J. Mater. Environ. Sci. 5 (6) (2014) 1885-1890
ISSN: 2028-2508
CODEN: JMESCN

Evaluation of essential oil and monoterpenes of peppermint (*Mentha piperita* L.) under humic acid with foliar nutrition.

A.E. EL- Gohary[1], S.E. El-Sherbeny[1], G. M.E.M. Ghazal[2], K.A. Khalid[1]*, M. S. Hussein[1]

[1]*Research of Medicinal and Aromatic Plants Department, National Research Centre, El Buhouth St., 12311, Dokki, Cairo, Egypt.*

[2]*Department of medicinal and aromatic plants, National Organization for Drug Control and Researches (NODCAR) Cairo Egypt*

Received 11 June 2014, Revised 26 Aug 2014, Accepted 26 Aug 2014.
** Corresponding author:ahmed490@gmail.com;Tel: (+20-1117727586)*

Abstract
Peppermint (*Mentha piperita* L.) is cultivated all over the world for its use in flavor, fragrance, medicinal, and pharmaceutical applications. Pot experiments were carried out to study the effect of different levels of humic acid, application of foliar nutrition and their interactions on the essential oil extracted from peppermint herb. Essential oil content (% or ml plant $^{-1}$) increased at all humic acid, foliar nutrition and humic acid with foliar nutrition levels. The highest accumulation of essential oil content (0.25 % or 0.19 ml plant $^{-1}$) was recorded at the highest humic acid level (0.4 g L $^{-1}$) with 10 g L $^{-1}$ of foliar nutrition interaction. It is clear that the highest amounts of major constituents were produced under the treatments of 0.4 g L $^{-1}$ of humic acid with 7.5 g L $^{-1}$ of foliar nutrition (for menthol), 0 g L $^{-1}$ of humic acid with 5 g L $^{-1}$ of foliar nutrition (for menthone), 0.4 g L $^{-1}$ of humic acid with 0 g L $^{-1}$ of foliar nutrition (for isomenthone) and 0.2 g L $^{-1}$ of humic acid with 0 g L $^{-1}$ of foliar nutrition (1, 8- cineole). It is evident that the concentration of monoterpene hydrocarbonsas highest in the essential oil of plants grown under 0.2 g L $^{-1}$ of humic acid with 10 g L $^{-1}$ of foliar nutrition. The highest values of oxygenated monoterpene resulted from the treatments of 0.4 g L $^{-1}$ of humic acid with 0 g L $^{-1}$ foliar nutrition.

Keywords:Humic acid, foliar nutrition, essential oil, menthol, monoterpenes.

1. Introduction
The well-known and widely used peppermint (*Mentha piperita* L.) (*Lamiaceae*) is a cultivated natural hybrid of *Mentha aquatica* L. (water mint) and *Mentha spicata* L. (spearmint). Although a native genus of the Mediterranean region, it is cultivated all over the world for its use in flavor, fragrance, medicinal, and pharmaceutical applications. Peppermint oil is one of the most widely produced and consumed essential oils [1-3]. Besides its uses in food, herbal tea preparations, and confectioneries, the medicinal uses of mint, which date back to ancient times, include carminative, antiinflammatory, antispasmodic, antiemetic, diaphoretic, analgesic, stimulant, emmenagogue, and anticatharrhal application. It is also used against nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints. Mint essential oils are generally used externally for antipruritic, astringent, rubefacient, antiseptic, and antimicrobial purposes, and for treating neuralgia, myalgia, headaches, and migraines [2 - 8]. Humates have long been used as a soil conditioner; fertilizer and soil supplement [9]. Humic acid (HA) is one of the major components of humus and can be used as growth regulate-hormone level improve plant growth and enhance stress tolerance [9 - 11]. Fortun [12, 13] reported that humic acid improve soil structure and change physical properties of soil, promote the chelating of many elements and make these available to plants, aid in correcting plant chlorisis, enhancement of photosynthesis density and plant root respiration has resulted in greater plant growth with humate application [14, 15]. Increase the permeability of plant membranes due to humate application resulted in improve growth of various groups of beneficial microorganisms, accelerate cell division, increased root growth and all plant organs for a number of horticultural crops and turf - grasses, as well as, the growth of some trees [16 - 18]. Oregano essential oil production increased significantly with K-humate application [19].
Nutrient available in the nutritional environment of plants are capable of changing essential oil yield and composition [20 - 23]. The highest basil essential oil yield was found at the highest NPK rate [20]. Nitrogen and

