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UTILIZATION OF ANTIOXIDANTS AND PLANT EXTRACTS IN CONTROLLING WILT DISEASE OF LUPINE (*Lupinus termis* forsk).

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ABSTRACT

Fusarium oxysporum f.sp. *Lupini* is a common fungal pathogen on lupine plants causing wilt disease resulting in serious economic losses. Therefore, this research was conducted in order to survey the wilt disease in five Governorates. Results showed that isolates obtained from Ismailia were the highest in the pathogenesis ability. Isolation and identification also showed that *Fusarium oxysporum* f.sp. *Lupini* is the main pathogen of this disease. The effect of soaking lupine seeds before planting in different concentrations of antioxidants such as ascorbic acid and citric acid, salicylic acid, sodium benzoate with four concentrations i.e. 25, 50, 100and200ppm, as well as five botanical extracts are leek leaves, thyme, basil, marigold, neem seeds with the concentrations 1, 2,4,6% under greenhouse conditions.

Pots experiments results showed that all antioxidants tested, as well as plant extracts significantly reduced the proportion of infected plants with wilt and increased the proportion of the remaining healthy plants, and also the various growth parameters compared to the control treatment. The results showed that the salicylic acid concentration 200ppm gave the best percentage of healthy plants and also the various growth parameters followed by the leek extract. Field results were similar to the results of the greenhouse experience where the each antioxidants tested, as well as plant extracts had increased the healthy plants ratio compared to the control has dovetailed increase in healthy plants percentage with the increase in the weight of the dry seed showing that the treatment of salicylic acid followed by ascorbic acid were the most influential, as well as the treatment of the balls, followed by neem were the best in plant extracts. The antioxidants are the best ever from plant extracts. The results indicate the possibility of using some chemical inducers and plant extracts in wilt resistance in a lupine in Egypt.

Keywords: Lupine, wilt disease, antioxidants, plant extract, controlling.

INTRODUCTION

(*Lupinus termis* Forsk) is considered one of the legumes with the promising future potential due to its high protein content as well as its adaptation to poor soil and dry climates. It has been used as a green manure, forage and seeds for human usage because of its high protein content.

Like other seed legumes, lupine plant is able to fix atmospheric nitrogen in the soil that increase soil fertility with no addition cost or effort particularly in reclaimed soils, therefore lupine have useful effect in such areas Jams *et al*.,(2002) and Campbell *et al*.,(2014).

Unfortunately in Egypt and several countries lupine legume plants are infected by several soil-borne fungi causing damping-off and wilt disease which effect both quantity and quality of the yield. Soil-borne fungal diseases are among the most important factors limiting the yield production of lupine resulting in serious economic losses. Several soil pathogens including *Fusarium oxysporum* f.sp. *Lupini* attack the root and stem base of lupine

plants El-Barougy and El-Sayed, (2003), Zian, (2005) and Baraka *et al*., (2011).

Some chemicals are affective in controlling these diseases. These chemicals are expensive and not environmental friendly. Many researchers have used chemical compounds and plant extracts as mean of protection against soil- borne diseases as an alternative control method to fungicides Nehal (2004), and Abd-el-Kader*, et al*., (2014).

The plant growth promoting chemical compound and plant extract has shown promising results for the control of various plant pathogen as well as growth promoters of some crops Abdel-Monaim M.F. and Ismail, M.E. (2010) and Islam and Faruq, (2012). Beneficial chemical compound and plant extract could be significant compound in the management of the soil environment so as to achieve attainable crop yield Waleed *et al*., (2011).

Application of many chemical compounds Nafie and Mazen, (2008) and extract of various plants have also been explored as natural resistance inducers such as, *Artemisia afra*, *Leucosidea sericea*, *Melia azedorach* and *Rhamnus prinoides* against *Rhizoctonia solani*, *p ythium ultimum and Fusarium oxysporum* Kena and Swart, (2011).

Therefore, the present study, was conducted to: 1, isolate pathogen (s) of lupine causing wilt diseases: 2, study the effect of the isolation of the pathogens chemical compounds and plant extracts on controlling wilt disease and some growth parameters of lupine plants variety Giza2 under greenhouse conditions, 3 the effect of same chemical inducers and plant extracts controlling wilt disease and seed yield of lupine plants variety Giza2 under field conditions.

MATERIALS AND METHODS

Survey of lupine wilt disease:

Tow months after lupine planting, survey of wilt was initiated and continued till three months. Wilted lupine plants (*L termis* L) that grown at different localities of Kalyobeia, Ismailia, Beheira, Sharkia and Assuit Governorates were surveyed during the growing season of 2011 and 2012. Three fields per each locality were concerned and the diseased plants showing typical symptoms of wilt were surveyed in the each location. Sampling sites were determined with a field map, 5 sampling sites were determined per field tested, one of each of the four corners plus one in the center of the field. Sampling sites were located at least 5m from the edge of the field. The average percentage of disease incidence was calculated as the number of wilted lupine plants in relative to the total number of examined plants.

