Minufiya J. Agric. Res. Vol. 32 No.3: 669 - 682 (2007)

BIOLOGICAL CONTROL OF *Rhizoctonia solani*, THE CAUSAL AGENT OF FABA BEAN ROOT-ROT AND STEM CANKER DISEASE

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(Received: Apr. 30, 2007)

ABSTRACT: ***Under green house conditions, Rhizoctonia solani caused damping-off and death of all Giza 3 Mohassan cv. faba bean seedlings. However the tested biocontrol agents resulted good biocontrol of the pathogenic fungus and Trichoderma harzianum was the best one. Application of T. harzianum and Rhizoblum leguminosarum to the soil infested with R. solani showed significant increase in leaves number of faba bean plants compared with the untreated plants or which treated with Bacillus megaterium var. phosphaticum after 40 days from seedings. Plant height and length were affected with the presence of the pathogenic fungus (R. solani) although the presence of the biocontrol agents. Application of T. harzianum and R. leguminosarum var.fabae gave the best result of plant growth while B. megaterium resulted the lowest plant height. The presence of the pathogenic fungus showed significant decrease in faba bean plants fresh and dry weight and also decreased the number of bacterial nodules on the roots. Application of R. leguminosarum resulted significant increase in the shoot and root total nitrogen content. Trichoderma harzianum increased the plant total nitrogen content by 4.7% while B. megaterium resulted total nitrogen content equal to that in the control. Combination of the three microorganisms increased the total nitrogen content by 23.7%. From the results of total protein analysis, it be concluded that R. solani + B. megaterium treatment gave the best result and showed 10 bands. R. solani + R. leguminosarum + B. megaterium+ T. harzianum treatment gave the worst result and showed 7 bands. Control, R. solani + R. leguminosarum and R. solani + T. harzianum showed 9,8 and 8 bands, respectively.***

Key words: ***Faba bean, Rhizoctonia solani, Rhizobium leguminosarum, Trichoderma harzianum, Bacillus megaterium, biocontrol agents.***

INTRODUCTION

Different bacterial and fungal species naturally occur in the soil were isolated and examined for their competieion to the pathogenic fungi. Gasoni *et al.*, (1998) showed that bacteria belonging to *Pseudomonas* and *Bacillus*

genera have been used as biocontrol agents. Yehia *et al.*, (1988) proved the antagonistic effect of *Trichoderma viride* against *Fusarium solani* of faba bean. Seed coating with *T. viride* increased fresh and dry weight of shoots, roots and nodules number. The population densities of fungi (including *Fusarium* spp.) were low in plants obtained from treated seeds. Nelson (1992) reported that *Trichoderma* spp. are specific biocontrol agents against fungal pathogens (from *Pythium* to *Rhizoctonia*) according to the type of antibiotic produced. He also mentioned that some species of *Pseudomonas* and *Bacillus* have significant antibiotic action. Under field conditions; Ehteshamul-Shaque and Ghaffar (1993) observed the antagonistic rhizobia and bradyrhizobia used as seed dressing or soil drench reduced infection of *Rhizoctonia solani* in both leguminous and nonleguminous plants. Baby (1998) mentioned that *Trichoderma harzianum* occurs widely in nature soil substrate and has the ability to compete with phytopathogenic fungi and produces toxins. The penetration of *Trichoderma harzianum* Rifai into the cell wall of other fungi is attributed to the production of enzymes that catalyze the breakdown of chitin (Zeillinger *et al.*, 1999). Harman (2000) recrded that *Trichoderma* spp. have been developed into several commercial biological control products in field crop and green house systems. Zheng and Sinclair (2000) showed that *Bacillus megaterium* is a potential bacterial biocontrol agent against *Rhizoctonia solani*. Lewis and Lumsdent (2001) cleared that *T. harzianum* and *T. viride* isolates reduced damping off of different plants caused by isolate R-23 of *R. solani*. Jensen *et al.*, (2002) evaluated the effect of *Bacillus subtils* and *T. harzianum* alone or in combination with Captan 400 and Vitavax 200 as biocontrol treatments against the dry bean root rot pathogens. They recorded that seed application of both biocontrol agents increased plant biomass and decreased disease severity, under green house conditions. Field experiments showed that seeds treated with *B. subtilis* reduced bean root rot and increased yield (31%) when compared to untreated plants. Bean seeds inoculated with *Rhizobium trobici* also increased yield (13%) over the untreated control. Cigdem and Merih (2003) showed that the filterates of *T. harzianum* (T9, T10, T15 and T19) were effective against plant pathogens: *Fusarium colmarum*, *F. oxysporum*, *F. moniliforme*, *Rhizoctonia solani* and *Sclerotium rolfsii*). Maria and Joseph (2006) showed that a *Trichoderma harzianum* strain was antagonistic; *In vitro*; to *Rhizoctonia solani* and *Verticillium dahliae* and may be considered a potential biocontrol agent.

