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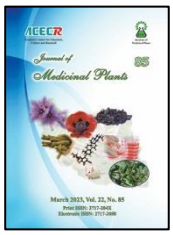

Research Article

Chemical composition of the essential oil of *in vitro* propagated *Mentha x piperita* L. and *Mentha pulegium* L. under methyl jasmonate and salicylic acid foliar application in field conditions

Ardeshir Qaderi[1], Shahmira Agahnia[2], Kamran Akbari Noghabi[2], Nassrin Qavami[1], Mohammad Reza Dehghani Meshkani[1], Hadi Kalantari[1], Javad Shaghaghi[3], Nasim Zarinpanjeh[1,*]

[1] *Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran*
[2] *Department of Horticulture, Faculty of Agricultural and Natural Resources, Islamic Azad University, Abhar, Iran*
[3] *Plant Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran*

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ABSTRACT

Background: The genus *Mentha* consists of several important medicinal plant species whose active ingredients are used for treating liver and prostate cancers, acute respiratory infection and allergic digestive problems, neuralgia, and migraines. **Objective:** This study aimed to investigate the effects of methyl jasmonate (MeJA) and salicylic acid (SA) in the field condition on the chemical composition of the essential oil of two *in vitro* propagated species of *Mentha* genus including *Mentha x piperita* and *Mentha pulegium*. **Methods:** *M. piperita* and *M. pulegium* species were propagated through the tissue culture method and the *in vitro* propagated plants were transferred to the farm after acclimatization. Then, the plants were treated with foliar application of MeJA and SA at four concentrations (0, 2, 4, and 6 mM) at three developmental stages. Chemical composition of the essential oils of samples were determined using a GC-MS analysis. **Results:** Both elicitors increased the level of menthol production in peppermint (*M. x piperita*), but in pennyroyal (*M. pulegium*), the level of pulegone production as the main secondary metabolite was decreased. Among different metabolites detected in the essential oil of peppermint, myrcene, octanal, *γ*-terpinene, ocimene, carvacrol, *iso*-menthol, *neo*-menthol, and oxygenated sesquiterpenes were the constituents with the lowest amounts under MeJA and SA treatment. **Conclusion:** The application of MeJA and SA significantly impacted on the quality and quantity of secondary metabolites of these species. The desired performance of valuable secondary metabolites can be achieved by determining the type and dosage of elicitors.

Abbreviations: BAP, 6-Benzyl Amino Purine; IBA, Indole-3-Butyric Acid; NAA, 1-Naphtaleneacetic Acid ; SA, Salicylic Acid ; MeJA, Methyl Jasmonate; JA, Jasmonic acid; mM, millimolar; ANOVA, Analysis of Variance; SE, Standard Error

*Corresponding author: zarinpanjeh@imp.ac.ir

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1. Introduction

Prescribing herbal medicines have been a useful resource for primary health care since ancient times [1]. The genus *Mentha* which is belonging to the family Lamiaceae include different species like *Mentha x piperita* and *Mentha pulegium*. The aerial parts of this genus produce essential oil containing a large number of aroma compounds such as menthol, menthone, *iso*-menthone and menthofuran which are used in pharmaceutical, food, flavour, cosmetics, beverages industries [2]. In search of alternative sources for the production of desirable medicinal compounds from plants, biotechnological approaches, specifically, plant tissue culture, have a great potential in the industrial production of bioactive plant metabolites [3]. In this regards, elicitation with various biotic and abiotic elicitors is a possible method to overcome various problems associated with large-scale production of the most commercially important secondary metabolites from wild and cultivated plants [4,5]. Also, elicitors can induce the production of primary metabolites and affect physiological processes like growth and yield [6]. MeJA along with its derivatives and SA are two key signal molecules widely used as secondary-metabolite-inducing compounds in many plants, plant cells, and callus cultures [7, 8]. They have been proposed as important signaling compounds in the elicitation process, regulating plant defense responses and promoting the production of various secondary metabolites in plant cell cultures [9]. For example, the foliar application of JA and SA in *Satureja hortensis* grown in green house condition showed significant impacts on some chemical components of essential oils. SA (50 µl) increased monoterpene hydrocarbons in *S. hortensis* oil, whereas oxygenated monoterpenes and sesquiterpenes decreased. JA (50 µl) significantly decreased carvacrol and γ-terpinene contents in the oils, whereas it improved (Z)-β-ocimene and α-pinene amounts [10]. Gharib [11] reported that SA improved the oil composition of *Ocimum basilicum* and *Majorana hortensis*. Ashrafi [12] reported that the foliar application of JA (100 µl) increased thymol and carvacrol content in the essential oil obtained from Thymus daenensis aerial parts compared with the control and other treatments. The purpose of this research is to investigate the role of foliar application of SA and MeJA (as elicitors) on the production of some photochemical compounds of *in vitro* propagated *M. piperita* and *M. pulegium* in field conditions.

2. Materials and Methods

2.1. Plant material and surface sterilization

Healthy young stems were collected from three-year-old *Mentha x piperita* L. and *Mentha pulegium* L. plants grown at the research farm of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran (56°35´ N and 50°58´ E; 1500 m of elevation). Stems (1.5-2 cm) having at least one lateral bud were excited and then washed with running tap water for 30 min. They were shaken with water having 2-3 drops of tween 20 for 5 min. Then, they were surface sterilized by treating modified methods including shaking with 70 % ethanol for 30 seconds and shaking with 0.1 % (v/v) mercury (II) chloride solution for 7 min. Explants were finally rinsed three times with sterile double-distilled water to remove any traces of the surfactants [13]. Then, they were cultured on a solid MS medium [14] and was incubated at 25 ± 2 ºC under the 16/8 hours photoperiod and light illumination of 3000 lux. The pH of the medium was adjusted to 5.8 and then autoclaved for 15 min at 121 °C. After two weeks, explants showing no fungal or bacterial contaminations were subculture on shoot induction media.

