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SUPPRESSION OF BEAN ROOT ROT BY VARIOUS AQUEOUS PLANT EXTRACTS AND SOME BENEFICIAL MICROORGANISMS *IN VITRO* AND *IN VIVO* CONDITIONS

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ABSTRACT

Three different aqueous plant extracts neem (*Azadirachta indica* L.) and camphor (*Eucalyptus deglupta* L.) leaves as well as garlic (*Allium sativum* L.) cloves and two beneficial microorganisms namely *Trichoderma harzianum* Rifai *and Bacillus subtilis* Cohn were investigated to evaluate their antagonistic effect against *Rhizoctonia solani* (Kuhn.) *and Fusarium solani* (Mart.) Sacc. under laboratory and greenhouse conditions. Garlic extract exhibited the best effect in reducing linear growth of both soil borne pathogens *R. solani* and *F. solani* comparing with the other two plant extracts. *T. harzianum* shows significant effect in reduction of pathogenic mycelial growth, *R. solani, F. solani*, compare with *B. subtilis* and control treatment. Different concentrations of plant extracts 10%, 5% and 2.5% were used to control damping off disease of bean under greenhouse conditions. Positive correlation between concentration of plant extracts and reduction in damping off disease, was observed. Garlic extract revealed the best result in reducing the disease incidence compare with other plant extracts or control treatment. Two bioagents were used at three different rates i.e. 100, 50, 25 ml/kg seeds. Obtained data revealed that, both bioagents reduced the disease incidence at all used rates. *B. subtilis* exhibited better results than *T. harzianum*. Positive correlation between doses of bioagents and percentage of surviving plants was noticed. Plant extracts or bioagents, under test, were used in two different forms, i.e. as powder or water preparation. Data obtained revealed that, plant extracts were more effective in powder form than liquid one. Garlic preparation gave the best result in this regards compare with other plant extracts or control treatment. On the contrary, in case of tested bioagents water preparation when used for bean seed soaking before planting gave better results compare with using the same antagonist in form of powder. *B. subtilis* shows more protection effect for bean seeds compare with *T. harzianum* or control treatment.

Keywords: Biological control, beans damping off, neem, camphor, garlic, *Trichoderma* spp., *Bacillus subtilis*.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important food legume crop grown worldwide (Wortmann and Allen, 1994; Wortmann *et al.*, 1998 and Buruchara, 2006). Beans are considered by many to be the perfect food as it contain high amount of protein, micronutrients, vitamins, dietary fiber, and also have a low glycemic index (Wortmann and Allen, 1994; Bennink, 2005; Widers, 2006 and Nzungize *et al.*, 2011). In world crop production, preharvest losses due to fungal diseases may reach 12% in developing countries (Lee *et al.*, 2001). Fungicides are widely used as seed or soil treatment to depress various root diseases. However, use of fungicides causes environmental hazards and development resistance pathogen varieties. Control diseases using chemical fungicides lead to pollution of atmosphere and negatively affect the properties of treated plants. Since the end of the Second World War, there has been a great boom in the use of fungicides throughout the world. After

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the great justified alarm in the early 60s about the dangerous consequences to man and environment in the area of phytotoxicity (Williams, 1975), there is an urgent need for alternative methods of plant disease control. In recent years, more emphasis has been given to the use of bioagents, plant growth promoting rhizobacteria (PGPRs) and plant extracts, either alone or in combination. Integrated management of root rot pathogens have been reported by several workers (Bowers and Locke, 2000; Momin and Nair, 2001; Suriachandraselvan and Seethuraman, 2002; Sharma and Gupta 2003, Sobti *et al.,* 2005; Yadav *et al.,* 2005 and Sitara *et al.,* 2008).

Garlic cloves is reported to contain volatile oils, alliin (S-allyl-L-cysteine sulfoxide ‹(S-methyl-L-cysteine sulfoxide and allinase). It is rich in vitamins like thiamine, riboflavine and niacin. Volatile oil contains allicin (diallyl thiosulphinate), an active odour principle of garlic. Other major compounds present are diallyl disulphide, diallyl trisulphide, allyl methyl trisulphide and allyl methyl disulphide (Husain *et al.,* 1992). Bulbs also have anti-bacterial and anti-fungal activity (Joy *et al.,*1998). Garlic extract was used as seed treatment to depress *Pythium aphanidermatum* growth .Inhibition of plant pathogen is due to the effect of plant products used, which inhibit hydrolytic enzymes production in the pathogen by different degrees (Kurucheve and Padmavathi, 1997). Garlic cloves extract is very effective against *F. solani, R. solani and Sclerotinia sclerotiorum* the causal organisms of watermelon and cantaloupe damping off (Shalaby and Atia, 1996). Soil amendment with neem (*Azadirachta indica*) seed cake, was significantly effective against *F. solani, Macrophomina phaseolina and R. solani* (Ehteshamul *et al.,* 1998). Moslem and El-Kholie (2009) reported that neem leaves and seed extracts were effective as antifungal against *Alternaria solani, F. oxysporum, R. solani* and *S. sclerotiorum.* Plant extracts of neem, camphor leaves and garlic cloves reduced linear growth of cucumber soil-borne pathogens. The most effective concentrations in this regard were 5% that led to significant reduction in damping off disease incidence (Tohamy *et al.,* 2002). *Trichoderma* spp. occur worldwide and are commonly associated with root soil and plant debris (Howell, 2003). Past research indicated that *Trichoderma* spp. can parasitize fungal pathogens and produce antibiotics. Weindling (1932) described in detail the mycoparasitism of a fungal pathogen causing damping off disease ,*Rhizoctonia solani,* by the hyphae of *Trichoderma*, including coiling around the hyphae, penetration, and subsequent dissolution of the host cytoplasm. He also described an antibiotic gliotoxin; which was toxic to both *R. solani* and *Sclerotinia americana.* In the following year similar results were reported by other plant pathologists. The mechanism of antibiosis was demonstrated in several studies. An antibiotic, gliovirin, from *T.virens* demonstrated strong inhibition of *Pythium ultimum* and the *Phytophthora* species (Howell and Stipanovic, 1995).

