Control of Root-Rot and Wilt Diseases of Lupine

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ABSTRACT

Root-rot and wilt diseases of lupine are considered as a disease problem in lupine production in Egypt. In this study plant extracts, biocontrol agents and fungicides were tested for their effect on root-rot and wilt of lupine. Root-rot disease caused by *Rhizoctonia solani, Fusarium solani* and *Fusarium moniliforme*. While wilt disease caused by *Fusarium oxysporum*. Laboratory experiments results indicated that, plant extracts of Neem and Eucalyptus, biocontrol agents (*Trichoderma harzianum, Trichoderma hamatum, Pseudomonas fluorescens* and *Bacillus subtilis*) and fungicides reduced the linear growth of the causal organisms. The fungicides, Moncerin and Rizolex-T gave the highest reduction in linear growth of different pathogens and Rizolex-T was the most effective than Moncerin. In greenhouse experiments, all treatments controlled significantly wilt and root-rot and indicated the effectiveness of the different treatments compared to the untreated plants. The most effective treatment was obtained by Neem and Eucalyptus extracts followed by *Trichoderma hamatum* and *Bacillus subtilis*. Agronomic characters i.e., plant height, number of branches, fresh weight, dry weight / plant, number of pods, seeds yield / plant and weight of 100 seeds were tested. Also, total N and crude protein % in seeds were determined.

Key words: Lupine (*Lupinus termis*), root-rot, wilt, plant extracts, biocontrol agents and fungicides.

INTRODUCTION

Lupine (*Lupinus termis. L.*) grown as a crop provides a variety of benefits, primarily because of their input of fixed N_2 improving the physical and chemical properties of the soil (Hoshikawa, 1991), lupine (*Lupinus termis* L.) is a legume crop contains high value of protein. The plant is subjected to the attack by several fungi causing great yield losses (Christinsen *et al.*, 1999). The most important fungal diseases are wilt and root-rot caused by *Rhizoctonia solani, Fusarium oxysporum, Fusarium solani, Verticillum albo-atrum, Macrophomina phaseolina* and *Sclerotium rolfsii* (Gowily *et al.*, 1995). Application of garlic extract resulted in a significant decrease in damping-off and root-rot incidence of cantaloupe caused by *Fusarium semitectum* (Michail and El-Khateeb, 1985) and root-rot of bean (Gaffar *et al.*, 1989). Soil amendments with Neem (*Azdirachta indica*) seed cake are effective against *F. oxysporum, M. phaseolina* and *R. solani* (Etheridge and Bateman 1998). Hussein (2005)

found that, plant extracts of Neem, lemon grass leaves and eucalyptus leaves reduced the linear growth of sugar beet root-rot pathogens. Plant extracts of garlic, onion and caraway have significantly reduced the linear growth of *R. solani*, *F. oxysporum*, *F. solani*, *F. semitectum* and *F. moniliforme* (Eisa *et al.*, 2006). Yehia *et al.* (1988) proved the antagonistic effect of *Trichoderma viride* against *F. solani* of faba been as seed coating and increased fresh and dry weight of shoots, root and nodules number. Mahmoud (2004) found that, in greenhouse and field trials, *P. fluorescens* and *B. subtilis* significantly reduced the incidence of all types of pod rots caused by *R. solani*, *S. rolfsii*, *M. phaspolinia* and *Fusarium* spp. and added that, *B. subtilis* induced the highest pod yield of peanut.

Maria and Joseph (2006) showed that, a *T. harzianum* strain was antagonistic, *in vitro*, to *R. solani* and *V. dahliae* and may be considered a potential biocontrol agent. Liu and Sindair (1991) observed that, seed treatment with Thiram and Graphite (2 g / kg seed) was effective to control wilt in winter. Peas in early stage. Zakharova *et al.* (1995) reported that, seed treatment with fundaza (*Benomy* L.) and the addition of Rhizotorfin gave significant control of root-rots of pea caused by *Fusarium* and *Aphanomyces* spp. El-Awadi *et al.* (1997) found that, lupine seeds dressing with fungicides Benlate 50 (2 g / kg seed) gave a good protection against the disease than untreated ones. Also, Benlate was the most effective fungicide in reducing fungal linear growth (Eisa *et al.*, 2006). The present work was conducted to investigate the effectiveness of plant extracts, biocontrol agents and fungicides for controlling root-rot and wilt diseases of lupine under laboratory, greenhouse and field conditions.

MATERIALS AND METHODS

1. Isolation, purification and identification of the causal organisms:

Rotten roots of lupine seedlings and while plants were collected from Minufiya and Giza, governorates at Egypt.

Infected parts were cut into small pieces, washed thoroughly with running water to remove any adhering soil particles. These pieces were surface sterilized by immersing in 5% sodium hypochlorite solution for 2 min, then washed several times in sterilized water then dried within sterilized filter papers. Four surface sterilized pieces were aseptically transferred onto Petri plates containing potato dextrose agar medium (PDA) containing 40 ppm streptomycin sulphate to avoid any bacterial

contamination. Plates were incubated at 25°C for 3 – 7 days and observations were recorded. Fungi grown were puzified using hyphal tip single spore techniques transferred and then identified according to their morphological and microscopically characters as described by Jans *et al.* (1991). Identification was confirmed by the Department of Mycology, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.

2. Pathogenicity test:

Pathogenicity tests were carried out under greenhouse conditions at (Sers El-Layian Agricultural Research Station at 2007growing season). In this study was a used pot (25 cm diameter).

