ISSN NO. 2320-5407

*Journal homepage: http://www.journalijar.com*

*INTERNATIONAL JOURNAL OF ADVANCED RESEARCH*

**RESEARCH ARTICLE**

# Effect of Foliar Spray with Salicylic Acid on Vegetative Growth, Stem and Leaf Anatomy, Photosynthetic Pigments and Productivity of Egyptian Lupine Plant (*Lupinus termis* Forssk.)

**[1]Elham F. Gomaa, [1] Rania M. A. Nassar and [2] Mahmoud A. Madkour**

**1.** Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, Egypt.

**2.** Department of Crops Physiology Research, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

## *Manuscript Info*

***Manuscript History:***

Received: 22 November 2014
Final Accepted: 25 December 2014
Published Online: January 2015

***Key words:***

Egyptian lupine, Lupinus termis Forssk., Salicylic acid, Growth, Yield, Chlorophyll pigments, Seed quality, Anatomy.

***Corresponding Author***

**Elham F. Gomaa**

## *Abstract*

Field experiments were carried out at the Agricultural Experiments and Researches Station, Faculty of Agriculture, Cairo University, Giza, Egypt during the two winter growing seasons of 2012/2013 and 2013/2014 in order to study the influence of foliar spray with various concentrations of salicylic acid (0, 25, 50, 75, 100 and 150 ppm) on flowering onset, morphological characters of vegetative growth, stem and leaf anatomy, photosynthetic pigments, yield characters and seed quality of Egyptian lupine 'Giza 2'. The obtained results revealed that the median used concentrations of Salicylic acid (SA) induced promotive effect on flowering onset, morphological characters of vegetative growth, photosynthetic pigments and yield characters as well as seed quality of Egyptian lupine 'Giza 2'. The maximum significant promotion was obtained when plants of Egyptian lupine 'Giza 2' were sprayed with 75 ppm SA. This treatment elicited beneficial changes in both vegetative and reproductive characters, which resulted in higher yield of seeds/ plant. These plants were characterized by earliness in flowering and had longer height which developed more primary branches and higher number of pods having more number of seeds of high specific weight and high protein content and low alkaloidal content. Such treatment (75 ppm SA) promoted chlorophyll pigments in leaves and induced favorable changes in anatomical structures of vegetative growth. Foliar application with 75 ppm SA induced prominent increase in stem diameter due mainly to the prominent increases induced in thickness of stem wall and in diameter of hollow pith. The increase in stem wall thickness could be attributed to the induced increase in thickness of epidermis, cortex, fiber strands, phloem and xylem tissues as well as in thickness of parenchymatous area of the pith and in vessel diameter. Likewise, such treatment increased thickness of both midvein and lamina of leaflets of Egyptian lupine 'Giza 2' due to increase induced in thickness of both palisade and spongy tissues as well as to increase induced in size of midvein bundle.



## INTRODUCTION

The Egyptian lupine (*Lupinus termis* Forssk.) is a fabaceous crop which is grown in Egypt for food, medical and industrial purposes. Lupine has a high protein content of their seeds varying between 24.8 to 49.9% (Blanco, 1980). The seeds are used for different foods, from vegan sausages to lupine tofu. Powdered dry seeds are taken against diabetes. The liquid left after soaking the bitter seeds in water is used as parasiticide, emollient of the skin for

scurf, tinea and itch. The alkaloids especially lupanine are occasionally employed as a stomach tonic and as hypnotic to promote sleep (Wallis, 1967). The plant is cultivated for its non-endospermic seeds, which contain alkaloids, protein, oil, cholesterol, lecithin, salts (phosphorus and potassium) and carbohydrates (Ibrahim *et al.*, 1990). Green plants are useful as green-manuring because of the high nitrogenous content (Roberto, 1984).

Salicylic acid (SA) is naturally occurs in plants in very low amounts and participates in the regulation of physiological processes in plant such as stomata closure, nutrient uptake, chlorophyll synthesis, and protein synthesis (Piatelli *et al*., 1969; Khan *et al*., 2003 and Shakirova *et al*. 2003). Salicylic acid is dependent signaling pathways regulate plant responses to both abiotic and biotic stress factors (Rao *et al*. 2000). It has shown many important functions in the plant and can change physiological behavior of plant. Foliar application with relatively low concentrations of salicylic acid also promoted and influenced the growth, development, differentiation of cells and tissues of plants and enhanced the plant's growth parameters (Helgi and Rolfe, 2005). Salicylic acid promotes some physiological processes and inhibiting others depending on its concentration, plant species, development stages and environmental conditions (Senaratna *et al*., 2000). The anti-oxidant capacity and phenolic content of tomato plants was increased at $10^{-4}$ M salicylic acid treatment (Khan *et al*., 2003). Thus salicylic acid could be expected to influence the growth and yield of lupine plants.

Therefore, the present investigation was undertaken to study the impact of spraying salicylic acid on some morphological criteria, anatomy and yield as well as some biochemical constituents of Egyptian lupine 'Giza 2' to improve growth, yield and nutritional value.

## MATERIALS AND METHODS

This study was performed at the Agricultural Experiments and Researches Station, Faculty of Agriculture, Cairo University, Giza, Egypt during the two growing successive seasons of 2012/2013 and 2013/2014 to study the effect of foliar application with salicylic acid (SA) on vegetative growth, yield and its components as well as photosynthetic pigments content in the leaves, protein and alkaloidal contents of lupine seeds and anatomical structure of Egyptian lupine 'Giza 2'.

