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Effect of Tryptophan and Ascorbic Acid on Yield and Some Chemical Constituents of Lupine (*Lupines termis* L.) Plants

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A FIELD experiment was carried out during two seasons 2017/2018 and 2018/2019 to study the effects of spraying Tryptophan and Ascorbic acid on yield components and chemical composition of plants using two cultivars Giza-1 and Giza-2 of lupine plants. Foliar spray of Tryptophan at rate (Trp.: 25 and 50mg/L), Ascorbic acid (AA: 100 and 200mg/L) either alone, or their combination and control. The effect of the previous treatments on yield and its components namely {plant height (cm), pod length (cm), number of branches/plant, weight of 100 seeds, seed yield/plant (g) and seed yield/fed (ton)}wear investigated as well as chemical composition such as, total nitrogen, crude protein (CP), total soluble sugars (TSS), crude lipids (CL), total alkaloids (TAs) and total phenolic compounds (TPCs).

Results indicated that foliar application Tryptophan and Ascorbic acid significantly improve yield and chemical composition. Trp. was more effective than A.A. in raising lupine yield, significantly increased yield of lupine plants, plant height (cm), pod length (cm), number of branches/plant, weight of 100 seeds, seed yield/plant(g) and seed yield/fed (ton).

Total nitrogen, crude protein (CP), total soluble sugars (TSS), crude lipids (CL), total alkaloid (TAs) and total phenolic compounds (TPCs) in seeds lupine were significantly enhancement by mounting Trp. from 25to 50mg/L and/or A.A. concentrations up to 100 and 200mg/L.

Generally, it is obvious that Giza-2 cultivar surpassed Giza-1 cultivar in the first and the second seasons, respectively in plant height (cm), Pod length (cm), number of branches/plant, weight of 100 seeds, seed yield per plant (g) and per fed (ton), crude lipids, total soluble sugars and total phenolic compounds and it is obvious that Giza-1 cultivar surpassed Giza-2 cultivar in both seasons in total nitrogen, crude protein and total alkaloid.

Keywords: Ascorbic acid, Chemical composition, *Lupinus termis* L., Tryptophan.

Introduction

Lupine (*Lupinus termis* L.) is cultivated in a wide range of conditions crosswise in Egypt. Its seed has a dietary quality like soybean seed and better than different legumes seed (Raza & Jrnsgard, 2005), and could be a significant wellspring of protein and oil. Actually, lupine seeds have been utilized for human utilization and as a medicinal plant in Egypt (Kattab, 1986; ARC, 1994) and other countries for thousands of years. Lupine is one of the ancient agricultural crops excessively utilized as a protein origin in feed production and for amelioration natural and chemical characteristic of soil (Maknickiene, 2001). Lupines are great wellspring of proteins and lipids and exceptionally low substance of protease inhibitors (Australia, 2001). Lupines

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are exceptionally esteemed as creature feed anyway have been underutilized as human sustenance yet the seeds are accounted for to be a rich wellspring of protein (33-47%) and oil (6-13%). There are additionally asserts that the seeds are wealthy in dietary fiber and beneficial phytochemical constituent's (William, 2000). The utilization of amino acids as a precursor of plant growth hormone is one way to deal with limit the impact of water stress on plant development and productivity. A typical precursor of plant hormone auxin is Tryptophan, which influences the physiological processes of plants after absorption directly or indirectly after converting into auxins (IAA) (Khalid et al., 2006). Tryptophan is an amazing amino acid, it might go about as an osmolyte, particle transport controller, adjusts stomatal opening and detoxify harmful effects of heavy metals (Orabi et al., 2014; Rai, 2002). In addition, the tryptophan pathway assumes a protective role in plants (Hussein et al. , 2014). It was also found that using of amino acids as foliar spray enhances some of chemical components such as total free amino acids and total soluble sugars in *Antholyza ethiopica* (Wahba et al., 2002). Also, utiliziation of Tryptophan, improves vegetative growth and photosynthesis process of *Iberis amara* L. (Attoa et al., 2002), *Chatharanthus roseus* L. (Talaat et al., 2005), *Philodendron erubescens* L. (Abou Dahab & Abd El-Aziz, 2006) and *Brassica napus* L. (Dawood & Sadak, 2007).

Ascorbic acid is one of the most significant noteworthy water dissolvable antioxidants in plants that effectively affect development, yield and it's components of many plants. Ascorbic acid affects rummage on scavenge the active oxygen that came about through respiration processes and photosynthesis (Foyer et al., 1991; Pastori et al., 2003). In higher plants D-glucose metabolism produce vitamin C which plays regulating role in development and advancement of plants, in addition to the role in electron transport (El-Kobisy et al., 2005). Ascorbic acid plays varied roles in plant development, such as regulation of biological process, cell membrane growth, chemical change, flowering, senescence (Davey et al., 2000; Barth et al., 2006). Undoubtedly, the ascorbate in leaves might manage the plant development through interaction with phytohormones (Pastori et al., 2003). Ascorbic acid is important co-factor within the synthesis of the many plant hormones, (ABA) (De Tullio & Arrigoni, 2003), such as gibberellin (GA), and Abscisic acid is available in living plant cells, the largest amount being for the initial half within the leaves and blooms, that effectively development vegetative growth and chemical components (Smirnoff et al., 2001; Ebrahim, 2005)

The main objectives of this studying are (1) To study the effect of Tryptophan and Ascorbic on yield and its components of *L. termis* plants. (2) To study the changes in chemical composition induced by foliar application of Tryptophan and Ascorbic acid on seed of Lupin plants.

