

The form, dose, and method of application of vermicompost differentiate the phenylpropene biosynthesis in the peltate glandular trichomes of methylchavicol chemotype of *Ocimum basilicum* L.

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ABSTRACT

Peltate glandular trichomes (PGTs) in basil (*Ocimum basilicum* L.) are ideal for studying phenylpropanoid metabolism, as they allow for the elimination of the effects of primary plant metabolism. In this study, we aimed to investigate the effects of 0, 10%, and 25% doses of both solid and tea forms of vermicompost on phenylpropanoid metabolism in PGTs of the methylchavicol chemotype of basil. The experiment based on the analysis of the gene expression (*PAL*, *4CL*, *EGS*, *EOMT*, *CVOMT*) and the accumulation of phenylpropene (eugenol, chavicol, methyleugenol, methylchavicol) in PGTs after vermicompost treatment. Our results showed that the application of solid vermicompost (SV) to the root zone of basil at 10% and 25% significantly reduced the expression of *EOMT* and *CVOMT* to below 0.1 from 2.43 and 3.85, respectively. The application of 10% solid vermicompost (SV) to basil soil resulted in a decrease in methyleugenol and methylchavicol accumulation approximately by 50% and 52%, respectively. Further application of 25% (SV) resulted in a total decrease of approximately 76% and 51% in methyleugenol and methylchavicol accumulation, respectively. Application of 10% vermicompost tea (VT) to basil leaves resulted in a significant increase in chavicol accumulation (243%) and methyleugenol accumulation (613%) in basil essential oil, as well as upregulation of *EOMT* and *CVOMT* gene expression approximately by 391-fold and 1,155-fold, respectively, compared to control plants. Furthermore, application of 25% VT resulted in a significant increase in eugenol accumulation (665%) and methylchavicol accumulation (56%), as well as downregulation of *EOMT* and *CVOMT* gene expression approximately by 31-fold and 9-fold, respectively, compared to control plants. These findings suggest that VT application can modulate the accumulation of important phenylpropenes in basil through regulation of gene expression. In addition, the use of vermicompost tea has been shown to significantly enhance the levels of phenylpropenes in basil, which are important contributors to the herb's aroma and antimicrobial activity. This suggests that the application of VT can play a crucial role in determining the quality of basil in terms of its secondary metabolites, making it a valuable tool in phytoremediation practices. Overall, our results demonstrate the effectiveness of vermicompost on phenylpropene biosynthesis and the utility of PGTs composed of a single cell type for the study of secondary metabolic processes in plants.

1. Introduction

Ocimum, an important genus of the Lamiaceae family, includes annual or perennial herbaceous plants and shrubs found in tropical and subtropical regions of Asia, Australia, and West Africa (Lal et al., 2023; Saran et al., 2017). Basil, *Ocimum basilicum* L., is cultivated for its essential oil, which is used as flavorant and odorizer in the food industry and in perfumery. It is also used in pharmaceutical industries as a source

of chemical raw materials (de de Elguea-Culebras et al., 2022; Telci et al., 2006). Basil has traditionally been used in folk medicine to treat headaches, coughs, diarrhea, constipation, warts, and kidney dysfunction, as well as to stimulate appetite and promote digestion (Klimánková et al., 2008; Milenković et al., 2019).

Like many other plant species, basil leaves have specialized glands called glandular trichomes that contain compounds that protect the plant against herbivores and microorganisms. Basil glandular trichomes

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has two types of morphology: peltate and capitate trichomes. The capitate glandular trichomes of basil are rich in terpenoid compounds synthesized from the mevalonic acid pathway, while the peltate glandular trichomes (PGTs) are rich in phenylpropenes such as eugenol, methyleugenol, chavicol and methylchavicol (estragole) (Gang et al.,

2001; Koeduka et al., 2006; Vassao et al., 2006; Xie et al., 2008). Although phenylpropanoid compounds are not common in plants whose essential oil is rich in terpenoid compounds, they are generally abundant in the essential oil of *O. basilicum* (Tavallali et al., 2019). Hydroxycinnamic acids and their derivatives, cinnamic aldehydes, monolignols,