Isolation, purification and identification of the pathogen (s):

Naturally infected plants showing wilt symptoms were collected from different fields in Kalyobeia, Ismailia, Beheira, Sharkia and Assuit Governorates and the tissue was cut into 5 mm long and 3 mm thick pieces. These pieces were surface sterilized with 1% NaOCL solution for 2 minutes then washed with sterilized water. These surface sterilized pieces were transferred into potato dextrose agar (PDA) in 9 cm diameter Petri dishes and incubated at 28ºC for 7days. Purification of the isolated fungus was carried

out using hyphal tip techniques according to Dhings and Sinclair (1985). Pure culture was identified to their morphological characters according to Nelson *et al.* (1983) and Barnett Hunter (1986) in Agric. Botany Dept., Fac. Agric., AL-Azhar Univ., Assuit.

Preparations of fungal inoculum:

Inoculum of the most frequent fungus was prepared from one week old culture of recovered fungi in flask (500ml) containing 150g autoclaved sorghum medium, (sorghum, sand and water in 1:1:4 ratio) according to Abdel –Rehim (1984) and incubated at 28±2ºC for 15days.

Then, the fungal inoculum was used for soil infestation under a greenhouse conditions for studying the pathogenicity test.

Pathogenicity test:

Pathogenicity test of *Fusarium oxysporum* f.sp. *Lupini* isolates which isolated from wilted lupine plants were carried out on the Giza2 variety under greenhouse conditions of Agric., Botany Dept., Fac. Agric., AL-Azhar Univ., Assuit. Pots (30cm in diameter) were always sterilized by immersing in 5% formalin for 15 minutes and left to dry the and filled with 5kg autoclaved sandy loam soil. The soil was infested with the fungal inoculum at the rate of 3% (w/w) of soil weight El-Barougy, (2008). Fungal inoculum was thoroughly mixed with the soil and regularly watered every three days for a tow weeks before planting.

Soil mixed alone with the same rate of the autoclaved sorghum medium served as a control. Lupine seeds (cv.Giza2) were surface sterilized by immersing them in 1% sodium hypochlorite solution (NaOCL) for 2 mines then washed several times with sterilized water. Five seeds were sown in each pot and maintained under greenhouse condition. A set of five replicates was used for each treatment. Percentage of early and late wilt were recorded at 45 and 90 days, respectively after sowing, while the numbers of the survived plants (healthy and infected) were recorded after 120days of sowing. Infected plants were evaluated by cutting longitudinally of stem and root, healthy survived plants = no visual evidence of the disease.

Disease severity of visual wilt symptoms and any discoloration of internal tissue were recorded to Ishikawa *et al.,* (2005) with some modification, based on 0-4 grades according to the percentage inside browning through stem and root:0=healthy, 1=0-25% browning, 2=>25-50% browning, 3=>50-75% browning and 4=>75-100% browning. The same fungus was reisolated from the diseased plants.

Effect of antioxidants and plant extracts on controlling lupine wilt disease on cv. Giza2 under greenhouse conditions:

Four antioxidant compounds, i.e. ascorbic acid (AA), citric acid (CA), salicylic acid (SA) and sodium benzoate (SB) and five plant extracts, i.e. leek leaves (*Allium porrum*), thyme leaves (*Thymus vulgaris*), basil leaves (*Ocium basillicum*), marigold leaves (*Tagetes erecta*) and neem seeds (*Azadirachta indica*). Four different concentrations of each tested compound i.e. 25ppm, 50ppm, 100ppm and 200ppm of antioxidants and 1, 2, 4and 6% of plant extracts were used. Only deionised distilled water (DDW) was served as control. Seeds of lupine cv. Giza2 were disinfested by dipping in 0.1% mercuric chloride solution for 2min, then washed thoroughly by (DDW) and

left to dry before soaking in the test solution. Sterilized seeds were soaked in the solution separately for 2.5 hrs before planting. Sterilized sorghum grain media was infested with the isolate *F. oxysporum* f.sp. *Lupini* Ismailia No (7) and incubated at 25C for 15days. Five lupine seeds per pot were sown in 30cm pots filled with *F. oxysporum* f.sp. *Lupini* infested sterilized soil at the rate of 3% (w/w) per pot as previously mentioned. The sterilized soil was used as check treatment. A-set of five pots for each treatment was used. Percentages of early wilt recorded after 45 days from sowing. Late wilt and survived plants were recorded after 90 days from sowing, while disease severity of wilt and any discoloration of internal tissue were recorded after 120 days of sowing. The wilted plants were evaluated by cutting longitudinally through each plant (stem and root), while healthy plants = no visual evidence of disease.

