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LENTIL (*Lens esculenta* L.) DAMPING-OFF AND ROOT-ROT DISEASES AND THEIR CHEMICAL AND BIOLOGICAL CONTROL IN EL-MINIA GOVERNORATE.

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ABSTRACT

Eleven isolates of *Rhizoctonia solani* and 15 isolates of *Fusarium spp* were isolated from lentil rotted roots and crowns. All isolates of *R.. solani* except two (R_2 and R_9) aggressively attacked lentil mostly during their seedling stages. On the other hand, three isolates of *F. solani* (F_{11} , F_{12} , F_{15}), out of the 15 tested isolates showed high pathological effect at all stages of the subjected plants causing pre- and post-emergence damping-off and root rot.

Isolates R_5 and R_8 of *R. solani* and isolate F_{11} of *F. solani* attacked six local cultivars of the lentil plants but the three Canadian cultivars tested showed great resistance.

Seven fungicides showed various inhibitory effect against *R. solani* and *F. solani in vitro*, the four more effective ones (Topsin-M, Rhizolex -T, Monceren and Homi) were tested *in vivo*.

Two antagonistic bacteria (*Bacillus spp.*) with great inhibitory effect towards *R. solani* and *F. solani* were secured from lentil rhizosphere and adequately used as a biocontrol agents against the two pathogens in pots experments.

INTRODUCTION

Lentils (*Lens esculenta* L.) is one of the most important legumincus crops in Egypt and in the world as well. The cultivated area in Egypt reach in 1998, 10.664 feddans. Most of this area were in upper Egypt especially Assiut Governorate. The cultivated area decreased to 5.025 feddans in 1999 (the Statistical Department of Ministry of Agriculture).

Lentil plants are prone to many diseases which attack the foliar parts of the plants causing mainly leaf blight and attack the root system causing seedling damping-off, root rot and vascular wilt to the more mature plants in the majority of lentil cultivated fields. In 1998-1999, it was noticed that severe disease was attacking lentil plantations in Beny-Abaid village, El-Minia Governorate.

In New Zealand, Fletcher *et al.* (1991) reported that four species of Fusarium were isolated from diseased Lentil plants. Of the 4 species isolated, *F. avenaceum* (*Gibberella avencacea*) was the most virulent pathogen.

Abdel-Kader (1977) isolated fungi belonging to six species of four genera from diseased Lentil plants obtained from different Localities of Upper Egypt during 1972 and 1973 seasons. The isolated fungi were *Fusarium solani* Marta, *F. oxysporum* Schecht ex fr., *F. moniliforme* Sheldon, *Gliocladium roseum* Bai, *Rhizoctonia solani* Kuhn, and *Pythium butleri* Subramanion. On the other hand, the most frequent fungus was *F. solani* followed by *R. solani*.

Yehia *et al.* (1985) reported that *F. solani*, *F. oxysporum*, *R. solani* and *Pythium debaryanum* were destructive on Lentil plants causing damping-off and root rot disease for lentil of both cvs. Giza 9 and Giza 370. On the other hand, *F. solani* and *F. oxysporum* proved the incitent of post-emergence damping-off for plants of both cultivars.

El-Garhy (2000) in a survey of fungal diseases of lentil in North Egypt and Upper Egypt found that *Macrophomina phaseolina*, *F. solani* and *R. solani* were prevalent in Upper Egypt Governorates.

Shatta *et al.* (1974) found that lentil cultivar Giza 9 was more resistant to root rot disease caused by *R. solani*, *F. solani* and *P. ultimum*. Kamaiyan and Nene (1975) reported that some cultivars of lentil were more resistant in the seedling stage when tested against *F.oxysporum*. f. sp. *lentis*. Rizk *et al.* (1992) testing 40 Lentil entries to root rot /wilt diseases under artificial inoculation in pots at Giza Agriculture Research Station, they found 5 entries were highly resistant, 10 were resistant, 13 were moderate susceptible, 4 were susceptible and 8 were highly susceptible. El-Garhy (2000) studied some other lentil cultivars, and he found that all cultivars he studied were highly susceptible to *R. solani* and *sclerotinia sclerotiorum*, except Giza 370 which was resistant only to *S. sclerotiorum*

Shatta *et al.* (1974) found that Topsin and Terraclcr fungicides were the most effective soil treatment at 21.8 kg/ha which decreased damping-off incidence in lentil plants caused by *R. sclani*, *F. solani* and *P. ultimum*. Bean seeds (cv. Contender) treated with Benlate, Moncerin, Moncerin combi and Quinolate fungicides reduced seedling mortalities caused by *R. solani* in pots and in the field (Raffat, Ferial, 1992). Also, under soil infestation in the field, all tested fungicides (Topsin-M 70%, Rhizolex-T, Homi 80, Tecto 60% and Quinolate 15%) increased percentage of survival Lentil plants in two grown seasons compared with the control (El-Garhy, 1994). Rhizolex-T 50% was the best fungicide in reducing disease incidence in peanut plants caused by *R. solani*, *F. oxysporum*, *F. solani*, *S. rolfsii* and *M. phaseolina* followed by Chlordosep and Amconil (El-Wakil and Ghonim, 2000).

The antagonistic microbes has long been isolated and their antagonistic action towards other microbes was recognized by some investigators. Some of those antagonists showed their inhibitory effect only on laboratory media and fewer proved to be of practical use as a plant disease control agent or in combination with other chemicals. Mew and Rosales (1986) found that fluorescent and non fluorescent bacteria inhibited mycelial growth of *R. solani* and affected there sclerotial viability. *In vivo* screening, pre-emergence damping-off disease controlled by *Bacillus subtilis* was excellent with plant stands being almost as high as non-diseaesed controls (Fiddaman and Rossall, 1995).

