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Effect of Some Fertilization and Micro-Nutrients Treatments on Growth and Chemical Constituents of *Echinacea purpurea* plant

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ABSTRACT

Two field experiments were carried out at special Farm at El Khatatba region Minofia Governorate , Egypt and in the Laboratory of Horticulture Department, Faculty of Agriculture at Moshtohor, Benha Univ., during 2014 and 2015 seasons to evaluate the effect of some fertilization treatments i.e. control (compost at 10 ton/fed.),100 % full chemical fertilizer dose, 75% chemical fertilizer (NPK) dose + compost at 10 ton/fed. + bio fertilizer (nitrobein + phosphorein) , 50% chemical fertilizer (NPK) dose + organic fertilizer (compost) + bio fertilizer (nitrobein + phosphorein) and 25% chemical fertilizer (NPK) dose + organic fertilizer (compost) + bio fertilizer(nitrobein + phosphorein) and some micro-nutrients i.e. Zn and B each at 100 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 tested treatments of fertilization and micro-nutrients treatments as well as their combination 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 suckers/plant, number of flowering heads/plant, fresh and dry weights of flowering heads/plant as compared with control plants in both seasons. Additionally, the enhanced growth of *Echinacea purpurea* plants due to the different treatments was accompanied by pronounced increase in leaves N, P and K content of treated plants in both seasons. Furthermore, total caffeic acid derivatives and total alkamides were increased by all fertilization and micro-nutrients treatments as well as their combinations, especially the combined treatment between 75% chemical fertilizer dose + compost at 10 ton/fed. + bio fertilizer and Zn or B each at 100 ppm. Conclusively, from the obtained results, it is preferable to fertilize *Echinacea purpurea* plants with 75% chemical fertilizer (NPK) + 10 ton compost/fed. + bio fertilizer(nitrobein + phosphorein) in combination with some micro-nutrients (Zn or B each at 100 ppm) as foliar spray to enhance plant growth and chemical composition which led finally to a safe product of high quality suitable for exportation and safe on human health

Keywords. *Echinacea purpurea*, chemical, organic and bio fertilization, micro-nutrients, growth , total caffeic acid derivatives and alkamides contents.

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INTRODUCTION

Medicinal and aromatic plants are use by 80% of global population for their medicinal therapeutic effects as reported by WHO (2008). Many of these plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Others contain alkaloids, glycosides, saponins and many secondary metabolites (Naguib, 2011)

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. Echinacea roots 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).

Recently, unconventional efforts are used to minimize the amounts of chemical fertilizers which applied to medicinal and aromatic plants in order to reduce production cost and environmental pollution without reduction of yield. Therefore, the trend now is using the bio and organic fertilizers. Bio-fertilizers are reasonably safer to the environment than chemical fertilizers and play an important role in decreasing the use of chemical fertilizers. Consequently, it causes a reduction in environmental pollution. Bio fertilizers are microbial inoculants consisting of living cells of micro-organism like bacteria, algae and fungi alone or in combination which may help in increasing crop productivity. Bio fertilizers can influence plant growth directly through the production of phytohormones such as gibberellins, cytokinins and IAA that act as growth regulators and indirectly through nitrogen fixation and production of bio-control agents against soil-borne phytopathogens and consequently increase formation of metabolites which encourage the plant vegetative growth and enhance the meristematic activity of tissues to produce more growth (Glick, 2003 and Ahmed and Kibret, 2014). The effect of bio-fertilizers on vegetative growth, yield and oil productivity in several studied was revealed by Badran and Safwat (2004) on fennel. Ismail (2007) on dragonhead plants, Amran (2013) on *Pelargonium graveolens* plants and El-Khyat (2013) on *Rosmarinus officinalis*.

Organic fertilizers are obtained from animal sources such as animal manure or plant sources like green manure. Continuous usage of inorganic fertilizer affects soil structure. Hence, organic manures can serve as alternative to mineral fertilizers for improving soil structure (Shahram and Ordookhani, 2011) and microbial biomass (Suresh *et al.* 2004). The addition of organic fertilizers to agricultural soils has beneficial effects on crop development and yields by improving soil physical and biological properties (Zheljazkov and Warman, 2004). Organic and bio fertilizers in comparison of the chemical fertilizers have lower nutrient content and are slow release but they are as effective as chemical fertilizers over longer periods of use (Naguib, 2011 and Mohamed *et al*., 2012).

Micro-nutrients, especially Zn and B 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; i.e. El-Khyat (2013) on *Rosmarinus officinalis* and Amran (2013) on *Pelargonium graveolens.* They indicated that foliar application of Fe, Zn and Mn improved the growth and chemical composition of the plants. Also, **Gomaa and Mady (2008)** found that boron foliar spray at 75 ppm increased growth parameter and oil productivity of chamomile plants. Therefore the purpose of this study was to evaluate the benefits of supplementing organic and bio fertilizers with chemical fertilizer in presence of Zn and B on growth and chemical composition of *Echinacea purpurea* plants and to minimize consuming of chemical fertilizers.

MATERIALS AND METHODS

Two field experiments were carried out at Special Farm at El Khatatba region Minofia Governorate , Egypt and in the Laboratory of Horticulture Department, Faculty of Agriculture at Moshtohor, Benha Univ., during 2014 and 2015 seasons to evaluate the effect of some fertilization treatments and some micro-nutrients i.e. Zn and B each at 100 ppm as well as their combinations on growth and chemical constituents of *Echinacea purpurea* plants.