One hundred grams of leek leaves (*Allium porrum*), thyme leaves (*Thymus vulgaris*), basil leaves (*Ocium basillicum*), marigold leaves (*Tagetes erecta*) and neem seeds (*Azadirachta indica*), were prepared as follows: A known weight (100g) of fresh leaves then plants were washed under running tap water, air dried for one hour under the laboratory conditions and homogenized in 100ml sterile distilled water using electric blender. The homogenates were centrifuged at 300rpm for 15 min. and filtered through filter paper (watman No.1). The supernatants were sterilized using bacteria proof seats filter and kept as stock solution 100% concentration in dark sterile bottle in a refrigerator until using.

Disease severity of wilt and any discoloration of internal tissue were recorded as mentioned before. Plant growth parameters (plant height, number of branches, and number of pods, seed dry weight and root length/plant) were recorded four months after planting.

Effect of some antioxidants and plant extract on lupine wilt and root rot diseases under field conditions:

An experiment was conducted in a farm of kalyobeia Governorate, Egypt during the first week of November for tow successive seasons of 2011/2012 and 2012/2013 in soil known to have a high inoculums density of the lupine wilt pathogen for controlling wilt disease of lupine as well as in a naturally infested field. Lupine seeds (cv.Giza2) previously soaked for thirty minutes in any of the tested four antioxidants solution and five plant extracts solution or untreated ones, were used for sowing. The treatments were arranged in a complete randomized block design with three replicates.

The field plot was 6m² (2m× 3m²) with five rows; each row contained 10 hills.100 seeds of cv.Giza2 were sown in each plot.

Percentages of early wilt and damping – off were recorded after 45 days of sowing, while infected plants and root –rotted plants as well as survived plants were recorded after 90 days of sowing and weight of seed yield /Fadden were recorded at harvest till all plants and seed dried.

Statistical Analysis

All experiments were performed twice. Analysis of variances was carried out used MSTA-C program (version 2.10, 1991). Least significant difference (LSD) was employed to test for significant difference between treatments at $p \leq 0.05$ (Gomez and Gomez, 1984)

RESULTS

Survey of lupine wilt disease in different the Governorates:

Data in Table (1) show the various percentage of infection of wilt disease after 120 days of sowing distributions of Governorates. The highest percentage of wilt disease was recorded in Ismailia (25%) followed by Kalyobeia (20%), moderate percentage was recorded in Behera while the least percentage of wilt infection (8%) was recorded in Sharkia Governorates.

Table 1. Mean percentage of natural infection of lupine wilt disease during2010/2011growing seasons.

Collection origin	Wilt (%)
Kalyobeia	20
Ismailia	25
Beheira	15
Sharkia	8
Assuit	10

Table 2. Pathogenicity of various *F. oxysporum* f.sp. *Lupini* isolates obtained from wilted lupine plants cv. Giza2.

Governorates	Isolates	Wilted plants %		Survived plants %		Disease Severity score
		45 days after sowing	90 days after sowing	Infected Plants %	Healthy Plants %	
Kalyobeia	1	16	28	20	36	1.4
	2	12	36	28	24	1.4
	3	20	32	20	28	1.8
	4	16	32	16	36	3.0
Ismailia	5	20	36	16	28	1.2
	6	16	32	12	40	1.8
	7	24	36	20	20	3.2
	8	16	24	12	48	2.4
Beheira	9	20	36	16	28	1.8
	10	16	32	24	28	2.0
	11	12	28	8	52	1.2
	12	20	28	8	44	0.8
Sharkia	13	16	36	16	32	2.0
	14	24	28	8	40	3.0
	15	16	28	12	44	1.6
	16	8	12	4	72	1.0
Assuit	17	16	32	4	48	1.2
	18	8	24	4	64	0.6
	19	4	24	4	68	0.8
	20	12	24	12	52	2.2
Control		0.0	0.0	0.0	100.0	0.0
L.S.D. at 5%		12.58	13.82	12.64	14.15	0.90

Isolation and identification of the causal pathogen and pathogenicity test:

Twenty *F. oxysporum* f.sp. *Lupini* isolates, isolated from roots of lupine plants showing typical symptoms of wilt disease, collected from different locations of Kaluobeia, Ismailia, Behera, Sharkia and Assuit Governorates were tested for the capabilities on lupine plants cv. Giza2. Data presented in Table (2) indicate that all the tested isolates were pathogenic and showed

significant variation in their pathogenicity in pot experiments. *Fusarium oxysporum* f.sp. *Lupini* isolate No.7 isolated from Ismailia was the most virulent causing 24% early wilted plants after 45 days and 36% late wilted plants after 90 days, followed by isolates No.(5) 20% and 36%, No.(9) 20% and 36%, No.(13) 16% and 36%, No.(3) 20% and 32%, and No.(17) 16% and 32%, respectively which were isolated from Ismailia, Beheira, Sharkia Kalyobeia and Assuit Governorates, respectively. In this respect, *F. oxysporum* f.sp. *Lupini* isolate No.7 was selected for further studies.

Table 3 .Effect of lupine seed soaking of some antioxidant on Controlling wilt disease in lupine plants grown in infested soil with *F. oxysporum* f.sp. *Lupini* under greenhouse conditions.

