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Unveiling the role of vermicompost in modulating phenylpropanoid metabolism in basil (*Ocimum basilicum* L.): A single-cell type PGT approach

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ABSTRACT

This research delves into phenylpropanoid metabolism, focusing on phenylpropene biosynthesis in the methyleugenol chemotype of basil (*Ocimum basilicum* L.). We isolated peltate glandular trichomes (PGTs) from basil leaves to eliminate primary metabolic influences, offering a unique perspective into these complex processes. Vermicompost, chosen for its eco-friendly composition and superiority in invigorating phenylpropanoid metabolism. In this study, we investigated the impacts of solid and tea-form vermicompost applications at 0%, 10%, and 25% doses on the methyleugenol chemotype of basil, focusing on the expression levels of *PAL*, *4CL*, *EGS*, *EOMT*, and *CVOMT* genes and phenylpropene accumulation in the peltate glandular trichomes. Results showed that 10% solid vermicompost (SV) application increased *4CL* expression level at 236%, while 25% SV application further enhanced *EOMT* and *CVOMT* expressions to towering values by 7,494-fold and 19,643-fold, respectively. SV applications did not significantly impact eugenol accumulation but suppressed chavicol biosynthesis. Methyleugenol and methylchavicol accumulation rose in a dose-dependent manner, with significant increases observed in the 25% SV application. A positive correlation was found between *CVOMT* expression and accumulation rates of methyleugenol and methylchavicol phenylpropenes following SV applications. Conversely, vermicompost tea (VT) applications led to mixed gene expression patterns and reduced eugenol and methyleugenol ratios in peltate glandular trichomes compared to control. In summary, the notably high gene expressions observed in the results of our preliminary study offer a new perspective in the field of phenylpropanoid metabolism. This underscores the value of utilizing single-cell type PGTs for examining secondary metabolic pathways in plants and demonstrates the impact of vermicompost on phenylpropene production.

1. Introduction

Single-cell transcriptomics is a notable technique, providing insights into cellular biological processes. It's used to study cell interactions, pathogen responses, gene expression, regulatory systems, and drug effects. Recently, it's been influential in plant and animal research, and with improved tools, its application has become easier [1]. Plant cells have intricate and dense cell walls, making their isolation challenging and necessitating extra preparation and thorough analysis. Despite these challenges, single-cell RNA sequencing has been effectively applied to some model plant species [2]. The use of terms defined in the Plant Ontology (PO) and the BRENDA (Braunschweig Enzyme Database) Tissue Ontology (BTO) has facilitated the description of plant organs, tissues, and cell types, aiding in the analysis of single-cell RNA sequencing data [3]. Moreover, the limited number of proteome atlas publications in plants highlights the need for comprehensive analysis covering multiple cell types and anatomical structures, which is essential for understanding the complex gene expression profiles in plant cells [4].

Basil serves as an umbrella term for all members of the *Ocimum* genus within the *Lamiaceae* family and is renowned as one of the most widely used herbs worldwide. Of the 65-plus species in this genus [5], sweet basil (*Ocimum basilicum* L.) holds the most economic significance in Europe and the United States and stands out as an incredibly valuable

Abbreviations: PAL, Phenylalanine ammonia-lyase; *4CL*, 4-Coumarate-CoA ligase; *EGS*, Eugenol synthase; *EOMT*, Eugenol *O*-methyltransferase; *CVOMT*, Chavicol *O*-methyltransferase.

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herb with numerous applications in the food, fragrance, and pharmaceutical sectors [6,7]. Each year, substantial amounts of basil are sent to European countries in the form of dried leaves and essential oil containing valuable chemical compounds like eugenol, methyleugenol, methylchavicol, linalool, germacrene A and D, elemicin, ß-elemene, and (*Z*)-ocimene [8]. *Ocimum basilicum* has a rich history of medicinal use, but also uses in aromatherapy, perfumery, cosmetics, and the manufacture of dietary supplements [9]. In the realm of medicine, basil serves as a remedy for conditions such as coughing, headaches, parasitic infections, diarrhea, and kidney issues. Additionally, it has been a staple in culinary practices for centuries. Its significance is particularly evident in Mediterranean cuisine, where it is used extensively to add flavor and depth to a range of dishes [10].

The leaves of basil plants contain specialized glandular trichomes

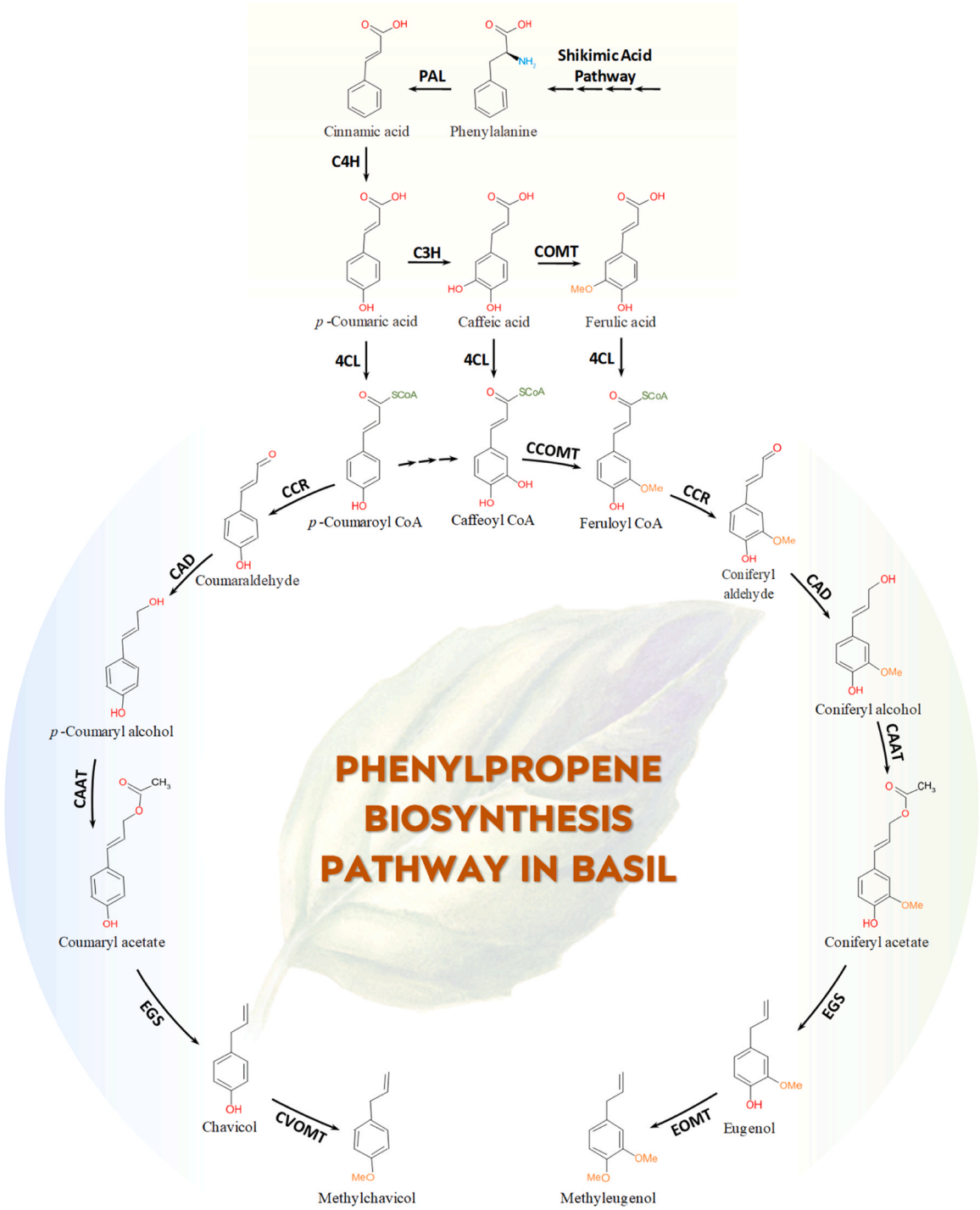


Fig. 1. The synthesis process of methyleugenol and methylchavicol via the phenylpropanoid pathway in *Ocimum basilicum*. Phenylalanine ammonia-lyase (PAL); Cinnamate 4-hydroxylase (C4H); 4-coumarate 3-hydroxylase (C3H); Caffeic acid *O*-methyltransferase (COMT); 4-Coumarate-CoA ligase (4CL); Cinnamoyl-CoA reductase (CCR); Cinnamoyl alcohol dehydrogenase (CAD); Coniferyl alcohol acethyltransferase (CAAT); Eugenol synthase (EGS); Chavicol *O*-metyltransferase (CVOMT); Shikimate *O*-metyltransferase (HCT); *p*-Coumaroyl 5-*O*-shikimate 3′-hydroxylase (CS3′H); Caffeoyl-CoA *O*-methyltransferase (CCOMT); and Eugenol *O*-methyltransferaz (EOMT). The double arrows point to the proposed intermediate reactions that are not explicitly shown in the diagram.

that produce a range of compounds to further defend the plant against herbivores and microorganisms. Two kinds of glandular trichomes are present in basil: capitate and peltate, with the previous rich in terpenoid compounds and the latter rich in phenylpropenes [11–14]. An examination of expressed sequence tags (ESTs) found in the peltate glandular trichomes of basil indicated that these structures display an exceptional upregulation of genes related to phenylpropanoid biosynthesis, which accounts for 13% of the total ESTs. Furthermore, 14% of all cDNAs encode the enzymes accountable for the formation of methylchavicol and methyleugenol [12]. This finding shed light on the significant role of these genes on phenylpropenes biosynthesis in basil peltate glands.

Phenylpropenes, which are generated as a result of a phenylpropanoid biosynthetic pathway branch, serve vital functions in drawing pollinators and safeguarding plants against both animals and microbes [15–17]. The economic significance of these compounds stems from their widespread use in industries such as pharmaceuticals, perfumery, and flavoring [18]. The biosynthesis of phenylpropanoids, encompassing phenylpropenes, comprises enzymes such as phenylalanine ammonia-lyase (PAL), 4-Coumarate-CoA ligase (4CL), eugenol synthase (EGS), eugenol *O*-methyltransferase (EOMT), and chavicol *O*-methyltransferase (CVOMT) (Fig. 1). PAL, in particular, is essential for phenylpropanoid synthesis, as it enables the creation of trans-Cinnamate through the deamination of phenylalanine. A significant juncture in this process involves the generation of 4-Coumaroyl--CoA from 4-Coumarate, which is mediated by the 4CL enzyme [19]. Later in phenylpropanoid metabolism, EGS catalyzes the formation of the initial phenylpropenes, chavicol from coumaryl acetate and eugenol from coniferyl acetate. In the concluding stages of phenylpropene biosynthesis, the enzymes EOMT and CVOMT modify the *para*-OH group of eugenol and chavicol to produce their methyl ether derivatives, methyleugenol and methylchavicol [11,13].

A significant number of plant-based phenolic compounds exhibit notable antioxidant properties, which can contribute to lowering the prevalence and death rates associated with cancer [20]. Research indicates that basil cultivated under organic farming conditions demonstrates a rise in phenolic compounds possessing such characteristics [21]. The content of essential oils in basil is influenced by several factors, including the growth and development stages of the plant tissues, geographical origin, and environmental conditions [22,23].