Materials and Methods

Samples

In the present study, two dry beans of lupine (*Lupinus* sp. L.) cultivars were provided from Agricultural Research Centre, Giza, Egypt. Two cultivars (Giza-1 and Giza-2) were bitter samples.

Experiment

This experiment was carried out in the Laboratory and Experimental Farm of Faculty of Agriculture, Al-Azhar University, Assiut, Egypt during the two successive seasons of 2017/2018 and 2018/2019 to study the effect of spraying Tryptophan and Ascorbic acid on yield and its component as well as chemical composition of seed plants. Experimental design was laid in a split plot arrangement in completely randomized block design with three replications, cultivars (Giza-1 and Giza-2) of lupine were established in main plots, foliar spray of Ascorbic and Tryptophan treatments were assigned in sub plots. Lupines seed were sown on October 25th of the two seasons. The experimental plot was 3.5 × 3m and contained 5 rows, 60cm apart, the distances between hills were 30cm. All agricultural practices were performed as recommended by Agriculture Ministry. At harvest the following data were recorded: Plant height (cm), pod length (cm), number of branches/plant, weight of 100 seeds and seed yield per plant (g) and per fed (ton) as well as chemical composition such as, total nitrogen, crude protein (CP), total soluble sugars (TSS), crude lipids (CL), total alkaloid (TAs) and total phenolic compounds (TPCs).

Spraying was performed using plastic atomizer and plants were sprayed twice with

Tryptophan (Try) and Ascorbic (AA) acid, the first spraying was applied after 45 days of sowing and the second spraying was applied on 15 days after. These treatments were as follows:
1- Control
2- Tryptophan at 25 and 50mg /L.
3- Ascorbic acid at 100 and 200mg /L.
4- Tryptophan + Ascorbic acid at: 25mg/L Try + 100mg/L AA, 25mg/L Try + 200mg /L AA) .
5- Tryptophan + Ascorbic acid at: 50mg/L Try + 100mg /L AA, 50 mg/L Try + 200mg/L A.A).

Soil physical and chemical characters are presented in Table 1 (A, B).

Approximate analysis

Chemical composition of *L. termis* seeds were carried out according to official methods of the Association of Official Analytical Chemists (AOAC, 1984). All determinations must perform in triplicates and means will be reported.

Determination of total nitrogen and crude protein (CP)

The Kjeldahl procedure is used to determine the total nitrogen content. This was performed by Rapid Nitrogen Apparatus Model-005 (RNAM-005). The crude protein was then calculated by multiplying nitrogen content by 6.25 as a factor.

Determination of crude lipids (CL)

Crude lipids is determined according to AOAC (1984) method as follows: Dried samples of 1-2g are accurately weighed, then extracted in a Soxhlet apparatus by petroleum ether (60-80°C) for 15hrs, the solvent then removed by evaporation under reduced pressure and the total lipids content was calculated.

Extraction and determination of total soluble sugars (TSS) in lupine seeds

Preparation of samples

100mg of dried seeds was placed in test tube then 10ml of H_2SO_4 (1N) was add, the test tube was placed in water bath at 100°C for 30min, then left to cool, and 0.1g of $BaCO_3$ was added. The sample was filtered through Whatman filter paper No 1 and washed several times with distilled water, then transferred to 100ml volumetric flask and completed to 100ml with distilled water. TSS content was determined using the phenol sulphuric acid method according to Dubois et al. (1956). A stranded curve was prepared using different concentration (10 to 100ug/ml) of pure glucose.

Determination of total alkaloids

Determination of alkaloids was done by the alkaline precipitation gravimetric method described by Harborn (1973). Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed. The absorbance was taken at 565nm against a blank. The experiment was carried out in triplicate.

TABLE 1. Physical and chemical properties of the soil.

Physical properties (A)

Depth (cm)	Percentage %			Texture class	O.M %	$Caco_3$ %
	Sand	Silt	Clay			
0-30	25	39.65	35.00	Clay loam	1.20	3.50
30-60	24.65	39.00	36.00	Clay loam	1.10	3.20

Chemical properties (B)

Depth (cm)	pH	Ece (ds/m)	Water soluble ions (mq/L) in the soil							Available nutrients in soil (ppm)		
			Co_3+HCO_3	Cl^-	So^-	Ca^{++}	Mg^{++}	Na^+	K^+	N	P	K
0-30	7.87	1.05	2.50	1.25	6.15	2.70	1.35	5.74	0.11	77	9.70	377
30-60	7.88	1.00	2.34	1.16	6.00	2.60	1.15	5.34	0.22	68	9.55	353

Extraction and determination of total phenolic compounds (TPCs)

TPCs was extracted from lupine sample (1.0g) by refluxing with 30ml of methanol containing 1% HCl for 10min, the extract was centrifuged at 8.000rpm. for 10min. The concentrations of total phenolic compounds in the methanolic extracts were determined by the method described by Singleton & Rossi (1965) with some modifications. One milliliter of sample was mixed with 1ml of Folin and Ciocalten's phenol reagent, after 3min, 1ml of saturated Na_2CO_3 (35%) was added to the mixture and completed to 10ml by adding distilled water. The reaction was kept in the dark for 90min, after which its absorbance was read at 725nm. A calibration curve was constructed with different concentrations of gallic acid (0.01–1mM) as standard.

Statistical analysis

Randomized complete block design was used with three replications for each treatment. Analysis of variance (ANOVA) was carried out using Proc Mixed of SAS package version 9.2 (SAS 2008) and means were compared by Duncan test at 5% level of significant (Steel & Torrie, 1981).

