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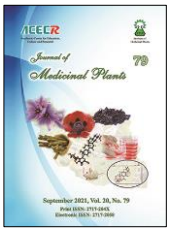

Research Article

Changes in essential oil composition of peppermint (*Mentha x piperita* L.) affected by yeast extract and salicylic acid foliar application

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ABSTRACT

Background: Peppermint (*Mentha x piperita* L.) is one of the most important medicinal plants which used in food, pharmaceutical, perfumery, and flavoring industry. **Objective:** This study was planned to investigate the effects of foliar application of salicylic acid and yeast extract on production of valuable essential oil components in peppermint. **Methods:** A completely randomized design experiment with nine treatments consisting salicylic acid (40, 80, 160 and 320 mg/l), yeast extract (0.25, 0.75, 1 and 1.5 g/l) and distilled water (control) with three replications was carried out under greenhouse conditions. **Results:** In total, forty compounds were identified in the essential oils of the plant aerial parts. Menthone, menthol, piperitone, isopulegol and *γ*-terpinene were the major compounds of the oils studied. Menthone and menthol were 16.69 % and 14.39 % of the essential oils, respectively. Salicylic acid and yeast extract were increased menthone, neomenthol, piperitone, *γ*-terpinene and isomenthol acetate production 42, 60, 39, 59 and 34 % higher than control plants, respectively. Foliar application with 320 mg/l salicylic acid gave the best result in the enhancement of the major essential oil components of treated plants. The results of correlation between essential oil constituents showed that the neomenthol content had a significant positive correlation with menthone ($r = 0.865^{**}$), *γ*-terpinene ($r = 0.848^{**}$) and negative correlation with isopulegol ($r = -0.886^{**}$). **Conclusion:** The quality of essential oil of *M. piperita* were influenced by the foliar application of salicylic acid and yeast extract at the appropriate concentrations. Elicitation by 320 mg/l salicylic acid was the optimum treatment for menthone, neomenthol, *γ*-terpinene and piperitone production.

1. Introduction

Secondary metabolites have complex structures to be manufactured by chemical synthesis and thus frequently extracted from naturally grown or cultivated plants [1]. Essential oils are natural complex volatile secondary metabolites that are often obtained from various aromatic plants using the hydro-distillation technique [2]. Peppermint (*Mentha x piperita* L.), a perennial herbaceous medicinal plant of Lamiaceae family is a natural hybrid from *M. aquatica* × *M. spicata*. The plant is cultivated

Abbreviations: GC-MS, Gas chromatography–mass spectrometry; SE, Standard error; C.V., Coefficient of variation

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in a temperate region of Europe, Asia, United States, India and Mediterranean countries due to their commercial value and distinct aroma [3]. It is an important medicinal and aromatic herb worldwide, in addition to its potential uses as a flavoring agent, in cosmetics, and pharmaceutical products among others [4, 5]. Leaves of *M. piperita* contain 1.2-3.9 % (v/w) of essential oils and more than 300 recognized components. The terpenes with about 52 % of monoterpenes and 9 % of sesquiterpenes are the most important components of peppermint leaves [6]. The composition of the essential oil isolated from aerial parts of *M. piperita* has been a subject of extensive studies [3, 7-11]. Peppermint essential oil containing high concentrations of menthol and menthone are used in traditional medicine to treat various conditions including infections and also as insect repellant. Various *in vitro* and *in vivo* studies have documented the biological properties of menthol such as its analgesic, antibacterial, antifungal, anaesthetic and penetration-enhancing effects as well as chemopreventive and immunomodulating actions [5, 12]. Menthol is one of the most important flavouring additives besides vanilla and citrus. The demand for menthol is high and it was previously estimated that the worldwide use of menthol was 30 - 32,000 metric tonnes per annum [12]. Most of the investigations have shown that the major constituents of essential oils in *M. piperita* were menthol and menthone [7, 9, 10]. The quality of medicinal plants used for the production of pharmacologically useful compounds is usually assessed by the content of biologically active compounds [13]. Several methods such as using of biotic or abiotic elicitor can be a suitable way to increase the production of valuable secondary metabolites in medicinal plants [14], which is currently being implemented extensively owing to its low cost and simplicity of usage [15]. Elicitation is the process of inducing or enhancing synthesis of secondary metabolites by the plants to ensure their survival, persistence and competitiveness [16]. Salicylic acid is a hormone-like substance that plays an important role in the regulation of plant growth and development [7, 17]. In several studies, the effect of salicylic acid on production of many bioactive compounds in medicinal plants was confirmed [13, 18-20]. Yeast extract is one of the biotic elicitors that can result in the improvement of secondary metabolites content. The stimulating influence of yeast extract on secondary metabolites was confirmed in several studies [20-22]. Therefore, in order to economically produce secondary metabolites, it is necessary to use elicitors optimally in medicinal plants. Because of the industrial use of *M. piperita,* it is important to develop an optimal method to obtain standardized plant material with specific quality parameters. Thus, the choice of proper concentration of salicylic acid or yeast extract foliar application can be a suitable strategy to increase the production of the main constituents of *M. piperita* essential oil. Due to the importance of these medicinal compounds, peppermint was therefore studied and explained and the correlation of these compounds was determined.