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2.2. Shoot and root induction

Aseptically explants from *M. piperita* and *M. pulegium* were cultured in solid MS with BAP at 3 mg/L and 1 mg/L for shoot induction, respectively. Then, they were kept for 30 days at the same environmental conditions mentioned above. For rooting, the regenerated shoots were transferred to MS supplemented with IBA at 1.5 mg/L for i and 1 mg/L for *M. pulegium* [15]. Finally, they were incubated under the same condition for 30 other days.

2.3. Acclimatization

Rooted plantlets of both *M. pulegium and M. piperita* were washed in running water to remove the nutrient media and then planted in small plastic pots (12 × 14 cm). The soil mix was prepared by mixing vermiculite, pittmoss, and perlite at a 1:1:1 ratio. The soil was autoclaved at 121 °C for 45 min before planting of the rooted plantlets. All the plantlets were acclimatized for 30 days before transferring them to the farm. To acclimatize the plantlets to the environmental humidity, all plantlets were kept in a plastic cap and every day one hole was made on it to reduce humidity gradually.

2.4. Elicitor application and sampling

The acclimatized regenerated plants of *M. piperita* and *M. pulegium* were transported to the main farm. Each plot consisted of 7 cultivating rows with 40 cm distance from each other. The distance between the cultivated plants in the rows was 20 cm. The soil was loam-silt with 0.071 % nitrogen, 48.9 mg/kg phosphorous, 33.6 mg/kg potassium, EC 2.71 dS/m, and pH 8.3.

Two elicitors including SA and MeJA (Sigma-Aldrich, USA) were used to study the elicitation effect on photochemical parameters. Both elicitors were foliar applied at final concentrations of 0, 2, 4, and 6 mM individually using water + methanol as solvent. Treatments were foliar sprayed three times at the six-to-eight-leaves stage, the ten-to-twelve-leaves stage, and early flowering stage. Sampling was carried out randomly one week after flowering stage.

2.5. Isolation and chemical analysis of essential oil

The essential oil was extracted by the hydro-distillation method in a clevenger-type apparatus according to the European Pharmacopoeia. Adequate amount (200 g) of each dried sample was well crushed and was boiled with water into a 500 ml flask until no more essential oil was obtained. The essential oil after cooling was collected by a syringe and dehydrated by adding sodium sulfate. Then, the essential oil was stored at 4 °C until analyzed by GC-MS apparatus. The experiment was repeated three times and their mean was reported as essential oil percentage on the dried plant. Phytochemical contents were determined using GC-MS device. GC-MS analysis was performed on an Agilent instrument coupled with a 5973 Mass system equipped with a flame ionization detector (FID) and a BPX5 capillary column (30 m × 0.25 mm; 0.25 μm film thicknesses). The temperature program includes oven temperature held for 5 min at 50 °C and was enhanced to 240 °C with 3 °C/min rate. Then, temperature enhancement was programmed up to 300 °C at 15 °C/min rate and this temperature was held for 3 min. Other operating conditions include carrier gas was with a flow rate of 1 ml/min; the injector temperature was 290 °C and split ratio was adjusted at 1:10. Mass spectrometer condition was as follow: ionized potential 70 eV and source temperature 200 °C.

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2.6. Statistical analysis

The experiments were set up on a randomized complete block factorial design with three replicates per treatment. Statistical differences were assessed based on ANOVA using SAS 9.4 software. Differences among means were analyzed using Duncan multiple range test at a probability level of $P < 0.01$. Variance homogeneity was evaluated by the Bartlett test. The values are expressed as the mean ± SE.

3. Results

In this study, the elicitation effects of MeJA and SA were investigated and 22 compounds were identified in *M. piperita* and *M. pulegium* essential oil using GC-MS. The whole stages including *in vitro* culturing of explants, shoot and root induction, acclimatization and elicitor application in the field experiment have been shown in Fig. 1.

According to variance analysis, the interaction effect of elicitors and the type of species on the production of phytochemical contents (%) which were assessed in this investigation including *α*-pinene, *β*-pinene, myrcene, octanal, limonene, cineole, ocimene, *γ*-terpinene, linalool, menthone, menthofuran, *iso*-menthone, *neo*-menthol, menthol, *iso*-menthol, *neoiso*-menthol, pulegone, carvacrol, monoterpene hydrocarbons, oxygeneted monoterpenes, sesquiterpene hydrocarbons and oxygeneted sesquiterpenes were significant at 1 % probability level (Table 1 to Table 6).