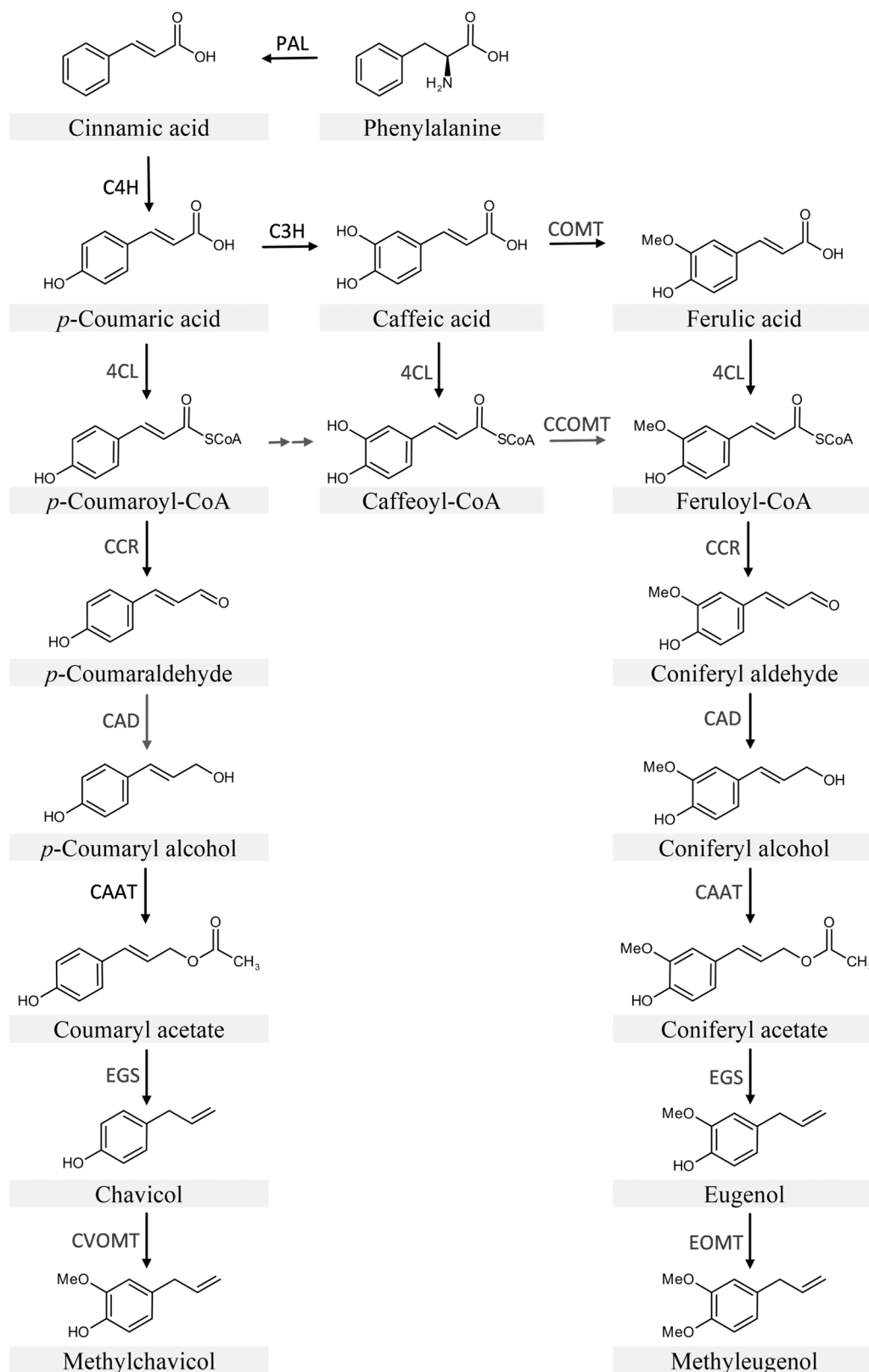


Fig. 1. Schematic representation of methyleugenol and methylchavicol synthesis through the phenylpropanoid pathway in *Ocimum basilicum*. PAL, Phenylalanine ammonia-lyase; C4H, Cinnamate 4-hydroxylase; C3H, 4-coumarate 3-hydroxylase; COMT, Caffeic acid O-methyltransferase; 4CL, 4-Coumarate-CoA ligase; CCR, Cinnamoyl-CoA reductase; CAD, Cinnamoyl alcohol dehydrogenase; CAAT, Coniferyl alcohol acetyltransferase; EGS, Eugenol synthase; CVOMT, Chavicol O-methyltransferase; HCT, Shikimate O-methyltransferase; CS3'H, *p*-Coumaroyl 5-O-shikimate 3'-hydroxylase; CCOMT, Caffeoyl-CoA O-methyltransferase; EOMT, Eugenol O-methyltransferase; CVOMT, Chavicol O-methyltransferase. Double arrows indicate intermediated suggested reactions not shown.

coumarins, isocoumarins, chromenes and phenylpropenes are synthesized in various branches and cascades of the phenylpropanoid pathway and are classified as "phenylpropanoids" (Tsimogiannis and Oreopoulou, 2019). Phenylpropenes, which are produced at the end of a branch of phenylpropanoid biosynthesis pathway, play an important role in attracting pollinators and in plant defense against animals and microorganisms. These compounds are commonly used in the flavor, perfume, and pharmaceutical industries, making them economically important along with terpenes (de de Elguea-Culebras et al., 2022). Many of the herbal phenolics have important antioxidant capacities that can reduce cancer incidence and mortality rates. It has been reported that basil grown in organic farming conditions shows an increase in phenolic compounds with these properties (Taie et al., 2010).

To date more than 140 compounds were detected in the essential oil of basil. The differences in the content of essential oils depend on the aroma, leaf and flower color, differences in the growth and development stages of plant tissues, geographical origins of the plants, and the environmental conditions in which they are grown (Čavar Zeljković et al., 2020; Khakdan et al., 2017; Kisa et al., 2021; Saran et al., 2023; Telci et al., 2006).

Phenylalanine ammonia-lyase (PAL) enzyme plays an important role in the biosynthesis of phenylpropanoids by catalyzing the synthesis of *trans*-Cinnamate through the deamination of phenylalanine (Fig. 1). Another important branching point in this pathway is the biosynthesis of 4-Coumaroyl-CoA from 4-Coumarate with the activity of 4-Coumarate ligase (4CL) enzyme (Rastogi et al., 2013). The eugenol synthase (EGS) enzyme performs both the synthesis of the first phenylpropenes; chavicol from coumaryl acetate and eugenol from coniferyl acetate in the later steps of phenylpropanoid metabolism (de de Elguea-Culebras et al., 2022; Koeduka et al., 2006; Vassao et al., 2006). In the final steps of the pathway towards phenylpropene biosynthesis, the eugenol O-methyltransferase (EOMT) and chavicol O-methyltransferase (CVOMT) enzymes methylate the *para*-OH group of eugenol and chavicol, forming their methyl ether derivatives methyleugenol and methylchavicol (Iijima et al., 2004a; Koeduka, 2014; Kumari et al., 2022).

Analysis of expressed sequence tags (ESTs) in PGTs of basil showed that the genes known to play a role in phenylpropanoid biosynthesis are expressed at extremely high levels in these glands, accounting for 13% of the total ESTs. In addition, 14% of the total cDNAs encode the enzymes catalyzing the biosynthesis of methylchavicol and methyleugenol using S-adenosyl-methionine as substrate (Gang et al., 2001). PGTs of basil are highly specialized structures for the synthesis and storage of phenylpropanoids and are an excellent research material for the investigation of phenylpropanoid biosynthesis. Among overall studies focused on PGTs of *O. basilicum* (Deschamps et al., 2006; Gang et al., 2002a; Gang et al., 2002b; Gang et al., 2002c; Gang et al., 2001; Iijima et al., 2004a; Iijima et al., 2004b; Koeduka et al., 2006; Vassao et al., 2006), Xie et al. (2008) carried out the first comprehensive study on the regulation of metabolism in PGTs by utilizing a proteomics-based approach with isolated trichomes of four different *Ocimum basilicum* L. lines holding very different metabolite profiles.

