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CONTROL OF CHARCOAL ROT DISEASE OF BEAN (*PHASEOLUS VULGARIS* L.) CAUSED BY *MACROPHOMINA PHASEOLINA* IN ISMAILIA GOVERNORATE, EGYPT

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Abstract

Bean plants are subject to attack by *Macrophomina phaseolina*, which was isolated from different localities in Ismailia governorate. It was frequently isolated from infected roots and less frequently from collected seeds. Nebraska bean cultivar was the least susceptible; Morgan cv. was moderately susceptible, while Xera cv. was the most susceptible. *Trichoderma harzianum* as a biocontrol agent and Plant guard as a biocide resulted in a great reduction in mycelial growth of *M. phaseolina* isolates (*In vitro*) and suppressed the disease in plants grown from dressed seeds or by soil drenching. The fungicides Vitavax and Rhizolex have completely prevented the mycelial growth of tested *M. phaseolina* isolates at 50 ppm. Benlate resulted in the same level of inhibition at 100 ppm. Vitavax reduced pre, post emergence damping-off and charcoal rot disease of Xera bean cultivar followed by Rhizolex and Benlate.

INTRODUCTION

Bean (*Phaseolus vulgaris. L.*) is one of the most important legumes grown in Egypt for both local consumption and exportation. It is consumed as green shelled, dried or canned. Snap bean pods are rich in protein, carbohydrate and other nutrients (calcium, phosphorus, potassium and vitamins).

Many pathogenic microorganisms attack bean plants at all stages of plant growth. Infection affects yield quality and quantity. Seed and soil-borne fungal pathogens are considered serious disease agents, which affect seed germination, seedling emergence and plant growth.

Common bean (*Phaseolus vulgaris* L.) is a good host for *M. phaseolina,* which causes a range of symptoms, depending on environmental conditions and age of plants. In addition to charcoal rot, the pathogen also causes damping-off and seedling blight

(Songa and Hillocks, 1996). Sarhan (2000) reported *M. phaseolina* associated with bean seed samples from Ismailia governorate at 9 and 9.51% in 1995 and 1996 growing seasons, respectively.

Biological control is an important control measure of soil and air-borne diseases. Considerable attention has been given to the control of plant pathogenic fungi using antagonistic microorganisms. Anti-fungal activity of many biocontrol agents was reported by many investigators (Dennis and Webster, 1971; Tschen, 1987 and Reddy *et al.*, 1994)

Diaz-Franco (1989) compared the effect of several fungicides in the greenhouse and the field against charcoal rot of bean and found that, thiabendazole, benomyl, chlorothalonil, quintozene, captan, RH 27180 and mancozeb protected the plants under greenhouse conditions but were ineffective in field trials.

The present work aimed to field survey the charcoal rot disease of bean plants and seed-borne fungi in Ismailia governorate and susceptibility of certain bean cultivars to *Macrophomina phaseolina*. Biological and chemical controls were studied *in vitro* and *in vivo*.

MATERIALS AND METHODS

1. Field survey, isolation and identification of the isolated organisms of bean root rot and associated fungi with seeds:

During the growing seasons of 1999/2000 and 2000/2001, survey of charcoal rot of bean was carried out. Naturally infected bean plants were collected from five locations in Ismailia governorate *i.e.* Ain Ghosin, Eltal-El kabeer, El Kassassin, El Mehsama and El Wasfia. Samples of naturally infected roots were thoroughly washed with running tap water, then cut into small portions and surface sterilized in 0.1% mercuric chloride solution for 2 minutes, then passed in several changes of sterilized water and dried between sterilized filter papers and transferred to Potato Dextrose Agar plates and incubated at 25 ± 2° C.

The method described by Neergaard (1979) was used to isolate fungi associated with seeds as follows: 20 bean seeds of each cultivar *i.e.* Bronco, Nebraska, Giza 6,

Baulista, Morgan, Coby, Xera and Royal Nel were collected from different locations in Ismailia governorate. Samples were picked up at random from each cultivar, then placed on three layers of sterilized wet filter papers in Petri dishes. Five seeds were placed in each dish, and then incubated for 7 days at 25 ± 2° C under fluorescent light (light/darkness rotations of 12/12 hrs.).

Resulting fungi were examined microscopically after 3-7 days and were identified by Plant Pathology Research Institute, Agriculture Research Center, Giza according to Clements and Chear (1957) and Gilman (1957). All isolated fungi were purified using hyphal tip technique. Percentage of frequency of seed-borne fungi was calculated.

