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Enhancement Quality and Quantity of Lupine Plant via Foliar Application of some Vitamins under Sandy Soil Conditions

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ABSTRACT

The physiological response of lupine to foliar application with ascorbic acid (vitamin C) (100,200,300ppm); thiamine (vitamin B1) (50, 75,100ppm) pyridoxine (vitamin B6) (50, 75, 100ppm) was studied during the two growing seasons of 2013/2014 and 2014/2015 at the Research and Production Station, National Research Centre, Nubaria Province, Behaira Governorate, Egypt. Data show that ascorbic acid, thiamine or pyridoxine at all doses caused significant increases in growth parameters, photosynthetic pigments, N percentage, seed yield and yield components, oil and protein contents. It is worthy to mention that; 100ppm thiamine is the most effective treatment in increases the dry weight of shoot /plant. Pyridoxine at 50 ppm caused increases in chlorophyll a by 141% and carotenoid by115% relative to control. Thiamine at 100ppm caused increases in chlorophyll b by 209% relative to control. Moreover, all treatments enhanced the uptake of macro-elements of lupine plants by different degrees. The highest significant increase in seed yield resulted from 50 and 75ppm pyridoxine compared to other treatments. While, ascorbic acid at 100ppm caused the lowest increase in seed yield/plant by 87% relative to control. It was observed that 50ppm pyridoxine and 100ppm thiamine caused the maximum increase in oil% by 36.81% and 31.43% respectively. Whereas, 100ppm thiamine and 50ppm pyridoxine caused the maximum increase in protein percentage by 11.13% and 9.90% respectively. All treatments showed more pronounced effect on degree of increments in oil content than protein content. Total essential amino acids decreased by increasing the doses of applied vitamins relative to control. 100ppm and 200ppm ascorbic acid and 50ppm thiamine caused increases in total non-essential amino acid.

It could be concluded that all treatments showed promotive effect on quality and quantity of lupine plant. The most promising treatments are 50 pm pyridoxine and 100 ppm thiamine.

Keywords: *Lupinus termis*, ascorbic acid, thiamine, pyridoxine, seed quality, yield.

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INTRODUCTION

Lupine (*Lupinstermis*L.) seeds contain valuable nutritive components (proteins, lipids, carbohydrates), as well as functional components (tocopherols, carotenoids, oligosaccharides, polyphenols, dietary fibers). Lupine is used for human consumption and ruminant feed as well as for pharmaceutical purposes (Sujak *et al.*, 2006).

Vitamins have several functions as growth regulators or hormone precursors that may enhance plant growth and yield (Oertli, 1987). Exogenous application of vitamins exerted a profound influence upon plant growth regulating factors that consequently influence many physiological processes and protect plant from harmful effects of environmental stress (Reda *et al*., 2005; Sadak and Dawood, 2014).

Ascorbic acid (vitamin C) is a product of D-glucose metabolism in higher plants that regulates plant growth and development, and plays an important role in electron transport system (El-Kobisy*et al*., 2005). Ascorbic acid has also been shown to play multiple roles in plant growth such as, regulation of cell division, cell wall expansion, photosynthesis, flowering, senescence and other developmental processes (Davey *et al*., 2000; Barth *et al.,* 2006). Indeed, the ascorbate in leaves could regulate the plant growth through interaction with phytohormones (Pastori *et al.,* 2003). Ascorbic acid serves as an important co-factor in the biosynthesis of many plant hormones, including ethylene, gibberellic acid (GA), and abscisic acid (ABA) (De Tullio and Arrigoni, 2003). Ascorbic acid participates in nodulation and nitrogen fixation (Garg and Kapoor, 1972), stimulates the synthesis of nucleic acid content especially RNA (Bharti and Garg, 1970), and encourages the emergence of lateral buds and flowering (Pbice, 1966).

Thiamine (vitamin B1) could serve as coenzyme in decarboxylation of α-keto acids, such as pyruvic acid and keto-glutamic acid and has important role in the metabolism of carbohydrates and fats (Oertli, 1987; Belanger *et al*., 1995). Thiamine is an important cofactor for the transketolation reactions of the pentose phosphate cycle, which provides pentose phosphate for nucleotide synthesis and to give rise reduced form of NADP required for various synthetic pathways (Jaleel *et al*., 2007). Leaf applied thiamine can transport in both acropetal and basipetal directions (Mozafar and Oertli, 1992 - 1993).

Pyridoxine (Vitamin B6) acts as essential coenzyme that involved in a wide range of biochemical reactions, including the metabolism of glycogen and amino acid synthesis (Di Salvo *et al.,* 2011; Boghdady, 2013). In addition, application of pyridoxine on different plants increased the cell division (Barakat, 2003), enhanced the root growth; enhanced nutrient uptake (Khan *et al.,* 1995) which increased efficiency of photosynthetic surface and enhanced dry matter production.