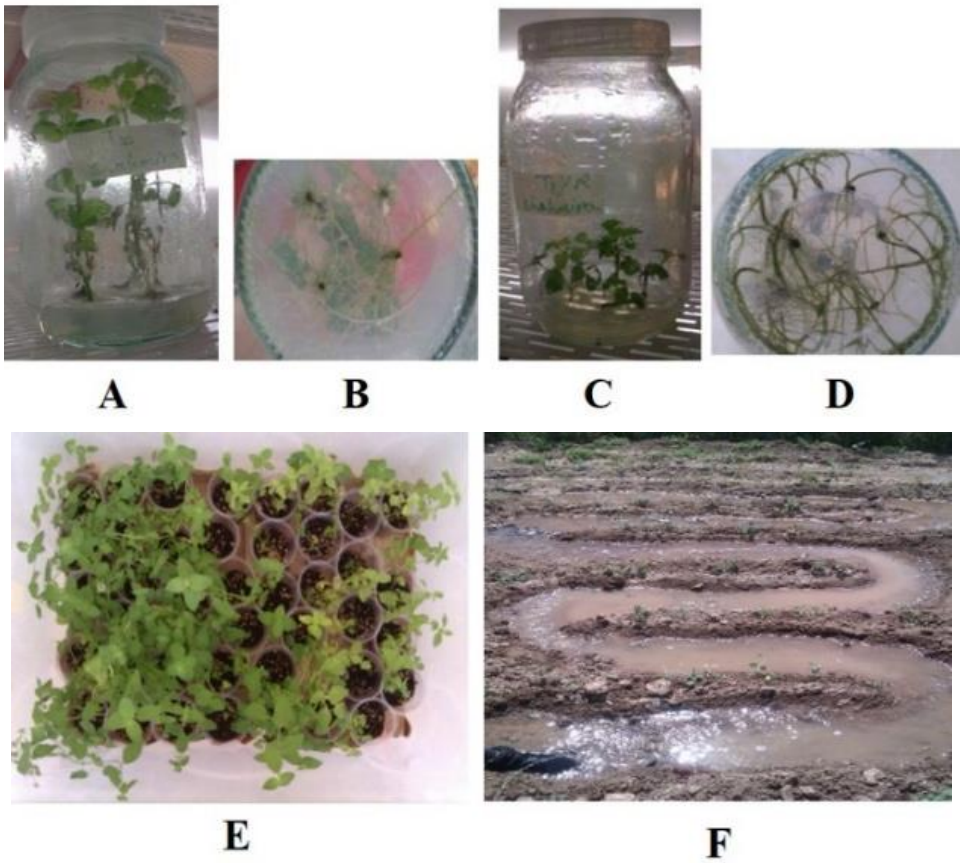

Fig. 1. **A** Shoot regeneration of *M. pulegium* **B** Root regeneration of *M. pulegium* **C** Shoot regeneration of *M. piperita*; **D** Root regeneration of *M. piperita*; **E** Transplanting of regenerated plantlets to the soil; **F** Elicitor application

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Table 1: The variance analysis of the effect of the combination of elicitors and the type of species on *α*-pinene, *β*-pinene, myrcene, and octanal.

Source of variation	df	Mean of square			
		***α*-Pinene**	***β*-Pinene**	**Myrcene**	**Octanal**
Repeat	2	0.016**	0.011**	0.002**	0.005**
Species	1	0.001ns	1.028**	5.490**	15.397**
Elicitor	6	0.009**	0.048**	0.0006*	0.004**
Elicitor × Species	6	0.003**	0.013**	0.027**	0.014**
Error	26	0.0002	0.00002	0.0002	0.0003
CV		2.21 %	0.46 %	3.31 %	3.038 %

**Significant at P ≤ 0.01; *Significant at P ≤ 0.05; nsNot significant at P ≤ 0.05

Table 2. The variance analysis of the effect of the combination of elicitors and the type of species on limonene, cineole, ocimene, and γ-terpinene

Source of variation	df	Mean of square			
		Limonene	**Cineole**	**Ocimene**	***γ*-Terpinene**
Repeat	2	0.005**	0.001**	0.0001ns	0.00003ns
Species	1	323.436**	289.459**	0.065**	0.004**
Elicitor	6	0.408**	0.005**	0.127**	0.004**
Elicitor × Species	6	0.094**	0.029**	0.065**	0.004**
Error	26	0.000	0.00001	0.00005	0.00003
CV		0	0.371 %	13.205 %	53.452 %

**Significant at P ≤ 0.01; *Significant at P ≤ 0.05; nsNot significant at P ≤ 0.05

Table 3. The variance analysis of the effect of the combination of elicitors and the type of species on linalool, menthone, menthofuran, and *iso*-menthone

Source of variation	df	Mean of square			
		Linalool	**Menthone**	**Menthofuran**	***iso*-Menthone**
Repeat	2	0.00001ns	0.037**	0.02**	0.009**
Species	1	0.003**	432.323**	3877.635**	10.055**
Elicitor	6	0.003**	3.665**	3.183**	1.412**
Elicitor × Species	6	0.003**	6.311**	3.183**	0.176**
Error	26	0.00001	0.0002	0.001	0.0006
CV		34.11 %	0.077 %	0.315 %	3.357 %

**Significant at P ≤ 0.01; *Significant at P ≤ 0.05; nsNot significant at P ≤ 0.05

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Table 4. The variance analysis of the effect of the combination of elicitors and the type of species on *neo*-menthol, menthol, *iso*-menthol, and *neoiso*-menthol

Source of variation	df	Mean of square			
		***neo*-Menthol**	**Menthol**	***iso*-Menthol**	***neoiso*-Menthol**
Repeat	2	0.03^{ns}	0.007**	0.009**	0.005^{ns}
Species	1	4724.421**	15179.105**	8.078**	1.213**
Elicitor	6	3.454**	3.05**	0.013**	0.236**
Elicitor × Species	6	14.293**	3.05**	0.013**	0.258**
Error	26	0.017	0.0005	0.00008	0.003
CV		1.17 %	0.122 %	2.086 %	27.761 %

**Significant at $P \leq 0.01$; *Significant at $P \leq 0.05$; ^{ns}Not significant at $P \leq 0.05$

Table 5. The variance analysis of the effect of the combination of elicitors and the type of species on pulegone, carvacrol, monoterpene hydrocarbons and oxygenated monoterpenes

Source of variation	df	Mean of square			
		Pulegone	**Carvacrol**	**Monoterpene hydrocarbons**	**Oxygenated monoterpenes**
Repeat	2	0.338 ^{ns}	0.00003 ^{ns}	0.005**	0.02**
Species	1	13236.89**	0.003**	8891.8**	8749.487**
Elicitor	6	9.829**	0.003**	1.246**	9.579**
Elicitor × Species	6	10.437**	0.003**	1.356**	2.040**
Error	26	0.211	0.00002	0.000	0.000
CV		2.456 %	62.36 %	0.008 %	0.006 %

**Significant at $P \leq 0.01$; *Significant at $P \leq 0.05$; ^{ns}Not significant at $P \leq 0.05$

Table 6. The variance analysis of the effect of the combination of elicitors and the type of species on sesquiterpene hydrocarbons and oxygenated sesquiterpenes.

