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INFLUENCE OF SOME AMINO ACIDS AND MICRO-NUTRIENTS TREATMENTS ON GROWTH AND CHEMICAL CONSTITUENTS OF *Echinacea purpurea* PLANT

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ABSTRACT

Two field trials were carried out during 2011 and 2012 seasons at the Experimental Farm, Horticulture Dept., of Agric Fac., Benha Univ., to evaluate the effect of some amino acid treatments i. e. tryptophan and glutamic acid each at 200 ppm and some micro-nutrients treatments i. e. Fe, Zn and Mn each at 150 ppm as well as their combinations on growth, total caffeic acid derivatives and total alkamides as well as chemical constituents of *Echinacea purpurea* plants. Results showed that different applied treatments of amino acid and/or micro-nutrients treatments led to significant increase of the studied growth parameters i.e. plant height, number of branches, fresh and dry weights of herb/plant, number of flowering heads/plant, fresh and dry weights of flowering heads/plant as compared with control plants in both seasons. However, the tallest plant, the highest number of flowering heads as well as their fresh and dry weights were recorded by 200ppm tryptophan-sprayed plants combined with Zn at 150ppm, whereas the highest number of branches and suckers/plant were scored by the combined treatment between glutamic acid at 200ppm and Fe at 150ppm in both seasons. Moreover, the heaviest fresh and dry weights of herb/plant were registered by 200ppm glutamic acid treatment combined with Zn at 150ppm in both seasons. In addition, the obtained vigrous growth of *Echinacea purpurea* plants with different treatments was accompanied by pronounced increase in leaves total free amino acids, Fe, Zn and Mn contents of treated plants in both seasons. Furthermore, total caffeic acid derivatives and total alkamides were increased by spraying all treatments of amino acids and micro-nutrients as well as their combinations, especially the combined treatment between glutamic acid at 200ppm and Fe or Zn each at 150ppm.

Consequently, it is preferable to spray *Echinacea purpurea* plants with tryptophan or glutamic acid each at 200 ppm as well as Fe or Zn each at 150 ppm and their combinations for enhancing the growth and chemical constituents of this plant.

Keywords. *Echinacea purpurea*, amino acids, micro-nutrients, growth , total caffeic acid derivatives and alkamides contents.

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INTRODUCTION

Echinacea purpurea (L.) Moench is an herbaceous perennial and a member of the Asteraceae family. Commonly called purple coneflower, it has a natural range extending from Michigan, Ohio, Illinois, and Iowa, to southeastern united States and west to Texas. *E. purpurea* grows at a rate of twelve to eighteen inches a year to a mature height of two to four feet. The leaves are ovate to lanceolate and the flowers are cone-shaped disks adorned with deep pink to purple ray flowers. Flowers bloom from June to August (Hendawy, 2000).

Echinacea is an herbal medicine, which is used by native Americans for enhancing the human immune system. In Europe and North America, they widely used the *Echinacea purpurea* as the herbal medicine for most of the remedies. Due to the increased market demand, economic value and its potential benefits to human health make increased cultivation of Echinacea. Beside, *Echinacea purpurea* is used for both ornamental and phytochemical in Europe, United States and Australia. Three groups of phytochemicals are determined such as caffeic acid derivatives, polysaccharides and liphophilic alkamides, which are responsible for the genus medicinal properties. Most of Americans and European have been used medicinal preparation of *Echinacea purpurea* for remedy of many diseases, including colds, toothaches, snake bites, headache and wound infections. It is the most effective antioxidant and it has immunoenhancing effects. The roots of Echinacea are used to treat blood poisoning, snake poisoning, skin disease, syphilis and rabbis. *Echinacea purpurea* herb is also used to treat chronic infections of respiratory tract and lower urinary tract (viral and bacterial origin). The polysaccharide from *Echinacea purpurea* is used to kill bacteria such as staphylococci. Arabinogalactan, a high molecular weight purified polysaccharide from plant cell cultures of *Echinacea purpurea* has potent to activate macrophage cytotoxicity actions against tumor cells and micro organisms. The main caffeic acid derivative (caftaric acid, chlorogenic acid and echinacoside) has been functionally linked to antiinflammatory and wound healing properties of Echinacea when applied topically. Caffeic acid derivatives are very effective antioxidants in free radical generation systems. Groups of phenolic compounds and alkamides, which have demonstrated antiviral and antifungal properties, respectively (Kumar and Ramaiah, 2011).

Amino acids have traditionally been considered as precursors and constituents of proteins. Many amino acids also act as precursors of other nitrogen containing compounds, e.g., nucleic acids. Amino acids can play wide roles in plants including acting as regulatory and signaling molecules. Amino acids also affect synthesis and activity of some enzumes, gene expression, and redoxhomeostasis (Rai, 2002). Many studies have reported that foliar application of amino acids caused an increase in the growth and development of plants. In this respect, Omer *et al.* (2013) reported that foliar spray with amino acids improved the growth and chemical composition of chamomile plant.

Micro-nutrients, especially Fe, Zn and Mn act either as metal components of various enzymes or as functional, structural, or regulatory cofactors. Thus, they are associated with saccharide metabolism, photo-synthesis and protein synthesis (Marschner, 1997).