This work aimed to compare the physiological changes occurred in lupine plant due to foliar application with some vitamins (ascorbic acid, pyridoxine and thiamine).

MATERIALS AND METHODS

Two field experiments were conducted at the Research and Production Station, National Research Centre, Nubaria Province, Behaira Governorate, Egypt during the two growing seasons of 2013/2014 and 2014/2015 to study the physiological response of Egyptian lupine to foliar application with different concentrations of ascorbic acid (vitamin C) (100,200, 300ppm); thiamine (vitamin B1) (50, 75, 100ppm) pyridoxine (vitamin B6) (50, 75, 100ppm). Seeds of lupine (Cultivar Giza 2) were secured from Legume Research Department, Field Crop Institute, Agricultural Research center, Giza, Egypt.

Experimental Procedure

Lupine seeds were sown on 18th November in the two seasons. The experiment was made in randomized complete block design with three replicates. Soil of the experimental site was sandy soil where mechanical and chemical analysis is reported in Table (1) according to Chapman and Pratt (1978). The experimental land is divided into ten plots, each contained one treatment. The plot was ridged, four meters long, 50cm apart, and hills were spaced at 20 cm distance, three seeds were sown in each hill. The plants were thinned to one plant per hill at 21 DAS. Soilpreparation, fertilizer application and cultural operations followed the normal practices of lupine cultivation in the vicinity. Plants were sprayed with different concentrations of

vitamins by means of an atomizer sprayed at 30 and 60 days after sowing (DAS). While, the control plants were sprayed with tap water.

Data recorded

A random sample of ten plants was assigned for investigation in each plot; total number of 30 plants was fixed for each treatment to study the morphological characters at the age of 75 days after sowing.

Morphological Characters:

1- Shoot height (cm).
2- Number of leaves / plant.
3- Stem fresh and dry weight / plant.
4- Leaves fresh and dry weight / plant.

Moreover, photosynthetic pigments (chlorophyll a,b and caortenoids) were determined in fresh leaves. Whereas, mineral contents (nitrogen, phosphorus and potassium) were determined in dried leaves.

Yield Characters:

A random sample of ten plants was assigned for investigation in each plot; total number of 30 plants was fixed for each treatment at harvest time (170 days from sowing date) to determine

1- Number of pods and seeds per plant.
2- Number of seeds per pod
3- Weight of pods and seeds per plant (g).
4- Weight of 100 seeds (g).
5- Seed yield (Kg/Feddan)

Moreover, protein, amino acid composition and oil contents were determined in the yielded seeds.

Biochemical Studies:

Determination of photosynthetic pigments

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in the fresh leaves at 75 days from sowing were determined as the method described by Moran (1982).

Determination of nitrogen, phosphorus and potassium

Nitrogen, phosphorus and potassium were determined in the leaves on the basis of dry weight according to Chapman and Pratt (1978).

Determination of oil

The oil content of the yielded seeds was determined according to the procedure reported by A.O.A.C. (1990).

Determination of protein

Nitrogen content of the yielded meals was determined and multiplied by 6.25 to calculate the crude protein content (A.O.A.C., 1990).

Determination of amino acid composition

Identification and determination of the amino acid composition of the yielded meals was carried out by using HPLC (Eppdrof, Germany) according to Gehrke *et al*., (1985).

Statistical Analysis

Data on 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). Since the trend was similar in both seasons the homogeneity test Bartlet's equation was applied and the combined analysis of the two seasons was calculated according to the method of Gomez and Gomez (1984). Means were compared by using least significant difference (LSD) at 5% levels of probability.

RESULTS AND DISCUSSION

Vegetative Growth Parameters

All treatments caused significant increases in growth parameters of lupine plant i.e. leaves number, fresh weight and dry weight of either stem or leaves/plant (Table, 2). The most pronounced treatments are 300ppm ascorbic acid, 100ppm thiamine and 50ppm pyridoxine that showed highest significant increments in dry weight of stem/plant by 208,225 and 201% respectively, and dry weight of leaves/plant by 176,270 and 167% respectively. Obviously, these results indicated that 100ppm thiamine is the most effective treatment in increases the dry weight of shoot /plant at the vegetative growth stage.

The increments in growth parameters due to ascorbic acid application may be attributed to its substantial role on many metabolic and physiological processes as mentioned by Shaddad*et al*. (1990). Ascorbic acid has been shown to play multiple roles in plant growth such as enhancement of cell division and cell wall expansion (Smirnoff, 2000; Pignocchi and Foyer, 2003; Athar *et al.*, 2008), promoting the cell elongation and cell proliferation (Arrigoni *et al*., 1997;Blokhina*et al.,* 2002). Dodds and Roberts (1995) reported that ascorbic acid can also serve as antioxidant which protects plant cell, regulates plant growth and reduces oxidative stress.

