Integrated Control of Pathogens Associated with Bean Seeds

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Abstract: The associated fungi of bean seeds were purified and identified as *Alternaria alternata, Aspergillus* spp*., Botrytis cinerea, Fusarium solani, Macrophomina phaseolina, Penicillium* sp*., Rhizoctonia solani, Sclerotinia sclerotiorum, Trichoderma viride and Verticillium* sp. Four tested fungi (*R. solani, M. phaseolina, F. solani,* and *S. sclerotiorum*) were proved to be pathogenic producing different degrees of pre-emergence (PRD), and post-emergence (PTD) damping-off and root rot symptoms on bean cultivars: Contender, Narina, Bolista and Giza-6. The most virulent isolates inducing PRD were *F. solani, M. phasiolina* and *R. solani* whereas *S. sclerotiorum* was the least virulent. Moreover, the highest levels of PTD were obtained with *S. sclerotiorum, F. solani,* and *R. solani,* whereas *M. phasiolina* was the least virulent. Bolista cultivar was the most compatible with PRD and PTD in all tested pathogenic fungi, whereas Giza-6 showed the lowest compatibility. The highest percentage of root-rot infection were obtained in Bolista cultivar with the tested fungi ranged from (70-80 %) and Narina (70-75 %), while the least infection was obtained in Giza-6 (55-60 %). Plant oils (Cinnamon, Clove, Spearmint and Lemon) were tested for their antifungal activities against the four tested pathogenic fungi *in vitro* and significantly reduced in their radial growth. Spearmint oil exhibited the highest antagonistic effect to the tested fungi followed by Clove and Cinnamon oils, while the least effect was Lemon oil compared with control. Hyphal growth of *R. solani* and *S. sclerotiorum* was completely inhibited with 100 % and 75 % conc. with Spearmint oil, while *R. solani* was completely inhibited by 100 % conc. with Cinnamon oil. In addition, the biological agents *Trichoderma viride*, *T. harzianum*, *T. koningii* and *Bacillus subtilis* were used *in vitro* to test their effectiveness against the four tested pathogenic fungi. *In vitro* experiment showed that all biological agents significantly reduced the linear growth of fungi. *T. harzianum* showed highly antagonistic effect, the growth reduction ranged from (75.6 to 77.8 %), while *B. subtilis* was the least, growth reduction ranged from (56.7 to 65.6 %) to the tested pathogenic fungi. Pot experiment In general, The tested treatments were evaluated in a pot experiments and resulted in significant effects on controlling pre- and post-emergence damping-off as well as root-rot diseases of bean caused by *R. solani*, *M. phaseoilina*, *F. solani*, and *S. sclerotiorum* when compared to the untreated control. In addition, treatment with *T. harzianum* was superior to the rest of the other treatments in controlling such studied diseases.
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1. Introduction

Bean (*Phaseolus vulgaris,* L.) is one of the important vegetables grown in Egypt and many parts of the world. Seeds play a vital role for the healthy production of the crop, but can be seedborne of important diseases, which causes considerable reduction in yield (EL-Ahmed 1996 and EL-Gali, 2003). Many reports about bean seedborne diseases were published and the principal pathogens associated were *A. alternata, F. solani, F. semitictum, F. equiseti, M. phaseolina, Penicillium* sp. and *R. solani,*. (Lazzaretti, *et al*., 1994 and Ramadan, 1989). Bean plants are commonly exposed to attack by many serious soilborne fungi i.e. *F. semitectum, F. oxysporum, F. solani, Pythium* spp*., R. solani, M. phaseolina, S. rolfsii, S. sclerotiorum* and *Verticillium* spp. (Lacicowa and Pieto, 1997 and EL-Gali, 2003). Most of these fungi cause damping-off and root rot diseases (Abada *et al*., 1992, El-Samra *et al*., 2006 c), leading to great economic losses in crop yield and quality. However, the modern approach in disease control was directed toward minimizing the fungicidal use to avoid environmental pollution (Oliveira *et al*., 1999). Thus, the approach of integrated pest management (IPM) was applied. One important compound of these strategies includes seed or soil treatments with extracts of some aromatic and medicinal plants which proved to be highly efficient in suppressing mycelial growth and spore germination of many plant pathogens (Hassanein and El-Doksch, 1997, El-Samra *et al*., 2006 b). Many of the essential plant oils were proved to be efficient in suppressing different pathogens whether *in vitro* or *in vivo*. Among these oils were Spearmint (El-Korashy, 1997), Clove oil (El-Safwani and Nasif 2002), Cinnamon and Lemon oils (Youssef, 2008). Sharaf El-Din *et al*., (2007) reported that antifungal activities were appeared by Cinnamon, Clove, Peppermint and

