

CONTROL OF SEED-BORNE BEAN PATHOGENS WITH SOME ANTAGONISTIC FUNGI AND NON FUNGICIDAL TREATMENTS.

Ibrahim, G. H. ; Laila Abd-El-Razek and Nadia A. El-Safawani
Plant Pathology Research Institute, ARC, Giza, Egypt

ABSTRACT

Ten associated fungi of bean seeds were isolated, purified and identified as *Alternaria alternata*, *Aspergillus* spp., *Botrytis cinerea*, *Fusarium solani*, *Macrophomina phaseolina*, *Penicillium* sp., *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Trichoderma viride* and *Verticillium* spp. Four tested fungal isolates (*R. solani*, *M. phaseolina*, *F. solani*, and *S. sclerotiorum*) were proved to be pathogenic producing different degrees of pre-emergence and post-emergence damping-off and root rot symptoms on bean cultivars, namely; Contender, Narina, Bolista and Giza-6. The most virulent isolates inducing PRD were *F. solani*, *M. phaseolina* 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. phaseolina* 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 in 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 *in vitro* tested for their antifungal activity against the four tested pathogenic fungi. They significantly reduced the radial growth was obtaining. Spearmint oil exhibited the highest antagonistic effect to the tested fungi followed by Clove and Cinnamon oils, while the least effect was Lemon oil as compared with control. Hyphal growth of *R. solani* and *S. sclerotiorum* was completely inhibited by 100% and 75% conc. of Spearmint oil, while *R. solani* was completely inhibited by 100% conc. of Cinnamon oil. Also, the biological agents *Trichoderma viride*, *T. harzianum*, *T. koningii* and *Bacillus subtilis* were used *in vitro* to evaluate their effectiveness against the four tested pathogenic fungi. Linear growth experiments showed that all biological agents were 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 four tested pathogenic fungi.

Keywords: Antagonistic fungi, Biological agents, Bean seeds, Damping-off pathogens, Plant oils, *Bacillus subtilis*, Root-rot pathogens, *Trichoderma* spp.

INTRODUCTION

Bean (*Phaseolus vulgaris*, L.) is one of the important legumes grown in Egypt and many parts of the world. Seeds play a vital role for the healthy production of the crop, but can be carriers of important diseases, which causes considerable reduction in yield of plant (EL-Ahmed 1996 and EL-Gali, 2003). Seed-borne fungi associated with bean seed affect plant character and yield and many reports about bean seed-borne diseases were published and the principal pathogens associated were *A. alternata*, *F. solani*, *F. semitectum*, *F. equiseti*, *M. phaseolina*, *Penicillium* sp. and *R. solani*.

(Lazzaretti, *et al.*, 1994 and Ramadan, 1989). Bean plants are commonly exposed to be attacked by many serious soil-borne fungi i.e. *F. semitectum*, *F. oxysporum*, *F. solani*, *Pythium spp.*, *R. solani*, *M. phaseolina*, *S. rolfsii*, *S. sclerotiorum* and *Verticillium spp.* (Lacicowa and Pioto, 1997 and EL-Gali, 2003). Most of them cause damping-off and root rot diseases (Abada *et al.*, 1992; Abdel-razek, 2005; 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, 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 (Abdel-razek, 2005; and 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 has succeeded in preventing 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 the bacteria *Bacillus subtilis* are among the most promising biocontrol agents who were applied against a wide range of plant pathogenic fungi (El-Kazzaz *et al.*, 2002). Abo Dakika and Zen El-Dein (2007) recorded that *T. harzianum* inhibited linear growth of *F. oxysporum* (82%). The objectives of this study were to (1) survey the most common damping-off and root rot fungal agents of bean include their isolation from seeds, purification, identification and inoculation, (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*.

MATERIALS AND METHODS

Isolation, purification and identification:

Seed samples of bean (*Phaseolus vulgaris*, L.), were collected from different localities in Egypt to test their mycoflora. Seed-borne fungi were isolated using agar-plate method (El-Ahmed, 1996; and EL-Gali, 2003). Seeds were surfaces sterilized by dipping in 1% sodium hypochlorite for two minutes, then rinsed thoroughly in several changes of 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 isolated on PDA medium and kept for purification and identification. Identification of the

isolated fungi was verified by the phytopathological staff of Plant Pathology Research Institute, Agricultural Research Center, Alexandria, Egypt. Using characteristics of mycelia and spores of fungi as described by Barnett and Hunter (1972), Booth (1971) and Ellis (1971).