Plant biostimulants are a new type of agricultural product that have been proposed as a way to modify genetic pathways involved in the production of secondary metabolites, making them especially beneficial for medicinal plants [24,25]. With the goal of reducing inputs and transitioning to ecologically and economically sustainable agri-environmental systems, there is a growing interest in identifying organic molecules and microorganisms that can influence primary and secondary plant metabolism [26,27]. Biostimulants for plants encompass both substances and microorganisms that, when applied to plants, boost their ability to cope with abiotic stress, enhance nutrient absorption, and elevate the quality of end products, independent of their nutrient composition [28]. One type of biostimulant, known as vermicompost, falls under the humic substances category and is created by feeding decomposed organic waste to worms during the vermicomposting process [29]. Over time, numerous studies have explored the impact of vermicompost on plant growth and development, with specific attention given to its hormone-stimulated activity mechanisms. It is deemed that the hormone-like impact of humic substances in vermicompost can be ascribed to either the biomass employed during production, or the hormones generated throughout the composting of biomass [30–32]. The chemical complexity of humic acids (HAs) 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 [33]. Vermicompost also contains soluble nutrients, free enzymes, a wide range of microorganisms, and water-soluble phenols that can easily diffuse into the water during the vermicompost tea production [31,34].

Basil is an important crop in essential oil production and is frequently used as a research material in the field of bioengineering [35]. However, previous studies on the effects of biostimulants, organic fertilizers, elicitors, or stress applications on basil have focused on one or more parameters, such as phenological parameters, total phenolic content, essential oil, and gene expression profile of the complete –intact, non--abraded– leaf. Therefore, it is essential to determine the response capability of PGTs as a result of an application –preferably vermicompost, which was chosen for this study– for the generation of phytochemicals. Another distinct feature of this study is its aim to simultaneously investigate the effects of solid and aqueous vermicompost applications on phenylpropene biosynthesis in the isolated PGTs of methyleugenol chemotype of basil, whereas all the previous studies that have used the complete leaf as research material.

In this study, we aimed to measure the expression range of important genes in phenylpropene biosynthesis in basil and hypothesized that applying mild (10%) and elevated (25%) doses of both solid and vermicompost tea will have a significant impact on the phenylpropene biosynthesis in the PGTs of the methyleugenol chemotype of basil.

2. Materials and methods

2.1. Plant materials and cultivation conditions

The basil seeds used in this study were sourced from the Department of Field Crops at Isparta University of Applied Sciences' Faculty of Agriculture. These seeds were germinated in a temperature-controlled incubator set at 35°C, as mentioned by Zhou et al. [36]. Following germination, the seedlings were placed in one-liter pots filled with a combination of peat, soil, and vermiculite in a 2:1:1 ratio. The specifications of peat and soil were shown in Table 1 and Table 2, respectively.

SV was incorporated into the mixture at 10% and 25% concentrations relative to the total volume of peat and soil. Starting from the second week, when true leaves appeared on all basil plants, VT was administered weekly at either 10% or 25% dilutions. A 25% concentrated VT solution was produced by combining solid vermicompost and distilled water in a 1:4 (w/v) proportion the day before its scheduled use. The blend was aerated for a full day utilizing an aeration pump designed for aquariums and a shaker, as described by Edwards et al. [31]. Following aeration, the mixture was passed through a 40 μm pore filter five times. To create a 10% concentration, the 25% solution was diluted at a 1:2.5 ratio. Both 10% and 25% concentrations were administered to the plants' leaves weekly via spraying, with a small amount of Tween® 80 added. Care was taken to ensure complete coverage of the leaf surfaces during the application of the VT solutions. Precautions were taken to avoid the VT on the leaves from dripping onto the soil and reaching the plant roots.

In the control group, basil leaves were treated with distilled water containing Tween® 80, using volumes equal to those employed in the 10% and 25% VT groups. The vermicompost for this study was sourced from the Agricultural Research and Application Center at Kırşehir Ahi

Table 1
The analysis results of the soil used in this research.

Analysis	Value	Method
pH	7.15	1:1 (w/v) soil:d-water pH-meter
EC (μS cm^{-1})	103.13	1:1 (w/v) soil:d-water EC-meter
Sand (%)	60.58	Bouyoucos hydrometer
Silt (%)	26.99	Bouyoucos hydrometer
Clay (%)	12.43	Bouyoucos hydrometer
Texture Class	Sandy loam	Soil texture triangle
$CaCO_3$ (%)	8.32	Shiebler calsimeter
Organic matter (%)	0,78	Walkey-Black Method
Total N (%)	0.11	Kjeldahl Method
Available P (%)	7.56	Olsen Method
K, me 100 g^{-1}	0.34	Ammonium acetate extraction
Ca+Mg, me 100 g^{-1}	14.5	Ammonium acetate extraction

Table 2
The declared specifications of the peat (Klasmann® TS1) used in this research by its manufacturer.

Composition	Sphagnum peat moss
pH	6.0
EC (ms/m)	35
Clay	-
Water holding capacity (%)	80
Dry matter (%)	30
Organic matter (%)	90
Fertilizer (NPK 14 10 18 kg/m3)	0.8

Evran University. Table 3 displays the characteristics of the vermicompost, which was derived from barnyard manure.

In the Department of Soil Science and Plant Nutrition at Kırşehir Ahi Evran University's Faculty of Agriculture, an automated climate-controlled growth chamber was used to cultivate the plants. The chamber provided 16 hours of light, 50%-60% humidity, and maintained temperatures between 25 and 18°C (Fig. 2).

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

Ten weeks post-seed germination, the Glass Bead Abrasion technique (Fig. 3) was implemented for PGT isolation from basil foliage, as described by Gang et al. [12]. Following this approach, young leaves shorter than 2 cm were carefully removed using forceps, and 15 g of leaf samples were collected.

The gathered samples were placed in a 300 mL beaker on ice, and ice-cold distilled water was added to submerge the leaves for 30–60 minutes. Once the water was drained, the swollen leaves were moved to a 300 mL Bead Beater device chamber. Subsequently, 40–50 g of 0.5 mm glass beads and 250 mL of ice-cold glandular trichome isolation buffer (containing 50 mM Tris-HCl, 200 mM d-Sorbitol, 20 mM Sucrose, 14 mM β-mercaptoethanol, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM K_2PO_4, 5 mM Succinic acid, 1 mM EGTA, 0.6% (w/v) Methylcellulose, and 1% (w/v) Polyvinylpyrrolidone) were introduced to the chamber of the bead beater.

In the bead beater device, the mixing process was executed three times, each lasting one minute, with one-minute breaks in between, and the spinning speed was reduced to 4200 rpm using an external voltage regulator. Post-mixing, the entire blend in the chamber was filtered through a 350 μm pore filter to eliminate the leaves. To remove any remaining leaf residues, the filtered mixture was passed through a 105 μm pore filter. About 300 mL of ice-cold isolation buffer, which did not include methylcellulose and polyvinylpyrrolidone, was utilized to facilitate flow through the filters. Peltate trichomes with an average diameter of 80 μm were collected by settling on the surface of a 40 μm pore filter (Fig. 4).

2.3. Determination of volatile organic compound content of PGTs

A 50 μL sample of PGTs was extracted using 100 μL of ethyl acetate twice. This extract was then concentrated in a 50°C water bath for around an hour until the final volume reached 20 μL. The concentrated extract was dissolved in 80 μL of ethyl acetate in preparation for GC-MS analysis, following the method by Gang et al. [12]. The volatile compounds in the extract were examined using a Thermo Scientific TRACE 1300 Gas Chromatograph connected to an ISQ Qd Mass Spectrometer, which was fitted with a TG-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 μm; Thermo Scientific). Helium served as the carrier gas with a flow rate of 1 mL/min. The oven temperature was initially set at 60°C for 2 minutes, then raised to 160°C at 5 °C/min and held for 2 minutes, followed by an increase to 260°C at 20 °C/min, maintained for 20 minutes. The injector and MS transfer line temperatures were set at 280°C, while the ion source temperature was 320°C. Mass spectra were recorded using electron ionization at 70 eV, with a scanning range of 35–550 *m/z*. The identification of volatile compounds in the extract, which was performed in triplicate, relied on comparing their retention indexes to the Wiley 9 mass spectral library search results. Mass spectrum match evaluations took into account the best match factor (SI, similarity index) or reverse match factor (RSI, reverse similarity index) scores exceeding 900 [37,38].

Table 3
Various chemical and biological characteristics of vermicompost employed in the research.

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

2.4. Total RNA extraction and cDNA synthesis

The supernatant (GeneAll® RibosaverTM RNA stabilization solution) was removed from the tubes via pipetting. Each tube containing PGTs was then treated with 5 mL of phosphate-buffered saline (PBS) solution prepared with RNase-free water. The tubes were centrifuged at 12,000 rpm for 1 minute, and the supernatant was discarded. This process was repeated. Total RNA isolation from PGTs was conducted following the EURX® GeneMATRIX Universal RNA Purification Kit protocol for plant tissues. The Biotium AccuLite™ Mini Fluorometer was used to quantify the isolated RNAs from PGTs. The reagents used in the device exhibit high specificity for RNA strands, minimizing the likelihood of interference from salts, solvents, free nucleotides, detergents, DNA, and protein contamination. This method allows RNA concentration measurements with a margin of error as low as 2%. The isolated RNA samples were capped and stored at −80°C until the cDNA synthesis stage. The EURX® NG dART RT kit (catalog number E0801–01) was employed 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 the RNA sample were combined in 0.2 mL RNase-free PCR tubes. The reverse transcription reaction was initiated by heating the mixture to 55°C for 60 minutes, followed by 85°C for 5 minutes. The cDNA samples were preserved at −20°C until the Real-Time qPCR step.

2.5. Real time qPCR analysis

Gene expression analysis was carried out using real-time qPCR to quantitatively assess the expression ratios of *PAL*, *4CL*, *EGS*, *EOMT*, and *CVOMT* genes. The primer sequence for each gene is presented in Table 4. GeneAll RealAmpTM SYBR qPCR Master Mix (catalog number 801–051) was employed during the Real-Time qPCR stage, with the following components: 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, and 3 μL RNase-Free Distilled Water. The Real-Time qPCR reaction was conducted using the Applied Biosystems™ 7500 Fast Real-Time PCR instrument. The reaction process started with a temperature profile of 5 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C for denaturation, and 60 seconds at 55–68°C for the specific annealing temperature of each primer. Melting curves were generated at 65–95°C with 2–5 seconds per step.

mRNA expression quantification was normalized to the control group by employing *GAPDH* transcript as the reference. The "$2^{-\Delta\Delta Ct}$ Method" was utilized for determining relative quantification [39].

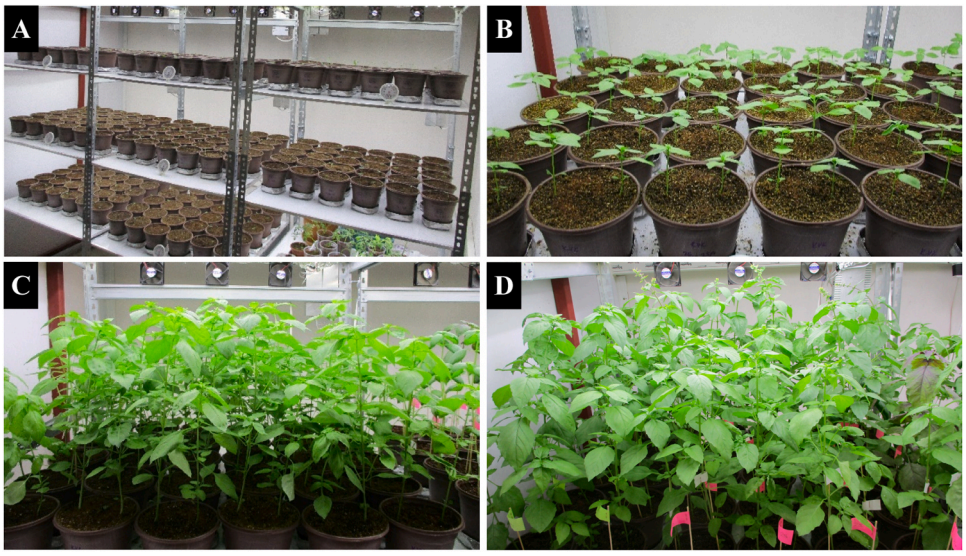


Fig. 2. The stages of basil plant development within the automated climate-controlled growth chamber's shelves. A: Basil plants during the first week. B: Basil plants during the fourth week. C: Basil plants during the eighth week. D: Basil plants during the tenth week.