These pots were sterilized by immersing them in 5% formaline solution for 15 minutes and then left for seven days before using. Soil (used for potting) was sterilized with 5% formaline solution and then left for two weeks for formalin evaporation. Soil infestation with each individual fungus was carried out at the rate of 3.5% of soil weight. Fungi were individually grown on sand-barley (SB) medium (25 g clean sand, 75 g barley and enough water to cover the mixture). Flasks contained sterilized medium were inoculated with each particular fungus and incubated at 25°C for two weeks. Soil of control pots was mixed with the same amount of sterilized sand-barley (SB) medium. Ten lupine seeds (Giza 2) cultivar were surface sterilized by sodium hypochlorite 5% for 2 min, washed several times with sterilized water, before sowing. Three pots were used for each treatment. Percentages of pre-and post-emergence damping-off as well as healthy survival plants in each treatment were determined 15 and 30 days after sowing, respectively using the next formula according to El-Helaly *et al.* (1970).

3. Effect of plant extracts and fungicides on linear growth of pathogen:

The antagonistic microorganisms such as *Trichoderma harzianum, T. hamatum, Bacillus subtilis* and *Pseudomonas fluorescens* were tested for their antagonistic abilities. All effective biocontrol agents were obtained from the collection of Department of Agricultural Botany, Faculty of Agriculture, Minufiya University, Egypt. In this respect, Petri plates containing PDA medium were inoculated with a disc (5 mm diameter) taken from 7 days-old cultures of the pathogenic fungi, i.e., *R. solani, F. oxysporum, F. solani* and *F. moniliforne*. The pathogenic fungi were inoculated at one side, whereas the opposite side, 5 mm apart was inoculated with both disc of each antagonistic fungus *T. harzianum* and *T. hamatum* or with streaking for antagonistic bacterium *B. subtilis* and *P.*

fluorescens. Plates only inoculated with pathogenic fungi at one side, 5 mm from the plate edge were kept as control. Three replicates were used for each treatment. Plates were incubated at 25°C. Linear growth of the tested fungi was measured when fungi completely covered the surface of the medium in the control treatment. The inhibition percent was calculated using the formula of Mohamed (1996).

Tissue extracts of neem and eucalyptus leaves were prepared by lending 10 g frozen plant materials with 100 ml water for 5 minutes. The resultant was strained through a double cheese cloth fabric and centrifuged for 10 minutes at 3000 rpm. Sterilization was made by 0.2 um milipore filters. The extract was added 1.5 m / plate to the gliotoxin fermentation medium (GFM) as described by Brain and Hemming (1945). Inoculation was done with *Rhizoctonia solani, Fusarium oxysporum, Fusarium solani* and *F. moniliforme*. Control (check) plates-devoiding extracts were incubated under similar conditions at 25°C. The fungicidal effect on fungal growth was tested. 200 ppm of concentration of Moncerin at the rate 250 ppm and Rizolex-T based on the active ingredient was prepared in PDA medium poured in Petri dishes. Petri dishes were inoculated with 5 mm disks for 7 days old fungal cultures. Three replicates were used for each treatment. All previous plates were incubated at 25°C for 7 days then examined. Percentage of reduction in fungal growth was calculated as the growth in different treatments relative to those in check treatment.

4. Effect of seed soaking in plant extracts, fungicides and antagonistic:

4.1. Effect of antagonistic bioagents:

This study was carried out in sterilized pots (25 cm diameter) containing sterilized clay soil in the greenhouse. Both of pots and soil were sterilized with 5% formalin solution. Inocula of the mentioned biocontrol agents (*Trichoderma harzianum* and *T. hamatum*) and the pathogenic fungi (*R. solani, F. oxysporum, F. solani* and *F. moniliforme*) were prepared by growing them separately on sand-barley medium (SB) for 15 days. While, *B. subtilis* and *P. fluorescens* were grown on liquid nutrient glucose broth medium for 4 days at 30°C (Abd El-Moity and Shatla, 1981), then applied to the pots at the rate of 50 ml / pot, 7 days before sowing. Pathogenic fungi were mixed thoroughly, ten days before sowing. Control treatment was inoculated only with pathogens. Ten seeds (Giza 1) were sown per pot and three replicates were used for each treatment percentages of pre- and post-emergence damping-off, survival plants, and plant height, fresh and

dry weight were assessed and recorded as mentioned before.

4.2. Effect of seed soaking in natural plant extracts:

This experiment was carried out in sterilized pots (25 cm diameter) containing sterilized clay soil in the greenhouse, both pots and soil were sterilized with 5% formalin solution. Soil was infested with each fungus *R. solani, F. oxysporum, F. solani and F. moniliforme* at the rate of 3% of soil weight.

Fungi were individually grown on sand-barley (SB) medium 25 g clean sand, 75 g barley and enough water to cover the mixture. Flasks contained sterilized medium were inoculated with each particular fungus and incubated at 25°C for two weeks. Potted soil was watered daily for a week to enhance fungal growth. Soil of control pots was mixed with the same amount of sterilized sand-barley (SB) medium were treated with 4% solution of carboxy methyl cellulose (CMC) as sticker, then air dried. Seeds were soaked for one hr in the plant extracts (neem, eucalyptus leaves) was prepared as follows (90 + 10) as sterilized water + plant extracts (Hussein, 2005). Seeds soaked in water only were used as control treatment. Ten seeds Giza 1 were sown per pot and three replicates were used for each treatment. Percentages of pre- and post-emergence damping-off, survival plant, plant height, fresh and dry weight were assessed an according to El-Helaly *et al.* (1970).

