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EFFICACY OF CHEMICAL AND BIOLOGICAL TREATMENTS FOR CONTROLLING SOIL-BORNE PATHOGENS OF SOYBEAN

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ABSTRACT

Several soil-borne fungal pathogens attack soybean plants, causing seedling damping-off and root rot diseases, in Egypt. Isolation trials from rooted rots of soybean, collected from various locations at Gharbiya, Kafr El-Sheikh and Minufiya governorates, revealed that *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina* were the most virulent and predominant pathogens. All the tested fungicides significantly reduced linear growth of the tested pathogens. Three known bioagents *i.e. Trichoderma harzianum*, *T. hamatum* and *Bacillus subtilis* were tested against such pathogens, and revealed remarkable effect in reducing mycelial growth on PDA medium. *T. hamatum*, mainly, grew over the mycelium of the tested pathogens. Under greenhouse conditions, both the fungicides and antagonists gave significant reductions of root rot severity, but fungicides were more effective than biocontrol agents in reducing the disease. Application of the selective antagonists and fungicides significantly decreased soybean root rot than untreated check, in naturally pathogen- infested fields. Plant growth and activity of nitrogenase enzyme were enhanced greatly, when the fungicides were applied compared with antagonists treatments.

INTRODUCTION

Soybean [*Glycine* max (L.) *meri*] is one of the most important legume crops worldwide, because it is considered a chief source of protein. In Egypt, soybean is subject to attack by a wide array of destructive pathogens belong to the genera *Pythium, Fusarium, Macrophomina, Rhizoctonia* and *Sclerotium*, which causing damping-off and root rot under field conditions **(Fadl & Hussein, 1978 and Mahrous & Ibrahim, 1984).** *Rhizoctonia solani* was the most virulent pathogen causing root rot disease **(Omar, 1986).** The fungus was pathogenic to several legume crops and its saprophytic ability was low in soil previously cultivated with maize and soybean **(Punja & Grogan, 1981; Singh, 1982; Galindo *et al* 1983; Dob & Dutta, 1991 and Carling *et al* 2001).** *Fusarium* spp., *Pythium* spp., *Phytophthora sojae* and *R. solani* isolated from diseased soybean seedlings in Iowa (USA) were the major causal fungi associated with seedling disease complex of soybean and control of these pathogens have depended mainly on fungicides **(Waraitch *et al* 1986 and Vyas, 1994). Vadhera *et al* (1997)** indicated that the fungicides Bavistin and Zinb, at all tested concentrations, completely inhibited *F. solani* on bean, in pot experiments. *Bacillus subtilis* and *Trichoderma harzainum*, alone, or in combination with Captan 400 and Vitavax 200, were effective against dry root rot pathogens of bean **(Jensen *et al* 2002).** Dressing treatment of faba bean seeds, with various fungicides, indicate that Benlate was the best fungicide followed by Vitavax T, Rizolex T and Moncerin **(El-Sayed, 2005).** The most biocontrol agents successfully used were *Trichoderma* spp., *Pseudomonas* spp. and *Bacillus* spp. **(Cook & Baker, 1983; Lewis & Papavizas, 1985; Weller, 1988 and Kumar, 1998). Maria and Joseph (2006)** showed that, *T. harzianum* was antagonistic, *in vitro,* to *R. solani* and *Verticillium dahliae* and considered a potential biocontrol agent.

MATERIALS AND METHODS

1. Isolation and identification of the causal organisms

Soybean plants, showing typical root rot symptoms, were collected from different growing loca-

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tions in three governorates in Egypt, *i.e.* Gharbiya, Kafr El-Sheikh and Minufiya, and were used for isolation of causal pathogens.

Infected roots and basal stems were cut into small pieces, and washed thoroughly with running water to remove adhering soil particles. These pieces were surface sterilized by immersing in 5% ethanol for 2 min, then washed several times in sterilized distilled water and dried with sterilized filter papers. Surface sterilized samples were aseptically transferred to potato dextrose agar medium (PDA) containing 40ppm streptomycin sulphate to avoid bacterial contamination. Plates were incubated at 25°C for 3–7 days and observed daily for fugal development. Hyphal-tips of the growing fungi were individually transferred to new PDA plates **(Riker and Riker, 1936)**. The obtained fungal isolates were identified on the basis of morphological and microscopical characteristics of mycelia and reproductive structures using the following taxonomic references, Barnett and Hunter, (1987) for genera of imperfect fungi, **Booth (1971)** for *Fusarium* spp., **Dhingra and Sinclair (1978)** for *Macrophomina phaseolina* and **Sneh *et al* (1991)** for *Rhizoctonia* spp. Identification was confirmed at Mycology and Disease Survey Dept., Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt.

