INDUCTION OF RESISTANCE IN LUPINE AGAINST ROOT ROT DISEASE

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ABSTRACT

Root rot caused by soil borne pathogenic fungi is the most sever disease attacks lupine plants. Isolation trials from diseased plants in some areas of Dakahlia province (Egypt) was carried out. Rhizoctonia solani and Fusarium solani proved to be the most dominant. Meanwhile, Fusarium oxysporum and Sclerotium rolfsii showed the least frequency.

The greenhouse experiments showed that S. rolfesii (No. 6) and R. solani (No. 2) followed by F. solani (No. 5) and F. oxysporum (No. 9) were the most aggressive root rot fungi. Efficacy of some plant resistance elicitors vis: chitosan (CHI), salicylic acid (SA) and hydroquinone (HQ) in comparing to the fungicide Rhizolex T-50 as seed treatments in reducing the severity of lupine root rot disease was investigated. Soaking susceptible lupine seeds (Giza 1) in each of the three selected elicitors showed a significant reduction in seedlings mortality. CHI at 8 g/l was superior in increasing the percentage of healthy plants to record 72.5, 80.9, 62.7and 64.3% when seeds were grown in soil infested with of F. solani, F. oxysporum, R. solani and S. rolfesii, respectively. These results were confirmed under field conditions in two locations i.e., Tag El-Ezz and El-Serow Research Stations. CHI 8 g/l proved to be the best elicitor after Rhizolex-T, in reducing lupine root rot disease. It showed 41 and 60% reduction in the plants mortality comparing to 56.37 and 69.13% in case of Rhizolex-T in Tag El-Ezz and El-Serow locations, respectively.

The treatments were accompanied with a significant increase in lupine growth parameters (plant height, No. of branches and No. of leaves / plant^{-1}), yield components (No. of pods and seed No./plant) and physiological aspects (photosynthetic pigments and total phenolic compounds). Application of CHI at 8 g/l or HQ at 1.2 g/l was the most potent in this respect.

Key words: *Elicitors, induced resistance, phenol contents, growth and yield parameters, lupine root rot*

INTRODUCTION

The Egyptian lupine (*Lupinus termis* Forsk) is a fabaceous crop which is grown in Egypt for food, medical and industrial purposes. The plant is cultivated for its non-endospermic seeds, which contains alkaloids, protein, oil, cholesterol, lecithin, salts (phosphorus and potassium), and carbohydrates (Ibrahim *et al.*, 1990). The alkaloids especially lupulin is occasionally emploied as a stomach tonic and as on hypnotic to promote sleep (Wallis, 1967). Green plants are useful as green-manuring because of the high nitrogenous content (Roberto Chiej, 1984).

Number of soil-borne fungi attacking lupine roots and stem base; causing serious losses in seed germination and plant stand including *Rhizoctonia solani, Sclerotium rolfsii, Fusarium solani* and *F. oxysporum*. (Fahim *et al.*, 1983 and Osman *et al.*, 1986).

Since the control of soil borne pathogens depends mainly on pesticides applications which increase the input of such pesticides into the environment, and might produce pesticide-resistant strains of pathogens. Efforts are being made to develop alternative protection strategies. One solution might be the activation of the plant's own defense system, known as induced resistance (Sticher *et al.* 1997). This induced systemic resistance is triggered by a number of chemicals such as salicylic acid (SA) and hydroquinone (HQ) (El-Mougy *et al.*, 2002; Matraux *et al.*, 1990, Shahda, 2000 and Amin *et al.*, 2007) or biologically active oligoglucosides, the so-called elicitors (Benhamou et al. 1994) which have been shown to induce systemic resistance in many crops like tomato, tobacco, pea, maize, cotton, rice, potato and other vegetables against viruses, fungi and bacteria (Oostendorp *et al.*, 2001). Among the most promising bioactive oligosaccharides is chitosan (CHI) which has attracted attention because of its unique biological properties of various pathogenic fungi and ability to be a potent elicitor of plant defense reactions against soil-borne pathogens (Benhamou *et al.*, 1998 and Prapagdee *et al.*, 2007).

