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RESPONSE OF EGYPTIAN LUPINE PLANT TO TREATMENT WITH STIGMASTEROL

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ABSTRACT

Field experiments were performed at the Agricultural Experiments and Researches Station, Faculty of Agriculture, Cairo University, Giza, Egypt during the two growing seasons of 2005/2006 and 2006/2007 to study the effect of foliar application with stigmasterol at various concentrations of 0, 20, 40, 80 and 160 ppm on flowering onset as well as on morphological, anatomical and yield characters of Egyptian lupine cv. Giza 2.

Results obtained could be summarized in the following:

1- Stigmasterol at relatively low used concentration of 20 ppm showed no significant effect on flowering onset of Egyptian lupine cv. Giza 2. While, the other three assigned concentrations (40, 80 and 160 ppm) of stigmasterol shortened significantly the period to flowering in both studied seasons without significant differences among them.

2- Stigmasterol at relatively low used concentration of 20 ppm or at relatively high used concentration of 160 ppm showed no significant effect on all studied morphological characters in the two investigated seasons. Whereas, foliar application with any of the two assigned median concentrations of 40 and 80 ppm stigmasterol induced significant promotive effects on plant height, main stem length and number of internodes of the main stem. While, number of branches was increased significantly when stigmasterol was sprayed only with 80 ppm in both studied seasons.

3- Stigmasterol at any of the two adopted median concentrations of 40 and 80 ppm had a significant promotive effect on yield and most of yield components (number of pods and seeds per plant) of Egyptian lupine cv. Giza 2 in the two growing seasons of investigation.

4- The highest percentage of crude protein (31.94%) was recorded in seeds of lupine plants sprayed with 80 ppm stigmasterol (the control recorded 26.47% crude protein in their seeds). By contrast, the lowest percentage of total alkaloids (3.68%) was recorded in seeds of lupine plants sprayed with 80 ppm stigmasterol (the control recorded 4.86% alkaloids in their seeds).

5- The increase in stem diameter of Egyptian lupine cv. Giza 2 due to foliar application with 80 ppm stigmasterol could be attributed to the prominent increase in stem wall thickness as well as in diameter of hollow pith. The increase in stem wall thickness could be attributed to the prominent increase in all included tissues (the thickness of epidermis, cortex, fiber strands, vascular tissue and parenchymatous area of the pith). Likewise, spraying stigmasterol at concentration of 80 ppm increased thickness of both midvein and lamina of leaflet blades of Egyptian lupine cv. Giza 2. The increase in lamina thickness was accompanied with increments in thickness of palisade and spongy tissues. Also, the main vascular undle of the midvein was increased in size as a result of spraying stigmasterol.

Keywords: Egyptian lupine , *Lupinus termis* Forssk. , Stigmasterol, Morphology, Anatomy , Yield.

INTRODUCTION

Fabaceae (pea family) consists of about 440 genera and 12000 species. It ranks second only to the Poaceae (grass family) in agricultural importance (Cronquist, 1981). The family is of considerable as a source of high – protein food, oil and forage as well as ornamentals and other uses.

Lupinus is a genus of the family consists of about 200 species (Cronquist, 1981 and Jones and Luchsinger, 1987). Martin *et al*. (1976) stated that l*upines* have been known for 2000 years. They are chiefly cultivated in the U.S.S.R. and Poland. Täckholm (1974) found that 4 species of *Lupinus* are included in the Egyptian Flora, *Lupinus termis* Forssk. (Egyptian lupine) is one of them. Boulos (1983) stated that Egyptian lupine could be used in many different aspects. Seeds are vermifuge. The liquid left after soaking the bitter seeds in water is used as parasiticide, emollient of the skin for scurf, tinea and itch. Cataplasm of seeds is emollient and resolvent. Powdered dry seeds are taken against diabetes.

Recently, more attention has been given to lupines because of the high protein content of their seeds. It was found that protein content of lupine seeds varying between 24.8 to 49.9% (Blanco, 1980). This is more than comparable with the protein level in soybean and superior to that in other major temperate legumes (Hill, 1986). Lupines adapt easily to poor soils and dry climates. Researches to promote their cultivation and to improve their nutritive qualities have increased in Europe, South America and Australia (Lopez-Bellido and Fuentes, 1986).

Likewise, increase of lupine yield in Egypt is highly recommended to meet the demand of human needs and livestock. Plant growth and development is known to be under the control of extremely minute quantity of endogenous hormones produced within the plant. Recently, a great attention has been focused on the possibility of using natural and safety substances in order to improve plant growth, flowering and fruit setting. Stigmasterol is considered as one of the mostly free or conjugated sterols that plays essential functions in plant growth such as structure component of lipid core of cell membrane and biogenetic precursor of numerous metabolites including steroid hormones (Gnus, 1978). It promoted assimilation of C14 in leaves and successive translocation of the assimilate into rice panicles (Fujii *et al*., 1991). Brassinolide and many other related compounds have been found to be widely distributed in the plant kingdom and have many effects on plant growth and development (Cutler *et al*., 1991). It was found that brassinosteroid caused pronounced elongation of hypocotyls, epicotyls and peduncles of dicots as well as coleoptile and mesocotyls of monocots (Sasse, 1991). Brassinosteroid promoted cell wall formation and resulted in hyperpolarization of cell membranes and accelerated growth cycle (Clouse and Sasse, 1998). In this regard, Abd-El-Wahed *et al*. (2000 and 2001) and Abd-El-Wahed (2001) reported that sitosterol had a stimulatory effect on growth, yield, photosynthetic pigments and some chemical composition of wheat and maize plants. Also, Ali *et al*. (2002) stated that stigmasterol had promotive effect on growth, yield and structure of rice plants. Likewise,

Nassar (2004) found that stigmasterol induced promotive effect on vegetative growth, anatomical structure and yield components as well as seed quality of soybean.

