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Effects of methyl jasmonate and salicylic acid on the production of camphor and phenolic compounds in cell suspension culture of endemic Turkish yarrow (*Achillea gypsicola*) species*

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Abstract: Elicitors have been widely used as biotic and abiotic stimulants in triggering the production of secondary metabolites in plant cell culture. The present study aimed to enhance the production of camphor and phenolic compounds and cell growth using methyl jasmonate (MeJA) and salicylic acid (SA) in the cell suspension culture of Turkish endemic species *Achillea gypsicola*. Various concentrations (0, 10, 50, and 100 µM) of MeJA and SA were applied to 8-day-old cell cultures. The camphor and phenolic compound contents were determined using a headspace gas chromatographic-mass spectrometer device and spectrophotometer. Increasing doses of MeJA and SA significantly enhanced the accumulation of the camphor and phenolic compounds in general. The highest amount of camphor accumulation occurred in cells treated with 100 µM MeJA (0.3449 µg/g) and 50 µM SA (0.3816 µg/g). Increasing concentrations of MeJA resulted in a significant decrease in the total anthocyanin when compared to the initial culture. The present study showed that MeJA and SA could effectively be used as potent elicitors to enhance the production of camphor and phenolic compounds, along with cell growth, in cell suspension cultures of the endemic Turkish yarrow species *Achillea gypsicola*.

Key words: Abiotic stress, elicitor, methyl jasmonate, salicylic acid, secondary metabolites

1. Introduction

The potential benefits of medicinal and aromatic plants are largely accounted for by naturally occurring bioactive compounds, called secondary metabolites (Bourgaud et al., 2001). The contents and constituents of these compounds vary greatly based on several factors such as plant health, parts used, growth stage, soil and climate, and harvesting time (Abdin et al., 2007; Biesalski et al., 2009). There is thus a need to develop some methods providing the production of high-value secondary metabolites with a certain level of quantity and quality (Hussain et al., 2012). Plant cell culture, in this respect, is of considerable importance and is widely used as an attractive alternative for producing valuable secondary metabolites (Georgiev et al., 2009; Wang et al., 2017). Despite its widespread usage, plant cell culture has a challenge of low yield of plant secondary metabolites (Zhao et al., 2005). Therefore, in vitro elicitor-

induced production of secondary metabolites has recently gained considerable interest worldwide to overcome the low yield of such plant phytochemicals (Namdeo, 2007; Matkowski, 2008; Patel and Krishnamurthy, 2013; Dias et al., 2016).

The synthesis and accumulation of secondary metabolites occurs in plants exposed to various biotic and abiotic stresses, as these compounds allow the plants to survive stress conditions prevailing in their surroundings. Based on this principle, some strategies for promoting in vitro production of secondary metabolites have recently been developed, including treatment with microbial, physical, or chemical factors, known as elicitors (Yue et al., 2016). It is well known that employing biotic and abiotic elicitors in plants effectively initiates or enhances the biosynthesis of defensive secondary metabolites (Rao and Ravishankar, 2002; Akula and Ravishankar, 2011).

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The effect of various elicitors (light and heavy metals, salts, yeast, methyl jasmonate (MeJA), salicylic acid (SA), etc.) on the accumulation of a wide range of secondary metabolites has been studied using plant cell cultures (Krzyzanowska et al., 2012; Wang et al., 2015). MeJA and SA have been proposed as important signaling compounds in the elicitation process, regulating the defense responses of plants and promoting the production of various secondary metabolites in plant cell cultures (Cheong and Choi, 2003; Singh and Dwivedi, 2018).

Turkey, located at the intersection point of 3 main floristic regions and having diverse climate and soil conditions, is one of the biologically richest countries in the world. Medicinal and aromatic plants, belonging to the families of Lamiaceae, Asteraceae, and Apiaceae in particular, represent an important constituent of this biodiversity (Baser, 2002). The genus *Achillea*, a member of the family Asteraceae, is widely distributed in Anatolia and represented by 48 species, 24 of which are endemic to Turkey (Baser, 2016). *Achillea* species, known as yarrow and called *civanperçemi* in Turkish, are important components in traditional and folk medicine due to their pharmaceutical, cosmetic, and fragrance properties (Nemeth and Bernath, 2008; Demirci et al., 2018).