2. Materials and Methods

2.1. Experimental field

The experiment was conducted under greenhouse conditions from March to July 2017 at the greenhouse of the Malayer Greenhouse Town, Malayer Municipality (latitude: 34° 19´ N, longitude: 48° 51´ E, altitude: 1725 m above sea level), located in the west of Iran and southeast of Hamadan province.

2.2. Plant materials, experimental set up and treatments

In this study, the plant rhizome (Code number: 13723) was obtained from Pakanbazr Company (Isfahan, Iran) (http://www.pakanbazr.com/). For cultivation, 54 clay pots with a height of 25 cm in diameter (radius) of 20 cm were used. All pots were containing a 1:1:1 uniform mixture of field soil, rotten leaf soil and sand. Three rhizomes of peppermint with 3 to 5 cm long were planted in each clay pot in depth of 3 to 5 cm and a thin layer of rotten manure was poured on them and irrigation was carried out immediately. Plants were grown in a naturally-lit greenhouse where relative humidity ranged of 50-55 % and average temperature was 25 ± 3°C during the experimental period. During the experimental period, plants were irrigated two to three times a week as required.

2.3. Experimental design and treatments

In this study, a completely randomized design experiment with nine treatments and three replications (two pots in each replication) was carried out under greenhouse conditions to investigate the effect of four salicylic acid doses (40, 80, 160 and 320 mg/l), four yeast extract doses (0.25, 0.75, 1 and 1.5 g/l) and distilled water (control) on major components of peppermint essential oil. For this purpose, yeast extract was dissolved in distilled water. Stock solution of salicylic acid was made by dissolving weighed quantity in minimum quantity of ethanol and final volume made by distilled water. Foliar application of the elicitors on *M. piperita* aerial parts was performed at 40 % flowering stage. In this experiment, distilled water foliar application was used as control. For each treatment, six pots having 3 plants per pot was used. Foliar sprays of the elicitors were done with a portable sprayer, early in the morning and one hour after sunrise. Spraying was done completely on aerial parts of the plants [19]. To evaluate the essential oil composition of *M. piperita*, the sampling was performed 5 days after the foliar application of the elicitors. The samples were dried in the shadow with proper ventilation and normal room temperature (25-30 °C) for 5 days. Each sample was placed in a plastic bag separately and their lids were closed. The characteristics such as amount of essential oil components were measured using gas chromatography-mass spectrometry (GC-MS).

2.4. Essential oil isolation and analysis procedure

Essential oil of each sample was isolated from chopped, dried aerial parts of *M. piperita* by hydro-distillation procedure. Briefly, 50 g of *M. piperita* was transferred into a 1 L round-bottom flask with Clevenger apparatus. Water distillation was performed for 3 h at 100 °C. The collected essential oil was dried over anhydrous Na_2SO_4 and stored in a dark bottle at 4 °C until tested and analyzed. The essential oil components of *M. piperita* affected by salicylic acid and yeast extract were determined by gas chromatography-mass spectrometry (GC-MS) using a Hewlett -Packard 5890 gas chromatograph equipped with a flame ionization detector (HP-5970 mass-selective detector-USA) and a 50 m × 0.20 mm HP-5 (cross-linked Phenyl–Methyl Silicon) column with a 0.25 µm film thickness. The ionization energy of the sample components was set to 70 eV. The flame ionization detector (FID) was maintained at 250 ºC. The temperature program was ranged over 100-250 °C at a rate of 4 °C/min. The carrier gas was helium while the flow through the column and the split ratio were set to 1 ml/min and 100:1, respectively. The individual constituents were identified by their identical

retention index and compared to those in reference books and articles using mass spectra of standard compounds and information contained in a computer library [23].

2.5. Statistical analysis

Data was statistically analyzed by one-way analysis of variance (ANOVA) using SPSS software version 16.0 (SPSS, Inc., Chicago, IL, USA). The mean values were compared using Duncan's multiple range test at $P < 0.01$ significant level. The values are presented as mean ± standard error (SE) of three replications. Correlation coefficient (r) between essential oil components was estimated by Pearson method.

