Influence of *Rhizobium leguminosarum* combined with *Trichoderma harzianum* on damping off diseases and growth parameters of faba bean and pea plants

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Received: *14/10/2009*

Abstract: The soilborne pathogens can cause severe economic losses to field crops. The effects of a combined inoculation of *Rhizobium leguminosarum* and a biocontrol fungus *Trichoderma harzianum* on damping-off diseases and plant growth parameters, *i.e.* root nodule mass, root biomass, and shoot biomass of broad bean and pea were studied under greenhouse conditions. Results indicated that *T. harzianum* and *R. leguminosarum* were more effective in decreasing the growth of the tested pathogenic fungi than other tested species of both microorganisms under laboratory conditions. Application of the microorganisms separately or in dual inoculation was assessed. All treatments reduced the infected plants as compared with untreated control. Application of the microorganisms in combinations decreased disease severity in most cases of the tested pathogens comparable with individual using treatment for each microorganisms. In addition to a positive effect on the plants production of both vegetative growth or dry weight. The results provide evidence of the potential of beneficial microorganisms in good protection against soil borne fungal damage.

Keywords: bioagents, damping off, *Rhizobium*, soilborne, *Trichoderma*

INTRODUCTION

Soilborne fungal diseases are among the most important factors limiting the yield of legume crops in many countries worldwide. Regarding to environmental and health concerns about extended use of chemicals, there is considerable interest in finding alternative control approaches for use in biological control strategies for crop diseases (Raupach and Kloepper, 1998.). The beneficial effect of *Rhizobium* sp. in terms of biological nitrogen fixation has been a main focus in the recent past (Deshwal *et al.*, 2003). Other reports (Nautiyal 1997 and Estevez de Jensen *et al.*, 2002) revealed that *Rhizobium* spp. can be used in control soilborne pathogens of legume crops, such as *Rhizoctonia* spp., *Fusarium* spp. and *Pythium* spp. A study by Bardin *et al.* (2004) proved that certain strains of *Rhizobium leguminosarum* Frank bv. *viceae* could fix nitrogen in an artificial medium, and were effective in reducing incidence of Pythium damping-off of field pea and sugar beet when applied as seed treatment. Trichoderma has long been known as effective antagonists against soilborne plant pathogenic fungi (Kumar and Mukerji, 1996). Combined inoculation of *Rhizobium* sp. and a biocontrol fungus *Trichoderma* spp. increased growth, nutrient uptake and yield of chickpea under glasshouse and field conditions (Rudresh, *et al.* 2005). However, little work has been done on the effect of combined inoculations of *Rhizobium* sp. and *Trichoderma* sp. on plant growth, nutrient uptake and yield in legumes crop. The study of combining these two organisms is of great potential value to organic agriculture in order to avoid the side effect of fertilizers and pesticides. In the present investigation, two groups of microorganisms viz., *Rhizobium* spp. and *Trichoderma* sp. were studied to prove their compatibility and their combined effects in controlling damping off and growth parameter of broad bean and pea plants under greenhouse conditions.

MATERIALS AND METHODS

Isolation and identification of the causal organisms:

Faba bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) plants showing symptoms of root-rot and damping off were collected in plastic bags from the different growing areas at Ismailia Governorate and brought to the laboratory. The infected samples were rinsed in tap water and the necrotic portions were excised and cut into 2-mm pieces, surface sterilized with 5% sodium hypochlorite for 30 s and rinsed in 4 successive changes of sterile distilled water, then plated on potato dextrose agar (PDA). This plats were and incubated at 25 ±2°C for up to 5 days under 12-h photoperiod. Hyphal tip transfer and the single spore techniques were adopted whenever possible. Pure cultures of fungal isolates were identified according to their cultural and morphological features with reference to Gilman (1957), Burnett and Hunter (1972) and Nelson *et al.* (1983).

Preparing Rhizobium inoculum:

Pure isolates of *Rhizobium* spp. were kindly obtained from the Department of Microbiology, Soils, Water and Environment Research Institute, ARC, Giza. Bacterial cultures were grown on tryptone yeast agar (TYA) in Petri dishes for 48 h and incubated at (25 ± 2_C) according to Beringer, 1974. The resulting colonies in each dish were suspended in 5 ml of 1% methyl cellulose (Sigma-Aldrich, Milwaukee, WI, USA) in sterile distilled water, and scraped gently with a spatula to obtain bacterial slurries.

