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Physiological Response of *Lupinus termis* to Trans-Cinammic Acid and Benzoic Acid Treatments under Sandy Soil Conditions.

Mohamed E. El-Awadi*, Yasser R. Abdel-Baky, Mervat Sh. Sadak , Abo Bakr A. Amin A, and Mona G. Dawood.

Botany Department, Research Department, National Research Centre, 33 El-Bohooth st., (former El-Tahrirs st.), Dokki, P.O. Code 12622, Cairo, Egypt.

ABSTRACT

The effect of soaking lupine seeds with phenolic acids (trans-cinnamic acid or benzoic acid at 10, 20 and 30 mg/l) on growth, photosynthetic pigments content, IAA and phenolic levels, yield and yield components in addition to some nutritional values of the yielded seeds was studied during two successive seasons 2013/2014 and 2014/2015 at the Research and Production Station of National Research Centre, Egypt. All trans-cinnamic acid and benzoic acid treatments promoted most of the studied growth parameters at both vegetative growth stage and flowering stage. These increments of growth parameters were associated with elevated level of photosynthetic pigments, growth promoter auxin in terms of indole acetic acid, and total phenolic contents of the treated plants as compared with untreated plants. All treatments led to marked increases in yield and yield components. Moreover, some chemical constituents as potassium, phosphorus, crude protein, carbohydrate and oil percentages of the yielded seeds were increased in treated plants with different concentrations of trans-cinnamic acid and benzoic acid. It could be concluded that either benzoic acid or trans-cinnamic acid at 30 mg/l are the most promising treatments in increasing quality and quantity of lupine plants grown under sandy soil condition.

Keywords: Lupine, phenolic acid, growth, IAA, oil percentage, yield.

***Corresponding author:** *el_awadi@yahoo.com*

INTRODUCTION

Lupine (*Lupinus termis*) is one of the oldest field crops and lupine seeds have a nutritional quality similar to soybean seed and superior to other legumes seed, since it could be considered as an important source of protein and oil (Raza and Jrnsgard, 2005). It contains 33-40% crude protein, 5-13% oil and relatively beneficial amino acids profile (Písaříková and Zralý, 2009). Further, proximate analysis for dehulled lupine flour gave 43.57% protein, 9.75% oil, 3.16% total ash, 12.45% crude fiber and 29% carbohydrate (Khalid and Elharadallou, 2013). Saturated fatty acids (11.35%) of bitter lupine seed oil were lower than unsaturated fatty acids (88.65) with oleic acid (52.22 %) as a predominant fatty acid. Bitter lupine seed oil is suitable for utilization in different food products due to presence of high antioxidants content (α- and δ-tocopherol (5.41 and 4.23 mg/100g oil, respectively) (Alamri, 2012).

Lupine plant like other seed legumes have the ability to fix atmospheric nitrogen in the soil that increase soil fertility, improve soil permeability and increase water storage (Wolko *et al*., 2011). Therefore lupine plant has the ability to grow well in poor sandy soils and used as additive to such soil in the form of green manure to increase its fertility. Lupine production can be increased by two means, either by vertical expansion (using antioxidant as phenolic acids) or by horizontal expansion (cultivation in sandy land).