2. Effect of Chemical and biological agents, *in vitro*

2.1. Effect of fungicides on fungal radial growth

Three fungicides namely: Rizolex-T (Tolclofos–methyl + thiram, WP50%), Vitavax-T (Carboxin 19.5% + thiram 19.5%) and Moncerin (Pencycuron 25%), were used throughout this study. Different concentrations were made for each fungicides *i.e.* 0, 50, 100, 200 and 400 ppm, and were added to PDA medium, directly before pouring in Petri plates (90 mm Ø). Plates were inoculated at the center by equal discs (5 mm Ø), taken from 7 days-old cultures of the pathogenic fungi *F. solani, R. solani* and *M. phaseolina*. Three replicates were used for each concentration. Plates were incubated at 28°C where linear growth of developed fungal colonies were measured when the check treatment covered plates and averaged.

2.2. Effect of bioagents on fungal radial growth

Three known antagonistic bioagents, i.e. *Trichoderma harzianum, T. hamatum* and *B. subtilis,* were kindly obtained from Legume Dis. Res. Dept., Plant Path. Res. Inst., ARC, Giza. They were tested for their antagonistic abilities against root rot pathogens of soybean. Petri plates containing PDA medium were inoculated with discs (5 mm Ø), which taken from 7 days old cultures of the pathogenic fungi. The pathogenic fungi were inoculated at 20mm of the Petri dish side, whereas the opposite side was inoculated with either disc of each antagonistic fungus or with streak of *B. subtilis*. Plates only inoculated with each pathogenic fungus at one side, 20 mm from the plate edge were kept as control. Three replicates were used for each treatment. Plates were incubated at 28°C. Linear growth of each tested fungi was measured when pathogenic fungi completely covered the surface of the medium in the control treatment. The inhibition percentage of pathogen growth was calculated using the formula adopted by **Mohamed (1996)** as follows:

$$\text{Reduction in linear growth \%} = \frac{R_1 - R_2}{R_1} \times 100$$

Where: R_1 = the radius of control growth.
R_2 = the radius of inhibited growth.

3. Greenhouse study

3.1. Effect of seed dressing fungicide treatment

This experiment was carried out in sterilized pots (25 cm Ø), containing sterilized clay soil in the greenhouse of Legume Dis. Res. Dept., Plant Path. Res. Inst., ARC at Giza. Inoculum for soil infestation was prepared by growing each fungal pathogen, on sand barley medium (25 g clean sand, 75 g barley grain and water was added to cover the mixture). Flasks containing sterilized medium were inoculated with each particular fungus and incubated at 25°C for two weeks. Soil was infested with each individual fungus at the rate of 2% of soil weight. Potted soil was watered every two days for a week to allow the fungal growth. Soybean seeds cv. Giza 35 were treated with the test fungicides, Vitavax T, Rizolex T and Moncerin at rates of 4, 3 and 3g / kg seeds, respectively. Treated seeds were shaked gently in a glass container containing 2 ml glue solution (supper-film 70) as a sticker material/kg seeds. Ten seeds were sown per each pot, and three replicates were used for each treatment. Percentages of pre- and post-emergence damping-off and survival plants were recorded after 15 and 30 days from seed sowing.

Meanwhile, fresh and dry weights of the growing plants were estimated after 45 days from sowing.

3.2. Effect of bioagents on seedling survival

This study was carried out in sterilized pots (25 cm Ø) containing sterilized clay soil in the greenhouse. Inocula of fungal bioagents were grown on sand barley medium and added to the soil 7 days before sowing at the rate of 5% of soil weight. While, *B. subtilis*, were grown on nutrient glucose broth **(Abd El-Moity and Shatla, 1981),** and the bacterial cell suspension were added at the rate of 50 ml / pot (10^9 cfu/ml), 7 days before sowing. The control treatment was infested with pathogen only, and three replicates were used for each treatment. Percentage of pre- and post-emergence damping-off and survival plants were recorded after 15 and 30 days. Fresh and dry weight of survival plants was recorded, 45 days after sowing.