2. Reaction of some bean cultivars to charcoal rot disease under greenhouse conditions:

Seeds of fifteen bean cultivars namely Bronco, Nebraska, Giza 6, Baulista, Morgan, Coby, Xera, Royal Nel, Giza 3, Samantha, Mexico 309, Helda, S1, Contender, and julta were obtained from the Central Administration of Seeds (CAS), Vegetable Research Department, Agricultural Research Center. Barley medium was inoculated with the tested isolate (Bean isolate) of *M. phaseolina* and added to the soil at the rate of 3 colonized barley grains /bean seed in pots (25cm in diameter). Five bean seeds were planted per pot and three replicates were employed for each treatment. Three pots containing healthy bean seeds were used as control.

Percentage of pre, post emergence damping-off, survived plants and charcoal rot was recorded.

3. Disease assessment:

Charcoal rot was recorded 60 days after sowing on a scale of 0-5 as described by Emara (1995).

4. Biological control:

The antagonistic effect of *Trichoderma harzianum, T. viride* and *Bacillus subtilis* (Biocontrol agents), as well as, Plant guard, Seeds guard and Rhizo N as biocides against three isolates of *M. phaseolina* (Sweet melon, Bean and Sesame isolates) was studied (Table 1).

Table 1. Trade name, active ingredient and doses of used three different biocides and fungicides.

Trade name	Active ingredient (a.i.)	Dose
Plant guard	*Trichoderma harzianum* (30 x10^6 spore /ml)	3ml/L
Seeds guard	*Trichoderma viride* (30 x10^6 spores /g)	6g/kg seeds
Rhizo N	*Bacillus subtilis* (30 x10^6 cell/g)	4 g/L
Benlate WP 50%	Methyl 1-(Butyl carbomoyl-2-benzimidazol carbamate	2 g /kg seed
Rhizolex-T 50%	0-(2,6-Dichloro 4-methyl) 0,0 dimethyl phosphorothioate + thiram	3 g /kg seed
Vitavax/ Thiram	5.6 dihydro-2-methyl-N-phenyl-1.4 Oxathiin 3-carboxamide+ Tetramethylthiuram disulfide.	3 g /kg seed

a. *In vitro*: Discs (5 mm in diameter) were taken from the marginal growth of *T. harzianum* and *M. phaseolina* grown for 5 days on PDA plates. Discs of *T. harzianum* were placed opposite to discs of *M. phaseolina* as described by (Ferreira *et al.*, 1991).

Bacillus subtilis was transferred to nutrient agar slants and incubated for 24hrs at 28 °C to obtain active culture. Discs 5 mm in diameter of 5 day old cultures of *M. phaseolina* was placed on opposite side to a streak of bacterial culture. The inoculated Petri dishes were incubated for 5 days at 25 °C. Three replicates were used for each treatment.

The percentage of growth reduction of *M. phaseolina* was determined using the formula stated by Ferreira *et al.* (1991).

$$\% \text{ G.R.} = \frac{A - B}{A} \times 100$$

Where, % G.R: Percentage of growth reduction. A: The length of mycelial growth of the pathogenic fungi on the distant side from the antagonist. B: The distance between mycelial growth of the pathogenic fungi and that of the antagonist.

The effect of different concentrations (0.0, 0.003, 0.03 and 0.3 g/100 ml medium) of three biocides *i.e.* Plant guard, Seeds guard and Rhizo N on the linear growth of three isolates of *M. phaseolina* was tested on PDA medium. The different concentra-

tions of biocides were added to PDA medium before being poured in Petri dishes (9 cm in diameter). The dishes were inoculated in the center, each with a disc (5 mm) taken from the marginal growth of *M. phaseolina.* Inoculated Petri dishes were incubated for 5 days at 25°C; three replicates were used for each treatment. The growth reduction percentage was calculated.

b. *In vivo*: A greenhouse experiment was carried out in a complete randomized block design with three replicates. Pots (25 cm in diameter) were sterilized in 5% formalin solution for 15 min. and left to dry for 7 days and filled with sandy clay soil (1:1) previously sterilized with 5% formalin solution and left to dry for 3 weeks. The pots were sown at the rate of 5 seeds/pot.

1. Seed treatment: Seeds of Xera bean cultivar were sterilized in 0.1% mercuric chloride solution for 2 minutes, then thoroughly washed with sterilized water and dried between sterilized filter papers. Surface sterilized seeds were immersed for one hour in a spore suspension of *T. harzianum* containing 6×10^3 spore/ml (Abdel-Kader, 1997).

