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HYDROGEN PEROXIDE AND ACETYLSALICYLIC ACID INDUCE THE DEFENSE OF LUPINE AGAINST ROOT ROT DISEASE

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ABSTRACT

Lupine is cultivated in Egypt for food, medical and industrial purposes. Root rot diseases caused by several soil-borne fungal pathogens are among the most destructive diseases attacking lupine plants. Greenhouse and field experiments were conducted to study the effect of hydrogen peroxide (H_2O_2) and acetylsalicylic acid (ASA) in addition to Rhizolex-T50 on lupine root rot disease, growth, certain physiological activities and productivity of lupine. Lupine seeds (cvs. Giza 1 and Giza 2) were examined. The data indicated that isolation of pathogenic fungi from both cultivars of diseased lupine was carried out in five locations of Dakahlia governorate. The high frequency isolated fungi presented in Temi El-Amdeed followed by Bani-Ebeed location. *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* whereas *F. solani* was 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. No significant differences between Rhizolex-T50 and H_2O_2 at 0.50 mM were detected. The high photosynthetic pigments and phenolic content were obtained from the application of ASA at moderate concentrate (15 mM) in both cultivars. Giza 2 gave the highest values in these parameters. Soaking in both tested materials increased significantly growth parameter examined, 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). Could be concluded 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.

Keywords: Lupine, Root rot disease, Hydrogen peroxide, Acetylsalicylic acid, *Fusarium solani, F. oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*

INTRODUCTION

Lupine (*Lupinus termis* Forks) is one of the most important crop which belonging to fabaceae family. Like other fabaceaus seeds it is good dietary sources of minerals (Trugo *et al*., 1993). Lupine seeds also contain chemical compounds i.e. protein, oil, cholesterol and alkaloids (lupulin. Luponine, lupuland, 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 and used in sustainable and environment–friendly agriculture because of its high potential for biological nitrogen fixation (Robinson *et al*., 2000). Lupine is cultivated in Egypt for food, medical and industrial purposes (Ibrahim *et al*., 1990).

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 and 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 phytoaluxin accumulation, phenols, lignifications and activation of peroxidase, polyphenoloxides, catalase and chitinase (Meena *et al*., 2001; Mahmoud *et al*., 2006 and Walters *et al*., 2007).

Some abiotic inducers i.e. acetyl salicylic 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 and Mahmoud *et al*., 2006).

Therefore, the present investigation aimed to study the effect of abiotic (ASA and H_2O_2) inducers on lupine root rot diseases, some morphological and physiological characters as well as on 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 inducer namely, hydrogen peroxide (H_2O_2) at 0.50,0.75 and 1.0 mM and acetyl salicylic acid (ASA) at 10,15 and20 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 locations of Dakahlia government. The infected roots were washed thoroughly with tap water, cut into small pieces (1cm) and surface disinfested with sodium hypochlorite 2% for two minutes, then re-washed several times with sterilized water and dried between folds of sterilized filter paper. They were placed onto potato dextrose agar (PDA) medium in petri-dishes supplemented with streptomycin sulfate (100µg/ml). Petri-dishes were incubated at 21^{o} C for five days. The developed

fungal colonies purified and identification was developed according to Ellis (1976), Sneh *et al*. (1991) 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±1º 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 the two cultivars were sown after surface satirized 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, Agric. Res. 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 seeds. Treated lupine seeds were sown in 30th and 10th of November in the 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 varieties, while sub-plots were occupied by treatments. The area of eachsub-plot was 3x3.5 m. Sowing was took place at the rate 180 seeds/plot.

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.

Morphological characters:

Samples were taken to estimate plant height, number of branches and number of leaves plant^{-1} at harvesting time (175 days from sowing in Giza 1 and 160 days in Giza 2)**.**

Physiological character:

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). In addition, total phenolic compounds were determined in fresh shoot after 75 days from sowing using the Folin-ciocalteau reagent according to Malik and Singh (1980).

Yield and its components:

Number of pods, plant yield and weight of 100-seed were recorded.Seed quality was estimated only in the second season. The seeds were dried at 70º C for 48 h, grounded and analyzed for alkaloid lupinine

(Dabbas, 1973) and total nitrogen by semi-micro-Kjldahle (Pregl, 1945). Protein % 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 locations of Dakahlia governorate, Egypt are shown in Table 1. It was observed that Giza1 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*.