Eucalyptus oils against *F. oxysporum* and *Aspergillus niger in vitro*. Also, biological control succeeded to prevent many plant diseases, offering an attractive alternative or supplement to pesticides, raising the plant growth and yield and reducing density of soil-borne pathogens (El-Samra *et al*., 2006 a). *Trichoderma* spp. and *Bacillis subtilis* are among the most promising biocontrol agents which applied against a wide range of plant pathogenic fungi (El-Kazzaz *et al*., 2002).

The objectives of this study were to (1) survey the most common damping-off and root rot fungi associated with bean seeds, (2) study the susceptibility of some bean cultivars to infection with damping-off and root rot pathogens, and (3) evaluate the antagonistic effect of some plant oils, fungal and bacterial bioagents on the radial growth of the tested damping-off and root rot pathogens *in vitro* in addition to their effect on controlling such pathogens in the greenhouse.

2. Material and Methods

Isolation, purification and identification from seed samples:

Seed samples of bean (*Phaseolus vulgaris,* L.), were collected from different seed lots of seed stores located in some governorates in Egypt. Seed-borne fungi were isolated using agar-plate method (ISTA, 1966 and EL-Gali, 2003). Seeds were surfaces sterilized by dipping in 1% sodium hypochlorite for two minutes, then rinsed thoroughly in several changes of autoclaved distilled water. The seeds were left to dry, then mounted on potato dextrose agar (PDA) medium (5 seeds per dish). The dishes were incubated at 25 °C and examined periodically. The developing fungi associated with seeds were transferred on PDA medium and kept for purification and identification. Purification was carried-out using either single-spore or hiphal-tip isolation techniques. Identification of the isolated fungi was carried-out according their morphological features under the compound microscope (Barnett and Hunter (1972), Booth (1971) and Ellis (1971). The identification was verified by the Mycological staff of Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Inoculation and determination of pathogenicity:

Throughout this study four fungal isolates, isolated from bean seed were used: *F. solani, M. phaseolina, R. solani,* and *S. sclerotiorum.* These isolates were individually tested for their pathogenic potential on Contender, Narina, Bolista and Giza-6 bean cultivars under greenhouse conditions. Clay pots (20 cm in diameter) were sterilized and filled with autoclaved aerated sand: clay soil (1:1 w/w). Fungal inocula were grown on sterilized barley grains-sand medium (30 gm barley grains, 10 gm sand, 30 ml water) at 25 °C for 2 weeks. Soil infestation was carried-out using the inoculum of each fungus at the rate of 4% of soil weight. Inoculum was mixed thoroughly with the soil in each pot, watered and left for one week to secure establishment of the tested fungi. Control pots were filled with the same soil mixed with the same amount of sterilized barley grains-sand medium (non-infested soil). A set of four pots with 10 seeds per pot, was used for each tested fungus. Bean seeds of each cultivar were surface-sterilized and sown, watered regularly every 3 days under greenhouse conditions. Determination of the number of pre- and post-emergence damping-off, seedling survival and root rot incidence were calculated as percentages and expressed as transformed arcsine numbers (Snedecor and Cochran, 1981).

Antagonistic effect of some plant oils:

(A) Sources of plant oils

Several plant oils were tested for their antifungal activities against damping-off and root rot diseases in bean seedlings: Cinnamon (*Cinnamomum zylanicum* L.), Clove (*Syzygium aromaticum* L.), Spearmint (*Mentha viridis* L.), and Lemon (*Citrus aurantifolia*). These plant oils were obtained from Horticulture Research Institute, Aromatic and Medicinal Plants Department, Giza, Egypt.