Many investigatores reported the stimulating effect of applied micronutrients as foliar spray on growth and flowering of different medicinal and aromatic plants; El-Khyat (2013) on *Rosmarinus officinalis* and Amran (2013) on *Pelargonium graveolens* indicated that foliar application of Fe, Zn and Mn improved the growth and chemical composition of the plants.

Therefore, the objective of this study was to evaluate the effect of foliar spray with some amino acids and mico-nutrients as well as their combinations on growth and chemical composition of *Echinacea purpurea* plants.

MATERIALS AND METHODS

Two field experiments were conducted at the Experimental Farm and in the Laboratory of Horticulture Department, Faculty of Agriculture at Moshtohor, Benha Univ., during 2011 and 2012 seasons to study the influence of some amino acids i.e. tryptophan (200 ppm) and glutamic acid (200ppm) and some micro-nutrients i.e. Fe, Zn and Mn each at 150ppm on growth and chemical constituents of *Echinacea purpurea* plants.

Echinacea purpurea seedlings (8-10cm height with 4-5 leaves) were obtained from Floriculture Farm, Horticulture Department, Faculty of Agriculture, Benha Univ., then seedlings were transplanted in clay loam soils on mid March in both seasons in beds (1x1 m) containing two rows (50 cm inbetween) each row contained two hills (50 cm apart). Soil was directly irrigated to provide suitable moisture for growth. All the traditional cultural practices for growing *Echinacea purpurea* plants were followed as recommended in this region.

Mechanical and chemical analyses of the experimental soils are presented in Tables (a) and (b), mechanical analysis was estimated according to Jackson (1973), whereas chemical analysis was estimated according to Black *et al.,* (1982).

Table (a): Mechanical analysis of the experimental soil.

Parameters	Unit	Season	
		2011	2012
Coarse sand	%	5.12	5.34
Fine sand	%	15.51	16.09
Silt	%	24.87	25.48
Clay	%	54.50	53.09
Textural class	------	Clay loam	Clay loam

Table (b): Chemical analysis of the experimental soil:

Parameters	Unit	Season	
		2011	2012
$CaCO_3$	%	0.91	0.86
Organic matter	%	1.32	1.41
Available nitrogen	%	0.94	0.98
Available phosphorus	%	0.61	0.67
Available potassium	%	0.71	0.78
E.C	ds/m	0.97	0.89
pH	--------------	7.49	7.62

Experimental layout.

The experimental treatments consisted of 12 treatments with three replicate (each replicate consisted of six beds, with four plants/bed), which represented all combinations between foliar application of amino acids treatments (control, glutamic acid at 200ppm and tryptophan at 200ppm) and foliar application of micro-nutrients (control, Fe at 150 ppm, Zn at 150 ppm and Mn at 150ppm). Amino acids treatments were sprayed at interval times of 50, 71 and 92 days, whereas the treatments of micro-nutrients were sprayed at interval times of 60, 81 and 102 days from transplanting. Treated plants were sprayed till run off, whereas control plants were sprayed with tap water. The plants were sprayed with a hand pump mister to the point of runoff. A surfactant (Tween 20) at a concentration of 0.01% was added to all tested solutions including the control. Common agricultural practices (irrigation, fertilization, manual weed control, … etc.) were carried out when needed.

Data recorded.

In both seasons, at harvest time (mid August) the following measurements were conducted as follow; plant height (cm.), number of branches/plant, fresh and dry weights of herb (leaves and stems)/plant (g), number of suckers/plant, number of flowering heads/plant, fresh and dry weights of flowering heads/plant (g). In addition, Fe, Zn, and Mn (%) were determined in the digested samples by atomic absorption as described by Chapman and Paratt (1961). Total free amino acids (mg/g F.W) were determined in the leaves according to Rosed (1957).

Total alkamides were quantitatively determined in *Echinacea purpurea* aerial parts (flowering heads, leaves and stems) in the second season (2012) using High- Performance Liquid Chromato-graphy (HPLC) according to Bauer and Remiger (1989).Total caffeic acid derivatives content in the plant organs (roots, herb and flowering heads) of *Echinacea purpurea* were determined as chicoric acid using spectrophotometer according to A.O.A.C (1980).

Statistical analysis

All data obtained in both seasons of study were subjected to analysis of variance as factorial experiments in a complete randomize block design. L.S.D. method was used to difference means according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Effect of amino acids and micro-nutrients treatments on growth and chemical composition of *Echinacea purpurea* plants.

I- Vegetative growth traits:

I-1- Plant height (cm)

Data in Table (1) declare that all applications of amino acids i.e., glutamic acid and trypyohan each at 200 ppm significantly increased the height of *Echinacea purpurea* plants as compared with control in both seasons, with the superiority of tryptophan treatment. This trend was true in both seasnons. Moreover, all tested applications of micro-nutrients i.e., Fe, Zn and Mn each at 150 ppm succeeded in increasing plant height, especially Zn treatment when compared with control in both seasons. However, most applied combinations between amino acids and micro-nutrients caused significant increments in plant height in the first and second seasons as compared with control. However, the highest value of plant height (98.12 and 94.72cm, in the first and second seasons, respectively) was recorded by tryptophan at 200ppm combined with Zn at 150ppm.