Results and Discussions

Yield and its components

Effect of Tryptophan and/or Ascorbic acid on the plant height (cm), pod length (cm), seed yield/ plant (g) and weight of 100 seeds

Data illustrated in Tables 1 and 2 indicate that foliar spray of Tryptophan either independently at Trp 25 and 50mg /L and the Ascorbic acid AA 100 and 200mg /L, or their incorporation, had raise significantly the lupine plant height (cm), pod length (cm), number of branches/plant, weight of 100 seeds, and seed yield per plant (g) and per fed (ton). The highest lupine yield and its contributing characters resulted from application of Tryptophan compare with Ascorbic acid .

The obtained data in Tables 2 and 3 reveal that plant height (cm), pod length (cm), number of branches/plant, weight of 100 seeds and seed yield per plant (g) and per fed (ton) of *Lupinus termis* L. had significantly affected in both experimental seasons. The results also obtained superiority of Giza-2 in all previous characters in both seasons.

The most encouraging results, obtained with 50mg/L Tryptophan. The highest increase in Lupine plant height (cm), pod length (cm), number of branches/plant, weight of 100 seeds, and seed yield per plant (g) and per fed (ton) were obtained by foliar spray with 50mg/L Tryptophan, followed by 200mg/L Ascorbic acid for all previous parametersas compared to untreated control in the two growing seasons. This may be due to the role of auxins produced from rhizosphere microflora derived from Tryptophan whcih could be taken up by plant roots and may be translocated to shoot resulting in physiological responses. These results agree with that was found by Martens & Frankenberger (1992).

Moreover, the increase in lupine yield result from mixing Trptophan with Ascorbic acid. The highest increment in lupine height and yield, i.e., pod length (cm), number of branches/plant, weight of 100 seeds and seed yield per plant (g) and per fed (ton) were obtained by using the rate 50mg/L Try + 200mg/L AA trailed by 50mg/L Try for all previous characters .

The present investigation demonstrated that use of Tryptophan up to 50 and Ascorbic acid up to 200mg/L, singly or together had enormously advanced the development by promote cell division and therefore get better yield and yield quality of seed; plant height (cm), pod length (cm), number of branches/plant, weight of 100 seeds and seed yield per plant (g) and per fed (ton). In other studies, Talaat et al. (2005) reported that Tryptophan increased plant height, branch number, fresh and dry weights of leaves of *Catharanthus roseus* L. plants. Amin et al. (2014) showed that Tryptophan at 25, 50 and 100m/L for each significantly increased yield of lupine plants at harvest such as plant height, number of branches, pods and seeds/plant, pod length (cm) and seed index (100 seed weight), seed and straw yield/plant and per fed, biological yield/ fed and harvest index. Amino acids treatment caused important increment in development, blooming properties and nature of inflorescences in lemon grass (Gamal El-Din et al., 1997), basil (Talaat & Youssef, 2002), *Pelargonium graveolens* (Mahgoub & Talaat, 2005), *Dianthus caryophyllus* (Bekheta & Mahgoub, 2005) and *Salvia farinacea* plants (Abdel–Aziz & Balbaa, 2007). Also, Tryptophan application up to 100ppm increased growth of *Iberis amara* L. (Attoa et al., 2002), *Chatharanthus roseus* L. (Talaat et al., 2005). Dawood & Sadak (2007) referenced that the probability of utilizing Tryptophan as an apparatus to get better yield of canola seeds/plant just as its oil, protein and carbohydrate content. The foliar spraying of Ascorbic acid positively affected the growth development characters of the *Lupinus termis* L. These outcomes were upheld by those of Ahmed & Morsy (2001) and Fayed (2010). These augmentations in the above parameters by using ascorbic this might be because of role of Ascorbic acid as an anti-oxidant as well as has an impact as plant growth hormone (Ahmed et al., 1997; Johnson et al., 1999). Shaddad et al. (1990) and Wassel et al. (2007) accepted that, the impact of Ascorbic acid on the plant development may be due to the auxinic activity of Ascorbic acid just as, it plays important role in numerous metabolic and physiological processes and raising the synthesis of carbohydrates. Maksoud et al. (2009) reported that foliar spraying Ascorbic acid may play a role in many metabolic and physiological processes which lead to advancing growth development and animate the root development to meet the requirements. However, some investigators reported that Ascorbic acid application resulted in increasing of seed yield per plant in some different plants and in this respect, many investigators reported that Ascorbic acid application resulted in enhancement of growth of some other different plant species, among them, Zahran (1993) and Abdel-Aziz (1999) on lentil, Mahmoud (1994) and Nofal et al. (1996) on faba bean, El-Kobisy et al. (2005) on pea, Nassar & Abdo (2009) on Egyptian lupine, Emam et al. (2011) and Nassar et al. (2016) on flax and Nassar (2013) on mungbean. All, are in harmony with the present findings.

The augmentations in growth characters due to the role of water-soluble vitamin which enhance metabolic and physiological processes as documented by Shaddad et al. (1990). Water-soluble vitamin has been perceived to assume numerous roles in plant growth like raising biological process and cell membrane enlargement (Smirnoff, 2000; Athar et al., 2008), encourage the cell elongation and cell proliferation (Arrigoni et al., 1997; Blokhina et al., 2003). Dodds & Roberts (1995) reported that water-soluble vitamin will likewise function inhibitor that ensures plant cell, regulates plant growth and reduce stress resulting from oxidation .

Data in Tables 2 and 3 showed that spraying *Lupinus termis* L. with Tryptophan (Trp) made significant increase in the plant height (cm), pod length (cm), number of branches/ plant, weight of 100 seeds, and seed yield (per plant (g) and per fed (ton) in lupine seeds. The highest value in cell character obtained from spraying application Tryptophan (Trp) at rate 50mg /L+ 200 mg/L AA.

