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BIOLOGICAL CONTROL OF BEAN DAMPING OFF CAUSED BY *RHIZOCTONIA SOLANI*

EL-KAFRAWY A.A.

Plant Pathology Research Institute, Agriculture Research Centre

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Abstract

Isolates of *Trichoderma* spp. reduced the radial growth of *Rhizoctonia solani* (Kuhn) by 59.6 – 78.4 % and *Gliocladium* spp. inhibited the radial growth of *R.solani* by 57.2 –70.1%. Soil treatment with *T.hamatum, T. harzianum, T.viride* and *Gliocladium virens* gave the maximum protection against pre and post-emergence damping-off and reduced the disease incidence from 50 to 6.6, 10, 10, and 10 % respectively, compared with the fungicide Rizolex at 10 %. Also, improved plant heights, fresh and dry weight, increased dry seeds yield from 4.5 with the control to 8.8, 8.7,8.6 and 8.5 gm in the above treatments, respectively, in comparison with Rizolex (8.2 gm) .The seed treatment with the bioagents was less effective than soil treatment. The soil treatment with antagonists remarkably reduced the population of *R.solani* in the soil from 30.5 to 0.01,0.03, 0.05, and 0.06 x 10^6 cfu respectively, in unsterilized soil after 6 weeks from sowing. The seed treatment with the antagonists was less effective. The bioagents gave persistent effect in reducing *R.solani* population for more than 6 weeks, while Rizolex was superior up to 3 weeks from sowing.

INTRODUCTION

Rhizoctonia. solani is a widespread, soilborne pathogen responsible for serious damage in many crops. Damping –off, root rot and seedling blight are among the most important diseases that may lead to yield losses reaching 60% in some growing areas (Sippell *et al.* 1985). The discovery of new biological control agents and the demonstration of their effects in reducing incidence and disease severity have opened a new promising avenues for practical application in agriculture and for promoting environmental safety (Boland, 1990). Among the fungal antagonists *Trichoderma* spp. and *Gliocladium* spp have shown satisfactory degrees of efficiency against soilborne pathogens and have been reported to successfully inhibit the pathogen growth (Papavizas, 1985). The ability of these antagonists to attack fungal pathogen at different stages of their development has led to the concept that they could be powerful biocontrol agents (Chet, 1987). *Trichoderma* sp. and *Gliocladium virens* have proven effective as

suppressant of damping–off disease incited by *Pythium ultimum* and *Rhizoctonia solani* (Hwang and Chakravarty, 1993) .The well known biocontrol fungi *G.virens* and *Trichoderma harzianum* have been used effectively against a number of pathogen, including *R.solani* on several crops (Lumsden& locke, 1989). Decline in soilborne inoculum of *R solani* is often caused by specific antagonists as *Trichoderma hamatum* and *G.virens* (Howell, 1987).

The aim of the present study was to evaluate antagonistic ability of some isolates of *Trichoderma* spp. and *Gliocladium* spp. applied either as soil or seed treatment in reducing damping-off of bean caused by *Rhizoctonia solani.*

MATERIALS AND METHODS

Rhizoctonia solani was isolated from bean roots and hypocotyls collected from damped-off or root rotted plants at Gemmeiza Research Station.

Antagonistic effect in vitro:

The antagonistic effect of *Trichoderma* spp. and *Gliocladium* spp. against isolates of *R. solani in vitro* was examined on Petri plates (9 cm) containing Potato Dextrose Agar (PDA) medium. A disc (6 mm) from a three day-old culture of the antagonists was transferred to one side of Petri plates containing solidified PDA medium and the other side was inoculated with mycelial disc (6mm) from edge of a three day-old culture of *R. solani.* Five plates were used for each particular antagonist, five plates were inoculated with the pathogen only serving as control and the inoculated plates were incubated at 25 ± 2 ℃ for 5 days. The radial growth inhibition percentage of the pathogen was calculated according to the formula of Zhou and Reeleder (1990).