More recent research indicated that certain strains of *Trichoderma* can induce systemic and localized resistance to several plant pathogens. In this respect, plants treated with *Trichoderma* in the root zone can produce higher levels of peroxidase, chitinase, deposition of callose-enriched wall appositions and pathogenesis-related proteins on the inner surface of cell walls. Moreover, some strains may enhance plant growth and development (Howell, 2003). Many researchers have demonstrated the potential of *Trichoderma* spp. in controlling damping-off and wilt diseases of crop plants caused by *R. solani* and *F.* spp. (Dubey *et al.,* 2007 and Rojo *et al.,* 2007). Abo-Elnaga, (2006) used *B. subtilis* as a biocontrol agent for controlling sugar beet damping-off disease. The usage of *B. subtilis* as seed treatment reduced the percentage of damping-off incidence of sugar beet under greenhouse conditions. Tomato plants treated with *B.subtilis* only and/or *T. harzianum* have shown biocontrol activity against damping off and root rot disease and gave high yield of tomato (Morsy, 2005; Zaghloul *et al.,* 2007 and Morsy *et al.* 2009). Both *Trichoderma* spp. and *Bacillus* spp. are wide spread throughout the world and have been recognized as the most successful biocontrol agents for soil borne pathogens. Several modes of action of the efficiency bioagents on reducing plant diseases have been described, including competition for nutrients, antibiosis, induced resistance, mycoparasitism, plant growth promotion and rhizosphere colonization capability (Bailey *et al.*, 2008; Hassanein *et al.*, 2006; Siddiqui and Akhtar, 2007 and Abd-El-Moneim, 2011).

The main objective of the current study is to evaluate using of *T. harzianum* and *B. subtilis* as biocontrol agents and three different aqueous plant extracts (neem, camphor leaves and garlic cloves) against root rot disease incidence of bean and its causal pathogens.

MATERIALS AND METHODS

The present work was carried out during season 2010 and repeated 2011 at the Central Lab. of Organic Agriculture, ARC, Giza, Egypt and in greenhouse belongs to Ministry of Agriculture, Dokki, Giza.

Isolation and Identification of the Causal Organisms

Samples of bean plants doubted to be infect with root rot and showing identical symptoms of damping off were collected from the naturally infected plants grown in greenhouse belongs to Ministry of Agriculture in Giza (Dokki). The collected root samples were washed using tap water then dried, and surface sterilized using 5% chlorine solution for 3 minutes. Sterilized surface plant materials were then washed several times with sterilized distilled water then dried between two sterilized filter papers. Dried sterilized plant materials were cut into small pieces with sterilized scalped and cultured on plain agar medium in Petri plates 9 cm in diameter. Plates were incubated at 28°C. Incubated plates were examined periodically and the developed mycelia were transferred to nutrient glucose agar (NGA) medium. The isolated fungi were purified using hyphal tip and/or single spore techniques (Hawker, 1960). Pathogenicity test of isolated fungi toward bean plants (cv. Giza 3) was estimated (Sallam *et al.*, 2008). Artificial inoculum of pathogenic fungi was prepared by growing each fungus on sorghum-sand medium as described by Abd El-Khair and El-Mougy (2003). The most aggressive isolate of each pathogenic fungus was used *in vitro* experiments.

Beneficial microorganisms

Different bio-control agents used in these studies i.e. (*Trichoderma harzianum and Bacillus subtilis*) were kindly obtained from the Central Lab. of Organic Agriculture ARC, Giza, Egypt. *T. harzianum* was grown for 9 days on liquid gliotoxin fermentation medium (GFM) developed by Brain and Hemming (1945) under complete darkness to stimulate toxin production (Abd-El-Moity and Shatla, 1981). *B.subtilis* was grown on nutrient glucose broth (NGB) medium developed by Dowson (1957) for 48 h. Different bio-control agents were formulated as suspension or powder using method developed by Abd-El-Moity (1985). Prepared suspension or powder were adjusted to be contain 30 x 10^6 cfu/ml or g for all used antagonists.

Aqueous plant extracts

Garlic (*Allium sativum* L.) cloves, neem (*Azadirachta indica* L.) leaves and camphor (*Eucalyptus deglupta* L.) leaves extracts, were prepared by mixing 10 g frozen plant materials with 100 ml of water using electric blender for 10 minutes.

The plant extracts were filtrated through double layer of cheesecloth. The filtrated plant extract was centrifuged at 3000 rpm for 10 minutes. The centrifuged plant extracts were sterilized using Millipore 0.2 µm. Each sterilized plant extract was adjusted to be 100 ml by using sterilized filtrated water as a final volume. Plant extracts for greenhouse treatment were used without any sterilization. Resulted extracts were considered as 10%.

In vitro Studies

Effect of different plant extracts on reduction percentage of the mycelial growth of the tested pathogenic fungi

Preparation of plant extracts

Three different plant extracts were used (garlic, neem and camphor). Different plant extracts were prepared as mentioned before. Each sterilized plant extract was added to plates, each contain 15 ml of gliotoxin fermentation medium (G F M) described by Brian and Hemming (1945) at the rate of 1.5 ml/plate. Plates having medium mixed with the plant extracts were inoculated with different pathogens. Different plates were inoculated with discs (6mm in diameter) obtained from the periphery of 4 days old pathogenic colonies. Five plates were used for each treatment. Plates recieved only GFM were inoculated with pathogens to serve as control. All inoculated plates were incubated at 25°C. Data were recorded when mycelial growth of pathogenic fungi cover all surface medium in control ones. Reduction percentages in mycelial growth due to adding plant extracts were calculated using the next formula:

$$X = 100 - [G2 / G1 \times 100]$$

Where

X: % of reduction

G1: growth of pathogenic fungi in control plates.

G2: growth of pathogenic fungi in treated plates.

Effect of the antagonistic microorganisms on the reduction percentage of the linear mycelial growth of pathogenic fungi

Different bioagents were evaluated under laboratory conditions for their antagonistic effect against the pathogenic fungi *(Rhizoctonia solani* (Kuhn.) *and Fusarium solani* (Mart.) Sacc.). Petri dishes 9 cm in diameter each contains 15ml of GFM were used to determine the antagonistic effect of the used antagonist on the isolated pathogenic fungi. Plates contain NGA medium were used to determine the effect of antagonistic bacterium against pathogenic fungi. Different plates were inoculated with discs (6mm in diameter) obtained from the periphery of 4 days old pathogenic colonies. The pathogenic fungus was inoculated at one side where the opposite side was inoculated with either disc of *T. harzianum* or with loop full of antagonistic bacteria grown for 48 hrs on liquid NGB medium. Five plates were used for each treatment. Plates inoculated only with the individual pathogenic fungi served as a control treatment. Inoculated plates were incubated at 25°C. When mycelial growth covers medium surface in control treatment, all plates were examined and reduction percentages in mycelial growth of pathogenic fungi were calculated using the formula mentioned before.