The genus *Achillea* has also been proven to have an important phytochemical, camphor, which has been used in medicine and the cosmetics industry for its antimicrobial and anticarcinogenic properties (Saeidnia et al., 2011). *Cinnamomum camphora*, known as camphor tree, grows in the Far East and is the main source of camphor, containing almost 68% camphor compound (Frizzo et al., 2000; Zuccarini, 2009). *Achillea gypsicola* is a yarrow species endemic to central Anatolia in Turkey and has 40% camphor content (Baser, 2016). *Achillea gypsicola* is placed in the vulnerable (VU) category in the Red Data Book of Turkish Plants. With its herbaceous growth habit and relatively short growing period, *Achillea gypsicola* is of considerable importance as an attractive and promising source of camphor.

To the best of our knowledge, there have been no reports concerning the in vitro use of elicitors on the biosynthesis of camphor and phenolic compounds in the endemic Turkish yarrow species *Achillea gypsicola*. The present study, therefore, will be the first attempt in this respect. In view of these points, this study aimed to enhance the accumulation of camphor and phenolic compounds and promote cell growth in cell suspension culture of *Achillea gypsicola* using MeJA and SA as elicitors.

2. Materials and methods

2.1. Collection of plant material

Seeds of *Achillea gypsicola* were collected from its natural habitat in the vicinity of the provinces of Çorum and Çankırı in central Anatolia, Turkey, with the written

permission of the Ministry of Food, Agriculture, and Livestock. The species diagnosis was performed by Assist Prof Dr Sevda Türkiş, a member of the Science Education Department, Faculty of Education of Ordu University. Voucher plant specimens were kept in the herbarium of the Field Crops Department of the Faculty of Agriculture, Ordu University.

2.2. Cell suspension cultures and elicitation process

In vitro plantlets obtained from these seeds were used as explant sources and cell suspension cultures of *A. gypsicola* were established using stem segment-derived callus tissues. They were maintained in B₅ medium (Gamborg et al., 1968) supplemented with 0.5 mg/L benzylaminopurine + 0.5 mg/L naphthalene acetic acid, and grown in three 250-mL Erlenmeyer flasks containing 50 mL of liquid medium and 2.5 g of green fragile calluses. Next, they were cultured on a rotary shaker at 105 rpm at 25 °C and a light/dark photoperiod 16/8. The MeJA was dissolved in pure 96% ethanol and passed through 0.2-µm filter paper, whereas the SA was dissolved in distilled water and the pH was adjusted to 5.8. The prepared elicitors were added separately to the suspension cultures 8 days after the beginning of cultivation using several concentrations and exposure times.

A total of 3 concentrations (10, 50, and 100 µM) of MeJA and SA were used, along with the control, to which equal volumes of ethanol and distilled water were added, respectively. Cell suspension cultures, incubated under the same conditions described above, were harvested at 8, 24, and 48 h after elicitation to determine the effect of MeJA and SA on cell growth and the accumulation of camphor and phenolic compounds. After aseptic filtering through Whatman No. 3 filter paper and washing with deionized water, the suspension cultures were kept in a deep-freezer (-20 °C) until extraction.

The cell suspension cultures were evenly crushed with a mortar and pestle before the chemical analysis. Extraction was performed using the method described by Dalar et al. (2012). In brief, 2 g of suspension, on a fresh weight basis, was mixed with 10 mL of 96% ethanol and homogenized for 2 min, and kept in a water bath at 45 °C for 1 night. The samples were then placed on a rotary shaker at 4000 rpm for 5 min, and supernatant portions of the extracts were collected in vials and evaporated until completely dry at 75 °C in a rotary evaporator. The residues were dissolved in 1 mL of methanol and used for chemical analysis (Dalar et al., 2012).