The aim of this investigation was to study the effect of *Rhizobium leguminosarum* var. *fabae*, *Bacillus megaterium* var. *phosphaticum* and *Trichoderma harzianum*; either single or mixed applications; to the potted soil previously infested with *Rhizoctonia solani* on the growth parameters, nitrogen content and protein analysis of faba bean plants. Both infested and noninfested control treatments were involved. The beneficial

microorganisms used were objectived as biofertilizers and biocontrol agents at the same time.

MATERIALS AND METHODS

This work has been carried out at the Environmental Biotechnology Dept., GEBRI, Sadat City, Minoufiya, Egypt.

1- Rhizosphere Microorganisms Tested:

Rhizobium leguminosarum var. *fabae* "Okkadeen biofertilizer" was obtained from Legume Crops Dept. Field Crops Research Institute, ARC, Giza, Egypt. *Bacillus megaterium* var.*phosphaticum* isolate was obtained from MERCIN, Fac. of Agric., Ain Shams Univ. An identified isolate of *Trichoderma harzianum* was achieved from Agricultural Botany Dept., Fac. of Agric., Minouf. Univ.

In order to isolate the causal organism(s) of stem canker and root rot disease; infected faba bean plants were collected from different cultivated areas at Sadat City. Roots and stem bases of the obtained samples were separatly washed by running tap water, surface sterilized by 70 % Ethanole and then left to dry on sterilized filter papers. The samples were cut into small pieces, plated on PDA medium and incubated at 25°C. Petri dishes were examined daily and hyphal tips abundant were individually transferred to new PDA plates. Some other root samples were used for isolation of the outer microorganisms "without surface sterilization". Obtained isolates were identified at the Agric. Botany Dept. Fac. of Agric., Min. Univ., Shebin El-kom, Egypt.

2- Laboratory Experiments:

Different trials of dual cultures have been carried out between the obtained bacterial and fungal isolates. A disk (4 mm in diameter) of 6 days old fungal growth or a bacterial lope (4 cm long line) were the assempled inocula which plated on PDA medium (5 cm in between). Control plates were inoculated with each isolate on PDA at the center of each dish. Four replicates were used for a separate treatment. Average diameter of each fungal growth was estimated when a control dish was full with the growth with the growth. Inhibition zone was also measured at the same time.

3- Pot Experiment:

Pots (20 cm in diameter) were sterilized by immersing them into 5 % Colorex for 15 min. and then left to dry in open air. Non-sterilized sandy-loam soil of Sadat City mixed thoroughly with peat moth at the rate of 1:1 were left for a week in the open air before using in this experiment.

Bacillus isolate was grown on Nutrient Broth medium for 48 hr. on a rotary shaker at 25 °C. The bacterial inoculum was applied as a soil treatment at the rate of 5 ml bacterial suspension per plant (1×10^8 cfu/ml).

However; application of fungal isolates was carried out on Barley medium at the rate of 3% of soil weight.

4- Chemical Analysis:

4- 1-Total nitrogen content:

Total nitrogen content was estimated using the modified semi-micro kjeldahl method according to Hassouna, 1962 and Rahal, 1978.

4-2- Protein electrophoresis:

For most proteins that are not secreted, expression levels are generally such that protein can be easily visualized by Comassie blue staining of total protein on SDS-polyacrylamide gels. Denaturing polyacrylamide gel electrophoresis is simple and powerful method for proteins separation according to their size. In the most commonly used procedure, the protein samples are first denatured by heating in the presence of (Sodium dodycyl sulphate) SDS as reducing agent and strong anionic detergent. The treatment dissociates virtually all protein complexes. The denatured proteins bind SDS and acquire negative charge. The amount of SDS bound is proportional to the molecular eeight of SDS-polyacrylamide complex during electrophoresis is denaturing on the size of the polypeptide.