Greenhouse experiment:

The soil used in this work was clay loam soil and it was divided into two parts, one was sterilized using formalin (25 ml) of 40 % /100 liter water) and the other was unsterilized soil infested, 7 days before the sowing with *R. solani* grown on sand wheat-bran medium (1:3V/V) at the rate of 3% w/w. The antagonistic fungi was applied in two ways :

a) Soil Treatment:

Inoculum of the antagonists was prepared on sand wheat-bran medium and used for greenhouse experiment. It was mixed with the soil at the rate of 3% w/w at sowing time.

b) Seed Treatment:

Spores of the antagonists were harvested from the surface of agar cultures after 7 days of incubation by adding 10 ml of sterile distilled water to each plate and the spore along with mycelial fragments were collected by soft brush, blended and filtrated through a muslin cloth. The filtrate containing conidia was centrifuged at 3000 rpm for 10 minutes. The supernatent was discarded and the conidial pellet was resuspended in 3 ml sterile distilled water. The spore suspension was adjusted to around $5x10^9$ conidia /ml using a haemocytometer. Three ml of the conidial suspension were mixed with 2ml of 0.1% carboxymethyl cellulose as a sticker to coat 10 gm of bean seeds, shade dried for 6 hours and sown in pathogen-inoculated sterilized and unsterilized soil. Pathogen-inoculated soil with no treatments served as control.

Seed treatment with fungicide:

Rizolex was used as seed dressing fungicide at the rate of 3 g/kg seeds. Seeds were treated with the fungicide 6 hours before sowing.

Ten seeds of the susceptible cultivar (Giza 6) were sown in each pot (25 cm in diameter) and 4 pots were used for each particular treatment. The percentages of pre and post-emergence damping-off were recorded after 15 and 30 days, respectively, from sowing. Plant height, dry and fresh weight and the yield as green pods and dry seeds were also recorded.

Effect of soil and seed treatment with antagonists on the soil population of *R. solani:*

Change in *R. solani* population were determined in pots infested with *Trichoderma* spp. and *Gliocladium* spp. applied either as soil or seed treatment. Rizolex was used as seed treatment, to compare the effect of antagonists. Serial dilutions using dilution plate technique were used where 5 gm of soil samples were collected from each partic-

ular treatment after 3 and 6 weeks of sowing and 0.1 ml of each dilution was spread over the surface of PDA plates containing 33 mg. rose Bengal /l to check bacterial contamination. The seeded plates were incubated at 25 ± 2 ℃ for 5 days. The population of *R. solani* was expressed as colony forming units (cfu) / g. soil.

RESULTS AND DISCUSSION

Antagonistic effect *in vitro*

The antagonistic effects of fungal isolates were measured by dual culture technique using PDA medium (Table 1). In general, all the antagonists inhibited the growth of *R. solani* significantly, when compared with the control. *Trichoderma* spp grew over the mycelium of *R.solani*. The inhibition zones were observed between *Gliocladium* spp. The radial growth of *R.solani* was inhibited by *T.hamatum* to 19.4 mm, *T. harzianum* (23.4), *T. viride* (26.2) and *G. virens* (26.9), and these values equal to 78.4, 74.0, 70.9 and 70.1% respectively in terms of % inhibition. These results are in line with that reported by Benhamou & Chet (1993) .

Greenhouse experiment:

The results in Table (2) indicate that, soil treatment, with the antagonists tested, generally gave higher protection against bean damping-off than seed treatment in both sterilized and unsterilized soil prior to infestation. This can be attributed to the fact that the antagonist is colonizing larger volume of the soil, consequently reaching more propagules of the pathogen. Moreover, adding *T.hamatum* to the soil gave the lowest percentage of pre and post- emergence damping- off (6.67%). Also, soil treatment with *T. harzianum*, *T. viride* and *G. virens* was found to be even as effective as Rizolex (10%) in checking the disease. The other antagonists, *T.koningii* (16.66%), *T.pseudokoningii* (20%), *G.deliquscens* (23.33%) and *T.polysporum* (26.67%) were less efficient in reducing the disease incidence, when compared with control (50%). The results obtained with sterilized soil were more or less similar to those of unsterilized soil; however, the survivals were always lower in the unsterilized soil. This can probably be attributed to the presence of additional inoculum and that the natural flora could have interfered with the antagonists. These results are in accordance with those obtained by Lewis *et al.* (1995) and Bazgir & Okhovvat (1997) who found that applica-

Table 1. Effect of antagonists on the radial growth of *R.solani in Vitro*

Antagonists	Growth (mm)	Inhibition (%)
T.harzianum	23.4	74.0
T.koningii	29.0	67.8
T.pseudokoningii	31.0	65.6
T.hamatum	19.4	78.4
T.viride	26.2	70.9
T.polysporum	36.4	59.6
G.virens	26.9	70.1
G.deliquescens	38.5	57.2
Control	90.0	-
L.S.D at 5%	7.5	-

T. = *Trichoderma* G. = *Gliocladium*

Table 2. Effect of seed and soil treatment with fungal antagonists and fungicide (Rizolex) on damping off of bean caused by *Rhizoctonia solani* in sterilized and unsterilized soil .