Surface sterilized Xera bean cultivar seeds were soaked for 5 hours before sowing in a liquid culture of *B. subtilis* (5×10^6 cfu/ml). The bean seeds were mixed with the recommended dose of biocide Plant guard, Seeds guard and Rhizo N (Table 1) in flasks containing 40 ml glue suspension as sticker /kg seeds, then left to dry prior to sowing in infested pots. Seeds of the control were left without treatment. Percentage of pre, post emergence damping-off, charcoal rot and survived plants were recorded.

2. Soil treatment: This experiment was carried out by preparing a suspension of the recommended dose of each bioagent at the concentration of 6×10^3 spore/ml for *T. harzianum,* 5×10^6 cfu/ml for *B. subtilis,* 30×10^6 spore/ml for Plant guard, 30×10^6 spore/ml for Seeds guard and 30×10^6 spore/ml for Rhizo N. Each concentration was added to the infested soil at the rate of 20 ml/Kg soil. Three pots were left without treatment as control. The application of each concentration was made either 7 days before inoculation, at the time of inoculation or 7 days after inoculation. The percentage of pre, post emergence damping-off, charcoal rot and survived plants were recorded.

5. Chemical control:

Three different fungicides *i.e.* Vitavax Thiram, Rhizolex-T 50% and Benlate WP 50% were used against *M. phaseolina in vitro* and *in vivo*:

a. *In vitro*: The aforementioned fungicides were tested for their effect on the linear growth of *M. phaseolina*. Four concentrations *i.e.* 0, 5, 10, 50 and 100 ppm of the active ingredient of each fungicide were used in PDA medium. The medium was poured in Petri dishes (9 cm in diameter) and three dishes were used for each concentration. The dishes were inoculated in the center with disks (5mm) of the three isolates of *M. phaseolina* (sweet melon, bean and sesame isolates) and incubated at 25°C. The linear growth (cm) of *M. phaseolina* was measured when the growth in the control treatment reached 9 cm in diameter. The growth reduction percentage was calculated.

b. *In vivo*: A greenhouse experiment was carried out to study the efficacy of fungicides to reduce the incidence of charcoal rot. The tested fungicides were used as seed dressing. Bean seeds (Xera) were mixed with the recommended dose of each fungicide in flasks containing 40 ml glue suspension as a sticker /kg seed. Seeds without treatment served as control. The treated seeds were left to dry and sown in the infested pots. The percentages of pre, post- emergence damping-off, charcoal rot and survived plants were recorded.

6. Statistical analysis:

The data were statistically analyzed using the analysis of variance procedure for completely randomized design. Treatment means were compared using the protected Least Significant Difference (L.S.D.) analysis according to Snedecor and Cochran (1967).

RESULTS

1. Field survey, isolation and identification of the causal organisms of bean root rot plants and associated fungi with seeds:

Isolation trials from rotted roots and seeds of bean plants yielded 77 fungal isolates. The isolated fungi were purified and identified as *Fusarium solani* (Burk), *Macrophomina phaseolina* (Tassi) Goid., *Rhizoctonia solani* (kuehn) and *Sclerotium rolfsii*

(Sacc.), from rotted roots and *Aspergillus niger, Alternaria solani* (Ell and G.Martin Sor.), *Botrytis cinerea* (Pers.ex.Fr.), *F. solani, M. phaseolina, Penicillium* spp., *R. solani* and *Rhizopus nigricans* (Ehrenb.ex Fr.) from seeds.

Data in Table (2) indicate that *M. phaseolina* recorded the highest percentage of frequency being 31.28 and 38.58% in 1999/2000 and 2000/2001 growing seasons, respectively. *M. phaseolina* recorded the highest percentage of frequency being 34.54% in Eltal-El kabeer in 1999/2000 season and 45.45% in El Mehsama in 2000/2001 season, while the lowest percentages of frequency being 28.57% and 30.77% were recorded in El Wasfia in 1999/2000 and 2000/2001 season, respectively. *F. solani* recorded the lowest percentages of frequency being 18.90 and 15.07% in 1999/2000 and 2000/2001 seasons, respectively. *R. solani* and *S. rolfsii* showed moderate frequencies being 29.86, 19.94 and 21.16, 25.17% in both seasons, respectively.

Table 2. Percentage of frequency of isolated fungi from bean roots (*Phaseolus vulgaris* L.) after 60 days of sowing from different locations in Ismailia governorate during 1999 and 2000 seasons.

