative control methods **(Cook, 1993)**. *Bacillus subtilis* and *Fluorescent pseudomonas*, isolated from the rhizosphere, are known to suppress several soil-borne diseases caused by phytopathoenic fungi and promote plant growth **(Mosa *et al.*, 2003). Yoshida *et al.* (2001)** demonstrated capability of *B. amyloliquefaciens* to inhibit mulberry anthracnose and secretion of several antifungal compounds. *Brevibacterium otitidis* has been joined as a bacterial biocontrol agent to antagonize two of powerful soilborne pathogenic fungi (*Macrophoamina phaseolina* and *Sclerotium rolfsii*) causing charcoal rot and southern blight of many crops **(Moussa *et al.*, 2006). Morsy (2005)** succeeded to prove the role of *Pseudomonas synxantha* as antagonistic bacterium against *R. solani* and *F. solani* in the rhizosphere of tomato plants.

Moussa, A. Lobna*; Ebtsam, M. Morsy *; Abeer, M.A. Shaltout ** and Soheir, S. Fahmy*
* Soils, Water and Environment Research Institute, ARC, Giza, Egypt
** Plant Disease Res. Institute, ARC, Giza, Egypt.

MATERIALS AND METHODS

- Peanut (*Arachis hypogaea* L.) seeds, cv. Giza 6 were provided from Horticulture Research Institute, ARC, Giza, Egypt.
- Sandy soil at Ismailia Agric. Res. Station was used during two summer seasons under sprinkler irrigation. Mechanical and chemical characteristics of the experimental soil are shown in Table1.

Table 1: Some mechanical and chemical characteristics of the experimental soil

(A) Mechanical properties				
Particle size distribution (%)				
Coarse sand	Fine sand	Silt	Clay	Textural class
31.82	61.61	1.22	5.35	Sandy

(B) Chemical properties										O.M. (%)	$CaCO_3$ (%)
EC	pH	Soluble cations (meq L^{-1})				Soluble anions (meq L^{-1})					
$dS\ m^{-1}$		Ca^{2+}	Mg^{2+}	Na^+	K^+	$CO_3^=$	HCO_3^-	Cl^-	$SO_4^=$		
0.33	7.68	1.61	1.28	1.02	0.18	---	1.53	1.92	0.64	0.44	1.42

- Rhizolex T (tolcofs-methyl thiram) was used as chemical fungicide.
- Biocontrol agents: Four of the potential biocontrol agents against limb rot and wilt disease were examined for their interaction with the pathogenic fungus *R. solani.* They were isolated from Egyptian soil *B. subtilis* and *Pseudomonas synxantha* were isolated by Morsey (2005), while *B. amyloliquefaciens* and *Brevibacterium otitidis* were isolated by **Moussa *et al.* (2006).**
- Pathogenic fungus: *Rhizoctonia solani* was isolated from the experimental soil (Ismailia Res. Station, ARC). It was identified on the basis of cultural and microscopic characteristics. Pathogencity of the strain toward peanut was estimated according to **Sneh *et al.* (1991).**

EXPERIMENTATION

Antibiosis of bioagents against phytopathogenic fungus

The antagonistic effect of the tested four biocontrol agents against *R. solani* was examined using agar plate inhibition zone technique. An explorative trial was made to study whether the biocontrol agents have the ability to grow and interact in medium of the tested fungus. All the tested biocontrol agents were succeeded to grow well on potato dextrose agar (PDA). A disc (5 mm. θ) of *R. solani* was inoculated at periphery of the plate against a steak of each tested bacterial strains at other periphery, which were firstly inoculated 24 hr before inoculation of the pathogenic fungus.

Inoculated plates were incubated at 25 °C for 3-5 days. The growth and reduction in mycelial growth of the pathogenic fungus were calculated using the following equation according to **Fokemma (1973):**

