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

ElpMetwally, M.A.; K.M. Abd ElHai and Naglaa T. Mohamed
Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

Corresponding Author: M.A. ElpMetwally, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt

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 (H₂O₂) 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-Ebread 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 H₂O₂ 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 H₂O₂ 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 H₂O₂ (0.50 mM). Could be concluded that the application of H₂O₂ 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 tonic. 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 (H₂O₂) 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 H₂O₂) 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 (H₂O₂) at 0.50, 0.75 and 1.0 mM and acetyl salicylic 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 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°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 inoculum preparation:**

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

**Pathogenicity test:**

The previously prepared fungal 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_{2}O_{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 each sub-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.
El-Metwally, M.A. et al.

(Dabbas, 1973) and total nitrogen by semi-micro-Kjeldahl (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 Dakahila governorate, Egypt are shown in Table 1. It was observed that Giza 1 cultivar was highly 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 Temai 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 rolfsii | 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              | 14.60 h            | 11.80 h            | 26.40 h         | 18.70 i            |
| El-Gamalia          | 12.00 j            | 10.00 j            | 24.00 i         | 20.50 h            |
| Dekernes            | 16.80 e            | 15.00 b            | 26.80 g         | 22.90 e            |
| Bani-Ebeed          | 20.10 a            | 12.70 f            | 30.50 c         | 26.40 b            |
| Temai El-Amdeed     | 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 tests:**

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 rolfsii* | 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 rolfsii* | 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 rolfsii* | 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$_2$O$_2$ then ASA in both varieties. The low concentration of H$_2$O$_2$(0.50 mM) was more effective in this respect. It is worthy to mention that there are no significant differences between H$_2$O$_2$ 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 under the application of ASA followed by H$_2$O$_2$. 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$_2$O$_2$.

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. 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. Acetyl salicylic acid at 15 mM appeared excellent superiority in all treatments on plant height, number of branches and leaves/plant followed by H$_2$O$_2$ at 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$_2$O$_2$ and the moderate concentration of ASA lead to the highest values. ASA at 15 mm was the most effective followed by H$_2$O$_2$ 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$_2$O$_2$ at 0.50 mM.
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$_2$O$_2$ and moderate ASA concentration gave significant effects in reducing percentage of disease parameters, in turn increasing % of healthy survival plants. The role of H$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ may remarkably inhibit the growth of pathogenic fungi and that H$_2$O$_2$ 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$_2$O$_2$ 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$_2$O$_2$ 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-amylose 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.

REFERENCES

Abdel-Kader, M.M. (1997). Field application of Trichoderma harzianum as biocide for control of bean root rot disease. Egypt. J. Phytopathol., 25: 19-25.
Abd-El-Kareem F.; M.A. Abd-Allah; N.G. El-Gamal and N.S. El-Mougy (2004). Integrated control of Lupine root rot disease in solarized soil under greenhouse and field condition. Egypt. J. Phytopathol., 32: 49-63.
El-Metwally, M.A. et al.

Ali, A.A.; K.M. Ghoneem; M.A. El-Metwally and K.M. Abd El-Hai (2009). Induce Systemic Resistance in Lupine Against Root Rot Diseases. Pakistan Journal of Biological Sciences, 12: 213-221.

CoStat (2005). CoStat program, Version 6.311. CoHort Software, Monterey, CA., USA., September 25, 2005.

Dabbas, W.A.S. (1973). The phytochemical investigation of some plants belonging to the families leguminous and solanaceae growing in Egypt. M. Sc. Thesis, Fac. of pharmacy, Alexandria Univ., Egypt.

Ellis, M.B. (1976). More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England. 507 pp.

El-Mougy, N.S. (2002). In vitro studies on antimicrobial activity of salicylic acid and acetylsalicylic acid as pesticidal alternatives against some soil-borne plant pathogens. Egypt. J. Phytopathol., 30: 41-55.

El-Mougy, N.S. (2004). Preliminary evaluation of salicylic acid and acetylsalicylic acid efficacy for controlling root rot disease of lupine under greenhouse conditions. Egypt. J. Phytopathol., 32(1-2): 11-21.

Filonow, A.B.; H.A. Melouk; M. Martin, and J. Sherwood (1988). Effect of calcium sulfate on pod rot of peanut. Plant Disease, 72: 589-593.

Galal, A.A. and E.S. Abdou (1996). Antioxidants for the control of fusarial disease in cowpea. Egypt. J. Phytopathol., 24: 1-12.

