The Accumulation of Essential Oils In Organ Culture of *Acinos Graveolens* Via Methyl Jasmonate And Salicylic Acid Exertion

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Abstract

The impact of Salicylic acid (SA) and Methyl jasmonate (MJ) on growth, and essential oil quantity and quality in organ culture of *Acinos graveolens* were investigated. In the present study, nodal segments were used as explants for shoot production using MS media with BA and NAA. Afterward, they were transferred to liquid MS media culture containing SA and MJ with 50, 100, and 150 μ M and their combinations (SA₂₅+MJ₂₅, SA₅₀+MJ₂₅, SA₂₅+MJ₅₀, and SA₅₀+MJ₅₀). After three weeks, samples were collected to assess the morphological and some growth parameters, quantitative changes in pigment amounts as well as volatile contents. Accordingly, almost all treatments led to a notable decrease in growth parameters in comparison with control. The highest amount of Chl a, b and carotenoids were achieved by MJ₁₀₀, SA₅₀, and SA₂₅+MJ₅₀, respectively. In addition, GC-MS results indicated 38 components of volatile products mainly alkane hydrocarbons and sesquiterpenes. The major identified compounds were Decane, Eicosane, and Germacrene D. Altogether, results asserted that SA₂₅+MJ₂₅ and SA₁₀₀ were more efficient in the enhancement of essential oil content among all treatments.

Key Message

Elicitors changed morphological features and pigment contents with different efficiency. They also improved the increase of the essential oils in a different pattern compared to the previously in vivo production.

Introduction

Acinos is one of the smaller but important genera in the Lamiaceae family from the economic point of view (Bonnier et al. 1927; Bown et al. 1995). This genus is represented by two species in the flora of Iran, *Acinos arvensis* and *Acinos graveolens* (Javidnia et al. 2010). *Acinos graveolens* is an annual, dwarf, evergreen, woody, small (10-45 cm), and clumpy herb growing in Iran and neighboring countries (Hedge 1982; Stojanović et al. 2009). Based on previous reports, the main component of essential oils of this plant is Germacrene D. It is also reported to be the major compound in the oil of *A. arvensis*, *A. hungaricus*, *A. alpinus*, and two subspecies of *A. troodi* (Javidnia et al. 2010). Some species of *Acinos* are reported to be used in medicine as an antiseptic, tonic, and antispasmodic since the efficacy effects on the melancholy, toothache, coughs, and gastrointestinal epizootic (Kaya et al. 1999; Soules et al. 1988; Velasco–Negueruela et al. 1993), however, more researches are needed to be accomplished about chemical composition, antibacterial and antioxidant activity as well as traditional applications of *Acinos* species in folk medicine to find out their value as medicinal plants (Stojanović et al. 2009).

The medicinal plants are used on a global scale owing to their valuable components known as secondary metabolites by which they gain pharmaceutical importance; hence most of the medicinal plants are threatened due to exploitation at a higher rate. For decades, scientists have been searching for new alternatives to conventional methods in order to overcome this problem, protect plants and simultaneously improve their bioactive compounds accumulation (Singh and Dwivedi 2018). Recently, the applications of signal molecules such as elicitors have provided an effective and appropriate strategy to promote changes in secondary metabolism in plant cell cultures (Zhao et al. 2005); therefore, the necessary conditions to stimulate the production of main biologically active ingredients can be clarified (Pasternak et al. 2005).

Salicylic acid (SA), phenolic in nature, has been found to increase plant growth, plant resistance against pathogens, flower induction, stomatal stroke, and the level of chlorophyll and carotenoid pigments. It also regulates the activity of some of the important enzymes as well as acts as a potent enhancer of some secondary metabolites (Arberg et al. 1981; Hayat et al. 2007; Pitta-Alvarez et al. 2000; Yin et al. 1997).