4. Efficacy of chemical and biological treatments, under field conditions

4.1. Effects of fungicides and bioagents

The experiments were conducted in naturally infested field at Sers El-Layian Agriculture Research Station, Minufiya, in 2007 and 2008 growing seasons. The experimental design was completely randomized block. The plot was divided to equal plots each one consisting of 4 rows (3.5 m long and 60 cm in width). Three replicates were used for each treatment.

Soybean seeds cv. Giza 35 were treated with three fungicides Vitavax T, Rizolex T and Moncerin at rates of 4, 3 and 3g / kg seeds, respectively. Meanwhile, bio-control agents were added as powder formula which prepared by growing each particular antagonist on 200 ml liquid potato dextrose medium for 7 days. Fungal growth was mixed with talc powder at the rate of 1: 1 (v: w) and the mixtures were left to dry. Arabic gum was used, as a sticker, before seed treatment with such agents. Disease parameters and yield components were recorded and estimated. Percentage of pre- and post emergence damping-off, survival plants and disease severity index were recorded up to 45 days after sowing. Root rot severity index was calculated according to **Soliman *et al* (1988)** as following:

$$\text{Disease severity} = [(a \times b \div N \times K) \times 100]$$

Where: a: Number of infected plants.
b: Grade of infection
N: Number of total plants
K: Maximum grade of infection.

4.2. Assay of nitrogenase activity in root nodules

Root samples of soybean plants with nodule were collected from greenhouse and field experiments for assay of nitrogenase activity according to the method of **Hardy *et al* (1973)**. For each bottle (containing nodule samples), 50 ml purified acetylene gas were injected using standard syringe (50 ml) to give 10% v/v. The injected bottles were incubated at 30°C for 2 hr and 1 hr, respectively. Two ml gas samples were taken out by a syringe from each bottle and assayed for the produced ethylene concentration using a Perkin Elmer Gas liquid chromatography (GLC) model-3920 B fitted with a flamer ionization detector (FID) and a stainless steel column (1.8 mm × 3.0 mm i.d.) packed with porapak R., 80 – 100 mesh, (air, 50 ml / min, H_2, 50 ml / min, N_2, 50 ml / min). The temperatures were 100, 60 and 150°C for injector, column and detector, respectively. The retention times of ethylene and acetylene were 1 and 2 min, respectively. Calibration curve was established using defined concentrations of pure ethylene in air. Pure ethylene (1.2 ml) was injected in 1200-ml serum-bottle, (1000 vpm final concentration), out of which serial dilutions were made. Two-ml gas of each solution was injected into the GLC under the previously mentioned conditions. A linear relation was obtained between ethylene concentrations and ethylene peak's height (k=0.08). The produced μmole C_2H_4 / hour were calculated using the following equation:

Peak height of sample (cm × μ moles C_2H_4 / hr = peak height of sample

$$\text{cm} \times \frac{\text{Vol. gas in sample container}}{\text{Vol. injected (ml)}} \times \frac{1}{\text{assay time}} \times \frac{1}{2.24}$$

Statistical analysis

Statistical analysis of data was done according to analysis of variance (ANOVA) procedures reported by **Clarke and Kenpson (1997)**. Treatment means were compared by the least significant difference test L.S.D at 5% level of probability.

RESULTS

1. Isolation and identification of the causal organisms

Twenty five fungal isolates were obtained from soybean plants showing root-rot symptoms, grown in different localities in Egypt **Table (1)**. Isolation trials showed that *Rhizoctonia solani* was the most predominant pathogen (52%); which showed the highest number of obtained isolates and their frequencies were 62.5, 55.5 and 37.5% at Gharbiya, Kafr El-Sheikh and Minufiya, respectively. Meanwhile, *Fusarium solani* and Macrophomina *phaseolina* occurred at frequencies 20 and 28%, respectively.

2. Effect of fungicides and bioagents

2.1. Effect of fungicides on fungal mycelial growth, *in vitro*

Three fungicides *i.e.* Rizolex T, Vitavax T and Moncerin were tested at different concentrations against the three pathogens, *R. solani, F. solani* and *M. phaseolina*. Results presented in **Table (2)** indicate that Vitavax T was superior to Moncerin. At 100 ppm, this compound could significantly inhibit growth of all tested pathogens.

However, at higher concentrations (400 ppm) of the tested fungicides, no growth for all tested fungi was recorded. Vitavax T was the most effective fungicide followed by Rizolex T and Moncerin, respectively.