In respect to thiamine effect, Yousef and Talaat (2003) stated that the pronounced increases in vegetative growth and chemical constituents of rosemary plants resulted from foliar application of thiamine through enhancing the endogenous level of various growth factors as cytokinins and gibberellins. Furthermore, Gamal El-Din (2005) and El-Shawy *et al.(*2008) reported that thiamine significantly increased growth parameters of sunflower and flax respectively.

Regarding stimulative effect of pyridoxine, Boghdady (2013) stated that 300ppm pyridoxine promoted significantly vegetative growth of Egyptian lupine cv. Giza 1.

Photosynthetic Pigments

All applied treatments caused significant increases in chlorophyll a,b and carotenoids (Fig. 1). Moreover, ascorbic acid at 300ppm, thiamine at 100ppm and pyridoxine at 50ppm caused the highest significant increases in chlorophyll (a)andchlorophyll (b). Pyridoxine at 50 ppm caused increases in chlorophyll (a) by 141% and carotenoids by115% relative to control. Thiamine at 100ppm caused increases in chlorophyll (b) by 209% relative to control.

The enhancement of pigment biosynthesis has been reported in higher plants exogenously supplied with vitamins (Shaddad *et al.,* 1989). Ascorbic acid is antioxidant and plays a central role against photo-inactivation (Siefermann and Yamamoto, 1974), protects plants against oxidative damage (Smirnoff, 2000), maintains the photosynthetic apparatus (Chen and Murata, 2002), delays premature senescence of leaves and protects the chlorophylls (Smirnoff, 1996). Ascorbate possibly regulates the plant senescence either by modulating the ROS accumulation or influencing the signal pathway of the phytohormones, such as GA, ABA and ethylene (Barth *et al*., 2006). Ebrahimian and Bybordi (2012) mentioned that foliar application of 50mM ascorbic acid had significant effect on total chlorophyll content but high concentrations of ascorbic acid (100 and 150mM) have some phytotoxic effect on chlorophyll function.

GopalaRao and Sastry (1972) reported that all B-group vitamins may be related to chlorophyll synthesis. In this respect, Hamad and Khulaef (2000) found that soaking seeds or spraying bean seedlings with 100ppm pyridoxine stimulated biosynthesis of photosynthetic pigment. Thiamine serves as a precursor of

thiamine diphosphate, which acts as a potential coenzyme in many key metabolic pathways, including plant pigment biosynthesis (Friedrich, 1987) and carbohydrate metabolism (Kawasaki, 1991). Exogenous application of thiamine or ascorbic acid showed an improvement in photosynthetic pigments, e.g., in pumpkin (Proebsting *et al*., 1990); *Brassica campestris*(Khan *et al.,* 2010); *Cicer arietinum* (Beltagi, 2008) and wheat (Amin *et al*., 2008).

Macro-elements

All treatments enhanced the uptake of macro-elements of lupine plants by different degrees as shown in (Fig. 2). All applied treatments caused significant increases in N percentage. The highest significant increases in N percentage resulted from 100ppm ascorbic acid followed by 75ppm pyridoxine, whereas the highest significant increase in P percentage resulted from 300ppm ascorbic acid. Regarding K percentage, 100ppm ascorbic acid, thiamine and pyridoxine caused significant increases in K percentage.

The increment in N concentration due to ascorbic acid application could be explained by Talaat (1995) who reported that accumulation of nitrate by ascorbic acid foliar application may be due to the positive effect of ascorbic acid on root growth which consequently increased nitrate absorption. In addition, ascorbic acid treatments might increase the organic acids excreted from the roots into the soil and consequently increase the solubility of most nutrients which release slowly into the rhizosphere zone where it may be utilized by the plants (Hanafy-Ahmed *et al.,* 1995).

In respect to thiamine effect, Yousef and Talaat (2003) reported that foliar application of thiamine increased the total N, P, K percentages in rosemary plants.

Regarding pyridoxine effect on mineral content, it was noted that absorption of N, P, K concentrations by wheat plant significantly enhanced by pyridoxine (Khan *et al*., 1996). Generally, Pyridoxine treatment increased the nutrients absorption from the soil and consequently increased the performance of agricultural plant (Lone *et al.,* 1999).

Yield and Yield Components

Data in Table (3) show clearly that seed yield and yield components (number of pods and seeds as well as weight of pods and seeds/plant) were gradually increased by increasing dose of ascorbic acid. Pyridoxine treatments showed opposite trend. Since, the lowest pyridoxine level (50ppm) showed the highest significant increase in seed yield and yield components than pyridoxine at 75 and 100ppm. Regarding thiamine effect, it was noted that 100ppm thiamine showed significant increase in seed yield and yield components followed by 50ppm thiamine. It is worthy to mention that the highest significant increase in seed yield resulted from 50 and 75ppm pyridoxine compared to other treatments. While, ascorbic acid at 100ppm caused the lowest increase in seed yield/plant by 87% relative to control.