2.3. Determination of the camphor and phenolic compounds

The total phenolic content of the cell suspension cultures was determined using the Folin-Ciocalteu reagent method as described by Slinkard and Singleton (1977). Absorbance values were measured at 765 nm. The total phenolic content, using a standard curve of gallic acid

solution (5–1000 mg, 0.1 mL⁻¹), was expressed as mg g⁻¹ fresh cell weight (FCW) in gallic acid equivalent (GAE). To determine the total flavanol contents in the cell suspension cultures, dimethylamino cinnamaldehyde (DMAC) chromogenic reagent was used, as proposed by Prior et al. (2010). The absorbance was recorded at 640 nm in the spectrophotometer and the results were given as mg g⁻¹ fresh cell weight in catechin equivalent (CTE). The total flavonol contents of the cell suspension cultures were determined using Neu solution as described by Dai et al. (1995). The extracts were mixed with methanol and 1% 2-aminoethyl diphenylborinate, and then the absorbance of the mixture was measured at 410 nm using a spectrophotometer and expressed as mg g⁻¹ FCW in rutin equivalent (RE). The total anthocyanin content was determined using the method of Qu et al. (2006), using McIlvaine's buffer. Absorbance of the solution was measured at 570 nm using a spectrophotometer. The color value (CV) of the extract, an indicator of anthocyanin content, was calculated using the following formula: $CV = 0.1 \times \text{absorbance} \times \text{dilution factor}$ (CV g⁻¹ FCW).

The camphor content was measured with a headspace gas chromatographic-mass spectrometer (GC-MS) (Innovatech Labs, LLC, USA) using a Shimadzu QP2010 Ultra integrated with a Shimadzu AOC-5000 plus autosampler (Shimadzu Scientific Inst., USA). Capillary column separation was observed with a 30-m RTX-5M. For the analysis, a camphor standard was given to the device and the mass fragments and retention times of the solution were determined. In order to increase the precision and accuracy of the method, 9 major ion peaks were chosen. Afterwards, a calibration curve was drawn, and using this curve the camphor content was expressed as µg g⁻¹. The experimental conditions of the GC-MS were as follows: carrier gas of helium, injection temperature of 250 °C, injection volume of 0.5 mL, ionization voltage of 70 eV, temperature of 100 °C, and heating period of 10 min.

2.4. Determination of the cell dry weight, number, and viability

Growth of the cell suspensions was evaluated by measuring the cell dry weight (g L⁻¹), number, and viability (%). The cell dry weight was measured by weighing the filtered cells, kept at 55 °C for 48 h in an oven. In order to determine cell viability, Trypan Blue solution (Thermo Fisher Scientific, USA) was used as described by Laloue et al. (1980). The number of cells was determined using a Nageotte Counting Chamber (Hausser Scientific, USA), as described by Moroff et al. (1994).

2.5. Statistical analysis

All of the applications were carried out in triplicate. The experimental layout was completely randomized and the data were analyzed by the 2-way analysis of variance (ANOVA) using the Minitab 17 statistical program (Minitab, LLC, USA). The Tukey test was used to

determine the differences among the means and statistical significance was considered as $P < 0.05$.

3. Results

Both the MeJA and SA generally influenced the synthesis of the camphor and phenolic compounds and cell growth. Treatment of the *Achillea gypsicola* cell suspension cultures with various doses of MeJA produced significant changes in the total phenolic, flavonol, anthocyanin, and camphor contents and cell number and dry weight (Tables 1 and 2). Increasing doses of MeJA, however, had no significant effect on the total flavanol content and cell viability. The effect of culture period was found to be significant for the total phenolic, flavanol, and camphor contents and cell number and dry weight, with the highest values obtained 72 h after the addition of MeJA to suspension cultures. The interaction effect between the elicitor concentrations and culture periods was not significant for any of the parameters evaluated.

An increase in camphor accumulation was recorded with all of the MeJA concentrations and the maximum accumulation (0.3449 µg g⁻¹) was measured 72 h after the addition of 100 µM, with an increase of 34.6% in comparison to the control culture (0.2561 µg g⁻¹). Similarly, a regular increase corresponding to increasing doses of MeJA was observed in the total phenolic content and the treatment of 100 µM MeJA for 72 h gave the highest value. Treatment with lower concentrations of MeJA (10 and 50 µM) decreased the total flavonol content when compared to the initial culture, but the treatment of 100 µM MeJA resulted in an increase, where its content was identical to that of the control. The application of MeJA to the cell suspension cultures, on the contrary, resulted in a significant reduction in the total anthocyanin content, with a decrease of 41.5% in the suspension treated with 100 µM MeJA for 72 h when compared to the initial culture. The suspension culture treated with 100 µM MeJA showed a significant increase in the cell number and dry weight when compared to the control.