Antagonists	Seed treatment						Soil treatment					
	Sterilized soil			Unsterilized soil			Sterilized soil			Unsterilized soil		
	Pre-emergence	Post-emergence	survival	Pre-emergence	Post-emergence	survival	Pre-emergence	Post-emergence	survival	Pre-emergence	Post-emergence	survival
T.harzianum	16.66	10.00	73.34	16.66	16.66	66.68	6.67	3.33	90.00	10.00	6.66	83.34
T.koningii	23.33	13.33	63.34	23.33	20.00	56.67	13.33	3.33	83.34	20.00	10.00	70.00
T.pseudokoningii	20.00	16.67	63.33	30.00	16.67	53.33	13.33	6.67	80.00	10.00	13.33	66.67
T.hamatum	16.66	6.66	76.67	20.00	10.00	70.00	6.67	0.00	93.34	10.00	3.33	86.67
T.viride	16.66	10.00	73.34	20.00	16.67	66.67	6.67	3.33	90.00	13.33	3.33	83.34
T.polysporum	23.33	16.67	60.00	26.67	23.33	50.00	16.67	10.00	73.33	20.00	13.33	66.67
G.virens	10.00	16.67	73.33	16.67	16.67	66.66	6.67	3.33	90.00	10.00	6.67	83.33
G.deliquescens	26.67	13.33	60.00	20.00	26.67	53.33	16.67	6.66	76.67	20.00	16.67	66.67
Rizolex	16.67	6.67	86.66	13.33	6.67	80.00	6.67	3.33	90.00	10.00	6.67	83.33
Control	40.00	16.67	43.33	46.67	13.33	40.00	36.67	13.33	50.00	43.33	16.67	40.00
L.S.D at 5%	9.09	5.05	12.20	10.00	6.28	13.10	8.35	3.21	14.15	11.28	5.49	15.02

T.= *Trichoderma* G.= *Gliocladium*

tion of wheat bran culture of antagonists were significantly more effective than seed dressing with Benomyl and PCNB in reducing disease incidence caused by *R.solani* on bean under field condition.

The plant height, fresh and dry weight of bean plants:

The effect of antagonists on plant growth is presented in Table (3). Soil treatment with the antagonists increased the plant height, fresh and dry weight of bean plants more than seed treatment in both sterilized and unsterilized soil. Data also revealed that treating the soil with *T.hamatum* and *T. harzianum* was the most effective one in improving the plant height from 22.6 to 42.6 and 40.3 cm., respectively, fresh weight from 9.25 to 23.45 and 22.55 gm. respectively and dry weight from 2.70 to 8.05 and 7.55 gm. respectively. *G. Deliquescens* and *T. polysporum* were the least effective, while other antagonists fall in between. The improvement of these parameters could be due to the control of the pathogen, on one hand, and the possible change in the hormonal behaviour of the plant itself and to the possible production of growth promoting substances by the antagonists, on the other hand.

The significance of results obtained with sterilized soil is similar to those of unsterilized soil and these results are in agreement with those obtained by Gandhikumar & Ranganathan (1997) and Joseph & Sivaprasad (1997) who found that *T.viride* showed positive influence on plant growth

The yield as green pods and dry seeds:

Data presented in Table (4) indicate that, soil treatment with the antagonists gave the maximum yield as green pods and dry seeds compared with seed treatment in both sterilized and unsterilized soil. Treating the soil with *T. hamatum* and *T. harzianum* increased the yield as green pods from 14.47 to 23.54 and 23.29 gm respectively, and dry seeds from 4.55 to 8.81 and 8.72 gm. respectively, while *G deliquescens* and *T. polysporum* gave the minimum yield, respectively, whereas other antagonists fall in between. This is probably a reflection of the better plant growth parameters as a result of disease control and the possible direct effect of metabolites.

Table 3. Effect of seed and soil treatment with fungal antagonists and fungicide(Rizolex)on the fresh ,dry weight and plant height of bean plant as affected by R.solani in sterilized and unsterilized soil.