Inoculation and determination of pathogenicity:

Four fungal isolates were isolated from bean seed used in this study. *F. solani*, *M. phaseolina*, *R. solani*, and *S. sclerotiorum*. These isolates individually were tested for their pathogenicity four bean cvs. named: Contender, Narina, Bolista and Giza-6 bean cultivars under greenhouse conditions. Pots (20 cm in diameter) were sterilized and filled with autoclaved aerated sandy 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 inoculums of each fungus at the rate of 4% of soil weight. Inoculums was mixed thoroughly with the soil in each pot, watered and left for one week to secure establishment of the inoculated 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 cultivar seeds were surface-sterilized and sown, watered regularly every 3-days. Determination of the number of pre- and post- emergence damping-off, seedling survival and root rot incidence were calculated as percentages and expressed in angular transformed values (Arcsine angular).

Antagonistic effect of some plant oils:

(A) Sources of plant oils

Four plant oils were tested for their antifungal activity against damping-off plus root rot diseases in bean seedlings, named, Cinnamon (*Cinnamomum zylanicum* L.), Clove (*Syzygium aromaticum* L.), Spearmint (*Mentha viridis* L.), and Lemon (*Citrus aurantifolia*). These plant oils were obtained from Horticulture Research Station, Aromatic and Medicine plants, Department of Sabahia, Alex., Egypt.

(B) In vitro experiments

Different concentrations of the tested plant oils, i.e., 0, 12.5, 25, 50, 75 and 100% were dissolved using acetone and adding tween 40 plus sterilized distilled water. Petri-plates (9 cm in diameter) with PDA media were used. Five mm disc of 7 days old culture of the 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 of the mycelial growth was calculated according to formula proposed by Ferreira *et al.*, (1991).

Antagonistic effect of some 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. Pure 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 at Taxonomy Department, Plant Pathology Research Institute, Agric. Res. Center, Giza. The bacterial bioagent *B. subtilis* was obtained from Plant Pathology Dept., Fac. of Agric. Alexandria Univ.

(B) In vitro experiments

Antagonistic effect of *T. viride*, *T. harzianum* and *T. koningii* on the linear growth of the four tested pathogenic fungi (*R. solani*, *M. phaseolina*, *F. solani* and *S. sclerotiorum*) were 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 either *T. viride*, *T. harzianum* or *T. koningii*, whereas the opposite half was inoculated with an equal disc of 7-days old culture of either of the four tested pathogenic fungi (Dhingra and Sinclair, 1985). Plates were then incubated at $25 \pm 2^\circ\text{C}$ 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 bioagent 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 $25 \pm 2^\circ\text{C}$. Control plates were streaked with sterilized distilled water instead of the bioagents. Four plates were served for each treatment. Linear growths of the pathogenic fungi were determined at the end of the experiment (cm) in each treatment and percentage of reduction in the linear growth of the tested fungi was calculated by using the formula proposed by Ferreira *et al.* (1991).

Statistical analysis:

Complete randomized designs with 4 replicates were used in the present study. Percentage data were transformed into arcsine numbers (Snedecor and Cochran, 1981), before carrying out analysis of variance (ANOVA). Least significant difference (LSD) at 5% level of probability was applied for comparing treatment means (Duncan, 1955).

RESULTS AND DISCUSSION

Survey of bean seed-borne fungi:

The most frequently isolated seed-borne fungi were *A. alternata*, followed by *Aspergillus* sp., *R. solani*, and *Penicillium* spp. However, 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* caused *Rhizoctonia* root rot (Godoy *et al.*, 1996). Mana Obkura and Christine (2009) reported that certain strains of *R. solani* infected bean in New York and had acquired the ability to infect corn, and a correlation between aggressiveness on corn and bean was observed. 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 varieties responses:

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

From data presented in Table (1) and Fig.1 (A), the followings could be concluded

- (1) All the tested isolates induced significant PRD symptoms on Contender bean 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), when compared with the control (9.1). The least virulent isolates were *M. phaseolina* and *S. sclerotiorum* (28.8).
- (2) In Narina bean cultivar, the highest infection values were obtained by *M. phaseolina*, *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 bean cultivar, the highest PRD occurred by *M. phaseolina* (43.1) followed by *R. solani* (37.2) then both *F. solani* & *S. sclerotiorum* (35.0).
- (4) Giza-6 bean cultivar was significantly affected by the tested isolates *F. solani* (31.0), *R. solani* (28.8) then both *M. phaseolina* & *S. sclerotiorum* (26.6).

