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# Effect of NaCl and iron oxide nanoparticles on *Mentha piperita* essential oil composition

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**Abstract**

*Mentha piperita* is a medicinal plant of the Labiatae family. this species produces valuable essential oil. In this study, the effect of eleven treatments of salinity and nanoparticles of iron oxide on the essential oil composition in *M. piperita* was investigated. Mature plants were collected at 90 days after planting. Essential oils were extracted from plant aerial parts using Clevenger-type apparatus, and the obtained oils were analyzed using gas chromatography and gas chromatography/mass spectrometry. The obtained results showed that the essential oil concentration differed between treatments. Application of iron nanoparticle increased essential oil amount, while salinity stress lovered its production. Menthone, menthol, 1,8-cineol, pulegone, menthofuran, *cis*-sabinene hydrate and germacrene D were the major essential oil compounds of control plants. The types and relative proportion of major components differed between studied treatments. Salt as well as iron nanoparticle stress had strong effects on essential oil production and composition.

**Key words:** essential oil, salinity, iron nanoparticles, *Mentha piperita*.
**Abbreviations:** GC, gas chromatograph.

## Introduction

Some medicinal and aromatic plants produce essential oil as secondary metabolites. The oil is very heterogeneous mixture of many compounds at various concentrations (Zouari et al. 2014). Factors such as climatic, and geographic conditions, as well as ontogeny of collected plants can affect formation of essential oil, its composition and its biological activities. For these reasons, studies of variations in chemical compounds of essential oil in relation to ecological parameters might provide information on causes of polymorphism in chemistry of essential oil. Furthermore, chemical variation in essential oil composition is a very important property for marketing and contributes to its commercialization as a main component in food and in pharmaceutical industries (Zouari 2013).

*Mentha piperita* L. is known as mint or peppermint. It is used as a medicinal and culinary herb (Lorenzi, Matos 2002). Cultivation of peppermint has economic value, due to production and accumulation of essential oil (Souza et al. 1991).

Peppermint aerial parts yield essential oils that contain many aromatic chemicals, like menthol, menthone, isomenthone and menthofuran, which are used in different industries, especially the two first mentioned components (Carmines 2002).

Menthol is used in products of oral hygiene, pharmaceutical and cosmetic industries. Studies have confirmed that this compound has high antifungal as well as antibacterial potential and is important in the scents and essences industry (Souza et al. 1991). Some investigators found that its antifungal activity is comparable to that of synthetic fungicides (Farkas et al. 2003, Hadian et al. 2008). Moraes (2000) believed that for these reasons, the essential oil of *M. piperita* ranks high in terms of total sales volume.

Some studies have been conducted on the effect of environmental factors on essential composition of *M. piperita* For example, in mint leaves infected with pathogene maximum menthofuran content of 4.9% was recorded (Gershenson et al. 2000). When plants of *M. piperita* were cultivated in nutrient solution with different levels of potassium, a mean menthofuran content of 11.79% was obtained (Valmorbida 2002).

No comparative study has been performed on the effect of salinity treatments on essential oil composition. In addition, we have not found any study on effects of iron oxide nanoparticles on essential oil composition. Iron nanoparticles can be useful in development of technological systems maximizing fertilizer and pesticide applications (Bakhtiari et al 2015).

Iron is significant trace element and is necessary for all living organisms (Rashno et al. 2013). Bakhtiari et al. (2015) suggested that iron nutrition can be a problem in plants growing in high pH as in calcareous soils. Iron is essential element in cell metabolism and it is involved in photosynthesis, respiration, etc. (Wiedenhoeft 2006; Rashno et al. 2013). Nanoparticles of iron oxide are smaller than typical iron oxide molecules and create more complexes

with higher iron availablity to plants (Mazaherinia et al. 2010). Therefore, the aim of the present investigation was to test the effect of effect of salinity and iron oxide nanoparticles treatment on the essential oil content of *M. piperita* plants.