3. Results

Essential oil of *M. piperita* aerial parts, were analyzed and quantified by gas chromatography-mass spectrometry (GC-MS) (Fig. 1). The effects of salicylic acid and yeast extract foliar application on essential oil components of the plant aerial parts were shown in Tables 1 & 2. Results of GC-MS indicated that a total of 40 compounds were identified in the essential oil from the aerial parts of peppermint under salicylic acid and yeast extract treatments. The chemical components are given in Table 2. The results showed that the major components of peppermint essential oil were menthone, menthol, piperitone, isopulegol and *γ*-terpinene, respectively (Totally 44.8 % to 54.3 %). In this study, menthone and menthol were 16.69 % and 14.39 % of the essential oils, respectively. The results of analysis of variance (ANOVA) showed that the effect of different concentrations of salicylic acid and different concentrations of yeast extract on production of 12 major compounds including menthone, neomenthol, isomenthol acetate, piperitone, isopulegol, *γ*-terpinene, *β*-pinene, myrcene, eucalyptol, dihydrocarvone, paramentol, hexyl isovalerate, caryophyllene oxide was highly significant at 1 % probability level ($P < 0.01$), two constituents including *β*-ocimene and dihydro carvyl acetate was significant at 5 % probability level ($P < 0.05$) (Table 1) and on 26 compounds (camphene, sabinene, *β*-pinene, (-)-*β*-pinene, octenol, *α*-phellandrene, *α*-terpinene, isoterpinolene, *p*-cymene, limonene, 1,8-cineole, linalool, *β*-terpineol, isomenthone, menthofuran, lavandulol, l-(+)-menthol, myrtenal, *p*-mentenol, carvone, borneol, dihydro carvyl actate, *β*-bourbonene, spathulenol, *β*-elmene, humulene epoxide) was not significant at 5 % probability level ($P > 0.05$) in *M. piperita* (Data not shown). As shown, salicylic acid and yeast extract significantly altered the amount of 14 constituents of essential oil of *M. piperita* (Table 1). Mean comparison for effects of salicylic acid and yeast extract on essential oil components (%) in *M. piperita* is shown in Table 2. The results of mean comparison showed that the amount of menthone varied from 12.13% to 17.20 %. The highest menthone production 17.20 % was obtained at 320 mg/l salicylic acid treated plants which was 42 % higher compared to control. This was followed by 160 mg/l salicylic acid (15.97 %) treatment. The lowest level of menthone production was observed in control (12.13 %). Also, the results showed that with increasing the amount of yeast extract from 0.75 to 1.5 g/l, the amount of menthone increased. It is observed that with increasing the amount of salicylic acid from 160 to 320 mg/l, the amount of menthone has increased from 15.97 to 17.20 % (Table 2). The highest neomenthol content (4.9 %) was approximately 60 % greater than control level, and was obtained in the plants treated with 320 mg/l salicylic acid. This was followed by 160 mg/l salicylic acid (4.6 %). The lowest level of neomenthol content (3.08 %) was observed in

control plants. The results showed that with increasing the concentration of salicylic acid from 40 to 320 mg/l, the amount of neomenthol increased (3.7, 4.2, 4.6 and 4.9 %, respectively). In other words, with increasing salicylic acid concentration, the amount of neomenthol has increased. The results of mean comparison showed that with increasing the amount of yeast extract from 0.25 to 1.5 g/l, the amount of neomenthol increased from 3.20 to 3.93 %. The results of mean comparison showed that the amount of piperitone varied from 6.40 to 8.90 %. The highest piperitone production (8.90 %) was obtained at 320 mg/l salicylic acid treated plants that was 39 % higher compared to control (6.40 %). This was followed by 160 mg/l salicylic acid (8.30 %). The effect of different concentrations of yeast extract on the amount of piperitone was not significant compared to the control. The results showed that with elicitation by 320 mg/l salicylic acid the accumulation of isopulegol was 27 % fewer than the control plants. The lowest amount of isopulegol production (5.1 %) was obtained at 320 mg/l salicylic acid treated plants and then the treatment of salicylic acid with a concentration of 160 mg/l was 5.4 % of essential oil. It is observed that with increasing salicylic acid concentration, the amount of isopulegol has a decreasing trend. Four levels of yeast extract did not have a significant effect on the production of isopulegol in peppermint in greenhouse conditions, but treatments of 80, 160 and 320 g/l salicylic acid reduced the amount of isopulegol. As shown, the highest γ-terpinene content (6 % of essential oil) was obtained at 320 mg/l salicylic acid treated plants which was 59 % higher compared to control. This was followed by 160 mg/l and 80 mg/l salicylic acid (5.63 and 5.27 %, respectively). These amounts, considering the amount of γ-terpinene in the control plants (3.77 %), could be considered as significant. It is observed that external application of salicylic acid has a positive effect on the production of γ-terpinene peppermint essential oil compared to yeast extract. Low concentrations of yeast extract are not significantly different from the control. It is observed that only 1.5 g/l of yeast extract has significantly different from control. The results of mean comparison for effects of salicylic acid and yeast extract on essential oil components (%) in *M. piperita* showed that the highest isomenthol acetate production 7.30 % was obtained at 40 mg/l salicylic acid treated plants that was 34 % higher compared to control (5.43 %). The results showed that with increasing the concentration of yeast extract, the percentage of isomenthol acetate increased and in contrast, with increasing the concentration of salicylic acid from 40 to 320 mg/l, it was observed that the amount of this essential oil compound decreased. Isomenthol acetate content increased significantly only with 40 mg/l salicylic acid application ($P < 0.01$).