phosphorus application contributes to quantitative changes in the essential oil of *Artemisia pallens* Wall. but it does not modify its composition [24]. The application of a higher NPK rate can increase essential oil content in *Tagetes patula* L., but only in some harvest periods [25]. Increased fertilization of summer savory plants with macro- and micro nutrient increases oil yield but also modifies its chemical composition [26]. Phosphorus application significantly increases basil essential oil content [27]. Phosphorus fertilization significantly increases oil yield in rose-scented geranium and also the content of citronellol and 10-epi-Û-eudesmol [28]. In turn, iron application in growing thyme has a repressive effect on essential oil content and chemical composition [29]. Differently, in basil grown under salt stress conditions foliar application of zinc and iron increases the linalool content in the oil [30]. Moreover, the above-mentioned metals contribute to increased tolerance of plants to salt stress. Oregano essential oil yield is higher by 31% compared to the control under the influence of foliar application of calcium and magnesium [31]. At the same time, volatile oil content is not dependent on the application of Mg^{2+}; these differences result primarily from a significant increase in dry matter yield under the influence of plant fertilizing [31]. Mg has a positive effect on the quality and quantity of chamomile essential oil production [32]. El-Wahab [33] indicated that *Trachysper-mum ammi* L. essential oil constituents were not largely affected by the applied Mg. 20 g L^{-1} $MgSO_4$ resulting in a significant increase in essential oil content and main components of essential oil [34]. Application of NP with micronutrient increased the essential oil of some aromatic plants [35].
The main objective of the present investigation was to study the effect of different levels of humic acid, application of foliar nutrition and their interactions on the essential oil extracted from peppermint herb.

2. Materials and methods

2.1. Experimental

Experiments were carried out in a greenhouse at the National Research Centre, Cairo, Egypt. During February of 2012 and 2013 seasons. Uniform suckers of (*Mentha piperita* L.) were transplanted into plastic pots (30cm diameter and 50 cm height). Each pot was filled with 10 kg of air-dried soil. Physical and chemical properties of the soil used in this study were determined according to Jackson [36, 37] and are presented in Table 1. Three weeks after transplanting, the seedlings were thinned to three plants per pot. All pots were divided into four main groups. The first group was subjected to different levels of humic acid: (0, 0.2 and 0.4 mg L^{-1}). The second, third and fourth groups were subjected to the same treatments of humic acid but complete commercial fertilizer (Green life) was added as foliar spray by levels of 5, 7.5 and 10 g L^{-1} for the second, third and fourth groups respectively. The foliar spray was added two times, the first one was added after 3 weeks from sowing while the second one was added after the first harvest by 2 weeks. All agriculture practices were conducted according to the main recommendations by the Egyptian Ministry of Agriculture. The analysis of humic acid was K_2O (10 – 12%), [Zn, Fe, Mn, etc.] (100 mg), pH (9 -10). Solubility (> 98%). Green life analysis was N (20%), P_2O_5 (20%), K_2O (20%), Zn (5 g L^{-1}), Mn (5 g L^{-1}), Fe (1 g L^{-1}).

Table 1. Physical and chemical properties of soil

Texture	pH	EC (dS m^{-1})	Available nutrients (mg g^{-2})			Soluble ions (Cmolc)				
			N	P	K	Ca	Mg	Fe	Zn	Mn
Clay loam	7.0	6.9	12.4	0.5	3.3	9.4	12.2	2.6	4.1	1.8

2.2. Harvesting

At the full bloom stage, all plants were harvested twice (first and second harvest) during the growing season, by cutting the plants 5 cm above the soil surface [after 45 days (during April) and 90 days (during May) from transplanting, respectively].