## Materials and methods

### *Plant material and treatment*

Seed samples of *M. piperita*, which had previously been described by Jamzad (2012), were used for this study. The study was set up in a growth chamber maintained at an air temperature of 20 to 25 °C (in night/ day respectively); light period was 14 h throughout the duration of the experiment, light intensity was 420 μmol $m^{-2}$ $s^{-1}$. Plastic flower pots (14 × 12 cm) filled with soil and perlite (1 : 1) were used for planting individual seeds.

Treatments with salt (NaCl, Merck, Germany) and iron solutions (as nanoparticles of iron oxide Sigma-Aldrich, United Kingdom and Fe-EDTA) were used. Combinations of four salt concentrations (0, 50, 100, and 150 mM) with three iron concentrations (0, 10 and 30 μm of $Fe_2O_3$ nanoparticles and normal iron-chelate), in total 12 solutions, were used (Table 1). The molecular concentrations of iron oxide nanoparticles were determined on the basis of iron concentrations in EDTA. Irrigation was performed weekly with 100 mL of complete Hoagland solution containing iron-chelate as Fe-EDTA or without iron-chelate and containing different concentrations of $Fe_2O_3$ nanoparticles and sodium chloride. Samples in the control treatment $Fe_2O_3$ no iron. Various concentrations of $Fe_2O_3$ nanoparticles were obtained as described by Prasad et al. (2012). Mature plants were collected 90 days after beginning of treatments.

### *Essential oil extraction*

Dry material from above ground plant parts were subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate and stored in sealed vials preserved from light at −20 °C until analysis.

Gas chromatograph (GC) analysis was performed on a Thermoquest-Finnigan Trace GC apparatus armed with a capillary DB-5 fused silica column (60 m × 0.25 mm i.d., thickness of film 0.25 μm). The carrier gas was nitrogen that was used at a stable flow of 1.1 mL $min^{-1}$. The oven temperature was maintained at 60 °C for 1 min, then raised to 250 °C at a rate of 4 °C $min^{-1}$ and held for 10 min. The injector temperature was maintained at 250 °C and detector temperature was at 280 °C. GC-MS analysis was carried out on a Thermoquest-Finnigan Trace GC-MS device, armed with a DB-5 fused silica column (60 m × 0.25 mm i.d., thickness of film 0.25 μm). The oven temperature was raised from 60 °C to 250 °C at a rate of 5 °C $min^{-1}$ and held at 250 °C for 10 min. The transfer line temperature was 250 °C. Helium was used as the bearer gas at a flow rate of 1.1 mL $min^{-1}$; split ratio was 1/50. The quadrupole mass spectrometer was scanned over the 40-460 AMU with an ionizing voltage of 70 eV. The ionization current was 150 μA.

The essential oil components were identified by calculation of retention indices under temperature-programmed situations for *n*-alkanes ($C_6$–$C_{24}$; Merck, Germany) and the retention data of essential oil components on the DB-5 column. Further information was made by comparison of component mass spectra with those of reference material or with prooved composition and confirmed by comparison of retention indices (Adams 2007).

One sample and paired sample *t*-tests were employed to determine significant differences in concentrations of essential oil and it's components among treatments, and Pearson's coefficient of correlation was used to determine significant correlations of preparations of essential oil components in relation to concentration of NaCl and iron oxide nanoparticles. SPSS ver. 9 (1998) software was used in the statistical analysis.

**Table 1.** Concentration of iron nanoparticles and NaCl used for treatments and essential oil yield obtained

| Treatment | Fe-nano (μm) | NaCl (mM) | Esseential oil (%) |
|---|---|---|---|
| Control | 0 | 0 | 2.19 |
| 1 | 10 | 0 | 3.31 |
| 2 | 30 | 0 | 3.56 |
| 3 | 0 | 50 | 1.81 |
| 4 | 10 | 50 | 1.92 |
| 5 | 30 | 50 | 2.03 |
| 6 | 0 | 100 | 1.46 |
| 7 | 10 | 100 | 1.57 |
| 8 | 30 | 100 | 1.69 |
| 9 | 0 | 150 | 1.08 |
| 10 | 10 | 150 | 1.17 |
| 11 | 30 | 150 | 1.32 |

## Results

In the present study, the effects of salt and iron oxide nanoparticles concentrations on the chemical composition of *M. piperita* essential oil were determined. The essential oil concentration differed between treatments. The concentration in control plants was 2.19%. One sample as well as paired sample *t*-tests ($p < 0.05$) showed significant differences between treatments. The highest concentration occurred in treatment No. 2 (3.56%), and the lowest in treatment No. 9 (1.08%) (Table 1).

