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Effects of Malic Acid and Salicylic Acid on Quality and Quantity of Essential Oil Components in *Rosmarinus officinalis*

[1]M. Kazemi and [2]E. Shirzadeh
[1]Young Researchers Club, Karaj Branch, Islamic Azad University, Karaj, Iran
[2]Young Researchers Club, Khorramabad Branch, Islamic Azad University, Khorramabad, Iran

Corresponding Author: M. Kazemi, Young Researchers Club, Karaj Branch, Islamic Azad University, Karaj, Iran

ABSTRACT

In the present study, effect of exogenous application of salicylic acid (1.5 and 3 mM) and malic acid (100 and 150 mg L^{-1}) on components of essential oils of *Rosmarinus officinalis* was evaluated. All SA and MA treatments enhanced limonene, 1.8-cineole, α-pinene, camphene and camphor, while terpinen-4-ol, sabinene, verbenol, germacrene-D, bornyl acetate, myrcene, α-phelandrene, α-terpinolene and β-caryophyllene decreased. The study demonstrated that SA and MA can be change secondary metabolites in *Rosmarinus officinalis*.

Key words: Malic acid, salicylic acid, *Rosmarinus officinalis*, essential oil

INTRODUCTION

Rosmarinus officinalis is a aromatic plant of the family Lamiaceae, essential oils of *Rosmarinus officinalis* are obtained by steam distillation of the fresh leaves and the yields range from 0.5 to 1.0% (Tewari and Virmani, 1987; Derwich *et al*., 2011). The essential oil of *Rosmarinus officinalis* has antioxidant activity (Peng *et al*., 2005; Wang *et al*., 2008), antibacterial (Moghtader and Afzali, 2009) and antifungal properties (Buchbauer, 2000; Ismail *et al*., 2011; Ahmad *et al*., 2005; Ganjewala and Luthra, 2007a-b; Reza and Abbas, 2007; Pozzatti *et al*., 2008; Swamy and Rao, 2008; Soltan *et al*., 2009; Fortes *et al*., 2011; Louis *et al*., 2011; Patra, 2011; Upadhyay and Patra, 2011; Dudareva *et al*., 2006). Major constituents described for the oil are α-pinene, 1,8-cineole and camphor (Bauer *et al*., 1997). Chalchat and Carry (1993) reported comparison Spain, Morocco and France Rosemary oils composition. For example the level of α-pinene (30-35%) was always higher than 1, 8-cineole (14-20%), which was in turn higher than camphor (7-12%). Salicylic acid is a hormone and its role in the defensive mechanism against biotic and abiotic stresses has been confirmed (Zahra *et al*., 2010). Exogenously added SA also increased the heat tolerance of mustard (Dat *et al*., 2000). MA is a well known organic acid that can reduced the number of bacteria in the solution and decrease ACC-oxidase activity cause delay the onset of hydrolysis of structural cell components, decrease ACC-oxidase activity and sensitivity (Kazemi *et al*., 2010). This study was designed to determine the exogenous application, of MA and SA on quantity and quality composition of essential oil in *Rosmarinus officinalis* in field conditions.

MATERIALS AND METHODS

Seeds of *Rosmarinus officinalis* 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 salicylic acid (1.5 and 3 mM) and malic acid (100 and 150 mg L^{-1}). All sprays solution were sprayed to the point of run off. One week after SA and MA application the aerial parts of *Rosmarinus officinalis* were harvested and air dried at ambient temperature in the shade. The essential oils were extracted by hydrodistilation 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 *Rosmarinus officinalis* essential oil has been listed in Table 1. 20 components were identified in untreated plants and 16 and 14 components in SA and MA treated plants, respectively (Table 1). In untreated plants, 20 volatile compounds, representing 95.37% of the total composition, were identified in the leaves oils (Table 1). Monoterpene hydrocarbons were found to be the major group of compounds, the main one being α-pinene (52.01%) followed Limonene (8.41%). The most abundant components found in the leaf oil were α-pinene (52.01%), other predominant components were terpinen-4-ol (1.01%), sabinene (1%), verbenol (4.12%), limonene (8.41%), 1.8-cineole (2.4 %), linalool (3.02%), camphene (3.17%), camphor (3.11%), germacrene-D (1.08%), β-pinene (2.01%), myrcene (2.11%), α-terpinolene (1%), β-caryophyllene (2.16%), borneol (3%) and cymene (3%). In SA (1.5 mM) treated plants, 16 volatile compounds, were identified in the leaves oils (Table 1). The most abundant components found in the leaf oil were α-pinene (58.36%), other predominant components were Terpinen-4-ol (0.87%), Sabinene (1%), Verbenol (3.12%), Limonene (9.12%), 1.8-Cineole (3.54%), Linalool (2.54%), Camphene (3.9 %), Camphor (3.74%), Germacrene-D (1%), β-pinene(2.12%), myrcene (1.78%), α-terpinolene (0.56%) and β-caryophyllene. In SA (3 mM) treated plants, 16 volatile compounds, were identified in the leaves oils (Table 1). The most abundant components found in the leaf oil were α-pinene (64.04%), other predominant components were Terpinen-4-ol (0.21%), Sabinene (0.92%), Verbenol (2%), Limonene (10.01%), 1.8-Cineole (5.6%), Linalool (2%), Camphene (4.58 %), Camphor (4.86%), Germacrene-D (0.35%), β-pinene (1.41%), myrcene (1.59%), α-terpinolene (0.21%) and β-caryophyllene (0.54%). In MA (100 mg L^{-1}) treated plants, 14 volatile compounds were identified in the leaves oils (Table 1). The most abundant components found in