Chemical constituents

Effect of Tryptophan and/or Ascorbic acid on primary and secondary metabolites

Results of the primary chemical metabolites in lupine seeds including 4 constituents, i.e. total nitrogen, crude protein, crude lipids, total soluble sugars, as well as, two secondary metabolites namely total alkaloid and total phenolic compounds are given in Tables 4, 5. These Data exhibited in Tables 3 and 4 explain that foliar spraying of either Tryptophan (Trp) or Ascorbic acid (AA) at any rates fundamentally increase the nitrogen, rough protein and total soluble sugar percentages just as unrefined crude lipids, total alkaloids (%) and total phenolic compounds, in the seed of lupine compared with their control at the harvest. Generally, utilization tryptophan was prevalent on ascorbic acid in increasing active substance ingredients in lupine seeds. The best treatment using 50mg/L of Trp or 200mg/L of AA separately and either their mix together (Trp) at rate 50mg/L+ 200mg/L AA. In our examination, foliar spraying of either Trp or AA impact the abundance of some substance and alter the metabolic frameworks because of their bio restrictive impact. Changes impact the morphology, physiology, protein action of plants and thus impact the translocation and assortment of building metabolites to seeds.

Data presented in Table 4 show significant increases in the total nitrogen, and crude protein and crude lipids of the yielded lupine seeds this resulting from incorporation of Tryptophan and Ascorbic acid compared with control (Table 4). The data given in Table 4 showed that using Tryptophan as foliar spray on lupine plants was more effective from Ascorbic acid at any rate as well as their interaction on total nitrogen and crude protein and crude lipids in lupine seeds compared with control and this increase was gradually positive that gave the highest increasing percentage. Spraying chamomile plant with amino acids raising oil %, nitrogen % and crude protein (Gamal El-Din & Abd El-Wahed, 2005; Reda et al., 2010).

TABLE 2. Effect of Tryptophan , Ascorbic acid and their interaction on the plant height (cm), pods length (cm) and branch number/plant in 2017/2018 and 2018/2019 seasons.

Seasons	2017/2018			2018/2019		
	Plant height (cm)					
Cultivars (A) / Conc. mg/L (B)	Giza-1	Giza-2	Mean	Giza-1	Giza-2	Mean
Control	92.20^{j}	99.73^{i}	95.97^{G}	87.03^{k}	94.67^{j}	90.85^{F}
25 Trp	97.90^{i}	108.27^{fg}	103.08^{F}	94.63^{j}	102.50^{ih}	98.57^{E}
50 Trp	110.33^{fg}	116.10^{c}	113.22^{C}	103.47^{ihg}	110.03^{dc}	106.75^{C}
100 AA	97.10^{i}	108.63^{fg}	102.87^{F}	92.90^{j}	103.93^{fhg}	98.42^{E}
200 AA	107.67^{g}	113.13^{de}	110.40^{D}	100.83^{i}	108.30^{de}	104.57^{D}
25 Trp+100 AA	104.03^{h}	108.50^{fg}	106.27^{E}	102.60^{ih}	106.40^{fe}	104.50^{D}
25 Trp+ 200 AA	108.70^{fg}	110.90^{fe}	109.80^{D}	106.20^{feg}	110.27^{dc}	108.23^{C}
50 Trp + 100 AA	114.53^{dc}	119.90^{b}	117.22^{B}	112.00^{c}	116.00^{b}	114.00^{B}
50 Trp + 200 AA	120.80b	124.97^{a}	122.88^{A}	116.30^{b}	119.37^{a}	117.83^{A}
Mean	**105.92^{B}**	**112.24^{A}**		**101.77^{B}**	**107.94^{A}**	
	Pod length (cm)					
Control	6.00^{j}	6.43^{h}	6.22^{F}	5.57^{l}	6.07^{jk}	5.82^{G}
25 Trp	6.23^{ihj}	6.83^{g}	6.53^{E}	6.10^{jki}	6.43^{ghi}	6.27^{E}
50 Trp	6.87^{g}	7.37^{e}	7.12^{D}	6.67^{gf}	6.93ef	6.80^{C}
100 AA	6.10^{ij}	6.43^{h}	6.27^{F}	5.831^{k}	6.27^{jhi}	6.05^{F}
200 AA	6.33^{ih}	6.97^{fg}	6.65^{E}	6.17^{ji}	6.70^{gf}	6.43^{ED}
25 Trp+100 AA	6.97^{fg}	7.23^{fe}	7.10^{D}	6.30^{jhi}	6.87^{f}	6.58^{D}
25 Trp+ 200 AA	7.25^{fe}	7.83^{d}	7.54^{C}	6.53^{gh}	7.23^{ed}	6.88^{C}
50 Trp + 100 AA	7.87^{d}	8.30^{c}	8.08^{B}	7.47^{cb}	7.63^{cb}	7.55^{B}
50 Trp + 200 AA	8.63^{b}	8.97^{a}	8.80^{A}	7.90^{b}	8.33a	8.12^{A}
Mean	**6.92^{B}**	**7.37^{A}**		**6.50^{B}**	**6.94^{A}**	
	Branch number/plant					
Control	20.33^{h}	22.33^{fg}	21.33^{H}	19.33^{j}	21.00^{hi}	20.17^{H}
25 Trp	22.67^{fg}	24.33^{de}	23.50^{F}	20.67^{i}	23.33^{dfe}	22.00^{FG}
50 Trp	24.33^{de}	27.00^{c}	25.67^{C}	23.00^{dgfe}	24.34^{dc}	23.67^{DC}
100 AA	21.67^{eg}	23.33^{fe}	22.50^{G}	21.00^{hi}	21.67hgi	21.33^{G}
200 AA	23.33^{fe}	25.67^{dc}	24.50^{DE}	22.33^{hgf}	22.67^{gfe}	22.50^{FE}
25 Trp+100 AA	23.00^{de}	24.33^{de}	23.67^{FE}	22.67^{gfe}	24.00^{dce}	23.33^{DE}
25 Trp+ 200 AA	23.67^{fe}	26.67^{c}	25.17^{DC}	23.67^{dfe}	25.33^{c}	24.50^{C}
50 Trp + 100 AA	26.33^{c}	28.33^{b}	27.33^{B}	25.33c	27.33^{b}	26.33^{B}
50 Trp + 200 AA	29.67^{a}	30.67^{a}	30.17^{A}	27.33^{b}	29.33^{a}	28.33^{A}
Mean	**23.89^{B}**	**25.85^{A}**		**22.81^{B}**	**24.33^{A}**	

