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Research Article
Hydrogen Peroxide and Acetylsalicylic Acid Induce the Defense of Lupine Against Root Rot Disease

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Abstract

Isolation of pathogenic fungi from both cultivars of diseased lupine was carried out in five districts of Dakahlia governorate. The high frequency isolated fungi presented in Temi El-Amdeed followed by Bani-Ebeed district. *Fusarium solani* and *F. oxysporum* proved to be the most dominate isolated followed by *Rhizoctonia solani*. In greenhouse, Giza 1 was high susceptible cultivar for infected with root rot pathogenic fungi. *Sclerotium rolfsii* followed by *R. solani* then *F. solani* were the most aggressive damping-off disease. In the field experiment, Giza 2 cultivar was the best in germination% and more tolerant of damping-off than Giza 1. The application of Rhizolex-T50 followed by H_2O_2 at low concentrate (0.50 mM) showed a highest percentage of germination within lowest percentage of damping-off. With considerable that, no significant differences between Rhizolex-T50 and low concentrate of H_2O_2. The high photosynthetic pigments and phenolic content were obtained from the application of acetylsalicylic acid (ASA) at moderate concentrate (15 mM) in both cultivars. Giza 2 gave the highest values in these parameters. Soaking both cultivars of lupine seeds in both tested materials increased significantly growth parameter, yield components and seed quality. The moderate concentration of ASA (15 mM) was the most effective followed by the low concentration of H_2O_2 (0.50 mM). The data suggested that the application of H_2O_2 at 0.50 mM and ASA at 15 mM as seed soaking could be considered as fungicide alternatives for controlling lupine root rot disease as well as improve growth and productivity.

Key words: Lupine, root rot disease, hydrogen peroxide, acetylsalicylic acid, *Fusarium solani, F. oxysporum*, *Rhizoctonia solani*, *Sclerotium rolfsii*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lupine (*Lupinus termis* Forsk) is one of the most important crop which belonging to Fabaceae family. Lupine, as other fabaceaus seeds is good dietary sources of minerals (Trugo *et al*., 1993). Lupine seeds also contains chemical compounds i.e., protein, oil, cholesterol and alkaloids (lupulin. Luponine, lupul and sparateine). lupulin is occasionally employed as stomachic tomic. Seeds can be eaten when the bitter components have been removed. Also, the seeds roasted can make a coffee substitute. Therefore, lupine is cultivated in Egypt for food, medical and industrial purposes (Chiej, 1984; Ibrahim *et al*., 1990). As well as, lupine plants used in sustainable and environment-friendly agriculture because of its high potential for biological nitrogen fixation (Robinson *et al*., 2000).

Damping-off and root rot diseases are among the most destructive diseases attacking lupine in Egypt. Several pathogens such as *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium solani* and *F. oxysporum* attacking lupine seeds, root and stem base causing serious losses in seed germination and plant stand (Abd-El-Kareem *et al*., 2004; El Mougy, 2004; Ali *et al*., 2009).

The application of fungicides is considered one of the most famous environmental pollutions. Therefore, it is urgent to alternative safe efficient methods against plant diseases. Induced resistance of plants against pathogens can be defined as the process of active resistance depended on the host plants physical or chemical barriers activated by abiotic and biotic agents. These agents sensitizes the plant to respond rapid after infection include phytoalexin accumulation, phenols, lignifications and activation of peroxidase, polyphenoloxidase, catalase and chitinase (Meena *et al*., 2001; Mahmoud *et al*., 2006; Walters, 2007).

Some abiotic inducers i.e., acetylsalicylic acid (ASA) on lupine and hydrogen peroxide (H_2O_2) on lentil and peanut have been shown to induce resistance in plants against damping-off and root rot diseases (El Mougy, 2004; Morsy, 2005; Mahmoud *et al*., 2006).

This study aimed to study the effect of abiotic (ASA and H_2O_2) inducers in reducing of lupine root rot diseases and enhancing of some morphological and physiological characters as well as yield and seed quality.

MATERIALS AND METHODS

Source of lupine seeds: Seed of two lupine cultivars (Giza 1 and Giza 2) were obtained from Legume Crop Research Department, Field Crop Research Institute, Agriculture Research Center, Giza, Egypt.

Abiotic inducers: Two abiotic chemical inducers namely, hydrogen peroxide (H_2O_2) at 0.50, 0.75 and 1.0 mM and acetylsalicylic acid (ASA) at 10, 15 and 20 mM were used as seed soaking to study their effects in inducing resistance in lupine plant against root-rot diseases.

Isolation, purification, identification of the causal pathogens: The causal pathogens were isolated from lupine plants showing typical symptoms of root rot disease from different districts of Dakahlia governorate. The infected roots were washed thoroughly with tap water, cut into small pieces (1 cm) and surface disinfested with sodium hypochlorite 2% for 2 min. Then re-washed several times with sterilized water, dried between folds of sterilized filter paper and were placed onto Potato Dextrose Agar (PDA) medium in petri-dishes supplemented with streptomycin sulfate (100 μg mL^{-1}). Petri-dishes were incubated at 21°C for five days. The developed fungal colonies purified and identified according to Ellis (1976), Sneh *et al*. (1992) and Nelson *et al*. (1983).

Fungal inoculums preparation: Inocula of *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium solani* and *Fusarium oxysporum* were prepared by growing each fungus on sorghum: coarse sand medium (1:1w/w and 40% water) for two weeks at 25±1°C according to Filonow *et al*. (1988).