Source of variation	df	Mean of square	
		Sesquiterpene hydrocarbons	**Oxygenated sesquiterpenes**
Repeat	2	0.013**	0.00003^{ns}
Species	1	10.570**	0.02**
Elicitor	6	0.268**	0.02**
Elicitor × Species	6	0.372**	0.02**
Error	26	0.000	0.00
CV		0.121 %	24.140 %

**Significant at $P \leq 0.01$; *Significant at $P \leq 0.05$; ^{ns}Not significant at $P \leq 0.05$

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Mean comparisons of the effect of elicitors and species on the content of *α*-pinene, *β*-pinene, myrcene and octanal were shown (Fig. 2). Results showed that the highest production of *α*-pinene (0.74 % ± 0.014) and *β*-pinene (1.16% ± 0.04) was observed in *M. piperita* species at 2 mM MeJA and 2 mM SA at treatments, respectively. Increasing SA enhanced the *α*-pinene production while increasing MeJA decreased the *α*-pinene production. Also, increased SA concentration led to the reduction of *β*-pinene production, while increased MeJA concentration led to the enhancement of *β*-pinene production. The highest amount of myrcene (0.8% ± 0.014) was related to *M. pulegium* when MeJA and SA were used. While foliar application of MeJA and SA in M. *piperita* reduced the production of myrcene. The production of octanal in *M. pulegium* was higher than M. *piperita*. The highest production of octanal (1.33% ± 0.017) was observed in 2 mM of MeJA. In *M. piperita*, foliar application of MeJA and SA reduced the Octane production.

Mean comparisons of the effect of elicitors and species on the content of limonene, cineole, ocimene, and *γ*-terpinene were shown in Fig. 3. The production of limonene in *M. pulegium* was higher than *M. piperita*. Also, using higher concentrations of MeJA and SA enhanced the production of l limonene in both species. The highest production of limonene was observed in *M. pulegium* in the treatment of 4 mM MeJA (8.47% ± 0). In the case of cineole, the production in *M. piperita* was higher than *M. pulegium* and an increasing trend was observed in cineole production in the treatment of 0 to 6 mM of MeJA. However, in *M. pulegium*, foliar application of MeJA and SA reduced the production of cineole. Applying SA and MeJA in both *M. piperita* and *M. pulegium* has caused a decrease in the production of ocimene. The highest production of ocimene in *M. pulegium* (0.66% ± 0.007) and *M. piperita* (0.11% ± 0.003) was observed in the control. In *M. pulegium*, the use and non-use of elicitors did not affect γ-terpinene production and the octanal content was zero in all treatments.

Mean comparisons of the effect of elicitors and species on the content of linalool, menthone, menthofuran, and *iso*-menthone on were shown in Fig. 4. According to the results applying both elicitors in *M. pulegium* did not produce linalool. While foliar application of elicitors in *M. piperita* has reduced linalool production. The highest production of menthone (22.37 % ± 0.014) was related to the treatment of MeJA at 2 mM in *M. pulegium*. The amount of menthofuran production in all elicitor treatments and also in control treatment for *M. pulegium* was negligible. However, in *M. piperita*, the elicitor treatment containing SA at 2 mM showed the highest menthofuran production (20.52 % ± 0.031). Foliar application of SA and MeJA reduced the *iso*-menthone production in both *M. pulegium* and *M. piperita*.

Mean comparisons of the effect of elicitors and species on the content of *neo*-menthol, menthol, *iso*-menthol, and *neoiso*-menthol were shown (Fig. 5). The maximum production of *neo*-menthol (24.96 % ± 0.13) was obtained by using MeJA at 2 mM in *M. pulegium*. The best treatment for maximum production of menthol (39.86 % ± 0.022) was related to the foliar application of SA at 6 mM in *M. piperita*. The maximum production of iso-menthol (0.98 % ± 0.008) was obtained in *M. piperita* by using SA at 2 and 4 mM. The use and non-use of elicitors did not affect menthol, and iso-menthol production in *M. pulegium*. About *neoiso*-menthol production, the highest amount (0.99 % ± 0.17) was obtained by applying SA at 2 mM.

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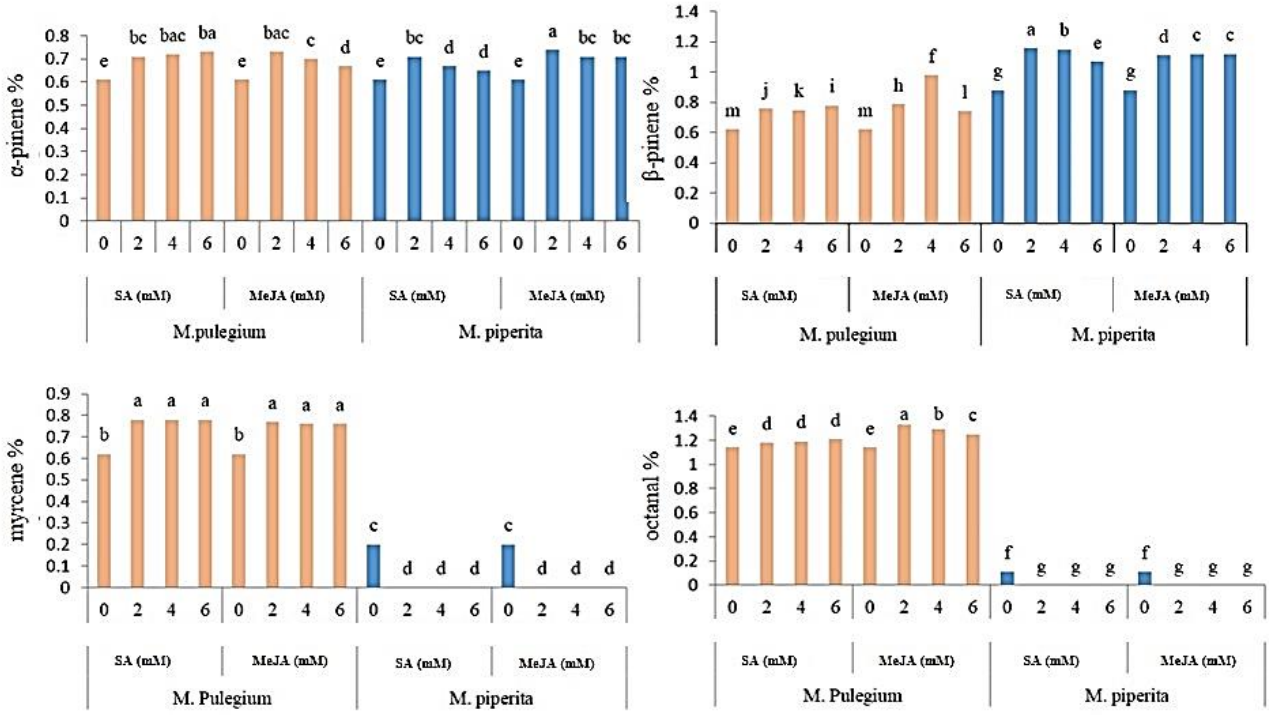