Greenhouse Experiments

In all greenhouse experiments unless indicated, a greenhouse (60m X 9m = 540 m^2) belongs to Central lab. for Agricultural Climate, Agricultural Research Center, Ministry of Agriculture and Land reclamation, Dokki, Giza, was used. Soil in this greenhouse is highly natural infested with *Rhizoctonia solani* (Kuhn.) and *Fusarium solani* (Mart.) Sacc. Selected bio-control agents *T. harzianum and B.subtilis* in addition to three different aqueous plant extracts (neem, camphor leaves and garlic cloves) were applied to determine their efficacy in controlling root rot disease of beans.

Bean (*Phaseolus Vulgaris* L.) var. Giza3 was cultivated. Experiments were designed in complete randomized plots .Each treatment contains 5 replicates and each replicate (2m X 2m) was cultivated using 20 bean seeds. Pre and post-emergence damping off were determined according to disease index (Categories 3, 4 includes pre- and post-emergence damping off) developed by (Ismail, 2011) The percentage of pre-emergence was determined after 8 and 11days from sowing seeds whereas percentage of post-emergence was determined after 30 days from sowing. Percentages of surviving plants were determined after 45 days from sowing time. All experiments were carried during season 2010 and repeated in 2011.

Effect of different concentrations of plant extracts on root rot disease incidence in bean for two seasons under greenhouse conditions

Three different plant extracts were examined (garlic, neem and camphor). Different plant extracts were prepared as mentioned before. Obtained extracts were considered as 10% and other concentration (5 and 2.5%) were prepared and adjusted by using distilled autoclaved water. Bean seeds were soaked in the different concentrations of the tested plant extracts for 30 minutes using tween 80 to ensure the distribution of the extracts on and in seeds. For each plant extract, three concentrations treatment were used. Seeds only soaked in water for the same period acted as control. Pre and post emergence damping off were determined as described before.

Effect of different doses of antagonistic bioagent (as seed spray) on percentage of bean root rot disease for two successive seasons under greenhouse conditions

Different antagonists were kindly obtained from the Central Lab. of Organic Agriculture ARC, Giza, Egypt. Bioagents were used as suspension in different doses. Used doses were 25, 50, 100 ml / kg seeds. Pre emergence and post emergence root rot disease incidence were determined, as described before.

Effect of using formulated plant extracts in powder or liquid form on the percentage of bean root rot disease incidence in two successive seasons under greenhouse conditions

Different plant extracts under test were formulated as liquid or as powder. The liquid was prepared by extracting 10 g in 100 ml of water as described before. Liquid form was used on bean seeds at the rate of 100 ml of each plant extract / kg seeds. Powder form was prepared by adding 250 ml from stock solution of each plant extract to 250 g of talk powder). Powder form was used as seed dressing by wetting by 5% of Arabic gum solution and then mixed with the powdered plant extract at the rate of 10 g/kg seed .Treated seeds with suspension or powder were sown in highly natural infested soil in the greenhouse. Seeds only received water act as control treatment. The disease assessment was recorded as mentioned before.

Effect of various formula of different bioagents on the percentage of bean root rot disease incidence in two successive seasons under greenhouse conditions

Two different forms suspension and powder of each bioagents were used. Suspension was used as soaking bean seeds at the rate of 100 ml/kg seed and left for 30 minuets before sowing. On the other hand, powder formula was used as seed dressing, where bean seeds were wetted using 5% solution of Arabic gum. Ten grams of each bio-control agent was formulated as powder then added to the wetted seeds and mixed thoroughly. All treated seeds were sown in heavily infested soil in the greenhouse. Seeds only received water act as control treatment. The disease assessment was recorded as mentioned before.

Statistical Analysis

Statistical analysis was carried out using the procedures ANOVA (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The Causal Pathogens

Isolated fungi were identified according to their culture and morphological characteristics according to keys developed by (Gilman 1957, Singh 1982 and Barnett and Hunter 1996). The purified isolated pathogens were identified as *Rhizoctonia solan* (Kuhn.) and *Fusarium solani* (Mart.) Sacc. Identification of pathogenic fungi were kindly confirmed through the Mycology and Plant Disease Survey Research Dept., Plant Pathology Research Institute, A.R.C., Egypt.

Effect of different plant extracts on reducing percentage of the mycelial growth of the tested pathogenic fungi

Data in Table 1 and Fig. 1 indicate that all plant extracts except camphor extract showed inhibition effect to the mycelial growth of *Rhizoctonia solani* and *Fusarium solani* compare with control treatment. Garlic extract exhibit high significant effect on linear growth of both *R. solani* and *F. solani* compare with the other two plant extracts or the control treatment. This might be due to the effective presence of certain disulfide amino acids in the garlic extract (Husain *et al.* 1992) which acts as antibacterial and antifungal activities (Joy *et al.* 1998). In addition, garlic extract might have special materials that inhibit the activity of hydrolytic enzymes production of the pathogen by different degrees (Kurucheve and Padmavathi 1997). Neem extract showed also significant effect on the reduction percentage of the mycelial growth of both investigated pathogens compare with control treatment. The explanation of the obtained results might confirm that neem leaves extract has antifungal, antibacterial activities (Jarvis and Morgan, 2000; Dai *et al.*, 2001; Mossini *et al.*, 2004; Amadioha, 2004; Sanjeet *et al.*, 2005; Moslem and El-Kholie, 2009). Camphor extract was the least effective one on mycelial linear growth of *F. solani* and has no effect on *R.solani.*

Table 1. Effect of different plant extracts on reducing percentage of the mycelial growth of the tested pathogenic fungi

Plant extracts	% of reduction in the linear growth of pathogenic fungi	
	Rhizoctonia solani	*Fusarium solani*
Garlic	55.5	66.6
Neem	33.3	44.4
Camphor	00.0	11.1
Control	00.0	00.0
L .S. D. at 5%	0.23	0.21