Seeds of Egyptian lupine 'Giza 2' were secured by courtesy of Field Crops Institute, Agricultural Research Center, Giza, Egypt. The growth promoter salicylic acid ($C_6H_4$ (OH) COOH) was obtained from Merck-Co. Five concentrations; namely, 25, 50, 75, 100 and 150 ppm were used as foliar application in both seasons. Foliar application of salicylic acid was carried out twice; the plants were sprayed with salicylic acid after 30 and 45 days from sowing. Control plants were sprayed with tap water and the volume of the spraying solution was maintained just to cover completely the plant foliage till drip.

Seeds of Egyptian lupine 'Giza 2' were sown on 11 $\underline{\text{th}}$ November 2012 in the first season and replicated on 10 $\underline{\text{th}}$ November 2013 in the second one. The experiment was made in a randomized complete block design with three replicates. The five levels of salicylic acid beside the control required that the experimental land of each replicate be divided into six plots, each contained one treatment. The plot was five ridges, 3.5 meter long, 60 cm apart and the hills were spaced at 25 cm distance. Three seeds were sown in each hill, and the stand was later thinned to one plant per hill. Land preparations, fertilizer application and agricultural operations followed the normal practices of lupine cultivation in the vicinity.

**Recording of data**

Investigations involved data pertaining to flowering onset, morphological characters of vegetative growth and yield components of Egyptian lupine 'Giza 2' as affected by different levels of salicylic acid in both studied seasons. Investigations involved also data pertaining to stem and leaf anatomy and photosynthetic pigments in leaves of the studied cultivar in addition to certain biochemical constituents of seeds yielded from treated and untreated plants of the second seasons 2013/2014.

In each growing season, a random sample of ten plants was assigned for investigation in each plot; *i.e*., a total number of 30 plants were fixed for each treatment. The plants were labeled at the middle region of the plot. Data were recorded on individual plants with respect to flowering onset and thereafter for the yield characters at harvest time. With respect to the data of morphological characters, another five plants were used for investigation in each plot at the age of 100 days from sowing date. The procedure of recording the various data was carried out in the following manner.

**A- Onset of flowering** (date of flowering), expressed as number of days elapsed from sowing till the appearance of the first flower on plant.

**B- Morphological characters of vegetative growth**

1- Plant height (cm).
2- Number of primary branches developed on the main stem/plant.

3- Number of leaves/plant.
4- Total leaf area ($cm^2$) /plant.
5- Shoot dry weight (g)/plant.

**C- Anatomical studies**

It was intended to carry out a comparative microscopically examination on plant material which showed the most prominent response of plant growth to investigated treatments. Specimens of Egyptian lupine 'Giza 2' were taken from the median internode of the main stem as well as from the terminal leaflet of the corresponding leaf. Plants used for examination were taken throughout the second growing season of 2013/2014 at the age of 100 days from sowing date. Specimens from stems and leaves were killed and fixed for at least 48 hrs. in F.A.A. (10ml formalin, 5ml glacial acetic acid and 85ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56ºC, sectioned to a thickness of 20 micrometer (µm), double stained with crystal violet-erythrosine, cleared in xylene and mounted in Canada balsam (Nasser and El-Sahara, 1998). Sections were read to detect histological manifestations of noticeable responses resulted from spraying with salicylic acid compared to control and photomicrography.

**D- Determination of photosynthetic pigments**

Photosynthetic pigments were determined quantitatively in upper most leaves developed on the main stem and on primary branches of treated and untreated plants in the second season of 2013/2014 at the age of 100 days from sowing date. For this purpose, a random sample of two plants was taken for investigation in each plot; *i.e*., a total number of six plants were fixed for each treatment. Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were extracted by using dimethyl form amide and determined according to Narnia (1982) as mg/g fresh weight of lupine leaves.

**E- Yield characters**

1- Number of pods /plant.
2- Number of seeds /plant.
3- Seed yield (g) /plant.
4- Specific weight of seeds (g), using ten random samples from each of the three replicates, each comprised of 100 seeds.

**F- Biochemical studies**

Chemical analysis of seeds (seed quality) was performed at harvest time (150 days from sowing date) on seeds obtained from treated and untreated plants of Egyptian lupine 'Giza 2' in the second growing season of 2013/2014. Percentages of crude protein and total alkaloids were determined as follows:

**1- Determination of crude protein**

Total nitrogen content was determined using the modified micro-Keelhaul method described by Pregl (1945). Nitrogen content of seeds was multiplied by 6.25 to calculate the crude protein content (Anon., 1990).

**2- Determination of total alkaloids**

The method of total alkaloids determination described by Ortiz and Mukherjee (1982) was used after certain modifications (Nasser *et al*., 1991). Lupine seed samples of 10g each was defatted by extraction with n-hexane (4x50ml). The defatted residue was dissolved in chloroform and filtered. The chloroformic filtrate contained the free alkaloid bases (Fraction 1). Subsequently, the combined salts of alkaloids retained in the defatted flakes were converted into free bases by treatment with ammonia solution and extracted with chloroform (Fraction 2). The two chloroformic fractions were combined and evaporated under vacuum till dryness. The produced residue was dissolved in 25ml of N/10 hydrochloric acid and back titrated with N/10 sodium hydroxide solution, using methyl orange as an indicator.

