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EFFECT OF FOLIAR APPLICATION WITH ACTIVE YEAST EXTRACT AND BENZYLADENINE ON SOME VEGETATIVE GROWTH CRITERIA AND CHEMICAL COMPOSITION OF LUPINE (*Lupinus termis, L.*) PLANTS

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ABSTRACT

In the present study, a field experiment was conducted during season, 2013/2014 to study the effect of spraying with active yeast extract and benzyladenine on chemical composition of *Lupinus termis* seeds using two cultivars namely Giza-1 and Giza-2 of lupine plants and their interactions. The effect of the same treatments on some vegetative growth criteria namely plant height (cm), seed yield (g/plant) and weight of 100 seeds was investigated as well as chemical composition such as, moisture, ash, total soluble sugars (TSS), crude lipids (CL), total nitrogen, crude protein (CP), total alkaloids (TAs), and total phenolic compounds (TPCs). The results indicated that foliar application with active yeast extract and benzyladenine significantly increased vegetative growth criteria and chemical composition. Seven chemical constituents of lupine dry seeds (total ash, total nitrogen, CP, TSS, CL, TAs and TPCs) were significantly affected by used treatments. Spraying application of yeast extract either foliar spraying or soil spraying caused significant increase in plant height (cm), seed yield (g)/plant and weight of 100 seeds of lupine. On the other hand, the effect of active yeast extract and benzyladenine on moisture (%) was insignificant in both treatments. In addition, treatment of plants with active yeast caused a significant decrease in total alkaloids content as compared with control treatment. The effect of active yeast extract on TPCs was insignificant.

Results of the chemical composition of lupine dry seeds indicated that the seeds of *L .termis*, contained higher crude protein level (37.5%) in sweet lupines than those reported for bitter lupines Giza-1 (34.13%), Giza-2 (33.06%), and Giza-3 (33.01 %). The CL in the seeds of bitter lupines reached to be 11.13% (Giza-1), 11.82% (Giza-2), and 12.42% (Giza-3) and 9.9 % (Giza-1) in sweet lupines. Seeds of sweet lupines (SL) contain higher TSS level (25.7%) than those reported for bitter lupines (BL). The elementary analyses of lupine dry seeds were showed that levels of sodium in bitter lupines Giza-3 (0.35%) were higher than those found in bitter lupines Giza-2 (0.30%). The potassium (K^+) content in bitter lupines Giza-1 was 204.3 ppm and this value is higher than those found in bitter lupines Giza-3 (178.6 ppm) and seeds of sweet lupine Giza-1 (169.5 ppm) and seeds of bitter lupines Giza-2 (163.7 ppm), while phosphorus content (P%) in seeds of bitter lupines Giza-1 (0.75%) was higher than those found in sweet lupines Giza-1 (0.74%) and seeds of bitter lupines Giza-2 (0.70%) and seeds of bitter lupines Giza-3 (0.68 %). Generally, these data indicate that studied seed of cultivars are rich and inexpensive sources of crude protein (33.06 -37.5 %), crude lipids (9.9-12.4%), TSS (23.03-25.7%) and total alkaloids (TAs, 1.19 to 1.378%).

Key words: Alkaloids in lupines; benzyladenine; bitter lupines; chemical composition of *Lupinus termis* plants; Spraying application of yeast extract; sweet lupines.

INTRODUCTION

Lupine (*Lupinus termis*, *L*.) is cultivated in a wide range of environments across Egypt. Its seeds have a nutritional quality similar to soybean seed and superior to other legumes seed (Raza and Jrnsgard, 2005), and could be an important source of protein and oil. In fact, lupine seeds have been used for human consumption and as a medicinal plant in Egypt (Kattab, 1986; and ARC, 1994) and other countries for thousands of years. Lupine (*L. termis*) is one of the oldest agricultural crops widely used in the world as a protein source in fodder production and for soil improvement (Maknickiene, 2001). Lupines are good source of protein and lipids and have no lectins and very low content of protease inhibitors (Australia New Zealand Food Authority, 2001).

In general, lupines are used for many purposes. These include pasture improvement, ornamentation, and erosion control and soil stabilization. It has also been used as a green manure and for fixing atmospheric nitrogen to the soil. Furthermore, it can be mixed in the soil during the flowering period in green houses to control some pests due to its alkaloids (Uzun *et al*, 2006).

Benzyladenine (BA) is one of the cytokinins which improve quantitatively and / or qualitatively the

yield of many plants (Gamal EI-Din and Talaat 1999 and Reda *et al*., 2010). BA plays permissive role in the regulation of various growth processes in the plants (Skoog *et al*., 1967 and Ibrahim *et al*., 2010). The improvement of growth of plants in response to foliar application of the treatments may result in improving quality of pods such as increased protein, nitrogen, total soluble sugars and oil content. Cytokinin including (BA) could induce cell division of excised root tissue and accompanied by great changes in protein (Butcher *et al.,* 1988 and Reynold, 1990). Mostafa *et al*., (1993) proved that (BA) treatment on soybean plants induced a highly significant increase in the oil percentage. El-Meleigy (1989) and Sun *et al*., (1996) reported that benzyladenine enhanced the accumulation of total carbohydrates and total N-contents in Roselle.