(B) *In vitro* experiment:

Different concentrations of the tested plant oils, i.e., 0, 12.5, 25, 50, 75 and 100% were diluted using acetone and few drops of Tween 40 and sterilized distilled water. Petri-plates (9 cm in diameter) with PDA medium were used. Five mm disc of 7 days old culture of each tested fungus was placed at the edge of the Petri-plate. On the opposite side 5 mm sterilized filter paper discs (Whatman No. 1) were saturated with 50 µl of plant oils and placed. Control treatment was carried-out using sterilized water instead of plant oils. Four replicates were used for each treatment. The plates were incubated at 25 ± 2°C for 7 days, linear growth and the percentage of reduction in the mycelial growth was calculated according to formula proposed by Ferreira *et al*., (1991).

Antagonistic effect of certain bioagents:

(A) Sources of bioagents

Certain antagonistic microorganisms, i.e. *Trichoderma viride*, *T. harzianum*, *T. koningii* and *Bacillus subtilis* were used *in vitro* to evaluate their effectiveness against the four tested pathogenic fungi. Purified and identified cultures of *T. viride* from bean seeds, *T. harzianum* from tomato plants, and *T. koningii* from strawberry plants were used. These isolates were verified by the Mycology staff, Plant Pathology Research Institute, Agric. Res. Center, Giza. An isolate *B. subtilis* has antagonistic effect capability

was obtained from Plant Pathology Dept., Fac. of Agric. Alexandria University.

(B) *In vitro* experiment

Antagonistic effect of *T. viride*, *T. harzianum* and *T. koningii* on the linear growth of the four tested pathogens, (*R. solani*, *M. phasiolina*, *F. solani* and *S. sclerotiorum*), was carried-out in Petri plates containing PDA medium, each plate was divided into two equal halves, one half was inoculated with a disc (5 mm in diameter) of 5 day-old culture of *T. viride* or *T. harzianum* or *T. koningii,* whereas the opposite half was inoculated with an equal disc of 7-days old culture of any of the four tested pathogenic fungi (Dhingra and Sinclair, 1985). Plates were then incubated at 25 ± 2°C for 7 days. Control plates were inoculated with discs of PDA medium instead of the bioagents. Four replicates were used for each treatment. In order to study the antagonistic effect of the bacterial isolate of *B. subtilis* on the growth of the four pathogenic fungi, the bacterium was streaked at one side on PDA medium plates and incubated for 24 hrs at 30°C, then one disc (5 mm in diameter) bearing 7-day old growth of one of the tested fungi was placed on the opposite side at 25 mm distance. Plates were incubated for 7 days at 25 ± 2°C. Control plates were streaked with sterilized distilled water instead of the bioagent. Four plates were served for each treatment. Linear growth (cm) of the pathogenic fungi was determined at the end of the experiment in each treatment and percentage of reduction in the linear growth of the tested fungi was calculated by using the formula as proposed by Ferreira *et al.* (1991).

The effect of plant oils and biological control agents on damping-off and root-rot diseases of bean in the greenhouse:

A greenhouse experiment was carried-out to test the both effect of plant oils (Cinnamon, Clove, Spearmint, and Lemon) at the concentration of 75 % each and the tested biological control agents (*T. viride*, *T. koningii*, and *T. harzianum*) on controlling damping-off and root-rot diseases of bean. Autoclaved clay pots, 20 cm in diameter, were filled with previously aerated autoclaved sand: clay soil (1:1 w/w). The tested pathogens, (*R. solani*, *M. phaseolina*, *F. solani*, and *S. sclerotiorum*), were separately grown on autoclaved barley grains medium in 500 ml glass bottles. Soil infestation was done by adding the previous inocula of each fungus to each pot at the rate of 4 % of soil weight. Pots were watered every other day for 8 days to ensure the establishment of isolates in the soil. Spore suspension of each of the tested *Trichoderma* spp., previously grown on Czapek-Dox liquid medium for 10 days at the rate of 10^6 / ml of each species was added to each pot, 3 days before sowing. Bean seeds (Bolista, cv.) were sown in each pot at the rate of 10 seeds each. Five pots were served for each treatment as replicates. The tested plant oils, 75 % each were added, (25 ml/pot), upon seed germination. A set of infested pots with the above mentioned pathogens and did not receive either the plant oils or the biological control agents were served as control. Data were recorded as percentage of pre-, post-emergence damping-off and root-rot incidence after 30 and 40 days from sowing date respectively.