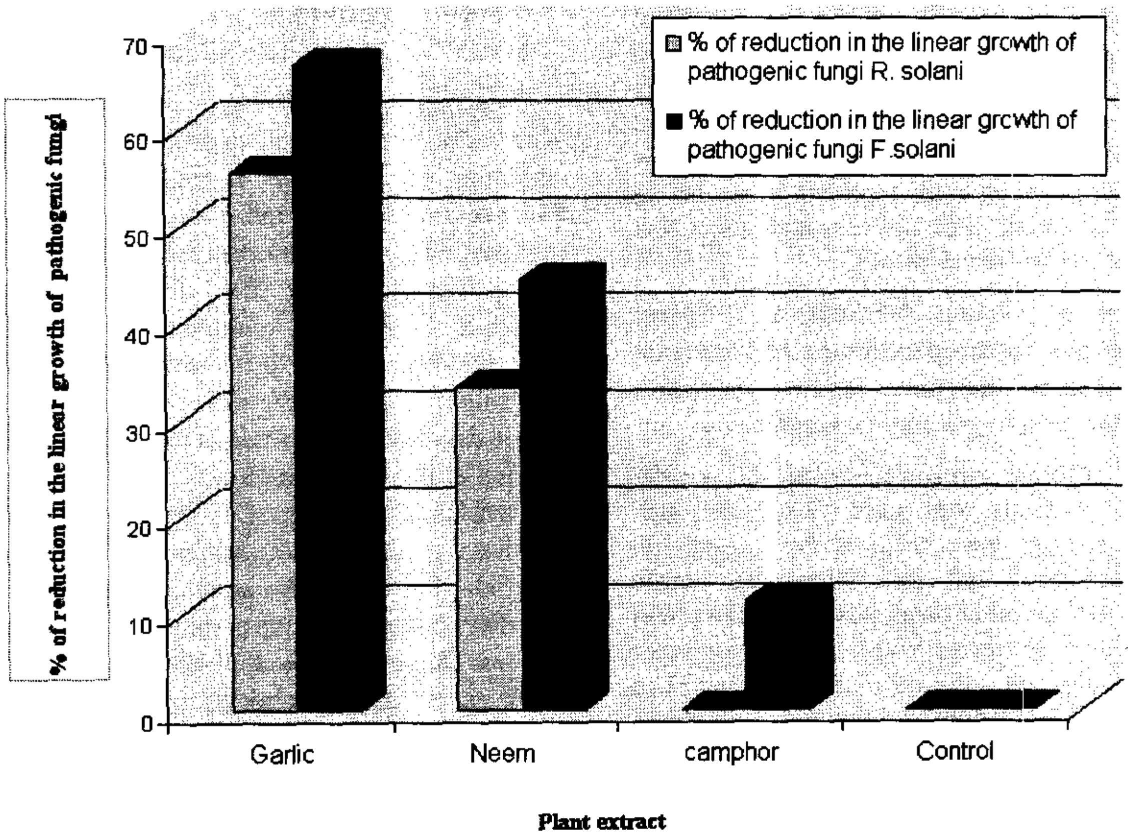

Fig. 1. Effect of different plant extracts on reducing percentage of the mycelial growth of the tested pathogenic fungi

Effect of the antagonistic microorganisms on reduction percentage of the linear mycelial growth of the pathogenic fungi

Data in Table 2 and Fig. 2 indicate that *T. harzianum* is the most effective antagonist against *Rhizoctonia solani and Fusarium solani* pathogens under investigation. This high potentiality in suppression might be due to that *Trichoderma* spp. act through different actions as production of antifungal substances (Turner, 1971; Abd - El-moity, 1981 and Hayes, 1992). It also acts through production of destructive enzymes i.e chitenase (Bolar *et al.,* 2000 and Harman, 2006), which attack and destroy pathogenic fungi structures. *Trichoderma* also attacks through mycoparasitism (Weindling, 1932; Abd-El-moity, 1981 and Abd-El-monaim, 2011). *Bacillus subtilis* also showed significant reduction on the growth of both tested pathogenic fungi. This might be due to that, *B. subtilis* act through production of number of antibiotics (Ferreria *et al.,* 1991 and Asaka and Shoda 1996).

Greenhouse Experiments

Effect of different concentrations of plant extracts on root rot disease incidence of bean for two seasons under greenhouse conditions

Data in Table 3 show positive correlation between different concentrations of plant extracts and efficacy of control damping off disease of bean plants. Plant extract at 10% concentration revealed the highest significant surviving plants if compared with 5% and 2.5%. The obtained results might be due to the increased amount of the active component in the high doses of plant extracts.

Garlic extract proved to be the most significant one in controlling damping off disease gave 80% and 83% surviving plants during both successive seasons compared with the control treatment. The explanation of such results might be due to that garlic extract has antibacterial and antifungal activities (Joy *et al.,* 1998). Also, it might inhibit hydrolytic enzymes produced by the pathogenic fungi genera during pathogenesis. In addition to the presence of certain disulfide amino acids in garlic extract as mentioned by Husain *et al.* (1992). Neem extract gave significant result in controlling damping off disease being 73% and 74% surviving plants of the two successive seasons, respectively compared with 40% and 35% in control one. This might be also due to that, neem extract has antibacterial and antifungal activities (Jarvis and Morgan, 2000; Dai *et al.,* 2001; Mossini *et al.,* 2004; Amadioha, 2004; Sanjeet *et al.,* 2005; Moslem and El-Kholie, 2009) Camphor extract proved to be the least significant one compared with garlic, neem or control treatment and/or the other plant extracts. The effect of camphor also might be attributed to the presence of volatile oils present in camphor leaves extract.