Table (1): Effect of some amino acids and micro-nutrients treatments on plant height and branches number / plant of *Echinacea purpurea* plants during 2011and 2012 seasons.

First season (2011)									
Parameters		**Plant height (cm)**				**branches number/plant**			
Amino acids / micronutrients		**Control**	**G. at 200 ppm**	**T. at 200 ppm**	**Mean**	**Control**	**G. at 200 ppm**	**T. at 200 ppm**	**Mean**
Control (0.0)		71.34	76.24	85.90	**77.83**	7.82	11.20	10.13	**9.72**
Fe at 150 ppm		77.10	81.07	92.36	**83.51**	9.08	16.24	14.92	**13.41**
Zn at 150 ppm		79.35	87.93	98.12	**88.47**	8.14	13.08	15.17	**12.13**
Mn at 150 ppm		75.03	83.41	91.17	**83.20**	8.37	14.21	11.70	**11.43**
Mean		**75.71**	**82.16**	**91.89**		**8.35**	**13.68**	**12.98.**	
L.S.D at 0.05 for	**Amino acids**	**4.17**				**2.34**			
	micronutrients	**4.81**				**2.70**			
	interaction	**8.34**				**4.64**			
Second season (2012)									
Control (0.0)		72.03	74.21	81.26	**75.83**	8.13	11.87	10.26	**10.09**
Fe at 150 ppm		76.19	87.14	91.50	**84.94**	10.14	17.25	16.07	**14.49**
Zn at 150 ppm		82.02	91.12	94.72	**89.29**	9.11	14.27	16.09	**13.16**
Mn at 150 ppm		78.20	84.62	89.33	**84.05**	10.07	14.93	13.26	**12.75**
Mean		77.11	84.27	89.20		9.36	14.58	13.92	
L.S.D at 0.05 for	**Amino acids**	**6.19**				**2.68**			
	micronutrients	**7.14**				**3.09**			
	interaction	**12.38**				**5.36**			

G.= Glutamic acid **T.= Tryptophan**

I-2- Number of branches/plant

Data in Table (1) indicate that number of branches/plant was increased due to amino acids treatments. However, the highest number of branches per plant was registered by glutamic acid treatment in both seasons. Moreover, all tested applications of micro-nutrients showed significant increaments in this respect. Anyway, the highest number of branchs/plant was gained by Fe treatment in both seasons as compared with control.

As for the interaction effect between amino acids and micro-nutrients, it was found that all combinations between amino acids and micro-nutrients led to increase the number of branches per plant in both seasons. However, the highest number of branches per plant (16.24 and 17.25) was recorded by the interaction between glutamic acid at 200ppm and Fe at 150ppm in the first and second seasons, respectively.

I-3- Fresh and dry weights of herb/plant

Data in Table (2) reveal that fresh and dry weights of herb per plant were positively affected by all amino acids and micro-nutrients treatments in both seasons. However, glutamic and tryptophan treatments succeeded in increasing the fresh and dry weights of herb per plant, with superiority for glutamic acid treatment in both seasons. Moreover, all treatments of micro-nutrients statistically increased the fresh and dry weights of herb per plant, especially the treatment of Zn in the first and second seasons. As for the interaction effect between amino acids and micro-nutrients, it was observed that all combinations of amino acids and micro-nutrients enhanced the fresh and dry weights of herb per plant as compared with control in both seasons. Generally, the heaviest fresh and dry weights of herb per plant in the first and seconed seasons were recoreded by the combined treatment between glutamic acid at 200ppm and Zn at 150ppm in both seasons.

Table (2): Effect of some amino acids and micro-nutrients treatments on fresh and dry weights of herb of *Echinacea purpurea* plants during 2011and 2012 seasons.

Parameters		Fresh weight of herb/plant (g)			Mean	Dry weight of herb/plant (g)			Mean
Amno acids / micronutrients		Control	G. at 200 ppm	T. at 200 ppm		Control	G. at 200 ppm	T. at 200 ppm	
First season (2011)									
Control (0.0)		905	1154	1138	1066	81.6	103.1	101.7	95.47
Fe at 150 ppm		1029	1466	1495	1330	92.4	124.3	128.9	115.2
Zn at 150 ppm		1111	1603	1372	1362	95.0	132.5	118.3	115.3
Mn at 150 ppm		1083	1432	1282	1266	91.8	121.4	112.5	108.6
Mean		1032	1414	1322		90.2	120.3	115.4	
L.S.D at 0.05 for	Amino acids	108				12.34			
	micronutrients	125				14.24			
	interaction	216				24.64			
Second season (2012)									
Control (0.0)		967	1295	1248	1170	86.4	111.7	109.5	102.5
Fe at 150 ppm		1109	1398	1512	1340	97.3	121.6	130.4	116.4
Zn at 150 ppm		1186	1628	1357	1390	104.1	139.2	117.0	120.1
Mn at 150 ppm		1110	1448	1282	1280	98.3	124.9	115.1	112.8
Mean		1093	1442	1350		96.5	124.4	118.0	
L.S.D at 0.05 for	Amino acids	118				13.58			
	micronutrients	136				15.67			
	interaction	236				27.16			

G.= Glutamic acid T.= Tryptophan

I-4- Number of suckers/plant:

Data presented in Table (3) illustrate that all tested amino acids significantly succeded in increasing the number of suckers/plant as compared with control in both seasons. Moreover, all micro-nutrients treatments significantly increased the number of suckers per plant, particularly Zn treatment in the first and second seasons. Referring to the interaction effect between amino acids and micro-nutrients, it was found that all interactions between amino acids and micro-nutrients increased the number of suckers per plant as compared with control in both seasons. Generally, the highest number of suckers/plant (12.34 and 12.22, in the first and second seasons, respectively) was recoreded by the combined treatment between 200ppm glutamic acid and 150ppm Fe. This may be due to the combined effects of both tryptophan as a precursor of indole acetic acid which induces cell division and enlargement and glutamic as a growth promoters, in addition to the effects of the studied micro- nutrients which supply the plant with the required nutrients necessary for well developed plant.