Jasmonic acid and its methyl ester (MJ), are fragrant volatile compounds (Demole et al. 1962) that play a role as a signal in plant responses and interactions (Cheong et al. 2003). These compounds function as important cellular regulators involved in various developmental processes, such as seed germination, root growth, and senescence (Creelman et al. 2002; Wasternack et al. 2002). Jasmonates activate plant defense mechanisms in response to biotic and abiotic stresses salinity (Wasternack et al. 1996). It has also been reported that Methyl jasmonate can induce the production of secondary metabolites under in vitro tissue cultures of various plants (Dathe et al. 1981; Ueda et al. 1980).

Based on the literature review, there is no extensive study on tissue culture and the production of secondary metabolites in *Acinos graveolens*. For this reason, the production of in vitro plants and the evaluation of essential oils in this medicinal species construct a new perspective on plant biotechnology. Therefore, the effect of different concentrations of methyl jasmonate and salicylic acid alone or their interaction on the chemical composition of the volatile compounds of *Acinos graveolens* was examined under in vitro conditions. Moreover, morphogenetic responses such as shoot, root and leaf proliferation as well as photosynthetic pigments content were evaluated.

Materials And Methods

Plant Material and Shoot Production

The seeds of *A. graveolens* were accumulated from the botanical garden of East-Azerbaijan province in Iran and were surface sterilized by diving in 70 % ethanol and 20 % sodium hypochlorite for 3 and 15 min, respectively. After 3 times rinses with sterile distilled water, the seeds cultivated in the Murashige and Skoog (MS) basal media (Murashige and Skoog 1962). The pH of all media was adjusted at 5.6-5.8 with NaOH before autoclaving at

121°C for 20 min. The media were solidified with 8 g l⁻¹ agar. Then all cultures were maintained under cool-white light illumination at 70 µmol m⁻² s⁻¹ with the daily photoperiod of 16 h at 25 ± 1°C. 35 days old seedlings grown on MS medium were used as the source explants.

In order to shoot proliferation, the nodal segments were cultured on 30 ml of hormone-free control medium and in MS medium supplemented with 1 mg l⁻¹ 6-benzyladenine (BA) and 0.2 mg l⁻¹ α-naphthalene acetic acid (NAA). All cultures were incubated under the situation explained above. After 25 days of culture, explants were transferred to liquid medium culture.

MS liquid medium preparation with the elicitors

Three different concentrations (50, 100, and 150 µM) of MJ and SA were added to MS liquid medium (pH 5.8) supplemented with 1 mg l⁻¹ BA and 0.2 mg l⁻¹ NAA. Samples were allowed to grow at constant shaking (100 rpm) in the above-mentioned medium for about four weeks. The synergistic effect of the two elicitors was also studied by applying them as 25 µM SA + 25 µM MJ (SA₂₅ + MJ₂₅), 25 µM SA + 50 µM MJ (SA₂₅ + MJ₅₀), 50 µM SA + 25 µM MJ (SA₅₀ + MJ₂₅), and 50 µM SA + 50 µM MJ (SA₅₀ + MJ₅₀). Control cultures had no elicitors. Finally, after four weeks of culture, shoot cultures were taken from the medium and washed thoroughly. Growth parameters including shoot and root percentage and fresh and dry weight (DW) of regenerated shoots per explants were determined.

Extraction of photosynthetic pigments

For the determination of total chlorophylls and carotenoids, 0.05 g of in vitro produced shoots were pressed in 2 ml of Dimethylsulphoxide using a pestle and mortar. The pigment extracts with a total volume of 2 ml were then centrifuged at 16300 x g for 15 minutes (Spectrafuge 24DF) in glass tubes to make the extract fully transparent. The resulting extracts (supernatant) were immediately assayed spectrophotometrically. Specific absorption coefficients of chl_a, chl_b, and carotenoids were used. The amount of these pigments was calculated by following formulas (Wellburn 1994).

1. $C_a = 12.19 A_{665} - 3.45 A_{649}$
2. $C_b = 21.99 A_{649} - 5.32 A_{665}$
3. $C_{(x+c)} = (1000 A_{480} - 2.14 C_a - 70.15 C_b) / 220$

Where C_a, C_b, and C_(x+c) are Chlorophyll a, Chlorophyll b, and total carotenoids, respectively. All of the pigments' content was expressed as mg g⁻¹ of fresh weight. Here A is the absorbance.