This research aims to develop and evaluate new and existing alternative safe control method for lupine root rot disease under greenhouse and field conditions. The research also was planed to study the effect of these treatment on the contents of phenolic compounds, and photosynthetic pigments in the coming up plants.

MATERIALS AND METHODS

Fungal pathogens isolation and identification:

Samples of lupine plants showing root rot disease symptoms were collected from different locations of Dakahlia regions in 2007 growing season. All samples were thoroughly washed in tap water, cut in small pieces (1 cm) and surface sterilized for two minutes in 2% sodium hypochlorite solution, then rinsed several times in sterilized distilled water and dried between a number folds of sterilized filter papers. The surface sterilized samples were plated onto Potato Dextrose Agar (PDA) medium supplemented with streptomycin-sulfate (100 mg/ml) and incubated

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at 27°C. After 4-5 days incubation period, the developed fungal colonies were purified by hyphal tip and single spore isolation techniques. The fungi then were identified according to cultural and microscopically characters described by Ellis (1976); Sneh *et al.* (1992) and Nelson *et al.* (1983). The number and frequency of each isolated fungus in each location were recorded.

Pathogenicity tests:

Pathogenicity tests were carried out for 20 isolates of *F. solani*, 17 isolates of *F. oxysporum*, 22 isolates of *R. solani* and 9 isolates of *S. rolfesii* to choose the most virulent strains of each fungal species.

Inocula of the isolated pathogens were prepared using sorghum: coarse sand: water (2:1:2 v/v) medium. The ingredients were mixed, bottled and autoclaved for 20 min. at 121°C and 1.5 air pressure. The sterilized medium was inoculated using agar discs obtained from the periphery of 5-day-old colony of each of the isolated fungi. The inoculated media were incubated at 28 ±1°C for 15 days. Plastic pots (25 cm diameter) filled up with 4 kg autoclaved clay soil and artificially infested with the previously prepared fungal inocula at the rate of 1.5% by weight. Pot soil were mixed thoroughly with inoculum then, watered and left for one week to ensure even distribution of the inoculated fungi. Five pots were used as replicates for each treatment as well as check treatment (uninfested soil). Apparently healthy lupine seeds (cv. Giza 1) were surface sterilized in 0.1% sodium hypochloride for two min. then washed in sterilized water. Dried lupine seeds were sown in infested soil (5 seed / pot). After 15 and 30 days from sowing, seedlings were rated for pre- and post-emergence damping-off, respectively.

Control of soil-borne fungi under greenhouse condition:

Controlling of the most aggressive pathogenic strains of lupine root rot i.e., *S. rolfesii* (No. 6), *R. solani* (No. 2), *F. solani* (No. 5) and *F. oxysporum* (No. 9) was carried out under greenhouse conditions. Pots of previously prepared as mentioned above were seeded with lupine seeds soaked in each of CHI, SA and HQ for 2 hours, just before sowing at the rate of 6 &8, 0.7 & 1.4 and 0.6 & 1.2 g/l water, respectively. Six treated seeds pot^{-1} were sown in infested soil in comparing with seeds of check and seeds treated with Rhizolex-T 50 fungicide. Soil treated with free medium of the fungus was served as check treatment. Percentages of pre- and post-emergence damping-off were assessed at 15 and 40 days from sowing.

Field experiment:

Filed experiment was carried out at Tag El-Ezz Research Station, Dakahlia province and El-Serow Research Station, Damietta province, Egypt during the growing winter season of 2007/2008. Elicitors treatments were used as seed soaking treatment while, Rhizolex-T 50 w.p. was used as seed coating. Treated lupine seeds (cv. Giza 1) were sown in the field at the 10^{th} of November 2007 in both locations and left under natural infection.

A complete randomized block design with three replicates was used in both experiments. The experimental plot contained 5 ridges occupying an area of 10.5 m^2 (3x3.5 m). Percentages of root rot incidence at pre- and post-emergence damping-off were recorded at 15 and 90 days from sowing.