Pathogenicity test:

Inocula of the more frequency isolated fungi, *i.e.* *Fusarium oxysporum* , *F. solani, Macrophomina phaseolina, Rhizoctonia solani* as well as *Sclerotium rolfsii* were prepared by growing each fungus on autoclaved maize-sand medium in glass bottles for 15 days at 25 °C. Soil infestation was achieved by mixing inoculum of each fungus with the soil at the rate of 1.5% (w/w) in sandy clay pots (25 cm diam.) and watered regularly for five days before planting. The same amount of autoclaved maize sand medium was added to the soil to serve as a control treatment. Five pots were used with each treatment to study the effect of tested fungi on the incidence of pre- and post emergence damping off of broad bean and pea plants. Each pot was sown by five seeds of broad bean cv. Giza 2 and pea cv. Lincoln and watered when needed. Percentages of pre- and post-emergence damping-off and survived plants were calculated at 15, 30 and 45 days after planting, respectively.

Antagonistic effects of the identified *Trichoderma* isolates:

Ten isolates of *Trichoderma harzianum*, which isolated from the rhizosphere and infected root samples, were subjected to evaluate their antagonistic effect against the five tested fungi, *i.e. F. oxysporum* , *F. solani, M. phaseolina, R. solani* as well as *S. rolfsii*. Petri dishes (9-cm in diam.) were used to detect the antagonistic effects between the isolated *T. harzianum* and each of the isolated fungi by measuring of the linear growth. All plates were inoculated with discs (5-mm in diam.) taken from 7-days-old culture of the antagonistic *T. harzianum* on one side of plate. Petri dishes plates were also inoculated with equal discs bearing growth of 7-days-old cultures of the pathogenic fungi on the opposite side of the plate. Each treatment was replicated three times. Plates were incubated at (25 ± 2 C). Cultures were observed daily and the degree of antagonism was calculated according to Kucuk and Kivanc (2003) as the percentage reduction in mycelial growth of the pathogen.

Antagonistic effects of Rhizobia on the fungal growth:

Four isolates of *Rhizobium* spp., *i.e., R. leguminosarum, R. phaseoli, R. trifolii,* and *R. melitoli* were tested to study their effect in reducing the mycelial growth of the tested fungi. Petri dishes containing 10 ml of the PDA medium were inoculated with equal disks (5-mm in diam.) of each of *F. oxysporum* , *F. solani, M. phaseolina, R. solani and S. rolfsii* obtained from 7-days-old cultures placed at the periphery of the plate. The antagonistic bacteria (*Rhizobium* spp.) were streaked at the opposite side of each plate by a loop loaded with 48 hr old culture grown at 25°C on yeast malt agar (YMA). Three replicates were used for each particular treatment. Antagonistic effect was determined as mentioned above. Petri dishes inoculated with each fungus alone were used as control treatment .

Greenhouse evaluation:

Experiments were carried out at the greenhouse of Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt, in artificially infested sandy clay soil as mentioned before. An infested pots for each tested fungus were divided into four groups. Pots of the first group were sown by treated seeds with spore suspension of *T. harzianum* isolate No.1. Second group, pots were sown by treated seeds with *R. leguminosarum*. Pots of the third group were sown by treated seeds with both *R. leguminosarum* and *T. harzianum*. Fourth group pots were sown in soil infested with each fungus alone (as control).

Seeds, broad bean cv. Giza 2 and pea cv. Lincoln, were surface sterilized with sodium hypochlorite (1%) then washed in sterilized water three times and soaked in prepared bacterial suspension for 20 min, spread on screen cloth with paper towel to absorb the excess slurry, and air-dried overnight (Rudresh, *et al.* 2005). Whereas, *T. harzianum* spore suspensions were quantified using a haemocytometer and diluted to approximately 6×10^6 conidia/ml with sterile distilled water and then applied as seed coating (Nawar, 2007). Enumeration of bacteria coated onto seeds was performed by placing five seeds of each crop in 5 ml of sterile distilled water in a test tube, vortexing for 30 s, and plating serial dilutions on TYA medium, 0.1 ml per 9-cm-diameter dish. After incubation at room temperature for 3 days, the bacterial colonies that developed in each dish were counted. There were three replicates for each treatment.