Echinacea purpurea seedlings (7-9 height with 4-5 leaves) were obtained from Floriculture Farm, Horticulture Department, Faculty of Agriculture, Benha Univ. The seedlings were transplanted in sandy soil on mid February of both seasons in beds (1x1 m) containing two rows (50 cm inbetween) and each row contained two hills (50 cm apart). The 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.

Physical and chemical analyses of the used soil are presented in Table (a). Mechanical and chemical soil analyses were determined according to Black *et al.*, (1982).

Table a: Mechanical and chemical analyses of the used soil.

Physical analysis		Chemical analysis			
		Cations (meq/l)		Anions (meq/l)	
Coarse sand	56.2%	Ca^{++}	1.34	CO_3^{--}	Zero
Fine sand	29.8%	Mg^{++}	0.81	HCO_3^-	2.23
Silt	6.2%	Na^+	1.94	Cl^-	1.48
Clay	7.8 %	K^+	0.12	SO_4^{--}	0.78
Texture class	Sandy				
		Soil pH	7.98		
		EC	0.43dS m^{-1}		
		Organic matter	0.81 g kg^{-1}		
		Available N	16.2 mg kg^{-1}		
		Available P	6.93 mg kg^{-1}		
		Available K	98 mg kg^{-1}		

Bio-fertilizer treatments

Echinacea purpurea seedlings were inoculated with a mixture of nitrobein + phosphorein contained efficient strains of nitrogen fixing bacteria namely, *Azotobacter chroococcum* + phosphate dissolving bacteria (*Bacillus megaterium* var phosphaticum) which supplied by the Department of Microbiology, Agric. Res. Center, Giza was used in this study as biological activators. The strains were characterized by a good ability to infect its specific host plant and by its high efficiency in N-fixation and phosphate solublizing. The roots of *Echinacea purpurea* seedlings were washed with water, thereafter the roots were soaked in cell suspension of the mixture of nitrobein and phosphorein (1ml contains 10^8 viable cell) for 30 min. Gum arabic (16 %) was added as an adhesive agent prior to soaking the roots. The inoculated roots were air dried at room temperature for one hour before planting. Another two applications were applied (1kg/fed) as an aqueous solution, the first one was applied just before irrigation after 60 days from planting date, whereas the second one was done after 90 days from planting date to increase the power ability of bacteria.

Organic fertilizer treatments

Organic fertilizer i.e. compost containing plant sources and cattle manure at the rate of 10 ton/fed., was thoroughly mixed with the soil before planting, the chemical properties of the tested compost are presented in Table (b).

Table (b): Chemical properties of the used compost:

Parameters	Ec $dS.m^{-1}$ (1:5)	pH (1:5)	Total C %	Total N %	Total P %	Total K %	Total Fe (ppm)	Total Zn (ppm)	C:N ratio
Values	2.81	6.69	24.21	1.34	0.77	1.62	1368	347	18:1

Chemical fertilizer treatments

The plants were fertilized with full chemical fertilizer dose as a recommended dose; where ammonium nitrate (33.5% N) was added at the rate of 100Kg N/fed., calcium superphosphate (15.5 % P_2O_5) was added at the rate of 150 Kg and potassium sulphate (48.5 % K_2O) at the rate of 150 Kg /fed. The amount of N and K fertilizers were divided into six equal portions as side dressing at 30, 45, 60, 75, 90 and 110 days after planting date of both seasons. However, the amount of P-fertilizer was added to the soil before seedlings plantins during soil preparation.

Micro-nutrients treatments

In the two seasons, the foliage was sprayed four times during the growth period with Zn and B each at concentration of 100 ppm at 60, 90, 120 and 150 days after planting date of both seasons. A surfactant (Tween 20) at a concentration of 0.01% was added to all tested solution treatments including the control.

Experiment layout

This experiment was set up in a split plot design with three replicates. The main plot was employed by five fertilization treatments i.e. control (compost at 10 ton/fed,(T1)), full chemical fertilizer dose (T2), 75% chemical fertilizer dose + compost at 10 ton/fed. + bio fertilizer (nitrobein + phosphorein) (T3), 50% chemical fertilizer dose + organic fertilizer (compost) + bio fertilizer (T4) and 25% chemical fertilizer dose + organic fertilizer + bio fertilizer (T5). Whereas, the sub plot was devoted to three micro-nutrients sprays i.e. control (tap water), Zn at 100 ppm and B at100 ppm.

Data recorded

In both seasons, at harvest time (Mid - September) 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, Total nitrogen, phosphorus and potassium were determined in *Echinacea purpurea* leaves at the flowering stage, according the methods described by Horneck and Miller (1998), Sandell (1950) and Horneck and Hanson (1998), respectively.

Total alkamides were quantitatively determined in *Echinacea purpurea* aerial parts (flowering heads, leaves and stems) in the second season (2015) 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:

The obtained data in both seasons of study were subjected to analysis of variance as a factorial experiment in split plot design. L.S.D. method was used to differentiate between means according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Effect of some fertilization and micro-nutrients treatments as well as their combination on growth and chemical composition of *Echinacea purpurea* plant.