Morphological characters of lupine, samples were recorded after 120 days after planting which included plant height, number of branches and number of leaves per plant. At harvest (165 days from sowing), number of pods, seed number / plant, weight of seeds / plants and weight of 100 seeds were also recorded.

Determination of total phenolic compounds:

Total phenols were determined 70 days after sowing in fresh shoots using the Folin-Ciocalteau reagent according to Singleton and Rossi (1965). Samples (2g) were homogenized in 80% ethanol at room temperature and centrifuged at 10000 rpm for 15 min. under cooling and the supernatants were saved. The residues were re-extracted twice in 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residues were dissolved in 5 mL of distilled water. One-hundred microlitres of this extract were diluted to 3 ml with water and 0.5 ml of Folin-Ciocalteau reagent was added. After 3 min., 2 ml of 20% of sodium carbonate was added and the contents were mixed thoroughly. The developed color was photometrically measured at 650 nm. after 60 min. using catechol as a standard. The results were expressed as mg catechol/ 100 g fresh weight material.

Determination of photosynthetic pigments:

The blade of the 3^{rd} leaf from plant tip (terminal leaflet) was taken at 70 days to determine photosynthetic pigments (chlorophyll a, b and carotenoids). Photosynthetic pigments were extracted in 90% methanol for 24 h at room temperature while traces of sodium carbonate were added (Robinson and Britz, 2000). The pigments were determined at the wave lengths (452.5, 650 and 665nm) as described by Mackinney, 1941.

Statistical analysis

The obtained data were statistically analyzed by "CoStat 3.4" software as the usual technique of analysis of variance (Gomez and Gomez, 1984). The means were compared using Least Significant Difference (L.S.D.) at $P\leq0.05$ as outlined by Duncan (1955).

RESULTS

Root rot fungi:

Sixty-eight fungal isolates representing four species of three genera were isolated from roots of lupine plants showing root rot symptoms including *Fusarium* spp. (37 isolate), *Rhizoctonia solani* (22 isolate) and *Sclerotium rolfsii* (9 isolate).

Results in Table (1) indicate that the most dominant fungi were *R. solani* (22%) and *F. solani* (20 %) followed by *F. oxysporum* (17.0 %). However, *S. rolfsii* was less frequent isolated fungus (9.0%).

On the other side, the high frequency of isolated fungi was in Temi El-Amdeed district (25.3 and 19.2%) followed by Sherbeen district (22.4 and 16.6%) for *F. solani* and *F. oxysporum*, respectively. However, *R. solani* was in Belkas district (38.5%) followed by Sherbeen district (36.1%). On the other hand, *S. rolfesii* occurred in Temi El-Amdeed district (21.1%) followed by El-Mansoura district (19.2%).

Tablel: Frequency of the isolated fungi from roots of lupine plants showing root rot disease symptoms at different locations in Dakahlia province.

Dakahlia location	Frequency of isolated fungi %							
	F. solani		*F. oxysporum*		*R. solani*		*S. rolfesii*	
	No. of isolates	%	No. of isolate s	%	No. of isolate s	%	No. of isolates	%
Temi El-Amdeed	6	25.3	4	19.2	3	31.4	2	21.1
Belkas	3	20.6	1	15.5	9	38.5	3	18
El-Mansoura	4	19	5	12	5	27.5	2	19.2
Sherbeen	2	22.4	3	16.6	2	36.1	1	10.4
Dekerns	5	17.2	3	14.6	3	25	1	14.8
Total	20	-	17	-	22	-	9	-

Samples were collected during 60 days of growing season.

Pathogenicity tests:

All tested fungi (68 isolates) were pathogenic and caused typical symptoms of pre- and post-emergence damping-off on lupine seedlings. *S. rolfesii* showed to be the most virulent fungus to cause 77.3% seedlings mortality, followed by *R. solani*, *F. solani* and *F. oxysporum* (67.7, 58.0 and 50.3%, respectively).

Results from the pathogenicity tests on the 30 days old lupine seedlings indicated that *F. solani* (No. 5), *F. oxysporum* (No. 9), *R. solani* (No. 2) and *S. rolfesii* (No. 6) were found to be the most aggressive fungi on the base of seedlings mortality. These pathogens were selected for further investigation.