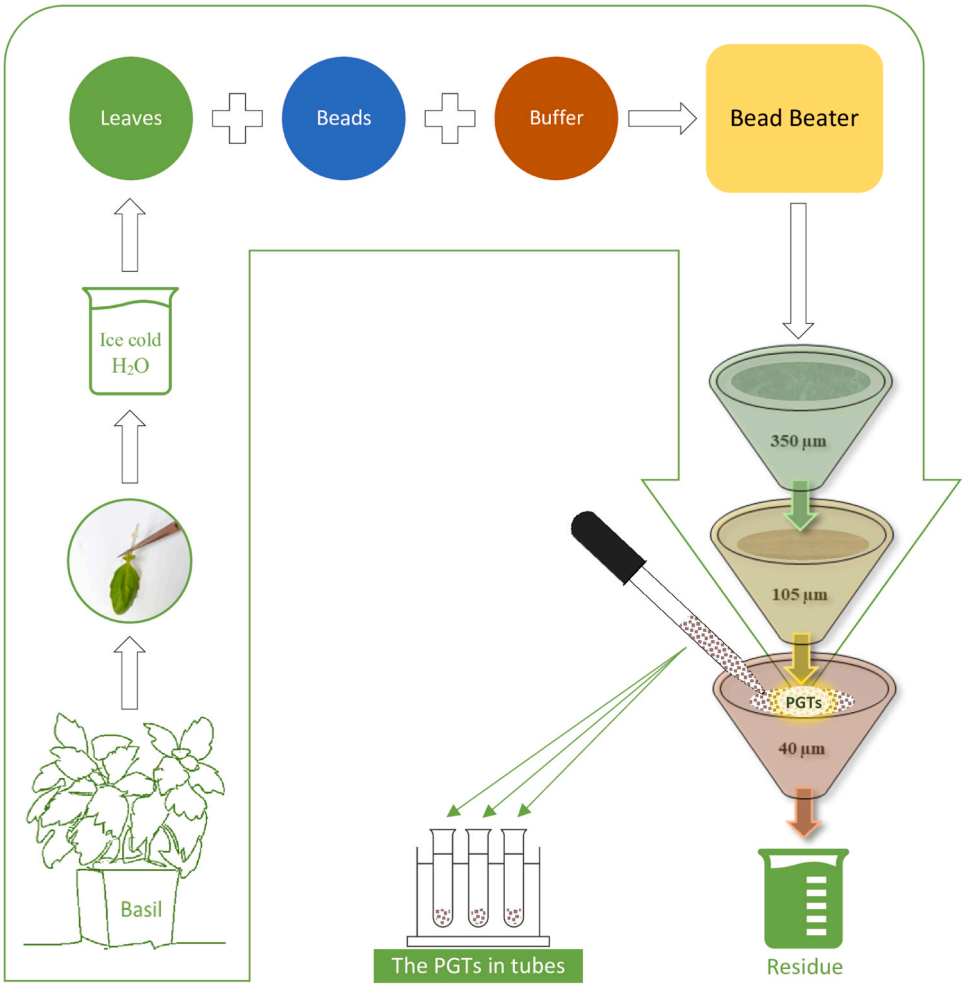


Fig. 3. The main stages of PGT isolation process from basil leaves.

2.6. Data analysis

The experimental outcomes are based on the average of three biological replicates. An analysis of variance (ANOVA) was conducted to assess the overall differences between groups. Post-hoc mean comparisons were performed using Duncan's test, and pairwise comparisons

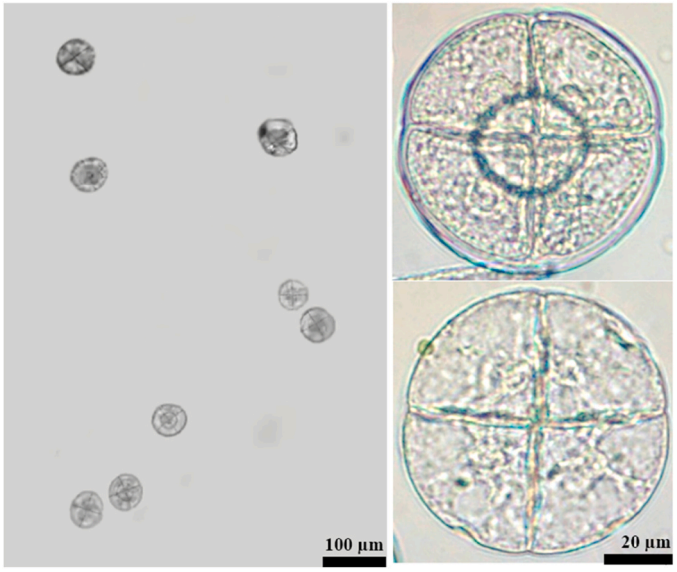


Fig. 4. Views of the PGTs, isolated by glass bead abrasion technique, under light microscope.

Table 4
Primer sequences employed in this research.

Primers		Sequences
GAPDH	Forward	AACATTATCCCCAGCAGCAC
	Reverse	TAGGAACTCGGAATGCCATC
PAL	Forward	GGCTACTCCGGCATAAGATTC
	Reverse	GTACGAGCTTCCGTCGAGGATG
4CL	Forward	AAGGCTGCTTGGATTTCTCG
	Reverse	TTTTCACCGTCGACTTGCTG
EGS	Forward	ATGGAGGAAAAAGGGTCGAAAAGC
	Reverse	TTATGCTGCTGAAGCAGGCGC
EOMT	Forward	TGAGGCAGCAAACGGATG
	Reverse	CCATCGTTCCATTACCACCAC
CVOMT	Forward	ACGCCACCCAGTTTGAGG
	Reverse	CCATTACCACCCCCAACATC

between specific groups were conducted using the T-test. Correlation analyses were also performed using the T-test. All statistical analyses were carried out using SPSS (version 15.0) [40].

3. Results

3.1. The effects of SV applications on the genes expression profile and the accumulation of phenylpropenes

The effects of vermicompost added at 0%, 10%, and 25% ratios to the root regions of methyl eugenol chemotype basil individuals on the expression levels of *PAL*, *4CL*, *EGS*, *EOMT*, and *CVOMT* genes in the peltate glandular trichomes of basil are shown in Fig. 5 and Fig. 6. It was found that applying 10% SV to methyleugenol chemotype basil resulted in approximately 2.5 times higher *4CL* expression, around 50% increase in *EOMT* expression, and approximately 77% increase in *CVOMT* expression compared to the SV control group. In the peltate glandular trichomes isolated from the leaves of basil in the 25% SV application group, it was observed that the *EGS* expression increased by 62%, while the expressions of *EOMT* and *CVOMT* genes encoding enzymes showing *O*-methyltransferase activity increased by 7,494 times and 19,643 times, respectively.

It has been determined that the application of different doses of SV (10% and 25%) to methyleugenol chemotypes does not have a significant effect on eugenol accumulation ($p < 0.05$) (Fig. 7). In contrast, it was observed that 10% and 25% SV applications suppress chavicol biosynthesis in peltate glandular trichomes isolated from basil leaves. It was found that the 10% SV application increased methylchavicol accumulation by up to 12%. SV applications have increased the accumulation of methyl eugenol and methyl chavicol in a dose-dependent manner. This increase is statistically significant in the 25% SV application ($p < 0.05$). According to the correlation analysis results, a positive correlation has been detected between the *CVOMT* expression and the accumulation rates of methyl eugenol and methyl chavicol phenylpropenes in peltate glandular trichomes as a result of increasing SV applications at higher doses (Table S1 in Supplementary material).

3.2. The effects of VT applications on the genes expression profile and the accumulation of phenylpropenes

As a result of the 25% VT application to methyl eugenol chemotype basil individuals, the expression of *PAL*, *4CL*, and *EOMT* genes increased, while the expression of *EGS* and *CVOMT* genes decreased (Fig. 8 and Fig. 9). In the 10% VT application group, the expression of the *PAL* gene increased, while the expression levels of the other genes decreased.

It was observed that VT applications to methyleugenol chemotype basil plants relatively reduced the amounts of eugenol and methyleugenol in peltate glandular trichomes (Fig. 10). Specifically, it was determined that the amount of methyleugenol in the 10% VT application group decreased by 53% compared to the VT control group. It was observed that VT applications to methylchavicol chemotype basil do not have a significant effect on the methylchavicol content.

According to the correlation analysis results, a negative correlation was identified between *PAL* expression and eugenol accumulation related to VT applications ($p < 0.01$). On the other hand, a positive correlation was determined between *EGS* expression and chavicol content, as well as between *CVOMT* expression and methylchavicol content ($p < 0.05$) (Table S2 in Supplementary material).

Fig. 11 demonstrates the percentage ratios of terpenes and phenylpropenes present in the essential oil derived from the PGTs of methyleugenol chemotype basil post-vermicompost.

4. Discussion

Applying 10% SV to basil plants heightened methylated phenylpropenes accumulation and the expression of *4CL*, *EOMT*, and *CVOMT* genes. Notably, *EOMT* and *CVOMT* expressions saw the highest increase in the 25% SV group (7,494-fold and 19,643-fold, respectively, $P < 0.05$). For methyleugenol chemotype basil, 10% and 25% SV didn't significantly alter eugenol ratio but reduced chavicol while increasing methylchavicol and methyleugenol significantly ($p < 0.05$). A positive correlation was observed between *CVOMT* expression and methylchavicol and methyleugenol content with SV applications (Table S1).

VT applications didn't significantly impact basil's methylchavicol content but led to decreased eugenol and methyleugenol ($p < 0.05$). The most significant change was a 50% reduction in methyleugenol in the 10% VT group, and an 80% increase in the 25% VT group compared to 10% VT group. Methyleugenol content changes mirrored *EOMT* expression fluctuations with VT applications: an 81% decrease in the 10% VT group and a 27-fold increase in the 25% VT group. Correlation analysis showed negative and positive correlations between various gene expressions and phenylpropene content, influenced by VT applications (Table S2).

Our study reaffirmed the significance of basil's peltate glandular trichomes (PGTs) for phenylpropanoid metabolism research, evidenced by high *EOMT* and *CVOMT* gene expression levels. This aligns with Gang et al. [41] and subsequent studies [13,16]which reported high EOMT and CVOMT enzyme activity in PGTs.