Galal and Saad (1995) found that three isolates of bacteria, two isolates belonging to *Pseudomonas fluorescens* sub group A and *P. carrugata*, and one *Bascillus subtilis* exhibited antagonistic activity to *F. solani*. *P. fluorescens* sub group A, was the most antagonist to mycelial growth of *Fusarium* species as it reduced incidence of stem, root rots and wilt diseases of Cowpea *in vivo*. Saleh (1997) reported that *B. subtilis* had strongest antagonistic effect against the tested fungi i.e. *R. solani* and *F.*

compactum *in vitro*. Also, this *B. subtilis in vivo* significantly decreased the incidence of root rot and wilt of Groundnut. Armanious, Hanaa (2000) found that one bacterial isolate *B. megaterium* was used as biocontrol agent against cotton plant pathogens (*Macrophomina phaseolina* and *F. oxysporum*). As well as the use of plant guard (*Trichoderma harzianum*), at 3 x 10^7 propagules/ gm or Rhizo-N (*B. subtilis*) at 3x10^7 cfu/gm, significantly controlled damping-off disease in rue (*Ruta graveolens* L.) (Helmy, Alia *et al.*, 2001).

MATERIALS AND METHODS

1. Isolation of the causal organisms :-

Diseased lentil plants (*Lens esculenta* L. cv. Balady) showing typical symptoms of damping-off and root rot were collected from Beni-Abaid village, Abou-Qurkas county, Minia Governrate, Egypt. Samples were thoroughly washed with running tap water, cut into small pieces, surface sterilized using 0.1% mercuric chloride ($HgCl_2$) solution for 2 minutes then washed three times in sterilized distilled water. Small portions of the disinfested roots (approximately 0.5-1 cm 1ong pieces) were plated in Petri dishes containing potato dextrose agar (PDA) medium without or with antibiotic (penicillin 20 Iu/ml). The inoculated plates were incubated at 25 °C for five days. The developing growth was then transferred separately to Petri dishes containing water agar or PDA medium and incubated for 2-5 days at room temperature (25°C). Pure cultures were obtained from the developing fungal colonies using the hyphal tip technique in case of *Rhizoctonia* and/or the single spore isolation technique in case of *Fusarium* on PDA medium. Subcultures of the obtained isolates were then kept on PDA slants and stored at 5 °C or room temperature for further studies.

2. Pathogenicity tests: -

Isolated fungi were tested for their pathogenicity on lentil plants sown in sterilized soil in pots (25 cm in diameter). Soil sterilization was carried out by autociaving for 2 hours at 15 kg/cm^2 pressure (121 °C) then left for 7 days before using for aerification.

The obtained isolates (*Rhizoctonia* and *Fusarium*) were grown separately on barley grain medium in conical flasks for 7-10 days to be used as a source of inoculum. Inocula of these tested fungi were applied separatly at the rate of 5% of the soil weight (Ragab *et al.*, 1997), mixed thoroughly with the soil then irrigated and left 7 days for establishment before sowing lentil seeds. Seeds of the local variety were disinfested by dipping in 0.1% mercuric chloride solution for 2 minutes, washed several times with sterilized distilled water before planting, then sown in the infested pots at the rate of 10 seeds /pot. Four pots were used for each isolate, (which were considered as replicates). Pots containing sterile soil mixed with barley grains free of any fungi were sown similarly with lentil seeds at the same rate to be used as a control treatment. All the pots were irrigated after soil infestation and later as needed.

The experiment was kept under careful observation and examined for pre-, post-emergence damping-off and root rot at 15, 30 and 90 days after sowing, respectively. Reisolation was carried out from some of the artificially

diseased plants to fulfill Koch's postulations and the developing fungi were compared with the original isolates. Final identification was kindly confirmed at Mycological Center, Ministry of Agriculture in Giza, to verify the suggested identification.

3. Varietal response :-

Response of six local lentil cultivars namely Giza 4, Giza 9, Giza 51, Giza 370, Sinaa 1 and Balady, in addition to three Canadian cultivars i.e. Eston, Laird and Richlea toward the disease infection was investigated using some selected fungal isolates which showed highly pathogenic ability through the pathogenicity test. The used isolates were two (R_5 and R_8) representative of *Rhizoctonia solani* and one (F_{11}) representative of *Fusarium solani*. This work was carried out in pots (25 cm in diameter) filled with sterile soil and infested with inocula prepared from the aforementioned isolates and sown with disinfested seeds of the cultivars to be tested (10 seeds/pot) and treated as described before. The density level of inoculum was 2.5% and 5% w/w. Each treatment was represented by four pots as replicates. Uninfested pots containing sterile soil were similarly sown with the tested cultivars to be used as a check treatment and kept under the same conditions.

Pots were fertilized once with ammonium nitrate (1g/pot) after 30 days of planting, irrigated as needed and kept under daily observation for disease incidence. Data were recorded for pre-and post-emergence damping-off at 15 and 30 days from sowing, respectively and after 90 days for root rot. Diseased plants were estimated as percentages to those of the healthy control treatments.

4. Disease management:-

4.1. Effect of some selected fungicides

Some of the commercial chemicals that are in common use as seed dressers were selected to be tested as protectants against the obtained lentil fungal isolates. This was carried out first in plates and then the more effect ones were used as seed dressers in pot trials.

4.1.1. Laboratory studies

The effect of seven selected fungicides, listed in Table (1), was tested *in vitro* on six *Rhizoctonia* and two *Fusarium* isolates in Petri dishes. Stock solutions of these chemicals were prepared (5000 , 1000 ppm) and volumes of which were added aseptically to melted Czapek 's agar medium in conical flasks to obtain serial of concentrations (2, 5, 10, 15, 25, 50, 100, 200,500 ppm) then dispenced in Petri dishes and left to solidify. Plates were then inoculated with the fungal isolates by placing cork porer made agar discs (5 mm) taken from the periphery of
fungal colonies grown after 48 h or 7 days in case of *Rhizoctonia* and *Fusarium*, respectivily. Plates containing Czapek 's agar medium without fungicides were inoculated similarly to be taken for comparison. There were two plates per concentration / isolate. Plates were then incubated at 25 °C. Data were recorded by measuring the diameter of the fungal growth (two perpendicular diameters) and found out their means. Efficacy of the tested chemicals was then evaluated according to their minimal inhibitory concentrations.

