

## Article

# Comprehensive Analysis of Volatile Organic Compounds and Their Impact on Apple Quality Following Some Essential Oil Treatments Against *Botrytis cinerea*

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**Abstract:** The susceptibility of apples to post-harvest decay by the fungus *Botrytis cinerea* has prompted innovative research into alternative preservation methods. In this regard, essential oils (EOs) have emerged as promising candidates due to their natural origin and potential antimicrobial properties. Investigating the biological significance of EO blends is crucial for understanding their potential antimicrobial mechanisms and evaluating their capacity to modulate metabolic responses that could inhibit post-harvest fungal decay in fruit tissues. This study delves into the intricate chemistry of apples when subjected to various EO treatments, shedding light on the profound changes in alcohols, esters, C6 compounds, terpenes, and volatile compounds. Based on our results, terpene concentrations exhibit significant variations with  $\alpha$ -Pinene ranging from  $13.4 \mu\text{g L}^{-1}$  in Fungus + Thymol + 1,8-Cineole treatment (Fun+Thy+Cin) to  $28.7 \mu\text{g L}^{-1}$  in Fungus + Thymol + 1,8-Cineole + Eugenol treatment (Fun+Thy+Cin+Eug), and  $\beta$ -Pinene concentrations spanning  $19.3 \mu\text{g L}^{-1}$  in Fungus + 1,8-Cineole + Eugenol treatment (Fun+Cin+Eug) to  $45.5 \mu\text{g L}^{-1}$  in Fungus + Thymol + 1,8-Cineole + Eugenol treatment (Fun+Thy+Cin+Eug). Ester elaboration presents marked changes, with ethyl octanoate peaking at  $715.7 \mu\text{g L}^{-1}$  in Fungus + Thymol + 1,8-Cineole + Eugenol treatment (Fun+Thy+Cin+Eug) and ethyl propionate reaching  $152.9 \mu\text{g L}^{-1}$  in Fungus + Thymol treatment (Fun+Thy). The volatile compound dynamics also demonstrate significant variations, with hexanoic acid concentrations ranging from 0.1 to 0.2 among treatments and 3-Methylbutanal displaying concentrations from 0.8 to 6.4, with the highest concentration observed in the Control. The essential oil combination of Thymol, Eugenol, and 1,8-Cineol (Fun+Thy+Cin+Eug) had the most significant impact on the volatile compound content in the fruits. The findings from this study unveil the intricate responses of apple chemistry to various EO treatments. These insights hold promise for enhancing post-harvest apple preservation strategies through the modulation of EO treatments.

**Keywords:** gray mold; biocontrol; Thymol; Eugenol; 1,8-Cineol; volatile organic compounds

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## 1. Introduction

Considering that apples, known scientifically as *Malus x domestica*, hold a significant place in the agricultural and culinary landscape worldwide, they are one of the most widely grown and consumed fruits globally, making them an indispensable part of our culture and diet [1]. Their rich history and widespread popularity have solidified the status of Golden Delicious apples as a cherished fruit [2]. They are cherished not only for their delicious taste but also for their remarkable versatility. From fresh consumption to being processed into juices, pies, jams, and even alcoholic beverages, apples have become a cornerstone of the global fruit industry [3]. Their nutritional value, high fiber content, and numerous health benefits further underscore

their importance in maintaining a balanced diet [2]. Among the vast array of apple varieties, the Golden Delicious apple cultivar stands out as one of the most iconic and beloved cultivar. Renowned for its delightful mellow flavor and distinctive yellow skin, Golden Delicious has won the hearts and palates of apple enthusiasts worldwide. This cultivar is a mainstay in the fruit industry, cherished for its crisp texture and harmonious, honey-like taste, making it a versatile choice for adding to salads or snacking and baking [4]. Nevertheless, like numerous apple cultivars, the Golden Delicious is susceptible to the menace of gray mold disease caused by the fungal pathogen *Botrytis cinerea*, which presents a substantial challenge to both the Golden Delicious cultivar and the apple industry [5]. Gray mold disease can render this delectable apple variety unsuitable for consumption owing to its characteristic symptoms of fuzzy gray mold soft rot, growth, and foul odor. Therefore, the importance of managing and mitigating gray mold disease in the Golden Delicious cultivar cannot be overstated. Recent research focusing on the chemical compounds associated with the disease's advancement offers a valuable foundation for comprehending the complex interplay between the *B. cinerea* fungus and apples [6]. The prospects of early detection and intervention alongside the potential utilization of apple-derived antifungal compounds provide optimism for mitigating the effects of gray mold disease.

