Impact of dual inoculation with *Rhizobium* and *Trichoderma* on damping off, root rot diseases and plant growth parameters of some legumes field crop under greenhouse conditions

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Damping off and root rot are a worldwide diseases, caused destructive yield losses of legumes field crop *of Vicia fabae, Cicer arietinum* and *Lupines terms*. The biological control of damping off and root rot diseases using microorganisms antagonistic to growth and development of the fungi were investigated under greenhouse conditions at the Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. The results exhibited that *Rhizobium* spp. and *Trichoderma* sp. fungi scoffed at the potential or ability to their compatibility and in combined effects in controlling the fungi, which caused the damping off and root rot. This resulted in an improvement in many plant growth parameters, which are ultimately expressed in increasing the yield components of branches plant^{-1}, pods plant^{-1}, seeds pod^{-1}, mean seed weight and then increase seed yield of the legume field crops broad bean, chickpea and lupine plants. The results suggested that the *Rhizobium* spp. and *Trichoderma* sp. can be used as biological control of some soil-borne fungal diseases causing significant yield losses in legumes field crops.

Keywords: Soil borne diseases, Faba bean, Chickpea, Lupine, yield characteristics

INTRODUCTION

Faba bean *(Vicia faba L.),* Chickpea *(Cicer arietinum L.)* and lupine *(Lupines terms* L.) are the most important legumes crops in the human feeding all over the world. Grain legumes are subject to numerous pathogens attacking both the roots and the aerial parts of the plant. Among biotic stresses, diseases are often the major limiting factor the yield of grain legumes, and they are economically important where these crops are grown (Puglia and Aragona, 1997). Soil-borne fungal diseases are the most important factors among all limiting the seed yield of legume crops in many countries worldwide.

On the other hand, regarding to environmental and health concerns about the extended use of effective chemicals, which are available to control a number of these diseases, we cannot rely upon them to a large extent for both economical and environmental reasons. The best approach is searching about a considerable interest in finding alternative control approaches for use in biological control strategies for crop diseases (Raupach and Kloepper, 1998; Pal and Garedener, 2006). The beneficial effect of *Rhizobium* sp. has been a main focus in terms of biological nitrogen fixation in the recent past (Deshwal et al., 2003). Other reports *via*, Nautiyal (1997), Estevez de Jensen et al. (2002) ; Baraka et al. (2009) revealed that *Rhizobium* spp. can be used in control soil-borne pathogens of legume crops, such as *Rhizoctonia* spp., *Fusarium* spp., *sclerotium rolfsii, Macrophomina phaseolina* and *Pythium* spp. As an extension to talk in this area, biological control of plant diseases, especially soil-borne plant pathogens, has been the subject of extensive research in the last two decades. *Trichoderma* spp. is well documented as effective biological control agents of plant diseases caused by soil-borne fungi (Sivan et al., 1984; Coley-Smith et al., 1991). Biological control of soil-borne plant pathogens can be achieved by seed treatment with antagonistic microorganisms.

In this connection, Hadar et al. (1979); Elad et al. (1980) found that the application of wheat bran colonized by *Trichoderma harzianum* to soils infested by *Rhizoctonia solani* and *Sclerotium rolfsii* reduced the incidence of disease caused by these pathogens in beans. Bardin et al. (2004) also 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 soil-borne 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 both of *Rhizobium* sp. and *Trichoderma* sp. on plant growth, nutrient uptake, seed yield and its components in legumes crop. Hence, a study of combining these two organisms together may have a great potential value in organic agriculture to avoid the side effect of chemical fertilizers and pesticides. Therefore, we established this study to prove compatibility and combined effects of *Rhizobium* spp. and *Trichoderma* sp. in controlling the damping off, root rot and growth parameters as well as seed yield with its components of broad bean, chickpea and lupine plants.

MATERIALS AND METHODS

Plant Materials

The plant materials used in this work represented three legumes crops viz. faba bean, chickpea and lupine. These crop cultivars were obtained from Food Legumes Research Department, Agriculture Research Center, Giza, Egypt. Legume crops, scientific name, cultivar name of each crop and their pedigree are present in Table (1).

Isolation and identification of the causal organisms

Legume crops (Faba bean, Chickpea and Lupine) plants with symptoms of root-rot and wilt infections 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, then surface sterilized with 5% sodium hypochlorite for 30s and rinsed in 4 successive changes of sterile distilled water. These were then plated on potato dextrose agar (PDA) and incubated at 25 ±2°C for up to 5 days under 12-h photoperiod. Hyphal tip transfer and the single spore technique were adopted whenever possible. Pure cultures of fungal isolates were identified using cultural and morphological feature with reference to Gilman (1957); Burnett and Hunter (1972); Nelson et al. (1983).

Preparing Rhizobium inoculum

Rhizobium leguminosarium was kindly obtained from a previous study (Baraka et al., 2009). Bacterial cultures were grown on tryptone yeast agar (TYA) (Beringer, 1974) in Petri dishes for 48 h at room temperature (25 ± 2°C). 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.

