J. Agric. Sci. Mansoura Univ., 31 (2): 963 - 980, 2006

SYMBIOTIC NITROGEN FIXATION PROCESS IN FABA BEAN AND CHICKPEA AS AFFECTED BY BIOLOGICAL AND CHEMICAL CONTROL OF ROOT-ROT

Hassanein, A.M.[1]; A.M.El-Garhy[1] and G.A.A.Mekhemar[2]

1- Plant pathology Res. Inst., Agric. Res. Center. Giza, Egypt.

2- Soils, Water and Environment Res. Inst., Agric. Res. Center, Giza, Egypt

ABSTRACT

Three biocontrol agents *Trichoderma harzianum*, *Gliocladium virens* and *Bacillus subtilis* were evaluated in controlling of damping – off caused by *Rhizoctonia solani* under laboratory and greenhouse conditions as well as their biocontrol agents effect on both nodulation, growth and nitrogen content of faba bean and chickpea plants. Results showed that all tested biocontrol agents significant reduction in mycelial radial growth of *R. solani* in all different agar media.

The obtained results showed that all tested biocontrol agents significantly reduced pre and post emergence damping-off and increased survival plants when combined with *Rhizobium* strain (ICARDA 441) for faba bean or *Rhizobium* strain (ICARDA 1148) for chickpea. The maximum protection of faba bean and chickpea plants were achieved when faba bean seed treatment with *Trichoderma harzianum* combined with *Rhizobium* and chickpea seed treatment with *Bacillus subtilis* combined with *Rhizobium*.

Concerning the effect of different biocontrol agents on nodulation, growth and N-content of faba bean and chickpea plants, the results indicated that the combined inoculation with *T. harzianum* and *Rhizobium* strain (ICARDA 441 for faba bean) gave the best results in terms of number, dry weight of nodules, shoots, roots dry weight, and their N- content with faba bean in case of non-infested or infested soil. While, *Bacillus subtilis* gave the best results in terms of number of nodules, dry weight of nodules, shoots dry weight, roots dry weight and their N-contents with chickpea in case of non-infested or infested soil when combined inoculation with *Rhizobium* strain (ICARDA 1148 for chickpea) at the first and the second season.

Taken together with evidence of obtained data it is clear that the beneficial effects exerted by symbiotic N_2-fixation in combination with disease protection afforded by biocontrol agent, had more compensated the loss occurred in N_2-fixation parameters caused by *R. solani* in both legume crops under investigation.

Generally, it is clear that favorite results were obtained in case of combined inoculation by three bioagent in combination with two *Rhizobium* strains in reducing damping – off, enhancing nodulation status, growth and N- content of faba bean and chickpea plants at the first and the second seasons.

Keywords: Faba bean, Chickpea, Damping–off, *Rhizoctonia solani, Trichoderma harzianum, Gliocladium virens, Rhizobium, Bacillus subtilis.*

INTRODUCTION

Legumes constitute a major portion of food human. In addition they also provide oils, fibers, timber and raw materials for many products. Furthermore, the incorporation of nitrogen rich leguminous crops is of great importance to soil fertility particularly under Egyptian conditions where soil is poor in its nitrogen content.

Inoculation of legumes with selective rhizobia lead to increment of seed yield and nitrogen content (Mekhemar *et al.*, 2005).

The symbiotic association between legumes and rhizobia is the most biocatalytic link for the flow of nitrogen between the largest potentially available N-reservoir, the atmosphere and the living world (Paau, 1989). Legumes also leave fixed nitrogen in the soil for succeeding crops.

However, legumes in most cultivated areas of Egypt are subjected to serious problems by soil borne fungi. These pathogens adversely affect the symbiotic N_2-fixation as well, they destroy the roots on which symbiotic take place, and hence reduce yields and affect product quality.

Damping-off, root-rot and wilt diseases of faba bean and chickpea crops are considered the most important diseases that affect plant stand, causing great losses in annual seed yield. Some of these diseases are caused by seed and or soil borne pathogens such *Rhizoctonia solani, Fusarium oxysporum* and *Fusarium* spp. (El-Awadi, 1993 and El-Garhy, 1994). Fungicide are the primary means of fungal disease control, but their use is becoming more and more controversial and faces severe restriction undesirable effect. In addition, some of these chemicals disrupt symbiosis occurring between plant and nitrogen fixing bacteria.

Therefore, safe control methods were required to avoid fungicidal hazards to the environment. Biological control of soil borne plant pathogens by addition of antagonistic microorganisms is a potential non-chemical means for a plant diseases control. Promising results were obtained by application of *Trichoderma* spp, *Gliocladium* spp, *Pseudomonas* spp and *Bacillus* spp in controlling several soil borne diseases under greenhouse or field conditions (Elad *et al.*, 1980 & 1982 and Nazim *et al.*, 1999). However, there have been relatively few attempts to obtain isolates of antagonistic microorganisms, which reduce disease severity and increase plant growth in absence or presence of the pathogen, therefore the aims of the present study are:

1- To investigate the ability of three different biocontrol agents as soil treatment to reduce or suppress damping-off of faba bean and chickpea caused by *R. solani* (the two important legume crops cultivated in Egypt, the first is produced on more 50 % of the total area under food legumes and serves as the main source of protein for the majority of Egyptian population).
2- To examine the effect of these biocontrol agents on enhancing nodulation and growth of faba bean and chickpea plants under greenhouse conditions.

MATERIALS AND METHODS

This investigation was carried out in the laboratory and the greenhouse of both Plant Pathology Research Institute and Soils, Water and Environment Research Institute (SWERI), Agriculture Research Center (ARC), Giza during two seasons (2003/04 and 2004/05).

Soil and Seeds:

Soil used in pot experiment was collected from the farm field of ARC, Giza. The texture of soil was clay loam (34.4 % Sand, 27.1 % Silt and 38.5 % Clay) pH (7.3) and E.C dS m^{-1} (1.30) (Page *et al.,* 1982).

Faba bean seeds (cv. Giza 3) and chickpea seed (cv. Giza 5) kindly supplied by Field Crops Research Institute, ARC were used in this study.

Preparation of inoculum:

- **Causal organism (*R. solani*):**

The fungal inocula were prepared by growing on autoclaved sorghum grain sand medium (75/25, w/w) in 500 ml bottles and inocubated at 25 ^{o}C for two weeks.

- **Rhizobial strains:**

Rhizobium leguminosarum bv. *viceae* strain ICARDA 441 for faba bean and *Rhizobium* strain ICARDA 1148 for chickpea kindly provided from Department of Microbiology, SWERI, ARC, Giza were used in this work. Four days old culture of *Rhizobium* grown on yeast mannitol broth YMB (Vincent, 1970) was suspended in sterilized water and thoroughly shaken to obtain a homogenous population of about 3 x 10^9 cell ml^{-1}. Fine peat was supplied by Micro Bio, UK, adjusted to pH 7 with 5 % finely ground $CaCO_3$. Fine peat was packed on polyethylene bag (300 g carrier per bag), then sealed and sterilized by gamma irradiation (5.0 x 10^6 rads). *Rhizobium* cultures were injected into peat carrier to satisfy 50 % of the maximal water holding capacity.