Applying solid vermicompost to the basil root zone may introduce vermicompost's microorganisms into plant tissues. Increased synthesis of antimicrobial methylated phenylpropenes in PGTs of basil leaves observed in this study, possibly in response to biotic stress from

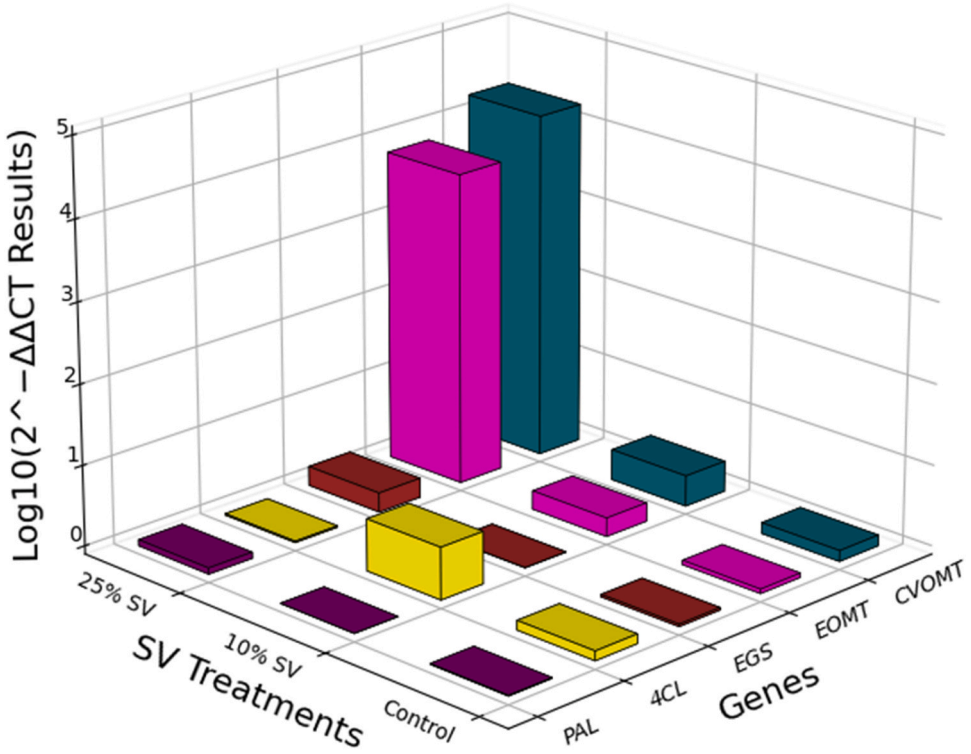


Fig. 5. The effects of SV applications on the expression of *PAL*, *4CL*, *EGS*, *EOMT*, and *CVOMT* genes in methyleugenol chemotype basil. The results of $2^{-\Delta\Delta CT}$ calculations were transformed to logarithmic values to better visualize them in one plot. The terms '10% SV' and '25% SV' denote the proportions of vermicompost incorporated into the pots. No vermicompost was added to the pots in the control group.

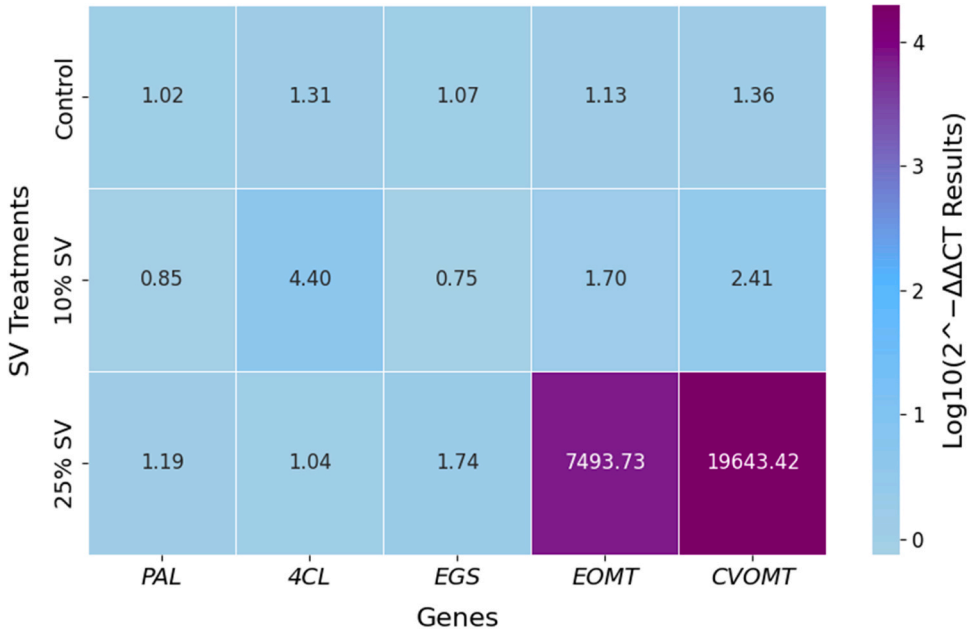


Fig. 6. The heatmap displays the relative expression values of the genes to preserve the original data integrity. However, for visual clarity and to effectively differentiate between varying levels of expression, the color intensity is determined by their log10-transformed values. Refer to the scale at the right of the heatmap for the color-coded log10 values, facilitating an intuitive interpretation of gene expression magnitude.

vermicompost microorganisms, validates Pauli and Kubeczka's [42] findings on their higher antimicrobial activity.

The phenylpropanoid pathway is a fundamental metabolic route responsible for the biosynthesis of a variety of plant secondary metabolites. This pathway is intricately regulated and highly responsive to environmental stimuli. One key enzyme in this pathway, 4CL, plays a pivotal role at a key branching point, directing substrates towards the synthesis of lignin, flavonoids, and other phenolic compounds [43–45]. The variability in 4CL expression under different SV application rates suggests a differential regulatory response aimed at optimizing the plant's metabolic output for growth and defense under varying nutrient and microbial load conditions. The increase in 4CL expression at the

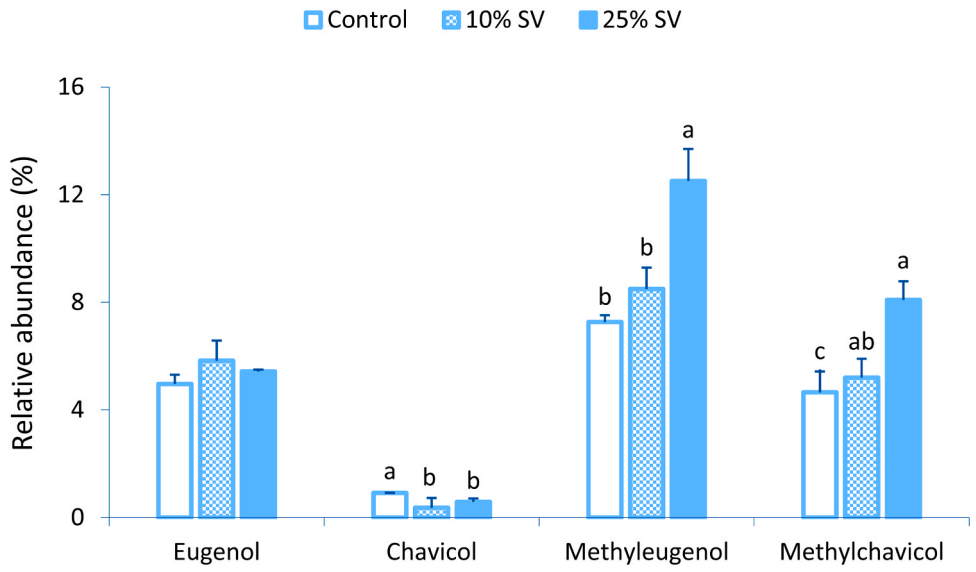


Fig. 7. The effects of SV applications on the phenylpropenes content of methyleugenol chemotype basil. Different letters indicate significant differences at $P < 0.05$.

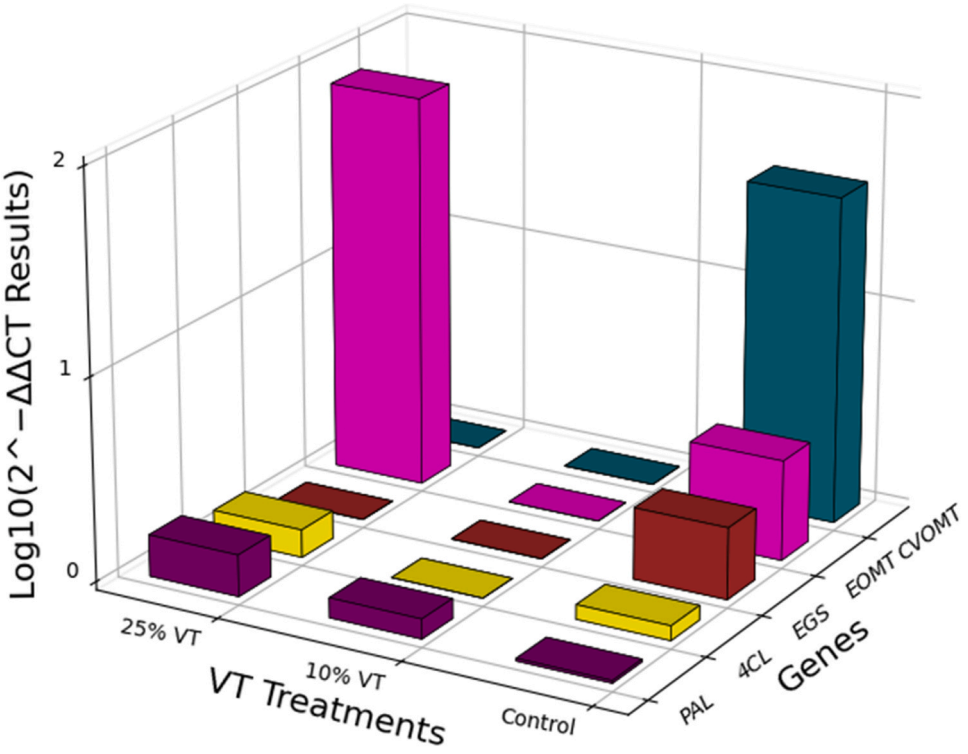


Fig. 8. The effects of SV applications on the expression of *PAL*, *4CL*, *EGS*, *EOMT*, and *CVOMT* genes in methyleugenol chemotype basil. The results of $2^{-\Delta\Delta CT}$ calculations were transformed to log10 values to better visualize them in one plot. The notations '10% VT' and '25% VT' correspond to the application of 10% and 25% vermicompost tea concentrations, respectively, by spraying onto the foliage of basil plants. In the control group, an equivalent volume of distilled water was applied to the basil leaves.

10% SV application group indicates an enhanced metabolic flux towards phenylpropanoid biosynthesis, potentially as a response to the nutrient availability or stress conditions mediated by the vermicompost application.

The observed decrease in chavicol content, despite a lesser increase in *CVOMT* expression at 25% SV, underscores the complexity of metabolic regulation within the phenylpropanoid pathway. This phenomenon can be attributed to the metabolic channeling effect, where increased 4CL activity may enhance the pool of CoA esters, diverting the pathway's flux towards other branches, such as lignin or flavonoid biosynthesis, rather than towards chavicol production. This redirection of metabolic flux is influenced by the availability of substrates, enzyme kinetics, and the spatial organization of enzymes within the cell, leading to differential accumulation of specific metabolites [46,47].

Similarly, the incomplete matching between the accumulation of chemicals and genetic changes observed in VT treatment highlights the dynamic nature of phenylpropanoid metabolism, where post-transcriptional and post-translational modifications, along with

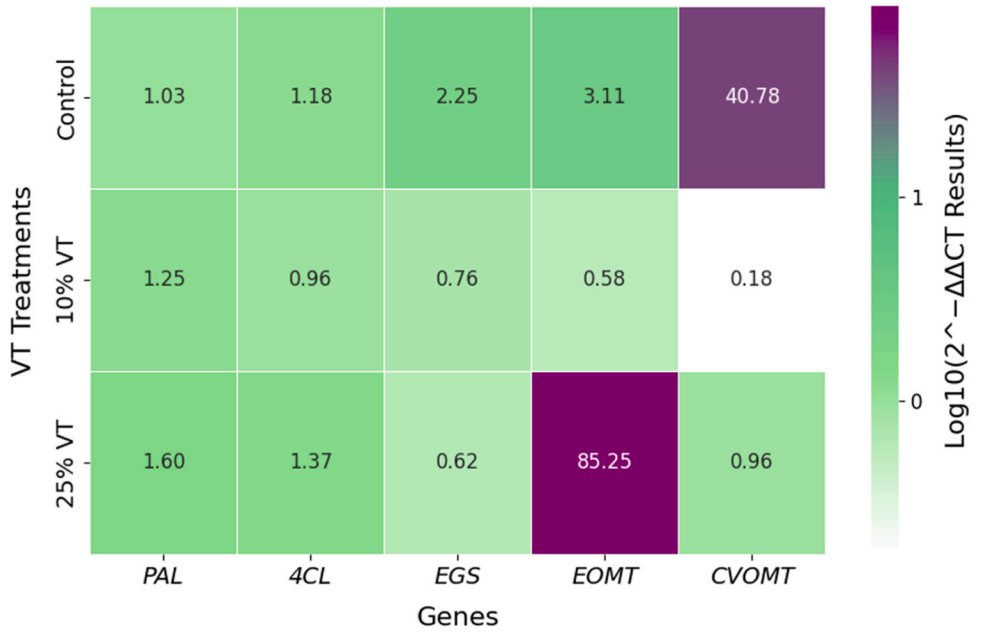


Fig. 9. The heatmap presents the relative expression values of the genes, with the aim of maintaining the authenticity of the expression data. The visualization of these expression levels, represented through color intensity, is based on log10-transformed values to accommodate a broad range of expression levels within a unified scale. The color-coded log10 scale provided on the right side of the heatmap aids in the clear interpretation of differential gene expression.