Thirty four components were identified in the control samples. Menthone (49.67%), menthol (22.19%), 1,8-cineol (7.90%), pulegone (2.86%), menthofuran (2.84%), *cis*-

sabinene hydrate (2.52%) and germacrene D (1.69%) were the major components. The types as well as proportions of major components differed between treatments (Table 2). When only iron nanoparticles were added (treatments No. 1 and 2; 10 and 30 μm respectively), the major components together constituting more than 80% of oils were similar to those of the control sample. However, the proportion of menthone increased and that of pulegone doubled in these treatments.

With increasing NaCl concentration and without iron nanoparticles (treatments No. 3, 6 and 9; 50, 100 and 150 mM NaCl, respectively), the three main components that respectively constituted 75.12, 71.24 and 60.93% of the essential oil contents were menthone, menthol and menthofuran. The proportions of menthone and menthol decreased in these treatments, while proportion of menthofuran increased from 2.84% in control plants to 11.45, 15.79 and 16.93%, respectively. In treatments No. 4, 5, 7, 8 and 10 with addition of Fe, the five major components that together constituted 74 to 85% percent of oil content were similar, but the concentration of each component differed.

With increased Fe concentration, menthone concentration decreased from 43.51% to 25.03%. Menthol concentration also decreased. Menthofuran and 1, 8-cineol concentrations increased from 10.42 to 21.42% and 6.92 to 14.30%, respectively. With highest concentration of NaCl and iron nanoparticles (treatment No. 11), the essential oil composition widely differed from that in other treatments. Isoborneol constituted 20% of essential oil and menthol was present as a trace compound, while it was the major compound for other treatments. Also menthone concentration was three times lower than in the control treatment (Table 3).

One-sample *t*-test showed significant differences ($p < 0.01$) in major essential oil components, such as menthone, methyl acetate, pulegone, 1,8-cineol and menthofuran, between treatments. Significant negative or positive correlations occurred between salt concentrations and the essential oil component concentrations. For example, significant negative correlation ($p = 0.01$, $r = -0.47$) occurred between salt concentration and menthone concentration, as well as menthol proportion, while other components like menthofuran, isoborneol and pulegone had positive significant correlation ($p = 0.05$, $r = 0.60$) with salt concentration. Also a positive correlation was observed for 1,8-cineol ($p = 0.01$, $r = 0.77$).

No significant correlation was found between concentration of iron oxide nanoparticles and essential oil component concentrations. In addition, significant correlation was seen between the component concentrations of essential oils. Menthone has a positive significant correlation with menthol concentration, but negative significant correlations with 1,8-cineol, pulegone and isoborneol.

## Discussion

Essential oils have various roles in plants. Bakkali et al. (2008) observed that essential oil has a crucial role in protection of plant from insects and pathogens. The oils act as attractants of pollinators, while providing a significant protection against herbivores and pathogenic fungi. In addition, essential oil also plays a prominent role in plant–plant interplays with an evolutionary origin for communication (Langenheim 1994; Batish et al. 2008). While essential oil biosynthesis in plants has genetic determination, environmental conditions also affect its production (McKiernan et al. 2012; Sadeghi et al. 2014).

In this study the effects of different stressors on essential oil composition were investigated. Salt as well as iron nanoparticle concentration had strong effect on essential oil production and its major components. The concentration of essential oils differed between treatments. Maximum essential oil concentration occurred in treatment No. 2, where plants were treated with 30 μm of iron nanoparticles. Increasing concentration of iron nanoparticles maximized essential oil production. Veronese et al. (2001) reported that biotic and abiotic agents affect essential oil composition in *M. piperita*. These findings were confirmed in other studies on fertilization effect on the development of this plant (Scavroni et al. 2005). Treatment with different levels of nitrogen affects essential oil formation (Piccaglia et al. 1993). In addition, absence of potassium causes a decrease of essential oil concentration in *M. piperita* plants (Praszna, Bernath 1993).