4.3. Effect of fungicides:

This experiment was carried out in pots (25 cm). Both pots and soil were sterilized as mentioned before. Lupine seeds (cv. Giza 1) were treated with the fungicides, Moncerin and Rizolex-T at rates (3 and 3 g / kg seeds, respectively). The same aforementioned methods were used without fungicides as control. Ten seeds were sown per pot and three replicates were used for each treatment. Percentages of pre and post emergence damping-off survival plants, plant height, fresh or dry weight were assessed and recorded as mentioned before.

5. Factors affecting lupine root-rot and wilt under field conditions:

These experiments were conducted in naturally infested soil at Sers El-Layian Agric. Res. Stat. Seeds were sown in 15th of November during 2007 / 2008 and 2008 / 2009 growing seasons. The experimental layout was in complete randomized block design with three replications for each

treatment. Each replicate had 3 rows 3 m long each and 60 cm in width.

5.1. Seed treatment:

Lupine seeds cv. Giza 1 were treated with *T. harzianum, B. subtilis* and *P. fluorescens* as powder form 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). The mixtures were left to dry out. Arabic gum was used as a sticker to dress the seed with any agent. While Moncerin and Rizolex-T were applied as mentioned before. Lupine seeds in the plant extract for two hour according to Hussein (2005). The dry method depends on mixing the seed with talcum powder laden with the extract and Arabic gum. The dried preparation was obtained by mixing 250 g of talc powder with 250 ml of the extract and spread till drying. Three replicates were used for each treatment. Disease assessment and yield components were recorded and estimated as mentioned before. Crude protein of seeds was calculated by multiplying total N % × 6.25

5.2. Disease assessment:

Pre-emergence damping-off was assessed after 15 days from sowing and post-emergence damping-off and survival plants were assessed after 30 days. Root rot severity index was calculated according to **Soliman *et al* (1988)** as following:

Disease severity = [$\left(a \times b \div N \times K \right)$**x 100]**

Where: a: Number of infected plants.

b: Grade of infection

N: Number of total plants

K: Maximum grade of infection.

Yield components infection of plant %, number of branches, number of seeds per plant, number of 100 seed and seed yield plot / kg were assessed.

6. Determination of protein:

Seed samples were taken, dried in an electric oven at 70° till constant weight and ground for determination of total N calorimetrically by using orange G dye method according to Hafez and Mikkelsen (1981). Crude protein of seeds was calculated by multiplying total N % × 6.25.

RESULTS

1. Isolation and identification of the causal organisms:

A. Sampling and isolation from infected seedlings and roots:

As shown in Table (1) twenty five isolate of different soil-borne fungi were isolated from wilted and rotten roots of lupine plants Giza 2 showed root-rot and wilt symptoms, cultivated in two Egyptian, Minoufiya and Giza. Identification of these isolates show that they were identified as *Rhizoctonia solani* (9 isolates) followed by *F. oxysporum* (6 isolates), *F. solani* (5 isolates) and *F. moniliforme* (5 isolates), respectively. As for the frequency % of the isolated fungi in the two governorates, *R. solani* followed by *F. oxysporum* were the most frequent fungi in the two governorates.

Table (1). Isolated fungi from rotten and wilted roots and their frequency (%) of two different locations.

Fungal isolate	Minufiya		Giza		Total
	Frequency	Frequency %	Frequency	Frequency %	
R.. solani	5	35.71	3	27.27	8.00
F. oxysporum	4	28.57	3	27.27	7.00
F. solani	3	21.43	3	27.27	6.00
F. moniliforme	2	14.28	2	18.18	4.00
Total	14		11		25

2. Pathogenicity tests:

In this experiment, 10 isolates only of root isolates 4-differnet fungi were tested for their pathogenic abilities.

Results presented in Table (2) reveal that, some tested isolates could be infect the roots of lupine cv. Giza 2 causing pre- and post-emergence damping-off and reduced the survived plants. The highest pre-emergence damping-off infection was recorded by *R. solani* from Minoufiya followed by *F. oxysporum* from Giza while *F. solani* and *F. moniliforme* isolated from Minoufiya, respectively. Also, all tested isolates without exception caused post-emergence damping-off infection %. Finally, it is concluded from the results that, the isolates i.e., *R. solani* (2) followed by *F. oxysporum* (4), *F. solani* (5) and *F. moniliforme* (7) were the more virulent ones.

Table (2). Pathogenicity tests of the fungi isolated from diseased lupine seedlings.

Isolated fungus	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survived plants (%)
R.. solani (1)	6.70	10.00	83.30
R.. solani (2)	25.00	13.33	61.67
R.. solani (8)	0.00	6.70	93.30
R.. solani (19)	3.30	6.70	90.00
F. oxysporum (12)	6.77	6.77	86.46
F. oxysporum (4)	13.33	16.66	70.01
F. solani (5)	13.33	13.33	73.34
F. solani (11)	6.77	3.33	89.90
F. moniliforme (16)	13.33	10.00	76.67
F. moniliforme (7)	3.30	6.77	89.93
L.S.D. at 0.05	11.20	9.81	15.32

3. Laboratory experiments:

Effect of plant extracts and fungicides on linear growth of *R.. solani, F. oxysporum, F. solani* and *F. moniliforme*.