Gomez, K.A. and A.A. Gomez (1984). Statistical Procedures for Agricultural Research. 2nd Edn., John Wiley and Sons Inc., New York, USA., pp: 229-308.

Guan L.M. and J.G. Scandalios (2000). Hydrogen peroxide-mediated catalase gene expression in response to wounding. Free Radical Biol. Med., 28(8): 1182-1190.

Gusui, W.U.; B.J. Shortt; E.B. Lawrence; J. Leon; K.C. Fitzsimmons; E.B. Levine; I. Raskin; D.M. Shah and G.S. Wu (1997). Activation of host defense mechanisms by elevated production of H₂O₂ in transgenic plants. Plant Physiol., 115: 427-435.

Hahlbrock, K. and D. Scheel (1989). Physiology and Molecular Biology of Phenylpropanoid Metabolism. Annual Review of Plant Physiology and Plant Molecular Biology, 40: 347-369.

Harper J.R. and N.E. Balke (1981). Characterization of the inhibition of K+ absorption in oats roots by salicylic acid. Plant Physiology, 68: 1349-1353.

Ibrahim, D.M.; M.A. Khafagy and A.M. Abo El–Kheer (1990). Some growth substances affecting the growth, chemical composition and alkaloidal content of Lupinus termis, L. Egypt. J. Applied Sci., 5: 367-381

Khalifa, M.M.A.; M.S. Abd-El-Megid and E.I.D. Eetmad (2007). Applying some chemical effectors for inducing systemic resistance against charcoal rot disease in Egypt. Egypt. J. Applied Sci., 22: 431-446.

Levine, A.; R. Tenhaken; R. Dixon and C.J. Lamb (1994). H₂O₂ from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell, 79: 583–593.

1504
Lu, H. and V.J. Higgins (1999). The effect of hydrogen peroxide on the viability of tomato cells and of the pathogen Cladosporium fulvum. Physiol. Mol. Plant Pathol., 54: 131–143.

Mackinney, G. (1941). Absorption of light by chlorophyll solution. J. Biol. Chem., 140: 315-322.

Macri, F., A. Vianello and S. Pennazio. 1986. Salicylate-collapsed membrane potential in pea stem mitochondria. Physiologia Plantarum, 67(2):136–140.

Mahmoud, E.Y.; S.Y.M. Shokry and Z.N. Hussein (2006). Induction of resistance in peanut plants against root rot diseases under greenhouse conditions by some chemical inducers. J. Agric. Sci. Mansoura Univ., 31: 3511-3524.

Malik, C.P. and M.B. Singh (1980). Plant enzymology and Histoenzymology. Kalyani Publishers. New Delhi, India, pp 53

Martinez C.; J.C. Baccou; E. Bresson; Y. Baissac; J.F. Daniel; A. Jalloul; J.L. Montillet; J.P. Geiger; K. Assigbetse and M. Nicole (2000). Salicylic acid mediated by the oxidative burst is a key molecule in local and systemic responses of cotton challenged by an avirulent race of Xanthomonas campestris pv. malvacearum. Plant Physiology, 122: 757-766.

Matta, A.; G.I. Abattista and L. Ferraris (1988). Stimulation of 1,3-glucanase and chitinase by stress that induce resistance to Fusarium wilt in tomato. Phytopathol. Medit., 27: 45-50.

Meena, B.; T. Marimuthu and R. Velazhahan (2001). Salicylic acid induced systemic resistance in groundnut against late leaf spot caused by Cercosporidium personatum. J. Mycology Plant Path., 31: 139-145.

Merzlyak, M.N.; I.V. Reshetnikova; O.B. Chivkunova; O.D. Ivanova and N.I. Maximova (1990). Hydrogen peroxide and superoxide dependent fatty acid breakdown in Phytophthora infestans zoospores. Plant Sci., 72: 207-212.

Morkunas, I.; Ł. Marczak; J. Stachowiak and M. Stobiecki (2005). Sucrose-induced lupine defense against Fusarium oxysporum: Sucrose-stimulated accumulation of isoflavonoids as a defense response of lupine to Fusarium oxysporum. Plant Physiology and Biochemistry, 43(4): 363–373.

Morsy, K.M.M. (2005). Induced Resistance against Damping-off, Root Rot and Wilt Diseases of Lentil. Egypt. J. Phytopathol., 33(2): 53-63.