Essential oils (EOs) show great promise in combating gray mold, a prevalent fungal disease affecting apples. EOs, extracted from diverse plant sources, house various bioactive compounds, including some with antifungal properties [7]. Compounds like Eugenol, 1,8-Cineole, and Thymol present in EOs like thyme, clove, and eucalyptus have demonstrated efficacy in restraining the growth and spread of *B. cinerea* [8]. When applied to apples, these EOs act as natural fungicides, forming a protective shield against the invading fungal pathogen. Beyond disease control, EOs offer an eco-friendly alternative to synthetic chemical fungicides [9]. Integrating EOs into apple cultivation practices could potentially reduce gray mold's impact, enhancing apple quality and sustainability. Nevertheless, the potential influence of these EOs on the flavor compounds and volatile aroma of apples remains an underexplored realm. While EOs exhibit effective fungicidal properties, their application might impact the characteristic volatile compounds in apple varieties, altering their aroma and flavor profiles. Volatile aroma compounds (VOCs) play a pivotal role in defining apples' distinct fragrance and flavor. Key VOCs responsible for the fruity, fresh, and sweet apple aroma include aldehydes, alcohols, and esters [10]. For instance, compounds such as ethyl acetate, ethyl butyrate, and hexanal contribute fruity and floral notes to apple aroma, while aldehydes like (*E*)-2-hexenal and nonanal introduce green, citrusy, and grassy nuances. Alcohols, namely hexanol and 2-heptanol provide subtle, sweet undertones. These intricate VOC combinations give each apple variety a unique sensory identity, elevating apples from a culinary delight to a sensorial experience [11]. Despite their potential benefits, limited research has explored the intricate interactions between EOs and the VOCs that shape apple sensory attributes [10]. Further investigations in this domain are essential to strike a balance between disease management and preserving the desirable sensory qualities of apples. By examining the impact of EOs on the VOCs in apples, we seek to provide valuable insights for apple growers and the agricultural industry at large. Ultimately, the goal is to develop more sustainable and environmentally friendly approaches to apple disease management without compromising the essential sensory experience that apples offer to consumers worldwide. Our recent investigation comprehensively examined the protective effects of essential oils (EOs) in biological control against *B. cinerea* infection in apples [12]. Despite these initial findings, significant knowledge gaps persist regarding the curative efficacy of EO components in *B. cinerea* control and their impact on volatile organic compounds (VOCs) contributing to fruit aroma. Therefore, this study aims to (i) evaluate the bio-fungicidal potential of three synthetic essential oils (Thymol, Eugenol, and 1,8-Cineole) against *B. cinerea*, (ii) provide detailed characterization of the volatile organic compound composition in post-harvest processed apples, and (iii) address the current limitations in understanding the mechanisms by which essential oils modulate fungal pathogenicity and fruit quality. By systematically investigating these critical aspects, this research seeks to advance our understanding of alternative agricultural

strategies for managing post-harvest fungal infections while preserving the sensory attributes of apple fruits.

## 2. Materials and Methods

### 2.1. Fruit Materials

This research spanned one year (2023) during the early fall, specifically from August to September, within an experimental apple orchard established in Erzincan (39°42' Eastern longitude and 39°42' Northern latitude, 1470 m, a.s.l.). The orchard covered an area of 1800 m<sup>2</sup> and featured 30 apple trees comprising the cultivars Golden Delicious (*Malus x domestica* L.). The trees were arranged in rows with 10 trees each, aligned in a southwest direction, maintaining a planting space of 5 × 6 m. The trees, grafted on MM106 rootstock, were trained using a steep leader system. Consistent practices, such as pruning, drip irrigation, plant protection, and fertilization, were uniformly implemented across all cultivars. Annual winter and spring pruning was performed while adhering to the prescribed training system. Additionally, light formative pruning took place in the spring during the initial leaf development phase. Drip irrigation was applied at four key fruit development stages: fruit set, pit hardening, fruit development, and fruit bud differentiation. A low-nitrogen fertilizer, specifically 5–10–10 (N, P, K), was administered once annually in the spring, approximately one month before blooming. The experimental design followed a complete randomized block structure, with six trees organized into three blocks for a cultivar. Only commercially ripe fruits free from physical damage, uniform in size, and devoid of pathogen infections were chosen for the experiments.