Extraction of volatile products

A quantity of 0.2 g of in vitro produced shoots in each treatment was used for the extraction of volatile products by crushing with n-hexane (Afify et al. 2011).

Gas chromatography-mass spectrometry (GC–MS)

GC-MS analysis was carried out to identify the composition of the essential oils. A volume of 1 ml of the clear extract was syringed into GC–MS (an Agilent 6890 gas chromatography) using HP5-MS capillary column (30 m, 0.25 mm, 0.25 µm). Injection initiation temperature was 150 °C, the column temperature was set at 70°C, for 3 min, and then heated to 120°C and kept for 5 min at a rate of 10 °C min⁻¹. Then reached 150 °C at a rate of 10 °C min⁻¹, after two minutes heated to 240 °C at a rate of 7 °C min⁻¹ for 5 min. The carrier gas was helium with a flow rate of 1 ml/min. Recognition of compounds was accomplished by comparing their retention indices and their mass spectra and with those reported in the literature (Adams, 1995).

Statistical analysis

The experiments were executed under a randomized complete block design (RCBD) for three replicates. The data were statistically analyzed using analysis of variance (ANOVA) and the means were compared by Duncan's Multiple Range Tests (DMRT) at the confidence level of P ≤ 0.05.

Results And Discussion

The effect of elicitors on shoot production and in vitro plant growth

Almost all growth parameters in terms of root and shoot number, average length, and fresh and dry weight were negatively affected by different concentrations of SA and MJ during experimentation. Detailed results have shown in Table 1 and Fig. 1. The results have been supported by the findings of Dong et al. (2010) that salicylic acid reduces the biomass significantly. Also, salicylic acid severely reduced biomass in the root culture of *Scopolia parviflora* (Kang et al. 2004) and hairy root culture of *Silybum marianum* (Khalili et al. 2009) that are consistent with the results of the present study. Coste et al. (2011) observed that the higher concentration of methyl jasmonate (MJ) significantly reduced biomass production in shoot cultures of *H. hirsutum* and *H. maculatum*. Spollansky et al. (2000) reported that the fresh weight of in vitro grown explants of *Brugmansia*

candida was increased by 200 μM of MJ, while it was reduced by 400 μM of MJ. In the present research, generally, MJ had negative impacts on growth parameters.

Quantification of photosynthetic pigments in the produced shoots

The content of photosynthetic pigments was affected by different concentrations of applied elicitors. Methyl jasmonate significantly enhanced the amount of chlorophyll a, b, and carotenoids compared to the control sample. The maximum amount of chlorophyll a, b and carotenoids were obtained by 100_{MJ}, 50_{SA}, and 25_{SA} + 50_{MJ}, respectively (Table 2). Reports in the case of the SA effect on pigments content have come to perplexing results ranging from negative to positive effects depending on its concentration, time of application, and used plant. However, its suitable concentrations support photosynthesis by reducing the degradation of chlorophyll, increasing the antioxidant capacity, and synthesizing new proteins (Belkhadi et al. 2010; Popova et al. 2003). In the present work, it has been observed that among different SA concentrations, 100 and 150 μM SA reduce the chlorophyll content in comparison with the control, but the lowest concentration of salicylic acid increases the chlorophyll content compared to the control.