Table 1: Chemical composition of essential oils investigated (*Rosmarinus officinalis* %)

Components	Control	SA (1.5 mM)	SA (3 mM)	MA (100 mg L^{-1})	MA (150 mg L^{-1})
Terpinen-4-ol	1.01	0.87	0.21	0.78	0.54
Sabinene	1.00	1.00	0.92	0.00	0.00
Verbenol	4.12	3.12	2.00	0.00	0.00
Limonene	8.41	9.12	10.01	8.41	9.00
1.8-Cineole	2.40	3.54	5.60	10.75	10.11
Linalool	3.02	2.54	2.00	2.04	1.36
α-Pinene	52.01	58.36	64.03	55.41	60.54
Camphene	3.17	3.90	4.58	3.78	5.45
Camphor	3.11	3.74	4.86	3.15	7.12
Germacrene-D	1.08	1.00	0.35	0.00	0.00
Bornyl acetate	1.00	0.26	0.11	0.46	0.20
β-pinene	2.01	2.12	1.41	2.14	2.89
Myrcene	2.11	1.78	1.59	1.00	0.34
α-phelandrene	0.50	0.21	0.09	0.10	0.09
para-cymene	0.84	0.00	0.00	0.76	0.24
α-terpinolene	1.00	0.56	0.21	0.64	0.24
Chrysanthenone	0.42	0.00	0.00	0.00	0.00
β-caryophyllene	2.16	1.05	0.54	2.00	1.04
Borneol	3.00	0.00	0.00	0.00	0.00
Geraniol	3.00	0.00	0.00	0.00	0.00
Total	95.37	93.17	98.51	91.42	99.16

the leaf oil were α-pinene (55.41%), other predominant components were Terpinen-4-ol (0.78%), Limonene (8.41%), 1.8-Cineole (10.75 %), Linalool (2.04%), Camphene (3.78 %), Camphor (3.15%), β-pinene(2.14%), myrcene (1%), α-terpinolene (0.64%) and β-caryophyllene (2%). In MA (150 mg L^{-1}) treated plants, 14 volatile compounds, were identified in the leaves oils (Table 1). The most abundant components found in the leaf oil were α-pinene (60.54%), other predominant components were Terpinen-4-ol (0.54%), Limonene (9%), 1.8-Cineole (10.11%), Linalool (1.36%), Camphene (5.45%), Camphor (7.12%), β-pinene(2.89%), myrcene (0.34%), α-terpinolene (0.24%) and β-caryophyllene (1.04%). The essential oils yield of *Rosmarinus officinalis* was 0.90%, it is relatively higher than other plants industrially exploited as a source of essential oils: *Artemisia herba-alba* (0.59%) and *Artemisia absinthium* (0.57%) (Derwich *et al.*, 2009a, b) and this yield is relatively lower than other plants: *Thymus* (1%) (Imelouane *et al.*, 2009) and *Rosmarinus officinalis* (0.48-1.75%) (Angioni *et al.*, 2004). Kadri *et al.* (2011) found that a-pinene, 1,8-cineole, camphor, verbenone and borneol constituted and represented about 77.32% of the total *R. officinalis* oil. Decrease in the proportion of Terpinen-4-ol, Verbenol, Linalool, Germacrene-D, Bornyl acetate, myrcene, α-phelandrene, α-terpinolene and β-caryophyllene have been found according to concentration of SA and MA. Some compounds such as para-cymene, chrysanthenone, borneol and geraniol detected in control (Table 1). Present result showed that Limonene, 1.8-Cineole, α-Pinene, Camphene and Camphor were significantly increased by SA and MA treatment, but Terpinen-4-ol, Sabinene, Verbenol, Germacrene-D, Bornyl acetate, myrcene, α-phelandrene, a-terpinolene and β-caryophyllene 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 martinii*. Among salicylates, free salicylic acid is considered to be the most biologically active from as a mediator of plant stress responses, including disease and systemic acquired resistance (Lee *et al*., 1995). The study demonstrated that SA and MA can be change secondary metabolites in Medicinal plants.

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