**- Statistical analysis**

Data on flowering onset, morphological characters, yield characters and seed quality were subjected to conventional methods of analysis of variance according to Snedecor and Cochran (1982). The least significant difference (L.S.D.) for each character was calculated at 0.05 level of probability.

## RESULTS AND DISCUSSION

**I- Flowering onset**

Data on flowering onset of Egyptian lupine 'Giza 2' as affected by spraying with various concentrations of salicylic acid (SA) in two growing seasons is given in Table (1).

It is obvious from Table (1) that the control plants recorded a period of 77.9 and 80.3 days from sowing date to flowering onset in the first and second season; respectively which differed significantly with most of tested concentrations of salicylic acid. It is realized that salicylic acid at 25 and 150 ppm showed no significant effect on flowering onset of Egyptian lupine 'Giza 2'. On the other hand, the other three used concentrations (50, 75 and 100 ppm) of salicylic acid shortened significantly the period to flowering. A maximum significant earliness of 6.4 days in the first season and of 7.4 days in the second one was recorded when salicylic acid was sprayed with 100 ppm.

In this connection, Metwally *et al*. (2003) reported that salicylic acid induces flowering earliness increases flower life, retards senescence and increases cell metabolic rate in barley seedlings.

**II - Morphological characters of vegetative growth**

Data presented in Table (1) clearly show that all assigned concentrations of salicylic acid except that of high used one (150 ppm) increased all investigated growth parameters of Egyptian lupine 'Giza 2' in both studied seasons. The most effective concentrations which induced significant increases in growth characters were the two median concentrations of 50 and 75 ppm salicylic acid. In this respect, the maximum significant increase was detected at 75 ppm salicylic acid, being 33.8 and 29.9% for plant height, 34.9 and 36.6% for number of primary branches/plant, 21.0 and 33.3% for number of leaves/plant, 33.8 and 22.3% for total leaf area/plant and 54.9 and 52.2% for shoot dry weight/plant more than those of the control plant in the first and second season; respectively. Worthy to note that the relatively high used concentration of 150 ppm salicylic acid induced significant retardation in all investigated growth characters of Egyptian lupine 'Giza 2' in both studied seasons. The decrements below the control were 10.8 and 9.9% for plant height, 20.9 and 19.5% for number of primary branches/plant, 9.2 and 12.4% for number of leaves/plant, 11.7 and 14.1% for total leaf area/plant and 14.5 and 16.7% for shoot dry weight /plant in the first and second season; respectively.

**Table 1. Flowering onset and morphological characters of vegetative growth of Egyptian lupine 'Giza 2' as affected by foliar spray with different concentrations of salicylic acid in two growing seasons (2012/2013 and 2013/2014)**

| Treatments | Conc. ppm | Flowering onset (days) | Morphological characters | | | | |
|---|---|---|---|---|---|---|---|
| | | | Plant height (cm) | No. of branches /plant | No. of leaves /plant | Total leaf area ($cm^2$)/plant | shoot dry weight (g)/plant |
| First season of 2012/2013 | | | | | | | |
| Control | 0 | 77.9 A | 92.3 D | 4.3 C | 19.5 D | 2364 B | 17.3 E |
| Salicylic acid | 25 | 76.2 AB | 99.8 C | 5.2 B | 20.3 CD | 2554 B | 19.5 D |
| | 50 | 74.5 BC | 110.4 B | 5.6 AB | 22.1 B | 3040 A | 24.4 B |
| | 75 | 73.8 CD | 123.5 A | 5.8 A | 23.6 A | 3162 A | 26.8 A |
| | 100 | 71.5 D | 101.2 C | 5.3 AB | 21.0 C | 2522 B | 22.1 C |
| | 150 | 76.7 AB | 82.3 E | 3.4 D | 17.7 E | 2087 C | 14.8 F |
| L.S.D (0.05) | | 2.4 | 7.5 | 0.53 | 0.96 | 210 | 1.90 |
| Second season of 2013/2014 | | | | | | | |
| Control | 0 | 80.3 A | 90.0 C | 4.1 C | 16.2 D | 2767 B | 18.6 D |
| Salicylic acid | 25 | 80.1 A | 100.6 B | 5.0 B | 18.3 C | 2945 B | 20.8 C |
| | 50 | 76.4 B | 107.3 B | 5.4 AB | 19.7 B | 3304 A | 24.5 B |
| | 75 | 75.5 BC | 116.9 A | 5.6 A | 21.6 A | 3385 A | 28.3 A |
| | 100 | 72.9 C | 104.7 B | 5.3 AB | 20.8 A | 2821 B | 22.3 C |
| | 150 | 80.0 A | 81.1 D | 3.3 D | 14.2 E | 2378 C | 15.5 E |
| L.S.D (0.05) | | 3.1 | 8.3 | 0.50 | 0.87 | 203 | 1.93 |
| Means having the same letter (S) are not significantly different at 0.05 level. | | | | | | | |

In this respect, some investigators confirmed these findings using other different plant species. For instance, Gutierrez *et al*. (1998) on soybean, Gharib (2006) on basil, Iqpal *et al*. (2006) on wheat, El-Mergawi and Abdel-Wahed (2007) on maize and Ali *et al*. (2009) on tomato. They stated, generally, that foliar application with salicylic

acid at relatively low used concentrations promoted significantly plant growth characters of the investigated species; all, being in harmony with the present findings.