SDS-polyacrylamide Gel Electrophoresis (SDS-Page) was carried out by using a discontinuous buffer system described by (Laemmli, 1970).

5- Statistical Analysis:

The data were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, inc, 1996).

RESULTS AND DISCUSSION:

A-Effect of Soil Microorganisms on Growth Characters:

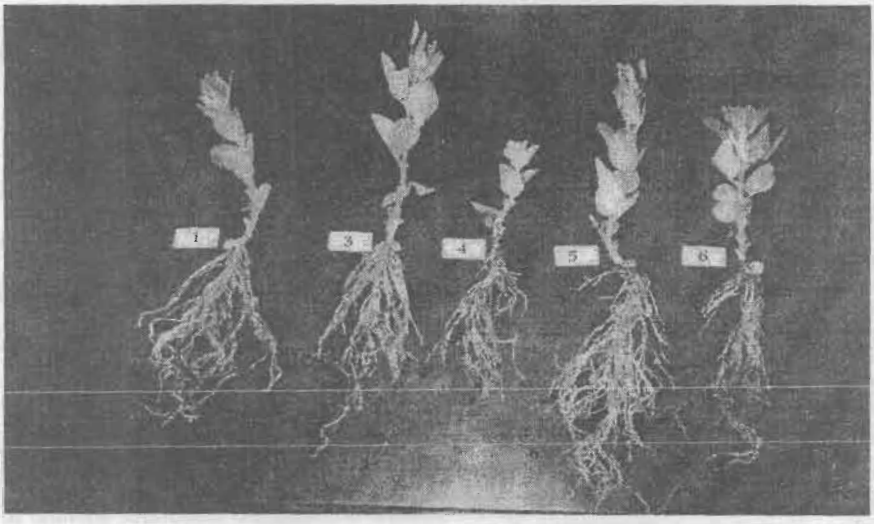
A-1- Effect of soil microorganisms on seed emergence and plant surviving:

Under green house and artificial inoculation conditions; results present in Table (1) and Figure (1) clear that *Rhizoctonia solani* caused pre-emergence damping off of all seeds of each faba bean cultivar. However; the untreated control pots resulted 90% emerged and survived plants. This result clear that Giza 3 Mohassan faba bean cultivar are highly succeptible to *R. solani.*

Application of the tested rhizosphere microorganisms to the potted soil infested with *R. solani* showed good control to the pathogen. The best was achieved with *Trichoderma harzianum* where all the seeds germinated and gave 100% plant survival *Bacillus megaterium* resulted 70% germination and 92.9% of them plant survival. *Rhizoblum leguminosarum* showed the least antagonistic effect to *R. solani* where 65% of the seeds emerged and 69.2% of them survived. The same results were achieved when the tested three microorganisms together were applied to *R. solani* infested soil.

Table (1): Effect of some rhizosphere microorganisms on seed germination of faba bean plants (10 days) and survived plants (30 days) infected with *Rhizoctonia solani* (Giza 3 Mohassan cv.):

Teeatments	Emerged plants		Survived plants	
	Number	%	Number of plants	%
Rhizoctonia solani+Rhizobium leguminosarum	13	65	9	69.2
Rhizoctonia solani+Bacillus megaterium	14	70	13	92.8
Rhizoctonia solani + Trichodrma harzianum	20	100	20	100
Rhizoctonia solani + Rhizobium leguminosarum + Bacillus megaterium	13	65	9	96.2
Rhizoctonia solani	0	0	0	0
Control	18	90	18	100



Figure(1): Effect of some rhizosphere microorganisms on growth of Giza 3 Mohassan faba bean plants (1- control, 3-*Rhizoctonia solani+ Rhizobium leguminosarum,4-R. solani+Bacillus megaterium,5-R. solani+Trichoderma harzianum*, 6-*R. solani+R. solani+R. leguminosarum+B. megaterium+T. harizianum*)

Application of the beneficial tested microorganisms; especially *Trichoderma harzianum*; gave good results in controlling *R. solani*. This could be due to their antagonistic effect as reported by Gassoni *et al.*, (1998), Nelson (1992), Ehteshamul-Shaque and Ghaffar (1993), Baby (1998), Harman (2000), Sinclair and Zheng (20002), Lewis and Lumsdent (2001), Jensen *et al.*, (2002) and Maria and Joseph (2006).

However, Cigdem and Merih (2003) showed that the filterates of *T. harzianum* strains were effective against different plant pathogens including *R. solani*.