Different results have been stated in the case of methyl jasmonate on the total amount of photosynthetic pigments. Jasmonic acid has been reported to have no impact on the plants' photosynthesis and chlorophyll content of *Populus* (Babst et al. 2005). However, in a study on *Arabidopsis thaliana*, seven days after treatment with 100 μM MJ, the content of chlorophyll a and b was dropped, and the electron transport of photosystem II was also affected (Jung 2004). Moreover, it has been reported that chlorophyll and Rubisco enzyme content showed a notable fall in barley leaves affected by MJ (Widhase et al. 1987). Although there are several reports on the role of methyl jasmonate in the degradation of photosynthetic pigments, the stimulated formation of chlorophyll a and b have been reported in the presence of light and applying methyl jasmonate in the tulip (Ueda and Saniewski 2006). Ueda and Saniewski (2006) have explained that methyl jasmonate is involved in the expression of a series of key enzyme genes in chlorophyll biosynthesis through the formation of Aminolevulinic Acid. It should be noted that this has been observed in the low concentrations of methyl jasmonate. Czerpak et al. (2006) have also reported the accumulation of chlorophyll in *Chlorella vulgaris* treated via jasmonate which is consistent with our results. In another study, jasmonate at a concentration of 0.1 μM has been shown to repair photosynthetic pigments, including chlorophyll a and carotenoids in *Wolffia arrhiza* (Piotrowska et al. 2009). It has also been demonstrated that the use of methyl jasmonate contributes to the protection of chlorophylls and enhancement of photosynthesis in barley under the influence of paraquat herbicide (Popova et al. 2003).

Composition of volatiles

The 38 identified components of volatile products induced from the in vitro shoots of *Acinos graveolense* are listed in Table 3. In control and major treatments except MJ₅₀, MJ₁₀₀, and SA₅₀ + MJ₂₅, alkane hydrocarbons were major volatile compounds, while most treatments were poor in the production of alcohol, fatty acid, oxygenated monoterpenes, monoterpenes, and sesquiterpenes. One diterpene, three oxygenated monoterpenes, two alcohol, and four fatty acids have been identified in all treatments. Among 11 studied treatments, SA₁₅₀ and MJ₁₀₀ had the highest number of compounds (12 compounds) and MJ₅₀, SA₅₀, and SA₅₀ + MJ₂₅ had the lowest number (5, 5, and 4, respectively). In the control treatment, Eicosane had the highest amount among all alkane hydrocarbons. Germacrene D was the main sesquiterpene found in all treatments except MJ₁₅₀. 1, 2-Diethylcyclohexadecane was the only identified diterpene in control and was not detected in other treatments. Among studied treatments, MJ₁₀₀ had favorable performance due to more sesquiterpene hydrocarbon compounds compared to other treatments. SA₅₀, SA₁₀₀, MJ₅₀, SA₅₀ + MJ₂₅ and SA₅₀ + MJ₅₀ did not produce any monoterpenes. Among all 11 examined treatments, SA₂₅ + MJ₂₅ and SA₁₀₀ were more effective in volatile compounds production, while control and SA₅₀ were poor in this case.

Based on the obtained results, alkane hydrocarbons and sesquiterpenes were the most plentiful of components and diterpenes had the least amount in all treatments. Decane and eicosane (two alkane hydrocarbon) and germacrene D (a sesquiterpene) were the major constituents of the oil obtained in treatments. However, survey literature showed some differences in the essential oil composition of *A. graveolens* from different origins. According to the studies in *A. graveolens* in the flora of Serbia, germacrene D and bicyclogermacrene were the compounds found in a significant amount (Golubovic et al. 2010). In another study on the *A. graveolens* in Iran, germacrene D and dillapiole were identified as the main compounds (Javidnia et al. 2010). In the present work, beta-elemene, delta-cadinene, and germacrene D were identified which was similar to Javidnia et al. (2010) and Golubovic et al. (2010) studies in *A. graveolens*. Other research on the Lamiaceae family such as *Salvia sclarea* L. (Nasermoadeli and Rowshan 2013), *Thymus caespitius* (Mendes et al. 2013), *Agastache rugosa* (Zielinska et al. 2011), and *Lantana camara* L. (Affonso et al. 2007) were also reported the mentioned compounds. Similarly, the content of sesquiterpene hydrocarbon, germacrene D, was comparable to its content in the composition of essential oils of *Thymus moroderi* (Marco-Medina and Casaa 2015), *Ziziphora persica* (Nadaf et al. 2013), and *Marrubium persicum* (Hamedeyazdan et al. 2013). Two identified compounds, docosane, and tridecane 1-iodo were similar to results obtained by Pourebadi et al. (2015) which studied secondary metabolites production in shoot and callus culture of *Lallemantia iberica* via Thidiazuron application. Previously accomplished work proposed some differences in quality or quantity of components could be related to variations in environmental conditions, time of harvesting, genotype, different parts of plant, growth phase of the plants, or in vitro ontogenetic changes (Price and Price 1999; Simões and Spitzer 2000; Lewinsohn et al. 2000).

