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Effects of Chemical Fertilizers on Growth and Active Constituents of *Echinacea paradoxa* L. Plants

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Abstract: Field experiments were conducted during the two successive seasons of 2008/2009 and 2009/2010 to investigate the response of *Echinacea paradoxa* L. plants to the nitrogen fertilizer (0, 100, 200 and 300 kg/fed. of ammonium sulphate, 20.5% N) and potassium fertilizer (0, 50 and 100 kg/fed. of potassium sulphate, 48% K) and their interactions. The response was measured in terms of plant height, herb fresh weight, herb dry weight, flower fresh weight, flower dry weight, whole plant fresh weight and whole plant dry weight. The study also dealt with the effects of these fertilizer treatments on the active constituents of this plant, polysaccharide, caffeic acid and alkamides. The recorded results showed that the highest levels of Nitrogen (N_3) or Potassium (K_2) and their interaction (N_3K_2) significantly improved the growth characters. The same treatments were the best in improving the contents of polysaccharides, caffeic acid and alkamides. The applied treatments did not change the characteristic feature of the alkamides spectrum, meaning that the alkamide No.3 (pentadeca-8Z, 13Z-diene-l1-yn.2.one) was the dominant one, followed by No.1 (tetradeca-8Z-ene-1 1, 13-diyn-2-one) and No.6 (pentadeca-8Z, 11Z-dien-2-one0. These three alkamides constituted about 80% of the total alkamides content of the plant.

Key words: *Echinacea paradoxa* · Chemical fertilization · Growth characters · Polysaccharide · Caffiec acid · Alkamides

INTRODUCTION

Echinacea species are members of the *Asteraceae* family [1]. *E. paradoxa* is the only yellow coneflower of the genus and is endemic to the Ozark Mountains of Missouri and Arkansas [2]. *Echinacea* is widely used in Europe and North America for the treatment of common cold [3, 4]. In 1998, *Echinacea* was the tenth most important medicinal plant sold in Europe with annual sales of about $120 million. In North America, Echinacea is listed as the first among 11 top-selling herbal extracts [5]. Retail sales of Echinacea products are more than $158 million annually in the USA and have been estimatedat $1,300 million annually worldwide Blumenthal [6]. Phenolic acids, alkamides, polyacetylenes, glycoproteins and polysaccharides have been detected as biologically active components in different *Echinacea* species [7]. Known phenolic compounds in *Echinacea* species include caffeic acid derivatives such as chicoric acid in *E. purpurea* and *E. pallida* and echinacoside in *E. angustifolia* [8]. Zhang *et al.* [9] reported that, *E. paradoxa* var. *paradoxa* has more potent anti-inflammatory activity as measured by inflammatory mediators. The alkamides have been shown to stimulate immune cell activity and inhibit enzymes involved in the production of inflammatory mediators Muller *et al.* [10] and Goel *et al.* [11]. Hudson *et al.* [12] reported that aqueous extracts of *E. purpurea* contained a relatively potent activity against herpes simplex virus (HSV) and influenza virus (FV). Results of Pellati *et al.* [13] and Dalby *et al.* [14] indicated that *Echinacea* is a good source of natural antioxidants and could be used to prevent free-radical-induced deleterious effects. Chicoric acid has been studied for its potential to inhibit HIV integrase [15, 16] and to enhance insulin secretion and glucose uptake [17].

Plant nutrition is one of the most important factors that increase plant production. Nitrogen plays the most recognized role in the plant for its presence in the structure of the protein molecule. In addition, nitrogen is found in such important molecules as purines,

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pyrimidines, porphyrines and coenzymes. Purines and Pyrimidines found in the nucleic acids RNA and DNA are essential for protein synthesis. The porphyrin structure is found in metabolically important compounds such as chlorophyll pigments and the cytochromes which are essential in photosynthesis and respiration. Potassium is essential for many physiological processes such as photosynthesis, translocation of photosynthetics into sink organs, activation of enzymes and protein synthesis and it mediates osmoregulation during cell expansion, stomatal movements and tropisms [18].

In Egypt, interest in *Echinacea* plant, regarding its cultivation, investigation and use in pharmaceutical preparations has started since 1990, accordingly several articles have been published in this regard, Hendawy [19], Shalaby *et al.* [20], Shalaby *et al.* [21] and El-Gengaihi *et al.* [22]. The previous studies were concentrated on *Echinacea purpurea*. As a result, standardized extracts and some phytomedicins are now produced locally. The present study aimed to further investigate the potentials of *E. paradoxa* under the local conditions. Experiments were carried out to investigate the effects of nitrogen and potassium fertilization and their interactions on the growth characters and chemical composition of *Echinacea paradoxa* L.