Data in Table (3: a, b, c and d) indicate that both plant extracts and fungicides significantly reduced the linear growth of the pathogenic fungi compared to control. Neem was the most effective plant extract followed by Eucalyptus compared to control. While the fungicides Moncerin and Rizolex-T revealed a significant reduction of fungal linear growth. Rizolex-T was the most effective fungicide in reducing fungal linear growth compared to control. All pathogenic fungi were sensitive to plant extract and fungicides compared to control.

Clear the antagonistic mode of action against the tested pathogenic fungi. *Trichoderma hamatam* grew over the mycelium of *R. solani, F. moniliforme* and *F. oxysporum*. The tested antagonists significantly reduced linear growth of all tested fungi compared to control. In general, *T. hamatum* and *T. harzianum* were the most effective bioagents followed by *B. subtilis* and *P. fluorescens* where the highest reduction % was by *F. moniliforme* in relation to their reaction to *T. hamatum* followed by *R. solani* and *F. oxysporum*, respectively. Meanwhile, lowest reduction % was *F. solani*. On the other hand, *T. harzianum* was different in its reaction with the tested pathogenic fungi where the highest reduction % was recorded with *F.*

moniliforme, *R. solani*, *F. oxysporum* and *F. solani*, respectively. Also, using *B. subtilis* and *P. fluorescens* as a natural antagonist were not highly effective in reducing the growth of tested pathogenic fungi comparing with *T. harzianum* and *T. hamatum*; all tested antagonists reduced the growth of the fungus.

4. Effect of plant extract, biocontrol agent and fungicides on lupine root-rot and wilt under greenhouse conditions:

Results in Table (4) indicated clearly that the antagonists reduced significantly pre-emergence damping-off and post-emergence damping-off infection of lupine plants infected with the causal pathogens comparing with control. While the fungicides more effective from antagonistic and plant extract compared to control. Rizolex-T was the best followed by *T. hamatum* and Neem, respectively. On the other hand, the lowest effective eucalyptus, *B. subtilis* and *P. fluorescens*. In this respect, the highest percentage of healthy survived plants was in case treatment against *T. hamatum* followed by *T. harzianum*, respectively. The best treatment was fungicides Rizelex-T and Moncerin compared to the control.

Table (3 a). Effect of biocontrol agents, plant extracts and fungicides on linear growth of *Rhizoctonia solani* in petri-dishes.

Treatment	Linear growth (cm)	Reduction (%)
Biocontrol agents:		
T. harzianum	3.50	61.11
T. hamatum	3.00	66.67
P. fluorescens	5.40	40.00
B. subtilis	5.00	44.44
Plant extracts:		
Neem	4.83	46.29
Eucalyptus	6.83	24.07
Fungicides:		
Moncerin	2.83	68.52
Rizolex-T	2.00	77.78
Control	9.00	0.00
L.S.D. at 0.05	0.29	3.24

Table (3 b). Effect of biocontrol agents, plant extracts and fungicides on linear growth of *Fusarium solani* in petri-dishes.

Treatment	Linear growth (cm)	Reduction (%)
Biocontrol agents:		
T. harzianum	4.80	46.67
T. hamatum	3.20	64.44
P. fluorescens	5.90	34.44
B. subtilis	6.00	33.33
Plant extracts:		
Neem	5.17	42.59
Eucalyptus	6.33	29.62
Fungicides:		
Moncerin	1.00	88.89
Rizolex-T	0.00	100.00
Control	9.00	0.00
L.S.D. at 0.05	0.66	7.55

Table (3 c). Effect of biocontrol agents, plant extracts and fungicides on linear growth of *Fusarium oxysporum* in petri-dishes.

Treatment	Linear growth (cm)	Reduction (%)
Biocontrol agents:		
T. harzianum	4.20	53.33
T. hamatum	3.00	66.67
P. fluorescens	5.00	44.44
B. subtilis	4.90	45.55
Plant extracts:		
Neem	3.50	61.11
Eucalyptus	4.67	48.14
Fungicides:		
Moncerin	2.33	74.07
Rizolex-T	1.67	81.48
Control	9.00	0.00
L.S.D. at 0.05	0.64	6.77

Table (3 d). Effect of biocontrol agents, plant extracts and fungicides on linear growth of *Fusarium moniliforme* in petri-dishes.

Treatment	Linear growth (cm)	Reduction (%)
Biocontrol agents:		
T. harzianum	2.50	72.22
T. hamatum	2.00	77.77
P. fluorescens	6.00	33.33
B. subtilis	5.30	41.11
Plant extracts:		
Neem	2.67	70.37
Eucalyptus	4.17	53.70
Fungicides:		
Moncerin	0.00	100.00
Rizolex-T	0.00	100.00
Control	9.00	0.00
L.S.D. at 0.05	0.24	

Data in Table (5) revealed that, there was a clear effect of treatment seeds on disease severity comparing with control (untreated). Lupine seeds pretreated with any tested fungicide lowered percentage of diseased plants than untreated one (control). Rizolex-T ranked as the best seed dressing fungicides judged by the lowest disease incidence followed by Moncerin, with *R. solani, F. oxysproum, F. solani* and *F. moniliforme*, respectively. Neem was the best followed by eucalyptus antagonists reduced significantly disease severity of lupine plant infected with the causal pathogen comparing with control treatment. *T. hamatum* was the best in this field followed by *T. harzianum, B. subtilis* and *P. fluorescens*, was the least effective one. Regarding fresh weight, dressing seeds with fungicides, plant extract and antagonist before sowing in soil infected with wilt and root-rot pathogens increased significantly the fresh weight of the lupine comparing with untreated. Concerning dry weight, of the results were obtained where, dressing seeds with fungicides, plant extract and antagonist increased significantly dry weight

in all tested treatments compared to the control.