Antioxidant	Con. Ppm	Wilted plants %		Survival plants %		Disease Severity Score	Reduction compared with Control %
		45 Days	90 days	Infected %	Healthy %		
Ascorbic acid	25	4.0	20.0	8.0	68.0	1.4	58.8
	50	0.0	16.0	4.0	80.0	1.0	70.6
	100	0.0	16.0	4.0	80.0	0.8	76.5
	200	0.0	12.0	4.0	84.0	0.4	88.2
Citric Acid	25	8.0	24.0	8.0	60.0	1.6	53.0
	50	8.0	20.0	8.0	64.0	1.4	58.8
	100	4.0	20.0	4.0	72.0	1.2	74.7
	200	4.0	16.0	4.0	76.0	0.8	76.5
Salicylic acid	25	4.0	16.0	8.0	72.0	1.2	74.7
	50	4.0	12.0	4.0	80.0	0.8	76.5
	100	0.0	8.0	4.0	88.0	0.4	88.2
	200	0.0	8.0	0.0	92.0	0.2	88.2
Sodium Benzoate	25	12.0	24.0	8.0	56.0	2.0	41.2
	50	8.0	24.0	4.0	64.0	1.8	47.1
	100	8.0	20.0	4.0	64.0	1.6	53.0
	200	8.0	20.0	4.0	68.0	1.4	58.8
Control		20.0	36.0	24.0	20.0	3.4	-
L.S.D at 5% Antioxidants (A)=		4.62	11.49	11.57	5.60	0.63	-
Concentration(c)=		4.33	3.78	4.69	4.21	0.55	-
Interaction (A×C)=		9.69	8.45	10.48	9.41	0.22	-

Effect of antioxidants and plant extracts for controlling lupine wilt disease caused with *F. oxysporum* f.sp. *Lupini* under greenhouse conditions.

a- Effect of some antioxidants:

Data presented in Table (3) show that all tested antioxidants significantly decreased the percentage of wilted plants 45 and 90 days after sowing, and increased survived plants compared with the control.

Data indicated that the cultivar Giza2 infected with *F. oxysporum* showed the highest severity score (3.4) control treatment and gave the lowest percentage of survived plant (20%) compared with the lowest disease severity score (0.2). Salicylic acid treatment at 200ppm, whereas the highest percentage of survived plants was recorded (92.0%) when the concentration of 100ppm and AA 200ppm (4.0 and 88.0) and (4.0 and 84.0) respectively. The highest percentage of disease reduction over the control was obtained

from SA when 200ppm (88%0) followed by each of SA when 100ppm and AA at 200ppm treatments, which recorded (88.2%).

Table 4. Effect of lupine seed soaking in some plant extract solution on controlling wilt disease grown in infested soil with *F. oxysporum* f.sp. *Lupini* under greenhouse conditions.

Plant extract	Con. %	Wilted plants %		Survival plants %		Disease Severity Score	Reduction compared with Control %
		45 days	90 days	Infected %	Healthy %		
Leek *(Allium Porrum)*	1	4.0	24.0	8.0	64.0	4.1	64.7
	2	0.0	16.0	4.0	80.0	1.2	66.7
	4	0.0	12.0	4.0	84.0	1.0	72.2
	6	0.0	8.0	4.0	88.0	0.8	77.7
Thyme *(Thymus vulgaris)*	1	8.0	24.0	12.0	56.0	2.4	33.3
	2	4.0	24.0	12.0	60.0	2.0	44.4
	4	4.0	20.0	8.0	68.0	1.6	55.6
	6	0.0	20.0	4.0	72.0	1.2	66.7
Basil *(Ocium basillicum)*	1	8.0	28.0	12.0	52.0	2.8	22.2
	2	8.0	28.0	8.0	56.0	2.4	33.3
	4	4.0	28.0	8.0	60.0	2.0	44.4
	6	4.0	24.0	8.0	64.0	1.4	64.7
Marigold *(Tagetes erecta)*	1	8.0	28.0	12.0	52.0	2.4	33.3
	2	8.0	24.0	0.8	60.0	2.0	44.4
	4	4.0	24.0	8.0	64.0	1.4	64.7
	6	4.0	20.0	8.0	68.0	1.2	66.7
Neem *Azadirachta indica)*	1	4.0	20.0	8.0	68.0	1.4	64.7
	2	4.0	16.0	8.0	72.0	1.2	66.7
	4	0.0	16.0	4.0	80.0	1.2	66.7
	6	0.0	12.0	4.0	84.0	1.0	72.2
Control		20.0	36.0	24.0	20.0	3.6	-
L.S.D at 5% Plant extract (E)=		4.62	6.96	7.61	5.05	0.61	-
Concentration(C)=		3.77	5.68	6.21	4.12	0.50	-
Interaction (E×C)=		9.25	13.92	15.22	10.10	1.23	-

b- Effect of some plant extracts:

Data in Table (4) indicated that soaking lupine seeds in solution of any tested plant extract decreased significantly wilted plants number after 45 days and three month from sowing and increased survived plants number. Whereas the highest percentage of survived plants (88.0%) was recorded with leek when used the concentration 6% followed by leek at 4% and neem at 6% (1.0 and 84.0%) and (1.0 and 84.0%) respectively. The highest percentage of disease reduction over the control was obtained from leek with the concentration of 6% (77.7%) followed by each of leek when used the concentration 4% and neem when used the concentration was 6% giving (72.2%).