Therefore, the present investigation is an attempt to bring to light more information about the influence of foliar application with stigmasterol on morphological, anatomical and yield characters of Egyptian lupine.

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 seasons of 2005/2006 and 2006/2007.

Seeds of Egyptian lupine cv. Giza 2 were secured by courtesy of Legume Research Department, Field Crops Institute, Agricultural Research Center, Giza, Egypt.

The growth promoter stigmasterol (Stigmasta- 5, 22- Diene- 3B- OL; (24S) 24 Ethylcholesta-5,22-Diene-3B-OL) was obtained from Merck-Co. Four concentrations; namely, 20, 40, 80 and 160ppm were used as foliar application. The control plants were sprayed with tap water.

Field work procedure:

Seeds of the Egyptian lupine cv. Giza 2 were sown on 16 th November 2005 in the first season and replicated on 20th November 2006 in the second one to provide the experimental plant materials. The experiment was made in a randomized complete block design with three replicates. The four levels of stigmasterol beside the control required that the experimental land of each replicate be divided into five plots, each contained one treatment. The plot was five ridges, 3.5 meter long, 60 cms apart and the hills were spaced at 20 cms 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.

Stigmasterol concentrations were applied by means of an atomizer sprayer at the age of 45 days from sowing date; i.e., the age of 8-9 leaf stage. Volume of spraying solution was 2.5 liters per plot. This volume was adequate to wet the plants of the plot thoroughly, and excess solution was dripping.

Recording of data:

Investigations involved data pertaining to flowering onset, morphology and yield performance of Egyptian lupine cv. Giza 2 as affected by different levels of stigmasterol in both studied seasons. Investigation involved also data pertaining to stem and leaf anatomy of the studied cultivar in addition to certain biochemical constituents of seeds yielded from treated and untreated plants of the second season 2006/2007.

A random sample of ten plants was assigned for investigation in each plot; i. e., a total number of 30 plants was fixed for each treatment. The plants

were labelled at the middle region of the plot. Data were recorded on individual plants with respect to flowering onset, and thereafter for the morphological and yield characters at harvest time (around mid May in both seasons). 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:

1- Plant height (cm).
2- Length of the main stem (cm).
3- Number of internodes developed by the main stem.
4- Number of primary branches developed on the main stem.

C- Yield characters:

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

D- Biochemical studies:

Chemical analysis of seeds (seed quality) was performed at harvest time on seeds obtained from untreated and treated plants of Egyptian lupine cv. Giza 2 in the second season. 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-Kjeldhahl 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 (Nassar *et al*., 1991). Lupine seed samples of 10 g each was defatted by extraction with n-hexane (4x50 ml). The defatted residue was dissolved in chloroform and filtered. The chloroformic filtrate contained the free alkaloidal 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 25 ml of N/10 hydrochloric acid and back titrated with N/10 sodium hydroxide solution, using methyl orange as an indicator.

E- Anatomical studies:

It was intended to carry out a comparative microscopical examination on plant material which showed the most prominent response of plant growth to investigated treatments. Specimens of Egyptian lupine cv. Giza 2 were taken from the tenth internode which resembled the median internode of the main stem as well as from the median leaflet of the corresponding leaf. Plants used for examination were taken throughout the second season of 2006/2007 at the age of three months (flowering stage). Specimens 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 microns, double stained with crystal violet-erythrosin, cleared in xylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998). Sections were read to detect histological manifestations of noticeable responses resulted from spraying with stigmasterol compared to control and photomicrographed.

Statistical analysis:

Data on flowering, morphological and yield characters as well as on 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:

Flowering onset of the investigated Egyptian lupine cv. Giza 2 was expressed as number of days elapsed from sowing of seeds up to appearance of the first flower on plant; i. e., opening of the first flower bud developed by the inflorescence of the main stem.

Data on flowering onset of Egyptian lupine cv. Giza 2 as affected by spraying with various levels of stigmasterol in two growing seasons are given in Table (1).

