

Article - Biological and Applied Sciences

Stimulating Effect of Melatonin on the Phytochemical Content of *Salvia officinalis* L. Callus Cultures

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Editor-in-Chief: Paulo Vitor Farago

Associate Editor: Jane Manfron Budel

Received: 17-Feb-2022; Accepted: 12-Oct-2022.

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HIGHLIGHTS

- Melatonin (*MEL*) affected the phytochemical content of sage in callus culture.
- *MEL* had the best stimulating effect at 100 µM concentration.
- The quantity and quality of volatile compounds depend on the concentration of it.
- The amount of rosmarinic acid was associated with callus color change.

Abstract: The use of melatonin (*MEL*) on plants has recently become widespread. Melatonin (*MEL*) was used as elicitor in the leaf-derived sage (*Salvia officinalis* L.) callus culture, which allows production in a short time regardless of environmental factors. *MEL* was applied to the calli in various concentrations, and the effect on the amount and quality of phytochemicals was determined. *MEL* stimulated the production of the maximum quantity of sage calli and the synthesis of secondary metabolites when applied as an elicitor at a certain ratio (100 M). The callus induction rate decreased while the *MEL* concentration increased. Among the phytochemicals scanned by HPLC and GC-MS, especially the amount of rosmarinic acid was found to increase by 75% (100 µM). The amount of rosmarinic acid decreased gradually in the 200 µM and control groups. The color differences of the callus cultures were also considerable. The color, which was quite dark brown at 100 µM *MEL*, turned into a light color as the amount of rosmarinic acid decreased. In addition, phytochemicals such as α-thujone (27.56%), 1.8-cineole (5.9%), camphor (16.84%) analyzed in 100 µM *MEL* application have the highest rates compared to other applications. Phytochemicals present in the control group but not in the *MEL* treatments, and components present in the treatments but not in the control were detected (1.8-cineol, some aldehyde groups). Therefore, stimulating the production of pharmacologically

valuable phytochemicals that can be obtained with a certain amount of *MEL* application in sage cell culture medium will provide an important commercial advantage.

Keywords: Callus; Melatonin; Rosmarinic acid; Sage; *Salvia*.

INTRODUCTION

Plants have to deal with various stress factors they are exposed to protect themselves and survive. Their most effective mechanism in this fight is to produce phytochemicals called secondary metabolites and thus to adapt to changing environmental conditions [1, 2]. More than 100,000 phytochemicals have been identified until yet in more than 250,000 plant species in nature. This number will reach enormous values as the molecular structure of phytochemicals is deciphered [3]. Many studies done conducted to resolve the extraction, biological activities, and chemical composition of phytochemicals. These chemicals, which are soldiers in the defense task of the plant, are widely used by people for various purposes in medicine, and pharmaceutical industries [4]. The limited plant resources in the face of this demand have led to efforts to increase the yield of phytochemicals [5]. The biosynthesis of phytochemicals generally occurs in an organ or specific tissue of the plant. This synthesis occurs after a particular developmental period of the plant. There are difficulties in the extraction of phytochemicals from some medicinal plants grown in their natural environment. When these plants are grown in in vitro culture medium competence of the cells synthesizing phytochemicals is increased. In this way, more efficient phytochemicals get by quality and quantity [6]. In vitro methods provide a high rate of phytochemical products from biological materials in a short time and offer easy and economical solutions due to the ease of manipulation of growing conditions. The use of the in vitro method is a turning point in this sense [7,8]. Plant structures (cell, tissue, or organ) produced by cell culture have a higher metabolic rate than differentiated mature plants. So, plant tissue cultures are also used in the industrial field to produce valuable compounds [9]. At the same time, the use of biotic and abiotic stimulators [10] and elicitors [11] in differentiated cell cultures has become widespread to stimulate the production of much more phytochemicals in vitro.

Plants can contend with abiotic stress sources, such as salinity, drought, temperature, and pH just as they can contend with biotic stress factors such as animal organisms, fungi, bacteria, and viruses. In this case, they show a series of morphological, physiological, biochemical and molecular responses to ensure the sustainability of their quality of life. The morphological and physiological responses of the plant exposed to biotic and abiotic stress factors, to protect itself from stress, physical such as high hydrostatic pressure (HP) [12], chemical such as organic and inorganic macromolecules [13,14] or biotic [15] are provided by elicitors [16]. The effect of elicitors in different biosynthetic pathways in a plant depends on its type and amount, but also varies according to both the plant in which the elicitor is used and the type of explant [17,18].

Phytomelatonin, a bio-stimulator that increases the stress tolerance of plants, was recently discovered in plants, and has attracted attention with its presence in very high concentrations in plants compared to vertebrates. In particular, the effective defense mechanism of plants that have to live dependent on the soil can be explained by the high levels of phytomelatonin they contain [19]. On the other hand, phytomelatonin, which is defined as the 'plant master regulator' since it is claimed to regulate all hormonal production and secretion of the plant, regulates the morphological, physiological and biochemical processes of the plant [20]. The impact of *MEL* (Melatonin), which is used exogenously in a various stress conditions, to the plant has been examined in recent studies [21, 22]. However, by adding to *MEL*'s in-vitro growth medium, the influence of Sage as an elicitor on the quantity and quality of phytochemicals was one of the subjects that needed to be investigated.

Sage (*Salvia officinalis* L.), a member of Lamiaceae, is an aromatic plant native to the Middle East and Mediterranean areas, which is used extensively in the food and pharmaceutical industry [23]. Sage is used in the treatment of many diseases such as nervous, circulatory, and respiratory system diseases thanks to the antioxidant and antimicrobial properties of the phytochemicals contained (phenolic, terpenoids, and volatile organic compounds) [24]. On the other hand, because it is antimicrobial, sage has become the sought-after product of the food industry, as it contains the most effective phytochemicals against food spoilage and foodborne pathogens [25]. This study, it was aimed to determine *MEL* as an elicitor of sage which has a wide range of use in the industrial field in the callus culture medium [26, 27]. For this purpose, the effect of exogen *MEL* applied, which have various concentrations, in vitro on the quantity and quality of sage phytochemicals were analyzed.