Greenhouse experiments:

The efficacy of CHI, SA and HQ at the rate of 6 & 8, 0.7 & 1.4 and 0.6 & 1.2 g/l, respectively, in form of seed soaking treatment and Rhizolex T-50 at concentration of 3 g/kg, for controlling damping-off was evaluated under greenhouse condition.

Data (Table 2) indicate that all tested elicitors significantly reduced pre- emergence damping-off syndromes as compared with the check. However, SA at 0.7 g/l was insignificant in decreasing damping-off caused by *F. oxysporum* and *R. solani*. As for post-emergence, the treatments have presented significant decreases than the check. CHI treatment at 8 g/l was superior in increasing healthy plants to reach (72.5, 80.9, 62.7 and 64.3% when *F. solani*, *F. oxysporum*, *R. solani* and *S. rolfesii* were found in the soil, respectively). In addition, the results show that Rhizolex T-50 gave the highest reduction in disease incidence.

Table (2): Effect of seed soaking in chitosan, salicylic acid, hydroquinone and Rhizolex T-50 on damping-off and seedling survivals of lupine, 40 days after sowing

Soil infestation	Damping-off (%)	Seed soaking							
		Check	Rhizolex T-50 (g/l)	Chitosan (g/l)		Salicylic acid (g/l)		Hydroquinone (g/l)	
			3	6	8	0.7	1.4	0.6	1.2
Check	Pre-emergence	2.0a*	0.0b	2.0a	1.4a	2.0a	1.9a	2.0a	1.5a
	Post-emergence	3.3a	0.0d	2.5ab	1.6c	3.1a	2.8ab	3.0a	2.0ab
	Survivals	94.7c	100.0a	95.3c	97.0b	94.9c	95.3c	95.0c	96.5b
F. solani (No. 5)	Pre-emergence	23.7a	5.5h	17.5d	11.2g	20.0b	16.3e	18.6c	14.3f
	Post-emergence	34.3a	8.9h	25.3d	16.3g	28.1b	22.6e	26.9c	19.2f
	Survivals	42.0h	85.6a	57.2e	72.5b	51.9g	61.1d	54.5f	66.5c
F. oxysporum (No. 9)	Pre-emergence	20.0a	4.5g	17.4c	8.6f	19.6a	15.3d	18.2b	10.8e
	Post-emergence	30.3a	2.6h	19.5d	10.5g	26.1b	18.9e	20.6c	16.3f
	Survivals	49.7h	92.9a	63.1e	80.9b	54.3g	65.8d	61.2f	72.9c
R. solani (No. 2)	Pre-emergence	27.7a	8.2g	24.2c	17.2f	27.6a	22.1d	24.9b	20.3e
	Post-emergence	40.0a	10.6h	28.3d	20.1g	35.2b	25.9e	31.2c	24.2f
	Survivals	32.3h	81.2a	47.5e	62.7b	37.2g	52.0d	43.9f	55.5c
S. rolfsii (No. 6)	Pre-emergence	33.0a	7.2h	22.7d	17.6g	26.1b	21.2e	24.2c	18.5f
	Post-emergence	44.3a	5.3h	28.0d	18.1g	33.5b	26.6e	30.4c	25.2f
	Survivals	22.7h	81.5a	49.3e	64.3b	40.4g	52.2d	45.4f	56.3c

*Values followed by the same letter(s) in each row do not differ significantly ($P \leq 0.05$).