Finally, it could be concluded that the most virulent isolates inducing PRD were *F. solani*, *M. phaseolina* and *R. solani*, whereas *S. sclerotiorum* was the least tested virulent isolate. Moreover, Bolista bean cultivar was the most compatible cultivar with PRD agents, especially with *M. phaseolina* and *R. solani*, as compared with the other tested cultivars, whereas Giza-6 bean cultivar showed the lowest compatibility.

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

Data were presented in Table (1) and Fig.1 (A), the obtained results revealed the followings:

- (1) The highest levels of infection percentage values of PTD were obtained with *S. sclerotiorum*, *F. solani* and *R. solani*, whereas *M. phaseolina* 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 bean cultivar showed the lowest compatibility.



Fig. (1) Showed in (A) Symptoms produced by artificial inoculation of bean with (1) *R. solani*; (2) *M. phaseolina*; (3) *F. solani*; (4) *S. sclerotiorum* showed seed decay, decaying of root symptom, lesion on root, stem and cotyledons, dropping of first leaf and wilt of seedling. In (B) Effect of some crude plant oils on growth of *R. solani* where: Cont: Control; (Be 11) Cinnamon; (Be 12) Clove; (Be 13) Spearmint; (Be 14) Lemon.

In (C) Antagonistic effect of some bioagents on growth of *F. solani* where; Cont: Control; (A3) *Trichoderma viride*; (B3) *T. koningii*; (C3) *T. harzianum*; (D3) *Bacillus subtilis*.

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					Cultivars					Cultivars				
	Contender	Narina	Bolista	Giza-6	Mean	Contender	Narina	Bolista	Giza-6	Mean	Contender	Narina	Bolista	Giza-6	Mean
<i>Rhizoctonia solani</i>	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
<i>Macrophomina phaseolina</i>	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
<i>Fusarium solani</i>	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
<i>Sclerotinia sclerotiorum</i>	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 Duncan's multiple range tests ($p \leq 0.05$).

(C) Seedling survival

Table (1) could be concluded, the following:

- (1) The highest survival rates were recorded when Giza-6, Contender bean cultivars were inoculated with all the tested isolates (from 52.8 to 39.2).
- (2) The moderate survival rates were recorded when Narina bean cultivar was inoculated with *F. solani* (33.0), *R. solani* and *M. phaseolina* (35.2).
- (3) The least survival rates were recorded in Bolista bean cultivar in all the tested isolates (from 28.1 to 30.3).

(D) Root rot incidence

Results in Fig. (2) Indicated that all the tested fungal isolates could induce root rot symptoms on all tested cultivars. However, root rot index differs according to the tested isolate and the inoculated cultivar. The highest infection percentage 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 were obtained in Giza-6 cultivar ranged from 55-60%.

The present study showed that *R. solani*, *F. solani* and *S. sclerotiorum* were highly pathogenic agents causing high rates of pre-emergence damping-off and post-emergence damping-off on bean seedling. These findings were similar to those found on bean seedling (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 this region are 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 isolates pathogens were in harmony with those recorded by (EL-Gali, 2003; Shama, 1989).

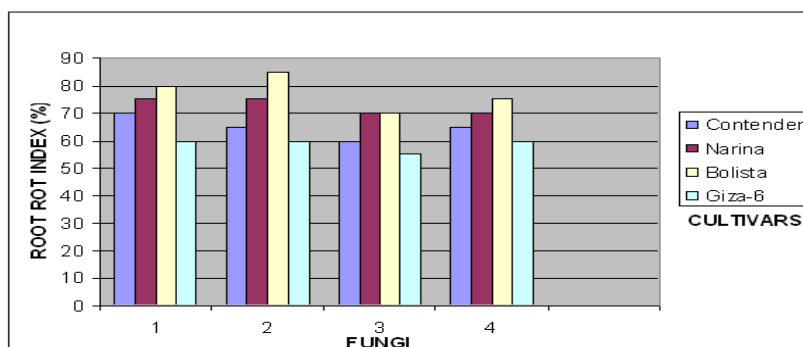


Fig. (2). Root rot index (%) of some bean damping-off and root-rot pathogens on different cultivars named: Contender, Narina, Bolista and giza-6 where: (1) *R. solani*; (2) *M. phaseolina*; (3) *F. solani*; (4) *S. sclerotiorum*.