Vegetative growth parameters:

Plant height (cm)

Data presented in Table (1) indicated that all tested fertilization treatments significantly increased the plant height of *Echinacea purpurea* as compared with control in both seasons, with superior for T2 treatment (100% chemical fertilizer), followed by T3 treatment (75% chemical fertuilizer+10 ton compost/fed+bio) in the two seasons. Moreover, all tested treatments of micro-nutrients i.e., Zn and B each at 100 ppm succeeded in increasing plant height, especially Zn treatment as compared with control in the two seasons. However, most tested combinations of fertrilization and micro-nutrients induced significant increments in plant height in the first and second seasons as compared with control. However, the highest value of plant height (91.3 and 82.4cm) were scored by the combined treatment of T2 and Zn at 100 ppm in the first and second seasons, respectively.

Number of branches /plant

Table (1) reveals that number of branches /plant was increased due to fertilization treatments as compared with control in the two seasons. However, the highest number of branches per plant was registered by T3, followed by T2 treatment, with non significant deferencess between them in both seasons. Moreover, all tested applications of micro-nutrients showed significant increaments in this regard. Anyway, the highest number of branchs/plant was gained by B at 100 ppm in both seasons as compared with control. Regarding the interaction effect between fertilization and micro-nutrients, it was found that all combinations of fertilization and micro-nutrients led to increase the number of branches per plant in both seasons. However, the highest number of branches per plant (9.16 and 8.61) was scored by the interaction between T3 and B at 100 ppm in the first and second seasons, respectively.

Fresh and dry weights of herb (g) / plant.

Table (2) shows that fresh and dry weights of herb per plant were significantly increased by all teted fertilizations and micro-nutrients treatments in both seasons. However, T3 treatment succeeded in producing the heaviest fresh and dry weights of herb per plant in both seasons. Moreover, all treatments of micro-nutrients statistically increased the fresh and dry weights of herb per plant, especially the treatment of B at 100 ppm in the two seasons. As for the interaction effect between fertilization and micro-nutrients, it was observed that all resulted combinations of fertilization and micro-nutrients increased the fresh and dry weights of herb per plant as compared with control in both seasons. In all, the heaviest fresh and dry weights of herb per plant in both seasons were recoreded by the combined treatment between T3 and B at 100 ppm in both seasons.

Table 1: Effect of some fertilization and micro-nutrients treatments on plant height number of branches /plant of *Echinacea purpurea* plants during 2014 and 2015 seasons.

Parameters / Micro-nutrients / Fertilization	Plant height (cm) Control	Zn at 100 ppm	B at 100 ppm	Mean	Number of branches /plant Control	Zn at 100 ppm	B at 100 ppm	Mean
First season (2014)								
T1: Control(compost at 10 ton/fed.)	68.3	74.6	71.9	71.6	5.64	6.26	7.39	6.43
T2: (chemical NPK)	86.2	91.3	89.3	88.9	7.84	8.16	8.94	8.31
T3:(75%NPK+ 10 ton compost/fed.)+bio	84.9	89.4	88.1	87.5	7.89	8.24	9.16	8.43
T4:(50%NPK+ 10 ton compost/fed.))+bio	79.8	84.1	81.7	81.9	7.16	8.16	8.43	7.91
T5:(25%NPK+ 10 ton compost/fed.))+bio	74.6	79.5	76.4	76.8	6.88	7.83	7.94	7.55
Mean	78.8	83. 8	81.5		7.08	7.73	8.37	
L.S.D at 0.05 for fertilization		3.12				0.32		
micronutrients		2.74				0.29		
interaction		6.61				0.69		
Second season (2015)								
T1: Control (compost at 10 ton/fed.)	65.6	68.9	67.1	67.2	5.14	6.72	6.94	6.26
T2: (chemical NPK)	78.4	82.4	81.0	80.6	8.12	8.24	8.36	8.24
T3:(75%NPK+ 10 ton compost/fed.)+bio	76.7	81.2	78.3	78.7	8.26	8.41	8.61	8.42
T4:(50%NPK+ 10 ton compost/fed.))+bio	73.1	76.3	74.9	74.8	7.64	7.91	8.21	7.92
T5:(25%NPK+ 10 ton compost/fed.))+bio	69.8	72.4	71.3	71.2	7.20	7.31	7.61	7.37
Mean	72.7	76.2	74.5		7.27	7.71	7.94	
L.S.D at 0.05 for fertilization		2.94				0.31		
micronutrients		2.63				0.27		
interaction		6.32				0.72		

Table 2: Effect of some fertilization and micro-nutrients treatments on fresh weight of herb/plant (g) and dry weight of herb/plant (g) of *Echinacea purpurea* plants during 2014 and 2015 seasons.