Field experiments:

a. Disease assessment:

Data in Table (3) show that soaking lupine seeds, in each of the three elicitors or coating with Rhizolex T-50, significantly decreased rotted seeds and root rot symptoms percentages as well as increased seedling survivals. Chitosan at 8 g/l came next to Rhizolèx T-50, in reducing lupine root rots. The percentage of reduction which could be recalculated from table 3 was 47.8 & 66.7%, while it was 87.98 & 78.46% in case of Rhizolex T-50 at pre emergence stage and 43 & 61.7% and 61.98&70% at post-emergence damping-off stage, in Tag El-Ezz and El-Serow locations, respectively. Hydraquinon at 1.2 g/l came the second in reducing plant mortality and increasing lupine plant survivals as compared to check treatment. On the other hand, no significant difference was observed in plant survivals due to Salcylic acid 0.7 or Hydroquinone 0.6 g/l applications as compared to check treatment

Table (3): Effect of 3 tested elicitors on survival of lupine seedling against root rot fungi under field conditions, 90 days after sowing

Treatment		Tag El-Ezz			El-Serow		
		Rotted seeds	Root rot	Survival	Pre emergence damping off	Post emergence damping off	Survival
Check (water)		20.30a*	26.30a	53.40e	19.50a	29.00a	51.50h
Rhizolex T-50	3 (g/kg)	6.50f	10.00f	83.50a	4.20f	8.70h	87.10a
Chitosan	6 (g/l)	11.60d	22.50c	65.90d	9.70c	20.30e	70.00d
	8 (g/l)	9.70e	15.00e	75.30b	6.50de	11.10g	82.40b
Salcylic acid	0.7 (g/l)	18.10b	24.30b	57.60e	14.70b	26.50b	58.80g
	1.4 (g/l)	13.00c	22.20c	64.80d	6.30e	25.40c	68.30e
Hydroqiunone	0.6 (g/l)	17.40b	23.00c	59.60e	14.80b	22.30d	62.90f
	1.2 (g/l)	11.00d	18.00d	71.00c	7.00d	14.0f	79.00c

*Values followed by the same letter(s) in each column do not differ significantly ($P \leq 0.05$).

b. Morphological characters:

The effects of the three tested elicitors and the Rhizolex T-50 on plant height, number of branches and leaves / plant at 120 days from sowing are shown in Table (4). It was clear that the use of high levels of CHI (8 g/l), HQ (1.2 g/l) and SA (1.4 g/l) gave the highest values of plant height. While, the low levels (6, 0.7 and 0.6 g/l) as well as fungicide at concentration of 3 g/kg had no significant effect on the tested parameter. Concerning, number of branches and leaves, data reveal that Rhizolex T-50 as well as the tested elicitors significantly increased the tested parameters. The highest means occurred under the application of CHI at 8 g/l followed by HQ at 1.2 g/l then CHI at 6 g/l. However, the fungicide came at the end compared with other treatments.

Table (4): Effect of the tested elicitors on the growth parameters of lupine plant, 60 days after sowing

Treatment		Tag El-Ezz			El-Serow		
		Plant height (cm)	Number of branches	Number of leaves	Plant height (cm)	Number of branches	Number of leaves
Check (water)		73.33d*	4.00e	109.00e	69.33d	3.00e	101.67d
Rhizolex T-50	3 (g/kg)	77.00cd	5.33d	124.33d	73.00b-d	4.33d	117.00d
Chitosan	6 (g/l)	78.33b-d	6.67bc	176.67b	74.33b-d	5.67bc	169.33b
	8 (g/l)	86.33a	8.33a	193.33a	82.33a	7.33a	186.00a
Salcylic acid	0.7 (g/l)	74.67d	6.33cd	142.67c	70.67cd	5.33cd	135.33c
	1.4 (g/l)	82.33a-c	7.33a-c	146.67c	78.33a-c	6.33a-c	139.33c
Hydroqiunone	0.6 (g/l)	78.00b-d	7.67ab	153.33c	75.67a-d	6.67ab	146.00c
	1.2 (g/l)	84.67ab	7.67ab	177.67b	80.67ab	6.67ab	171.00ab

*Values followed by the same letter(s) in each column do not differ significantly ($P \leq 0.05$).

c. Yield and its components:

The data in Table (5) indicate that the number of pods / plant was significantly increased by enhancind elicitors concentrations. The highly significant increase occurred under the high concentration of CHI followed by HQ then SA. Application of CHI at 8 g/l caused a significant increase in the number of seeds and weight / plant as well as weight of 100-seeds. However, the other treatments had no significant effects in tested parameters except, the weight of 100-seeds, which showed significant increases when high conc. of HQ and SA were used.