Plant biostimulant products are novel products in agricultural science, and are particularly effective in medicinal plants, as they can enable genetic manipulation of the synthesis pathways of secondary metabolites. One of the most important goals for today's agricultural activities is to significantly reduce inputs and move towards environmentally and economically sustainable agri-environmental systems. The identification of organic molecules and microorganisms that can modulate the primary and secondary metabolism of plants is relatively a new field of research in achieving this goal (Ciriello et al., 2022; Rouphael et al., 2021).

Plant biostimulants are any substance or microorganisms applied to plants to improve nutritional efficiency, abiotic stress tolerance and/or quality characteristics of products, regardless of their nutritional content (du Jardin, 2015). Seaweed extracts are the most widely used

biostimulants among protein hydrolysates (PHs), plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) (Ciriello et al., 2022). Vermicomposts are produced at the end of vermicomposting process using worms which are fed decomposed organic waste (Kızılkaya and Hepşen, 2004) and were categorized in the humic substances group of biostimulants by du Jardin (2015). The effects of vermicompost on plant growth and development were investigated by numerous researchers and the "hormone-stimulated activity" mechanisms of vermicompost also stated by them in those studies (Aguir et al., 2012; Arancon et al., 2006; Cabanillas et al., 2013; Canellas et al., 2010; Maji et al., 2017; Pant et al., 2011; Singh et al., 2010; Zhang et al., 2014). The hormone-like activity of vermicompost humic substances is thought to come from either the raw material used in the production of vermicompost or the hormones produced during the composting of biomass (Arthur et al., 2007; Scaglia et al., 2016). The chemical complexity of humic acids (HA) has made it difficult to identify auxin-like molecules that cause biostimulant effects, leading researchers to determine that organic fractions containing HAs have auxin-like properties (Canellas et al., 2015; Hernandez et al., 2014; Olivares et al., 2015; Schiavon et al., 2010). Vermicomposts also contain soluble nutrients, free enzymes, a wide range of microorganisms, and water-soluble phenols (Kızılkaya et al., 2012) which can easily diffuse to the water during the vermicompost tea production (Sherman, 2010).

Basil is frequently used as a research material in the field of bioengineering due to its economic importance in essential oil production. However, there are many studies reporting the effects of biostimulants or organic fertilizers (Bączek et al., 2019; Copetta et al., 2006; G.R et al., 2019; Javanmardi and Ghorbani, 2012; Khan et al., 2023; Klimánková et al., 2008; Mohamed et al., 2016; Shokooh et al., 2013; Singh et al., 2013; Taie et al., 2010; Verma et al., 2016), elicitors (Kahveci et al., 2021; Koca and Karaman, 2015; Li et al., 2007; Mirzajani et al., 2015; Tavallali et al., 2019; Zlotek et al., 2016) or stress applications (Abdollahi Mandoulakani et al., 2017; Khakdan et al., 2017; Lung et al., 2016; Zhan et al., 2016) on basil, but all of them are focused on one or more parameters as phenological parameters, total phenolic content, essential oil and gene expression profile of complete leaf (non-abraded, intact leaf including the whole tissues). It is more reasonable solely to focus on to PGTs rather than the complete leaf with all tissues, in order to eliminate the interference of the data which would be retrieved from primary metabolic activities that occur in the other tissues of the leaves.

The use of chemical fertilizers is gradually being reduced increasingly to ensure better food safety and soil health. Therefore, it is important to determine the potential of vermicompost on plant secondary metabolite production. This study, which focused on the phenylpropene content and the expression profiles of *PAL*, *4CL*, *EGS*, *EOMT* and *CVOMT* genes in PGTs basil, rather than the complete leaf, will contribute to the interests of bioengineering researchers and sustainable agriculture practices. Another distinct feature of this study is its aim to simultaneously investigate the effects of solid and aqueous vermicompost applications on phenylpropene content and gene expression profiles of isolated PGTs of basil, whereas all the previous studies that have used the complete leaf as research material.

The aim of this study was to assess the impact of moderate (10%) and high (25%) doses of solid and tea vermicompost applications on phenylpropene biosynthesis in the PGTs of methylchavicol chemotype of *Ocimum basilicum* L.

2. Materials and methods

2.1. Plant materials and cultivation conditions

Basil seeds were obtained from the collection of the Department of Field Crops, Faculty of Agriculture at Isparta University of Applied Sciences. Germination of seeds was carried out in an incubator at a constant temperature of 35 °C (Zhou et al., 2016). The germinated seeds were transferred to 1000 cc pots containing a mixture of peat, soil and

vermiculite (2:1:1). Vermicompost was added as 10% and 25% of the total volume of peat and soil for solid vermicompost (SV) application groups. Vermicompost tea (VT) at 10% or 25% concentrations were applied once a week, starting from the second week of culture when true leaves were formed in all basil plants. One liter of vermicompost tea at a 25% concentration was prepared using a solid vermicompost:distilled water mixture at a ratio of 1:4 (w/v) on the day before the application day. The mixture was aerated for 24 h using an aquarium-type aeration pump and a shaker (Edwards et al., 2010). The resulting mixture was filtered five times through a filter with a 40 µm pore opening. The VT solution at a 10% concentration was prepared by diluting a sufficient volume taken from the 25% concentrated VT solution at 1:2.5 ratio. The 10% and 25% concentrated VT solutions were applied to the leaves once a week by spraying, with a few drops of Tween® 80 added. It was ensured that the leaf surfaces were completely wet during VT applications. Care was taken to use a sufficient amount to prevent the VT covering the leaf surface from dripping into the soil and reaching the root zone. The leaves of the basil plants in the control group were sprayed with Tween® 80 added distilled water in the same amount as the VT consumed in the 10% and 25% VT application groups. The vermicompost used in this research was obtained from the Agricultural Research and Application Center at Kırşehir Ahi Evran University. The specifications of vermicompost produced from barnyard manure are shown in Table 1.