Pathogenicity test: The previously prepared fungal in inocula were tested for their pathogenicity on lupine under greenhouse conditions. Inoculum of each isolate was mixed thoroughly with autoclaved soil in plastic pots (25 cm diam.) at the rate of 5% by weight (Abdel-Kader, 1997). Four pots were used as replicates for each fungus as well as check (uninfested soil). Healthy lupine seeds for tow cultivars (Giza 1 and Giza 2) were sown after surface sterilized at the rate of 6 seeds/pot. The percentage of root rot disease incidence was calculated as pre- and post-emergence damping off after 15 and 40 days of sowing, respectively.

Field experiments: Two field experiments were carried out at Tag El-Ezz, Agriculture Research Station, Dakahlia, Egypt during 2012/2013 and 2013/2014 seasons.

Lupine seeds were soaked for 3 h in abiotic inducers (H_2O_2 at 0.50, 0.75 and 1.0 mM and ASA at 10, 15 and 20 mM) while, Rhizolex-T 50 w.p. was used as seed coating at the rate of 3 g kg^{-1} seeds. Treated lupine seeds (cvs. Giza 1 and Giza 2) were sown in 30th and 10th of November in two seasons, respectively and left under natural infection. A split plot design with three replicates was used in these experiments.

The main plots were occupied by cultivars, while sub-plots were occupied by treatments. The area of each sub-plot was 3×3.5 m, sowing with 180 seeds.

Germination and disease assessment: Germination percentage and pre-emergence damping-off were recorded at 20 days from sowing while post-emergence damping-off was determined at 80 days from sowing.

Physiological characters: At 75 days from sowing, photosynthetic pigments (chlorophyll a, b and carotenoids) were extracted in methanol 90% from the blade of the third leaf from plant tip (terminal leaflet) according to Robinson and Britz (2000) then determined spectrophotometrically according to Mackinney (1941).

Total phenolic compounds were determined in fresh shoot using the Folin-ciocalteau reagent according to Malik and Singh (1980).

Morphological and yield characters: At harvest, 175 days from sowing for Giza 1 cultivar and 160 days for Giza 2 cultivar, samples were taken to estimate plant height, number of branches and leaves per plant, number of pods, plant yield and weight of 100-seed.

Seed quality: In the second season, lupine seeds were dried at 70°C for 48 h, grounded and analyzed for alkaloid lupinine (Dabbas, 1973) and total nitrogen by semi-micro-Kjldahle (Pregl, 1945). Protein percentage was calculated by multiplying the N% by 6.25.

Statistical analysis: All data were statistically analyzed by the Software; CoStat (2005) in consultation with the analysis of variance (Gomez and Gomez, 1984).

RESULTS

Isolation of pathogenic fungi: Infected lupine cvs. Giza 1 and Giza 2 with typical symptoms of root rot diseases collected from different districts of Dakahlia governorate, Egypt are shown in Table 1. It was observed that Giza 1 cultivar was high susceptible for infected with root rot pathogenic fungi except, *Rhizoctonia solani* as compared with Giza 2 cultivar. The high frequency isolated fungi were found in Temi El-Amdeed district followed by Bani-Ebeed then Senblaween, while Dekerns came late. *Fusarium solani* was isolated at high percentage followed by *F. oxysporum* then *Rhizoctonia solani*.

Pathogenicity testes: Data presented in Table 2 show that Giza 1 lupine cultivar was more sensitive to the infection of

Table 1: Frequency of the isolated fungi from lupine roots at different districts in Dakahlia governorate

Treatments	*Rhizoctonia solani*	*Sclerotium rolfesii*	*Fusarium solani*	*Fusarium oxysporum*
Cultivars				
Giza 1	16.22^{b}	13.20^{a}	29.50^{a}	23.90^{a}
Giza 2	16.48^{a}	12.54^{b}	27.16^{b}	20.96^{b}
Districts				
El-Gamalia	15.20^{d}	12.00^{d}	28.30^{c}	19.90^{d}
Dekernes	12.35^{e}	10.40^{e}	26.45^{d}	21.85^{c}
Bani-Ebeed	16.40^{c}	15.30^{a}	24.25^{e}	24.50^{b}
Temai El-Amdeed	19.66^{a}	13.00^{c}	32.55^{a}	27.45^{a}
Senblaween	18.15^{b}	13.65^{b}	30.10^{b}	18.45^{e}
Interaction				
Giza 1				
El-Gamalia	15.80^{g}	12.20^{g}	30.20^{d}	21.10^{f}
Dekernes	12.70^{i}	10.80^{i}	28.90^{e}	23.20^{d}
Bani-Ebeed	16.00^{f}	15.60^{a}	21.70^{j}	26.10^{c}
Temai El-Amdeed	19.20^{b}	13.30^{d}	34.60^{a}	28.50^{a}
Senblaween	17.40^{d}	14.10^{c}	32.10^{b}	20.60^{g}
Giza 2				
El-Gamalia	14.60^{h}	11.80^{h}	26.40^{h}	18.70^{j}
Dekernes	12.00^{j}	10.00^{j}	24.00^{i}	20.50^{h}
Bani-Ebeed	16.80^{e}	15.00^{b}	26.80^{g}	22.90^{e}
Temai El-Amdeed	20.10^{a}	12.70^{f}	30.50^{c}	26.40^{b}
Senblaween	18.90^{c}	13.20^{e}	28.10^{f}	16.30^{j}

Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range tests

Table 2: Pathogenicity test of isolated fungi from lupine plants under greenhouse conditions