Antagonistic effects of *R. leguminosarium* on the fungal growth

Rhizobium leguminosarium was tested to study their effect in reduction the mycelial growth of isolated fungi. Petri dishes containing 10 ml of the PDA medium were inoculated with equal disks (5-mm in diam.) of *F. oxysporum*, *F. solani, M. phaseolina, R. solani and S. rolfsii* obtained from 7 days old culture which placed at the periphery of the plate. The antagonistic bacterium (*R. leguminosarium*) was streaked at the opposite side of each plate by a loop loaded with 48 hr old culture grown at 25°C on YMA. Five Petri plat were used as a replicate. Each particular treatment was replicated three times. Antagonistic effect was determined according to Kucuk and Kivanc, (2003). Petri dishes inoculated with a fungus only were used as control treatment.

Antagonistic effects of the identified Trichoderma isolates

Isolate of *T. harzianum* in controlling soil-borne pathogens from the mentioned study above was subjected to evaluate their antagonistic effect against five isolates *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 *T. harzianum* and all fungal isolates by measurement of linear growth. Different plates were inoculated with discs (5-mm in diam.) taken from 7 days old culture of antagonistic *T. harzianum*. Pathogenic isolates were inoculated with either an equal discs bearing growth of seven days old culture (Abd el-Moity, 1985). Five Petri plat were used as a replicate. 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 reduction percentage in mycelial growth of the pathogen.

Greenhouse evaluation

Pathogenicity test

Experiments were carried out in artificially infested sandy clay soil at the greenhouse of Agronomy Department, in the Experimental Farm of Faculty of Agricultural, Suez Canal University in Ismailia, Egypt. 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 ºC. 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 ×25 × 30 cm^3) 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. Each pot was sown by five seeds of each tested plant cultivars and watered when

Table 1. Scientific names, cultivar names and pedigree of the legume crops.

No.	Name of legume crop	Scientific name	Cultivar name	Pedigree
1	Faba bean	*Vicia faba* L.	Giza 2	Individual plant selection from land races
2	Chickpea	*Cicer arietinum* L	Giza 531	Cross (Giza 1 x N A 29).
3	Lupine	*Lupines terms* L.	Giza 1	Not available

needed. Five pots were used with each treatment to study the effect of tested fungi on the incidence of pre- and post emergence damping off, percentages of pre- and post-emergence damping-off and survived plants were calculated at 15, 30 and 45 days after planting, respectively

Effect of seed treatment with *R. leguminosarum*

Inocula of more pathogenic isolates were prepared and mixed in the soil as mentioned above. Infested pots with each tested fungus were divided into four groups as the following; first group was inoculated with Trichoderma (*T. harzianum*) spore suspension, second group was inoculated with Rhizobium (*R. leguminosarium*) as treated seeds, third group was inoculated with both Rhizoium (treated seeds) and Trichoderma (spore suspension) and fourth group was infested soil as control.

Seeds of Faba bean (Cv. Giza 2), Chickpea (Cv. Giza 531) and Lupine (Cv. Giza 1) were first surface sterilized with sodium hypochlorite (1%) and soaked in prepared bacterial suspension as mentioned before for 20 min, spread on screen cloth with paper towel to absorb the excess slurry, and air-dried overnight (Rudresh et al., 2005). *T. harzianum* was applied as seed coating by 6 x 10^{10} conidia/ml (Nawar, 2007).

The treated eight seeds of each of cv. Giza 2 (broad bean), cv. 531 (chickpea) and cv.1 (lupine), were planted per pot. Three pots were used as replicate and three replicates were used for each treatment. Percentages of pre-or post-emergence damping-off, root rot and survival plants were calculated at 15, 30, and 60 days after planting, respectively. The pots treatments were arranged in a randomized complete block design (RCBD) with three replicates.

Measurement of studied characters

Root nodule mass, root and shoot biomass were recorded at sixty days after seed sowing. On the other hand, in concerning seed yield and its components characteristics such as plant height (cm), number of branches (plant^{-1}), number of pods (plant^{-1}), 100-Seed weight (g) and seed yield (g plant^{-1}) were determined as average number of potted plants at the harvest date of each crop cultivar.

Statistical analyses

Statistical analyses of the data were performed using SAS Computer Software, version 8.2 (SAS Institute, 2001). The differences among treatments for all the studied characteristics mentioned above were analyzed for statistical significance using variance analysis (ANOVA). Treatment were compared by using Duncan's multiple range (LSR) test at the P = 0.05 significance level.

RESULTS AND DISCUSSION

The seed yield of grain legumes is the result of many plant growth processes, which are ultimately expressed in the yield components of branches plant^{-1}, pods plant^{-1}, seeds pod^{-1} and mean seed weight. The highest seed yields are generally obtained when all obvious characters are maximized (Ayaz, 2001). The components of seed yield approach has been used extensively to explain variations in the yield of grain legumes such as *Vicia faba* (Husain *et al*., 1988), *Phaseolus vulgaris* (Dapaah *et al*., 2000), *Cicer arietinum* (Verghis, 1996); *Lupines terms* (Julier *et al.*, 1995).

Isolation of the causal organisms

The fungi isolated and identified in the samplings of the studied legumes crops showing clear symptoms of root rot as well as their percentage of occurrence were recorded. The associated fungi of the legumes crops affected by damping off or root rot were isolated and identified. Data presented in Figure 1 showed that *R. solani* was the dominant pathogen in all studied locations, followed by *F. oxysporum*, *S. rolfsii*, *F. solani* and *M. phaseolina* respectively. These fungi were previously reported to be associated with beans damping off or root-rot disease in other different studies (Vishwa and Gurha, 1998; Infantin, et al., 2006**;** Mazen et al., 2008 ; Baraka et al., 2009).