Antagonists	Seed treatment						Soil treatment					
	Sterilized soil			Unsterilized soil			Sterilized soil			Unsterilized soil		
	Fresh weight	Dry weight	Plant height	Fresh weight	Dry weight	Plant height	Fresh weight	Dry weight	Plant height	Fresh weight	Dry weight	Plant height
T.harzianum	16.250	5.654	34.50	15.401	5.050	31.50	22.550	7.550	40.30	19.815	6.325	37.70
T.koningii	14.353	4.605	30.60	14.304	4.204	28.50	19.755	6.005	37.90	15.454	5.255	32.50
T.pseudokoningii	13.805	4.305	28.50	13.800	4.356	28.00	18.256	5.882	36.30	14.305	4.250	30.30
T.hamatum	17.104	5.900	36.50	15.655	5.158	34.50	23.454	8.051	42.60	19.906	6.355	37.80
T.viride	14.902	4.952	32.30	14.752	4.757	30.30	20.409	6.800	38.50	18.902	6.050	36.50
T.polysporum	12.652	3.853	25.50	10.507	3.055	24.60	14.952	4.953	30.60	12.724	3.958	26.50
G.virens	15.851	5.502	34.00	15.105	5.005	31.00	21.418	7.207	39.90	19.158	6.100	37.00
G.deliquescens	12.950	3.951	26.90	11.204	3.204	25.30	15.357	5.406	30.90	13.307	4.058	27.00
Rizolex	20.253	6.755	38.00	19.720	6.452	37.30	20.400	6.800	38.20	19.609	6.232	37.50
Control	7.850	2.055	20.40	6.456	1.603	18.30	9.250	2.702	22.60	7.552	1.750	19.20
L.S.D at 5%	2.12	0.91	3.55	3.93	1.42	4.26	4.48	2.31	3.36	3.01	1.20	4.00

T. =*Trichoderma* G.= *Gliocladium*

Table 4. Effect of seed and soil treatment with fungal antagonists on yield of bean as green bods and dry seeds (gm)in sterilized and unsterilized soil infested by *R.solani*

Antagonists	Seed treatment				Soil treatment			
	Sterilized soil		Unsterilized soil		Sterilized soil		Unsterilized soil	
	Green pods	Dry seeds	Green pods	Dry seeds	Green pods	Dry seeds	Green pods	Dry seeds
T.harzianum	19.990	6.685	18.420	5.890	23.290	8.720	22.210	7.675
T.koningii	16.215	5.660	14.500	4.995	22.275	7.700	18.690	6.110
T.pseudokoningii	15.350	5.410	13.990	4.740	22.050	7.450	16.510	5.895
T.hamatum	20.120	6.945	18.680	6.690	23.540	8.810	22.460	7.910
T.viride	18.785	6.655	16.410	5.850	22.950	8.525	22.100	7.280
T.polysporum	16.295	5.590	12.800	4.545	18.820	6.695	16.465	5.790
G.virens	18.790	6.825	16.580	6.340	23.080	8.590	22.110	7.315
G.deliquescens	14.985	5.370	13.885	4.650	20.210	6.780	16.225	5.435
Rizolex	22.270	7.920	21.995	7.125	22.275	8.200	22.160	7.580
Control	12.845	3.455	10.780	3.050	14.470	4.545	11.155	3.110
L.S.D at 5%	4.12	1.50	3.64	1.39	4.35	1.74	3.44	1.30

T. =*Trichoderma* G.= *Gliocladium*

Table 5. Effect of seed and soil treatment with the fungal antagonists on population *R. solani* at different intervals (week) cfu x 10^6 per gm / soil.

Antagonists	Seed treatment						Soil treatment					
	Sterilized soil			Unsterilized soil			Sterilized soil			Unsterilized soil		
	0	3 w	6 w	0	3 w	6 w	0	3 w	6 w	0	3 w	6 w
T.harzianum	26.25	18.00	12.25	21.00	13.50	6.75	22.50	10.25	0.08	18.25	6.75	0.03
T.koningii	27.00	23.50	17.50	18.75	13.75	7.00	28.75	13.50	2.00	15.75	5.75	0.25
T.pseudokoningii	29.75	24.75	18.75	20.50	14.50	9.50	30.50	15.00	2.25	19.50	7.50	0.75
T.hamatum	24.50	16.25	10.00	19.75	10.75	2.25	24.25	8.50	0.06	20.00	5.50	0.01
T.viride	28.00	20.75	14.00	18.25	13.50	8.25	22.00	15.00	0.75	17.75	7.75	0.05
T.polysporum	20.75	18.50	16.25	19.00	15.75	10.75	26.75	16.50	3.50	18.75	8.25	1.75
G.virens	21.00	17.75	12.50	18.50	11.00	4.50	28.75	13.00	0.80	16.25	6.50	0.06
G.deliquescens	23.50	20.50	18.00	21.00	16.25	10.00	22.75	14.75	3.00	18.00	8.75	1.75
Rizolex	27.50	8.25	12.75	17.00	4.25	7.50	25.25	6.50	10.50	17.50	5.25	8.50
Conterol	28.50	32.25	42.50	25.75	29.50	45.00	26.50	33.50	38.75	19.00	24.25	30.50
L.S.D at 5%	2.41	5.42	4.59	2.02	4.46	3.91	1.74	4.05	2.78	2.12	2.97	2.26

