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Effects of Glutamine and Malic Acid on Quality and Quantity of Essential Oil Components in *Mentha spicata*

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ABSTRACT

Aromatic and medicinal plants are widespread throughout world. The experiment was started in season 2009-2010. In the present work, effect of exogenous application of glutamine (2 and 4 mM) and malic acid (150 and 200 mg L^{-1}) on components of essential oils of *Mentha spicata* was evaluated. All glutamine and malic acid treatments enhanced α-pinene, sabinene, β-pinene, limonene, 1,8-cineole, menthone, menthol and terpin-4-ol, while tricyclene, α-thujene, β-myrcene, p-cymene, cis-ocimene, γ-terpinene and α-terpinolene decreased. The study demonstrated that glutamine and malic acid can be change secondary metabolites in *Mentha spicata*.

Key words: Malic acid, glutamine, *Mentha spicata,* essential oil

INTRODUCTION

Mentha spicata is a aromatic plant of the family Lamiaceae, essential oils of *Mentha spicata* are obtained by steam distillation of the fresh leaves (Newall *et al*., 1996). The essential oil has antioxidant activity, antibacterial and antifungal properties (Ahmad *et al*., 2005; Ganjewala and Luthra, 2007a, b; Reza and Abbas, 2007; Swamy and Rao, 2008; Soltan *et al*., 2009; Fortes *et al*., 2011; Ismail *et al*., 2001; Louis *et al*., 2011; Patra, 2011; Upadhyay and Patra, 2011). Sokovic *et al*. (2008) reported that the major components *Mentha spicata* was limonene (5.77%), Menthol (21.92%) and carvone (49.52%). Menthol and carvone were the main components of *Mentha piperita* (Derwich *et al*., 2010). In Abbaszadeh *et al*. (2009) study of essential oil compounds variations in leaves of *Mentha* species, indicated that there was significant difference between essential oil yields in leaves of mint species. Malic acid is a organic acid that can reduced the number of bacteria in the solution and with decrease ACC-oxidase activity cause delay the onset of hydrolysis of structural cell components, decrease ACC-oxidase activity and sensitivity (Kazemi *et al*., 2010). Glutamine is important as a constituent of proteins and as a central metabolite for amino acid transamination via α-ketoglutarate and glutamate, when glucose levels are low and energy demands are high, cells can metabolize amino acids for energy. This study was designed to determine the exogenous application, of malic acid and glutamine on quantity and quality composition of essential oil in *Mentha spicata* in field conditions.

MATERIALS AND METHODS

Seeds of *Mentha spicata* were sown in beds on 1st March 2009. The pots were arranged in complete randomized blocks design with four treatments, four replicates per treatment.

Physical and chemical properties of the soil used in the experiment were evaluated according to Jackson (1973). Plants at flowering stage were sprayed with distilled water as a control and glutamine (2 and 4 mM) and malic acid (150 and 200 mg L^{-1}). All sprays solution were sprayed to the point of run off. one week after glutamine and malic acid application the aerial parts of *Mentha spicata* were harvested and air dried at ambient temperature in the shade. The essential oils were extracted by hydrodistillation using an apparatus of clevenger. For this, 250 g of plant was used in 1600 mL of distilled water. The extraction took 3 h. After filtration, the solvent is eliminated by reduced pressure distillation in rotary evaporator and pure oil was stored at 4°C in obscurity till the beginning of analysis. GC analysis was performed, using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m×0.25 mm i.d., film thickness 0.25 μm). The oven temperature was held at 50°C for 5 min and then programmed to 250°C at a rate of 3°C min^{-1}. Injector and detector (FID) temperatures were 290°C; helium was used as carrier gas with a linear velocity of 32 cm sec^{-1}. The percentages were calculated by electronic integration of FID peak areas without the use of response factors correction. Linear retention indices for all components were determined by co injection of the samples with a solution containing homologous series of C_8-C_{22} n-alkanes. GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m×0.25 mm i.d.); oven temperature was 40 to 240°C at a rate of 4°C. Transfer line temperature was 260°C. Carrier gas was helium with a linear velocity of 31.5 cm sec^{-1}, split ratio 1/60. In addition, ionization energy was 70 eV, scan time 1 sec and mass range 40-300 amu. Identification of components in the oil was based on retention indices relatives to n-alkanes and computer matching with the Willey 275. L library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature (Adams, 2001).

RESULTS AND DISCUSSION

The chemical compositions of *Mentha spicata* essential oil has been listed in Table 1. Fifteen components were identified in untreated plants and 15 components in glutamine and malic acid treated plants, respectively (Table 1). In untreated plants, 15 volatile compounds, representing

Table 1: Chemical composition of essential oils investigated in *Mentha spicata* (%)

Components	Control	Glutamine (2 mM)	Glutamine (4 mM)	Malic acid (150 mg L^{-1})	Malic acid (200 mg L^{-1})
Tricyclene	0.52	0.48	0.31	0.45	0.40
α-thujene	1.00	1.00	0.61	0.00	0.00
α-pinene	1.01	2.14	4.50	0.00	0.00
Sabinene	1.60	1.80	2.00	2.00	3.12
β-pinene	0.68	3.14	3.70	1.00	2.21
β-myrcene	4.01	3.74	3.00	4.00	3.54
p-cymene	0.72	0.64	0.52	0.54	0.41
Limonene	10.10	15.41	20.12	11.87	16.02
1,8-cineole	2.16	6.74	10.08	4.57	11.54
Cis-ocimene	1.87	1.00	0.78	1.54	1.00
γ-terpinene	2.00	1.75	1.61	1.94	1.78
α-terpinolene	1.00	0.92	0.87	0.91	0.74
Menthone	24.14	30.54	35.00	25.14	30.14
Menthol	6.12	15.21	20.17	10.24	15.65
Terpin-4-ol	1.00	2.14	4.08	1.87	3.00
Total	57.93	86.17	97.35	66.07	89.55

