Turkish Journal of Agriculture - Food Science and Technology, 11(10): 1882-1888, 2023
DOI: https://doi.org/10.24925/turjaf.v11i10.1882-1888.6328

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Callus Formation and Camphor Accumulation in Response to Sorbitol Stimulated Osmotic Stress in Yarrow

Muhammed Akif Açıkgöz[1,a], Ahmet Aygün[2,b], Ebru Batı Ay[3,c], Şevket Metin Kara[1,d]

[1]*Ordu University, Faculty of Agriculture, Department of Field Crops, Ordu, Türkiye*
[2]*Kocaeli University, Faculty of Agriculture, Department of Horticulture, Kocaeli, Türkiye*
[3]*Amasya University, Suluova Vocational School, Medicinal and Aromatic Plants Program, Amasya, Türkiye*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 01-09-2023
Accepted : 04-10-2023

Keywords:
Asteraceae
Cell suspension cultures
Elicitor
Terpenoid
Camphor

ABSTRACT

Sorbitol is an important source of abiotic stress that is used to increase osmolality in cell cultures. It increases the antioxidant enzymes of defense catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in the stress state of cells. Sorbitol plays an important role in stimulating these enzymes in cells and increasing phenylalanine ammonium lyase (PAL) activity. The aim of this study was to apply increasing doses of sorbitol elicitor to cell suspension cultures to determine the changes in cell number, viability, dry weight, and camphor content. *In vitro* plantlets were obtained from plant seeds and stem segments of these plants were used as explant source. Cell cultures were established after callus formation. Then, 0 (control), 5, 25, and 50 g L^{-1} sorbitol was dissolved in distilled water and cultured. Samples were taken three times in total, starting from day 1 to day 3. The content of camphor was detected by gas chromatography-mass spectrometry (GC-MS). Cell number, viability,dry weight, and camphor content increased significantly with increasing doses of sorbitol compared to sampling times. Compared to the initial culture, the amount of camphor increased by 40% at the 5 g L^{-1} dose, 82% at the 25 g L^{-1} dose, and 154% at the 50 g L^{-1} dose. In *A. gypsicola* cell cultures, increasing doses of sorbitol have clearly demonstrated the secondary metabolite accumulation and its positive effect on cell growth.

[a] *makifacikgoz@gmail.com* *https://orcid.org/0000-0003-2436-5605*
[b] *ahmet.aygun@kocaeli.edu.tr* *https://orcid.org/0000-0002-6321-0350*
[c] *ziraatciebru@hotmail.com* *https://orcid.org/0000-0002-9210-6907*
[d] *smkara58@hotmail.com* *https://orcid.org/0000-0001-7755-1394*

Introduction

The *Achillea* genus, a significant member of the Asteraceae family, boasts a global representation of over 100 species. Within this genus, the essential oil harbors the camphor terpene, yielding medicinal benefits such as antimicrobial, antitussive, antinociceptive, antimutagenic, anticarcinogenic, and cardiovascular effects. Moreover, this versatile oil finds application in diverse industries including pesticide, cosmetics, plastics, and anti-rust coatings (Lin et al., 2007; Cheng et al., 2009; Sherkheli et al., 2009; Abdel-Rahman et al., 2015).

A dominant source of the camphor compound is the *Cinnamomum camphora* tree, renowned as the camphor tree, thriving in the far eastern regions. This tree comprises around 68% camphor in its chemical makeup (Frizzo et al., 2000). In contrast, the endemic *Achillea gypsicola* Hub, with its herbaceous form, exhibits a remarkable 61.8% camphor content (Açıkgöz, 2019). Studies demonstrate the *Achillea* genus's heightened camphor concentration compared to other medicinal and aromatic plants. Notable camphor contents among these species are: 0.6% (*Achillea filipendulina*), 1.3% (*A. magnifica*), 2.1% (*A.millefolium*), 3.17% (*A. aleppica*), 5.9% (*A. crithmifolia*), 6.7% (*A.santolina*), 7.1% (*A. tenuifolia*), 8.6% (*A.biebersteinii*), 15.92% (*A. tenuifolia*), 16.6% (*A. wilhelmsii*), 17.7% (*A. micrantha*), 22.8% (*A. grandifolia*), 23.21% (*A. magnifica*), 32.65% (*A. cucullata*), and 61.8% (*A. gypsicola*) (Pavlović et al., 2008; Smelcerovic et al., 2010; Toncer et al., 2010; Khiyari et al., 2014; Almadiy et al., 2016; Sampietro et al., 2016; Ahmadi-Dastgerdi et al., 2017; Ghasemi, 2017; Demirci et al., 2017; Açıkgöz, 2020b).

Considering its growth cycle, *A. gypsicola* emerges as a notable camphor-rich species within the plant kingdom, even rivaling tree forms (Açıkgöz, 2017). Ecological conditions significantly influence plants, resulting in diverse effects on the quality and consistency of their secondary metabolites. Stress plays a pivotal role in shaping the chemical composition of medicinal and aromatic plants. Plant stress is categorized into two groups: biotic and abiotic. Abiotic stress encompasses non-biological factors like physical, chemical, and hormonal influences. Physical stressors include light variations, UV

exposure, osmotic stress, drought, salinity, and thermal fluctuations. Osmotic stressors such as mannitol or sorbitol inhibit mineral absorption, slowing plant growth and development (Dodds and Roberts, 1985; Thompson et al., 1986). Various studies employ substances like polyethylene glycol (PEG) or sorbitol to simulate artificial drought stress, as these substances reduce osmotic potential, creating water stress non-metabolized by plants (Rai et al., 2011; Bidabadi et al., 2012; Placide et al., 2012; Vanhove et al., 2012).