The plants were grown in an automatic climate-controlled plant growth room at the Department of Soil Science and Plant Nutrition, Faculty of Agriculture at Kırşehir Ahi Evran University 16 h of light, 50%–60% humidity, and a temperature range of 25–18 °C (Fig. 2).

2.2. Isolation of peltate glandular trichomes (PGTs) from leaves

Ten weeks after germination of the seeds, the Glass Bead Abrasion protocol was carried out to isolate PGTs from basil leaves (Gang et al., 2001). According to this method, young leaves smaller than 2 cm in length were cut using forceps and 15 g leaf samples were collected (Fig. 3).

The collected material was transferred to a 300 mL beaker on ice. Ice-cold distilled water was poured into the beaker and the leaves were soaked in the water for 30–60 min. After the water was drained, the swelled leaves were transferred to a 300 mL chamber of a Bead Beater device. Then, 40–50 g of 0.5 mm diameter glass beads and 250 mL of ice-cold glandular trichome isolation buffer (50 mM Tris-HCl, 200 mM D-Sorbitol, 20 mM Sucrose, 14 mM β-mercaptoethanol, 10 mM KCl, 5 mM MgCl₂, 0.5 mM K₂PO₄, 5 mM Succinic acid, 1 mM EGTA, 0.6% (w/v) Methylcellulose, 1% (w/v) Polyvinylpyrrolidone) were added to the chamber.

Mixing process was performed three times for one minute each, with one-minute intervals, in the Bead Beater device whose spinning speed was retarded to 4200 rpm by an external voltage regulator. After the mixing process, the entire mixture in the chamber was passed through a filter with a pore opening of 350 µm and the leaves were removed. The filtered mixture was passed through a 105 µm pore filter in order to remove the leaf residues. Approximately 300 mL of isolation buffer at

ice temperature was used to ensure the flow through the filters, which did not contain methylcellulose and polyvinylpyrrolidone. Peltate trichomes with an average diameter of 80 µm were obtained by depositing on the surface of the filter with a pore opening of 40 µm. (Fig. 4).

2.3. Determination of volatile organic compound content of PGTs

50 µL of PGTs were taken and extracted twice with 100 µL of ethyl acetate. The extract was concentrated in a water bath at 50 °C for approximately 1 h until the final volume was 20 µL. The final extract was redissolved in 80 µL of ethyl acetate and made ready for GC-MS analysis (Gang et al., 2001). Volatile compounds in the extract were analyzed on a Thermo Scientific TRACE 1300 Gas Chromatograph connected to an ISQ Qd Mass Spectrometer and equipped with a TG-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm; Thermo Scientific). Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed at 60 °C for 2 min, then increased to 160 °C at 5 °C/min and hold for 2 min. It was then raised to 260 °C at 20 °C/min and maintained for 20 min. The temperature of the injector and the MS transfer line was set to 280 °C. The temperature of the ion source was set at 320 °C. Mass spectra were recorded by electron ionization at 70 eV. The scanning range was from 35 to 550 *m/z*. Identification of volatile compounds in the extract, which repeated three times, is based on comparison of their retention indexes with the search results of the Wiley 9 mass spectral library. Evaluation of mass spectrum match was carried out by considering the best match factor (SI, similarity index) or reverse match factor (RSI, reverse similarity index) score above 900 (Gujar et al., 2018; Sparkman et al., 2011).

2.4. Total RNA extraction and cDNA synthesis

The supernatant (GeneAll® Ribosaver™ RNA stabilization solution) in the tubes was removed by pipetting. 5 mL of phosphate buffered saline (PBS) solution prepared with RNase-free water was added to each tube containing PGTs, centrifuged at 12 000 rpm for 1 min, the supernatant was removed, and this process was repeated. Total RNA isolation from PGTs was performed according to the EURX® GeneMATRIX Universal RNA Purification Kit RNA isolation protocol from plant tissues. Quantification of RNAs isolated from PGTs were made with Biotium AccuLite™ Mini Fluorometer. The consumables used in the device show remarkably high specificity for the RNA strand. Therefore, it is unlikely that salts, solvents, free nucleotides, detergents, DNA, and protein contamination will affect the measurement results. RNA concentration measurements can be performed with a 2% smaller margin of error by this method. The isolated RNA samples capped and stored at –80 °C until to cDNA synthesis stage. EURX® NG dART RT kit with catalog number E0801–01 was used for cDNA synthesis. For synthesis reactions, 4 µL of 5X NG cDNA Buffer, 1 µL of 50 µM Oligo(dT)₂₀, 1 µL of NG dART RT mix and 14 µL of RNA sample were added to RNase-free PCR tubes of 0.2 mL volume. Reverse transcription reaction was started by heating it to 55 °C for 60 min and 85 °C for 5 min. The cDNA samples were stored at –20 °C until to Real-Time qPCR step.

2.5. Real Time qPCR analysis

Gene expression analyses were implemented through real-time qPCR to quantitatively measure the expression ratios of *PAL*, *4CL*, *EGS*, *EOMT* and *CVOMT* genes. The primer sequence of each gene was shown at Table 2. GeneAll RealAmp™ SYBR qPCR Master Mix with catalog number 801–051 (10 µL 2X MasterMix with SYBR-Green, 1 µL ROX Dye, 1 µL Forward Primer (10 µM), 1 µL Reverse Primer (10 µM), 4 µL cDNA Template, 3 µL RNase-Free Distilled Water) was used in the Real-Time qPCR stage. The Real-Time qPCR reaction was performed on the Applied Biosystems™ 7500 Fast Real-Time PCR instrument. The reactions began with a temperature profile of 5 min at 95 °C, followed by 40 cycles of 15 s at 95 °C for denaturation, 60 s at 55–68 °C for specific

Table 1

Some chemical and biological properties of vermicompost used in this study.