Treated faba bean and pea seeds were planted at the rate of 5 seeds per pot. Three pots were used for each treatment. Percentages of pre- and post-emergence damping-off and survived plants were recorded. Treatments were arranged in a complete randomized design with three replicates.

Seedlings growth parameters were determined for peas and broad bean at 6-node and 5-node growth stages, respectively. Plants, including the root mass, were excavated with a trowel and taken to the laboratory. Excess soil was removed from the roots by placing the root mass in a sieve and washing with running tap water. Cleaned plants were then separated into nodules, roots (everything below the first node except nodules) and shoots (everything above the first node). Nodules, roots and shoots from each plant were placed in paper bags, and dried in an oven at 60 °C for 48 h and then their dry weights were determined (Rudresh, *et al.* 2005).

Differences among treatments for incidence of damping-off, seedling height, root nodule mass, root biomass, and shoot biomass data were analyzed for statistical significance using analysis of variance (ANOV). Treatment means for each set of data were separated using LSD at the P = 0.05 level. All statistical analyses were performed using SAS Computer Software, version 8.2 (Anonymous, 2001).

RESULTS AND DISSECTION

Isolation of the causal organisms:

Associated fungi from affected broad bean and pea plants by damping off or root rot were isolated and identified. Data presented in Fig. (1) show that *Rhizoctonia solani*, *Fusarium oxysporum*, and *Sclerotium rolfsii* were more frequent than other fungi isolated from both broad bean and pea plants by means 21.1, 15.7 and 13.4%, respectively, followed by *F. solani* and *Macrophomina phaseolina* by means 10.6 and 9.3%, respectively. On the other hand, *T. harzianum* were isolated by the 6.9% from the isolated fungi from the rhizosphere. These fungi were previously reported to be associated with beans damping off or root-rot (Vishwa and Gurha 1998; Infantin, *et al.*, 2006 and Mazen, *et al.* 2008).

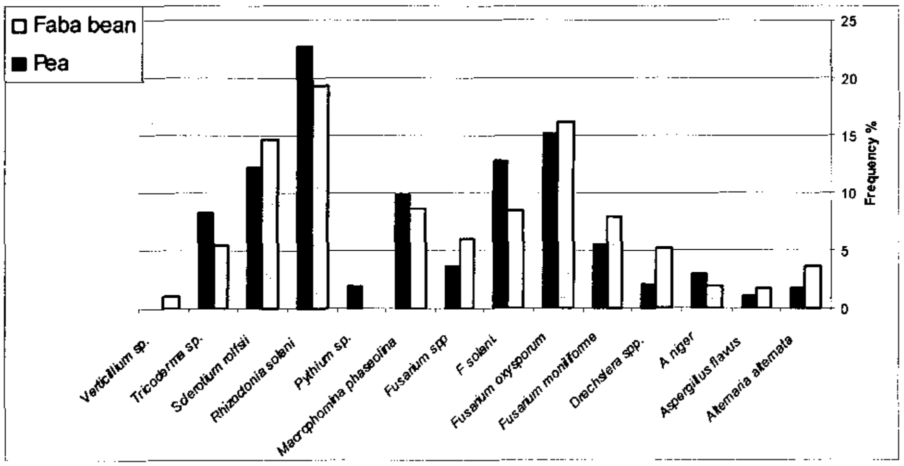

Fig.(1): Frequency of fungi isolated from broad bean and pea plants showing symptoms of damping off or root rot.