Table (1): Flowering onset and morphological characters of Egyptian lupine cv. Giza 2 as affected by foliar application with different levels of stigmasterol in two growing seasons (2005/2006 and 2006/2007)

Treatments Conc. ppm	Flowering onset (days)		Morphological characters							
			Plant height (cm)		Main stem length (cm)		Number of internodes / main stem		Number of branches / plant	
	First season	Second season	First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control 0	77.9	81.3	89.5	96.7	32.8	33.4	20.4	21.2	3.3	3.2
Stigmasterol 20	78.2	80.6	92.7	95.1	34.6	33.9	20.3	21.5	3.2	3.1
40	74.5	77.1	108.4	113.8	41.7	42.5	21.6	22.8	3.6	3.5
80	73.8	76.4	117.9	125.3	48.1	49.7	22.8	23.9	4.2	4.1
160	75.7	77.9	95.2	101.6	35.5	36.3	20.5	21.7	3.4	3.3
L.S.D(0.05)	2.4	2.1	8.7	9.9	5.2	5.7	0.87	0.96	0.52	0.55

It is obvious from Table (1) that the untreated plants recorded a period of 77.9 and 81.3 days from sowing date to flowering onset in the first and second seasons; respectively which differed significantly with most of

tested concentrations of stigmasterol. It is realized that stigmasterol at 20ppm showed no significant effect on flowering onset of Egyptian lupine cv. Giza. On the other hand, the other three used concentrations (40, 80 and 160 ppm) of stigmasterol shortened significantly the period to flowering without significant differences among them. A maximum significant earliness of 4.1 days in the first season and of 4.9 days in the second one was recorded when stigmasterol was sprayed with 80 ppm.

As far as the authors are aware, previous information about the effect of spraying various levels of stigmasterol on flowering onset of Egyptian lupine or of any other pulse crop are not available in the literature.

II- Morphological characters:

Data on morphological characters of Egyptian lupine cv. Giza 2 as affected by spraying with different concentrations of stigmasterol in two growing seasons are presented in Table (1). The investigated characters included plant height (cm), length of the main stem (cm), number of internodes of the main stem and number of branches per plant.

1- Plant height:

Data presented in Table (1) reveal that the relatively low used concentration of 20 ppm stigmasterol as well as the relatively high used concentration of 160 ppm stigmasterol showed no significant effect on height of Egyptian lupine plant cv. Giza 2 in both studied seasons. At the same time, application of stigmasterol at any of the two assigned median concentrations (40 and 80 ppm) induced significant increase in plant height of Egyptian lupine cv. Giza 2 and the difference between these two median concentrations proved significant in both studied seasons. The maximum height was recorded at 80 ppm stigmasterol, being 31.7 and 29.6% more than height of untreated plants in the first and second season; respectively.

2- Length of the main stem:

It is clear from Table (1) that the length of the main stem of Egyptian lupine cv. Giza 2 was increased with increasing the sprayed concentration of stigmasterol up to 80 ppm. The significant increase in the main stem length was detected at the relatively median used concentration of 40 or 80 ppm stigmasterol in both studied seasons. The maximum increase in main stem length occurred at 80 ppm stigmasterol, being 46.7 and 48.8% more than control in the first and second season; respectively. Worthy to note that, the difference between the two used median concentrations (40 and 80 ppm) of stigmasterol proved significant in this respect. On the other hand, it is obvious that the relatively high used concentration of 160 ppm stigmasterol like the relatively low used concentration of 20 ppm stigmasterol showed no significant effect on length of the main stem of Egyptian lupine cv. Giza 2 and the difference between the low and high used concentration proved insignificant in both studied seasons.

3- Number of internodes of the main stem:

Results given in Table (1) indicate that foliar application with stigmasterol at relatively low used concentration of 20 ppm or at relatively

high used concentration of 160 ppm showed no statistical effect on number of internodes of the main stem of Egyptian lupine cv. Giza 2 in both studied seasons. By contrast, foliar application with stigmasterol at any of the two used median concentrations (40 and 80 ppm) increased significantly number of internodes of the main stem of Egyptian lupine cv. Giza 2 with significant difference between these two median concentrations in both studied seasons. The highest number of 22.8 internodes in the first season and of 23.9 internodes in the second one was recorded at treatment of 80 ppm stigmasterol, being 11.8 and 12.7% more than number of internodes of the main stem of untreated plant of Egyptian lupine cv. Giza 2 in the first and second season; respectively.

4- Number of primary branches per plant:

It is realized from Table (1) that the untreated plants of Egyptian lupine cv. Giza 2 recorded a mean number of 3.3 and 3.1 branches per plant in the first and second season; respectively which proved significant difference with only the second used median concentration of 80 ppm stigmasterol. Whereas, the relatively low used concentration of 20 ppm as well as the first used median concentration of 40 ppm and the relatively high used concentration of 160 ppm stigmasterol showed no significant effect on number of primary branches developed on the main stem of Egyptian lupine cv. Giza 2 in both studied seasons. It is obvious that foliar application with stigmasterol at concentration of 80 ppm increased significantly number of branches. The highest number of 4.2 and 4.1 branches per plant in the first and second season; respectively was recorded at treatment of 80 ppm stigmasterol, being 27.3 and 32.3% more than number of branches developed per untreated plant of Egyptian lupine cv. Giza 2 in the first and second season; respectively.

From the aforementioned results concerning the effect of foliar application with different concentrations of stigmasterol on morphological characters of Egyptian lupine cv. Giza 2, it could be stated generally that stigmasterol at relatively low used concentration of 20 ppm or at relatively high used concentration of 160 ppm showed no significant effect on all studied morphological characters in the two growing seasons of study. On the other hand, foliar application with any of the two assigned median concentrations of 40 and 80 ppm stigmasterol induced significant promotive effects on plant height, main stem length and number of internodes of the main stem, while number of branches was increased significantly when stigmasterol was sprayed only with 80 ppm in both studied seasons.