Classical plant production methods and the extraction of secondary metabolites prove more resource-intensive and time-consuming compared to cell cultures. Hence, tissue culture techniques, particularly callus culture, emerge as popular methods for secondary metabolite production (Açıkgöz et al., 2018a; Açıkgöz et al., 2018b; Açıkgöz et al., 2019; Açıkgöz et al., 2022; Dağlioğlu et al., 2022; Ebru et al., 2022; Açıkgöz et al., 2023). Callus culture efficiently generates products of specific quality and standards, enhances genetic diversity, introduces new compounds absent in the mother plant, optimizes space utilization, and utilizes minimal resources. This study investigates the impact of abiotic stress through sorbitol elicitation on callus cultures, focusing on changes in cell number, viability, dry weight, and camphor content.

Materials and Methods

Plant Material

Seeds of *A. gypsicola* were procured from their native environment near the regions of Çorum, located in central Anatolia, Turkey. The Ministry of Food, Agriculture, and Livestock granted written permission for the collection. Validation of the species was carried out by Prof. Dr. Hayri Duman. The herbarium of the Field Crops Department at the Ordu University Faculty of Agriculture securely housed voucher plant specimens. Plant seeds were harvested by sampling from all plant clusters available in the area and were kept in storage until they were cleaned and planted in cork-stopped glass jars. The slope of the land where the plants were collected was 32.0–37.2%, the altitude 743–760 meters, and the aspect was determined as south-southwest (Figure 1).

Cultivation of Callus and Elicitation Sorbitol

In vitro plantlets derived from the collected seeds (pre-treatments) (Açıkgöz and Kara, 2019) served as the source explants (Figure 2). The establishment of *A. gypsicola* cell suspension cultures was initiated using callus tissues obtained from stem segments. The cultures were sustained in B5 medium, fortified with 0.5 mg L^{-1} of benzylaminopurine and 0.5 mg L^{-1} of naphthalene acetic acid. These cultures were maintained in three 250-mL Erlenmeyer flasks, each containing 50 mL of liquid medium and 2.5 g of delicate green calluses. Incubation was carried out on a rotary shaker at 105 rpm, with the temperature set at 25 °C and a photoperiod of 16 hours light/8 hours dark. Three concentrations (5, 25, and 50 g L^{-1}) of sorbitol were tested, alongside the control groups receiving equivalent volumes of ethyl alcohol and distilled water, respectively. The cell suspension cultures, following consistent incubation conditions as mentioned earlier, were harvested at 8-, 24-, and 48-hours post-elicitation. This aimed to assess the influence of sorbitol on cell growth, as well as the accumulation of camphor compounds. After aseptic filtration using Whatman No. 3 filter paper and subsequent washing with deionized water, the filtered suspension cultures were stored in a deep freezer at -20 °C for subsequent extraction. For chemical analysis, the cell suspension cultures were homogenized using a mortar and pestle. The extraction followed the methodology outlined by Açıkgöz (2021). Specifically, 2 g of suspension on a fresh weight basis were mixed with 10 mL of 96% ethyl alcohol and homogenized for 2 minutes. This mixture was maintained at 45°C in a water bath for one night. Subsequently, the samples were placed on a rotary shaker at 4000 rpm for 5 minutes, and the resulting supernatant was collected in vials. The collected extracts were evaporated fully dry at 75 °C using a rotary evaporator. The dried residues were dissolved in 1 mL of methanol for subsequent chemical analysis (Açıkgöz, 2021).

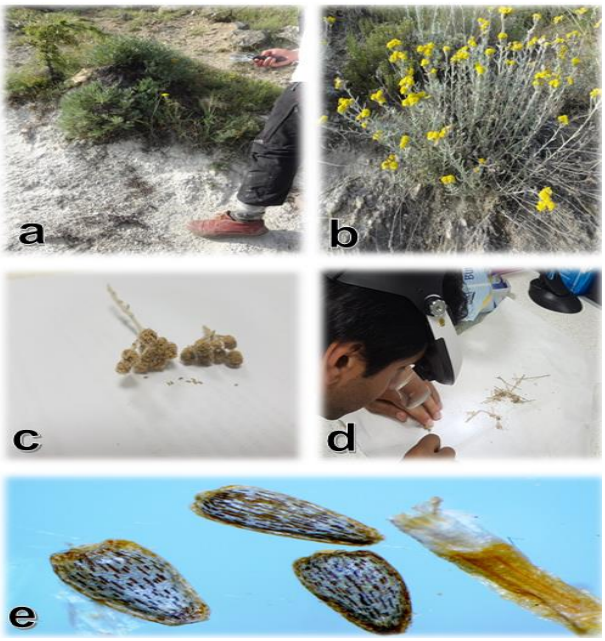

Figure 1. Image of the soil structure of *A. gypsicola* species
(a), view of the plant in the field (b), image of seeds in the laboratory (c), image of pest control (d) and view of seeds under a binocular microscope (e)