Pathogenicity test

Pathogenicity test with the fungi showing high frequency from isolation process, *i.e. F. oxysporum, F. solani, R. solani, M. phaseolina and S. rolfsii* were studied under greenhouse conditions compared with the untreated control. Data presented in Table (1) show that *R. solani* caused the highest damping off incidence with pre and post emergency damping off on broad bean and pea plants, being 46.67, 26.67, 40.0 and 33.33%, respectively. Whereas, *M. phaseolina* and *S. rolfsii* were the lowest destructive for seedlings or causing damping off disease incidence. Our results are in agreement with other investigators (Bardin, *et al.* 2004; Infantin, *et al.*, 2006 and Mazen, *et al.* 2008)

***In-vitro* test for antagonistic activity:**

Agar plates inoculated with the tested fungi of *F. oxysporum, F. solani, M. phaseolina, R. solani and S. rolfsii* and the isolates of *T. harzianum* Table (2) reveal the presence of clear antagonistic action between them. Marked inhibition in the linear growth of the tested fungi was occurred. The highest mean of inhibition values being 88.11, 81.11, 87.00 and 79.67% were obtained between *T. harzianum* isolate No.1 and *F. oxysporum, M. phaseolina, S. rolfsii and R. solani*, respectively, whereas the highest reduction for *F. solani* was obtained with *T. harzianum* isolate No.5.

Trichoderma spp. are well known for their biological control capabilities against a wide range of commercially important plant pathogens (Whipps and Lumsden, 2001; McLean *et al.* 2004). They are known to produce a number of antibiotics, such as trichodermin, trichodermol A and harzianolide (Claydon *et al.*, 1991). These compounds are responsible for the inhibition of most fungal phytopathogens (Nawar, 2007).

Antagonistic effects of Rhizobia on the fungal growth were studied against fungal pathogens. Data in Table (3) reveal that the inhibition zone area fluctuated significantly in response to antagonists species with the pathogenic fungi. *Rhizobium leguminosarium* recorded the highest effect against all tested fungi followed by *R. melitoli* against *F. solani* and *R. trifolii* against *F. oxysporum* and *M. phaseolina* as well as *R. phaseoli* against *Rhizoctonia solani*

The results of the present investigation are in agreement with those reported by different investigators who reported that Rhizobia are able to inhibit significantly the growth of pathogenic fungi such as *M. phaseolina, Rhizoctonia spp, Fusarium* sp. and *Pythium* spp. in leguminous plants (Estevez de Jensen, *et al.*, 2002 and Bardin, *et al.* 2004).

Greenhouse studies:

Enumeration of bacteria coated onto seeds revealed similar numbers of bacteria per seed for *R. leguminosarum*, for both broad bean and pea. The number of colony-forming units per seed ranged from 2.5×10^5 to 2.9×10^5 for broad bean, and from 2.2×10^5 to 3.1×10^5 for pea. Combined application with *R. leguminosarum* and *T. harzianum* and their individual effects in controlling damping off disease were studied. Data in Table (4) show that treatment of broad bean seeds with *R. leguminosarum* under soil infestation conditions with different pathogenic fungi resulted in significant reduction in damping-off compared with the untreated control. The lowest incidence of damping-off and highest percentage of plant survival were obtained when seeds were treated with *R. leguminosarum* against *F. oxysporum* being 86.6% followed by *M. phaseolina* and *S. rolfsii*, 40.0 and 60.0%, respectively. Whereas, the lowest percent of plant survival was recorded with *R. solani* treatment being 46.6%. In this regard, treated seeds with *T. harzianum* without adding *R. leguminosarum* showed increasing in plant survival compared with the control for different pathogenic fungi, *i.e.* 73.4, 60.0, 53.4. 53.4 and 63.4 % for *F. oxysporum, F. solani, R. solani, M. phaseaolina and S. rolfsii*, respectively. The combined effect between both *Trichoderma* and *Rhizobium* showed the maximum effect in controlling damping-off for various tested pathogens. This treatment, also, increased the percentage of plant survival, *i.e.* 86.6, 86.6, 80.0, 60.0 and 60.0% with *F. oxysporum, S. rolfsii, F. solani, R. solani,* and *M. phaseaolina*, respectively. Combination between *T. harzianum* and *R. leguminosarum* showed the greatest effect on pea seeds against *R. solani*, since survival recorded 80.0% compared with 20.4% for check treatment (without bioagents). In general, *R. leguminosarum*, as seed treatment, plus *T. harzianum*, as soil treatment sharply decreased damping off compared with other treatment. The current study is confirmed by the findings of Bardin, *et al.* (2004) who reported that seeds treated with *R. leguminosarum* bv. *viceae* was effective in controlling damping-off of pea. On the other hand, Huang and Erickson (2007) confirmed that besides the effect on disease control, seed treatment with *R. leguminosarum* also improved plant growth in the current study, as shown by increased plant height, root-nodule formation, root biomass and shoot biomass of broad bean and pea. Thus, treatment of broad bean and pea seeds with effective strain of *R. leguminosarum* may be preferable than using fungicides, because of the potential for the nitrogen-fixing bacteria to control damping-off, improvement soil fertility, increasing crop productivity and reducing the negative environmental impact associated with chemical use (Huang and Erickson, 2007). Other investigators observed the benefits on chickpea by combined inoculation of *R. leguminosarum*, and *Trichoderma* spp. seems likely to be due to cumulative effect on processes such as the supply of N and P to the crop in addition to the growth promoting substances produced by these organisms and the biological control of soilborne fungal pathogens by *Trichoderma* spp. (Windham *et al.*, 1986 and Alagawadi and Gaur, 1988). Similar reports of increasing growth, nodulation, nutrient uptake and yield parameters in chickpea were reported when Rhizobium and phosphate solubilizing bacteria were inoculated together (Rudresh *et al.*, 2005).