Treatments	Pre-emergency damping off	Post-emergency damping off	Survival plants
Cultivars			
Giza 1	22.80^{a}	20.60^{a}	56.6^{b}
Giza 2	19.53^{b}	17.07^{b}	63.4^{a}
Fungi			
Check	0.00^{e}	0.00^{d}	100.00^{a}
Rhizoctonia solani	31.33^{b}	20.83^{c}	47.83^{d}
Sclerotium rolfesii	39.50^{a}	30.67^{a}	29.83^{e}
Fusarium solani	19.00^{c}	22.67^{b}	58.33^{c}
Fusarium oxysporum	16.00^{d}	20.00^{c}	64.00^{b}
Interaction			
Giza 1			
Check	0.00^{h}	0.00^{e}	100.00^{a}
Rhizoctonia solani	33.33^{c}	21.33^{c}	45.33^{f}
Sclerotium rolfesii	42.33^{a}	35.67^{a}	22.00^{h}
Fusarium solani	20.67^{e}	24.33^{b}	55.00^{d}
Fusarium oxysporum	17.67^{ef}	21.67^{c}	60.67^{c}
Giza 2			
Check	0.00^{h}	0.00^{e}	100.00^{a}
Rhizoctonia solani	29.33^{d}	20.33^{cd}	50.33^{e}
Sclerotium rolfesii	36.67^{b}	25.67^{b}	37.67^{g}
Fusarium solani	17.33^{fg}	21.00^{c}	61.67^{c}
Fusarium oxysporum	14.33^{g}	18.33^{d}	67.33^{b}

Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range tests

Table 3: Effect of inducers on germination percentage and damping off disease of lupine plants under field conditions

	2012/2013				2013/2014			
Treatments	Germination (%)	Pre-emergency damping off	Post-emergency damping off	Survival plants	Germination (%)	Pre-emergency damping off	Post-emergency damping off	Survival plants
Cultivars								
Giza 1	88.38^{b}	11.63^{a}	14.13^{a}	74.24^{b}	85.54^{b}	14.54^{a}	13.38^{a}	72.08^{b}
Giza 2	91.08^{a}	8.92^{b}	12.46^{b}	78.62^{a}	88.54^{a}	11.46^{b}	11.17^{b}	77.37^{a}
Treatments								
Check	85.33^{f}	14.67^{a}	20.17^{a}	65.16^{f}	82.83^{f}	17.17^{a}	19.00^{a}	63.83^{g}
H_2O_2 (0.50 mM)	92.50^{b}	7.50^{e}	9.33^{f}	83.17^{b}	90.83^{b}	9.17^{e}	8.00^{e}	82.83^{b}
H_2O_2 (0.75 mM)	90.67^{c}	9.33^{d}	11.50^{e}	79.17^{c}	87.17^{cd}	12.83^{d}	10.00^{d}	77.17^{c}
H_2O_2 (1.00 mM)	85.67^{f}	14.33^{a}	16.50^{b}	69.17^{e}	84.17^{e}	15.83^{b}	15.50^{b}	68.67^{f}
ASA (10 mM)	86.67^{j}	13.33^{b}	13.67^{ef}	73.00^{ef}	82.67^{i}	17.33^{b}	13.67^{de}	69.00^{g}
ASA (15 mM)	91.00^{c}	9.00^{d}	12.83^{d}	78.17^{c}	87.83^{c}	12.17^{d}	12.00^{c}	75.83^{d}
ASA (20 mM)	88.00^{e}	12.00^{b}	13.83^{d}	74.17^{d}	84.67^{e}	15.33^{b}	13.00^{c}	71.67^{c}
Rhizolex T-50	95.17^{a}	4.83^{f}	7.00^{g}	88.17^{a}	92.67^{a}	7.33^{f}	6.17^{f}	86.50^{a}
Interaction								
Giza 1								
Check	83.33^{k}	16.67^{a}	22.33^{a}	61.00^{i}	80.67^{j}	19.33^{a}	21.33^{a}	59.34^{i}
H_2O_2 (0.50 mM)	91.00^{d-f}	9.00^{f-g}	10.00^{g}	81.00^{c}	90.00^{cd}	10.00^{hi}	9.00^{h}	81.00^{c}
H_2O_2 (0.75 mM)	89.67^{f-g}	10.33^{d-f}	13.00^{ef}	76.67^{d}	86.00^{gh}	14.00^{de}	11.33^{fg}	74.67^{e}
H_2O_2 (1.00 mM)	84.67^{k}	15.33^{a}	17.33^{bc}	67.34^{h}	82.33^{i}	17.67^{b}	16.33^{b}	66.00^{h}
ASA (10 mM)	86.67^{j}	13.33^{b}	13.67^{ef}	73.00^{ef}	82.67^{i}	17.33^{b}	13.67^{de}	69.00^{g}
ASA (15 mM)	89.67^{f-h}	10.33^{d-f}	13.33^{ef}	76.34^{d}	86.67^{g}	13.33^{e}	13.33^{de}	73.34^{e}
ASA (20 mM)	88.33^{hi}	11.67^{cd}	15.67^{cd}	72.66^{f}	85.00^{h}	15.67^{c}	15.33^{bc}	69.00^{g}
Rhizolex T-50	93.67^{bc}	6.33^{ij}	7.67^{hi}	86.00^{b}	91.00^{bc}	9.00^{ij}	6.67^{i}	84.33^{b}
Giza 2								
Check	87.33^{ij}	12.67^{bc}	18.00^{b}	69.33^{gh}	85.00^{h}	15.00^{cd}	16.67^{b}	68.33^{g}
H_2O_2 (0.50 mM)	94.00^{b}	6.00^{j}	8.67^{gh}	85.33^{b}	91.67^{b}	8.33^{j}	7.00^{i}	84.67^{b}
H_2O_2 (0.75 mM)	91.67^{de}	8.33^{gh}	10.00^{g}	81.67^{c}	88.33^{ef}	11.67^{fg}	8.67^{h}	79.66^{cd}
H_2O_2 (1.00 mM)	86.67^{j}	13.33^{b}	15.67^{cd}	71.00^{fg}	86.00^{gh}	14.00^{de}	14.67^{cd}	71.33^{f}
ASA (10 mM)	89.33^{gh}	10.67^{de}	14.00^{d-f}	75.33^{de}	86.67^{g}	13.33^{e}	12.33^{ef}	74.34^{e}
ASA (15 mM)	92.33^{cd}	7.67^{hi}	12.33^{f}	80.00^{c}	89.00^{de}	11.00^{gh}	10.67^{g}	78.33^{d}
ASA (20 mM)	90.67^{e-g}	9.33^{e-g}	14.67^{de}	76.00^{d}	87.33^{fg}	12.67^{ef}	13.67^{de}	73.66^{e}
Rhizolex T-50	96.67^{a}	3.33^{k}	6.33^{j}	90.34^{a}	94.33^{a}	5.67^{k}	5.67^{i}	88.66^{a}

Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range tests

pre- and post-emergence damping-off than Giza 2 cultivar. Generally, *Sclerotium rolfsii* showed highest percentage of pre- and post-emergence damping-off in both lupine cultivars than other pathogenic fungi. *Rhizoctonia solani* came second followed by *F. solani* then *F. oxysporum.* With considerable that, all tested fungi were pathogenic and causes typical symptoms of pre- and post-emergence damping-off of lupine seedlings.

Field experiments

Germination and disease assessment: Data of germination percentage and damping-off of lupine plants as affected by inducers under field conditions were recorded in Table 3. Giza 2 cultivar was the best in generation% and was more tolerant of damping-off than Giza 1. Soaking of lupine seeds in each one of both inducers significantly increased germination percentage within decreased pre and post-emergence damping-off in both seasons compared with check.

Concerning the effects of treatments and its interacted with cultivars, data show that Rhizolex-T50 was the most effective followed by H_2O_2 then ASA in both cultivars. The low concentration of H_2O_2 (0.50 mM) was more effective in this respect. It is worthy to mention that there is no significant difference between H_2O_2 at 0.50 mM and Rhizolex-T50 treatments.

Physiological characters: Photosynthetic pigments and total phenols are not only a good parameters reflecting the health conditions of plant but also, carotenoids and phenols are known that a highly effective antioxidants. As shown in Table 4, Giza 2 cultivar gave the highest values of photosynthetic pigments (Chl. a, b and carotenoids) and total phenol content as compared with Giza 1 cultivar. There is a positive relationship among chlorophyll a, b and total phenols content. Both tested inducers increased significantly photosynthetic pigment and phenols. The maximum increase in chlorophyll a and b as well as phenolic content occurred

Table 4: Effect of inducers on germination percentage and damping off disease of lupine plants under field conditions