Yeast is a natural source of cytokinins and has stimulatory effects on bean plants (Amer, 2004). Yeast extract was suggested to participate in a beneficial role during vegetative and reproductive growth through improving flower formation and their set in some plants due to its high auxin and cytokinins content and enhancement of carbohydrates accumulation (Barnett *et al.,* 1990). Also, its stimulatory effects on cell division and enlargement, protein and nucleic acid synthesis and chlorophyll formation was reported (El-Desouky *et al.,* 1998 and Wanas 2002, Wanas, 2006), in addition to its content of cryoprotective agent, i.e. sugars, protein, amino acids and also several vitamins (Mahmoud 2001). Moreover, the improving growth, flowering and fruit set by using foliar application with yeast extract was reported by Fathy *et al*., (2000); Abou-Aly, (2005) and Wanas (2006).

The main objectives of the present investigation are:

1. To study the effect of spraying application with active yeast extract either spraying of foliar or spraying of soil and use of different concentrations of benzyladenine on chemical composition of *Lupinus termis* plants.
2. To study the changes in chemical composition induced by foliar application of active yeast extract and benzyladenine on *L. termis* plants.

MATERALS AND METHODS

Samples:

Four dry beans of lupine (*Lupinus spp*. L.) cultivars were provided from Agricultural Research Centre, Giza, Egypt. Three varieties were bitter samples and one cultivar was sweet sample. These samples were kindly provided by Prof. Dr. Ahmed Mekheimar, professor in Agricultural Research center, Giza.

Experiment:

This experiment was carried out in the Laboratory and Experimental Farm of Faculty of Agriculture, Al-Azhar University, Assiut, Egypt during the season of 2013/2014 to study the effect of spraying with active yeast extract and benzyladenine on chemical composition of *Lupinus*

termis, L. plants. Experimental design was laid in a split plot arrangement in completely randomized block design with three replications, and cultivars (Giza-1 and Giza-2) of lupine, *Lupinus termis* L. plants of Lupine, *Lupinus termis* L. plants considered the main plots, spray treatments were assigned as sub plots. Lupines seed were sown on October 25th of the first season. The experimental plot was 3.6 × 3 m and contained 5 rows, 60 cm apart. The distances between the hills were 30 cm. All agricultural practices were performed as usual. At the end of the experiment, the following data were recorded: (plant height (cm), seed yield (g/plant) and weight of 100 seeds) and 8 constituents were assayed; moisture (%), total ash (%), total nitrogen (TN %), crude protein, Total soluble sugars (TSS) (%), crude lipids (%), total alkaloids (%) and total phenolic compounds.

Spraying was performed using plastic atomizer, and plants were sprayed twice with yeast and benzyladenine, the first spraying was applied after 45 days of sowing and the second spraying was applied on 15 days later. These treatments were as follows: -

1- Yeast extract (spraying foliar – spraying soil) at (control – 90 ml/L).

2- Benzyladenine was applied foliarly twice at (control, 20 ppm and 40 ppm).

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 were performed in triplicates and means were reported.

Determination of moisture and total ash content (TAC):

Lupine samples were firstly dried at 60°C for 6 hours and the temperature was increased to be 105°C until a constant weight was reached (AOAC;1984). TAC is determined by heating the samples (1.0-2.0 g) in a muffle furnace at about 600±10°C for 3 hrs. until they were completely ashed (AOAC, 1984).

Determination of crude lipids (CL):-

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

Determination of total nitrogen and crude protein (CP):

The Kjeldahl procedure was 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.

Extraction and determination of total soluble sugars (TSS):-

Preparation of samples: A portion of 100 mg of dried seeds was placed in a test tube, then 10 ml of H_2SO_4 (1N) were added, then the test tube was placed in water bath at 100°C for 30 min. then, the test tube was left to cool, and 0.1 g 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 100 ml volumetric flask and completed to 100 ml 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 concentrations (10 to 100 g/ml) of pure glucose.

Determination of total reducing sugars (TRS):

Eight ml of the ethanolic extract containing total soluble sugars were transferred into a 25 ml measuring flask, and completed to mark with distilled water, then 5 ml of the solution were pipetted in a test tube, followed by addition of 2 ml of 3,5-dinitrosalicylic acid. The test tube was then heated and the developed color was measured after cooling to room temperature and reducing sugars were calculated from a standard calibration curve for glucose and calculated as mg glucose/100 g dry matter (Bernfeld, 1955 and Miller, 1959).

The difference between the percentage of total soluble sugars and total reducing sugars was taken as the percentage of non-reducing sugars.

% Non-reducing sugars = % Total soluble sugars - % Total reducing sugars

Determination of vitamin C (L – ascorbic acid):-

The indophenol method (2,6-dichlorophenol indophenol, 50 mg in 250 ml H_2O), as described by Mondy and Ponnampalam (1986), was used for determination of L-ascorbic acid concentration in *L. termis***.** Vitamin C was extracted using 1.25 % oxalic acid solution. All determinations were performed in triplicates and the mean values are recorded.

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 565 nm against a blank. The determination was carried out in triplicates.

Extraction and determination of total phenolic compounds (TPCs):-

TPCs were extracted from lupine sample (1.0g) by refluxing with 30 ml of methanol containing 1 % HCl for 10 min, and then the extract was centrifuged at 8,000 r.p.m. for 10 min. The concentrations of total phenolic compounds in the methanolic extracts were determined by the method described by Singleton and Rossi (1965) with some modifications. One milliliter of sample was mixed with 1 ml of Folin and Ciocalten's phenol reagent. After 3 min. 1 ml of saturated Na_2CO_3 (35 %) was added to the mixture and this was made up to 10 ml by adding distilled water. The reaction was kept in the dark for 90 min, after which its absorbance was read at 725 nm. A calibration curve was constructed with different concentrations of gallic acid (0.01 – 1 mM) as standard.