**III- Anatomical studies**

**1- Anatomy of the main stem**

Microscopically measurements of certain histological features in transverse sections through the twelfth internode of the main stem of Egyptian lupine 'Giza 2' sprayed with 75 ppm salicylic acid (the most effective concentration induced maximum promotion in vegetative growth) and those of control are presented in Table (2) and illustrated in Figure (1).

It is realized from Table (2) and Figure (1) that foliar application with 75 ppm salicylic acid increased the diameter of the main stem at its twelfth internode by 16.0% over that of the control.

The increment in stem diameter could be attributed mainly to the prominent increase in stem wall thickness and in diameter of hollow pith by 13.2 and 22.1% more than those of the control; respectively.

**Table 2. Measurements in micrometer (µm) of certain histological characters in transverse sections through the twelfth internode of the main stem of Egyptian lupine 'Giza 2' aged 100 days as affected by foliar application with 75 ppm salicylic acid**

| Histological characters | Treatments | | |
|---|---|---|---|
| | Control | 75 ppm salicylic acid | ± % to control |
| Stem diameter | 7544 | 8752 | +16.0 |
| Stem wall thickness | 2569 | 2908 | +13.2 |
| Epidermis thickness | 24 | 27 | +12.5 |
| Cortex thickness | 203 | 244 | +20.2 |
| Fiber strands thickness | 88 | 106 | +20.5 |
| Phloem tissue thickness | 162 | 192 | +18.5 |
| Xylem tissue thickness | 336 | 467 | +39.0 |
| Vessel diameter | 48 | 56 | +16.7 |
| Parenchymatous pith thickness | 1752 | 1863 | +6.3 |
| Hollow pith diameter | 2414 | 2948 | +22.1 |

The increase in stem wall thickness could be attributed mainly to the prominent increases in all included tissues. The thickness of epidermis, cortex, fiber strands, phloem, xylem and parenchymatous area of the pith were increased over those of the control by 12.5, 20.2, 20.5, 18.5, 39.0 and 6.3%; respectively. Likewise, vessel diameter was increased by 16.7% over the control.

In this connection, Maddah *et al*. (2007) working on chickpea stated that foliar application with 0.1 mM salicylic acid increased parenchyma tissue and sclerenchyma tissue in stems as well as increased xylem tissue in roots, being partially in harmony with the present findings.

**2- Anatomy of the leaf**

Microscopically measurements of certain histological characters in transverse sections through the blade of the terminal leaflet of the twelfth compound leaf developed on the main stem of control plants of Egyptian lupine 'Giza 2' and of those sprayed with 75 ppm salicylic acid are presented in Table (3). Likewise, microphotography's illustrating these treatments are shown in Figure (2).

It is obvious from Table (3) and Figure (2) that spraying salicylic acid at concentration of 75 ppm increased thickness of both midvein and lamina of leaflet blades of Egyptian lupine 'Giza 2' by 19.5 and 21.4% more than those of the control; respectively.

It is noted that the increase in lamina thickness was accompanied with 19.1 and 23.5% increments in thickness of palisade and spongy tissues compared with the control; respectively. Results also indicated that the main vascular bundle of the midvein was increased in size as a result of spraying salicylic acid. The increment was mainly due to

the increase in length by 10.3% and in width by 7.1% more than the control. Moreover, xylem vessels had wider cavities, being 14.0% more than the control, which amounted to more total active conducting area to cope with vigorous growth resulting from treatment with 75 ppm salicylic acid.