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, namely Cinnamon, Clove, Spearmint and Lemon against bean damping-off and root rot pathogens, (*R. solani*, *M. phaseolina*, *F. solani* and *S. sclerotiorum*). Linear growth of the tested fungi was determinate in Table (2) and illustrated in Fig.1 (B).

- (1) The obtained results showed significant antagonistic effect of Spearmint oil followed by Clove and Cinnamon oils.
- (2) Crude Spearmint oil proved to be the most effective against in all the tested pathogens, followed by crude Clove and Cinnamon oils. While the least effect was obtained on crude Lemon oil compared with the control.

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

Treatment (Oils)	Concentration %	<i>Rhizoctonia solani</i>		<i>Macrophomina phaseolina</i>		<i>Fusarium solani</i>		<i>Sclerotinia sclerotiorum</i>	
		Mycelial growth (cm)	% Reduction	Mycelial growth (cm)	% Reduction	Mycelial growth (cm)	% Reduction	Mycelial growth (cm)	% Reduction
1- Cinnamon	100	0.1	99.9	6.0	33.3	6.0	33.3	6.0	33.3
	33.3	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	-
2- 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}	-
3- 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 Duncan's multiple range tests (p≤ 0.05).

- (3) In this respect, hyphal growth of both *R. solani*, and *S. sclerotiorum* was completely inhibited by crude spearmint oil. Also crude Cinnamon oil was completely inhibited *R. solani*.
- (4) Moreover, it were efficient in reducing the hyphal growth of each of *M. phaseolina* (66.7%), *S. sclerotiorum* (53.3%) and *F. solani* (50.0%) by crude Clove oil, whereas reducing *F. solani* (66.7%) and *M. phaseolina* (50.0%) by crude Spearmint oil.
- (5) Highly effect of Spearmint oil at conc.75% to reduce the percentage of hyphal growth of both *R. solani* and *S. sclerotiorum*, it was completely inhibited the growth,

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

Treatments	Linear growth of mycelia (cm)							
	<i>Rhizoctonia solani</i>		<i>Macrophomina phaseolina</i>		<i>Fusarium solani</i>		<i>Sclerotinia sclerotiorum</i>	
	Mycelia growth (cm)	% Reduction	Mycelia growth (cm)	% Reduction	Mycelia growth (cm)	% Reduction	Mycelia growth (cm)	% Reduction
<i>T. viride</i>	2.67 ^b	70.0	2.27 ^b	74.4	2.0 ^a	76.7	2.33 ^a	74.4
<i>T. koningii</i>	2.67 ^b	70.0	2.87 ^c	67.8	1.83 ^a	80.0	2.83 ^a	64.4
<i>T. harzianum</i>	2.0 ^a	77.8	2.0 ^a	77.8	2.0 ^a	77.8	2.17 ^a	75.6
<i>B. subtilis</i>	3.0 ^c	65.6	3.93 ^d	56.7	3.3 ^b	63.3	3.07 ^a	65.6
Control	9.0 ^d	0.0	9.0 ^e	0.0	9.0 ^c	0.00	9.0 ^b	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 Duncan's multiple range tests ($p \leq 0.05$).

REFERENCES

- Abada, K.A.; Aly, H.Y; and Mansour, M.S. (1992). Phytopathological studies on damping-off and root-rot diseases of Pea in A.R.E. Egypt. *J. Appl. Sci.*, 7: 242-261.
- Abdel-Aal, A.A.Z. (2001). Biological Control of Some Vegetable Diseases in Greenhouse. M.Sc. Thesis. Submitted to Univ. of Alexandria. pp. 83.
- Abdel-Razek, L. K. (2005). Studies on Seedlings Damping-Off and Root Rot Diseases of Pea. Ph. D. Thesis, Faculty of Agric. Saba Basha Alex. Univ.pp.192.
- Abo Dakika, M. F. and Zen El-Dein, M. (2007). Biocontrol of pea Fusarium pod rot. *J. Agric. Sci. Mansoura Univ.*, 32:1837-1849.
- Amer, G.A. and El-Desouky, Sh.M. (2000). Suppression of bean damping-off caused by *Sclerotium rolfsii* using *Trichoderma* and *Gliocladium* species. *Minufiya J. Agric. Res.*, 25: 921- 932.
- Barnett, H.L. and Hunter, B.B. (1972). *Illustrated Genera of Imperfect Fungi*. Burgess publishing company. Minneapolis, Minnesota, U.S.A., pp. 241.
- Bilgi, V. N.; Bradley, C. A. and Khot, S.D. (2008). Response of dry bean genotypes to Fusarium root rot caused by *Fusarium solani* under field and controlled conditions. *Plant Dis.* 92:1197-1200.
- Booth, C. (1971). *The Genus Fusarium*. Commw. Mycol. Inst., Kew, Surrey, England.