The present results are in harmony with those reported by Krizek and Mandava (1982) on bean plants and by Braun and Wild (1984) on wheat and mustard plants as well as by Wang and Wang (1997), Chon *et al.* (2000) and Ali *et al.* (2002) on rice plants. Likewise, Abd-El-Wahed *et al.* (2000) confirmed these findings on maize plants. Also, Nassar (2004) reached to similar conclusion on soybean plants.

III- Yield characters:

The mean values of yield characters of Egyptian lupine cv. Giza 2 as affected by foliar application with various concentrations of stigmasterol in two seasons are given in Table (2).

1- Number of pods per plant:

Results presented in Table (2) reveal that foliar application with stigmasterol at relatively low used concentration of 20 ppm or at relatively high used concentration of 160 ppm showed no significant effect on number of pods per plant of Egyptian lupine cv. Giza 2 in both studied seasons. At the same time, foliar application with stigmasterol at any of the two used median concentrations (40 and 80 ppm) increased significantly number of pods per plant of Egyptian lupine cv. Giza 2 with significant difference between these two median concentrations in both studied seasons. The highest number of pods (36.8 and 38.3 pods in the first and second season; respectively) was detected at 80 ppm stigmasterol, being 36.8 and 35.3% more than the number of pods per untreated plant (26.9 and 28.3 pods) in the first and second season; respectively.

Table (2): Yield characters of Egyptian lupine cv. Giza 2 as affected by foliar application with different levels of stigmasterol in two growing seasons (2005/2006 and 2006/2007)

Treatments Conc. ppm		Number of pods/plant		Number of seeds/ plant		Yield of seeds (g)/plant		Weight of 100 seeds (g)	
		First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control	0	26.9	28.3	103.6	109.2	30.7	34.1	29.6	31.2
Stigmasterol	20	27.2	28.6	106.4	112.4	31.8	35.3	29.9	31.4
	40	32.5	33.9	128.7	134.9	40.0	43.8	31.1	32.5
	80	36.8	38.3	154.2	152.8	48.1	49.9	31.2	32.7
	160	28.1	29.7	111.3	116.1	33.2	36.6	29.8	31.5
L.S.D. (0.05)		3.2	3.6	10.8	12.5	4.8	5.3	N.S	N.S

2- Number of seeds per plant:

It is clear from Table (2) that the effect of foliar application with stigmasterol on number of seeds per plant of Egyptian lupine cv. Giza 2 showed the same trend that previously mentioned about the effect of stigmasterol on number of pods per plant. It is noted that the relatively low used concentration of 20 ppm stigmasterol as well as the relatively high used concentration of 160 ppm stigmasterol showed no statistical effect on number of seeds per plant of Egyptian lupine cv. Giza 2 in both studied seasons. Whereas, application of stigmasterol at any of the two used median concentrations (40 and 80 ppm) induced significant increase in number of seeds per plant and the difference between these two used concentrations

proved significant in both studied seasons. The highest number of 154.2 seed in the first season and of 152.8 seeds in the second one was recorded at treatment of 80 ppm stigmasterol, being 48.8 and 39.9% more than number of seeds per untreated plant in the first and second season; respectively.

3- Specific weight of seeds (average weight of 100 seeds):

Data presented in Table (2) clearly show that foliar application with stigmasterol at any of the four assigned concentrations (20, 40, 80 and 160ppm) had no significant effect on specific weight of seeds of Egyptian lupine cv. Giza 2 in both studied seasons.

4- Yield of seeds per plant:

It is noted from Table (2) that seed yield per plant of Egyptian lupine cv. Giza 2 was increased with increasing the concentration of stigmasterol up to 80 ppm. The significant increase in seed yield was recorded at the relatively median tested concentration of 40 or 80 ppm stigmasterol in both studied seasons. The maximum significant increase in seed yield was obtained at 80 ppm stigmasterol, being 56.9 and 46.7% more than seed yield per untreated plant in the first and second season; respectively. Worthy to note that the difference between the two sprayed median concentrations (40 and 80 ppm stigmasterol) was significant in this respect. By contrast, it is obvious that the relatively high used concentration of 160 ppm stigmasterol like the relatively low used concentration of 20 ppm stigmasterol showed no significant effect on seed yield per plant of Egyptian lupine cv. Giza 2 in both studied seasons.

From the above mentioned results concerning the effect of foliar application with different concentrations of stigmasterol on yield characters of Egyptian lupine cv. Giza 2, it could be stated that seed yield per plant being the outcome of various components; i. e., number of pods per plant, number of seeds per plant and specific weight of seeds showed high response to investigated treatments except that of specific weight of seeds which was not affected. It is realized that stigmasterol at any of the two assigned median concentrations of 40 and 80 ppm had a significant promotive effect on yield and most of yield components (number of pods and number of seeds per plant) of Egyptian lupine cv. Giza 2 in the two growing seasons of investigation. These results are generally in accordance with those recorded by Abd-El-Wahed *et al*. (2000) on maize plants and by Ali *et al*. (2002) on rice plants as well as by Nassar (2004) on soybean plants.

