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Effect of Foliar Organic Fertilization on the Growth, Yield and Oil Content of *Mentha piperita* var. *citrata*

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ABSTRACT

This study was carried out to evaluate the response of *Mintha piperita* var. *citrata* (Eau de Cologne mint) to foliar fertilization under Egyptian conditions. This study was carried out on *Mintha piperita* var. *citrata* (Eau de Cologne mint) at Sekem Experimental field. Fresh, healthy, insect and disease free suckers were transplanted in furrow at a depth of 4-5 cm as per the treatments. Three weeks later after transplanting, the plants were sprayed with aqueous solution of the test nutrient compounds humic acid (0. 2.5 and 5 g L^{-1}) and amino spot (0, 1 and 1.5 mL L^{-1}). The crop was harvested in mid-May (First cutting) and mid-August (Second cutting). Growth and yield characters were measure. The essential oil percentage was determined in both cuts from fresh herb. The essential oil was analyzed by GC/Mass. It can be observed that, humic acid and/or amino spot fertilizer (Algae extract) had a significant effect on growth characters during both cuts. Increasing amino spot doses increased growth characters (plant height, herb fresh and dry weight) at all doses in the two cuts. The results show that there were clear significantly positive trend in increasing growth characters by spraying of humic acid. The interaction effect was significant in both cuts, the highest values of plant height, herb fresh and dry weight (g $plant^{-1}$) were produced from the treatment sprayed with humic acid at 5 g L^{-1}+amino spot at 1.5 mL L^{-1}, followed by the treatment sprayed with 2.5 g L^{-1} humic acid+1.5 mL L^{-1} amino spot at the two cuts. During the 1st cut, humic acid or amino spot fertilizer had a significant effect on essential oil percentage and yield (mL $plant^{-1}$) while, all treatments produced significant effect on oil percentage and oil yield (mL $plant^{-1}$) except the interaction treatments, which had no significant effect on essential oil percentage during second cut. Linalool and linalyl acetate were the main constituents of essential oil of this plant. All treatments or cuttings had a pronounced effect on essential oil constituents. Based on the experimental results it is recommended to treat *Mintha piperita* var. *citrata* (Eau de Cologne mint) plants with humic acid at 5 g L^{-1}+amino spot at 1.5 mL L^{-1} to produce high mass production and oil yield.

Key words: *Mintha piperita* var. *citrata*, foliar fertilization, humic acid, amino spot fertilizer and essential oil

INTRODUCTION

Mint is a perennial plant and productive up to 15 years. Primarily, it is grown for oil production from its leaves. The life span of a commercial mint field is 3-5 years and mainly depends on genetic and environmental factors. Generally the growth, development and the quality of aromatic plants are affected by the genetic background, environment and cultivation practices as well as the processing and storage of plant tissues (Clark and Menary, 1980a, b). The concentrations of secondary metabolites such as essential oils in plants result from their continuous formation and breakdown during plant growth. *Mentha piperita* var. *citrata* is a fully hardy deciduous herb. It is best planted in moist but well drained soil and grows to an ultimate height of 0.1-0.5 m in 1-2 years. Eau de Cologne mint, like many other members of this genus, is often used as a domestic herbal remedy, being valued especially for its antiseptic properties and its beneficial effect on the digestion. Like other members of the genus, it is best not used by pregnant women because large doses can cause an abortion. The leaves and flowering plant are anodyne, antiseptic, antispasmodic, carminative, cholagogue, diaphoretic, refrigerant, stomachic, tonic and vasodilator. A tea made from the leaves has traditionally been used in the treatment of fevers, headaches, digestive disorders and various minor ailments. The medicinal uses of this herb are more akin to lavender (*Lavandula* spp.) than the mints. It is used to treat infertility, rapid heartbeat, nervous exhaustion etc. The leaves are harvested as the plant comes into flower and can be dried for later use. The essential oil in the leaves is antiseptic, though it is toxic in large doses. The highest content of essential oil (1%) was obtained at 120 days while, the highest content the majority compounds-fenchol (49.92%) and cis-myrtanol (30.03%) reached its highest value at 120 and 150 days after transplanting, respectively (De Oliveira *et al*., 2012). The total oil accumulation depends on the genetic composition of the plant and it varies between genera and species (Franz *et al*., 1984).

Plant nutrition is one of the most important factors that increase plant production. Nitrogen (N) is the most recognized in plant for its presence in the structure of the protein molecule. Accordingly, N plays an important role in synthesis of the plant constituents through the action of different enzymes (Jones *et al*., 1991). Nitrogen fertilization has been reported to reduce essential oil content in creeping juniper (*Juniperus horizontalis*) (Robert, 1986), although it has been reported to increase total essential oil yield in thyme (*Thymus vulgaris* L.) (Baranauskiene *et al*., 2003). Baranauskiene *et al*. (2003) found that N fertilizer increased herb yield, but essential oil content was not remarkable of thyme (*Thymus vulgaris*). Ashraf *et al*. (2006) showed that N fertilization had a significant increase in the oil content of black cumin (*Nigella sativa* L.) seeds. Phosphorous (P) plays an important role in various metabolic processes. It is a constituent of nucleic acid, phospholipids, the coenzymes, DNA and NADP and most importantly ATP. It activates coenzymes for amino acid production used in protein synthesis and it decomposes carbohydrate production in photosynthesis; as well as involved many other metabolic processes required normal growth, such as photosynthesis, glycolysis, respiration, fatty acid synthesis. Application of 100 kg ha^{-1} P significantly increased the fresh and dry weights in feverfew (*Tanacetum parthenium* L.) Schultz Bip) plant while, all P levels (50, 100 and 150 kg ha^{-1}) as compared with the control significantly enhanced the EO concentration (Saharkhiz and Omidbaigi, 2008). Application of 50 kg P_2O_5 ha^{-1} significantly increased the total dry matter and EO yield in mint (*Mentha longifolia*) plant (Alsafar and Al-Hassan, 2009). The leaf biomass of garden sage (*Salvia officinalis* L.) and EO content increased with adding P fertilizer (Nell *et al*., 2009). Potassium (K) is one of the most important elements for plant nutrition, which content occupies 1-5% of crops' dry matter, it plays a very important role in the growth, yield and quality of crops, K is involved in the activities of over 60 kinds of plant enzymes, potassium affects the metabolism of N and carbohydrates and the synthesis of lipid, starch and protein. Potassium also plays a very important role in substance transportation inside plants (Zahra *et al*., 1984). Adding K fertilizer increased herb, EO and its constituents in rue (*R. graveolens* L.)