Table (5): Effect of elicitors on the yield components of lupine plant after 90 days from sowing

Treatment		Tag El-Ezz				El-Serow			
		Number of pods plant^{-1}	Number of seeds plant^{-1}	weight of seeds plant^{-1}	Weight of 100 seed (g)	Number of pods plant^{-1}	Number of seeds plant^{-1}	weight of seeds plant^{-1}	Weight of 100 seed (g)
Check (water)		8.00e*	26.30bc	7.40b	25.60b	6.00e	24.30bc	7.00b	24.30b
Rhizolex T-50	3 (g/kg)	11.33d	26.47bc	7.83b	32.40ab	9.33d	24.47bc	7.43b	27.77ab
Chitosan	6 (g/l)	14.00bc	24.77c	8.77ab	30.67ab	12.00bc	22.77c	8.37ab	29.37ab
	8 (g/l)	17.67a	32.30a	10.63a	37.73a	15.67a	30.30a	10.23a	35.03a
Salcylic acid	0.7 (g/l)	12.33cd	26.30bc	8.67ab	30.53ab	10.33cd	24.30bc	8.27ab	29.23ab
	1.4 (g/l)	14.33bc	27.40a-c	8.80ab	34.267a	12.33bc	25.40a-c	8.40ab	29.77ab
Hydroqiunone	0.6 (g/l)	13.33cd	29.57a-c	8.83ab	31.57ab	11.33cd	27.56a-c	8.43ab	30.27 ab
	1.2 (g/l)	16.00ab	31.10ab	9.30ab	36.33a	14.00ab	29.10ab	8.90ab	32.97ab

*Values followed by the same letter(s) in each column do not differ significantly ($P \leq 0.05$).

d. Total phenol and photosynthetic pigments contents:

Data in Table (6) reveal that Rhizolex T-50 and three tested elicitors increased the concentration of total phenols and the photosynthetic pigments in fresh leaves of lupine plants. The high concentration of total phenols occurred in the treatment where CHI 8 g/l was used, followed by fungicide with no significant differences. The recorded increases were 36.25 & 38.26% and 44.65 & 45.88%, respectively in Tag EL-Ezz and El-Serow localities. HQ at 1.2 g/l treatment came in the second order in increasing lupine shoots content of total phenols as compared to check treatment.

It was also observed that chlorophyll a, b and caroteinoids concentrations significantly increased when CHI 8 g/l, fungicide, HQ 1.2 g/l and SA 1.4 g/l were applied. On the other hand, no significant differences were recorded between these treatments.

Table (6): Effect of the tested elicitors on total phenols and photosynthetic pigment contents of lupine plant 60 days after sowing

Treatment		Tag El-Ezz				El-Serow			
		Total phenol (mg/100g)	Photosynthetic pigments mg/100g fresh weight			Total phenol (mg/100g)	Photosynthetic pigments mg/100g fresh weight		
			Chl. a	Chl. b	Caroteinoids		Chl. a	Chl. b	Caroteinoids
Check (water)		356.4d*	1.10c	0.72b	0.21b	346.41d	0.90c	0.55c	0.19b
Rhizolex T-50	3 (g/kg)	485.60ab	1.54ab	1.09a	0.31a	478.94ab	1.38a	0.93a	0.30a
Chitosan	6 (g/l)	428.33c	1.37a-c	0.86ab	0.27ab	418.33c	1.17a-c	0.70bc	0.25a
	8 (g/l)	515.52a	1.58a	1.09a	0.32a	505.53a	1.34a	0.93ab	0.29a
Salcylic acid	0.7 (g/l)	363.58d	1.27a-c	0.99a	0.21b	353.58d	1.07a-c	0.82ab	0.19b
	1.4 (g/l)	418.15c	1.44a-c	0.99a	0.31a	408.15c	1.24ab	0.83ab	0.29a
Hydroqiunone	0.6 (g/l)	403.38cd	1.17bc	1.01a	0.21b	393.38cd	0.97bc	0.85ab	0.18b
	1.2 (g/l)	449.67bc	1.48a-c	1.05a	0.30a	439.67bc	1.28ab	0.89ab	0.28a

*Values followed by the same letter(s) in each column do not differ significantly ($P \leq 0.05$).