Treatment of the *A. gypsicola* cell suspensions with various doses of SA exerted a significant effect on the total flavonol, camphor, and anthocyanin contents and cell number and dry weight (Tables 3 and 4). Increasing doses of SA, on the other hand, resulted in no significant changes in the total phenolic and flavanol contents and the cell viability. The culture period resulted in a significant effect on the total flavanol and camphor contents and the cell number and viability. The interaction effect among the SA doses and culture periods, however, was nonsignificant.

The highest accumulation of camphor, 0.3816 µg g⁻¹, was noticed 72 h after the addition of 50 µM SA, indicating an increment of 49.0% in comparison to the initial culture. Treatment with 10 and 50 µM SA resulted in a decrease in the total flavanol content when compared

Table 1. Total phenolic, flavanol, flavonol, anthocyanin, and camphor contents recorded in *Achillea gypsicola* cell suspension cultures subjected to different MeJA doses for various hours.

| MeJA doses (μM) | Culture periods (h) | | | Mean |
|--|---------------------|-----------|----------|-----------|
| | 8 | 48 | 72 | |
| Total phenolic (mg g^{-1}) | | | | |
| 0 (control) | 0.6369 | 0.6382 | 0.6418 | 0.6390 c* |
| 10 | 0.6408 | 0.6332 | 0.6435 | 0.6392 c |
| 50 | 0.6502 | 0.6539 | 0.6546 | 0.6529 b |
| 100 | 0.6530 | 0.6624 | 0.6641 | 0.6598 a |
| Mean | 0.6452 B* | 0.6469 AB | 0.6510 A | |
| Total flavanol (mg g^{-1}) | | | | |
| 0 (control) | 0.0099 | 0.0111 | 0.0115 | 0.0108 |
| 10 | 0.0102 | 0.0127 | 0.0124 | 0.0118 |
| 50 | 0.0104 | 0.0099 | 0.0114 | 0.0106 |
| 100 | 0.0104 | 0.0115 | 0.0120 | 0.0113 |
| Mean | 0.0102 B* | 0.0113 AB | 0.0118 A | |
| Total flavonol (mg g^{-1}) | | | | |
| 0 (control) | 0.0039 | 0.0030 | 0.0037 | 0.0035 a* |
| 10 | 0.0026 | 0.0027 | 0.0032 | 0.0028 b |
| 50 | 0.0029 | 0.0025 | 0.0022 | 0.0025 b |
| 100 | 0.0039 | 0.0030 | 0.0037 | 0.0035 a |
| Mean | 0.0033 | 0.0028 | 0.0032 | |
| Total anthocyanin (CV g^{-1}) | | | | |
| 0 (control) | 0.0053 | 0.0058 | 0.0055 | 0.0056 a* |
| 10 | 0.0039 | 0.0030 | 0.0037 | 0.0035 b |
| 50 | 0.0026 | 0.0027 | 0.0032 | 0.0029 b |
| 100 | 0.0029 | 0.0025 | 0.0022 | 0.0025 b |
| Mean | 0.0037 | 0.0035 | 0.0037 | |
| Camphor ($\mu\text{g g}^{-1}$) | | | | |
| 0 (control) | 0.2561 | 0.2624 | 0.2740 | 0.2642 c* |
| 10 | 0.2592 | 0.2630 | 0.2742 | 0.2654 c |
| 50 | 0.3057 | 0.3091 | 0.3107 | 0.3085 b |
| 100 | 0.3346 | 0.3413 | 0.3449 | 0.3403 a |
| Mean | 0.2889 B* | 0.2940 AB | 0.3009 A | |

* Means with the same letters in the same row and column are not significantly different from each other ($P < 0.05$).

to the initial culture, whereas the application of 100 μM SA increased the total flavonol content to a level higher than that of the control culture. The total anthocyanin content significantly increased in the SA-treated suspensions when compared to the initial culture and an increment of 33.9% was observed with treatment of 50 μM SA. The suspension culture treated with 100 μM SA ended up with a significant

increase in cell number and dry weight when compared to the control.