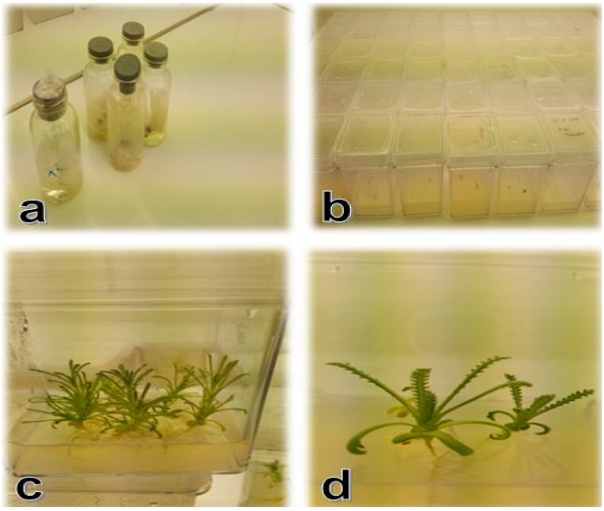

Figure 2. Image of the pre-treatments of *A. gypsicola* species
(a) and images of *in vitro* plantlets germinating in the climate chamber in magenta pots (b, c, and d)

Assessment of Cell Dry Weight, Number, and Viability

The progression of cell suspensions was assessed by gauging key parameters encompassing cell dry weight (g L^{-1}), cell number, and cell viability (%). Cell dry weight was ascertained by carefully weighing the filtered cell samples, which had been exposed to a controlled temperature of 55 °C for 48 hours within an oven. This meticulous process allowed for the precise determination of cell mass. To evaluate cell viability, a Trypan Blue solution from Thermo Fisher Scientific, USA, was utilized in accordance with the methodology delineated by Laloue et al. (1980). This approach facilitated the differentiation between viable and non-viable cells based on dye exclusion. The quantification of cell numbers was carried out utilizing a Nageotte Counting Chamber (Hausser Scientific, USA), employing the procedure elucidated by Moroff et al. (1994). This technique ensured accurate assessment of cell population densities.

Measurement of Camphor Content

The camphor content quantification was accomplished through the utilization of a headspace gas chromatographic-mass spectrometer (GC-MS) system provided by Innovatech Labs, LLC, USA. This analytical setup was integrated with a Shimadzu QP2010 Ultra mass spectrometer and a Shimadzu AOC-5000 plus autosampler from Shimadzu Scientific Instruments, USA. The separation of compounds was achieved using a capillary column with a 30-meter length known as RTX-5M. The analysis commenced by introducing a camphor standard into the instrument. This enabled the determination of mass fragments and retention times associated with the camphor solution. To enhance the method's accuracy and precision, a selection was made of nine prominent ion peaks. A calibration curve was subsequently this data. Utilizing this curve, the camphor content within the samples was expressed in micrograms per gram (µg g^{-1}). generated employing During the GC-MS analysis, specific experimental parameters were maintained as follows: helium served as the carrier gas, an injection temperature of 250 °C was applied, an injection volume of 0.5 mL was employed, ionization voltage was set to 70 eV, a temperature of 100°C was sustained, and a heating duration of 10 minutes was observed. This comprehensive analytical approach allowed for the precise determination of camphor content, facilitating a comprehensive assessment of its presence within the *A. gypsicola* cell suspension cultures under diverse elicitation conditions and time points.

Statistical Analysis

The entire experimental procedures were conducted in triplicate to ensure robustness and reliability. The experimental design adopted a fully randomized layout. The collected data underwent thorough analysis through a 2-way analysis of variance (ANOVA) employing the Minitab 17 statistical software (Minitab, LLC, USA). To discern significant variations among means, the Tukey test was employed, and statistical significance was determined at a threshold of $p<0.05$. This analytical framework enabled the identification of noteworthy disparities and trends within the data.

Results and Discussion

Cell number, cell dry weight (g/L), and cell viability (%)

The variance analysis of cell number indicated significant differences ($p<0.01$) among the sorbitol treatment doses and sampling times (Figure 3). Accordingly, the application of sorbitol at concentrations of 5 and 25 g L^{-1} significantly increased cell numbers compared to the initial culture; however, these two doses exhibited a similar effect on cell numbers. The average cell count, initially at 82.900 cells, increased to 84.390 with the 5 g L^{-1} dose, but there was no further increase with the 25 g L^{-1} dose. On the other hand, the highest average cell count was achieved with the 50 g L^{-1} sorbitol treatment, reaching 90.010 cells. According to the relevant table, sampling times showed a significant effect on cell numbers. No statistically significant change in cell count was observed in samples taken on the first two days (84.865 and 85.350 cells, respectively), while a significant increase was seen in samples taken on the third day (86.060 cells).