2.3 Essential oil isolation

Fresh mass was collected from each treatment during the first and second harvest, and then 300 g from each replicate of all treatments was subjected to hydro-distillation for 3 h using a Clevenger-type apparatus [38]. The essential oil content was calculated as a relative percentage (v/w). In addition, total essential oil g plant^{-}1was calculated by using the fresh mass. The essential oils extracted from mint were collected during the first and second harvests from each treatment and dried over anhydrous sodium sulphate to identify the chemical constituents of the essential oil.

2.4. GC/MS

The essential oil was analyzed on a VG analytical 70 - 250S sector field mass spectrometer, 70 eV, using a SPsil5, 25 m x 30 m, 0.25 μm coating thickness, fused silica capillary column, injector 222°C, detector 240 °C, linear temperature 80–270°C at 10 °C/min. Diluted samples (1/100, v/v, in n - pentane) of 1 ml were injected, at 250 °C, manually and in the split less mode flame ionization detection (FID) using the HP Chemstation software on a HP 5980 GC with the same type column as used for GC/MS and same temperature program.

2.5 Qualitative and quantitative analyses

Identifications were made by library searches [39] combining MS and retention data of authentic compounds by comparison of their GC retention indices (RI) with those of the literature [39] or with those of standards available in our laboratories. The retention indices were determined in relation to a homologous series of n-alkanes (C8–C22) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature [39]. Component relative concentrations were calculated based on GC peak areas without using correction factors.

2.6. Statistical analysis

In this experiment, two factors were considered: humic acid and foliar nutrients. For each treatment there were 3 replicates, each of which had 10 pots; in each pot 3 individual plants were planted. The experimental design followed a complete random block design according to Snedecor [40]. The averages of data were statistically analyzed using 2-ways analysis of variance (ANOVA-2). The applications of that technique were according to the STAT-ITCF program [41].

3. Results and discussion

3.1. Effect of humic acid, green life fertilizer and their interactions on essential oil content

As shown in Table 2, essential oil content (% or ml plant $^{-1}$) increased at all humic acid, foliar nutrition and humic acid with foliar nutrition levels.

Table 2. Effect of humic acid, green life fertilizer and their interactions on essential oil content (means of two seasons)

Treatments		Essential oil content	
Green life(g L $^{-1}$)	Humic acid(g L $^{-1}$)	%	ml Plant $^{-1}$
0	Control	0.12	0.05
	0.2	0.15	0.07
	0.4	0.18	0.09
Overall 0		0.15	0.07
5	Control	0.14	0.06
	0.2	0.17	0.09
	0.4	0.21	0.13
Overall 5		0.17	0.09
7.5	Control	0.19	0.06
	0.2	0.22	0.09
	0.4	0.22	0.13
Overall 7.5		0.21	0.09
10	Control	0.19	0.13
	0.2	0.21	0.15
	0.4	0.25	0.19
Overall 10		0.22	0.16
Overall humic acid	Control	0.16	0.07
	0.2	0.19	0.10
	0.4	0.22	0.14
F value			
Humic acid		83.3**	98.6**
Green life		84.9**	70.5**
Humic acid x Green life		2.6*	1.0

* $P \leq 0.05$ according to F-values of the 2-way analysis of variance (ANOVA-2).

** $P < 0.01$ according to F-values of the 2-way analysis of variance (ANOVA-2).

The highest accumulation of essential oil content (0.25 % or 0.19 ml plant $^{-1}$) was recorded at the highest humic acid level (0.4 g L $^{-1}$) with 10 g L $^{-1}$ of foliar nutrition interaction compared with control Treatment. ANOVA indicted that the changes in essential oil content were highly significant for humic acid or foliar nutrition treatments. The changes in essential oil percentage were significant for humic acid with foliar nutrition while the changes in essential oil per plant were insignificant for humic acid and foliar nutrition interaction treatments.