IV- Seed quality:

Chemical analysis was performed on mature dried seeds, at harvest time of the second season, of Egyptian lupine cv. Giza 2 as affected by different concentrations of stigmasterol. For each treatment, chemical analysis was done to determine the percentage of crude protein and of total alkaloids. Such quantitative determinations were used to disclose the qualitative changes in Egyptian lupine seeds as a result of spraying plants with stigmasterol.

The percentages of these fractions in seeds of treated and untreated plants of Egyptian lupine cv. Giza 2 are given in Table (3).

1- Crude protein:

Data presented in Table (3) indicate that the relatively low used concentration of 20 ppm stigmasterol showed no significant response on the percentage of crude protein in seeds of Egyptian lupine cv. Giza 2. At the same time, foliar application with stigmasterol at any of the two assigned median concentrations (40 and 80 ppm) and at the relatively high used concentration of 160 ppm increased significantly the percentage of crude protein in seeds of Egyptian lupine cv. Giza 2. The highest percentage of crude protein (31.94%) was recorded in seeds of lupine plants sprayed with 80 ppm stigmasterol, being 20.7% more than crude protein in seeds of untreated plants which recorded a percentage of 26.47% crude protein.

Table (3): Percentages of crude protein and total alkaloids in mature dried seeds of Egyptian Lupine cv. Giza 2 as affected by foliar application with different levels of stigmasterol in the second season (2006/2007)

Biochemical analysis	Stigmasterol treatments (ppm)					L.S.D. (0.05)
	Control 0	20	40	80	160	
Crude protein %	26.47	27.93	30.52	31.94	29.87	1.52 %
Total alkaloids %	4.86	4.75	3.94	3.68	4.13	0.46 %

In this connection, Abd-El-Wahed *et al*. (2000) stated that foliar application with stigmasterol at concentration of 20, 40 and 80 ppm on maize plants increased the percentage of crude protein in grains of treated plants. Likewise, Ali *et al.* (2002) using stigmasterol at concentrations of 50, 100 and 150 ppm on rice plants found that total protein content of rice grains were increased with all stigmasterol concentrations especially at treatments with higher concentrations of 100 and 150 ppm. Also, Nassar (2004) using stigmasterol at concentrations of 25, 50, 100 and 200 ppm on soybean plants found that foliar application with stigmasterol at any of the two assigned median concentrations (50 and 100 ppm) increased significantly the percentage of crude protein in seeds of soybean cv. Giza 2.All, being generally in accordance with the present findings.

2- Total alkaloids:

It is clear from Table (3) that the relatively low used concentration of 20 ppm stigmasterol showed no significant effect on the percentage of total alkaloids in seeds of Egyptian lupine cv. Giza 2. By contrast, foliar application with stigmasterol at any of the two tested median concentrations of 40 and 80 ppm as well as at the relatively high used concentration of 160 ppm induced significant decrease in the percentage of total alkaloids in seeds of Egyptian lupine cv. Giza 2. Worthy to note that the differences among these used three concentrations (40, 80 and 160 ppm) proved insignificant in this respect. The maximum decrease in total alkaloids content was recorded at 80 ppm stigmasterol, being 24.3% less than total alkaloids in seeds of untreated plants.

As far as the authors are aware, previous information about the effect of spraying various concentrations of stigmasterol on alkaloidal content in seeds of Egyptian lupine or of any other species of the genus *Lupinus* are not available in the literature.

V- Anatomical studies:

1- Anatomy of the main stem:

Microscopical measurements of certain histological characters of the tenth internode which resembled the median internode of the main stem of Egyptian lupine cv. Giza 2 sprayed with 80 ppm stigmasterol and those of control are given in Table (4). Likewise, microphotographs illustrating these treatments are shown in Figure (1).

It is obvious from Table (4) and Figure (1) that foliar application with stigmasterol at concentration of 80 ppm increased the diameter of the main stem at its median portion (at the tenth internode) of Egyptian lupine cv. Giza 2 by 14.7% more than that of the control. The increase in stem diameter, due to foliar application with 80 ppm stigmasterol, could be attributed to the prominent increase in stem wall thickness as well as in diameter of hollow pith by 17.2 and 9.2% over the control; respectively.

Table (4): Measurements in microns of certain histological characters in transverse sections through the middle part of the tenth internode of the main stem of Egyptian lupine cv. Giza 2, at the age of three months, as affected by foliar application with 80 ppm stigmasterol (Means of three sections from three specimens)

Characters	Treatments		
	Control (0)	80 ppm Stigmasterol	± % to control
Stem diameter	8694.2	9968.6	+14.66
Stem wall thickness	2975.8	3486.4	+17.16
Epidermis thickness	26.9	29.7	+10.41
Cortex thickness	318.5	476.9	+49.73
Fiber strands thickness	99.2	121.4	+22.38
Vascular tissue thickness	685.8	793.5	+15.70
Phloem tissue thickness	139.7	158.8	+13.67
Xylem tissue thickness	518.2	604.7	+16.69
Vessel diameter	32.6	37.2	+14.11
Parenchymatous pith thickness	1845.4	2064.9	+11.89
Hollow pith diameter	2742.6	2995.8	+9.23

The increase in stem wall thickness could be attributed to the prominent increase in all included tissues. The thickness of epidermis, cortex, fiber strands, vascular tissue and parenchymatous area of the pith were 10.4, 49.7, 22.4, 15.7 and 11.9% more than those of the control; respectively. It is clear that the prominent increase which was observed in the thickness of

vascular tissue of the main stem of Egyptian lupine cv. Giza 2 as affected by foliar application with 80 ppm stigmasterol could be attributed mainly to the increase in thickness of phloem tissue and of xylem tissue by 13.7 and 16.7% more than those of the control; respectively. Moreover, vessel diameter in stem of treated plant was also increased by 14.1% over the control.