MATERIALS AND METHODS

A field experiment was carried out at the Experimental Farm of Agriculture Faculty, Cairo University, Giza Governorate, Egypt during two successive seasons of 2008/2009 and 2009/2010. The physical and chemical properties of the soil were determined. The field soil was sandy loam, having a physical composition as follows: 24.3 % sand, 53.7 % silt, 22 % clay and 0.49% organic matter. Soil chemical analysis was as follows: pH= 8.08, E.C (mmhos/cm) = 1.35, available N, P and K were 1.12, 0.71 and 0.23%, respectively [23]. Seeds of *Echinacea paradoxa* L. plant were obtained from Jellitto Standensamen Gmbh, Schwarmstedt, Germany. The seeds were sown in seed beds in a greenhouse on 22nd of October, during both seasons. The seedlings were transplanted in rows on the 25th of March of each season, in 2×3 m plots, 40 cm apart and 50cm between. Two months later, the seedlings were thinned to two plants per hill. Four levels of nitrogen were applied as ammonium sulphate (20.5% N) at N_0, N_1, N_2 and N_3 levels corresponding to 0, 100, 200 and 300 kg/feddan (one feddan = 0.42 ha). Meanwhile, three levels of potassium were added as potassium sulphate (48%K_2O) at K_0, K_1 and K_2 levels corresponding to 0, 50 and 100 kg/fed. Each of the used fertilizers was either added separately or in combination with another element so, a total of 19 fertilizing treatments were applied. The plants received a basal dose of 100 kg/fed of phosphorus as calcium superphosphate (15.5%P_2O_5). All plants received the usual agricultural practices of weeding and irrigation.

The plants were assessed at the maturity stage (in September) when 70 % of the plants bloomed and seeds started to be mature. Ten plants from each replicate were assessed and the mean value was recorded for that replicate in the two seasons.

The following vegetative parameters were recorded:

- Plant height (cm)
- Fresh and dry weight of herb (g/plant)
- Fresh and dry weight of flowers (g/plant)
- Whole plant fresh and dry weights (g/plant)

Chemical Analysis: Since the commercial preparations of *Echinacea* are mostly prepared from the whole aerial parts of the plant (leaves, stems and flowers), plant samples (whole plant) representing the different treatments were collected. They were air dried at room temperature (27°C ± 3) and kept in paper pages in a dark place. They were ground to fine powder (20 mesh) just before chemical analysis.

Total Polysaccharides Content (%): The total polysaccharide content was determined according to the method reported by Wu *et al.* [24]. Two grams of the powdered aerial parts (whole plant) were extracted by 80% methanol, the sediment was collected and desiccated in an oven at 60°C. 0.2 g of the sediment was re-suspended in 5 ml of 5% sulphuric acid and placed in boiling water for 2 hours. After acidic hydrolysis, the liquid-solid mixture was diluted to 50 ml with distilled water. The supernatant was separated by sedimentation and the polysaccharide in the supernatant was assayed according to the carbazole reaction method as follows. A sample of 0.2 ml taken from the above supernatant was mixed with 6 ml concentrated sulphuric acid, held in a boiling water bath for 20 min and cooled. Then, 0.2 ml carbazole-absolute ethanol (125 mg of carbazole in 100 ml of absolute ethanol) was added and the contents were vigorously mixed. After the reaction time of 2 h in darkness at room temperature a purplish red color was developed. The absorbance was measured at 530 nm against β-Galacturonic acid as a standard.

Caffeic Acid Derivatives Content (%): Caffeic acid derivatives content (measured as chicoric acid) was determined in the whole plant (% of dry matter) according to the method reported by Bauer and Wagner [25]. A 0.2g the powdered herb was vigorously shaken in 50ml chloroform, left for 15 min, then filtered and washed with chloroform several times. The defatted powder was left till dryness. The powder was transferred to 100 ml round flask and extracted with 20 ml methanol (80%) under reflux for 30min., then cooled, filtrated and adjusted to a final volume of 50ml. A 1ml portion of this solution was completed to 10ml with methanol 80%. The absorbance was read at 330nm against chicoric acid standard curve.

Total Alkamides Content (%): The total alkamides content (%) was determined in the aerial parts in samples collected from the second season. It was determined according to the method reported by Letchamo *et al.* [26]. A 0.5g sample was extracted three times with 8 ml portions of 70% ethanol using ultrasound for 5 min, followed by centrifugation and collection of the subsequent supernatant. The extract volume was adjusted to 25ml and filtered through a 0.45µm PTFE membrane prior to injection of 5µl into HPLC system. The liquid extract was centrifuged to delete any particulate matter and the supernatant was filtered as above prior to injection. The oily sample was diluted with ethanol (ca 1/20 for the lipophilic analysis) and filtered as above prior to injection.