3.2. Effect of the interactions between humic acid and foliar nutrition on essential oil constituents

Eleven constituents amounting 86.5 - 98.5 % of the oil were found in the peppermint essentialoil extracted by the hydro - distillation method. Different levels of humic acid with foliar nutrition levels had no effect on the number of chemical components of mint essential oil (Table 3). The main components were menthol (45.3 % - 66.8 %), menthone(7.8% - 25.5%), isomenthone(4.4% - 11.8%) and 1, 8- cineole (2.6% - 4.1%). It is clear that the highest amounts of major constituents were produced under the treatments of 0.4 g L $^{-1}$ of humic acid with 7.5 g L $^{-1}$ of foliar nutrition fertilizer (for menthol), 0 g L $^{-1}$ of humic acid with 5 g L $^{-1}$ of foliar nutrition fertilizer (for menthone), 0.4 g L $^{-1}$ of humic acid with 0 g L $^{-1}$ of foliar nutrition fertilizer (for isomenthone) and 0.2 g L $^{-1}$ of humic acid with 0 g L $^{-1}$ of foliar nutrition (1, 8- cineole). Also Table3 represents the compounds obtained from peppermint essential oil grouped into class's i.e monoterpenes hydrocarbons (1.2% - 2.5%) and oxygenated monoterpenes (80.3% - 96.8%). It is evident that the concentration of monoterpene hydrocarbonsas highest in the essential oil of plants grown under 0.4 g L $^{-1}$ of humic acid with 5 g L $^{-1}$ of foliar nutrition. The highest values of oxygenated monoterpenes resulted from the treatments of 0.4 g L $^{-1}$ of humic acid + 0 g L $^{-1}$ of foliar nutrition. ANOVA indicated that the changes in α-pinene, β-pinene, limonene and 1, 8- cineole were insignificant while the changes in menthone, menthofuran, isomenthone, menthol and pulegone were highly significant. On the other hand the changes in carvone and methyl acetate were insignificant. Regarding to the changes in monoterpene hydrocarbons and oxygenated monoterpenes were significant.

Table. 3. Effect of the interactions between humic acid and foliar nutrition on essential oil constituents (means of two seasons)

No.	Components (%)	RI*	Humic acid treatments (0, 0.2 and 0.4 mg L $^{-1}$)												F value
			Green life treatments (0, 5, 7.5 and 10 g L $^{-1}$)												
			0			5			7.5			10			
			0	0.2	0.4	0	0.2	0.4	0	0.2	0.4	0	0.2	0.4	
1	ὰ-Pinene	934	0.5	0.1	0.6	0.5	0.5	0.6	0.6	0.5	0.5	0.5	0.6	0.5	1.0
2	β-Pinene	980	0.7	1.1	1.1	1.2	1.3	1.9	1.2	1.1	1.2	1.4	1.4	1.3	2.2
3	Limonene	1031	2.7	3.1	3.1	1.3	2.2	3.7	4.2	3.9	2.8	1.4	1.1	2.2	2.1
4	1,8- Cineole	1033	3.4	4.1	3.2	3.1	3.8	3.8	3.6	2.6	3.3	2.8	2.8	2.9	2.2
5	Menthone	1154	23.6	19.3	15.1	25.5	12.8	20.2	16.1	12.5	7.8	15.3	16.5	12.3	9.3**
6	Menthofuran	1163	0.5	1.1	1.3	1.0	0.7	0.9	1.2	1.7	0.7	9.8	0.7	0.7	5.2**
7	Isomenthone	1164	6.9	9.4	11.8	11.0	5.1	5.6	4.4	8.0	8.7	8.4	11.5	7.1	8.7**
8	Menthol	1173	48.5	46.9	57.5	45.3	57.3	53.3	56.0	45.8	66.8	53.1	57.1	54.3	7.4**
9	Pulegone	1237	1.4	1.3	1.1	0.7	1.1	1.4	1.3	1.0	1.7	1.2	1.0	1.5	5.5**
10	Carvone	1242	1.4	1.5	1.7	1.3	1.6	1.3	1.7	1.9	1.5	2.0	1.0	2.1	2.9*
11	Methyl acetate	1256	3.1	3.0	2.0	2.7	5.5	3.4	2.5	2.9	2.3	2.7	2.8	3.0	3.0*
MH= Monoterpene hydrocarbons			1.2	1.2	1.7	1.7	1.8	2.5	1.8	1.6	1.7	1.9	2.0	1.8	2.7**
OM= Oxygenated Monoterpenes			91.5	89.7	96.8	91.9	90.1	93.6	91.0	80.3	95.6	96.7	94.5	86.1	4.6**
Total identified			92.7	90.0	98.5	93.6	91.9	96.1	92.8	81.9	97.3	98.6	86.5	87.9	