Lowest concentration of essential oil was recorded in plans in the 150 mM salt treatment. Thus, salt decreases production of essential oil. Charles et al. (1990) confirmed that salinity stress reduced essential oil formation in *M. piperita*. There are many reasons for this, but the main reason is related to lower cytokinin supply from the roots to the aerial parts, altered ratio between abscisic acid and cytokinin in leaves. Razmjoo et al. (2008) showed that nearly all growth factors in *Nigella sativa* decreased with increased salinity. Similar observations were recorded for *Matricaria chamomila* (Dadkhah 2010). The production of essential oil in *Melissa officialis* decreased in response to salinity (Ozturk et al. 2004). We observed that iron nanoparticles can decrease the effect of salinity in plants. The concentration of essential oil in combined treatments (combination of iron and salt) were higher in comparison to plants that received only salt treatments. Investigations showed that other environmental factors such as growing location, year, shading, fertilizer, water availability, as well as time of harvest affect essential oil production (Burbott, Loomis 1967).

There was no significant correlation between concentration of iron nanoparticles and major essential oil components. However, the concentration menthone was higher in iron oxide treatments. The types of major

**Table 2.** Concentration of different chemical compounds (%) in essential oils from treated plants

| Compound | Control | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (E)-β-Farnesene | 0.2413 | 0.2862 | 0.4194 | 0.3728 | 0.4318 | 0.2471 | 0.3286 | 0.2008 | 0.2547 | 0.3981 | 0.1241 | 0.5814 |
| 1,8-Cineol | 7.9068 | 5.662 | 6.2413 | 4.7036 | 6.6927 | 8.7812 | 6.8813 | 8.8987 | 9.9772 | 10.4529 | 14.3078 | 16.728 |
| *trans*-α- Bergamotene | 0.0174 | 0.0195 | 0.0246 | 0.0193 | 0.0417 | 0.0228 | 0.0299 | 0.0245 | 0.0687 | 0.0631 | 0.011 | 0.0264 |
| Bicyclogermacrene | 0.168 | 0.2288 | 0.305 | 0.2785 | 0.2902 | 0.1121 | 0.3301 | 0.1327 | 0.1805 | 0.3058 | 0.0943 | 0.4526 |
| Bornyl acetate | tr | tr | tr | tr | tr | tr | tr | 0.2915 | tr | tr | tr | 0.0125 |
| Caryophyllene oxide | tr | 0.0256 | 0.0186 | 0.0271 | 0.0217 | tr | 0.0683 | 0.0137 | 0.0634 | 0.142 | 0.0458 | 0.1493 |
| *cis*-Sabinene hydrate | 2.5251 | 1.4372 | 2.5285 | 0.809 | 2.3221 | 2.1173 | 2.4155 | 2.1713 | 2.1488 | 2.363 | 2.4032 | 4.5215 |
| β-Elemene | tr | tr | 6.15E-03 | tr | 0.0118 | tr | 0.0135 | tr | 0.0223 | 0.0489 | tr | 0.022 |
| Germacrene A | 0.2284 | 0.0585 | 0.2273 | 0.1305 | 0.2229 | 0.0688 | 0.1318 | 0.1824 | 0.0511 | 0.2404 | 0.1021 | 0.2634 |
| Germacrene D | 1.6968 | 1.0466 | 1.8643 | 1.7893 | 3.051 | 2.767 | 2.8445 | 1.3267 | 1.7677 | 2.8843 | 1.9594 | 4.5018 |
| Isoborneol | 1.1792 | 0.7001 | 0.6894 | 1.7674 | 0.0904 | 2.5211 | 2.592 | 3.8257 | 2.6877 | 2.6689 | 11.1076 | 20.0285 |