2.2. Assay of antagonism, in *vitro*

Results shown in **Table (3)** indicate that all tested bioagents significantly reduced radial growth of the three fungal pathogens. *T. hamatum* showed the highest reduction effect against *R. solani*, while *T. harzianum* showed the highest effect against *F. solani*. Although, *B. subtilis* isolate reduced *R. solani* growth by 89.4%, it was less effective against *F. solani* and *M. phaseolina*. It was also observed that mycelia of *T. hamatum* mainly grew over the mycelium of all tested pathogens.

2.3. Effect of fungicides on seedling damping–off and seedling growth, in pots

Results in **Table (4)** show the effect of certain fungicides, used as seed dressing, for controlling root rot of soybean. All tested fungicides significantly reduced the development of damping-off and root-rot disease, under greenhouse conditions, compared with control. Vitavax T, at rate of 4g/kg seeds, was the best fungicide for reducing pre- and post-emergence damping-off. Rizolex T and Monceren were the least effective fungicide.

As for the effect of dressing seeds with fungicides on some growth characters of soybean grown in soil artificially infested with with root rot pathogens, data in **Table (5)** reveal that, there were clear effects of dressing seeds with fungicides on dry weight and fresh weight compared with untreated plants. Vitavax T and Rizolex T were the best fungicides, respectively. Meanwhile, Monceren was the least effective one. Concerning dry weight, similar trend were also obtained where, dressing seeds with fungicides increased significantly fresh weight and dry weight in all tested treatments compared to the control (untreated seeds).

2.4. Effect of bioagents on seedling damping–off and seedling growth, in pots

Results in **Table (6)** indicate clearly that the three tested bioagents significantly reduced pre- and post-emergence damping-off of soybean seedlings compared with untreated control. The highest disease reduction was achieved in case of *T. hamatum* treatment. However, *B. subtilis* was the least effective bioagent.

Remarkable effects of bioagents, on some growth characters of soybean plants, grown in pathogen-infested soil, were recorded **Table (7)**. All tested bioagents increased fresh and dry weight of plants, especially when used against *R. solani* and *M. phaseolina*.

3. Field Experiment

3.1. Efficacy of fungicides and biocontrol agents in disease suppression

Data in Table (8) clearly indicate that application of either bioagents or fungicides, under field conditions, significantly reduced pre- and post-emergence damping-off compared with untreated control. *T. hamatum*, Vitavax-thiram and Rizolex-T were the best treatment in reducing damping–off of seedlings and consequently increased survival plants in both growing seasons (2007 and 2008).

3.2. Effects on plant growth

As for the effect of antagonists and fungicides on growth characters of soybean under field conditions, the obtained data **Table (9)** show that the

Table 1. Frequency of isolation of pathogenic fungi from rotted roots of soybean, collected from three different governorates in Egypt

Fungal Isolates	Gharbiya		Kafr RI-Sheikh		Minufiya		Total	
	No.	Frequency %	No.	Frequency %	No.	Frequency %	Total	Frequency %
R. solani	5	62.5	5	55.5	3	37.5	13	52
F. solani	1	12.5	3	33.3	3	37.5	7	28
M. phaseolina	2	25.0	1	11.1	2	37.5	5	20
Total	8	-	9	-	8	-	25	-

Table 2. Effect of fungicides on linear growth of three tested fungal pathogens of soybean roots, *in vitro*

Fungicide and Concentration (ppm)	*R. solani*		*F. solani*		*M. phaseolina*	
	Linear growth (mm)	% Reduction	Linear growth (mm)	% Reduction	Linear growth (mm)	% Reduction
Rizolex T 0	90.0	-	90.0	-	90.0	-
50	50.0	44.4	60.0	33.3	40.15	55.4
100	35.17	60.9	50.0	44.4	30.0	66.7
200	0.0	100.0	20.0	77.8	0.0	100.0
400	0.0	100.0	0.0	100.0	0.0	100.0
Vitavax T 0	90.0	-	90.0	-	-	-
50	30.0	66.7	35.0	61.1	35.0	61.1
100	10.0	88.9	25.0	72.2	20.0	77.8
200	0.0	100.0	15.0	83.3	10.0	88.9
400	0.0	100.0	0.0	100.0	0.0	100.0
Mancerin 0	90.0	-	90.0	-	-	-
50	80.0	11.1	70.0	22.2	80.52	10.5
100	60.0	33.3	50.0	44.4	70.0	22.2
200	40.0	55.6	20.0	77.8	50.0	44.4
400	20.0	77.8	0.0	100.0	30.0	66.7