* P ≤ 0.05 according to F-values of the 2-way analysis of variance (ANOVA-2).
** P < 0.01 according to F-values of the 2-way analysis of variance (ANOVA-2).
*RI = Confirmed by comparison with Retention indices on DB5 column

The variations in essential oil content and composition could be due to its effect of different treatments on enzymes activity and metabolism improvements [42]. Our results agree with those obtained by some previous literature i.e. Oregano essential oil production increased significantly with K-humate application [19]. Fortun [12, 13] reported that humic acid improve soil structure and change physical properties of soil, promote the chelating of many elements and make these available to plants, aid in correcting plant chlorisis, enhancement of photosynthesis density and plant root respiration has resulted in increasing the plant growth and essential oil content with humate application [14, 15].Increased fertilization of summer savory plants with macro- and micronutrientincreases oil yield but also modifies its chemical composition [26]. Differently, in basil grown under salt stress conditions foliar application of zinc and iron increases the linalool content in the oil [30]. Moreover, the above-mentioned metals contribute to increased tolerance of plants to salt stress. Oregano essential oil yield is higher by 31% compared to the control under the influence of foliar application of calcium and magnesium [31]. Mg has a positive effect on the quality and quantity of chamomile essential oil production [32]. El-Wahab [33] indicated that *Trachysper-mum ammi* L. essential oil constituents were not largely affected by the applied Mg. 20 g L^{-1} $MgSO_4$ resulting in a significant increase in essential oil content and main components of essential oil [34]. Application of NP with micronutrient increased the essential oil of some aromatic plants [35]. Foliar application of zinc and iron increases the linalool content in the oil extracted from oregano plants [30].

Conclusion

It may be concludedthe peppermint essential oiland its constituents were affected by changinghumic acid with or without foliar nutrition. The highest accumulation of essential oil content was recorded with the interaction between humic acid (0.4 g L^{-1}) with 10 g L^{-1} of foliar nutrition at the highest level. The main constituents and essential oil classes were changed under humic acid with or without foliar nutrition.