Biocontrol agents used in this study :

Three biocontrol agents of *Trichoderma harzianum, Gliocladium virens* and *Bacillus subtilis* were obtained from the Plant Pathology Research Institute, ARC, Giza. *Trichoderma harzianum* and *Gliocladium virens* were grown on sterilized sorghum grains and sand medium (75/25, W/W) for 15 days at 25^{O} C. Whereas, *Bacillus subtilis* was grown on nutrient broth for 48 hr at 25^{O} C. One fungicide was used in this study Rizolex-T at rate 3 g/kg seeds.

***In vitro* study :**

Trichoderma harzianum, *Gliocladium virens* and *Bacillus subtilis* were tested using four solid media namely potato dextrose agar (PDA), nutrient agar (NA), yeast mannitol agar (YMA) and King's B medium (KBM) as an antagonistic to *Rhizoctonia solani.* Agar disks culture of *R. solani* were cut from periphery of 7days old culture and streaked a loop full from 48 hr old culture of antagonistic bacteria were placed opposite to each other at 5cm apart and incubated at 25^{O} C for 7days (Hassanein *et al.*, 1996). Four Petri dishes were used for each antagonistic test and the same dishes number was kept as control by plating the pathogen alone on one side of the Petri dish. After incubation period, colony diameter of *R. solani* was recorded. The percentage of the mycelial radial growth reduction was also measured.

***In vivo* study:**

Two pot experiments were carried out in greenhouse at Soils, Water and Environment Research Institute, ARC, Giza during two winter seasons of 2003/2004 and 2004/2005 to evaluate the effect of biocontrol agent on nodulation and growth of faba bean and chickpea plants in the presence or absence of the tested pathogen (*R. solani*) using pots 25 cm in diameter each. Pots were then sterilized with 5 % formalin solution and left to dry before use. Potted soil was infested with the inoculum of *R. solani* at the rate of 5 % (W/W) with irrigation and left for seven days. Soil application with the fungal antagonists was done by mixing the inoculum with soil at the rate of 10 g / kg soil. *B. subtilis* suspension was diluted by water to 50 % and thoroughly mixed and then added to soil at the rate of 100 ml / pot. Seeds were treated with 16 % Arabic gum and thoroughly mixed with the *Rhizobium*-containing carrier at the rate of 400g inoculum /30 kg seeds. 8 & 12 seeds each of faba bean (Giza 3 cv.) and chickpea (Giza 5 cv.) were used with each pot, respectively. The infested soil planted with non-treated as well as *R. solani* free soil was served as controls. The experiments comprise five treatments besides the control one. The treatments were in four replicates and were arranged in randomized complete block design (Gomez and Gomez, 1984). These treatments were as follows:

1. Control (Soils received no inoculation).
2. Soil inoculated with *Rhizobium.*
3. Soil inoculated with *Rhizobium* + Rizolex-T.
4. Soil inoculated with *Rhizobium* + *Trichoderma harzianum.*
5. Soil inoculated with *Rhizobium* + *Gliocladium virens.*
6. Soil inoculated with *Rhizobium* + *Bacillus subtilis.*

The percentage of Pre- and Post- emergence damping-off were recorded after 15 and 45 days after planting in infested soil only, respectively. The survival plants were recorded after 60 days from planting and plants were uprooted to determined root rot disease severity on a scale 0-5 according to (Cardoso *et al.*, 1997) where 0 = no lesions on hypocotyl, 1 = lesions ≤ 2.5 mm long, 2 = lesions 2.5 - 5.0 mm long, 3 = lesions ≥ 5.0 mm long, 4 = lesions girdling plant and wilting visible on leaves, and 5 = seedlings damped- off or dead and to estimate number and dry weight of nodules, shoots and roots dry weight. Nitrogen contents were estimated in shoots and roots according to the methods described by (Page *et al.*, 1982). Data were subjected to analysis of variance using SAS program.

RESULTS AND DISCUSSION

In vitro test:

Data in Table (1) indicate the effect of three different bioagents for their efficiency to inhibit the mycelial radial growth of *R. solani* on different agar media. Results revealed that all tested biocontrol agents significantly reduction in colony diameter of *R. solani* compared on all tested medium compared with control (*R. solani* alone). *T. harzianum* was highly antagonistic effect against of *R. solani* when tested on potato dextrose agar and yeast extract mannitol agar media which gave 73.3 %, 62.2 % reduction in colony diameter, respectively. *T. harzianum* grew over the mycelium of *R. solani*, *B. subtilis* caused significant reduction on potato dextrose agar and nutrient agar media to 67.8 %, 58.9 % and less reduction on King's B medium to 32.2 %, respectively. Also, show that *G. virens* caused reduction on yeast extract mannitol agar medium to 48.9 % more than *B. subtilis*. The inhibition zones were observed only between bioagent *G. virens, B. subtilis* and the pathogen (*R. solani)*. In general, all the antagonists inhibited the growth of *R. solani* significantly, when compared with the control. These results are in agreement with Abd El-Moity *et al.* (1992) proved that linear growth of *F. oxysporum* and *R. solani* was greatly affected in the presence of the antagonistic fungus *T. viride, T. spiralis* or *B. subtilis*. Trichodermin and peptide antibiotics are reported to be produced by *Trichoderma* spp. These antibiotics can inhibit the growth of several fungus (Dennis and Webster, 1971a). Some *Trichoderma* spp. was able to produce acetaldehyde or other acidic volatiles (Dennis and Webster, 1971b). Hassanein *et al.* (2000) reported that *T. harzianum* reduced the mycelial radial growth of *R. solani* by 53%, *B. subtilis* and Mycostop (*Streptomyces griseovirridis*) retarded colony growth of the pathogens at a distance by producing a clear inhibitory zone against the pathogens and inhibited their growth by 36.1 and 59.4%, respectively. These antibiotics can inhibit the growth of several fungus (Dennis and Webster, 1971a). *Trichoderma* was reported to suppress the germination of *S. rolfsii, Sclerotia* to attack many fungi by coiling around and penetrating into the hyphae and lyse mycelia fungi by producing B (1-3) gluconase and chitinase (Papavizas and Lewis, 1989). El-Kafrawy (2002) reported that isolates of *Trichoderma* spp. reduced the radial growth of *Rhizoctonia solani* (Kuhn) by 59.6 -78.4% and *Gliocladium* spp. inhibited the radial growth of *Rhizoctonia solani* by 57.2 –70.1%. (Hassanein and Mekhemar, 2003) tested *Pseudomonas fluorescens* and *Pseudomonas putida* against *S. rolfsii* on different agar media they found that significant reductions in colony diameter on King's B medium only.

Table 1. Effect of antagonistic microorganisms on mycelial radial growth of *Rhizoctonia solani* on different media.