T. = *Trichoderma* G.= *Gliocladium* w = week

There are not much differences between the results obtained with sterilized soil and those of unsterilized soil. Results obtained support those of Gandhikumar & Ranganathan (1997).

Effect of soil and seed treatment with antagonists on the soil population of R. solani:

Data presented in Table (5) show that, treating the soil with the antagonistic fungi significantly decreased the population of *R. solani* by time and was comparable to seed treatment with antagonists in both sterilized and unsterilized soil. Noticeable reduction in *R.solani* population was noticed, when *T. hamatum, T. harzianum, T.viride and G. virens* were added to the soil from 30.50 x 10^6 to 0.01, 0.03, 0.05 and 0.06 x 10^6 cfu in unsterilized soil, respectively, and from 38.75 x 10^6 to 0.06, 0.08, 0.75 and 0.80 x 10^6 in sterilized soil respectively, after 6 weeks of sowing. The other antagonists also decreased the population of *R. solani* compared with Rizolex, that led to reduction of the population of *R. solani* up to 3weeks after sowing, then started increasing. The mechanism of bioagents against plant pathogens could be categorized under five general categories: parasitism, antibiosis, competition, induced resistance and lysis (Elad *et al.* 1982). Sivan & Chet (1986) found that the population of *Fusarium* spp. decreased in soil treated with *Trichoderma* preparation. Joseph & Sivapasad (1997) also found that *T.viride* treated pots significantly reduced the population of *Pythium aphanidermatum*. Results reported herein indicate the feasibility of bioagents application, as one tactic of the integrated management program dealing with damping off of beans.

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المكافحة الحيوية لمرض موت البادرات فى الفاصوليا المتسبب عن فطر رايزوكتونيا سولانى بواسطة أنواع من فطرى التريكودرما والجليوكلاديوم

احمد أبو ريا الكفراوى

معهد بحوث أمراض النباتات

تم تشبيط نمو فطر الريزوكتونيا سولانى بواسطة عزلات من فطرى التريكوديرما والجليوكلاديوم فى أطباق بترى وكانت نسبة التثبيط ٧٨,٤، ١٠٠، ٧٠ ٪ على التوالى .

أعطت معاملة التربة بفطرى الترايكوديرما هاماتم وهارزياتم وفيردى وجليوكلاديوم فيرنس اقصى حماية ضد مرض موت البادرات وأدى إلى انخفاض نسبة حدوث المرض من ٥٠٪ إلى ٦,٦ ،١٠٠،١٠٠،١٠٠٪ على التوالى مقارنة بالمبيد الفطرى الريزولكس الذى أعطى ١٠٪ وأدت هذه المعاملة إلى تحسين الصفات الخضرية للنبات وكذلك زيادة إنتاج القرون الخضراء والبذور الجافة ,وأما معاملة البذور فكانت اقل كفاءة من معاملة التربة فى هذا الخصوص.

كما أدت معاملة التربة بالكائنات المضادة إلى خفض تعداد الفطر المرض ريزوكتونيا سولانى فى التربة بأعداد تتراوح من ٣٠,٥×١٠^{٦} إلى ١٠٠,٠٠٠، ٣٠٠,٠٠٠، ٥٠٠,٠٠٠، ٦٠٠,٠٠٠ × ١٠^{٦} وحدة تكوين مستعمرة على التوالى فى التربة غير المعقمة بعد ٦ أسابيع من الزراعة وكذلك كانت معاملة البذرة بتلك الكائنات المضادة اقل كفاءة من معاملة التربة فى هذا الخصوص.

كما أظهرت الكائنات المضادة أثراً متبقياً لمدة طويلة فى التربة وأدت إلى خفض أعداد الفطر المرض (ريزوكتونيا سولانى) لمدة اكبر من ٦ أسابيع مقارنة بالمبيد الفطرى ريزولكس والذى انخفضت كفاءته بعد ثلاث أسابيع من الزراعة .