*Means with the same letter within each column are insignificantly different according to Duncan multiple range test at 5% probability level.

TABLE 3. Weight of 100 seeds, seed yield /plant (g), and seed yield/fed (ton) affected by Tryptophan, Ascorbic acid and their interaction in 2017/2018 and 2018/2019 seasons.

Seasons	2017/2018			2018/2019		
	Weight of 100-seeds					
Cultivars (A) / **Conc. mg/L(B)**	**Giza-1**	**Giza-2**	**Mean**	**Giza-1**	**Giza-2**	**Mean**
Control	33.70^{n}	36.06^{lkj}	34.88^{G}	32.84^{j}	34.43^{ih}	33.64^{G}
25 Trp	35.33l^{m}	38.12^{fg}	36.73^{E}	34.89h	36.88fe	35.88^{E}
50 Trp	36.59^{ikj}	38.86^{fe}	37.72^{D}	35.97^{g}	38.98^{c}	37.48^{C}
100 AA	34.73^{m}	36.88^{ihj}	35.81^{F}	33.76^{i}	36.15^{fg}	34.95^{F}
200 AA	35.84^{lk}	37.71^{hg}	36.77^{E}	34.98^{h}	37.25^{e}	36.11^{ED}
25 Trp+100 AA	37.21^{ih}	38.54^{feg}	37.88^{D}	35.84^{g}	37.37e	36.60^{D}
25 Trp+ 200 AA	39.05^{e}	42.28^{c}	40.67^{C}	36.27f^{g}	39.29^{c}	37.78^{C}
50 Trp + 100 AA	40.65^{d}	44.66^{b}	42.66^{B}	38.17d	40.83^{b}	39.50^{B}
50 Trp + 200 AA	44.23^{b}	46.87^{a}	45.55^{A}	41.17^{b}	42.28^{a}	41.73^{A}
Mean	**37.48^{A}**	**40.00^{B}**		**35.99^{A}**	**38.16^{B}**	
	Seed yield/ plant (g)					
Control	17.94^{n}	19.89^{m}	18.92^{H}	17.24^{m}	19.08^{l}	18.16^{I}
25 Trp	21.58^{kl}	23.10^{ih}	22.34^{F}	20.92^{j}	21.50^{i}	21.21^{G}
50 Trp	24.43^{gf}	25.91^{e}	25.17^{D}	23.04^{h}	24.07^{g}	23.56^{E}
100 AA	20.89^{l}	21.89^{kj}	21.39^{G}	19.95^{k}	20.48^{j}	20.22^{H}
200 AA	22.76^{ij}	23.95^{gh}	23.35^{E}	21.91^{i}	23.27^{h}	22.59^{F}
25 Trp+100 AA	25.00^{f}	26.21^{e}	25.61^{D}	23.42^{h}	24.61^{f}	24.01^{D}
25 Trp+ 200 AA	26.82^{e}	28.39^{d}	27.61^{C}	24.86^{f}	26.43^{e}	25.64^{C}
50 Trp + 100 AA	28.89^{d}	30.89^{c}	29.89^{B}	26.92^{d}	29.27^{c}	28.10^{B}
50 Trp + 200 AA	32.16^{b}	33.95^{a}	33.06^{A}	30.31^{b}	32.04^{a}	31.17^{A}
Mean	**24.50^{B}**	**26.02^{A}**		**23.17^{B}**	**24.53^{A}**	
	Seed yield /fed (ton)					
Control	0.936^{h}	0.957^{g}	0.947^{H}	0.923^{i}	0.947^{ih}	0.935^{G}
25 Trp	0.977^{f}	0.993^{f}	0.985^{F}	0.963^{h}	0.980^{h}	0.972^{F}
50 Trp	1.143^{d}	1.167^{c}	1.155^{C}	1.063^{fe}	1.137^{bc}	1.100^{C}
100 AA	0.957^{g}	0.980^{f}	0.969^{G}	0.950^{h}	0.953^{ih}	0.952^{GF}
200 AA	0.983^{f}	1.070^{e}	1.027^{E}	0.977^{g}	1.017^{g}	1.00^{E}
25 Trp+100 AA	1.087^{e}	1.137^{d}	1.112^{D}	1.053^{f}	1.08^{fe}	1.067^{D}
25 Trp+ 200 AA	1.140^{d}	1.180^{c}	1.160^{C}	1.093^{ed}	1.117^{cd}	1.105^{C}
50 Trp + 100 AA	1.173^{c}	1.200^{b}	1.187^{B}	1.127^{bcd}	1.153^{ba}	1.140^{B}
50 Trp + 200 AA	1.203^{b}	1.223^{a}	1.213^{A}	1.177^{a}	1.187^{a}	1.182^{A}
Mean	**1.067^{B}**	**1.103^{A}**		**1.036^{B}**	**1.063^{A}**	

*Means with the same letter within each column are insignificantly different according to Duncan multiple range test at 5% probability level..