Table (3): Effect of some amino acids and micro-nutrients treatments on the number of suckers and flowering heads of *Echinacea purpurea* plants during 2011and 2012 seasons.

First season (2011)									
Parameters		**Number of suckers/plant**			**Mean**	**Number of flowering heads/plant**			**Mean**
Amino acids / micronutrients		**Control**	**G. at 200 ppm**	**T. at 200 ppm**		**Control**	**G. at 200 ppm**	**T. at 200 ppm**	
Control (0.0)		5.39	8.64	8.27	7.43	14.28	18.24	17.81	16.78
Fe at 150 ppm		7.06	12.34	9.24	9.55	16.08	28.19	24.80	23.02
Zn at 150 ppm		7.94	9.14	11.82	9.63	17.18	21.96	28.93	22.69
Mn at 150 ppm		7.28	9.08	9.80	8.72	15.94	18.63	19.21	17.93
Mean		**6.92**	**9.80**	**9.78**		**15.87**	**21.76**	**22.69**	
L.S.D at 0.05 for	Amino acids	1.24				4.17			
	micronutrients	1.43				4.81			
	interaction	2.48				8.34			
Second season (2012)									
Control (0.0)		5.92	9.07	9.18	8.06	15.29	19.78	21.23	18.77
Fe at 150 ppm		8.25	12.22	10.11	10.19	18.11	29.20	27.75	25.02
Zn at 150 ppm		9.04	10.20	12.16	10.47	19.24	23.24	32.16	24.88
Mn at 150 ppm		8.12	9.85	10.03	9.33	17.36	21.43	27.11	21.97
Mean		7.83	10.34	10.37		17.50	23.41	27.06	
L.S.D at 0.05 for	Amino acids	1.43				4.85			
	micronutrients	1.65				5.60			
	interaction	2.83				9.70			

G.= Glutamic acid T.= Tryptophan

The aforementioned results of amino acids are in agreement with Hendawy (2000) on *Echinacea purpurea*, Wahabe *et al.,* (2002) on *Antholyza aethiopica*, Youssef *et al.,* (2004) on Datura plants, Gamal El-Din and Abd El-Wahed (2005*)* on chamomile plant, Balbaa and Talaat (2007) on rosemary plants, Gomaa and Mady (2008) on *Matricaria chamomilla*, Youssef (2009) on rosemary plant, Sarojnee *et al.,* (2009) on hot peppers, El-Awadi and Hassan (2010) on fennel plant, Behzad (2011) on flixweld (*Descurainia sophia* L.), Haj Seyed Hadi *et al.,* (2011) on chamomile plant, Datir *et al.,* (2012) on *Capsicum annum* L., Hussein (2013) on chamomile plant, Rahimi *et al.,* (2013) on basil plant and Omer *et al.,* (2013) on chamomile plant. The abovementioned results of micro-nutrients are nearly similar to those obtained by Hendawy (2000) on *Echinacea purpurea,* Kuntal *et al.,* (2005) on *Stevia rebaudiana*, Gomaa (2008) on *Hibiscus sabdariffa*, Youssef (2009) on rosemary plant, Nasiri *et al.*, (2010) on chamomile plant, Ajay *et al.,* (2010) on *Mentha arvensis* L., Said-Al Ahl and Mahmoud (2010) on sweet basil, Amuamuha *et al.,* (2012) on marigold plant., Khalid (2012) on anise plant, Shilpa and Dhumal (2012) on *Cassia angustifolia*, Saeid Zehtab *et al.,* (2012) on Psyllium plant, Amran (2013) on *Pelargonium graveolens* and El-Khyat (2013) on *Rosmarinus officinalis.*

II-Flowering growth traits:

Data in Tables (3 and 4) indicate that all tested sprays of amino acids resulted in significant increments in the number, fresh and dry weights of flowering heads/plant, with superiority for tryptophan treatment as compared with control in both seasons. Also, all tested micro-nutrients statistically increased the number, fresh and dry weights of flowering heads/plant, especially those received Fe treatment in both seasons.

Table (4): Effect of some amino acids and micro-nutrients treatments on fresh and dry weights of flowering heads of *Echinacea purpurea* plants during 2011and 2012 seasons.