Table (1): Effect of some fungi on the incidence of pre- and post emergence damping off of faba bean and pea plant under greenhouse conditions.

Pathogens	Broad bean (cv. Giza2)			Pea (cv. Lincoln)		
	Damping-off %		% Survived plants	Damping-off %		% Survived plants
	Pre-emergence	Post-emergence		Pre-mergence	Post-mergence	
F. oxysporum	13.33	20.00	66.67	6.67	13.33	80.00
F. solani	20.00	26.67	53.33	13.33	13.33	73.34
M. phaseolina	13.33	13.33	73.34	6.67	20.00	73.33
R. solani	46.67	26.67	26.66	40.00	33.33	26.67
S. roffsii	6.67	20.00	73.33	26.67	26.67	46.67
Control	0.00	0.00	100.00	0.00	0.00	100.00
L.S.D. 5%.	1.19	2.7	1.39	0.97	3.1	0.94

Table (2): Effect of ten isolates of *Trichoderma harzianum* on the linear growth of some fungi isolated from diseased broad bean and pea plants

Isolates of *T. harzianum*	Mean linear growth (mm)									
	F. oxysporum		*F. solani*		*M. phaseolina*		*S. rolfsii*		*R. solani*	
	R.G.	R. %	R.G.	R. %	R.G.	R. %	R.G.	R. %	R.G.	R. %
No. 1	10.7	88.11	27.7	69.22	17.0	81.11	11.7	87.00	18.3	79.67
No. 2	13.0	85.56	18.7	79.22	19.0	78.89	12.7	85.89	29.0	67.78
No. 3	13.3	85.22	26.7	70.33	18.3	79.67	12.3	86.33	44.3	50.78
No. 4	15.3	83.00	23.3	74.11	20.3	77.44	13.3	85.22	29.0	67.78
No. 5	15.0	83.33	15.0	83.33	17.3	80.78	14.0	84.44	21.3	76.33
No. 6	12.3	86.33	21.0	76.69	17.3	80.78	17.0	81.11	20.7	77.00
No. 7	12.7	85.89	19.0	78.89	17.3	80.78	21.0	76.67	34.7	61.44
No. 8	13.3	85.29	21.0	76.67	17.3	80.78	12.7	85.89	38.0	57.78
No. 9	11.7	87.00	17.0	81.11	19.0	80.89	17.3	80.78	30.3	66.33
No. 10	18.3	79.67	18.3	79.67	17.3	80.78	16.3	81.89	42.0	53.33
L.S.D. at 5%	0.48		0.32		0.65		0.19		1.54	

Control = 90.0 mm R.G. = radial growth; R % = reduction relative to control

Table (3): Effect of four Rhizobia groups on the linear growth of some fungi isolated from diseased broad bean and pea plants.