$$\text{Reduction\%} = R_1 - R_2 / R_1 \times 100$$

where: R_1= liner growth of control and R_2= liner growth of treatment

Field experiment

A field trial was conducted in an attempt to practices controlling of limb-rot disease biologically. The used experimental soil has history of limb-rot disease causing by *R.solani*. The experimental layout comprised peanut (*Arachis hypogaea* L., Giza 6), *Bradyrhizobium* sp. (R617) **** added by seed coating, four biocontrol agents (*B. subtilis, B. amyloliquefaciens, Pseudomonas synxantha* and *Brevibacterium otitidis),* beside the fungicide (Rhizolex-T). To avoid the possibility of earlier negative interaction between the introduced *Bradyrhizobium* and biocontrol agents, they were alternately applied by two different ways, while, biocontrol agents were applied as soil drench individually or in combined mixtures at rates of 1:1 after 15, 30 and 45 days of sowing. The fungicide (Rhizolex-T) was used with recommended dose (3 g/kg seeds). The NPK fertilizers were incorporated into soil at the rate of 200 kg/fed of superphosphate (15.5% P_2O_5), 45 kg N/fed and 50 kg K_2O/fed potassium sulphate (48% K_2O). Seeds were drilled in rows of 30 cm apart. The experimental design was a complete randomized block with three replicates.

After peanut maturity, shoots and roots dry weights were determined. While pods and 100 seed weights as well as the shelling percent were recorded. Data obtained were subjected to the statistical analysis according to **Snedecor and Cochran (1989).**

Biological determinations

Fungal count

The isolated fungi were counted and identified and estimated in the Mycology Res. and Plant Disease Survey Dept., Plant Pathol. Res. Instit. ARC. The frequency of each individual fungal group was calculated as according to **Martin, (1950)** as follows:

Fungi (%) = (No. colonies of each group/plate)/ (No. total appeared fungal colonies) x 100.

Nitrogenase activity of peanut root nodules was determined by assaying acetylene reduction as described by **Dilowarth (1970).** In addition, dehydrogenase activity in the rhizosphere was assayed according to **Thalmann (1967).**

RESULTS AND DISCUSSION

Antagonistic efficacy of the tested bacteria

Four bacterial strains were evaluated for their potentiality to antagonize growth of the pathogenic fungus (*Rhizoctonia solani*). Data presented in Table 2 showed that all the tested bacterial strains succeeded to reduce radial growth of the pathogenic fungus. The most effective one was *Bacillus amyloliquefaciens* which gave 48.8%

reduction followed by *B. subtilis* (47.8%). *Brevibacterium otitidis* and *Pseudomonas synxantha* resulted in reduction of the growth being 37.8 and 35.3%, respectively in comparison with control. These findings are in agreement with those obtained by **Yoshida *et al.* (2001)** who attributed potentiality of *Bacillus amyloliquefaciens* St-RC-2 against *Colletotrichum dematium* (which cause mulberry anthracnose), to the antifungal compounds produced in the culture filtrate. Potentiality of *Ps. synxantha* and *Brevibacterium otitidis* to retard the fungal growth could be attributed to their antibioses towards the tested fungus. Similar results were reported by **Turner and Messenger (1986),** who estimated the ability of *Pseudomonas*, *Streptomyces*, and *Brevibacterium* to produce phenazine compounds.

Table (2): Influence of bacterial bioagents on mycelial growth of *Rhizoctonia solani (in vitro).*

Bacteria	Mean radial growth (cm)	
	Radial growth	Reduction %
B. amyloliquefaciens	4.6	48.8
Pseudomonas snyxantha	5.8	35.5
B. subtilis	4.7	47.4
Brevibacterium otitidis	5.6	37.8
Control	9.0	0.0

Efficiency of the bacteria on limb rot incidence on peanut

Data presented in Figure 1, showed that all the tested bacterial strains succeeded to suppress the incidence disease on peanut plants as well as the fungicide Rizolex T. The most effective bacteria were *B. subtilis* and *B. amyloliquefaciens* which caused 84.1% reduction in disease incidence followed by *Brevibacterium otitidis* (74.3%) and *Ps. synxantha* (56.6%).

Data also cleared that using a combined mixture of the four strains or mixture of *Brevibacterium otitidis* with either *B. amyloliquefaciens* or *Ps. synxantha* completely suppressed the incidence disease on peanut plants (100% reduction). These results are in harmony with those obtained by **Yu and Sinclair (1996)**. On the other hand, **Jetiyanon *et al.* (2003)** indicated that *B. amyloliquefaciens* induced systemic resistance (ISR) in several plants against different plant pathogens. These (ISR) include lignification, peroxidase and superoxide dismutase production. Strains of *B.mojavensis*, *B. amyloliquefaciens*, *B.subtilis* and *Brevibacterium halotolerans* are antagonistic to the fungus *Fusarium* moniliforme **(Bacon and Hinton, 2002).**