MATERIAL AND METHODS

Source of Explants and Culture Conditions

Sage calli (*Salvia officinalis* L.) were produced from leaf explants, grown in a controlled growth cabinet in a tissue culture laboratory for 6 weeks (PAR: $135 \mu\text{mol m}^{-2} \text{s}^{-1}$; photoperiod: 16/8 h day/night; temperature: $24 \pm 2^\circ\text{C}$; relative humidity: 51–54%). Sage leaves were washed with running water for 1 h and then waited in 70% (v/v) ethanol solution for 20 s and 10% (v/v) sodium hypochlorite solution for 5 min. for surface sterilization. For callus induction of leaf explants ($0.5 \times 0.5 \text{ cm}$), MS medium (Sigma-Aldrich® M0404, St. Louis, MO) containing 1 mg L^{-1} 6-benzylaminopurine (BAP) and 2 mg L^{-1} 1-naphthaleneacetic acid (NAA) was solidified with 7.0 g L^{-1} agar and 30 g L^{-1} sucrose (pH: 5.7 ± 1). 0.0 (as control), 100 μM , and 200 μM amounts of MEL (Melatonin) were added to the MS medium and autoclaved for 20 min under 103 kPa at 121°C . All groups (with three replications) were incubated at $25 \pm 1^\circ\text{C}$ in darkness for six weeks in a growth chamber. The formula for calculating the callus induction frequency:

$$\text{Callus induction frequency (\%)} = [(\text{Number of explants with callus}) / (\text{Total number of explants})] \times 100 \quad (1)$$

Quantification of Secondary Metabolites in Callus

The phytochemicals of sage calli applied with different concentrations of MEL were determined by GC/MS and HPLC.

Sample Preparation: Sage calli (1g) were homogenized with 10 mL methanol and were centrifuged at 4000 rpm for 5 min. The supernatant was dried in a vacuum rotary evaporator at 40°C . Dry residues were dissolved in 500 μL methanol and filtered a 0.02 μm Millipore filter.

HPLC Analysis: The HPLC system was equipped with LC-10 ADvp pump, SIL-10A Dvpauto-sampler, and CTO-10 Avp column oven (Shimadzu, Kyoto, Japan). Agilent Eclipse XDB-C18 ($250 \times 4.60 \text{ mm}$, 5 μm) column and a mobile phase consisting of methanol and acetic acid (3%v/v) in water were used. The flow rate was 0.8 mL min^{-1} and the injection volume was 20 μL . The column temperature was adjusted to 30°C . Diode Array Detector (DAD) was working at a value of $\lambda_{\text{max}}=278 \text{ nm}$, and chromatograms were acquired at different wavelengths according to the absorption maxima of the analyzed compounds.

GC-MS Analysis: Aromatic content was measured by gas chromatography/mass spectrometry (GC/MS). The name of the system was fused silica SPME fiber CAR/PDMS with a column of Restek Rx-5Sil MS ($30 \text{ m} \times 0.25 \text{ mm i.d.}$, 0.25 μm film thickness). The flow rate of helium as carrier gas was 1.61 mL/min. The injector temperature was 250°C , set for splitless injection. After waiting 2 min at 40°C , the system reached 250°C in 4°C increments per min and waited for 5 min at 250°C . Mass spectra were taken at 70 eV. The sample was kept to stand for 30 min with fiber, for 15 min without fiber at 60°C , and desorbed at 250°C . Relative percentage amounts of the separated aromatic compounds were calculated from total ion chromatograms by the computerized integrator.

Statistical Analysis

The obtained data were statistically analyzed using SPSS version 19 (Chicago, IL, USA). All applications were repeated three times, and the results were expressed as mean $\pm$ standard deviation (SD) values. One-way analysis of variance (ANOVA) and Duncan's multiple range test was performed for comparing the means of different applications ($P < 0.05$). Different letters indicate statistically significant differences.

RESULTS AND DISCUSSION

In in vitro culture media, in a short time, the qualitative and quantitative of phytochemicals generally increase significantly with the effect of different inducing materials and biotic and abiotic elicitors that trigger defense [28]. Well-developed calli were obtained from young and healthy leaf explants of sage and they were placed in MS medium at concentrations of 100 and 200 μM MEL (Melatonin), together with the control group (0.0). The callus induction rate decreased in parallel with the increase in MEL concentration, 98%, 56.2% and, 35.4%, and 0.0, 100, 200 μM MEL concentrations, respectively. The best callus growing was in MEL application at 100 μM concentration. Morphological changes that occur in vitro with callus induction can be identified to a significant extent [29]. In particular, the callogenesis process is affected by some elicitors [30]. In addition, changes in callus induction rates and callus sizes also help determine the optimal components of the cultures. While very low rates of some bioactive compounds such as α -pinene, α -thujone, and camphor were detected in undifferentiated callus tissues in *A. spicigera*, the rate of callus induction increased with the application of 1 mg L^{-1} NAA (naphthalene acetic acid) to the MS medium. This event demonstrates the

existence of a relationship between in vitro shoot induction and the production of volatile compounds [31]. The maximum determination of callus size at 100 μM MEL concentration (14.57 mm, 21.83mm, 19.33 mm; 0.0, 100, 200 μM MEL concentrations, respectively) ($P < 0.05$) can be defined as a clear indicator of the optimum effect of MEL in the callus medium (Figure 1). The highest rate of callus size at 100 μM MEL concentration may indicate that MEL alters some physiological processes such as phytochemical synthesis this concentration.



Figure 1. Callus induction; a: control (0.0); b: 100 μM MEL; c: 200 μM MEL.