TABLE 4. Influence of Tryptophan, Ascorbic acid and their interaction on the total nitrogen (%) and crude protein (%), crude lipids (%) of seeds lupine in 2017/2018 and 2018 /2019 seasons.

Seasons	2017/2018			2018/2019		
	Total nitrogen (%)					
Cultivars (A) / **Conc. mg/L(B)**	**Giza-1**	**Giza-2**	**Mean**	**Giza-1**	**Giza-2**	**Mean**
Control	5.38^{k}	5.30^{k}	5.34^{F}	5.44^{i}	5.27^{j}	5.36^{H}
25 Trp	5.94^{hi}	5.70j	5.82^{E}	5.91^{fg}	5.75^{hg}	5.83^{F}
50 Trp	6.54^{dc}	6.19^{fg}	6.37^{C}	6.40^{d}	6.13^{e}	6.26^{D}
100 AA	5.75^{ji}	5.79^{ji}	5.77^{E}	5.62^{h}	5.76^{hg}	5.69^{G}
200 AA	6.06^{hg}	6.13^{fg}	6.10^{D}	6.10^{e}	6.14^{e}	6.12^{E}
25 Trp+100 AA	6.31^{fe}	5.91^{hi}	6.11^{D}	6.42^{ed}	6.01^{fe}	6.22^{ED}
25 Trp+ 200 AA	6.70^{c}	6.15^{fg}	6.42^{C}	6.86^{c}	6.17e	6.51^{C}
50 Trp + 100 AA	7.05^{b}	6.46^{de}	6.76^{B}	7.03a^{b}	6.42^{d}	6.73^{B}
50 Trp + 200 AA	7.27^{a}	6.93^{b}	7.10^{A}	7.12^{a}	6.89c	7.01^{A}
Mean	6.33^{A}	6.06^{B}		6.32^{A}	6.06^{B}	
	Crude protein (%)					
Control	33.61^{ml}	32.52^{m}	33.07^{F}	34.02^{h}	32.00h	33.51^{H}
25 Trp	37.10i^{gh}	34.84^{kl}	35.97^{E}	36.94^{ef}	35.92^{gf}	36.43^{F}
50 Trp	40.79^{dc}	38.67^{fg}	39.73^{C}	39.98^{c}	38.29^{d}	39.14^{D}
100 AA	35.96^{kj}	36.34^{j}	36.15^{E}	35.13^{g}	36.00^{gf}	35.56^{G}
200 AA	37.92^{igh}	38.25^{fijh}	38.08^{D}	38.10^{d}	38.40^{d}	38.25^{E}
25 Trp+100 AA	39.42^{fe}	36.96^{ij}	38.19^{D}	40.12^{c}	37.58^{fd}	38.85^{ED}
25 Trp+ 200 AA	41.87^{c}	38.42^{fgh}	40.15^{C}	42.86^{b}	38.56^{d}	40.71^{C}
50 Trp + 100 AA	44.00^{b}	40.40^{de}	42.20^{B}	44.42^{a}	40.11^{c}	42.26^{B}
50 Trp + 200 AA	45.46^{a}	43.32^{b}	44.39^{A}	44.52^{a}	43.06^{b}	43.79^{A}
Mean	39.57^{B}	37.75^{A}		39.57^{B}	37.88^{A}	
	Crude lipids (%)					
Control	11.60^{j}	11.98^{i}	11.79^{F}	11.84^{j}	12.12^{ij}	11.98^{G}
25 Trp	12.84^{gf}	12.96^{f}	12.90^{ED}	12.95^{gf}	13.29f	13.12^{E}
50 Trp	13.69^{de}	14.08^{d}	13.89^{C}	14.03^{de}	14.30^{d}	14.17^{C}
100 AA	12.07^{gh}	13.25^{ih}	12.66^{E}	12.67^{gh}	12.31^{ih}	12.49^{F}
200 AA	12.40^{gf}	14.10^{gf}	13.25^{D}	12.94^{gf}	13.06^{gf}	13.00^{E}
25 Trp+100 AA	13.53^{f}	13.92^{e}	13.73^{C}	13.21^{f}	13.77^{e}	13.49^{D}
25 Trp+ 200 AA	13.98^{de}	14.25^{d}	14.12^{C}	14.03^{de}	14.36^{d}	14.20^{C}
50 Trp + 100 AA	14.65^{dc}	14.96^{bc}	14.80^{B}	14.52^{dc}	14.95^{c}	14.73^{B}
50 Trp + 200 AA	15.57^{ba}	15.99^{a}	15.78^{A}	15.35^{ba}	15.68^{a}	15.52^{A}
Mean	13.37^{B}	13.94^{A}		13.50^{B}	13.76^{A}	

*Means with the same letter within each column are insignificantly different according to Duncan multiple range test at 5% probability level.

TABLE 5. Impact of Tryptophan and/or Ascorbic acid on the total soluble sugars, total alkaloids (%) and total phenolic compounds of seeds lupine in 2017/2018 and 2018 /2019.