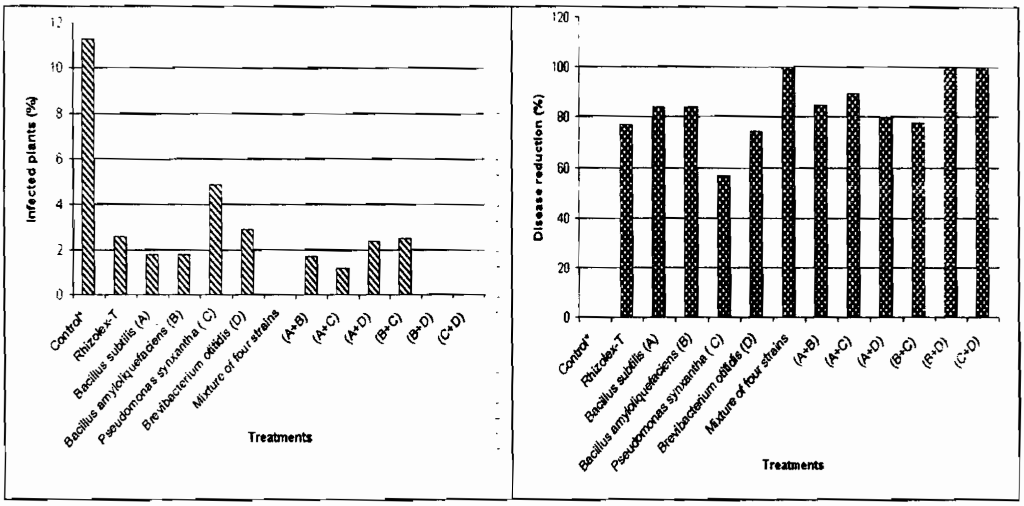

Fig. 1: Effect of the four tested bacteria on incidence of peanut limb rot caused by *Rhizoctonia solani*

Effect of the tested bacteria on beneficial fungi in the rhizosphere of peanut

Effect of the tested bacteria on the frequency percentage of beneficial fungi such as *Trichoderma harzianum* and *T. hamatum* in the rhizosphere of peanut through growth stages was investigated. Data in Table 3 indicated that frequency of the two beneficial fungi was increased with adding the four tested bacteria compared to control and the fungicide (Rhizolex-T) treatments.

Table (3): Effect of tested bacteria on beneficial fungi frequency in peanut rhizosphere.

Treatments	Total fungal Counts (cfu x 10^3)			Frequency of beneficial fungi %					
				Trichoderma harzianum			*Trichoderma harzianum*		
	V	F	M	V	F	M	V	F	M
Control	25	28	22	15	18	20	12	10	12
Rhizolex -T	5	10	10	0	10	10	0	10	20
Bacillus subtilis	12	15	10	25	26	30	17	20	10
Bacillus amyloliquefaciens	14	17	13	24	24	23	24	14	15
Pseudomonas synxantha	12	16	11	16	25	27	16	18	18
Brevibacterium otitidis	13	15	10	15	20	20	15	18	10
Mixture of four strains	10	12	9	30	33	33	20	16	22
B. subtilis + B. amyloliquefaciens	14	16	10	28	30	30	14	18	20
B. subtilis + Ps. synxantha.	8	10	10	25	25	30	12	20	20
B. subtilis + Br. otitidis	10	13	11	20	23	27	10	15	18
B. amyloliquefaciens + Ps. synxantha.	13	15	12	15	20	25	15	20	20
B. amyloliquefaciens + Br. otitidis	13	15	12	15	20	25	15	26	25
Ps. synxantha.+ Br. otitidis	12	14	10	25	28	30	16	21	20
Before sowing	20			15			10		

V= Vegetative stage F= Flowering stage M= Maturity

Also, it was shown that the most frequencies of *T. harzianum* were recorded due to *B. subtilis* and *B. amyloliquefaciens*. However, *T. hamatum* frequencies varied with the tested bacteria and growth stages. Also, the mixture of the four tested bacteria dramatically increased the frequency percentages of the two fungi compared with using either. This was also shown when adding mixtures of *Brevibacterium otitidis* with each of *B. amyloliquefaciens* and *Ps. synxantha. T. harzianum* is known to produce extracellular cell wall degrading enzymes such as chitinase, β-1-3-glucanases and cellulases which are important features of mycoparasites for the colonization of their host fungi (Di Pietro, 1995). Here, it is suggest that *Trichoderma* species found in peanut rhizosphere could be also having an antagonistic effect against the tested pathogen.