The present findings are generally in agreement with those obtained by Ali *et al*. (2002) using 100 or 150 ppm stigmasterol on rice plants as well as by Nassar (2004) using 100 ppm stigmasterol on soybean plants. They recorded favourable anatomicalchanges in stem anatomy due to the effect of stigmasterol.

2- Anatomy of the leaf:

Microscopical counts and measurements of certain histological characters in transverse sections through the blade of the median leaflet of the tenth leaf on the main stem of control plants of Egyptian lupine cv. Giza 2 and of those sprayed with 80 ppm stigmasterol are presented in Table (5). Likewise, microphotographs illustrating these treatments are shown in Figure (2).

It is realized from Table (5) and Figure (2) that spraying stigmasterol at concentration of 80 ppm increased thickness of both midvein and lamina of leaflet blades of Egyptian lupine cv. Giza 2 by 9.7 and 8.3% more than the control; respectively. It is noted that the increase in lamina thickness was accompanied with 12.2 and 4.6% increments in thickness of palisade and spongy tissues compared with the control; respectively. The main vascular bundle of the midvein increased in size as a result of spraying stigmasterol. The increment was mainly due to the increase in length by 7.5% and in width by 14.3% more than the control. Also, average number of vessels per midvein bundle was increased by 64.2% over the control. Moreover, xylem vessels had wider cavities, being 2.2% more than the control.

In this connection, Ali *et al.* (2002) stated that histological examination of flag leaf cross sections revealed favourable anatomical effects for stigmasterol treatments at the concentration of 100 and 150 ppm when foliarly sprayed twice to rice plants at leaf tube and tillering stages. Such favourable effects resulted in increasing leaf thickness, upper and lower epidermal layers, mesophyll tissue and dimensions of both main and smaller leaf vascular bundles. Likewise, Nassar (2004) using stigmasterol as foliar application with 100 ppm on soybean plants found that such treatment increased thickness of both midvein and lamina of leaflet blades of treated plants, being in accordance with the present findings.