5. Effect of treating lupine plants with fungicides, plant extract and biocontrol agents on root-rot and wilt disease:

Data in Table (6) clearly show the influence of both fungicides, plant extract and antagonist treatment on root-rot and wilt diseases of lupine under field conditions during two seasons. The results cleared with, treatments application reduced significantly pre- and post-emergence damping-off compared to control. Rizolex-T was the best in reducing pre- and post-emergence damping-off and consequently increased survival plant compared to control. Neem and *T. hamatum* were the best in reducing pre- and post-emergence compared to control. *P. fluorescens* and eucalyptus ranked as the lowest effective. In general, the protection degree offered by fungicides and plant extract (Neem) application were much better than antagonistic.

Table (4). Influence of biocontrol agents, plant extracts and fungicides on lupine root-rot and wilt under greenhouse conditions.

Treatments	Pre-emergence damping-off (%)					Post-emergence damping-off (%)					Survival plants					Disease severity				
	R. solani	*F. solani*	*F. oxysporum*	*F. moniliforme*	Mean	*R. solani*	*F. solani*	*F. oxysporum*	*F. moniliforme*	Mean	*R. solani*	*F. solani*	*F. oxysporum*	*F. moniliforme*	Mean	*R. solani*	*F. solani*	*F. oxysporum*	*F. moniliforme*	Mean
T. harzianum	36.7	25.0	40.0	20.0	30.42	20.0	15.0	15.0	10.0	15.00	43.3	60.0	45.0	70.0	54.57	30.11	24.44	25.30	20.00	24.96
T. hamatum	34.0	22.0	33.0	18.0	26.79	20.0	15.0	15.0	10.0	15.00	46.0	63.0	52.0	72.0	58.25	25.20	25.60	25.20	20.00	24.00
P. fluorescens	40.0	30.0	40.0	25.0	33.75	20.0	20.0	30.0	15.0	21.25	40.0	50.0	30.0	60.0	45.00	35.16	38.16	40.18	30.12	35.91
B. subtilis	50.0	40.0	46.7	35.0	42.92	30.0	25.0	25.0	20.0	25.0	20.0	35.0	28.3	45.0	32.10	33.12	30.11	31.15	25.00	29.84
Neem	40.0	30.0	36.0	25.0	32.75	20.0	10.0	10.0	15.0	13.75	40.0	60.0	54.0	60.0	53.50	24.18	18.17	19.16	15.00	19.13
Eucalyptus	46.7	40.0	50.0	30.0	41.67	15.0	10.0	20.0	20.0	16.25	38.3	50.0	30.0	50.0	42.10	35.16	30.11	31.14	28.12	31.13
Moncerin	25.0	20.0	30.0	20.0	23.75	10.0	10.0	10.0	0.0	7.50	65.0	70.0	60.0	80.0	68.75	30.11	17.40	18.10	15.00	20.15
Rizolex-T	20.0	10.0	10.0	15.0	13.75	10.0	0.0	10.0	0.0	5.00	70.0	90.0	80.0	85.0	81.25	20.17	15.33	16.11	12.00	15.90
Control	60.0	50.0	60.0	40.0	52.50	30.0	20.0	30.0	20.0	25.00	10.0	30.0	10.0	40.0	22.50	77.40	68.18	65.18	60.00	67.69
Mean	39.15	29.11	38.41	25.33		19.44	13.89	18.33	12.22		41.40	56.44	43.25	62.44		34.51	29.72	30.17	26.03	
L.S.D at 0.05	Pathogen = 7.9 Treatment = 8.4 Interaction = 13.1					Pathogen = 4.1 Treatment = 4.9 Interaction = 10.9					Pathogen = 9.7 Treatment = 10.6 Interaction = 21.3					Pathogen = 8.86 Treatment = 6.12 Interaction = 12.07				

Table (5). Effect of biocontrol agents plant extract and fungicides on some growth parameters of lupine plants under greenhouse conditions.

Treatment	Fresh weight / plant					Dry weight / plant				
	R. solani	*F. solani*	*F. oxysporum*	*F. moniliforune*	mean	*R. solani*	*F. solani*	*F. oxysporum*	*F. moniliforune*	mean
T. harzianum	11.00	12.90	12.50	13.00	12.35	2.00	1.90	2.30	2.00	2.05
T. hamatum	13.00	13.00	12.00	13.00	12.75	2.50	2.00	2.00	2.30	2.20
P. fluorescens	10.50	12.00	11.00	12.50	11.50	1.30	1.40	1.40	2.00	1.52
B. subtilis	8.50	10.70	9.63	12.00	10.21	0.95	1.10	0.97	1.50	1.13
Neem	10.12	12.30	11.30	13.00	11.68	2.30	2.30	2.40	2.50	2.40
Eucalyptus	7.90	10.50	9.25	10.00	9.41	0.86	0.93	0.90	1.30	0.99
Moncerin	9.27	10.00	11.76	11.00	10.51	1.73	1.93	1.90	2.40	1.99
Rizolex-T	13.00	15.00	14.00	12.00	13.50	2.10	2.30	2.30	2.70	2.35
Control (pathogen)	3.20	6.12	5.27	7.00	5.40	0.53	0.83	0.74	1.20	0.82
Mean	9.61	11.39	10.74	11.50		1.59	1.63	1.66	1.99	
L.S.D. p = 0.05	Pathogen = 1.30 Treatment = 1.50 Interaction = 2.20					Pathogen = 0.26 Treatment = 0.39 Interaction = 0.62				

Table (6). Effect of treating lupine seeds with plant extracts, fungicides and antagonists on root-rot and wilt disease during two seasons under field conditions.