Phenolic compounds are plant secondary metabolites, having great significance role in plant development (Curir *et al.,* 1990). These biomolecules exert an essential role in weed management, mineral nutrition, and act as defense molecules against soil pests and pathogens (Makoi and Ndakidemi, 2007). Additionally, they serve as flower pigments, signal molecules, allelopathic compounds (Ndakidemi and Dakora, 2003), pesticides (Beier, 1990) and protect plants agents against biotic and abiotic stress (Deladonde*et al.,* 1996). They have long been recognized as allelochemicals for weed control (Putnam and Tang, 1986) and plant defense molecules (Vidhyasekaran, 1988). They act as important precursors for the synthesis of soil humic substances (Haider*et al.,* 1975). The role of trans-cinnamic acid in stimulating growth and activating plants was studied by many investigators who reported that plants synthesize large amounts of phenylpropanoid acids, mainly hydroxyl cinnamic acids and hydroxyl benzoic acid, which are often found in conjugated forms, such as glycosides or glycosides esters. These conjugates have been identified in numerous plants (Molgaard and Ravn, 1988, and Dixon, 2001). In addition, trans-cinnamic acid is a key intermediate in shikimate and phenylpropanoid pathways. Shikimic acid is a precursor of many alkaloids, aromatic amino acids, and indole derivatives. Benzoic acid is a biosynthetic precursor of salicylic acid and has been tested in different crops (Raskin, 1992). Benzoic acid is naturally synthesized by plants and classified in the group of carboxylic acids. These organic compounds are exudated toward the rhizosphere to facilitate the assimilation of mineral nutrients, also associated with the elevation of soil weathering and mineral lixiviation rate (van Hees *et al.,* 2000). The concentration of organic acids in the soil solution may be very low, less than 50 µmolar (van Hees *et al.,* 2005), however, some plants accumulate benzoic acid in the soil insignificant quantities working as an allelochemical that interferes with the growth of plant competitors (Kaur *et al.,* 2005). In addition, benzoic acid seems to work like a stress signaling compound, when it is applied in a low concentration range (less than one millimolar) it induces tolerance to saline stress in cabbage and tomato plants (Benavides-Mendoza, 2002).

The present study aimed to investigate the role of cinnamic acid or benzoic acid in improving growth, development, some physiological attributes, yield, and metabolic constituents of the yielded lupine seeds grown under sandy soil conditions.

MATERIALS AND METHODS

Experimental Procedure:

Two field experiments was carried out at the Experimental Station of the National Research Centre (Research and Production Station, Nubaria region, Behira Governorate, Egypt) during the two successive seasons of 2013/2014 and 2014/2015. The experiments were carried out under sandy soil conditions the physical and chemical proprieties of the soil are presented in Table (1) according to Chapman and Pratt (1978).

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Table (1): Soil mechanical and chemical analysis characters.

Character	Value	Character	Value
Sand %	88	K	10.18
Silt %	4	Ca mg/100g	92.0
Clay %	7.2	Mg	18.4
Texture	Sandy	Na	12.36
pH (1: 2.5 water)	8.83	Fe	8.92
E.C(mmhos/cm)(1:2.5)	0.12	Mn mg/kg	8.34
CaCO3 %	4.8	Zn	0.13
O.M %	0.24	Cu	0.10
P	0.22		

Lupine (*Lupinus termis* L. cv. Giza 2) seeds were secured from Agriculture Research Centre, Egypt, cleaned and soaked with trans cinnamic acid or benzoic acid at 10,20 ,30 mg/l for 12 hours and left to dry in open air. The soaked seeds were inoculated with nitrogen fixing bacteria (*Rhizobia*) and sown on 10^{th} and 12^{th} November 2013 and 2014, respectively. The experimental design was a randomize complete block design with four replications. The plot area was 10.5 m^2 (3.0 m x 3.5 m) and consisted of four rows 60 cm apart and the distance between hills along the row 25 cm apart. Calcium super-phosphate (15.5% P_2O_5) at rate of 30 kg/fed was applied to the soil prior sowing. While, nitrogen fertilizer was applied at the rate 120 units of N/fed in form of ammonium nitrate (33.5%N) and was divided in four equal portions. The dosage was added before the irrigation.