Nelson, P.E.; T.A. Tousoun and W.F.O. Marason (1983). Fusarium spp. An Illustrated Manual of Identification. Pennsylvania University Press, USA, pp: 218.

Nescli, A.; M. Rodriguez and M. Etcheverry (2003). Control of Aspergillus growth and aflatoxin production using antioxidants at different conditions of water activity and pH. Journal of Applied Microbiology, 95(2): 279–287.

Peng, M., and J.Kuc (1992). Peroxidase-generated hydrogen peroxide as a source of antifungal activity in vitro and on tobacco leaf discs. Phytopathology, 82: 696-699.
دف نباتات الترسم لمقاومة مرض عفن الجذور باستخدام فقود أكسيدي الهيدروجين
وحمض أسيتيل ساليسليك

محمد أحمد العوللي ، محمد عبد الحليم ولاء محمد طلعت

 medidas و دراسات بحثية لإنتاج النباتات

Prof. K.O. Robinson, J. D.A. Beyene, P. Van Berkum, M.A.R.K Mason and H.L. Pregl, F. (1945). "Quantitative Organic Micro-Analysis, 4th Ed." J. and A. Churchill Ltd., London, p: 203.

Prof. J.M. Robinson, J.A. Britz (2000). "Tolerance of a field grown soybean cultivar to elevated ozone level is concurrent with higher leaflet ascorbic acid level, higher ascorbate-dehydroascorbate redox status and long term photosynthetic productivity. Photosynth. Res., 64: 77-87.

Prof. K.O. Robinson, J.A. Beyene, P. Van Berkum, M.A.R.K Mason and H.L. Pregl, F. (1945). "Quantitative Organic Micro-Analysis, 4th Ed." J. and A. Churchill Ltd., London, p: 203.

Prof. J.M. Robinson, J.A. Britz (2000). "Tolerance of a field grown soybean cultivar to elevated ozone level is concurrent with higher leaflet ascorbic acid level, higher ascorbate-dehydroascorbate redox status and long term photosynthetic productivity. Photosynth. Res., 64: 77-87.

Prof. K.O. Robinson, J.A. Beyene, P. Van Berkum, M.A.R.K Mason and H.L. Pregl, F. (1945). "Quantitative Organic Micro-Analysis, 4th Ed." J. and A. Churchill Ltd., London, p: 203.