Fig. 2. Effect of SA and MeJA foliar application on the concentrations of *α*-pinene, *β*-pinene, myrcene and octanal contents in *M. piperita* and *M. pulegium*. Values followed by different letters in each trait are significantly different at P ≤ 0.01

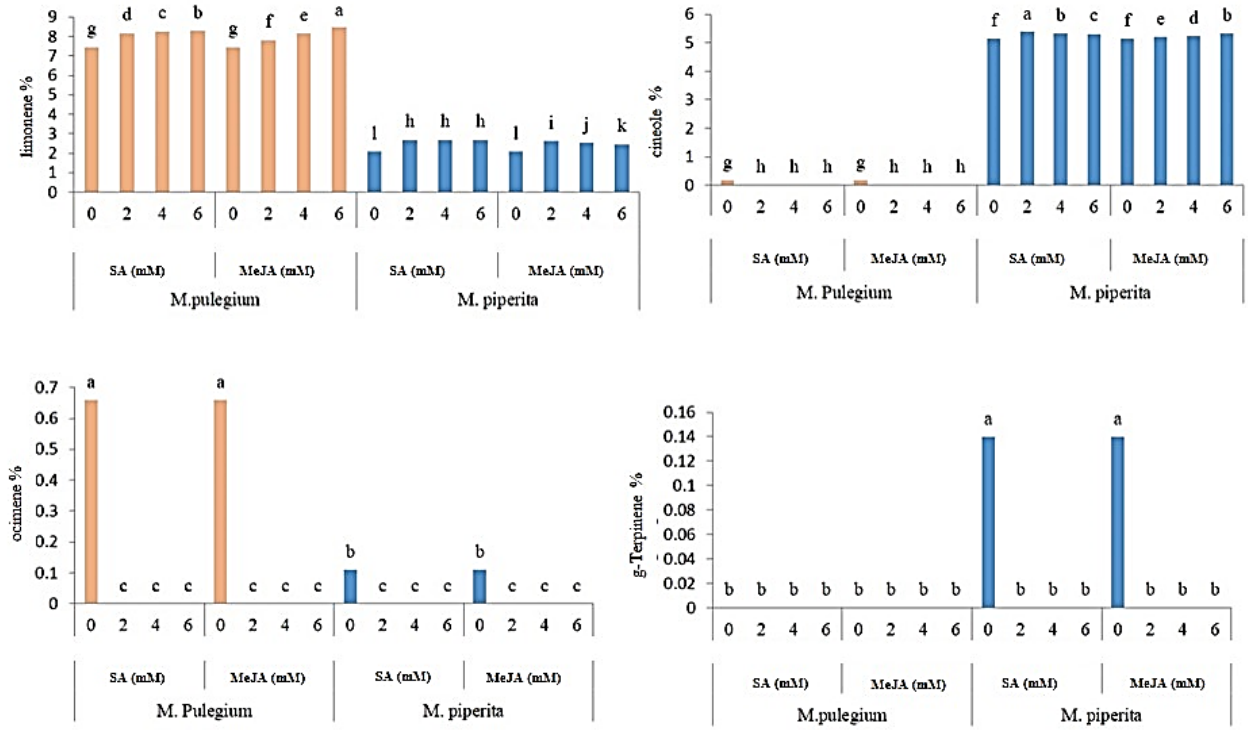

Fig. 3. Effect of SA and MeJA foliar application on the concentrations of limonene, cineole, ocimene, and *γ*-terpinene contents in *M. piperita* and *M. pulegium.* Values followed by different letters in each trait are significantly different at P ≤ 0.01

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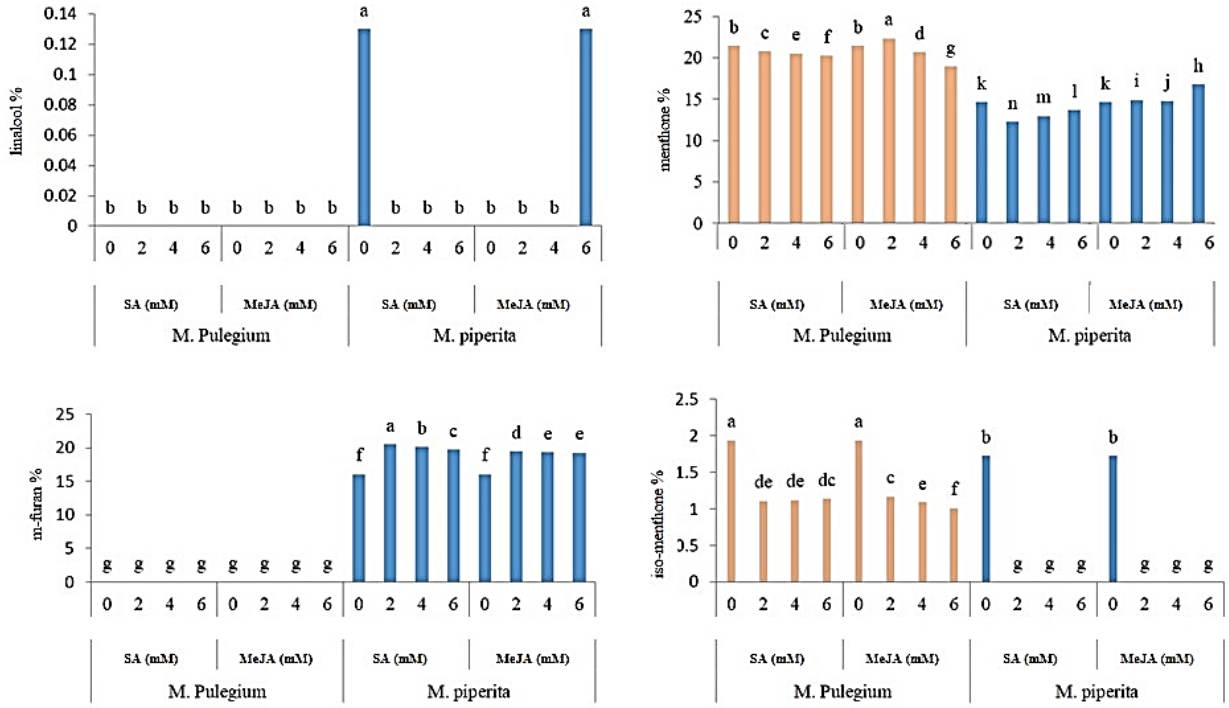