Parameters / Micro-nutrients / Fertilization	Fresh weight of herb/plant (g) Control	Zn at 100 ppm	B at 100 ppm	Mean	Dry weight of herb/plant (g) Control	Zn at 100 ppm	B at 100 ppm	Mean
First season (2014)								
T1: Control (compost at 10 ton/fed.)	841	992	1184	1006	92.5	109.0	131.0	110.8
T2: (chemical NPK)	1248	1296	1468	1337	149.6	155.5	176.2	160.4
T3:(75%NPK+ 10 ton compost/fed.)+bio	1262	1312	1483	1352	151.4	157.4	177.9	162.2
T4:(50%NPK+ 10 ton compost/fed.))+bio	1152	1296	1365	1271	132.4	149.0	156.9	146.1
T5:(25%NPK+ 10 ton compost/fed.))+bio	1088	1251	1286	1208	121.8	140.1	144.0	135.3
Mean	1118	1230	1357		129.5	142.2	157.2	
L.S.D at 0.05 for fertilization		84.6				24.6		
Micro-nutrients		74.7				21.6		
interaction		178.5				51.9		
Second season (2015)								
T1: Control (compost at 10 ton/fed.)	719	1072	1124	972	79.1	117.9	123.6	106.9
T2: (chemical NPK)	1218	1312	1354	1295	146.1	157.4	162.4	155.3
T3:(75%NPK+ 10 ton compost/fed.)+bio	1229	1344	1361	1311	147.4	161.2	163.2	157.3
T4:(50%NPK+ 10 ton compost/fed.))+bio	1130	1264	1328	1241	129.9	145.3	152.7	142.6
T5:(25%NPK+ 10 ton compost/fed.))+bio	1071	1168	1231	1157	119.9	130.8	137.8	129.5
Mean	1073	1232	1280		124.5	142.5	147.9	
L.S.D at 0.05 for fertilization		73.1				18.9		
Micro-nutrients		64.3				16.6		
interaction		154.3				39.8		

Number of suckers/plant

Table (3) declares that all tested fertilization treatments succeded in increasing number of suckers/plant as compared with control in both seasons. Moreover, all micro-nutrients treatments significantly increased number of suckers per plant, particularly B treatment in the two seasons. As for the interaction effect between fertilization and micro-nutrients treatments, it was observed that all interactions of fertilization and micro-nutrients treatments increased the number of suckers per plant as compared with control in both seasons. In general, the highest number of suckers/plant (8.43 and 8.62) were recoreded by the combined treatment between T3 and B at 100 ppm in the first and second seasons, respectively.

The aforementioned results of tested fertilization treatment are in agreement with those obtained by Kandeel (2004) on *Ocimum basilicum*, Niakan *et al.* (2004) on *Mentha piperita*, El-Maadawy (2007) on *Amaranthus tricolor*, El-Maadawy and Moursy (2007) on jojoba, Gomaa and Youssef (2007a) on fennel, Badran *et al.* (2007) on cumin, Gomaa and Youssef (2007b) on lovage, El-Shora (2009) on *Mentha piperita*, Abou El-Ghait *et al.* (2012) on indian fennel, Gendy *et al.* (2012) on roselle plants, Gendy *et al.* (2013) on guar plants, Mohamed *et al.* (2012) on *Stevia rebaudiana*, Amran (2013) on *Pelargonium graveolens* and El-Khyat (2013) on *Rosmarinus officinalis*. Whereas, 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.*

Flowering growth parameters:

Tables (3) and (4) illustrate that all tested fertilization treatments resulted in significant increments in number, fresh and dry weights of flowering heads/plant, with superiority for T3 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 B treatment in both seasons.

As for the interaction effect between fertilization and micro-nutrients, it was found that all resulted interactions of fertilization 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 (23.60 and 25.30), the heaviest fresh weight of flowering heads/plant (542.8 and 581.7 g) and the heaviest dry weight of flowering heads/plant (74.2 and 79.1 g) were recorded by T3 treatment combined with B at 100 ppm, in the first and second seasons, respectively.

The aforementioned results of fertilization treatment are in parallel with those obtained by El-Maadawy (2007) on *Amaranthus tricolor*, El-Maadawy and Moursy (2007) on jojoba, Gomaa and Youssef (2007a) on fennel, Badran *et al.* (2007) on cumin, Gomaa and Youssef (2007b) on lovage, Abou El-Ghait *et al.* (2012) on indian fennel, Gendy *et al.* (2012) on roselle plants, Gendy *et al.* (2013) on guar plants, Mohamed *et al.* (2012) on *Stevia rebaudiana* and Amran (2013) on *Pelargonium graveolens*. While, 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.

Table 3: Effect of some fertilization and micro-nutrients treatments on number of suckers and flowering heads / plant of *Echinacea purpurea* plants during 2014 and 2015 seasons.

Parameters / Micro-nutrients / Fertilization	Number of suckers/plant: Control	Zn at 100 ppm	B at 100 ppm	Mean	Number of flowering heads/plant: Control	Zn at 100 ppm	B at 100 ppm	Mean
First season (2014)								
T1: Control (compost at 10 ton/fed.)	4.21	4.62	4.92	4.58	11.24	12.31	15.17	12.90
T2: (chemical NPK)	7.26	7.84	8.39	7.83	18.17	19.26	22.92	20.13
T3:(75%NPK+ 10 ton compost/fed.)+bio	7.39	7.89	8.43	7.90	18.29	19.21	23.60	20.36
T4:(50%NPK+ 10 ton compost/fed.))+bio	5.36	6.14	6.81	6.10	15.36	17.31	20.11	17.59
T5:(25%NPK+ 10 ton compost/fed.))+bio	4.94	5.26	5.84	5.34	13.98	15.62	18.21	15.93
Mean	5.83	6.35	6.87		15.40	16.74	20.00	
L.S.D at 0.05 for fertilization		0.45				1.23		
L.S.D at 0.05 for Micro-nutrients		0.39				1.08		
L.S.D at 0.05 for interaction		0.94				2.59		
Second season (2015)								
T1: Control (compost at 10 ton/fed.)	4.82	5.04	5.86	5.24	13.46	14.50	16.94	14.97
T2: (chemical NPK)	7.84	8.19	8.57	8.20	19.46	21.27	24.91	21.88
T3:(75%NPK+10 ton compost/fed.)+bio	7.94	8.23	8.62	8.26	19.36	21.36	25.30	22.00
T4:(50%NPK+10 ton compost/fed.))+bio	6.92	6.98	7.30	7.06	17.21	19.24	21.46	19.30
T5:(25%NPK+10 ton compost/fed.))+bio	5.64	5.93	6.74	6.10	15.92	17.21	19.20	17.44
Mean	6.63	6.87	7.41		17.08	18.72	21.56	
L.S.D at 0.05 for fertilization		0.34				1.44		
L.S.D at 0.05 for Micro-nutrients		0.29				1.26		
L.S.D at 0.05 for interaction		0.71				3.00		