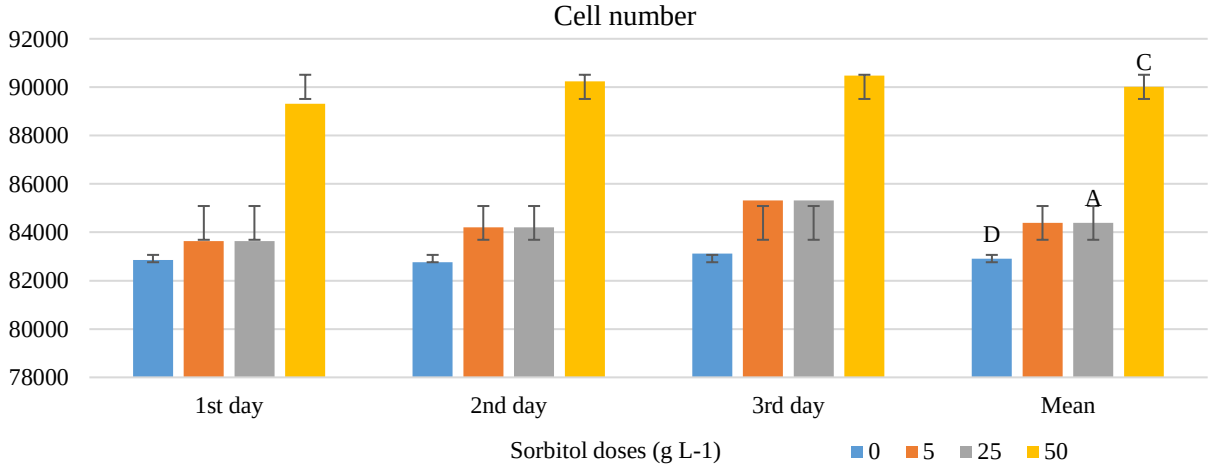

Figure 3. Effects of sorbitol doses (g L^{-1}) and sampling times (days) on cell number in *A. gypsicola* cell suspension cultures

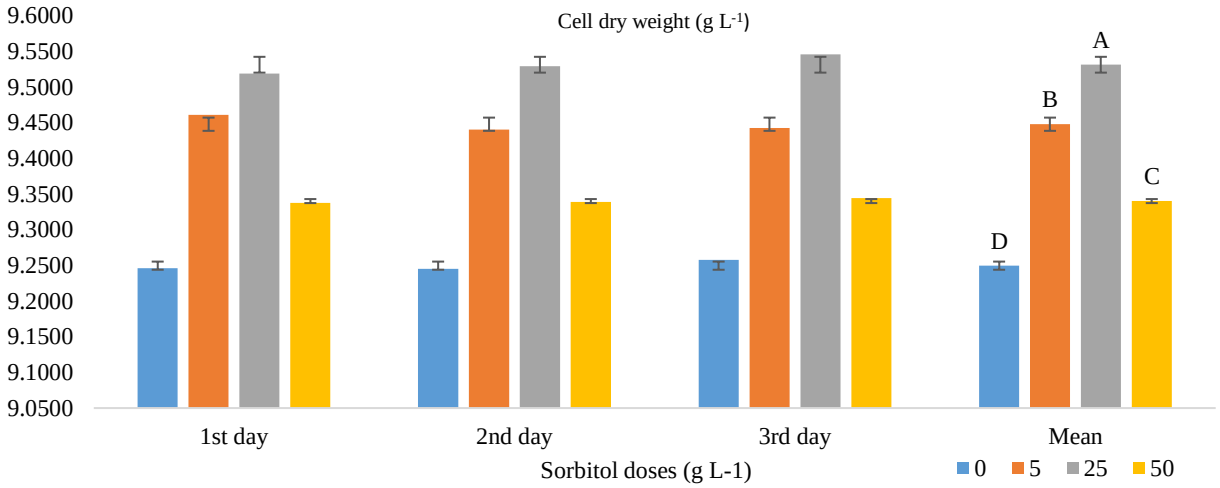

Figure 4. Effects of sorbitol doses (g L^{-1}) and sampling times (days) on cell dry weight (g L^{-1}) in *A. gypsicola* cell suspension cultures

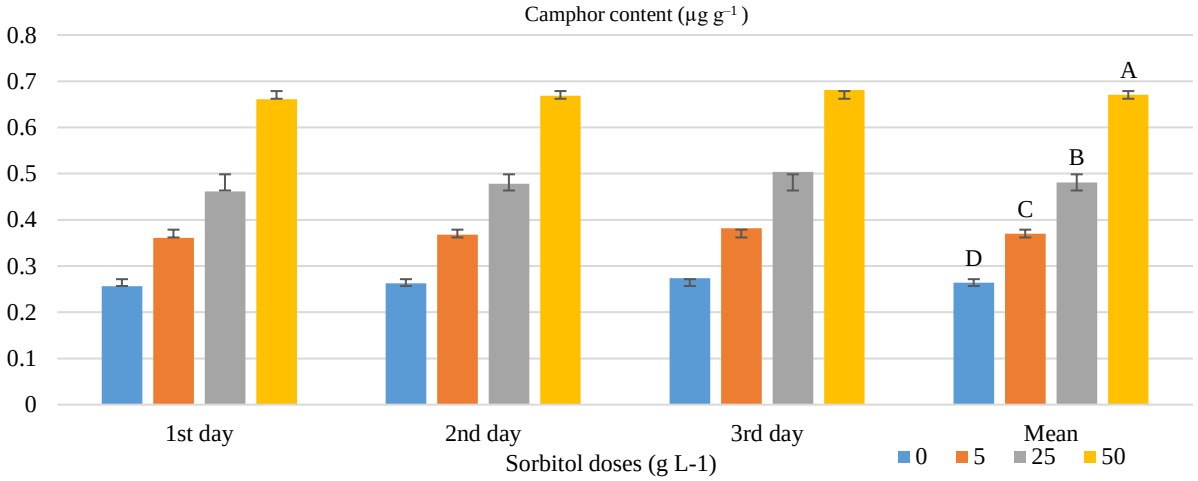

Figure 5. Effects of sorbitol doses (g L^{-1}) and sampling times (days) on camphor content (µg g^{-1}) in *A. gypsicola* cell suspension cultures

For cell dry weight (g L^{-1}), variance analysis revealed significant differences between sorbitol doses ($p<0.01$) and sampling times ($p<0.05$). The initial culture's cell dry weight of 9.25 g L^{-1} increased to 9.34, 9.34, and 9.35 g L^{-1} with the doses of 5, 25, and 50 g L^{-1}, respectively. On the other hand, sampling times significantly influenced cell dry weight. The cell dry weight of 9.31 g L^{-1} in samples taken on the first day (8 hours after application) increased to 9.32 g L^{-1} on the second day and further to 9.32 g L^{-1} on the third day (Figure 4).