Table 5. Effect of some antioxidants on growth parameters of lupine cv. Giza2 under greenhouse conditions.

Antioxidants	Con. Ppm	P.H.cm	N.B.	N.P.	W.S.gm	R.L. cm
Ascorbic acid	25	107.6	8.4	17.0	13.8	2.31
	50	115.0	9.2	17.2	14.4	32.4
	100	123.2	9.6	19.4	15.2	40.4
	200	133.2	12.2	24.4	17.2	51.0
Citric Acid	25	100.0	7.6	14.6	13.6	27.2
	50	107.8	8.0	15.6	14.0	29.0
	100	114.8	8.4	18.2	14.8	32.6
	200	121.4	10.4	20.2	15.4	45.6
Salicylic acid	25	119.4	10.2	19.4	14.6	34.6
	50	127.4	10.4	21.0	15.4	35.6
	100	137.8	11.4	22.6	16.7	39.8
	200	143.2	12.6	25.6	19.6	54.4
Sodium Benzoate	25	99.0	5.6	13.6	13.4	25.8
	50	100.0	.6.8	14.8	13.6	26.4
	100	101.0	7.2	15.0	14.0	32.8
	200	110.6	7.4	16.2	14.0	36.2
Control		67.8	2.2	6.2	7.6	17.8
L.S.Dat5%Antioxidants(A)=		2.68	0.77	1.13	1.01	2.25
Concentration(A)=		2.39	0.69	1.01	0.90	2.01
Interaction (A×C)=		5.35	1.55	2.27	2.02	4.49

P.H.= Plant height (cm), N.B.=Number of branches, N.P.=Number of pods/plant, W.S.=Weight of dry seeds/plant (gm), R.L.=Root length(cm).

Effect of antioxidants and plant extracts on some growth parameters of lupine plants cv. Giza2.

a- effect of some antioxidants:

Data presented in Table (5) revealed that the application of AA, CA, SA and SB caused a significant increase in the values of plant height, number of branches, number of pods, seed weight and root length over the control. The obtained data indicate that the greatest values (143.2cm height, 12.6 branches, 25.6 pods, 19.6gm/plant and 54.4cm/plant, respectively) of plant height, number of branches, number of pods, seed weight and root length of lupine plants were recorded in infested soil with *F. oxysporum* f.sp. *Lupini* from when used SA at 200ppm compared with the control treatment which exhibited 67.8cm height, 2.2 branches, 6.2pods,7.6gm/plant and 17.8cm height/plant, respectively, obtained from untreated lupine seeds grown in infested soil control.

b- Effect of some plant extracts:

Data presented in Table (6) revealed that the application of leek, thyme, basil, marigold and neem caused a significant increase in the values of plant height, number of branches, number of pods, seed weight and root length over the control. The obtained data indicated that the greatest values (118.8cm height, 9.6branch, 20.0pods,16.2gm/plant and 45.8cm height /plant, respectively) of plant height, number of branches, number of pods, seed weight and root length of lupine plants grown in infested soil with *F. oxysporum* f.sp. *Lupini* were recorded from leek treatment when the concentration of 6% was used compared with the control.

Table 6. Effect of some plant extracts on some growth parameters of lupine Giza2cv. under greenhouse conditions.

Plant extracts	Con. (%)	P.H.cm	N.B.	N.P.	W.S.gm	R.L. cm
Leek (Allium Porrum)	1	102.6	6.2	16.8	10.0	32.2
	2	106.6	7.0	18.0	11.6	36.2
	4	108.6	8.0	18.6	14.2	36.6
	6	118.8	9.6	20.0	16.2	45.8
Thyme (Thymus vulgaris)	1	86.0	3.6	15.8	9.0	24.2
	2	93.0	4.6	12.0	10.4	28.2
	4	98.0	5.6	14.2	12.0	30.6
	6	100.8	6.4	15.0	13.8	34.0
Basil (Ocium basillicum)	1	69.6	2.4	6.8	7.8	19.2
	2	71.0	3.6	7.4	8.8	21.0
	4	71.8	4.0	8.8	9.8	23.6
	6	83.6	4.4	10.2	10.6	24.8
Marigold (Tagetes erecta)	1	83.2	3.0	9.2	8.0	21.0
	2	85.6	3.6	10.0	7.6	24.2
	4	86.8	4.4	11.2	10.0	0.26
	6	91.4	5.0	12.6	12.0	30.4
Neem (Azadirachta indica)	1	93.6	4.4	14.8	9.0	26.0
	2	99.8	5.4	16.6	10.0	30.0
	4	104.4	6.6	17.0	12.4	33.8
	6	112.8	8.6	17.8	14.8	39.6
Control		67.8	2.2	6.2	7.6	17.8
LSD at 5% Extracts(E)		5.35	0.96	1.27	1.56	2.70
Concentration(E)=		3.72	0.55	0.89	1.06	1.81
Interaction (E×C)=		9.11	1.34	2.19	2.60	4.44