Table 4: Effect of some fertilization and micro-nutrients treatments on fresh and dry weights of flowering heads of *Echinacea purpurea* plants during 2014 and 2015 seasons.

Parameters / Micro-nutrients / Fertilization	Fresh weight of flowering heads/plant (g) Control	Zn at 100 ppm	B at 100 ppm	Mean	Dry weight of flowering heads/plant (g) Control	Zn at 100 ppm	B at 100 ppm	Mean
First season (2014)								
T1: Control (compost at 10 ton/fed.)	224.2	258.3	319.3	267.3	29.1	34.1	43.1	35.4
T2: (chemical NPK)	380.6	422.1	503.6	435.4	51.3	56.9	67.9	58.7
T3:(75%NPK+ 10 ton compost/fed.)+bio	399.7	416	542.8	452.8	54.6	56.9	74.2	61.9
T4:(50%NPK+ 10 ton compost/fed.))+bio	321.4	363.9	442.3	375.9	43.1	48.6	59.2	50.3
T5:(25%NPK+ 10 ton compost/fed.))+bio	280.7	312.8	401.7	331.7	37.2	41.5	53.3	44.0
Mean	321.3	354.6	441.9		43.1	47.6	59.5	
L.S.D at 0.05 for fertilization		25.3				8.6		
L.S.D at 0.05 for Micro-nutrients		22.2				7.5		
L.S.D at 0.05 for interaction		53.4				18.1		
Second season (2015)								
T1: Control (compost at 10 ton/fed.)	281.6	319.4	372.7	324.6	36.8	41.7	48.7	42.4
T2: (chemical NPK)	426.3	466.8	572.6	488.6	57.5	62.9	77.2	65.9
T3:(75%NPK+ 10 ton compost/fed.)+bio	443.4	489.4	581.7	504.8	60.2	66.5	79.1	68.6
T4:(50%NPK+ 10 ton compost/fed.))+bio	378.0	422.3	471.5	423.9	50.6	56.4	63.1	56.7
T5:(25%NPK+ 10 ton compost/fed.))+bio	349.7	378.5	422.6	383.6	46.1	49.8	55.7	50.5
Mean	375.8	415.3	484.2		50.2	55.5	64.8	
L.S.D at 0.05 for fertilization		38.4				6.2		
L.S.D at 0.05 for Micro-nutrients		33.8				5.4		
L.S.D at 0.05 for interaction		81.1				13.1		

Leaf chemical composition:

Table (5) declares that all tested treatments fertilization and micro-nutrients increased leaf N, P and K content of *Echinacea purpurea* leaves in both seasons. In this concern, the richest leaf N, P and K content ws recorded by T2 and B at 100 ppm treatments as well as their combinations in both seasons as compared with control and the rest treatments. This may be due to the combined effects of both fertilization treatments which induces cell division and enlargement, in addition to the effects of the tested micro-nutrients which supply the plant with the required nutrients necessary for healthy growth.

Total alkamides (%)

Table (6) demonstrates that all tested fertilization and micro-nutrients treatments 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 (0.082 %) was recorded by T3 treatment combined with Zn at 100 ppm, followed by the combined with treatment between T3 and B at 100 ppm treatments as it gave 0.079 against to 0.034% for control.

Total caffeic acid derivatives (%)

It is clear from Table (7) that the flowering heads of *Echinacea purpurea* were the richest plant part with total caffeic acid derivatives as they contained 0.75 and 0.83 % , followed by the herb (0.43 and 0.50 %), then the roots (0.29 and 0.31%) in the first and second seasons, respectively. However, T3 treatment showed to be the most effective one in inducing the highest values of total caffeic acid derivatives of *Echinaceae purpurea* plants (flowering heads, herb and roots), in both seasons. Also, all tested micro-nutrients sprays increased total caffeic acid derivatives,especially Zn at 100 ppm in case of flowering heads, herb parts and root parts in both seasons. In general the highest values of total caffeic acids derivatives in the flowering heads (0.92 and 0.94 %), in the herb (0.59 and 0.64%) and in the roots (0.43 and 0.41%) were recorded by the combined treatment between T3 treatment and Zn at 100 ppm , in the first and second seasons, respectively**.**

Table 5: Effect of some fertilization and micro-nutrients treatments on leaf nitrogen , phosphorus and potassium contents of *Echinacea purpurea* plants during 2014 and 2015 seasons.