### 2.2. Pathogen

*B. cinerea* was isolated from each plant, molecularly identified, and utilized in this study based on the previous study [12]. Before the experiments, *B. cinerea* was incubated in Potato Dextrose Agar (PDA) medium for seven days at a constant temperature of 25 °C.

### 2.3. Chemicals

Essential oils, including Thymol, Eugenol, and 1,8-Cineole, were purchased from Sigma-Aldrich, Shanghai, China, and stored at 4 °C in a dark environment. Various chemicals and reagents, including glucose, sodium chloride, tartaric acid, citric acid, sodium hydroxide (NaOH), ethanol, sodium dihydrogen phosphate, dichloromethane, methanol, and several chemical standards used in quantification and identification, were sourced from Sigma-Aldrich and other suppliers.

### 2.4. Fruit Inoculation and Storage

A 10% stock solution of each EO (Thymol, Eugenol, and 1,8-Cineole) was prepared, and this solution was further diluted by adding 5 mL of the stock solution to 400 mL of water. The fruits underwent a washing procedure in a 10 mL L<sup>-1</sup> sodium hypochlorite solution for 5 min, followed by rinsing with tap water and drying at room temperature. Wounds were created on disinfected apple fruits by using a sterile puncture needle, generating two wounds at the equator, each measuring 3 mm in depth and 3 mm in width [13]: Fungus (%10<sup>5</sup> conidia mL<sup>-1</sup>) + Thymol (%1.25 µL) + 1,8-Cineole (%1.25 µL); Fungus (%10<sup>5</sup> conidia mL<sup>-1</sup>) + Thymol (%1.25 µL) + 1,8-Cineole (%1.25 µL) + Eugenol (%1.25 µL). The experiment included multiple treatments: Fungus (spore suspension of the pathogen; 1 × 10<sup>5</sup> conidia mL<sup>-1</sup>), encompassing control (distilled water), individual EO at 1.25 µL each, various EO combinations, and different concentrations (ranging from 1.25 µL to 3.75 µL). The experiment comprised 9 treatments, with each protective and treatment procedure replicated three times, using three apples per repetition, following a completely randomized design.

### 2.5. Curative Measures

The fruits were immersed in EO solutions for 30 min and then dried at room temperature for 24 h. Subsequently, the wound sites were inoculated with 125 µL of a conidial suspension of *B. cinerea* at 1 × 10<sup>5</sup> spores/mL. The inoculated apples were placed in a

storage room inside transparent plastic boxes and incubated at +4 °C with high humidity (90 ± 5%). The apples were incubated in the dark for one week at 4 °C in 90 ± 5% humidity. The infected fruits were checked 7 days after incubation [14], which allowed for a comprehensive assessment of disease progression and essential oil treatment efficacy.

### 2.6. Sample Pretreatment

Each treatment was prepared in triplicate, and three apples were kept in water at 4 °C. The apples were homogenized, and the resulting pulps were subjected to centrifugation three times at 8,000 rpm for 10 min at 4 °C to obtain the supernatant.

### 2.7. Preparation of Free- and Bound-Form Volatiles

VOCs from the apples were extracted using headspace solid-phase micro-extraction (HS-SPME) and determined via gas chromatography–mass spectrometry (GC–MS). The extraction and preparation process followed optimized methods from previous studies [15,16]. The VOCs were separated into free-form and bound-form volatiles, with the latter extracted by enzymatic hydrolysis in citrate/phosphate buffer at pH 5.0 [15,16].

### 2.8. SPME Conditions

The free- and bound-form VOCs for the samples were extracted from the apple samples using HS-SPME under specific conditions, including equilibration at 60 °C for 40 min with agitation, the use of CAR/PDMS/DVB extraction coating fiber, and subsequent injection into the GC port.

### 2.9. GC/MS Analysis

Gas chromatography–mass spectrometry analysis (GC–MS) was conducted using an Agilent 7890 GC (Santa Clara, CA, USA) equipped with an Agilent 5975 MS. The analysis employed a capillary column (HP-INNOWAX) and helium as the carrier gas. The temperature conditions for the GC–MS analysis were set as per established protocols [17].

### 2.10. Quantification and Odor Activity Values (OAVs) Calculation

The quantification of VOCs in the fruits was performed using HPLC grade ethanol and known concentrations of standard VOCs. The concentration of VOCs in apples without standards was estimated by considering VOCs with similar functional groups and carbon atom numbers. The VOCs were quantified using characteristic ion peak areas relative to the internal standard 4-methyl-2-pentanol [17].