(Khalid *et al*., 2007). The highest peppermint (*Mentha piperita*) EO production was in K at 218 mg L^{-1} concentration (Mollafilabi *et al*., 2010). Addition of K at 123 kg ha^{-1} $year^{-1}$ enhanced the total EO yield of palmarosa (*Cymbopogon martini* [Roxb.] Wats. var. Motia burk) plant by 23.7% in comparison to no K application (Singh, 2008). Potassium fertilization increased EO and anethole content of anise (*Pimpinella anisum* L.) plant (Al-Awak, 2010). 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 enzymes, gene expression and redox-homeostasis (Rai, 2002). Talaat and Youssef (2002) found a pronounced increase in vegetative growth of basil plant as a result of lysine and ornithine treatments. However, S-adenosyl-methionine play a role via the methyl group as a donor to produce estragole and t-anethole in cell-free extracts of the bitter fennel plant (Gross *et al*., 2002). Maxwell and Kieber (2004) indicated the link of methionine to the biosynthesis of growth regulating substances, e.g cytokinins, auxins and brassinosteroids in plants. Whereas, the link of tryptophan to the biosynthesis of auxins, the phytoalexin camalexin, phenylpropanoids and other related natural products in plants was recently reported (Tao *et al*., 2008). Humic acid provides numerous benefits to crop production. It has been reported to influence the plant growth both directly and indirectly. The indirect effects of humic acid compounds have been attributed to the improvement of physical, chemical and biological conditions of soil. It breaks up clay and compacted soils, assists in transferring micronutrients from the soil to the plant, enhances water retention, increases seed germination rates, improves nutrient absorption, plant growth and penetration and stimulates the development of microflora populations. The direct effects on plant growth is to the increase the cell chlorophyll content, the acceleration of the respiration process, hormonal growth responses, increasing substances penetration to plant membranes, affect the dry matter production and the uptake of nutrients by plants (Rao *et al*., 1987). Also, humic acid has positive effects on the promotion of root development. It increases the root/shoot ratio as well as the product ion of thin lateral roots of some plants (Tattini *et al*., 1990, 1991).

So far, there is no report on the yield and composition of the essential oil of *Mentha piperita* f. *citrata* plants cultivated under this experimental condition in literature. This study was carried out to evaluate the response of this variety to foliar fertilization under Egyptian conditions especially there is no enough data on this variety.

MATERIALS AND METHODS

The experiment was carried out on *Mintha piperita* var. *citrata* (Eau de Cologne mint) at Sekem experimental field during two successive seasons (2012 and 2013). The soil characteristics and nutrient concentration in soil of experiment (0-30 cm depth) were shown in Table 1. The experiment was laid out in Factorial Randomize Block Design (FRBD) with three replications. Fresh, healthy, insect and disease free suckers were transplanted in furrow at a depth of 4-5 cm as per the

Table 1: Physical and chemical properties of the soil

Texture			Sand (g kg^{-1})				Silt (g kg^{-1})				Clay (g kg^{-1})
Sand			858				118				22.5
Available (mg g^{-2})		Total (mg g^{-2})	Soluble anions (C mol)			Soluble cations (C mol)					
P	K	N	CO_3	HCO_3	CL	SO_4	Na	Mg	Ca	EC (dS m^{-1})	pH
9.25	16	45	-	2.79	5.81	2.50	5.10	1.57	4.03	1.11	8.15

Table 2: Guaranteed analysis and physical data of humic total

Guaranteed analysis	Values
Humic acid (%)	80
Potassium (K_2O) (%)	10-12
Zn , Fe , Mn , etc. (ppm)	100
Physical data	
Appearance	Black powder
pH (%)	9-10
Water solubility (%)	>98

Table 3: Some chemical properties of amino spot

Amino acids	Percentage
Total amino acids	25
K	8
P	5
N	8

treatments. Three weeks later after transplanting, the plants were sprayed with aqueous solution of the test nutrient compounds, humic acid and amino spot. Foliar application was repeated after two weeks from 1st cut. Humic acid which produced by Leili Agrochemistry Co., LTD, China and its properties are shown in Table 2. Amino spot which is produced by Keymand Co. for Fertilizers and Chemical Industries-2nd Industrial Zone-El-Nobarya (Special formula abstracted from the algae which is very rich with-free amino acids and cytokinin+major elements as shown in Table 3).

Treatments which were carried out can be summarized as following:

- Control (Foliar application with water)
- Foliar application with humic acid at 2.5 g L^{-1}
- Foliar application with humic acid at 5 g L^{-1}
- Foliar application with amino spot at 1 cm L^{-1}
- Foliar application with amino spot at 1.5 cm L^{-1}
- Foliar application with humic acid at 2.5 g L^{-1}+amino spot at1 cm L^{-1}
- Foliar application with humic acid at 2.5 g L^{-1}+amino spot at 1.5 cm L^{-1}
- Foliar application with humic acid at 5 g L^{-1}+amino spot at 1 cm L^{-1}
- Foliar application with humic acid at 5 g L^{-1}+amino spot at 1.5 cm L^{-1}

The crop was harvested in mid-May (First cutting) and mid-August (Second cutting). The data on plant growth, fresh and dry herb yield, oil content and oil yield were recorded at the each cutting. The extracted essential oil was dehydrated over anhydrous sodium sulphate and stored at freezer till used for gas chromatography-mass spectrometry (GC-MS) analysis.