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 uncontaminated control. Data presented in Table 2 showed that *R. solani* caused the highest damping off and root rot incidence on faba bean (Giza 2), chickpea (Giza 531) and lupine (Giza 1) plants, and showing the less healthy surviving plants at 63.5, 57.43 and 53.97%, respectively. Whereas, *M. phaseolina* showed the lowest destructive for seedlings or causing damping off or root rot disease incidence. Our results are in agreement with other investigators (Bardin et al., 2004; Infantin, et al., 2006; Mazen, et al., 2008 ; Baraka et al., 2009).

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* against to *T. harzianum* showed a clear

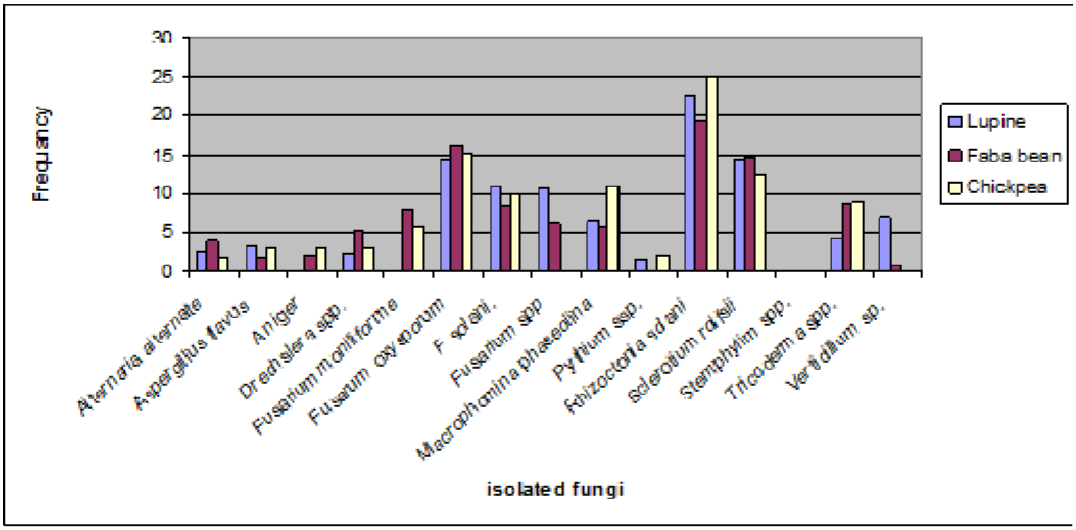

Figure 1. Spelling of frequency is wrong, legend of the figure should be“frequency (%)”

antagonistic action between the pathogenic fungi and *T. harzianum*. Marked inhibition in the linear growth of the tested fungi was observed. The highest mean of inhibition rates 88.77, 70.33, 81.11, 87.66 and 80.0% were obtained between *T. harzianum* and each of *F. oxysporum, S. rolfsii, M. phaseolina R. solani*, and *F. solani* respectively. *Trichoderma* spp. are well known as to be high-capabilities and highly efficient in biological control in a wide range of commercially important plant pathogens (Whipps and Lumsden, 2001; McLean et al., 2004). However it is known phenomena that *Thrichoderma* spp. 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 also studied against the fungal pathogens. Data in Table 3 revealed 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 *i.e. F. solani*, *F. oxysporum S. rolfsii* and *M. phaseolina* as well as *R. solani.* The results of the present investigation are in harmony 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 ; Bardin et al., 2004).

Nodules were observed on the roots of Giza 2 (faba bean), Giza 531 (chickpea) and Giza 1 (lupine) 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 plant^{-1} for the treatment of *T. harzianum* was significantly higher than without added *Trichoderma* (Table 4). Results in the current investigation indicated that the antagonistic effect between bioagents and pathogenic fungi can cause more nodules production in different tested legumes crops under greenhouse conditions. In the contrary, the absent of the bioagent (*T. harzianum*) the competition on the roots between the pathogens and *R. leguminosarum* decreased the nodule production and caused lesser number of nodules. In this respect, root nodules in plants treated with *R. leguminosarum* were larger in size and pinkish in color, whereas root nodules in the untreated plants control were smaller in size and brownish in color. In an earlier study, Appleby (1974) reported that there was leghaemoglobin in the root nodules with a pinkish color and this indicated a healthy nodules with a high rate of nitrogen fixation.

Greenhouse studies

Effect of seed treatment with *R. leguminosarum* and *T. harzianum* in disease studies

Data in Table 5 showed that treatment of broad bean seeds with *R. leguminosarum* under soil infestation conditions by *R. solani* resulted significant reduction in damping-off as compared to the untreated control. The lowest incidence of damping-off and root rot as well as highest percentage of survival plant were obtained when

Table 2. Effect of some soil-borne fungi on the incidence of pre- and post emergence damping off of Broad bean, Chickpea and Lupine plant under greenhouse conditions.