Treatments	Season 2007-2008			Season 2008-2009		
	Pre-emergence (%)	Post-emergence (%)	Survival (%)	Pre-emergence (%)	Post-emergence (%)	Survival (%)
T. harzianum	3.33	6.67	90.00	6.67	10.00	83.33
T. hamatum	0.00	6.67	93.33	6.67	3.33	90.00
P. fluorescens	16.67	13.33	30.00	13.33	13.33	73.33
	13.33	13.33	73.33	6.67	6.67	86.67
B. subtilis	3.33	6.67	90.00	3.33	3.33	93.33
Neem	6.67	13.33	80.00	10.00	10.00	80.00
Eucalyptus	3.33	6.67	90.00	0.00	3.33	96.67
Moncerin	0.00	6.67	93.33	3.33	3.33	93.33
Rizolex-T	20.00	16.67	56.67	16.67	16.67	66.67
Control						
L.S.D. at 5%	10.89	10.72	24.62	8.10	8.96	15.04

Effect of plant extract, antagonists and fungicides on growth characters and yield component of lupine under field conditions, the obtained data in Table (7) cleared the efficacy of plant extract, antagonists and fungicides significant were shown in plant height, fresh weight, dry weight. While infection of plant reduced significantly on infection percentage compared to untreated. The obtained results show that all treatments increased the number of pods, seeds weight / plant, 100 seed weight and seed yield / plot. Rizolex-T was the best treatment among *T. hamatum* and Neem, respectively. All tested treatment showed also a significant protection against the disease over the control.

Data in Table (8) showed that, during two studied seasons total N and crude protein concentration in Giza 1 lupine seeds were increased significantly each of total N and protein comparing to the control.

DISCUSSION

Lupine (*Lupinus termis L.*) is a legume crop contains high value of protein. It is infected with many fungal pathogens causing considerable yield losses where root-rot and wilt diseases (Christinsen et al. (1999). *However R. solani, F. solani, F. oxysporum and F. moniliforune* were virulent and predominant.

On the other hand, they found that, *in vitro* using antagonistic bioagents significantly reduced the linear growth of all examined fungi, where *T. hamatum* and *T. harzianum* were the most effective bio-agents followed by *B. subtilis* and *P. fluorescens*. *T. hamatum* and *T. harzianum* were the best in this regard, while *B. subtilis* and *P. fluorescens* were the least effective ones. Moreover, application of the antagonistic under bioagents greenhouse conditions reduced significantly pre-and post-emergence damping-off infection and increased healthy survived plants of lupine plants comparing with control. In this respect, *T. hamatum* was the most effective one followed by *T. harzianum* meanwhile, *P. fluorescens*, was the least effective one. Also, these increased plant height, fresh weight and dry weight compared with the control as mentioned by Mahmoud (2004).

The results indicated that neem extract was the most effective plant extract followed by eucalyptus. Meanwhile, the most sensitive fungus to neem was *F. moniliforme* and *R. solani*. Whereas, under greenhouse conditions, using neem and eucalyptus extracts reduced significantly, pre-emergence and post-emergence damping-off infection as well as increased significantly the healthy survived plants compared with control treatment as well as increased the fresh and dry of plant growth weight compared with untreated. Similar results were mentioned by Hussein (2005) and Eisa *et al.* (2006).

Concerning the effect of fungicides, in vitro results decreased gradually the fungal linear growth of the tested pathogenic isolates. Rizolex-T was the most effective fungicides all pathogenic fungi were sensitive to Rizolex-T and Moncerin under greenhouse conditions, all the used fungicides reduced significantly pre-and post-emergence damping-off infection pathogens. Rizolex-T treatment was the best effective one in increasing plant fresh and dry weight followed by Moncerin. It could be concluded that, application of antagonistic, plant extracts and fungicides under field conditions reduced significantly pre-and post-emergence damping-off compared to un-treated plants (control). The results of 2007/2008 and 2008/2009 seasons, revealed a significant effect of plant extracts, antagonistic and fungicides comparing to the control on plant height, fresh weight, dry weight, number of pods, pods / weight / plant and 100 seed weight. In this respect, all obtained results of Zakharova *et al.* (1995), El-Awadi *et al.* (1997) and Eisa *et al.* (2006).

Table (7). Effect of treating lupine seeds with biocontrol agents, plant extracts and fungicides on some yield component of cv. Giza 1 grown under field conditions.