### 2.11. Data Analysis

Data analyses were performed using SPSS software (version 25.0) and JMP pro 13, with ANOVA used to analyze the variables, followed by the Duncan test ( $p \leq 0.01$ ) to separate the means. Principal component analysis (PCA) was employed to explore the relationships among the variables based on the treatments using average data in each case.

## 3. Results

Table 1 showcases the terpene contents ( $\mu\text{g L}^{-1}$ ) of the apples subjected to curative applications of individual and combined essential oils (EOs) against *B. cinerea*. The concentration of  $\alpha$ -Pinene varied across treatments, with the highest recorded for the Fungus + Thymol + 1,8-Cineol + Eugenol treatment ( $28.71 \mu\text{g L}^{-1}$ ) and the lowest for the Fun+Thy+Cin treatment ( $13.4 \mu\text{g L}^{-1}$ ). D-Limonene values ranged from  $21.7 \mu\text{g L}^{-1}$  in the CT group to  $17.0 \mu\text{g L}^{-1}$  in the Fun+Thy+Cin treatment. For  $\beta$ -Pinene, the highest concentration was noted in Fun+Thy+Cin ( $45.5 \mu\text{g L}^{-1}$ ) and the lowest in Fun+Cin+Eug ( $19.3 \mu\text{g L}^{-1}$ ). Phellandrene content ranged from  $53.7 \mu\text{g L}^{-1}$  in Fun+Thy+Cin to a maximum of  $104.1 \mu\text{g L}^{-1}$  in Fun+Thy+Cin+Eug. Neral content was lowest in Fun+Thy+Eug ( $1.4 \mu\text{g L}^{-1}$ ) and peaked in Fun+Cin+Eug ( $4.7 \mu\text{g L}^{-1}$ ). Geraniol displayed a considerable variation across treatments, with Fun+Thy+Cin+Eug showing the highest concentration of  $65.9 \mu\text{g L}^{-1}$  and

Fun+Thy+Eug showing the least at  $29.6 \mu\text{g L}^{-1}$ . For geranic acid, the highest concentration was in Fun+Cin+Eug ( $58.7 \mu\text{g L}^{-1}$ ), and the lowest was in Fun+Thy+Eug ( $22.9 \mu\text{g L}^{-1}$ ). For E-Nerolidol, the highest was observed for Fun+Thy+Cin+Eug at  $253.6 \mu\text{g L}^{-1}$ , while the control had a value of  $165.1 \mu\text{g L}^{-1}$ . Cedrol ranged from  $31.4 \mu\text{g L}^{-1}$  in Fun+Thy+Cin to  $64.0 \mu\text{g L}^{-1}$  in Fun+Thy+Cin+Eug. In this study, the ester contents ( $\mu\text{g L}^{-1}$ ) of the harvested apples following curative applications of individual and combined EOs against *B. cinerea* were examined, as shown in Table 2. The analysis of ester contents in the harvested apples following various EO treatments against *B. cinerea* revealed noteworthy findings. Among these treatments, propyl acetate demonstrated its highest concentration in the Fun+Thy+Cin+Eug combination, measuring  $76.5 \mu\text{g L}^{-1}$ . For ethyl isobutyrate, the Fun+Cin+Eug combination yielded the highest concentration at  $79.7 \mu\text{g L}^{-1}$ . In the case of ethyl acetate, the highest concentration was determined in the Fun+Cin+Eug combination, reaching  $58.3 \mu\text{g L}^{-1}$ .