Pearson correlation coefficient was performed by SPSS software to calculate the relationship between the major constituents of peppermint essential oil (Table 3). In the present study, it was found that salicylic acid and yeast extract, while affecting the amount of some of the major constituents of peppermint essential oil, led to a significant reduction in a number of essential oil constituents. The most significant negative correlation coefficients between isopulegol composition and other major essential oil constituents were neomenthol ($r = -0.886^{**}$), γ-terpinene ($r = -0.879^{**}$), menthone ($r = -0.813^{**}$) and piperitone ($r = -0.712^{**}$). In other words, as the amount of major components of the essential oil increased, the amount of isopulegol had an inverse correlation and decreased. The results showed that the menthone content had a

significant positive correlation with *γ*-terpinene (r = 0.881**, at P < 0.01 significant level) and had a significant negative correlation with isopulegol, *β*-pinene, myrcene, eucalyptol and *β*-ocimene. The results of correlation between essential oil constituents showed that the neomenthol content had a significant positive correlation with menthone (r = 0.865**, at P < 0.01 significant level), *γ*-terpinene (r = 0.848**) and had a significant negative correlation with the compounds isopulegol, menthofuran, eucalyptol and *β*-ocimene; So that it had a negative correlation with isopulegol (r = -0.886**). The results showed that the *γ*-terpinene content had a significant positive correlation with menthone (r = 0.881**, at P < 0.01 significant level), neomenthol (r = 0.848**), piperitone (r = 0.713**) and hexyl isovalerate (r = 0.613**) and had a significant negative correlation with isopulegol (r = -0.879**). In this study, the major components of peppermint essential oil often had a positive and significant relationship with each other.

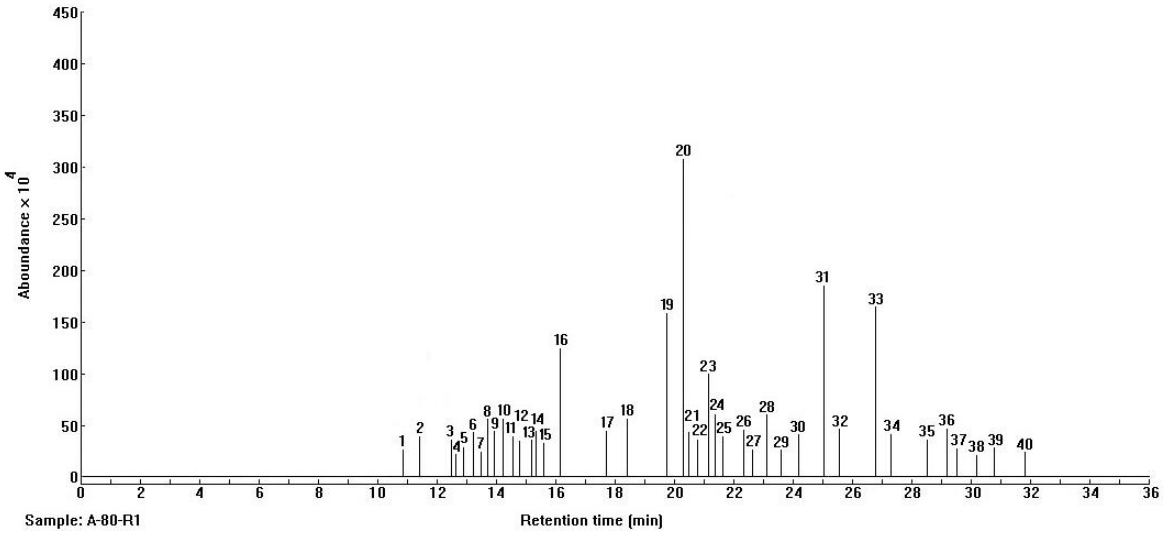

Fig. 1. GC-MS chromatogram of *M. piperita* essential oil treated with 80 mg/l salicylic acid foliar application (horizontal axis is the time diagram and vertical axis is the frequency)

Table 1. Analysis of variance for the effect of salicylic acid and yeast extract foliar application on essential oil components (%) in *M. piperita*

Source of variation (S.O.V)	df	Mean of Squares						
		β-Pinene	Myrcene	Eucalyptol	*γ*-Terpinene	Isopulegol	Menthone	Neomenthol
Treatment	8	0.148**	0.213**	0.328**	1.695**	2.012**	7.302**	1.169**
Error	18	0.036	0.023	0.055	0.098	0.122	0.262	0.067
Total	26							
C.V. (%)		16.47	14.42	19.12	6.75	5.45	3.48	6.73
Source of variation (S.O.V)	**df**	**Dihydrocarvone**	**Hexyl isovalerate**	**Piperitone**	**Isomenthol acetate**	**Caryophyllene oxide**	***β*-Ocimene**	**Dihydrocarvyl actate**
Treatment	8	0.162**	0.519**	2.097**	1.251**	0.141**	0.227*	0.101*
Error	18	0.029	0.024	0.250	0.129	0.034	0.063	0.031
Total	26							
C.V. (%)		9.62	22.85	6.90	19.99	13.64	22.17	18.08

*, ** Significantly different at the 5 and 1 % probability level, respectively

Table 2. Mean comparison for effects of salicylic acid and yeast extract on essential oil components (%) in *M. piperita*