Pathogens	Broad bean (Giza 2)				Chickpea (Giza 531)				Lupine (Giza 1)			
	Damping-off %				Damping-off %				Damping-off %			
	Pre-emergence	Pre-emergence	Root rot	Survival rate (%)	Pre-emergence	Post-emergence	Root rot	Survival rate (%)	Pre-mergence	Post-mergence	Root rot	Survival rate (%)
F. oxysporum	6.67	13.33	8.2	78.47	13.33	20.00	17.2	62.8	6.67	13.33	6.1	80.57
F. solani	13.33	13.33	7.3	79.37	20.00	26.67	13.1	60.23	13.33	13.33	7.5	79.17
M. phaseolina	6.67	6.67	3.6	89.73	13.33	13.33	6.7	79.97	6.67	20.00	9.6	70.4
R. solani	26.67	20.00	16.5	63.5	46.67	26.67	15.9	57.43	40.00	33.33	12.7	53.97
S. roffsii	6.67	13.33	18.7	67.97	6.67	20.00	11.8	68.2	26.67	26.67	11.3	62.03
Control	0.00	0.00	00	100.00	0.00	0.00	00	100.00	0.00	0.00	00	100.00
L.S.D. 5%	**3.6**	**2.9**	**2.1**	**4.17**	**2.19**	**2.7**	**2.1**	**4.39**	**1.97**	**3.31**	**2.7**	**3.94**

Table 3. Effect of *T. harzianum* and *R. leguminosarum* on the linear growth of some fungi isolated from diseased broad bean and pea plants

Antagonistic Isolates	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. %
T. harzianum	10.1	88.77	26.7	70.33	17.0	81.11	11.1	87.66	18.0	80.00
R. leguminosarum	10.2	88.66	13.5	85.00	15.4	82.89	13.0	85.55	15.6	82.67
L.S.D. 5%	**0.58**		**0.30**		**0.65**		**0.29**		**1.56**	

R G: means radial growth
R : means growth reduction

Table 4. Effect of the *R. solani* on nodules production of faba bean, chickpea and lupine treated with *R. leguminosarum* (dry weight of nodules).

	Treatment	Nodules Dry weight (mg plant^{-1})		
		Faba bean	Chickpea	Lupine
Rhizoctonia solani	With *Trichoderma*	169.12	131.53	153.29
	Without *Trichoderma*	11.93	10.24	13.69
	Control	**9.1**	**6.3**	**6.7**
	LSD 5%	**4.71**	**4.72**	**3.14**

Table 5. Effect of some fungi on the incidence of pre-and post emergence root rot of Giza 2 (Broad bean) plants under greenhouse conditions during 2008/09 & 2009/10 seasons.

Treatments			Broad bean (Cv. Giza2)							
						2008/09				2009/10
			Pre-	Post-	Root rot	Survival	Pre-	Post-	Root rot	Survival
R. solani	With Trichoderma	With Rhizobium	6.60	6.60	6.1	80.7	8.0	8.7	11.4	71.9
		Without Rhizobium	13.40	6.6	8.2	71.8	6.0	8.5	15.4	70.1
	Without Trichoderma	With Rhizobium	18.0	9.7	10.9	61.5	18.0	8.7	11.6	61.7
		Without Rhizobium	20.00	6.60	8.33	56.07	16.0	11.9	13.1	59.0
LSD 5%			1.2	0.8	1.1	3.2	2.6	1.5	2.2	4.7

seeds treated with *R. leguminosarum* with 80.7 and 71.9% in both seasons 2008/09 and 2009/10, respectively. However, the lowest percent of survival plants was recorded with *R. solani* treatment and scored 56.07 and 59.0% in both seasons in the control. In this regard, seeds treated with *T. harzianum* without adding *R. leguminosarum* showed increasing in survival plant compared to the control, where scored 71.8 in season 2008/09 and 70.1 % in season 2009/10. The combined effect of *Trichoderma* and *Rhizobium* showed a maximum effect in controlling damping-off and root rot. Similar finings were obtained in chickpea and lupine plants (Table 3). On the other hand, the combined treatment of *T. harzianum* and *R. leguminosarum* also increased the percentage of survival plant and possessed 80.59, 80.3, 77.9, and 75.5% for chickpea and lupine plants through the two seasons, respectively. In general, *R. leguminosarum*, as seed treatment plus *T. harzianum*, as soil treatment sharply decreased damping off and root rot disease compared with other treatment. These results in the current study is confirmed by the findings of Bardin et al. (2004) who reported that seeds treated by *R. leguminosarum* bv. *viceae* was effective in controlling damping-off in 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. Thus, treatment of broad bean, chickpea and lupine 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 via. Windham et al. (1986); Alagawadi and Gaur, (1988), observed the benefits on chickpea, when treated by combined inoculation of *R. leguminosarum*, and *Trichoderma spp.* where, seems likely as cumulative effect on processes due to supply of N and P to the crop considered. In addition to the growth promoting substances, which in turn produced by these organisms and the biological control of soil-borne fungal pathogens. 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).

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Seed yield and its components characteristics

Seed yield and its components approach has been used extensively to give details variations in the yield of grain legumes such as *Vicia faba* (Husain *et al*., 1988), *Phaseolus vulgaris* (Dapaah *et al*., 2000), *Cicer arietinum* (Verghis, 1996); *Lupines terms* (Julier *et al.*, 1995). Therefore, the data in this ingredient shows the effect of *T. harzianum* and *R. leguminosarum* against *R. solani* root rot disease on some legume crops, such as faba bean, chickpea and lupine as described in Tables 6, 8 and 10, receptively. In addition to added contain a combination innate of the three fungi and one of them how the affect are on seed yield and some of its components characters through the three crops under the greenhouse conditions over the two seasons (2008/09 & 2009/10).