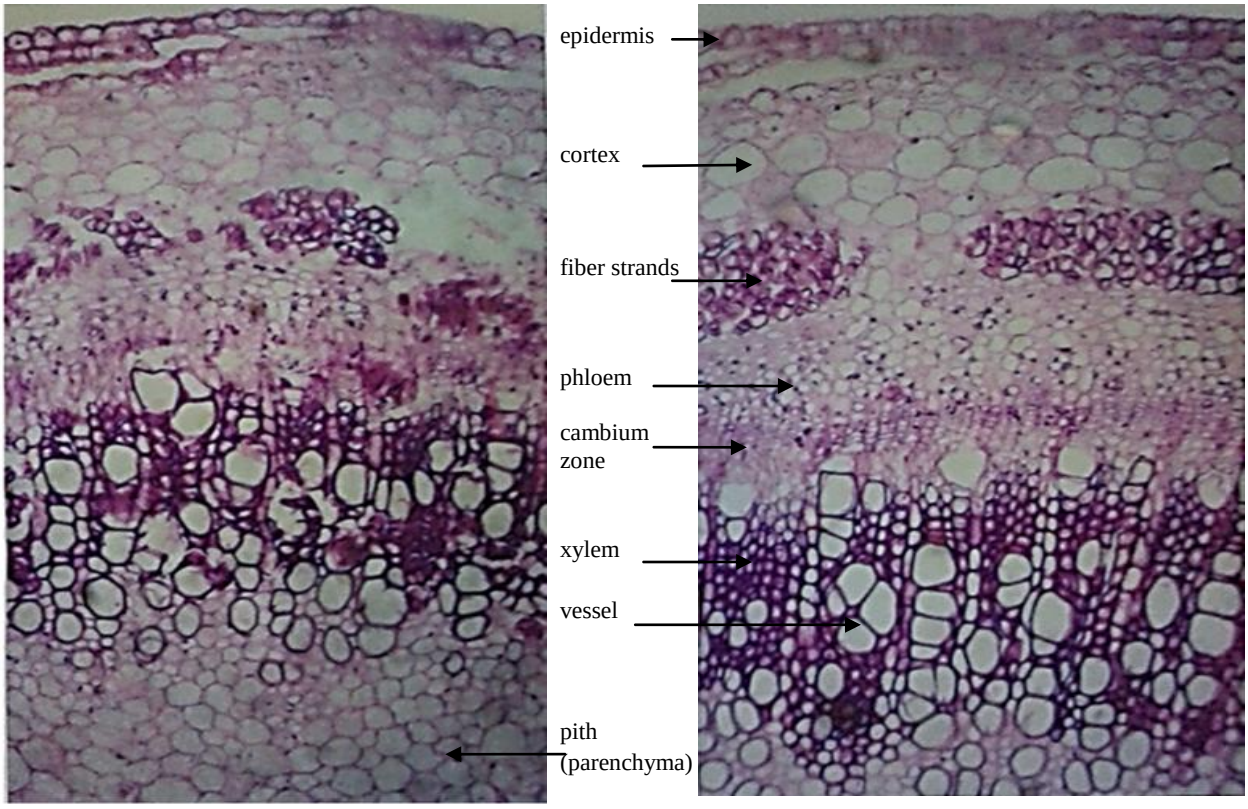


Fig 1. Transverse sections through the twelfth internode of the main stem of Egyptian lupine 'Giza 2' aged 100 days as affected by foliar application with salicylic acid. (X 125)

A- From untreated plant (control).

B- From plant treated with 75 ppm salicylic acid.

**Table 3. Measurements in micrometer (µm) of certain histological characters in transverse sections through the blade of the terminal leaflet of the compound leaf developed on the median portion of the main stem of Egyptian lupine 'Giza 2' at the age of 100 days from sowing date as affected by foliar application with 75 ppm salicylic acid (Means of three sections from three specimens)**

| Histological characters | Treatments | | |
|---|---|---|---|
| | Control | 75 ppm Salicylic acid | ± % to control |
| Thickness of midvein | 522 | 624 | +19.5 |
| Thickness of lamina | 364 | 442 | +21.4 |
| Thickness of palisade tissue | 162 | 193 | +19.1 |
| Thickness of spongy tissue | 136 | 168 | +23.5 |
| Dimensions of midvein bundle: | | | |
| Length | 184 | 203 | +10.3 |
| Width | 226 | 242 | +7.1 |
| Vessel diameter | 15.7 | 17.9 | +14.0 |

In this connection, Nour *et al*. (2012) found that foliar application with 50 ppm salicylic acid on bean plants resulted in thicker leaflets due mainly to the prominent increase induced in thickness of both midvein and lamina. The thicker lamina could be attributed to the increase in thickness of both palisade and spongy tissues. Likewise, midvein bundle was increased in size as a result of spraying salicylic acid. These results are in harmony with the present findings.