Seasons	2017/2018			2018 /2019		
	TSS (%)					
Cultivars (A) / Conc. mg/L(B)	Giza-1	Giza-2	Mean	Giza-1	Giza-2	Mean
Control	23.42^{k}	24.84^{j}	24.13^{G}	23.97^{j}	25.03^{h}	24.50^{I}
25 Trp	25.31^{ji}	26.30^{h}	25.81^{E}	25.42^{h}	27.12^{f}	26.27^{F}
50 Trp	27.50^{f}	28.03^{e}	27.77^{C}	26.95^{f}	28.53^{d}	27.74^{D}
100 AA	23.42^{k}	25.00^{ji}	24.21^{G}	23.93^{j}	25.93^{g}	24.93^{H}
200 AA	23.68^{k}	25.48^{i}	24.58^{F}	24.55^{i}	26.75f	25.65^{G}
25 Trp+100 AA	26.14^{h}	27.47^{f}	26.81^{D}	26.17^{g}	28.01^{e}	27.09^{E}
25 Trp+ 200 AA	26.95^{dg}	28.33^{ed}	27.64^{C}	26.89^{f}	29.94^{c}	28.42^{C}
50 Trp + 100 AA	28.81^{d}	29.99^{b}	29.40^{B}	28.19^{ed}	31.04^{b}	29.62^{B}
50 Trp + 200 AA	30.95^{b}	31.96^{a}	31.45^{A}	31.07^{b}	32.94^{a}	32.01^{A}
Mean	26.24^{B}	27.49^{A}		26.35^{B}	28.37^{A}	
	Total alkaloids (%)					
Control	1.357^{gh}	1.192^{i}	1.274^{G}	1.353^{j}	1.178^{l}	1.266^{F}
25 Trp	1.543^{cb}	1.426^{ef}	1.485^{D}	1.467h^{g}	1.375^{ji}	1.421^{D}
50 Trp	1.662^{a}	1.494^{cd}	1.578^{B}	1.616^{a}	1.491^{efgd}	1.554^{B}
100 AA	1.429^{ef}	1.321^{h}	1.375^{F}	1.404^{hi}	1.30^{k}	1.349^{E}
200 AA	1.487^{d}	1.380^{gf}	1.434^{E}	1.446^{hg}	1.339^{jk}	1.393^{D}
25 Trp+100 AA	1.544^{cb}	1.460^{ed}	1.503^{DC}	1.508^{efcd}	1.448^{hg}	1.478^{C}
25 Trp+ 200 AA	1.565^{b}	1.482^{d}	1.524^{C}	1.529^{bcd}	1.477e^{fg}	1.503^{C}
50 Trp + 100 AA	1.642^{a}	1.548^{b}	1.594^{B}	1.57b^{a}	1.519e^{cd}	1.548^{BA}
50 Trp + 200 AA	1.690^{a}	1.588^{b}	1.639^{A}	1.617^{a}	1.547^{bc}	1.578^{A}
Mean	1.547^{A}	1.432^{B}		1.502^{A}	1.408^{B}	
	Total phenolic compounds (%)					
Control	2.327^{h}	2.302^{h}	2.314^{F}	2.374^{h}	2.336^{h}	2.354^{F}
25 Trp	2.548^{g}	2.673^{ef}	2.611^{E}	2.471^{g}	2.50^{g}	2.484^{E}
50 Trp	2.667^{ef}	2.793^{dc}	2.730^{C}	2.627^{f}	2.676^{ed}	2.652C
100 AA	2.802^{dc}	2.667^{ef}	2.734^{C}	2.752^{b}	2.50^{g}	2.624^{DC}
200 AA	2.911^{ba}	2.849^{bac}	2.880^{A}	2.854^{a}	2.669^{ed}	2.762^{B}
25 Trp+100 AA	2.550^{g}	2.692^{ef}	2.621^{DE}	2.519^{g}	2.664^{ed}	2.592^{D}
25 Trp+ 200 AA	2.620^{gf}	2.735^{fd}	2.675^{DC}	2.604^{f}	2.702^{ed}	2.654^{C}
50 Trp + 100 AA	2.838^{bc}	2.80^{dc}	2.817^{B}	2.785^{b}	2.775^{b}	2.779^{B}
50 Trp + 200 AA	2.90b^{a}	2.926^{a}	2.912A	2.855^{a}	2.884^{a}	2.869^{A}
Mean	2.684^{B}	2.714^{A}		2.649^{B}	2.633^{A}	

*Means with the same letter within each column are insignificantly different according to Duncan multiple range test at 5% probability level.

Dawood & Sadak (2007) referenced that the credibility of utilizing tryptophan as instrument to enhance the oil, protein and carbohydrate content of canola seeds/plant. Amino acids can serve as a wellspring of carbon and vitality once sugars become insufficient within the plants. Amino acids are, releasing the ammonia and organic acid form which the amino acid was originally formed. The organic acids then enter the Kerb's cycle, to be separated and discharge vitality through respiration (Goss, 1973). Amino acids provide plant cells a quickly open wellspring of chemical element, that by and enormous will be taken by the cells more than inorganic chemical element (Thon et al., 1981).

The augmentation in N fixation due to vitamin C application may well be processed by Talaat (1995) who declared that accumulation of nitrate by vitamin C would possibly be due to the constructive outcome of vitamin C on root development that so enlarged nitrate uptake. What is additional, would possibly increase the organic acids sweat from the roots into the soil and so increment the financial condition of most supplements gradually into the rhizosphere zone where it might be used by the plants (Hanafy-Ahmed et al., 1995).

The raising in protein content because of vitamin C application is also because of its role of in scavenging free radical oxygen and preventing protein oxidation and denaturation (Noctor & Foyer, 1998). El-Bassiouny et al. (2005) referenced that faba bean plants foliar sprayed at rate 400mg/L Ascorbic acid make enhancement to the total carbohydrate, rough protein and some elements such as K, P and Ca in seeds. In addition, exogenous use of vitamin C improve the protein moreover as starch contents in chick pea (Beltagi, 2008).