Parameters Micro-nutrients Fertilization	N% Con.	N% Zn at 100 ppm	N% B at 100 ppm	N% Mean	P% Con.	P% Zn at 100 ppm	P% B at 100 ppm	P% Mean	K% Con.	K% Zn at 100 ppm	K% B at 100 ppm	K% Mean
First season (2014)												
T1: Control (compost at 10 ton/fed.)	1.86	1.92	1.98	1.92	0.216	0.221	0.219	0.219	1.26	1.29	1.34	1.30
T2: (chemical NPK)	2.42	2.49	2.51	2.47	0.237	0.252	0.250	0.246	1.48	1.57	1.68	1.58
T3:(75%NPK+ 10 ton compost/fed.)+bio	2.46	2.41	2.48	2.45	0.234	0.249	0.248	0.244	1.46	1.54	1.64	1.55
T4:(50%NPK+ 10 ton compost/fed.))+bio	2.19	2.34	2.41	2.31	0.229	0.236	0.234	0.233	1.39	1.42	1.53	1.45
T5:(25%NPK+ 10 ton compost/fed.))+bio	1.94	1.99	2.17	2.03	0.221	0.228	0.226	0.225	1.32	1.36	1.42	1.37
Mean	2.17	2.23	2.31		0.227	0.237	0.235		1.38	1.44	1.52	
L.S.D at 0.05 for fertilization		0.14				0.011				0.11		
L.S.D at 0.05 for Micro-nutrients		0.12				0.009				0.09		
L.S.D at 0.05 for interaction		0.29				0.023				0.23		
Second season (2015)												
T1: Control (compost at 10 ton/fed.)	1.79	1.84	1.94	1.86	0.221	0.232	0.229	0.227	1.31	1.33	1.39	1.34
T2: (chemical NPK)	2.30	2.39	2.49	2.39	0.251	0.281	0.273	0.268	1.61	1.64	1.73	1.66
T3:(75%NPK + 10 ton compost/fed.) + bio	2.31	2.36	2.46	2.38	0.253	0.283	0.270	0.269	1.58	1.61	1.71	1.63
T4:(50%NPK + 10 ton compost/fed.))+bio	2.13	2.18	2.21	2.17	0.247	0.261	0.258	0.255	1.47	1.56	1.62	1.55
T5:(25%NPK + 10 ton compost/fed.)) + bio	1.91	1.92	2.08	1.97	0.236	0.254	0.251	0.247	1.39	1.42	1.49	1.43
Mean	2.09	2.14	2.24		0.241	0.262	0.256		1.47	1.51	1.59	
L.S.D at 0.05 for fertilization		0.16				0.012				0.09		
L.S.D at 0.05 for micro-nutrients		0.14				0.010				0.07		
L.S.D at 0.05 for interaction		0.33				0.025				0.18		

Table 6: Effect of some fertilization and micro-nutrients treatments on total alkamides (%) of *Echinacea purpurea* during 2015 season.

Micro-nutrients Fertilization	Control (0.0 ppm)	Zn at 100 ppm	B at 100 ppm
T1: Control (compost at 10 ton/fed.)	0.034	0.046	0.041
T2: (chemical NPK)	0.051	0.071	0.067
T3:(75%NPK+10 ton compost/fed.)+bio	0.058	0.082	0.079
T4:(50%NPK+10 ton compost/fed.)+bio	0.049	0.064	0.062
T5:(25%NPK+10 ton compost/fed.)+bio	0.043	0.053	0.051

Table 7: Effect of some fertilization and micro-nutrients treatments on total caffeic acid derivatives (g/100g dry weight) of *Echinacea purpurea* plants during 2014 and 2015 seasons.

Season / Organ Micronutrients Fertilization	Flowering heads Con.	Flowering heads Zn at 100 ppm	Flowering heads B at 100 ppm	Flowering heads Mean	Herb Con.	Herb Zn at 100 ppm	Herb B at 100 ppm	Herb Mean	Roots Con.	Roots Zn at 100 ppm	Roots B at 100 ppm	Roots Mean
First season (2014)												
T1: Control (compost at 10 ton/fed.)	0.62	0.68	0.65	0.63	0.31	0.39	0.36	0. 35	0.19	0. 25	0.21	0.21
T2: (chemical NPK)	0.71	0.80	0.79	0.76	0.40	0.48	0.44	0.44	0.28	0.32	0.31	0.30
T3:(75%NPK+10 ton compost/fed.)+bio	0.84	0.92	0.88	0.88	0.49	0.59	0.56	0.54	0.34	0.43	0.39	0.38
T4:(50%NPK+10 ton compost/fed.))+bio	0.73	0.81	0.80	0.78	0.41	0.49	0.46	0.45	0.29	0.34	0.32	0.31
T5:(25%NPK+10 ton compost/fed.))+bio	0.69	0.74	0.71	0.71	0.37	0.42	0.40	0.39	0.24	0.29	0.26	0.26
Mean	0.71	0.78	0.76	0.75	0.39	0.47	0.44	0.43	0.26	0.32	0.29	0.29
L.S.D at 0.05 for fertilization		0.022				0.018				0.014		
L.S.D at 0.05 for micro-nutrients		0.019				0.015				0.012		
L.S.D at 0.05 for interaction		0.045				0.041				0.031		
Second season (2015)												
T1: Control (compost at 10 ton/fed.)	0.69	0.78	0.73	0.73	0.36	0.43	0.41	0.40	0.21	0.26	0.23	0.23
T2: (chemical NPK)	0.81	0.89	0.88	0.86	0.48	0.57	0.52	0.52	0.28	0.37	0.33	0.32
T3:(75%NPK+10 ton compost/fed.)+bio	0.84	0.94	0.91	0.89	0.51	0.64	0.61	0.58	0.31	0.41	0.39	0.37
T4:(50%NPK+10 ton compost/fed.))+bio	0.79	0.91	0.88	0.86	0.49	0.58	0.53	0.53	0.29	0.38	0.34	0.33
T5:(25%NPK+10 ton compost/fed.))+bio	0.76	0.86	0.81	0.81	0.42	0.51	0.49	0.47	0.26	0.31	0.28	0.28
Mean	0.77	0.87	0.84	0.83	0.45	0.54	0.51	0.50	0.27	0.34	0.31	0.31
L.S.D at 0.05 for fertilization		0.032				0.021				0.011		
L.S.D at 0.05 for micro-nutrients		0.028				0.018				0.009		
L.S.D at 0.05 for interaction		0.064				0.045				0.025		