Table (1): Frequency of the isolated fungi from lupine roots at different locations in Dakahlia province

Treatments		*Rhizoctonia solani*	*Sclerotium rolfesii*	*Fusarium solani*	*Fusarium oxysporum*
Variety					
Giza 1		16.22 b*	13.2 a	29.50 a	23.90 a
Giza 2		16.48 a	12.54 b	27.16 b	20.96 b
Location					
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 i
	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

***Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05**

Pathogenicity testes:

Data presented in Table 2 show that Giza 1 lupine cultivar was more sensitive to the infection of 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. *R. 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.

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

Treatments		Pre-emergency damping off	Post-emergency damping off	Survival Plants
Variety				
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

***Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05**

Field experiments:

Germination and disease assessment:

Data of germination percentage and damping-off of lupine plants as affected by inducers under field conditions are presented 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 with decreasing 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 varieties.The tow concentration of H_2O_2(0.50 mM) was more effective in this respect. It is worthy to mention that there are no significant differences between H_2O_2 at 0.50 mM and Rhizolex-T50 treatments.

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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 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 ASA followed by H_2O_2.

Growth and yield:

As shown in Table 5 and 6, there were a significant differences between treatments of both lupine cultivars regarding lupine growth (plant height, number of branches and leaves 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. Soakinglupine seeds in both tested inducers increased significantly plant height, number of branches and leaves/plant in both cultivars during the two growing seasons. Acetyl salicylic acid at 15 mM appeared excellent superiority in all treatments on plant height, number of branches and leaves/plant followed by H_2O_2at 0.50mM.

Data concerning yield components in relation to the effect of tested inducers are presented in Table 6. It can easily notice that Giza 2 cultivar gave the highest average of pods number plant^{-1}, 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. 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:

Data in Table 7 show that Giza2 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.

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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 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 and Khalifa *et al*., 2007). 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 peroxidase and chitinase enzymes (Gusui *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%) enhanced the activity of oxidative enzymes and increased the content of phenols compounds (Mahmoud *et al*., 2006). On contrast, increasing of hydrogen peroxide concentration led to decrease its positively affect due to the role of H_2O_2 in rapid generation of active oxygen species (AOS) called the oxidative burst (Levine *et al*., 1994)). 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_2may 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 Scandalios, 1999 and 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 and 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-Shi Gong *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). Acetyl salicylic 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 to 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, it caused the collapse of the transmembrane electrochemical potential of mitochondria which had effect on ATP- production (Macri *et al*., 1986). Generally it was reported that, the antimicrobial effect of inducers may be due to one or more the following reasons: a) inhibit the functions of several enzymes 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).

The stimulating effects of both inducers used in this study on photosynthetic pigments, phenol content, growth and yield as well as seed quality may be due to the increase in photosynthesis process and carbohydrate content. Carbohydrates include 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. Markunas *et al*. (2005) indicated that soluble carbohydrates may 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 acetyl salicylic acid at 15 mM as seed soaking is recommended for reducing root rot in lupine plants as well as improving growth, yield and its components as well as seed quality.

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دفع نباتات الترمس لمقاومة مرض عفن الجذور بإستخدام فوق أكسيد الهيدروجين وحمض أسيتيل ساليسيليك