L.S.D. 0.01 Fungicides = 1.60 Fungi = 1.32 Interaction = 3.09

Table 3. Effect of the selected bioagents, on linear growth of three fungal pathogens of soybean roots

Bioagents	*R. solani*		*F. solani*		*M. phaseolina*	
	Linear growth (mm)	Reduction (%)	Linear growth (mm	Reduction (%)	Linear growth (mm	Reduction (%)
Control	90.00	-	90.00	-	90.00	-
T. hamatum	20.0	89.72	30.0	66.66	22.50	75.00
T. harzianum	35.00	51.11	50.00	89.44	44.50	50.55
B. subtilis	50.00	89.44	62.50	30.55	55.00	38.88

L.S.D. 0.01 Antagonistic = 1.33 Pathogens = 2.52 Interaction = 4.55

Table 4. Effect of different fungicides on incidence of seedling damping-off and survival of soybean plants, under greenhouse condition

Fungicide	Rate g//kg seeds	Pathogens	Damping-off (%)		Survival plants*	Root rot severity index (%)**
			Pre-emergence	Post-emergence		
Control (Nontreated)	0	*R. solani*	40.00	40.00	20.00	70.15
		F. solani	30.33	26.77	42.90	40.18
		M.phaseolina	33.33	33.33	33.34	62.11
Rhizolex T	3	*R. solani*	21.33	13.33	65.34	28.17
		F. solani	11.33	11.33	77.34	31.15
		M.phaseolina	13.33	13.33	73.34	35.15
Vitavax- Thiram	4	*R. solani*	0.00	26.77	73.23	29.35
		F. solani	0.00	0.00	100.00	0.00
		M.phaseolina	0.00	20.00	80.00	0.00
Moncerin	3	*R. solani*	26.77	21.33	51.90	60.35
		F. solani	21.33	18.35	60.32	29.35
		M.phaseolina	21.33	21.33	57.34	40.15
L.S.D 0.05%			5.50	4.20	8.67	9.50

*30 days after sowing. ** 45 days after sowing.

Table 5. Effect of different seed dreesing fungicides on fresh and dry weight of soybean plants grown in pathogen-infested soil, under greenhouse conditions, 45 days after sowing

Fungicide	Rate g / kg seeds	Pathogen	Fresh weight gm / plant	Dry weight gm / plant
Control (Nontreated)	0	*R. solani*	4.70	1.10
		F. solani	5.00	1.50
		M.phaseolina	4.30	1.30
Rhizolex-T	3	*R. solani*	7.90	3.00
		F. solani	7.50	2.50
		M.phaseolina	5.80	2.00
Vitavax- Thiram	4	*R. solani*	7.20	3.00
		F. solani	6.00	2.50
		M.phaseolina	7.00	3.40
Moncerin	3	*R. solani*	5.30	2.10
		F. solani	5.70	3.00
		M.phaseolina	5.20	2.90
L.S.D 0.05%			1.70	1.30

Table 6. Effect of certain bioagents on soybean seedling survival, under greenhouse conditions

Bio-agent / Pathogen	Pre emergence damping-off (%)				Post-emergence damping-off (%)*				Survival plant (%)*			
	Control	*T. harzianum*	*T. hamatum*	*B. subtilis*	Control	*T. harzianum*	*T. hamatum*	*B. subtilis*	Control	*T. harzianum*	*T. hamatum*	*B. subtilis*
R. solani	51.30	30.33	20.00	26.77	30.00	33.33	26.77	30.00	18.70	36.34	53.23	43.23
F. solani	40.00	20.66	10.33	20.33	40.00	20.33	20.66	30.66	20.00	59.01	69.01	49.01
M.phaseolina	50.00	33.33	26.77	28.33	35.50	20.00	20.00	26.77	14.50	46.67	53.23	44.90
L.S.D. at 5%	(T) Bioagent = 3.90 (F) Fungi = 1.00 T × F = 2.30				T = 3.00 F = 0.50 T × F = 0.90				T = 4.00 F = N.S T × F = 0.90			

*30 days after sowing.