HPLC Analysis: Separation and quantitative analysis of the components was based on a modified method of Livesey *et al.* [27], based on Bauer and Remiger [28]. HPLC (C-18 column, 75 x 4.6 mm, 3 µm) was used to determine the lipophilic constituents.

Statistical Analysis: Treatments were arranged in completely randomized blocks design with three replicates. Data of each season were statistically analyzed applying the ANOVA test (MS DOS/ Costat Exe Program) according to Gomez and Gomez [29]. The least significant difference (LSD at level of 5%) was used to compare between different means according to Snedecor and Cochran [30].

RESULTS AND DISCUSSION

Effects of Fertilization Treatments on Growth Characters: The obtained results regarding effects of the fertilization treatments on the growth characters are summarized in Tables 1 and 2 for both seasons, respectively. It is obvious from data of the first season (Table 1), that all the applied nitrogen treatments significantly increased all the growth parameters compared with the untreated plants. The effect was concentration dependent. The maximum mean values of different growth characters were obtained as a result of N_3 treatment compared with other treatments. Similar results were reported by Hendawy [19] on *Echinacea purpurea,* El-Seifi *et al.* [31] on garlic and Ezz El-Din *et al.* [32] on *Carum carvi*. The favorable effect of nitrogen fertilization may be due to that nitrogen is the most essential element for the plants, this leads to the production of extra protein and allows the leaves of the plant to grow larger and hence to have larger surface available for synthesis [33]. Moreover, nitrogen gave more proliferation of root biomass, resulting in the higher absorption of nutrients and water from the soil leading to the production of higher vegetative biomass [34, 35]. Regarding the effects of potassium fertilization, it could be noticed that the two levels of potassium (K_1 and K_2) increased all the growth characters, compared with the untreated plants however the differences between K_0 and K1 were insignificant in case of plant height and herb dry weight and consequently the whole plant dry weight. On the other hand, the differences between the effects of K_1 and K_2 were significant in all cases where K_2 was superior in this regard. Similar results were reported by Said-Al Ahl *et al.* [36] on *Origanum vulgare* and Zaghloul *et al.* [37] on *Thuja orientalis* who indicated that potassium fertilization increased the plant growth and yield.

Regarding the interaction treatments (N + K), the data clearly show that treatment with the highest levels of both elements (N_3K_2) gave the highest effect on the growth characters under investigation. Treatments with the N_3K_1 came in the second rank regarding the effect on the vegetative parameters *i.e.* plant height, fresh and dry weight of flower and whole plant fresh weight. However, the treatment with N_3 K_0 came in the second rank in case of fresh and dry weight of herb as well as whole plant dry weight. On the other hand, the treatment with N_0 even with K_1 was the least effective on both the vegetative and/or the flowering capacity of this plant. Similar results were reported by Azzaz and Hassan [38] recorded that, vegetative growth and parameters yield on fennel plants were increased when applied with different mineral fertilizers.

Results of the second season presented in Table 2 almost followed the same trends observed in the first season. Treatment with either N_3 or K_2 or their combination N_3 K_2 remained the most effective treatments on the vegetative growth and flowering of *E. paradoxa* plants.

Table 1: Effects of nitrogen and potassium fertilizers, as well as their interactions on growth characters of *Echinacea paradoxa* L. during the first season (2008/2009)

Treatments	Growth characters: Plant height (cm)	Herb* fresh weight (g/plant)	Herb dry weight (g/plant)	Flower fresh weight (g/plant)	Flower dry weight (g/plant)	Whole** plant fresh weight (g/plant)	Whole plant dry weight (g/plant)
	a) Effect of Nitrogen fertilizers						
N_0	47.06	90.68	37.88	38.33	14.31	129.00	52.19
N_1	62.02	108.13	42.11	46.03	16.44	154.15	58.55
N_2	66.76	139.88	53.23	55.58	19.38	195.46	72.61
N_3	73.47	154.59	64.11	63.46	22.18	218.05	86.29
LSD 5%	1.13	2.91	1.18	1.13	0.35	3.99	1.52
	b) Effect of Potassium fertilizers						
K_0	60.19	117.3	47.60	46.34	16.94	163.65	64.54
K_1	60.03	120.33	47.27	50.28	18.18	170.60	65.45
K_2	66.76	132.32	53.14	55.93	19.10	188.25	72.24
LSD 5%	0.87	1.80	0.75	1.09	0.25	2.87	0.95
	c) Effect of interaction treatments						
N_0K_0	42.33	86.93	35.90	30.45	11.49	117.39	47.39
N_0K_1	43.53	90.45	38.18	41.75	15.48	132.20	53.66
N_0K_2	55.31	94.65	39.56	42.78	15.94	137.44	55.51
N_1K_0	61.33	98.65	40.32	46.61	16.38	145.27	56.70
N_1K_1	60.07	103.35	40.50	39.90	15.74	143.25	56.24
N_1K_2	64.67	122.38	45.51	51.57	17.20	173.95	62.72
N_2K_0	66.03	131.11	51.05	51.51	18.42	182.62	69.47
N_2K_1	62.73	137.18	48.76	55.75	19.63	192.93	68.39
N_2K_2	71.53	151.37	59.89	59.47	20.09	210.84	79.98
N_3K_0	71.07	152.52	63.12	56.80	21.48	209.32	84.60
N_3K_1	73.80	150.34	61.64	63.70	21.88	214.04	83.52
N_3K_2	75.53	160.90	67.58	69.89	23.18	230.79	90.76
LSD 5%	2.29	5.87	2.38	2.29	0.71	8.06	3.06