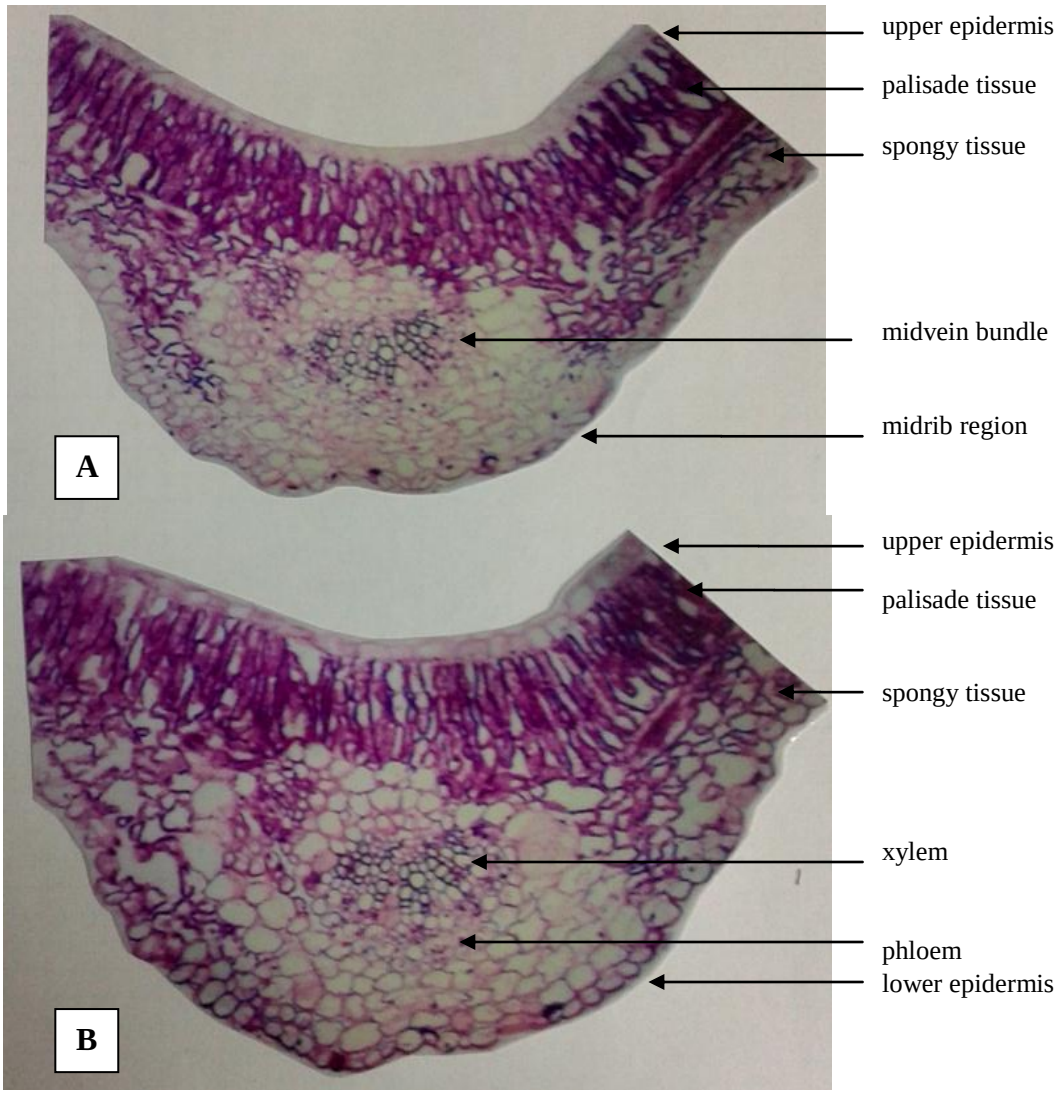


Fig 2. Transverse sections through the blade of terminal leaflet of the compound leaf developed on the median portion of the main stem of Egyptian lupine 'Giza 2' aged 100 days as affected by foliar application with salicylic acid. (X125)

A- From untreated plant (control).

B- From plant sprayed with 75 ppm salicylic acid.

**IV- Photosynthetic pigments**

Data presented in Table (4) reveal that foliar application with salicylic acid at any of the first three assigned concentrations (25, 50 and 75ppm) showed promotive effect on the mean value of chlorophyll a, chlorophyll b and carotenoids in leaves of Egyptian lupine 'Giza 2' compared with the control. By contrast, salicylic acid at the relatively high used concentrations of 100 and 150 ppm inhibited chlorophyll contents in leaves of Egyptian lupine 'Giza 2'.

Claims to the promoting effect of salicylic acid on photosynthetic pigments of lupine leaves were recorded by Haroun *et al* (1998). Other reviewers confirmed these findings using other plant species. For instance, Kord and Hathout (1992) on tomato, Shehata *et al*. (2001) on maize, Iqpal *et al*. (2006) on wheat and Nadeem *et al*. (2012) on fennel. However, Gopal and Surendra (2011) found that salicylic acid induced slight decreases in photosynthetic pigments of wheat leaves.

**Table (4): Photosynthetic pigments (mg/g fresh weight) in leaves of Egyptian lupine 'Giza 2'aged 100 days as affected by foliar application with different concentrations of salicylic acid in the second growing season of 2013/2014**

| Treatments | Conc. ppm | Photosynthetic pigments (mg/g F.W.) | | |
|---|---|---|---|---|
| | | Chlorophyll a | Chlorophyll b | Carotenoids |
| Control | 0 | 3.129 | 1.313 | 1.411 |
| Salicylic acid | 25 | 3.310 | 1.379 | 1.535 |
| | 50 | 3.625 | 1.405 | 1.574 |
| | 75 | 3.770 | 1.531 | 1.615 |
| | 100 | 2.223 | 1.305 | 1.323 |
| | 150 | 2.031 | 1.263 | 1.311 |

**V- Yield characters**

The mean values of yield characters of Egyptian lupine 'Giza 2' as affected by foliar application with different concentrations of salicylic acid in two growing seasons are given in Table (5). The investigated characters included number of pods/plant, number of seeds/plant, specific weight of seeds (average weight of 100 seeds in grams) and yield of seeds (g)/plant.

**Table 5. Yield characters of Egyptian lupine 'Giza 2' as affected by foliar spray with different concentrations of salicylic acid in two growing seasons (2012/2013 and 2013/2014)**

| Treatments | Conc. ppm | Yield characters | | | |
|---|---|---|---|---|---|
| | | Number of pods/plant | Number of seeds/plant | Weight of 100 seeds (g) | Seed yield (g)/plant |
| First season of 2012/2013 | | | | | |
| Control | 0 | 27.3 B | 98.0 C | 30.48 B | 29.87 C |
| Salicylic acid | 25 | 28.9 B | 108.1 C | 32.36 B | 34.98 BC |
| | 50 | 33.5 A | 123.9 B | 32.91 B | 40.77 B |
| | 75 | 36.4 A | 142.7 A | 36.47 A | 52.04 A |
| | 100 | 29.5 B | 107.9 C | 32.83 B | 35.42 BC |
| | 150 | 22.8 C | 76.4 D | 27.55 C | 21.05 D |
| L.S.D (0.05) | | 3.59 | 11.79 | 2.69 | 5.89 |
| Second season of 2013/2014 | | | | | |
| Control | 0 | 29.6 B | 109.3 C | 31.64 B | 34.58 C |
| Salicylic acid | 25 | 31.4 B | 120.6 C | 33.29 B | 40.15 C |
| | 50 | 36.2 A | 138.4 B | 33.85 B | 46.84 B |
| | 75 | 39.1 A | 154.8 A | 36.92 A | 57.15 A |
| | 100 | 32.2 B | 119.7 C | 33.04 B | 39.56 C |
| | 150 | 24.9 C | 88.1 D | 28.71 C | 25.29 D |
| L.S.D (0.05) | | 3.74 | 12.48 | 2.82 | 6.43 |
| Means having the same letter are not significantly different at 0.05 level. | | | | | |