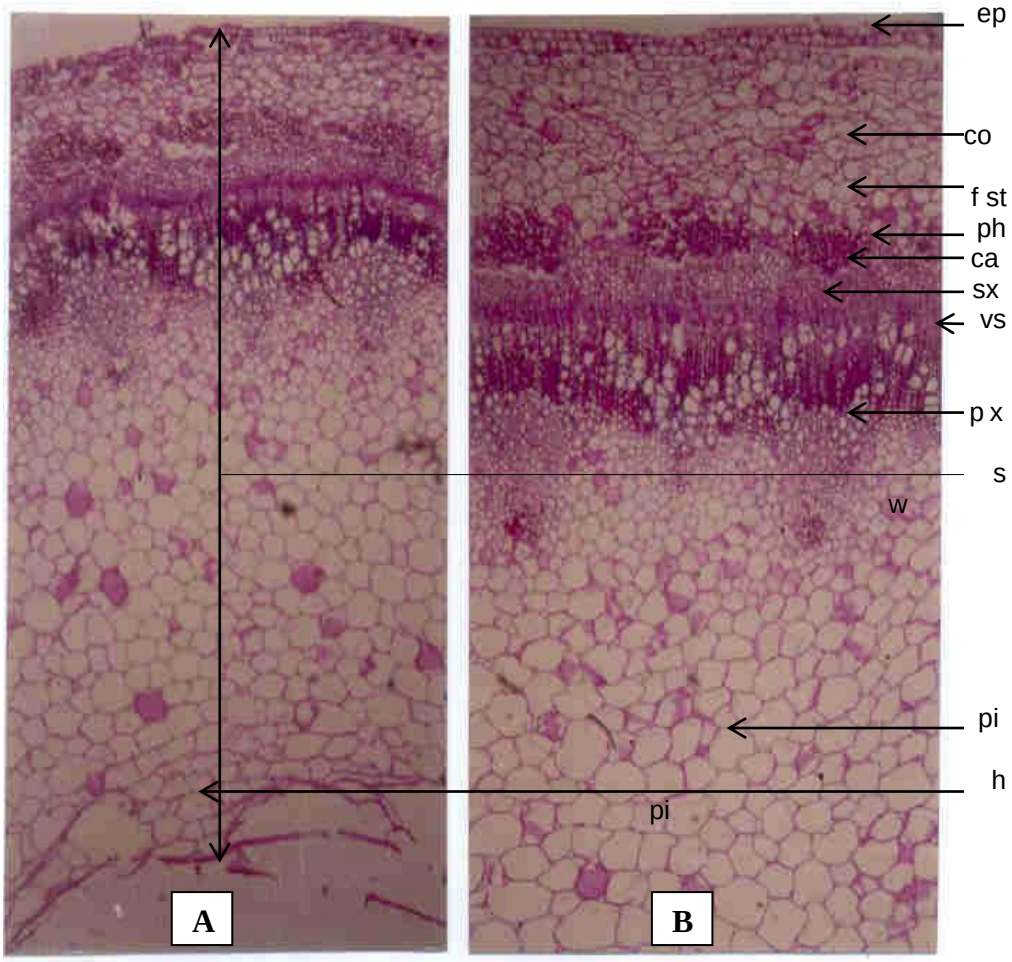

Fig. (1): Transverse sections through the tenth internode of the main stem of Egyptian lupine cv. Giza 2, at the age of 90 days, as affected by spraying with stigmasterol. (X 52)
A-From untreated plant (control).
B-From plant treated with 80 ppm stigmasterol.

Details: ca, cambium; co, cortex; ep, epidermis; fi st, fibre strands; h pi, hollow pith; ph, phloem; pi, pith; p x, primary xylem; s w, stem wall; s x, secondary xylem and vs, vessel.

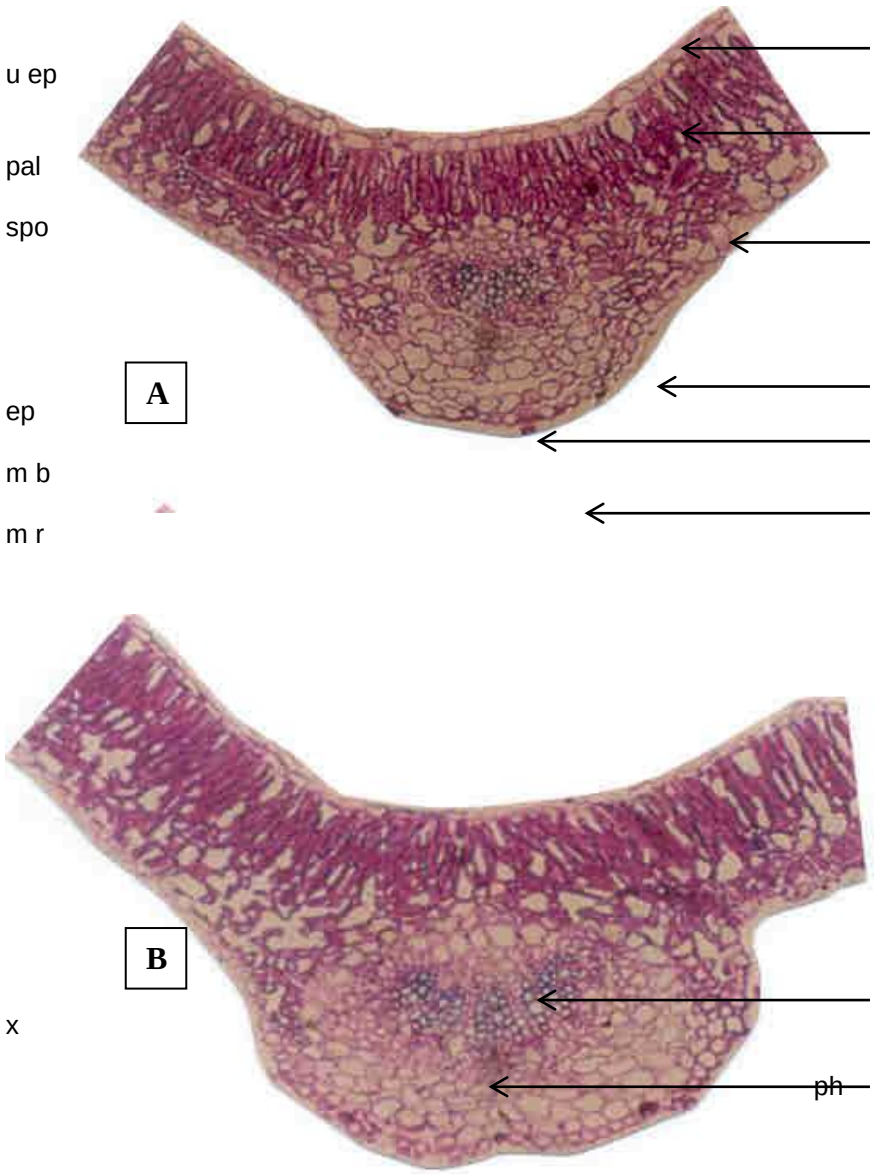

Fig. (2): Transverse sections through the blade of terminal leaflet of the tenth compound leaf developed on the main stem of Egyptian lupine cv. Giza 2, at the age of 90 days, as affected by spraying with stigmasterol. (X 52)

A-From untreated plant (control).

B-From plant treated with 80 ppm stigmasterol.

Details: l ep, lower epidermis; m b, midvein bundle; m r, midrib region; pal, palisade tissue; ph, phloem; spo, spongy tissue; u ep, upper epidermis and x, xylem.