Rhizobia Groups	Mean linear growth (mm)									
	F. solani		*F. oxysporum*		*R. solani*		*M. phaseolina*		*S. rolfsii*	
	L.G	R%	L.G	R%	L.G	R%	L.G	R%	L.G	R%
R. leguminosarum	11.2	87.56	14.1	84.33	15.4	82.89	13.1	85.44	15.6	82.67
R. phaseoli	19.1	78.78	27.3	69.67	22.9	74.56	20.0	77.77	27.3	69.67
R. trifolii	20.4	77.33	20.2	77.56	27.1	69.89	15.4	82.89	24.2	73.11
R. melitoli	13.9	84.56	35.4	60.67	39.3	56.33	35.0	60.11	30.4	66.22
Control	90.0	0.00	90.0	0.00	90.0	0.00	90.0	0.00	90.0	0.00
L.S.D. 5%	3.16		2.68		2.39		4.15		3.29	

L.G. = Linear growth R%= G1 – G2 / G1 X 100, (G1 : growth of control , G2 :growth of treatment).

Table (4): The Effect of *T. harzianum* and *R. leguminosarum* each alone or in combination on controlling damping off of Broad bean and pea plants.

Treatments			Broad bean (cv. Giza2)			Pea (cv. Lincoln)		
			Damping-off %		**% plant Survival**	**Damping-off %**		**% plant Survival**
			Pre-	Post.		Pre.	Post.	
F. oxysporum	With Trichoderma	+ Rhizobium	6.60	6.60	86.80	0.00	26.6	73.4
		- Rhizobium	20.00	6.60	73.40	20.2	13.4	66.4
	Without Trichoderma	+ Rhizobium	13.40	6.6	80.0	40.0	6.6	53.4
		- Rhizobium	26.60	6.6	66.8	33.4	20.0	46.6
F. solani	With Trichoderma	+ Rhizobium	20.00	0.00	80.00	20.00	6.6	73.4
		- Rhizobium	26.60	13.40	60.00	40.0	00.0	60.0
	Without Trichoderma	+ Rhizobium	26.60	20.00	53.40	20.0	33.4	46.6
		- Rhizobium	46.60	6.60	46.80	26.6	20.0	53.4
R. solani	With Trichoderma	+ Rhizobium	20.00	13.40	66.60	6.6	6.6	86.8
		- Rhizobium	26.60	20.00	53.40	13.4	13.4	76.2
	Without Trichoderma	+ Rhizobium	40.00	13.40	46.60	6.6	20.0	73.4
		- Rhizobium	53.40	6.60	40.00	26.6	53.0	20. 4
S. rolfsii	With Trichoderma	+ Rhizobium	26.60	13.40	60.00	20.0	13.4	66.6
		- Rhizobium	33.40	13.40	53.20	26.6	13.4	60.0
	Without Trichoderma	+ Rhizobium	53.40	6.60	40.00	6.6	33.4	60.0
		- Rhizobium	33.40	33.40	33.20	13.4	33.2	53.4
M. phaseolina	With Trichoderma	+ Rhizobium	13.40	00.0	86.6	6.6	6.6	86.8
		- Rhizobium	20.00	13.60	66.40	0.0	13.4	86.6
	Without Trichoderma	+ Rhizobium	33.40	6.60	60.00	0.0	13.4	86.6
		- Rhizobium	44.60	33.40	22.00	13.4	13.4	73.2
Control			00	00	100	00	00	100
L.S.D. 5%			1.04	2.30	2.59	0.35	0.23	2.41

(+) = with, (-) = without

Nodules were observed on the roots of broad bean and pea plants in all treatments including seeds treated with or without Trichoderma in addition to, *R. leguminosarum* and the untreated control. The root nodule mass as dry weight per plant for the treatment of *R. leguminosarum* plus *T. harzianum* treatment was significantly higher than the treatment of Rhizobium alone (Table, 5). Results in the present investigation indicate that the antagonistic effect between bioagents and pathogenic fungi can cause more nodules production per plant for both tested crops under greenhouse conditions. In the contrary, in the absent of the bioagent (*T. harzianum*) the competition on the roots between the pathogens and *R. leguminosarum* decreased the nodulation production and caused less nodules number. In this respect, root nodules from plants treated with *R. leguminosarum* were larger in size and pinkish in colour, whereas root nodules from plants of the untreated control were smaller in size and brownish in colour. Appleby (1974) found that the presence of leghaemoglobin in root nodules resulted in a pinkish colour that was indicative of healthy nodules with a high rate of nitrogen fixation.