* Leaves and stems

** Leaves, stems and flowers

Effects of Fertilization Treatments on Chemical Composition

Effects on Polysaccharides Percentage: Table 3 compiles results of the effects of nitrogen and potassium fertilizers, as well as their interactions on the polysaccharides content of *E. paradoxa* plants in the two seasons. All levels of nitrogen gradually and significantly increased the polysaccharides percentage during both seasons. The N_3 level gave the highest values of polysaccharides. Similar findings were previously revealed by Abd El-Salam [39] on *Pimpinella anisum*. In this respect, Jacoub [40] and Abd El-Aziz [41] found that spraying basil plants with urea increased the percentage of total carbohydrates in the leaves of *Ociumum basilicum*.

It is obvious from the same data that, applying the lower level of potassium (K_1) slightly decreased the polysaccharide percentage of the herb, compared with the control treatment K_0. But again, the polysaccharide content increased due to application of the higher level of K_2 resulting in the highest values of polysaccharides. The pronounced effect of potassium fertilization on the polysaccharide percentage was clearly shown by many investigators. Abd El-Azim [42] on *Salvia officinalis* found that fertilizing the plant with 100 kg potassium sulphate/fed caused a significant increment in total carbohydrates contents in the plant.

The interaction between nitrogen and potassium fertilization resulted in remarkable increments of polysaccharide content. Again it was observed that under any of the nitrogen levels, applying the lower level of potassium (K_1) decreased the polysaccharide content in comparison with the K_0 level. But increasing the potassium to the K_2 level again increased the polysaccharides content. That was followed in both seasons where the treatment N_3K_0 was the best followed by N_3K_2. Similar results were reported by Essa [43] on chamomile plant and Jacoub [40] on *Ocimum basilicum*. Ibrahim [44] reported that chemical fertilization with ammonium sulphate and potassium sulphate increased the total carbohydrates in the herb of *Ammi visnaga* and *Foeniculum vulgare*.

Table 2: Effects of nitrogen and potassium fertilizers, as well as their interactions on growth characters of *Echinacea paradoxa* L. during second season (2009/2010)

Treatments	Second season (2009/2010) Growth characters Plant height (cm)	Herb* fresh weight (g/plant)	Herb dry weight (g/plant)	Flower fresh weight (g/plant)	Flower dry weight (g/plant)	Whole** plant fresh weight (g/plant)	Whole plant dry weight (g/plant)
	a) Effect of Nitrogen fertilizers						
N_0	52.87	86.58	35.73	35.34	14.14	121.92	49.86
N_1	64.62	112.58	44.54	48.67	17.22	161.24	61.76
N_2	70.59	141.88	55.04	58.41	19.99	200.29	75.03
N_3	77.98	168.34	66.94	70.18	22.75	238.52	89.68
LSD 5%	2.46	3.55	1.35	1.49	0.38	5.03	1.73
	b) Effect of Potassium fertilizers						
K_0	63.11	120.61	47.08	49.3	18.07	169.91	65.16
K_1	66.27	120.8	47.17	51.03	17.65	171.82	64.81
K_2	70.18	140.63	57.44	59.12	19.84	199.75	77.28
LSD 5%	2.25	2.61	1.35	1.19	0.26	3.79	1.61
	c) Effect of interaction treatments						
N_0K_0	48.57	83.20	34.21	33.53	14.21	116.73	48.42
N_0K_1	52.42	84.84	34.55	34.68	14.22	119.52	48.77
N_0K_2	57.63	91.71	38.43	37.81	13.97	129.52	52.40
N_1K_0	59.93	102.73	42.58	43.23	16.62	145.96	59.20
N_1K_1	65.27	103.05	39.63	45.23	16.18	148.28	55.81
N_1K_2	68.67	131.95	51.43	57.53	18.85	189.49	70.27
N_2K_0	67.62	139.24	50.55	57.88	20.53	197.12	71.07
N_2K_1	68.49	130.03	50.34	53.49	17.97	183.52	68.31
N_2K_2	75.67	156.36	64.23	63.86	21.46	220.22	85.69
N_3K_0	76.33	157.27	61.00	62.57	20.93	219.84	81.93
N_3K_1	78.89	165.27	64.14	70.69	22.21	235.96	86.35
N_3K_2	78.73	182.49	75.67	77.28	25.10	259.77	100.77
LSD 5%	N.S.	7.16	2.73	3.00	0.76	10.15	3.48