According our findings in Table 6, there was a significant increase for each of the parameters measured {plant height (cm), number of branches plant^{-1}, number of

Table 6. Effect of *R. solani* fungi on seed yield and some of its components characters of Giza 2 (Broad bean) plants under greenhouse conditions during 2008/09 & 2009/10 seasons.

Treatments			Broad bean (Cv. Giza2) 2008/09					2009/10				
			h	of es t-1)	Number of pods (plant-1)	100-Seed weight (g)	yield (g plant-1)	Plant height (cm)	branches (plant-1)	Number of pods (plant-1)	100-Seed weight (g)	Seed yield (g plant-1)
R. solani	With Trichoderma	With Rhizobium	110.39	5.02	18.04	71.08	56.28	110.09	5.12	19.01	70.18	54.21
		Without Rhizobium	106.32	4.61	16.64	67.25	50.36	108.22	4.71	15.94	65.55	51.06
	Without Trichoderma	With Rhizobium	103.47	4.21	15.01	66.69	50.10	100.40	4.01	16.21	61.49	49.12
		Without Rhizobium	91.15	3.35	10.58	54.91	42.31	91.16	3.24	11.50	52.13	42.30
LSD 5%			10.98	1.26	6.09	9.49	7.41	12.63	1.39	5.64	12.28	7.98

Table 7. Effect of *R. solani* on the incidence of pre- and post emergence root rot of chickpea (Giza 531) plants under greenhouse conditions during 2008/09 & 2009/010 seasons.

Treatments			Chickpea (Cv. Giza 531) 2008/09				2009/010			
			Pre-	Post-	Root rot	Survival	Pre-	Post-	Root rot	Survival
R. solani	With Trichoderma	With Rhizobium	10.0	3.41	6.0	80.59	8.0	6.5	5.2	80.3
		Without Rhizobium	16.60	6.60	13.80	63.0	14.0	8.3	6.7	71.0
	Without Trichoderma	With Rhizobium	20.60	13.40	8.2	57.8	12.6	15.4	13.1	58.9
		Without Rhizobium	26.60	20.00	11.7	41.7	23.7	15.3	25.2	35.8
LSD 5%			1.10	1.80	1.9	3.8	1.8	2.3	2.2	3.7

pods plant^{-1}, 100-seed weight (g) and seed yield g plant^{-1}} in faba bean (*Vicia faba*) plants. This increase was upward from the bottom to the top through both seasons (2008/09 & 2009/10) at the level of the studied characters. These increases was at the maximum range of treatment with *T. harzianum* and *R. leguminosarum*, while this increasing reached the lowest in the treatment of without *T. harzianum* and *R. leguminosarum*

Table 8. Effect of *R. solani* on seed yield and some of its components characters of chickpea (Giza 531) plants under greenhouse conditions during 2008/09 & 2009/010 seasons.

Treatments			Chickpea (Cv. Giza 531)									
			2008/09					2009/010				
			h	of es t^{-1})	Number of pods (plant^{-1})	100-Seed weight (g)	yield (g plant^{-1})	Plant height (cm)	branches (plant^{-1})	Number of pods (plant^{-1})	100-Seed weight (g)	Seed yield (g plant^{-1})
R. solani	With Trichoderma	With Rhizobium	117.23	4.47	32.32	36.28	20.67	123.20	4.51	30.92	35.26	23.67
		Without Rhizobium	112.32	4.12	30.98	35.89	20.13	122.02	4.02	32.91	35.88	21.13
	Without Trichoderma	With Rhizobium	107.92	4.08	31.01	30.41	16.99	111.32	4.18	27.57	32.31	17.79
		Without Rhizobium	100.99	3.59	22.87	25.28	14.01	107.55	3.13	20.67	23.22	13.99
LSD 5%			11.95	0.60	7.44	10.19	5.93	12.47	0.97	10.23	12.17	7.03

Table 9. Effect of *R. solani* on the incidence of pre- and post emergence root rot of Lupine (Giza 2) plants under greenhouse conditions during 2008/09 & 2009/010 seasons.

Treatments			Lupine (Cv. Giza 2)							
			2008/09				2009/010			
			Pre-	Post-	Root rot	Survival	Pre-	Post-	Root rot	Survival
R. solani	With Trichoderma	With Rhizobium	7.2	6.6	8.3	77.9	6.2	00.0	18.3	75.50
		Without Rhizobium	10.0	4.3	9.4	76.3	12.8	6.6	7.4	73.2
	Without Trichoderma	With Rhizobium	14.5	13.4	11.6	60.5	20.0	13.40	16.1	50.50
		Without Rhizobium	13.4	20.0	26.9	39.7	26.6	20.0	8.5	44.90
LSD 5%			1.10	1.80	1.90	3.80	1.50	3.00	2.10	4.20

under *R. solani* infestation conditions. The heights values were 110.39, 5.02, 18.04, 71.08, 56.28 (2008/09) and 110.09, 5.12, 19.01, 70.18, 54.21 (2009/10) for plant height (cm), number of branches plant^{-1}, number of pods plant^{-1}, 100-seed weight (g) and seed yield g plant^{-1}, respectively.

