BIOLOGICAL CONTROL OF PEANUT DAMPING - OFF DISEASE BY *BACILLUS SPHAERICUS* SOIL TREATMENT.

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Abstract

Three isolates of *Bacillus sphaericus* were isolated from the rhizosphere of white rot free-onion plants. All isolates exhibited antagonistic potential against some fungi infecting peanuts. The highest antagonism to *Fusarium solani* was detected with isolate B_1. Isolate B_2 was the most antagonistic to *Rhizoctonia solani* , whereas *Sclerotium rolfsii* was most antagonized by isolate B_4 . The three bacterial isolates were not significantly different with respect to antagonizing *Macrophomina phaseolina.*

Application of these bacterial isolates (3×10^{8} cfu/ml) to potted soil resulted in a 57–71 % reduction of damping-off in the greenhouse when used at a rate equivalent to 4 l //fed.

Field application at the rate of 4.5 l / fed. after sowing resulted in a 48–77 % reduction in peanut damping–off with a 22.7–54.54 % increase in yield over the control treatment. The bio–control potentiality was slightly increased when the rate of application was raised to 6 l / fed. accompanied with 44–56 % increase in yield over the control treatment . Soil spray with *B. sphaericus* at the rate of 6 l / fed. after planting seed could be recommended.

INTRODUCTION

Rhizoctonia solani, Fusarium solani, Sclerotium rolfsii and *Macrophomina phaseolina* are mostly the main pathogens isolated from rotted peanut roots (El-Deeb, 1977, Porter *et al.*, 1990). Losses in the stand may reach 35% in some fields. Biological control of these pathogens on peanut and other crops was tried as seed treatments using fungal and bacterial bioagents (Mathivanan *et al.*, 2000, Jagadeesh and Geeta, 1994, Turner and Backman, 1991 and Ganesan and Gnanamanickam, 1987).

The present research was carried out to evaluate the efficacy of three isolates of *Bacillus sphaericus,* isolated from onion plants rhizosphere, for controlling peanut damping–off. Evaluation was made in the laboratory , greenhouse and in the field.

MATERIALS AND METHODS

Laboratory experiments:

Bacteria were isolated by the first author from the rhizosphere of white rot - free onion plants grown at Mallawy Experimental Research Station, El-Minia Governorate. The isolates were identified at the Fermentation Technology and Applied microbiology unit, Fac. of Science, AL–Azhar University.

Rhizotonia solani, Sclerotium rolfsii, Fusarium solani and *Macrophomina phaseolina* were previously isolated from damped-off peanut seedlings collected from a field at Beheira Governorate in 1997. The isolates were identified by the first author according to Porter *et al.*, (1990), Domesch *et al.*, (1980), Gilman (1975) and Barnett (1955).

Antagonism of the bacterial isolates to *R. solani, S. rolfsii. F. solani* and *M. phaseolina* was tested on PDA plates. Two disks of 10 day-old fungal culture were oppositely placed at sides of each plate and then bacterial isolates were longitudinally streaked between disks. Plates were incubated at 26° C for 10 days and the width of inhibition zones was recoded in cm.

Greenhouse experiments:

Bacterial isolates were grown in potato dextrose broth (pH 7) at 26^5C for 2 weeks. *R. solani, S. rolfsii. F. solani* and *M. phaseolina* were cultured on sorghum grains and incubated at 26^5c for 15 days. Soil was infested with a mixture of these fungi at the rate of 1% (w/w) for each fungus. Pots (20 cm in diam.) sown with 5 peanut seeds each were treated with bacterial suspension (3×10^8 cfu / ml) at rates equivalent to 1, 2, 3 and 4 l / fed. Each treatment was replicated three times. Percentages of pre, post–emergence damping–off and healthy survived plants were recorded. Percent reduction in disease incidence, percentage of increase in yield and treatments efficacies were calculated .

Field trials:

Trials were carried out in naturally infested fields located at (Beheira governorate) in May 2001 and 2002. Soil was sprayed with the PD broth cultures (3×10^8 cfu/ml) at the rates of 1.5, 3 and 4.5 l / fed. in 2001 and 3 and 6 l/fed in 2002. Pre–emergence, post-emergence damping–off and survivals were recorded for each 10.5 m^2 plot . Each treatment included 3 plots. Each plot was planted with 88 – 91 peanut seeds. Crop was harvested at 110-120 days after planting and yields were weighed.