Compounds (%)	RT (min)	Treatment								
		Control	YE-0.25	YE-0.75	YE-1	YE-1.5	SA-40	SA-80	SA-160	SA-320
***α*-Pinene**	10.87	1.40^{a}	1.20^{a}	1.27^{a}	1.07^{a}	1.20^{a}	1.13^{a}	0.97^{a}	0.97^{a}	1.10^{a}
Camphene	11.33	1.83^{a}	1.70^{a}	1.63^{a}	1.60^{a}	1.77^{a}	1.63^{a}	1.63^{a}	1.57^{a}	1.50^{a}
Sabinene	12.43	2.10^{a}	1.73^{a}	1.77^{a}	1.83^{a}	1.63^{a}	1.83^{a}	1.77^{a}	1.73^{a}	1.67^{a}
***β*-Pinene**	12.64	1.63^{a}	1.30^{ab}	1.03^{b}	1.00^{b}	1.13^{b}	1.23^{ab}	1.03^{b}	1.13^{b}	0.87^{b}
(-)-*β*-Pinene	12.96	1.73^{a}	1.83^{a}	1.47^{a}	1.33^{a}	1.63^{a}	1.63^{a}	1.47^{a}	1.37^{a}	1.43^{a}
Octenol	13.27	2.13^{a}	1.80^{a}	1.87^{a}	1.60^{a}	1.67^{a}	1.50^{a}	1.80^{a}	1.67^{a}	1.67^{a}
Myrcene	13.50	1.43^{a}	1.57^{a}	1.07^{b}	0.90^{b}	0.97^{b}	0.80^{b}	0.90^{b}	0.97^{b}	0.87^{b}
***α*-Phellandrene**	13.76	2.47^{a}	2.57^{a}	2.30^{a}	2.77^{a}	2.87^{a}	2.63^{a}	2.30^{a}	2.43^{a}	2.37^{a}
***α*-Terpinene**	13.95	1.83^{a}	1.93^{a}	1.93^{a}	1.90^{a}	1.60^{a}	1.43^{a}	1.93^{a}	1.63^{a}	1.57^{a}
Isoterpinolene	14.26	2.27^{a}	2.20^{a}	2.07^{a}	1.90^{a}	2.10^{a}	2.17^{a}	2.10^{a}	2.03^{a}	2.03^{a}
***p*-Cymene**	14.69	1.90^{a}	2.07^{a}	1.80^{a}	1.67^{a}	1.57^{a}	1.70^{a}	1.63^{a}	2.07^{a}	1.37^{a}
Eucalyptol	14.88	1.90^{a}	1.57^{ab}	0.90^{c}	1.20^{bc}	1.20^{bc}	1.17^{bc}	1.17^{bc}	1.13^{bc}	0.80^{c}
Limonene	15.16	2.03^{a}	1.90^{a}	2.07^{a}	1.97^{a}	1.73^{a}	1.83^{a}	1.73^{a}	1.60^{a}	1.53^{a}
1,8-Cineole	15.30	2.03^{a}	2.00^{a}	1.97^{a}	1.93^{a}	2.00^{a}	1.90^{a}	1.90^{a}	1.90^{a}	1.90^{a}
***β*-Ocimene**	15.66	1.57^{a}	1.47^{a}	1.30^{ab}	1.10^{abc}	0.80^{c}	1.17^{abc}	1.10^{abc}	0.80^{c}	0.90^{bc}
***γ*-Terpinene**	**16.19**	**3.77^{f}**	**4.03^{ef}**	**4.23^{def}**	**4.37^{def}**	**4.70^{cde}**	**4.87^{bcd}**	**5.27^{abc}**	**5.63^{ab}**	**6.00^{a}**
Linalool	17.78	2.00^{a}	1.90^{a}	1.90^{a}	1.90^{a}	1.70^{a}	1.97^{a}	1.77^{a}	1.87^{a}	1.80^{a}
***β*-Terpineol**	18.38	1.93^{a}	1.80^{a}	2.17^{a}	2.00^{a}	1.87^{a}	1.67^{a}	2.10^{a}	1.80^{a}	1.63^{a}
Isopulegol	**19.81**	**7.00^{ab}**	**7.63^{a}**	**7.10^{ab}**	**6.77^{abc}**	**6.40^{bc}**	**6.30^{bc}**	**6.00^{cd}**	**5.40^{de}**	**5.10^{e}**
Menthone	**20.18**	**12.13^{f}**	**13.03^{ef}**	**13.83^{de}**	**14.57^{cd}**	**15.70^{bc}**	**14.70^{bcd}**	**15.40^{bc}**	**15.97^{b}**	**17.20^{a}**
Isomenthone	20.50	2.10^{a}	1.90^{a}	1.93^{a}	2.10^{a}	1.73^{a}	2.10^{a}	1.93^{a}	1.90^{a}	1.97^{a}
Menthofuran	20.86	2.00^{a}	1.90^{a}	2.07^{a}	1.90^{a}	1.83^{a}	1.77^{a}	1.70^{a}	1.93^{a}	1.33^{a}
Neomenthol	21.11	3.07^{e}	3.20^{e}	3.40^{de}	3.60^{cde}	3.93^{cd}	3.70^{cde}	4.20^{bc}	4.60^{ab}	4.90^{a}
Lavandulol	21.40	2.30^{a}	2.53^{a}	2.70^{a}	2.53^{a}	2.53^{a}	2.30^{a}	2.53^{a}	2.53^{a}	2.40^{a}
l-(+)-Menthol	21.73	1.97^{a}	1.87^{a}	1.73^{a}	1.87^{a}	1.67^{a}	1.77^{a}	1.80^{a}	1.67^{a}	1.83^{a}
Myrtenal	22.31	2.070^{a}	1.80^{a}	2.00^{a}	1.93^{a}	1.70^{a}	1.87^{a}	1.87^{a}	1.73^{a}	1.77^{a}
Dihydrocarvone	22.66	1.13^{b}	1.70^{a}	1.17^{b}	1.00^{b}	1.10^{b}	0.93^{b}	1.03^{b}	1.10^{b}	0.93^{b}
***p*-Mentenol**	23.08	2.90^{a}	2.40^{a}	2.63^{a}	2.87^{a}	2.83^{a}	3.00^{a}	2.67^{a}	2.57^{a}	2.87^{a}
Hexyl isovalerate	23.60	0.00^{c}	0.00^{c}	0.70^{ab}	1.03^{a}	0.57^{b}	1.00^{a}	1.00^{a}	0.80^{ab}	1.00^{a}
Carvone	24.11	1.83^{a}	1.93^{a}	1.83^{a}	1.90^{a}	1.93^{a}	1.80^{a}	1.90^{a}	1.87^{a}	1.90^{a}
Piperitone	**25.05**	**6.40^{c}**	**6.60^{c}**	**6.87^{c}**	**6.80^{c}**	**7.50^{bc}**	**6.87^{c}**	**7.00^{c}**	**8.30^{ab}**	**8.90^{a}**
Borneol	25.60	1.93^{a}	1.87^{a}	1.83^{a}	1.80^{a}	1.73^{a}	1.67^{a}	1.73^{a}	1.73^{a}	1.60^{a}
Isomenthol acetate	**26.81**	**5.43^{de}**	**5.23^{e}**	**5.73^{bcde}**	**6.20^{bcd}**	**6.40^{bc}**	**7.30^{a}**	**6.43^{b}**	**5.83^{bcde}**	**5.50^{cde}**
Dihydro carvyl actate	27.21	1.93^{abc}	1.73^{bcd}	2.07^{a}	2.00^{ab}	1.67^{bcd}	1.63^{cd}	1.67^{bcd}	1.70^{bcd}	1.53^{d}
***β*-Bourbonene**	28.52	1.90^{a}	1.83^{a}	1.77^{a}	1.73^{a}	1.83^{a}	1.73^{a}	1.40^{a}	1.33^{a}	1.47^{a}
Spathulenol	29.14	1.77^{a}	1.63^{a}	2.00^{a}	1.90^{a}	1.70^{a}	1.60^{a}	2.00^{a}	1.70^{a}	1.77^{a}
***β*-Elmene**	29.52	0.97^{a}	1.10^{a}	1.33^{a}	1.13^{a}	0.90^{a}	0.77^{a}	0.87^{a}	0.90^{a}	0.87^{a}
Dihydrocarvyl acetate	30.18	1.33^{a}	1.30^{a}	0.87^{a}	0.87^{a}	0.77^{a}	1.03^{a}	1.00^{a}	0.77^{a}	0.83^{a}
Caryophyllene oxide	30.86	1.63^{a}	1.63^{a}	1.27^{ab}	1.00^{b}	1.10^{b}	1.30^{ab}	1.33^{ab}	1.47^{ab}	1.43^{ab}
Humulene epoxide	31.90	1.37^{a}	1.43^{a}	1.27^{a}	1.17^{a}	1.30^{a}	1.30^{a}	0.87^{a}	1.07^{a}	1.00^{a}