Parameters		Fresh weight of flowering heads/plant (g)			Mean	Dry weight of flowering heads/plant (g)			Mean
First season (2011)									
Amino acids / micronutrients		Control	G. at 200 ppm	T. at 200 ppm		Control	G. at 200 ppm	T. at 200 ppm	
Control (0.0)		318	480	451	416	27.13	40.12	37.38	34.88
Fe at 150 ppm		365	716	670	584	30.72	59.22	54.56	48.17
Zn at 150 ppm		382	582	726	563	32.49	48.18	62.17	47.61
Mn at 150 ppm		349	480	542	457	29.41	39.10	44.16	37.56
Mean		354	565	597		29.94	46.66	49.57	
L.S.D at 0.05 for	Amino acids	83.47				11.09			
	micronutrients	96.32				12.80			
	interaction	166.9				22.18			
Second season (2012)									
Control (0.0)		319	453	504	425	27.52	38.41	42.40	36.11
Fe at 150 ppm		424	741	783	649	35.47	61.32	63.71	53.50
Zn at 150 ppm		452	599	792	614	37.75	48.72	66.04	50.84
Mn at 150 ppm		408	569	786	588	34.37	47.14	65.04	48.85
Mean		400.8	591	716.3		33.78	48.90	59.30	
L.S.D at 0.05 for	Amino acids	79.24				12.18			
	micronutrients	91.44				14.06			
	interaction	158.5				24.36			

G.= Glutamic acid T.= Tryptophan

As for the interaction effect between amino acids and micro-nutrients, it was noticed that all interactions between amino acids and micro-nutrients increased the values of the number, fresh and dry weights of flowering heads/plant. However, the highest number of flowering heads/plant (28.93 and 32.16), the heaviest fresh weight of flowering heads/plant (726 and 792g) and the heaviest dry weight of flowering heads/plant (62.17 and 66.04g) were recorded by 200ppm tryptophan treatment combined with Zn at 150ppm, in the first and second seasons, respectively.

The aforementioned results of amino acids are in agreement with Hendawy (2000) on *Echinacea purpurea*, Wahabe *et al.,* (2002) on *Antholyza aethiopica*, Youssef *et al.,* (2004) on Datura plants, Gamal El-Din and Abd El-Wahed (2005) on chamomile plant, Gomaa and Mady (2008**)** on *Matricaria chamomilla*, Sarojnee *et al.,* (2009) on hot peppers, El-Awadi and Hassan (2010) on fennel plant, Haj Seyed Hadi *et al.,* (2011) on chamomile plant, Datir *et al.,* (2012) on *Capsicum annum* L., Behzad (2011) on flixweld (*Descurainia sophia* L.), Hussein (2013) on chamomile plant and, Omer *et al.,* (2013) on chamomile plant. The aforementioned results of micro-nutrients are in parallel with those obtained by Hendawy (2000) on *Echinacea purpurea,* Gomaa (2008) on *Hibiscus sabdariffa*, Nasiri *et al.,* (2010) on chamomile plant, Amuamuha *et al.,* (2012) on marigold plant., Khalid (2012) on anise plant, Shilpa and Dhumal (2012) on *Cassia angustifolia*, and Saeid Zehtab *et al.,* (2012) on Psyllium plant.

III- Leaf chemical composition:

III-1. Leaf total free amino acids, Fe, Zn and Mn contents:

Data in Tables (5 and 6) declare that all tested amino acids and micro-nutrients treatments increased total free amino acids, Fe, Zn and Mn contents in *Echinacea purpurea* leaves in both seasons. In this respect, the richest leaf total free amino acids and Zn contents were recorded by tryptophan at 200ppm and Zn at 150ppm treatment as well as their combinations in both seasons as compared with control and the rest treatments. Besides, spraying *Echinacea purpurea* plants with glutamic acid and Fe treatments as well as their combinations proved to be the most pronounced treatments for producing the highest leaf Fe content in both seasons. Also, 200ppm glutamic acid or 150ppm Mn-sprayed plants and their interactions induced the greatest values in this respect in both seasons. However, the highest values of leaf total free amio acids (24.17 and 21.94 mg/g FW) and Zn (0.034 and 0.036%) contents were scored by tryptophan at 200ppm combined with Zn at 150ppm, in the first and second seasons, respectively. In addition, the highest leaf Fe content (0.071 and 0.078%) was registered by 200ppm glutamic acid combined with Fe at 150ppm, whereas the highest leaf Mn content (0.019 and 0.018%) was scored by the combined treatment between glutamic acid at 200ppm and Mn at 150ppm, in the first and second seasons, respecively. This may be due to the combined effects of both tryptophan as a precursor of indole acetic acid which induces cell division and enlargement and glutamic as a growth promoters, in addition to the effects of the studied micro-nutrients which supply the plant with the required nutrients necessary for healthy growth.