Bioagents	Mean linear growth (mm)				Reduction (%)			
	A	B	C	D	A	B	C	D
Trichoderma harzianum	24.0	65.0	49.0	34.0	73.3	27.78	45.6	62.2
Gliocladium virens	56.0	71.0	76.0	46.0	37.9	21.11	15.56	48.9
Bacillus subtilis	29.0	37.0	61.0	51.0	67.8	58.9	32.22	43.33
R.S.441 for faba bean	90.0	90.0	90.0	90.0	0.0	0.0	0.0	0.0
R.S.1148 for chickpea	90.0	90.0	90.0	90.0	0.0	0.0	0.0	0.0
Rhizoctonia solani	90.0	90.0	90.0	90.0	0.0	0.0	0.0	0.0

A = Potato dextrose agar (PDA) B =Nutrient agar (NA)
C = King's B medium (KBM) D = Yeast extract mannitol agar (YMA)
R.S = *Rhizobium* strain
L.S.D at 5 % for :
Bioagents (B) = 1.52 Medium (M) =3.67 B x M = 7.33

In-vivo **test :**

Effect of *Trichoderma harzianum, Gliocladium virens, Bacillus subtilis* and two strains of *Rhizobium* on damping-off of faba bean and chickpea plants:

Results presented in Table (2) indicate the efficiency of *T. harzianum, G. virens* and *B. subtilis* in controlling faba bean and chickpea damping-off caused by *R. solani.* It was revealed that *T. harzianum, G. virens* and *B. subtilis* significantly reduced pre and post-emergence damping-off and increased survival plants compared with the control (untreated). No significant difference was observed in case of interaction between the two crops and treatments in post emergence damping-off during first season and in case of survival plants during the second season, while observed significant difference between the three bioagents and two crops in pre emergence damping-off during two seasons and survival plants in the first season.

Table 2. Effect of interaction between *Rhizobium* inoculation and biocontrol agents on faba bean and chickpea damping-off and root-rot caused by *R. solani*.

Treatments	Season 2003 / 2004								Season 2004 / 2005							
	Faba bean				Chickpea				Faba bean				Chickpea			
	% Pre-	% Post-	% Sur-	DSI*	% Pre-	% Post-	% Sur-	DSI*	% Pre-	% Post-	% Sur-	DSI*	% Pre-	% Post-	% Sur-	DSI*
R. solani	43.75	12.50	43.75	2.33	66.56	27.19	6.25	4.00	34.37	15.63	50.00	2.17	72.80	18.88	8.33	3.58
Rhizobium	40.63	12.50	46.87	2.25	66.56	27.19	6.25	3.89	34.37	15.63	50.00	2.11	72.80	18.88	8.33	3.55
Rhizobium + Rizolex– T	9.38	0.00	90.62	0.67	22.88	6.24	70.88	0.89	3.13	3.13	93.74	0.50	27.04	4.32	68.64	1.11
Rhizobium + *T. harzianum*	25.00	3.12	71.88	1.22	43.68	10.40	45.92	2.67	28.13	3.12	68.75	1.17	41.60	8.40	50.00	2.25
Rhizobium + *G. virens*	28.13	6.25	65.25	1.40	45.76	12.57	41.67	3.00	28.13	9.38	62.50	1.84	49.92	14.56	35.52	3.17
Rhizobium + *B. subtilis*	37.50	9.38	53.12	2.00	41.52	8.48	50.00	2.55	31.25	9.38	59.37	1.89	39.52	4.32	56.16	2.17

L.S.D at 5 % for:

Treatment (T) = 2.76 1.58 1.13 0.06 1.70 N.S 5.30 0.04
Crop (C) = 1.93 2.03 2.50 0.18 2.83 2.60 4.60 0.14
C x T = 3.90 N.S 3.53 0.25 4.01 3.70 N.S 0.20

DSI * = Disease Severity Index : were rated on a scale of 0 – 5 where :
0 = no lesion on hypocotyls, 1 = lesions ≤ 2.5 mm long, 2 = lesions 2.5 – 5.0 mm long, 3 = lesions ≥ 5.0 mm long, 4 = lesions gridling plant and wilting visible on leaves and 5 = Seedlings damped-off or dead.

The lowest percentage of damping off, disease severity index and highest percentage of survival plants (71.88% and 68.75%) in the two growing seasons of faba bean were obtained along with *Trichoderma harzianum* and *Rhizobium* application, followed by combination of both *G. virens* and *Rhizobium*. For chickpea the combination of both *Bacillus subtilis* and *Rhizobium* was the most effective in controlling damping off and root rot and increased survival plants (50.00 % and 56.16 %) followed by combination of both *Trichoderma harzianum* and *Rhizobium*. In general, the highest percentage of survival plants was obtained with *T. harzianum* in faba bean followed by *G. virens* while the highest percentage of survival plants was obtained with *B. subtilis* in chickpea followed by *T. harzianum* but *G. virens* was lowest effect. In all cases the lowest percentage of pre and post emergence damping-off and highest percentage of survival plants was obtained with fungicide (Rizolex-T). Papavizas *et al.* (1968) mentioned that from the voluminous accumulated rhizospheric data, which support the presence of antagonism in soil, it is reasonable to *believe* that antagonism is an important factor affecting the population density and survival of the population in the rhizosphere. Schreiber *et al.* (1988) mentioned that *B. subtilis* produce the cyclic peptide antibiotics mycobacillin; iturin A; bacillomycin; mycosubtilin; fungistatin; subsporin; bacilysin and fengymycin. Fiddaman and Rossal (1993) found that growth of *Rhizoctonia solani* and *P. ultimum* was severely impaired in the presence of the volatiles produced by *B. subtilis*. Lewis *et al.* (1996) and Bazgir & Okovvat (1997) who found that application of wheat bran culture of antagonists were significantly more effective than seed dressing with Benomyl and PCNB in reducing disease incidence caused by *R. solani* on bean under field condition. Mansour (1997) reported that the emerged plants of broad bean, chickpea, common bean, cowpea and fenugreek increased when *B. subtilis* isolates were added to the soil infested with *C. lunata* or *F. solani*. Moreover, root-rots of these tested crops were decreased. El – Kafrawy (2002) reported that adding *T. hamatum* to the soil gave the lowest percentage of pre and post- emergence damping – off (6.67 %). Also, soil treatment with *T. harzianum*, *T. viride* and *G. virens* was found to be even as effective as Rizolex (10 %) in checking the disease. Abou-Zeid *et al.* (2003) reported that seed treated with *G. virens*, *B. subtilis*, and *T. harzianum* could be considered as an effective biocontrol against for pre- and post-emergence damping-off, in faba bean, lentil and chickpea crops especially in early planting. Also, they concluded that the application of antagonistic microorganisms to seed has been employed not only to control pre- diseases of the leguminous crop and to promote plant growth and yield, but also to reduce the density of soil-pathogens also, prospective application of biocontrol technique may help to decrease the use of fungicide and avoid environmental pollution.