Complete randomized design in a factorial arrangement was applied for laboratory experiments. while a complete randomized design was used for greenhouse experiments. Field trials were designed in complete randomized blocks. Results were statistically analyzed using "F" test and LSD values to compare treatments.

RESULTS AND DISCUSSION

Laboratory experiments:

Three bacterial isolates identified as *Bacillus sphaericus* were found antagonistic to the isolates of *S. rolfsii, R. solani, F. solani* and *M.* phaseolina isolated from diseased peanut plants. Means of inhibition zone values were significantly different (Table 1).

The most sensitive fungus was *S.rolfsii* followed by *F.solani, R.solani* and *M. phaseolina* in a descending order. The bioagents–fungi interaction was variably significant, depending on the isolate of the antagonist.

Table 1. Antagonism of bacterial isolates to peanut fungal pathogens expressed as a width of inhibition zones in cm.

pathogens	Inhibition zone in cm of bacterial isolates			Mean of inhibition zone for each pathogen (cm)
	B_1	B_2	B_4	
S. rolfsii	1.75	1.65	2.00	1.80
F. solani	1.30	0.95	0.75	1.00
R. solani	0.35	0.55	0.50	0.47
M. phaseolina	0.25	0.20	0.30	0.25
Mean of each isolate antagonist	0.91	0.84	0.89	

L.S.D's at 0.05

treatments = 0.2 pathogens (P) = 0. 1

bacteria (b) = N. S. pxb interaction = 0. 2

S. rolfsii was most suppressed by isolate B_4, while *F. solani* was most antagonized by isolate B_1, and *R. solani* by the isolate B_2. On the other hand, the three isolates were not significantly different against *M. phaseolina*. Differences in antagonism of bacterial isolates across the four fungi, were not significantly different.

Antagonism as an important aspect of biological control (Baker and Cook, 1974) has been reported with respect to mycelial growth of the same fungal pathogens by different bioagents on different host plants such as *Pseudomonas fluorescens* against *S. rolfsii* in peanut (Ganesan and Gnanamanickam, 1987), *T. harzianum* and *Penicillium pinophilum* for *S. rolfsii* in sesame (Gaikwad and Kapgat, 1990), *T. harzianum* against *R. solani, M. phaseolina, S. rolfsii* and *F. oxysporum* in sesame (Hamed *et al.*, 1996) and *T. viride* against *R. solani* in peanut , cotton , okra, eggplant and sunflower (Mathivanan *et al.*, 2000).

Greenhouse experiments:

Potted soil separately treated with cultures of B_1, B_2 and B_4 isolates at rates equivalent to 1, 2, 3 and 4 l/fed. significantly reduced pre–emergence damping–off except B_4 isolate at the rate of 1 l / fed . (Table 2)

B_1 isolate at the rate of 4 l/fed., B_2 and B_4 isolates at the rates of 2, 3 and 4 l /fed. were significantly superior to other treatments, exhibiting a range of 57.1–71. 4% reduction in disease incidence. B_1 isolate at the rate of 1, 2 and 3 l/fed. and B_2 isolate at the rate of 1 l/fed. were significantly less effective exhibiting 28.6–42.9 % reduction in disease incidence. Values of post–emergence damping–off were not significantly different as almost all treatments showed no disease incidence. All treatments significantly increased healthy survived plants ranging between 42. 8 and 85.7 % . The highest applied rate (4 l/fed.) resulted in the highest percentage of survived plants. It is obvious that the increase in survivals is a direct result of reducing pre-emergence damping off .