Elementary analysis of lupine seeds:-

All elements except (Na, K, P) were determined according to the official method (AOAC, 1984), using the ICPES (Inductively Coupled Plasma Emission Spectrometer) (type ICAP 6200), Na and K were determined by flame photometer and Phosphorus was determined using the Spectrophotometer using flame photomrssi. All determinations were done in Faculty of Agriculture, Assiut University, Assiut, Egypt

Amino Acid Analysis

The analysis of amino acid was performed in Central Service Unit, Research Centre, Giza, Egypt, using LC3000 amino-acid analyzer (Eppendorf-Biotronik, Germany).

Statistical Analysis

Experimental design was laid in a split plot. The obtained data were subjected to the analysis of variance procedure and treatment means were compared to the LSD test according to Gomez and Gomez (1984).

RESULTS AND DISCUSSIONS

(1)-Effect of application treatments with AYE and/or BA on the plant height (cm), seed yield (g/plant) and weight of 100 seeds

Data presented in Table (1) show that foliar spraying with active yeast extract (AYE) and/or benzyladenine (BA) significantly increased the plant height (cm), weight of 100 seeds and seed yield (g/plant) in lupines seeds. These results are in agreement with those obtained by Amer (2004) who found that foliar application with active yeast increased weight of 100 seeds of bean plants. Also, Tartoura (2001) stated that foliar application with active yeast extract induced significant increase in number of seeds/pod and weight of 100 green seeds of pea plants. These findings are in agreement with the results reported by Mahmoud, *et al.,* (2013) on pea plants and Abo-El-Hamd *et al*., (2015) on faba bean they found that the application of yeast extract increased yields and their component and. It could be concluded that, yeast extract treatments were suggested to participate beneficial role during vegetative and reproductive growths through improving flower formation and their set in some plants due to its high auxins and cytokinins contents and its beneficial effect on carbohydrate accumulation (Barnett *et al.,* 1990). The trend of these results is supported by El-Tohamy *et al* (2008), Abou EL-Yazied and Mady (2011 and 2012), Kamal and Ghanem (2012) and Mahmoud, *et al.,* (2013). Ayad and Gamal El–Din (2011) reported that spraying foliar application by benzyladenine significantly increased plant height, weight of 1000 seeds and seed yield (g/plant) in lupines seeds. Hussein (2005) reported that the spraying of *Catharanthus roseus*, L. with BA significantly increased plant height, fresh and dry weight of leaves. Gamal EI-Din and Talaat, (1999) found that spraying of benzyladenine on sugar-beet enhanced vegetative growth and yield. Dawood and Sadak (2007) indicated that exogenous application of (BA) increase plant height, fresh and dry weights of *Brassica napus*, L. plant. These results are in agreement with those reported

by Mansour *et al*., (1994) who stated that foliar application of benzyladenine caused significant increase of soybean plant height; fresh and dry weight/plant; leaf area and leaf area index. Moreover, benzyladenine application to pepper plants induced significant increase in plant height; number of branches and number of leaves/plant (Abdel-Hamid, 1997). Such increase in growth parameters in response to benzyladenine application can be attributed to the increase of cell elongation and/or cell division (Li and Liu 2003, Hopkins and Huner 2004).

Table (1): Effect of application treatments with active yeast extract (AYE) and benzyladenine (BA) on the plant height (cm), seed yield (g/plant) and weight of 100 seeds (gm).

Treatments	Plant height (cm) in Cultivar (A)		
	Giza-1	Giza-2	Mean
Control	101.57	106.37	103.97
Foliar application with AYE	114.87	121.33	118.12
Soil treatment with AYE	108.23	114.80	111.52
Treatment with BA, 20 ppm	115.40	122.07	118.74
Treatment with BA, 40 ppm	118.30	124.83	121.57
$LSD_{0.05}$	A N.S	B 21.025	AB N.S
	Seed yield (g)/plant in Cultivar (A)		
Control	17.34	19.92	18.63
Foliar application with AYE	25.29	26.71	26.00
Soil treatment with AYE	22.83	24.16	23.50
Treatment with BA, 20 ppm	25.29	27.53	26.41
Treatment with BA, 40 ppm	27.16	28.94	28.05
$LSD_{0.05}$	A N.S	B 10.917	AB 1.132
	Weight of 100-seeds		
Control	33.07	35.91	34.49
Foliar application with AYE	34.92	37.08	36.00
Soil treatment with AYE	33.85	36.53	35.19
Treatment with BA, 20 ppm	35.01	36.92	35.97
Treatment with BA, 40 ppm	35.23	37.35	36.29
$LSD_{0.05}$	A 0.723	B 2.477	AB 2.531

In addition, yeast extract treatments were suggested to participate in a beneficial role during vegetative and reproductive growth through improving flower formation and their set in some plants due to its high auxins and cytokinins content and its beneficial effect on protective agents i.e. sugars and amino acids as well as, several vitamins (Shady, 1978 and Mahmoud, 2001). The effect of foliar application with yeast extract on

production and quality of two faba beans was recently investigated by Abo-El-Hamd, *et al*., (2015). They found that faba bean varieties exhibited significant differences in 100-seed weight, seed yield and straw yield/fed and protein percentage in faba bean seeds in both seasons, except seed yield /plant in the first season only. Application of yeast extract induced significant increases in number of branches, number of pods, seed yield/plant, 100-seed weight as well as seed and straw yield /fed. Also, such treatment increased phosphorus and protein percentage of faba bean seeds.