Statistical analysis:

Completely randomized design was used in the above expriments. Percentage data were transformed into arcsine angles (Snedecor and Cochran, 1981) before carrying out analysis of variance (ANOVA) to produce approximately constant variance. Least significant difference (LSD) and standard deviation (SD) value at 5% level of probability were applied for comparing treatment means (Snedecor and Cochran, 1981).

3. Results and Discussion

Survey of bean seed-borne fungi:

Seed-borne fungi were isolated from bean seed samples as previously described in the section of materials and methods. The most frequently isolated fungi were *A. alternata*, followed by *Aspergillus* sp., *R. solani*, and *Penicillium spp.*, while the lowest isolated fungi were *M. phaseolina* followed by *F. solani, S. sclerotiorum, Trichoderma viride, Verticillium* spp. Moreover, most of these fungi were recorded as damping-off and root rot pathogens of bean. *F. solani* has been shown to reduce seed germination and seedling damping-off in bean (Ziedan, 1980 and EL-Gali, 2003). Whereas, *M. phaseolina* cause ashy stem blight and *R. solani* cause Rhizoctonia root rot (Godoy-Lutz *et al*., 1996). Obkura *et al*., 2009 reported that of *R. solani* infecting bean in New York have acquired the ability to infect corn, and a correlation between aggressiveness on corn and bean was observed. Moreover, *S. sclerotiorum* causes white mould of bean (Ziedan, 1980). Also, most of these fungi were isolated from many vegetable and field crops, other than bean, as damping-off and root rot pathogens, (Ibrahim, 1996, Mao, *et al*., 1998 and El-Samra *et al*., 2006 c).

Pathogenicity and varietals responses:

(A) Pre-emergence damping-off (PRD)

Data presented in Table (1) revealed that: (1) All the tested isolates induced significant PRD symptoms on Contender cultivar, however, the infection percentage differed according to the tested isolate. Infection values were significantly higher in case of *F. solani* and *R. solani* (33.2, 31.0 respectively), compared with control (9.1). The least virulent isolates were *M. phasiolina* and *S. sclerotiorum* (28.8). (2) In Narina cultivar, the highest infection values were obtained by *M. phasiolina*, *F. solani* and *R. solani*

(41.1, 37.2 and 35.0 respectively), whereas *S. sclerotiorum* treatment gave the least infection values (30.9). (3) In Bolista cultivar, the highest PRD occurred by *M. phasiolina* (43.1) followed by *R. solani* (37.2) and *F. solani*, *S. sclerotiorum* (35.0). (4) Giza-6 cultivar was significantly affected by the tested isolates *F. solani* (31.0), *R. solani* (28.8) and *M. phasiolina, S. sclerotiorum* (26.6). Finally, it could be concluded that the most virulent isolates inducing PRD were *F. solani*, *M. phasiolina* and *R. solani,* whereas *S. sclerotiorum* was the least virulent isolate tested. Moreover, Bolista cultivar was the most compatible cultivar with PRD agents, especially with *M. phasiolina* and *R. solani,* compared with the other tested cultivars, whereas Giza-6 cultivar showed the lowest compatibility.

(B) Post-emergence damping-off (PTD)

Data presented in Table (1) showed the following: (1) The highest levels of infection percentage values of PTD were obtained with *S. sclerotiorum, F. solani* and *R. solani,* whereas *M. phasiolina* isolate was the least virulent. This was true for all the tested cultivars. (2) In addition, each of Bolista and Narina cultivars showed relatively higher rates of compatibility to *S. sclerotiorum* (41.1 and 33.2 respectively), *F. solani* and *R. solani* (39.2 and 35.2 respectively). Giza-6 cultivar showed the lowest compatibility.

(C) Seedling survival

From data presented in Table (1) the following could be concluded:

(1) The highest survival rates were recorded when Giza-6, and Contender cultivars were inoculated with all the tested isolates (from 52.8 to 39.2).