The phytochemicals of sage calli applied with different concentrations of MEL were analyzed by GC/MS and HPLC. Phytochemical contents of sage calli (mono-, sesquiterpene hydrocarbons and oxygenated mono-sesquiterpenes) have different responses according to MEL concentration, compared to control ($P < 0.05$). The main components commonly used to evaluate phytochemicals for essential oil are of sage: α -pinene, camphene, β -pinene and myrcene (monoterpene hydrocarbons), β -caryophyllene and α -humulene (sesquiterpene hydrocarbons), 1,8-cineole, camphor, α -thujone, β -thujone, borneol, bornyl acetate (oxygenated monoterpenes), viridiflorol, manool (oxygenated sesquiterpenes). The amount of these components obtained from sage shows the quality of the sample in terms of essential oil [32, 33, 34, 35]. Mono- and sesquiterpene hydrocarbons at 100 μM MEL concentration in tissue culture medium supplemented with different concentrations of MEL; from α -pinene (1.33%), camphene (2.8%), α -humulene (6.2%) and oxygenated monoterpenes; α -thujone (27.56%), 1,8-cineole (5.9%), camphor (16.84%) were defined highest ratios content (Figure 2). These ratios decreased in the control group and 200 μM MEL concentration, respectively ($P < 0.05$). 27.56% of the volatile compounds of sage calli at 100 μM MEL is α -thujone, the highest proportion of all content. α -thujone, a volatile monoterpene ketone, can only be produced by a few plants in nature. Besides being used to sweeten foods, this compound is most well known for its neurotoxicity. Thereby, it uses in the cure of various diseases (hepatoprotection, bronchial catarrh, rheumatism, psoriasis and uterine carcinomas) [36] antiviral and antitumor effects, as well as showing different wound healing and pain relief properties [37] and stimulates the immune response with its immunomodulatory properties [38]. Although the α -thujone ratio was low in the control group calli of sage (4.23%), this ratio increased approximately seven times to 27.56% with the addition of 100 μM MEL. The fact that the α -thujone ratio in the volatile content of in-vitro grown sage is higher than the naturally collected samples [39] is an indication of the contribution of in-vitro MEL application as an elicitor. However, at 200 μM MEL, the rate of α -thujone was close to control (6.81%). For example, the α -thujone ratios of *Salvia officinalis* L. collected from 5 different natural habitats were determined as 14.8-19.0% [33]. Sage volatiles, collected from various European countries, contains α -thujone 6.8-26% [34]. This result shows that when a certain MEL concentration is being used can significant amounts of the α -thujone be synthesized. This concentration (100 mM of MEL) might be the one that may be raised the most by interfering with MEL's process for synthesizing α -thujone. On the other hand, this can also be explained by the fact that sabinene, which is responsible for the synthesis of the main monoterpenes in sage, was the lowest in 100 μM MEL (0.22%, 0.05% and 0.12% at 0.0, 100, 200 μM MEL, respectively). Because sabinene is the precursor compound responsible for the formation of monoterpenes such as α - and β -thujone [40]. Similarly, camphor (16.84%) from oxygenated monoterpenes was determined as the 2nd highest amount of compound at 100 μM MEL, compared to control. Interestingly, this compound was not detected in the control group (0.0 MEL) but at 200 μM MEL, it decreased by 58%, with a value of 7.17%, showing a 1.3-fold reduction, compared to 100 μM MEL ($P < 0.05$). The antiviral and antimicrobial effect of sage is directly proportional to the amount of α -thujone and camphor, in its phytochemical content [35]. The increase of α -thujone and camphor content with MEL

application (100 μM), may indicate that *MEL* is a considerable elicitor to improve the phytochemical quality of sage. In addition, the stimulating and positive effect of *MEL* can promote the synthesis of different compounds under various biotic/abiotic stresses by stimulating the biosynthesis of substances and by inducing different metabolic and physiological pathways [41]. For example, 1,8-cineole and β -thujone from oxygenated sesquiterpenes were not detected at the control but in the 100 μM and 200 μM of *MEL* applications. It has decreased in the 200 μM application (1,8-cineole decreased from 5.9% to 1.5%, β -thujone decreased from 2.3% to 1.98%), compared to 100 μM *MEL* ($P < 0.05$). It is matter to increase the quantity and quality of these products with various applications, especially for the industrial field. 1,8-cineole is used as a food preservative active ingredient in the food and flavoring industry, and has attracted attention for its insecticidal properties as a bioactive component of volatile-based 'green pesticides' in recent years [42]. Increasing the quantity and quality of these compounds means an increase in green pesticide sources, and serious environmental and health problems caused by synthetic pesticides can be solved to a great extent. Furthermore, by the examination of sage phytochemicals, the content that were been in the control group but not in the applications was found. In particular, the detection of some compounds from the aldehyde group (isobutanal, p-dichlorobenzene, hexanal, n-octanal) just at the control group may be due to the effect of *MEL* on some enzymes involved in the volatile biosynthesis.