- Dhingra, O.D. and Sinclair, J.B. (1985). Basic Plant Pathology Methods. CRC Press, Inc. Boca Raton, Florida, USA, 353 PP. [C.F. Egypt. J. Phytopathol. 26 (2): 109-119. 1998].
- Duncan, D.B. (1955). Multiple Ranges and Multiple F. test. Biometrics, 11: 142.
- El-Ahmed, A. (1996). Seed health and storage issues. ICARD seed health policy in seed production. Aleppo, Syria, Pages 140-194.
- El-Farnawany, M. and Shama, S. (1996). Biological control of *Rhizoctonia solani* affecting bean seedlings damping-off. Alex. J. Agric. Res., 41: 253-260.
- El-Gali, Z. (2003). Histopathological and Biochemical Studies on *Phaseolus vulgaris* Seeds Infected by Some Seed-borne Fungi. Ph.D. Thesis. Fac. of Agric. (Saba Basha). Alex. Univ. pp. 293.
- El-Kazzaz, M.K.; Ghoniem, K.E. and Hammouda, S.M.H. (2002). *In vitro* effect of some bacterial and fungal antagonists on certain soil borne fungal isolated from diseased tomato and pepper plants. J Agric. Res. Tanta Univ., 28: 9-22.
- El-Korashy, M. (1997). Effect of some plant extracts against damping-off disease of peanut plants J. Agric. Sci. Mansoura Univ., 22: 1912-1929.
- Ellis, M.B. (1971). Dematiaceous Hyphomycetes. C.M. Institute, Kew. Surrey England, 608 PP.
- El-Safwani, A. Nadia. and Nasif, O.B. (2002). Antifungal activity of some plant extracts against damping-off disease of lupin and chick pea seedlings. J. Agric. Sci. Mansoura Univ., 27: 2945-2953.
- El-Samra, I. A.; M. El-Farnawany; N. A. El-Safawani and L. Abd-El-Razek. (2006 a) Studies on seedlings damping-Off and root rot diseases of pea. Biological control. J. Agric. Res. Tanta Univ., 32:300-318.
- El-Samra, I. A.; M. El-Farnawany; N. A. El-Safawani and L. Abd-El-Razek. (2006 b) Studies on seedlings damping-Off and root rot diseases of pea. Control by essential plant oils. J. Adv. Agric. Res. (Fac. Agric. Saba Basha). 11:343-358.
- El-Samra, I. A.; M. El-Farnawany; N. A. El-Safawani and L. Abd-El-Razek. (2006 c) Studies on seedlings damping-Off and root rot diseases of pea. Disease agents and varieties responses. J. Agric. Res. Tanta Univ., 32:286-299.
- El-Sharkawy, T.A.; El-Barougy, E. and Gaafer, E.M. (1998). Biological, chemical control and susceptibility of alfalfa to damping-off (*M. phaseolina*) in Egypt. Egypt J. Appl. Sci., 13: 19-34.
- Ferreira, J.H.S.; Matthee, F.N. and Thomas, A.C. (1991). Biological control of Eutypalata on Grapevine by an antagonistic strain of *Bacillus subtilis*. Phytopathology, 81: 283-287.
- Godoy, L. G.; Arias, J.; Steadman, J. and Eskridge, K. (1996). Role of natural seed infection by the web blight pathogen in common bean seed damage, seedling emergence and early disease development. Plant Dis. 80:887-890.