Essential oil production: The percentages of volatile oil were determined in the fresh herb using 100 g samples for each cut per plant. Water distillation of the volatile oil according to Guenther (1961).

GC-MS analysis: The GC-Ms analysis of the essential oil samples was carried out in the second season using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: A TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m×0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL min^{-1} and a split ratio of 1:10 using the following temperature program: 40°C for 1 min; rising at 4.0°C min^{-1} to 160°C and held for 6 min; rising at 6°C min^{-1} to 210°C and held for 1 min. The injector and detector were held at 210°C. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were always injected. Mass spectra were obtained by Electron Ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using two different analytical methods: (a) KI, Kovats indices in reference to n-alkanes (C9-C22) and (b) mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

Statistical analysis: Data was combined over the two seasons for statistical analysis. All recorded data was subjected to analysis of variance procedures and treatment means were compared using LSD at 5% described by Snedecor and Chochran (1980).

RESULTS AND DISCUSSION

Growth characters: Concerning variance analysis results in Table 4 and 5, it can be observed that, humic acid and/or amino spot fertilizer had a significant effect on growth characters during both cuts.

Data presented in Table 6 and 7 indicated that humic acid and/or amino spot fertilizer affected growth characters in both cuts.

Increasing amino spot doses increased growth characters (plant height, herb fresh and dry weight) at all doses in the two cuts. These doses had increased growth characters compared to the control treatment. Nitrogen, is the structural component of cell constituents and metabolically active element, helps in maintenance of energy transformation, enzyme action and improves quality of leafy crop. The beneficial effect of phosphorus in influencing herbage yield is due to its role in various metabolic activities of plant which might have resulted in increased herbage yield.

Table 4: Summary of analysis of variances for morphological traits and qualitative and quantitative yield of eau de cologne mint plant (1st cut) mean values of two successive seasons

		Means of squares				
Source	df	Plant height	Herb fresh weight	Herb dry weight	Oil (%)	Oil yield
Replication	2	1.333^{ns}	1.11e-5^{ns}	0.113^{ns}	1.77e-4^{ns}	0.009^{ns}
Humic acid	2	17.65**	50.60**	43.30**	0.003**	0.005^{ns}
Amino spot	2	3.25**	343.13**	106.20**	0.001**	0.017^{ns}
Humic×amino spot	4	16.53**	58.187**	8.55**	1.33e-4^{ns}	0.007^{ns}
Error	16	0.75	0.75	0.611	1.028e-4	0.009
CV (%)		1.744	2.014	3.267	7.299	119.11

ns: Not significant, *Significant, **Moderate significant, ***Highest significant

Table 5: Summary of analysis of variances for morphological traits and qualitative and quantitative yield of eau de cologne mint plant (2nd cut) mean values of two successive seasons

		Means of squares				
Source	df	Plant height	Herb fresh weight	Herb dry weight	Oil (%)	Oil yield
Replication	2	1.00^{ns}	0.111^{ns}	0.111^{ns}	9e-6^{ns}	1.37e-6^{ns}
Humic acid	2	150.70***	1523.6***	629.57**	0.002**	0.006**
Amino spot	2	182.14***	957.92***	877.57**	0.001**	0.006**
Humic×amino spot	4	26.08***	154.76***	88.114**	1.25e-4^{ns}	7.70e-4**
Error	16	0.75	0.8611	0.8611	8.65e-5	4.95e-7
CV (%)		1.24	0.97	1.97	6.341	0.499

ns: Not significant, *Significant, **Moderate significant, ***Highest significant

Table 6: Effect of amino spot fertilizer and humic acid on yield characters and essential oil content of eau de cologne mint plant (1st cut) mean values of two successive seasons

Humic acid and amino spot	Plant height (cm)	Herb fresh weight (g plant^{-1})	Herb dry weight (g plant^{-1})	Oil (%)	Oil yield (mL plant^{-1})
0					
0	45.20	34.34	18.54	0.11	0.038
1	49.60	41.94	20.34	0.13	0.055
1.5	50.00	42.80	25.34	0.13	0.056
Mean value of 0	48.27	39.69	21.41	0.12	0.048
2.5					
0	47.60	35.78	21.34	0.13	0.047
1	49.60	42.38	23.94	0.13	0.055
1.5	51.60	50.38	26.66	0.15	0.076
Mean value of 2.5	49.60	42.85	23.98	0.14	0.060
5					
0	50.00	41.68	23.00	0.14	0.058
1	51.20	42.34	25.06	0.16	0.068
1.5	52.00	55.44	31.06	0.17	0.094
Mean value of 5	51.07	46.48	26.37	0.16	0.074
Mean value of amino spot					
0	47.60	37.27	20.96	0.13	0.047
1	50.13	42.22	23.11	0.14	0.059
1.5	51.00	49.54	27.69	0.15	0.075
F ratio					
Humic acid	4.34*	67.46***	70.88***	24.65***	0.50^{ns}
Amino spot	23.54***	457.50***	173.86***	12.00***	1.92^{ns}
Interaction	22.04***	77.58***	14.00***	1.30^{ns}	0.81^{ns}

ns: Not significant, *Significant, **Moderate significant, ***Highest significant

Table 7: Effect of amino spot fertilizer and humic acid on yield characters and essential oil content of eau de cologne mint plant (2nd cut) mean values of two successive seasons