(2)-Effect of application treatments with AYE and/or BA on primary and secondary metabolites: -

Results of the primary chemical metabolites in lupine seeds including six constituents i.e. dry matter accumulation, total ash content, total nitrogen, crude protein, crude lipids, total soluble sugars, as well as, two secondary metabolites namely total alkaloids and total phenolic compounds are given in Table (2). These results indicate that chemical constituents in the treated lupine seeds were gradually increased with increasing the concentrations of yeast or benzyladenine. It is worthily to mention that treatment with benzyladenine at 40 ppm/L showed the highest increases in the chemical constituents of yielded seeds than the other treatments.

Data presented in Table (2) show a significant increase in the crude lipids of the yielded lupine seeds. These results are in agreement with those obtained by Mostafa *et al*. (1993) and Abdel-Rahim *et al*. (2000) who pointed that foliar application of benzyladenine to soybean and datura induced significant increase in oil percentage. The increase in oil percentage as a result of benzyladenine treatments may be due to its promotive effect on nutrient transport to the developing seeds (El-Meleigy, 1989; Talaat and Youssef, 1998).

Benzyladenine foliar application (Table 2) caused a marked increase in the protein percentage and total carbohydrates. This result is similar to those reported by El-Abagy *et al*. (2003) on faba bean. El-Meleigy (1989) and Sun *et al*., (1996) reported that benzyladenine enhanced the accumulation of total carbohydrates and total N-contents in Roselle. Cytokinins are known to activate enzymes which regulate carbohydrate metabolism. The increase in carbohydrate accumulation may be due to the decline in the carbohydrate degradation (Cao and Shannon, 1997). Hopkins and Huner (2004) reported that benzyladenine promoted RNA and protein synthesis, while it inhibited certain proteolytic enzymes (Shibaoka and Thimann 1970). Tarraf (1999) found that spraying sugar beet plants with benzyladenine (BA) significantly increased T.S.S. %. Dawood and Sadak (2007) reported that spraying canola plant with benzyladenine caused significant increases in the oil%, protein and phenolic compounds.

Data presented in Table (2) show significant increases in the total nitrogen, crude protein and total soluble sugars of the yielded lupine seeds. The effect of yeast application on protein, nitrogen and carbohydrates was previously studied in other lupine plant species (Mady, 2009).

The improving of AYS treatments for proline and soluble sugars under different water supplies could be related to its ability to produce these substances in the surrounding environment (Mahmoud, 2001) and to increase the efficiency of photosynthesis which enhances all metabolic pathways.

Foliar application with yeast extract was sufficient for reducing the harmful effect of cadmium and improved the percentage of crude protein and total lipids in seeds of soybean plants with polluted cadmium (Abdo *et al*., 2012).

Data presented in Table (2) also show that foliar application with yeast ((90 ml) or benzyladenine (40 ppm/l) caused a significant increase in phenolic compounds percentage in the yielded lupines seeds. The effect of benzyladenine on the phenolic content was reported by Abdel-Al *et al*., (1988) who showed that cytokinins increased phenolic content in cotton plants. The increase in phenolic content may be attributed to the increase in carbohydrate synthesis. This result was confirmed by Maksoud and Dadoura (1997) in *Helianthus annuus* and Shehata *et al.*, (2001) in maize. The increase in total phenolic content was concured with the increases in IAA contents in shoots and led to the suggestion that most of phenolic compounds are diphenols and polyphenols which may inhibit IAA-oxidase activity resulting in auxin accumulation, which reflected in stimulating the growth and yield of plant as reported by Mervat, (2005)**.** The effect of AYS on phenolic compounds was insignificant in both seasons as reported by Ibrahim (2014).

Biochemical evaluation of lupine dry seeds:-

To evaluate the four dry lupine seeds, the chemical composition is undertaken including ten chemical constituents and the results are given in Table (3). These data showed that lupine seeds contain the highest crude protein level (37.5%) in sweet lupine cultivar Giza-1 and lower levels as reported in bitter lupines Giza-1 (34.13%), Giza-2 (33.06 %), and Giza-3 (33.01%). The crude lipids in the seeds were found to be 11.13% (Giza-1), 11.82 (Giza-2), 12.42% (Giza-3) in bitter lupines and 9.9 % (Giza-1) in sweet lupines. The results indicated that the lupine seeds contained higher protein levels such results are close to those reported for soybean seeds by Abd El-Raheem *et al*., (2016).

The seeds of lupine cultivars have been used with increasing frequency as a source of proteins replacing proteins of animal origin or soybean in feed compounds. It contains 33-40% crude protein, 5-13% oil and relatively beneficial amino acids profile (Písaříková and Zralý, 2009).

Results also showed that concentrations of total soluble sugars (TSS) ranged from 25.7 to 23.03% and the highest level is determined for

sweet lupines and the lowest one for bitter lupines (Giza-3). These data indicate that the lupine seeds are rich and inexpensive sources of crude protein (33.06 -37.5%), crude lipids (9.9-12.42%) and T.S.S (23.03-25.7%). Moisture content in bitter lupine Giza-2 seeds is lower than those reported in bitter lupines Giza-1 and Giza-3 and sweet lupine. Levels of L-ascorbic acid (vitamin C) are higher in bitter lupines Giza-1 (16 mg /100g) followed by sweet lupine (15 mg/100g); bitter lupines Giza-2 (14 mg /100g) and bitter lupine Giza-3 (13 mg /100g). *lupines* seed meal, however, may be used as an animal feed ingredient because of the presence of several nutrients.