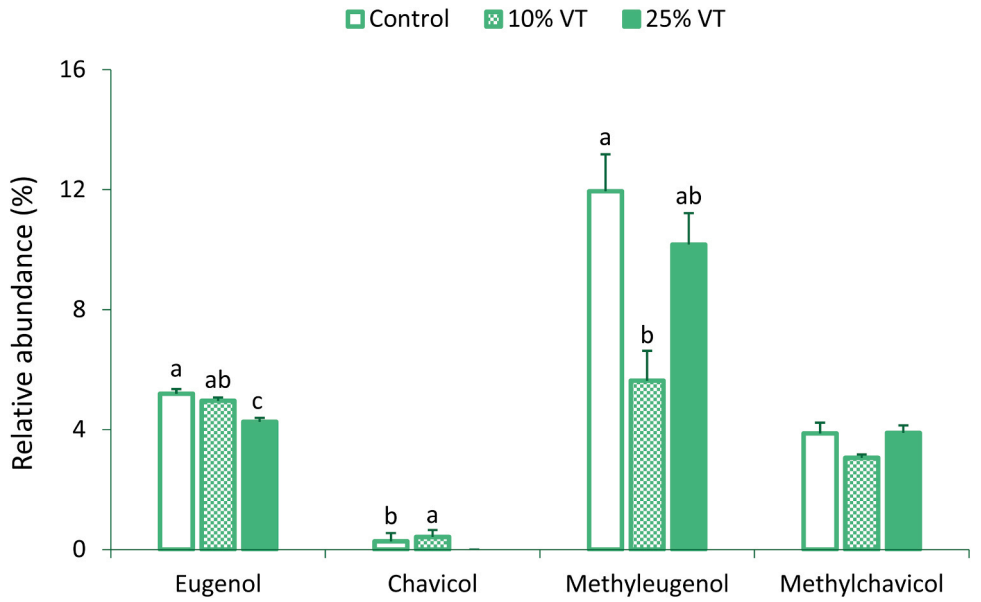
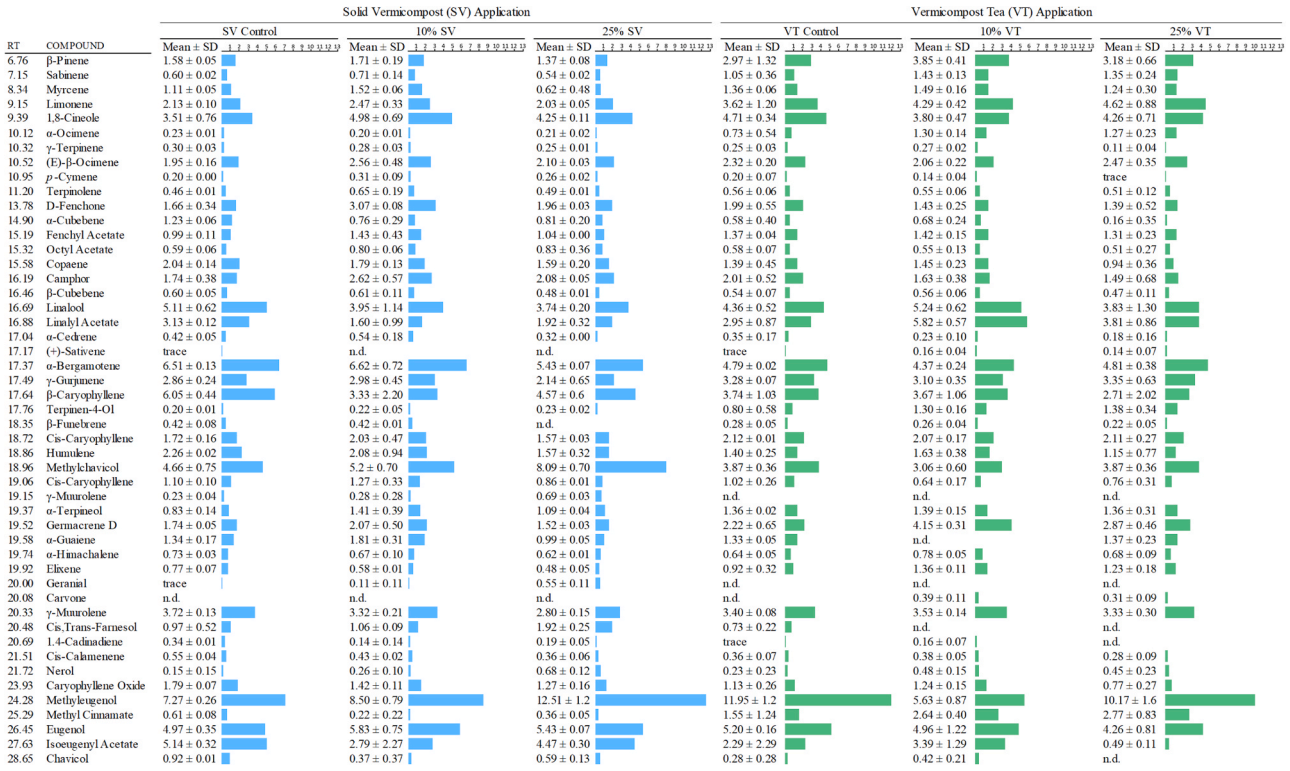


Fig. 10. The effects of VT applications on the phenylpropenes content ratios of methyleugenol chemotype basil. Different letters indicate significant differences at $P < 0.05$.

enzyme localization and substrate availability, play significant roles in determining the final metabolic profile. Environmental factors introduced through VT could modulate enzyme activities or affect the transport and compartmentalization of intermediates and end-products, leading to outcomes that are not directly predictable from gene expression levels alone.

The regulation of phenylpropanoid metabolism, particularly at branching points mediated by 4CL, involves a complex interplay of genetic, enzymatic, and environmental factors [48–50]. The discrepancies observed in metabolite accumulation versus gene expression rates under different vermicompost treatments underscore the need for a holistic approach in studying plant secondary metabolism, taking into account the entire metabolic network, regulatory mechanisms, and environmental influences to fully understand the biosynthesis and accumulation of specific phenolic compounds.

In the results of this study, notable differences were also observed in the composition of volatile oils, particularly methyleugenol, extracted from the peltate glandular trichomes (PGTs) of basil plants subjected to different treatment conditions. Specifically, the methyleugenol content exhibited significant variance between the SV control and VT control groups, with the latter showing a markedly higher ratio. The SV treatment involved the addition of solid vermicompost to the soil at varying concentrations, without any corresponding foliar application. In contrast, the VT control group received foliar applications of distilled water containing Tween® 80, intended to simulate the physical action of vermicompost tea spraying without introducing its biochemical