While, the lowest values were in the same sequencecharacters 91.15, 3.35, 10.58,

Table 10. Effect of *R. solani* on the on seed yield and some of its components characters of Lupine (Giza 2) plants under greenhouse conditions during 2008/09 & 2009/010 seasons

Treatments			Lupine (Cv. Giza2)									
			2008/09					2009/010				
			Plant height (cm)	Number of branches (plant^{-1})	Number of pods (plant^{-1})	100-Seed weight (g)	Seed yield (g plant^{-1})	Plant height (cm)	Number of branches (plant^{-1})	Number of pods (plant^{-1})	100-Seed weight (g)	Seed yield (g plant^{-1})
R. solani	With Trichoderma	With Rhizobium	109.27	4.33	31.08	28.97	26.01	112.09	4.83	34.00	29.99	27.62
		Without Rhizobium	103.78	4.35	26.98	30.14	26.41	108.70	4.45	29.90	30.34	28.40
	Without Trichoderma	With Rhizobium	99.69	3.89	27.40	25.69	20.5	104.19	3.83	27.80	25.40	22.39
		Without Rhizobium	102.09	3.10	20.34	22.90	21.09	105.80	3.25	24.33	23.00	21.15
LSD 5%			7.59	0.63	7.21	4.18	5.75	7.04	1.05	8.7	6.18	5.18

54.91, 42.31 in the first season (2008/09) and 91.16, 3.24, 11.50, 52.13, 42.30 in the second season (2009/10). The increase in these studied characters under a combination of *T*. *harzianum* and *R. leguminosarum* due to to the attributable significant impact of such a combination, which discourage the growth inhibitors of damping-off and root rot putrefy fungi. It is here then draw the activity of leguminous plants to form a radical, which in turn helps to increase the growth of leguminous plant (faba bean) and speed of split cells, resulting in resistance to damping-off and root rot pathogens and increase the seed yield and its components characteristics. In this regard, Khalequzzaman and Hossain (2007) found that Length/breadth of green pod, number of green pods, weight of green pods, weight of seeds, healthy looking seeds and discoloured seeds were significantly influenced when seeds were treated with *Rhizobium* strains. These results were in harmony with the finding of Al-Mahareeq (2005). Farzana et al. (1991) also showed that seed treatment with *Rhizobium meliloti* was significantly effective in reducing infection of root infecting fungi (*Fusarium* spp.) of other legemose crop (soybean plant).

The results of chickpea (Giza 531) grown in soil containing *T. harzianum* with *Rhizobium* and infested with R. solani were presented in Table 8. The results exhibited that combination of *T. harzianum* with *Rhizobium* enhanced plant height (cm), number of branches plant^{-1}, number of pods plant^{-1}, 100-seed weight (g) and seed yield g plant^{-1} toward the positive direction in both seasons (2008/09 and 2009/10). The above mentioned characters were supported the highest potential resistance or tolerance to the effect of *R. solani* infestation with combination of *T. harzianum* with *Rhizobium* in compared *Trichoderma* without *Rhizobium*. Results showed that the combination of *Trichoderma* with *Rhizobium*, more effective comparison combination *of Rhizobium* without *Trichoderma* sp., in both first season and second season under greenhouse conditions (Table 8). The increase in yield of chickpea can be attributes the positive effect of the *T*. *harzianum* and *R. leguminosarum* together as stimulating fungi the growth of chickpea as a result of inhibition of plant pathogenic fungi. It is in this plant draws his?? entire efforts to increase the seed yield and its components. Rhizobium strain with *Trichoderma* sp. may be produced maximum healthy looking and minimum discoloured seeds. These findings were in harmony with the finding noted in earlier by Khalequzzaman and Hossain (2007). In the same line, Rashid and Singh (2000**)** in a pot culture and field experiment found that *Cicer arietinum* seed inoculated with different *Rhizobium* strains increased seed yields by 24.0–26.0 percent, while Sandhu (1984) reported that inoculation with Rhizobium bacteria led to a significant increase in seed yield of chickpea compared with uninoculated control. Sattar (1997) observed that inoculation with *Rhizobium* strain CC 1192 increased seed yield of chickpea by 29 percent and Siddiqui et al. (2000) reported that 8-22 percent of yield increased in lentil from seed inoculation with Rhizobium culture. Each of Pal and Gardener, (2006); and also Somasegaran and Hoben (1994) reported that seed inoculation with *R. leguminosarum* strain L 25 and L 20 increased seed yield by 59.8 percent in lentil and up to 38.87 percent in chickpea, in recpectively. Seed yield increase has also been reported by other workers (Farzana et al., 1991, Gomez and Gomez. 1984; Hossain et al., 2000).

In concerning of lupine (Cv. Giza 2), the same trend with faba bean, (Giza 2) and chickpea (Giza 531) was observed in studied traits (Table 10). These characters were different significantly from each other, where plant height ranged from 99.69 to 109.27, branches plant^{-1} from 3.10 to 4.35, pods plant^{-1} from 20.34 to 31.08, 100-seed weight from 22.90 to 30.14 and seed yield from 20.5 to 26.41 in the first season (2008/09). While in the second season (2009/10) they ranged in the same sequence from 105.80 to 112.09 (plant height), from 3.25 to 4.83 (branches plant^{-1}), from 24.33 to 34.00 (pods plant^{-1}), 23.00 to 30.34 (100-seed weight) and from 21.15 to 28.40 (seed yield). Seed yield and its components can be affected by diseases management, genotype and environment, and may help to explain why a reduction in yield has occurred (Gardner et al., 1985). Management the damping-off and root rot diseases to improve each yield component aim to maximize the total seed yield of grain legume crops via lupine crop. On the other hand, due to the potential effects of nitrogen-fixing, which improvement the soil fertility, *Rhizobium* sp. can control the damping-off or root rot diseases, and consequently increasing crop productivity with reducing the negative environmental impact , which associated chemical use (Huang and Erickson, 2007).