Prof. J.M. Robinson, J.A. Britz (2000). "Tolerance of a field grown soybean cultivar to elevated ozone level is concurrent with higher leaflet ascorbic acid level, higher ascorbate-dehydroascorbate redox status and long term photosynthetic productivity. Photosynth. Res., 64: 77-87.
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   | 13.38 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$_2$O$_2$ (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$_2$O$_2$ (0.75)        | 90.67 c    | 9.33 d    | 11.50 e              | 79.17 c          | 67.17 cd  | 12.83 d   | 10.00 d              | 77.17 c          |
| 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          |
| H$_2$O$_2$ (0.50)        | 91.00 d-f  | 10.00 f-q | 10.00 g              | 81.00 c          | 90.00 cd  | 14.00 h   | 11.33 fg             | 74.67 e          |
| H$_2$O$_2$ (0.75)        | 89.67 f-g  | 10.33 d-f | 13.00 e-f            | 76.67 d          | 86.00 gh  | 14.00 de  | 11.33 fg             | 74.67 e          |
| 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 h-f | 13.33 c-f | 13.33 ef             | 76.34 d          | 86.67 g   | 13.33 c-f | 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 i    | 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          |
| H$_2$O$_2$ (0.50)        | 94.00 b    | 6.00 j    | 6.67 gh              | 85.33 b          | 91.67 h   | 8.33 j    | 7.00 i               | 84.67 b          |
| H$_2$O$_2$ (0.75)        | 91.67 de   | 8.33 gh   | 10.00 g              | 81.67 c          | 88.33 ef  | 11.67 fg  | 6.67 h               | 79.66 cd         |
| Acetyl salicylic acid (ASA) (10) | 86.67 j | 13.33 b   | 13.67 cd             | 71.00 fg         | 86.00 gh  | 14.00 de  | 14.67 cd             | 71.33 f          |
| Acetyl salicylic acid (ASA) (15) | 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) (20) | 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               | 86.66 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               | Chlorophyll a | Chlorophyll b | Carotenoids | Total Phenols (mg/100g) | Chlorophyll a | Chlorophyll b | Carotenoids | Total Phenols (mg/100g) |
|--------------------------|---------------|---------------|-------------|-------------------------|---------------|---------------|-------------|-------------------------|
|                          | 2012/2013     | 2013/2014     |             |                         | 2012/2013     | 2013/2014     |             |                         |
| Giza 1                   | 1.05 b*       | 0.52 b        | 0.37 b      | 403.67 b                | 1.10 b        | 0.55 b        | 0.32 b      | 411.98 b                |
| Giza 2                   | 1.13 a        | 0.58 a        | 0.39 a      | 416.54 a                | 1.19 a        | 0.65 a        | 0.35 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                |
| H₂O₂ (0.50)              | 1.20 b        | 0.62 b        | 0.46 a      | 454.00 b                | 1.26 b        | 0.69 b        | 0.40 b      | 465.00 a                |
| H₂O₂ (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                |
| Acetyl salicylic acid (ASA) (10) | 1.07 e | 0.53 d | 0.36 de | 389.50 f | 1.07 f | 0.55 de | 0.30 e | 393.67 f |
| 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 Tq50            | 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                   | 0.89 h        | 0.46 i        | 0.29 i      | 347.33 l                | 0.95 k        | 0.49 kl       | 0.22 j      | 350.00 m                |
| H₂O₂ (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                |
| H₂O₂ (0.75)              | 1.08 de       | 0.52 fn       | 0.36 fg     | 417.67 fg               | 1.14 g        | 0.54 h        | 0.31 ef      | 425.00 h                |
| Acetyl salicylic acid (ASA) (10) | 0.97 g | 0.49 h | 0.36 fg | 383.00 j | 1.04 i | 0.51 yk | 0.28 gh | 390.00 k |
| Acetyl salicylic acid (ASA) (15) | 1.04 ef | 0.50 gh | 0.37 ef | 406.67 h | 1.10 h | 0.52 i-x | 0.31 ef | 414.33 i |
| Acetyl salicylic acid (ASA) (20) | 1.24 b | 0.61 cd | 0.46 a | 464.00 b | 1.27 c | 0.65 de | 0.43 a | 473.33 c |
| Rhizolex Tq50            | 0.86 h        | 0.44 i        | 0.33 h      | 347.33 l                | 0.90 f        | 0.46 i        | 0.25 i      | 351.33 m                |
| Giza 2                   | 1.00 fg       | 0.51 gh       | 0.34 gh     | 358.33 k                | 1.07 hi       | 0.56 hi       | 0.28 h      | 372.00 l                |
| H₂O₂ (0.50)              | 1.24 b        | 0.67 b        | 0.42 bc     | 465.00 b                | 1.31 b        | 0.75 b        | 0.33 de      | 480.33 a                |
| H₂O₂ (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                |
| Acetyl salicylic acid (ASA) (10) | 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) (15) | 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) (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 Tq50            | 0.97 g        | 0.48 h        | 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                          | Variety | Plant height (cm) | No. of branches/ plant | No. of Leaves/ plants | Plant height (cm) | No. of branches/ plant | No. of Leaves/ plants |
|-----------------------------------|---------|-------------------|------------------------|-----------------------|-------------------|------------------------|-----------------------|
|                                   | 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               |
|                                   | H₂O₂ (0.50) | 112.50 d       | 14.83 b                | 49.00 b               | 111.83 d         | 12.83 b                | 44.67 b               |
|                                   | H₂O₂ (0.75) | 107.67 e         | 13.67 c                | 46.50 c               | 108.17 e         | 11.50 c                | 41.33 c               |
|                                   | H₂O₂ (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 Tq50 | 105.17 ef       | 11.17 ef               | 39.17 e               | 106.33 f         | 8.67 fg                | 36.00 e               |
| Interaction                       | Giza 1  | 98.33 k           | 9.67 j                 | 33.67 h               | 89.67 k          | 7.67 j                 | 30.67 l               |
|                                   | Check   | 107.00 h          | 13.33 df               | 44.67 ef              | 104.67 h         | 13.00 bc               | 40.67 e-g             |
|                                   | H₂O₂ (0.50) | 100.67 i         | 12.33 e-g              | 42.33 fg              | 99.67 l          | 11.00 e-g              | 37.33 hi              |
|                                   | H₂O₂ (0.75) | 94.67 j          | 11.33 g-i              | 41.00 g               | 95.33           | 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) | 125.33 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 hj               | 41.67 fg              | 117.33 de        | 9.33 hi                | 36.00 h               |
|                                   | Rhizolex Tq50 | 98.33 jj         | 10.00 jj               | 35.33 h               | 96.33           | 8.00 jj                | 33.00 kl              |
|                                   | Check   | 109.00 gh         | 12.00 f-h              | 42.33 fg              | 112.67 g         | 9.33 hi                | 37.33 hi              |
|                                   | H₂O₂ (0.50) | 118.00 c-e       | 16.33 ab               | 53.33 b               | 119.00 d         | 11.67 de               | 43.33 cd              |
|                                   | H₂O₂ (0.75) | 114.67 d-f       | 15.00 bc               | 50.67 bc              | 116.67 ef        | 12.67 cd               | 48.67 b               |
|                                   | Acetyl salicylic acid (ASA) (10) | 112.33 fg       | 14.33 cd               | 48.00 cd              | 114.67 fg        | 12.00 c-e              | 45.33 c               |
|                                   | Acetyl salicylic acid (ASA) (15) | 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) (20) | 138.00 a       | 17.33 a                | 60.33 a               | 132.00 a         | 14.33 a                | 51.67 a               |
|                                   | Rhizolex Tq50 | 125.67 b       | 13.67 c-e               | 49.00 cd              | 129.33 b         | 11.33 ef               | 41.67 d-f             |
|                                   | Rhizolex Tq50 | 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**