Data of plant growth parameters studied show that the vegetative characters of broad bean plants were affected by the infection with the studied causal pathogens. Data presented in Table (6) show that plants shoot fresh and dry weight showed the lowest values when the seeds were plated in soil infested by *S. rolfsii,* together with the bioagent *T. harzianum,* with or without applying *R. leguminosarum.* Whereas, they were reached the minimum values in pea plants grown in soil infested by *R. solani.* Generally, we can conclude that using the bioagent *T. harzianum* increased significantly the fresh and dry weights of plants. Furthermore, lytic enzymes (e.g. protease, chitinase, cellulase, amylase, glucanase, Whipps and Lumsden 2001) and antimicrobial compounds (e.g.

Table (5): Effect of the isolated tested fungi on nodules production by *Rhizobium leguminosarum* (dry weight of nodules).

Treatment		Broad bean cv. (Giza)	Pea cv. (Lincoln)
		Nodules Dry weight (mg $plant^{-1}$)	Nodules Dry weight (mg $plant^{-1}$)
With Trichoderma	*F. oxysporum*	180.2	155.18
	F. solani	150.19	135.10
	M. phaseolina	129.00	156.00
	R. solani	169.12	141.33
	S. rolfsii	175.10	131.12
Without Trichoderma	*F. oxysporum*	25.19	20.00
	F. solani	20.00	18.11
	M. phaseolina	21.09	14.05
	R. solani	11.93	9.14
	S. rolfsii	18.75	10.00
Control		9.1	7.3
L.S.D. 5%		4.71	4.92

Table (6): Effect of *T. harzianum* and *R. leguminosarum* on some growth parameters of broad bean and pea plants inoculated with damping off pathogens

Treatments			Broad bean (cv. Giza2)				Pea (cv. Lincoln)			
			Roots		Shoots		Roots		Shoots	
			Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
F. oxysporum	With Trichoderma	+ Rhizobium	17.4	2.1	17.4	2.2	6.1	0.6	6.1	0.6
		- Rhizobium	10.4	1.1	17.1	1.7	3.9	0.4	5.4	0.6
	Without Trichoderma	+ Rhizobium	17.1	1.8	17.4	2.2	5.4	0.6	3.9	0.4
		- Rhizobium	7.1	0.8	17.1	1.8	3.7	0.3	3.7	0.3
F. solani	With Trichoderma	+ Rhizobium	22.3	2.1	22.3	2.1	6.5	0.8	6.5	0.8
		- Rhizobium	19.3	1.9	23	1.9	5.3	0.6	5.5	0.6
	Without Trichoderma	+ Rhizobium	23.0	1.9	19.3	1.9	5.5	0.6	5.3	0.6
		- Rhizobium	14.6	0.8	17.6	1.8	4.1	0.3	4.1	0.3
R. solani	With Trichoderma	+ Rhizobium	18.1	2.2	19.4	2.2	4.5	0.7	4.5	0.7
		- Rhizobium	19.5	1.8	12.2	1.5	4.1	0.1	4.1	0.5
	Without Trichoderma	+ Rhizobium	12.2	1.5	18.1	1.8	4.1	0.1	2.8	0.8
		- Rhizobium	11.1	1.5	11.7	1.1	0.8	0.1	1.3	0.5
S. rolfsii	With Trichoderma	+ Rhizobium	18.7	1.9	14.8	1.4	3.3	1.4	7.6	0.7
		- Rhizobium	18.1	1.9	9.3	1.0	2.1	0.7	6.7	0.6
	Without Trichoderma	+ Rhizobium	18.1	1.8	10.9	0.9	2.8	1.8	1.3	1.2
		- Rhizobium	15.1	1.6	8.0	0.7	2.5	1.6	1.0	1.0
M. phaseolina	With Trichoderma	+ Rhizobium	19.7	2.23	17.5	2.3	2.8	0.3	6.9	2.0
		- Rhizobium	17.1	1.7	16.4	1.9	0.5	0.1	5.9	1.7
	Without Trichoderma	+ Rhizobium	19.0	1.6	11.3	2.1	1.9	0.3	6.1	1.6
		- Rhizobium	17.1	1.5	9.7	1.77	1.8	0.3	5.9	1.5
Control			20.1	2.4	23.6	2.2	2.4	0.81	0.7	1.0
L.S.D. 5%			0.51	0.11	2.3	0.59	0.12	0.03	0.15	0.08