Humic acid and amino spot	Plant height (cm)	Herb fresh weight (g plant^{-1})	Herb dry weight (g plant^{-1})	Oil (%)	Oil yield (mL plant^{-1})
0					
0	62.80	75.90	35.20	0.13	0.095
1	63.42	81.84	35.94	0.15	0.119
1.5	69.00	84.08	44.94	0.15	0.126
Mean value of 0	64.74	80.72	38.69	0.14	0.113
2.5					
0	68.00	96.88	38.66	0.13	0.126
1	71.2	96.94	46.54	0.13	0.126
1.5	73.20	122.16	56.40	0.16	0.189
Mean value of 2.5	70.80	105.90	47.20	0.14	0.147
5					
0	65.60	85.52	42.86	0.15	0.128
1	72.40	101.36	50.54	0.17	0.167
1.5	81.00	113.14	72.86	0.17	0.192
Mean value of 5	73.00	100.01	55.42	0.16	0.162
Mean value of amino spot					
0	65.47	86.10	38.91	0.14	0.116
1	69.01	93.38	44.34	0.15	0.137
1.5	74.40	106.46	58.07	0.16	0.169
F ratio					
Humic acid	200.9***	1769.35***	731.11***	17.63***	11358.00***
Amino spot	242.85***	1112.42***	1019.12***	14.16***	12827.00***
Interaction	34.776***	179.72***	102.33***	1.45^{ns}	1555.70***

ns: Not significant, *Significant, **Moderate significant, ***Highest significant

Phosphorus, also a structural component of nucleic acid, plays an important role in energy transfer and protein metabolism and potassium helps in osmotic and ionic regulation.

The results in Table 6 and 7 show that plant growth is a function of nutrients supply providing, there were clear significantly positive trend in increasing growth characters by spraying of humic acid. Similar results were reported by Zaghloul *et al*. (2009), they indicated that spraying

Thuja orientalis plants with humic acid increased growth compared with control plants due to the direct effect of humic acid on solubilization and transport of nutrients. These results are in accordance with those obtained by Arancon *et al*. (2004) on marigolds, peppers and number of fruits of strawberries. Chen and Avaid (1990) added that humic substances have a very pronounced influence on the growth of plant roots and enhance root initiation and increased root growth which known root stimulator. Humic acid improve growth of plant foliage and roots. Vaughan (1974) proposed that humic acids may primarily increase root growth by increasing cell elongation or root cell membrane permeability, therefore increased water uptake by increased plant roots and added that it can produce root systems with increased branching and number of fine roots, as a result potentially increase nutrients uptake by increase root surface area (Rauthan and Schnitzer, 1981).

The interaction effect was significant in both cuts, the highest values of plant height, herb fresh and dry weight (g plant^{-1}) were produced from the treatment sprayed with 1% humic acid at 5 g L^{-1}+amino spot at 1.5 mL L^{-1}, followed by the treatment sprayed with 2.5 g L^{-1} humic acid+1.5 mL L^{-1} amino spot at the two cuts.

For performing necessary physiological function to buildup different yield attributes these nutrients, inorganic and organic which have been integrated in present study were possibly responsible for synthesizing necessary enzymes, proteins, energy (ATP and NADP), chlorophyll and other for the translocation of photosynthates and perhaps only because of these factors.

Essential oil production: The ANOVA results indicated that, during the 1st cut, Humic acid or amino spot fertilizer had a significant effect on essential oil percentage and yield (mL plant^{-1}), while all treatments produced significant effect on oil percentage and oil yield (mL plant^{-1}) except the interaction treatments which had no significant effect on essential oil percentage during second cut.

The mean values of essential oils due to amino spot application at 1 and 1.5 mL L^{-1} increased in both cuts. Fertilization might enhance the essential oil biosynthesis processes through its direct or indirect role in plant metabolism resulting in more plant metabolites. These finding are in agreement with those of Omer (1998) and Omer *et al*. (2008), who said that nitrogen fertilizer was effective in increasing essential oil of *Origanum syriacum* and *Ocimum americanum*, respectively. Essential oil percent in mint fresh herb were significantly affected as a result of foliar application with humic acid (Table 6 and 7). Zaghloul *et al*. (2009) reported that humate application lead to increase oil content in *Thuja orientalis*.

From the above mentioned results, it could be concluded that foliar application of humic acid promoted growth and possessed the best oil percentage in mint plant.

Generally, the maximum essential oil content (%) was observed in the fresh herb of plants that sprayed with 5 g L^{-1} humic acid and 1.5 mL L^{-1} in the two cuts.

The oil yield of mint (mL plant^{-1}) was affected by foliar application of humic acid and/or amino spot in both cuts. The higher the dose of amino spot, the higher was the oil yield. The response of volatile oil content to amino spot fertilization might be attributed to de novo meristemic cell metabolism in building dry matter with essential oil production. These results agree with those of Omer (1998) and Omer *et al*. (2008), who found a positive correlation between fertilizer and essential oil content in herbage of *Origanum syriacum* and *Ocimum americanum*, respectively in all cuttings. In addition, spraying mint plants with humic acid caused an increase in the essential oil yield (Table 6 and 7). Generally, the highest essential oil yield (mL plant^{-1}) was obtained from plants sprayed with amino spot at 1.5 mL L^{-1} and humic acid at 5 g L^{-1} in both cuts.