Analysis	Value
pH	7.00
Ec (dS/m)	0.05
Organic matter (%)	33–35
Total nitrogen (%)	1.5–1.75
C/N	9–11
Moisture	25–30
Heavy metal contamination	None
Pathogen microorganism	None

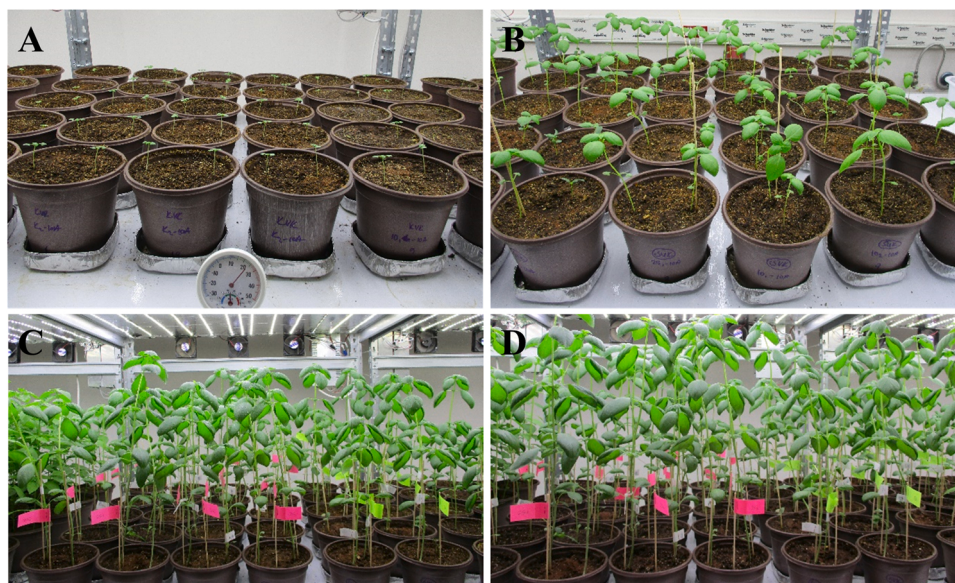


Fig. 2. Views of the growth stages of basil plants in the shelves of the automatic climate-controlled plant growth room. A: View of the basil plants at the first week. B: View of the basil plants at the fourth week. C: View of the basil plants at the eighth week. D: View of the basil plants at the tenth week.



Fig. 3. Collecting leaves (less than 2 cm) using forceps for PGT isolation.

annealing temperature of each primer. Melting curves were obtained at 65–95 °C and 2–5 s/step.

2.6. Data analysis

The results of the experiment were the mean of three times replicated analyses. Data analysis was performed using analysis of variance (ANOVA), and mean comparisons and correlation analyses were performed using Duncan's test and T-test (SPSS).

3. Results

3.1. Effects of vermicompost applications on the accumulation of phenylpropenes

GC-MS analysis was used to examine the effect of solid vermicompost (SV) and vermicompost tea (VT) on the accumulation of phenylpropenes in the essential oil content of isolated PGTs from basil leaves. An analysis of variance was conducted to assess the impact of vermicompost applications on the levels of chavicol, eugenol, methylchavicol, and methyleugenol phenylpropenes. Our findings indicated that addition of 10% SV to the soil, the accumulation of methyleugenol and methylchavicol in basil decreased by 49.7% and 51.5%, respectively. Application of 25% SV resulted in a 50.7% reduction in methylchavicol, and an even more

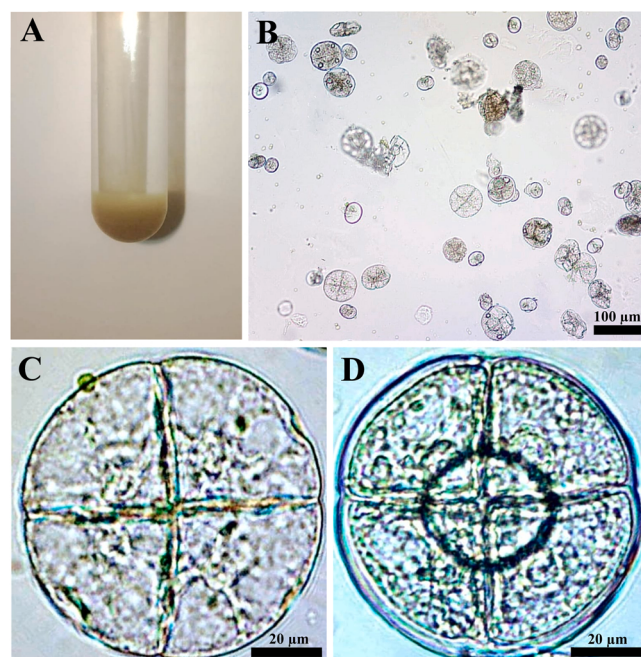


Fig. 4. Views of PGTs. A, view of isolated PGTs in tube; B, C, D, view of PGTs under light microscope.

substantial decrease of 75.8% was observed in the accumulation of methyleugenol ($P < 0.05$) (Fig. 5).

Applying 10% vermicompost tea (VT) to basil leaves had a substantial impact on the accumulation of chavicol and methyleugenol in basil essential oil, resulting in an increase of 242.6% and 613.2%, respectively, compared to control plants (Fig. 6). On the other hand, the application of 25% VT led to a significant increase in the accumulation of eugenol (664.5%) and methylchavicol (56.1%).

The ratios of some terpenes in the essential oil of methylchavicol chemotype basil, following vermicompost applications were shown in Table S1 (Supplementary material). The dose and form of vermicompost application to basil affected the ratio of terpenes in essential oil of PGTs, with the exception of linalool which was relatively low compared to

Table 2

Primers sequences utilized for this study.

Primers		Sequences	Reference
PAL	Forward	GGCTACTCCGGCATAAGATTC	(Ziaei et al., 2012)
	Reverse	GTACGAGCTTCGTCGAGGATG	
4CL	Forward	AAGGCTGCTTGGATTCTCG	(Khakdan et al., 2017)
	Reverse	TTTTCACCGTCGACTTGTCTG	
EGS	Forward	ATGGAGGAAAAAGGTCGAAAAGC	(Anand et al., 2016)
	Reverse	TTATGCTGTCTGAAGCAGGCGC	
EOMT	Forward	TGAGGCAGCAACGGATG	(Khakdan et al., 2017)
	Reverse	CCATCGTTCCATTACCCAC	
CVOMT	Forward	ACGCCACCCAGTTTGAGG	(Khakdan et al., 2017)
	Reverse	CCATTACCACCCCAACATC	
GAPDH	Forward	AACATTATCCCAGCAGCAC	(Kwon et al., 2019)
	Reverse	TAGGAACCTCGGAATGCCATC	

Quantification of mRNA expressions was normalized to the control group using the *GAPDH* transcript as a reference. “ $2^{-\Delta\Delta C_T}$ Method” was used in the calculation of relative quantification (Livak and Schmittgen, 2001).