Table 7. Effect of certain bioagents on fresh and dry weight of soybean seedlings grown in soil infested with grown in pathogen-infested soil, under greenhouse conditions, 45 days after sowing

Bio-agent / Pathogen	Fresh weight / plant				Dry weight / plant			
	Control	*T. harzianum*	*T. hamatum*	*B. subtilis*	Control	*T. harzianum*	*T. hamatum*	*B. subtilis*
R. solani	3.00	4.00	4.50	3.90	1.00	1.1	1.90	1.30
F. solani	4.00	6.00	6.70	5.85	1.50	3.00	3.20	2.90
M. phaseolina	3.50	5.90	6.00	4.90	1.70	2.30	2.50	2.00
L.S.D. at 5%	T = 1.12 F = 1.17 T × F = 3.40				T = 0.17 F = 0.13 T × F = 0.39			

Table 8. Effect of certain bioagents and fungicide seed treatments on incidence of seedling-damping-off and survival of soybean plants, during two growing seasons (2007 and 2008), under field conditions

Treatments	Season 2007			Season 2008		
	% Pre-emergence	% Post-emergence	Survival plants%*	%Pre-emergence	%Post-emergence	Survival plants%*
Bioagents:						
T. harzianum	12.3	4.6	83.1	13.7	9.5	76.8
T. hamatum	8.9	5.5	85.6	10.5	5.9	83.6
B. subitilus	13.7	4.2	82.1	14.8	8.7	76.5
Fungicides						
Rhizolex T	4.5	3.9	90.7	7.9	4.3	87.8
Vitavax T	6.0	3.3	90.7	6.7	5.9	87.4
Moncerin	8.4	6.7	84.4	9.4	8.7	81.9
Control	14.6	9.5	75.9	15.5	12.2	72.3
L.S.D 0. 05%	1.6	0.77	1.90	1.73	0.83	2.00

*45 days after sowing.

Table 9. Nodulation, fresh weight and dry weight of soybean plants under field conditions

Treatments	Season 2007			Season 2008		
	Nodules No./plant*	Fresh weight (g)*	Dry weight (g)*	Nodules No./plant.*	Fresh weight (g)*	Dry weight (g)*
T. harzianum	63.5	103.9	68.3	65.6	105.9	70.7
T. hamatum	65.7	110.14	72.5	67.7	113.7	76.3
B. subitilus	60.0	100.0	65.3	62.2	105.3	68.2
Rhisolex T	68.8	115.22	73.2	70.0	117.2	75.8
Vitavax T	67.6	117.8	75.7	68.9	119.6	78.3
Moncerin	62.2	105.3	70.0	65.3	108.3	71.2
Control	40.3	72.7	38.0	36.8	75.5	40.5
L.S.D 0. 05%	3.75	9.8	4.9	4.0	8.9	5.0

*45 days after sowing.

Table 10. Effect of treating soybean seeds with antagonists and fungicides on nitrogenase enzyme activity in root bacterial nodules

Treatments	Nitrogenase enzyme activity*		
	Season 2007	Season 2008	Mean
T. harzianum	25.8	30.3	28.05
T. hamatum	30.2	35.8	33.00
B. subitilus	22.3	25.8	24.05
Rhizolex T	38.2	42.4	40.30
Vitavax T	40.5	44.7	42.60
Moncerin	35.3	40.2	37.75
Control	18.7	23.8	21.25
L.S.D 0. 05%	4.21	3.12	

*45 days after sowing

tested fungicides and biocontrol agents significantly reduced damping-off of seedling and increasead plant survivals. These treatments also increased the number of nodules/plant, fresh weight and dry weight of soybean plants, 45 days after sowing. Similar results for the two experiments (2007 and 2008) ware recorded. However, Vitavax T and Rhizelex T were the best treatment among the tested fungicides. On the other hand, *T. hamatum* gave a good performance than *B. subtilis* for improving soybean plant growth parameters.

3.3. Effects on nitrogenase activity of bacterial nodule

Data in **Table (10)** reveal that nitrogenase activity was increased significantly in soybean roots grown from seeds treated with either fungicides or biocontrol agents, respectively, compared with untreated control. Moreover, Vitavax T and Rhizolex T showed a higher increase in nitrogenase activity than obtained with *T. hamatum* and *T. harzainum* treatments.

DISCUSSION

Seedling damping-off and root-rot diseases of soybean seedlings are considered a critical factor in soybean production worldwide. The results of this study revealed that such diseases are caused by several soil borne pathogens which include mainly, *Rhizoctonia solani, Fusarium solani* and *Macrophomina phaseolina*. These soil borne pathogens have been widely reported as the causal organism of soybean damping-off (**Fadl & Hussien, 1978; Deb & Dutta, 1991 and Carling *et al* 2001**).