The results of amino acids are in accordance with the findings of Mohamed (1992) on *Dahlia pinnata*, Gamal El-Din *et al.,* (1997) on lemongrass plants, Hendawy (2000) on *Echinacea purpurea*, El-Khayat (2001) on *Hibiscus sabdariffa*, Wahabe *et al.,* (2002) on *Antholyza aethiopica*, Youssef *et al.,* (2004) on Datura plants and Balbaa and Talaat (2007) on rosemary plant, Youssef (2009) on rosemary plant, Amran (2013) on *Pelargonium graveolens* and El-Khyat (2013) on *Rosmarinus officinalis.*

The aforementioned results of micro-nutrients are in conformity with those obtained by Hendawy (2000) on *Echinacea purpurea*, Gomaa (2008) on *Hibiscus sabdariffa*, Youssef (2009) on rosemary plant, Nasiri *et al.,* (2010) on chamomile plant, Ajay *et al.,* (2010) on *Mentha arvensis* L., Amuamuha *et al.* (2012) on marigold plant., Shilpa and Dhumal (2012) on *Cassia angustifolia*, Saeid Zehtab *et al.,* (2012) on Psyllium plant, Amran (2013) on *Pelargonium graveolens* and El-Khyat (2013) on *Rosmarinus officinalis.*

Table (5): Effect of some amino acids and micro-nutrients treatments on leaf total free amino acids and Fe contents of *Echinacea purpurea* plants during 2011and 2012 seasons.

First season (2011)									
Parameters		**Total free amino acids (mg/g FW)**			**Mean**	**Fe (%)**			**Mean**
Amino acids / micronutrients		**Control**	**G. at 200 ppm**	**T. at 200 ppm**		**Control**	**G. at 200 ppm**	**T. at 200 ppm**	
Control (0.0)		14.34	16.14	17.24	15.91	0.054	0.058	0.057	0.056
Fe at 150 ppm		16.97	20.84	23.03	20.28	0.062	0.071	0.068	0.067
Zn at 150 ppm		17.23	19.63	24.17	20.34	0.057	0.064	0.062	0.061
Mn at 150 ppm		16.42	18.80	21.41	18.88	0.059	0.061	0.060	0.060
Mean		16.24	18.85	21.46		0.058	0.064	0.062	
L.S.D at 0.05 for	Amino acids	1.34				0.009			
	micronutrients	1.55				0.010			
	interaction	2.68				0.018			
Second season (2012)									
Control (0.0)		13.52	16.81	18.03	16.12	0.046	0.056	0.052	0.051
Fe at 150 ppm		15.41	17.87	20.32	17.87	0.069	0.078	0.074	0.074
Zn at 150 ppm		16.01	19.81	21.94	19.25	0.051	0.072	0.065	0.063
Mn at 150 ppm		15.92	18.45	19.21	17.86	0.054	0.068	0.062	0.061
Mean		15.22	18.24	19.88		0.055	0.069	0.063	
L.S.D at 0.05 for	Amino acids	1.19				0.007			
	micro-nutrients	1.37				0.008			
	interaction	2.38				0.014			

G.= Glutamic acid T.= Tryptophan

Table (6): Effect of some amino acids and micro-nutrients treatments on leaf Zn and Mn contents of *Echinacea purpurea* plants during 2011and 2012 seasons.

First season (2011)									
Parameters		**Zn (%)**			**Mean**	**Mn (%)**			**Mean**
Amino acids / micronutrients		**Control**	**G. at 200 ppm**	**T. at 200 ppm**		**Control**	**G. at 200 ppm**	**T. at 200 ppm**	
Control (0.0)		0.021	0.023	0.025	0.023	0.012	0.013	0.013	0.013
Fe at 150 ppm		0.027	0.024	0.031	0.027	0.015	0.018	0.016	0.016
Zn at 150 ppm		0.028	0.030	0.034	0.031	0.014	0.017	0.015	0.015
Mn at 150 ppm		0.025	0.026	0.029	0.027	0.016	0.019	0.018	0.018
Mean		0.025	0.026	0.030		0.014	0.017	0.016	
L.S.D at 0.05 for	Amino acids	0.006				0.0025			
	micronutrients	0.007				0.0028			
	interaction	0.012				0.0050			
Second season (2012)									
Control (0.0)		0.023	0.024	0.026	0.024	0.013	0.015	0.015	0.014
Fe at 150 ppm		0.025	0.026	0.028	0.026	0.014	0.015	0.014	0.014
Zn at 150 ppm		0.030	0.034	0.036	0.033	0.014	0.015	0.014	0.014
Mn at 150 ppm		0.026	0.029	0.031	0.029	0.015	0.018	0.016	0.016
Mean		0.026	0.028	0.030		0.014	0.016	0.015	
L.S.D at 0.05 for	Amino acids	0.007				0.0012			
	micronutrients	0.008				0.0014			
	interaction	0.014				0.0024			

G.= Glutamic acid T.= Tryptophan

III-2 Total alkamides (%):

Data presented in Table (7) show that all tested treatments of amino acids and micro-nutrients as well as their interactions succeeded in increasing total alkamides (%) in the above ground organs (leaves , stems and flowering heads) of *Echinacea purpurea* plant. However, the highest value of total alkamides (0.072 %) was scored by glutamic acid at 200ppm combined with Zn at 150ppm, followed by the combined treatment between glutamic acid at 200ppm and Fe at 150ppm as it gave 0.69 against to 0.034% for control.

Table (7): Effect of some amino acids and micro-nutrients treatments on total alkamides (%) of *Echinacea purpurea* during 2012 season.