It is clear from Table (5) that the relatively low used concentration of 25 ppm as well as the relatively high used concentration of 100 ppm salicylic acid showed no significant effect on all investigated yield characters of Egyptian lupine 'Giza 2' in both studied seasons. By contrast, the median used concentration of 50 or 75 ppm salicylic acid induced significant increase in all investigated yield characters, except that of specific seed weight (weight of 100 seeds) at treatment of 50 ppm salicylic acid, in both studied seasons. The maximum significant increase in any of the investigated yield characters was detected at 75ppm salicylic acid, being 33.3 and 32.1% for number of pods /plant, 45.6 and 41.6% for number of seeds/plant, 19.7 and 16.7% for specific seed weight and 74.2 and 65.3% for seed yield/plant more than the control in the first and second season; respectively. However, the relatively high used concentration of 150 ppm salicylic acid induced significant decrease in all investigated yield characters in both studied seasons, being 16.5 and 15.9 for number of pods/plant, 22.0 and 19.4% for number of seeds/plant, 9.6 and 9.3% for specific seed weight and 29.5 and 26.9% for yield of seeds /plant less than the control in the first and second season; respectively. In this respect, Haroun *et al*. (1998) agreed that foliar spray with relatively high doses of salicylic acid decreased yield of lupine plant.

Claims to the promoting effect of relatively median concentrations of salicylic acid on yield characters were recorded by Gutierrez *et al*. (1998) on soybean plant. Similar results were also reported by Nadeem *et al*. (2012) on fennel plant; all, being in accordance with the present findings.

**VI- Seed quality**

Chemical analysis was performed on mature dried seeds, at harvest time of the second season, of Egyptian lupine 'Giza 2' as affected by foliar application with various concentrations of salicylic acid. For each treatment, the percentages of crude protein and total alkaloids were determined to disclose the qualitative changes in Egyptian lupine seeds as a result of spraying plants with salicylic acid.

The percentages of these fractions in seeds of treated and untreated plants of Egyptian lupine 'Giza 2' are presented in Table (6).

**1- Crude protein**

It is realized from Table (6) that foliar application with salicylic acid at any of the assigned concentrations (25, 50, 75, 100 and 150 ppm) increased significantly the percentage of crude protein in seeds of Egyptian lupine 'Giza 2'. The highest percentage of crude protein (28.5%) was recorded in seeds of lupine plants which sprayed with 75 ppm salicylic acid, being 22.53% more than crude protein in seeds of untreated plants which recorded a percentage of 23.26% crude protein. In this connection, Sanaa *et al*. (2001) reported that the foliar spray of broad bean with salicylic acid increased total protein content in leaves and fruits. Also, Ali *et al*. (2009) noticed that spraying tomato plants with salicylic acid at 100 ppm increased total protein content in leaves and fruits; all, being in harmony with the present findings.

**Table 6. Percentages of crude protein and total alkaloids in mature dried seeds of Egyptian lupine 'Giza 2' as affected by foliar application with different concentrations of salicylic acid in the second growing season of 2013/2014**

| Treatments | Conc. (ppm) | Crude protein % | Total alkaloids % |
|---|---|---|---|
| Control | 0 | 23.26 | 5.2 |
| Salicylic acid | 25 | 25.33 | 5.0 |
| | 50 | 28.35 | 4.7 |
| | 75 | 28.50 | 4.3 |
| | 100 | 27.66 | 4.6 |
| | 150 | 26.25 | 4.8 |
| L.S.D (0.05) | | 1.3 | 0.30 |

**2- Total alkaloids**

It is clear from Table (6) that the low used concentration of 25 ppm salicylic acid showed no significant effect on the percentage of total alkaloids in seeds of Egyptian lupine 'Giza 2'. By contrast, foliar application with salicylic acid at any concentrations of 50, 75, 100 and 150 ppm induced significant decrease in the percentage of total alkaloids in seeds of Egyptian lupine 'Giza 2'. The maximum decrease in total alkaloids content was recorded at 75 ppm salicylic acid, being 17.31% less than total alkaloids in seeds of untreated plants.

As far as the authors are aware, previous information about the effect of foliar application with salicylic acid on total alkaloids percentage in seeds of lupine plants are not available.

## REFERENCES


Ali, A. A.; Ali, T. B. and Nour, K. A. M. (2009). Antioxidants and some natural compounds applications in relation to tomato growth, yield and chemical constituents. Ann. Agric. Sci., Moshtohor, 47 (4): 469-477.

Anon., (1990). Official methods of analysis of the association of the official analytical chemists (A.O.A.C.).15th Edit., Published by A.O.A.C., Washington, D.C., USA.

Blanco, O.G. (1980). Genetic variability of Tarwi (*Lupinus mutabilis*). Proc. 1st. Int. Lupine Conf., Lima (Peru), p. 34-49.

El-Mergawi, R. and Abdel-Wahed, M. (2007). Diversity in salicylic acid effects on growth criteria and different indole acetic acid forms among faba bean and maize. International Plant Growth Substances Association.19 th Annual meeting, Puerto Vallarta, Mexico. pp: 21-25.

Gharib, F.A. (2006). Effect of salicylic acid on the growth, metabolic activities and oil content of basil and marjoram. Int. J. Agr. Biol. 4: 485-492.