Means followed by similar letter (s) in each row are not significantly different by Duncan's multiple range test at $P < 0.01$. The values are mean of three replicates ± standard error (SE). RT: Retention time (min). YE: Yeast extract (g/l). SA: Salicylic acid (mg/l).

Table 3. Pearson's correlation coefficients between some essential oil compositions in peppermint under salicylic acid and yeast extract foliar application

Compounds	Piperitone	Hexyl isovalerate	Neomenthol	Menthofuran	Menthone	Isopulegol	γ-Terpinene	β-Ocimene	Eucalyptol	Myrcene	β-Pinene
β-Pinene	-0.409*	-0.697**	-0.519**	0.421*	-0.531**	0.385*	-0.478*	0.229	0.632**	0.506**	1
Myrcene	-0.405*	-0.826**	-0.640**	0.378	-0.701**	0.636**	-0.600**	0.487**	0.550**	1	-
Eucalyptol	-0.361	-0.633**	-0.576**	0.311	-0.638**	0.460*	-0.545**	0.430*	1	-	-
β-Ocimene	-0.618**	-0.464*	-0.588**	0.090	-0.758**	0.561**	-0.631**	1	-	-	-
γ-Terpinene	0.713**	0.613**	0.848**	-0.482*	0.881**	-0.879**	1	-	-	-	-
Isopulegol	-0.712**	-0.554**	-0.886**	0.425*	-0.813**	1	-	-	-	-	-
Menthone	0.739**	0.683**	0.865**	-0.357	1	-	-	-	-	-	-
Menthofuran	-0.387*	-0.244	-0.493**	1	-	-	-	-	-	-	-
Neomenthol	0.748**	0.578**	1	-	-	-	-	-	-	-	-
Hexyl isovalerate	0.375	1	-	-	-	-	-	-	-	-	-
Piperitone	1	-	-	-	-	-	-	-	-	-	-