P.H.= Plant height (cm), N.B.=Number of branches, N.P.=Number of pods/plant, W.S.=Weight of dry seeds/plant (gm), R.L.=Root length(cm).

a- Effect of some antioxidants:

The effects of antioxidants (AA, CA, SA and SB) on lupine wilt disease under field conditions during 2011/2012 and 2012/2013 grown seasons were shown in Table(7).

Data show in table (7) that all the tested antioxidants treatments increased the percentage of survived plants compared with the control. The highest percentage of survival plant (97.3 and 95.0%) was recorded during the first and second season, respectively by using SA treatment at the concentration 200ppm, followed by 93.3 and 93.0%, 91.0 and 86.0% and 90.o and 80.3% respectively recorded from AA,CA and SB comparing with 72.3 and 69.7% which obtained from the control treatment.

However, the differences among the treatments were not significant. Similar results were noticed with seed yield, the highest values of seed yield (2.942 and 2.633kg/plot) were recorded during the first and second season, respectively by using SA at the concentration 200ppm. It is also clear from data in (Table7) that SA treatment increased the percentage of survived plants compared with the (control)

from 71.0 to 96.15 as means during the tow successive seasons. The same trend was noticed with yield weigh, the highest mean of yield weigh (2.788kg/plot) was recorded by SA treatment compared with 0.993 kg / plot in the control treatment.

Table 7. Effect of some antioxidants on survival of plants and seed yield of lupine plants in naturally infested soil during 2011-2012 and 2012-2013 under field conditions.

Antioxidants	Con. Ppm	Survived plants (%)		Mean	Increasing over the control %	Seed yield(kg/plot)		Mean	Increasing over the control %
		2011-2012	2012-2013			2011-2012	2012-2013		
Ascorbic	100	91.3	86.7	89.0	25.35	2.541	2.125	2.333	134.94
Acid	200	93.3	93.3	93.15	31.20	2.801	2.258	2.530	78.154
Citric	100	85.3	81.0	83.15	17.11	2.300	2.041	2.171	118.63
Acid	200	91.0	86.0	88.5	24.64	2.500	2.130	2.315	133.13
Salicylic	100	95.0	90.7	92.85	30.77	2.617	2.458	2.538	155.59
Acid	200	97.3	95.0	96.15	35.42	2.942	2.633	2.788	180.77
Sodium	100	82.7	75.3	79.0	11.27	2.065	1.908	1.987	100.10
Benzoate	200	90.0	80.3	85.15	19.93	2.133	2.083	2.108	112.29
Control		72.3	69.7	71.0	-	1.008	0.977	0.993	-
L.S.D at 5% antioxidants (A)=		2.80	3.56	-	-	0.21	0.11	-	-
Concentration(c)=		1.26	1.29	-	-	0.06	0.07	-	-
Interaction (A×C)=		2.82	2.89	-	-	0.14	0.15	-	-

Effect of antioxidants and plant extracts on wilt and root rot diseases of lupine plants under field conditions.

Table 8. Effect of some plant extracts on survival of plants and seed yield of lupine planted grown in naturally infested soil during 2011-2012 and 2012-2013 under field conditions.

Plant extracts	Con. %	Survived plants (%)		Mean	Increasing over the control	Seed yield(kg/plot)		Mean	Increasing over the control%
		2011-2012	2012-2013			2011-2012	2012-2013		
Leek (Allium Porrum)	4	88.0	85.3	86.65	22.04	2.383	2.175	2.279	111.80
	6	92.0	91.7	92.0	29.58	2.783	2.408	2.596	141.26
Thyme (Thymus Vulgaris)	4	80.0	77.3	78.65	10.77	2.225	1.808	2.017	87.45
	6	89.0	83.3	86.30	21.55	2.508	2.150	2.329	124.22
Basil (Ocium basillicum)	4	77.3	71.3	74.30	4.65	2.050	1.367	1.709	58.83
	6	82.3	73.3	77.80	9.58	2.167	1.616	1.892	75.84
Marigold (Tagetes erecta)	4	78.0	73.3	75.65	6.55	2.108	1.516	1.812	68.40
	6	86.0	80.7	83.35	17.39	2.425	2.100	2.262	110.22
Neem (Azadirachta indica)	4	83.3	80.3	81.80	15.21	2.292	2.085	2.189	103.44
	6	90.0	87.3	88.65	24.86	2.625	2.225	2.425	125.37
Control		72.3	69.7	71.0	-	1.175	0.977	1.076	-
LSD at 5% Extracts(E)=		3.25	3.14	-	-	0.70	0.14	-	-
Concentration(C) =		1.26	1.74	-	-	0.38	0.07	-	-
Interaction (E×C)=		3.08	4.27	-	-	0.92	0.16	-	-

b- Effect of some plant extracts:

The effect of plant extracts (leek, thyme, basil, marigold and neem) on lupine wilt disease incidence under field conditions during 2011/2012and2012/2013 growing seasons was shown in (Table 8).