Gopal, S. K. and Surendra, S. C. (2011). Changes in growth, pigment content and antioxidants in the root and leaf tissues of wheat plants under the influence of exogenous salicylic acid. Brazilian Journal of Plant Physiology. vol. 23 no. 3.

Gutierrez-Coronado, M.A.; Trejo-Lopez, C. S. and Karque-Saavedra, A. (1998). Effect of salicylic acid on the growth of roots and shoots in soybean. Plant Physiol. Biochem. 36(8): 563.

Haroun, S.A.; Al-desuqy, H.S.; Shukry, W.M. and Gaber, A.M. (1998). Regulation of growth and metabolism in *lupinus termis* plant by sodium salicylate. Egypt J. Physiol. Sci. 22:75-79.

Helgi- Öpik, S. and Rolfe, A. (2005). The physiology of flowering plants. Cambridge University Press Plant Physiology: pp. 191.

Iqpal, M.; Ashraf, M.; Jamil, A. and Shafiq, U.R. (2006). Does seed priming in L. duce changes in the levels of some endogenous plant hormones in hexaploid wheat plant under salt stress. J. Plant Biol. 48(2): 181-189.

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

Khan, W.; Balakrishnan, P. and Smith, D.L. (2003). Photosynthetic responses of corn and soybean to foliar application of salicylate. J. Plant Physiol.160 (5): 485- 492.

Kord, M. and Hathout, T. (1992). Changes on some growth criteria, metabolic activities and endogenous hormones in tomato plants consequent to spraying with different concentrations of salicylic acid. Egypt. J. Physiol. Science 16: 117.

Maddah, S.M.; Falahian, F. A.; Sabaghpour, S. H. and Chalabian, F. (2007). Effect of salicylic acid on yield, yield components and anatomical structures of Chickpea (*Cicer arietinum* L.). Journal of Sciences, V. 16 (62): 61-70.

Metwally, A.; Finkemeier, I.; Georgi, M. and Dietz, K. (2003). Salicylic acid alleviates the cadmium toxicity in barley seedlings. Plant Physiol. 132: 272-281.

Nadeem, H. M.; Masroor, A. K.; Moinuddin, M.I. and Tariq, A. (2012). Exogenous salicylic acid stimulates physiological and biochemical changes to improve growth, yield and active constituents of fennel essential oil. Plant growth Regulation, Vol. 68 (11): 281-291.

Nour, K. A. M; Mansour, N. T. S. and Eisa, G. S.A. (2012). Effect of some antioxidants on some physiological and anatomical characters of snap bean plants. New York Science Journal; 5 (5): 1-9.

Nassar, M.A. and El-Sahhar, K.F. (1998). Botanical preparations and microscopy (Microtechnique). Academic Bookshop, Dokki, Giza, Egypt, 219 pp. (In Arabic).

Nassar, M.A.; Taha, K.F.; Nofal, F.H.; Abdel-Aal, M.S. and Hassan, H. R. (1991). Morphological, histological and phytochemical studies on mutations induced in *Lupinus termis* Forssk. by chemical mutagens. xx11 Conf. of Pharmaceutical Sci., Egypt, p. 309-335.

Nornai, R. (1982). Formulae for determination of chlorophylls pigments extracted with N.N. Dimethyl formamide. Plant Physiol., 69: 1371-1381.

Ortiz, J.G. and Mukherjee, K.D. (1982). Extraction and determination of alkaloids and oil from bitter lupine seeds. J. Am. Oil Chem. Soc., 59 (5): 241-244.

Piatelli, M.; Denicola, M. and Castrogiovanni, V. (1969). Photo control of maranthin synthesis in *Amaranthus tricolor*. Phytochem 8: 731-736.

Pregl, F. (1945). Quantitative organic microanalysis. 4th Edit. J. and A. Churchill Ltd., London.

Rao, M.V.; Lee, H. I.; Creelman, R. A.; Mullet, J. A. and Davis, K.R. (2000). Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. Plant Cell 12:1633-1646.

Roberto, C. (1984). The macdonald encyclopedia of medicinal plants, Mac Donald, London, pp: 447.

Sanaa, A. M. Z.; Ibrahim, S. I. and Eldeen, H. M. S. (2001). The effect of naphthalene acetic acid (NAA), salicylic acid on growth, fruit setting, yield and some correlated components in dry bean. Ann. Agric. Sci., Cairo. 46 (2):451 463.

Senaratna, T.; Touchell, D. H.; Bunn, E. and Dixon, K.W. (2000). Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. J. Plant Growth Regul. 30: 157-161.

Shakirova, F. M.; Sakhabutdinova, A. R.; Bezrukova, M.V.; Fathkutdinova, R.A. and Fatkhutdinova, D.R. (2003).Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Sci. 164:317.

Shehata, S.A.M.; Ibrahim, S.I. and Zaghlool, S.A.M. (2001). Physiological response of flag leaf and ears of maize plant induced by foliar application of kinetin and acetyl salicylic acid (ASA). Ann. Agric. Sci. Ain Shams Univ. Cairo, 46(2): 435-49.

Snedecor, G.W. and Cochran, W.G. (1982). Statistical Methods. The Iowa State Univ., 7th Ed., 507 pp.

Wallis, T.E. (1967). Text of Pharmacogognosy. 5th Edn., J. and A. Churchill Ltd., London.