(2) The moderate survival rates were recorded when Narina cultivar was inoculated with *F. solani* (33.0), *R. solani* and *M. phasiolina* (35.2).

(3) The least survival rates were recorded in Bolista cultivar in all the tested isolates (from 28.1 to 30.3).

Table (1): Infection index of some bean damping-off and root rot pathogens on different bean cultivars.

Fungi	Index values														
	Pre-emergence damping- off					Post-emergence damping- off					Survival				
	Cultivars														
	Contender	Narina	Bolista	Giza-6	Mean	Contender	Narina	Bolista	Giza-6	Mean	Contender	Narina	Bolista	Giza-6	Mean
Rhizoctonia solani	31.0 ^{b}	35.0 ^{b}	37.2 ^{b}	28.8 ^{b}	33.0	31.0 ^{b}	35.2 ^{b}	39.2 ^{bc}	28.8 ^{b}	33.6	43.1 ^{a}	35.2 ^{a}	28.8 ^{a}	46.9 ^{a}	38.5
Macrophomina phasiolina	28.8 ^{b}	41.1 ^{b}	43.1 ^{b}	26.6 ^{b}	34.9	28.8 ^{b}	28.8 ^{b}	30.8 ^{b}	23.7 ^{b}	28.1	49.9 ^{a}	35.2 ^{a}	30.3 ^{a}	52.8 ^{a}	41.3
Fusarium solani	33.2 ^{b}	37.2 ^{b}	35.0 ^{b}	31.0 ^{b}	34.1	33.2 ^{b}	35.2 ^{b}	39.2 ^{bc}	26.6 ^{b}	33.6	39.2 ^{a}	33.0 ^{a}	30.3 ^{a}	46.9 ^{a}	37.5
Sclerotinia sclerotiorum	28.8 ^{b}	30.9 ^{b}	35.0 ^{b}	26.6 ^{b}	30. 3	31.0 ^{b}	33.2 ^{b}	41.1 ^{c}	31.0 ^{b}	34.1	45.0 ^{a}	41.1 ^{a}	28.1 ^{a}	46.9 ^{a}	40.3
Control	9.1 ^{a}	9.1 ^{a}	9.1 ^{a}	9.1 ^{a}	9.1	9.1 ^{a}	9.1 ^{a}	9.1 ^{a}	9.1 ^{a}	9.1	80.9 ^{b}	80.9 ^{b}	80.9 ^{b}	80.9 ^{b}	80.9

* Values are means of 4 replicates. * Values are the arcsine square root of transformation percentage of data.

* Values within the same column and followed by the same letter are not significantly different from each other according to L.S.D. ($p \leq 0.05$).

(D) Root-rot incidence

Results were presented in Fig. (1). Data indicated that all the tested isolates could induce root rot symptoms on all the tested cultivars. However, root-rot index differs according to the tested isolate and the inoculated cultivar. The highest infection percentages were obtained in Bolista cultivar in all tested isolates ranged from 70-85% and Narina cultivar ranged from 70-75%, while the least infection percentage was obtained in Giza-6 cultivar ranged from 55-60%.

The present study showed that *R. solani, F. solani* and *S. sclerotiorum* were highly pathogenic and causing high rates of PRD and PTD on bean seedlings. These findings were similar to those found on bean seedlings (El-Farnawany and Shama, 1996). Bilgi, *et al*., 2008 mentioned that Fusarium root-rot of bean, caused by *F. solani* is a major yield-limiting disease in North Dakota and Minnesota in USA and most commercial bean cultivars grown in those regions were susceptible. Giza-6 cultivar was the most incompatible with many of the tested pathogens, whereas Bolista and Narina cultivars were the most compatible. The detailed symptoms produced due to inoculation with the tested pathogens were in harmony with those recorded by (EL-Gali, 2003, Shama, 1989).

