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BIOLOGICAL CONTROL OF PEAS (*Pisum sativum* L.) DAMPING-OFF DISEASE CAUSED BY FOUR FUNGI

Eisa, Nour-Jehan M. M.
Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt.

ABSTRACT

Four biocontrol agents, namely Rizo-N (*Bacillus subtilis*), Plant Guard (*Trichoderma harzianum* and *T. konigii*) and Pseudomonas fluorescents, were tested for their antagonistic action against four fungi causing pea damping-off, i.e. *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani* and *Macrophomina phaseolina*. Through *In vitro* studies, Promot caused the highest percentage decrease in linear mycelial growth of all the tested pathogenic fungi, followed by Rizo-N and Plant Guard whereas low effect was recorded for *P. fluorescents*. Rizo-N (*B. subtilis*) gave best results in controlling pea damping-off disease *In vivo*.

Keywords: Rizo-N, Promot, biocontrol, pea damping-off, *Rhizoctonia solani, Sclerotium rolfsii, Macrophomina phaseolina, Fusarium solani* f.sp. *pisi, Pseudomonas fluorescens.*

INTRODUCTION

Fusarium solani (Mart), *Rhizoctonia solani* (Sacc.), *Macrophomina phaseolina* (Maubl.) and *Sclerotium rolfsii* (Kühn) are considered the most pathogenic fungi attacking legume crops in Egypt (Omar, 1977, Nofal, *et al.*, 1982, El-Gantiry, *et al.*, 1994 and Omar and Abd, 2000).

Application of antagonistic microorganisms to the seeds before sowing has been successfully used for controlling damping-off and root-rot diseases of several plants (Walther and Cindral, 1988, Sabet, *et al.*, 1991 and Abd El-Moity, 1992).

Damping-off caused by some fungi is one of the most serious diseases affecting bean seedlings (Al-Jurifani, Amal, 1996). Among the most destructive fungi causing broad bean damping-off disease is *R. solani* (Rusuku, *et al.*, 1997).

MATERIALS AND METHODS

1. Isolation and identification of the causal fungi:

Samples of naturally infected legume plants (pea and cowpea) showing different degrees of root-rot and damping-off symptoms were collected from four governorates, i.e. Giza, Ismaillia, Sharkia and Kalubia. Discolored roots were cut into small fragments, surface sterilized by immersing them in 3% sodium hypochlorite for 2 minutes and then washed several times in sterilized distilled water. Surface sterilized root fragments were dried between two folds of sterilized filter paper. Then transferred onto potato dextrose agar (PDA) medium (4 pieces per a dish). Plates were incubated for 5 days at 25°C. Any developed fungus was transferred to new

PDA plates, purified and identified following Gilman (1957) and Barnett and Hunter (1972).

Bacterial colonies appearing during isolation were also picked up. Purified by streaking on nutrient agar (NA) medium in Petri dishes (Dhingra and Sinclair, 1985). Identification procedures were carried out in Plant Dis. and Biological Control Dept. Plant Pathology Research Institute, Giza, Egypt, according to Claus and Berkeley (1984).

2. Pathogenicity test:

The isolated fungi, i.e. *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani,* and *Macrophomina phaseolina,* were individually tested for their pathogenicity on Progars peas and Dokkie 331 cowpeas cultivars under greenhouse conditions. Pots (25cm in diameter) were sterilized by immersing them in 5% formalin solution for 15 minutes and left to dry. The clay soil, used for planting in pots, was also sterilized with 5% formalin solution. Then, pots and soil were left for 3 weeks to allow formaldhyde evaporation.

Inoculum of the isolated fungi was separately grown on moistened sterilized corn-sand medium for 20 days at 25°C. Soil infestation was carried out using the inoculum of each fungus at the rate of 2% of soil weight. Inoculum was mixed thoroughly with the soil in each pot, watered and left for one week to ensure even distribution of the inoculum. Control pots were filled with the same soil mixed with the same amount of sterilized corn-sand medium (non infested soil). A set of five pots with ten seeds per pot, were used for each tested fungus.