Regarding cell viability (%), variance analysis indicated significant differences ($p<0.05$) among sorbitol doses and sampling times. The corresponding descriptive statistical values and Tukey test results, demonstrated that at concentrations of 5 and 25 g L^{-1} of sorbitol, there was a significant increase in viable cell counts compared to the initial culture. However, both doses had a similar effect on the increase in viable cell count. In contrast, the application of 50 g L^{-1}sorbitol resulted in a decrease in the average viable cell count from 97.7% to 97.4% compared to the initial culture. Upon examining the relevant table, it is evident that sampling times had a significant effect on the average viable cell count. The viability, which was 97.6% on the first day, increased to 98.1% on the second day and then returned to 97.6% on the third day.

Numerous researchers have documented the substantial efficacy of elicitor treatments in enhancing cell growth and the production of secondary metabolites. Factors such as the developmental stage of the cell culture (Namdeo, 2007; Kang et al., 2009), duration of elicitor exposure, and the specific elicitor employed play pivotal roles in optimizing the outcomes of these treatments (Kubeš et al., 2014; Nazir et al., 2019; Açıkgöz, 2020a). Prior investigations have demonstrated that, akin to our findings, sorbitol treatment can significantly stimulate cell growth at appropriate concentrations (Al-Khayri and Al-Bahrany, 2002; Wu and Shi, 2008; de Costa et al., 2013; Zaker et al., 2015; Singh et al., 2017). Nonetheless, there exist studies in which sorbitol treatment has exhibited inhibitory effects on cell growth, leading to reductions in cell dry weight (Patil et al., 2013; Salehi et al., 2019; Ramulifho et al., 2019). Conversely, certain studies have emphasized the critical role of selecting suitable concentrations for maintaining cell viability during sorbitol treatments, highlighting that, as observed in this study, high concentrations of sorbitol may result in cell death (Hong et al., 2012; Valayil et al., 2015; Sarmadi et al., 2019).

Camphor Content (µg g^{-1}).

According to the variance analysis conducted for the camphor content (µg g^{-1}), while the difference between sorbitol doses was found to be significant ($p<0.01$), the variation among sampling times was deemed insignificant. The camphor content (µg g^{-1}) exhibited a significantly proportional increase with rising sorbitol doses (Figure 4). The initial culture's camphor content, which was 0.264 µg g^{-1}, increased by 40% to 0.370 µg g^{-1} at a dose of 5 g L^{-1}, by 82% to 0.4810 µg g^{-1} at a dose of 25 g L^{-1}, and by a remarkable 154% to 0.670 µg g^{-1} at a dose of 50 g L^{-1} (Figure 5). In cell cultures, sorbitol, which is commonly used to elevate osmolality, serves as a significant source of abiotic stress. It is well known that under stress conditions, cells increase their defense-oriented antioxidant enzyme levels, including CAT, POD, and SOD (Sytar et al., 2013; Vuleta et al., 2016; Azarabadi et al., 2017). Previous studies have demonstrated that sorbitol plays a crucial role in stimulating these enzymes and enhancing PAL activity within cells (Wu et al., 2008). Particularly, PAL and similar enzymes are the most active in the production of secondary metabolites within cells.

Numerous studies have reported that sorbitol elicitor promotes the accumulation of secondary metabolites and cell growth in cell cultures (Ling et al., 2008; Wu and Shi, 2008; Zhao et al., 2010; Patil et al., 2013; Valayıl et al., 2015; Zaker et al., 2015; Razavizadeh and Adabavazeh, 2017; Sing et al., 2017; Yang et al., 2017). Consistent with these findings, our own research has shown that the doses of sorbitol used in our study positively stimulate secondary metabolite accumulation and cell growth in cell cultures, underscoring its significance as an osmotic stress inducer.

Cells undergoing various growth stages in plant tissue culture systems exhibit distinct levels of mRNA and proteins, as indicated by Chong et al. (2005). This variance in cellular composition leads to differential responses to elicitor treatments, consequently influencing the accumulation of bioactive compounds, as demonstrated by Kang et al. (2009). As a result, precise calibration of the dosage and duration of elicitor treatments becomes crucial to effectively stimulate the generation of signaling molecules within the cells. Notably, specific concentrations of sorbitol employed in this investigation exhibited superior performance in enhancing the accumulation of camphor.