Faba bean plants :

Effect of Soil treatments with some biocontrol agents on nodulation status:

The effect of soil treatment with each tested bioagents, i.e. *Trichoderma harzianum*, *Gliocladium virens* and *Bacillus subtilis* on nodulation of faba bean plants was evaluated in presence or absence of

Rhizoctonia solani after 60 days from planting under greenhouse conditions. Results in Table (3) showed that in the first season 2003 / 2004 un-inoculated faba bean plants recorded reasonable number and dry weight of nodules to be 37.0 and 26.0 nodules plant^{-1} as well as 227.0 and 202.0 mg plant^{-1} in non-infested and infested soil, respectively. Indicating that the occurrence of specific indigenous rhizobia in this soil. The same trend was observed in the second season 2004 / 2005 which recorded 33 and 29 nodules/plants as well as 219 and 201 mg / plant. Generally, inoculation of faba bean seeds with *R. leguminosarum* form effective nodules in presence or absence of *R. solani.* These results are in agreement with those obtained by (Abo El-Soud *et al.*, 2004 and Mekhemar, 2005). Data in Table (3) revealed also that treating soil with *R. leguminosarum* in combination with any biocontrol agents used improved production of nodules on roots of faba bean plants. Yet these treatment in *R. solani* infested soil were less effective in producing the nodules comparable to the non-infested soil.

Table 3. Effect of inoculation with *Rhizobium* on nodulation status of faba bean plants grown in non-infested and infested soil under biological and chemical control.

Bioagents	Season 2003/2004				Season 2004/2005			
	Non-infested soil		Infested soil		Non-infested soil		Infested soil	
	Number of nodules plant^{-1}	Dry weight of nodules mg plant^{-1}	Number of nodules plant^{-1}	Dry weight of nodules mg plant^{-1}	Number of nodules plant^{-1}	Dry weight of nodules mg plant^{-1}	Number Of nodules plant^{-1}	Dry weight of nodules mg plant^{-1}
Uninoculated (Control)	37.0	227.0	26.0	202.0	33.0	219.0	29.0	201.0
Rhizobium	78.0	517.0	60.0	318.0	84.0	600.0	67.0	338.0
Rhizobium + Rizolex-T	60.0	324.0	47.0	282.0	61.0	395.0	53.0	290.0
Rhizobium + T. harzianum	107.0	809.0	76.0	566.0	114.0	867.0	94.0	611.0
Rhizobium + G. virens	90.0	668.0	70.0	514.0	92.0	684.0	81.0	548.0
Rhizobium + B. subtilis	60.0	454.0	52.0	443.0	61.0	489.0	54.0	456.0
L.S.D at 0.05 %	7.18	36.77	4.77	31.95	6.48	35.47	5.38	45.54

From data in Table (3), it is obvious that the maximum number and dry weight of nodules were obtained in plants treated with both *R. leguminosarum* and *Trichoderma harzianum* followed by plants treated with both *R. leguminosarum* and *Gliocladium virens* in presence or absence of *R. solani* in the first and the second season. Whereas, applying *R. leguminosarum* along with *Bacillus subtilis* was less effective in improving root nodulation than using *R. leguminosarum* alone. This is correct in both *R. solani* infested or non-infested soil (Table 3). These results are in agreement with those obtained by (Smith, 1996 and Al-Kahal &El-Garhy, 2003) they reported that nodulation of faba bean plants treated with *Trichoderma harzianum* or *Gliocladium virens* increased compared with the non treated control.

Effect of biocontrol agents and *R. leguminosarum* on plant dry weight and N-content in relation to faba bean damping-off:

Data presented in Table (4) show that treating with any of biocontrol agents in combination with *R. leguminosarum* caused an obvious increase in the shoots dry weight, roots dry weight and N-content of faba bean. Combination of biocontrol agents and *R. leguminosarum*, however, were more effective in this respect if compared with the uninoculated (control). This is true in non-infested or infested soil with the pathogen. But, these treatments were more effective in increasing the plant dry weight of shoots, dry weight of roots and N-content in the non-infested soil than in the infested one. There are some evidence that most of microorganisms used in biological control produce some growth stimulatory factors that positively affect the plant growth of the host, whether fungus or higher plant (Klopper and Schroth, 1981). One possible mechanism for increase the efficiency of plant growth by *Trichoderma* spp. is that *Trichoderma* increase the efficiency of nutrient transfer from the soil to the roots in a way analogues to mycorrhizal effect (Brundrett, 1991). Data also revealed that combined inoculation of *R. leguminosarum* and *Bacillus subtilis* led to decrease in shoot dry weight, root dry weight and N-contents compared to the other combination may be attributed to the sensitivity of *R. leguminosarum* to the antibiotic complex containing bacilysim and lengymysim (Fengycim) produced by *Bacillus subtilis* as suggested by Burla *et al.* (1996) who found that *Bacillus subtilis* inhibited the growth of *Rhizobium phaseoli.*

Chickpea plants:

Effect of some biocontrol agents and *Rhizobium* on nodulation status of chickpea:

Data presented in Table (5) indicated that uninoculated chickpea plants produced the lowest number and dry weight of nodules to be 11.0, 8.0 nodules / plant with 47.0, 32.0 mg / plant in non-infested and infested soil in the first season respectively. Similar trend also observed in the second season which recorder number and dry weight of nodules were 17.0 and 15.0 nodules / plant with 62.0 and 53.0 mg / plant in non- infested and infested soil, respectively. These indicating the occupancy of specific indigenous *Rhizobium* in this soil. This observation was also, reported by (Abdel -Wahab *et al.*, 1994 and Mekhemar *et al.,* 2005). In the first season inoculation with *Rhizobium* alone caused increase in number and dry weight of nodules over the uninoculated (control) by 163, 225% with 217 and 215 % in non-infested and infested soil respectively. The corresponding increases at the second season were 117 and 106 % with 241 and 247% in non-infested and infested soil. These results are in harmony with those obtained by (Hussein *et al.*, 1997 and Mekhemar *et al.,* 2005) but in infested soil number and dry weight of nodules were less values than those of disease free soil. This indicated that soil infection with *R. solani* had suppressive effect on nodulation, this finding was also reported by (Al- Kahal and El-Garhy, 2003). Combination between biocontrol agents and *Rhizobium* caused significant increases in number and dry weight of nodules compared with the uninoculated plants and plants treated with fungicide in non-infested and infested soil during the two seasons.

Table 4. Effect of inoculation with *Rhizobium* on plant growth and N-content of faba bean plants grown in non-infested and infested soil under biological and chemical control.

Treatments	Season 2003 / 2004								Season 2004 / 2005							
	Non-infested soil				Infested soil				Non-infested soil				Infested soil			
	D.W.S	S.N.C	D.W.R	R.N.C	D.W.S	S.N.C	D.W.R	R.N.C	D.W.S	S.N.C	D.W.R	R.N.C	D.W.S	S.N.C	D.W.R	R.N.C
(Uninoculated Control)	2.97	62.3	1.83	27.1	2.03	36.0	1.61	22.8	3.08	70.3	1.96	32.1	2.23	45.0	1.70	25.9
Rhizobium	3.72	97.7	2.25	42.8	2.93	65.7	1.88	34.4	4.21	135.3	2.35	49.5	3.28	77.7	2.29	44.8
Rhizobium + Rizolex-T	3.48	82.7	1.89	32.6	2.74	63.0	1.80	30.2	3.91	117.3	2.10	41.0	3.53	96.0	1.91	36.2
Rhizobium + *T. harzianum*	4.42	133.0	2.45	51.6	3.41	104.0	2.29	44.3	4.97	176.0	2.49	63.3	4.36	141.3	2.37	54.7
Rhizobium + *G. virens*	4.22	120.3	2.28	46.3	3.50	92.7	2.23	41.1	4.89	162.0	2.46	56.1	4.19	126.0	2.34	48.7
Rhizobium + *B. subtilis*	3.99	107.7	2.17	40.3	3.18	76.0	1.91	34.0	4.05	149.0	2.33	47.0	4.01	114.7	2.19	42.2
L.S.D at 0.05 %	0.19	6.17	0.11	3.18	0.1	6.15	0.11	1.85	0.15	8.03	0.06	3.14	0.18	4.62	0.16	2.88