The aforementioned results of fertilization concerning chemical constituents are in parallel with those obtained by El-khayat (2001) on roselle plants, Kandeel (2004) on *Ocimum basilicum*, Niakan *et al.* (2004) on *Mentha piperita*, El-Maadawy (2007) on *Amaranthus tricolor*, El-Maadawy and Moursy (2007) on jojoba, Gomaa and Youssef (2007a) on fennel plant, Badran *et al.,* (2007) on cumin plant, Gomaa and Youssef (2007b) on lovage plants, El-Shora (2009) on *Mentha piperita*, Abou El-Ghait *et al.* (2012) on indian fennel plant, Gendy *et al.* (2012) on roselle plants, Mohamed *et al.* (2012) on *Stevia rebaudiana*, 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.*

The obtained results of this study may be due to the role of fertilization and micro-nutrients in growth and development of the plants; where the use of N-fixing bacteria (nitrobein) as a bio-fertilizer product containing nitrogen fixing bacteria, e.g. Azotobacter and Azospirillum was found to have not only the ability to fix nitrogen but also to release certain phytohormones of cytokinins, gibberellins and auxins which could enhance plant growth through absorption of nutrients and so on enhancing photosynthesis process (Hegde *et al.,* 1999). Microorganisms used as bio-fertilizers may affect the integrity of growing plants by one mechanism or more such as nitrogen fixation production of growth promoting substances or organic acids, enhancing nutrients uptake or protection against plant pathogens (Hawaka, 2000) Also, N-fixers synthesize stimulatory compounds such as, gibberellins, cytokinins and IAA. They act as growth regulators, which increased the surface area per unit of root length and were responsible for root hair branching with an eventual increase in the uptake of nutrients from the soil (Sperenat, 1990 and Dadarwal *et al.,* 1997). Besides, the use of Phosphate dissolving bacteria (phosophorein) as a bio-fertilizer product containing very active phoshphate dissolving bactcria has proved its efficiency in enhancing different aspects of growth and development of many plant species including medicinal and aromatic ones. Establishment of a strong root system is related to the level of available phosphate in the soil. Phosphate dissolvers or vesicular arbuscular mycorrhizae and silica bacteria are capable of converting tricalcium phosphate to monocalcium phosphate ready for plant nutrition. Phosphate also increased mineral uptake and water use efficiency (Hawaka, 2000). Moreover, when organic manures (compost) added as fertilizer, it led to decrease soil pH which in turn increasing solubility of nutrients for plant uptake, in some cases organic materials may act as low release fertilizer. Recently, on the way of sustainable agriculture with minimum effects, the use of organic manures (compost or chicken manure, ..etc) as natural soil amendments is recommended to replace the soluble chemical fertilizers. They improve the structure of weak-structured sandy soils and increase their water holding capacity. Also, they improve soil fertility, and stimulate root development, induce active biological conditions and enhancing activities of micro-organisms especially those involved in mineralization (Suresh *et al*., 2004). Furthermore, to interpret and evaluate the effect of chemical fertilization concerned in this study, on augmenting the different tested vegetative growth parameters, yield component parameters and chemical constituents of *Echinacea purpurea* plants. It is important to refer to the physiological roles of nitrogen, phosphorus and potassium in plant growth and development. Such three macronutrient elements are the common elements usually included in fertilizers. Plant supplement with these macronutrients in form of fertilizers is necessary because the soil is usually in deficient of them due to plant removal leaching or they are not readily available for plants. Therefore, such addition of well balanced NPK fertilization quantities insured production of high productivity and chemical constituents of *Echinacea purpurea* plants.

The role of NPK fertilization in promoting vegetative growth characters, enhancing yield component parameters and increasing growth, as well as stimulating the chemical constituents content of *Echinacea purpurea* plants could be explained by recognizing their fundamental involvement in the very large number of enzymatic reaction that depend on NPK fertilization . NPK reflected directly on increasing the content of total carbohydrates, total sugars and total free amino acids as well as NPK % in the leaves were indirectly the cause for enhancing the augmenting of all other vegetative growth traits and chemical constituents of *Echinacea purpurea* plants (Cooke, 1982).

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). Zn and B 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 positive effects of the tested micro-nutrients may be due to the role of Zinc which is consider 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).