The results obtained in the present study suggest that SA and MJ improved the increase of the essential oils. The present results are in agreement with the results of Rowshan *et al.* (2010) and Lee and Yang (2005) who demonstrated that SA spray increased terpenes in *Chrysanthemum* and essential oil in *Carum copticum*, respectively. Exogenous application of SA has earlier been indicated to promote growth, yield, and essential oil content in important species of Lamiaceae such as basil, marjoram, and summer savory (Gharib 2007; Marandi *et al.* 2011). Moreover, Yao and Tian (2005) suggested that SA stimulates phenolic compounds and the synthesis of new polyphenolic substances in sweet cherry fruit. It is also evident from the reports of Hashmi *et al.* (2012) that exogenous applications of SA could have influenced the synthesis of major components of fennel essential oil. In addition, an increased percentage of essential oil components via using SA was reported by Jalal *et al.* (2012) in Shara (*P. tenuiflorus*) culture even under drought stress conditions.

Present work showed that among the subjected of explants with different concentrations of MJ (50, 100, and 150 μ M), the samples treated with 100 μ M showed a higher amount of essential oil content in comparison with the others which conformed with Fard *et al.* (2012) findings who suggested MJ function as an elicitor in *A. foeniculum*. Similarly, Deschamps *et al.* (2008) and Talebi *et al.* (2018) reported that elicitation of MJ increased methyl chavicol production and total essential oil content in the leaves of basil and the essential oil yield in the Rubi, respectively.

Corresponding to our results and literature review as mentioned above, the positive effects of different plant growth regulators (PGRs) and elicitors such as SA and MJ, on the essential oil yield and its main components have been demonstrated by many researchers (Ghorbani *et al.* 2021; Sangwan *et al.* 1993; Zare-Hassani *et al.* 2019). It has appeared that plant secondary metabolite's pathways are activated during some particular growth and development stages and or in periods of stress caused by excess or deficiency of some factors of the environment (Mann 1987). Despite other factors, it has been demonstrated that signal molecules such as elicitors are very potential for induction of plant secondary metabolites (Zhao *et al.* 2005). When plant cells are exposed to the exogenous application of elicitors, a signal transduction initiates from the surface of the plasma membrane that triggers ROS production, which in turn induces plant defense response, subsequently, cause the rise of key enzymes activity, catalyzing the biosynthesis of target secondary metabolites (Zhao *et al.* 2005). It can be concluded that the increment in essential oil contents has occurred as a defense mechanism.

Conclusion

It could be concluded that the application of SA and MJ, as an appropriate strategy, can be used to improve the volatile oil ratio in *A. graveolens* in vitro culture. This work provides the basis for further research on improving the quality of *A. graveolens* components using elicitors.

Abbreviations

SA Salicylic acid

MJ Methyl jasmonate

Declarations

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Authors' Contribution

The work was conducted under the first author's supervision, GC-MS analysis was done with him. Doing experiments and writing the manuscript was done with second and third authors. Experiments belong to organ cultures designed with the fourth author and were guided with him.

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval This article does not contain any human or animal studies performed by any of the authors.

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Tables

Table 1 The influence of different concentrations of SA and MJ on growth parameters