Fig. 4. Effect of SA and MeJA foliar application on the concentrations of linalool, menthone, menthofuran and *iso*-menthone contents in *M. piperita* and *M. pulegium*. Values followed by different letters in each trait are significantly different at P ≤ 0.01

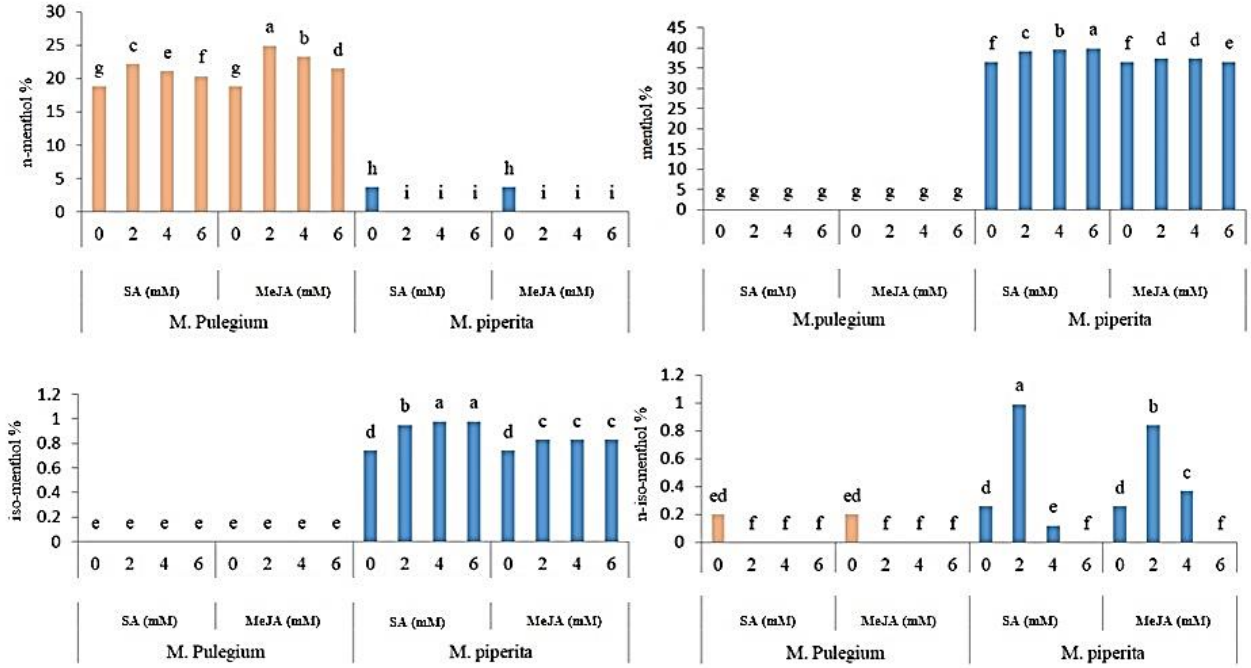

Fig. 5. Effect of SA and MeJA foliar application on the concentrations of *neo*-menthol, menthol, *iso*-menthol and *neoiso*-menthol contents in *M. piperita* and *M. pulegium*. Values followed by different letters in each trait are significantly different at P ≤ 0.01

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Mean comparisons of the effect of elicitors and species on the content of pulegone, carvacrol, monoterpene hydrocarbons, and oxygenated monoterpenes were shown (Fig. 6). Pulegone production in *M. pulegium* was higher than *M. piperita*. Although in *M. pulegium*, increasing the concentration of MeJA and SA has increased pulegone production, the highest amount was related to the control (40.49 % ± 0.45). While the use and non-use of elicitor did not affect carvacrol production in *M. pulegium*, but using elicitor in *M. piperita* has reduced the carvacrol production. The maximum monoterpene hydrocarbons (35.33 % ± 0.0) and oxygeneted monoterpenes (92.44 % ± 0.0) production were obtained by using MeJA at 2 mM in *M. pulegium* and control treatment in *M. piperita*, respectively.

Mean comparisons of the effect of elicitors and species on the content of sesquiterpene hydrocarbons and oxygeneted sesquiterpenes were shown (Fig. 7). The highest sesquiterpene hydrocarbons production (2.16 % ± 0.0) was related to the application of SA at 6 mM. In the case of oxygeneted sesquiterpenes, the use and non-use of the elicitor did not affect the production in *M. pulegium*. However, the highest oxygeneted sesquiterpenes (0.31 % ± 0.0) has been shown in the control treatment of *M. piperita*.