Vegetative growth characters

Growth characters (plant height (cm), number of leaves /plant, fresh and dry weights of shoot (g $plant^{-1}$) was recorded during the vegetative stage (A). Plant height (cm), number of leaves and flowers/plant, fresh and dry weights of leaves, stem/plant and dry weight of flowers / plant were recorded at flowering stages (B). During stage A and B (90 and 105 days after sowing) fresh samples were taken to determine photosynthetic pigments, while, IAA and phenol contents were determined only in stage A. At harvest time, ten guarded plants were taken out at random from the middle two ridges of each plot to determine the mean values of yield and its related parameters, i.e., number of pods/plant, number of seeds/pod, number of seeds/plant, ,dry weight of pods and seeds/plant and 100 seed weight. N, P, K, crude protein, carbohydrates and oil percentage were determined in the yielded seeds.

Chemical analysis:

Photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) in fresh leaves were determined according to Lichtenthaler and Buschmann (2001). Indole acetic acid content were extracted and analyzed by the method of Larsen *et al*. (1962). Total phenolic content was extracted as IAA extraction, and measured as the method described by Danil and George (1972). Seed oil contents were determined using Soxhlet apparatus and petroleum ether (40-60^o C) as a solvent according to A.O.A.C. (1981). The resultant defatted meal used for determination of phosphorus, potassium, nitrogen, carbohydrates, proteins. Phosphorus (P) and potassium (K^+) were measured according to the methods described by Chapman and Pratt (1978). Total nitrogen (N) was determined using the micro-Kjeldahl method, as described by the A.O.A.C. (1970) then crude protein percentage was calculated by multiplying the values of total nitrogen by 6.25 (A.O.A.C., 1988). A total carbohydrate was determined according to Herbert *et al*. (1971).

Statistical Analysis:

Data were statistically analyzed using the least significant difference at 5% level of probability according to Snedecor and Cochran (1990)The Duncan multiple range test was used to compare the treatment means (Duncan, 1955). The MSTATC (1989) program was used in this connection.

RESULTS AND DISCUSSION

Effect of trans-cinnamic acid or benzoic acid on growth parameters:

All trans-cinnamic acid and benzoic acid treatments caused increases in growth parameters in terms of plant height, number of leaves/plant, fresh and dry weight of shoot/plant at stage (A) (Table 2a) and plant height, number of leaves and flowers/plant, fresh and dry weights of leaves and stems /plant at stage (B) (Table 2b). Either trans-cinnamic acid or benzoic acid at 30 mg $^{L-1}$ stimulated most of the morphological parameters compared with untreated control. Moreover, benzoic acid was more effective than trans-cinnamic acid as it caused the highest increases in the dry weight of leaves, stem and flowers/plant. In this concern, Einhellig and Leather (1988) demonstrated that, the increase in growth parameters is attributed to the influence of phenolic compounds on physiological processes such as cellular expansion, membrane permeability, nutrient uptake and chlorophyll synthesis. Moreover, the promotive effect of benzoic acid (as a precursor of salicylic acid) could be attributed to its bioregulator effects on physiological and biochemical processes in plants. Talaat and Balbaa (2010) reported that, exogenous application of trans-cinnamic acid on basil plants considerably increased plant growth at both the two cuttings. Meanwhile, Anjum *et al.* (2013) and Sadak *et al.* (2013) stated that benzoic acid treatment increased growth parameters of soybean plant. Talaat *et al.* (2014) reported that foliar treatment of trans-cinnamic acid and benzoic acid enhanced the vegetative growth of geranium plants, especially at 100 µM concentration.

Table (2a): Effect of trans-cinnamic acid or benzoic acid on growth parameters of *Lupinus termis* plant (vegetative stage) grown under sandy soil conditions.

Stage A:

Treatments		Plant height (cm)	No of leaves /plant	Fresh wt. of shoot/plant (g)	Dry wt. of shoot /plant (g)
Control		17.89C	15.00C	7.67B	1.53C
Cinnamic acid (mg/l)	10	23.33B	15.67BC	10.00A	1.73C
	20	26.33A	17.44A	11.07A	2.07AB
	30	26.89A	16.89AB	10.63A	2.17A
Benzoic acid (mg/l)	10	23.56B	15.67BC	10.10A	1.80BC
	20	27.00A	17.33AB	10.50A	1.83BC
	30	26.22A	16.33ABC	12.43A	2.20A

Table (2b): Effect of trans-cinnamic acid or benzoic acid on growth parameters of *Lupinus termis* plant (flowering stage) grown under sandy soil conditions.