Essential oil constituents: The essential oil composition varies according to cuts and/or different treatments and was characterized by high percentage of oxygenated compounds ranged from 61.07-79.96% in the 1st cut and from 71.43-84.27 in the second one. The components of essential oil in herb for different treatments during two cuts were shown in Table 8 and 9. The identified components ranged from 28-30 in the 1st cut and from 27-30 in the 2nd one representing about 96.14-100% as a result of different treatments during both cuts. L. Linalool was identified as the

Table 8: Essential oil constituents of eau de Cologne mint plant as affected by amino spot fertilizer and humic acid (1st cut of the 2nd season)

Constituents	RT	1	2	3	4	5	6	7	8	9
α-pinene	3.88	0.44	0.71	0.99	1.12	0.88	0.77	1.23	0.98	1.46
Camphene	4.52	-	0.63	0.84	0.94	0.80	0.74	1.11	0.87	1.33
β-pinene	5.24	-	0.65	0.89	0.99	0.77	0.67	1.09	0.84	1.32
Sabinene	5.57	-	0.35	0.48	0.52	0.41	0.36	0.60	0.44	0.75
β-myrcene	6.61	1.18	1.84	1.62	2.13	1.88	1.61	2.61	1.76	2.28
D- limonene	7.31	-	0.79	0.80	1.01	0.83	0.74	1.26	0.89	1.21
Eucalyptol	7.61	5.75	5.25	6.24	7.10	5.77	4.93	7.29	6.22	7.72
Trans-β-ocimene	8.42	2.31	2.36	2.02	2.48	2.14	1.96	3.05	2.22	2.67
γ-terpinene	8.56	-	0.33	0.34	0.38	-	-	0.46	0.35	-
β- ocimene	8.87	1.08	1.79	1.42	1.88	1.60	1.47	2.41	1.59	1.93
α-terpinolene	9.56	-	0.40	-	0.43	0.37	0.34	0.59	0.36	0.45
n-amyl isovalerate	10.46	-	-	-	-	0.31	0.30	-	0.38	0.54
1-octen-3-ol- acetate	12.87	1.26	1.81	1.50	1.56	1.21	1.40	2.05	1.52	1.97
1-octen-3-ol	15.15	6.18	-	-	-	-	-	-	-	-
Dihydroedulan ii	15.85	0.66	0.35	0.44	0.38	0.39	0.54	0.51	-	0.51
p-menthan-3-one,cis	16.19	0.90	-	-	-	-	-	-	-	-
L-linalool	18.19	23.51	19.48	17.24	22.22	21.09	19.44	18.30	20.83	14.68
Linalyl acetate	18.33	22.43	23.37	28.51	17.86	22.43	26.37	12.49	21.98	15.32
(-)-bornyl acetate	18.77	2.10	4.76	4.52	3.70	4.16	4.44	4.54	4.53	5.58
Caryophyllene	18.99	3.52	6.18	6.48	6.24	7.21	6.86	7.02	6.44	7.93
(±) lavandulyl acetate	19.86	2.40	5.12	5.00	5.15	4.50	4.44	4.99	4.71	5.68
Pulegone	20.87	-	-	-	-	-	-	-	-	-
Humulene	21.08	-	0.63	0.68	0.62	0.70	0.76	0.81	0.68	0.87
cis-β-farnesene	21.36	0.67	1.02	1.13	0.92	1.09	1.18	1.19	1.04	1.35
Germacrene-D	22.24	2.16	2.50	2.57	2.53	2.53	2.54	2.81	2.46	2.85
β-fenchyl alcohol	22.53	5.07	5.16	4.16	5.69	4.99	4.76	6.56	5.16	5.86
Bicyclogermacrene	22.92	1.10	1.36	1.38	1.39	1.40	1.39	1.58	1.30	1.55
Neryl acetate	23.26	1.27	1.83	1.33	1.69	1.67	1.56	2.04	1.64	1.92
Carvone	23.59									
Geranyl acetate	24.12	2.23	3.50	2.53	3.30	3.23	3.03	3.77	3.18	3.47
Methyl salicylate	24.78	0.62		0.37						
Nerol	25.45	0.64	0.89	0.69	0.92	0.85	0.81	1.13	0.87	1.01
Trans-3-caren-2-ol	26.31	0.84	1.06	0.90	0.82	0.96	0.95	0.98	0.93	1.09
Geraniol	26.77	1.96	2.59	2.06	2.67	2.55	2.38	3.13	2.54	2.81
(-)-caryophyllene oxide	29.80									
Veridiflorol	32.72	0.84	0.82	0.78	0.82	0.72	0.73	0.90	0.69	0.84
Elemol	32.88	3.71	2.47	2.10	2.57	2.55	2.52	3.48		
(+)-aromadendrene	35.78	0.69								
α-eudesmol	37.90	0.56								
β-eudesmol (CAS)	38.22	0.55								
Total identified compounds		96.63	100.00	99.91	99.94	99.99	99.99	99.98	97.40	96.95
Total hydrocarbon compounds		16.67	27.72	28.12	29.82	29.82	28.25	34.84	28.66	35.88
Total oxygenated compounds		79.96	72.28	71.79	70.12	70.17	71.74	65.14	68.74	61.07
Sesquiterpene hydrocarbon compounds		8.14	11.69	12.24	11.70	12.93	12.73	13.41	11.92	14.55
Sesquiterpene oxygenated compounds		5.66	3.29	2.88	3.39	3.27	3.25	4.38	0.69	0.84

1: Control (Foliar application with water), 2: Foliar application with humic acid at 2.5 g L^{-1}, 3: Foliar application with humic acid at 5 g L^{-1}, 4: Foliar application with amino spot at 1 cm L^{-1}, 5: Foliar application with amino spot at 1.5 cm L^{-1}, 6: Foliar application with humic acid at 2.5 g L^{-1}+amino spot at 1 cm L^{-1}, 7: Foliar application with humic acid at 2.5 g L^{-1}+amino spot at 1.5 cm L^{-1}, 8: Foliar application with humic acid at 5 g L^{-1}+amino spot at 1 cm L–$^{-1}$, 9: Foliar application with humic acid at5 g L^{-1}+amino spot at 1.5 cm L^{-1}

Table 9: Essential oil constituents of eau de Cologne mint plant as affected by amino spot fertilizer and humic acid (2nd cut of the 2nd season)