Ascorbic acid has a regulatory role in promoting productivity in pepper (Shehata *et al.,* 2002). As mentioned previously; pyridoxine application promotes root growth which consequently enhances nutrient uptake from soil. This in turn improves plants' vegetative and reproductive growth, as well as increased amount of active ingredients, and leads to higher economic yield (Prasad *et al*., 1985; Samiullah *et al.* 1988; Lone *et al*., 1999; Asghari *et al.,* 2013). Khoshlahjeh *et al.* (2013) stated that pyridoxine treatments have significant positive effects on yield of alkekengi.

Nutritive Value of Seeds

All applied treatments caused significant increases in oil and protein contents relative to control (Fig. 3). Oil and protein contents were increased gradually and significantly by increasing the doses of ascorbic acid and thiamine and by decreasing the doses of pyridoxine. It was observed that 50ppm pyridoxine and 100ppm thiamine caused the maximum increase in oil content by 36.81% and 31.43% respectively. Whereas, 100ppm thiamine and 50ppm pyridoxine caused the maximum increase in protein percentage by 11.13% and 9.90% respectively. It is important to mention that all applications showed more pronounced effect on degree of increments in oil content than protein content. Moreover, 100ppm ascorbic acid caused the minimum significant increases in oil content and protein content by 13.99% and 3.75% respectively.

The increments in protein content due to ascorbic acid application may be due to the its role of in scavenging reactive oxygen species and preventing protein oxidation and degradation (Noctor and Foyer, 1998). Price (1966) reported that ascorbic acid increased nucleic acid content, especially RNA and protein content of wheat grains. Jyotsna and Srivastava (1988) stated that soaked pigeon pea seeds with 50ppm ascorbic acid improved protein, free amino acids and soluble sugars contents. El-Bassiouny *et al*. (2005) mentioned that faba bean plants foliar sprayed with 400mg/L ascorbic acid increased the total carbohydrate, crude protein, K, P and Ca contents in seeds. Moreover, exogenous application of ascorbic acid increased the protein as well as starch contents in chick pea (Beltagi, 2008).

Regarding oil content, Tarraf *et al.* (1999) stated that ascorbic acid treatment caused pronounced increases in lemongrass essential oil percent and oil yield per plant. Also, Gamal El-Din (2005) reported that ascorbic acid treatment significantly increased oil percentage of the yielded sunflower seeds.

On the other hand, Hendawy and Ezz EL-Din (2010) reported that vitamin B complex act as co-enzymes in the enzymatic reactions by which carbohydrates, fats and proteins are metabolized. In this respect, thiamine could serve as coenzyme in decarboxylation of α-keto acids, such as pyruvic acid and keto-glutamic acid which has important role in the metabolism of carbohydrates and fats (Bidwell, 1979). In addition, thiamine is an important factor for the translocation reaction of the pentose phosphate cycle, which provides pentose phosphate for nucleotide synthesis and for the role of NADP required for various synthetic pathways (Jaleel *et al*., 2007). Makled (1995) reported that application of thiamin enhanced protein accumulation in *vulgaris vulgaris*.

On the other hand, Ansari *et al.*(1990) mentioned that pyridoxine solution significantly enhanced mung bean protein concentration of the yielded seeds. They added that pyridoxine application not only enhanced nutrients to plants but also was responsible for the maintenance of favourable source-sink relationship, thus ensuring more nutritious seeds of mung bean. Dolatabadian and Sanavy(2008) stated that pyridoxine treatments significantly increased protein content in sunflower and rapeseed.

Amino Acid Composition

Data in Table (4) show that leucine and lysine are the most predominant essential amino acid under all treatments. Methionine is the minor essential amino acid. Total essential amino acids decreased by increasing the doses of applied vitamins relative to control. On the other hand, aspartic acid is the predominant non-essential amino acid followed by glutamic acid under all applied treatments. Proline, serine and glycine are presented as minor non-essential amino acid. 100ppm and 200ppm ascorbic acid and 50ppm thiamine caused increases in total non-essential amino acid. While other treatments caused decreases in total non-essential amino acid and consequently in total amino acid content of the yielded meals.

Pyridoxine is an essential cofactor for numerous metabolic enzymes including amino acid metabolism and is a requirement for growth and differentiation of some plant species (Dolatabadian and Sanavy, 2008). Mengel and Kirkby (1982) stated that the enhancement in mustard oil production by pre-sowing seed treatment with pyridoxine seems to have been favoured by the synthesis of mustard oil precursor amino acids i.e., glutamate, aspartate, alanine, or serine at the expense of α-keto acids produced during the Kreb's cycle as a result of well-established role of pyridoxine as co-enzyme in various amino transferase system (Lehninger, 1984).