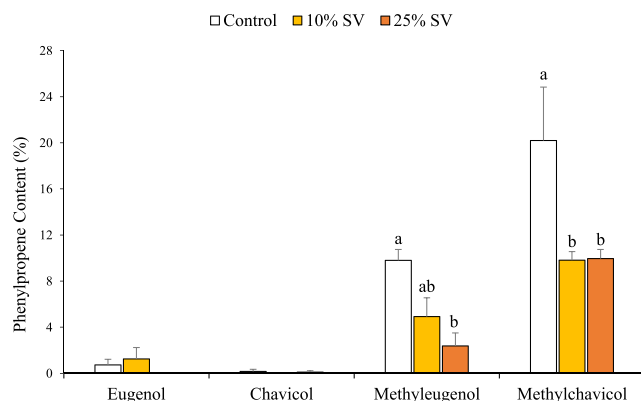


Fig. 5. The effects of solid vermicompost application on the content of chavicol, eugenol, methylchavicol (estragole) and methyleugenol. ‘10% SV’ and ‘25% SV’ refer to ratios of vermicompost added to pots. Pots in the control group did not have any vermicompost added. Different letters indicate significant differences at $P < 0.05$.

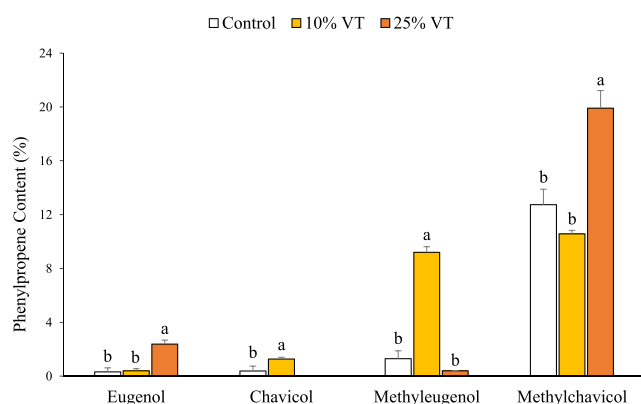


Fig. 6. The effects of vermicompost tea application on the content of chavicol, eugenol, methylchavicol (estragole) and methyleugenol. ‘10% VT’ and ‘25% VT’ refer to vermicompost tea applications by spraying 10% and 25% concentration of vermicompost tea to the leaves of basil plants, respectively. An equal amount of distilled water was sprayed onto the leaves of basil plants in the control group. Different letters indicate significant differences at $P < 0.05$.

content of essential oils extracted from dried leaves of basil in previous studies by Lawrence (1993), Telci et al. (2006), Giachino et al. (2014) and Varga et al. (2017).

3.2. Effects of vermicompost applications on genes expression level

Vermicompost addition as 10% and 25% SV applications to the root zone of basil plants caused no significant changes in the expression rates of *PAL* and *EGS* genes. In contrast, both the SV application doses resulted in a significant decrease in the expression of *4CL*, *EOMT*, and *CVOMT* genes, independently of dose increase ($P < 0.05$) (Fig. 7). Most notably, the expression levels of *EOMT* and *CVOMT* were reduced from 2.43 and 3.85, respectively, to below 0.1.

The results of the correlation analysis between the expression rates of *PAL*, *4CL*, *EGS*, *EOMT* and *CVOMT* genes and the abundance of phenylpropenes in the essential oil depending on SV applications were shown in Table S2 (Supplementary material). According to the analysis results, the strongest and most positive correlation was found between *CVOMT* gene expression and methylchavicol accumulation ($P < 0.01$). In addition, a positive correlation was found between the methyleugenol and methylchavicol accumulation and expression rate of the *4CL* gene, which is at the most important branching point in the phenylpropanoid biosynthesis pathway ($P < 0.05$). A positive correlation was also found between the accumulation of methyleugenol in PGTs and the expression of both the *EOMT* and *CVOMT* genes.

Fig. 8 reveals the effect of VT applications on genes expression level of methylchavicol chemotype basil. It was found that *4CL* expression decreased by 56%, while *EOMT* and *CVOMT* genes expressions increased 391-fold and 1155-fold, respectively, compared to the control group as a result of 10% VT application ($P < 0.05$).

However, significant decreases in the expressions of all genes were determined at PGTs in the 25% VT application group, except *EGS* ($P < 0.05$). The decrease in the expression of *EOMT* and *CVOMT* genes were approximately by 31-fold and 9-fold, respectively, compared to

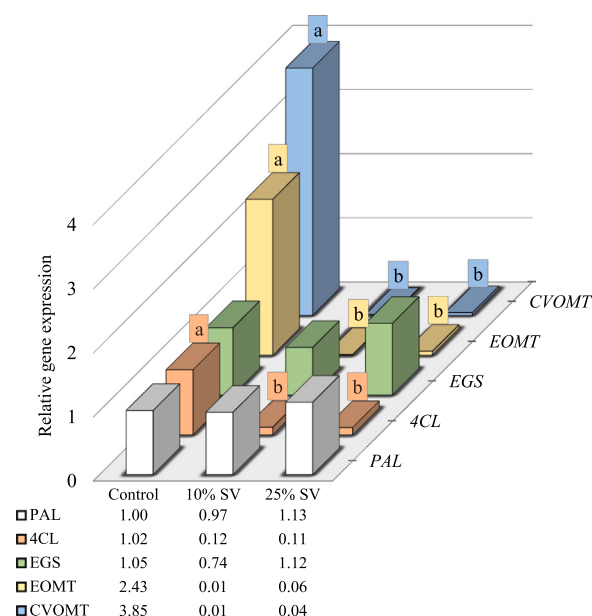


Fig. 7. The effects of solid vermicompost (SV) applications on the expressions of *PAL*, *4CL*, *EGS*, *EOMT* and *CVOMT* genes of methylchavicol chemotype basil.