Conclusion

In this study, it has been determined for the first time that increasing doses of sorbitol applications in *A. gypsicola* cell suspension culture significantly enhance cell count, cell dry weight, and camphor content. The concentrations of sorbitol at 5 and 25 g L^{-1} were found to notably increase cell count compared to the initial culture, and cell dry weight increased in response to all three sorbitol doses. The initial camphor content of 0.264 µg g^{-1} increased by 40% to 0.370 µg g^{-1} at the dose of 5 g L^{-1}, by 82% to 0.481 µg g^{-1} at the dose of 25 g L^{-1}, and by an impressive 154% to 0.670 µg g^{-1} at the dose of 50 g L^{-1}. When evaluating cell count and cell dry weight based on sampling times, no statistically significant changes were observed in cell count for samples taken within the first two days, but a significant increase was seen in samples taken on the third day. On the other hand, sampling times were found to significantly affect cell dry weight. It was determined that cell dry weight increased in samples taken on the first, second, and third days. This research has demonstrated that the sorbitol doses used positively stimulate secondary metabolite accumulation and cell growth, thus highlighting its importance as a source of osmotic stress in cell cultures.

Acknowledgments

The authors extend their gratitude to the TUBITAK for their invaluable financial support, which was instrumental in facilitating this research. The support was provided under Project No. 1001-114O564. Additionally, it's noteworthy that this study constitutes a significant segment of Dr. Muhammed Akif Açıkgöz's doctoral thesis.

Declaration of Competing Interest

The authors report no declarations of interest.

References

Abdel-Rahman R, Alqasoumi S, El-Desoky A, Soliman G, Paré G, Hegazy ME. 2015. Evaluation of the anti-inflammatory, analgesic and anti-ulcerogenic potentials of *Achillea fragrantissima* (Forssk.). South African Journal of Botany, 98: 122-127.

Açıkgöz MA. 2020a. Establishment of cell suspension cultures of *Ocimum basilicum* L. and enhanced production of pharmaceutical active ingredients. Ind Crops Prod 148:112278.

Açıkgöz MA. 2020b. Determination of essential oil compositions and antimicrobial activity of *Achillea gypsicola* Hub.-Mor. at different plant parts and phenological stages. Journal of Essential Oil Research, 32(4), 331-347.

Açıkgöz MA. 2021. Effects of sorbitol on the production of phenolic compounds and terpenoids in the cell suspension cultures of *Ocimum basilicum* L. Biologia, 76(1), 395-409.

Açıkgöz MA. 2017. *Achillea gypsicola* türünde kallus kültürü ile sekonder metabolit üretim potansiyelinin belirlenmesi. Ordu University (Doctoral dissertation, PhD Thesis (Printed), Ordu).

Açıkgöz MA, Kara ŞM. 2019. Effect of various pretreatments on germination of Turkish endemic *Achillea gypsicola* Hub.-Mor. species under in vivo and in vitro conditions. Journal of the Institute of Science and Technology, 9(4), 2321-2329.

Açıkgöz MA, Ebru BA, Aygün A, Kara ŞM. 2022. Light-Mediated Biosynthesis of Phenylpropanoid Metabolites in Cell Suspension Cultures of Turkish Yarrow (*Achillea gypsicola* Hub. Mor.). Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 25(6), 1234-1242

Açıkgöz MA, Ebru BA, Kara ŞM, Aygün A. 2023. Influence of Selected Heavy Metals on Cell Growth and Camphor Secretion in *Achillea gypsicola* Hub. Mor. *In vitro* Cell Cultures. Journal of Agricultural Faculty of Gaziosmanpaşa University (JAFAG), 40(1), 11-18.

Açıkgöz MA, Kara ŞM, Ebru BA, Odabaş S. 2018b. Effect of light on biosynthesis of alkamide, caffeic acid derivatives and echinacoside in *Echinacea purpurea* L. callus cultures. Akademik Ziraat Dergisi, 7(2), 179-184.

Açıkgöz MA, Yarılgaç T, Kara ŞM. 2018a. Enhancement of phytochemical compounds using biotic and abiotic elicitors in purple coneflower (*Echinacea purpurea* L.). Indian J Pharm Educ Res, 52, 140-145.

Açıkgöz MA, Kara SM, Aygun A, Ozcan MM, Ay EB. 2019. 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. Turkish Journal of Agriculture and Forestry

Açıkgöz MA. 2019. Evaluation of phytochemical compositions and biological properties of *Achillea gypsicola* at different phenological stages. Chemistry & Biodiversity, 16(12), e1900373.

Ahmadi-Dastgerdi A, Ezzatpanah H, Asgary S, Dokhani S, Rahimi E. 2017. Phytochemical, Antioxidant and Antimicrobial Activity of the Essential Oil from Flowers and Leaves of *Achillea millefolium* subsp. *millefolium*. Journal of Essential Oil Bearing Plants, 20(2): 395-409.

Al-Khayri JM, Al-Bahrany AM. 2002. Callus growth and proline accumulation in response to sorbitol and sucrose-induced osmotic stress in rice. Biol Plant 45:609–611.

Almadiy AA, Nenaah GE, Al Assiuty BA, Moussa EA, Mira NM. 2016. Chemical composition and antibacterial activity of essential oils and major fractions of four *Achillea* species and their nanoemulsions against foodborne bacteria. LWT-Food Science and Technology, 69: 529-537.

Azarabadi S, Abdollahi H, Torabi M, Salehi Z, Nasiri J. 2017. ROS generation, oxidative burst and dynamic expression profiles of ROS-scavenging enzymes of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in response to *Erwinia amylovora* in pear (*Pyrus communis* L). Eur J Plant Pathol 147:279–294.