Isolates / % of frequency	1999/2000						2000/2001					
	Ain Ghosine	EL-Kassassin	EL-Tal EL kabeer	El-Mehsama	El-Wasfia	Means	Ain Ghosine	EL-Kassassin	EL-Tal EL kabeer	El-Mehsama	El-Wasfia	Means
F. solani	21.05	19.04	20.00	15.38	19.05	18.90	8.33	20.13	14.71	9.09	23.08	15.07
M. phaseolina	31.57	30.95	34.54	30.76	28.57	31.28	41.66	36.91	38.23	45.45	30.77	38.58
R. solani	26.31	30.95	32.72	30.76	28.57	29.86	16.66	13.42	17.64	27.27	30.77	21.16
S. rolfsii	21.05	19.04	12.72	23.07	23.81	19.94	33.33	29.53	29.41	18.18	15.38	25.17

Data in Table (3) show the fungi associated with seeds of some bean cultivars. *Rhizopus nigricans* recorded the highest frequency followed by *Aspergillus niger* and *Penicillium spp.* However, *M. phaseolina* recorded a moderate frequency but *R. solani* and *F. solani* were of low frequency. On the other hand, *M. phaseolina* recorded the highest frequency (15.14%) on Xera bean cultivar, followed by Bronco, Nebraska, and Giza 6 cultivars 6.34, 2.73 and 1.1% respectively; however, it was not recorded on some cultivars *i.e.* Baulista, Morgan and Royal Nel.

Table 3. Percentage of frequency of seed-borne fungi associated with some bean cultivars from Ismailia Governorate.

Isolates / % of frequency	Bronco	Nebraska	Giza 6	Baulista	Morgan	Coby	Xera	Royal Nel	Mean
Aspergillus niger	14.29	21.91	27.77	15.25	23.84	24.99	37.88	25.8	23.97
Alternaria solani	25.39	24.67	0.0	27.11	20.18	25.97	0.0	16.12	17.43
Botrytis cinerea	6.34	1.35	0.0	0.0	0.0	1.9	0.0	0.0	1.20
Fusarium solani	0.0	0.0	0.0	0.84	0.0	0.0	0.0	0.0	0.11
Macrophomina phaseolina	6.34	2.73	1.1	0.0	0.0	0.95	15.14	0.0	3.28
Penicillium spp	15.87	17.12	33.34	27.97	20.18	0.0	4.54	25.8	18.1
Rhizoctonia solani	4.76	6.16	0.0	0.0	0.0	0.0	0.0	3.21	1.77
Rhizopus nigricans	26.99	26.02	37.77	28.81	35.79	46.17	42.42	29.04	34.13

2. Reaction of some bean cultivars to charcoal rot disease under greenhouse conditions:

This study was carried out using fifteen bean cultivars to evaluate their susceptibility to infection with *M. phaseolina* under greenhouse conditions. Data in Table (4) indicate that (Nebraska) was less susceptible when sown in infected soil showing 12.5, 0.0, 13.32 and 87.5% of pre, post emergence damping-off, charcoal rot and survived plants, respectively. The same trend was observed in other cultivars *i.e.* Royal Nel, Contender and Bronco.

Morgan bean cultivar was moderately susceptible when sown in infested soil showing 33.33, 0.0, 30.58 and 66.66% of pre, post emergence damping-off, charcoal rot and survived plants, respectively. Giza 3, Giza 6, Baulista and Samantha cultivars showed the same trend. On the contrary, (Xera) was highly susceptible and recorded 37.5, 12.5, 44.4 and 50.0% for pre, post emergence damping-off, charcoal rot and survived plants, respectively. Also, Coby, Julta and Helda cv. were highly susceptible cultivars.

Table 4. Percentage of pre, post emergence damping-off, charcoal rot and survived plants of some bean cultivars under artificial inoculation with *M. phaseolina*.

Cultivars	% of emergence damping-off		% of charcoal rot	% of survived plants
	Pre.	Post.		
Giza 6	12.5	4.16	25.33	83.33
Nebraska	12.5	0.0	13.32	87.5
Giza 3	12.5	8.33	34.54	79.16
Baulista	8.33	8.33	30.84	83.33
Morgan	33.33	0.0	30.58	66.66
Coby	25.0	0.0	32.74	75.0
Xera	37.5	12.5	44.4	50.0
Royal Nel	12.5	0.0	30.84	87.5
Contender	12.5	0.0	32.06	87.5
Samantha	20.83	4.16	33.3	75.0
Mexico 309	16.66	0.0	14.8	83.33
Helda	16.66	0.0	35.77	83.33
S1	8.33	12.5	33.33	79.16
Bronco	12.5	0.0	14.8	87.5
Julta	29.16	4.16	40.47	66.66
L.S.D. 5%	20.17	6.87	20.66	23.01

3. Biological control:

Three isolates of *M. phaseolina* were isolated from samples of naturally infected plants *i.e.* Sweet melon, Bean and Sesame from different localities of Ismailia governorate (Eltal-El kabeer, Ain Ghosin, and Wasfia), respectively.