Table (1): Soil Mechanical-chemical characters

Mechanical analysis	2013/2014	2014/2015
Sand %	91.7	90.5
Silt %	3.5	4.2
Clay %	4.8	5.3
Chemical analysis:		
CaCo3 %	3.53	3.51
Organic matter %	0.18	0.17
EC. mmhos/cm	0.4	0.3

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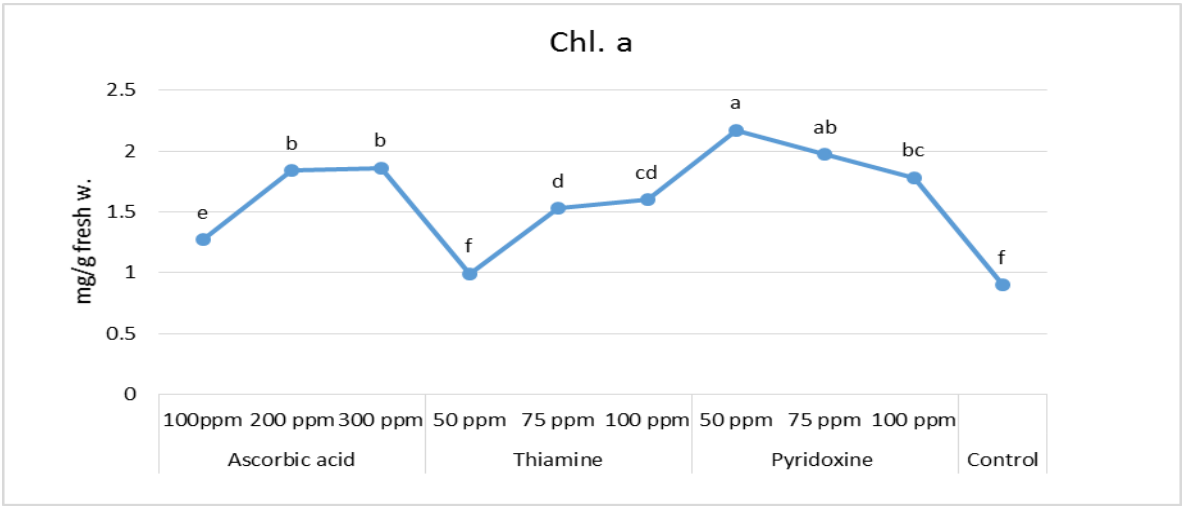
pH	7.6	7.8
Soluble N%	7.7	8.2
Available P (ppm)	12.0	11.5
Available K (ppm)	15.08	18.5

Table (2): Effect of ascorbic acid, thiamine and pyridoxine on vegetative growth parameters of lupine plant

Treatments (ppm)		Shoot height (Cm)	Leaves number	Fresh weight/plant (g)		Shoot fresh weight/plant (g)	dry weight/plant(g)		Shoot dry weight/plant (g)
				Stem	Leaves		Stem	Leaves	
A	100	24.50 ab	26.00 b	10.91 ab	17.03 bcd	27.94 c	1.47 abc	2.66 bc	4.13 ab
	200	32.00 a	24.00 bcd	11.64 ab	15.07 d	26.71 cd	1.54 abc	2.03 d	3.57 b
	300	27.50 bcd	24.50 bcd	11.87 a	18.43 b	30.3 b	1.79 a	2.74 b	4.53 ab
T	50	21.50 cde	30.00 a	11.22 ab	15.34 d	26.56 cd	1.21 c	2.39 bcd	3.6 b
	75	20.00 de	23.50 cd	8.56 b	16.16 bcd	24.72 e	1.75 ab	2.51 bc	4.26 ab
	100	26.00 bc	24.00 bcd	11.71 ab	25.73 a	37.44 a	1.89 a	3.67 a	5.56 a
P	50	29.00 ab	25.00 bc	12.15 a	18.17 bc	30.32 b	1.75 ab	2.65 bc	4.4 ab
	75	21.50 cde	20.50 e	10.20 ab	15.67 cd	25.87 de	1.52 abc	2.28 cd	3.8 b
	100	25.50 bc	22.50 de	9.96 ab	15.51 cd	25.47 de	1.30 bc	2.48 bc	3.78 b
Control		19.00 e	17.00 f	3.81 c	6.68 e	10.49 f	0.58 d	0.99 e	1.57 c
L.S.D		4.90	2.17	3.22	2.69	1.53	0.49	0.41	1.65

A (ascorbic acid), T (thiamine), P (pyridoxine)
Data are combined of two seasons -Means followed by the same letter for each tested parameter are not significantly different by Duncan's test (P <0.05).



