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The Effect of Methyl Jasmonate on Monoterpene Composition of the Essential Oil in Shoot Cultures of *Sautreja khuzistanica* Jamzad

Fatemeh Farzi[1*], Hamid Fahimi[1], Mozafar Sharifi[2] and Amir Mousavi[3]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Department of Plant Biology, Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran.
[3]National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. All authors were responsible for design of the study. Author FF performed the analyses and wrote the first draft of the manuscript. Authors HF, MS and AM reviewed and improved the manuscript. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Background and Objective: Numerous monoterpenes are present in the essential oil of *Sautreja khuzistanica* Jamzad. In this study, the effect of methyl jasmonate (MJ) applied in the LS medium of shoot cultures of *S. khuzistanica* on individual monoterpene percentages were evaluated.
Results: Our results showed that 44 h after elicitation with 250 and 500 µM MJ, several monoterpenes including α-Pinene, β-Pinene, γ-Terpinene, α-Terpinene, β-Myrcene, p-Cymene, α-Thujene, Carvone significantly decreased ($p \leq 0.001$). Linalool significantly decreased by treatment with 250 µM MJ. A significant increase in carvacrol and α-Terpineol concentration was observed in 250 and 500 µM MJ treatments ($p \leq 0.001$). Cis-sabinene hydrate concentration was significantly increased by treatment with 500 µM MJ ($p \leq 0.001$).

**Corresponding author: E-mail: fatemehfarzi@gmx.com;*

Discussion and Conclusion: Changes in individual monoterpene percentages were observed in MJ treated versus control plants. The results of this study indicated that MJ application may affect essential oil monoterpene composition. The information is beneficial to our further investigation and understanding of MJ elicitation on the essential oil quality, and it may be also helpful to the manipulation of the essential oil composition.

Keywords: Satureja khuzistanica; methyl jasmonate; monoterpene; carvacrol.

1. INTRODUCTION

Many plant species constitutively produce large quantities of terpenoid rich essential oils [1], that are complex mixtures of numerous molecules. Monoterpenes, the C_{10} members of the terpenoid family of secondary metabolites are components of plant essential oils [2], that are widely used in medicine, industry and agriculture [3]. Essential oils have been largely employed for their antibacterial, antifungal and insecticidal activities [4].

Satureja khuzistanica Jamzad (Lamiaceae) is an endemic plant of Iran [5]. *S. khuzistanica* is a medicinal aromatic plant that in the folk medicine has been used as an analgesic and antiseptic agents among the inhabitants of southern parts of Iran [6]. There are several monoterpenes in the essential oils of *S. khuzistanica* jamzad [7]. Among them, carvacrol is the most abundant monoterpene [5], a monoterpenic phenol [8]. Carvacrol has a wide spectrum of antimicrobial activity [9]. It is possible that the activity of the main component of the essential oil is modulated by other minor components [4].

Jasmonic acid and its methyl ester methyl jasmonate (MJ) are involved in plant development and defense responses [10]. MJ has been used as a means to induce terpene based responses in *Picea abies* L. Karst [11], *Gossypium hirsutum* L. [12], and *Ocimum basilicum* L. plants [13].

The seeds of *S. khuzistanica* are dormant and have a very low germination rate, thus shoot culture would be an alternative method for production of this plant [14]. In this study, we analysed the effect of methyl jasmonate treatment on individual monoterpene concentrations in the oil of shoot cultures of *Satureja khuzistanica* Jamzad.

2. MATERIALS AND METHODS

2.1 Plant Materials and Treatments

Nodal segments of shoots of *S. khuzistanica* cultured on LS medium [15] were subcultured for shoot proliferation on LS medium containing benzylaminopurine and 30 g/L sucrose. The medium was solidified with 8 g/L agar. The shoots from explants were transferred to LS medium lacking plant growth regulators in order to promote shoot elongation. When the plants attained the height of 5-7cm, they were used as experimental material for control and elicitor treatment. After excision of their roots, these plants were transferred to liquid LS medium containing different concentrations of methyl jasmonate. Methyl jasmonate was dissolved in ethanol, filter-sterilized and added to the subcultures of *S. khuzistanica* at a final concentration of 250 and, 500 µM. Ethanol controls were made by supplementing the medium with an amount of ethanol equivalent to that present in 500 µM MJ treatments. The induction of volatiles biosynthesis pathways by methyl jasmonate treatment (0 h–48 h) in different species has been carried out [16,17]. The time course of experiment was 44 h. All treatments were performed in triplicate.