| Z-Jasmone | 0.0523 | 0.0597 | 0.1154 | 0.092 | 0.113 | 0.3426 | 0.0876 | 0.0443 | 0.0559 | 0.0992 | 0.0242 | 0.1381 |
| Limonene | 1.1934 | 0.8513 | 0.7514 | 0.8944 | 0.0524 | 0.7769 | 0.6786 | 0.9655 | 1.0578 | 1.244 | 1.0072 | 1.6958 |
| Linalool | 0.5336 | 0.5572 | 0.5751 | 0.403 | 0.4592 | 0.522 | 0.4922 | 0.4637 | 0.4183 | 0.5093 | 0.3758 | 0.9414 |
| Menthofuran | 2.8488 | 2.5697 | 2.602 | 11.4563 | 10.4264 | 8.4236 | 15.7976 | 14.0987 | 21.4965 | 16.9356 | 15.1961 | 5.3604 |
| Menthol | 22.1954 | 20.5189 | 19.1808 | 18.766 | 18.5643 | 17.9305 | 15.5021 | 15.9384 | 14.6493 | 14.4205 | 12.1413 | tr |
| *iso*-Menthol | 0.0692 | 0.0336 | 0.0344 | 0.0156 | 0.0324 | 0.0732 | 0.0247 | 0.0879 | 0.0809 | 0.0739 | 0.0369 | 0.0194 |
| Menthone | 49.6702 | 53.8015 | 53.7652 | 44.9057 | 43.5183 | 40.285 | 39.9488 | 36.3939 | 30.5831 | 29.5769 | 25.0316 | 15.275 |
| Menthyl acetate | 1.2672 | 1.1679 | 1.1614 | 1.1552 | 2.0733 | 2.9537 | 1.3802 | 2.5028 | 3.5495 | 3.537 | 3.487 | tr |
| *neo*-Menthyl acetate | 0.526 | 0.282 | 0.4012 | 0.2415 | 0.0256 | 0.3748 | 0.3047 | 0.1016 | 0.0858 | 0.1286 | 0.3583 | tr |
| Methyl salicylate | 0.0406 | tr | tr | tr | 0.0723 | 0.0758 | 0.0444 | 0.1053 | 0.1918 | 0.1699 | 0.0352 | tr |
| Piperitone | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | tr | 0.023 |
| Pulegone | 2.8632 | 5.8326 | 4.2665 | 7.7181 | 5.9733 | 5.8365 | 4.9058 | 7.536 | 5.5668 | 7.3253 | 7.3413 | 1.68E+01 |
| Sabinene | 0.5957 | 0.6093 | 0.5274 | 0.55 | 0.5565 | 0.5931 | 0.5086 | 0.6427 | 0.5787 | 0.6561 | 0.5514 | 1.2514 |
| α-Terpineol | 0.193 | 0.1799 | 0.1836 | 0.1252 | 0.1582 | 0.1897 | 0.1743 | 0.2019 | 0.1901 | 0.2476 | 0.1134 | 2.97E-01 |
| *trans*-Caryophyllene | 0.9617 | 1.1139 | 1.5367 | 1.3894 | 1.7073 | 1.544 | 1.4387 | 0.8484 | 1.3866 | 2.0381 | 1.4958 | 2.1571 |
| Verbenone | 0.1078 | 0.0984 | 0.0809 | 0.0998 | 0.1152 | 0.1075 | 0.1923 | 0.1019 | 0.1729 | 0.2735 | 0.0964 | 0.3445 |
| Viridiflorol | 0.5062 | 0.469 | 0.5471 | 0.5923 | 0.6936 | 0.4205 | 0.4748 | 0.3957 | 0.3827 | 0.6256 | 0.2918 | 0.8732 |
| α-Cadinol | 0.0695 | 0.0811 | 0.0712 | 0.2471 | 0.0573 | 0.0621 | 0.4913 | 0.066 | 0.1361 | 0.1339 | 0.1033 | 0.2444 |
| α-Pinene | 0.7568 | 0.7604 | 0.573 | 0.6573 | 0.6702 | 0.7481 | 0.5929 | 0.8229 | 0.6966 | 0.7914 | 0.6961 | 1.6475 |
| α-thujene | 0.0337 | 0.0265 | 0.0283 | 0.022 | 0.0288 | 0.0337 | 0.0253 | 0.0294 | 0.0271 | 0.0297 | 0.0286 | 0.0764 |
| β-Myrcene | 0.2993 | 0.2727 | 0.2416 | 0.2243 | 0.2883 | 0.2882 | 0.2505 | 0.2716 | 0.2571 | 0.3071 | 0.2572 | 0.6202 |
| β-Pinene | 1.224 | 1.0168 | 0.9952 | 1.0958 | 1.1849 | 1.2465 | 1.0241 | 1.2932 | 1.1859 | 1.2787 | 1.1543 | 2.6212 |
| δ-Cadinene | 0.0122 | 0.014 | 0.0157 | 0.0262 | 0.0457 | 0.017 | 0.0159 | 0.0116 | 0.021 | 0.0219 | 0.0139 | 0.0354 |