Treatment (μM)	Mean Number			Mean Length (cm)	Weight (g)		
	Shoots	Roots	Leaves	Shoot	Root	Fresh	dry
Control	3.444 $\pm$ 0.530 ^a	5.305 $\pm$ 0.745 ^a	32.111 $\pm$ 4.897 ^a	2.391 $\pm$ 0.382 ^{ab}	0.902 $\pm$ 0.172 ^{ab}	181.972 $\pm$ 4.365 ^a	14.67 $\pm$ 0.667 ^a
SA ₅₀	2.443 $\pm$ 0.338 ^{ab}	2.773 $\pm$ 0.113 ^{bc}	16.886 $\pm$ 3.386 ^b	1.726 $\pm$ 0.188 ^{bc}	1.056 $\pm$ 0.101 ^a	57.333 $\pm$ 18.427 ^b	12.00 $\pm$ 1.155 ^a
SA ₁₀₀	2.138 $\pm$ 0.734 ^b	2.222 $\pm$ 0.547 ^{bc}	18.694 $\pm$ 0.573 ^b	1.340 $\pm$ 0.182 ^{bc}	0.866 $\pm$ 0.134 ^{abc}	66.583 $\pm$ 4.370 ^b	13.33 $\pm$ 1.333 ^a
SA ₁₅₀	2.222 $\pm$ 0.309 ^b	1.888 $\pm$ 0.771 ^{bc}	12.499 $\pm$ 3.237 ^b	1.108 $\pm$ 0.191 ^c	0.592 $\pm$ 0.261 ^{abc}	36.222 $\pm$ 7.946 ^b	12.00 $\pm$ 0.000 ^a
MJ ₅₀	2.388 $\pm$ 0.454 ^{ab}	2.861 $\pm$ 0.628 ^{bc}	25.805 $\pm$ 5.93 ^{ab}	1.908 $\pm$ 0.339 ^{abc}	0.855 $\pm$ 0.190 ^{abc}	136.638 $\pm$ 38.847 ^a	15.33 $\pm$ 2.404 ^a
MJ ₁₀₀	2.805 $\pm$ 0.327 ^{ab}	3.416 $\pm$ 0.173 ^{ab}	26.055 $\pm$ 3.644 ^{ab}	2.408 $\pm$ 0.526 ^{ab}	0.863 $\pm$ 0.200 ^{abc}	140.500 $\pm$ 15.223 ^a	16.00 $\pm$ 2.000 ^a
MJ ₁₅₀	2.944 $\pm$ 0.055 ^{ab}	3.777 $\pm$ 1.019 ^{ab}	18.666 $\pm$ 8.505 ^b	2.813 $\pm$ 1.120 ^a	0.763 $\pm$ 0.220 ^{abc}	148.360 $\pm$ 33.026 ^a	11.33 $\pm$ 2.404 ^a
SA ₂₅ +MJ ₂₅	2.305 $\pm$ 0.100 ^b	0.888 $\pm$ 0.587 ^c	15.388 $\pm$ 0.963 ^b	1.483 $\pm$ 0.148 ^{bc}	0.466 $\pm$ 0.233 ^{abc}	56.694 $\pm$ 5.366 ^b	14.00 $\pm$ 3.055 ^a
SA ₅₀ +MJ ₂₅	2.722 $\pm$ 0.147 ^{ab}	1.750 $\pm$ 0.721 ^{bc}	17.333 $\pm$ 1.766 ^b	1.536 $\pm$ 0.112 ^{bc}	0.572 $\pm$ 0.964 ^{abc}	64.805 $\pm$ 3.209 ^b	16.00 $\pm$ 5.033 ^a
SA ₂₅ +MJ ₅₀	2.666 $\pm$ 0.440 ^{ab}	1.111 $\pm$ 0.389 ^c	16.305 $\pm$ 1.178 ^b	0.924 $\pm$ 0.173 ^c	0.277 $\pm$ 0.14 ^{6c}	59.722 $\pm$ 3.525 ^b	15.33 $\pm$ 0.667 ^a
SA ₅₀ +MJ ₅₀	2.999 $\pm$ 0.254 ^{ab}	1.888 $\pm$ 0.818 ^{bc}	17.055 $\pm$ 3.292 ^b	1.172 $\pm$ 0.474 ^c	0.355 $\pm$ 0.100 ^{bc}	63.555 $\pm$ 18.878 ^b	13.33 $\pm$ 1.333 ^a

Data represent the mean $\pm$ standard error (SE). Mean values followed by the same letters within a column are not significantly different according to Duncan's multiple range test at 5% level (P = 0.05)