A-2- Effect of soil microorganisms on leaves number:

Results shown in Table (2) indicate that there was no significant variation between the average number of leaves formed on faba bean plants up to 30 days of sowing, in response to different biocontrol agent(s) application. However, *Trichoderma harzianum* caused significant increase of leaves number as compared with *Bacillus megaterium* 35 days after seeding. The same result was noticed for *T. harzianum* and *Rhizobium leguminosarum* 40 days after planting, where they resulted significantly more number of leaves than control and/or *B. megaterium* treatments.

Table (2): Effect of some rhizosphere microorganisms on the average number of leaves plant (Giza 3 Mohassan cv.,) infected with *Rhizoctonia solani* after 20-40days from seeding:

Treatments	Plant age (days)/no. of leaves per plant				
	20	25	30	35	40
Rhizoctonia solani+Rhizoblum leguminosarum	3.1	4.1	5.2	6.5	7.8
Rhizoctonia solani+Bacillus megaterium	3.1	4.1	5.1	5.6	6.3
Rhizoctonia solani + Trichodrma harzianum	3.0	4.0	5.2	7.0	7.5
Rhizoctonia solani + Rhizobium leguminosarum + Bacillus megaterium	3.0	4.0	5.0	6.1	6.8
Rhizoctonia solani	-	-	-	-	-
Control	3.0	4.0	5.0	6.1	6.3
L.S.D $_{0.05}$	0.79	2.09	0.77	1.07	1.13

These results could be attributed to the competition between the biocontrol agent(s) and *R. solani* as reported also by Baby (1998), Zeilinger *et al.*, (1999) and Ammar (2003).

A-3- Effect of soil microorganisms on plant height:

Faba bean plant height has been affected by soil infestation with *Rhizoctonia solani*, even in the presence of the biocontrol agent(s). the untreated control pots resulted healthy and significantly higher plants than most of the combined *R. solani* and biocontrol agent (s) (Table 3). These results had been shown as early as 20 days and stayed up to 40 days of

sowing. Generally, application of either *Trichoderma harzianum* or *Rhizobium leguminosarum* to the soil infected with *R. solani* showed the best plant growth.While application of *Bacillus megaterium* and/ or the three biocontrol agents resulted the worst results of plant height.

Table (3): Effect of some rhizosphere microorganisms on Giza 3 Mohassan cv., faba bean plants height infected with *Rhizoctonia solani* after 20-40 days from seeding:

Treatments	Plant age (days)				
	20	25	30	35	40
Rhizoctonia solani+Rhizobium leguminosarum	5.5*	7.8	13.3	16.4	18.5
Rhizoctonia solani+Bacillus megaterium	4.3	7.5	10.8	15.2	15.2
Rhizoctonia solani + Trichodrma harzianum	6.2	8.6	11.6	16.0	18.7
Rhizoctonia solani + Rhizobium leguminosarum + Bacillus megaterium	4.9	7.1	9.5	12.9	16.6
Rhizoctonia solani	-	-	-	-	-
Control	7.4	10.7	13.7	18.4	21.8
L.S.D $_{0.05}$	0.9	3.33	4.14	2.25	3.78

*: Plant height Cm.

A-4-Effect of soil microorganisms on plant length:

Results in Table 4 indicate that the average total length of faba bean plants severly affected with soil infestation with *Rhizoctonia solani.*

Control plants, sown in nonsterilized soil, gave the best plant length (58.0 cm) after 45 days from seeding. Complete death was obtained when Giza 3 Mohassan faba bean cv. was seeded in *R. solani* infested pots. Significant reduction of total plant lenghth was noticed in all treatments contained *R. solani* and one or all of the beneficial soil microorganisms except that of *Trichoderma harzianum* which resulted insignificant reduction (12.9%).

However, Nelson (1992) reported that *Trichoderma* spp. are specific biocontrol agents against *Rhizoctonia solani.*

A-5- Effect of soil microorganisms on plant fresh weight:

Shoot system fresh weight of Giza 3 Mohassan cv. plants was significantly less than control in response to soil infestation with *Rhizoctonia solani,* in most cases (Table 5). The most fresh weight reduction of shoots was achieved with application of the three microorganisms to the infested soil with *R. solani* (4.5/8.8%). However; the competitive saprophytic ability could minimized the antagonistic role of each tested biocontrol agent itself (Ammar, 2003). *Bacillus megaterium* application caused (50.5%) reduction in fresh weight.