Table (1): List of fungicides used in physiological and control experiments of lentil damping –off and root rot inducing fungi.

Commercial name	Chemical name	Chemical constitution	Active ingredient	Recommended dose (g/kg seed)	Manufacture
Rhizolex-T 50 % WP	Tolclofos methyle + thiram	Tolclofos methyl (20 %) + Tetramethyle thiram disulfide (30 %)	50 %	3	Sumitomo Chemical Co. LTD Osaka Japan
Moncerin 25 % WP	Pencycuron 25 %	Pencycuron 25 %	25%	3	Bayer Garmany
Homai 80 % WP	Thiophonate + TMTD	1,2- Bis (3-Methoxycarbanyl –2-thioreido) benzene (50 %) + Tetra methyle thiram disulfide (30 %)	80 %	3	Nippon Soda Co. Japan
Vitavax /Captan 75 % WP	Carboxin + Captan	5,6- dihydro- 2- methyl-1-4 oxathin- 3 carboxinilide (37.5 %) + N- trichloromythyl mer-capto 4- cycloh exane 1, 2 dicarboximide 37.5 %	75 %	3	California Spray chemical Crop., USA
Topsin-M 70 % W.P	Methoxy-carbonyl	1,2 bio (3- methoxycarbonyl –2- thio ureido) benzene	70 %	3	(Nisso) N ippo nsoda Co., LTD
Mancoper 69.5 % WP	Mancozeb + Cupper	Mancozeb 52 % + copper 17.5 %	69.5 %	1.5 g / L	Qufer- Elziate Co. Egypt
Tri-Miltox fort 41.5 % WP	Mancozeb	Mancozeb 20 % + Cu (Cu oxychloride + Cu carbonate + Cu sulphate 21.5 %)	41.5 %	2.5 g /L	Sandoz Agrotto CH-4002 Basle-Switzerland

4.1.2. Pot trials

The most effective fungicides on the *in vitro* tests were then selected to be used as seed protectant in pot trials. Healthy lentil seeds were dressed with each of Rhizolex-T, Moncerin, Topsin-M and Homi-80 at the rate of 3 g/Kg seed in presence of carboxymethyl cellulose solutions 0.1 % to serve for adhesiveness of the chemicals with the seed surfaces.

The treated seeds were then sown in sterile soil previously infested with either *Rhizoctonia* or *Fusarium* at the rate of 2.5 % and / or 5 %. For comparison, pots infested with inocula of either pathogens at the same rates were sown with lentil healthy seeds (10 seeds / pot) without perior dressing with any of the chemicals. There were four pots per each treatment. Pots were then irrigated shortly after sowing and later as needed.

The experiment was carried out during November, 2000 and lasted for 3 months during which period damped-off (pre- or post-) and root rot incidence were recorded after 15, 30 and 90 days, respectively. Percentages of healthy survived plants / unit were calculated as compared to the chemically untreated control to be taken as indication to the efficiency of the used chemicals. Also, the survived plants per each pot were dried at 80 °C for 24 h. tc serve as additional data to reflect how healthy were the chemically treated plants in comparison with each other.

4.2. Biological control

4.2.1. Isolation of antagonistic microorganisms and *in vitro* effect

Biolcgical control of soil borne plant pathogens has been used recently as an effective mean in disease control. It had been recognized that antagonistic microorganisms isolated from the rhizosphere of different plants might be ideal for use as biocontrol agent. Hence, isolation of antagonistic microorganisms was carried out from healthy lentil rhizosphere.

To accomplish this, 5 g soil adhering to healthy lentil roots was collected and suspended in 200 ml sterile water in conical flask and shaked vigorously for 10 min. Basic dilutions were prepared to obtained 10^{-2} , 10^{-3} volumes. One - two ml of the prepared soil dilutions were added aseptically to 200 ml PDA melted medium at 50 °C, shack thoroughly and poured in Petri dishes and left to solidify.

Cork borer made discs (5mm) taken from the pirephary of 48 h. old growth of *Rhizoctonia solani* isolate (R_5) were plated over the center of the solidified medium containing the rhizosphere microorganisms and left to grow at 25 °C for 3-7 days. Plates were then examined for development of antagonism. The antagonistic organisms were recognized according to apperance of wide inhibitory area around the developing colonies. These colonies were selected accordingly, transferred and purified then their antagonistic action was further tested against both pathogens (*Rhizoctonia solani* and *Fusarium solani*). This was carried out by streaking the antagonist to be tested on one side of the plate containing nutrient sucrose agar medium, left for 48 h., then agar discs (5 mm) of either pathogens were placed on plate center at a short distance of the tested antagonists. Plates were then incubated for 3-7 days at 25 °C and checked for antagonistic actions on the tested fungi. These microorganisms that showed the widest

inhibitory area were selected and maintained on agar slopes to be used for further studies.

4.2.2. Biocontrol of lentil root diseases

Two rhizosphere bacterial isolates that proved the most antagonistics towards both pathogens, according to their inhibitory zones in plates were selected to be used in this study. The antagonistic bacteria were administered in two ways.