4. Discussion

Achillea gypsicola is a perennial herbaceous-wooly plant that grows in steppe-limestone areas of central Anatolia (Akcin and Akcin, 2010). The plant has erect stems, growing

Table 2. Cell number, dry weight, and viability recorded in *Achillea gypsicola* cell suspension cultures subjected to different MeJA doses for various hours.

| MeJA doses (μM) | Culture periods (h) | | | Mean |
|---------------------------------------|---------------------|----------|---------|----------|
| | 8 | 48 | 72 | |
| Cell number | | | | |
| 0 (control) | 82.84 | 82.76 | 83.12 | 82.90 b* |
| 10 | 83.40 | 83.50 | 85.00 | 83.96 a |
| 50 | 83.32 | 83.84 | 84.44 | 83.86 ab |
| 100 | 83.80 | 83.80 | 84.88 | 84.16 a |
| Mean | 83.34 B* | 83.48 B | 84.36 A | |
| Cell dry weight (g L^{-1}) | | | | |
| 0 (control) | 9.246 | 9.245 | 9.258 | 9.250 b* |
| 10 | 9.252 | 9.257 | 9.266 | 9.258 b |
| 50 | 9.250 | 9.259 | 9.270 | 9.260 b |
| 100 | 9.338 | 9.338 | 9.345 | 9.340 a |
| Mean | 9.272 B* | 9.275 AB | 9.285 A | |
| Cell viability (%) | | | | |
| 0 (control) | 97.973 | 97.747 | 97.347 | 97.689 |
| 10 | 97.747 | 98.630 | 97.827 | 98.068 |
| 50 | 98.050 | 98.233 | 97.660 | 97.981 |
| 100 | 97.540 | 98.067 | 98.140 | 97.916 |
| Mean | 97.828 | 98.169 | 97.743 | |

* Means with the same letters in the same row and column are not significantly different from each other ($P < 0.05$).

up to 20 cm in height, with imbricate and 3-partite leaves, and golden yellow disk flowers. Previous studies reported that camphor (40%), 1,8-cineole (22%), piperitone (11%), borneol (10%), and α -terpineol (2%) were found as the main constituents in *A. gypsicola* essential oil (Kordali et al., 2009; Tozlu et al., 2011; Baser, 2016).

Plants have been found to develop various defensive responses when subjected to in vitro stress factors called elicitors, which are signals triggering the formation of secondary metabolites. In the present study, both MeJA and SA produced almost the same effect on the camphor, phenolic compounds, and cell growth, except for anthocyanin content. There was a significant increase in the total flavonoid and camphor contents and the cell number and dry weight in response to MeJA and SA applications. The effects of MeJA and SA were much more pronounced for camphor content, as the treatments of both elicitors resulted in increases of 34.6% and 49.0%, respectively, when compared to the initial culture. There are several reports in respect to the content and constituents of essential oils and total phenolic compounds along with the antimicrobial

and antioxidant activity of some *Achillea* species (Baser, et al., 2001; Toncer et al., 2010; Mohsen, 2015; Tabanca et al., 2016; Mohammadhosseini et al., 2017). No data, however, were available in the scientific literature regarding elicitor-induced camphor accumulation with which to compare the results of the present study.

The anthocyanin content showed a significant decrease in accordance with the increasing concentrations of MeJA, whereas it resulted in a significant increment with SA treatments. The decrease in anthocyanin content in cell suspension cultures treated with MeJA could be attributed to the shifting of biosynthetic pathways from anthocyanins to phenolic compounds. Ghanati et al. (2014) reported similar results with *Achillea millefolium*, as they indicated that treatment with MeJA at the flowering stage increased the flavonoid content and yield of essential oils but reduced the anthocyanin content of the plants when compared to the control plants. Gorni and Pachedo (2016) reported that, in *Achillea millefolium*, the total chlorophyll content significantly increased in 1.00 mM SA-treated plants when compared with the control. However, they did not observe

Table 3. Total phenolic, flavanol, flavonol, anthocyanin, and camphor content recorded in *Achillea gypsicola* cell suspension cultures treated with different SA doses for various hours.