Antagonistic effect of some plant oils and their concentrations *in vitro*

This investigation was carried out to study the antagonistic effect of four plant oils: Cinnamon, Clove, Spearmint and Lemon against bean damping-off and root rot pathogens, (*R. solani*, *M. phasiolina*, *F. solani* and *S. sclerotiorum*). Linear growth of the tested fungi was recorded in Table (2). The following could be concluded:

(1) Significant antagonistic effect of Spearmint oil followed by Clove and Cinnamon oils. (2)

Undiluted Spearmint oil proved to be the most effective against in all the tested pathogens, followed by undiluted Clove and Cinnamon oils. While the least effect was obtained on undiluted Lemon oil compared with the control. (3) In this respect, hyphal growth of both *R. solani,* and *S. sclerotiorum* was completely inhibited by undiluted spearmint oil. Also, undiluted Cinnamon oil was completely inhibited *R. solani.* (4) Moreover, it was efficient in reducing the hyphal growth of each of *M. phasiolina (*66.7%), *S. sclerotiorum* (53.3%) and *F. solani* (50.0%) by undiluted Clove oil, whereas reducing *F. solani* (66.7%) and *M. phasiolina* (50.0%) by undiluted Spearmint oil. (5) Highly effect of Spearmint oil at conc.75% that completely inhibited the growth of both *R. solani* and *S. sclerotiorum*, while inhibited *F. solani* (66.7%) and *M. phasiolina* (38.9). Moreover, conc. 75% of Clove oil inhibited *R. solani* (62.2%), *S. sclerotiorum* (50.0%) and *F. solani* (41.1%). (6) Spearmint oil (50 %) affected the radial growth of *R. solani*, *S. sclerotiorum* (55.6%) and *F. solani* (41.1%). These findings were in agreement with those of Pattnaik *et al*., (1996), who found that Spearmint oil was among the most effective oils tested against *F. solani, F. oxysporum* and *M. phasiolina in vitro*. Similar results were found by many investigators on many plants (El-Samra *et al*., 2006 b). Youssef, 2008 mentioned that undiluted Spearmint oil completely inhibited growth of *S. sclerotiorum, F. solani, R. solani* and *M. phasiolina.* Efficacy of medicinal and aromatic plants against mycelial growth and spore germination of different pathogens was documented by Zedan *et al*., (1994) and Hassanein and El-Doksch (1997). The present results on the antifungal activity of Clove and Cinnamon oils on bean were in agreement with those obtained by El-Safwani and Nasif (2002), on the effect of these oils on the growth of damping-off fungal pathogens.

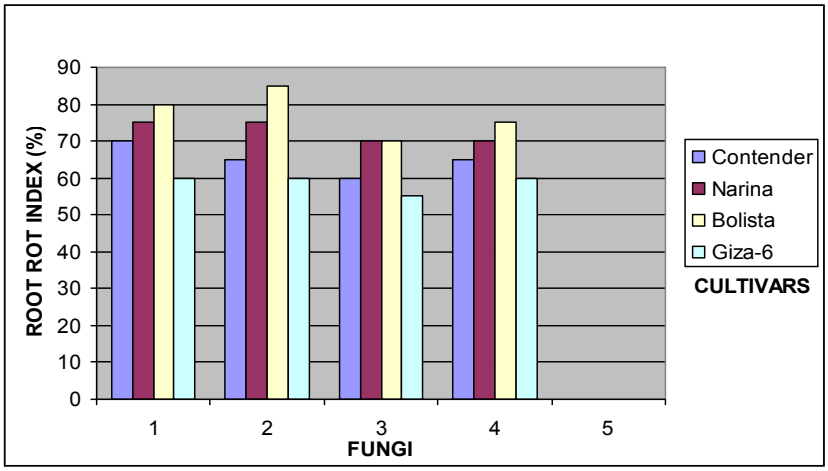

Fig. (1). Root-rot index (%) of the tested bean damping-off and root-rot pathogens on different cultivars where: (1) *R. solani*, (2) *M. phasiolina*, (3) *F. solani*, (4) *S. sclerotiorum* and (5) Control.