Constituents	RT	1	2	3	4	5	6	7	8	9
α-Pinene	3.88	0.98	0.62	0.59	0.70	0.90	1.23	0.97	1.03	1.32
Camphene	4.52	1.01	0.69				1.35	0.87	1.07	1.44
β-Pinene	5.24	0.97	0.56	0.58		0.83	1.03	0.89	0.90	1.08
Sabinene	5.57	0.53								0.53
β-Myrcene	6.61	2.16	1.74	1.61	1.23	1.58	1.98	1.88	2.59	2.66
D- Limonene	7.31	1.07	0.90	1.49	0.97	1.10	1.63	1.10	1.40	1.48
Eucalyptol	7.61	6.49	4.80	5.00	2.01	6.31	7.25	5.16	7.33	7.27
Trans-β-Ocimene	8.42	2.48	1.66	1.64	1.35	1.45		1.74	1.83	
γ-Terpinene	8.56									
β-Ocimene	8.87	2.00	1.19	1.29	0.88	1.01		1.25	1.38	
α-Terpinolene	9.56	0.48		0.59		0.80	1.41	0.72	0.80	0.83
n-Amyl isovalerate	10.46	0.51					0.57			
1-Octen-3-ol- acetate	12.87	1.70	1.19	1.22		1.71	2.20	1.98	1.90	2.16
1-Octen-3-ol	15.15		0.60		0.69	0.75	0.68			
Dihydroedulan ii	15.85	0.47			0.78					
p-Menthan-3-one, cis	16.19									
L-Linalool	18.19	16.51	25.23	23.56	17.14	18.02	19.71	12.67	21.23	20.72
Linalyl acetate	18.33	16.50	16.71	14.12	10.08	12.49	8.08	10.30	8.50	11.36
(-)-Bornyl acetate	18.77	5.55	3.58	3.73	3.12	4.69	6.26	4.84	4.29	5.56
Caryophyllene	18.99	7.45	7.14	7.38	12.14	8.12	5.08	7.32	7.03	6.16
(±)Lavandulyl acetate	19.86	5.56	3.18	3.36	2.92	4.72	5.36	5.21	3.91	4.70
Pulegone	20.87			0.55	1.36	1.60	1.85	1.20		
Humulene	21.08	0.80	0.75	0.77	1.49	0.97	0.79	1.55	0.86	0.77
cis-β-Farnesene	21.36	1.30	1.19	1.26	2.32	1.50	1.23	2.48	1.30	1.09
Germacrene-D	22.24	2.68	1.71	2.38	3.74	2.66		3.47	1.48	0.56
β-Fenchyl alcohol	22.53	6.11	7.33	7.03	7.92	6.73	8.65	7.40	8.94	7.91
Bicyclogermacrene	22.92	1.49	0.94	1.19	2.22	1.53		2.14	0.78	
Neryl acetate	23.26	2.08	1.97	2.23	2.16	2.26	3.25	3.19	2.56	2.76
Carvone	23.59			3.01			0.85	0.69		0.57
Geranyl acetate	24.12	3.93	3.86	4.37	4.21	4.14	5.11	5.17	4.73	5.01
Methyl salicylate	24.78		3.54	1.26	1.59	2.53	0.90	1.79	3.09	1.88
Nerol	25.45	1.08	1.18	1.25	1.69	1.31	1.39	2.05	1.53	1.30
trans-3-Caren-2-ol	26.31	1.10	0.56	0.66	0.77	0.72		1.26		
Geraniol	26.77	3.08	3.38	3.57	4.98	3.64	3.48	4.73	4.30	3.57
(-)-Caryophyllene oxide	29.80						3.12			1.36
Veridiflorol	32.72	0.76	0.65	0.69	1.55	1.47	0.75	1.60	0.65	0.75
Elemol	32.88			3.07	6.12	3.62	4.15	4.38	2.55	
(+)-Aromadendrene	35.78									
α-Eudesmol	37.90		0.48		1.28				0.65	0.56
β-Eudesmol (CAS)	38.22		0.56	0.55	1.44	0.83	0.66		0.79	0.78
Total identified compounds		9664.83	97.89	100.00	98.85	99.99	100.00	100.00	99.40	96.14
Total hydrocarbon compounds		25.40	19.09	20.77	27.04	22.45	15.73	26.38	22.45	17.92
Total oxygenated compounds		71.43	78.80	79.23	71.81	77.54	84.27	73.62	76.95	78.22
Sesquiterpene hydrocarbon compounds		13.72	11.73	12.98	24.98	17.78	7.10	16.96	11.45	8.58
Sesquiterpene oxygenated compounds		0.76	1.69	4.31	10.39	5.92	8.68	5.98	4.64	3.45

1: Control (Foliar application with water), 2: Foliar application with humic acid at 2.5 g L^{-1}, 3: Foliar application with humic acid at 5 g L^{-1}, 4: Foliar application with amino spot at 1 cm L^{-1}, 5: Foliar application with amino spot at 1.5 cm L^{-1}, 6: Foliar application with humic acid at 2.5 g L^{-1}+amino spot at 1 cm L^{-1}, 7: Foliar application with humic acid at 2.5 g L^{-1}+amino spot at 1.5 cm L^{-1}, 8: Foliar application with humic acid at 5 g L^{-1}+amino spot at 1 cm L^{-1}, 9: Foliar application with humic acid at 5 g L^{-1}+amino spot at 1.5 cm L^{-1}

major compound in the different treatments ranging from 14.68-23.51% in the 1st cut and from 12.67-25.23% in the 2nd one. Linalyl acetate, the second main component, ranged from 12.49-26.37% and from 8.08-16.71% in the 1st and 2nd cuts, respectively. In the oil of the 1st cut, the third main component was eucalyptol which ranged from 4.93-7.72% followed by β-Fenchyl alcohol which ranged from 4.16-6.56%. On the other hand, the third main component in the 2nd cut was caryophyllene which ranged from 5.08-12.14% followed by β-Fenchyl alcohol (6.11-8.94%). In this respect, chemical composition of essential oil of mint herb is very variable depending on the