Bidabadi SS, Meon S, Wahab Z, Subramaniam S, Mahmood M. 2012. *In vitro* selection and characterization of water stress tolerant lines among ethyl methanesulphonate (EMS) induced variants of banana (*Musa spp*., with AAA genome). Australian Journal of Crop Science, 6(3), 567-575.

Cheng LH, Eugene ICW, Yu CS. 2009. Essential Oil Compositions and Bioactivities of the Various Parts of *Cinnamomum camphora* Sieb.var, *linaloolifera* Fujuta, Journal of Forestry Research, 2: 77-96.

Chong TM, Abdullah MA, Lai OM, Nor'Aini FM, Lajis NH. 2005. Effective elicitation factors in *Morinda elliptica* cell suspension culture.

Dağlioğlu Y, Açıkgöz MA, Özcan MM, Kara ŞM. 2022. Impact of application of alumina oxide nanoparticles on callus induction, pigment content, cell damage and antioxidant enzyme activities in *Ocimum basilicum*. Journal of International Environmental Application and Science, 17(1), 22-33.

de Costa F, Yendo ACA, Fleck JD, Gosmann G, Fett-Neto AG. 2013. Accumulation of a bioactive triterpene saponin fraction of *Quillaja brasiliensis* leaves is associated with abiotic and biotic stresses. Plant Physiol Biochem 66:56–62.

Demirci B, Başer KHC, Aytaç Z, Khan SI, Jacob MR, Tabanca N. 2017. Comparative Study of Three *Achillea* Essential Oils from Eastern Part of Turkey and their Biological Activities.

Dodds JH, Roberts LW. 1985. Experiments in plant tissue culture. International Potato Center.

Ebru BA, Kocaman B, Öner EK, Açıkgöz MA. 2022. Effects of elicitors on secondary metabolite (SM) production and antioxidant activity in sweet basil (*Ocimum basilicum* L.) cell suspension cultures. Notulae Scientia Biologicae, 14(2), 11246-11246.

Frizzo CD, Santos AC, Paraul N, Serafini LA, Dellacassa E, Lorenzo D, Moyna P. 2000. Essential Oils of Camphor Tree (*Cinnamomum camphora* Nees and Eberm) Cultivated in Southern Brazil. Journal of Brazilian Archives of Biology and Technology, 43(3): 313-316.

Ghasemi Pirbalouti A. 2017. Chemical Composition of the Essential Oils from the Leaves and Flowers of Two *Achillea* species from Iran. Journal of Essential Oil Bearing Plants, 20(1): 205-214.

Hong MLK, Bhatt ARVIND, Ping NS, Keng CL. 2012. Detection of elicitation effect on *Hyoscyamus niger* L. root cultures for the root growth and production of tropane alkaloids. Rom Biotech Lett 17:7340–7351

Kang SM, Min JY, Kim YD, Karigar CS, Kim SW, Goo GH, Choi MS. 2009 Effect of biotic elicitors on the accumulation of bilobalide and ginkgolides in *Ginkgo biloba* cell cultures. J Biotechnol 139:84–88.

Khiyari MEA, Kasrati A, Jamali CA, Zeroual S, Markouk M, Bekkouche K, Abbad A. 2014. Chemical composition, antioxidant and insecticidal properties of essential oils from wild and cultivated *Salvia aucheri* subsp. *blancoana* (Webb. & Helder)), an endemic, threatened medicinal plant in Morocco. Industrial Crops and Products, 57: 106-109.

Kubeš J, Tůmová L, Martin J, Vildová A, Hendrychová H, Sojková K. 2014 The production of isoflavonoids in *Genista tinctoria* L. cell suspension culture after abiotic stressors treatment. Nat Prod Res 28:2253–2263.

Laloue M, Courtois D, Manigault P. 1980 Convenient and rapid fluorescent staining of plant cell nuclei with '33258'Hoechst. Plant Sci Lett 17:175–179.

Lin TC, Chu FH, Tseng YH, Tsai JB, Chang ST, Wang SY. 2007. Bioactivity Investigation of Lauraceae Trees Grown in Taiwan. Pharmaceutical Biology, 8: 638-644.

Ling OS, Kiong ALP, Hussein S. 2008. Establishment and optimisation of growth parameters for cell suspension cultures of *Ficus deltoidea*. American-Eurasian Journal of Sustainable Agriculture, 2(1): 38-49.

Moroff G, Eich J, Dabay M. 1994. Validation of use of the nageotte: hemocytometer to count low levels of white cells in white cell reduced platelet components. Transfusion, 34: 35-38.

Namdeo AG. 2007. Plant cell elicitation for production of secondary metabolites: a review. Pharmacogn Rev 1:69–79

Nazir M, Tungmunnithum D, Bose S, Drouet S, Garros L, Giglioli-Guivarc'h N, Abbasi BH, Hano C. 2019. Differential production of phenylpropanoid metabolites in callus cultures of *Ocimum basilicum* L. with distinct *in vitro* antioxidant activities and in vivo protective effects against UV stress. J Agric Food Chem 67:1847–1859.

Patil JG, Ahire ML, Nitnaware KM, Panda S, Bhatt VP, Kishor Kavi PB, Nikam TD. 2013. *In vitro* propagation and production of cardiotonic glycosides in shoot cultures of *Digitalis purpurea* L. by elicitation and precursor feeding. Applied microbiology and biotechnology, 97(6): 2379-2393.