Ethyl butyrate showed its highest concentration of  $136.6 \mu\text{g L}^{-1}$  when employing the Fun+Thy+Cin+Eug combination. Conversely, ethyl hexanoate reached its highest concentration of  $26.7 \mu\text{g L}^{-1}$  in the Fun+Cin+Eug combination. Ethyl heptanoate showed an intriguing pattern, achieving its peak concentration of  $314.1 \mu\text{g L}^{-1}$  with the Fun+Thy+Cin+Eug combination. The ethyl propionate concentration reached its peak at  $152.9 \mu\text{g L}^{-1}$  when using the Fun+Thy combination. In the Fun+Thy+Cin+Eug combination, ethyl pentanoate achieved its maximum concentration, measuring  $84.7 \mu\text{g L}^{-1}$ . A similar pattern emerged for ethyl 3-methylbutanoate, with Fun+Eug resulting in the highest concentration at  $57.1 \mu\text{g L}^{-1}$ . Hexyl acetate also had its highest concentration,  $86.2 \mu\text{g L}^{-1}$ , in the Fun+Cin+Eug combination. (Z)-3-hexenyl acetate displayed its peak concentration of  $134.7 \mu\text{g L}^{-1}$  in the Fun+Cin+Eug combination. When applying the Fun+Cin+Eug combination, butyl acetate exhibited its highest concentration at  $79.1 \mu\text{g L}^{-1}$ . Lastly, ethyl octanoate exhibited its maximum concentration at  $715.7 \mu\text{g L}^{-1}$  in the Fun+Thy+Cin+Eug combination. Notably, ethyl 3-hydroxybutyrate showed consistent concentrations of  $173.4 \mu\text{g L}^{-1}$  across multiple treatment combinations. Table 3 presents the concentration of various C6 compounds and alcohols in the harvested apples after they underwent different treatments to combat *B. cinerea*. Notably, the highest concentrations of hexanal were observed in the Fun+Cin and Fun+Cin+Eug groups, both reporting  $6.2 \mu\text{g L}^{-1}$ . Hexanal concentrations ranged from  $4.7 \mu\text{g L}^{-1}$  in the CT group to a high of  $6.2 \mu\text{g L}^{-1}$  in the Fun+Cin+Eug group. For the three isomers of hexenal, the variations were as follows: (Z)-3-hexenal concentrations were highest in the Fun+Thy+Eug group ( $2.3 \mu\text{g L}^{-1}$ ) and lowest in the Fun+Thy+Cin+Eug group ( $1.4 \mu\text{g L}^{-1}$ ). Hexanol concentrations peaked at  $3.5 \mu\text{g L}^{-1}$  for the Fun+Cin+Eug group and were at their lowest for the Fun+Thy+Cin+Eug group ( $2.7 \mu\text{g L}^{-1}$ ). (E)-2-hexenal showed minimal variation across all treatments, maintaining concentrations around the range of  $1.9$  to  $2.4 \mu\text{g L}^{-1}$ . Among the alcohols, 2-heptanol maintained relatively stable concentrations across all treatments, roughly averaging  $0.5 \mu\text{g L}^{-1}$ . 1-octen-3-ol exhibited its highest concentration in the Fun+Thy+Eug group with  $2.5 \mu\text{g L}^{-1}$  and the lowest in several groups at  $0.6 \mu\text{g L}^{-1}$ .

Interestingly, 2-ethyl hexanol showed significant variation, with its highest concentration in the Fun+Thy+Eug group ( $2.3 \mu\text{g L}^{-1}$ ) and the lowest in the Fun+Eug group ( $1.1 \mu\text{g L}^{-1}$ ). Heptanol ranged from  $6.1 \mu\text{g L}^{-1}$  (CT group) to a high of  $6.5 \mu\text{g L}^{-1}$  in the Fun+Thy+Cin group. Octanol, benzyl alcohol, nonanol, and phenylethyl alcohol also showed varied concentrations across the different treatments, with notable peaks in specific groups. The analysis of volatile compounds in the harvested apples following various treatments yielded notable findings (Table 4). The statistical analysis revealed no significant difference ( $p$ -value = 0.3171) among the treatments for this compound. The highest concentrations were observed in the “Fun+Thy+Eug” and “Fun+Thy+Cin” treatments. For hexanoic acid, the concentrations showed a range from 0.1 to 0.2 among the different treatments. Importantly, a statistically significant difference was noted, with a  $p$ -value of 0.0055\*, indicating the influence of the treatments on the levels of hexanoic acid. In contrast, 2-hexenoic acid exhibited more consistent concentrations, hovering around 0.2 across all treatments. Moving on to octanoic acid, the concentrations exhibited a wider variation,