The composition of *Lupinus termis* L. oil from Egypt consists of many fatty acids such as palmitic acid 9.13 %, oleic acid 36.31 %, linoleic acid 17.3% , linolenic acid 7.29 % and Unsaturated fatty acid 60.9 % (Ayad and Gamal El–Din (2011).

Elementary analysis of *lupines* seeds .

The elements analysis of seeds of *L. termis L* are presented in Table (4). The results show that the levels of some elements are higher in seeds of *L. termis L* than those found in seeds of legumes. Sodium contents in lupine bitter Giza-3 (0.35%) are higher than those reported in lupines bitter Giza-2 (0.30%), seeds lupines sweet Giza-1 (0.28%) and seeds lupines bitter Giza-1 (0.25%). Potassium (K^+) contents in dry lupine seeds ranged from 204.3 to 163.7 ppm and the highest content is found in the seeds of bitter lupine Giza-1 followed by Giza-3 (178.6 ppm) and the lowest one is found in bitter lupine Giza-2 (163.7 ppm), whereas, sweet lupine Giza-1 contains 169.5 ppm. Phosphorus percent (P %) in seeds of bitter lupine Giza-1 (0.75%) are higher than those reported in all lupine samples. The heavy metals determined, such as Co and Pb are exits in all tested cultivars, but these seeds are free from Ba, these results indicated that *L. termis* seeds are capable to store some heavy metals in own tissues.

Phosphorus is always found with calcium in the body, both contributing to the blood formation and supportive structure of the body. Modern foods rich in animal protein and phosphorus can promote the loss of calcium in urine (Shills and Young, 1992). This led to the concept of calcium phosphorus ratio (Ca/P). If the Ca/P ratio is low, calcium will be low and there will be high phosphorus intake which leads to calcium loss in the urine more than normal. If the Ca/P of any food is above one that food is considered "good" and "poor" if the ratio is less than 0.5. A Ca/P ratio above two helps to increase the absorption of calcium in the small intestine. The results of Ca/P ratio in the studied samples Giza-1, Giza-2, Giza-3 and sweet Giza-1 were 1.093, 1.704, 1.529 and 1.297, respectively. Our results show that lupine seeds are good sources of many elements when compared with other legumes such as soybean.

Table (2): Effect of application treatments with active yeast extract (AYE) and benzyladenine (BA) on the levels of some chemical constituents of the yielded lupine seeds.

Treatments	Moisture (%) in Cultivar (A)		
	Giza-1	Giza-2	Mean
Control	6.896	6.862	6.879
Foliar application with AYE	6.898	6.849	6.874
Soil treatment with AYE	6.859	6.824	6.862
Treatment with BA, 20 ppm	6.896	6.828	6.862
Treatment with BA, 40 ppm	6.893	6.833	6.863
LSD0.05	A 0.097	B NS	AB NS
	Total ash content (%) in Cultivar (A)		
Control	3.467	3.700	3.584
Foliar application with AYE	3.764	3.884	3.824
Soil treatment with AYE	3.590	3.799	3.705
Treatment with BA, 20 ppm	3.750	3.823	3.787
Treatment with BA, 40 ppm	3.757	3.917	3.837
LSD0.05	A 0.061	B 0.315	AB NS
	Total nitrogen (%) in cultivar (A)		
Control	5.46	5.29	5.38
Foliar application with AYE	6.01	5.76	5.89
Soil treatment with AYE	5.82	5.57	5.70
Treatment with BA, 20 ppm	6.19	5.88	6.04
Treatment with BA, 40 ppm	6.37	6.11	6.24
LSD0.05	A NS	B 0.979	AB NS
	Crude protein (%) in Cultivar (A)		
Control	34.15	33.09	33.62
Foliar application with AYE	37.54	36.02	36.78
Soil treatment with AYE	36.35	34.83	35.59
Treatment with BA, 20 ppm	38.71	36.73	37.72
Treatment with BA, 40 ppm	39.79	38.21	39.00
LSD0.05	A NS	B 6.115	AB NS
	Crude lipids (%) in Cultivar (A)		
Control	11.13	11.82	11.48
Foliar application with AYE	12.11	13.57	12.84
Soil treatment with AYE	11.82	12.82	12.32
Treatment with BA, 20	12.62	13.95	13.29
Treatment with BA, 40 ppm	13.61	14.78	14.20
LSD0.05	A NS	B 3.032	AB 0.634
	TSS (%) in Cultivar (A)		
Control	23.19	24.70	23.95
Foliar application with AYE	25.31	26.70	26.01
Soil treatment with AYE	24.72	26.16	25.44
Treatment with BA, 20 ppm	25.29	27.53	26.41
Treatment with BA, 40 ppm	26.56	28.24	27.40
LSD0.05	A NS	B 3.801	AB 0.729
	Total alkaloids (%) in Cultivar (A)		
Control	1.350	1.217	1.284
Foliar application with AYE	1.347	1.195	1.271
Soil treatment with AYE	1.349	1.211	1.280
Treatment with BA, 20 ppm	1.364	1.224	1.294
Treatment with BA, 40 ppm	1.378	1.238	1.308
LSD0.05	A 0.009	B NS	AB NS
	Total phenolic compounds (mg/100g) in Cultivar (A)		
Control	2.119	2.192	2.156
Foliar application with AYE	2.128	2.207	2.163
Soil treatment with AYE	2.220	2.198	2.209
Treatment with BA, 20 ppm	3.208	3.269	3.239
Treatment with BA, 40 ppm	3.262	3.295	3.279
LSD0.05	A NS	B 1.787	AB NS

NS= Non-Significant, AYE = Active Yeast Extract, BA= Benzyladenine

Table (3): Analytical data for proximate composition of lupine seeds.