RT	COMPOUND	SV Control Mean ± SD	10% SV Mean ± SD	25% SV Mean ± SD	VT Control Mean ± SD	10% VT Mean ± SD	25% VT Mean ± SD
6.76	β-Pinene	1.58 ± 0.05	1.71 ± 0.19	1.37 ± 0.08	2.97 ± 1.32	3.85 ± 0.41	3.18 ± 0.66
7.15	Sabinene	0.60 ± 0.02	0.71 ± 0.14	0.54 ± 0.02	1.05 ± 0.36	1.43 ± 0.13	1.35 ± 0.24
8.34	Myrcene	1.11 ± 0.05	1.52 ± 0.06	0.62 ± 0.48	1.36 ± 0.06	1.49 ± 0.16	1.24 ± 0.30
9.15	Limonene	2.13 ± 0.10	2.47 ± 0.33	2.03 ± 0.05	3.62 ± 1.20	4.29 ± 0.42	4.62 ± 0.88
9.39	1,8-Cineole	3.51 ± 0.76	4.98 ± 0.69	4.25 ± 0.11	4.71 ± 0.34	3.80 ± 0.47	4.26 ± 0.71
10.12	α-Ocimene	0.23 ± 0.01	0.20 ± 0.01	0.21 ± 0.02	0.73 ± 0.54	1.30 ± 0.14	1.27 ± 0.23
10.32	γ-Terpinene	0.30 ± 0.03	0.28 ± 0.03	0.25 ± 0.01	0.25 ± 0.03	0.27 ± 0.02	0.11 ± 0.04
10.52	(E)-β-Ocimene	1.95 ± 0.16	2.56 ± 0.48	2.10 ± 0.03	2.32 ± 0.20	2.06 ± 0.22	2.47 ± 0.35
10.95	*p*-Cymene	0.20 ± 0.00	0.31 ± 0.09	0.26 ± 0.02	0.20 ± 0.07	0.14 ± 0.04	trace
11.20	Terpinolene	0.46 ± 0.01	0.65 ± 0.19	0.49 ± 0.01	0.56 ± 0.06	0.55 ± 0.06	0.51 ± 0.12
13.78	D-Fenchone	1.66 ± 0.34	3.07 ± 0.08	1.96 ± 0.03	1.99 ± 0.55	1.43 ± 0.25	1.39 ± 0.52
14.90	α-Cubebene	1.23 ± 0.06	0.76 ± 0.29	0.81 ± 0.20	0.58 ± 0.40	0.68 ± 0.24	0.16 ± 0.35
15.19	Fenchyl Acetate	0.99 ± 0.11	1.43 ± 0.43	1.04 ± 0.00	1.37 ± 0.04	1.42 ± 0.15	1.31 ± 0.23
15.32	Octyl Acetate	0.59 ± 0.06	0.80 ± 0.06	0.83 ± 0.36	0.58 ± 0.07	0.55 ± 0.13	0.51 ± 0.27
15.58	Copaene	2.04 ± 0.14	1.79 ± 0.13	1.59 ± 0.20	1.39 ± 0.45	1.45 ± 0.23	0.94 ± 0.36
16.19	Camphor	1.74 ± 0.38	2.62 ± 0.57	2.08 ± 0.05	2.01 ± 0.52	1.63 ± 0.38	1.49 ± 0.68
16.46	β-Cubebene	0.60 ± 0.05	0.61 ± 0.11	0.48 ± 0.01	0.54 ± 0.07	0.56 ± 0.06	0.47 ± 0.11
16.69	Linalool	5.11 ± 0.62	3.95 ± 1.14	3.74 ± 0.20	4.36 ± 0.52	5.24 ± 0.62	3.83 ± 1.30
16.88	Linalyl Acetate	3.13 ± 0.12	1.60 ± 0.99	1.92 ± 0.32	2.95 ± 0.87	5.82 ± 0.57	3.81 ± 0.86
17.04	α-Cedrene	0.42 ± 0.05	0.54 ± 0.18	0.32 ± 0.00	0.35 ± 0.17	0.23 ± 0.10	0.18 ± 0.16
17.17	(+)-Sativene	trace	n.d.	n.d.	trace	0.16 ± 0.04	0.14 ± 0.07
17.37	α-Bergamotene	6.51 ± 0.13	6.62 ± 0.72	5.43 ± 0.07	4.79 ± 0.02	4.37 ± 0.24	4.81 ± 0.38
17.49	γ-Gurjunene	2.86 ± 0.24	2.98 ± 0.45	2.14 ± 0.65	3.28 ± 0.07	3.10 ± 0.35	3.35 ± 0.63
17.64	β-Caryophyllene	6.05 ± 0.44	3.33 ± 2.20	4.57 ± 0.6	3.74 ± 1.03	3.67 ± 1.06	2.71 ± 2.02
17.76	Terpinen-4-Ol	0.20 ± 0.01	0.22 ± 0.05	0.23 ± 0.02	0.80 ± 0.58	1.30 ± 0.16	1.38 ± 0.34
18.35	β-Funebrene	0.42 ± 0.08	0.42 ± 0.01	n.d.	0.28 ± 0.05	0.26 ± 0.04	0.22 ± 0.05
18.72	Cis-Caryophyllene	1.72 ± 0.16	2.03 ± 0.47	1.57 ± 0.03	2.12 ± 0.01	2.07 ± 0.17	2.11 ± 0.27
18.86	Humulene	2.26 ± 0.02	2.08 ± 0.94	1.57 ± 0.32	1.40 ± 0.25	1.63 ± 0.38	1.15 ± 0.77
18.96	Methylchavicol	4.66 ± 0.75	5.2 ± 0.70	8.09 ± 0.70	3.87 ± 0.36	3.06 ± 0.60	3.87 ± 0.36
19.06	Cis-Caryophyllene	1.10 ± 0.10	1.27 ± 0.33	0.86 ± 0.01	1.02 ± 0.26	0.64 ± 0.17	0.76 ± 0.31
19.15	γ-Muurolene	0.23 ± 0.04	0.28 ± 0.28	0.69 ± 0.03	n.d.	n.d.	n.d.
19.37	α-Terpineol	0.83 ± 0.14	1.41 ± 0.39	1.09 ± 0.04	1.36 ± 0.02	1.39 ± 0.15	1.36 ± 0.31
19.52	Germacrene D	1.74 ± 0.05	2.07 ± 0.50	1.52 ± 0.03	2.22 ± 0.65	4.15 ± 0.31	2.87 ± 0.46
19.58	α-Guaiene	1.34 ± 0.17	1.81 ± 0.31	0.99 ± 0.05	1.33 ± 0.05	n.d.	1.37 ± 0.23
19.74	α-Himachalene	0.73 ± 0.03	0.67 ± 0.10	0.62 ± 0.01	0.64 ± 0.05	0.78 ± 0.05	0.68 ± 0.09
19.92	Elixene	0.77 ± 0.07	0.58 ± 0.01	0.48 ± 0.05	0.92 ± 0.32	1.36 ± 0.11	1.23 ± 0.18
20.00	Geranial	trace	0.11 ± 0.11	0.55 ± 0.11	n.d.	n.d.	n.d.
20.08	Carvone	n.d.	n.d.	n.d.	n.d.	0.39 ± 0.11	0.31 ± 0.09
20.33	γ-Muurolene	3.72 ± 0.13	3.32 ± 0.21	2.80 ± 0.15	3.40 ± 0.08	3.53 ± 0.14	3.33 ± 0.30
20.48	Cis,Trans-Farnesol	0.97 ± 0.52	1.06 ± 0.09	1.92 ± 0.25	0.73 ± 0.22	n.d.	n.d.
20.69	1.4-Cadinadiene	0.34 ± 0.01	0.14 ± 0.14	0.19 ± 0.05	trace	0.16 ± 0.07	n.d.
21.51	Cis-Calamenene	0.55 ± 0.04	0.43 ± 0.02	0.36 ± 0.06	0.36 ± 0.07	0.38 ± 0.05	0.28 ± 0.09
21.72	Nerol	0.15 ± 0.15	0.26 ± 0.10	0.68 ± 0.12	0.23 ± 0.23	0.48 ± 0.15	0.45 ± 0.23
23.93	Caryophyllene Oxide	1.79 ± 0.07	1.42 ± 0.11	1.27 ± 0.16	1.13 ± 0.26	1.24 ± 0.15	0.77 ± 0.27
24.28	Methyleugenol	7.27 ± 0.26	8.50 ± 0.79	12.51 ± 1.2	11.95 ± 1.2	5.63 ± 0.87	10.17 ± 1.6
25.29	Methyl Cinnamate	0.61 ± 0.08	0.22 ± 0.22	0.36 ± 0.05	1.55 ± 1.24	2.64 ± 0.40	2.77 ± 0.83
26.45	Eugenol	4.97 ± 0.35	5.83 ± 0.75	5.43 ± 0.07	5.20 ± 0.16	4.96 ± 1.22	4.26 ± 0.81
27.63	Isoeugenyl Acetate	5.14 ± 0.32	2.79 ± 2.27	4.47 ± 0.30	2.29 ± 2.29	3.39 ± 1.29	0.49 ± 0.11
28.65	Chavicol	0.92 ± 0.01	0.37 ± 0.37	0.59 ± 0.13	0.28 ± 0.28	0.42 ± 0.21	n.d.

Fig. 11. Percentage composition of terpenes and phenylpropenes in basil essential oil extracted from PGTs. (RT, Retention time; SD, Standard deviation; trace < 0.1%; n.d., not detected).

constituents. This methodological distinction between the SV and VT treatments is critical for understanding the differential responses observed in the secondary metabolite profiles of the treated plants.

Furthermore, the presence of Tween® 80 in the distilled water used for the VT control foliar applications may also play a role in modulating plant responses. Surfactants like Tween® 80 can affect membrane permeability and might influence the uptake or transport of molecules within the plant, potentially affecting metabolic processes [51–53]. This observation underscores the complexity of plant metabolic responses to environmental stimuli and highlights the importance of considering all aspects of experimental treatments in phytochemical research.

Biostimulants may affect secondary metabolites production by modulating their synthesis, accumulation, and degradation [24,29]. For instance, specific biostimulants may enhance phytoalexins production, plants' stress-induced antimicrobial molecules [54]. Biostimulants' different concentrations and mechanisms may impact secondary metabolites content in medicinal and aromatic plants through pathways involving kinases activation, reactive oxygen species production, ion movement, and cytoplasm acidification [25].

G-proteins, involved in various cellular processes, may also respond to biostimulants [55]. Evidence suggests PLC/IP3-DAG/PKC pathway involvement in plant responses to elicitors [56,57]. Elicitor treatments may increase cAMP levels, potentially aiding phytoalexins production [58,59]. Additionally, in eukaryotes, MAPK/ERK cascades crucial for converting external signals into intracellular reactions are activated in plants by stimuli such as wounding, pathogen attack, salt stress, and UV radiation [25]. However, identifying the specific processes activated by biostimulants is challenging due to the complex nature of the extracts used and the multitude of molecules present in the solution, as highlighted by Bulgari et al. [33] and Posmyk and Szafranska [24].

The EGS enzyme contributes to eugenol biosynthesis, with EGS transcripts found in basil roots [60]. Similarly, certain basil chemotypes have been found to contain eugenol, methyleugenol, and methylchavicol phenylpropenes in their roots [61]. These findings coincide with our observation of increased methylated phenylpropenes ratios in PGTs after vermicompost application, suggesting potential inter-organ signaling. Plants have evolved networks for inter-organ communication involving diverse signals triggered by environmental changes, transmitted through vascular tissues like the xylem and phloem [62]. Numerous chemical signals, including hormones, peptides, metabolites, and RNAs, facilitate this signaling [62–65]. Indications of alternative communication via ion movements, reactive oxygen species (ROS), and electric signals are also emerging [66]. Thus, increased methylated phenylpropenes biosynthesis in basil PGTs may be a defense response to vermicompost's microbial load.

SV and VT applications had contrasting effects on the methylated phenylpropenes ratios of different basil chemotypes' PGTs. SV applications increased methylated phenylpropenes in methyleugenol chemotype PGTs, while VT applications led to a decrease. Conversely, SV applications caused a decrease and VT applications resulted to an increase in methylchavicol chemotype PGTs according to the results of a previous research [34]. These varied responses underscore chemotype differences' role in shaping basil plants' treatment reactions. Further research is needed to understand these chemotype-specific responses. The differential response of *Ocimum basilicum* L. to various biotic and abiotic stressors, elicitors, and fertilizer applications observed across the cultivar, line, and chemotype spectrum is a testament to the intricate dynamics of plant-environment interactions and the potential for optimization within plant cultivation practices [8,22,67,68]. Such diversity in response can be attributed to the genetic variability embedded in the different cultivars, lines, and chemotypes, which results in differential gene expression under various stress conditions [69]. This, in turn, influences the plant's capacity to produce secondary metabolites, alter its physiological functions, and modify its growth and developmental patterns. Interestingly, the responses are not only stress-dependent but also highly cultivar, line, or chemotype-specific. For instance, a particular basil cultivar may exhibit enhanced response to a specific biofertilizer yet may demonstrate greater susceptibility to a particular abiotic

stressor such as drought or nutrient deficiency [10,70–72]. Furthermore, elicitors and fertilizers differentially affect the performance of various basil lines [73], thereby adding another layer of complexity. These findings highlight the importance of carefully selecting the cultivar, line, or chemotype of basil for cultivation, with considerations not only to the desired agronomic traits but also the environmental conditions and farming practices to maximize productivity and resilience.

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 [30,31,74,75], as well as its microbial population [76]. 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. Additional studies are needed to pinpoint the specific component (or potentially interacting components) within vermicompost that positively influence phenylpropanoid or secondary metabolic processes.

Plants possess the capability to generate a broad spectrum of chemical compounds, referred to as secondary metabolites. These compounds don't directly contribute to plant growth or development, but they may offer beneficial impacts on the plant or its surroundings. The metabolic regulations towards the biosynthesis of a wide variety of phenolic molecules ranging from lignin synthesis to flavonoids, stilbenoids, diarylheptanoids, and gingerols can occur by using intermediate products at many points in the phenylpropanoid biosynthesis pathway [77]. The vast branching and final product diversity in the phenylpropanoid biosynthesis pathway are attractive to many researchers but also pose many difficulties in discovering the dynamics of the pathway. To overcome this difficulty, studies on phenylpropanoid metabolism should be planned in a multidisciplinary manner. It is thought that future studies aimed at investigating the biosynthesis of phenylpropenes, which are the end products at one end of the pathway, should also include the biosynthesis of lignin, an important biomaterial gain indicator, and/or flavonoids synthesized in response to various stress factors.

5. Conclusion

This study demonstrated that solid vermicompost (SV) applications significantly enhance the accumulation of methylated phenylpropenes and the expression of key genes (*4CL*, *EOMT*, *CVOMT*) involved in the phenylpropanoid pathway in methyleugenol chemotype of basil. In contrast, vermicompost tea (VT) showed no significant effect on methylchavicol levels but led to a reduction in eugenol and methyleugenol content. These results highlight the chemotype-specific responses of basil to SV and VT, suggesting that SV may trigger a defense mechanism against microbial invasion by promoting the synthesis of compounds with higher antimicrobial activity.

The observed differential gene expression and volatile oil composition underline the importance of genetic factors in plant responses to environmental treatments, indicating the necessity for selective cultivation practices based on chemotype characteristics. Moreover, our findings underscore the potential of vermicompost as a biotic stress modulator, warranting further investigation into its components and their impact on plant secondary metabolism. Future research should adopt a multidisciplinary approach to elucidate the complex interactions within the phenylpropanoid biosynthesis pathway, facilitating optimized basil cultivation and expanding our understanding of plant secondary metabolism's role in stress response and adaptation. By clarifying the influence of vermicompost on basil's gene expression and volatile oil profiles, this study paves the way for improved agricultural practices and targeted phenylpropanoid research, contributing to the broader field of plant biochemistry and phenylpropanoid metabolism.

CRediT authorship contribution statement

İlker TÜRKAY: Conceptualization, Methodology, Software, Data curation, Writing- Original draft preparation, Visualization, Investigation. **Lokman Öztürk:** Supervision, Validation, Reviewing and Editing. **F.Ş.H. Türkay:** Methodology, Data curation, Validation

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.cpb.2024.100335.