Table (5): Counts and measurements in micron of certain histological features in transverse sections through the blade of the median leaflet of the tenth leaf on the main stem of Egyptian lupine cv. Giza 2, at the age of three months, as affected by foliar application with 80 ppm stigmasterol (Means of three sections from three specimens)

Characters	Treatments		
	Control 0	80 ppm Stigmasterol	± % to control
Thickness of midvein	639.4	701.4	+9.7
Thickness of lamina	358.2	387.9	+8.3
Thickness of palisade tissue	164.9	185.1	+12.2
Thickness of spongy tissue	138.8	145.2	+4.6
Dimensions of midvein bundle:			
- Length	172.3	185.2	+7.5
- Width	269.5	308.1	+14.3
Number of vessels/ midvein bundle	27.9	45.8	+64.2
Vessel diameter	18.6	19.0	+2.2

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استجابة نبات الترمس المصرى للمعاملة بالاستيجماستيرول

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أجريت تجارب حقلية بمحطة البحوث و التجارب الزراعية بكلية الزراعة جامعة القاهرة بالجيزة خلال موسمى النمو 2006/2005 و 2007/2006 لدراسة تأثير الرش بتركيزات مختلفة من الاستيجماستيرول (صفر ، 20 ،40 ، 80 ، 160 جزء فى المليون) على بداية التزهير وعلى الصفات المورفولوجية و التشريحية و المحصولية لنبات الترمس المصرى صنف جيزة 2.

ويمكن ايجاز أهم النتائج المتحصل عليها فيما يلى:

1- رش الاستيجماستيرول بالتركيز المنخفض نسبيا (20 جزء فى المليون) لم يكن له أى تأثير معنوى على بداية التزهير لنبات الترمس المصرى صنف جيزة 2. بينما أدى الرش بأى من التركيزات الأخرى (40، 80 ، 160جزء فى المليون) الى التبكير فى بداية التزهير فى كلا موسمى الدراسة.

2- رش الاستيجماستيرول بالتركيز المنخفض نسبيا (20 جزء فى المليون) أو بالتركيز المرتفع نسبيا (160 جزء فى المليون) لم يكن له تأثير معنوى على جميع الصفات المورفولوجية للنمو الخضرى لنبات الترمس المصرى صنف جيزة 2 فى كلا موسمى الدراسة.و على العكس من ذلك أدى الرش بأى من التركيزين المتوسطين (40 و 80 جزء فى المليون) من الاستيجماستيرول الى حدوث تأثيرات معنوية منشطة مرغوبة على صفات ارتفاع النبات ، طول الساق الرئيسية وعدد سلاميتها ، بينما حدثت زيادة معنوية فى عدد الأفرع على الساق الرئيسيةعند رش الاستيجماستيرول بتركيز 80 جزء فى المليون فقط وذلك فى كلا موسمى الدراسة.

3- فيما يتعلق بتأثير الاستيجماستيرول على الصفات المحصولية ، وجد أن الرش بأى من التركيزين المتوسطين (40 و 80 جزء فى المليون) كان له تأثير منشط معنوى حيث كان هناك زيادة معنوية فى محصول النبات من البذور و كذلك فى عدد قرون و بذور النبات للترمس المصرى صنف جيزة 2 فى كلا موسمى الدراسة.

4- أعلى نسبة بروتين خام (94ٍ31%) سجلت فى بذور النباتات المعاملة بتركيز 80 جزء فى المليون استيجماستيرول مقارنة بنسبة بروتين خام47ٍ 26% سجلت فى بذور نباتات الترمس المصرى صنف جيزة 2 الغير معاملة (الكنترول). فى نفس الوقت أدى استخدام الرش بنفس التركيز (80 جزء فى المليون استيجماستيرول) الى الحصول على أقل نسبة قلويدات كلية (68ٍ 3 %) فى بذور النباتات المرشوشة مقارنة بنسبة قلويدات 86ٍ 4% فى بذور النباتات الغير معاملة.

5- يتضح أن أقصى تأثير منشط للنمو الخضرى لنبات الترمس المصرى صنف جيزة 2 قد تم الحصول عليه عند استخدام الاستيجماستيرول رشا على النباتات بتركيز 80 جزء فى المليون ، لذلك تم دراسة تأثير الرش بهذا التركيز على التركيب التشريحى للمجموع الخضرى (الساق الرئيسية و الأوراق) لنبات الترمس المصرى صنف جيزة 2حيث أظهرت هذه المعاملة زيادة فى قطر الساق الرئيسية نتيجة حدوث زيادة واضحة فى سمك كل من البشرة و القشرة و الآلياف و الأنسجة الوعائية (اللحاء و الخشب) وكذلك المساحة البارنشيمية من النخاع. كما أدى الرش بنفس هذا التركيز (80 جزء فى المليون استيجماستيرول) الى حدوث زيادة واضحة فى سمك كل من العرق الوسطى و نصل الوريقة ، وقد صاحب الزيادة فى سمك النصل زيادة فى سمك كل من النسيج العمادى و الاسفنجى ، كذلك كان هناك زيادة فى حجم الحزمة الوعائية الرئيسية للعرق الوسطى كنتيجة للرش بالاستيجماستيرول.