REFERENCE

Abd El Moity TH (1985). Effect of single and mixture of *Trichoderma harzianum* isolates on controlling three different soil borne pathogens. Egypt. J. Microbiol., Special Issue, 111-120.

Al-Mahareeq FAA (2005). Biological Control of *Rhizoctonia Solani* and *Sclerotium Rolfsii* by using Local Isolates of *Trichoderma* Spp. This Ph. D Studies Faculty of Graduate, An-Najah National University, Pgg, 109.

Alagawadi AR, Gaur AC (1988). Associative effect of Rhizobium and phosphate-solubilizing bacteria on the yield and nutrient uptake of chickpea. Plant Soil 105, 241–246.

Anonymous (2001). SAS Institute. Statistical Analysis Software, Version 8.2. North Carolina, Cary, 2001.

Appleby CA (1974). Leghemoglobin. In: Quispel A (ed.), The Biology of Nitrogen Fixation, New York, Elsevier/North Holland, pp. 521–554.

Ayaz S (2001). Variability of harvest index in four grain legume species. Ph.D. thesis, Lincoln University, Canterbury.

Bardin SD, Huang HC, Pinto J, Amundsen EJ and Erickson RS (2004). Biological control of Pythium damping-off of pea and sugar beet by *Rhizobium leguminosarum* bv. *viceae*. Can. J. Bot. 82: 291–296.

Baraka MA, Shaban WI, Abd EL-Moneim H (2009). Influence of *Rizobium* sp. combined with *Trichoderma* sp. on damping off diseases and growth parameter of some legume crops. Agric. Res. J. Suez Canal University, 9 (3): 87-96.

Beringer JE 1974. R-factor transfer in *Rhizobium leguminosarum*. J. Gen. Microbiol. 84:188–198.

Burnett HL and Hunter BB 1972. Illustrated Genera of Imperfect Fungi. Burgess Pub. Co. Minneapolis, Minnesota, USA. pp: 241.

Claydon N; Hanson JR; Truneh A and Avent AG 1991. Harzianolide, a butenolide metabolite from cultures of *Trichoderma harzianum*. Phytochem. 30: 3802-3803.

Coley-Smith JR; Ridout CJ; Mitchell CM and Lynch JM 1991. Control of button rot disease of lettuce (*Rhizoctonia solani*) using preparations of *Trichoderma viride*, *T. harzianum* or tolclofosmethy. *Plant Pathology 40*, 359-366.

Dapaah HK; Mckenzie BA and Hill GD 2000. Influence of sowing date and irrigation on the growth and yield of pinto beans (*Phaseolus vulgaris*) in a sub-humid temperate environment. Journal of Agricultural Science, Cambridge 134, 33–43.

Deshwal VK; Dubey RC and Maheshwari DK 2003. Isolation of plant growth-promoting strains of *Bradyrhizobium* (*Arachis*) sp. with biocontrol potential against *Macrophomina phaseolina* causing charcoal rot of peanut. Current Sci., 84: 443-448.

Elad Y; Chet I; and Katan J 1980. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Journal of Phytopathology 70, 119-121.

Estevez De; Jensen C; Percich JA and Graha PH 2002. The effect of *Bacillus subtilis* and Rhizobium inoculation of dry bean seed on root rot severity and yield in Minnesota. Annu. Rep. Bean Improv. Coop. 45:98–99.

Farzana A; Gaffer A and Ali F 1991. Effect of seed treatment with biological antagonists on rhizosphere mycoflora and root infecting fungi of soybean. Pakistan J. Bot., 23 (2): 183–188.

Gilman CJ 1957. A Manual of Soil Fungi. 2nd ed. Iowa State College Press. USA pp. 450.

Gardner FP; Pearce RB and Mitchell RL,1985. Physiology of Crop Plants. Ames, IA: Iowa State University Press.

Gomez KA and Gomez AA 1984. Statistical Procedures for Agricultural Research, Int. Rice Res. Inst. John Willy and Sons, New York, Chickester, Brisbane, Torento, Singapore. Pp.643.

Hadar Y; Chet I and Henis Y 1979. Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum*. *Journal of Phytopathology 69*, 64-68.

Hossain I; Jalil M.A; Khan MAI and Aminuzzaman FM 2000. Seed treatment with *Rhizobium* and N P K nutrition on disease incidence and yield of chickpea (*Cicer arietinum* L.). Bangladesh J. Seed Sci. Tech. 4 (1&2) : 1–6.

Huang HC and Erickson RS 2007. Effect of seed treatment with *Rhizobium leguminosarum* on Pythium damping-off, seedling height, root nodulation, root biomass, shoot biomass, and seed yield of pea and lentil. J. Phytopathology 155: 31–37.