	2012/2013				2013/2014			
Treatments	Chlorophyll a	Chlorophyll b	Carotenoids	Total phenols (mg/100 g)	Chlorophyll a	Chlorophyll b	Carotenoids	Total phenols (mg/100 g)
Cultivars								
Giza 1	1.05^{b}	0.52^{b}	0.37^{b}	403.67^{b}	1.10^{b}	0.55^{b}	0.32^{b}	411.58^{b}
Giza 2	1.13^{a}	0.58^{a}	0.39^{a}	416.54^{a}	1.19^{a}	0.65^{a}	o.35^{a}	479.79^{a}
Treatments								
Check	0.94^{g}	0.49^{ef}	0.32^{f}	352.83^{g}	1.01^{g}	0.52^{e}	0.25^{g}	363.50^{g}
H_2O_2 (0.50 mM)	1.20^{b}	0.62^{b}	0.46^{a}	454.00^{b}	1.26^{b}	0.69^{b}	0.42^{a}	665.00^{a}
H_2O_2 (0.75 mM)	1.12^{d}	0.55^{c}	0.40^{c}	421.17^{d}	1.17^{d}	0.61^{c}	0.32^{d}	433.33^{d}
H_2O_2 (1.00 mM)	1.01^{f}	0.50^{e}	0.36^{de}	389.50^{f}	1.07^{f}	0.55^{de}	0.30^{e}	393.67^{f}
ASA (10 mM)	1.07^{e}	0.53^{d}	0.36^{de}	410.50^{e}	1.14^{e}	0.57^{d}	0.33^{d}	417.83^{e}
ASA (15 mM)	1.28^{a}	0.66^{a}	0.43^{b}	473.17^{a}	1.32^{a}	0.74^{a}	0.40^{b}	481.67^{b}
ASA (20 mM)	1.17^{c}	0.58^{c}	0.38^{d}	427.17^{c}	1.21^{c}	0.63^{c}	0.36^{c}	450.83^{c}
Rhizolex T-50	0.92^{g}	0.46^{f}	0.35^{e}	352.50^{g}	0.96^{h}	0.48^{f}	0.28^{f}	359.67^{h}
Interaction								
Giza 1								
Check	0.89^{h}	0.46^{ij}	0.29^{j}	347.33^{l}	0.95^{k}	0.49^{kl}	0.22^{j}	355.00^{m}
H_2O_2 (0.50 mM)	1.16^{c}	0.58^{de}	0.44^{ab}	443.00^{c}	1.21^{de}	0.62^{ef}	0.40^{bc}	449.67^{e}
H_2O_2 (0.75 mM)	1.08^{de}	0.52^{f-h}	0.36^{fg}	417.67^{fg}	1.14^{g}	0.54^{h-j}	0.31^{ef}	425.00^{h}
H_2O_2 (1.00 mM)	0.97^{g}	0.49^{hi}	0.36^{fg}	383.00^{j}	1.04^{ij}	0.51^{vjk}	0.28^{gh}	390.00^{k}
ASA (10 mM)	1.04^{ef}	0.50^{gh}	0.37^{ef}	406.67^{h}	1.10^{h}	0.52^{i-k}	0.31^{ef}	414.33^{i}
ASA (15 mM)	1.24^{b}	0.61^{cd}	0.46^{a}	464.00^{b}	1.27^{c}	0.65^{de}	0.43^{a}	471.33^{c}
ASA (20 mM)	1.12^{cd}	0.53^{fg}	0.39^{de}	420.33^{ef}	1.17^{fg}	0.56^{hi}	0.35^{d}	436.00^{g}
Rhizolex T-50	0.86^{h}	0.44^{j}	0.33^{h}	347.33^{l}	0.90^{l}	0.46^{l}	0.25^{i}	351.33^{m}
Giza 2								
Check	1.00^{fg}	0.51^{gh}	0.34^{gh}	358.33^{k}	1.07^{hi}	0.56^{hi}	0.28^{h}	372.00^{l}
H_2O_2 (0.50 mM)	1.24^{b}	0.67^{b}	0.42^{bc}	465.00^{b}	1.31^{b}	0.75^{b}	0.33^{de}	880.33^{a}
H_2O_2 (0.75 mM)	1.16^{c}	0.59^{cd}	0.37^{ef}	424.67^{e}	1.20^{ef}	0.67^{cd}	0.41^{ab}	441.67^{f}
H_2O_2 (1.00 mM)	1.04^{ef}	0.51^{gh}	0.36^{fg}	396.00^{i}	1.10^{h}	0.58^{gh}	0.40^{bc}	397.33^{j}
ASA (10 mM)	1.10^{d}	0.55^{ef}	0.40^{d}	414.33^{g}	1.18^{ef}	0.61^{fg}	0.31^{ef}	421.33^{h}
ASA (15 mM)	1.32^{a}	0.71^{a}	0.46^{a}	482.33^{a}	1.37^{a}	0.82^{a}	0.30f^{g}	492.00^{b}
ASA (20 mM)	1.21^{b}	0.63^{c}	0.41^{cd}	434.00^{d}	1.24^{cd}	0.70^{c}	0.38^{c}	465.67^{d}
Rhizolex T-50	0.97^{g}	0.48^{hi}	0.36^{fg}	357.67^{k}	1.02^{j}	0.49^{kl}	0.35^{d}	368.00^{l}

Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range tests

under the application of ASA followed by H_2O_2. The moderate concentrate of ASA (15 mM) was more effective. Whilst, Rhizolex-T50 had no significant effect on photosynthetic pigments and total phenols in lupine plants. On the other side, the highest increase in carotenoids content was observed with low level of H_2O_2 followed by moderate level of ASA.

Lupine growth and yield components: As shown in Table 5 and 6, there were significant differences between treatments of both lupine cultivars regarding lupine growth (plant height, branches and leaves number per plant) and yield components (number of pods/plant, plant yield and weight of 100-seeds).

Data in Table 6 show that Giza 2 cultivar recorded the highest values of plant height, branches and leaves number per plant. Soaking lupine seeds in both tested inducers increased significantly plant height, number of branches and leaves/plant in both cultivars during the two growing seasons. Acetylsalicylic acid at 15 mM appeared excellent superiority in all treatments on plant height, number of branches and leaves/plant followed by H_2O_2 at 0.50 mM.

Data concerning yield components in relation to the effect of tested inducers are present in Table 6. It can easily notice that Giza 2 cultivar gave the highest average of pods number per plant, plant yield and weight of 100-seed.

Moreover, all treatments increased significantly the same parameters in both cultivars. Generally, the low concentration of H_2O_2 and the moderate concentration of ASA lead to the highest values. In this respect ASA at 15 mM was the most effective followed by H_2O_2 at 0.50 mM. Meanwhile, Rhizolex-T50 had no significantly effect on the pervious parameters when compared with check.

Seed quality: Lupine seed quality was determined as protein and lupinine (alkaloids) percentages in the second season only. Data in Table 7 show that Giza 2 cultivar seeds were contains protein percentage more than Giza 1 while, Giza 1 contains lupinine percentage more than Giza 2. The maximum values of protein and lupinine in both lupine cultivars occurred under the application of ASA at moderate concentration followed by H_2O_2 at 0.50 mM.