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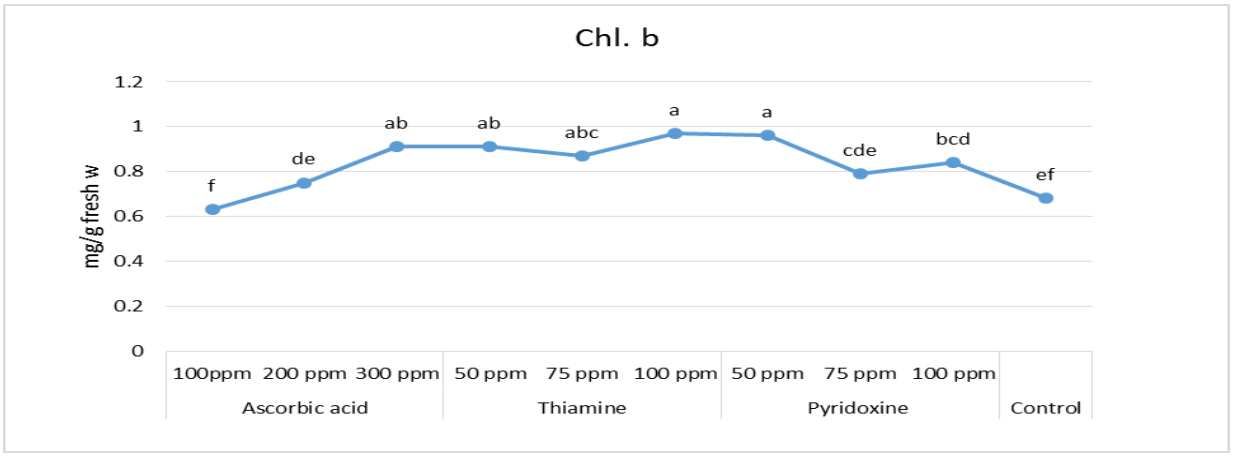

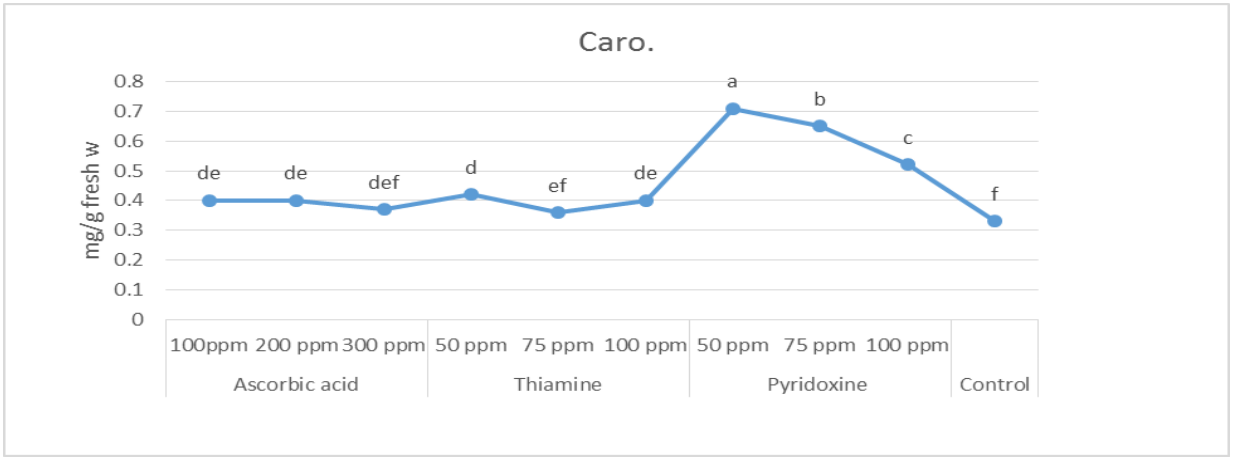

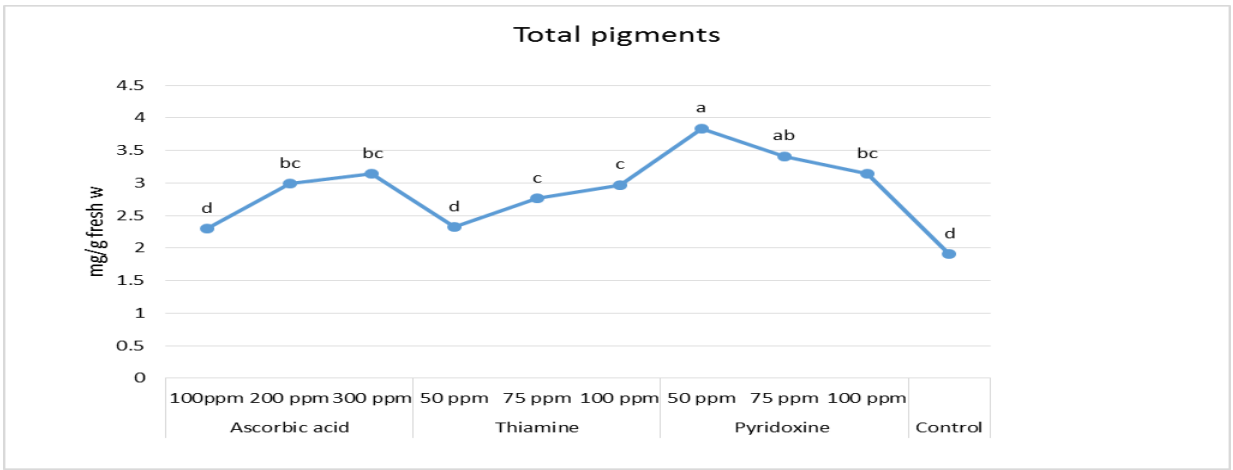

Fig. (1): Effect of ascorbic acid (A), thiamine (T) and pyridoxine (P) on photosynthetic pigments of fresh lupine leaf Data are combined of two seasons -Means followed by the same letter for each tested parameter are not significantly different by Duncan's test (*P* <0.05).