Amino acids / Micro-nutrients	Control (0.0 ppm)	Glutamic at 200 ppm	Tryptophan at 200 ppm
Control (0.0 ppm)	0.034	0.059	0.049
Fe at 150 ppm	0.046	0.069	0.052
Zn at 150 ppm	0.051	0.072	0.056
Mn at 150 ppm	0.049	0.063	0.054

III-3 Total caffeic acid derivatives (%):

It is clear from Table (8) that the flowering heads of *Echinacea purpurea* are the richest plant part with total caffeic acid derivatives as they contained 0.82 and 0.78 % , followed by the herb (0.45 and 0.48 %), then the roots (0.31 and 0.31%) in the first and second seasons, respectively. However, 200 ppm glutamic- sprayed plants showed to be the most effective one for inducing the highest values of total caffeic acid derivatives of *Echinaceae purpurea* plants (flowering heads, herb and roots), in both seasons. All tested sprays of micro-nutrients increased total caffeic acid derivatives,especially Fe at 150 ppm in case of flowering heads and herb parts and Zn at 150 ppm in case of root parts in both seasons. In general the highest values of total caffeic acids derivatives in the flowering heads (0.98 and 0.94 %) and in the herb (0.61 and 0.64%) were recorded by the combined treatment between glutamic acid at 200 ppm and Fe at 150ppm , whereas the highest values of total caffeic acid derivatives in the roots (0.41 and 0.39%) were scored by 200 ppm glutamic acid-sprayed plants combined with 150 ppmZn treatment, in the first and second seasons, respectively.

The obtained results of this study may be due to the role of amino acids and micro-nutrients in growth and development of the plants; where amino acids are essential constituents in all cells. In addition to their role in protein synthesis, they participate in both primary and secondary metabolic processes associated with plant development and in responses to stress. Amino acids contribute to the tolerance of plants against biotic and abiotic stresses either directly or indirectly by serving as precursors to secondary compounds and hormones. Moreover, amino acids are particularly important for cell growth stimulation. They act as buffers which help to maintain favorable pH value within the plant cell. They protect the plants from ammonia toxicity. They can serve as a source of carbon and energy, as well as protect the plants against pathogens. Amino acids, also function in the synthesis of other organic compounds, such as protein, amines, purines and pyrimidines, alkaloids, vitamins, enzymes, terpenoids and others. Amino acids are critical to life, and have many functions in metabolism (Behzad, 2011). Furthermore, amino acids affect synthesis and activity of some enzumes, gene expression, and redoxhomeostasis (Rai, 2002).

Table 8: Effect of some amino acids and micro-nutrients treatments on total caffeic acid derivatives (g/100g dry weight) of *Echinacea purpurea* plants during 2011and 2012 seasons.

Season		First season (2011)											
organ		Flowering heads			Mean	Herb			Mean	Roots			Mean
Amino acids / Micro-nutrients		Con.	G. at 200 ppm	T. at 200 ppm		Con.	G. at 200 ppm	T. at 200 ppm		Con.	G. at 200 ppm	T. at 200 ppm	
Control (0.0)		0.62	0.86	0.82	0.77	0.31	0.49	0.38	0.39	0.19	0.31	0.28	0.26
Fe at 150 ppm		0.74	0.98	0.87	0.86	0.40	0.61	0.46	0.49	0.27	0.38	0.29	0.31
Zn at 150 ppm		0.71	0.92	0.82	0.82	0.39	0.54	0.42	0.45	0.31	0.41	0.33	0.35
Mn at 150 ppm		0.81	0.89	0.79	0.83	0.38	0.52	0.44	0.45	0.28	0.34	0.29	0.30
Mean		0.72	0.91	0.83	0.82	0.37	0.54	0.43	0.45	0.26	0.36	0.30	0.31
L.S.D at 0.05 for	**Amino acids**	0.042				0.034				0.012			
	micronutrients	0.048				0.039				0.013			
	interaction	0.084				0.068				0.024			
		Second season (2012)											
Control (0.0)		0.56	0.89	0.72	0.72	0.28	0.51	0.46	0.42	0.18	0.32	0.28	0.26
Fe at 150 ppm		0.70	0.94	0.84	0.83	0.42	0.64	0.55	0.54	0.28	0.36	0.28	0.31
Zn at 150 ppm		0.68	0.89	0.73	0.76	0.38	0.56	0.52	0.49	0.32	0.39	0.35	0.35
Mn at 150 ppm		0.66	0.92	0.81	0.79	0.39	0.58	0.48	0.48	0.29	0.34	0.29	0.31
Mean		0.65	0.91	0.78	0.78	0.37	0.57	0.50	0.48	0.27	0.35	0.30	0.31
L.S.D at 0.05 for	**Amino acids**	0.033				0.027				0.014			
	Micronutrients	0.038				0.030				0.016			
	Interaction	0.066				0.54				0.028			

G.= Glutamic acid T.= Tryptophan

Tryptophan (a precursor of indole acetic acid) is known as growth regulating factor that influence many biological processes. Phillips (1971) suggested several alternative roles of IAA synthesis in plants, all starting from tryptophan, thus when tryptophan is supplied to most plant tissues, IAA was formed. Glutamic acid connect the two important metabolic cycles of the plant, the carbon and nitrogen cycles, and has an influence on sugars and on proteins. The tryptophan inhibits the precocious flower and fruit fall and it is important in the process of production of enzyme that catalyses synthesis reaction of auxin. The glutamic acid is important for the synthesis of the auxin and fruit set (Taiz and Zeiger, 2002).