References

[1] V.R. Netla, H. Shinde, G. Kumar, A. Dudhate, J.C. Hong, U.S. Kadam, A comparative analysis of single-cell transcriptomic technologies in plants and animals, Curr. Plant Biol. 35-36 (2023), https://doi.org/10.1016/j.cpb.2023.100289.

[2] I. Efroni, K.D. Birnbaum, The potential of single-cell profiling in plants, Genome Biol. 17 (2016) 1–8, https://doi.org/10.1186/s13059-016-0931-2.

[3] D. Szakonyi, S.V. Landeghem, K. Baerenfaller, L. Baeyens, J. Blomme, R. Casanova-Sáez, S.D. Bodt, D. Esteve-Bruna, F. Fiorani, N. González, J.T. Grønlund, R.G. H. Immink, S. Jover-Gil, A. Kuwabara, T. Muñoz-Nortes, A.D.Jv Dijk, D. Wilson-Sánchez, V. Buchanan-Wollaston, G.C. Angenent, Y.Vd Peer, D. Inzé, J.L. Micol, W. Gruissem, S. Walsh, P. Hilson, The knownleaf literature curation system captures knowledge about arabidopsis leaf growth and development and facilitates integrated data mining, Curr. Plant Biol. (2015), https://doi.org/10.1016/j.cpb.2014.12.002.

[4] J.W. Walley, S.P. Briggs, Dual use of peptide mass spectra: protein atlas and genome annotation, Curr. Plant Biol. (2015), https://doi.org/10.1016/j.cpb.2015.02.001.

[5] WFO. World Flora Online, Version 2022.12. 2022 [cited 2023 05.05]; Available from: ⟨https://wfoplantlist.org/plant-list/taxon/wfo-4000026511-2022-12?page=1⟩.

[6] J.E. Simon, J. Quinn, R.G. Murray, Basil: a source of essential oils, Adv. N. Crops (1990) 484–489.

[7] O.S. Dorr, S. Brezina, D. Rauhut, H. Mibus, Plant architecture and phytochemical composition of basil (Ocimum basilicum L.) under the influence of light from microwave plasma and high-pressure sodium lamps, J. Photochem. Photobio. B 202 (2020) 111678, https://doi.org/10.1016/j.jphotobiol.2019.111678.

[8] R.K. Lal, P. Gupta, C.S. Chanotiya, A. Mishra, A. Kumar, The nature and extent of heterosis, combining ability under the influence of character associations, and path analysis in basil (Ocimum basilicum L.), Ind. Crops Prod. 195 (2023), https://doi.org/10.1016/j.indcrop.2023.116421.

[9] L. Lenti, D. Rigano, S.L. Woo, A. Nartea, D. Pacetti, F. Maggi, D. Fiorini, A rapid procedure for the simultaneous determination of eugenol, linalool and fatty acid composition in basil leaves, Foods 11 (21) (2022) 3315, https://doi.org/10.3390/foods11213315.

[10] M. Burducea, V.D. Zheljazkov, I. Dincheva, A. Lobiuc, G.-C. Teliban, V. Stoleru, M.-M. Zamfirache, Fertilization modifies the essential oil and physiology of basil varieties, Ind. Crops Prod. 121 (2018) 282–293, https://doi.org/10.1016/j.indcrop.2018.05.021.

[11] S. Rastogi, S. Shah, R. Kumar, A. Kumar, A.K. Shasany, Comparative temporal metabolomics studies to investigate interspecies variation in three Ocimum species, Sci. Rep. 10 (1) (2020) 5234, https://doi.org/10.1038/s41598-020-61957-5.

[12] D.R. Gang, J. Wang, N. Dudareva, K.H. Nam, J.E. Simon, E. Lewinsohn, E. Pichersky, An investigation of the storage and biosynthesis of phenylpropenes in sweet basil, Plant Physiol. 125 (2) (2001) 539–555, https://doi.org/10.1104/pp.125.2.539.

[13] Z. Xie, J. Kapteyn, D.R. Gang, A systems biology investigation of the MEP/terpenoid and shikimate/phenylpropanoid pathways points to multiple levels of metabolic control in sweet basil glandular trichomes, Plant J. 54 (3) (2008) 349–361, https://doi.org/10.1111/j.1365-313X.2008.03429.x.
[14] V. Tavallali, M. Kiani, S. Hojati, Iron nano-complexes and iron chelate improve biological activities of sweet basil (Ocimum basilicum L.), Plant Physiol. Biochem 144 (2019) 445–454, https://doi.org/10.1016/j.plaphy.2019.10.021.
[15] T. Koeduka, The phenylpropene synthase pathway and its applications in the engineering of volatile phenylpropanoids in plants_review, Plant Biotechnol. 31 (5) (2014) 401–407, https://doi.org/10.5511/plantbiotechnology.14.0801a.
[16] Y. Iijima, R. Davidovich-Rikanati, E. Fridman, D.R. Gang, E. Bar, E. Lewinsohn, E. Pichersky, The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil, Plant Physiol. 136 (3) (2004) 3724–3736, https://doi.org/10.1104/pp.104.051318.
[17] B. Vanholme, I. El Houari, W. Boerjan, Bioactivity: phenylpropanoids' best kept secret (review), Curr. Opin. Biotechnol. 56 (2019) 156–162, https://doi.org/10.1016/j.copbio.2018.11.012.
[18] S.M. Brooks, C. Marsan, K.B. Reed, S.F. Yuan, D.D. Nguyen, A. Trivedi, G. Altin-Yavuzarslan, N. Ballinger, A. Nelson, H.S. Alper, A tripartite microbial co-culture system for de novo biosynthesis of diverse plant phenylpropanoids, Nat. Commun. 14 (1) (2023) 4448, https://doi.org/10.1038/s41467-023-40242-9.
[19] S. Rastogi, R. Kumar, C.S. Chanotiya, K. Shanker, M.M. Gupta, D.A. Nagegowda, A. K. Shasany, 4-coumarate: CoA ligase partitions metabolites for eugenol biosynthesis, Plant Cell Physiol. 54 (8) (2013) 1238–1252, https://doi.org/10.1093/pcp/pct073.
[20] K. Kumari, S. Kumar, Prashant, A.K. Jha, N. Kumar, Biotechnological intervention in genetic improvement and regulation of secondary metabolites production in Ocimum sanctum L, Ind. Crops Prod. 187 (2022), https://doi.org/10.1016/j.indcrop.2022.115329.
[21] H. Taie, D.Z. Salama, R. Samir, Potential activity of basil plants as a source of antioxidants and anticancer agents as affected by organic and bio-organic fertilization, Not. Bot. Horti Agrobot. Cluj. -Napoca 38 (2010) 119–127, https://doi.org/10.15835/nbha3813534.
[22] F. Varga, K. Carović-Stanko, M. Ristić, M. Grdiša, Z. Liber, Z. Šatović, Morphological and biochemical intraspecific characterization of Ocimum basilicum L, Ind. Crops Prod. 109 (2017) 611–618, https://doi.org/10.1016/j.indcrop.2017.09.018.
[23] E. Klimánková, K. Holadová, J. Hajšlová, T. Čajka, J. Poustka, M. Koudela, Aroma profiles of five basil (Ocimum basilicum L.) cultivars grown under conventional and organic conditions, Food Chem. 107 (1) (2008) 464–472, https://doi.org/10.1016/j.foodchem.2007.07.062.
[24] M.M. Posmyk, K. Szafranska, Biostimulators: a new trend towards solving an old problem, Front Plant Sci. 7 (2016) 748, https://doi.org/10.3389/fpls.2016.00748.
[25] H. Rafiee, H. Naghdi Badi, A. Mehrafarin, A. Qaderi, N. Zarinpanjeh, A. Sekara, E. Zand, Application of plant biostimulants as new approach to improve the biological responses of medicinal plants- a critical review, J. Med. Plants 3 (59) (2016) 6–39.
[26] S.G. Gandhi, V. Mahajan, Y.S. Bedi, Changing trends in biotechnology of secondary metabolism in medicinal and aromatic plants, Planta 241 (2) (2015) 303–317, https://doi.org/10.1007/s00425-014-2232-x.
[27] Y. Rouphael, P. Carillo, F. Cristofano, M. Cardarelli, G. Colla, Effects of vegetal-versus animal-derived protein hydrolysate on sweet basil morpho-physiological and metabolic traits, Sci. Hortic. 284 (2021) 110123, https://doi.org/10.1016/j.scienta.2021.110123.
[28] M. Ciriello, M.C. Kyriacou, S. De Pascale, Y. Rouphael, An appraisal of critical factors configuring the composition of basil in minerals, bioactive secondary metabolites, micronutrients and volatile aromatic compounds, J. Food Compos. Anal. (2022), https://doi.org/10.1016/j.jfca.2022.104582.
[29] P. du Jardin, Plant biostimulants: definition, concept, main categories and regulation, Sci. Hortic. 196 (2015) 3–14, https://doi.org/10.1016/j.scienta.2015.09.021.
[30] N.Q. Arancon, C.A. Edwards, S. Lee, R. Byrne, Effects of humic acids from vermicomposts on plant growth, Eur. J. Soil Biol. 42 (2006) S65–S69, https://doi.org/10.1016/j.ejsobi.2006.06.004.
[31] C.A. Edwards, N.Q. Arancon, R.L. Sherman. Vermiculture Technology: Earthworms, Organic Wastes, and Environmental Management, 1st Edition ed., CRC Press, Boca Raton, FL, 2010 https://doi.org/10.1201/b10453.
[32] B. Scaglia, R.R. Nunes, M.O.O. Rezende, F. Tambone, F. Adani, Investigating organic molecules responsible of auxin-like activity of humic acid fraction extracted from vermicompost, Sci. Total Environ. 562 (2016) 289–295, https://doi.org/10.1016/j.scitotenv.2016.03.212.
[33] R. Bulgari, G. Cocetta, A. Trivellini, P. Vernieri, A. Ferrante, Biostimulants and crop responses: a review, Biol. Agric. Hortic. 31 (1) (2015) 1–17, https://doi.org/10.1080/01448765.2014.964649.
[34] İ. Türkay, L. Öztürk, 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, Ind. Crops Prod. 198 (2023) 116688, https://doi.org/10.1016/j.indcrop.2023.116688.
[35] Shao, D.D. and M.Y. Tian, A qPCR approach to quantify the growth of basil downy mildew pathogen during infection. Current Plant Biology, 2018. 15: p. 2-7, https://doi.org/10.1016/j.cpb.2018.09.003.
[36] D. Zhou, J. Barney, M.A. Ponder, G.E. Welbaum, Germination response of six sweet basil (Ocimum basilicum) cultivars to temperature, Seed Technol. 37 (1) (2016) 43–51.
[37] I. Koo, S. Kim, X. Zhang, Comparative analysis of mass spectral matching-based compound identification in gas chromatography-mass spectrometry, J. Chromatogr. A 1298 (2013) 132–138, https://doi.org/10.1016/j.chroma.2013.05.021.
[38] O.D. Sparkman, Z. Penton, F.G. Kitson, Gas Chromatography and Mass Spectrometry, Academic press, 2011, https://doi.org/10.1016/c2009-0-17039-3.
[39] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method, methods 25 (4) (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.
[40] SPSS, S., for windows release 15.0, SPSS Inc. 2006, Chicago-USA.
[41] D.R. Gang, J. Simon, E. Lewinsohn, E. Pichersky, Peltate glandular trichomes ofocimum basilicuml. (Sweet Basil) contain high levels of enzymes involved in the biosynthesis of phenylpropenes, J. Herbs Spices Med. Plants 9 (2-3) (2002) 189–195, https://doi.org/10.1300/j044v09n02_27.
[42] A. Pauli, K.H. Kubeczka, Antimicrobial properties of volatile phenylpropanes, Nat. Prod. Commun. 5 (9) (2010) 1387–1394, https://doi.org/10.1177/1934578×1000500910.
[43] W.L. Dolan, B.P. Dilkes, J. Stout, N.D. Bonawitz, C. Chapple, Mediator complex subunits MED2, MED5, MED16, and MED23 genetically interact in the regulation of phenylpropanoid biosynthesis, Plant Cell (2017), https://doi.org/10.1105/tpc.17.00282.
[44] H. Tak, S. Negi, T.R. Ganapathi, Overexpression of MusaMYB31, a R2R3 Type MYB transcription factor gene indicate its role as a negative regulator of lignin biosynthesis in banana, Plos One (2017), https://doi.org/10.1371/journal.pone.0172695.
[45] V. Ayyappan, V.R. Sripathi, V. Kalavacharla, M.C. Saha, J. Thimmapuram, K. P. Bhide, E. Fiedler, Genome-wide identification of histone methylation (H3K9me2) and acetylation (H4K12ac) marks in two ecotypes of switchgrass (Panicum virgatum L.), BMC Genom. (2019), https://doi.org/10.1186/s12864-019-6038-x.
[46] T. Liu, R. Yao, Y. Zhao, S. Xu, C.H. Huang, J. Luo, L.Y. Kong, Cloning, Functional characterization and site-directed mutagenesis of 4-coumarate: coenzyme a ligase (4CL) involved in coumarin biosynthesis in peucedanum praeruptorum dunn, Front. Plant Sci. (2017), https://doi.org/10.3389/fpls.2017.00004.
[47] M. Alariqi, M. Ramadan, Q. Wang, Z. Yang, H. Xi, X. Nie, A.M.M. Ahmed, Q. Chen, W. Yan-yin, L. Zhu, X. Zhang, S. Jin, Cotton 4-coumarate-CoA Ligase 3 enhanced plant resistance to verticillium dahliae by, Plant J. (2023), https://doi.org/10.1111/tpj.16223 promoting jasmonic acid signaling-mediated vascular lignification and metabolic flux,.
[48] Y. Cao, S. Hu, S. Huang, P. Ren, X. Lü, Molecular cloning, expression pattern, and putative cis-acting elements of a 4-coumarate:coa ligase gene in bamboo (Neosinocalamus Affinis), Electron. J. Biotechnol. (2012), https://doi.org/10.2225/vol15-issue5-fulltext-10.
[49] C. Fritz, N. Palacios-Rojas, R. Feil, M. Stitt, Regulation of secondary metabolism by the carbon–nitrogen status in tobacco: nitrate inhibits large sectors of phenylpropanoid metabolism, Plant J. (2006), https://doi.org/10.1111/j.1365-313x.2006.02715.x.
[50] Y. Zhao, Q. Xi, Q. Xu, M. He, J. Ding, Y.-C. Dai, N.P. Keller, W. Zheng, Correlation of nitric oxide produced by an inducible nitric oxide synthase-like protein with enhanced expression of the phenylpropanoid pathway in inonotus obliquus cocultured with phellinus morii, Appl. Microbiol. Biotechnol. (2015), https://doi.org/10.1007/s00253-014-6367-2.
[51] Zhou, M., J. Wu, L.-X. Hu, W. Hu, J.-B. Huang, X.-L. Huang, X.-L. Gao, Y.-N. Luo, Z. Xue, and Y. Liu, Improvement of Vitamin K2 Production by Bacillus Subtilis Mutant With Leakage Fermentation. 2022, https://doi.org/10.21203/rs.3.rs-2351994/v1.
[52] J.M. Miller, A. Beig, B.J. Krieg, R. Carr, T.B. Borchardt, G.E. Amidon, G.L. Amidon, A. Dahan, The solubility–permeability interplay: mechanistic modeling and predictive application of the impact of micellar solubilization on intestinal permeation, Mol. Pharm. (2011), https://doi.org/10.1021/mp200181v.
[53] Y. Chen, F. Qiao, Y. Fan, Y. Han, Y. Wang, Interactions of phospholipid vesicles with cationic and anionic oligomeric surfactants, J. Phys. Chem. B (2017), https://doi.org/10.1021/acs.jpcb.7b05297.
[54] A. Kumar, J.P. Verma, Does plant-microbe interaction confer stress tolerance in plants: a review? Microbiol Res. 207 (2018) 41–52, https://doi.org/10.1016/j.micres.2017.11.004.
[55] A. Ferrero-Serrano, D. Chakravorty, Plants and heterotrimeric G proteins: expect the unexpected, Mol. Plant 16 (3) (2023) 506–508, https://doi.org/10.1016/j.molp.2022.12.017.
[56] Y. Hong, J. Zhao, L. Guo, S.C. Kim, X. Deng, G. Wang, G. Zhang, M. Li, X. Wang, Plant phospholipases D and C and their diverse functions in stress responses, Prog. Lipid Res. 62 (2016) 55–74, https://doi.org/10.1016/j.plipres.2016.01.002.
[57] J. Wang, M. Fan, S. Yin, X. Xu, B. Fu, R. Jiang, L. Sun, Ginseng oligosaccharides (GSO) inhibit C48/80-stimulated pseudoallergic mechanisms through the PLC/Ca2+/PKC/MAPK/c-Fos signaling pathway, J. Funct. Foods 96 (2022) 105211, https://doi.org/10.1016/j.jff.2022.105211.
[58] A. Jia, S. Huang, S. Ma, X. Chang, Z. Han, J. Chai, TIR-catalyzed nucleotide signaling molecules in plant defense, Curr. Opin. Plant Biol. 73 (2023) 102334, https://doi.org/10.1016/j.pbi.2022.102334.
[59] H. Zhang, J. Zhu, Z. Gong, J.-K. Zhu, Abiotic stress responses in plants, Nat. Rev. Genet. 23 (2) (2022) 104–119, https://doi.org/10.1038/s41576-021-00413-0.
[60] V.A. Reddy, C. Li, K. Nadimuthu, J.G. Tjhang, I.-C. Jang, S. Rajani, Sweet basil has distinct synthases for eugenol biosynthesis in glandular trichomes and roots with different regulatory mechanisms, Int. J. Mol. Sci. 22 (2) (2021) 681, https://doi.org/10.3390/ijms22020681.
[61] A. Anand, R.H. Jayaramaiah, S.D. Beedkar, P.A. Singh, R.S. Joshi, F.A. Mulani, B. B. Dholakia, S.A. Punekar, W.N. Gade, H.V. Thulasiram, A.P. Giri, Comparative