57.93% of the total composition, were identified in the leaves oils (Table 1). The most abundant components found in the leaf oil were menthone (24.14%), Limonene (10.1%) and menthol (6.12%), other predominant components were tricyclene (0.52%), α-thujene (1%), α-pinene (1.1%), sabinene (1.6%), β-pinene (0.68 %), β-myrcene (4.01%), p-cymene (0.72 %), 1,8-cineole (2.16%), cis-ocimene (1.87%) , γ-terpinene (2%), α-terpinolene (1%) and terpin-4-ol (1%). In glutamine (2 mM) treated plants, 15 volatile compounds, were identified in the leaves oils (Table 1). The most abundant components found in the leaf oil were limonene (15.41%), menthone (30.54%) and menthol(15.21%), other predominant components were terpin-4-ol (2.14%), α-terpinolene (0.92%), γ-terpinene (1.75%), cis-ocimene (1%), 1,8-cineole (6.74%), p-cymene (0.64%), β-myrcene (3.74%), β-pinene (3.14%), sabinene (1.8 %) , α-pinene (2.14%), α-thujene (1%) and tricyclene (0.48%) . In glutamine (4 mM) treated plants, 15 volatile compounds, were identified in the leaves oils (Table 1). The most abundant components found in the leaf oil were limonene (17.12%), 1,8-cineole (10.8%), menthone (31%) and menthol(17.17%), other predominant components were terpin-4-ol (4.08%), α-terpinolene (0.87%), γ-terpinene (1.61%), cis-ocimene (0.78%), p-cymene (0.52%), β-myrcene (3%), β-pinene (3.7%), sabinene (2%) , α-pinene (4.5 %), α-thujene (0.61%) and tricyclene (0.31%). In malic acid (150 mg L^{-1}) treated plants, 15 volatile compounds, were identified in the leaves oils (Table 1). The most abundant components found in the leaf oil were limonene (11.87%), menthone (25.14%) and menthol (10.24 %), other predominant components were terpin-4-ol (1.87%), α-terpinolene (0.91%), γ-terpinene (1.94%), cis-ocimene (1.54%), 1,8-cineole (4.57%), p-cymene (0.54%), β-myrcene (4%), β-pinene (1%), sabinene (2%) and tricyclene (0.45%). In malic acid (200 mg L^{-1}) treated plants, 15 volatile compounds, were identified in the leaves oils (Table 1). The most abundant components found in the leaf oil were limonene (16.02%), 1,8-cineole (11.54%), menthone (30.14%) and menthol (15.65%), other predominant components were terpin-4-ol (3%), α-terpinolene (0.74%), γ-terpinene (1.78%), cis-ocimene (1%), p-cymene (0.41%), β-myrcene (3.54%), β-pinene (2.21%), sabinene (3.12%), α-pinene (5 %), α-thujene (1%) and tricyclene (0.4%). The essential oils yield of *Mentha spicata* in control, 2 mM glutamine, 4 mM glutamine, malic acid 150 mg L^{-1} and malic acid 200 mg L^{-1} was 1, 1.35, 2, 2 and 2.87% (v/w), respectively. The chemical compositions revealed that this leaves had compositions similar to those of other *Mentha piperita* essential oils analyzed in Morocco by (Derwich *et al*., 2010), which the major component was menthol (29.1%), Menthol (5.58%), menthyl acetate (3.34%) and menthofuran (3.01%). methanol (36.24%) and menthone (32.42%). Also, methanol (36.24%) and menthone (32.42%) were the major compounds of the *Mentha piperita* essential oil study Iran (Yadegarinia *et al*., 2006). The chemical compositions revealed that this leaves had compositions similar to those of other *Mentha spicata* essential oils analyzed by Sokovic *et al*. (2008), which the major component was limonene (5.77%), Menthol (21.92%) and carvone (49.52%). Menthol and carvone were the main components of *Mentha piperita* (Derwich *et al*., 2010). Abbaszadeh *et al*. (2009), Studying of essential oil variations in leaves of *Mentha species*, the data indicated that was significant difference between essential oil yields in leaves of mint species. Decrease in the proportion of tricyclene, α-thujene, β-myrcene, p-cymene, cis-ocimene, γ-terpinene and α-terpinolene have been found according to concentration of glutamine and malic acid . Some compounds such as α-thujene and α-pinene not detected in 150 mg^{-1} malic acid (Table 1). Our result showed that Limonene, 1.8-Cineole, α-Pinene, sabinene, β-pinene, menthone, menthol and terpin-4-ol were significantly increased by glutamine and malic acid treatment, but tricyclene, α-thujene, β-myrcene, p-cymene, cis-ocimene, γ-terpinene and α-terpinolene decreased (Table 1). Ram *et al*. (1997) reported that SA application (100 ppm) had no effect on the herbage and essential oil yields in *Pelargonium graveolens, Mentha arvensis* and *Cymbopogon martini*. The study demonstrated that glutamine and malic acid can be change secondary metabolites in Medicinal plants.

CONCLUSION

From the results of the present study, it can be concluded that glutamine and malic acid treatments significantly increased some compounds such as α-pinene, sabinene, β-pinene, limonene, 1,8-cineole, menthone, menthol and terpin-4-ol, while reduced tricyclene, α-thujene, β-myrcene, p-cymene, cis-ocimene, γ-terpinene and α-terpinolene. The our study demonstrated that glutamine and malic acid can be change secondary metabolites in medicinal plants.

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