*, ** Significantly different at the 5 and 1 % probability level, respectively

4. Discussion

The quality of plants used for production of pharmacological compounds is usually assessed by secondary metabolite content [13]. High-quality oils of *M. piperita* are characterized by a complex compositional balance of monoterpenes with high menthol, moderate menthone, and low pulegone and menthofuran quantities [26]. In this

study, the identified essential oil compounds of *M. piperita* aerial parts are listed in Table 2. Elicitation effects of salicylic acid and yeast extract were investigated and 40 compounds were identified in peppermint essential oil using GC-MS that menthone and menthol were major components (16.69 % and 14.39 % of the essential oils, respectively). To our best knowledge, results are consistent with other studies, wherein menthol and menthone were the most abundant constituents in *M. piperita* essential oils [7-10, 12]. Similarly, it was observed that in the aerial parts of cultivated *M. piperita* plants, a total of 39 compounds were identified in essential oil by hydro-distillation method. The main components in the hydro-distillation procedure were menthol (45.34 %), menthone (16.04 %) and menthofuran (8.91 %) [9]. It was reported that a total of 28 compounds were identified in the essential oil from peppermint aerial parts under salicylic acid treatments and the major constituents (more than 70 %) were menthol, menthone, and isomenthone, respectively [7]. It was observed that a total of 29 compounds were identified in the essential oil from aerial parts of peppermint and the main constituents in the essential oil were camphane (14.01 %), menthone (13.89 %), menthol (12.37 %) *β*-pinene (7.62 %) and pulegone (6.41 %) [8]. The results of an investigation showed that the major components of the oil were menthone, menthol, menthofuran, pulegone, 1,8-cineole, and menthyl acetate [10]. In another experiment, it was found that the major compounds identified were isomenthone (27.4 %), menthol (24.3 %), menthone (9.2 %), limonene (5.8 %), 1,8-cineole (5.6 %), menthofuran (4.4 %) and isomenthol (3.2 %) [12]. In addition, results of an investigation indicated that a total of 33 compounds were detected in the essential oil samples of peppermint treated with different salicylic acid concentrations with menthone (15.8-18.1 %), menthol (46.3-47.4 %), methyl acetate (8.5-9.7 %) and 1,8-cineole (4.3-4.6 %) being the major ones [19]. It is observed that in all researches, menthol and menthone were the main constituents of peppermint essential oil. A comparison of our results with the previous reports suggests some variation in quantities and quality of components within the essential oil of the plants. The phytochemical variability of *M. piperita* oils is may be due to the geographical conditions of the plant sample, agronomic practices, climatic, harvesting time and drying method as well as essential oils extraction procedure [3, 24-25]. The variation in the percentage of some constituents within the essential oil showed trend as a result of the salicylic acid and yeast extract exposure treatment. Our experimental results show that essential oil compounds of *M. piperita* aerial parts can be significantly stimulated by both biotic and abiotic elicitors. Salicylic acid from 40-320 mg/l and yeast extract from 0.25-1.5 g/l exhibited different eliciting effects on major components of *M. piperita*. The lowest level of major components production was observed in control plants that distilled water was used for foliar application. In present study, with increasing salicylic acid concentration, the amount of menthone, neomenthol, piperitone, *γ*-terpinene have increased. The highest percentage of those components were obtained at 320 mg/l salicylic acid treated plants that were 42, 60, 39 and 59 % higher compared to control. Several reports have shown that exogenous application of salicylic acid has a positive effects on production of many bioactive compounds in medicinal plants [7, 13, 18, and 19]. Similarly, it was reported that the foliar application by 10 mM of salicylic acid and 1.5 mg/l indol-3-acetic acid increased major essential oil components of