* Leaves and stems

** Leaves, stems and flowers

Effects of Fertilization Treatments on Caffeic Acid Percentage: The obtained data in this regard are presented in Table 3. It is obvious from the data that nitrogen fertilization resulted in significant increments of caffeic acid percentage in both seasons. The N_3 level gave the highest values followed by the N_2 level while, the untreated plants showed the lowest values. These findings are in agreement with those obtained by Montanari *et al.* [45], on *Echinacea angustifolia* who reported that the concentration of caffeic acid was significantly higher in plants grown with nitrate as a source of nitrogen. The same data revealed that the caffeic acid percentage significantly responded to the increase in potassium fertilization. The two levels K_1 and K_2 increased the caffeic acid content in the plant. In the two seasons, K_2 treatment resulted in the highest values, while, the untreated plants showed the lowest values.

In case of the interaction between nitrogen and potassium fertilization, it could be noticed that under any level of nitrogen, a gradual increase in the caffeic acid content was parallel to the increase in potassium levels. It can be noticed that, the differences were insignificant in the second season. The highest values were recorded from the interaction treatment (N_3K_2) in both seasons. Similar results were reported by Berbec *et al.* [46] who mentioned that the highest dose of fertilization increased the phenolic acids content of *Echinacea purpurea* L. plant.

Effects of Fertilization Treatments on Alkamides Content and Composition: It could be seen from data in Table 4 that nitrogen and potassium fertilization increased the total alkamides content (%) in the whole herb of plant of *Echinacea paradoxa* L. This positive effect was noticed

Table 3: Effects of nitrogen and potassium fertilizers, as well as their interactions on polysaccharide and caffeic acid contents of *Echinacea paradoxa* L. during the two seasons (2008/2009) and (2009/2010)

	First season (2008/2009)		Second season (2009/2010)	
Treatments	Polysaccharides (%)	Caffeic acid (%)	Polysaccharide (%)	Caffeic acid (%)
	a) Effect of Nitrogen fertilizers			
N_0	2.45	0.79	3.00	0.80
N_1	3.40	1.19	3.47	1.46
N_2	4.23	1.56	3.66	1.71
N_3	5.41	1.88	5.82	1.93
LSD 5%	0.82	0.05	0.89	0.19
	b) Effect of Potassium fertilizers			
K_0	4.09	1.17	4.00	1.29
K_1	3.41	1.44	3.61	1.53
K_2	4.12	1.45	4.35	1.61
LSD 5%	N.S	0.04	N.S	0.12
	c) Effect of interaction treatments			
N_0K_0	1.37	0.72	3.21	0.78
N_0K_1	2.63	0.76	3.47	0.79
N_0K_2	3.34	0.88	3.73	0.83
N_1K_0	4.46	0.97	3.53	1.20
N_1K_1	3.60	1.29	2.26	1.53
N_1K_2	4.63	1.33	3.24	1.66
N_2K_0	4.47	1.21	3.70	1.41
N_2K_1	2.63	1.74	3.53	1.80
N_2K_2	3.10	1.72	3.76	1.92
N_3K_0	6.04	1.79	6.78	1.79
N_3K_1	4.77	1.88	4.00	2.00
N_3K_2	5.43	1.97	6.68	2.02
LSD 5%	1.66	0.10	1.79	N.S

Table 4: Effect of nitrogen and potassium fertilization, as well as their interactions on Alkamides content of *Echinacea paradoxa* L. during 2009/2010 season