Influence of repeated inoculation with different bacterial strains on growth and yield

Data presented in Table 4 shows that, introduced of antagonistic bacteria significantly increased the dry weights of peanut shoot and root. The increase percentages were 92.7 and 87.3% for shoot, 100 and 85.7% for root with *B. amyloliquefaciens* and *B. subtilis* respectively. The combined mixtures effect of the four strains recorded increase of 76.6 and 71.4% for shoot and root, respectively compared with control and soil treated with the fungicide Rizolex-T. The obtained results indicated potentialities of antagonistic bacteria to suppress the plant pathogens and stimulated its growth.

Bai *et al.* (2002) pointed out that *B. subtilis* when applied as co-inoculation to soybean plant provided the most consistent increases in shoot and root weights. The application of antagonistic bacteria alone significantly increased the pod peanut yields up to 48.9, 44.9 and 38.9% for *B. amyloliquefaciens*, *B. subtilis* and *Brervibacterium otitidis*, respectively, than the control and soil treated with Rizolex T. The combined mixture of *Ps. synxantha* + *Brevi. otitidis* dramatically increased the pod yield of peanut.

Table (4): Influence of repeated inoculation with different bacterial strains and fungicide on shoot, root biomass and yield of peanut plants

Treatments	Shoot dry weight		Root dry weight		Pods yield		100 Seed Weight (g)	Shelling %
	g/ plant	Increase %	g/ plant	Increase %	Ardeb/ fed.	Increase %		
Control*	44.9		1.4	-	14.9	-	92.5	64.5
Rizolex T	61.0	35.9	2.3	64.3	15.7	5.4	86.3	65.8
Bacillus subtilis	84.1	87.3	2.6	85.7	21.6	44.9	99.6	66.5
Bacillus amyloliquefaciens	86.5	92.3	2.8	100	22.2	48.9	98.7	67.1
Pseudomonas synxantha	68.3	52.1	2.5	78.6	18.2	22.1	96.1	64.6
Brevibacterium otitidis	73.8	64.4	2.4	71.4	20.7	38.9	98.2	64.9
Mixture of four strains	79.3	76.6	2.4	71.4	19.3	29.5	93.9	66.3
B. subtilis + B. amyloliquefaciens	68.2	51.4	2.3	64.3	18.1	21.5	95.6	66.9
B. subtilis + Ps. synxantha	61.2	36.3	2.7	92.9	18.3	22.8	93.7	66.3
B. subtilis + Brevi. otitidis	70.4	56.8	2.6	85.7	19.7	32.2	92.7	66.6
B. amyloliquefaciens + Ps. Synxantha	65.0	44.8	2.5	78.6	15.5	4.0	93.8	68.1
B. amyloliquefaciens + Brevi. Otitidis	61.5	35.6	2.2	57.1	18.1	21.5	93.7	67.1
Ps. Synxantha + Brevi. Otitidis	63.2	40.8	2.8	100	20.3	36.2	95.4	66.4
LSD at 0.05	18.4		0.4		5.3		6.4	

* Uninoculated treatment ** Soil treated with Rizolex T fungicide

This result is in harmony with that of Turner and Backman (1991) who recorded increased yield of carrots (48%), oats (33%) and peanuts (37%) when plants were inoculated with *B. subtilis*.

Concerning dry weights of 100 seeds and shelling percentages, data showed significant increases in all treatments as compared with those of the control and treatment with Rizolex T.

Effect of different bacterial strains on enzyme activity and dry weight of nodules in soil infected with *R. solani:*

Table 5 shows that all treatments inoculated with antagonistic bacteria remarkably recorded increases in DHA activity rather than control (uninoculated treatment).

Soil inoculated with *B. amyloliquefaciens*, *B. subtilis* and mixture of *B. subtilis* and *Ps. synxantha* gave the highest DHA enzyme activity.