2.2 Isolation of the Essential Oil

Plant materials were air-dried at room temperature in the shade and hydrodistilled using a Clevenger type apparatus for 90 min. The oils were dried over anhydrous sodium sulfate and stored at 4°C until analysed by GC and GC - MS. All analyses were performed three times.

2.3 Study Using Gas Chromatography/ Mass Spectrometry

GC analysis was carried out using a GC (Agilent, 6890) fitted with a HP-5 (5% phenyl methyl siloxan) capillary column (30 m × 320 µm × 0.25 µm film thickness) and flame ionisation detector (FID). The treatment program started at 50°C (hold 1 min), followed by 3°C/min to 125°C (hold 5 min) and 8°C/min to 240°C (hold 3 min), and finally 10°C/min until 280°C (hold 3 min). The injector and detector temperatures were 275°C and 285°C, respectively. The injection volume for all samples was 1 µl of the oil. Split ratio was 30:1. The carrier gas was helium with flow at 1 ml min^{-1}. The GC/MS analyses were

performed using an Agilent 6890N-5973 equipped with a HP-5MS (5% methyl siloxan) capillary column (30 m × 320 µm × 0.25 µm film thickness). The oven temperature was identical to that used in analysis with the HP-5 column mentioned above. The injector temperature was 275°C. the injection volume was 1 µl of the oil. Split ratio was 30:1. The carrier gas was helium with flow at 1 ml min $^{-1}$.

2.4 Identification of Components

The identification of monoterpenes was based on a comparison of their mass spectra and retention indices obtained using *n*-alkanes (C8-C28) as standard with those of NIST and literature. Monoterpene composition (% of total volatile terpenes) of the essential oil was evaluated from the total peak area without the use of an internal standard or correction factors [18,19].

2.5 Statistical Analysis

All statistical analyses were conducted with SPSS software. One-way analysis of variance (ANOVA) was used to compare the means. The differences between control and treatments were considered at $p \leq 0.001$.

3. RESULTS

Analyses of monoterpenes from MJ-treated and control plants 44 h after treatment showed that monoterpene profile of treated and control plants were similar.

A total of 24 different monoterpenes were identified in the essential oil of *S. khuzistanica* plantlets. Several monoterpenes including α-Pinene, β-Pinene, γ-Terpinene, α-Terpinene, β-Myrcene, p-Cymene, α-Thujene and Carvone significantly decreased in 250 and 500 µM MJ treatments. Linalool significantly decreased by treatment with 250 µM MJ. A significant increase in carvacrol and α-Terpineol concentration was observed in 250 and 500 µM MJ treatments. Cis-sabinene hydrate concentration was significantly increased by treatment with 500 µM MJ.

4. DISCUSSION

As shown in Table 1, changes in individual monoterpene percentages were observed in MJ treated versus control plants. Rodriguez-Saona, et al. [12] showed that exogenous methyl jasmonate induced changes in the amount of volatile compounds such as linalool and β-ocimene. MJ application to sweet basil plants increased eugenol and linalool [13]. The key enzymes responsible for the biosynthesis of terpenoids are the terpene synthases [20]. Crocoll, et al. [21] reported that terpene synthase expression levels of *Origanum vulgare* L. directly control the composition of the essential oil. According to the Ohara, et al. [22] and van Schie, et al. [23], jasmonic acid application can induce terpene synthase in tabacco and tomato, respectively. So, the altered essential oil composition can be attributed to terpene synthases. It will be interesting to investigate the effect of MJ on terpene synthase gene expression and enzyme activity of *S. khuzistanica* plantlets.