DISCUSSION

Identifying the defense activators (elicitors) that can substitute conventional chemical fungicides is a valuable contribution to lupine disease management, especially for the prevalence and destructive soil borne wilt and root rot pathogens. Isolation trials for the causal pathogens of lupine root rot symptoms showed that, *R. solani* and *F. solani* were the most dominant fungi isolated from infected roots in Dakahlia province. Meanwhile, *F. oxysporum* and *S. rolfesii* were less frequent. From 68 fungal isolates, *F. solani* (No. 5), *F. oxysporum* (No. 9), *R. solani* (No. 2) and *S. rolfsii* (No. 6) were most aggressive in causing root rot of lupine seedlings.

Soaking seeds of lupine in each tested elicitor had great values in decreasing pre- & post-emergence damping-off incidence, hence increasing survivals under greenhouse conditions. These positive results in decreasing lupine root rot severity were approved in two locations i.e., Dakahlia and Damietta provinces. Several reports indicated that treating seeds of various crops with the elicitors such as CHI effectively controlled soil-borne diseases (Benhamou et al., 1998; Dasgupta *et al.*, 1998; Jiang *et al.*, 1999 and Hilal *et al.*, 2006). Field application of CHI for inducing resistance against late and early blight diseases of potato and root rot disease of lupine plants was reported by Abd-El-Kareem *et.al.*, (2001 and 2004a). CHI was suggested to increase lignin biosynthesis and plant cell wall lignifications as well as its effects on enzyme biosynthesis which associated with the development of resistance (Tiuterev *et al.*, 1996). Several studies have demonstrated that over expression of chitinases and *B*-1, 3-glucanase in plants is associated with enhanced resistance to various fungal pathogens (Chen *et al.*, 1999 and Abd-El-Karem *et al.*, 2004b).

Soaked lupine seeds showed significant increase in lupine growth parameters, especially these soaked with CHI at 8 g/l, followed by HQ at 1.2 g/l. CHI was reported to enhance plant growth of other crops similar to the present results (Dasgupta *et al.* 1998 and Hilal *et al.*, 2006). These increases may be attributed to their effect on physiological processes in plant such as ion uptake, cell elongation, cell division, enzymatic activation and protein synthesis (Shakirova et al., 2003, Farouk, 2005 and Amin et al., 2007).

Treatment of lupine seeds with CHI at 8 g/l and HQ at 1.2 g/l had led to increase in plant shoots and its content of total phenols. Phenolics are well-known as antifungal, antibacterial and antiviral compounds occur naturally in plants (Sivaprakasan & Vidhyasekaran, 1993). According to Matern & Kneusal (1988), the first step of the defence mechanism in plants involves a rapid accumulation of phenols at the infection site, which restricts or slows the growth of the pathogen. This effect might be due to the impact of these substances on enzymatic activity and translocation of the metabolites to lupine plant. The results presented here are in agreement with those obtained by Hilal *et al.*, 2006 who reported that, total, free and conjugated phenols content in caraway and fennel leaves significantly increased by CHI application. This accumulation in phenolic compounds in leaves may be due to inhibition of catalase activity, which in turn induces phenylalanine lyase gene expression and synthesis of phenolic compounds (Vermerris and Nicholson, 2006). Yet total phenols have long been considered as important defense-related compounds whose levels are naturally high in resistant varieties of many crops (Gogoi *et al.*, 2001 and Khaleifa *et al.*, 2006).

Chlorophyll content was determined in lupine plants since it is a good parameter reflects the health condition of plant. Noticeable increments in chlorophyll content were observed in plants developed from lupine seeds soaked in tested elicitors. This increment in chlorophyll content is by stimulating pigment formation and enhancing the efficacy of photosynthetic apparatus with a better potential for resistance and decrease in photophosphorylation rate usually occurring after infection (Amaresh and Bhatt, 1998). The previous discussion may introduce an explanation for the high yield and growth parameters in lupine plants.