Antagonistic effect of some bioagents *in vitro*

Data obtained on the antagonistic effect of the bioagents against the tested damping-off and root rot pathogens were presented in Table (3). It was evident that more antagonistic effect on *F. solani* was induced by *T. koningii*, where growth was reduced by 80%. *S. sclerotiorum* proved to be less sensitive to the effect of *T. koningii* than the other tested pathogens (64.4%). These findings assured similar results published by several authors (El-Sharkawy *et al*., 1998, Abd El-Aal, 2001, El-Samra *et al*., 2006 a). *T. harzianum* also showed highly significant antagonistic effect on all the tested fungi, however, growth reduction ranged from (75.6 to 77.8%). Although *T. viride* effectively reduced the growth rates of all the tested fungal pathogen (70.0 – 76.7%), however, reduction was more pronounced in *F. solani* than the other tested pathogens. The least antagonistic effect was against *R. solani* (70.0%). Similar results of the effect of *T. viride* on different soil-borne fungi were recorded (Ismail, 1998, Amer and El-Desouky, 2000 and El-Samra *et al*., 2006 a). The antagonistic effect of *Trichoderma* spp. against fungal pathogens may be attributed to the fast growth and better saprophytic activity which suppressed the growth of the host (Iqbal and Akhtar, 1987). Moreover, this effect may be due to substances secreted in the growth media by *Trichoderma* spp. or toxic compounds which were identified as antifungal and antibacterial compounds, i. e., viridian, sesquiterpen, gliotoxin, gliovirin, gliocladic acid, heptelidic acid, viridol and valinotvicin (El-Kazzat *et al*., 2002, El-Samra *et al*., 2006 a). The bacterial bioagent *B. subtilis* exhibited more antagonistic effect on *R. solani*, *S. sclerotiorum* and *F. solani* reduced the growth rates from (65.6 to 63.3%). Moreover, *M. phasiolina* growth was greatly affected (56.7%).

Similar results on the effect of *B. subtilis* on different soil-borne fungi were found (EL-Gali, 2003, El-Samra *et al*., 2006 a). Antagonistic effect of *B. subtilis* was suggested to be due to secretion of dipeptide compounds namely, bacilycin and fengymycin (Loeffer *et al*., 1986) or cyclic peptide antibiotics mycobacillin, bacillomycin, mycosubtilin, fungislatin, subsporin (Schreiber *et al*., 1988).

The effect of plant oils and biological control agents on damping-off and root-rot diseases of bean in the greenhouse:

The obtained data were presented in Table (4). In general, the tested treatments had significant effects on controlling pre- and post-emergence damping-off as well as root-rot diseases of bean caused by *R. solani*, *M. phaseoilina*, *F. solani*, and *S. sclerotiorum* when compared to the untreated control. In addition, treatment with *T. harzianum* was superior to the rest of the other treatments in controlling such studied diseases.

In conclusion, from the above mentioned results and discussion, it is clear that using the biological control agents and certain plant oils could be added as considerable safe alternative elements to the integrated pest management approach in order to minimize the hazardous effects of chemical fungicides.

Table (2): Effect of different concentrations of four tested oils on the mycelial growth of some bean damping-off and root-rot pathogens.

Treatment **(Oils)**	**Concentration %**	*Rhizoctonia solani*		*Macrophomina phasiolina*		*Fusarium solani*		*Sclerotinia sclerotiorum*	
		Mycelial growth (cm)	% Reduction	Mycelial growth (cm)	% Reduction	Mycelial growth (cm)	% Reduction	Mycelial growth (cm)	% Reduction
Cinnamon	100	0.1	99.9	6.0	33.3	6.0	33.3	6.0	33.3
	75	6.1	32.2	8.0	11.1	6.2	31.1	7.2	20.0
	50	7.1	21.1	8.3	7.8	6.8	24.4	9.0	0.0
	25	7.5	16.7	8.5	5.6	7.6	15.6	9.0	0.0
	12.5	9.0	0.0	9.0	0.0	7.8	13.3	9.0	0.0
	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	0.0
Mean		6.47^{ab}	-	8.13^{ab}	-	7.2^{b}	-	8.2^{b}	-
Clove	100	3.0	66.7	5.5	38.9	4.5	50.0	4.2	53.3
	75	3.4	62.2	7.7	14.4	5.3	41.1	4.5	50.0
	50	4.1	54.4	8.0	11.1	7.0	22.2	6.0	33.3
	25	5.9	34.4	9.0	0.0	7.3	18.9	6.5	27.8
	12.5	8.4	6.7	9.0	0.0	7.5	16.7	7.0	22.2
	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	0.0
Mean		5.63^{a}	-	8.03^{ab}	-	6.76^{b}	-	6.2^{ab}	-
Spearmint	100	0.1	99.9	4.5	50.0	3.0	66.7	0.1	99.9
	75	0.1	99.9	5.5	38.9	3.5	61.1	0.1	99.9
	50	4.0	55.6	7.3	18.9	5.3	41.1	4.0	55.6
	25	7.5	16.7	9.0	0.0	7.0	22.2	7.5	16.7
	12.5	8.1	10.0	9.0	0.0	7.5	16.7	9.0	0.0
	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	0.0
Mean		4.8^{a}	-	7.38^{a}	-	5.88^{a}	-	4.95^{a}	-
Lemon	100	6.0	33.3	8.4	6.7	5.5	38.9	5.5	38.9
	75	7.4	17.8	9.0	0.0	6.4	28.9	9.0	0.0
	50	8.0	11.1	9.0	0.0	7.0	22.2	9.0	0.0
	25	9.0	0.0	9.0	0.0	7.3	18.9	9.0	0.0
	12.5	9.0	0.0	9.0	0.0	7.5	16.7	9.0	0.0
	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	0.0
Mean		8.06^{b}	-	8.9^{b}	-	7.12^{b}	-	8.42^{b}	-