Treatments	Season 2007/2008								Season 2008/2009							
	Plant height (cm)	Fresh weight (g)	Dry weight (g)	No. of branches / plant	Infection (%)	No. of pods / plant	100 seed weight (g)	Seed yield plot (kg)	Plant height (cm)	Fresh weight (g)	Dry weight (g)	No. of branches / plant	Infection (%)	No. of pods / plant	100 seed weight (g)	Seed yield plot (kg)
T. harzianum	29.67	15.67	2.23	4.00	23.33	21.33	24.67	5.43	33.67	17.67	3.17	4.33	22.00	23.67	24.67	4.93
T. hamatum	31.33	15.72	2.00	4.00	20.67	22.00	25.33	5.90	34.00	18.00	3.30	4.67	20.00	24.33	24.67	5.00
P. fluorescens	27.33	13.67	1.97	3.33	23.33	18.33	22.67	4.93	30.67	14.33	2.27	3.67	26.67	21.33	23.67	4.10
B. subtilis	28.33	13.33	2.03	3.33	21.67	18.67	23.00	4.88	32.33	15.00	2.90	4.00	23.33	23.00	23.33	4.83
Neem	31.67	15.50	2.83	3.07	10.00	21.33	24.67	5.67	34.00	17.33	3.17	3.67	11.67	25.33	24.00	5.10
Eucalyptus	28.67	12.83	1.83	3.00	20.00	19.33	22.33	4.80	30.33	13.67	2.33	3.00	16.67	21.00	20.00	4.47
Moncerin	31.67	16.17	2.93	4.00	16.67	22.33	24.67	5.33	35.33	17.33	3.27	4.67	10.00	25.33	22.67	5.00
Rizolex-T	34.33	18.06	3.37	4.33	11.00	25.33	26.33	5.90	37.00	19.00	3.60	5.00	6.67	27.00	25.67	5.43
Control	25.00	12.50	1.27	2.00	33.33	14.67	19.33	4.37	28.33	13.33	1.37	2.67	33.67	15.33	18.00	4.00
L.S.D at 0.05	1.20	0.96	0.25	0.68	7.64	1.64	1.01	0.39	1.53	0.42	0.37	0.82	7.79	1.84	1.02	0.34

Table (8). Total nitrogen and protein constitutes of lupine dry seeds as affected by plant extract, fungicides and antagonists.

Treatments	Season 2007-2008		Season 2008-2009	
	Total nitrogen (%)	Protein (%)	Total nitrogen (%)	Protein (%)
T. harzianum	5.22	32.67	5.36	33.50
T. hamatum	5.29	33.12	5.19	32.44
P. fluorescens	4.65	29.12	4.60	28.73
B. subtilis	4.55	28.44	4.75	29.67
Neem	4.87	30.44	5.12	32.00
Eucalyptus	4.75	29.67	4.80	30.00
Moncerin	4.91	30.70	4.99	31.19
Rizolex-T	5.30	33.14	5.48	34.22
Control	4.34	27.15	4.51	28.16
L.S.D. at 5%	0.33	2.90	0.51	2.25

REFERENCES

Abd El-Moity, T. H. and Shatla, M. N. (1981). Biological control of white rot diseases of onion (*Sclerotium cepivorum*) by *Trichoderma harzianum*. Phytopathology. Zeitschr., 100: 29 – 35.

Brain, P. W. and Hemming, H. G. (1945). Gliotoxin a fungistatic metabolic product of *Trichoderma viride*. Ann. Appl. Biol., 32: 214 – 220.

Christinsen, J. L.; Raza, S. and Ortiz, R. (1999). White lupine (*Lupinus albus* L.) germplasm collection and preliminary in situ diversity assessment in Egypt. Genetic Resources and Crop Evolution, 46 (1): 169 – 174.

Eisa, Nawal A.; El-Habaa, G. M.; Omar, S. M. and El-Sayed, Sahar A. (2006). Efficacy of antagonists, natural plant extracts and fungicides in controlling wilt, root-rot and chocolate spot pathogens of faba bean in vitro. Annals of Agric. Sci., Moshtohor, 44 (4): 1547 – 1570.

El-Awadi, F. A.; Ismail, I. A. and A., Nadia E. (1997). Evaluation of lupine entries and fungicides against root-rot and wilt disease of lupine 8th Congress of the Egyptian Phytopathol. Soc., Cairo, pp. 393 – 395.

El-Helaly, A. F.; Elarosi, H. M.; Assawah, M. W. and Abol-Wafa, M. T. (1970). Studies on damping-off and root-rots of bean in UAR (Egypt). Egypt. J. Phytopathol., 2: 41 – 57.

Etheridge, J. V. and Bateman, G. L. (1998). Fungal diseases of white lupine (*Lupinus albus*) and their control. Brighton crop protection conference. Pests & Diseases, 1998 volume 3: Proceedings of an International Conference, Brighton, UK, 16 – 19 November, 1998, pp. 1005 – 1010.

Gaffar, A. A.; El-Khateeb, Sanaa R.; Khalifa, E. Z. and Mousa, M. M. (1989). Control of bean damping-off and root-rot by plant extracts growth regulators and fungicides in relation to root nodules bacteria. Minufiya J. Agric. Res., 14: 1989.

Gowily, A. M.; Mostafa, M. A.; Soliman, G. L. and Omer, S. A. (1995). Role of copper and zinc sulphates as seed treatment in controlling damping-off disease caused by *Rhizoctonia solani* in lupine plants. Egypt. J. Appl. Sci., 10 (6): 462 – 472.