Effect of different doses of antagonistic bioagent (as soil drench) on bean root rot disease percentage for two successive seasons under greenhouse conditions

Three different doses of bioagents are tested to determine the most effective one in controlling bean damping off disease. Data in Table 4 indicate a positive correlation between doses and efficacy of bioagents in controlling damping off. Data showed that, *B. subtilis* was better than *T. harzianum* in controlling damping off disease especially in the second season. This might be due to that, these bacteria produce antibiotics (bacteriacin and subtilisin) which act as inhibitors to the pathogenic fungi (Ferreria *et al.* 1991 and Asaka and Shoda 1996). In addition, *B. subtilis* also grows very fast and occupies the court of infection and consumes all available nutrients, preventing the pathogens to invade the plant (Wolk and Sorkar 1994). On the other hand, the destructive effect of *T. harzianum* could be explained as direct mycoparasitism or through inducing enzymes and/or antifungal substances that affect pathogenic fungi growth and pathogenesis (Turner, 1971; Abd-El-moity, 1981; Martin and Hancock, 1987; and Padares *et al.,* 1992).

Effect of using formulated plant extracts in powder or liquid form on the percentage of bean root rot disease incidence in two successive seasons under greenhouse conditions:

Data in Table 5 showed that bean plants previously tested with different formula (powder and /or suspension) of various plant extracts resulted significant control of disease incidence

Table 2. Effect of the antagonistic microorganisms on reduction percentage of the linear mycelial growth of the pathogenic fungi

Different antagonistic microorganisms	% of Reduction in the linear growth of the pathogenic fungi	
	Rhizoctonia solani	*Fusarium solani*
Trichoderma harzianum	82.1	85.5
Bacillus subtilis	32.2	38.8
Control	00.0	00.0
L. S. D .at 5%	0.23	0.21

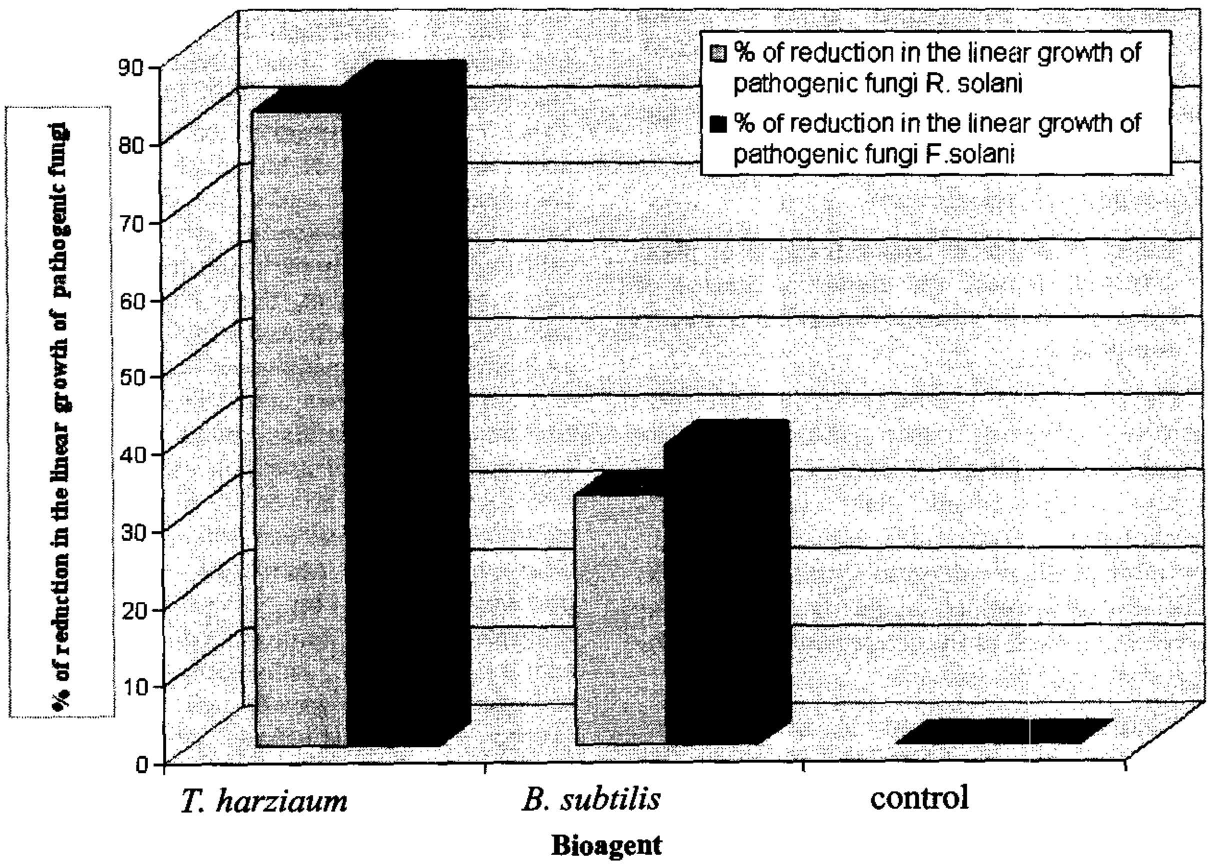

Fig. 2. Effect of the antagonistc microorganisms on reduction percentage of the linear mycelial growth of the pathogenic fungi

Table 3. Effect of different concentrations of plant extracts on root rot disease incidence of bean for two seasons under greenhouse conditions

Different treatments	First season 2010			Second season 2011		
	damping off (%)		Surviving plant (%)	damping off (%)		Surviving plant (%)
	Pre	Post		Pre	Post	
Garlic						
10 %	12	8	80	11	6	83
5 %	17	9	74	15	7	78
2.5 %	25	11	64	24	11	65
Neem						
10 %	15	12	73	14	12	74
5 %	20	14	66	20	13	67
2.5 %	29	20	51	30	19	51
Camphor						
10 %	35	13	52	35	10	55
5 %	38	18	44	39	17	44
2.5 %	40	19	41	40	18	42
control	45	15	40	47	18	35
L.S.D.	0.31	0.22	0.24	0.32	0.21	0.23

Table 4. Effect of different doses of antagonistic bioagent (as soil drench) on bean root rot disease percentage for two successive seasons under greenhouse conditions

Different Treatments	First season 2010			Second season 2011		
	damping off (%)		Surviving plant (%)	damping off (%)		Surviving plant (%)
ml /kg seed	Pre	Post		Pre	Post	
Trichoderma harzianum						
100	6	2	92	5	0	95
50	12	4	84	12	5	83
25	15	5	80	14	4	82
Bacillus subtilis						
100	4	3	93	0	0	100
50	6	6	88	6	7	87
25	11	7	82	13	6	81
controll	45	15	40	47	18	35
L.S.D.	0.41	0.32	0.23	0.26	0.31	0.33