Stage B:

Treatments		Plant height	No of leaves /plant	No of flowers /plant	Fresh wt of leaves /plant	Fresh wt. of stem /plant	Dry wt. of leaves /plant	Dry wt. of stem /plant	Dry wt. of flowers /plant
Control		31.75D	11.50C	10.50C	8.04B	7.04C	1.067	0.67B	0.19B
Cinnamic acid (mg/l)	10	33.75CD	21.50B	11.75BC	9.38AB	8.67BC	1.133	0.75AB	0.45AB
	20	38.75AB	23.75AB	16.00AB	10.77A	10.82A	1.200	0.97AB	0.46AB
	30	37.75AB	27.50A	16.75A	11.35A	10.91A	1.533	1.10AB	0.57A
Benzoic acid (mg/l)	10	36.25BC	19.00B	11.75BC	10.01AB	8.58BC	1.233	0.73AB	0.56A
	20	36.25BC	22.25B	12.50ABC	10.34AB	9.32AB	1.533	1.13AB	0.57A
	30	40.00A	28.00A	13.00ABC	11.53A	9.35AB	1.633	1.20A	0.61A

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).

Effect of trans-cinnamic acid or benzoic acid on photosynthetic pigments:

The changes of photosynthetic pigments due to trans-cinnamic acid and benzoic acid treatments at two growth stages are shown in Figures (1 a, b and c). Chlorophyll a, chlorophyll b and carotenoids increased gradually with increasing trans-cinnamic acid or benzoic acid concentrations at stage A and B as compared with untreated control plants. The maximum increase in chlorophylls and carotenoids recorded at 30 mg^{L-1} benzoic acid followed by 30 mgL^{-1} trans-cinnamic acid treatments. The increase in the level of photosynthetic pigments may be attributed to the promotion of their synthesis and/or retardation of pigment degradation. Talaat *et al.* (2014); Singh and Chaturvedi (2014) who found that, Khella plant and maize plants treated with t-cinnamic acid respectively, retained more chlorophyll compared to control. Where, they supposed that cinnamic acid could increase the functional state of the photosynthetic machinery in plants either by the mobilization of internal tissue nitrate or chlorophyll biosynthesis. The obtained results of benzoic acid are in concurrent with those obtained by Anjum *et al.* (2013) & Sadak *et al.* (2013) on soybean and Talaat *et al.* (2014) on khella plant, these increases in photosynthetic pigments may be attributed to the effective role of benzoic acid in increasing gas exchange attributes, stomatal conductance, transpiration rate and photosynthetic rate.