Husain MM; Hill GD and Gallagher JN 1988. The response of field beans (Vicia faba L.) to irrigation and sowing date. I. Yield and yield components. Journal of Agricultural Science, Cambridge 111, 221–232.

Infantin A; Kharrat M; Riccioni L; Coyne CJ; Mcphee K; Niklaus J and Grunwald NJ 2006**.** Screening techniques and sources of resistance to root diseases in cool season food legumes. Euphytica, 147: 201-221.

Julier B; Huyghe C; Papineau J; Billot C; and Deroo C 1995. Genetic and environmental variation in architecture and yield components in determinate white lupine (*Lupinus albus* L.). Eaphytica, 81: 171-179.

Khalequzzaman KM and Hossain I. 2007. Effect of seed treatment with Rhizobium strains and biofertilizers on foot/root rot and yield of bushbean in *fusarium solani* infested soil. J. Agric. Res., 2007, 45(2).

Kucuk C, Kivanc M (2003). Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features. Turk Biol. 22: 247-253.

Kumar RN, Mukerji KG (1996). Integrated disease management future perspectives. In: Mukerji, K.G., Mathur, B., Chamola, B.P., and Chitralekha, C. (Eds.), Advances in Botany, APH Publishing Corp, New Delhi, pp. 335–347.

Lindsey DL, Baker R (1967). Effect of certain fungi on dwarf tomatoes grown under gnotobiotic conditions. Phytopathology. 57:1262-1263.

Mazen MM, El-Batanony Nadia H, Abd El-Monium MM, Massoud ON (2008). Cultural filtrate of *Rhizobium* spp. and Arbuscular Mycorrhiza are potential biological control agents against root rot fungal diseases of faba bean. Global J. Biotech. and Biochem. 3: 32-41.

Mclean KL, Dodd SL, Sleight BE, Hill RA, Stewart A (2004). Comparison of the behavior of a transformed hygromycin resistant strain of *Trichoderma atoviride* with the wild-type strain. New Zealand Plant Protection, 57: 72-76.

Nautiyal CS (1997). Rhizosphere competence of *Pseudomonas* sp. NBRI9926 and *Rhizobium* sp. NBRI9513 involved in the suppression of chickpea (*Cicer arietinum* L.) pathogenic fungi. FEMS Microbiol. Ecol. 23:145–158

Nawar Lobna S(2007). Pathological and rhizospherical studies on root-rot disease of squash in Saudi Arabia and its control. Afric. J. of Biotech. 6: 219-226.

Nelson PE, Toussoum TA, Marasas WF (1983). *Fusarium* spp. An Illustrated Manual For Identification. The Pennsylvania University, Parl, USA. pp: 198.

Pal KP, Gardener BM (2006). Biological control of plant pathogens. The plant Health Instructor: DOI.10.1094/PHI-2006-117-02.

Puglia AP, Aragona M (1997). **I**mprovement of grain legumes Volume 53, Issues 1-3 July 1997, Field Crops Research general part: diseases, Pages 17-30

Rashid MA, Singh DP (2000). A Manual on Vegetable Seed Production in Bangladesh. AVRDC–USAID–Bangladesh Project, Horticulture Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh. p. 73.

Raupach GS, Kloepper JW (1998). Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathol. 88: 1158-1164.

Rudresh DL; Shivaprakash MK; and Prasad RD (2005). Effect of combined application of Rhizobium, phosphate solubilizing bacterium and *Trichoderma* spp. on growth, nutrient uptake and yield of chickpea (*Cicer aritenium* L.). Applied Soil Ecol. 28: 139–146.

Sandhu PS (1984). Effect of sowing date, phosphorus level and herbicides on the response of *Rhizobium* inoculation in lentil. Lentil Abst. 1986 (1) 28 : 3.

Sattar MA (1997). Jeebanu Sar : Parichiti O Prayog (Bangla), A Training Manual. Bangladesh Institute of Nuclear Agriculture, Mymensingh. Pp.75.

Siddiqui IA, Haque SE, Zaki MJ, Ghaffar A (2000). Greenhouse evaluation of Rhizobia as biocontrol agent of rootinfecting fungi in okra. *Acta Agrobotanica*. 53 (1) : 13-22.

Sivan A, Elad Y, Chet I (1984). Biological control effects of a new isolate of *Trichoderma harzianum* on *Pythium aphanidermatum*. J. Phytopathol. 74: 498-501.

Somasegaran P, Hoben HJ (1994). Handbook for Rhizobia : Methods in Legume-*Rhizobium* Technology. Springer-Verlag New York, Inc., 175 Fifth Avenue, New York, NY 10010, USA. p.337-415.

Verghis TI (1996). Yield and yield development of chickpea (*Cicer arietinum* L.). Ph.D. thesis, Lincoln University, Canterbury.

Vishwa D, Gurha SN (1998). Seed mycoflora of French bean in relation to the seed coat colour. Indian Journal of Pulses Research (ICAR), *Rhizoctonia solani*, a seed borne pathogen of French bean in Malaysia. Pertanika 2: 11-15

Whipps JM, Lumsden RD (2001). Commercial use of fungi as plant disease biological control agents: status and prospects. CABI Publishing, Wallingford, United Kingdom. pp: 9-22.

Windham MT, Elad Y , Baker R (1986). A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathol. 76: 518–521.