The increase in lupine yield may also be due to the role of elicitors in stimulation of physiological processes which reflect on improving vegetative growth that followed by active translocation of the photoassimilates. In this concern, SA might be regulating plant growth by increasing enzyme activity as α-amylase and nitrate reductase, which accelerate the sugar translocation from the leaves to developing fruit (Sharma *et al.*, 1986). In addition, application of SA inhibits ethylene production leading to an increase in fruit number and consequently increases fruit yield per plant (Leslie and Romani, 1986).

This research recommended the use of different plant elicitors as alternative method for seed treatment and to avoid the hazards produced when using the toxic synthetic chemicals.

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الملخص العربي

إستحثاث مقاومة الترمس ضد مرض عفن الجذور

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يعتبر عفن الجذور من أخطر الأمراض التي تهاجم نبات الترمس ويسببه العديد من فطريات التربة. في هذه الدراسة تم عزل المسببات المرضية الفطرية من نباتات الترمس المصابة بمرض عفن الجذور من أماكن مختلفة بمحافظة الدقهلية. ولقد وجد أن فطرى الريزوكتونيا سولانى والفيوزاريم سولانى هما العزلتان السائدتان من العزلات الفطرية، في حين أن فطرى الفيوزاريم اوكسيسبورم والاسكلرشيم رولفزياى كانا الأقل تكرارا. ولقد تم دراسة تأثير معاملة البذور ببعض المحثات النباتية (الكيتوزان ، حامض السالسيليك و الهيدروكينون) مقارنة بالمطهر الفطري ريزوليكس تى-٥٠ على حدوث مرض عفن الجذور في الترمس وقد أظهرت النتائج مايلى:-

-في تجارب الصوبة: في اختبارات القدرة المرضية وجد أن فطرى الأسكليرشيم رولفزياى (رقم ٦) والريزوكتونيا سولانى (رقم ٢) كانا الأكثر عدوانية تبعهما الفيوزاريم سولانى (رقم ٥) و الفيوزاريم اوكسيسبورم (رقم ٩). ولقد أدى نقع بذور الترمس القابلة للإصابة (جيزة ١) في المحثات الثلاثة إلى حدوث نقص معنوي في موت البادرات. وكان الكيتوزان بتركيز ٨ جم/لتر هو الأعلى تأثيرا في زياد النسبة المئوية للنباتات السليمة والتى تراوحت بين (٧٢,٥ ، ٨٠,٩ ، ٦٢,٧ و ٦٤,٣%) وذلك في التربة الملقحة بفطريات فيوزاريم سولانى و فيوزاريم اوكسيسبورم وريزوكتونيا سولانى واسكليرشيم رولفزياى على الترتيب.

-في تجارب الحقل: تم الحصول على نتائج مشابهة لتجارب الصوبة تحت الظروف الحقلية وذلك في موقعين مختلفين (محطة بحوث تاج العز- دقهلية ومحطة بحوث السرو بدمياط) ، حيث وجد أن الكيتوزان بتركيز ٨ جم/لتر هو أعلى المحثات قدرة على خفض النسبة المئوية لعفن الجذور (٤١ و ٦٠%) مقارنة بمبيد الريزوليكس (٥٦,٣٧ و ٦٩,١٣%) في تاج العز والسرو على الترتيب.

وقد تلازم النقص في المرض والناتج عن معظم تركيزات المحثات المستخدمة مع حدوث زيادة معنوية في صفات النمو الخضري (ارتفاع النبات ، عدد الأفرع وعدد الأوراق للنبات) ومكونات المحصول (عدد القرون وعدد البذور للنبات ووزن ١٠٠ بذرة) وكذلك الصفات الفسيولوجية (محتوى الفينولات وصبغات البناء الضوئي). وكانت المعاملة بالكيتوزان بتركيز ٨ جم/لتر والهيدروكينون بتركيز ١,٢ هما الأعلى تأثيرا.