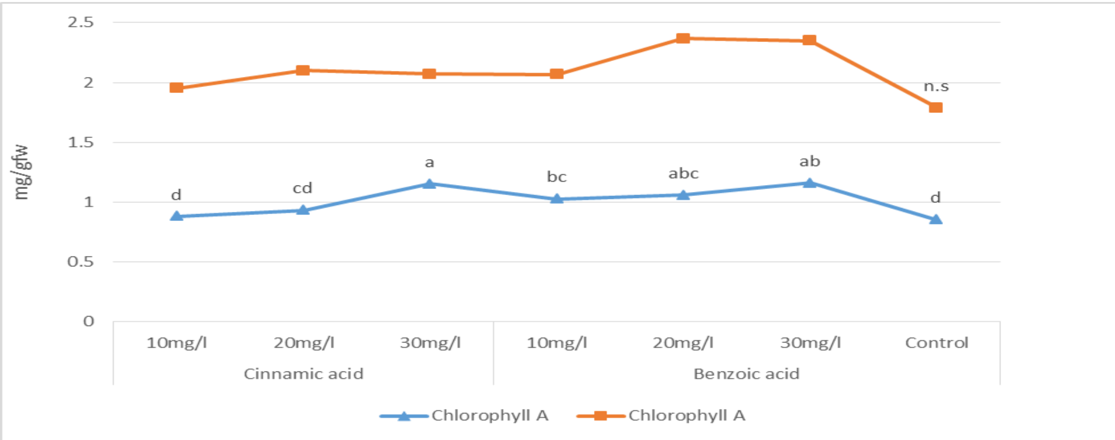

Fig (1 a): Chlorophyll a content (mg/g fresh leaves) as affected by cinnamic acid and benzoic acid treatments during stage A(blue) and B (red).

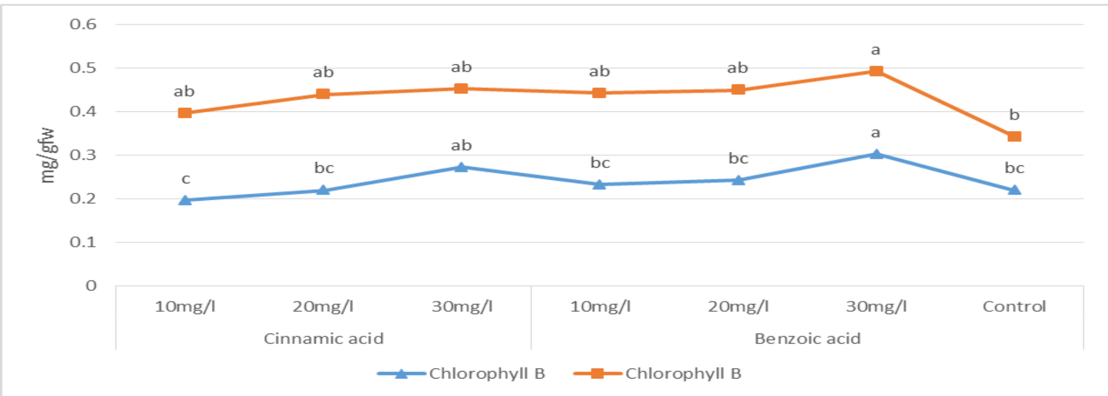

Fig (1 b): chlorophyll b (mg/g fresh leaves) as affected by cinnamic acid and benzoic acid treatments during stage A(blue) and B (red).

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).

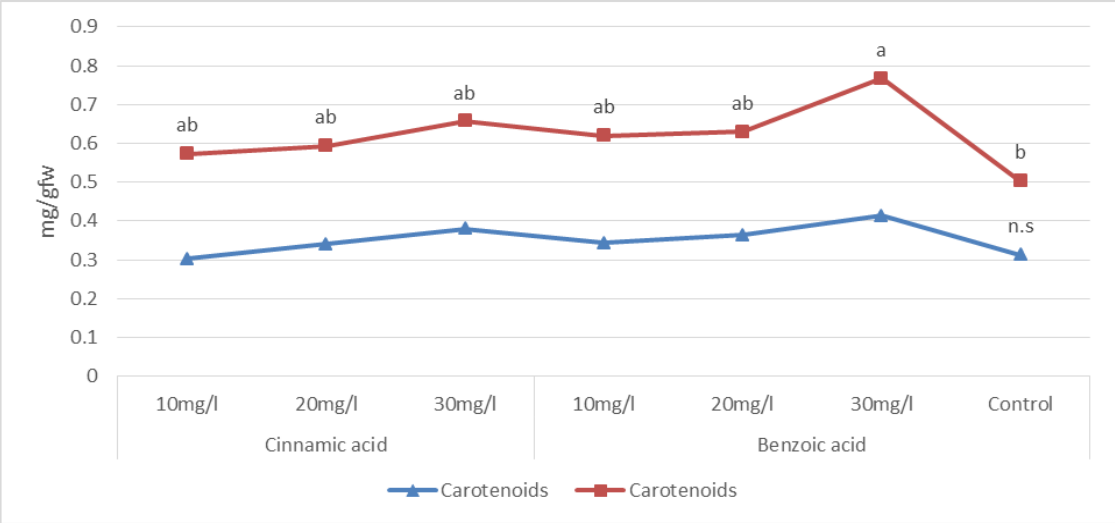

Fig (1 c): Carotenoids (mg/g fresh leaves) as affected by cinnamic acid and benzoic acid treatments at stage A(blue) and B (red).