	Proportional content (%) of the Individual alkamides								
Treatments	7	A	A	1	2	3	4+5	6	Total alkamides (%)
	a) Effect of Nitrogen fertilizers								
N_0	1.69	1.33	1.99	18.42	0.99	62.89	2.63	10.05	0.024
N_1	1.54	3.01	4.59	18.42	3.07	46.03	8.33	15.03	0.060
N_2	2.43	2.10	4.09	23.19	1.93	46.03	9.52	10.70	0.082
N_3	2.65	3.17	7.96	20.31	3.15	36.27	10.91	15.59	0.122
	b) Effect of Potassium fertilizers								
K_0	1.72	3.49	2.79	21.00	1.96	52.60	5.06	11.39	0.051
K_1	2.09	1.17	5.31	20.65	1.86	45.45	7.91	15.58	0.077
K_2	2.42	2.55	5.87	18.61	3.03	45.37	10.58	11.56	0.088
	c) Effect of interaction treatments								
N_0K_0	2.40	1.34	3.14	15.08	0.76	64.28	3.89	9.11	0.021
N_0K_1	1.57	1.04	1.32	20.32	1.11	54.22	1.91	18.51	0.025
N_0K_2	1.10	1.61	1.51	19.87	1.10	70.16	2.09	2.54	0.026
N_1K_0	1.77	4.83	1.53	22.76	1.36	43.88	4.90	18.97	0.044
N_1K_1	0.40	1.35	1.23	17.19	0.51	60.07	5.11	14.15	0.066
N_1K_2	2.46	2.84	11.00	15.30	7.33	34.15	14.97	11.96	0.070
N_2K_0	2.23	3.11	1.00	24.30	0.71	57.01	5.78	5.86	0.062
N_2K_1	1.28	0.29	6.10	30.49	3.38	34.78	10.26	13.40	0.079
N_2K_2	3.77	2.91	5.16	14.79	1.70	46.3	12.52	12.84	0.104
N_3K_0	0.47	4.67	5.49	21.84	5.02	45.22	5.66	11.63	0.075
N_3K_1	5.12	1.98	12.58	14.60	2.42	32.71	14.35	16.25	0.139
N_3K_2	2.35	2.85	5.80	24.48	2.00	30.88	12.73	18.90	0.152

HPLC profile of the lipophilic constituents of *Echinacea paradoxa*. Compound name [2]
1 = tetradeca-8Z-ene-1 1, 13-diyn-2-one, 2 = pentadeca-8Z.ene-l 1, 13-diyn-2-one,
3= pentadeca-8Z, 13Z-diene-l1-yn.2.one, 4= pentadeca- 8Z, 11Z, 13E-trien-2-one,
5 = pentadeca-8Z, 11E, 13Z-trien-2-one, 6 = pentadeca-8Z, 11Z-dien-2-one,
7 = dodeca-2E, 4Z-diene-8,1O.diynoic acid isobutylamide, A= unknown alkamide.

either they were applied separately or in combination. The N_3 and K_2 treatments were the most effective treatments, they gave the values of 0.122 and 0.088%, respectively. However, the combined treatment N_3K_2 was the best, giving the value of 0.152% followed by N_3K_1 which recorded 0.139%. The applied treatments did not change the characteristic feature of the alkamides spectrum, meaning that the alkamide No.3 remained the dominant one, followed by alkamides No.1 and No.6, respectively. These three alkamides constituted about 80% of the total alkamides. These results came in agreement with those of Bauer and Foster [2]. Similar results were reported by El-Gengaihi *et al.* [22] who stated that treating *Echinacea purpurea* plants with relatively high level of nitrogen and low potassium level increased the alkamide content in the plant tissues.

CONCLUSION

It could be concluded that potassium and nitrogen fertilizers and their interactions increased growth characters and chemical composition of *Echinacea paradoxa*. It could be recommended to fertilize *Echinacea paradoxa* plants with either 300 kg/fed of ammonium sulphate or 100kg/fed of potassium sulphate or a combination of them to get the best growth and active constituents.