These results agree with Gamal El-Din (2005) on sunflower plants, who notify that antioxidant considerably raising oil % and proteins % of seeds. Moreover, Mohamed (2013) showed that foliar application of antioxidant such as vitamin C, vitamin B12 and Folic acid had important encouraging impact on proteins % and fat % of wheat seeds compared with control. Pastori et al. (2003) illustrated that antioxidant is one amongst the foremost important water soluble antioxidants in plants, going concerning as a modulator of plant advancement through thormone flagging and as coenzyme in responses by which starches, fats and proteins are processed. Also, Tarraf et al. (1999) stated that vitamin C caused considerable increase in lemongrass oil and oil yield/plant. This could also be because of the raising N concentration in seeds that agree with finding by Talaat (2003),who indicated that the aggregation of nitrate by antioxidant application may be as a result of the beneficial outcome of ascorbic on root development which thusly expanded nitrate ingestion. Also, Dolatabadian et al. (2010) found that application of Ascorbic acid at concentration 150ppm enhancing protein percentage of grain corn.

Data in Table 4 showed that foliar application with 25, 50mg /L was more positive effect from Ascorbic acid at 100, 200mg /L, singly or together that caused a significant increase in total soluble sugar percentages and total alkaloids (%) in the yielded lupines seeds.

Generally, it is obvious that Giza-2 cultivar surpassed Giza-1 cultivar in both seasons, in crude lipids, total soluble sugars and total phenolic compounds and it is obvious that Giza-1 cultivar surpassed Giza-2 cultivar both in total nitrogen, crude protein and total alkaloid. Thee effect of Tryptophan and Ascorbic acid on total soluble sugar percentages, total alkaloids (%) and phenolic content was reported by Dawood & Sadak, (2007) indicated that foliar spray of Tryptophan of canola seeds/plant significantly increased total carbohydrate %. Hendawy (2000) on *Echinacea* plants, reported that the utilization of amino acids (Tryptophan, Tyrosine Asparatic and Glutamic acids) significantly increased total carbohydrate percentage, caffeic acid derivatives production and total lipid content. Wahba et al. (2002) on *Antholyza aethiopica,* showed that Tryptophan at 25, 50 and 75ppm for each enhances total soluble sugars and free amino acids Abou Dahab & Abdel -Aziz (2006) reported that using of Tryptophan and phenylalanine at rate 100ppm significantly improve photosynthesis proses and total sugar in *Iberis amara* L. (Attoa et al., 2002), *Salvia farinacea* (Abdel -Aziz & Balbaa, 2007) and *Antirrhinum majus* L. because of their important impact on the synthesis of pigment molecules that therefore influenced total soluble sugars (Abdel -Aziz et al., 2009). Talaat et al. (2005) announced that Tryptophan enlarged soluble and total insoluble sugars, total proteins and total alkaloids contents of *Catharanthus* *roseus* L. plants. World Health Organization expressed that everyone starting from amino acids in addition add the consolidation of alternative compound, like macromolecule amines, alkaloids and enzymes (Goss, 1973).

El-Quesni et al. (2009) shown that exploitation of vitamin C to hibiscus plants significantly enhance total carbohydrate %. Antioxidant prevents carbohydrates, fats, proteins and nucleic acids (DNA and RNA) from damage motivated by aerobic stress and different responsive species (Higdon & Victoria, 2012). Ascorbic acid plays important role in plant advancement through as co- enzyme in responses by which carbohydrates, fats and proteins are metabolized (Pastori et al., 2003).

Data illustrate in Table 4 also show that foliar spray with Ascorbic acid at rates 100, 200mg/L was more positive in case of Tryptophan 25, 50mg/L, separately or in incorporated, it greatly promoted and caused a significant increase in total phenolic constituents % in the yielded lupines seeds.

These results explained the constructive effect of vitamin C on enhancing the total phenolic compounds. These results are in concurrence with the resulting of previous researches that concentrated n the impact of Ascorbic acid as foliar spray on total phenol components. Also, show that raising Ascorbic acid concentration from 50 to 200ppm improveme total phenol ingredients compared with control (Abdel -Aziz et al., 2007). Likewise, these results are in agreement with that reported in literature, which concluded that total soluble sugar content significantly increased when plants were treated with Ascorbic acid at 200ppm (Abdel Aziz, et al. 2009). The present data in aharmony with Youssef & Talaat (2003), on *Khya senegalensis* L. plants (Abdel -Aziz et al., 2007), on *Syngonium podyphyllum* L. plants (Abdel -Aziz et al., 2007) and on *Cupressus sempetrirens* L. (Farahat et al., 2007). This constructive outcome of vitamin C might be because of the gainful impact of vitamin C on advancing the biosynthesis of starches and take-up of most supplements (Masoud & El-Sahrawy, 2012) particularly; N, P and K and soluble sugars (Talaat, 2003; Abdel -Aziz et al., 2009).

Conclusion

Tryptophan (Trp) and Ascorbic acid (AA) can be effectively applied to lupine plants in the field. It could be inferred that foliar utilization of Trp and AA could assume an upgrade roles on numerous metabolic and physiological processes of lupine *(Lupines termis* L.) that reflected in expanding seed quality processes of lupine (*Lupines termis* L.). Use of Trp and AA separately, brought about a critical increment in each morphological trait and healthy benefit. They improved the yield contributing characters and nutritional estimation of seeds as a promising potential well spring of negligible exertion protein, minerals, amino acids and oils for possible use as sustenance/feed supplements. This seed quality represented in increasing of protein content, oil content, total soluble sugars, total alkaloid and total phenolic compounds of seeds.

Recommendation

In general, the study recommended Giza-2 cultivar with in this concern was tryptophan (Trp) at rate 50mg/L+ 200mg/L (AA) of Ascorbic acid combination for agriculture under Assiut conditions.

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