D.W.S. = Dry weight of shoot g / plant
D.W.R. = Dry weight of root g / plant
S.N.C. = Shoot N-content mg / plant
R.N.C. = Root N-content mg / plant

In the first season the maximum number of nodules (59.0 and 47.0 nodules / plant) and dry weight of nodules (207.0 and 190.0 mg / plant) were observed in plants treated with *Rhizobium* and *T. harzianum* followed by plants inoculated with *Rhizobium* and *B. subtilis* which recorded number of nodules (47.0 and 40.0 nodules / plant) and dry weight of nodules (198.0 and 167.0mg / plant) followed by plants inoculated with *Rhizobium* and *G. virens*. Similar trend also observed in the second season, where the highest number and dry weight of nodules were obtained in the co-inoculated treatment with *Rhizobium* and *T. harzianum* followed by plants treated with *Rhizobium* and *B. subtilis* followed double inoculation with *Rhizobium* and *G. virens* in presence or absence of *R. solani*. These results are in harmony with those obtained by Al-Kahal and El-Garhy (2003) who reported that co-inoculation of *T. harzianum* and *G. virens* with *Rhizobium* gave the highest number and dry weight of nodules. Smith.(1996) reported that nodulation of bean plants treated with *G. virens* was obviously increased compared with the non-treated (control). Also Srinivasan *et al.* (1996) found that co-inoculation with *Rhizobium* and *Bacillus* isolates resulted increased nodules number, nodules fresh weight, nitrogenase activity, leghaemoglobin content and total soluble protein content in the root nodules of *Phaseolus vulgaris*.

Table 5. Effect of inoculation with *Rhizobium* on nodulation status of chickpea plants grown in non-infested and infested soil under biological and chemical control.

Bioagents	Season 2003/2004				Season 2004/2005			
	Non-infested soil		Infested soil		Non-infested soil		Infested soil	
	Number of nodules plant^{-1}	Dry Weight of nodules mg plant^{-1}	Number of nodules plant^{-1}	Dry weight of nodules mg plant^{-1}	Number of nodules plant^{-1}	Dry weight of nodules mg plant^{-1}	Number Of nodules plant^{-1}	Dry Weight of nodules mg plant^{-1}
Uninoculated (Control)	11.0	47.0	8.0	32.0	17.0	62.0	15.0	53.0
Rhizobium	29.0	149.0	26.0	101.0	37.0	212.0	31.0	184.0
Rhizobium + Rizolex-T	19.0	89.0	12.0	81.0	24.0	203.0	17.0	141.0
Rhizobium + *T. harzianum*	59.0	207.0	47.0	190.0	65.0	372.0	55.0	305.0
Rhizobium + *G. virens*	33.0	186.0	27.0	115.0	41.0	230.0	31.0	203.0
Rhizobium + *B. subtilis*	47.0	198.0	40.0	167.0	50.0	285.0	42.0	233.0
L.S.D at 0.05 %	2.77	10.5	3.22	4.91	4.60	32.63	3.86	44.36

Effect of some biocontrol agents and *Rhizobium* on dry weight of shoot, root and N-content in reiation to chickpea damping-off :

Data presented in Table (6) indicated that Rhizobial inoculation gave significant increases in shoot and root dry weight in non-infested and infested soil during the two seasons as compared to the uninoculated plants (control). On the other hand, *Rhizobium* alone increased dry weight of shoots and roots by 34.0 and 10.7 % in non-infested and by 43.6 % and 15.2 % in infested soil over the control in the first season, respectively. The same trend was observed in the second season. These results could be attributed to the role of *Rhizobium* increasing the growth and N_2 fixation. Abo El-Soud *et al.* (2004) and Mekhemar *et al.* (2005). Also data presented in Table (6) show that treating with any biocontrol agents combined with *Rhizobium* caused an obvious increase in shoot and root dry weight of chickpea compared with the applications of *Rhizobium* alone. This is true in non-infested or infested soil with the pathogen, but these treatments were more efficient in increasing dry weight of shoots and roots in the non-infested soil than in the infested one. These results are in agreement with those obtained by Klopper and Schroth (1981) who found that most microorganisms used in biological control produce some growth stimulatory factors that positively affect the plant growth of the host. Brundrett (1991) also found that *Trichoderma* increase the efficiency of nutrient transfer from soil to the roots in away analogous to mycorrhizal effect. Tang (1994) and Kilian *et al.* (2001) who found that a number of *B. subtilis* isolates have the ability to form phytohormones such as zeatin, gibberellic acid and abscisic acid which could stimulate plant growth, absorption of nutrients and their efficiency as well as the metabolism of photosynthates. Also, *B. subtilis* lead to stronger root growth there may also be an increased synthesis of plant cytokinins, which also cause delayed senescence and higher yield.

Concerning the N-content of shoots and roots, data presented in Table (6) shows that uninoculated plants recorded the lowest N-content in shoots and roots in non-infested and infested soil at the two seasons. Inoculation with *Rhizobium* only increased N-content in shoots and roots compared with the uninoculated plants in the non-infested and infested soil at the first and the second seasons. These could be due to the role of *Rhizobium* for improving growth and N_2 –fixation (Abo El-Soud, 2004 and Mekhemar *et al.* 2005). Combined application of biocontrol agents with *Rhizobium* caused significant increase in shoot and roots N. content at the two soil compared with the plants inoculated with *Rhizobium* only. Data presented in Table (6) revealed also that the highest nitrogen content was obtained by plant treated with double inoculation (*Rhizobium* + *T. harzianum*) followed by inoculated (*Rhizobium* + *B. subtilis*) followed by inoculated with (*Rhizobium* + *G. virens*) in non-infested and infested soil at the first and second season. These results are in agreement with those obtained (Dileep-Kumar *et al.*, 2001 and Al-Kahal and El-Garhy, 2003) who found that seed treated with biocontrol agents and *Bradyrhizobium japonicum* enhanced nodulation, growth and N-content of soybean plants. Also, chickpea inoculated with *Rhizobium* and other rhizobacteria enhanced nodules weight, root length, shoot biomass and total N- content (Parmar and Dadarwal, 1999). Srinivasan *et al.* (1996) also revealed that co-inoculation of *Bacillus* isolates with *Rhizobium* increased nitrogenase activity and nodules protein content.