REFERENCES

1. Perry, B., E. Burges and V. Glennie, 2001. Echinacea standardization: analytical methods for phenolic compounds and typical levels in medicinal species. Journal of Agric. Food Chem., 49: 1702-1706.
2. Bauer, R. and S. Foster, 1991. Analysis of Alkamides and Caffeic Acid Derivatives from *Echinacea simulata* and *E. paradoxa* roots. Planta Med., 57: 447-449.
3. Binns, S.E., J.T. Arnason and B.R. Baum, 2004. Taxonomic History and Revision of the Genus *Echinacea*. In: S. Miller, (Ed) Echinacea. The Genus Echinacea. CRC Press, Boca Raton, FL, pp: 3-11.
4. Doeve, A. and T.K. Basu, 2007. Echinacea: a Critical Analysis of its Immunomodulating Potential. Advances in Medicinal Plant Research, pp: 41-50.
5. Yu, H.C. and M. Kaarlas, 2004. Popularity, Diversity and Quality of *Echinacea*, In: S. Miller, (Ed) *Echinacea*. The Genus *Echinacea*. CRC Press, Boca Raton, FL., pp: 29-52.
6. Blumenthal, M., 2003. The ABC Clinical Guide to Herbs. American Botanical Council. Thieme, New York.
7. Bauer, R. and H. Wagner, 1991. *Echinacea* Species as Potential Immunostimulatory Drugs. In: H. Wagner and N.R. Farnsworth, (eds.), Economic and Medicinal Plant Research. Academic Press, New York, 5(8): 253-321.
8. Harborne, J.B. and C.A. Williams, 2004. Phytochemistry of the Genus *Echinacea*. In: S. Miller, (Ed) Echinacea. The Genus *Echinacea*. CRC Press, Boca Raton, FL, pp: 55-71.
9. Zhang, X., L. Rizshsky, C. Hauck, L. Qu, M.P. Widrlechner, B.J. Nikolau, P.A. Murphy and D.F. Birt, 2012. Bauer ketones 23 and 24 from *Echinacea paradoxa* var. *paradoxa* inhibit lipopolysaccharide-induced nitric oxide, prostaglandin E2 and cytokines in RAW264.7 mouse macrophages. Phytochemistry, 74: 146-158.
10. Muller, J.B., M. Muller, A. Probstle, T.A. Johns and R. Bauer, 1993. Anti inflammatory Activity of *Zanthoxylum chalybeum* Extracts and Identification of protoberberine and Benzophenanthridine Alkaloids by GC-MS and HPLC. Planta Med., 59: 664.
11. Goel, V., C. Chang, J.V. Slama, R. Barton, R. Bauer, R. Gahler and T.K. Basu, 2002. Alkylamides of *Echinacea purpurea* stimulate alveolar macrophage function in normal rats. Int. Immunopharmacol., 2(2-3): 381-387.
12. Hudson, J., V. Selvarani, L. Kang, V.T. Amiguet, J. Livesey and J.T. Arnason, 2005. Characterization of antiviral activities in Echinacea root preparations. Pharmaceutical Biology, 43(9): 790-796.
13. Pellati, F., S. Benvenuti, L. Magro, M. Melegari and F. Soragni, 2004. Analysis of phenolic compounds and radical scavenging activity of *Echinacea spp*. Journal of Pharmaceutical and Biomedical Analysis, 35(2): 289-301.
14. Dalby, B.L., H. Barsett, A.K.R. Landbo, A.S. Meyer and P. Molgaard, 2005. Synergistic antioxidative effects of alkamides, caffeic acid derivatives and polysaccharide fractions from *Echinacea purpurea* on in vitro oxidation of human low-density lipoproteins. Journal of Agricultural and Food Chemistry, 53(24): 9413-9423.
15. Charvat, T.T., D.J. Lee, W.E. Robinson and A.R. Chamberlin, 2006. Design, synthesis and biological evaluation of chicoric acid analogs as inhibitors of HIV- a integrase. Bioorganic and Medicinal Chemistry, 14: 4552-4567.