Both mentioned treatments also resulted significant reduction in roots fresh weight(6 / 12.4 and 5.8 / 12.4% respectively).

Average fresh weight of faba bean whole plant was significantly less than control of all tested treatments (Table 5).

Table (4): Effect of some rhizosphere microorganisms on the length of Giza 3 Mohassan cv., faba beant plant infected with *Rhizoctonia solani*:

Treatments	Length (cm)			
	Shoots	Roots	Total	Length%
Rhizoctonia solani+Rhizobium leguminosarum	22.8	25.1	47.9	-17.4
Rhizoctonia solani+Bacillus megaterium	18.4	24.0	42.4	-16.0
Rhizoctonia solani + Trichodrma harzianum	21.4	29.1	50.5	-12.9
Rhizoctonia solani + Rhizobium leguminosarum + Bacillus megaterium	18.9	25.3	44.2	-23.8
Rhizoctonia solani	-	-	-	-
Control	25.6	32.4	58.0	-
L.S.D $_{0.05}$	3.47	6.32	9.32	

Table (5): Effect of some rhizosphere microorganisms on the fresh weight of Giza 3 Mohassan cv., faba bean plant infected with *Rhizoctonia solani*:

Treatments	Average fresh weight (gm)			
	Shoots	Roots	Total	Fresh weight%
Rhizoctonia solani+Rhizobium leguminosarum	5.1	7.7	12.8	-39.6
Rhizoctonia solani+Bacillus megaterium	4.7	5.8	10.5	-50.5
Rhizoctonia solani + Trichodrma harzianum	5.8	7.9	13.7	-35.4
Rhizoctonia solani + Rhizobium leguminosarum + Bacillus megaterium	4.5	6.0	10.5	-50.5
Rhizoctonia solani	-	-	-	-
Control	8.8	12.4	21.2	-
L.S.D $_{0.05}$	3.5	6.0	3.7	

A-6- Effect of soil microorganisms on plant dry weight:

Results shown in Table 6 nearly clear the same response of plants dry weight as found in fresh weight.

The above results clear the aggressivness of tested *Rhizoctonia solani* isolate and its high tolerance to the tested biocontrol agents. This pathogen cause complete pre-emergence damping off of all faba bean seedlings. It also affected the growth characters of those survived in the presence of the biocontrol agents, which had a good role in this investigation.

Table (6): Effect of some rhizosphere microorganisms on the dry weight of Giza 3 Mohassan cv., faba bean plant infected with *Rhizoctonia solani*:

Treatments	Average dry weight (gm)			
	Shoots	Roots	Total	Fresh weight%
Rhizoctonia solani+Rhizobium leguminosarum	0.8	0.5	1.3	-27
Rhizoctonia solani+Bacillus megaterium	0.4	0.4	0.8	-55.5
Rhizoctonia solani + Trichodrma harzianum	0.6	0.5	1.1	-38.0
Rhizoctonia solani + Rhizobium leguminosarum + Bacillus megaterium	0.5	0.4	0.9	-50.0
Rhizoctonia solani	-	-	-	-
Control	0.9	0.9	1.8	-
L.S.D $_{0.05}$	0.25	0.7	0.05	

B- Effect of soil microorganisms on flowering:

Results present in Table (7) clear that flowers number of faba bean plants didn't affect by various beneficial biocontrol agents. There were no significant differences between the average number of flowers emerged on the plants of various treatments up to 45 days after seeding. It is of interesting to notice that individual treatment with *Rhizoctonia solani* resulted complete pre-emergence damping-off.

Table (7): Effect of some rhizosphere microorganisms on the average number of flowers formed on Giza 3 Mohassan faba bean cultivar plants infected with *Rhizoctonia solani*:

Treatments	Flowers number
Rhizoctonia solani+Rhizobium leguminosarum	4.3
Rhizoctonia solani+Bacillus megaterium	4.0
Rhizoctonia solani + Trichodrma harzianum	4.3
Rhizoctonia solani + Rhizobium leguminosarum + Bacillus megaterium	4.5
Rhizoctonia solani	-*
Control	4.5
L.S.D $_{0.05}$	N.S

C-Effect of soil microorganisms on nodules formation:

Average number of nodules significantly decreased in response to the soil infested with *Rhizoctonia solani* even in the presence of the biocontrol agent(s). This was noticed in comparison with the non-infested control soil (nonsterilized soil). However, number of *Rhizobium leguminosarum* nodules was the worst when the three microorganisms were applied to the soil

infested with *R. solani*. This could be due to the antagonistic effect (s) of the tested microorganisms to R. solani as mentioned before. On the other hand, the best number of nodules was achieved when *Bacillus megaterium* applied to the infested soil (Table 8). Both bacteria could compete each other in root colonization as mentioned by Anusuya and Sullia (1985).