Table 1. Monoterpene compounds of essential oil in *S. khuzistanica* plantlets

No.	Compound	RI*
1	α-Thujene	921
2	α-Pinene	928
3	Camphene	943
4	Sabinene	967
5	β-Pinen	970
6	β-Myrcene	985
7	3-Octanol	992
8	α-Phellandrene	999
9	δ-3-Carene	1005
10	α-Terpinene	1011
11	p-cymene	1020
12	β-Phellandrene	1023
13	β-cis-ocimene	1042
14	γ-Terpinene	1054
15	cis-sabinene hydrate	1062
16	α-Terpinolene	1082
17	linalool	1094
18	endo borneol	1160
19	***-4***Terpineol	1172
20	α-Terpineol	1226
21	Carvone	1244
22	Thymol	1291
23	Carvacrol	1333
24	Thymol acetate	1376

**: Retention indices calculated based on a series of n-alkanes (C8-C28) on HP 5MS column*

The phenolic monoterpenes including carvacrol are especially known for their anti-herbivore, antimicrobial, pharmaceutical and antioxidant activities [21]. Our results showed that MJ treatment increased carvacrol concentration. The biosynthesis of carvacrol involves hydroxylation step catalysed by cytochrome P450 monooxygenase. It has been shown that MJ treatment increases geranyl 10-hydroxylase activity in *Catharanthus roseus* cell cultures [24]. Probably such an effect may be involved in the carvacrol increasement upon MJ treatment.