For adequate plant growth and production, micronutrients are needed in small quantities in balance of macronutrients; however, their deficiencies cause a great disturbance in the physiological and metabolic processes in the plant. Plants normally take up nutrients from soils through their roots although nutrients can be supplied to plants as fertilizers by foliar sprays (Baloch *et al.,* 2008). Fe, Zn and Mn acts either as metal components of various enzymes or as functional, structural, or regulatory cofactors. Thus, it is associated with saccharide metabolism, photosynthesis, and protein synthesis (Marschner, 1997). The effects of the tested micro-nutrients may be due to the role of iron as it incorporated directly into the cytochromes, into compounds necessary to the electron transport system in mitochondria and into ferredaxin. Ferredaxin is indispensable to the light reactions of photosynthesis. Iron is essential for the synthesis of chlorophyll and it plays an essential chemical role in both the synthesis and degradation of chlorophyll (Nason and Mc-Elory, 1963). Fe is a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions. Hence, iron has many essential roles in plant growth and development including chlorophyll synthesis and chloroplast development. Additionally, iron has important functions in plant metabolism, such as activating catalase enzymes associated with superoxide dismutase, as well as in photorespiration, the glycolate pathway and chlorophyll content (Granick, 1950). Moreover, Zinc is one of the essential microelements for growth and flowering of plants (Chandler, 1982). Zinc is an important micronutrient that is closely involved in the methabolism of RNA and ribosomal content in plant cells, leading to stimulation of carbohydrates, proteins and the DNA formation. It is also, required for the synthesis of tryptophan, a precursor of IAA which acts as a growth promoting substance. Zinc has three functions: catalytic, cocatalytic (coactive) and structural (Amberger, 1974). Therefore, sufficient amount of these nutrients in the plant is necessary for normal growth, in order to obtain satisfactory yield (Yassen *et al.,* 2010). So, micronutrients such as iron, zinc and manganese have important roles in growth and chemical composition of *Echinacea purpurea* plant.

Conclusively, from the obtained results, it is preferable to spray *Echinacea purpurea* plants with amino acids (tryptophan or glutamic acid at 200ppm) in combination with some micro-nutrients (Fe, Zn or Mn each at 150ppm) to enhance plant growth and chemical composition which led finally to a product of high quality suitable for exportation and safe on human health.

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تأثير بعض معاملات الأحماض الأمينية والعناصر الصغري على النمو والمحتوي الكيماوي لنبات الأيشنيسيا

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أجريت تجربه حقلية لمدة عامين خلال موسمي ٢٠١١ & ٢٠١٢ بمزرعة الزينة بقسم البساتين – كلية الزراعة- جامعة بنها. بهدف دراسة تأثير بعض معاملات الأحماض الأمينية (التربتوفان والجلوتاميك بتركيز ٢٠٠ جزء في المليون لكل منهما) منفرده والعناصر الصغري (حديد- زنك- منجنيز بتركيز ١٥٠ جزء في المليون لكل منهما) منفرده، وايضا التفاعل بين معاملات الأحماض الأمينية والعناصر الصغري على النمو الخضري والمحتوى الكيماوي لنبات الأيشنيسيا. أوضحت النتائج أن المعاملات المختبره من الأحماض الأمينيه أو العناصر الصغري أدت إلي زيادة معنوية في قياسات النمو الخضري (طول النبات ، عدد الأفرع/ نبات، الوزن الطازج والجاف للعشب/ نبات وعدد الخلفات / نبات). وأعطت جميع المعاملات المختبره زيادة كبيرة في النمو مصحوبه بزيادة في نسبة محتوى الأوراق من الأحماض الأمينيه الحره الكليه، الحديد ، الزنك والمنجنيز . وبصفه عامه أعطت المعامله بالتربتوفان بتركيز ٢٠٠ جزء في المليون المدعمه بالرش بالزنك بتركيز ١٥٠ جزء في المليون أكبر طول للنبات ،أكبر عدد ووزن طازج وجاف للأزهار في حين تم الحصول علي أكبر عدد من الأفرع والخلفات للنبات بمعامله النبات بالجلوتاميك بتركيز ٢٠٠ جزء في المليون مدعمه بالحديد بتركيز ١٥٠ جزء في المليون في كلا الموسمين. بالأضافه الي ذلك أدي الرش بالجلوتاميك بتركيز ٢٠٠ جزء في المليون مدعما بالرش بالزنك بتركيز ١٥٠ جزء في المليون الي انتاج أكبر وزن طازج وجاف للعشب (أوراق وسيقان). قد أدت جميع معاملات الأحماض الأمينيه والعناصر الصغري المختبره وتفاعلاتهم الي زياده محتوي النبات من مشتقات حامض الكافيك الكليه (في الجذور والأوراق والأزهار) والألكاميدات الكليه (للعشب) وخاصه المعامله المختلطه بين الجلوتاميك والحديد أو بين الجلوتاميك والزنك .

وبناءا على النتائج المتحصل عليها فانه يفضل الرش بالتربتوفان أوالجلوتاميك بتركيز ٢٠٠ جزء في المليون مدعما بالحديد أوالزنك بتركيز١٥٠ جزء في المليون لتحسين النمو والمحتوى الكيماوي لنبات الأيشنيسيا.

قام بتحكيم البحث

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