Table (8): Effect of some rhizosphere microorganisms on the nodules number formed on Giza 3 Mohassan faba bean cultivar roots infected with *Rhizoctonia solani*:

Treatments	Average of nodules number
Rhizoctonia solani+Rhizobium leguminosarum	10.1
Rhizoctonia solani+Bacillus megaterium	7.8
Rhizoctonia solani + Trichodrma harzianum	10.1
Rhizoctonia solani + Rhizobium leguminosarum + Bacillus megaterium	16.5
Rhizoctonia solani	-*
Control	37.6
L.S.D $_{0.05}$	18.24

D- Effect of soil microorganisms on Total nitrogen content:

Results present in Table(9) clear that combination of *Rhizobium leguminosarum* to faba bean plants sharbly increased nitrogen content in both of plant roots and shoots. This may be due to their antagonistic effects against R. solani. The other two microorganisms; i.e., *Bacillus megaterium* and *Trichoderma harzianum* consequently resulted the same and 4.7 % more nitrogen levels when compared with the untreated control plants. Application of the hree microorganisms to the soil gave 23.7 % more total nitrogen content of the whole plant than control.

Table (9): Effect of some rhizosphere microorganisms on total nitrogen content of Giza 3 Mohassan cv., faba bean plant infected with *Rhizoctonia solani*

Treatments	Total nitrogen content (mg/g dry matter)			
	Shoots	Roots	Total	%
Rhizoctonia solani+Rhizobium leguminosarum	2.08	2.08	4.16	+41
Rhizoctonia solani+Bacillus megaterium	1.29	1.66	2.95	-
Rhizoctonia solani + Trichodrma harzianum	1.43	1.66	3.09	+4.7
Rhizoctonia solani + Rhizobium leguminosarum + Bacillus megaterium	1.57	2.08	3.65	+23.7
Rhizoctonia solani	-	-	-	-
Control	1.29	1.66	2.95	-

E- Effect of soil microorganisms on protein analysis:

SDS-PAGE were achieved to screen the water soluble leaf protein extracted from Giza 3 Mohassan cv. faba bean plants cultivated in the soil infested with *Rhizoctonia solani* and treated with the rhizosphere microorganisms after 45 days are presented in Fig. (2) and Table (10).

From the results of total protein analysis Fig. (2) and Table (10) it be concluded that *R. solani* + *B. megaterium* treatment gave the best result and showed 10 bands. *R. solani* + *R. leguminosarum* + *B. megaterium*+ *T. harzianum* treatment gave the worst result and showed 7 bands. Control, *R. solani* + *R. leguminosarum* and *R. solani* + *T. harzianum* showed 9,8 and 8 bands respectively.

Table (10): Presence (1) versus absence (0) of SDS-PAGE protein bands of soluble protein extracted from faba bean plants infected with *Rhizoctonia solani*.

No	MW.(kd)	*Rhizoctonia solani*+Rhizobium leguminosarum	*Rhizoctonia solani*+Bacillus megaterium	*Rhizoctonia solani*+ Trichodrma harzianum	*Rhizoctonia solani*+ Rhizobium leguminosarum +Bacillus megaterium	Control
1	215.290	1	0	0	0	0
2	204.020	0	0	0	0	1
3	193.340	0	1	1	0	0
4	188.210	0	0	0	1	0
5	104.170	0	0	1	0	0
6	098.718	1	0	0	0	0
7	092.766	0	1	0	1	1
8	077.670	0	1	1	0	0
9	073.641	1	0	0	0	0
10	070.763	0	0	0	1	1
11	053.783	1	1	1	1	1
12	045.958	0	1	0	1	1
13	042.336	1	0	1	0	0
14	036.435	0	0	1	1	0
15	034.863	1	0	1	1	1
16	031.285	1	1	1	1	1
17	028.740	0	1	0	0	0
18	024.328	0	0	1	0	0
19	021.158	1	1	1	0	0
20	010.294	1	0	0	0	0