Although the seed dressing with Rizolex significantly reduced the disease severity from 2.55 to 0.585 of faba bean and from 3.7 to 1.0 of chickpea plants (average of two seasons, Table 2); but negatively affect *Rhizobium* and deprive the legumes from beneficial effect of rhizobial inoculation. The harmful effect on nodule formation on both faba bean and chickpea plants was markedly significant. The number of nodules per plant dropped from 73.0 to 56.0 and from 31.0 to 19.0, respectively. The dry weight of nodular tissues followed the same trend, as it reduced from 443.5 to 323.0 mg/plant and from 162.0 to 128.5 mg/plant of faba bean and chickpea plant, respectively (Table 7).

Table 6. Effect of inoculation with *Rhizobium* on plant growth and N-content of chickpea plants grown in non-infested and infested soil under biological and chemical control.

Treatments	Season 2003 / 2004								Season 2004 / 2005							
	Non-infested soil				Infested soil				Non-infested soil				Infested soil			
	D.W.S	S.N.C	D.W.R	R.N.C	D.W.S	S.N.C	D.W.R	R.N.C	D.W.S	S.N.C	D.W.R	R.N.C	D.W.S	S.N.C	D.W.R	R.N.C
(Uninoculated control)	2.18	54.3	0.95	11.3	1.96	36.6	0.88	8.9	2.50	64.6	1.12	14.6	2.06	54.0	1.05	12.9
Rhizobium	2.94	74.0	1.06	14.9	2.56	62.6	1.02	13.4	3.36	94.0	1.24	18.8	2.96	84.6	1.21	17.4
Rhizobium + Rizolex-T	2.72	55.4	1.0	13.9	2.58	50.6	0.98	13.5	3.42	92.0	1.19	16.9	2.76	82.0	1.13	15.8
Rhizobium +*T. harzianum*	3.82	115.4	1.41	21.9	3.48	92.6	1.26	17.9	4.58	127.4	1.52	24.4	3.82	107.4	1.39	21.3
Rhizobium + *G. virens*	3.02	84.3	1.16	16.6	2.82	70.6	0.99	13.6	3.64	102.0	1.28	19.2	3.42	92.0	1.25	17.8
Rhizobium + *B. subtilis*	3.54	100	1.29	19.2	3.12	77.4	1.17	15.4	3.70	104.6	1.36	21.3	2.62	98.6	1.31	19.6
L.S.D at 0.05 %	0.27	8.62	0.04	0.78	0.19	9.94	0.05	0.92	0.26	8.6	0.03	0.76	0.18	6.74	0.05	0.79

D.W.S. = Dry weight of shoot g / plant
D.W.R. = Dry weight of root g / plant
S.N.C. = Shoot N-content mg / plant
R.N.C. = Root N-content mg / plant

Table 7: Percentage of changes in N_2-fixation parameter due to rhizobial inoculation and some biocontrol agents of faba bean and chickpea grown in non-infested and infested soil (Average of two seasons of 2003/04 and 2004/05).

Treatments	No. of nodules per plant		D.W. of nodules (mg/plant)		D.W. of biomass (shoot + root) (g/plant)		Total N-content of biomass (g/plant)		Loss (%) due to disease				Increases (%) due to rhizobial inoculation and diseases control			
	Non-infes.	Infes.	Non-infes.	Infes.	Non-infes.	Infes.	Non-infes.	Infes.	No. of nod.	D.W. of nod	D.W. (S+R)	T-N (S+R)	No. of nod.	D.W. of nod	D.W. (S+R)	T-N (S+R)
Faba bean																
(Uninoculated Control)	35.0	28.0	223.0	202.0	2.46	1.89	47.95	32.42	25.0	10.40	30.2	47.9	-	-	-	-
Rhizobium	81.0	64.0	559.0	328.0	3.13	2.85	81.33	55.65	26.6	70.40	10.0	46.1	130.2	103.7	37.2	71.4
Rhizobium + Rizolex-T	61.0	50.0	360.0	286.0	2.85	2.50	68.40	56.35	22.0	25.90	14.0	21.4	76.2	52.0	22.7	55.2
Rhizobium + *T. harzianum*	111.0	85.0	838.0	589.0	3.58	3.36	106.2	86.10	30.6	41.80	6.5	23.3	211.1	235.7	50.17	139.3
Rhizobium + *G. virens*	91.0	76.0	676.0	531.0	3.46	3.06	96.18	77.13	19.7	27.30	13.1	24.7	165.1	184.0	49.5	115.6
Rhizobium + *B. subtilis*	61.0	53.0	472.0	450.0	3.14	2.82	86.00	66.73	15.1	4.90	11.3	28.9	80.9	117.0	36.7	90.0
Chickpea																
(Uninoculated Control)	14.0	12.0	55.0	43.0	1.69	1.49	36.20	28.1	16.7	27.9	13.4	28.80	-	-	-	-
Rhizobium	33.0	29.0	181.0	143.0	2.20	2.19	50.50	44.5	13.8	26.6	0.46	13.50	138.5	230.6	38.1	47.7
Rhizobium + Rizolex-T	22.0	15.0	146.0	111.0	2.10	1.86	44.60	40.5	46.7	31.5	12.90	10.12	42.3	162.8	24.5	32.3
Rhizobium + *T. harzianum*	62.0	51.0	289.0	248.0	2.83	2.40	72.28	59.8	21.6	16.5	17.92	20.86	334.6	447.9	64.2	105.4
Rhizobium + *G. virens*	37.0	29.0	208.0	159.0	2.28	2.12	55.5	48.5	27.6	30.8	7.55	14.43	153.8	274.5	38.4	61.7
Rhizobium + *B. subtilis*	49.0	41.0	242.0	200.0	2.47	2.31	61.3	52.8	19.5	21.0	6.93	16.10	246.2	351.0	50.3	77.4

$$\text{Loss (\%) due to diseases} = \frac{\text{Non-infested} - \text{Infested}}{\text{Infested}} \times 100$$

$$\text{Increases (\%) due to both rhizobial inoculation and diseases control} = \frac{\Sigma\,(\text{non-infested treatments} + \text{infested treatments}) - \Sigma\,(\text{non-infested control} + \text{infested control})}{\Sigma\,(\text{non-infested control} + \text{infested control})} \times 100$$

According to Tan and Tan (1986).

This inhibition in nodule formation is a consequence of the apparently toxicity of this chemical exerted on *Rhizobium* cells (Graham *et al.*, 1980 and Ghobrial *et al.*, 1996). They reported that many fungicides tend to reduce or eliminate nodulation of legume plants.

Comparing the mean values of interacting of the three factors (*Rhizobium*, *R. solani* and biocontrol agents). The percentage of changes in N_2-fixation parameter are determined. The calculated data are presented in Table (7) from which the following could be observed:

1- Irrespective of biocontrol agents, an increase of about 130.0, 108.7, 37.3 and 71.0 in the number and dry weight of nodules, dry weight of plant biomass (shoot + root) and their total N- content, respectively as a direct effect of inoculation with *Rhizobium* of faba bean plants grown in non-infested and infested soils as compared with control treatments (uninoculated).
2- Rhizobial inoculation was greatly enhanced when it applied in combination with biocontrol agent. Both of them achieve high levels of protection against pathogen and permit meanwhile reasonable symbiotic N_2-fixation. The stimulation varied, however, according to the type of biocontrol agent. The relative improving effect of dual inoculation with *Rhizobium* and *Trichoderma* showed a considerable response. The relative increases were 211.0, 235.7, 128.5 and 139.0%, respectively of faba bean plant grown in non-infested and infested soil. As shown from data presented in Table (7).
3- It is worthy to note that chickpea plants behaved similarity to all the applied treatments as did in faba bean plants (Table 7).
4- Taken together with evidence presented above, it is clear that the beneficial with disease protection afforded by biocontrol agent, had more compensated the loss occurred in N_2-fixation parameters caused by *R. solani* in both legume crops under investigation (Table 7).