A) Seed inoculation

Two bacterial antagonists (B_1 and B_2) were separetely streaked on nutrient sucrose agar medium and left to grow for 48 h at 25 °C after which they were suspended in 0.1 % methyl cellulose solution to obtain dense inoculum (1-1.2 $x10^8$ cell/ml). The prepared cell suspensions of each bacterial antagonist were then mixed thoroughly with previously disinfested lentil seeds (Giza 9) and left for one hour. The inoculated seeds were then sown in pots (25 cm in diameter) containing sterile soil previously infested with either pathogens *Rhizoctonia solani* isolate R5 and *Fusarium solani* isolate F11 grown on barley grain medium at the rate of 2.5 and 5 % (w/w). The inocula of both pathogens were prepared as described before.

B) Soil drench

The antagonists were grown in shaked liquid sucrose nutrient medium for 48 h at 25 °C then the liquid cultures of each antagonist were used to inoculate the prepared soil in pots containing the pathogens.

Each pot was inoculated with 100 ml of broth medium of each antagonist to attain complete distributions of the bacterial cells. Pots were wisely irrigated to secure moisture maintenance and left for a week to let the pathogen and the antagonist to react together after which period the pots were sown with healthy disinfested seeds (10 seeds / pot). There were 4 pots / treatment. Control pots containing the pathogens alone were sown with lentil seeds at the same rate and kept under the same circumstances.

The plants were irrigated as needed and kept under observation at the greenhouse for 3 months. Data were then recorded considering damped-off plants before and after emergence and root rot after 15, 30 and 90 days after sowning.

Dry weight of the experimental units was estimated and compared with those of the control (without antagonists).

Statistical analysis:

In all experiments the least significant difference (LSD) at 0.05 confidence was determined (Gomez and Gomez, 1984) to compare the variance between them.

RESULTS AND DISCUSSION

1. Isolation , identification and pathogenicity test :

Isolation trails carried out from deseased lentil plants suffering from root and crown rot resulted in securing 11 *Rhizoctonia solani* and 15 *Fusarium* isolates.

In pathogenecity test as shown in Table (2) the majority of *Rhizoctonia solani* isolates agressively attacked lentils mostly during their

seedling stages causing 90-100% damping-off (pre or post- emergence). On the other hand, only three *Fusarium* isolates out of 15 isolates showed high pathological effect at all stages of the subjected plants causing pre- and post-emergence damping-off (52-57%) and root-rot (15-25%) while the remainder isolates were weakly parasite.

Table 2: Pathogenicity tests of fungal isolates obtained from naturally diseased lentil plants (cv. Balady).

Isolates No.	% Damping –off		Root rot %	Survival plants %
	Pre-emergence	Post-emergence		
Rhizoctonia solani No.				
1	74.4	15.6	0.0	10.0
2	22.8	2.8	7.8	66.6
3	87.5	6.3	0.0	6.2
4	85.0	7.5	7.5	0.0
5	100.0	0.0	0.0	0.0
6	87.0	13.0	0.0	0.0
7	90.9	3.0	6.1	0.0
8	95.0	5.0	0.0	0.0
9	10.0	2.5	2.5	85.0
10	78.4	13.6	0.0	8.0
11	91.6	2.5	0.0	5.9
Fusarium sp No.				
1	5.6	2.8	0.0	91.6
2	11.8	2.8	0.0	85.4
3	25.0	2.5	0.0	72.5
4	20.3	0.0	5.3	74.4
5	20.3	2.5	5.0	72.2
6	18.6	0.0	0.0	81.4
7	12.5	2.5	5.3	79.7
8	10.0	2.5	0.0	87.5
9	25.0	5.0	2.5	67.5
10	25.0	0.0	0.0	75.0
11	62.5	5.0	25.0	7.5
12	17.5	2.5	2.5	77.5
13	25.0	2.5	2.5	70.0
14	47.5	7.5	15.0	30.0
15	45 .0	7.5	20.5	27.0

Values are means of 40 plants

Subcultures representative of *Fusarium* isolates were sent to Mycological Center, Ministry of Agriculture in Giza for verification and were kindly identified as *Fusarium solani*.

2.Varietal response :

Tables (3,4) show that *Rhizoctonia solani* isolates agressively attacked the six local lentil cultivars causing pre- and post-emergence damping-off to 90-100% of the subjected plants after 30 days.

Table 3: Varietal response of 9 lentil cultivars towards two effective isolates of *Rhizoctoni solani* obtained from diseased lentil plants

Cultivars	Rhizoctonia isolates	Concentration of inoculum								Mean of Survival plants
		2.5 %				5 %				
		% Damping - off		% Root rot	% Survival plants	% Damping -off		% Root rot	% Survival plants	
		Pre-	Post-			Pre-	Post-			
Giza 4	R 5	97.5	2.5	0.0	0.0	95.0	2.5	2.5	0.0	0.0
	R 8	90.0	0.0	0.0	10.0	97.5	0.0	0.0	2.5	6.25
	Mean	93.75	1.25	0.0	5.0	96.25	1.25	1.25	1.25	
Giza 9	R 5	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
	R 8	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
	Mean	100.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	
Giza 51	R 5	97.5	0.0	0.0	2.5	97.5	0.0	2.5	0.0	1.25
	R 8	92.5	0.0	2.5	5.0	97.5	0.0	2.5	0.0	2.5
	Mean	95.0	0.0	1.25	3.75	97.5	0.0	2.5	0.0	
Giza 370	R 5	92.5	0.0	0.0	7.5	97.5	0.0	0.0	2.5	5.0
	R 8	100.0	0.0	0.0	0.0	97.5	2.5	0.0	0.0	0.0
	Mean	96.25	0.0	0.0	3.75	97.5	1.25	0.0	1.25	
Sinaa 1	R 5	92.5	2.5	5.0	0.0	97.5	2.5	0.0	0.0	0.0
	R 8	92.5	0.0	0.0	7.5	97.5	0.0	0.0	2.5	5.0
	Mean	92.5	1.25	2.50	3.75	97.5	1.25	0.0	1.25	
Balady	R 5	95.0	2.5	2.5	0.0	100.0	0.0	0.0	0.0	0.0
	R 8	95.0	0.0	0.0	5.0	100.0	0.0	0.0	0.0	2.5
	Mean	95.0	1.25	1.25	2.5	100.0	0.0	0.0	0.0	
Eston	R 5	3.4	0.0	0.0	96.6	10.3	3.4	0.0	86.3	91.45
	R 8	2.5	0.0	0.0	97.5	6.9	0.0	0.0	93.1	95.3
	Mean	2.95	0.0	0.0	97.05	8.6	1.7	0.0	89.7	
Laird	R 5	10.0	2.5	2.5	85.0	12.5	2.5	2.5	82.5	83.75
	R 8	5.0	2.5	0.0	92.5	5.0	2.5	2.5	90.0	91.25
	Mean	7.5	2.5	1.25	88.75	8.75	2.5	2.5	86.25	
Richlea	R5	10.0	2.5	2.5	85.0	10.0	0.0	7.5	82.5	83.75
	R8	7.5	0.0	2.5	90.0	10.0	2.5	2.5	85.0	87.5
	Mean	8.75	1.25	2.5	87.5	10.0	1.25	5.0	83.75	
Control		0.0	0.0	0.0	100.0	0.0	0.0	0.0	100.0	100.0
Mean		62.3	0.8	0.9	36.0	64.9	1.0	1.1	33.0	