1. *in vitro* studies:

a. Biocontrol agent: Data in Table (5) reveal that *T. harzianum* caused growth reduction of 67.68, 83.68 and 88.71% for the three isolates of the pathogen (Sweet melon, Bean and Sesame) respectively; however, *T. viride* was less inhibitive causing 54.4, 65.63 and 76.4% of growth reduction. *B. subtilis* reduced radial mycelial growth of the pathogen by 56.4, 76.4 and 85.0% for the three isolates, respectively.

Table 5. Effect of antagonistic microorganisms on percentage of growth reduction of three isolates of *M. phaseolina* after 5 days of incubation at 25°C.

Isolates (A) / Biocontrol agent (B)	% Growth reduction		
	Trichoderma harzianum	*Trichoderma viride*	*Bacillus subtilis*
Sweet melon isolate	67.68	54.40	56.40
Bean isolate	83.68	65.63	76.40
Sesame isolate	88.71	76.40	85.00

L.S.D. at 5% for : Isolates (A) = 6.1
Biocontrol agent (B) = 3.9
Interaction (AXB) = NS

Generally, *T. harzianum* was the most suppressive followed by *B. subtilis* and *T. viride* in a descending order.

b. Biocides: Effect of different biocides formulation on the growth of three isolates of *M. phaseolina* was investigated (Table 6). Data show that Plant guard reduced growth by 33.09, 42.84 and 51.85 % for the three isolates (sweet melon, bean and sesame) respectively. Seeds guard and Rhizo N showed similar trend; however, slightly less inhibitory compared to Plant guard. Plant guard applied at 0.3 g/100 ml PDA medium was the most effective biocide compared with the lower concentration 0.003 and 0.03 g/100 ml medium, where the percentage of growth reduction were 54.7, 60.74 and 75.55% for the three isolates, respectively.

Seeds guard decreased mycelial growth by 52.22, 58.52 and 74.82% at 0.3 g/100 ml medium for the three isolates, respectively. Rhizo N caused growth reduction of 48.88, 57.41 and 70.74% at 0.3 g/100 ml in the three pathogen isolates. Less inhibition was observed with the lower concentration of the biocides.

Generally, Plant guard was the most antagonistic biocide in reducing mycelial growth of *M. phaseolina* followed by Seeds guard and Rhizo N.

2. *In vivo* studies:

a. Seed treatment: Effect of antagonistic microorganisms as seed dressing on disease incidence under greenhouse conditions is shown in Table (7). Plant guard decreased disease incidence to 6.66, 0.0 and 9.62% of pre, post emergence damping off

Table 6. Effect of tested biocides on percentage of growth reduction in the three isolates of *M. phaseolina* after 5 days of incubation at 25 °C *in vitro*.

Isolates of *M. phaseolina* (I) / Biocides (B)		% Growth reduction (g/100ml) (C)				Mean
		0.0	0.003	0.03	0.3	
Sweet melon isolate	Plant guard	0.0	15.93	29.26	54.7	33.09
	Seeds guard	0.0	13.71	26.3	52.22	30.74
	Rhizo N	0.0	12.60	18.88	48.88	25.78
	Mean	0.0	14.08	24.81	51.22	
Bean isolate	Plant guard	0.0	31.11	36.66	60.74	42.84
	Seeds guard	0.0	31.11	34.82	58.52	41.48
	Rhizo N	0.0	28.52	32.22	57.41	39.38
	Mean	0.0	30.25	34.57	59.89	
Sesame isolate	Plant guard	0.0	35.93	44.07	75.55	51.85
	Seeds guard	0.0	35.18	40.74	74.82	50.24
	Rhizo N	0.0	32.22	38.15	70.74	47.04
	Mean	0.0	34.44	40.99	73.70	

L. S. D. at 5% for:

Isolates (I)	=1.11	Interaction	(I x B) = NS
Biocides (B)	= 0.96		(I x C) = 1.21
Concentrations (C)	= 0.7		(B x C) = 1.21

Table 7. Effect of tested antagonistic microorganism and biocides as seed dressing on %emergence damping-off, charcoal rot and survived plants of Xera bean cultivar under greenhouse conditions.