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).

Effect of trans-cinnamic acid or benzoic acid on IAA content:

Data represent in Figure (2) showed significant increments in indole acetic acid (IAA) in plants treated with trans-cinnamic acid and benzoic acid. High concentrations of indole acetic acid (IAA) were detected in lupine plants treated with benzoic acid at different concentrations compared with trans-cinnamic acid acids. The highest values of IAA were recorded in plants exposed to 30 mg L^{-1} benzoic acid. These increases in the levels of endogenous growth promoter IAA could be attributed to the increases in their biosynthesis and/or decreases in their degradation and conjugation (Hopkins and Huner, 2004). In addition, Gharib (2006) mentioned that phenolic acids may be a prerequisite for the synthesis of auxin and/or cytokinin. These results are in accordance with those obtained by Talaat *et al.* (2014) on khela plant and Sadak *et al.* (2015) on Roselle plant.

Effect of trans-cinnamic acid or benzoic acid on phenolic content:

Data in Figure (2) clearly show that, foliar application of trans-cinnamic acid and benzoic acid at different concentrations (10, 20 and 30 mgL^{-1}) significantly increased phenolic content as compared with control plant. Benzoic acid at 30 mg $^{L-1}$ was the most effective treatment in increasing the tested parameter. These increments in phenolic content may be due to the phenolic nature of trans-cinnamic acid and benzoic acid (Curir *et al.,* 1990). Therefore, it could be expected that their application on plants increased phenolic concentration as mentioned by Sadak *et al.* (2013) using benzoic acid on soybean plant and Sadak *et al.* (2015) using t-cinnamic acid on roselle plant. The increase in total phenolic contents in response to all treatments may be due to the increases in carbohydrate synthesis (Youssef, 1993). Moreover, Dawood and Sadak (2007) showed that the increase in total phenolic contents was concur with the increase in IAA contents and led to the suggestion that most of phenolic compounds are diphenols and polyphenols which may inhibit IAA oxidase activity and leading to auxin accumulation, and reflected in stimulating the growth and yield of plant.