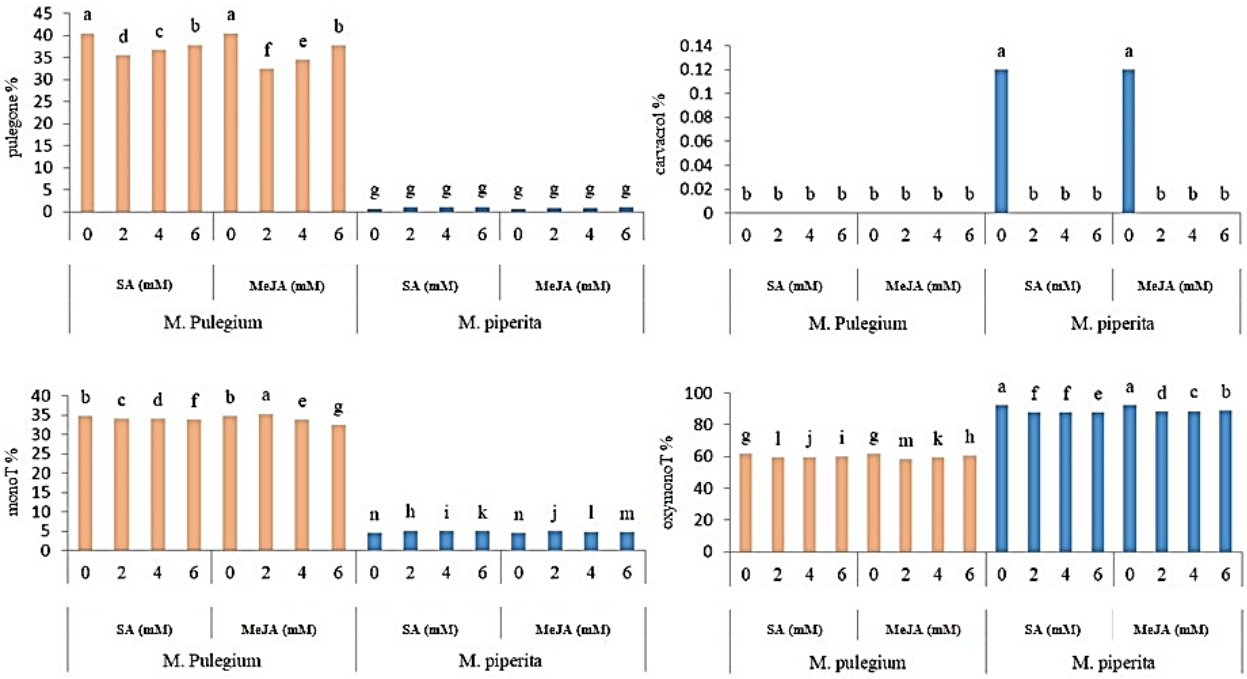

Fig. 6. Effect of SA and MeJA foliar application on the concentrations of pulegone, carvacrol, monoterpene hydrocarbons and oxygeneted monoterpenes contents in *M. piperita* and *M. pulegium*. Values followed by different letters in each trait are significantly different at P ≤ 0.01

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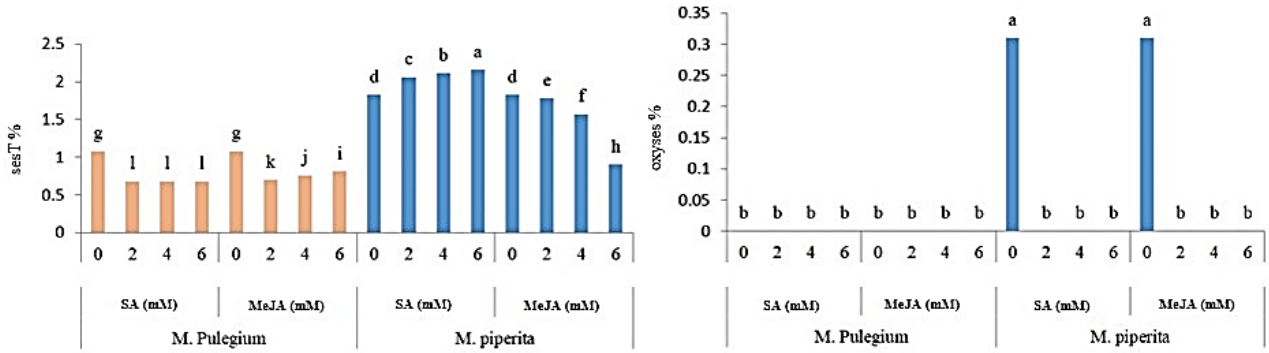

Fig. 7. Effect of SA and MeJA foliar application on the concentrations of sesquiterpene hydrocarbons and oxygeneted sesquiterpenes contents in *M. piperita* and *M. pulegium*. Values followed by different letters in each trait are significantly different at P ≤ 0.01.

4. Discussion

The quality of plants used for the production of medicinal compounds is usually evaluated by their secondary metabolite contents. High-quality oils of *Mentha* species are characterized by a complex combination balance of monoterpenes with high menthol, moderate menthone, and low amounts of pulegone and menthofuran [16]. Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavors, and other industrial materials. Accumulation of such metabolites often occurs in plants that are under stresses such as various elicitors or signal molecules [17]. Exogenous application of JA signaling compounds stimulates the biosynthesis of secondary metabolites including a wide variety of plant secondary products such as terpenoids, flavonoids, alkaloids, and phenylpropanoids [18, 19], while MeJA application has been reported to be both safe and inexpensive [20,21]. SA is one of the most important plant phenolics that not only affects seed germination, photosynthesis, enzyme activities, nutrient uptake, flowering induction and so on, but also can efficiently recuperate the biosynthesis of secondary metabolites in plants [22]. Salicylic acid increases plant productivity and usually low quantities of SA are needed to establish positive responses in the plants. In horticultural species, the reported effect is to increase yield without affecting fruit quality. It is suggested that the increase is mainly due to the positive effect of SA on root length and density [23]. Even low concentrations of SA delay potassium influx and increase calcium and alter proton influx. [24].