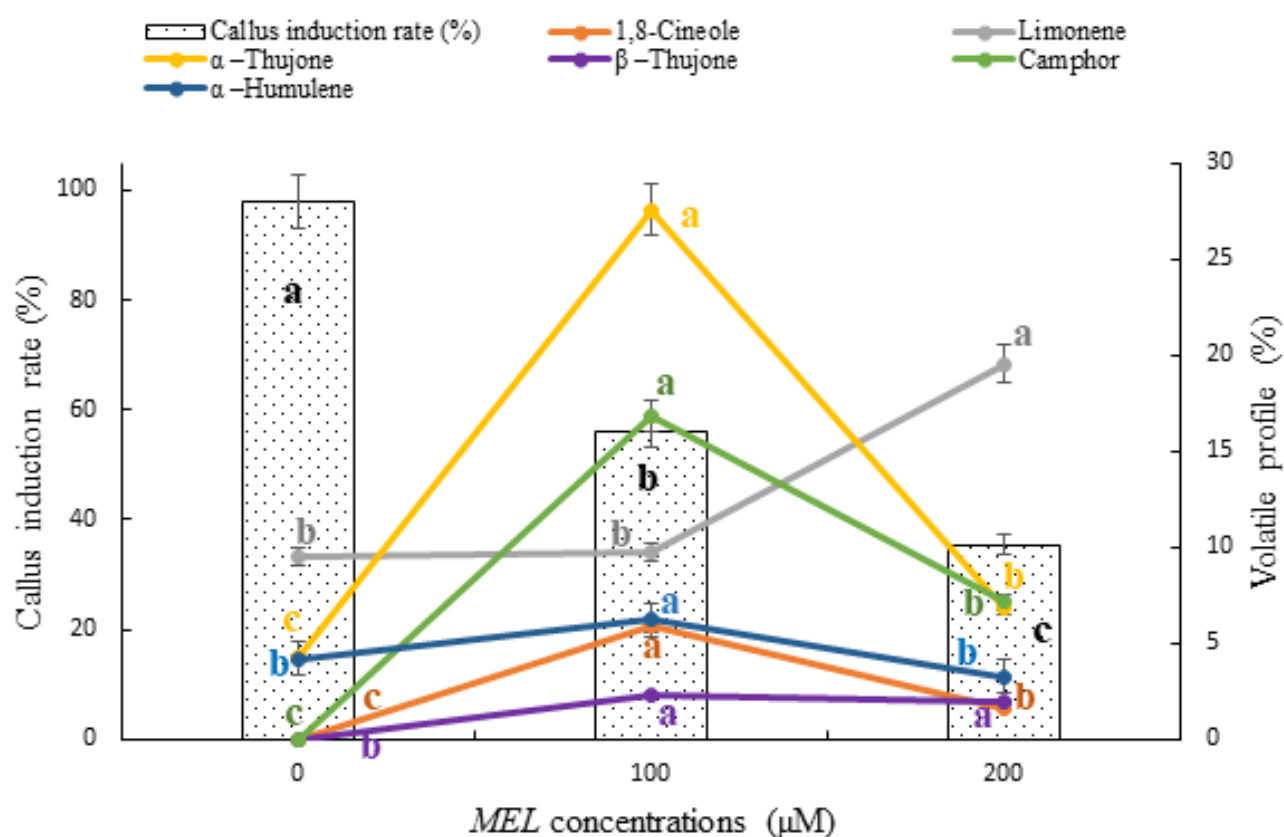


Figure 2. Callus induction rates and volatile profile varying according to MEL concentration of sage. Bars indicate standard errors of the means. Separately for each measured parameter, different letters indicate statistically significant difference (Duncan post-hoc test; $P < 0.05$).

When the phenolic content of calli grown in *MEL* supplemented MS medium was investigated, it was found that rosmarinic acid was affected by the change in *MEL* concentration, and its amount was very different from the control group ($P < 0.05$). The amount of rosmarinic acid was found to be the highest with $475 \mu\text{g g}^{-1}$ at 100 μM *MEL* concentration. It was determined as $170 \mu\text{g g}^{-1}$ in 200 μM *MEL*, and $120 \mu\text{g g}^{-1}$ in the control group. This difference between applications is quite significant ($P < 0.05$). This difference between the amounts of rosmarinic acid was highest in 100 μM *MEL* application, with a 75% increase, compared to the control (Figure 3). Rosmarinic acid has very important biological activities such as antiviral, antibacterial, antiinflammatory and antioxidant. One of the main parameters of using sage as a medicinal plant is that the phytochemical it produces contains high levels of rosmarinic acid [43]. Studies to increase the synthesis of this chemical, which is a significant phenolic, show that the tissue culture method accumulates more rosmarinic acid than the *in vivo* method [44]. On the other hand, the callus colors of sage in applications were

also different from each other. Especially, dark brown dominated the MS medium at 100 μM *MEL* and color, lightened from 200 μM to the control (Figure 3). Browning occurring in callus cultures is a main morphological character in determining the success of phytochemical accumulation in plant tissue culture. Because phenolics such as rosmarinic acid have been produced in large quantities in callus culture, it is characterized by high browning [45]. Since *MEL* stimulates phenolic compound production by inducing specialized metabolite production [46], the ratios of rosmarinic acid and α -thujone changed as a result of *MEL* treatments. On the other hand, when *MEL* was applied to propagate *Salvia* plant with tissue culture technique, 1.5 g L⁻¹ melatonin was determined to be the most effective in promoting callus formation and secondary metabolite production [47]. Additionally, when the phytochemical content of *Salvia absconditiflora* Greuter & Burdet was examined, nine different phenolic compounds were detected, and five of them, including rosmarinic acid, were found to have substantial antioxidant effects. It was emphasized that the plant should be cultured in order to isolate the bioactive compounds of these compounds for use in the pharmaceutical and food industries [48].