Antagonistic microorganisms and Biocides	% Emergence damping-off		% Charcoal rot (60 days)	% Survived plants (60 days)
	Pre. (15 days)	Post. (30 days)		
Trichoderma harzianum	6.66	0.0	11.1	93.33
Bacillus subtilis	13.33	0.0	14.8	86.66
Plant guard	6.66	0.0	9.62	93.33
Seeds guard	13.33	0.0	11.1	86.66
Rhizo N	13.33	0.0	18.5	86.66
Control	53.33	13.33	44.4	33.33
L. S. D. at 5 %	11.5	8.58	7.58	18.41

and charcoal rot, respectively. *T. harzianum* and Seeds guard led to 6.66, 0.0 and 11.1%, as well as, 13.33, 0.0 and 11.1% of pre, post emergence damping-off and charcoal rot, respectively. Also, Rhizo N decreased incidence of the disease to 13.33, 0.0 and 18.5% of pre, post emergence damping-off and charcoal rot, respectively. On the other hand, *B. subtilis* decreased infection to 13.33, 0.0 and 14.8% of pre, post emergence damping-off and charcoal rot respectively, compared with control being 53.33, 13.33 and 44.4% for pre, post emergence damping-off and charcoal rot, respectively. As to the survivals, all treatments resulted in a tremendous increase reaching 86.66 % with Rhizo N, Seeds guard and *B. subtilis* and 93.33 % with *Trichoderma harzianum* and Plant guard compared with the control of only 33.33 % survival.

b. Soil treatment: *T. harzianum*, *B. subtilis*, Plant guard, Seeds guard and Rhizo N were used as soil drench prior to, after or at the time of infestation to define their effect on disease incidence.

Data in Table (8) show that *T. harzianum* decreased the disease incidence to 6.6, 0.0 and 4.44% of pre, post emergence damping-off and charcoal rot, respectively if applied 7 days before soil infestation, followed by that made at time of soil infestation and 7 days after soil infestation. *B. subtilis* treatment recorded 13.33, 0.0 and 7.03% of pre, post emergence damping-off and charcoal rot, respectively when added before soil infestation; however, these values were 26.66, 6.66 and 18.5% when used at the time of infestation. Adding antagonistic bacteria at 7 days after soil infestation recorded higher disease percentages.

Rhizo N added 7 days before soil infestation reduced the disease incidence to 20.0, 0.0 and 9.62% and 33.33, 0.0 and 34.63% when used simultaneously with infesting the soil with the pathogens. The percentage of disease incidence 7 days after inoculation were 40.0, 13.33 and 42.03% of pre, post emergence damping-off and charcoal rot. Seeds guard and Plant guard resulted in lower disease incidence compared with Rhizo N.

Generally, the most affective bioagent was *T. harzianum*, while Rhizo N was less effective. On the other hand, the best time for application of biocontrol agents was 7 days before soil infestation with *M. phaseolina*.

Table 8. Effect of tested antagonistic microorganisms and biocides as soil drench on percentage of emergence damping-off, charcoal rot and survival plants of Xera bean cultivar under artificial infection.

Bioagents (B)	Timing of biocontrol agents (I) application	% emergence damping-off		%Charcoal rot (60 days)	% survival plants (60 days)
		Pre (15 days)	Post (30 days)		
Trichoderma harzianum	7 days before inoculation.	6.66	0.0	4.44	93.33
	At same time of inoculation.	20.0	0.0	14.8	80.0
	7 days after inoculation.	26.66	6.66	34.63	66.66
Bacillus subtilis	7 days before inoculation.	13.33	0.0	7.03	86.66
	At same time of inoculation.	26.66	6.66	18.5	66.66
	7 days after inoculation.	33.33	6.66	39.66	60.0
Plant guard	7 days before inoculation.	6.66	0.0	8.14	93.33
	At same time of inoculation.	26.66	0.0	22.2	73.33
	7 days after inoculation.	33.33	13.33	39.66	53.33
Seeds guard	7 days before inoculation.	13.33	0.0	3.33	86.66
	At same time of inoculation.	26.66	0.0	18.5	73.33
	7 days after inoculation.	33.33	6.66	37.3	60.0
Rhizo N	7 days before inoculation.	20.0	0.0	9.62	80.0
	At same time of inoculation.	33.33	0.0	34.63	66.66
	7 days after inoculation.	40.0	13.33	42.03	46.66
Control		53.33	13.33	44.4	33.33
L.S.D.at 5%	Bioagent (B)	6.49	NS	6.33	NS
	Inoculum times (I)	8.04	5.6	4.15	9.38
	Interaction (B x I)	10.21	NS	9.62	NS

4. Chemical control:

1. *In vitro* experiments: The effect of some fungicides on the linear growth of three isolates of *M. phaseolina* were evaluated under laboratory conditions.