Healthy seeds of Progars peas and Dokkie 331 cowpeas cultivars were then sown at a depth of 2 cm (Gonzalez-Avila and Marrero-Gonzalez, 1981) and watered regularly every 3 days under greenhouse conditions at Plant Pathology Research Institute, Agric. Res. Center, Giza Governorate. The atmospheric temperature ranged between 22°C to 29.5°C. Percentages of pre-, post-emergence damping-off and root rot were recorded after 15, 30 and 60 days from planting, respectively.

3. Biological control:

- In vivo:

This investigation was carried out for studying the efficacy of some biocontrol agents, prepared by the commercial companies namely Plant Guard (*Trichoderma harzianum*, 30 x 10^6 cells per ml), Promot (*Trichoderma harzianum* and *T. konigii*, 50 x 10^6 cells per g) Rizo-N (*Bacillus subtilis*, 30 x 10^6 cells per g and *Pseudomonas fluorescens* 30 x 10^6 cells per g) in controlling pre-, post-emergence damping-off and root rot diseases of Progars peas cultivar in the soil previously infested with either *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium solani* f.sp. and *Macrophomina phaseolina*, under greenhouse conditions. These biocontrol agents individually added to the pots (25cm in diameter) at the rate of 500ml solution prepared from Plant Guard (5ml per liter of water), Promot (5g per liter of water), Rizo-N (5g per liter of water) or *P. fluorescens* (5ml per liter of water) directly at sowing time as recommended doses of the producing company for each biocontrol agent. Ten seeds were sown per pot and each treatment contained five pots. Control treatment was carried out without adding biocontrol agent. Disease assessment was recorded as percentage of pre- and post-emergence damping-off after 15 and 30 days from sowing, respectively. Whereas, root rot and survival plants were calculated 60 days after sowing.

3. Biological control:

- In vivo:

3.1. Antagonistic effect of bacteria:

In order to study the antagonistic effect of bacteria on the growth of the four pathogens, each bioagent was streaked at one side on PDA medium in plates (Dhingra and Sinclair, 1985). Plates were incubated for 24 hrs. at 30°C, then one disc (5mm in diameter) bearing 7-days-old growth of each described fungus isolate was placed on the opposite side at 25 mm distance. Plates were incubated for 5 days at 25°C. Control plates were streaked with sterilized distilled water instead of the bioagents. The number of replicates was 4 plates for each treatment. The decrease percentages that occurred in linear growth of the pathogenic fungi were determined at the end of the experiment in each treatment.

3.2. Antagonistic effect of fungi:

Antagonistic effect of both *T. harzianum* and *T. konigii* on the linear growth of the four tested pathogenic fungal isolates were carried out in Petri dishes containing PDA medium. Each plate was divided into two equal halves, one half was inoculated with a disc (5mm in diameter) of 5-days-old cultivar of either *T. harzianum* or *T. konigii* where as, the opposite half was inoculated with an equal disc of 7-days-old culture of any of the four tested pathogenic fungal isolates (Dhingra and Sinclair, 1985). Plates were then incubated at 25°C for 5 days. Control plates were inoculated with discs of PDA medium instead of the bioagents. Four replicate plates were used for each treatment. Decrease percentages in the linear growth of the tested pathogenic fungi were calculated at the end of the experiment.

RESULTS AND DISCUSSION

1. Isolation and identification of the causal fungi:

A total number of 221 fungal and bacterial isolated obtained during isolation trials are shown in (Table 1). Identification studies showed that the isolated fungi belong to 8 different genera namely, *Macrophomina*, *Fusarium*, *Rhizoctonia*, *Sclerotium*, *Aspergillus*, *Penicillium*, *Fusarium oxysporum* and *Trichoderma* spp.