Table 5: Effect of inducers on some morphological characters of lupine plants under field conditions

	2012/2013			2013/2014		
Treatments	Plant height (cm)	No. of branches/plant	No. of leaves/plants	Plant height (cm)	No. of branches/plant	No. of leaves/plants
Cultivars						
Giza 1	106.17^{b}	11.71^{b}	41.29^{b}	104.88^{b}	10.21^{b}	35.96^{b}
Giza 2	118.96^{a}	14.17^{a}	49.13^{a}	120.29^{a}	11.38^{a}	43.33^{a}
Treatments						
Check	98.67^{g}	10.83^{f}	38.00^{e}	101.17^{g}	8.50^{g}	34.00^{f}
H_2O_2 (0.50 mM)	112.50^{d}	14.83^{b}	49.00^{b}	111.83^{d}	12.83^{b}	44.67^{b}
H_2O_2 (0.75 mM)	107.67^{e}	13.67^{c}	46.50^{c}	108.17^{e}	11.50^{c}	41.33^{c}
H_2O_2 (1.00 mM)	103.50^{f}	12.83^{cd}	44.50^{cd}	105.00^{f}	10.83^{cd}	38.83^{d}
ASA (10 mM)	118.17^{c}	11.33^{ef}	43.67^{d}	117.33^{c}	9.50^{ef}	36.17^{e}
ASA (15 mM)	132.17^{a}	16.67^{a}	55.50^{a}	127.50^{a}	14.17^{a}	47.33^{a}
ASA (20 mM)	122.67^{b}	12.17^{de}	45.33^{cd}	123.33^{b}	10.33^{de}	38.83^{d}
Rhizolex T-50	105.17^{ef}	11.17^{ef}	39.17^{e}	106.33^{f}	8.67^{fg}	36.00^{e}
Interaction						
Giza 1						
Check	88.33^{k}	9.67^{j}	33.67^{h}	89.67^{k}	7.67^{j}	30.67^{l}
H_2O_2 (0.50 mM)	107.00^{h}	13.33^{d-f}	44.67^{ef}	104.67^{h}	13.00^{bc}	40.67^{e-g}
H_2O_2 (0.75 mM)	100.67^{i}	12.33^{e-g}	42.33^{fg}	99.67^{i}	11.00^{e-g}	37.33^{hi}
H_2O_2 (1.00 mM)	94.67^{j}	11.33^{g-i}	41.00^{g}	95.33^{j}	10.00^{gh}	34.33^{jk}
ASA (10 mM)	114.33^{ef}	10.33^{ij}	41.00^{g}	113.00^{g}	8.67^{ij}	32.67^{kl}
ASA (15 mM)	126.33^{b}	16.00^{ab}	50.67^{bc}	123.00^{c}	14.00^{ab}	43.00^{c-e}
ASA (20 mM)	119.67^{cd}	10.67^{h-j}	41.67^{fg}	117.33^{de}	9.33^{hi}	36.00^{ij}
Rhizolex T-50	98.33^{ij}	10.00^{ij}	35.33^{h}	96.33^{j}	8.00 j	33.00^{kl}
Giza 2						
Check	109.00^{gh}	12.00^{f-h}	42.33^{fg}	112.67^{g}	9.33^{hi}	37.33^{hi}
H_2O_2 (0.50 mM)	118.00^{c-e}	16.33^{ab}	53.33^{b}	119.00^{d}	11.67^{de}	43.33^{cd}
H_2O_2 (0.75 mM)	114.67^{d-f}	15.00^{bc}	50.67^{bc}	116.67^{ef}	12.67^{cd}	48.67^{b}
H_2O_2 (1.00 mM)	112.33^{fg}	14.33^{cd}	48.00^{cd}	114.67^{fg}	12.00^{c-e}	45.33^{c}
ASA (10 mM)	122.00^{bc}	12.33^{e-g}	46.33^{de}	121.67^{c}	10.33^{f-h}	39.67^{f-h}
ASA (15 mM)	138.00^{a}	17.33^{a}	60.33^{a}	132.00^{a}	14.33^{a}	51.67^{a}
ASA (20 mM)	125.67^{b}	13.67^{c-e}	49.00^{cd}	129.33^{b}	11.33^{ef}	41.67^{d-f}
Rhizolex T-50	112.00^{f-h}	12.33^{e-g}	43.00^{fg}	116.33^{ef}	9.33^{hi}	39.00^{gh}

Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range tests

DISCUSSION

Abiotic inducers are considered one of the alternative methods to decrease the use of fungicides in plant disease control. Soaking lupine seeds in both inducers, especially at low concentration of H_2O_2 and moderate ASA concentration gave a significant effects in reducing percentage of disease parameters, in turn increasing% of healthy survival plants. The role of H_2O_2 in induced disease resistance may be due to activation of peroxidase, polyphenol oxidase, catalase and B-1, 3-glucanase enzymes, which protect plants against pathogen infection (Morsy, 2005; Khalifa *et al*., 2007). Moreover, Martinez *et al*. (2000) stated that H_2O_2 positively influences one the local and systemic accumulation of salicylic acid which correlated with enhancement of peroxidase activity. Hydrogen peroxide also increased lignin and suberin content as well as activated of peroxidase and chitinase enzymes (Wu *et al*., 1997), which activities the defense mechanisms. In addition to, H_2O_2 inhibites pathogens directly and/or it may generate other reactive free radicals that are antimicrobial (Peng and Kuc, 1992). Hydrogen peroxide at lowest concentration (0.25%) enhancement the activity of oxidative enzymes and increases the content of phenols compounds (Mahmoud *et al*., 2006). On contrast, increasing of hydrogen peroxide concentration led to decrease its positively effect due to the role of H_2O_2 in rapid generation of Active Oxygen Species (AOS) called the oxidative burst (Lamb and Dixon, 1997). Active Oxygen Species (AOS) gives opposite effect on physiological processes in plants in increased its concentration, especially the role of hydrogen peroxide in accumulation of SA (Martinez *et al*., 2000). While, Lu and Higgins (1999) stated that H_2O_2 may remarkably inhibit the growth of pathogenic fungi and that H_2O_2 concentration effective in killing the fungus is considerably lower than the concentration causing plant cell death. Some studies have shown that acting at a relatively low concentration of H_2O_2 could be a factor inducing the expression of defence-related genes, including genes coding for catalase (Polidoros and