Table 2 The influence of the production of photosynthetic pigments of SA and MJ concentration (μM) on the regenerated shoot

Treatment (μM)	Chlorophyll a (mg g ⁻¹)	Chlorophyll b (mg g ⁻¹)	Carotenoids (mg g ⁻¹)
Control	0.2677 $\pm$ 0.0001 ^f	0.1037 $\pm$ 0.0002 ^f	0.0603 $\pm$ 0.0022 ^d
SA ₅₀	0.3323 $\pm$ 0.0002 ^c	0.1458 $\pm$ 0.0001 ^a	0.0722 $\pm$ 0.0000 ^c
SA ₁₀₀	0.0908 $\pm$ 0.0000 ^h	0.0662 $\pm$ 0.0002 ^h	0.0204 $\pm$ 0.0000 ^g
SA ₁₅₀	0.044 $\pm$ 0.0000 ^k	0.0315 $\pm$ 0.0000 ^k	0.0085 $\pm$ 0.0000 ⁱ
MJ ₅₀	0.3104 $\pm$ 0.0001 ^d	0.1159 $\pm$ 0.0000 ^d	0.0712 $\pm$ 0.0000 ^c
MJ ₁₀₀	0.3472 $\pm$ 0.0001 ^a	0.1237 $\pm$ 0.0000 ^c	0.0731 $\pm$ 0.0000 ^c
MJ ₁₅₀	0.3057 $\pm$ 0.0001 ^e	0.1114 $\pm$ 0.0000 ^e	0.0775 $\pm$ 0.0001 ^b
SA ₂₅ +MJ ₂₅	0.0731 $\pm$ 0.0000 ^j	0.0374 $\pm$ 0.0003 ^j	0.0232 $\pm$ 0.0001 ^f
SA ₅₀ +MJ ₂₅	0.1811 $\pm$ 0.0000 ^g	0.0869 $\pm$ 0.0003 ^g	0.054 $\pm$ 0.0001 ^e
SA ₂₅ +MJ ₅₀	0.3354 $\pm$ 0.0001 ^b	0.1326 $\pm$ 0.0002 ^b	0.0847 $\pm$ 0.0000 ^a
SA ₅₀ +MJ ₅₀	0.080 $\pm$ 0.0000 ⁱ	0.0592 $\pm$ 0.0000 ⁱ	0.0124 $\pm$ 0.0001 ^h

Data represent the mean $\pm$ standard error (SE). Mean values followed by the same letters within a column are not significantly different according to Duncan's multiple range test at 5% level (P = 0.05)

Table 3 Composition of volatiles from the shoot culture of *A. graveolens* under SA and MJ concentrations (μM) treatments