| SA doses (μM) | Culture periods (h) | | | Mean |
|--|---------------------|----------|----------|-----------|
| | 8 | 48 | 72 | |
| Total phenolic (mg g^{-1}) | | | | |
| 0 (control) | 0.6369 | 0.6382 | 0.6418 | 0.6390 |
| 10 | 0.6318 | 0.6314 | 0.6363 | 0.6332 |
| 50 | 0.6351 | 0.6364 | 0.6383 | 0.6366 |
| 100 | 0.6363 | 0.6318 | 0.6348 | 0.6343 |
| Mean | 0.6350 | 0.6345 | 0.6378 | |
| Total flavanol (mg g^{-1}) | | | | |
| 0 (control) | 0.0099 | 0.0111 | 0.0115 | 0.0108 |
| 10 | 0.0102 | 0.0127 | 0.0124 | 0.0118 |
| 50 | 0.0104 | 0.0109 | 0.0114 | 0.0109 |
| 100 | 0.0117 | 0.0123 | 0.0123 | 0.0121 |
| Mean | 0.0105 B* | 0.0118 A | 0.0119 A | |
| Total flavonol (mg g^{-1}) | | | | |
| 0 (control) | 0.0426 | 0.0436 | 0.0429 | 0.0430 b* |
| 10 | 0.0427 | 0.0428 | 0.0423 | 0.0426 b |
| 50 | 0.0415 | 0.0427 | 0.0436 | 0.0426 b |
| 100 | 0.0448 | 0.0459 | 0.0476 | 0.0461 a |
| Mean | 0.0429 | 0.0437 | 0.0441 | |
| Total anthocyanin (CV g^{-1}) | | | | |
| 0 (control) | 0.0053 | 0.0058 | 0.0055 | 0.0056 b* |
| 10 | 0.0055 | 0.0054 | 0.0056 | 0.0055 b |
| 50 | 0.0064 | 0.0069 | 0.0071 | 0.0068 a |
| 100 | 0.0059 | 0.0062 | 0.0059 | 0.0060 ab |
| Mean | 0.0058 | 0.0061 | 0.0060 | |
| Camphor ($\mu\text{g g}^{-1}$) | | | | |
| 0 (control) | 0.2561 | 0.2624 | 0.2740 | 0.2642 c* |
| 10 | 0.2592 | 0.2630 | 0.2742 | 0.2654 c |
| 50 | 0.3613 | 0.3680 | 0.3816 | 0.3703 a |
| 100 | 0.3440 | 0.3378 | 0.3542 | 0.3454 b |
| Mean | 0.3052 B* | 0.3078 B | 0.3210 A | |

* Means with the same letters in the same row and column are not significantly different from each other ($P < 0.05$).

significant differences in carotenoids and anthocyanins between SA-treated and control yarrow plants. The results of these and other studies imply that various elicitors exert species-specific and metabolite-dependent responses in plant cell cultures.

The increase in exposure time caused a significant increase in many of the parameters in the MeJA and SA-

treated cell suspensions when compared to the control culture, with the highest generally obtained 72 h after the addition of both elicitors. Krzyzanowska et al. (2012) reported that an increase in rosmarinic acid accumulation in *Mentha piperita* was noticed for all of the applied jasmonic acid and MeJA doses, but the maximum was observed at various times. These findings imply that elicitor

Table 4. Cell number, dry weight, and viability recorded in *Achillea gypsicola* cell suspension cultures treated with different SA doses for various hours.