Table 6: Effect of inducers on some yield components of lupine plants under field conditions

	2012/2013			2013/2014		
Treatments	No. of pods/plant	Plant yield (g)	Weight of 100-seeds (g)	No. of pods/plant	Plant yield (g)	Weight of 100-seeds (g)
Cultivars						
Giza 1	31.92^{b}	25.20^{b}	27.26^{b}	30.21^{b}	23.69^{b}	27.12^{b}
Giza 2	36.88^{a}	29.82^{a}	28.74^{a}	35.75^{a}	28.59^{a}	28.55^{a}
Treatments						
Check	25.83^{g}	23.47^{g}	25.02^{g}	24.00^{f}	21.57^{g}	22.87^{g}
H_2O_2 (0.50 mM)	38.50^{c}	29.33^{c}	29.05^{c}	35.83^{c}	28.62^{c}	30.50^{c}
H_2O_2 (0.75 mM)	30.33^{e}	25.53^{e}	27.22^{e}	32.00^{d}	24.87^{e}	27.50^{e}
H_2O_2 (1.00 mM)	28.50^{f}	24.02^{f}	26.27^{f}	29.33^{e}	23.25^{f}	26.55^{f}
ASA (10 mM)	33.67^{d}	27.62^{d}	28.17^{d}	33.50^{d}	27.03^{d}	29.35^{d}
ASA (15 mM)	47.33^{a}	34.42^{a}	32.40^{a}	43.50^{a}	31.90^{a}	32.00^{a}
ASA (20 mM)	43.00^{b}	31.96^{b}	31.02^{b}	40.17^{b}	30.10^{b}	31.18^{b}
Rhizolex T-50	28.00^{f}	23.70^{fg}	24.88^{g}	25.50^{f}	21.80^{g}	22.72^{g}
Interaction						
Giza 1						
Check	22.00^{i}	22.37^{k}	24.57^{k}	20.33^{j}	20.40^{k}	22.20^{k}
H_2O_2 (0.50 mM)	36.67^{d}	26.47^{g}	28.10^{f}	34.00^{de}	25.60^{g}	30.00^{e}
H_2O_2 (0.75 mM)	28.33^{gh}	23.70^{j}	27.27^{gh}	31.00^{fg}	23.20^{i}	26.77^{h}
H_2O_2 (1.00 mM)	26.33^{h}	22.70^{k}	26.30^{i}	26.33^{i}	22.37^{j}	25.70^{i}
ASA (10 mM)	32.00^{e}	24.57^{i}	27.60^{g}	31.00^{fg}	23.87^{h}	28.10^{f}
ASA (15 mM)	44.33^{b}	30.83^{d}	30.33^{c}	40.67^{b}	27.30^{e}	31.50^{bc}
ASA (20 mM)	41.67^{c}	28.27^{e}	29.53^{d}	37.00^{c}	26.20^{f}	30.57^{d}
Rhizolex T-50	24.00^{i}	22.67^{k}	24.40^{k}	21.33^{j}	20.60^{k}	22.13^{k}
Giza 2						
Check	29.67^{fg}	24.57^{i}	25.47^{j}	27.67^{hi}	22.73^{ij}	23.53^{j}
H_2O_2 (0.50 mM)	40.33^{c}	32.20^{c}	30.00^{c}	37.67^{c}	31.63^{c}	31.00^{cd}
H_2O_2 (0.75 mM)	32.33^{e}	27.37^{f}	27.17^{h}	33.00^{ef}	26.53^{f}	28.23^{f}
H_2O_2 (1.00 mM)	30.67^{ef}	25.33^{h}	26.23^{i}	32.33^{e-g}	24.13^{h}	27.40^{g}
ASA (10 mM)	35.33^{d}	30.67^{d}	28.73^{e}	36.00^{cd}	30.20^{d}	30.60^{d}
ASA (15 mM)	50.33^{a}	38.00^{a}	34.47^{a}	46.33^{a}	36.50^{a}	32.50^{a}
ASA (20 mM)	44.33^{b}	35.65^{b}	32.50^{b}	43.33^{b}	34.00^{b}	31.80^{b}
Rhizolex T-50	32.00^{e}	24.73^{i}	25.37^{j}	29.67^{gh}	23.00^{i}	23.30^{j}

Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range tests

Scandalios, 1999; Guan and Scandalios, 2000). Moreover, Levine *et al*. (1994) suggested that H_2O_2 directly or indirectly, plays as a signal for inducing systemic acquired resistance. Hydrogen peroxide and other activated oxygen species in the plant cell wall and in plasma membrane is often considered to be a defensive oxidative barrier to phytopathogenic fungi (Merzlyak *et al*., 1990; Galal and Abdou, 1996).