L SD at 0.05 for

		Pre-	Post-	Root rot	Survival plants
Cultivars	(A)	0.633	0.199	0.193	1.345
Isolates	(B)	0.676	0.148	0.151	0.754
Concentrations	(C)	0.570	NS	0.195	0.915
Interaction	(ABC)	1.144	0.301	0.267	2.368

Also few individual plants showed root-rot symptoms later on after 90 days. On the other hand, the three Canadian cultivars showed high resistance and the percentage of the diseased plants did not exceed 7-17%. Also the majority of the local cultivars showed high suseptibilty towards *Fusarium solani* infection. In spite of great percentage of the subjected plants were damped off still others resisted the pathogen. That was clear in case of

cultivar Giza 4 which proved the most tolerant one as 20-45% of the plants remained apparently healthy. The three Canadian cultivars again showed great resistance towards *Fusarium* and the infection ranged between 15-21%. This indicates that all Canadian unlike the local cultivars, were resistant to both *Rhizoctonia solani* and *Fusarium solani* isolates.

The two isolated pathogens has been recognized as the main causitive agents of lentil damping-off and root-rot diseases in the majority of lentil cultivated area in the world (Sharma and Agnihotri, 1972; Punder *et al.* 1991 and Kaiser, 1992). In Egypt the two pathogens were also isolated from lentils suffering from root-rot or seedling diseases (El-Shanawani, 1973; Abdel-Kader, 1977; Abd-El-Kader *et al.* 1978; Yehia *et al.* 1985; Abou-Zeid *et al.* 1990; El-Garhy, 1994; Yihia *et al.* 1994 a and b; Hamdi and Hassanein, 1996; Abou-Zeid *et al.* 1997; Hassanein *et al.* 1997 and El-Garhy, 2000).

Table 4: Varietal response of 9 lentil cultivars to one effective isolate of *Fusarium solani* obtained from diseased lentil plants

Cultivars	Concentration of inculum							
	2.5 %				5 %			
	% Damping -off		% Root rot	% Survival plants	% Damping -off		% Root rot	% Survival plants
	Pre-	Post-			Pre-	Post-		
Giza 4	20.0	30.0	5.0	45.0	47.5	25.0	7.5	20.0
Giza 9	50.0	15.0	20.0	15.0	57.5	5.0	30.0	7.5
Giza 51	60.0	5.0	7.5	27.5	62.5	5.0	20.0	12.5
Giza 370	45.0	17.5	12.5	25.0	67.5	10.0	10.0	12.5
Sinaa 1	77.5	5.0	7.5	10.0	77.5	2.5	10.0	10.0
Balady	82.5	2.5	5.0	10.0	80.0	15.0	5.0	0.0
Eston	10.4	0.0	6.9	82.7	13.8	3.5	3.5	79.2
Laird	10.0	2.5	2.5	85.0	12.5	2.5	0.0	85.0
Richlea	10.0	2.5	2.5	85.0	17.5	2.5	2.5	77.5
Control	0.0	0.0	0.0	100.0	0.0	0.0	0.0	100.0
Mean	36.5	8.0	6.9	48.6	43.6	7.1	8.9	40.4

LSD at 0.05 for		Pre-	Post-	Root rot	Survival plants
Cultivars	(A)	1.991	1.458	1.546	5.564
Inoculum concentration	(B)	0.811	0.604	0.181	2.177
Interaction	(AxB)	2.033	1.913	1.855	6.530

3.Effect of some selected fungicides :

3.1.Laboratory test :

Data presented in Tables (5,6) show various inhibitory effect of the seven selected fungicides towards the linear growth of six *Rhizoctonia solani* and two *Fusarium solani* isolates.