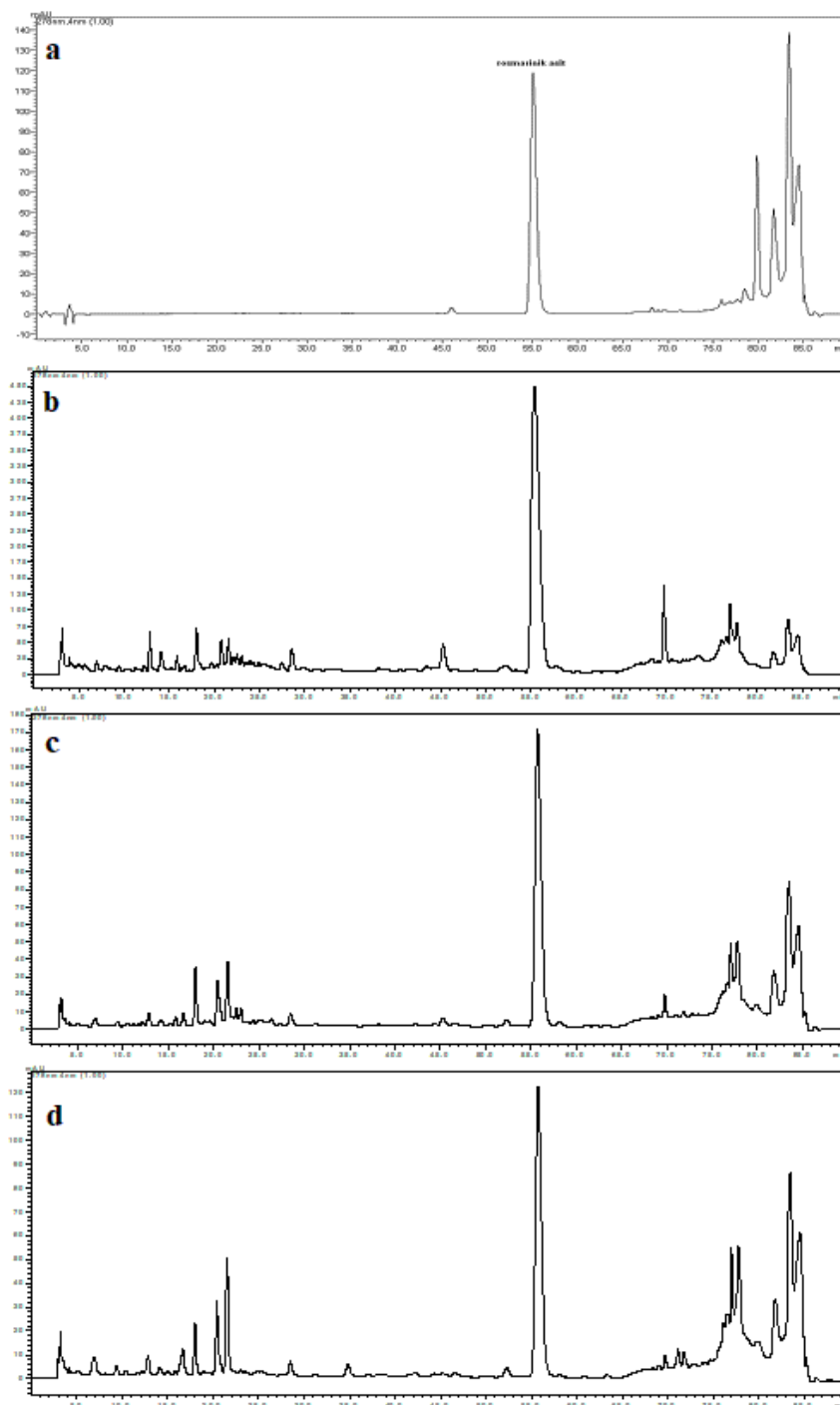


Figure 3. Rosmarinic acid profile of sage calli: a:standart chromatogram; b: control; c: 100 μM *MEL*; d: 200 μM *MEL*.

In summary, 100 μM *MEL* application increases the synthesis of terpenes and ketones but decreases the synthesis of aldehydes, whereas 200 μM *MEL* application shows an increase in these rates. However the favorable impact of *MEL* was demonstrated by the rise in terpenes and ketones production across all *MEL* applications.

CONCLUSION

Very significant results have got in the analysis of the contribution of different concentrations of *MEL* to the phytochemical content and amount of sage calli grown in MS medium. Especially the 100 μM concentration of *MEL* changed the phytochemical content and amount. While terpenes and ketones increased, aldehydes decreased at 100 μM *MEL*. Although the proportion of these compounds at 200 μM *MEL* decreased (compared to 100 μM *MEL*), they were higher than the proportions at the control (Figure 4). On the other hand, the presence of some phytochemicals found in the control group but not found in *MEL* applications or not found in the control group, but found in *MEL* applications indicates that *MEL* changes some synthesis mechanisms in phytochemical production. These results reflect the effect of the application of *MEL* at a certain concentration (100 μM) in MS medium on the quantity and quality of phytochemicals by activating or suppressing some enzymes that carry out the phytochemical synthesis of sage. Therefore, for future studies, it is crucial to define the contribution of *MEL* to enzymatic reactions in phytochemical synthesis.

In our study, we applied *MEL* to sage, a plant produced for its aromatic contents, in cell culture, thus increasing the phytochemical content and quality of the plant significantly. Rosmaniric acid, in particular, has been synthesized in large quantities. Furthermore, several phytochemicals, such as 1.8-cineol, were only clearly produced in *MEL* application as a result of our research. These findings show that transforming sage phytochemicals generated in vitro and enhanced by *MEL* into commercial products would be more advantageous than the classical method. Because the processes of growing plants that produce secondary metabolites, which are essential components of the pharmaceutical, perfumery, and food industries, as well as obtaining products, are lengthy and complicated. In-vitro cultures provide significant advantages for high biomass production, enabling rapid growth and consistent metabolite productivity to keep up with rising demand [49].