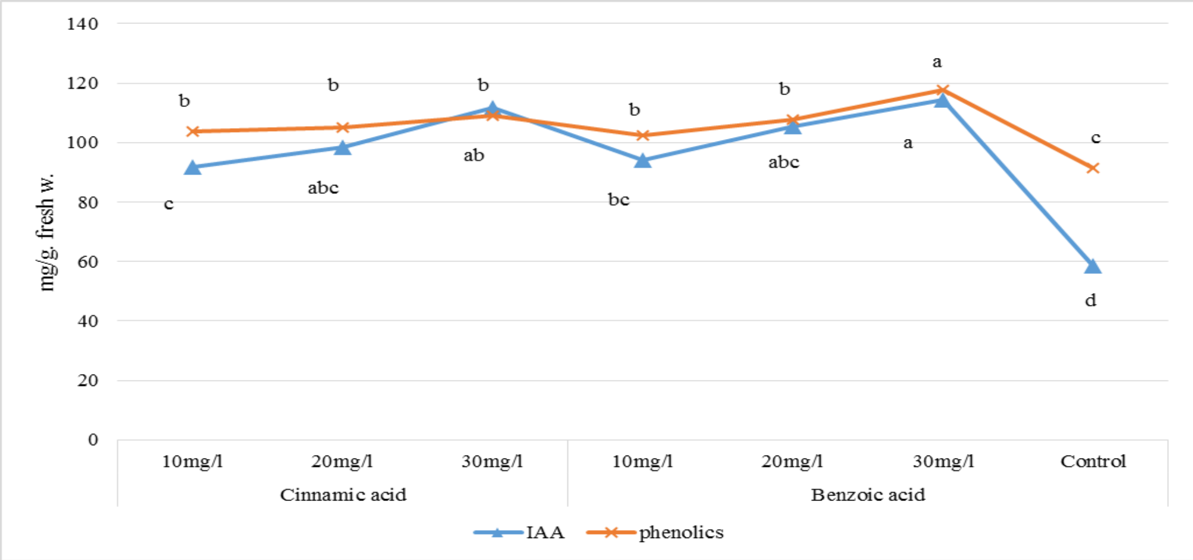

Fig (2): IAA and phenolics contents (mg/g fresh weight) as affected by trans cinnamic acid and benzoic acid treatments

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).

Effect of trans-cinnamic acid or benzoic acid on yield and yield components:

Data in Table (3) revealed the effect of trans-cinnamic acid or benzoic acid at different concentrations on yield and yield components of lupine. The data indicated that different concentrations of both trans-cinnamic acid and benzoic acid induced increases in number of pods/plant, number of seeds/ pod, number of seeds /plant, weight of pods/plant and weight of seeds/plant and 100seeds weight. In addition, the data indicated that there was a gradual increase in most of the yield components with increasing the concentrations of both trans-cinnamic acid and benzoic acid. The most effective treatments were at 30 mgL^{-1} benzoic acid and 30 mgL^{-1}trans-cinnamic. The increase in the seed yield could be a reflection of the effect of both phenolic acids on growth and development. It might be due to (a) marked increases in growth criteria of plant (Table 2 a,b) which gave a chance to the plant to carry more flowers, pods and hence more seeds. (b) and marked increases in the photosynthetic pigments content (Fig. 1 a,b,c), which could lead to increase in photosynthesis, resulting in greater transfer of photo-assimilates to the seeds and causing increases in their weights. A possible explanation of increasing yield components is the capacity of the phenolic acids to diminish pH and form metal complexes that increase their absorption in soils, which tend chemically to fix these ion metals (Lopez- Bucio *et al.,* 2000).

Table (3): Effect of trans-cinnamic acid or benzoic acid on yield and yield components of *Lupinus termis* plant grown under sandy soil conditions.

Treatments		No. of Seeds/pods	No. of Pods/plant	Weight of Pods/plant (g)	Weight of Seeds/plant (g)	No. of Seeds/plant (g)	Weight of 100 seeds (g)
Control		3.50B	3.00D	3.52C	2.30C	9.30C	29.90CD
Cinnamic acid (mg/)	10	4.00AB	5.00BC	6.13ABC	4.12ABC	15.50AB	31.75BCD
	20	4.20AB	4.70BC	6.55ABC	4.35ABC	14.60AB	39.25AB
	30	4.70A	5.50AB	8.61A	5.81A	19.10A	37.15ABC
Benzoic acid (mg/l)	10	4.00AB	4.30C	4.60BC	2.90BC	12.20BC	27.50D
	20	4.30AB	4.60BC	7.25AB	4.83ABC	14.60AB	37.90ABC
	30	4.00AB	6.30A	7.88A	5.27AB	17.50A	41.45A

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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Nutritive value of the yielded seeds

Figures (3 a,b) show that foliar application of either trans-cinnamic acid or benzoic acid at different concentrations increased the phosphorus, potassium, crude protein, carbohydrates and oil percentages in the yielded lupine seeds compared with control plants. Generally, the maximum levels of phosphorus, potassium, crude protein, carbohydrates and oil percentages were recorded in seeds due to 30 mg $^{L-1}$ benzoic acid and trans-cinnamic treatments. These increments in yield components, oil% and carbohydrate% might be due to the increase in vegetative growth (Table 2) and nutrient uptake. Similar results were reported by Christen and Lovett (1993) on spring barley, Benavides-Mendoza *et al.* (2012) on tomato plant, Anjum *et al.* (2013) and Sadak *et al.* (2013) on soybean and Talaat *et al.* (2014) on Khella plant.