| SA doses (μM) | Culture periods (h) | | | Mean |
|---------------------------------------|---------------------|----------|----------|-----------|
| | 8 | 48 | 72 | |
| Cell number | | | | |
| 0 (control) | 82.860 | 82.720 | 83.120 | 82.900 c* |
| 10 | 83.400 | 83.500 | 85.100 | 84.000 b |
| 50 | 83.640 | 84.200 | 85.320 | 84.386 b |
| 100 | 92.920 | 93.240 | 94.640 | 93.600 a |
| Mean | 85.705 B* | 85.915 B | 87.045 A | |
| Cell dry weight (g L^{-1}) | | | | |
| 0 (control) | 9.246 | 9.245 | 9.245 | 9.250 c* |
| 10 | 9.252 | 9.257 | 9.257 | 9.258 c |
| 50 | 9.333 | 9.335 | 9.335 | 9.338 b |
| 100 | 9.461 | 9.441 | 9.441 | 9.448 a |
| Mean | 9.323 | 9.319 | 9.328 | |
| Cell viability (%) | | | | |
| 0 (control) | 97.973 | 97.747 | 97.347 | 97.689 |
| 10 | 97.747 | 98.630 | 97.827 | 98.068 |
| 50 | 97.747 | 98.630 | 97.827 | 98.068 |
| 100 | 98.207 | 98.067 | 98.140 | 98.138 |
| Mean | 97.918 AB* | 98.268 A | 97.785 B | |

* Means with the same letters in the same row and column are not significantly different from each other ($P < 0.05$).

concentration and the time of incubation with the elicitor are of considerable importance for the accumulation of secondary metabolites.

This study resulted in a significant increase in the cell number and dry weight in the MeJA- and SA-treated cell suspensions when compared to the control culture. These results are in agreement with those of Ali et al. (2015), who showed that a 2.6-fold, 2.9-fold, and 3-fold increase in dry biomass was observed in *Artemisia absinthium* cultures treated with 0.5, 1.0, and 2.0 mg L^{-1} MeJA, respectively. However, Ali et al. (2007) reported different results, in which the fresh and dry weights decreased significantly after 9 days of exposure to SA and MeJA in *Panax ginseng* root suspension culture. Similarly, it was reported that suspension cultures of *Mentha piperita* treated with MeJA showed a significant decrease in biomass accumulation when compared to the control, which was proportional to the applied concentration of MeJA (Krzyzanowska et al., 2012).

Elicitor concentration and induction time are key factors that affect the cell growth and product yield for

plant cell suspension culture (Ali et al., 2007; Wang et al., 2015). The effects of both MeJA and SA were dependent on elicitor concentration and length of exposure. With the lowest MeJA and SA concentrations, i.e. 10 μM , these effects were not significantly different than that of the control. On the other hand, they were mostly significant in the case of suspension cultures elicited with higher concentrations of elicitor (50 and 100 μM), especially at 72 h after treatment. A similar observation was reported in *Mentha piperita* suspension culture (Krzyzanowska et al., 2012). A number of factors, including the elicitor concentration and selectivity, duration of elicitor exposure, age of culture, cell line, growth regulation, and nutrient composition, should be considered in the correct evaluation of the elicitation process regarding cell growth and product yield for plant cell suspension cultures (Smetanska, 2008; Wang et al., 2017).

To the best of our knowledge, this is the first report regarding in vitro elicitor-induced accumulation of camphor and phenolic compounds in *Achillea gypsicola*, an endemic Turkish yarrow species. The elicitor

concentration and time of incubation with the elicitor proved to be crucial for the accumulation of camphor and the phenolic compounds. The addition of MeJA and SA significantly enhanced the camphor accumulation in cell suspension culture compared with the untreated control. The highest accumulation of camphor, $0.3449 \mu\text{g g}^{-1}$, was detected 72 h after the addition of $100 \mu\text{M}$ MeJA. In the case of SA treatment, however, the highest concentration, $0.3816 \mu\text{g g}^{-1}$, was detected 72 h after the application of $50 \mu\text{M}$ SA. Those values were 34.6% and 49.0% higher when compared to the initial culture, respectively. The camphor contents were higher in the SA-treated suspension cultures than in the MeJA-treated ones.

The production of phenolic compounds and camphor was promoted by MeJA and SA, along with an increase in cell growth, which is very preferable for obtaining valuable plant secondary metabolites from in vitro culture.

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- However, increasing concentrations of MeJA resulted in a significant decrease in total anthocyanin content, probably due to the shifting of biosynthetic pathways from anthocyanins to other phenolic compounds.
- The present study demonstrated that MeJA and SA could effectively be used as potent elicitors to enhance the synthesis of camphor and phenolic compounds in cell suspension cultures of the endemic Turkish yarrow species *Achillea gypsicola*.

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