Table 5: Effect of different concentrations of some selected fungicides on the linear growth of six *Rhizoctonia solani* isolates pathogenic to lentil calculated as percentage of the inhibitory action

Fungicides	Concentrations (ppm)	% inhibition						
		R_1	R_3	R_5	R_6	R_8	R_{11}	Mean
Control	0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rhizolex -T	2	78.88	81.21	85.38	68.61	82.51	81.55	97.69
50 % WP	5	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	5	45.33	40.11	42.2	45.55	50.00	60.00	47.20
	10	71.66	56.31	47.77	50.00	57.77	67.77	58.54
Vitavax \	15	71.66	56.31	47.77	51.50	59.77	69.97	59.50
captan	25	76.66	61.40	50.00	62.22	67.77	72.22	65.05
70 % WP	50	77.77	86.11	51.66	81.66	71.11	87.77	76.01
	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	5	0.00	37.77	82.22	76.66	78.88	81.66	56.53
Moncerin	10	61.66	63.33	100.00	87.22	84.44	83.33	79.99
25 % WP	15	91.11	86.11	100.00	91.11	88.88	84.44	90.27
	25	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Topsin -M	5	74.44	73.11	82.55	75.00	82.22	83.33	78.43
50 % WP	10	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	5	0.00	0.00	0.000	0.00	0.00	0.00	0.00
Homi -	10	0.00	17.40	0.00	74.44	90.00	0.00	30.31
80 % WP	15	85.55	88.88	81.11	83.00	100.00	86.11	87.44
	25	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mancober	500	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Trimeltox-forte	500	0.00	0.00	0.00	0.00	0.00	0.00	0.00

The results obtained could be summarized as follows. The fungicide Homi-80 showed the highest inhibitory efect on the linear growth of the six *Rhizoctonia* and the two *Fusarium* tested isolates as it completely inhibited the fungal growth at concentrations as low as 25 ppm in case of *Rhizoctonia solani* and 10 ppm in case of *Fusarium solani*.

Rhizolex-T exerted high inhibitory effect at 5 ppm on the tested *Rhizoctonia* isolates but reduced only 71% of *Fusarium* linear growth at 500 ppm.

Topsin-M and Moncerin were effective on both tested fungi and their inhibitory effect being more effective on *Rhizoctonia* isolates as their minimal inhibitory concentrations were at 10 ppm in case of Topsin-M and 25 ppm in case of Moncerin. However the two chemicals showed their inhibitory effect on *Fusarium* isolates at rather higher concentration (200 ppm).

Vitavax-captan was slightly less effective on *Rhizoctonia* and *Fusarium* isolates as it completely retarded the fungal growth at 100 ppm but reduced only 61% of *Fusarium* linear growth at concentration as high as 500 ppm.

On the other hand, neither Tri-Meltoxforte nor Moncober had any inhibitory effect on the two tested fungi.

Table 6: Effect of different concentrations of some selected fungicides on the linear growth of two *Fusarium solani* isolates pathogenic to lentil calculated as percentage of the inhibitory action

Fungicides	Isolates No.	Cont.	Concentration of fungicides (ppm)							
			5	10	15	25	50	100	200	500
Rhizolex-T 50 % WP	F_{11}	0.0	35.61	38.61	40.51	42.61	45.62	52.52	60.63	70.21
	F_{14}	0.0	37.81	38.52	42.51	41.61	48.65	56.52	62.01	72.21
	Mean	0.0	36.71	38.57	41.51	42.11	47.14	54.52	61.32	71.21
Vitavax \ captan 70 % WP	F_{11}	0.0	25.65	28.61	30.21	35.56	39.62	50.91	55.21	60.15
	F_{14}	0.0	26.51	29.61	32.21	36.81	41.81	49.81	53.81	62.16
	Mean	0.0	26.08	29.11	31.21	36.19	40.72	50.36	54.51	61.16
Moncerin 25 % WP	F_{11}	0.0	50.55	55.55	56.64	56.66	58.88	80.05	100.0	100.0
	F_{14}	0.0	48.56	58.56	53.65	57.51	58.88	81.51	100.0	100.0
	Mean	0.0	49.56	57.06	55.15	57.09	58.88	80.78	100.0	100.0
Topsin –M 50 % WP	F_{11}	0.0	45.82	45.92	46.66	50.33	60.61	75.51	100.0	100.0
	F_{14}	0.0	46.61	46.95	47.51	50.98	62.61	72.51	100.0	100.0
	Mean	0.0	46.22	46.29	47.07	50.66	61.61	74.01	100.0	100.0
Homi 80 % WP	F_{11}	0.0	50.61	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	F_{14}	0.0	51.77	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Mean	0.0	51.19	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Mancober	F_{11}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	F_{14}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trimeltox-forte	F_{11}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	F_{14}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

3.2.Pot trails :

Four effective fungicides namely : Rhizolex-T, Moncerin, Topsin-M and Homi-80 which proved more effective in retarding fungal radial growth were selected to be used as seed dresser against *Rhizoctonia* (isolate R_5) and *Fusarium* (isolate F_{11}). This was carried out in pcts containing steril loamy soil and inoculated with either fungi as described before at the rate of 2-5 and/or 5%.

Data presented in Table (7) show that all the four fungicides proved effective as seed portectant against the two pathogenic fungi as they reduced significantly disease incidence (pre-, post-emergence damping-off and root-rot) irrespective of inoculum density. However there were significant differnces between the four chemicals as regards their effecacy against damping-off incidence being Homi-80 the most effective protectant.

Rhizolex-T treated seeds proved more effective in case of *Rhizoctonia* than in case of *Fusarium*. It increased the number of plant stands in *Rhizoctonia* infested pots up to 90% whereas it did not exceed more than 50% in presence of *Fusarium*. Topsin-M and Moncerin reacted differently towards the two pathogens. While they reduced disease incidence in case of *Rhizoctonia*, pre- and post-emergence damping-off, to 17% and 30% respectively, they had a weak inhibitory action against *Fusarium* as they slightly reduced the percentage of the diseased plants. It could also be noted that plants emerged from Homi-80 treated seeds attained the highest dry weight mean over all the other chemically treated plants. On the contrary

plants emerged from Topsin-M or Moncerin treated seeds had the lowest dry weight. Hence it could recommended Homi-80 to be used as effective seed dresser against the damping-off causing pathogens of lentils particularly *Rhizoctonia* and *Fusarium*.

Such rsults has been reported by many investigators dealing with such seed dressers against damping-off pathogens mostly by either *Rhizoctonia solani* or *Fusarium* spp. (Amr, Afaf *et al.* 1987, Abada, 1995; Hassanein *et al.*, 1997; Ragab *et al.* 1999; El-Wakil and Ghonim, 2000 and Helmy, Alia *et al.*, 2001).