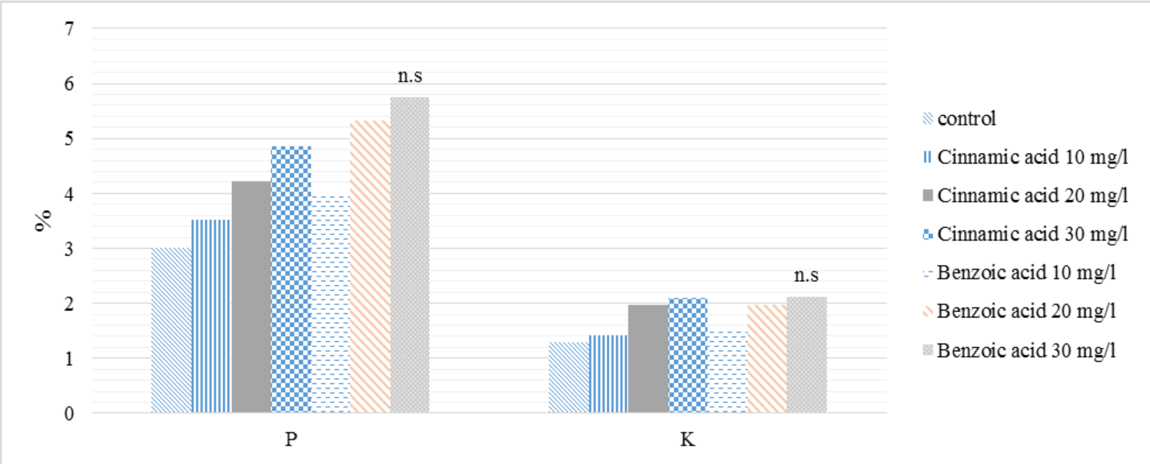

Fig (3 a): Phosporous and potassium content in the yielded lupine seeds as affected by trans cinnamic acid and benzoic acid treatments.

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).

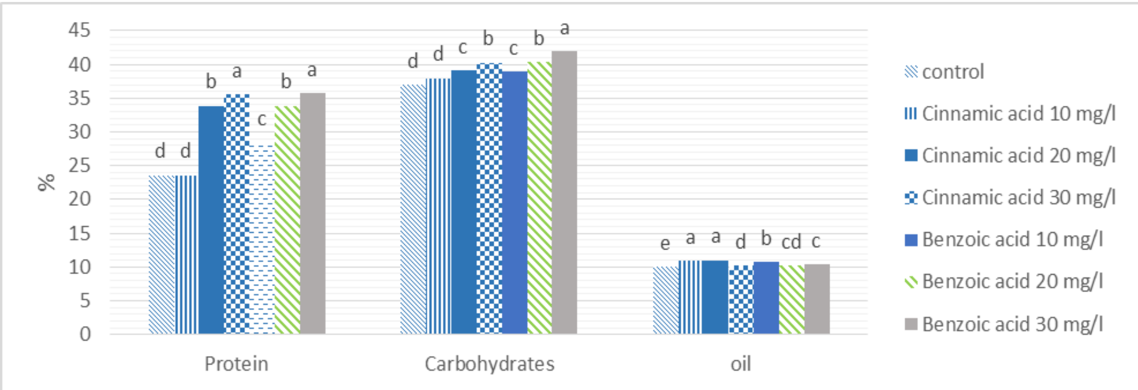

Fig (3 b): protein, carbohydrate and oil content in the yielded lupine seeds as affected by trans cinnamic acid and benzoic acid treatments

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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