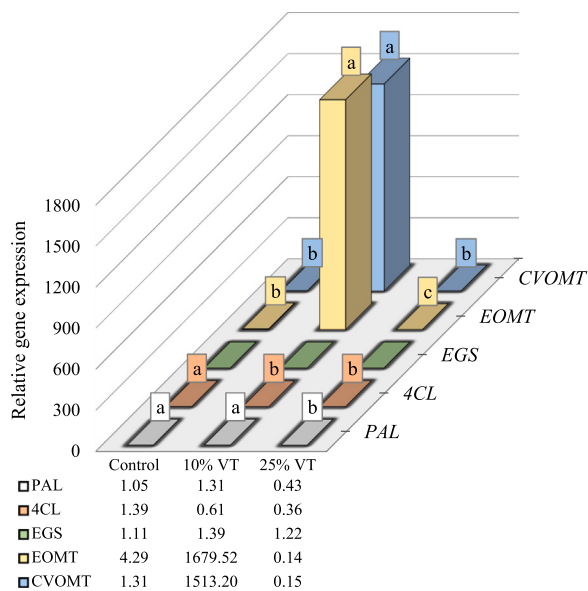


Fig. 8. The effects of vermicompost tea (VT) applications on the expressions of PAL, 4CL, EGS, EOMT and CVOMT genes of methylchavicol chemotype basil.

control plants. According to the results of the correlation analysis, positive correlations were found between the accumulation of chavicol and methyleugenol and the expression rates of *EOMT* and *CVOMT* genes in peltate glandular trichomes, following VT applications (Table S3, Supplementary material).

4. Discussion

It was determined that the phenylpropene content and biosynthesis in the PGTs were variable according to the results of this study which focuses on the effectiveness of moderate (10%) and higher (25%) doses of SV and VT applications. The application of 10% SV and 25% SV to the root region of methylchavicol chemotype basil had an effect on reducing the content of phenylpropene compounds in the PGTs isolated from the leaves. The methylchavicol content in both the 10% and 25% SV treatment groups was reduced by approximately 50% compared to the control group. Taie et al. (2010) reported a similar decrease in methylchavicol content of basil leaves following the application of compost and compost+biofertilizer to the root zone of basil grown in pots, which is consistent with the results of the current study. Verma et al. (2016) reported that methylchavicol in basil essential oil decreased in organically grown cultivar within their study. The reported decreases in methylchavicol content of basil essential oil as a result of organic fertilizer applications to soil within the studies mentioned above is consistent with our results.

Another notable result of this study was the gradual decrease in methyleugenol accumulation inversely with the increase in SV application dose. It was also determined that the expression of the *4CL* gene, which codes for an important enzyme at the branching point in phenylpropanoid metabolism, and the expressions of the *CVOMT* and *EOMT* genes, were significantly reduced by both 10% and 25% SV applications ($P < 0.05$).

The application of SV obviously seems reduced the expression of *EOMT* and *CVOMT* genes and the accumulation of the end products, both methyleugenol and methylchavicol. Therefore, the results of GC-MS and qRT-PCR analyses were found to be compatible with each other. The positive correlation between the accumulation of methylchavicol and methyleugenol phenylpropenes in the PGTs and the expression rates of *4CL*, *EOMT* and *CVOMT* genes following SV applications was also confirmed by the results of correlation analysis (Table S2, Supplementary material). Abdollahi Mandoulakani et al.

(2017), Khakdan et al. (2017) and Rezaie et al. (2020) observed a correlation, which is compatible with our results, between the expression rates of *EOMT* and *CVOMT* genes and the content of methylated phenylpropene in their studies on the impacts of drought stress, water-deficit stress, and cold stress, respectively.

Although there was no significant change in the methylchavicol ratio of the 10% VT application group in present study, an increase (approximately 6-fold) in the methyleugenol ratio was detected. It is hypothesized that the increase in methyleugenol ratio was due to the significant upregulation *EOMT* gene together with *CVOMT* genes, as previous research by Gang et al. (2002b) has shown that *EOMT* enzyme has a lower affinity for chavicol compared to its affinity for eugenol. However, it was determined that eugenol was accumulated approximately 6.5 times more than the control group as a result of 25% VT application in the results of this study. In other words, eugenol was not methylated by *EOMT* enzyme and the methyleugenol ratio inherently decreased by 70%. Therefore, methylchavicol chemotype basil increased the accumulation of methylchavicol which is the dominant phenylpropene yield of its regular metabolism in response to 25% VT application. Based on our research results, the most probable explanation for this phenomenon is that the effects of phenolics or hormones in the vermicompost dominate the effect of microbial load in vermicompost on the secondary metabolism of basil and regulates it in a way that increases the accumulation of methylchavicol instead of methyleugenol. Besides, possible post-transcriptional and post-translational regulation of these enzymes shouldn't be ignored, which is another remarkable determinant on the overall regulation of basil secondary metabolism as previously stated by Xie et al. (2008).

Microorganisms in the vermicompost have the potential to penetrate plant tissues as a result of VT applications to methylchavicol chemotype basil. Therefore, the increase in methylated phenylpropenes compared to the control group is thought to be due to the high microbial load of vermicompost. Pauli and Kubeczka (2010) reported that methylated phenylpropenes have higher antimicrobial activity and are synthesized more during biotic stress. In their study, Pauli and Kubeczka (2010) showed that the methylated forms of eugenol and chavicol phenylpropene compounds have much higher antimicrobial activities (methyleugenol > methylchavicol > eugenol > chavicol) compared to their unmethylated forms. Thus, it is possible that the increase in *EOMT* gene expression and methyleugenol ratio in the phenylpropene content of basil following 10% VT application was in response to the microorganisms in the vermicompost, in order to provide more effective defense. The determination of highest rate in methylchavicol accumulation following 25% VT application reveals that the response to biotic stress continues, although the expression rates of genes other than *EGS* are downregulated. According to Tavallali et al.'s (2019) findings, it appears that the use of Fe sources lacking microbial populations (such as Fe-arginine, Fe-glycine, Fe-histidine nanocomplexes, and Fe-EDDHA) for foliar application led to a reduction in the phenylpropene content of basil essential oil, aligning with the hypothesis regarding the increase of methylated phenylpropenes in PGTs following VT application in current study.