It is clear that much more is going on these association as it provide a natural, safe, effective, persistent and durable type of protection beside the symbiotic system by rhizobia that siphon an appreciable amount of nitrogen from the atmospheric reservoir and enrich the soil with this important but scarce nutrient.

REFERENCES

Abdel- Wahab, S.M.; M.E. Hassan, and B.M. Rabeia. 1994. Response of chickpea to *Rhizobium* inoculation in the traditional and new reclaimed areas.Nile Valley Regional program on cool season Food legumes and Cereals. Six Annual Coordination Meeting Cairo, 11- 15 September, P. 366-368.

Abd-El-Moity, T.H.; A. A. Khaled and S.A. Omar. 1992. Biological treatments for control root-rot / wilt disease of faba bean in Egypt.J. Appl.Sci., 7: 13-21.

Abo El-Soud, A. A.; M. A. El- Deeb and Kh. M. El- Yamani.2004. Improving of faba bean production by application of organic compost and rhizobial inoculation under newly reclaimed soil.Egypt.J. Appl. Sci. 19: 333- 344.

Abou-Zeid, N.M.; M.K. Arafa and Sabah Attia. 2003. Biological control of pre and post- emergence diseases on faba bean, lentil and chickpea in Egypt. Egypt. J. Agric. Res., 18: 1491-1503.

Al-Kahal, A. A. and A. M. El- Garhy. 2003. Suppression of soybean damping-of caused by *Sclerotium rolfsii* using some biocontrol agents. Egypt. J. Appl. Sci; 18: 137-151.

Bazgir, E. and M. Okovvat. 1997. Biological control of *Rhizoctonia solani* by *Trichoderma* spp. and *Gliocladium virens*. Indian Phytopath. Society-Golden Jubilee Inter. Confer. New Delhi, India. AB. No. 9.

Brundrett, M.1991.Mycorrhizas natural ecosystem.Adv.Ecol. Res.2: 171- 313.

Burla, M.; M. Goverde; F. J. Schwinn and A. Wiem–Ken.1996. Influence of biocontrol organisms on root pathogenic fungi and on the plant symbiotic microorganism, *Rhizobium phaseoli* and *Glomus mosseoe*. Zeitschrift fuer pflanzen krankh eiten und pflauzeuschutz.,103:156-163.

Cardoso, J.E., and Echandi, E. 1987. Biological control of Rhizoctonia root-rot of snap bean with binucleate *Rhizoctonia* – like fungi. Plant Dis. 71: 167-170.

Dennis, L. and J. Webster. 1971a. Antagonistic properties of specific groups of *Trichoderma*. 1- production of non- volatile antibiotics. Trans. Brit. Mycol. Soc., 57: 25-39.

Dennis, L. and J. Webster. 1971b. Antagonistic properties of specific groups of Trichoderma. 11- production of volatile antibiotics. Trans. Brit. Mycol. Soc., 57: 41- 48.

Dileep-Kumar, B.S.; I. Berggren and A. M. Martensson. 2001. Potential for improving pea production by co-inoculation with *Fluorescent pseudomonas* and *Rhizobium*. Plant and soil, 229: 25 – 34.

Elad, Y.; I. Chet, and Y. Henis.1982. Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Can. J. Microbiol., 28: 719 – 725.

Elad, Y; I. Chet and J.I. Katan. 1980. *Trichoderma harzianum*, a biological control agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70: 119 – 121.

El-Awadi, F.A.1993. Sources and mechanism of resistance to root-rot and wilt diseases complex in chickpea at sandy soil. Ph. D. Thesis, Fac. Agric. Suze Canal Univ. Egypt.

El-Garhy, A.M. 1994. Studies on root-rot and wilt of lentil. M.Sc. Thesis, Fac. Agric. Al-Azhar Univ., Egypt.

El-Kafrawy, A. A. 2002. Biological control of damping-off caused by *Rhizoctonia solani*. Egypt. J. Agric. Res., 80: 57-70.

Fiddaman, P. J. and S. Rossal. 1993. The production of antifungal volatiles by *Bacillus subtilis*. J. Appl. Bacteriology, 74: 119-126.

Ghobrial, W.N.; R.Y. Rizk and Dawlat Abadi. 1996. Symbiotic nitrogen fixation process in *Phaseolus vulgaris* as affected by biological and chemical control of bean root-rot. J. Agric. Sci. Mansoura Univ., 21: 1515-1524.

Gomez, K.A. and Arturo A. Gomez.1984. Statistical procedures for Agricultural Research., (2^{nd} ed.). pp. 20-29.

Graham, P.H.; G. Ocmpa; L.D. Ruiz and A. Duque. 1980. Survival of *Rhizobium phaseolii* in contact with chemical seed protectants. Agron. J., 72: 625-627.

Hassanein, A. M and G. A. A. Mekhemar. 2003. Biological control of soybean damping-off caused by *Sclerotium rolfsii* using *Pseudomonas fluorescens* and *Pseudcmonas putida*. Egypt, J. Appl. Sci. 18: 73-86.

Hassanein, A.M.; Ebtehag El-Barougy; A.M. El-Garhy; Paviparikka and T. A. El-Sharkawy. 2000. Biological control of damping-off, root- rot/wilt diseases of alfalfa in Egypt. Egypt. J. Agric. Res., 78: 63-71.

Hassanein, A.M; G. A. El – Morsy and N.M. Abou-Zeid. 1996. Biological control of damping – off, root rot and wilt diseases of lentil. Proceeding Symposium Regional Sur Les Maladies des Ceral et des Legumineues Alimentaires 11-14 Nov., Rabat, Maroc.

Hussein, A.H.A; S.A. Saleh, M. A. EL- Deeb and W. Kadry (1997). Effect of *Rhizobium* inoculation, phosphorus and potassium fertilization on growth, nodulation and yield of faba bean cultivated in the newly reclaimed soils of middle Egypt.Bull.Fac.Agric.Univ.,Cairo.48:201- 214.

Kilian, M.; M. Steiner; B. Krebs; H. Junge ; G. Schmiedeknecht and R. Hain. 2001. F B Z 24 *Bacillus subtilis* mode of action of a microbial agent enhancing plant Vitality, Pflanzenschutz Nachrichten Bayer 1: 72- 93.

Kloepper, J.W. and M.N. Schroth. 1981. Relationship of *In-Vitro* antibiosis of plant growth promoting rhizobacteria to plant growth and the displacement of root microflora. Phytopathol. 71: 1020-1024.