- Hassanein, M. Ferial and El-Doksch, H.A. (1997). Antibacterial action of carvone and some plant extracts on certain phytopathogenic bacteria and pathogenicity of *Agrobacterium tumefaciens*. Alex. J. Agric. Res., 42: 127-136.
- Ibrahim, M. Mona (1996). Studies on Sclerotium Blight of Soybean in Egypt. M. Sc. Thesis, Fac. Agric., Minufiya Univ. Egypt. (8th congress of the Egyptian phytopathol. Soc., Cairo, 1997).
- Iqbal, S.M. and Akhtar, C.M. (1987). Biological control of sugarcane red rot (*Colletotrichum flactum* went.). J. Agric. Res., 25: 195-202.
- Ismail, B.R. (1998). The use of some fungicides and *Trichoderma* spp. in controlling some soil pathogenic fungi. Egypt J. Appl. Sci., 13: 57-64.
- Lacicowa, B.; Pieta, D. (1997). Efficacy of micro biological dressing of bean seed (*Phaseolous coccineus* L.) in conditions of disease risk arising from fungi living in soil. [C.F. Rev. Pl. Path. 76: 273].
- Lazzaretti, E.; Menten, J. O. and Billiol, W. (1994). *Bacillus subtilis* antagonistic to the principal pathogens associated with bean and wheat seeds. Fitopatologia Venezolana. 7:42-46.
- Loeffler, W.; Tschen, J.S.M.; Vanittlankom, N.; Kugler, M.; Knorpp, E. and Hsieh, T.S. (1986). Antifungal effects of Bacilysin and Fengymycin from *Bacillus subtilis* F-29-3. A comparison with activities of other *Bacillus* antibiotics. J. Phytopathology, 115: 204-213.
- Mana Ohkura, G. S. and Christine, D. S. (2009). Diversity and aggressiveness of *Rhizoctonia solani* and *Rhizoctonia*-like fungi on vegetables in New York. Plant Dis. 93:615-624.
- Mao, W.; Lumsden, R.D.; Lewis, J.A. and Hebbbar, P.K. (1998). Seed treatment using pre-infiltration and bio-control agents to reduce damping-off of corn caused by species of *Pythium* and *Fusarium*. Plant Dis. 82: 294-299.
- Oliveira, A.E.A.; Gomes, V.M.; Sales, M.P.; Fernades, K.V.S.; Carlini, C.R. and Xavier-Fitho, J. (1999). The toxicity of Jack bean [*Canavalia ensiformis* (L.) DC.] canatoxin to plant pathogenic fungi. Revist Brasileira de biologia, 59: 59-62 (C.F. Rev. Pl. Path. 78: 4390].
- Pattnaik, S.; Subramanyam, V.R. and Kole, C. (1996). Antibacterial and antifungal activity of ten essential oils *in vitro*. Microbes, 86: 237-246. (C.F. Rev. Pl. Path. 78:5118).
- Ramadan, N. A. (1989). Studies on Certain Seed-borne Disease of Leguminous Crops. Ph. D. Thesis. Submitted to Univ. of Alexandria. pp 151.
- Schreiber, L.R.; Gregory, G.F.; Krause, C.R. and Jehida, J.M. (1988). Production, Partial Purification and antimicrobial activity of a novel antibiotic produced by *Bacillus subtilis* isolate from *Ulmus americana*. Can. J. Bot., 66: 2338-2346. [8th Congress of The Egyptian Phytopathol., Soc. Cairo, 1997].
- Shama, S.M. (1989). Transmission of *Rhizoctonia solani* (Kuhn) in seeds of bean (*Phaseolus vulgaris* L.) Curr. Sci., 58: 972-974.
- Sharaf El-Din, A.; Osman, A. I. and Saleh, A. M. (2007). Effect of post-harvest essential oils application on resistance of onion and garlic on storage rot diseases. Minufiya J. Agric. Res., 32:335-346.

- Snedecor, G.W. and Cochran, W.G. (1981). Statistical Methods. 7th ed. Iowa. Stat Univ. Press, Ames. Iowa, USA.
- Youssef, L. G. (2008). Studies on Faba Bean Root Rot Diseases. M. Sc. Thesis, Faculty of Agric. Saba Basha Alex. Univ.
- Zedan, A.M.; El-Toony, A.M. and Awad, N.G.H. (1994). A comparative study on antifungal activity of certain plant extracts, essential oils and fungicides on tomato wilt pathogens. Al-Azhar J. Agric. Res. 20: 217-236.
- Ziedan, M. I. (1980). Index of Plant Disease in Egypt. Inst. Plant Pathol. Agric. Res., Cairo, Egypt, pp. 95.