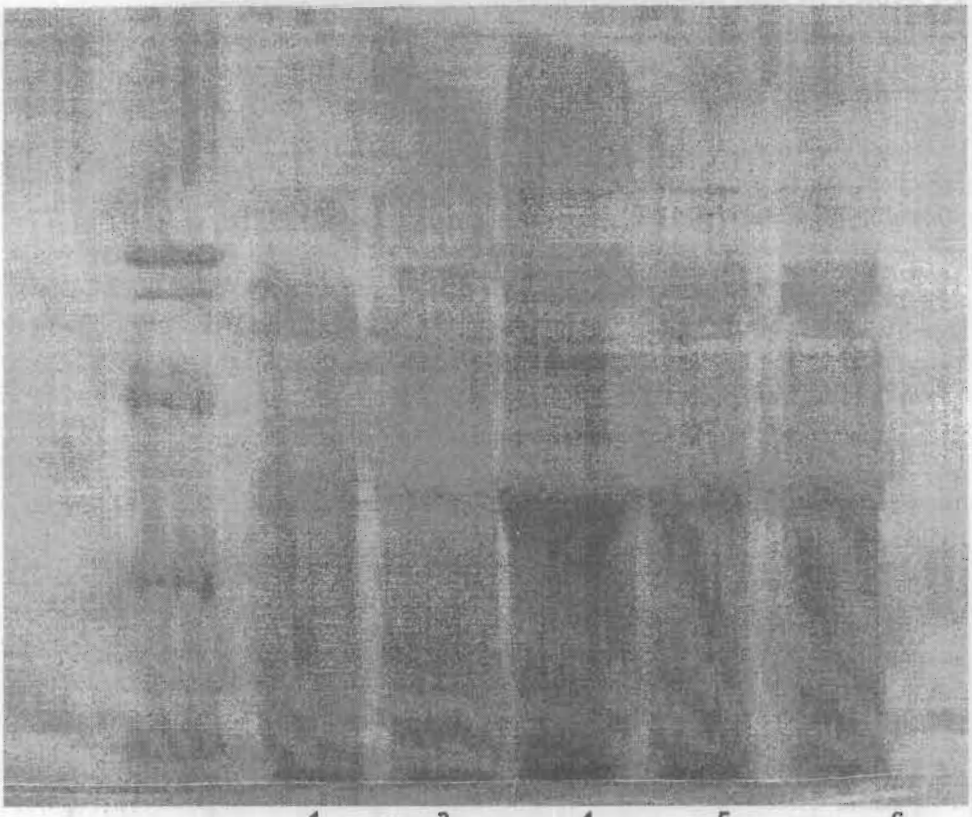


Figure 2: Effect of rhizosphere microorganisms on protein analysis of Giza 3 Mohassan faba bean plants. (1- control, 3-*Rhizoctonia solani*+ *Rhizobium leguminosarum*,4-*R. solani*+*Bacillus megaterium*,5-*R. solani*+*Trichoderma harzianum*, 6-*R. solani*+*R. solani*+*R. leguminosarum*+*B. megaterium*+*T. harizianum*)

REFRENCES

Ammar, M. M. (2003). Fungi (second part): physiology, reproduction and their relation to human and environment. El-dar El-Arabia for press and distribution. Cairo, pp.597.

Anusuya, D. and S. B. Sullia (1985). Interaction between *Rhizobium* and three soil fungi. Trop. Agric., 62: 13-14.

Baby, U.I. (1998). Biocontrol potential of fungicide resistant mutanmts of Trichoderma spp. Indian Journal of Microbiology. 38(3): 165-166.

Cigdem Kucuk and Merih Kivanc (2003). Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features. Turk. J. Biol., 27:247-253.

Ehteshmul. Shaque and A. Ghaffar (1993). Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mungbean. J. Phytopathol., 138: 157-163.

Gasoni, L., J. Cozzi, K. Kobayashi, V. Yossen, G. Zumelzu and S. Babbit (1998). Supressive effect of antagonistic agents on *Rhizoctonia* on lettuce and potato in Argentina field plots. In: International congress of Plant Pathology (9^{th}-16^{th} August, 1998, Edinburgh, Scotland), p.5.2-44.

Harman, G. H. (2000). Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease, 84:377-393.