components of essential oil of plants treated with nanoparticles were similar to these of control plants. Therefore, application of nanoparticles of iron oxide not only maximized essential oil concentration in the treated plants, but also increased the proportion of useful components. Studies have shown that use of fertilizers can produce similar results. For example, the mean amount of menthol in mint samples grown in nutritive medium with varying levels of potassium was 27.8% (Valmorbida 2002). Essential oils of *M. piperita* samples grown under different levels of fertilizer were investigated by Zheljazkov and Margina (1996). Their results showed that menthol constituted 60.9% of essential oil when grown without fertilizer, while when mineral fertilizer was added its poroprtion decreased to 58.1%.

In contrast, salt concentration had major effect on the composition of essential oil. Environmental factors can have strong effect on this species and cause differences in plant features. Studies showed that some members of Labiatae family, such as *M. piperita*, can quickly adapt to different ecological conditions (Scora, Chang 1997). This is also true for other medicinal plants. Stresses due to abiotic environmental factors like salinity as well as drought can have prominent effect on medicinal herbs (Heidari et al. 2008), as observed for chemical plasticity of essential oil composition. Significant correlations were found between salt concentrations with major essential oil compounds such as menthone, menthol, menthofuran, isoborneol and pulegone. Menthone and menthol are among the most economical important components, which constituted more than 70% percent of essential oil in control plant samples. Various experimental studies have confirmed our findings. For example, Mahmoud and Croteau (2003) suggested that menthone and also menthol, from a qualitative view point, constitute the main compounds of the *M. piperita* essential oil. The economic value of mint essential oil depends on the amount of the mentioned constitutes and also the low percentage of other compounds as menthofuran. The findings of other investigations (Charles et al. 1990; Tabatabaie et al. 2007) on mint grown in various osmotic stress levels produced similar results. Scavroni et al. (2005) showed that biosolid was harmful for formation of menthol. Tounekti et al. (2008) studied the effect of increasing concentrations of NaCl on the essential oil composition of *Rosmarinus officinalis*. The obtained results showed that the content of 1,8-cineole decreased with increasing concentrations of salt, concomitant with a slight increase in content of borneol. Alaei et al. (2014) investigated effects of different concentrations of salinity on the essential composition of *Dracocephalum moldavica*. Their study showed that geranyl acetate and geraniol were the main components and by increased salinity caused low concentration of geranyl acetate.

About 3.52-fold variation was observed in menthone concentration between the used treatments. The differences in menthol concentrations between the samples was also high. Menthol is an organic component made artificially or acquired from essential oils of *M. piperita* or other species of the Labiatae family. This compound is waxy and crystalline; its color is clear or white. Menthol is solid at room temperature and melts slightly above this temperature. The basic form of menthol present in nature is (−)menthol. Investigations confirmed that this compound has local anesthetic and counter irritant intimacy, and it is broadly applied to relieve minor throat irritation (Moghtader 2013).

Since concentrations of major compounds of mint oil such as menthol as well as menthone highly differed under different stress conditions, it can be presumed that the economic importance of its oils widely depend on growing region. We observed that in treatment No. 11 (30 μM Fe nano; 15 mM NaCl) the amounts of useful constitutes were lower, while other unsuitable ones were higher.

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