Meanwhile, bacterial isolates were belonging to 2 different genera, i.e. *Bacillus* and *Pseudomonas*.

Among the isolated fungi, were identified as *R. solani* (19.2%), *S. rolfsii* (16.7%) *M. phaseolina* (6.8%) *F. solani* (12.2%). Similar results were obtained by Nofal, *et al.* (1982) and El-Gantiry, *et al.* (1994).

Table (1) : Occurrence and frequency of microorganisms isolated from two legume crops showing symptoms of root-rot and damping-off, collected from four governorates.

Isolated microorganisms	Giza				Ismaillia				Sharkia				behera				#	%
	Pea		Cowpea		Pea		Cowpea		Pea		Cowpea		Pea		Cowpea			
	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%		
S. rolfsii	12	50.0	4	13.3	6	19.4	3	8.6	4	23.5	2	15.4	8	47.1	0	0.0	39	16.7
R. solani	8	26.7	6	17.1	7	22.6	8	53.3	9	90.0	5	21.7	2	11.8	0	0.0	45	19.2
M. phaseolina	6	20.0	4	11.4	3	23.2	0	0.0	3	32.0	0	0.0	0	0.0	0	0.0	16	6.8
F. solani	4	13.3	2	25.0	7	20.0	5	16.1	1	6.7	1	10.0	6	26.1	2	18.2	28	12.2
Aspergillus sp.	2	6.7	2	8.3	6	17.1	4	12.9	2	13.3	3	13.0	2	25.0	2	11.8	23	9.5
Penicillium	5	21.7	0	0.0	3	25.5	0	0.0	2	20.0	0	0.0	0	0.0	0	0.0	10	4.3
F. oxysporum	4	13.3	4	11.4	2	6.5	2	13.3	1	4.3	2	25.0	3	17.6	2	15.4	20	8.5
T. harzianum	2	6.7	4	16.7	2	5.7	2	6.5	2	13.3	1	4.3	3	27.3	4	29.4	20	8.5
Total fungi	43		26		36		24		24		14		24		10		211	
B. subtilis	0	0.0	2		0		0		0		0		2		2		0	
P. fluoresneces	3	0.0	0		1		0		0		0		0		0		4	
Total microorganisms	46		38		37		24		24		14		25		12		221	

 # Number of counted colonies.

It is also obvious from data in Table (1) that the occurrence and frequency of the mentioned fungi varied according to locality and the kind of the crop. The highest number of the isolated fungi was obtained from samples collected from Giza governorate meanwhile, those collected from Behera were the least ones. On the other hand, the highest number of fungal colonies (127 colonies) were obtained from cowpea plants, followed by pea (74 colonies) respectively. Differences between frequencies of isolated fungi obtained from different governorates and crops might be due to plant exudates and soil moisture content as mentioned by Belmar *et al.* (1987) and Rusuku *et al.* (1997).

2. Pathogenicity test:

Data in (Table 2) indicate that all tested fungi isolates caused various degrees of pre-emergence damping-off to the sown pea seeds. Pea fungal isolates as *R. solani* (R-1), *S. rolfsii* (S-1), *F. solani* (F-1) were the most pathogenic isolates, which caused (78.1, 68.7% and 16.3%) pre-emergence damping-off, respectively. Meanwhile, cow pea isolate of *R. solani* (R-2) gave the lowest percentage of infection in the previously mentioned stage, being 37.3% while no infection was recorded from the cow pea isolate of *M. phaseolina* (M-2), 3.2%.

Table (2): Pathogenicity and effect of the fungi, isolated from roots of diseased pea and cowpea plants.