Results given in Table (9) show that Vitavax and Rhizolex at 50 ppm completely inhibited the mycelial growth of the tested isolates. Benlate at 100 ppm completely inhibited the growth of the Sweet melon, Bean and Sesame isolates. At 10 ppm, Vitavax was the most inhibitive fungicide to *M. phaseolina* isolates, as it caused growth reduction of 66.66, 77.44 and 81.88% of the three isolates, respectively. Generally, Vitavax was the most effective followed by Rhizolex and Benlate in a descending order.

2. *In vivo* experiments: Vitavax, Benlate and Rhizolex were evaluated as seed dressing on the incidence of pre, post emergence damping-off and charcoal rot. Data in

table (10) show that Vitavax significantly reduced pre and post emergence damping-off, followed by Benlate and Rhizolex compared with 40.0 and 20.0% in the control. Also, Vitavax decreased the charcoal rot to a low percentage of 6.66%, followed by Rhizolex and Benlate being 8.14 and 9.62%, respectively, compared with the control (44.4%). Generally, Vitavax was superior in controlling damping-off and charcoal rot of bean and showed higher percentage of survived plants, thus conforming with the *in vitro* results.

Table 9. Effect of different fungicides on percentage of reduction of linear growth of three isolates of *M. phaseolina* grown on PDA medium *in vitro*.

Isolates(I) and Fungicides (F) \ Concentrations (C)		Growth reduction (%) of 3 isolates					Mean
		0 ppm	5 ppm	10 ppm	50 ppm	100 ppm	
Sweet melon isolate	Benlate	0.0	38.22	50.0	91.11	100.0	55.86
	Rhizolex	0.0	42.66	55.22	100.0	100.0	59.57
	Vitavax	0.0	44.11	66.66	100.0	100.0	62.15
	Mean	0.0	41.66	57.29	97.04	100.0	
Bean isolate	Benlate	0.0	46.0	61.11	93.33	100.0	60.09
	Rhizolex	0.0	51.55	70.77	100.0	100.0	64.46
	Vitavax	0.0	55.55	77.44	100.0	100.0	66.59
	Mean	0.0	51.03	69.77	97.77	100.0	
Sesame isolate	Benlate	0.0	58.88	73.77	100.0	100.0	66.53
	Rhizolex	0.0	64.44	77.1	100.0	100.0	68.3
	Vitavax	0.0	66.66	81.88	100.0	100.0	69.71
	Mean	0.0	63.33	77.58	100.0	100.0	

L. S. D. at 5% for: Isolates (I) = 0.41 Interaction (I x F) = NS (IxC) = 1.11
Fungicides (F) = 0.65 (FxC) = 1.11 (I xF xC) = 1.92
Concentrations (C) = 0.64

Table 10. Effect of different fungicides as seed dressing on percentage of emergence damping-off, charcoal rot and survived plants of Xera bean cultivar under artificial infection *in vivo*.

Fungicides	% Emergence damping-off		% Charcoal rot	% Survived plants
	Pre. (15 days)	Post. (30 days)	(60 days)	(60 days)
Rhizolex T	13.33	0.0	8.14	86.66
Benlate	20.0	0.0	9.62	80.0
Vitavax	6.66	0.0	6.66	93.33
Control	40.0	20.0	44.4	40.0
L. S. D. 5 %	18.83	11.5	.3.31	18.8

DISCUSSION

Bean productivity is often affected by different diseases. Charcoal rot caused by *M. phaseolina* is one of the more destructive diseases as it commonly reduces bean yield and quality and high losses occur every year. The pathogen is worldwide in distribution and has a wide host range, causing charcoal rot disease in more than 500 plant species (Dhingra and Sinclair, 1977).

Isolation from naturally infected roots of bean plants collected from five localities in Ismailia governorate *i.e.* Ain Ghosin, El Kassassin, El- Tal- El-Kabeer, El Mehsama and El Wasfia, during two successive seasons (1999/2000 and 2000/2001) yielded 77 fungal isolates. Fungi associated with diseased roots showed that *M. phaseolina* was the most frequent followed by *R. solani, Sclerotium rolfsii* and *F. solani. M. phaseolina* causes pre, post emergence damping-off and charcoal rot resulting in serious losses in bean crop. These data are in harmony with those reported by Eisa (1998).