Table 5. Effect of using formulated plant extracts in powder or liquid form on the percentage of bean root rot disease incidence in two successive seasons under greenhouse conditions

Different treatments	First season 2010			Second season 2011		
	damping off (%)		Surviving plant (%)	damping off (%)		Surviving plant (%)
	Pre	Post		Pre	post	
Garlic ex.						
Suspension	11	7	82	10	7	83
Powder	10	5	85	9	4	87
Neem ex.						
Suspension	14	10	76	15	9	76
Powder	12	9	79	11	10	79
Camphor ex.						
Suspension	35	11	54	35	11	54
Powder	30	12	58	30	8	62
control	45	15	40	47	18	35
L.S.D.	0.31	0.22	0.42	0.32	0.25	0.44

if compared with the control treatment. Data also showed that powder form of different plant extracts was the most effective in reducing root-rot disease incidence of bean. Thus, it could be explained that food base or it's formula played an important role on efficacy of the treatment (Abd-El-moity, 1981). However, garlic extract was the most significant in depressing disease incidence if compared with neem and camphor extracts.

Effect of various formulae of different bioagents on the percentage of bean root rot disease incidence in two successive seasons under greenhouse conditions

Data in Table 6 showed that, suspension form of the different bioagents used for seed spraying was more effective than powder form used as seed dressing in controlling root-rot disease of bean. Data also indicated that, *B. subtilis* had better effect than *T. harzianum* in reducing the disease incidence. An action of *B. subtilis* is due to that, the bacterium grown faster and occupy the court of infection and producing high level of antibiotics (Ryder *et al.*, 1999) that delay pathogenic fungal growth or its action. This fast growth and production of antibiotics act as barrier surrounded the root tissues and prevent pathogens to invade these healthy plant tissues. *Trichoderma* spp. Also act through certain mechanisms including mycoparesitism (Harman, 2006) and may also act through enzyme and/or antifungal substances production (Hayes, 1992 and Bolar *et al.*, 2000).

Table 6. Effect of various formulae of different bioagents on the percentage of bean root rot disease incidence in two successive seasons under greenhouse conditions

Different Treatments	First season 2010			Second season 2011		
	damping off (%)		Surviving plant (%)	damping off (%)		Surviving plant (%)
	Pre	Post		Pre	Post	
			Trichoderma harzianum			
Seed dressing	11	3	86	10	3	87
Seed spraying	7	2	91	6	2	92
			Bacillus subtilis			
Seed dressing	8	7	85	7	5	88
Seed spraying	4	4	92	4	1	95
control	45	15	40	47	18	35
L.S.D.	0.31	0.41	0.42	0.41	0.20	0.42

REFERENCES

Abd El-Khair, H. and N.S. El-Mougy (2003). Field biological approach under organic cultivation conditions for controlling garlic black mould disease infection during storage. Egypt. J. Appl. Sci., 18 (6): 50-69.

Abd-El-Moity, T.H. (1981). Further studies on the biological control of white rot disease of onion. Ph D. Thesis, Fac. Agric., Minufiya Univ., Egypt.

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

Abd-El-Moity, T.H. and M.N. Shatla (1981). Biological control of white rot disease of onion *sclerotium cepivorum* by *Trichoderma harzianum*. Phytopathology Z., 100: 29-35.

Abd-El-Monaim, M. F. (2011). Integrated management of damping-off, root and/or stem rot diseases of chickpea and efficacy of the suggested formula. Nat. Sci. Biol., 3 (3): 80-88.

Abo-Elnaga,H.I.G. (2006). *Bacillus subtilis* as a bio-control agent for controlling sugar beet damping off disease. Egypt. J. Phytopathology, 34 (1): 51-59.

Amadioha, A. (2004). Control of black rot of potato caused by *Rhizoctonia bataticola* using some plant leaf extracts. Arch. Pathol. Plant Prot., 37: 111-117.

Asaka, O. and M. Shoda (1996). Biocontrol of *Rhizoctonia solani* root rot on tomato with *Bacillus subtilis* RB14. Applied and Environmental Microbiology, 62:4081-4085.

Bailey, B.A., H. Bae, M.D. Strem, J. Crozier, S.E. Thomas, G.J. Samuels, B.T. Vinyard and K.A. Holmes (2008). Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. Bio Control, 46: 24-35.

Barnett, H.L. and B.B. Hunter (1996). Illustrated genera of imperfect fungi. Burgess Publ. Co., Minnesota.

Bennink, M. (2005). Eat beans for good health. Annual Report Bean Improvement Cooperative, 48 : 1-5.

Bolar, J.P., J.L. Norelli, K.W. Wong, C.K. Hayes, E. Harman and H.S. Aldwlnckle (2000). Expression of endochitinase from *Trichoderma harzianium* in transgenic apple increase resistance to apple scab and reduce vigor. Phytopathology, 90: 72-77.

Bowers, J.H. and J.C. Locke (2000). Effect of botanical extracts on population density of *Fusarium oxysporum* in soil and control of *Fusarium* wilt in the greenhouse. Plant Dis., 88: 300-305.

Brain, P.W. and H.G. Hemming (1945). Gliotoxin a fungistatic metabolic product of *Trichoderma viride*. Ann. Appl. Biol., 32: 214-220.

Buruchara, R.A. (2006). Background information on common beans (*Phaseolus vulgaris* L). Biotechnology, Breeding and Seed Systems for African Crops. The Rockefeller Foundation, Nairobi, Kenya.

Dai, J., V. Yaylayan, G.S.V. Raghavan, J.P.R. Pare and Z. Liu (2001). Multivariate calibration for the determination of total azadirachtin related limonoids and simple terpenoids in neem extracts using vanillin assay. J. Agric. Food Chem., 49: 1169-1174.

Dowson, W.J. (1957). Plant diseases due to bacteria. Second Ed., Cambridge the University Press, London.

Dubey, S.C., M. Suresh and S. Birendra (2007). Singh Evaluation of *Trichoderma* species against *Fusarium oxysporum* f.sp. *ciceris* for integrated management of chickpea wilts. Biological Control, 40: 118–127.