Table 7: Reaction of different fungicides used as seed dresser against damping-off and root rot pathogens at inoculum concentration of 2.5 % and 5% in pots

Fungicides	Fungi	Concentration of inoculum									
		2.5%					5%				
		% Damping-off		% Root rot	% survival plants	Dry weight gm/pot	% Damping-off		% Root rot	% survived plants	Dry weight gm/pot
		Pre-	Post-				Pre-	Post-			
Topsin-M	R_5	10.0	0.0	0.0	90.0	6.7	10.0	5.0	2.5	82.5	2.2
50 % WP	F_{11}	10.0	15.0	40.0	35.0	1.7	15.0	2.5	70.0	12.5	0.3
Rhizolex	R_5	7.5	0.0	0.0	92.5	8.4	7.5	2.5	0.0	90.0	4.2
50 % WP	F_{11}	10.5	0.0	20.0	69.5	3.6	20.0	10.0	20.0	50.0	3.2
Monceren	R_5	15.0	0.0	0.0	85.0	5.5	15.0	5.0	10.0	70.0	3.9
25 % WP	F_{11}	20.0	0.0	32.5	47.5	1.5	15.0	17.0	52.5	15.5	0.3
Homi	R_5	10.0	0.0	10.0	80.0	7.9	16.5	2.5	2.5	78.5	4.5
80 % WP	F_{11}	15.0	0.0	0.0	85.0	11.6	15.0	0.0	5.0	80.0	4.6
Mean		12.2	1.8	12.8	73.2	5.9	14.3	5.6	20.3	59.8	2.9
Control	R_5	100.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
	F_{11}	47.5	12.5	30.0	10.0	0.3	60.0	10.0	25.0	5.0	0.2

LSD at 0.05 for:		Pre-	Post-	Root rot	Survived plants	Dry weight
Fungicides	(A)	NS	1.03	3.19	3.26	0.13
Fungi	(B)	NS	1.46	1.59	2.85	0.09
Concentrations	(C)	NS	1.69	3.56	3.62	0.16
Interaction	(ABC)	NS	4.78	8.05	8.21	0.44

4.Isolation of antagonstic microorganisms :

Several bacterial colonies developed from the diluted suspension of rhizosphere of healthy lentil plants and some showed marked inhibitory action against the test organism (*Rhizoctonia*). Of those antagonistis two bacterial isolates (B_1 & B_2) were selected according to their wide inhibitory zones and their antagonistic action was further verivied against *Rhizoctonia* and *Fusarium* isolates when tested in plates (Fig. 1,A and B).

The two selected antagonists were rods, gram positive, facultative anaerabes and spore formers. As their physiological activities of both bacteria did not agree with any of the known species of *Bacillus*, hence they could be tentatively identified at the generic level as *Bacillus* spp.

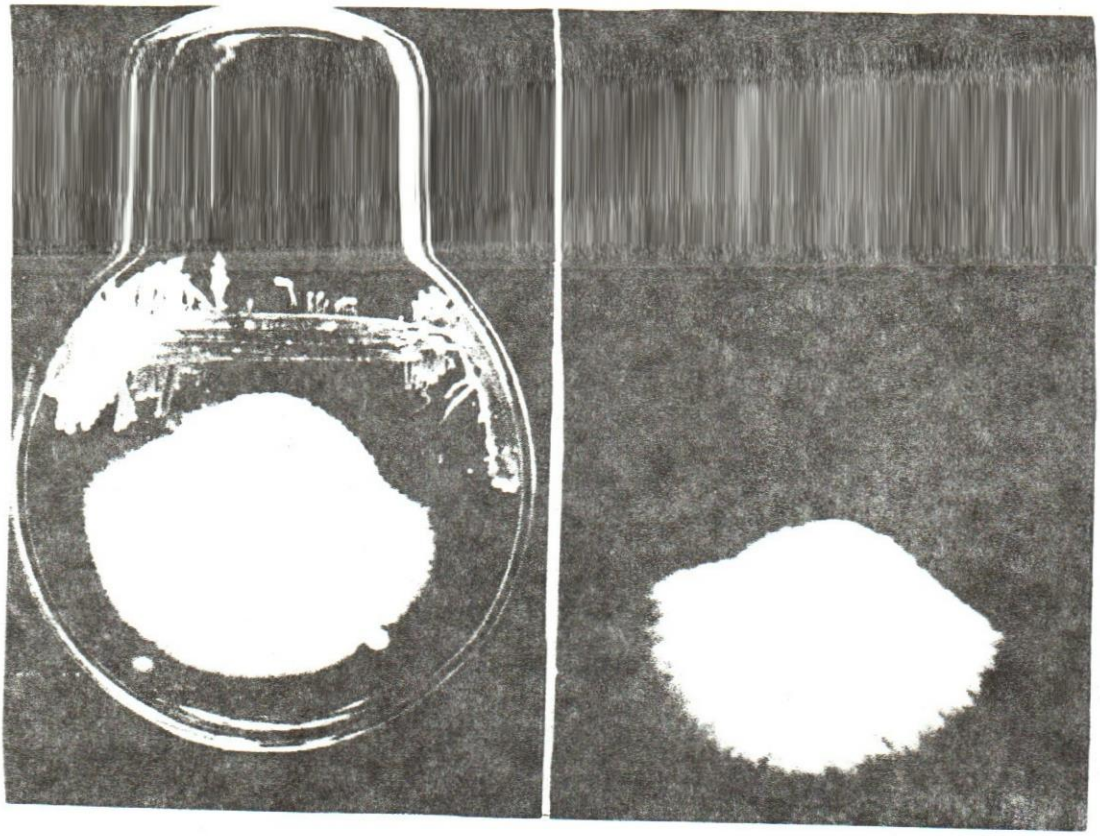

Fig.1 (A) The antagonistic actions of *Bacillus* on *Rhizoctonia solani* and B) on *Fusarium solani* showing powerful inhibitory actions.