Constituents	Giza-1 (bitter)	Giza-2 (bitter)	Giza-3 (bitter)	Giza-1 (sweet)
Moisture	6.89±0.68	6.86±0.68	7.2±0.72	7.29±0.72
Total ash content, TAC	3.47±0.34	3.62±0.36	3.51±0.35	3.90±0.39
Crude fiber, CF	9.37±0.93	9.62±0.96	9.51±0.95	10.75±1.07
Crude lipid, CL	11.13±1.45	11.82±1.18	12.42±1.24	9.99±0.99
Total nitrogen, N	5.62±0.56	5.29±0.52	5.15±0.51	6.00±0.6
Crude protein CP	34.13±3.51	33.06±3.31	33.01±3.31	37.5±3.75
Total soluble sugars, TSS	23.19±2.32	24.69±2.47	23.03±2.30	24.18±2.42
Total reducing sugars, TRS	13.03±1.30	13.88±1.39	12.29±1.23	12.78±1.28
Total non-reducing sugars, TNRS*	10.16±1.02	10.81±1.08	10.60±1.06	11.40±1.14
Vitamin C, mg /100g	16±1.6	14±1.4	13±1.3	15±1.5

* Total non-reducing sugars TNRS was calculated by differences between TSS-TRS; Each value represents the mean ±Standard deviation

Table (4): Elementary analysis of lupine seeds

Element	Giza-1 (bitter)	Giza-2 (bitter)	Giza-3 (bitter)	Giza-1 (sweet)
Cu, ppm	12	10	13	13
Fe , ppm	78	80	77	75
Pb, ppm	17	18	16	14
Li, ppm	1.1	1.4	1.5	1.4
Zn, ppm	75	70	68	70
Co ppm	16	14	16	10
K^+, ppm flame photometer	204.3	163.7	178.6	169.5
M (%)	0.72	0.67	0.60	0.72
Mn (%)	86	88	90	87
Ba	0.0	0.0	0.0	0.0
Na (%) (flame photometer	0.25	0.30	0.35	0.28
Ca (%)	0.82	1.21	1.04	0.96
P (%)	0.75	0.71	0.68	0.74
Ca/P ratio	1.093	1.704	1.529	1.297

All elements except (Na, K, P) were determined using the ICP (Inductively Coupled Plasma Emission Spectrometer) (iCAP 6200), Na and K were determined by flame photometer and Phosphorus was determined using the Spectrophotometer*

Calcium (0.82-1.21%) makes lupine seeds suitable for bone formation for children. Potassium is the most abundant mineral in agricultural products. Processing (boiling, cooking, roasting, sprouting and fermentation) significantly reduced the content of some minerals (Mg, P, Ca, and Na) (Audu and Aremu, 2011).

Amino acid analysis of lupine seeds:

The amino acids compositions of four lupine seeds were determined and the results are given in Table (5). These results reveal the existence of 17 amino acids in all studied samples. The lupine seeds contained a lower amount of sulphur-containing amino acid (cystine + methionine). Regarding the non-essential amino acid, glutamic acid was the most abundant one followed by aspartic acid and arginine. However, E/T ratio indicated a misleading conclusion for the nutritional quality of the protein. An excess or deficiencies of certain amino acids in protein have deleterious effects on nutrition. Protein quality depends on the concentration and ratios of amino acids making up a specific protein. The greater ratio of indispensable amino acids, is the greater biological value or quality. The provisional amino acid scoring pattern proposed by the World Health organization of the United Nations (FAO/WHO, 1973) qualified an ideal protein one in which 36% of the total residues are essential amino acid. The protein of both BL Giza-1 and SL Giza-1 had higher amounts of EAA than those observed for BL Giza-2 and BL Giza-3. The ratios of E/T % were the highest in proteins of BL Giza-1 39.54%, which had lower level of essential amino acids (13.69%).

Sulfur containing amino acids SAA (Methionine+cystine) in BL Giza-1 are higher than those reported for the other three cultivars. Aromatic amino acids ARAA (phenylalanine + tyrosine) ranged from 2.94 to 3.56% and the highest level is found in BL Giza-1 and the lowest one for BL Giza-3.

Amino acids comparison among serum albumin, soybean and lupine seeds indicate that all lupine seed amino acids were under their levels in corresponding protein samples (Table 6). These results are in good agreement with those reported by Torun *et al*, (1981) for soybean seeds.

Lysine levels in powder of lupine seeds:-

The results indicate that the levels of positively charged basic amino acid lysine ranged from 1.53% to 1.82% for bitter lupine seed Giza-2 and sweet lupine seed Giza-1. These results also indicate that levels of lysine in sweet lupine are 118.95% higher than those determined in Giza-2 (100%). Lysine is essential amino acid and very important amino acid in the defence mechanism of quinolizidine alkaloids (QA). Cultivar containing the highest level of lysine is considered desired and favored on the view of point of legume breeders. It became well-documented that isolated QA are biosynthesized from lysine. It was established by Wink (1987) that lysine and its decarboxylation product

cadaverine serve as the only precursors for the bi- and tetracyclic QA. Since plants must have mechanisms for their protection against pathogens and herbivorous, QA might be a suitable means for defence, since most legumes have soft tissues which are not defended by thorns or stining hairs. Legume species which contain QA usually do not accumulate other secondary metabolites to a similar degree. QA-free legumes on the other hand accumulate toxic non-protein amino acids, pyrrolizidine alkaloids, cyanogenic glycosides, proteinase inhibitor, or lectins instead, for which a defensive roles is also assumed (Wink, 1985, 1987; Polhill and Raven 1981 and Franco *et al*., 2002).