Ehteshamul, H.S., M.J. Zaki, A.A. Vahidy and Abdil A. Ghaffar (1998). Effect of organic amendments on the efficacy of *Pseudomonas aeruginosa* in the control of root rot disease of sunflower. Pak. J. Bot., 30:45-50.(C.F.Rev .Pl. Pathol.,78:1486).

Ferreria, J.H.S., F.N. Matthee and A.C. Thomas (1991). Biological control of *Eutypa lota* on grapevine by antagonistic strain *Bacillus subtilis*. Phytopathology, 81: 283-287.

Gilman, J.C. (1957). A manual of soil fungi-Iowa state University press, Ames, Iowa, U.S.A.

Harman, G.E. (2006). Overview of mechanisms of uses of *Trichoderma* spp. Phytopathology, 96: 190-194.

Hassanein, A.M., A.M. El-Garhy and G.A.A. Mekhemar (2006). Symbiotic nitrogen fixation process in faba bean and chickpea as affected by biological and chemical control of root-rot. J. Agric. Sci., Mansoura Univ., 31 (2): 963-980.

Hawker, L.E. (1960). Physiology of fungi. Univ. of London Press, LTD War-Wich Square, London.

Hayes, C.K. (1992). Improvement of *Trichoderma* and *Gliocladium* by genetic manipulation. 227-266 pp. in biological control of plant diseases progress and challenges for the future. Thomas, E. C.; G. C. Papuvizas and R. J. cook (eds). Plenum Press, New York and London Published in Cooperation with NATO Scientific Affairs Division.

Howell, C.R. (2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The History and evolution of current concepts. Plant Dis., 87: 4-10.

Howell, C.R. and R.D. Stipanovic (1995). Mechanism in the biocontrol of *Rhizoctonia solani* induced cotton seedling disease by *Gliocladium virens*: Antibiosis. Phytopathology, 85: 469-472.

Husain, A., O.P. Virmani, S.P. Popli, L.N. Misra, M.M. Gupta, G.N. Srivastava, Z. Abraham and A.K. Singh (1992). Dictionary of Indian Medicinal Plants. CIMAP, Lucknow, India.

Ismail, A.A. (2011). Epidemics of *Fusarium* species on cucumber and their control. PhD Thesis, Fac. Agric., Minufiya Univ., Egypt.

Jarvis, A.P.E. and D. Morgan (2000). Analysis of small samples of limonoids of neem (*Azadirachta indica*) using solid phase extraction from tissue culture. Photochem. Anal., 11: 184-189.

Joy, P.P., J. Thomas, S. Mathew and B.P. Skaria (1998). Medicinal Plants,Kerala Agri. Unv. Aromatic and Medicinal Plants Research Station, Odakkali, Asamannoor P.O., Ernakulam District, Kerala, India. Kurucheve, V. and R. Padmavathi (1997). Fungi toxicity of selected plant products against *Pythium aphanidermatum*. Indian Phytopathol., 50: 529-535.

Lee, S.E., J.E. Kim and H.S. Lee (2001). Insecticide resistance in increasing interest. Agric. Chem. Biotechnol., 44: 105-112.

Martin, F.N. and J.C. Hancock (1987). The use of *Pythium oligandrum* for biological control of pre emergence damping off caused by *P. ultimum*. Phytopathology, 77: 1013- 1020.

Momin, R.A. and M.G. Nair (2001). Mosquitocidal, nematicidal and antifungal compounds from *Apium graveolens* L., seeds. J. Agric. Food Chem., 49: 142-145.

Morsy, E.M. (2005). Role of growth promoting substances producing microorganisms on tomato plant and control of some root rot fung. Ph.D. Thesis, Fac. of Agric.; Ain Shams Univ., Cairo.

Morsy, E.M., K.A. Abdel-Kawi and M.N.A. Khalil (2009). Efficiency of *Trichoderma viride* and *Bacillus subtilis* as a bio-control agents against *Fusarium solani* on tomato plants. Egypt. J. Phytopathology, 37 (1) : 47-57.

Moslem, M.A. and E.M. El-Kholie (2009). Effect of neem (*Azardirachta indica* A. Juss) seeds and leaves extract on some plant pathogenic fungi. Pakistan Journal of Biological Sciences, 12: 1045-1048.

Mossini, S.A., K.P. Oliveira and C. Kemmelmeier (2004). Inhibition of patulin production by *Penicillium expansum* cultured with neem (*Azadirachta indica*) leaf extracts. Basic Microbiol., 44: 106-113.

Nzungize, J., P. Gepts, R. Buruchara, A. Male, P. Ragama, J.P. Busogoroand and J.P. Baudoin (2011). Introgression of *Pythium* root rot resistance gene into Rwandan susceptible common bean cultivars. African J. Plant Sci., 5 (3): 193-200.

Padares, D.F., J. Hockenhull, D.F. Jensen and S.B. Marthur (1992). *In vivo* screening of *Trichoderma* isolated for antagonism against *Sclerotium rolfsii* using rice seedlings. Bulletin oil B/SROP, 15: 33-35. (C.F.Rev. P1. Pathol., 72 (I): 208).

Rojo, F.G., M.M. Reynoso, M.F. Sofia, N. Chulze1 and A.M. Torres (2007). Biological control by *Trichoderma* species of *Fusarium solani* causing peanut brown root rot under field conditions. Crop Protection, 26 : 549-555.

Ryder, M.H., Z.H. Yan, T.E. Terrace, A.D. Rovira, W. Tang., R.L. Correll, Z.H. Yan and W. Tang (1999). Use of strains of *Bacillus* isolated in China to suppress take all and *Rhizabacteria* root rot and to promote wheat seedling growth of glasshouse grown wheat in Australian soils. Soil Biology and Biochemistry, 31 (1): 19-29.

Sallam, N.M.A., K.A.M. Abo-Elyousr and M.A.E. Hassan (2008). Evaluation of *Trichoderma* species as biocontrol agents for damping-off and efficacy of suggested formula. Egypt. J. Phytopathol., 36 (1-2):81-93.

Sanjeet, K., J.P. Upadhyay and S. Kumar (2005). Evaluation of plant extracts for control of *Alternaria* leaf spot of *Vicia faba*. Annals Pl. Protect. Sci., 13: 258-259.