| Variety          | 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) |
| **Giza 1**       | 31.92 b*       | 25.20 b     | 27.28 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               | 21.57 g           | 24.00 f       | 21.27 g               |
| H$_2$O$_2$ (0.50)| 38.50 c       | 29.33 c     | 29.05 c               | 28.62 c           | 30.50 c       | 27.50 e               |
| H$_2$O$_2$ (0.75)| 30.33 e       | 25.53 e     | 27.22 e               | 32.00 d           | 24.87 e       | 27.50 e               |
| Acetyl salicylic acid (ASA) (10)| 33.67 d       | 27.82 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 Tq50    | 28.00 f       | 23.70 fg    | 24.88 g               | 25.50 f           | 21.80 g       | 22.72 g               |
| **Interaction**  |            |            |                       |                  |              |                       |
| Check            | 22.00 i       | 22.37 k     | 24.57 k               | 20.33 j           | 20.40 k       | 22.20 k               |
| H$_2$O$_2$ (0.50)| 36.67 d       | 26.47 g     | 28.10 f               | 34.00 de          | 25.60 g       | 30.00 e               |
| H$_2$O$_2$ (0.75)| 28.33 gh      | 23.70 j     | 27.27 gh              | 31.00 fg          | 22.20 i       | 26.77 h               |
| Acetyl salicylic acid (ASA) (10)| 32.00 e       | 24.67 f     | 26.30 i               | 26.33 i           | 22.37 j       | 28.70 i               |
| 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 Tq50    | 24.00 i       | 22.67 k     | 24.40 k               | 21.33 j           | 20.60 k       | 22.13 k               |
| **Giza 2**       |            |            |                       |                  |              |                       |
| Check            | 28.67 fg      | 24.57 f     | 27.67 h               | 21.73 j           | 23.53 j       |                       |
| H$_2$O$_2$ (0.50)| 40.33 c       | 32.20 c     | 30.00 c               | 37.67 c           | 31.63 c       | 31.00 cd              |
| H$_2$O$_2$ (0.75)| 32.33 e       | 27.37 f     | 27.17 h               | 33.00 ef          | 26.53 f       | 28.25 f               |
| Acetyl salicylic acid (ASA) (10)| 30.67 ef      | 25.33 h     | 26.23 i               | 32.33 e-g         | 24.13 e-f     | 27.40 g               |
| Acetyl salicylic acid (ASA) (15)| 35.33 d       | 30.67 d     | 28.73 e               | 36.00 od          | 30.20 d       | 30.60 d               |
| Acetyl salicylic acid (ASA) (20)| 50.33 a       | 38.00 a     | 34.47 a               | 46.33 a           | 36.50 a       | 32.50 a               |
| Rhizolex Tq50    | 44.33 b       | 35.65 b     | 32.50 b               | 43.33 b           | 34.00 b       | 31.80 b               |