Moreover, boron (B) is one of the important micronutrients, which has basic role in stabilizing certain constituents of cell walls structure and function and activity of plasma membrane, enhancement of cell division, tissue differentiation. Thus, boron could be directly associated with cell growth (Goldbach *et al.,* 1990). Also, boron has been involved in metabolism of nucleic acid, carbohydrate, protein, auxin and phenol. Moreover, boron has been role in sugar translocation, nucleic acids synthesis and pollen tube growth. Also, boron plays a key role in higher plants by facilitating the short- and long- distance transport of sugar via the formation of borate- sugar complexes. However, such a proposal is unacceptable because, the prevalent sugar transport in the phloem forms only weak complexes with boron, and in the mechanisms of phloem loading of sucrose boron is not involved (Marschner, 1997, Goldbach and Wimmer, 2007 and Ganie *et al.,* 2013). Besides, more than 90% of the boron is found in cell walls. Its functions are also related to cell wall synthesis, lignifications and maintenance of cell wall structure (Hansch and Mendel, 2009). In addition, Ganie *et al.* (2013) reported that application of boron increase net photosynthetic rate which may be attributed to the increase in chlorophylls content of leaves. Furthermore, application of boron increased the activity of catalase and glutathione reductase, which act as antioxidants thus saving the electron transport mechanism of plant from getting oxidized by free radicals like superoxide radicals, singlet oxygen radicals (Wojcik *et al.,* 2008).

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 zinc and boron have important roles in growth and chemical composition of *Echinacea purpurea* plant.

Conclusively, from the obtained results, it is preferable to fertilize *Echinacea purpurea* plants with 75% of chemical fertilizer (NPK) + 10 ton compost/fed. + bio fertilizer (nitrobein + phosphorein) in combination with some micro-nutrients (Zn or B each at 100 ppm) as foliar spray to enhance plant growth and chemical composition which led finally to a safe product of high quality suitable for exportation and safe on human health.

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تاثير بعض معاملات التسميد ومعاملات التغذية بالعناصر الصغري علي النمو والمحتوي الكيماوي لنبات الاشينيسيا

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اجريت تجربة حقلية بمزرعة خاصة بمنطقة الخطاطبة – محافظه المنوفية – مصر ومعمل قسم البساتين – كلية الزراعة بمشتهر - جامعه بنها لمده عامين خلال موسمي ٢٠١٤ &٢٠١٥بهدف دراسة تاثير بعض معاملات التسميد المختلفه وقد تم استخدام سماد الكمبوست بمعدل ١٠ طن /للفدان كمقارنة وجرعه كامله من السماد المعدني المكون من النتروجين والفوسفور والبوتاسيوم منفرده، (١٠٠%) وثلاث ارباع جرعه من السماد المعدني المكون من النتروجين والفوسفور والبوتاسيوم + التسميد الحيوي المكون من النتروبين + الفوسفورين + الكمبوست بمعدل ١٠ طن / فدان كما تم استخدام نصف جرعه من السماد المعدني المكون من النتروجين والفوسفور والبوتاسيوم + التسميد الحيوي المكون من النتروبين + الفوسفورين + التسميد العضوي (الكمبوست) بالاضافه الي استخدام ربع جرعه من السماد المعدني باستخدام النتروجين والفوسفور والبوتاسيوم +التسميد الحيوي المكون من النتروبين + الفوسفورين +التسميد العضوي .مع استخدام معاملات الرش بالعناصر الصغري ممثله في عنصري الزنك والبورون بمعدل ١٠٠ جزء في المليون والتداخلات المختلفه بينهم وتاثيره علي النمو ومشتقات حامض الكافيك الكليه والالكاميدات الكليه والمحتوي الكيماوى لنباتات الاشينيسيا.وقد اوضحت النتائج ان المعاملات المختلفه من التسميد ومعاملات الرش بالعناصر الصغري بالاضافة الي التداخل بينهم ادت الي زيادة معنوية كبيرة في معاملات النمو المختلفه تحت الدراسه كارتفاع النبات ، عدد الافرع ،والوزن الطازج والجاف للعشب،وعدد الخلفات، وعدد الرؤس الزهرية والوزن الطازج والجاف لها مقارنه بالكنترول في كلا الموسمين بالاضافة الي ذلك فان الزياده في النمو الحادثه والمتحصل عليها لنباتات الاشينيسيا باستخدام المعاملات المختلفة كانت مصحوبه بزياده واضحه في محتوي الاوراق من النتروجين والفوسفور والبوتاسيوم للنباتات المعامله في كلا الموسمين .وعلاوه علي ذلك فان مشتقات حامض الكافيك والالكاميدات الكلية قد زادت زياده واضحه باستخدام معاملات التسميد المختلفة ومعاملات الرش بالعناصر الصغري فضلا عن التداخلات المختلفه بينهم وخاصة عند استخدام معامله التداخل بين ثلاث ارباع جرعه من السماد المعدني باستخدام النتروجين والفوسفور والبوتاسيوم + الكمبوست بمعدل ١٠ طن / فدان + التسميد الحيوي المكون من النتروبين + الفوسفورين والزنك والبورون بتركيز ١٠٠ جزء في المليون.وبناء علي نتائج هذه الدراسة ، فانه يفضل تسميد نباتات الاشينيسيا بثلاث ارباع جرعه من التسميد المعدني النتروجين والفوسفور والبوتاسيوم (٧٥%) + ١٠ طن كمبوست / للفدان + التسميد الحيوي المكون من النتروبين + الفوسفورين ،مدعما بالرش بعنصري الزنك او البورون عند تركيز ١٠٠ جزء في المليون لزياده نمو النبات والمحتوي الكيماوي والذي يؤدي في النهاية الي الحصول علي منتج ءامن ذ ات جوده عالية مناسب للتصدير وامن علي صحة الانسان.