functional characterization of eugenol synthase from four different Ocimum species: implications on eugenol accumulation, Biochim Biophys. Acta 1864 (11) (2016) 1539–1547, https://doi.org/10.1016/j.bbapap.2016.08.004.
[62] T. Kuromori, M. Fujita, F. Takahashi, K. Yamaguchi-Shinozaki, K. Shinozaki, Inter-tissue and inter-organ signaling in drought stress response and phenotyping of drought tolerance, Plant J. 109 (2) (2022) 342–358, https://doi.org/10.1111/tpj.15619.
[63] F. Takahashi, K. Hanada, T. Kondo, K. Shinozaki, Hormone-like peptides and small coding genes in plant stress signaling and development, Curr. Opin. Plant Biol. 51 (2019) 88–95, https://doi.org/10.1016/j.pbi.2019.05.011.
[64] H.R. Thomas, M.H. Frank, Connecting the pieces: uncovering the molecular basis for long-distance communication through plant grafting, N. Phytol. 223 (2) (2019) 582–589, https://doi.org/10.1111/nph.15772.
[65] D. Tsikou, Z. Yan, D.B. Holt, N.B. Abel, D.E. Reid, L.H. Madsen, H. Bhasin, M. Sexauer, J. Stougaard, K. Markmann, Systemic control of legume susceptibility to rhizobial infection by a mobile microRNA, Science 362 (6411) (2018) 233–236, https://doi.org/10.1126/science.aat6907.
[66] S. Johns, T. Hagihara, M. Toyota, S. Gilroy, The fast and the furious: rapid long-range signaling in plants, Plant Physiol. 185 (3) (2021) 694–706, https://doi.org/10.1093/plphys/kiaa098.
[67] S. Ćavar Zeljković, K. Komzáková, J. Šišková, E. Karalija, K. Smékalová, P. Tarkowski, Phytochemical variability of selected basil genotypes, Ind. Crops Prod. 157 (2020), https://doi.org/10.1016/j.indcrop.2020.112910.
[68] R. Maurya, P. Gupta, C.S. Chanotiya, S.S. Dhawan, S. Srivastava, A. Yadav, A. Kumar, Y. Swamy, R.K. Lal, Investigation of monoterpenoids rich essential oils of two Ocimum basilicum L. varieties at different agro-climatic conditions in India, Acta Ecol. Sin. (2020), https://doi.org/10.1016/j.chnaes.2020.11.002.
[69] F. Khakdan, J. Nasiri, M. Ranjbar, H. Alizadeh, Water deficit stress fluctuates expression profiles of 4Cl, C3H, COMT, CVOMT and EOMT genes involved in the biosynthetic pathway of volatile phenylpropanoids alongside accumulation of methylchavicol and methyleugenol in different Iranian cultivars of basil, J. Plant Physiol. 218 (2017) 74–83, https://doi.org/10.1016/j.jplph.2017.07.012.
[70] K.A. Khalid, M.A. Mohamed, M.E. Ibrahim, H.E. Wahba, Yield and essential oil of sweet basil affected by chemical and biological fertilizers, Res. J. Med. Plants 10 (3) (2016) 246–253, https://doi.org/10.3923/rjmp.2016.246.253.
[71] H. Kosem, M.Z. Kocak, M.G. Kaysim, F. Celikcan, M. Kulak, Liquid Leachate produced from vermicompost effects on some agronomic attributes and secondary metabolites of sweet basil (ocimum basilicum l.) exposed to severe water stress conditions, Horticulturae 8 (12) (2022) 1190, https://doi.org/10.3390/horticulturae8121190.
[72] F. Celikcan, M.Z. Kocak, M. Kulak, Vermicompost applications on growth, nutrition uptake and secondary metabolites of Ocimum basilicum L. under water stress: a comprehensive analysis, Ind. Crops Prod. 171 (2021), https://doi.org/10.1016/j.indcrop.2021.113973.
[73] T.P. Gurav, B.B. Dholakia, A.P. Giri, A glance at the chemodiversity of Ocimum species: trends, implications, and strategies for the quality and yield improvement of essential oil, Phytochem. Rev. 21 (3) (2022) 879–913, https://doi.org/10.1007/s11101-021-09767-z.
[74] R. Kızılkaya, F.Ş.H. Türkay, Vermicomposting of anaerobically digested sewage sludge with hazelnut husk and cow manure by earthworm eisenia foetida, Compost Sci. Util. 22 (2) (2014) 68–82, https://doi.org/10.1080/1065657X.2014.895454.
[75] M.H. Khan, M.K. Meghvansi, R. Gupta, V. Veer, L. Singh, M.C. Kalita, Foliar spray with vermiwash modifies the Arbuscular mycorrhizal dependency and nutrient stoichiometry of Bhut Jolokia (Capsicum assamicum), PLoS One 9 (3) (2014) e92318, https://doi.org/10.1371/journal.pone.0092318.
[76] L.F. Santos, F.L. Olivares, Plant microbiome structure and benefits for sustainable agriculture, Curr. Plant Biol. 26 (2021), https://doi.org/10.1016/j.cpb.2021.100198.
[77] D. Tsimogiannis, V. Oreopoulou, Classification of Phenolic Compounds in Plants, in: R.R. Watson (Ed.), in Polyphenols in Plants, Academic Press, 2019, pp. 263–284, https://doi.org/10.1016/B978-0-12-813768-0.00026-8.