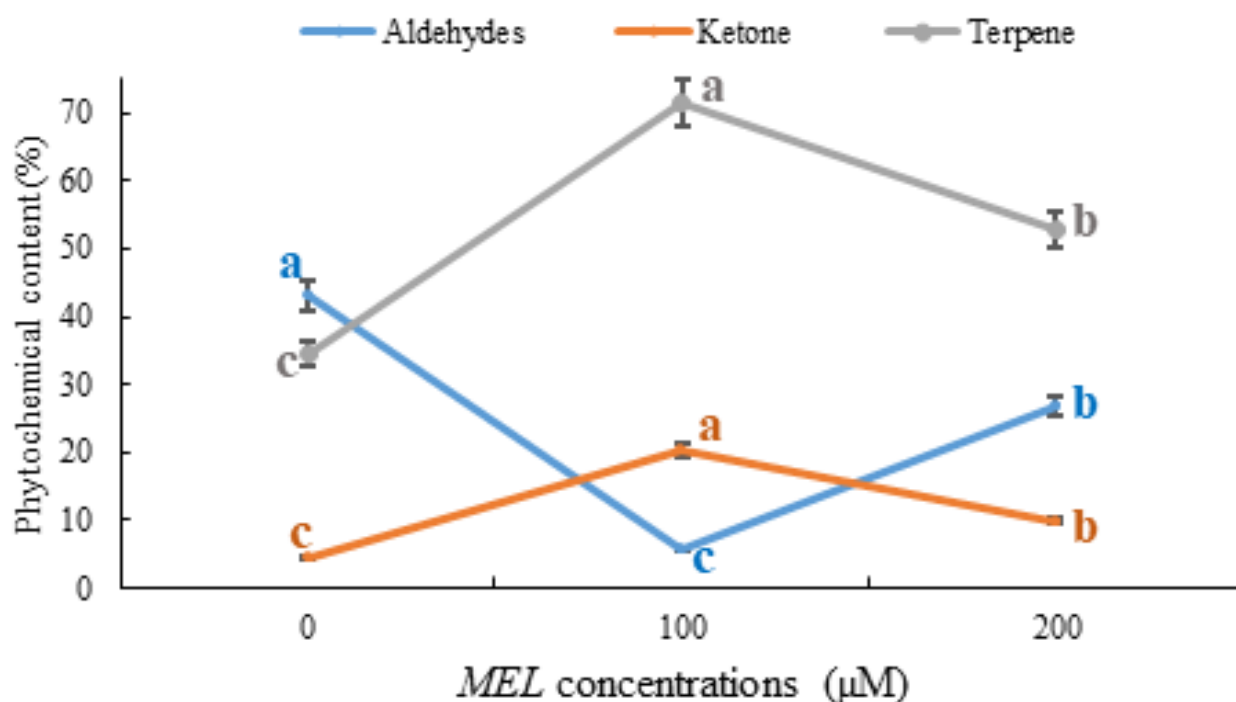


Figure 4. Changes in the phytochemical content of sage. Bars indicate standard errors of the means. Separately for each measured parameter, different letters indicate statistically significant difference (Duncan post-hoc test; $P < 0.05$).

Funding: This research received no external funding

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Moore BD, Andrew RL, Külheim C, Foley WJ. Explaining intraspecific diversity in plant secondary metabolites in an ecological context. *New Phytol.* 2014; 201(3): 733-50. Doi: <https://doi.org/10.1111/nph.12526>.
2. Sharma A, Shahzad B, Rehman A, Bhardwaj R, Landi M, Zheng B. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules.* 2019;24(13):2452. Doi: <https://doi.org/10.3390/molecules24132452>
3. Defosse E, Pitteloud C, Descombes P, Glauser G, Allard PM, Walker TW, et al. Spatial and evolutionary predictability of phytochemical diversity. *Proc Natl Acad Sci.* 2021;118(3). Doi: <https://doi.org/10.1073/pnas.2013344118>.
4. Leicach SR, Chludil HD. Plant secondary metabolites: Structure–activity relationships in human health prevention and treatment of common diseases. In: *Studies in Natural Products Chemistry.* 2014; 42: 267-304 Elsevier. Doi: <https://doi.org/10.1016/B978-0-444-63281-4.00009-4>.
5. Moscatiello R, Baldan B, Navazio L. Plant Cell Suspension Cultures. In: *Plant Mineral Nutrients.* 2013; 77-93 Humana Press, Totowa, NJ. Doi: https://doi.org/10.1007/978-1-62703-152-3_5.
6. Nagella P, Murthy HN. Effects of macroelements and nitrogen source on biomass accumulation and withanolide-A production from cell suspension cultures of *Withania somnifera* (L.) Dunal. *Plant Cell Tissue Organ Cult.* 2011; 104(1): 119-24. Doi: <https://doi.org/10.1007/s11240-010-9799-0>.
7. Hussain MS, Fareed S, Ansari MS, Rahman A, Ahmad IZ, Saeed M. Current approaches toward production of secondary plant metabolites. *J Pharm Bioallied Sci.* 2012;4(1):10. Doi: <https://doi.org/10.4103/0975-7406.92725>.
8. Niazian M. Application of genetics and biotechnology for improving medicinal plants. *Planta.* 2019; 249(4): 953-73. doi: <https://doi.org/10.1007/s00425-019-03099-1>.
9. Steingroewer J, Bley T, Georgiev V, Ivanov I, Lenk F, Marchev A, et al. Bioprocessing of differentiated plant in vitro systems. *Eng Life Sci.* 2013; 13(1): 26-38. Doi: <https://doi.org/10.1002/elsc.201100226>.
10. Amoo SO, Van Staden J. Influence of plant growth regulators on shoot proliferation and secondary metabolite production in micropropagated *Huernia hystrix*. *Plant Cell Tissue Organ Cult.* 2013; 112(2): 249-56. Doi: <https://doi.org/10.1007/s11240-012-0230-x>.
11. Giri CC, Zaheer M. Chemical elicitors versus secondary metabolite production in vitro using plant cell, tissue and organ cultures: recent trends and a sky eye view appraisal. *Plant Cell Tissue Organ Cult.* 2016;126(1):1-18. Doi: <https://doi.org/10.1007/s11240-016-0985-6>.
12. Cai Z, Riedel H, Saw NMMT, Mewis I, Reineke K, Knorr D, et al. Effects of elicitors and high hydrostatic pressure on secondary metabolism of *Vitis vinifera* suspension culture. *Process Biochem.* 2011;46(7):1411-6. Doi: <https://doi.org/10.1016/j.procbio.2011.03.015>.
13. Sabater-Jara AB, Souliman-Youssef S, Novo-Uzal E, Almagro L, Belchí-Navarro S, Pedreño MA. Biotechnological approaches to enhance the biosynthesis of ginkgolides and bilobalide in *Ginkgo biloba*. *Phytochem Rev.* 2013; 12(1): 191-205. Doi: <https://doi.org/10.1007/s11101-013-9275-7>.
14. Kiselev KV, Tyunin AP, Karetin YA. Salicylic acid induces alterations in the methylation pattern of the VaSTS1, VaSTS2, and VaSTS10 genes in *Vitis amurensis* Rupr. cell cultures. *Plant Cell Rep.* 2015;34(2):311-20. Doi: <https://doi.org/10.1007/s00299-014-1708-2>.
15. Kumar V, Rajauria G, Sahai V, Bisaria VS. Culture filtrate of root endophytic fungus *Piriformospora indica* promotes the growth and lignan production of *Linum album* hairy root cultures. *Process Biochem.* 2012;47(6):901-7. Doi: <https://doi.org/10.1016/j.procbio.2011.06.012>.
16. Schreiner M, Huyskens-Keil S. Phytochemicals in fruit and vegetables: health promotion and postharvest elicitors. *Crit Rev Plant Sci.* 2006; 25(3): 267-78. Doi: <https://doi.org/10.1080/07352680600671661>.
17. Narayani M, Srivastava S. Elicitation: a stimulation of stress in in vitro plant cell/tissue cultures for enhancement of secondary metabolite production. *Phytochem Rev.* 2017;16(6):1227-52. Doi: <https://doi.org/10.1007/s11101-017-9534-0>.
18. Halder M, Sarkar S, Jha S. Elicitation: A biotechnological tool for enhanced production of secondary metabolites in hairy root cultures. *Eng Life Sci.* 2019;19(12):880-95. Doi: <https://doi.org/10.1002/elsc.201900058>.
19. Moustafa-Farag M, Almoneafy A, Mahmoud A, Elkelish A, Arnao MB, Li L, et al. Melatonin and its protective role against biotic stress impacts on plants. *Biomolecules.* 2020;10(1):54. Doi: <https://doi.org/10.3390/biom10010054>.
20. Arnao MB, Hernández-Ruiz J. Is phyto-melatonin a new plant hormone?. *Agronomy.* 2020;10(1):95. Doi: <https://doi.org/10.1016/j.scienta.2003.07.006>.
21. Murch SJ, Saxena PK. Melatonin: a potential regulator of plant growth and development?. *In Vitro Cell Dev Biol Plant.* 2002;38(6):531-6. Doi: <https://doi.org/10.1079/IVP2002333>.
22. Nawaz K, Chaudhary R, Sarwar A, Ahmad B, Gul A, Hano C, Abbasi BH, Anjum S. Melatonin as master regulator in plant growth, development and stress alleviator for sustainable agricultural production: Current status and future perspectives. *Sustainability.* 2021;13(1):294. Doi: <https://doi.org/10.3390/su13010294>.