Fungal isolate	Host	Damping-off (%)		Survival (%)
		Pre-emergence	Post-emergence	
R. solani (R-1)	Pea	78.1	18.7	3.2
R. solani (R-2)	Cow pea	37.3	23.9	38.8
S. rolfsii (S-1)	Pea	68.7	21.9	9.4
S. rolfsii (S-2)	Cow pea	41.7	12.5	45.8
F. solani (F-1)	Pea	16.3	21.5	62.2
F. solani (F-2)	Cow pea	18.3	33.3	48.4
M. phaseolina (M-1)	Pea	6.2	9.4	84.4
M. phaseolina (M-2)	Cow pea	3.2	0.0	96.8
Control (free soil)		0.0	0.0	100.0
L.S.D. at 5%		8.0	7.0	23.7

On the other hand, all the tested fungal isolates, except the cowpea isolate of *R. solani* (R-2) caused post-emergence damping-off to pea seedlings. Cowpea isolates of *F. solani, R. solani* (F-2, R-2) and *S. rolfsii* (S-1) gave the highest percentage of post-emergence damping-off being 33.3, 23.9% and 21.9% respectively.

Whereas, the lowest percentage infection at this stage was recorded by (S-1) isolate of *S. rolfsii* and (M-1) isolate of *M. phaseolina* being 9.4% for both. These findings indicate that the aggressiveness of the tested fungi in pathogenicity test might be correlated with the host of which they were isolated from.

3. Biological control:

- In vitro tests;

Effect of five antagonistic microorganisms on the linear growth of the four pathogenic fungal isolates:

Obtained data in (Table 3) clearly indicate that all the antagonists effectively decreased the mycelial growth of the four pathogenic fungal isolates. Promot gave the highest growth reduction to *S. rolfsii* and *F. solani* being 45.0 and 64.8% respectively. While *B. subtilis* was the most effective one on *R. solani* and *M. phaseolina*. The least effective bioagent was Plant Guard against all the pathogens.

These results were expected for Rizo-N, Promot and Plant Guard, as it had been stated before that such bioagents during their growth might secrete some antagonistic substances (Podile *et al.*, 1988 and Fiddaman and Rossal, 1993).

The low antagonistic activities of *P. fluorescence* towards the growth of the pathogenic fungi might be due to low rates of some antifungal metabolites diffused from the bioagent to the hyphae of the pathogens. This conclusion was previously reported by Walter and Cindral (1988) who found that *In vitro*, antagonistic activities of *C. globosum* towards *R. solani* were due to hyphal coiling and some diffusible antifungal metabolites.

3.2. In vivo tests:

Tabulated data in (Table 4) showed that all the tested bioagents significantly decreased both stages of damping-off incited by any of the four pathogenic fungi in comparison with the control treatments. The lowest percentage of pre-emergence damping-off was obtained when B. subtilis was used (6.3%). This treatment increased the percentage of survived plants, reaching 85.9%.

In addition, *R. solani* was the most affected pathogen by these treatments followed by *S. rolfsii*. Whereas, *F. solani* and *M. phaseolina* were the least affected. The corresponding means of survived plant percentages were 83.3, 73.3, 70.6 and 66.0% respectively. Both Tschen (1987) and Reddy *et al.* (1994) obtained promising results on controlling bean damping-off diseases when Promot was applied in the from of wheat barn to soil infested with *R. solani* or *S. rolfsii*. Whereas, in case of the infection with *S. rolfsii* or *M. phaseolina*, Promot (*T. harzianum* and *T. konigii*) was the superior followed by Rizo-N, Plant Guard and *P. fluorescens*. This may be due to Promot containing two species of *Trichoderma* spp. which were more effective than each antibiotic agent sued singly (Reborti *et al.*, 1993 and Montealegre and Leranas, 1995). Also, these results were confirmed by the results obtained by many investigators (Benhamou and Chet, 1993 and Al-Jurifani, Amal, 1996). Therefore, it was though that the use of biological control, either singly or combined in an integrated control program, will be of more success in controlling the disease.

Table (3) : Effect of four antagonistic microorganisms on the linear mycelial growth (mm) of the four tested pathogenic fungi and growth decrease percentage after 5 days of incubation at 25°C.