As to the fungi associated with bean seeds, *Rhizopus nigricans* was the most frequent, followed by *Aspergillus niger, Penicillium spp.* and *Alternaria solani. M. phaseolina* was of moderate frequency followed by *Botrytis cinerea* and *Rhizoctonia solani.* Also, *Fusarium oxysporum* f.sp *phaseoli, F. semitectum, F. solani, F. moniliforme, M. phaseolina, Rhizoctonia solani, Alternaria alternata, Stemphylium sp., Trichothecium roseum, Aspergillus spp., Nigrospora oryza,* and *Penicillium spp.* were previously isolated by Sarhan (2000) from bean seeds.

Fifteen bean cultivars were evaluated as to their reaction against *M. phaseolina.* The obtained results revealed that all the tested cultivars were susceptible to different degrees to infection with *M. phaseolina.* (Nebraska) cultivar showed the lowest percentage of disease incidence, followed by Royal Nel, Contender and Bronco cultivars. Xera, Coby, Julta and Helda exhibited the highest percentage of disease incidence. Morgan, Giza 3, Giza 6, Baulista and Samantha cultivars showed moderate levels of disease incidence. The differences between cultivars in infection may be attributed to mechanical or physiological resistance (Songa and Hillocks, 1996).

Biological control trials were carried out with some antagonistic microorganisms *i.e. Trichoderma harzianum, T. viride* and *Bacillus subtilis* and some commercial biocides

i.e. Plant guard, Seeds guard and Rhizo N, *in vitro* and *in vivo*. Antagonistic microorganisms and biocides caused growth reduction for all tested isolates of *M. phaseolina in vitro* and caused remarkable decrease in disease incidence, in both treated seeds and soil. These results are in harmony with those reported by Dennis and Webster (1971), who reported the ability of the bioagents to produce mycolytic enzyme *i.e.* β-1,3 gluconase and chitinase degrading the mycelium of *M. phaseolina*. Also, many isolates may produce volatile, and nonvolatile antibiotics.

Rhizo N and Plant guard were effective in controlling charcoal rot. This may be due to the sensitivity of *M. phaseolina* to an antibiotic complex containing bacilysin and fungymycin produced by *B. subtilis* (Rhizo N), (Tschen, 1987 and Reddy *et al.*, 1994).

Chemical control was studied using three fungicides to evaluate their effect on the linear growth of *M. phaseolina in vitro* at concentrations varying from 5 to 100 ppm. All tested fungicides have completely inhibited the mycelial growth of the tested isolates at 100 ppm. A concentration of 50 ppm was sufficient to obtain complete inhibition in most cases. Tested fungicides significantly decreased the disease when applied as seed dressing *in vivo* at the rate 3g/kg seeds. Using the biocontrol agents as soil drench resulted in a noticeable reduction in disease incidence; however, the highest effect was observed with the application prior to soil infestation with the pathogen. At such a timing, the biocontrol agent had the opportunity to get established in the soil and reach an effective population. The results showed some variation among fungicides *in vitro* and *in vivo*. These conclusions are in agreement with Singh and Sardinia (1990), who speculated that differences in reaction might be due to selective effect between the fungicide and the fungus.

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مكافحة مرض العفن الفحمي في الفاصوليا في محافظة الإسماعيلية

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تتعرض نباتات الفاصوليا للإصابة بالفطر ماكروفومينا فاصيولينا والذي تم عزله من مناطق مختلفة من محافظة الإسماعيلية (عين غصين - القصاصين- التل الكبير - المحسمة - الواصفية) حيث سجل الفطر أعلى نسبة تكرار على الجذور المصابة ونسبة متوسطة على البذور لأصناف مختلفة من الفاصوليا وكان الصنف نبراسكا أقل حساسية للإصابة بالمرض بينما الصنف مورجان متوسط الحساسية أما الصنف أكسيرا فأظهر حساسية عالية للإصابة عند زراعته في تربة ملوثة صناعيا بالفطر. سجل الفطر ترايكودرما هارزيانم والمبيد الحيوي بلانت جارد أعلى نسبة تثبيط للنمو الميسليومى للفطر ماكروفومينا فاصيولينا في المعمل وتحت ظروف الصوبة أدت معاملة البذور أو التربة قبل الزراعة إلى مكافحة المرض . المبيدات الفطرية فيتافاكس وريزولكس و بنليت ٥٠ ٪ منعت تماما النمو الميسليومى لعزلات الفطر ماكروفومينا فاصيولينا عند تركيزات ٥٠ جزء في المليون .المبيد الفطري فيتافاكس قلل معدل موت البادرات قبل وبعد ظهورها فوق سطح التربة وكذلك مرض عفن الجذور الفحمي في صنف الفاصوليا اكسيرا يليه المبيد ريزولكس ثم بنليت .