Table (5): Effect of different bacterial strains on enzyme activity and dry weight of nodules in soil infected with *R. solani*

Treatments	Enzyme activity						Dry weight of nodules g/plant		
	Dehydrogenase *u*g TPF/g dry soil/day			Nitrogenase *u*mol C_2H_4/plant/hr					
	45 *d	75 *d	100 *d	45 *d	75 *d	100 *d	45 *d	75 *d	100 *d
Control*	23.3	35.0	46.2	0.75	0.76	1.40	0.13	0.33	0.700
Rizolex T	27.4	31.3	54.0	1.50	2.12	3.40	0.15	0.23	0.730
Bacillus subtilis	66.9	53.5	56.4	2.83	3.00	9.30	0.17	0.52	0.800
Bacillus amyloliquefaciens	59.1	51.2	57.1	3.51	7.34	19.3	0.16	0.49	0.832
Pseudomonas synxantha	46.3	46.3	54.5	2.89	2.92	7.40	0.19	0.25	0.860
Brevibacterium otitidis	20.3	55.4	54.2	5.10	6.30	7.90	0.18	0.45	0.763
Mixture of four strains	22.2	49.5	48.9	3.90	9.54	10.10	0.17	0.84	0.900
B. subtilis + B. amyloliquefaciens	36.9	42.8	64.1	4.60	6.12	5.10	0.18	0.47	0.780
B. subtilis + Ps. synxantha	54.7	50.8	76.3	2.32	5.23	12.54	0.17	0.51	0.950
B. subtilis + Brevi. otitidis	41.4	37.0	77.4	5.40	5.82	13.12	0.19	0.30	0.995
B. amyloliquefaciens + Ps. Synxantha	28.8	57.0	49.3	2.53	2.16	8.97	0.16	0.37	0.874
B. amyloliquefaciens + Brevi. otitidis	34.0	43.8	69.6	4.97	6.60	15.10	0.18	0.42	0.950
Ps. Synxantha + Brevi. otitidis	38.2	45.3	61.4	3.60	4.60	14.91	0.20	0.45	0.880

* After 45 days, 75 days and 100 days

LSD at 5% for:	Dehydrogenase	Nitrogenase	Dry weight of nodules
Treatment	17.3	2.27	0.16
Periods	9.37	1.11	0.11
T x P	30.1	3.93	0.27

The increase in DHA activity as a result of soil inoculation with antagonistic bacteria may be due to the synergistic effect between the native soil micro organisms and the introduced ones.

Bacterial inoculation increased nodulation as well as N_2-ase activity but *B. amyloliquefaciens*, coupling of *B.amyloliqufaciens+ Brevi. otitidis* and coupling of *B. subtilis* + *Brevi. otitidis* gave higher N_2-ase activity and dry weights of nodules than remainder of treatments. In their investigation, a strain of B. can play a role in enhanced nitrogenous enzyme in soil. Bai *et al.* (2002) found that, enhanced nodulation and subsequent nitrogen fixation by soybean plants infested by three Bacillus strains.

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كفاءة بعض السلالات البكتيرية فى المكافحة الحيوية لمرض

عفن جذور الفول السودانى فى الأراضى الرملية

لبنى عبدالعزيز موسى[1]، إبتسام محمد مرسى[1]، عبير أحمد شلتوت[2]، سهير سيد محمد فهمى[1]

١ - معهد بحوث الأراضى والمياه والبيئة، مركز البحوث الزراعية، الجيزة، مصر

٢ - معهد بحوث أمراض النبات، مركز البحوث الزراعية، الجيزة، مصر

تم إختبار كفاءة أربعة سلالات بكتيرية فى المكافحة الحيوية لفطر ريزوكتونيا سولانى الذى يصيب الفول السودانى وهى باسلس ساتلس، باسلس أميلوليكوفيشنس، سيدوموناس ساينسانسا، بريفيباكتريم أوتيتداس وأظهرت النتائج أن كل السلالات أدت إلى تثبيط نمو الفطر وأظهر ميكروب باسلس أميلوليكوفيشنس أعلى نسبة تثبيط لنمو هذا الفطر. وفى تجربة حقلية بمحطة بحوث الأسماعيلية تم تقييم كفاءة هذه السلالات كلاً على حدة وبخليط منهم حيث ظهرت كفاءتهم فى مكافحة المرض. وقد تم تقدير نشاط إنزيمى الدهيدروجينيز فى التربة والنيتروجينيز فى العقد الجذرية وتكوين العقد الجذرية والمحصول المنتج من الفول السودانى. وأظهرت النتائج أن كل السلالات المختبرة أدت إلى زيادة معنوية فى الأوزان الجافة للنباتات وكذلك محصول الفول السودانى، بينما كانت السلالاتين باسلس ساتلس، باسلس أميلوليكوفيشنس أكثرهما كفاءة.