Pavlović M, Petrović S, Milenković M, Kukić J, Couladis M, Tzakou O, Niketić M. 2008. Composition and antiradical capacity of *Achillea grandifolia* essential oil from Serbia. Planta Medica, 74(9): 1191-1191.

Placide R, Carpentier S, Swennen R. 2012. Development of *in vitro* technique to screen for drought tolerant banana varieties by sorbitol induced osmotic stress. African Journal of Plant Science, 6(15), 416-425. Process Biochem 40:3397–3405

Rai MK, Kalia RK, Singh R, Gangola MP, Dhawan AK. 2011. Developing stress tolerant plants through in vitro selection-an overview of the recent progress. Environmental and Experimental Botany, 71(1), 89-98.

Ramulifho E, Goche T, Van As J, Tsilo TJ, Chivasa S, Ngara R. 2019. Establishment and characterization of callus and cell suspension cultures of selected *Sorghum bicolor* (L.) Moench varieties: a resource for gene discovery in plant stress biology. Agronomy 9:218.

Razavizadeh R, Adabavazeh F, Chermahini MR. 2017. Adaptive Responses of *Carum copticum* to *in vitro* Salt Stress. World Academy of Science, Engineering and Technology, International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 11(1): 37-42.

Salehi M, Karimzadeh G, Naghavi MR. 2019 Synergistic effect of coronatine and sorbitol on artemisinin production in cell suspension culture of *Artemisia annua* L. cv. Anamed. Plant Cell Tissue Organ Cult 137:587–597.

Sampietro DA, Lizarraga EF, Ibatayev ZA, Omarova AB, Suleimen YM, Catalán CA. 2016. Chemical composition and antimicrobial activity of essential oils from *Acantholippia deserticola*, *Artemisia proceriformis*, *Achillea micrantha* and *Libanotis buchtormensis* against phytopathogenic bacteria and fungi. Natural product research, 30(17): 1950-1955.

Sarmadi M, Karimi N, Palazón J, Ghassempour A, Mirjalili MH. 2019. Improved effects of polyethylene glycol on the growth, antioxidative enzymes activity and taxanes production in a *Taxus baccata* L. callus culture. Plant Cell Tissue Organ Cult 137:319–328.

Sherkheli MA, Benecke H, Doerner JF. 2009. Monoterpenoids induce agonist-specific desensitization of transient receptor potential vanilloid-3 (TRPV3) ion channels, Journal Pharmaclogy Science, 12: 116-128.

Singh B, Sahu PM, Sharma RA. 2017. Effect of elicitors on the production of pyrroloquinazoline alkaloids by stimulating anthranilate synthase activity in *Adhatoda vasica* Nees cell cultures. Planta, 1-13.

Smelcerovic A, Lamshoeft M, Radulovic N, Ilic D, Palic R. 2010. LC–MS Analysis of the Essential Oils of Achillea millefolium and *Achillea crithmifolia*. Chromatographia, 71(1-2): 113-116.

Sytar O, Kumar A, Latowski D, Kuczynska P, Strzałka K, Prasad MNV. 2013. Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiol Plant 35:985–999.

Thompson MR, Douglas TJ, Obata-Sasamoto H, Thorpe TA. 1986. Mannitol metabolism in cultured plant cells. Physiologia Plantarum, 67(3), 365-369.

Toncer O, Basbağ S, Karaman S, Diraz E, Basbağ M. 2010. Chemical composition of the essential oils of some *Achillea* species growing wild in Turkey. International Journal of Agriculture and Biology, 12(4): 527-530.

Valayıl JM, Kuriakose GC, Jayabaskaran C. 2015. Modulating the biosynthesis of a bioactive steroidal saponin, cholestanol glucoside by *Lasiodiplodia theobromae* using abiotic stress factors. International Journal of Pharmacy and Pharmaceutical Sciences, 7: 114-117.

Vanhove AC, Vermaelen W, Panis B, Swennen R, Carpentier, SC. 2012. Screening the banana biodiversity for drought tolerance: can an *in vitro* growth model and proteomics be used as a tool to discover tolerant varieties and understand homeostasis. Frontiers in Plant Science, 3, 176.

Vuleta A, Jovanović SM, Tucić B. 2016. Adaptive flexibility of enzymatic antioxidants SOD, APX and CAT to high light stress: the clonal perennial monocot *Iris pumila* as a study case. Plant Physiol Biochem 100:166–173.

Wu JY, Shi M. 2008. Ultrahigh diterpenoid tanshinone production through repeated osmotic stress and elicitor stimulation in fed-batch culture of *Salvia miltiorrhiza* hairy roots. Applied Microbiology and Biotechnology, 78(3): 441-448.

Yang Y, Pu X, Qu X, Chen F, Zhang G, Luo Y. 2017. Enhanced production of camptothecin and biological preparation of N1-acetylkynuramine in *Camptotheca acuminata* cell suspension cultures. Applied microbiology and biotechnology, 101(10): 4053-4062.

Zaker A, Sykora C, Gössnitzer F, Abrishamchi P, Asili J, Mousavi SH, Wawrosch C. 2015. Effects of some elicitors on tanshinone production in adventitious root cultures of *Perovskia abrotanoides* Karel. Industrial Crops and Products, 67: 97-102.

Zhao JL, Zhou LG, Wu JY. 2010. Effects of biotic and abiotic elicitors on cell growth and tanshinone accumulation in *Salvia miltiorrhiza* cell cultures. Applied Microbiology and Biotechnology, 87(1): 137-144.