Table 2. Percent damping off and survivals of peanut plants in response to potted soil treatment with different isolates of *B. sphaericus.*

Bacterial isolates	Rates l/ fed*	% damping – off			% Survivals	
		Pre -	% reduction in pre-	Post -	incidence	Increase over control
B_1	1	33.33	28.6	0.00	66.67	42.8
	2	26.66	42.9	0.00	73.34	57.1
	3	26.66	42.9	0.00	73.34	57.1
	4	20.00	57.1	0.00	80.00	71.4
B_2	1	33.33	28.6	0.00	66.67	42.8
	2	20.00	57.1	6.60	80.00	71.4
	3	20.00	57.1	0.00	80.00	71.4
	4	20.00	57.1	0.0	80.00	71.4
B_4	1	40.00	14.3	0.00	60.00	28.5
	2	20.00	57.1	0.00	80.00	71.4
	3	20.00	57.1	0.00	80.00	71.4
	4	13.33	71.4	0.00	86.67	85.7
Control	0.0	46.66	-	6.66	46.68	-
L.S.D at 0.05		12.4	-	Ns	11.38	-

* 1 ml= $3X10^8$ cfu

Field experiments:

Efficacy of the B_1, B_2 and B_4 isolates of *B. sphaericus* in controlling peanut damping–off in a naturally infested field was evaluated in 2001. All tested rates signifcantly reduced the disease except for the B_4 isolate at 1.5 l/fed. Disease incidence was significantly suppressed when the rates of B_1 and B_4 isolates were increased. The two bioagents at 4.5 l / fed could reduce the disease by 77.4 and 60.72%, respectively. Disease incidence obtained with B_2 isolate at 4.5 l/fed. was significantly higher than that obtained with B_1 isolate at the same rate. Values of post–emergence damping–off were not significantly different from the control.

Table 3. Effect of field soil treatment with bacterial isolates on controlling peanut damping – off (Beheira, 2001)

isolates	Rates l / fed	% damping – off			% Survivals		Yield	
		Pre -	% reduction in pre-	Post -	incidence	% increase	Kg/plot	% increase
B_1	1.5	22.84	26.6	4.86	72.30	11.5	5.5	25.0
	3.0	15.15	51.3	5.68	79.17	22.1	5.9	34.1
	4.5	7.03	77.4	4.07	88.90	37.1	6.8	54.5
B_2	1.5	21.11	32.1	4.07	74.82	15.4	5.6	27.3
	3.0	16.28	47.7	4.92	78.80	21. 6	5.5	25.0
	4.5	16.10	48.2	2.24	81.66	26.0	5.4	22.7
B_4	1.5	28.88	7.2	3.33	67.79	4.6	5.2	18.2
	3.0	14.81	52.4	4.81	80.38	24.0	5.8	31.8
	4.5	12.22	60.7	4.81	82.97	28.0	6.0	36.4
Control	0.0	31.11	-	4.07	64.82	-	4.4	-
LS D at 0.05		6.8	-	N. S.	5.94	-	0.85	-

Percentage of healthy survived plants were highly increased when B1 was used at the rate of 4.5 l/fed. more than other treatments, exhibiting 88.9% healthy survived plants. While B2 at the same rate displayed less percentage of survived plants, which may be clear the lower efficacy of B2 than B1 at this rate. B4 at the rate of 4.5 l/fed exhibited 82.97% healthy survived plants without significant differences with B_1 and B_2

Peanut yield was also affected by soil treatments with the bacterial isolates. The highest yield was obtained with B_1 and B_4 isolates at the rate of 4.5 l/fed. exhibiting 54.5% and 36.4 % increase in yield over control value, respectively. Other treatments displayed lower increase in yield, except B_4 isolate at the rate of 1.5 l / fed. which was not significantly different from the control.

In the season (2002), the experiment was carried out using 3 and 6 l / fed. of the bacterial preparations (Table 4).

Table 4. Evaluation of field soil treatments with bacterial isolates for controlling peanut damping – off (Beheira, 2002)

Bacterial isolates	Rates l / fed	% damping – off		Survival	Yield kg / plot
		Pre -	Post -		
B 1	3	17.77	3.33	78.90	5.5
	6	5.49	6.59	87.92	6.5
B 2	3	12.35	4.86	82.79	5.5
	6	11.23	4.86	83.91	6.0
B 4	3	13.48	4.49	82.03	5.3
	6	9.89	6.96	83.15	6.4
Control	0.0	26.37	10.98	62.65	4.16
LSD at 0.05		5.86	3.99	5.12	0.72

All treatments significantly reduced pre-emergence damping off. The lowest value of pre–emergence damping–off (5.49 %) was obtained with B_1 isolate at the highest rate (6 l/fed.), which was not significantly different from those obtained with of B_2 and B_4 isolates applied at the same rate. The lower rate of 3 l/fed. resulted in lower control potentials .