Hassouna, M. G. (1962). Soil microorganisms and nutrient relations in plant colonization. Ph.D. Theses. University CIlege of wales.

Jensen, C. Estivez, J. A. Percich and P. H. Graham (2002). Integrated management strategies of bean root rot with *Bacillus subtilis* and *Rhizobium* in Minnesota. Field Crops Research, 74 (2-3): 107-115.

Laemmile, K. (1970). Cl;eavage of structural protein during the assembly of the head of bacteriophage T4. Nature (London), 227:680-685.

Lewis, J. A. and R. D. Lundsen (2001). Biocontrol of damping off green house grown crops caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp.Crop Protection, 22 (9): 49-56.

Maria Pilar santamarina and Joseph roselló (2006). Influence of temperature and water activity on the antagonism of *Trichoderma harzianum* to *Verticillium* and *Rhizoctonia*. Crop Protection, 25 (10): 110-121.

Nelson, E. B. (1992). Rapid germination of sporangia of *Pythium* species. Phytopathology, 77: 1108-1112.

Rahal, M. M. (1978). Control of plant parasitic nematodes using chemical methods. M. Sc. Theses. Fac. of Agric., Alex. Univ., Egypt.

SAS Institute (1996). SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute Inc., Cary, NC 27512-8000. 1028 pp.

Yehia, A. H., S. A. El-Hassan and A. H. Bahdali (1988). Biological seed treatment to control *Fusarium* root rot of broad bean. Egyptian Journal of Phytopathology, 14 (1-2):59-66.

Zeilinger, S., G. Galhaub, K. Payer, S. L. Woo, R. L. Mach, C. Fekete, M. Lorito and C. P. Kupicek, (1999). Chitinase gene expression during mycoparasitic interaction of *Trichoderma harzianum* with its host fungal. Fungal Genet. Biol., 26:131-140.

Zheng, X. Y. and J. B. Sinclair (2000). The effect of traits of *Bacillus megaterium* on seed and root colonization root rot of soybean. Biocontrol, 45 (2): 223-243.

المقاومة الحيوية لفطر *Rhizoctonia solani* المسبب لمرض عفن الجذور و تقرح الساق فى الفول البلدى

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الملخص العربى

تحت ظروف الصوبة و العدوى الصناعية أدت عزلة رايزوكتونيا سولانى إلى موت جميع بادرات الفول البلدى صنف جيزة ٣ محسن قبل ظهورها فوق سطح التربة. فى حين أظهرت كائنات التضاد الحيوى المختبرة مكافحة جيدة للفطر الممرض, و كان أفضلها الفطر ترايكودرما هارزيانم. أدى إستخدام الفطر ترايكودرما هارزيانم و كذلك بكتيريا رايزوبيم ليجيومينوزارم فى تربة معداة بالفطر رايزوكتونيا سولانى إلى زيادة معنوية فى عدد أوراق نباتات الفول مقارنة بالنباتات غير المعاملة أو تلك المنزرعة فى تربة محتوية على باسيلس ميجاتيريم بعد ٤٠ يوم من الزراعة. تأثر إرتفاع النباتات بوجود الفطر الممرض (رايزوكتونيا سولانى) رغم وجود كائنات التضاد الحيوى ؛ حيث كانت نباتات المقارنة أكثر إرتفاعا. و كانت أفضل نتائج نمو النبات عند إضافة الفطر ترايكودرما هارزيانم أو بكتيريا رايزوبيم إلى التربة فى حين كان إرتفاع النبات أقل ما يمكن بإضافة باسيلس ميجاتيريم إلى التربة المعداه بالفطر الممرض. أدى وجود الفطر الممرض إلى نقص معنوى فى الوزن الغض و الوزن الجاف لنباتات الفول. أدى وجود الفطر الممرض جذور نباتات الفول إلى زيادة معنوية فى أى من الجذور أو المجموع الخضرى فى حين أدى إستخدام الفطر ترايكودرما هارزيانم ككائن تضاد حيوى إلى زيادة قدرها ٤,٧% فى محتوى النيتروجين فى النبات. أما النباتات المنزرعة فى تربة تحوى بكتيريا باسيلس بالإضافة إلى الفطر الممرض فكان محتواها من النيتروجين مماثلا لنباتات المقارنة. وأدى إستخدام الكائنات مجتمعة إلى زيادة قدرها ٢٣,٧%.