Table 10: Regression analysis and correlation coefficient of the relation between fertilizers treatments and mint characters (1st cut)

Characters	Intercept	Regression coefficient	Adjusted determination coefficient (R^2)	Correlation
Plant height	46.64	0.600	0.600	+0.77
Herb fresh weight	34.84	1.632	0.470	+0.69
Herb dry weight	13.09	2.710	0.445	+0.67
Oil (%)	0.108	0.006	0.848	+0.92
Oil yield	0.036	0.004	0.665	+0.82

Table 11: Regression analysis and correlation coefficient of the relation between fertilizers treatments and mint characters (2nd cut)

Characters	Intercept	Regression coefficient	Adjusted determination coefficient (R^2)	Correlation
Plant height	61.44	1.635	0.625	0.79
Herb fresh weight	75.67	3.928	0.497	0.70
Herb dry weight	29.76	3.467	0.642	0.81
Oil (%)	0.128	0.004	0.498	0.71
Oil yield	0.091	0.010	0.662	0.81

habitat and climate. In the 1st cut untreated plants produced the maximum value of linalool (23.51%) followed by foliar application treatment with amino spot at 1.5 cm L^{-1}, which gave 21.09. The highest value of linalyl acetate (28.15%) as a result of foliar application with humic acid at 5 g L^{-1} followed by foliar application with humic acid at 2.5 g L^{-1}+amino spot at1 cm L^{-1}, which gave 26.37%. In the 2nd cut linalool reached to its maximum value (25.23%) as a result of foliar application with humic acid at 2.5 g L^{-1} followed by foliar application with humic acid at 5 g L^{-1} which gave (23.56%). On the other hand, foliar application with humic acid at the 2.5 g L^{-1} produced the highest relative percentage (16.71%) followed by untreated plants which gave (16.50%). The effect of different treatments on EO yield and constituents may be due to their effect(s) on enzyme activity and metabolism of EO production. It is already known that fertilizers affect the growth and essential oil synthesis in medicinal plants. These components influence the levels of enzymes that are very important in the biosynthesis of important terpenoides.

Regression analysis and correlation coefficient of the relation between fertilizers treatments and mint characters: Results of the regression analysis of the relation between growth characters, essential oil percentage and essential oil yield of mint and all fertilizers treatments are presented in Table 10 and 11. The regression coefficients of all characters were significant at $p \leq 0.0001$ and had the positive trend. The adjusted determination coefficients ranged from 0.470 for herb fresh weight to 0.848 for essential oil percentage for the 1st cut while, it ranged from 0.497 for herb fresh weight to 0.662 for 2nd cut.

As, a conclusion from this regression analysis that as fertilizer increased, essential oil percentage increased by 0.006 and 0.004% as well as essential oil yield by 0.004 and 0.010% increased during 1st and 2nd cuts, respectively.

Moreover, data in Table 10 and 11 clear that, there are positive relationship between fertilizers treatments and different characters under study. The correlation coefficient values ranged from 0.67 for herb dry weight to 0.92 for essential oil percentage and from 0.70 for herb fresh weight to 0.81 for herb dry weight and essential oil yield during 1st and 2nd cuts, respectively.

These results showed the positive effect of fertilizer treatments on growth characters and essential oil content.

CONCLUSION

From the above mentioned results, it could be concluded that foliar application of humic acid and/or amino acids promoted growth and possessed the best oil percentage and yield in mint plant.

Based on the experimental results it is recommended to treat *Mintha piperita* var. *citrata* (Eau de Cologne mint) plants with humic acid at 5 g L^{-1}+amino spot at 1.5 mL L^{-1} to produce high mass production and oil yield.

REFERENCES

Al-Awak, R.H., 2010. The effect of sowing dates and potassium fertilization on the yield and the quality of the produced essential oil of anise plant (*Pimpinella anisum* L.). Ph.D. Thesis, Faculty of Agriculture, Damascus University, Syria.

Alsafar, M.S. and Y.M. Al-Hassan, 2009. Effect of nitrogen and phosphorus fertilizers on growth and oil yield of indigenous mint (*Mentha longifolia* L.). Biotechnology, 8: 380-384.

Arancon, N.Q., A. Stephenlesa, C.A. Edwardsa and R. Atiyeha, 2004. Effect of Humic Acid Derived from Cattle, Food and Paper-Waste Vermiconiposts on Growth of Greenhouse Plants. The Ohio State Univ., Columbus, OH., USA..

Ashraf, M., Q. Ali and Z. Iqbal, 2006. Effect of nitrogen application rate on the content and composition of oil, essential oil and minerals in black cumin (*Nigella sativa* L.) seeds. J. Sci. Food Agric., 86: 871-876.

Baranauskiene, R., P.R. Venskutonis, P. Viskelis and E. Dambrauskiene, 2003. Influence of nitrogen fertilizers on the yield and composition of thyme (*Thymus vulgaris*). J. Agric. Food Chem., 51: 7751-7758.

Chen, Y. and T. Aviad, 1990. Effect of Humic Substances on Plant Growth. In: Humic Substances in Soil and Crop Sciences: Selected Reading, MacCarthy, P., C.E. Clapp, R.L. Malcolm and P.R. Bloom (Eds). Soil Science Society America, Madison, WI., pp: 161-187.

Clark, R.J. and R.C. Menary, 1980a. Environmental effects on peppermint (*Mentha piperita* L.). I. Effect of daylength, photon flux density, night temperature and day temperature on the yield and composition of peppermint oil. Aust. J. Plant Physiol., 7: 685-692.