*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 | Protein % | Lupinine |
| Variety                            |           |          |           |           |           |          |
| Giza 1                             | 32.88 b*  | 1.285 a  | 33.88 b   | 1.331 a   | 33.88 b   | 1.331 a  |
| Giza 2                             | 34.50 a   | 1.197 b  | 35.33 a   | 1.226 b   | 35.33 a   | 1.226 b  |
| Treatments                         |           |          |           |           |           |          |
| Check                              | 32.00 d   | 1.220 d  | 33.17 e   | 1.252 d   | 33.17 e   | 1.252 d  |
| H$_2$O$_2$ (0.50)                  | 34.33 c   | 1.257 b  | 35.00 c   | 1.293 bc  | 35.00 c   | 1.293 bc |
| H$_2$O$_2$ (0.75)                  | 33.83 c   | 1.240 c  | 34.17 d   | 1.272 cd  | 34.17 d   | 1.272 cd |
| H$_2$O$_2$ (0.75)                  | 31.33 d   | 1.237 c  | 32.33 f   | 1.267 d   | 32.33 f   | 1.267 d  |
| Acetyl salicylic acid (ASA) (10)   | 35.17 b   | 1.270 b  | 35.67 bc  | 1.272 cd  | 35.67 bc  | 1.272 cd |
| Acetyl salicylic acid (ASA) (15)   | 36.17 a   | 1.295 a  | 37.33 a   | 1.347 a   | 37.33 a   | 1.347 a  |
| Acetyl salicylic acid (ASA) (20)   | 35.17 b   | 1.240 c  | 36.17 b   | 1.310 b   | 36.17 b   | 1.310 b  |
| Rhizolex T-50                      | 31.50 d   | 1.170 e  | 33.00 ef  | 1.218 e   | 33.00 ef  | 1.218 e  |
| Interaction                        |           |          |           |           |           |          |
| Giza 1                             | Check     | 31.00 f  | 1.260 de  | 32.33 jk  | 1.300 de  | 32.33 jk |
|                                    | H$_2$O$_2$ (0.50) | 34.00 d | 1.310 b  | 34.33 f-h | 1.350 bc  | 34.33 f-h |
|                                    | H$_2$O$_2$ (0.75) | 33.00 e | 1.300 bc  | 33.00 ij  | 1.330 cd  | 33.00 ij |
|                                    | H$_2$O$_2$ (0.75) | 30.00 g | 1.270 de  | 31.33 k   | 1.310 de  | 31.33 k |
|                                    | Acetyl salicylic acid (ASA) (10) | 34.33 d | 1.280 cd  | 35.00 e-g | 1.320 cd  | 35.00 e-g |
|                                    | Acetyl salicylic acid (ASA) (15) | 35.33 bc | 1.340 a  | 37.00 ab  | 1.410 a   | 37.00 ab |
|                                    | Acetyl salicylic acid (ASA) (20) | 34.67 cd | 1.320 ab  | 35.67 c-d | 1.370 b   | 35.67 c-d |
|                                    | Rhizolex T-50 | 30.67 fg | 1.200 fg  | 32.33 jk  | 1.260 fg  | 32.33 jk |
| Giza 2                             | Check     | 33.00 e  | 1.180 g   | 34.00 g-i | 1.203 ij  | 34.00 g-i |
|                                    | H$_2$O$_2$ (0.50) | 34.67 cd | 1.203 f  | 35.67 c-e | 1.237 g-i | 35.67 c-e |
|                                    | H$_2$O$_2$ (0.75) | 34.67 cd | 1.203 f  | 35.33 d-f | 1.223 hi  | 35.33 d-f |
|                                    | H$_2$O$_2$ (0.75) | 32.67 e  | 1.180 g   | 33.33 h-j | 1.213 i   | 33.33 h-j |
|                                    | Acetyl salicylic acid (ASA) (10) | 35.67 b | 1.200 fg  | 36.33 b-d | 1.223 hi  | 36.33 b-d |
|                                    | Acetyl salicylic acid (ASA) (15) | 37.00 a | 1.250 e  | 37.67 a   | 1.283 ef  | 37.67 a |
|                                    | Acetyl salicylic acid (ASA) (20) | 36.00 b | 1.220 f  | 36.67 a-c | 1.250 h  | 36.67 a-c |
|                                    | Rhizolex T-50 | 32.33 e  | 1.140 h   | 33.67 hi  | 1.177 j   | 33.67 hi |

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