16. Healy, E.F., J. Sanders, P.J. King and W.J.R. Edwards, 2009. A docking study of E-chicoric acid with HIV-1 integrase. Journal of Molecular Graphics and Modelling, 27: 584-589.
17. Tousch, D., A. Lajoix, E. Hosy, J. Azay-Milhau, K. Ferrare, C. Jahannault, G. Cros and P. Petit, 2008. Chicoric acid, a new compound able to enhance insulin release and glucose uptake. Biochemical and Biophysical Research Communi Cations, 377: 131-135.
18. Marschner, H., 1995. Mineral Nutrient of Higher Plants. 2nd Ed., Academic Press Limited. Harcourt Brace and Company, Publishers, London, pp: 347-364.
19. Hendawy S.F., 1995. Agriculture and Chemical Studies on *Echinacea purpurea*. M.Sc. Thesis, Fac. Agric. Zagazig Univ. Egypt, pp: 184.
20. Shalaby, A.S., A.E. Agina, S.E. El-Gengihi and S.F. Hendawy, 1997a. Response of Echinacea to some agricultural practices. Journal of Herbs, Spices and Medicinal Plants, 4(4): 59-68.
21. Shalaby, A.S., S.E. El-Gengihi, E.A. Agina, A.S. Elkhayat and S.F. Hendawy, 1997b. Growth and yield of *Echinacea purpurea* L. as influenced by planting density and fertilization. Journal of Herbs, Spices and Medicinal Plants, 5(1): 69-76.
22. El-Gengaihi, S.E., A.S. Shalaby, E.A. Agina and S.F. Hendawy, 1998. Alkamides of *Echinacea purpurea* as influenced by plant ontogony and fertilization. Journal of Herbs, Spices and Medicinal Plants, 5(4): 35-42.
23. Jackson, M.L., 1973. Soil Chemical Analysis Prentice-Hall of India, pp: 144-197.
24. Wu, C.H., H.N. Murthy, E.J. Hahn, H.L. Lee and K.Y. Paek, 2008. Efficient extraction of caffeic acid derivatives from adventitious roots of *Echinacea purpurea*. Czech J. Food Sci., 26: 254-258.
25. Bauer, R. and H. Wagner, 1988. Echinacea der Sonnehut Stand Der for Schung Zeitschriftfier Der Phytotherapie, 9: 151-159.
26. Letchamo, W., J. Livesey, T.J. Arnason, C. Bergeron and V.S. Krutilina, 1999. Chicoric Acid and Isobutylamide Content in *Echinacea purpurea* as Influenced by Flower Developmental Stages, pp: 494-498.
27. Livesey, J., C. Bergeron, J. Rana, D.V.C. Awang, W. Letchamo and J. Arnason, 1998. Developing of a method for identity assurance and quality control in *Echinacea spp*. Paper presented at the conference of the American Society of Pharmacognosy, Orlando, Florida.
28. Bauer, R. and P. Remiger, 1989. TLC and HPLC analysis of alkamides in *Echinacea* drugs. Planta Med., 55: 367-371.
29. Gomez, K.A. and A.A. Gomez, 1984. Statistical Procedures for Agriculture Research 2nd Ed. pp: 180 John Wiley and Sons, New York U.S.A.
30. Snedecor, G.W. and W.G. Cochran, 1982. Statistical Method.7th Edition, Iowa State Univ., Press. Ames. Iowa, U.S.A., pp: 325-330.
31. El-Seifi, S.K., S.M.H. Sarg, A.I. Abdel-Fattah and M.A. Mohamed, 2004. Effect of biofertilizers and nitrogen levels on the productivity and quality of Chinese garlic under sandy soil condition. Zagazig Journal of Agric. Res., 31(3): 889-914.
32. Ezz El-Din, A.A., S.F. Hendawy, E.E. Aziz and E.A. Omer, 2010. Enhancing growth, yield and essential oil of caraway plants by nitrogen and potassium fertilizers. International Journal of Academic Res., 2(3): 192-197.
33. Arisha, H.M. and A. Bardisi, 1999. Effect of nitrogen fertilization and plant spacing on growth, yields and bud quality of common bean under sandy soil conditions. Zagazig Journal of Agric. Res., 26: 407-419.
34. Taylor, H.M. and B. Klepper, 1978. The role of rooting characteristics in the supply of water to plants. Adv. Agron., 30: 99-125.
35. Hamblin, A.P., 1985. The influence of soil structure on water movement, crop root growth and water uptake. Adv. Agron., 38: 95-112.
36. Said-Al Ahl, H.A.H., S.A. Hasnaa and S.F. Hendawy, 2009. Effect of potassium humate and nitrogen fertilizer on herb and essential oil of oregano under different irrigation intervals. Ozean Journal of Applied Sci., 2(3): 319-329.
37. Zaghloul, S.M., F.E.M. El-Quesni and A.A.M. Mazhar, 2009. Influence of Potassium Humate on Growth and Chemical constituents of *Thuja orientalis,* L seedlings. Ozean Journal of Applied Sci., 2(1): 73-78.
38. Azzaz, N.A.E. and E.A. Hassan, 2008. The chemical constituents and vegetative and yielding characteristics of fennel plants treated with organic and biofertilizer AS A. substitute of mineral fertilizer. Journal of Biol. Chem. Environ. Sci., 3(1): 411-454.
39. Abd El-Salam, I.Z., 1994. Effect of chemical fertilization and planting distances on growth and chemical composition on *Pimpinella anisum,* L. plant. M.Sc. Thesis, Fac. of Agric. Cairo Univ. Egypt.

40. Jacoub, W.R., 1995. Effect of chemical fertilization on growth and oil yield of sweet basil (*Ocimum basilicum*, L.) plants. M.Sc. Thesis, Fac. of Agric. Cairo Univ. Egypt.
41. Abd El-Aziz, A.H., 2000. Effect of some fertilizers treatments on the growth and oil yield of basil plants. Ph.D. Thesis, Fac. of Agric. Zagazig Univ. Egypt.
42. Abd El-Azim, W.M., 2003. Production of *Salvia officinalis*, L. plant under Sinai conditions. Ph.D. Thesis, Fac. Agric. Cairo Univ. Egypt.
43. Essa, A.M., 1999. Effect of some cultural treatments on growth and yield of *Marticaria chamomilla*, L. plant. Ph.D. Thesis, Fac. Agric. Ain Shams Univ. Cairo, Egypt.
44. Ibrahim, S.M., 2000. Effect of seeding rate and biofertilization on fennel (*Foeniculum vulgare*, Miller) plants. M.Sc. Thesis, Fac. of Agric. Cairo Univ. Egypt.
45. Montanari, M., D.I. Elena, R. Maggini, S. Pacifici, A. Pardossi and L. Guidi, 2008. Effect of nitrate fertilization and saline stress on the contents of active constituents of *Echinacea angustifolia* DC. Food Chemistry, 107: 1461-1466.
46. Berbec, S., B. Krol and T. Wolski, 1998. The effect of soil and fertilization on the biomass and phenolic acids content in coneflower (*Echinacea purpurea*, Moench). Herba-Polonica, 44(4): 397-402.