Clark, R.J. and R.C. Menary, 1980b. Environmental effects on peppermint (*Mentha piperita* L.). II. Effects of temperature on photosynthesis, photorespiration and dark respiration in peppermint with reference to oil composition. Aust. J. Plant Physiol., 7: 693-697.

De Oliveira, A.R.M.F., C.N. Jezler, R.A. Oliveira and L.C. do Bomfim Costa, 2012. Influence of plant age on production and quality of essential oil of peppermint. Revista Ceres, 59: 241-245.

Franz, C., J. Holzl, A. Ceylan and A. Vomel, 1984. Influence of the growing site on the quality of *Mentha piperita* L. oil. Acta Hortic., 144: 145-150.

Gross, M., J. Friedman, N. Dudai, O. Larkov and Y. Cohen *et al*., 2002. Biosynthesis of estragole and t-anethole in bitter fennel (*Foeniculum vulgare* Mill. var. *vulgare*) chemotypes. Changes in SAM: Phenylpropene O-methyltransferase activities during development. Plant Sci., 163: 1047-1053.

Guenther, E., 1961. The Essential Oils. Vol. IV, Nostrand Co. Inc., New York.

Jones, Jr., J.B., B. Wolf and H.A. Mills, 1991. Plant Analysis Handbook: A Practical Sampling, Preparation, Analysis and Interpretation Guide. Micro Macro Publishing, Athens, USA., ISBN-13: 9781878148001, Pages: 213.

Khalid, K.A., S.E. El-Sherbeny and A.M. Shafei, 2007. Response of *Ruta graveolens* L. to rock phosphate and/or feldspar under biological fertilizers. Arab Univ. J. Agri. Sci., 15: 203-213.

Maxwell, B. and J. Kieber, 2004. Cytokinin Signal Transduction. In: Plant Hormones: Biosynthesis, Signal Transduction, Action, Davies, P.J. (Ed.). Kluwer Academic Publishers, Dordrecht, The Netherland, ISBN: 978-1-4020-2686-7, pp: 321-349.

Mollafilabi, A., K.H.Z. Rad, H. Aroiee, R.S. Haghighi, S.J. Pour and M.T. Ebadi, 2010. Effect of optimizing nitrogen and potassium application in Johnson nutrient solution on essential oil content of peppermint in hydroponics culture. Acta Hortic., 853: 157-160.

Nell, M., M. Votsch, H. Vierheilig, S. Steinkellner, K. Zitterl-Eglseer, C. Franz and J. Novak, 2009. Effect of phosphorus uptake on growth and secondary metabolites of garden sage (*Salvia officinalis* L.). J. Sci. Food Agric., 89: 1090-1096.

Omer, E.A., 1998. Response of wild Egyptian oregano to nitrogen fertilization in sandy soil. Egypt. J. Hortic., 25: 295-307.

Omer, E.A., A.G.A. ElSayed, A. El-Lathy, M.A. Khattab and S.A. Sabra, 2008. Effect of the nitrogen fertilizer forms and time of their application on the yield of herb and essential oil of *Ocimum americanum* L. Herba Polonica, 54: 34-46.

Rai, V.K., 2002. Role of amino acids in plant responses to stresses. Biol. Planta., 45: 481-487.

Rao, M.M., R. Govindasamy and S. Chandrasekaran, 1987. Effect of humic acid on *Sorghum vulgare* var. CSH-9. Curr. Sci., 56: 1273-1276.

Rauthan, B.S. and M. Schnitzer, 1981. Effects of a soil fulvic acid on the growth and nutrient content of cucumber (*Cucumis sativus*) plants. Plants Soil, 63: 491-495.

Robert, M.D., 1986. Plant Physiology. Robert Johnson Publisher, Boston, USA., Pages: 756.

Saharkhiz, M.J. and R. Omidbaigi, 2008. The effect phosphorus on the productivity of feverfew (*Tanacetum parthenium* (L.) Schultz Bip). Adv. Natl. Applied Sci., 2: 63-67.

Singh, M., 2008. Effect of nitrogen and potassium fertilizer on growth, herbage and oil yield of irrigated palmarosa (*Cymbopogon martinii* [roxb.] wats. var. motia burk) in a semi-arid tropical climate. Arch. Agron. Soil Sci., 54: 395-400.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Edn., Iwa State University Press, Ames, Iowa.

Talaat, I.M. and A.A. Youssef, 2002. The role of the amino acids lysine and ornithine in growth and chemical constituents of basil plant. Egypt. J. Applied Sci., 17: 83-95.

Tao, Y., J.L. Ferrer, K. Ljung, F. Pojer and F. Hong *et al*., 2008. Rapid synthesis of auxin via a new tryptophan-dependent pathway is required for shade avoidance in plants. Cell, 133: 164-176.

Tattini, M., A. Chiarini, R. Tafani and M. Castagneto, 1990. Effect of humic acids on growth and nitrogen uptake of container-grown olive (*Olea europaea* L. Maurino). Acta Hortic., 286: 125-128.

Tattini, M., P. Bertoni, A. Landi and M.L. Traversim, 1991. Effect of humic acids on growth and biomass partitioning of container-grown olive plants. Acta Hortic., 294: 75-80.

Vaughan, D., 1974. A possible mechanism for humic acid action on cell elongation in root segments of *Pisum sativum* under aseptic conditions. Soil Biol. Biochem., 6: 241-247.

Zaghloul, SM., 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 J. Applied Sci., 2: 73-78.

Zahra, M.K., M. Monib, Sh.I. Abbdel-Al and A. Heggo, 1984. Significance of soil inoculation with silicate bacteria. Zentralblatt fur Mikrobiologie, 139: 349-357.