All treatments significantly reduced post–emergence damping–off compared with control treatment. No significant differences were detected between post–emergence disease incidence observed in different bacterial treatments. Increase of applied rates from 4.5 to 6.0 l/fed. in 2002 resulted in a slight increase in healthy survivals . Increasing rates over 6.0 l/fed. may result in less efficacy in controlling the disease as was previously detected with *Penicillium janthinellum* in controlling white rot disease of onion and garlic. This phenomenon was attributed to self toxicity by the bioagent condensed metabolites excreted and accumulated at excessive levels (El-Shehaby, 2000).

Yield of peanut was significantly increased at the higher rate (6 L /fed) of the bioagent compared to the lower rate (3 l / fed.). The increase in yield obtained by

B_1, B_2 and B_4 isolates at the rate of 6l/fed. were 65.25 , 44.23 and 53.84% over the control treatment, respectively.

Based on the results reported herein , soil spray with *B.spaericus* at the rate of 6 l/fed (3.0 × 10^8 cfu/ml) after seeding and directly before irrigation can be easily applied and is highly recommended to control damping–off disease in peanut fields.

These results are in agreement with those obtained by some investigators for biologically controlling damping–off on peanut and other crops (Ganesan and Gnanamanickam, 1987), Farzana Ali *et al.*, 1991), Turner and Backman, 1991), Rossal and Mcknight, 1992), Jagadeesh and Geeta, 1994, Wuik *et al.,* 1998, Ehteshamul – Haque, *et al.,* 1998 and Mathivanan *et al.*, 2000) .

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المقاومة الحيوية لمرض موت البادرات فى الفول السودانى بالبكتريا باسيلس سفاريكس

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أمكن الحصول على ثلاث عزلات للبكتريا باسيلس سفاريكس من ريزوسفير نباتات بصل غير مصابة بمرض العفن الأبيض، وقد اختبرت قدرة العزلات على التضاد مع بعض المسببات المرضية التى تصيب الفول السودانى . وقد اظهرت العزلات تباينا في قدرتها التضادية لهذه المسببات المرضية، وكان اكثر العزلات تضادا مع الفطر فيوزاريوم سولانى هى العزلة ب١، بينما كانت العزلة ب٢ هى الأقوى فى تضادها مع الفطر رايزوكتونيا سولانى ، كما كانت العزلة ب٤ هى الاكثر تضادا للفطر سكليروشيم رولفزياى ، وقد أظهرت العزلات الثلاثة تضادا مع الفطرماكروفومينا فاسيولينا ، وكانت قيم هذا التضاد متقاربة حيث لم يظهر اختلافا معنويا فيما بينها .

وفي تجارب الصوبة، اظهرت هذه العزلات كفاءه في مقاومة مرض موت البادرات للفول السودانى قدرها ٥٧ – ٧١ %، عندما أضيفت البكتريا إلى التربة بمعدل يكافئ ٤ لتر / للفدان (يحتوى ١ مل على ٣ × ١٠^٨ خلية نشطة).

وقد اظهرت تجارب المكافحه الحيويه فى الحقل التى اجريت بمحافظة البحيرة ٢٠٠١ كفاءة في مقاومة المرض قدرها ٤٨ – ٧٧ %، مع زيادة في المحصول قدرها ٢٢,٧ – ٥٤,٥ % من محصول الكنترول ، وذلك عندما أضيفت البكتريا إلى التربة بمعدل ٤,٥ لتر / للفدان، وقد زادت كفاءة هذه العزلات زيادة ضئيله عندما ازداد معدل الاستخدام في العام التالى إلى ٦ لتر / للفدان، حيث انخفض المرض بنسبة ٥٧ – ٧٩ % مع زيادة في محصول الفول السودانى قدرها ٤٤ – ٥٦ % عن محصول الكنترول.

وتعتبر معاملة التربة بالبكتريا باسيلس سفاريكس. بمعدل ٦ لتر / للفدان توصية يمكن تطبيقها بعد زراعة البذور وقبل الرى مباشرة لمقاومة مرض موت البادرات في حقول الفول السودانى.