23. Trivellini A, Lucchesini M, Maggini R, Mosadegh H, Villamarin TSS, Vernieri P, et al. Lamiaceae phenols as multifaceted compounds: bioactivity, industrial prospects and role of "positive-stress". *Ind Crops Prod*. 2016;83:241-54. Doi: <https://doi.org/10.1016/j.indcrop.2015.12.039>.
24. Vergine M, Nicoli F, Negro C, Luvisi A, Nutricati E, Accogli RA, et al. Phytochemical profiles and antioxidant activity of *Salvia* species from southern Italy. *Rec Nat Prod*. 2019;13(3):215. Doi: <http://doi.org/10.25135/rnp.96.18.07.119>.
25. Sharifi-Rad M, Ozcelik B, Altin G, Daşkaya-Dikmen C, Martorell M, Ramírez-Alarcón K, et al. *Salvia* spp. plants-from farm to food applications and phytopharmacotherapy. *Trends Food Sci Technol*. 2018;80:242-63. Doi: <https://doi.org/10.1016/j.tifs.2018.08.008>.
26. Jakovljević M, Jokić S, Molnar M, Jašić M, Babić J, Jukić H, et al. Bioactive profile of various *Salvia officinalis* L. preparations. *Plants*. 2019;8(3):55. Doi: <https://doi.org/10.3390/plants8030055>.
27. Ayoub I, George MY, Menze ET, Mahmoud M, Botros M, Essam M, et al. Insights on the neuroprotective effects of *Salvia officinalis* L. and *Salvia microphylla* Kunth in memory impairment rat model. *Food Funct*. 2022; 13: 2253-68. Doi: <https://doi.org/10.1039/D1FO02988F>.
28. Niazian M, Howyeh MS, Sadat-Noori SA. Integrative effects of stress-and stress tolerance-inducing elicitors on in vitro bioactive compounds of ajowan [*Trachyspermum ammi* (L.) Sprague] medicinal plant. *Plant Cell Tissue Organ Cult*. 2021;1-16. Doi: <https://doi.org/10.1007/s11240-021-02096-1>.
29. Tůmová L, Tůma J, Megušar K, Doležal M. Substituted pyrazinecarboxamides as abiotic elicitors of flavolignan production in *Silybum marianum* (L.) gaertn cultures in vitro. *Molecules*. 2010;15(1):331-40. Doi: <https://doi.org/10.3390/molecules15010331>.
30. Khan MA, Ali A, Mohammad S, Ali H, Khan T, Mashwani ZUR, Jan A, Ahmad P. Iron nano modulated growth and biosynthesis of steviol glycosides in *Stevia rebaudiana*. *Plant Cell Tissue Organ Cult*. 2020; 143(1): 121-30. Doi: <https://doi.org/10.1007/s11240-020-01902-6>.
31. Ghorbani S, Kosari-Nasab M, Mahjouri S, Talebpour AH, Movafeghi A, Maggi F. Enhancement of in vitro production of volatile organic compounds by shoot differentiation in *Artemisia spicigera*. *Plants*. 2021; 10(2): 208. Doi: <https://doi.org/10.3390/plants10020208>.
32. Hazzoumi Z, Moustakime Y, Joutei KA. Essential Oil and Glandular Hairs: Diversity and Roles. In: *Essential Oils - Oils of Nature*. IntechOpen. 2019. Doi: <https://doi.org/10.5772/intechopen.86571>.
36. Akkol EK, İlhan M, Demirel MA, Keles H, Tumen I, Suntar I. *Thuja occidentalis* L. and its active compound, α -thujone: Promising effects in the treatment of polycystic ovary syndrome without inducing osteoporosis. *J Ethnopharmacol*. 2015;168:25-30. Doi: <https://doi.org/10.1016/j.jep.2015.03.029>.
37. Pudełek M, Catapano J, Kochanowski P, Mrowiec K, Janik-Olchawa N, Czyż J, et al. Therapeutic potential of monoterpene α -thujone, the main compound of *Thuja occidentalis* L. essential oil, against malignant glioblastoma multiforme cells in vitro. *Fitoterapia*. 2019;134:172-81. Doi: <https://doi.org/10.1016/j.fitote.2019.02.020>.
38. Zhou Y, Liu JQ, Zhou ZH, Lv XT, Chen YQ, Sun LQ, et al. Enhancement of CD3AK cell proliferation and killing ability by α -thujone. *Internat Immunopharmacol*. 2016; 30: 57-61. Doi: <https://doi.org/10.1016/j.intimp.2015.11.027>.
39. Arikat NA, Jawad FM, Karam NS, Shibli RA. Micropropagation and accumulation of essential oils in wild sage (*Salvia fruticosa* Mill.). *Sci Hortic*. 2004;100(1-4):193-202.
33. Mockutė D, Nivinskienė O, Bernotienė G, Butkienė R. The cis-thujone chemotype of *Salvia officinalis* L. essential oils. *Chemija*. 2003;14(4):216-20.
34. Raal A, Orav A, Arak E. Composition of the essential oil of *Salvia officinalis* L. from various European countries. *Nat Prod Res*. 2007;21(5):406-11. Doi: <https://doi.org/10.1080/14786410500528478>.
40. Schmiderer C, Grausgruber-Gröger S, Grassi P, Steinborn R, Novak J. Influence of gibberellin and daminozide on the expression of terpene synthases and on monoterpenes in common sage (*Salvia officinalis*). *J Plant Physiol*. 2010; 167(10): 779-86. Doi: <https://doi.org/10.1016/j.jplph.2009.12.009>.
35. JD Craft, P Satyal, WN Setzer. The chemotaxonomy of common sage (*Salvia officinalis*) based on the volatile constituents. *Medicines*. 2017;4(3):47. Doi: <https://doi.org/10.3390/medicines4030047>.
41. Tan DX, Hardeland R, Manchester LC, Korkmaz A, Ma S, Rosales-Corral S, et al. Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science. *J Exp Bot*. 2012;63(2):577-97. Doi: <https://doi.org/10.1093/jxb/err256>.
42. Saroj A, Oriyomi OV, Nayak AK, Haider SZ. Phytochemicals of plant-derived essential oils: A novel green approach against pests. in: *Natural Remedies for Pest, Disease and Weed Control*. pp. 65-79. Academic Press. 2020. Doi: <https://doi.org/10.1016/B978-0-12-819304-4.00006-3>.
43. Santos-Gomes PC, Seabra RM, Andrade PB, Fernandes-Ferreira M. Phenolic antioxidant compounds produced by in vitro shoots of sage (*Salvia officinalis* L.). *Plant Sci*. 2002; 162(6): 981-7. Doi: [https://doi.org/10.1016/S0168-9452\(02\)00052-3](https://doi.org/10.1016/S0168-9452(02)00052-3).
44. Petersen M. Rosmarinic acid: new aspects. *Phytochem Rev*. 2013;12(1):207-27. Doi: <https://doi.org/10.1007/s11101-013-9282-8>.
45. Hesami M, Tohidfar M, Alizadeh M, Daneshvar MH. Effects of sodium nitroprusside on callus browning of *Ficus religiosa*: an important medicinal plant. *J For Res*. 2020;31(3):789-96. Doi: <https://doi.org/10.1007/s11676-018-0860-x>.