Data showed that all the tested plant extracts increased the percentage of survived plants compared with the control. The highest percentage of plants survival (92.3 and 91.7%) was recorded during the first and second season respectively with the leek treatment at the concentration 6% followed by (90.0 and 87.3%, 89.3 and 83.3%, 86.0 and 80.7% and 82.3 and 73.3% respectively) which recorded from neem, thyme, marigold and basil compared with 72.3 and 69.7% from the control treatment.

However, the differences among the treatments were not significant. Similar results were noticed with seed yield, the highest values of seed yield (2.783 and 2.408 kg/plot) were recorded during the first and second season, respectively by using leek at the concentration 6%. It is also clear from data (Table 8) that leek increased the percentage of survived plants compared with the non-treated treatment of 71.0 to 92.0 as means of the two seasons. Similar trend was noticed with yield weight, the highest mean of yield weight (2.596kg/plot) was recorded by leek treatment compared with (1.076 kg/plot) in the control treatment

DISCUSSION

White lupine is suffering from infection with many disease especially wilt disease caused by *F. oxysporum* f.sp. *Lupini* which considered the most serious disease of white lupine in Egypt, causing a considerable damage and loss in seed yield Abou zeid *et al.,* (2002). Isolation's trials from wilted lupine plants yielded *F. oxysporum* f.sp. *Lupini* which was the most isolated fungus, confirming to other reports of (Baraka *et al*., (2011). Zian *et al*.,(2013).

Pathogenicity test on cv. Giza2 was conducted with twenty *F. oxysporum* f.sp. *Lupini* isolates led to symptoms which were almost similar to those noticed under field conditions. All the *F. oxysporum* f.sp. *Lupini* isolates were pathogenic to lupine with some variations when amended into the pot soil. The present investigation demonstrated that *F. oxysporum* f.sp. *Lupini* isolated from naturally infected field could increase wilted plants and reduce percentages of healthy survival plants and could directly affect the yield. *Fusarium oxysporum* f.sp. *Lupini* isolate no. (7) showed to by high virulent, it gave the highest percentage of wilted plant. These results were in agreement with those reported by Zian, (2005) and Baraka *et al*., (2011). They were found that the application of inoculums of *F. oxysporum* f.sp. *Lupini* caused significant increase of losses in plants and yields of white lupine.

Recently, an increasing desire to reduce the used of pesticides is seen through the attempts to develop integrated pest management approaches. Antioxidants and plant extracts when used to control is high on the list of potential alternative control methods. The effects of some chemical compounds and plant extracts were evaluated against *F. oxysporum* f.sp. *Lupini* infection of lupine plants as alternative control methods.

Generally, the best control was applied as seed soaking with high concentration (200ppm) of antioxidants and plant extracts at 6%. Salicylic acid was better than the other antioxidants followed by ascorbic acid. Similar result were reported by Galal and Abdou(1996) who found that application of salicylic acid or ascorbic acid gave the best control of fusarial diseases of cowpea Ismail *et al.,* (2011). Abdel-Monaim (2008) showed that soaking of lupine seeds in antioxidants solution reduced damping - off and root rot diseases caused by *Fusarium solani* and *Macrophomina phaseolina.*

The mode of action of antioxidants was reported in many host - pathogen interaction, i.e. many oxidative enzymes such as peroxidase, catalase, ascorbic acid oxidase and polyphenol oxidase were detected as a result of infection with many pathogen Clark *et al*., (2002) and Abdel-Monaim, (2008) or as a result of treatments with different antioxidants Ragab *et al.*, 2009 and Abdel-Monaim *et al.,* (2011).

The efficacy of various plant extracts to induce resistance was studied in these experiments; overall results showed that leek and neem leaf extracts at the rate of 6% rates can provide protection against lupine wilt disease. The role of plant extracts to induce resistance against plant pathogen has been reported by different researchers. Islam and Faruq (2012) showed that garlic clove extract allamonda leaf extract gave the best result followed by neem leaf extract in reducing percent damping –off of tomato, eggplant and chilli seedlings. Ghazanfer *et al.,* (2011) found that the maximum disease reduction (43.5%) against disease in the plant extracts was observed by applying *Azadirachta indica* leaf extract, but *Datura metel* and *Allium sativum* extracts gave the least effect. Guleria and Kumar (2006) found that aqueous leaf extract of neem controlled Alternaria leaf spot of sesame. The efficacy of neem leaf extract has been reported by Paul and Sharma (2000) and it had the same effect as bavistin fungicide in managing leaf stripe pathogen of parley.