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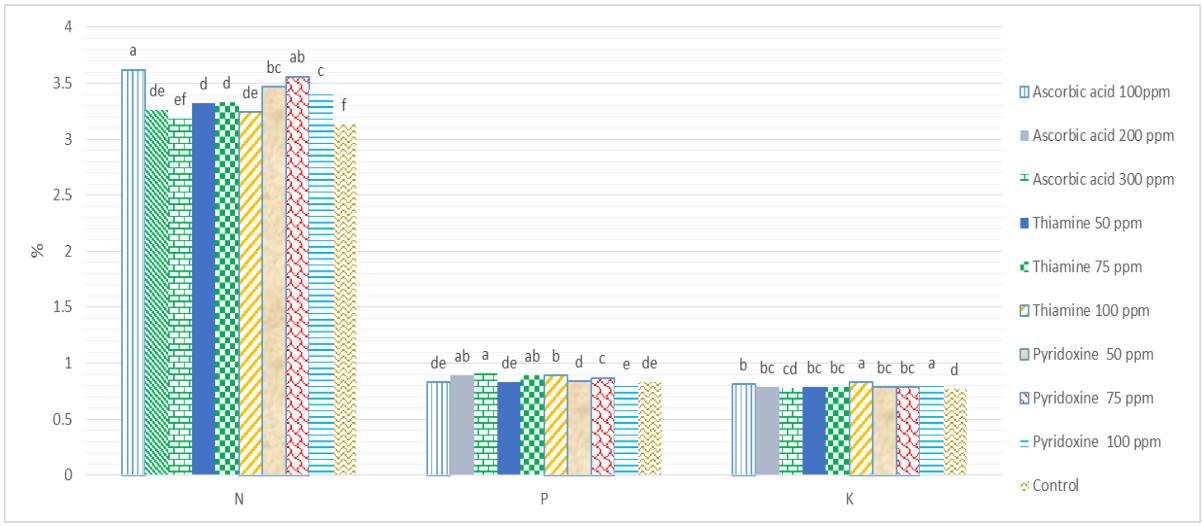

Fig. (2): Effect of ascorbic acid (A), thiamine (T) and pyridoxine (P) on macro-elements of dry lupine leaf
Data are combined of two seasons -Means followed by the same letter for each tested parameter are not significantly different by Duncan's test (*P* <0.05).

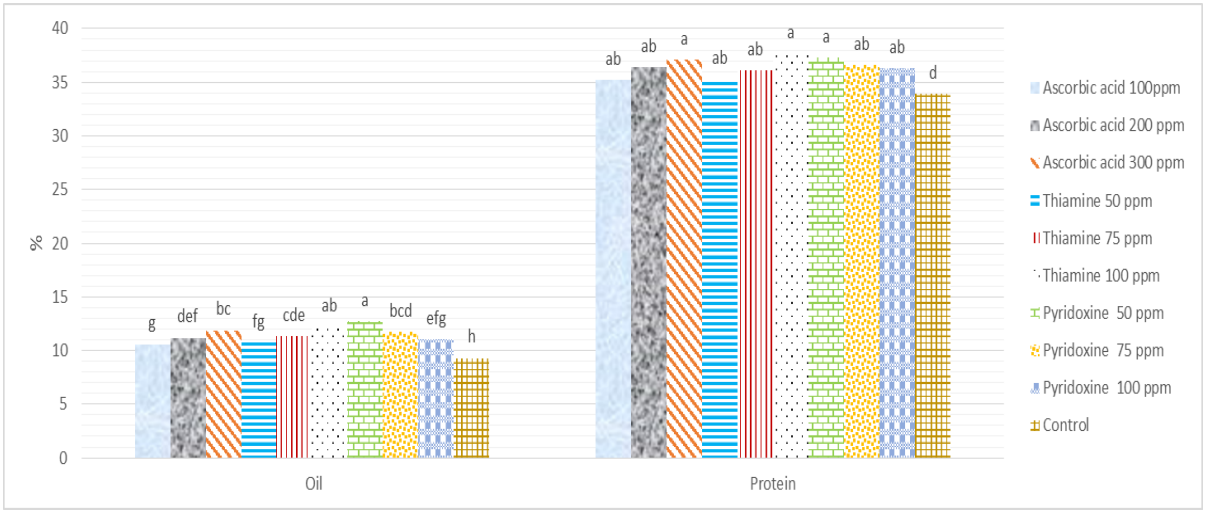

Fig. (3): Effect of ascorbic acid (A), thiamine (T) and pyridoxine (P) on oil content of lupine seed and protein content of lupine meal
Data are combined of two seasons -Means followed by the same letter for each tested parameter are not significantly different by Duncan's test (*P* <0.05).