* Values are means of 4 replicates.

* Values within the same column and followed by the same letter are not significantly different from each other according to L.S.D. ($p \leq 0.05$).

Table (3): Antagonistic effect of three *Trichoderma* species and *Bacillus subtilis* on some bean damping-off and root- rot pathogens.

Treatments	Linear growth							
	Rhizoctonia solani		*Macrophomina phasiolina*		*Fusarium solani*		*Sclerotinia sclerotiorum*	
	Mycelial growth (cm)	% Reduction	Mycelial growth (cm)	% Reduction	Mycelial growth (cm)	% Reduction	Mycelial growth (cm)	% Reduction
T. viride	2.67 [b]	70.0	2.27 [b]	74.4	2.00 [a]	76.7	2.33 [a]	74.4
T. koningii	2.67 [b]	70.0	2.87 [c]	67.8	1.83 [a]	80.0	2.83 [a]	64.4
T. harzianum	2.00 [a]	77.8	2.00 [a]	77.8	2.00[a]	77.8	2.17 [a]	75.6
B. subtilis	3.00 [c]	65.6	3.93 [d]	56.7	3.30 [b]	63.3	3.07 [a]	65.6
Control	9.00 [d]	0.00	9.00 [e]	0.00	9.00 [c]	0.00	9.0 [0b]	0.00

* Values are means of 4 replicates. * Values within the same column and followed by the same letter are not significantly different from each other according to L.S.D. ($p \leq 0.05$).

Table (4): The effect of plant oils and biological control agents on damping-off and root-rot diseases of bean in the greenhouse:

Treatment	Pathogens												Mean
	R. solani			*M. phaseolina*			*F. solani*		*S. sclerotiorum*				
	Pre-emergence	Post-emergence	Root-Rot	Pre-emergence	Post-emergence	Root-Rot	Pre-emergence	Post-emergence	Root-Rot	Pre-emergence	Post-emergence	Root-Rot	
Lemon oil	6	20	10	12	11	17	0.00	10	30	7	25	11	13.25[bc]
Cinnamon oil	11	20	12	15	15	21	17	15	20	11	18	10	15.42[bc]
Clove oil	17	13	11	0.0	18	40	16	16	20	25	33	14	18.58[b]
Spearmint oil	21	20	32	17	24	25	26	33	11	8	35	24	23.00[b]
T. viride	2	17	10	23	10	10	6	6	3	8	21	31	12.25[bc]
T. harzianum	7	11	9	8	9	14	2	5	11	5	13	27	10.00[c]
T. koningii	3	3	17	11	11	17	4	9	14	14	12	32	12.25[bc]
Control	30	31	25	37	23	45	33	23	47	66	19	13	32.67[a]

* Values are means of 5 replicates. * Values within the same column and followed by the same letter are not significantly different from each other according to standard deviation ($p \leq 0.05$).
$SD_{0.05} = 7.50$

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