At the present investigation, the foliar application of SA and MeJA as elicitors on the phytochemical content of *in vitro* propagated *M. piperita* and *M. pulegium* was studied. The results showed that the maximum production of menthol (39.86 %) was related to the foliar application of SA at 6 mM in *M. piperita* and the highest production of menthone (22.37 %) was related to the treatment of MeJA at 2 mM in *M. pulegium*. Similarly, the most promising impact of SA on the elevation of the levels of total essential oil, menthol, and menthyl acetate in *M. piperita* was recorded [25]. Also, monoterpene concentration in peppermint essential oil increased in response to the exogenous SA treatment [26]. In another experiment, 0.1 mM of SA was able to increase the menthol concentration significantly, when applied exogenously to peppermint [27]. In contrast, the foliar application of MeJA and SA had no significant effect on the ratio of menthol and menthone, menthofuran and sesquiterpene

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hydrocarbons in *M. piperita*. Only the ratio of pulegone was reduced after spraying 2 mM of SA by approximately 43 % [28]. A comparison of our results with the previous reports suggests some variation in quantities and quality of components within the essential oil. The phytochemical variability of oils 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 [16].

5. Conclusion

The bioactive compound in the intact plant is less in quantity to meet the commercial demands of pharmaceuticals, food additives, flavors and other industrial materials. The accumulation of such metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. The present study reports that foliar application of MeJA and SA as elicitors can be a promising technique for the enhancement of phytochemical contents of *M. piperita* and *M. pulegium* in field condition.

Author Contribution

Experiment Performing and Data Gathering: Sh. A., Data Analysis: A.Q., Writing: N.Z., A.Q., H.K., & J. Sh., Editing: K. AN., N.Q & MR. DM.

Conflicts of interest

Authors declare that there is no conflict of interest.

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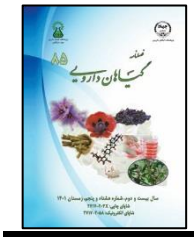

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مقاله تحقیقاتی

بررسی ترکیبات شیمیایی اسانس گونه‌های نعناع فلفلی و پونه معطر تکثیر شده به روش درون شیشه‌ای تحت محلول‌پاشی با متیل جاسمونات و سالسیلیک اسید در شرایط مزرعه

اردشیر قادری[۱]، شهمیرا آگاه نیا[۲]، کامران اکبری نوقابی[۲]، نسرین قوامی[۱]، محمد رضا دهقانی مشکانی[۱]، هادی کلانتری[۱]، جواد شقاقی[۳]، نسیم زرین پنجه[۱،*]

[۱] *مرکز تحقیقات گیاهان دارویی، پژوهشکده گیاهان دارویی جهاد دانشگاهی، کرج، ایران*

[۲] *گروه باغبانی، دانشکده کشاورزی و منابع طبیعی، دانشگاه آزاد اسلامی، ابهر، ایران*

[۳] *مرکز ملی ذخایر ژنتیکی و زیستی ایران، جهاد دانشگاهی، کرج، ایران*

اطلاعات مقاله

گل‌واژگان:
متیل جاسمونات
نعناع فلفلی
پونه معطر
سالسیلیک اسید
متابولیت‌های ثانویه

چکیده

مقدمه: جنس نعناع شامل چندین گونه گیاه دارویی مهم است که مواد موثره آن برای درمان سرطان‌های کبد و پروستات، عفونت حاد تنفسی و مشکلات آلرژی گوارشی، درد عصبی و میگرن مورد استفاده قرار می‌گیرد. **هدف:** این مطالعه با هدف بررسی اثرات متیل جاسمونات و سالیسیلیک اسید در شرایط مزرعه بر ترکیبات شیمیایی اسانس دو گونه از جنس نعناع شامل نعناع فلفلی و پونه معطر تکثیر شده به روش درون شیشه‌ای انجام شد. **روش بررسی:** گونه‌های نعناع فلفلی و پونه معطر از طریق کشت بافت تکثیر شدند و پس از سازگاری به مزرعه منتقل شدند. سپس گیاهان با متیل جاسمونات و سالیسیلیک اسید در چهار غلظت (۰، ۲، ۴ و ۶ میلی‌مولار) در سه مرحله رشد تحت تیمار محلول پاشی قرار گرفتند. ترکیبات شیمیایی اسانس نمونه‌ها با استفاده از کروماتوگرافی متصل به طیف‌سنج جرمی تعیین شد. **نتایج:** هر دو عامل باعث افزایش سطح تولید منتول در نعناع فلفلی شدند، اما در پونه معطر تولید پولگون به عنوان متابولیت ثانویه اصلی این گونه، کاهش یافت. از بین متابولیت‌های مختلف شناسایی‌شده در اسانس نعناع، میرسن، اکتانال، گاما- ترپینن، اوسیمن، کارواکرول، ایزو- منتول، نئومنتول و سزکوئی ترپن‌های اکسیژنه کمترین مقدار را تحت تیمار متیل جاسمونات و سالیسیلیک اسید داشتند. **نتیجه‌گیری:** کاربرد متیل جاسمونات و سالیسیلیک اسید به طور قابل توجهی بر کیفیت و کمیت متابولیت‌های ثانویه این گونه‌ها تأثیر گذاشت. عملکرد مطلوب متابولیت‌های ثانویه ارزشمند را می‌توان با تعیین نوع و غلظت الیسیتورها به دست آورد.

مخففها: BAP، ۶- بنزیل آمینو پورین؛ IBA، ایندول-۳- بوتیریک اسید؛ NAA، ۱- نفتالن استیک اسید؛ SA، سالسیلیک اسید؛ MeJA، متیل جاسمونات؛ JA، جاسمونیک اسید؛ mM، میلی‌مولار؛ ANOVA، تجزیه واریانس؛ SE، خطای استاندارد

* نویسنده مسؤول: zarinpanjeh@imp.ac.ir

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