It has been reported that application of fungicidal seed treatment is essential for manage such diseases. The results of this study revealed that seed dressing treatments, with three recommended fungicides in Egypt, significantly reduced damping-off disease of seedling and increased plant stand. These results are in agreement with other results obtained by **Vadhora *et al* (1997)** and **Jensen *et al* (2002).** Over two seasons, Vitavax Thiram and Rhizolex T were the best fungiceds in reducing incidence of soybean damping – off and root rot under field conditions. It is logic that these specific fungicides have a good role in disease control. Such fungicides showed also effective results with faba bean plants as reported by **El-Syed (2005)**.

The results of this study demonstrate the efficacy of certain bioagents, as seed treatments, for controlling distinct three soil-borne pathogens of soybean, either under greenhouse and field conditions. The bioagents *T. hamatum* and *T. harzianum* were the best antagonists that significantly reduced seedling damping–off of soybean. These results support earlier studies that certain bicontrol agent are promising factors for controlling soil-borne diseases on various plants (**Cook & Baker, 1983; Lewis & Papavizas, 1985; Weller, 1988 and Kumar, 1998).** However, all tested fungicides and bioagents showed positive effects in reducing the harmful effects of the pathogens and increased growth of plants grown in pathogen infested soils and induced a high increase in nitrogenase enzyme activity in bacterial nodules on soybean roots, that may lead to more crop yield.

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فاعلية المعاملات الكيماوية والحيوية لمكافحة فطريات التربة التى تصيب فول الصويا

[١٣]

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الموجـــــز

يصاب فول الصويا بالعديد من فطريات التربة التى تسبب مرض موت البادرات وعفن الجذور فى مصر. وقد أجريت هذه الدراسة بهدف تحديد المسببات المرضية الرئيسية للإصابة بمرض وموت البادرات وعفن الجذور فى فول الصويا فى بعض مناطق الزراعة حالياً فى مصر ومقاومته، تم عزل العديد من الفطريات الممرضة من بادرات ونباتات فول صويا مصابة بعفن الجذور من محافظات الغربيه وكفر الشيخ والمنوفية. وكان أكثر الفطريات عند العزل هى فطريات *Rhizoctonia solani* (٥٢%) ، *Fusarium solani* (٢٨%) *Macrophomina Phaseolina* (٢٠%) والتى كانت سائدة وأشد ضراوة.

أختبرت ثلاثة من المبيدات الفطرية ، تم دراسة كفاءة التضاد الحيوى لثلاثة عوامل حيوية معروفة وهى *Trichoderma harzianum* & *Trichoderma hamatum* & *Bacillus subtils* فى قدرتها التضادية للنمو الميسليومى للفطريات الممرضة على أطباق الأجار فى المعمل. وأظهرت النتائج أن لتلك المبيدات والعوامل الحيوية قدرة عالية على تثبيط النمو الميسليومى للفطريات الممرضة فى المعمل.

وبالنسبة لنتائج الصوبة فقد تم اختبار تلك العوامل الحيوية مقارنة بثلاثة من المبيدات المستخدمة فى معاملة البذوربمعدل 4-3 جم / كجم بذور والتى أعطت نتيجة إيجابية فى المعمل ، حيث أدت أيضاً إلى انخفاض شدة الإصابة فى الصوبة مقارنة بالكنترول. وكانت المبيدات أكثر كفاءة من كائنات التضاد الحيوى فى تقليل شدة الإصابة.

أثبتت التجارب الحقلية أن استخدام أياً من المبيدات الفطرية أوالعوامل الحيوية كان لها تأثير ملحوظ على الإصابة الفطرية بمرض موت البادرات وعفن الجذور حيث أدت معاملة البذور وتغليفها بالمبيدات أوكائنات التضاد الحيوى إلى اختزال الإصابة وزيادة عدد النباتات السليمة الحية وكذلك زيادة عدد العقد البكتيرية على النباتات المعاملة. وعند تقدير إنزيم النتروجينيز وجد أن معاملة البذور بالمبيدات أو كائنات التضاد الحيوى قد أدت إلى زيادة فى إنزيم النتروجينيز مقارنةً بالكنترول. وكانت المبيدات أكفأ من كائنات التضاد الحيوى فى هذا المجال.

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