The present investigation revealed that ASA increased lupine germination percentage and decreased per- and post-emergence damping-off. These results are in harmony with Zhang *et al*. (1999), who stated that the addition of SA and ASA on wheat seeds not only increase germination rate but also increase germination and activities of alpha-amylase and proteinase in endosperm and their contents of soluble sugars, protein and free amino acids. Rizolex-T decrease root rot incidence due to the expected degradation of fungicide when introduced into the soil and exposed to the environmental conditions (Abdel-kader, 1997). Treated lupine seeds with ASA or Rizolex-T provide such protection to seed bed region against soil-borne pathogens reflected on the observed lower disease incidence at pre- emergence stage before exposure to degradation factors (El Mougy, 2004). Acetylsalicylic acid reduced lupine root rot incidence might be attributed to the act of ASA as plant defense inducers or to their direct effect on soil borne plant pathogens (El Mougy, 2002). Also, ASA induced resistance in various plants is associated with enhancing the activities of chitinase and B-1, 3-glucanase which hydrolysis hyphal cell wall of fungi (Matta *et al*., 1988). The effect of ASA on damping-off decreased with increasing concentration from 15-20 mM may be due to the damage effects of SA at high concentration on physiological processes, includes inhibited phosphorus uptake and potassium absorption (Harper and Balke, 1981). In addition to, it caused the collapse of the transmembrane electrochemical potential of mitochondria which had effect on ATP-production (Macri *et al*., 1986). Generally, the antimicrobial effect of inducers may be due to one or more the following reasons: a) inhibit the functions of several enzymes

Table 7: Effect of inducers on protein (%) and lupinine (%) of lupine seeds under field conditions

	2012/2013		2013/2014	
Treatments	Protein (%)	Lupinine (%)	Protein (%)	Lupinine (%)
Cultivars				
Giza 1	32.88^{b}	1.285^{a}	33.88^{b}	1.331^{a}
Giza 2	34.50^{a}	1.197^{b}	35.33^{a}	1.226^{b}
Treatments				
Check	32.00^{d}	1.220^{d}	33.17^{e}	1.252^{d}
H_2O_2 (0.50 mM)	34.33^{c}	1.257^{b}	35.00^{c}	1.293^{bc}
H_2O_2 (0.75 mM)	33.83^{c}	1.240^{c}	34.17^{d}	1.272^{cd}
H_2O_2 (1.00 mM)	31.33^{d}	1.237^{c}	32.33^{f}	1.267^{d}
ASA (10 mM)	35.17^{b}	1.270^{b}	35.67^{bc}	1.272^{cd}
ASA (15 mM)	36.17^{a}	1.295^{a}	37.33^{a}	1.347^{a}
ASA (20 mM)	35.17^{b}	1.240^{c}	36.17^{b}	1.310^{b}
Rhizolex T-50	31.50^{d}	1.170^{e}	33.00^{ef}	1.218^{e}
Interaction				
Giza 1				
Check	31.00^{f}	1.260^{de}	32.33^{jk}	1.300^{de}
H_2O_2 (0.50 mM)	34.00^{d}	1.310^{b}	34.33^{f-h}	1.350^{bc}
H_2O_2 (0.75 mM)	33.00^{e}	1.300^{bc}	33.00^{ij}	1.330^{cd}
H_2O_2 (1.00 mM)	30.00^{g}	1.270^{de}	31.33^{k}	1.310^{de}
ASA (10 mM)	34.33^{d}	1.280^{cd}	35.00^{e-g}	1.320^{cd}
ASA (15 mM)	35.33^{bc}	1.340^{a}	37.00^{ab}	1.410^{a}
ASA (20 mM)	34.67^{cd}	1.320^{ab}	35.67^{c-d}	1.370^{b}
Rhizolex T-50	30.67^{fg}	1.200^{fg}	32.33^{jk}	1.260^{fg}
Giza 2				
Check	33.00^{e}	1.180^{g}	34.00^{g-i}	1.203^{ij}
H_2O_2 (0.50 mM)	34.67^{cd}	1.203^{f}	35.67^{c-e}	1.237^{g-i}
H_2O_2 (0.75 mM)	34.67^{cd}	1.203^{f}	35.33^{d-f}	1.223^{hi}
H_2O_2 (1.00 mM)	32.67^{e}	1.180^{g}	33.33^{h-j}	1.213^{i}
ASA (10 mM)	35.67^{b}	1.200^{fg}	36.33^{b-d}	1.223^{hi}
ASA (15 mM)	37.00^{a}	1.250^{e}	37.67^{a}	1.283^{ef}
ASA (20 mM)	36.00^{b}	1.220^{f}	36.67^{a-c}	1.250^{f-h}
Rhizolex T-50	32.33^{e}	1.140^{h}	33.67^{hi}	1.177^{j}

Mean within a column followed by the same letter(s) is not significantly different according to Duncan's multiple range tests

by the oxidized compounds, b) dissolve in membrane lipids and interfere with membrane functions, c) interfere with the synthesis of protein, RNA and DNA and, d) act on the sites and number of hydroxyl groups on the phenol compounds which increase toxicity to microorganisms (Nesci *et al*., 2003).

In the present study, it could be stated that both inducers had a stimulatory effects on photosynthetic pigments, phenol content, lupine plant growth and yield components as well as seed quality. These effect may be due to the increase in photosynthesis process, consequently increase carbohydrate content. Carbohydrates includes cellulose, hemicelluloses and pectin which consider as a barrier against pathogen invasion (Hahlbrock and Scheel, 1989). They added that, phenolic compounds are associated with structural carbohydrates, which play major role in plant defense. Moreover, Morkunas *et al*. (2005) indicated that soluble carbohydrates my be involved in the mechanism of resistance, because it can be used as carbon skeletons for synthesis of isoflavonoids, which are important elements of the defense system of legumes.

CONCLUSION

It could be concluded that application of hydrogen peroxide at 0.50 mM and acetylsalicylic acid at 15 mM as seed soaking is recommended for reducing root rot in lupine plants as well as improving growth, yield and seed quality.

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