Tested Pathogen / Tested Bioagent	Mean linear growth (mm) on PDA							
	R. solani		*S. rolfsii*		*F. solani*		*M. phaseolina*	
	Linear growth (mm)	Decrease %	Linear growth (mm)	Decrease %	Linear growth (mm)	Decrease %	Linear growth (mm)	Decrease %
Rizo-N	25.4	71.2	50.8	43.8	58.8	34.7	47.3	46.4
Promot	35.1	61.0	49.5	45.0	31.7	64.8	66.6	26.0
Plant Guard	38.0	57.8	52.9	41.2	58.8	34.7	48.3	41.7
P. fluorescents	69.3	23.0	71.8	20.2	44.7	50.3	70.2	22.3
Control	90.0	0.0	90.0	0.0	90.0	0.0	90.0	0.0

Rizo-N = *B. subtilis*
Promot = *Trichoderma harzianum + T. konigii*
Plant Guard = *T. harzianum*

Table (4): Effect of four biocontrol agents on pea damping-off caused by four fungi.

Treatment	Pre- and post-emergence damping-off % in soil infested with												Treatment means		
	R. solani			*S. rolfsii*			*F. solani*			*M. phaseolina*					
	% pre	% post	% survival	% pre	% post	% survival	% pre	% post	% survival	% pre	% post	% survival	% pre	% post	% survival
Rizo-N	0.0	9.4	90.6	6.3	0.0	93.7	0.0	21.9	78.1	18.8	0.0	81.2	6.3	78	85.9
Promot	15.7	12.5	71.8	5.2	0.0	94.8	6.3	21.9	71.8	15.6	6.3	78.1	10.7	10.2	79.1
Plant Guard	12.5	8.3	79.2	12.5	0.0	87.5	18.8	8.3	72.9	18.8	12.5	68.7	15.7	7.3	77.1
P. fluorescens	12.5	0.0	87.5	9.4	6.0	90.6	28.1	12.5	59.4	25.0	12.5	68.8	18.8	7.8	76.6
Control	12.5	50.0	37.5	37.5	12.5	50.0	18.8	33.3	47.7	8.0	35.8	56.2	19.2	32.9	47.9
Mean	14.2	3.7	83.3	10.6	16.0	73.3	17.2	13.4	70.0	14.4	19.6	66.0			

L.S.D at 5% for:	Pre-emergence	Post-emergence	Survival
Treatment (T)	0.8	0.6	2.1
Fungi (F)	0.4	0.6	2.0
T x F	0.9	1.3	4.5

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المقاومة الحيوية لمرض موت البادرات فى البسلة المتسبب عن أربعة من الفطريات

نورجيهان محمد عيسى
معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة – مصر

تم إختبار التأثير التضادى لأربعة من العوامل الحيوية هى الريزو-إن (باسيلس ساتلس) ، البرموت (تريكودرما هارزيانم + تريكودرما كونجيى) وبلانت جارد (تريكودرما هارزيانم) وسيدوموناس فلوريسنس ضد أربعة من الفطريات المسببة لمرض موت البادرات فى البسلة وهى ريزوكتونيا سولانى وفيوزاريوم سولانى وفيوزاريوم سولانى وسكليروشيوم رولفزياى وماكروفومينا فاصولينا.

ففى الدراسة المعملية ، أحدث البرموت (تريكودرما هارزيانم + تريكودرما كونجيى) أعلى نسبة مئوية للنقص فى النمو الطولى لجميع الفطريات الممرضة تلاه البكتريا ريزو-إن (باسيلس ساتلس) ثم البلانت جارد التريكودرما هارزيانم. أما أقل تأثير على نمو الفطريات الممرضة فقد أحدثته البكتريا سيدوموناس فلوريسنس. أما فى الدراسة التى أجريت بالأصص فقد أعطى الريزو-إن (باسلس ساتلس) أحسن النتائج فى مقاومة مرض موت البادرات فى البسلة.