Lewis, J.A.; R.D. Lumesden and J.C. Lock. 1996. Biocontrol of damping- off diseases caused by *Rhizoctonia solani* and *Pythium ultimum* with alginate pellets of *Gliocladium virens*, *Trichoderma hamatum* and various food bases. Biocontrol Sci. & Technol., 6: 163-173.

Mansour, M.S. 1997. *Bacillus subtilis* an antagonist against some soil – borne pathogenic fungi. Egyptian Congress of the Egyptian Phytopathological Society, Cairo, Egypt.

Mekhemar, G.A.A.; M. Shaaban, A. A. Ragab and A.M.M. Biomy. 2005. Response of faba bean to inoculation with *Rhizobium leguminosarum* bv. *viceae* and plant growth promoting rhizobacteria under newly reclaimed soil. Egypt, J. Appl. Sci., 20: 126-144.

Nazim, M.; F. Z. Khalifa; Sh. M. El- Desouky and G. A. Amer. 1999. Effect of time and method of application of *Trichoderma harzianum* on controlling soybean damping-off caused by *Macrophomin phaseolina* and *Sclerotium rolfsii.* Minufiya J. Agric. Res. 24: 1871-1879.

Paau, A.S. (1989). Improvement of *Rhizobium* inoculants. Appl. Environ. Microbiol., 55: 862-865.

Page, A. L.; R. H. Miller and D.R.Keeney.1982. Methods of soil Analysis. 11 Chemical and Microbiological Properties. 2nd edition. Madion. Wisconsin. USA.

Papavizas, G.C; P.B. Adams and J.A. lewis. 1968. Survival of root- infecting fungi in soil. V. Saprophytic multiplication of *Fusarium solani* f. sp. *phaseoli* in soil. Phytopathology, 58: 414.

Papavizas, G.C. and J.A. Lewis. 1989. Effect of *Gliocladium* and *Trichoderma* on damping-off and blight of snapbean caused by *S. rolfsii* in the greenhouse. Plant Pathology, 38: 277-286.

Parmar, N. and K.R. Dadarwal.1999. Stimulation of nitrogen fixation and induction of flavonoid like compounds by rhizobacteria. J. Appl. Microbiol. 86: 36-44.

Schreiber, L.R.; G.F. Gregory; C.R. Krause and J.M. Jchida. 1988. production, partial purification and antimicrobial activity of a noval antibiotic produced by *Bacillus subtilis* isolate from Ulmus Americana. Can. J. Bot., 66: 2338-2346.

Smith, V. L. 1996. Enhancement of snap bean emergence by *Gliocladium virens*. Hort. Science, 31: 984-985.

Srinivasan, M.; D. J. Petersen and F.B. Holl. 1996. Influence of indole acetic acid – producing Bacillus isolates on the nodulation of *Phaseolus vulgaris* by *Rhizobium etli* under gntobiotic conditions Can. J. Microbiol. 42: 1006- 1014.

Tan, G.Y. and W.K. Tan. 1986. Effect of nodulation on resistance to alfalfa sickness among ten alfalfa cultivars. Plant and Soil, 94: 133-141.

Tang, W.H., 1994. Yield increasing bacteria (YIB) and biocontrol of sheath blight of rice. Proceeding of the 3rd international workshop on plant growth – promoting rhizobacteria. 267-273.

Vincent, J.M. 1970. A manual for the practical study of the root- nodule bacteria. International Biological program handbook No.15. Blackwell Scientific Publications. Ltd., Oxford and Edinburgh U. K.

التثبيت التكافلى للنتروجين ومدى تأثره بالمقاومة البيولوجية والكيماوية لمرض عفن الجذور فى نباتات الفول البلدى والحمص

احمد محمد حسنين١ ، عبد الله متولي الجارحى١ جمال عبد الفتاح أحمد مخيمر٢

١- معهد بحوث أمراض النباتات - مركز البحوث الزراعية - جيزة - مصر.

٢- معهد بحوث الأراضي والمياه والبيئة- مركز البحوث الزراعية - جيزة - مصر.

تم تقيم ثلاث عوامل حيوية هي الترايكودرما هيرزيانم ، جلوكلوديوم فيرنز و الباسيلس ساتلس فى مقاومة فطر الرايزوكتونيا سولانى المسبب لمرض موت البادرات و عفن الجذور في الفول البلدى والحمص وذلك تحت ظروف المعمل والصوبة بجانب دراسة تأثير استخدام هذه العوامل الحيوية على عملية التعقيد الجذرى والنمو ومحتوى النبات من النيتروجين.

وقد أظهرت النتائج المتحصل عليها تحت ظروف المعمل أن كل العوامل الحيوية أحدثت تثبيط لنمو ميسيليوم الفطر الممرض (الرايزوكتونيا سولانى) وذلك على كل البيئات المستخدمة.

كذلك أظهرت النتائج المتحصل عليها تحت ظروف الصوبة أن كل العوامل الحيوية كان لها تأثير معنوى فى خفض النسبة المئوية لمرض موت البادرات وعفن الجذور وزيادة النسبة المئوية للنباتات السليمة وذلك عند خلط العوامل الحيوية بسلالات الريزوبيم (ايكاردا ٤٤١) بالنسبة للفول البلدى ، (ايكاردا ١١٤٨) بالنسبة للحمص وكانت أعلى مقاومة عندما عوملت بذور الفول البلدى بالترايكودرما هيرزيانم وريزوبيم الفول البلدى وعند معاملة بذور الحمص بالباسيلس ساتلس وريزوبيم الحمص.

أما بالنسبة لتأثير عوامل المقاومة الحيوية على عملية تكوين العقد الجذرية والنمو ومحتوى النباتات من النيتروجين لكل من الفول البلدى والحمص فقد أوضحت النتائج أن معاملة بذور الفول البلدى بالترايكودرما هيرزيانم وسلالة الريزوبيم (ايكاردا ٤٤١) أعطت زيادة معنوية فى عدد العقد الجذرية والوزن الجاف لها والوزن الجاف للمجموع الخضرى والجذرى ونسبة النيتروجين فيهما وذلك فى التربة المعدية بالفطر (رايزوكتونيا سولانى) أو الغير معدية. بينما أعطى العامل الحيوى باسيلس ساتلس زيادة معنوية فى عدد العقد الجذرية والوزن الجاف لها والوزن الجاف للمجموع الخضرى والجذرى ونسبة النيتروجين فيهما وذلك عند خلطه بسلاله الريزوبيم (ايكاردا ١١٤٨) الخاص بالحمص وذلك فى كل من الموسم الأول والثانى. هذا وقد وجد أن استخدام اللقاحات البكتيرية مع بعض عوامل المقاومة الحيوية أدى إلى تعويض الفقد الناتج من تأثير المرض في بعض القياسات الخاصة بتثبيت الأزوت الحوى فى كل من المحصولين.

بصفة عامة : فقد اتضح من النتائج المتحصل عليها أن التلقيح بأى من أحد العوامل الحيوية الثلاث مرتبطا مع سلالات الريزوبيم أدى إلى تقليل نسبة موت البادرات وعفن الجذور بجانب زيادة التعقيد الجذرى والنمو ومحتوى النباتات من النيتروجين لكل من محصول الفول البلدى والحمص فى كل من الموسم الأول والموسم الثانى.