(+) = with, (-) = without

tricholin, trichodermin, 6-pentyl-a-pyrone, gliovirin, gliotoxin, viridian, and viridiol, Worasatit *et al.* 1994) produced by *Trichoderma* spp. are well documented and play a major role in biocontrol and plant growth promotion (Lindsey and Baker 1967). This may be due to its antagonistic action on the studied pathogens. In addition, it was also obvious that applying the bioagent *T. harzianum* combined with *R. leguminosarum* gained higher fresh and dry weights of the studied plants. *Trichoderma harzianum.* have been found to show potential as biological agents against seed and root rotting pathogens witch are an agreement with Okigbo and Ikediugwu (2000). Moreover, the *R. leguminosarum* in this study has greater commercial potential for legume crops because it can be applied as biocontrol agent when damping off is rampant or as a biofertilizer when the disease is not a problem on these crops (Bardin *et al.*, 2004). On the other hand, earlier studies (Meyer *et al.*, 2001) indicated that incompatibility between microorganisms may occur, causing a mixture of biocontrol organisms to be less effective than single species application. In this respect, the increase in growth and yield parameters of broad bean and pea by combined inoculation of *Rhizobium* sp. and *Trichoderma* sp. may be due to cumulative effects, such as enhanced supply of nitrogen to the crops in addition to, growth promoting substances produced by these organisms. In addition to the biocontrol activity of *T. harzianum* against soil borne fungal pathogens (Alagawadi and Gaur, 1988). The increase in growth and yield of tested crops could also be due to nutrient supplementation among the inoculated organisms, which might have enhanced their efficiencies like N fixation by *Rhizobium* sp. and effective pathogen suppression by *Trichoderma* sp. Similarly increased nitrogen fixation by *Rhizobium* sp. due to phosphorus supplementation was reported (Manjunath and Bagyaraj, 1984).

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تأثير الجمع بين بكتيريا الريزوبيوم و فطر التريكودرما على موت البادرات ومؤشرات النمو فى نباتات الفول البلدى والبسلة

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تسبب الإمراض الكامنة بالتربة العديد من الخسائر الاقتصادية بالنسبة لمحاصيل الحقل وبخاصة المحاصيل البقوليه. درس تأثير الجمع بين بكتيريا الريزوبيوم والمقاوم الحيوى فطر التريكودرما على كل من مقاومة موت البادرات ومقاييس النمو (إنتاج العقد الجذرية والنمو الخضري والنمو الجذري) على كل من نباتات الفول البلدى ونباتات البسلة تحت ظروف الصوبة. أوضحت النتائج المعملية القدرة العالية لفطر التريكودرما هارزيانم وبكتريا ريزوبيوم لجيومينوزارم في تثبيط النمو الفطري للفطريات الممرضة المختبرة. اختبر تأثير إضافة كلا الميكروبين منفصلا أو كلاهما معا حيث ادت المعاملات المختلفة إلى انخفاض نسبة حدوث مرض موت البادرات مقارنة بالكنترول. أشارت النتائج أن أضافت فطر التريكودرما مع بكتيريا الريزوبيوم ادى الى خفض كبير في شدة المرض مع مختف الفطريات الممرضة مقارنة بتأثير كل منهما منفرد بالإضافة إلى تاثيرهما الايجابي على إنتاج النبات سواء النمو الخضري أو الوزن الجاف. تقدم النتائج دليل واضح على أمكان استخدام الكائنات الحية النافعة في عمل حماية جيدة ضد فطريات التربة الممرضة.