peak No	constituent	RI	MS ₀	SA ₅₀	SA ₁₀₀	SA ₁₅₀	M _{J50}	MJ ₁₀₀	MJ ₁₅₀	SA ₂₅ +MJ ₂₅	SA ₅₀ +MJ ₂₅	SA ₂₅ +MJ ₅₀	SA ₅₀ +MJ ₅₀
1	Nonane, 3-methyl	976	-	-	-	-	-	-	-	+	-	-	-
2	Decane	1000	-	-	+	-	-	-	+	+	-	-	+
3	Benzene, 1-methyl-3-(1-methylethyl)	1022	-	-	-	-	-	+	-	-	-	-	-
4	dl-Limonene	1033	+	-	-	-	-	-	+	-	-	+	-
5	Benzene, 2-ethyl-1,4-dimethyl	1077	-	-	-	+	-	+	-	-	-	-	-
6	Benzene, 1-ethyl-2,4-dimethyl	1078	-	-	-	-	-	+	-	-	-	-	-
7	Benzene, 4-ethyl-1,2-dimethyl	1083	-	-	-	+	-	+	-	-	-	-	-
8	Undecane	1100	-	-	+	+	-	-	-	-	-	-	-
9	Benzene, 1,4-diethyl-	1106	-	-	-	-	-	+	-	-	-	-	-
10	Benzene, 1,2,4,5-tetramethyl	1133	-	-	-	+	-	+	-	-	-	-	-
11	Undecane, 5-methyl	1150	-	-	-	-	-	-	-	+	-	-	-
12	Cyclopentane, 1-hexyl-3-methyl	1219	-	-	-	-	-	-	-	+	-	-	-
13	m-Thymol	1262	-	-	-	+	+	-	-	-	+	-	-
14	Tridecane	1300	-	+	+	-	-	-	+	+	-	+	+
15	1-Tridecene	1304	+	-	-	-	-	-	-	-	-	-	-
16	1,5,5-Trimethyl-6-methylene-cyclohexene	1338	-	-	-	-	-	+	-	-	-	-	-
17	Dodecane, 2,6,10-trimethyl	1379	-	-	-	-	-	-	-	-	-	-	+
18	Beta. elemene	1391	+	-	-	-	+	+	-	-	-	-	-
19	Tetradecane	1400	-	+	+	-	-	-	+	+	-	+	+
20	Beta gurjunene	1428	-	-	-	-	+	-	-	-	+	+	-
21	Epi-bicyclosquiphellandrene	1448	-	-	-	-	-	+	-	-	-	-	-
22	Germacrene D	1485	+	+	+	+	+	+	+	-	+	+	+
23	Delta-Cadinene	1528	-	-	-	-	-	+	-	-	-	-	-
24	7-Hexadecene, (Z)	1630	-	-	+	-	-	-	-	-	-	-	-
25	1-Heptadecene	1701	-	-	-	-	-	-	-	+	-	-	-
26	Tridecane, 1-iodo	1728	-	-	+	-	-	-	-	-	-	-	-
27	1-Octadecene	1788	+	-	-	-	-	-	+	-	-	-	-
28	1-Nonadecene	1885	-	-	-	+	-	-	-	-	-	-	-
29	Hexadecanoic acid	1975	-	-	+	-	-	-	-	-	-	-	-
30	Hexadecanoic acid, ethyl ester	1993	-	-	-	+	-	-	-	-	-	-	-
31	Eicosane	2000	+	+	+	+	-	-	+	-	+	+	+
32	Ethyl linoleate	2159	-	-	-	+	-	-	-	-	-	-	-
33	Octadecanoic acid, ethyl ester	2193	-	-	-	+	-	-	-	-	-	-	-
34	Docosane	2200	+	-	-	+	-	-	+	-	-	-	+

35	Cyclohexadecane, 1,2-diethyl	2239	+	-	-	-	-	-	-	-	-	-	-
36	Docosane, 2,21-dimethyl	2279	-	+	+	-	-	-	-	-	-	-	-
37	Ethanol, 2-(dodecyloxy)	2328	-	-	-	-	+	-	-	-	-	-	+
38	Triacontane, 1-bromo	3299	-	-	-	-	-	+	-	-	-	-	-

RI retention index, MS₀: MS media free plant growth regulators, SA₅₀: 50 µM of salicylic acid, SA₁₀₀: 100 µM of salicylic acid, SA₁₅₀: 150 µM of salicylic acid, MJ₅₀: 50 µM of methyl jasmonate, MJ₁₀₀: 100 µM of methyl jasmonate, MJ₁₅₀: 150 µM of methyl jasmonate, SA₂₅+MJ₂₅: 25 µM of salicylic acid + 25 µM of methyl jasmonate, SA₅₀ + MJ₂₅: 50 µM of salicylic acid + 25 µM of methyl jasmonate, SA₂₅ + MJ₅₀: 25 µM of salicylic acid + 50 µM of methyl jasmonate, SA₅₀ + MJ₅₀: 50 µM of salicylic acid + 50 µM of methyl jasmonate

Figures

Figure 1

The effect of different concentrations of SA and MJ on *A. graveolens* plant

Control, SA₅₀, SA₁₀₀, SA₁₅₀, MJ₅₀, MJ₁₀₀, MJ₁₅₀, SA₂₅+MJ₂₅, SA₂₅+MJ₅₀, SA₅₀+MJ₂₅ and SA₅₀+MJ₅₀ (from left to right, respectively)