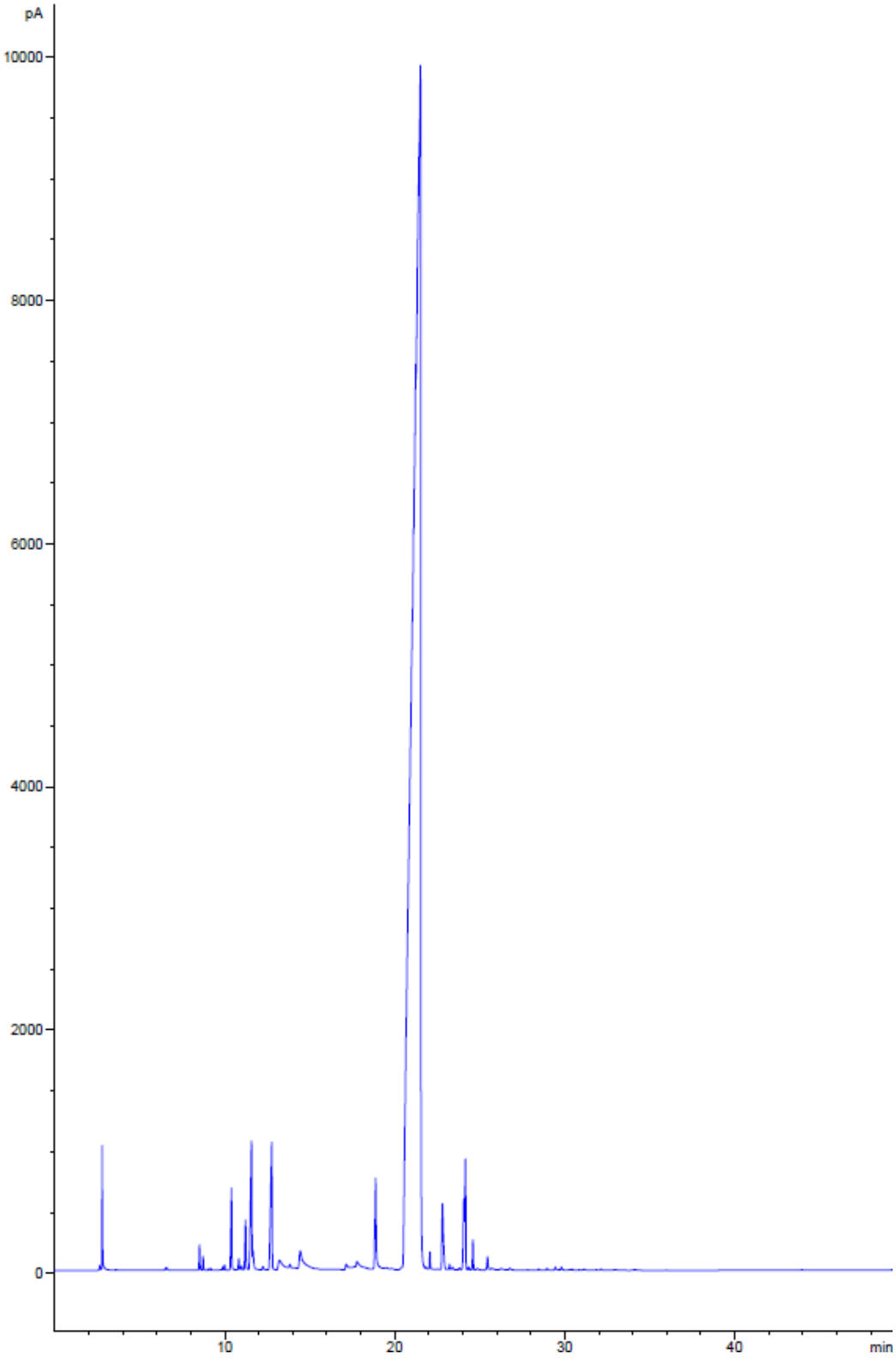

Fig. 1. Example of chromatogram showing essential oil from 500 µM MJ treated plant

Table 2. Monoterpene composition of % of total volatile terpenes of essential oil in treated and control *S. khuzistanica* plantlets

Mnoterpenes	Control	250 µM MJ	500 µM MJ
α-Thujene	0.23 ± 0/0014	0.2 ± 0/0028	0.19 ± 0/0003
α-Pinene	0.13 ± 0/0006	0.1 ± 0/0002	0.1 ± 0/0005
Camphene	0.02	0.02	0.02
Sabinene	0.03	0.03	0.03
β-Pinene	0.05 ± 0/0004	0.04 ± 0/0003	0.04 ± 0/0001
β-Myrcene	0.84 ± 0/0005	0.7 ± 0/0106	0.7 ± 0/0028
3-Octanol	tr	tr	tr
α-Phellandrene	0.12	0.11	0.09
δ-3-Carene	0.04	0.03	0.03
α-Terpinene	0.58	0.49	0.45
p-Cymene	1.78 ± 0/0025	1.22 ± 0/0155	1.59 ± 0/0031
β-Phellandrene	0.23	0.2	0.21
β-cis-Ocimene	tr	tr	tr
γ-Terpinene	2.13 ± 0/0021	2.13 ± 0/0231	1.84 ± 0/0083
cis-Sabinene hydrate	0.31± 0/0015	0.32± 0/0075	0.39± 0/0025
α-Terpinolene	0.07	0.06	0.06
Linalool	0.62 ± 0/0075	0.51 ± 0/0028	0.61 ± 0/0072
Endo borneol	0.1	0.08	0.09
4-Terpineol	tr	tr	tr
α-Terpineol	0.02 ± 0/0053	0.1± 0/0054	0.15± 0/0028
Carvone	1.66 ± 0/0155	1.38 ± 0/0095	1.12 ± 0/0075
Thymol	tr	tr	tr
Carvacrol	87.2 ± 0/0086	89.8 ± 0/2568	89.2 ± 0/0086
Thymol acetate	0.12	0.12	0.12

Note: value of compounds with statistically significant differences ($p \leq 0.001$) are given as means±SE. tr, trace

5. CONCLUSION

The results of this study indicated that MJ application affects essential oil monoterpene composition. The information is beneficial to our further investigation and understanding of MJ elicitation on the essential oil quality, and it may be also helpful to the manipulation of the essential oil composition.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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