Results in this study are in harmony with those reported by Roth *et al*., (2000) who studied the effects of an extract of *Lychnis viscaria* L. seed that contains brassinosteroids, an aqueous application enhanced by 36% resistance to tobacco, cucumber, and tomato against viral and fungal pathogens increasing PR-proteins, peroxidase,chitinase, and β-1,3-glucanase.

The reduction in disease incidence reflected on plant height, number of branches, root length, weight of dry seeds and number of pods of plants grown in infested soil with *F. oxysporum* f.sp. *Lupini* and treated with antioxidants and plant extracts which were greater compared with the control. These results are similar to those obtained by Abdel-Monaim and Ismail (2010) also in the present study shows that all tested antioxidants reduced the area of the root rot and wilt and increased the plant vigor and pod yield per plant.

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). Abdel-Monaim (2008) found that lupine seed soaking in antioxidant solution increases of chlorophyll and carotenoids content in leaves and this reflects the health condition of the plant.

Waleed *et al*., (2011) found that the application of mustard seed meal resulted in significant reduction of wilted plants after 30 and 90 days of sowing and increased survived plants compared to the control. The reduction in disease incidence was reflected on lupine growth parameters. Increases in plant height, number of branches, number of pods, weight of seeds and root length of plants grown, Islam and Faruq (2012).

Effect of antioxidants and plant extracts on survival lupine plants under field conditions was also studied. The obtained results showed that seed treated with soaking in antioxidants (salicylic acid, ascorbic acid, citric acid and sodium benzoate) and plant extracts (leek, neem, thyme, marigold and basil) with the concentration 200ppm antioxidants and 6% plant extracts increased survival plant by 35.42, 31.20, 24.64 and19.93% and 29.58, 24.86, 21.55, 17.39 and 9.58% respectively, as a means of two seasons compared with control treatment. Such increasing in survival plants were accompanied by an increase in yield weight.

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الاستفادة من مضادات الأكسدة والمستخلصات النباتية في مقاومة مرض ذبول الترمس

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يعتبر فطر الفيوزاريم اوكسيسبورم من الفطريات الممرضة التي تصيب نباتات الترمس مسببا مرض الذبول الوعائي والذي يؤدي بدوره إلي خسائر إقتصادية في المحصول لذلك تم اجراء هذا البحث بهدف حصر مرض الذبول في خمس محافظات وأظهرت النتائج أن العزلات المتحصل عليها من الأسماعليه كانت الأعلي في القدرة المرضية . كما أظهر العزل والتعريف أن الفطر فيوزاريم اوكسيسبورم هو السبب الرئيسي لهذا المرض .تم دراسة تأثير نقع بذورالترمس قبل الزراعة في تركيزات مختلفة من المواد المضادة للاكسدة مثل حمض الاسكوربيك وحمض السيتريك، والساليسيلك، بنزوات الصوديوم في أربعة تركيزات ٢٥، ٥٠، ١٠٠، ٢٠٠ جزء في المليون، فضلا عن خمسة مستخلصات نباتية هي أوراق الكرات والزعتر والريحان والقطيفة وبذور النيم بتركيزات ١،٢،٤،٦٪ تحت ظروف الصوبة .

وقد أوضحت نتائج تجربة الأصص أن كل مضادات الأكسدة المختبرة وكذلك المستخلصات النباتية خفضت بشكل معنوي نسبة النباتات المصابة بالذبول وزادت من نسبة النباتات السليمة الباقية وأيضا مقاييس النمو المختلفة مقارنة بمعاملة الكنترول الملقح بالفطر.كذلك أوضحت النتائج أن حمض الساليسيلك بتركيز ٢٠٠ جزء فى المليون كان الأفضل في الحصول علي أعلي نسبة نباتات سليمة وأيضا مقاييس النمو المختلفة وكان أفضل المستخلصات النباتية هومستخلص الكرات.

كانت نتائج تجربة الحقل مشابهة لنتائج الصوبة حيث أن كل مضادت الأكسدة المختبرة وكذلك المستخلصات النباتية زادت من نسبة النباتات السليمة مقارنة بالكنترول زيادة نسبة النباتات السليمة أدت إلى زيادة وزن البذرة الجافة وقد أظهرت النتائج أن المعاملة بالساليسيلك يليها الأسكوربيك كانت الأكثر تأثيرا وكذلك معاملة الكرات يليها النيم كانت الأفضل في المستخلصات النباتية وكانت مضادات الأكسدة هي الأفضل علي الأطلاق مقارنة بالمستخلصات النباتية.

وتشير النتائج الي امكانية أستخدام بعض المستحثات الكيماوية والمستخلصات النباتية في مقاومة الذبول في الترمس في مصر.