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يزرع الترمس في مصر للتغذية ولأغراض طبية وصناعية أخرى. ولأمراض أعفان الجذور التي تسببها العديد من مسببات الأمراض الفطرية المنقولة عن طريق التربة آثار مدمرة لهذا المحصول. ولهذا الغرض أجرى هذا البحث لدراسة تأثير كل من فوق أكسيد الهيدروجين (H_2O_2) وحمض أسيتيل ساليسيليك (ASA) و Rhizolex-T50 على درجة الإصابة ومعدل نمو النبات وبعض أنشتطة الفسيولوجية ومدى تأثر المحصول وإستخدم لهذا الغرض أصناف جيزة 1 وجيزة 2 وتم زراعتها فى مواقع مختلفة من محافظة الدقهلية. وقد أوضحت النتائج إمكانية عزل الفطريات المسببة للمرض من كلا الصنفين المصابين تحت الدراسة والتى أخذت من خمسة مواقع وكانت أعلى نسب الإصابة من هذه الفطريات المعزولة في مركز تمى الأمديد يليه مركز بنى عبيد. كما أشارت النتائج إلى أن الفطر فيوزاريوم سولانى والفطر فيوزاريوم أوكسيسبورم هما أكثر الفطريات المعزولة يليها فطر الرايزوكتونيا سولاني. وفى تجارب الصوبة، وجد أن الصنف جيزة 1 هو الأكثر قابلية للإصابة بفطريات عفن الجذر المسببة للمراض مثل فطريات الإسكلوروشيم رولفسياى يليه فطر الرايزوكتونيا بينما كان فطر الفيوزاريوم سولاني هو الأكثر عدوانية فى إحداث أعراض سقوط البادرات. وأوضحت التجربة الحقلية، أن الصنف جيزة 2 كان الأفضل في نسبة الإنبات وكان الأكثر تحملاً لأعراض سقوط البادرات عن الصنف جيزة 1. وقد حققت المعاملة بالمبيد الفطرى Rhizolex-T50 أو H_2O_2 (0.50 ملليمول) أعلى نسبة إنبات مع أقل نسبة لأعراض سقوط البادرات ولم يلاحظ وجود فروق معنوية بينهما ومن ناحية أخرى أعطت المعاملة بحمض الأستيل سالسيليك ASA (15 ملليمول) أعلى محتوى للنبات من صبغات البناء الضوئي والفينولات الكلية في كلا الصنفين. مع تفوق الصنف جيزة 2 فى ذلك. كما وجد أنه في المعاملة بكلا المادتين المختبرتين أعطت زيادة معنوية فى صفات النمو وكمية المحصول ومكوناته مع جودة البذور. وكانت أفضل المعاملات فى ذلك هى حمض الأستيل سالسيليك ASA عند تركيز (15 ملليمول) وتلاه التركيز المنخفض من فوق أكسيد الهيدروجين H_2O_2 (0.50 ملليمول). وتوصى هذه الدراسة بإستخدام نقع البذور بفوق أكسيد الهيدروجين H_2O_2 بتركيز 0.50 ملليمول وحمض الأسيتيل ساليسليك ASA بتركيز 15 ملليمول بدلاً من إستخدام المبيدات الفطرية لمقاومة مرض عفن الجذر فى نباتات الترمس وتحسين نموه وإنتاجيته.

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

Treatments		2012/2013				2013/2014			
		Germination %	Pre-emergency damping off	Post-emergency damping off	Survival Plants	Germination %	Pre-emergency damping off	Post-emergency damping off	Survival Plants
Variety									
Giza 1		88.38 b*	11.63 a	14.13 a	74.24 b	85.54 b	14.54 a	13.38a	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)		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)		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 (0.75)		85.67 f	14.33 a	16.50 b	69.17 e	84.17 e	15.83 b	15.50 b	68.67 f
Acetyl salicylic acid (ASA) (10)		86.67 j	13.33 b	13.67 ef	73.00 ef	82.67 i	17.33 b	13.67 de	69.00 g
Acetyl salicylic acid (ASA) (15)		91.00 c	9.00 d	12.83 d	78.17 c	87.83 c	12.17 d	12.00 c	75.83 d
Acetyl salicylic acid (ASA) (20)		88.00 e	12.00 b	13.83 d	74.17 d	84.67 e	15.33 b	13.00 c	71.67 e
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)	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)	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)	84.67 k	15.33 a	17.33 bc	67.34 h	82.33 i	17.67 b	16.33 b	66.00 h
	Acetyl salicylic acid (ASA) (10)	86.67 j	13.33 b	13.67 ef	73.00 ef	82.67 i	17.33 b	13.67 de	69.00 g
	Acetyl salicylic acid (ASA) (15)	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
	Acetyl salicylic acid (ASA) (20)	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)	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)	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)	86.67 j	13.33 b	15.67 cd	71.00 fg	86.00 gh	14.00 de	14.67 cd	71.33 f
	Acetyl salicylic acid (ASA) (10)	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
	Acetyl salicylic acid (ASA) (15)	92.33 cd	7.67 hi	12.33 f	80.00 c	89.00 de	11.00 gh	10.67 g	78.33 d
	Acetyl salicylic acid (ASA) (20)	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 i	90.34 a	94.33 a	5.67 k	5.67 i	88.66 a

***Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05**

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

Treatments		2012/2013				2013/2014			
		Chlorophyll a	Chlorophyll b	Carotenoids	Total Phenols (mg/100g)	Chlorophyll a	Chlorophyll b	Carotenoids	Total Phenols (mg/100g)
Variety									
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)		1.20 b	0.62 b	0.46 a	454.00 b	1.26 b	0.69 b	0.40 b	665.00 a
H_2O_2 (0.75)		1.12 d	0.55 c	0.43 b	421.17 d	1.17 d	0.61 c	0.32 d	433.33 d
H_2O_2 (0.75)		1.01 f	0.50 e	0.36 de	389.50 f	1.07 f	0.55 de	0.30 e	393.67 f
Acetyl salicylic acid (ASA) (10)		1.07 e	0.53 d	0.36 de	410.50 e	1.14 e	0.57 d	0.33 d	417.83 e
Acetyl salicylic acid (ASA) (15)		1.28 a	0.66 a	0.40 c	473.17 a	1.32 a	0.74 a	0.42 a	481.67 b
Acetyl salicylic acid (ASA) (20)		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 i	347.33 l	0.95 k	0.49 kl	0.22 j	355.00 m
	H_2O_2 (0.50)	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)	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 (0.75)	0.97 g	0.49 hi	0.36 fg	383.00 j	1.04 ij	0.51vjk	0.28 gh	390.00 k
	Acetyl salicylic acid (ASA) (10)	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
	Acetyl salicylic acid (ASA) (15)	1.24 b	0.61 cd	0.46 a	464.00 b	1.27 c	0.65 de	0.43 a	471.33 c
	Acetyl salicylic acid (ASA) (20)	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)	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)	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 (0.75)	1.04 ef	0.51 gh	0.36 fg	396.00 i	1.10 h	0.58 gh	0.40 bc	397.33 j
	Acetyl salicylic acid (ASA) (10)	1.10 d	0.55 ef	0.40 d	414.33 g	1.18 ef	0.61 fg	0.31 ef	421.33 h
	Acetyl salicylic acid (ASA) (15)	1.32 a	0.71 a	0.46 a	482.33 a	1.37 a	0.82 a	0.30 fg	492.00 b
	Acetyl salicylic acid (ASA) (20)	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

***Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05**

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

Treatment		2012/2013			2013/2014		
		Plant height (cm)	No. of branches/ plant	No. of Leaves/ plants	Plant height (cm)	No. of branches/ plant	No. of Leaves/ plants
Variety							
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)		112.50 d	14.83 b	49.00 b	111.83 d	12.83 b	44.67 b
H_2O_2 (0.75)		107.67 e	13.67 c	46.50 c	108.17 e	11.50 c	41.33 c
H_2O_2 (0.75)		103.50 f	12.83 cd	44.50 cd	105.00 f	10.83 cd	38.83 d
Acetyl salicylic acid (ASA) (10)		118.17 c	11.33 ef	43.67 d	117.33 c	9.50 ef	36.17 e
Acetyl salicylic acid (ASA) (15)		132.17 a	16.67 a	55.50 a	127.50 a	14.17 a	47.33 a
Acetyl salicylic acid (ASA) (20)		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)	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)	100.67 i	12.33 e-g	42.33 fg	99.67 i	11.00 e-g	37.33 hi
	H_2O_2 (0.75)	94.67 j	11.33 g-i	41.00 g	95.33 j	10.00 gh	34.33 jk
	Acetyl salicylic acid (ASA) (10)	114.33 ef	10.33 ij	41.00 g	113.00 g	8.67 ij	32.67 kl
	Acetyl salicylic acid (ASA) (15)	126.33 b	16.00 ab	50.67 bc	123.00 c	14.00 ab	43.00 c-e
	Acetyl salicylic acid (ASA) (20)	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)	118.00 c-e	16.33 ab	53.33 b	119.00 d	11.67 de	43.33 cd
	H_2O_2 (0.75)	114.67 d-f	15.00 bc	50.67 bc	116.67 ef	12.67 cd	48.67 b
	H_2O_2 (0.75)	112.33 fg	14.33 cd	48.00 cd	114.67 fg	12.00 c-e	45.33 c
	Acetyl salicylic acid (ASA) (10)	122.00 bc	12.33 e-g	46.33 de	121.67 c	10.33 f-h	39.67 f-h
	Acetyl salicylic acid (ASA) (15)	138.00 a	17.33 a	60.33 a	132.00 a	14.33 a	51.67 a
	Acetyl salicylic acid (ASA) (20)	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

***Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05**

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

Treatment		2012/2013			2013/2014		
		No. of pods/plant	Plant yield (g)	Weight of 100-seeds (g)	No. of pods/plant	Plant yield (g)	Weight of 100-seeds (g)
Variety							
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)		38.50 c	29.33 c	29.05 c	35.83 c	28.62 c	30.50 c
H_2O_2 (0.75)		30.33 e	25.53 e	27.22 e	32.00 d	24.87 e	27.50 e
H_2O_2 (0.75)		28.50 f	24.02 f	26.27 f	29.33 e	23.25 f	26.55 f
Acetyl salicylic acid (ASA) (10)		33.67 d	27.62 d	28.17 d	33.50 d	27.03 d	29.35 d
Acetyl salicylic acid (ASA) (15)		47.33 a	34.42 a	32.40 a	43.50 a	31.90 a	32.00 a
Acetyl salicylic acid (ASA) (20)		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)	36.67 d	26.47 g	28.10 f	34.00 de	25.60 g	30.00 e
	H_2O_2 (0.75)	28.33 gh	23.70 j	27.27 gh	31.00 fg	23.20 i	26.77 h
	H_2O_2 (0.75)	26.33 h	22.70 k	26.30 i	26.33 i	22.37 j	25.70 i
	Acetyl salicylic acid (ASA) (10)	32.00 e	24.57 i	27.60 g	31.00 fg	23.87 h	28.10 f
	Acetyl salicylic acid (ASA) (15)	44.33 b	30.83 d	30.33 c	40.67 b	27.30 e	31.50 bc
	Acetyl salicylic acid (ASA) (20)	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)	40.33 c	32.20 c	30.00 c	37.67 c	31.63 c	31.00 cd
	H_2O_2 (0.75)	32.33 e	27.37 f	27.17 h	33.00 ef	26.53 f	28.23 f
	H_2O_2 (0.75)	30.67 ef	25.33 h	26.23 i	32.33 e-g	24.13 h	27.40 g
	Acetyl salicylic acid (ASA) (10)	35.33 d	30.67 d	28.73 e	36.00 cd	30.20 d	30.60 d
	Acetyl salicylic acid (ASA) (15)	50.33 a	38.00 a	34.47 a	46.33 a	36.50 a	32.50 a
	Acetyl salicylic acid (ASA) (20)	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

***Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05**

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

Treatment		2012/2013		2013/2014	
		Protein %	Lupinine %	Protein %	Lupinine %
Variety					
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)		34.33 c	1.257 b	35.00 c	1.293 bc
H_2O_2 (0.75)		33.83 c	1.240 c	34.17 d	1.272 cd
H_2O_2 (0.75)		31.33 d	1.237 c	32.33 f	1.267 d
Acetyl salicylic acid (ASA) (10)		35.17 b	1.270 b	35.67 bc	1.272 cd
Acetyl salicylic acid (ASA) (15)		36.17 a	1.295 a	37.33 a	1.347 a
Acetyl salicylic acid (ASA) (20)		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)	34.00 d	1.310 b	34.33 f-h	1.350 bc
	H_2O_2 (0.75)	33.00 e	1.300 bc	33.00 ij	1.330 cd
	H_2O_2 (0.75)	30.00 g	1.270 de	31.33 k	1.310 de
	Acetyl salicylic acid (ASA) (10)	34.33 d	1.280 cd	35.00 e-g	1.320 cd
	Acetyl salicylic acid (ASA) (15)	35.33 bc	1.340 a	37.00 ab	1.410 a
	Acetyl salicylic acid (ASA) (20)	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)	34.67 cd	1.203 f	35.67 c-e	1.237 g-i
	H_2O_2 (0.75)	34.67 cd	1.203 f	35.33 d-f	1.223 hi
	H_2O_2 (0.75)	32.67 e	1.180 g	33.33 h-j	1.213 i
	Acetyl salicylic acid (ASA) (10)	35.67 b	1.200 fg	36.33 b-d	1.223 hi
	Acetyl salicylic acid (ASA) (15)	37.00 a	1.250 e	37.67 a	1.283 ef
	Acetyl salicylic acid (ASA) (20)	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

***Means followed by different letter (s) in the column are significantly different according to Duncan's multiple range test at p = 0.05**

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