5-Biocontrol of lentil root diseases :

Lentil plants grown in soil infested with either pathogens *Rhizoctonia solani* (R_5) and *Fusarium solani* (F_{11}) together with or without the antagonists showed great differences as regards disease incidence. The two antagonistic bacteria B_1 & B_2 exerted great inhibitory effect through the two methods of applications regardless of pathogen inoculum density (Table 8). While *Rhizoctonia* and *Fusarium* caused high disease incidence (100% and 90-95% respectively) in pots freed of the antagonists, the disease was greatly controlled in presence of both antagonists. However, seed ioculation with the antagonists proved more practical and logic.

It had long been reported that antagonistic bacteria had been widely used as a biocontrol agants against many diseases. The reported antagonistic organisms that are successfully used for biological control are belonging mostly to the genus Bacillus (Weller, 1988; Pleban *et al.*, 1995; Liang *et al.*, 1996; Saleh, 1997; Gabr *et al.*, 1998; Podile and Loxmi, 1998; Felaifel *et al.*, 1999; Abd-El-Latif *et al.*, 2000 and Helmy, Alia *et al.*, 2001).

Table(8): Effect of two Bacilli isolates (B_1 and B_2) antagonistic to *Rhizoctonia solani* and *Fusarium solani* pathogens of lentil plants on disease severity when applied as seed inoculation or soil drench

Treatments	Antagonists isolates	Pathogens	Concentration of inoculum %									
			2.5 %					5 %				
			% Damping-off		% Root rot	% survivals	Dry weight gm/pot	% Damping-off		% Root rot	% survivals	Dry weight gm/pot
			Pre-	Post-				Pre-	Post-			
Seed Inoculation	B_1	R_5	10.0	0.0	0.0	90.0	8.8	10.0	0.0	0.0	90.0	4.3
		F_{11}	15.0	0.0	0.0	85.0	10.4	25.0	0.0	0.0	75.0	8.6
	B_2	R_5	17.5	0.0	0.0	82.5	11.8	17.5	0.0	0.0	82.5	8.2
		F_{11}	27.5	0.0	0.0	72.5	5.6	27.5	0.0	0.0	72.5	3.8
Mean			17.5	0.0	0.0	82.5	9.1	20.0	0.0	0.0	80.0	6.2
Soil Drench	B_1	R_5	25.0	0.0	0.0	75.0	15.0	32.5	0.0	0.0	67.5	9.9
		F_{11}	10.0	2.5	0.0	87.5	10.1	10.0	2.5	0.0	87.5	7.8
	B_2	R_5	27.5	0.0	0.0	72.5	15.4	35.0	5.0	0.0	60.0	11.9
		F_{11}	15.0	0.0	2.5	82.5	15.7	22.5	0.0	0.0	77.5	11.8
Mean			19.4	0.6	0.6	79.4	14.1	25.0	1.9	0.0	73.1	10.4
Control		R_5	100.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
		F_{11}	47.5	12.5	30.0	10.0	0.33	60.0	15.0	20.0	5.0	0.2

LSD at 0.05 for:		Pre-	Post-	Root rot	Survived plants	Dry weight
Treatments	(A) Bacterial	1.56	NS	NS	1.56	0.13
	(B)	0.57	NS	NS	2.65	0.21
Fungi	(C)	0.10	1.57	2.5	1.65	0.15
Concentrations	(D)	1.31	1.09	0.18	2.56	0.11
Interaction	(ABCD)	2.52	3.37	0.72	4.89	0.46

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عفن جذور وسقوط بادرات العدس وطرق مقاومتها الكيماوية والبيولوجية في محافظة المنيا

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قسم أمراض النبات-كلية الزراعة-جامعة المنيا

في دراسة أجريت على مرضى عفن جذور وسقوط بادرات العدس في قرية بني عبيد بمحافظة المنيا أمكن التحصل على احدى عشر عزلة من فطر الريزوكتونيا سولاني وخمسة عشر عزلة من فطر الفيوزاريوم من الأنسجة المصابة لعينات مريضة من نبات العدس.

وفي إختبار القدرة المرضية أظهرت معظم عزلات الريزوكتونيا قدرة مرضية عالية حيث هاجمت نباتات العدس المختبرة خصوصا في طور البادرة مسببة سقوط البادرات وموتها بينما أظهرت ثلاث عزلات فقط من فطر الفيوزاريوم قدرة مرضية عالية دون باقي العزلات والتي عرفت على أنها فيوزاريوم سولاني. وفي دراسة لإختبار حساسية ستة أصناف عدس محلية وثلاثه كندية أظهرت الأصناف المحلية حساسية فائقة للإصابة بكل من الريزوكتونيا والفيوزاريوم بينما كانت الأصناف الكندية شديدة المقاومة لكلا الفطرين.

هذا وقد أجري تقييم سبعة مبيدات فطرية تستخدم معظمها كواقيات للبذور وجد بعضها مثل التوبسين-إم والريزولكس-تي والمونسرين والبوماي-٨٠ ذات فاعلية عالية وإن اختلفت في أقل تركيز مثبط للنمو وأيضا تفاوتت هذه المواد في مدى فاعليتها كواقيات للبذور ضد الكائنين الممرضين في اختبار الأصص.

كذلك أمكن الحصول من منطقة الريزوسفير لنبات العدس على عزلتين بكتيريتين تابعتين للجنس باسيلس اظهرتا نشاط تضادي عالى للفطرين وقد استعملتا بكفاءة في مقاومة الاصابة بالفطرين في تجارب الأصص.