46. Vafadar F, Amooaghaie R, Ehsanzadeh P, Ghanadian M, Talebi M, Ghanati F. Melatonin and calcium modulate the production of rosmarinic acid, luteolin, and apigenin in *Dracocephalum kotschy* under salinity stress. *Phytochem.* 2020;177:112422. Doi: <https://doi.org/10.1016/j.phytochem.2020.112422>
47. Bano AS, Khattak AM, Basit A, Alam M, Shah ST, Ahmad N, et al. Callus Induction, Proliferation, Enhanced Secondary Metabolites Production and Antioxidants Activity of *Salvia moorcroftiana* L. as Influenced by Combinations of Auxin, Cytokinin and Melatonin. *Braz Arch Biol Technol.* 2022;65. Doi: <https://doi.org/10.1590/1678-4324-2022210200>
48. Koysu P, Genc N, Elmastas M, Aksit H, Erenler R. Isolation, identification of secondary metabolites from *Salvia absconditiflora* and evaluation of their antioxidative properties. *Nat Prod Res.* 2019; 33(24): 3592-5. Doi: <https://doi.org/10.1080/14786419.2018.1488700>
49. Hashim M, Ahmad B, Drouet S, Hano C, Abbasi BH, Anjum S. Comparative effects of different light sources on the production of key secondary metabolites in plants in vitro cultures. *Plants.* 2021; 10(8):1521. Doi: <https://doi.org/10.3390/plants10081521>



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