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Table (3): effect of ascorbic acid, thiamine and pyridoxine on yield and yield components of lupine plant

Treatments (ppm)		Pods number /plant	Seeds number /plant	Seeds number /pod	Weight of Pods/plant	Weight of seeds/plant	Weight of 100 seeds	Seed yield (Kg/feddan)
					(g)			
A	100	8.7 ef	30.5 d	3.4 ab	10.3 ef	6.5 cde	27.92 a	668.2 de
	200	9.0 ef	28.9 d	3.3 ab	9.9 efg	5.8 def	26.38 b	572.8 ef
	300	10.2 de	30.9 d	3.1 b	10.7 de	6.6 cde	27.96 a	680.8 de
T	50	12.1 c	39.0 c	3.1 b	12.4 cd	7.0 cd	23.92 c	735.4 cd
	75	8.9 ef	26.6 d	3.1 b	8.5 g	4.7 f	23.90 c	436.6 g
	100	15.2 b	49.9 b	3.3 ab	17.0 b	9.7 b	25.89 b	907.3 b
P	50	23.6 a	66.1 a	3.2 b	27.2 a	14.4 a	25.49 bc	1033.6 a
	75	11.8 cd	38.2 c	3.1 b	13.7 c	7.5 c	26.17 b	812.0 bc
	100	8.3 f	27.8 d	3.6 a	9.0 fg	5.4 ef	25.59 bc	519.7 fg
Control		4.7 g	11.3 e	2.4 c	3.9 h	2.3 g	26.78 ab	308.2 h
L.S.D		1.65	4.88	0.40	1.60	1.54	1.73	116.3

A (ascorbic acid), T (thiamine), P (pyridoxine)
Data are combined of two seasons -Means followed by the same letter for each tested parameter are not significantly different by Duncan's test (P <0.05).

Table (4): effect of ascorbic acid, thiamine and pyridoxine on amino acid composition (mg/g) of the lupine meal

Amino acids	Treatments ppm									
	A			T			P			
	100	200	300	50	75	100	50	75	100	Control
Essential amino acids										
Threonine	0.42	0.44	0.41	0.49	0.42	0.52	0.33	0.29	0.41	0.53
Valine	0.74	1.95	0.83	1.11	1.86	1.13	1.48	0.97	0.83	0.99
Methionine	0.07	0.00	0.00	0.17	0.21	0.00	0.00	0.00	0.27	0.35
Isoleucine	0.24	0.37	0.27	0.33	0.39	0.34	0.26	0.25	1.51	2.09
Leucine	1.74	1.83	1.57	1.73	1.99	2.15	1.58	1.56	0.66	0.98
Phenyalanine	0.38	0.05	0.28	1.39	0.64	0.72	0.36	0.22	0.24	0.33
Histidine	1.10	0.00	0.00	0.64	0.00	0.39	0.00	0.00	0.87	1.18
Lysine	1.59	1.07	1.12	1.34	1.52	1.34	0.94	0.75	1.09	1.44
Total essential amino acids	6.28	5.71	4.48	7.2	7.03	6.59	4.95	4.04	5.88	7.89
Non-Essential amino acids										
Agrginine	2.28	1.49	1.66	1.65	1.64	2.34	1.27	1.16	0.20	0.52
Proline	0.89	0.37	0.16	0.53	0.00	0.00	0.00	0.00	0.20	0.52
Aspartic	27.85	35.30	19.05	25.27	20.70	22.46	15.85	14.28	17.48	23.99
Serine	0.94	0.81	0.76	0.92	0.59	0.89	0.57	0.46	0.66	0.88
Glutamic Acid	11.83	7.45	7.06	10.83	7.14	6.79	4.93	4.47	7.68	9.34
Glycine	0.72	7.41	0.62	0.44	0.57	0.51	0.44	0.48	0.38	0.41
Alanine	1.90	9.65	1.55	1.71	2.29	1.86	1.65	1.69	1.07	1.99
Tyrosine	1.14	0.67	1.26	0.53	0.00	1.45	0.56	0.81	0.49	0.92
Aspartic	1.85	1.75	1.49	1.45	1.43	2.04	1.46	1.16	0.94	1.53
Total non-essential amino acids	49.4	64.9	33.61	43.33	34.36	38.34	26.73	24.51	29.1	40.1
Total Essential and non-essential	55.68	70.61	38.09	50.53	41.39	44.93	31.68	28.55	34.98	47.99

A (ascorbic acid), T (thiamine), P (pyridoxine)

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