مقاومة الكائنات الممرضة المصاحبة لبذور الفاصوليا باستخدام الكائنات الفطرية المضادة وبعض المعاملات الأخرى غير المبيدات الفطرية جمال الدين حامد إبراهيم , ليلي عبد الرازق و نادية الصفواني معهد بحوث أمراض النباتات- مركز البحوث الزراعية-الجيزة

تم عزل وتنقية وتعريف عشرة فطريات مصاحبة لبذور الفاصوليا وشملت الترناريا الترناريا و جنس الاسبرجلس وبوتريتس سينريا وفيوزاريم سولاني و ماكرو فومينا فاسيولينا و جنس البنيسيليوم و الريزوكتونيا سولاني و سكليروتينيا سكليروشيورم و تريكودرما فيردى و جنس الفريسيولوم. اثبتت اختبارات القدرة المرضية ان اربعة فطريات هي الريزوكتونيا سولاني و ماكرو فومينا فاسيولينا و فيوزاريم سولاني و سكليروتينيا سكليروشيورم تحدث مستويات مختلفة من اعراض الذبول الطرى ما قبل و مابعد ظهور البادرات وكذلك اعراض افغان الجذور وذلك على اربعة اصناف من الفاصوليا هي كونتندر ، نارينا ، بوليستا وجيزه-6.

وجد ان فطريات فيوزاريم سولاني و ماكرو فومينا فاسيولينا و ريذوكتونيا سولاني هي اكثر الفطريات قدرة على احداث ذبول ما قبل ظهور البادرات، بينما كان الفطر سكليروتينيا سكليروشيورم اقلها. كذلك وجد ان فطريات سكليروتينيا سكليروشيورم و فيوزاريم سولاني و ريذوكتونيا سولاني هي اكثر الفطريات مقدره على احداث ذبول مابعد ظهور البادرات، بينما كان الفطر ماكرو فومينا فاسيولينا هو اقلها.

كان الصنف بوليستا هو اكثر الاصناف اصابة بالذبول الطرى، بينما كان الصنف جيزه-6 هو اقل الاصناف توافقا مع مسببات الذبول الطرى. ايضا كان الصنف بوليستا هو اكثر الاصناف اصابة بعفن الجذور وذلك على جميع الفطريات المختبرة و تراوحت نسبة الاصابة من 70-85%، يليه صنف نارينا 70-75%، بينما كان الصنف جيزه-6 هو اقلها 60-65%.

تم اختبار عدد من الزيوت النباتية وهي زيت القرقة، القرنفل، النعناع والليمون فى المعمل لمقاومة الكائنات الاربعة الممرضة السابقة للفاصوليا. ومن النتائج اتضح أن جميع الزيوت المختبرة ادت الى انخفاض معنوي فى النمو القطرى للفطريات المختبرة.

وكان زيت النعناع هو اكثر الزيوت فعالية فى تثبيط نمو الفطريات المختبرة، يليه زيت القرنفل و القرقة، بينما كان زيت الليمون اقلها وذلك بالمقارنة بالكونترول. وكان زيت النعناع بتركيز 75 و 100% فعال ضد فطريات الريزوكتونيا سولاني و سكليروتينيا سكليروشيورم حيث ادى الى تثبيط كامل للنمو، وكذلك زيت القرقة بتركيز 100% ادى الى تثبيط كامل لنمو الفطر ريذوكتونيا سولاني.

تم اختبار عدد من كائنات المقاومة الحيوية وهي تريكودرما فيردى، تريكودرما هرزيانم، تريكودرما كونجياى و باسيلس ساتلس معمليا لمقاومة الكائنات الاربعة الممرضة للفاصوليا. و اوضحت تجارب النمو القطرى ان جميع الكائنات الحيوية المستخدمة ادت الى انخفاض معنوي فى نمو الكائنات الممرضة المختبرة. وكان الفطر تريكودرما هرزيانم أكثر تأثيرا فى الفطريات الممرضة حيث ادى الى انخفاض فى النمو يتراوح من 75,8-77%، بينما البيكتيريا باسيلس ساتلس كانت اقل كائنات المقاومة الحيوية تأثيرا حيث تراوح انخفاض النمو من 67,6-65,6%.

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة
كلية الزراعة – جامعة الإسكندرية

أ.د / محمود أحمد المزاتي
أ.د / محمد سعد أبو السعود