M. piperita and *Melissa officinalis* aerial parts [7]. In another experiment, it was found that foliar treatment of lemon balm plants with salicylic acid considerably enhanced the monoterpene oxygenated and sesquiterpenes secondary metabolites [18]. It was found that the main oil components were not markedly affected by different salicylic acid treatments. However, the maximum concentration of menthol (47.4 %) was observed when 150 mg/l salicylic acid applied [19] which this is not in agreement with the results obtained in this study. The findings of the present study substantiated the role of salicylic acid as an elicitor that with increasing the amount from 160 to 320 mg/l the content of main constituents of essential oil in aerial parts of *M. piperita* except isopulegol increased. The results of GC-MS analysis of peppermint aerial parts showed that yeast extract significantly altered the amount of some of the main constituents. In present study, the results showed that with increasing the concentrations of yeast extract from 0.75 to 1.5 g/l, the amount of menthone, neomenthol and *γ*-terpinene increased. Also, four levels of yeast extract did not have a significant effect on the production of piperitone and isopulegol compared to the control. The stimulating influence of yeast extract on secondary metabolites was confirmed in several studies [1, 20-22]. The majority of biotic elicitors are recognized by specific receptors bound to the cell membrane. These stimulants are then transferred to the cell by a signal transduction system, producing changes that ultimately lead to the formation of phytoalexins [27]. In general, it was clearly observed from Table 2 that different types of elicitors exhibited distinct influences on the phytochemical accumulation in *M. piperita*. This indicated that the sensitivity of biologically active compounds biosynthesis toward elicitation varied with the elicitors used in *M. piperita*. In this research, correlation coefficient of essential oils compositions in peppermint under salicylic acid and yeast extract foliar application was calculated. In general, often the major constituents of peppermint essential oil in the conditions of this study had a positive and significant relationship with each other. In another experiment, it was found that most of the major constituents of the essential oil of *M. piperita* and *M. officinalis* both had significant positive correlation with each other [7] which was in agreement with the results of this study.

5. Conclusion

In conclusion, the quality of essential oil of *M. piperita* were influenced by the foliar application of salicylic acid and yeast extract at the appropriate concentrations. Forty compounds were identified in peppermint essential oil that the major components were menthone, menthol, piperitone, isopulegol and *γ*-terpinene, respectively. Salicylic acid was effective at high concentration (320 mg/l) for menthone, *γ*-terpinene, neomenthol and piperitone production. In this study, the major components of peppermint essential oil had a positive and significant relationship with each other, generally. The results can be useful to apply in modern agriculture for enhancing the quality of peppermint.

Author contributions

M. M. carried out the experiment and contributed in data gathering. M. A. supervised the research, assisted in data analysis and wrote the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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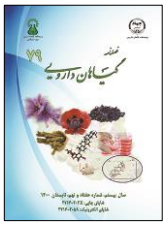

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مقاله تحقیقاتی

تغییرات اجزای اسانس نعناع فلفلی تحت تأثیر محلول پاشی با عصاره مخمر و سالیسیلیک اسید

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اطلاعات مقاله

گل‌واژگان:
نعناع فلفلی
محرک
متابولیت‌های ثانویه
گیاه دارویی
منتون
منتول

چکیده

مقدمه: نعناع فلفلی (*Mentha x piperita* L.) یکی از مهمترین گیاهان دارویی است که در صنایع غذایی، دارویی، عطرسازی و طعم‌دهنده‌ها مورد استفاده قرار می‌گیرد. **هدف:** این پژوهش، با هدف بررسی اثر محلول پاشی سالیسیلیک اسید و عصاره مخمر بر افزایش تولید اجزای ارزشمند اسانس نعناع فلفلی انجام شد. **روش بررسی:** این تحقیق به صورت طرح کاملاً تصادفی با ۹ تیمار شامل سالیسیلیک اسید (۴۰، ۸۰، ۱۶۰ و ۳۲۰ میلی‌گرم در لیتر)، عصاره مخمر (۰/۲۵، ۰/۷۵، ۱ و ۱/۵ گرم در لیتر) و آب مقطر (شاهد) در سه تکرار تحت شرایط گلخانه‌ای انجام شد. **نتایج:** در مجموع، ۴۰ ترکیب در ساختار هوایی نعناع فلفلی شناسایی شد. اجزای اصلی اسانس به ترتیب منتون، منتول، پیپریتون، ایزوپولگول و گاماترپینن بودند. منتون و منتول به ترتیب با ۱۶/۶۹ و ۱۴/۳۹ درصد، ترکیب‌های اصلی اسانس بودند. محرک‌های سالیسیلیک اسید و عصاره مخمر باعث افزایش تولید منتون، نئومنتول، پیپریتون، گاماترپینن و ایزومنتول استات (به ترتیب ۴۲، ۶۰، ۳۹، ۵۹ و ۳۴ درصد) نسبت به گیاهان شاهد شدند. محلول‌پاشی با تیمار ۳۲۰ میلی‌گرم در لیتر سالیسیلیک اسید، بهترین تیمار برای افزایش درصد ترکیبات عمده اسانس گیاهان تیمار شده بود. نتایج همبستگی بین اجزای اسانس نشان داد که میزان نئومنتول همبستگی مثبت معنی‌داری با منتون ($r = ۰/۸۶۵^{**}$)، گاما ترپینن ($r = ۰/۸۴۸^{**}$) و همبستگی منفی با ایزوپولگول ($r = -۰/۸۸۶^{**}$) داشت. **نتیجه‌گیری:** کیفیت اسانس نعناع فلفلی تحت تأثیر غلظت‌های مناسب سالیسیلیک اسید و عصاره مخمر بود. برای تولید منتون، نئومنتول، گاما ترپینن و پیپریتون، ۳۲۰ میلی‌گرم در لیتر سالیسیلیک اسید تیمار مناسبی بود.

مخففها: GC-MS، کروماتوگرافی گازی – طیف سنجی جرمی؛ SE، خطای استاندارد؛ C.V.، ضریب تغییرات

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