The acquisition of remarkably high expression rates in *EOMT* and *CVOMT* genes following 10% VT application within the current study was very coherent with the EST analysis results of previous researchers (Berim et al., 2012; Deschamps et al., 2006; Gang et al., 2002a; Gang et al., 2002b; Gang et al., 2002c; Gang et al., 2001; Iijima et al., 2004a; Xie et al., 2008).

Plants have the ability to produce a variety of chemical compounds known as secondary metabolites, which are not directly involved in their growth or development but may have beneficial effects on the plant or the environment. Some studies have suggested that plant biostimulants, which are substances that enhance plant growth and development, may influence the production of secondary metabolites by modifying the rates of synthesis, accumulation, and degradation of these compounds (du Jardin, 2015; Posmyk and Szafranska, 2016). For example, the

application of certain biostimulants may stimulate the synthesis and accumulation of phytoalexins, which are small molecules with antimicrobial properties that are produced by plants in response to stress. It is thought that plants are able to recognize specific molecules as signals, and the perception of these signals may trigger a series of reactions that ultimately lead to the production of secondary metabolites. It has been reported that the application of biostimulants to plants at various concentrations and their mechanism of action cause very different effects on the secondary metabolite content of medicinal and aromatic plants. These reactions may include the activation of kinases, the synthesis of reactive oxygen species, the movement of ions, and the acidification of the cytoplasm (Rafiee et al., 2016). G-proteins, a type of protein found in plants, have been found to be involved in various cellular processes such as growth, hormone signaling, and defense responses (Ferrero-Serrano and Chakravorty, 2023). Additionally, previous research has indicated that G-proteins may also play a role in the response of plants to biostimulants. There is evidence that the PLC/IP3- DAG/PKC pathway, a series of chemical reactions, occurs in plants and may contribute to the responses of plants to elicitors (Hong et al., 2016; Wang et al., 2022). It has been observed that treatment with elicitors can lead to an increase in levels of cAMP, a chemical messenger, in certain plant species (Jia et al., 2023; Zhang et al., 2022). This increase in cAMP may facilitate the production of phytoalexins, a class of compounds with antimicrobial properties. In eukaryotes, MAPK/ERK cascades are central to the transduction of external signals, such as those received by receptors or sensors, into intracellular reactions. These cascades, which consist of a series of chemical reactions, are activated in plants by a variety of stimuli including wounding, pathogen attack, salt stress, drought, temperature changes, reactive oxygen species and UV irradiation (Rafiee et al., 2016). Nevertheless, it's challenging to determine the specific processes triggered by biostimulants due to the intricate nature of the extracts used and the diverse array of molecules present in the solution, as noted by Bulgari et al. (2015).

On the other hand, research has indicated that plant growth-stimulating elements such as humic, fulvic, and other organic acids, auxin-like substances, and cytokinin-like substances are present in vermicompost and vermicompost tea (Edwards et al., 2010; Khan et al., 2014), as well as its microbial population. The complex nature of vermicompost limits this study's ability to conclusively identify the specific fragment of vermicompost responsible for the change in phenylpropene biosynthesis. Based on our results, the phenylpropene content in PGTs was higher following direct application of VT as a foliar spray on leaves, compared to the SV application groups. The microorganisms in VT can easily penetrate the leaves, whereas SV application targets the soil (root zone). Therefore, the main factor contributing to the change in phenylpropene biosynthesis found in this study can be attributed to the microorganisms in VT with their ability to easily penetrate the leaves. However, further research is required to identify the responsible fragment (or fragments having synergistic interactions) present in vermicompost that are effective on phenylpropanoid metabolism or secondary metabolism.

Our results contribute not only to the understanding of the potential of vermicompost on phenylpropene biosynthesis in basil PGTs, but also to the potential of PGTs as a site for the O-methylation process of phenylpropenes. Therefore, future research should focus on the re-evaluation of the effectiveness of various elicitors, biotic/abiotic stress factors, or organic/inorganic fertilizers on phenylpropanoid or secondary metabolism by isolating PGTs.

5. Conclusion

In this study, methylchavicol chemotype of *Ocimum basilicum* L. was grown in six conditions namely 0% SV, 10%SV, 25% SV addition to soil and 0% VT (distilled water), 10% VT and 25% VT application to leaves as foliar spray. The phenylpropene content and the expression rates of PAL, 4CL, EGS, EOMT and CVOMT genes were determined in the PGTs

isolated from basil leaves of all application groups. The results showed that both the SV applications caused a significant decrease in methylated phenylpropenes as well as in the O-methyltransferase genes (EOMT and CVOMT) expression. When 10% VT was applied to the leaves, there was a significant increase in chavicol and methyleugenol accumulation, as well as a towering increase in the expression of EOMT and CVOMT genes. However, applying 25% VT resulted in an increase in the ratio of eugenol to methylchavicol and a sharp decrease in the expression of EOMT and CVOMT genes. The decrease in phenylpropene biosynthesis in SV application groups can be deduced to the activation of primary metabolic activities instead of secondary metabolism. On the contrary, it can be inferred that the rise in expression of O-methyltransferase genes and the methyleugenol ratio in 10% VT application, as well as the methylchavicol ratio in 25% VT application, were attributed to the microbial burden of VT, which has the potential to infiltrate leaf tissues and trigger a defensive reaction. In the light of these results, it can be inferred that the method of organic fertilization in basil farming have a capital importance for its aroma and also for its antimicrobial activity, which is a very important feature in the field of phytoremediation practices. Moreover, the results of this study reveal the prominence of basil PGTs as research material in the field of phenylpropanoid metabolism or even more so in bioengineering research and applications.

CRedit authorship contribution statement

İlker Türkay: Conceptualization, Methodology, Validation, Investigation Writing – original draft. **Lokman Öztürk:** Resources, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2023.116688.

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