Table (5): Amino acid concentrations (%) in lupine seeds.

Amino acid % (AA)	Cultivars			
	BL Giza-1	BL Giza-2	BL Giza-3	SL Giza-1
Non-essential (NEAA)				
Glycine	1.41	1.59	1.36	1.52
Alanine	1.20	1.31	1.12	1.31
Aspartic	3.72	3.49	3.49	3.95
Arginine	3.40	3.32	3.36	4.37
Glutamic	7.29	7.49	7.19	7.48
Serine	1.85	1.68	1.70	1.93
Proline	1.18	1.22	1.24	1.42
Histidine	0.88	0.81	0.82	0.91
Total NEAA	20.93	20.91	20.28	22.89
Essential amino acids (EAA)				
Cystine	0.75	0.61	0.56	0.55
Tyrosine	1.73	1.59	1.61	1.79
Valine	1.85	1.35	1.44	1.62
Methionine	0.31	0.18	0.23	0.30
Leucine	2.62	2.46	2.47	2.65
Isoleucine	1.56	1.47	1.47	1.60
Threonine	1.28	1.20	1.22	1.39
Phenylalanine	1.83	1.39	1.33	1.62
Lysine	1.76	1.53	1.61	1.82
Total of EAA (E)	13.69	11.78	11.94	13.34
Total EAA+TNAA (T)	34.62	32.69	32.22	36.23
E/T %	39.54	36.04	37.06	36.82

Phenylalanine levels in lupine seeds powder:-

The results of phenylalanine concentrations (PHE) are given in Table (6) and Fig. (1). The highest level of PHE is found in bitter lupine seed Giza-1 (1.83%) and the lowest one (1.33%) was found in Giza-3.

Results also, showed that the lupine cultivars could be ordered according to PHE levels as follows: BL Giza-1 (137%) > SL Giza-1 (121.8%) > BL Giza-2 (104.5%) > BL Giza-3 (100%). Comparison of Lysine and PHE levels showed that the lysine level are higher than those reported for PHE except BL Giza-1 (Fig.1).

Cultivars have the highest level of phenylalanine are considered desired and favored on the view of point of legume breeders. Phenylalanine is essential amino acid and play very important role in biosynthesis of salicylic acid (SA) which mediated several compounds in shikimic cycle. SA is plant hormone in several organs of plant and plays an important role in both local and systemic resistance. SA is an endogenous regulator of disease resistance. Two key enzymes are involved in SA biosynthesis and metabolism: benzoic acid 2-hydroxylase. The importance of SA as a component of a signal transduction pathway in disease resistance and as a regulator of thermogenesis has stimulated interest in its biosynthesis and metabolism (Lee *et al*., 1995, War *et al*., 2011).

It can be concluded that yeast active extract and benzyladenine improve quantitatively and/or qualitatively the yield of many lupine plants

Table (6): Amino acids comparison among serum albumin, soybean and lupine seeds

Amino acid	Serum albumin*	Soybean*	Lupine Cultivars			
			BLGiza-1	BLGiza-2	BLGiza-3	SLGiza-1
Glycine	2.7	4.0	1.41	1.59	1.36	1.52
Alanine	8.9	5.0	1.20	1.31	1.12	1.31
Aspartic	7.3	8.0	3.72	3.49	3.49	3.95
Arginine	7.6	5.8	3.40	3.32	3.36	4.37
Glutamic	9.6	21.0	7.29	7.49	7.19	7.48
Serine	4.4	6.0	1.85	1.68	1.70	1.93
Proline	4.4	8.0	1.18	1.22	1.24	1.42
Histidine	7.7	2.3	0.88	0.81	0.82	0.91
Cystine	0.9	0.9	0.75	0.61	0.56	0.55
Tyrosine	3.0	4.0	1.73	1.59	1.61	1.79
Valine	5.0	4.2	1.85	1.35	1.44	1.62
Methionine	2.1	2.0	0.31	0.18	0.23	0.30
Leucine	8.1	6.6	2.62	2.46	2.47	2.65
Isoleucine	2.0	4.7	1.56	1.47	1.47	1.60
Threonine	4.8	4.1	1.28	1.20	1.22	1.39
Phenylalanine	3.5	5.7	1.83	1.39	1.33	1.62
Lysine	11.7	5.4	1.76	1.53	1.61	1.82