Shalaby, M.S. and M.M. Atia (1996). Biological and chemical control of watermelon and cantaloupe root-rot in the reclaimed soils. Zagazig J. Agric. Res., 22: 1113-1130.

Sharma, M. and S.K. Gupta (2003). Ecofriendly method for the management of root rot and web blight (*Rhizoctonia solani*) of French bean. Journal of Mycology and Plant Pathology, 33: 345–361.

Siddiqui, Z.A. and M.S. Akhtar (2007). Biocontrol of a chickpea root rot disease complex with phosphate-solubilizing microorganisms. J of Plant Pathology, 9 (1): 67-77.

Singh, R.S. (1982). Plant Pathogens "The Fungi" Oxford and IBH Publishing New Delhi, Bombay, Calcutta.

Sitara, N.U.I., J. Naseem and N. Sultana (2008). Antifungal effect of essential oils on *in vitro* growth of pathogenic fungi. Pak. J. Bot., 40 (1): 409-419.

Snedecor, G.W. and W.G. Cochran (1980). Statistical Methods 7th edition Oxford and J.BH Publishing Com.

Sobti, A.K., R.L. Meena and A.K. Nag (2005). Evaluation of rhizobacteria against downy mildew of pearl millet. Journal of Mycology and Plant Pathology, 35: 341–342.

Suriachandraselvan, M. and K. Seethuraman (2002). Efficacy of *Trichoderma viride* in combination with fungicides against *M. phaseolina* (Tassi) Goid. infection in blackgram. Madras Agric. J., 89: 512–514.

Tohamy, M.R.A., A.Z. Aly, T.H. Abd-El-Moity, M.M. Atia and M.L. Abd-El-Moneim (2002). Evaluation of some plant extracts in control damping-off and mildew diseases of cucumber. Egypt. J. Phytopathl., 30 (2):71-80.

Turner, W.B. (1971). Fungal Metabolites. Academic press, London, New York.

Weindling, R. (1932). *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology, 22: 837-845.

Widers, I.E. (2006). The beans for health alliance: A public-private sector partnership to support research on the nutritional and health attributes of beans. Annual Report Bean Improvement Cooperative, 49: 3-5.

Williams, R.J. (1975). Disease of cowpea *Vigna unguiculata* (L.) Walp. In Nigeria PANS, 21: 253-267.

Wolk, M. and S. Sorkar (1994). Antagonism *in vivo* of *Bacillus* spp. against *Rhizocotonia solani* and *Pythium* spp. Angeiger fur schanding skundey PF lanzenchutz, umweltschutz, 67:1-5 (C.F. Rev Pl. Path, 73 (6): 4601.

Wortmann, C.S. and D.J. Allen (1994). African bean production environments. Their definition, characteristics, and constraints. Network on Bean Research in Africa. Occasional Publication Series No. 11, Dar esSalaam, Tanzania, p: 47.

Wortmann, C.S., R.A. Kirkby, C.A. Aledu and D.J. Allen (1998). Atlas of common bean (*Phaseolus vulgaris* L.) production in Africa. Centro Internacionalde Agricultura Tropica, Cali, Colombia, p: 131.

Yadav, B., R.P. Gupta and R.V. Singh (2005). Effect of neem kernel extract on mycelial growth and wilt management in pigeonpea. Journal of Mycology and Plant Pathology, 35: 578.

Zaghloul, R.A., E.A. Hanafy, N.A. Neweigy and N.A. Khalifa (2007). Application of biofertilization and biological control for tomato production. 12th Conference of Microbiology; Cairo, Egypt, (18-22) March, 198-212.

تثبيط عفن جذور الفاصوليا باستخدام بعض المستخلصات النباتية والكائنات النافعة تحت ظروف المعمل والصوبة

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تم استخدام ثلاثة مستخلصات مائية وهي (أوراق النيم والكافور وفصوص الثوم) واثنين من الكائنات النافعة وهما (فطر الترايكوديرما هازيانم وبكتريا الباسيلس ساتلس) وذلك لتقييم قدرتهما التضاديه لفطر ريزوكتونيا سولاني وفيوزاريم سولاني وذلك تحت ظروف المعمل والصوبة. اعطى مستخلص الثوم أعلى تثبيط في النمو الميسليومي لكلا الفطرين ريزوكتونيا سولاني وفيوزاريم سولاني و ذلك مقارنة بمعاملة المقارنة.اظهرت الترايكوديرما هارزيانم تأثير معنوى فى خفض النمو الميسليومي لكل من فطر ريزوكتونيا سولاني وفيوزاريم سولاني وذلك مقارنة بمعاملة المقارنة. تم استخدام تركيزات مختلفة من المستخلصات النباتية وهي ١٠٪، ٥٪، ٢,٥٪ لمقاومة مرض موت البادرات في الفاصوليا وذلك تحت ظروف الصوب التجارية. تم ملاحظة علاقة طردية بين تركيز المستخلصات النباتية ونسبة مقاومة مرض موت البادرات. اعطى مستخلص الثوم اعلي درجة مقاومة لمرض موت البادرات و ذلك مقارنة بالمستخلصات الاخرى او معاملة المقارنة. تم استخدام جرعات مختلفة من كائني المقاومة الحيوية وذلك بمعدل ١٠٠، ٥٠، ٢٥ مل/ كجم بذرة، وأظهرت النتائج أن كلا الكائنين نجحا في مقاومة المرض ولوحظ أن بكتريا الباسيلس ستلس أعطت نتائج افضل من الترايكوديرما هارزيانم .كما لوحظ وجود علاقة طردية بين الجرعة المستخدمة من كلا الكائنين ونسبة النباتات الباقيه حيه. تم استخدام كل من المستخلصات النباتيه والكائنات الحيه تحت الدراسة في صورتين مختلفتين وهما معلق ومسحوق، ولقد اعطى مسحوق المستخلصات النباتيه نتيجة افضل من المعلق، كما اعطى مسحوق الثوم أفضل نتيجة لمقاومة المرض و ذلك مقارنة بالمستخلصات الأخرى أو معاملة المقارنة. أعطى معلق الكائنات الحيه الدقيقة نتيجة افضل من المسحوق لمقاومة المرض، كما لوحظ ان نتائج بكتريا الباسيلس ستلس كانت أفضل إذ ما قورنت بنتائج الفطر التريكوديرما هارزيانم في مقاومة المرض.