References

1. Heywood V. H., Flowering Plants of the World. Oxford University Press: *Oxford, UK* (1979).
2. Foster S., Peppermint, *Menthapiperit*a. In Botanical Series; American Botanical Council: *Austin, TX, no 306*. (1990).
3. Brown D., Encyclopaedia of Herbs and Their Uses. Dorling Kindersley: *London, UK* (1995).
4. Bisset N. G., Herbal Drugs. *Medpharm Scientific Publishers: Stuttgart, Germany* (1995).
5. Tyler V. E., The Honest Herbal. Pharmaceutical Products Press*: New York* (1993).
6. Baytop T., Therapy with Medicinal Plants in Turkey-Past and Present, 2nd ed., Nobel *Tıp Basımevi: Istanbul, Turkey*, pp 348-349. (1999).
7. Hendriks H., 1998. *Perf. Flav.*, 23 (1998) 15.
8. Cowan M. M., *Clin. Microbi. Rev*., 12 (1999) 564.
9. Albayrak S., Camas N.,*J. Agro*., 4 (2005) 130.
10. Piccola A., Nardi S., Concheri G.,*Soil Biochem*., 24(1992) 373.
11. Tan K.H., Nopamornbodi V.,*Plant and Soil*, 51(1979) 283.
12. Fortun C., Fortun A., Almendros G.,*The Sci. Environ.*, 81/82 (1989) 561.
13. Kononova M.M., Soil organic matter. Its role in soil formation and in soil fertility. *Pergamon Press, Oxford*(1966).
14. Chen Y., and Avaid, T., Effect of humic substances on plant growth. Pp. 161-186., *Madison, WI* (1990).
15. Smidova M., The influence of humus acid on the respiration of plant roots. *Biol. Plant.*, 2(1960) 154.
16. Rsso R.O., Berlyn G.P.,*J. Sust. Agric*., 1(1990) 19.
17. Sanders D.C., Ricotta J.A., Hodges L.,*Hortsci*., 25(1990) 181.
18. Pioncelot R.P.,*J. Sust. Agric*., 3(1993) 99.
19. Said-Al Ahl H.A.H., Hussein M.S.,*Ozean J. App. Sci.*, 3(2010) 125.
20. NurzyĚska-Wierdak R., *J. Ess. Oil Res*., 24 (2012) 21.
21. Sharafzadeh S., Esmaeilli M., Mohammadi A.H., *Adv. Environ. Biol.* 5 (2011) 1285.
22. Sharafzadeh S., Khosh-Khui M., *Adv. Environ. Biol*. 5 (2011) 639.
23. Sakr W.R.A., El-Sayed A.A., Hammouda A.M., Saad El Deen F.S.A., *J. Hortic. Sci. Ornamen. Plants* 4 (2012) 34.
24. Kumar T.S., Swaminathan V., Kumar S.,. EJEAFChe 8 (2009) 86.
25. Stojanova A., Primova T., Anastassov Ch., *J. Essent. Oil Res*. 12 (2000) 609.
26. Alizadeh A., Khoshkhui M., Javidnia K., Firuzi O., Tafazoli E., Khalighi A., *J. Med. Plants Res.*, 4 (2010) 33.
27. Ramezani S., Rezaei M.R., Sotoudehnia P., *J. App. Biol. Sci.* 3 (2009) 96.

28. Prasad A., Kumar S., Pandey A.,. *Biol. Fertil. Soils*, 48 (2012) 117.
29. Jabbari R., Dehaghi M.A., Sanavi A.M.M., Agahi K., *Adv. Environ. Biol.* 5 (2011) 433.
30. Said-Al Ahl H.A.H., Mahmoud A.A., *Ozean J. Appl. Sci.* 3 (2010) 97.
31. Dordas C., *Ind. Crops Prod*., 29 (2009) 599.
32. EvaS.,EmoK.,MaD.,SandorA.,KissC.,LaraS.,EvaL.,*J.Amer.Coll.Nut*.,23 (2004)763S.
33. El–WahabA.,MohamedA.,J.App. Sci.Res.,3 (2007)781.
34. Khalid A.K., Zaghloul S.M., Abd-Elazim A.Y.,*Med. Arom. plant Sci. Biotech.*, 3 (2009) 52.
35. Khalid A. K.,*J. Soil Sci. Plant Nutri.*,12 (2012) 617.
36. Jackson M. L., Soil Chemical Analysis. *Prentice Hall of India Pvt., Ltd., M.97, Connght Citrus, New Delhi* (1973).
37. CottenieA., VerlooM., Kiekens L., Velghe G., CamerlynckR., ChemicalAnalysis of Plant and Soil. Laboratory of Analytical and Agrochemistry, *State Univ., Ghent, Belgium* (1982).
38. Clevenger J.F., J. Amer. Pharma. Assoc., 17 (1928) 346.
39. Adams R.P., Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th ed. *Allured Publ. Corp., Carol Stream, IL.*(2007).
40. Snedecor G. W., Cochran W. G., Statistical Methods. 11th Ed. Iowa State Univ., Press. *Ames, Iowa, USA* (1990).
41. Foucart T., Analyse factorielle, programmation sur micro- ordinateur. *Masson, ITCF, Paris* (1982).
42. Burbott A. J., Loomis D., *Plant Phys*., 44 (1969) 173.