***** according to Torun *et al*, (1981)

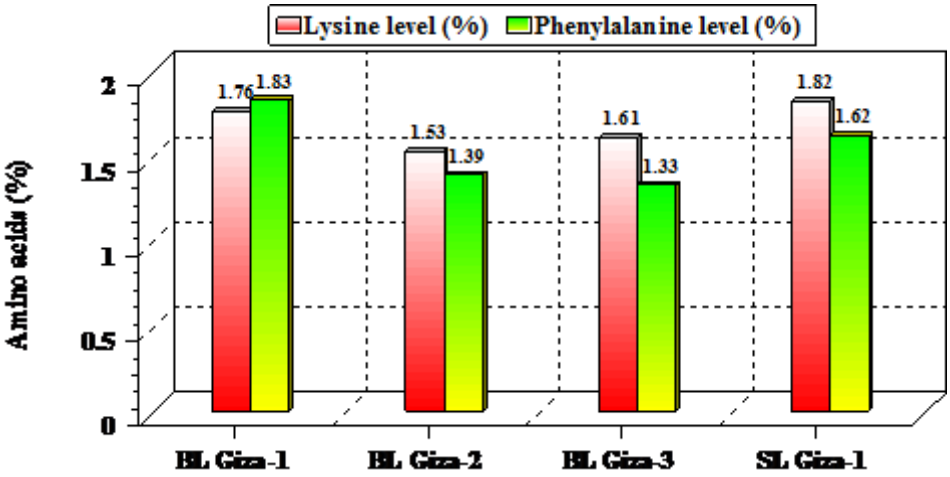

Fig. (1): Levels of lysine and phenylalanine in lupine seeds

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الملخص العربي

تأثير الرش بمستخلص الخميرة النشط والبنزيل أدينين على بعض مقاييس النمو الخضرية والتركيب الكيميائي لنباتات الترمس (*Lupinus termis*, L.)

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في هذه الدراسة أجريتْ تجربة حقلِية أثناء موسم 2014/2013 وذلك لدِراسَة تأثيرِ الرَش بمستخلص الخميرةِ النشطِ والبنزيل ادينين على التركيبِ الكيميائي لبذور صنفين من أصناف التّرمس وهما جيزة–1 و جيزة–2 و تداخلاتها. و كذلك تم دراسة تأثير المعاملات السابقة علي صفات النمو الخضرية و منها إرتفاع النباتِ (سم)، محصول البذور (جم/نبات) ووزن الـ 100 بذرة هذا بالإضافة إلى التركيب الكيميائي مثل الرطوبة، الرماد، السكريات الذائبة الكلية TSS، الليبيدات الخام CL، النيتروجين الكلي TN، البروتين الخام CP، القلويدات الكلية TAs، المركّباتَ الفينولية الكلية TPCs. تشير النَتائِج إلي أن استخدام الرش الورقي بمستخلص الخميرةِ النشطِ والبنزيل ادينين أدي إلي زيادة معنوية في الصفات الخضرية و المكونات

الكيميائية. تأثرت سبعة مكونات في بذور الترمس الجافة تأثراً معنوياً باستخدام الرش بمستخلص الخميرة النشط والبنزيل ادينين و هي الرماد الكلي و النيتروجين الكلي و البروتين الخام و السكريات الذائبة الكلية TSS و الليبيدات الخام و القلويدات الكلية TAs و المواد الفينولية TPCs. استخدام الرش بمستخلص الخميرة سواء كان الَرش الورقي أَو رش التربة أدي إلي زيادةَ معنوية في صفة إرتفاعِ النباتِ (سم)، محصول البذور (جم/نبات) و وزن الـ 100 بذرة مِن بذور نبات الترمس. من ناحية أخرى، كان تأثير مستخلص الخميرة النشط والبنزيل أدينينِ على محتوي البذور من الرطوبة (%) غير معنوياً في كلتا المعاملتين بالإضافة إلي أن المعاملة بمستخلص الخميرة النشط تسبّبت في نقص معنوي في محتوى البذور من القلويدات الكلية عند المُقارنة بالكنترول، و كان تأثير المعاملة بمستخلص الخميرة النشط على تركيز المركبات الفينولية الكلية TPCs معنوي.

تشير نتائج تقدير التركيب الكيميائي للبذور الجافة للترمس إلي أن بذور الترمس الحلو تحتوي علي مستويات أعلي من البروتين الخام (37.5 %) مِن تلك المقدرة في بذور التّرمس المر جيزة – 1 (34.13 %)، جيزة – 2 (33.06 %)، جيزة – 3 (33.01 %) وصل تركيز الليبيدات الخام في بذورِ الترمس الي 11.13 % في جيزة –1، 11.82 % في بذور جيزة – 2 ،12.42 % في جيزة– 3 في نباتات التّرمس المر، و9.9 % في بذور التّرمس الحلوِ. أشارت النتائج إلي أن بذور الترمس الحلو تحتوي علي مستويات أعلي من السكريات الذائبة الكلية (25.7 %) من تلك المقدرة في بذور التّرمس المر.

تطرقت الدراسة إلي تقدير العناصر المعدنية في بذورالترمس الجافّة و النَتائِج تشير الي ان مستويات الصوديومِ في بذور التّرمس المر جيزة – 3 (0.35 %) أعلى قليلاً من بذور التّرمس المر جيزة –2 (0.30 %). محتوى البوتاسيوم (K^+) في بذور التّرمس المر جيزة –1 (204.3 جزء في المليون ppm) وأن هذه القيمة أعلى مِن تلك المقدرة في بذور التّرمس المر جيزة –3 (178.6 ppm) و بذور الترمس الحلو جيزة – 1 (169.5 ppm) و بذور التّرمس المر جيزة –2 (163.7 ppm)، بينما محتوى الفوسفورِ (P) في بذورِ نباتات التّرمس المر جيزة –1 (0.75 %) و هذه المستويات أعلى مِن تلك المقدَرة في بذور التّرمس الحلو جيزة –1 (0.74 %) و بذور التّرمس المر جيزة –2 (0.70 %) و بذور التّرمس المر جيزة – 3 (0.68%).

عموما تشير هذه النتائج إلي أن بذور اصناف التّرمس محل الدراسة مصادر غنية و رخيصة للبروتين الخام (33.06 –37.5 %) و الليبيدات الخام (9.9– 12.42 %) و السكريات الكلية الذائبة (23.03– 25.7 %) و القلويدات الكلية (1.19 الي 1.378 %).