Hafez, A. and Mikkelsen, D. S. (1981). Colorimetric determination of nitrogen for evaluating the nutritional status of rice. Commnu., 12 (1): 61 – 69.

Hoshikawa, K. (1991). Significance of legume crops in improving the productivity and stability of cropping system. In: Johans, C.; K. K. Lee and K. L. sahrawat (eds). Phosphorus nutrition of legumes in the semi-arid tropics, pp. 173 – 182. ICRISAT, Patancheru, India.

Hussein, Manal Y. (2005). Evaluation of some plant extracts in controlling damping-off and root-rot of sugar beet. Minufiya J. Agric. Res., 3 (3): 867 – 876.

Jans, C. F.; Thrane, V. and Mathur, S. B. (1991). An illustrated manual on identification of some seed-borne Aspergilli, Fusaria, Penicilia and their Mycotoxins. Danish Government, Institute of Seed Pathology for Developing Countries, Ryvans Alle 78, DK, 2900 Hellerue, Denmark. (C.F. CABI Database Abstracts).

Liu, Z. and Sindair, J. B. (1991). Effect of seed coating with *Bacillus spp.* on *Rhizoctonia* damping-off, root and stem rot of soybeans. Biological and Cultural Tests for Control of Plant Diseases, 6: 62.

Mahmoud, E. Y. (2004). Intergrated control of pod rot diseases of peanut. Ph.D. Thesis, Fac. of Agric., Ain-Shams Univ., p. 154.

Maria, P. S. and Joseph, R. (2006). Influence of temperature and water activity on the antagonism of *Trichoderma harzianum* to *Verticillium* and *Rhizoctonia*. Crop protection, 25 (10).

Michail, S. H. and El-Khateeb, Sanaa, R. (1985). *Fusarium semitectum* a causal organism of fruit rot, damping-off and root-rot of cantaloupe, with reference to biological control. Minufiya J. Agric. Res., Rot., 10: 631 – 646.

Mohamed, F. G. (1996). Biological control of some bacterial and fungal diseases on beans (*Phaseolus vulgaris* L.). Ph.D. Thesis, Fac. Agric., Moshtohor, Zagazig Univ., Benha, Zagazig University.

Soliman N.K.; M.S. Mikhaili, P.K. Harb and E.M. Khalil (1988). Response of broad bean plants infected with *Rhizoctonia solani* to application of growth regulators and calcium. Egypt. J. Phytopathol., **20 (1): 1-11.**

Yehia, A. H.; El-Hassan, S. A. and El-Bahadi, A. H. (1988). Biological seed treatment to control *Fusarium* root-rot of broad bean. Egyptian Journal of Phytopathology, 14 (1/2): 59 – 66.

Zakharova, T. B.; Gortlevskii, A. A. and Zazimko, M. I. (1995). Biological control against root-rots of pea. *Zashchita Rastenii* Moskva, 7: 32.

الملخص العربى

مقاومة أمراض عفن الجذور والذبول فى الترمس

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معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة

وجد أن مرض عفن الجذور والذبول فى الترمس يُسبب مشكلة كبيرة فى مصر وللبحث عن حل لهذه المشكلة فقد تم استخدام المستخلصات النباتية وكائنات التضاد الحيوى والمبيدات للحد من هذه الكائنات المُمرضة المُسببة لمرض عفن الجذور والذبول فى الترمس مثل فطر رايزوكتونيا سولانى وفيوزاريوم

سولانى وفيوزاريوم مونيليفورم و فيوزاريوم أوكسيسبورم المُسبب لمرض الذبول فى الترمس .

ولقد أوضحت النتائج المتحصل عليها ما يلى :

١- **فى المعمل** : وجد أنه عند استخدام مُستخلص النيم والكافور (مستخلصات نباتية) وترايكودرما هارزيانم وترايكودرما هاماتم وبستدموناس فلوروسينس وباسيلس سنلس ضد النمو الميسليومى للفطريات المُمرضة ، وجد أن أفضل المعاملات هو استخدام النيم كمستخلص نباتى ثم يتبعه ترايكودرما هاماتم كأفضل كائنات التضاد . أما عند استخدام المبيدات فقد وجد أن رايزوليكس تى كان أفضل من مونسرين ، كما لوحظ أن المبيدات بصفة عامة كان تأثيرها أقوى من المستخلصات النباتية وكائنات التضاد الحيوى .

٢- **فى الصوبة** : أعطت المستخلصات النباتية وكائنات التضاد الحيوى والمبيدات تأثيراً معنوياً مقارنةً بالكنترول وكان النيم أفضل المستخلصات وكانت الترايكودرما هاماتم أفضل كائنات التضاد ورايزوليكس تى أفضل المبيدات مقارنةً بالكنترول .

٣- **فى الحقل** : أظهر رايزولكس تى تفوقه كمبيد فى تجارب الحقل وأظهر النيم تفوقه فى انخفاض الإصابة بأعفان الجذور والذبول وكذلك معاملة البذور بالترايكودرما هاماتم والترايكودرما هارزيانم والباسلس ساتلس .

كما أوضحت النتائج أيضاً أن هذه المعاملات أثرت على زيادة عدد النباتات السليمة وزيادة عدد القرون والبذور وكذلك زيادة فى وزن القطعة التجريبية ووزن الـ ١٠٠ بذرة وزيادة المحصول وكذلك وجد زيادة فى النسبة المئوية لكل من النيتروجين والبروتين فى البذور الجافة .