ranging from 0.6 to 3.7. In the case of 3-methylbutanal, the concentrations varied from 0.8 to 6.4, with the highest concentration observed in the “CT” treatment. The differences among the treatments were highly significant ( $p$ -value  $< 0.0001^*$ ). Similar trends were observed for the other aldehydes within this category. Notably, the “Fun+Thy+Eug” treatment yielded the highest concentration. The differences among the treatments were highly significant ( $p$ -value  $< 0.0001^*$ ), highlighting the substantial impact of various treatments on octanoic acid levels. Shifting the focus to aldehydes, 2-methylbutanal displayed concentrations ranging from 0.1 to 0.4, with a highly significant difference noted among the treatments ( $p$ -value = 0.0006\*). Finally, considering the C13-norisoprenoids group,  $\beta$ -Damascenone displayed concentrations ranging from 34.6 to 70.5, with a statistically significant difference among the treatments ( $p$ -value = 0.0003\*).  $\beta$ -Ionone exhibited concentration variations, ranging from 44.0 to 86.5. The statistical analysis revealed a highly significant difference among the treatments ( $p$ -value  $< 0.0001^*$ ). Similarly, geranyl acetone values varied between 138.1 and 309.3, with a highly significant difference among the treatments ( $p$ -value  $< 0.0001^*$ ).

The colors within the heatmap represent the concentration or abundance of various compounds. Typically, darker colors, such as purple or deep blue, indicate higher levels of these compounds, whereas lighter colors, like yellow or green, signify lower levels. This visual representation indicated that the compounds exhibited varying degrees of presence across the different treatments. Some compounds appeared to be more abundant under specific treatment conditions, while others maintained relatively consistent levels across all treatments. The horizontal dendrogram positioned at the top of the heatmap was used to group the compounds based on their similarity in expression patterns across the different treatment conditions. The compounds that clustered closely together were those with similar expression profiles. On the other hand, the vertical dendrogram situated on the left side of the heatmap clustered the treatment conditions based on the similarity of compound expression within each treatment. The conditions that were closely grouped together in the dendrogram displayed similar compound profiles. For example, Fun+Thy+Eug and Fun+Thy+Cin were closely grouped in the dendrogram, indicating that they shared similar compound expression patterns. At the bottom of the heatmap, a series of compounds were listed and represented by different color bands. These compounds encompassed a range of chemical categories, including various types of aldehydes, acids, and others. These compounds were the focal points of measurement within each of the treatment conditions. In addition, at the bottom right of the heatmap, an additional plot or graph was present, appearing as a line graph or curve (Figure 1).

The plot in Figure 2 is a visual representation of various treatment conditions presented as labeled points. The relative distances between these points offers valuable insights into the differences and similarities among these conditions, relying on the underlying multivariate data. Points positioned closer to each other on the plot are indicative of profiles in the original data or shared characteristics, signifying a higher degree of similarity, while points located farther apart represent more distinct profiles. The Y and X axes within the plot correspond to the first two principal components, which effectively capture the maximum variance within the dataset. Typically, the X-axis accounts for more variance compared to the Y-axis. Consequently, the positioning of points along these axes conveys the degree of similarity or distinction between the conditions. Notably, the Fungus and Control conditions appear closely situated on the plot, indicating a degree of similarity in their profiles. In contrast, conditions marked in blue, such as Fun+Cin, Fun+Thy, Fun+Thy+Cin+Eug, and Fun+Eug, display an arch-shaped trajectory on the plot. This trajectory indicates a gradient of variations among these treatments, with conditions positioned along this path showing incremental differences in their profiles. Conversely, conditions marked in green, including Fun+Thy+Cin, Fun+Cin+Eug, and Fun+Thy+Eug, form a distinct cluster on the PCA plot, suggesting that these conditions share unique characteristics that distinguish them from the other treatments (Figure 2).

**Table 1.** Terpene contents ( $\mu\text{g L}^{-1}$ ) of harvested apples of curative applications of individuals and combinations of EOs against *B. cinerea*.

Compounds	CT	Fun	Fun+Thy	Fun+Cin	Fun+Eug	Fun+Cin+Eug	Fun+Thy+Eug	Fun+Thy+Cin	Fun+Thy+Cin+Eug	p-Value
<b>Terpenes</b>										
$\alpha$ -Pinene	14.5 $\pm$ 1.1 <sup>d</sup>	16.4 $\pm$ 0.9 <sup>cd</sup>	22.8 $\pm$ 1.2 <sup>bc</sup>	25.4 $\pm$ 1.2 <sup>ab</sup>	21.5 $\pm$ 4.2 <sup>bc</sup>	22.0 $\pm$ 2.2 <sup>bc</sup>	14.1 $\pm$ 0.6 <sup>d</sup>	13.4 $\pm$ 2.5 <sup>d</sup>	28.7 $\pm$ 1.3 <sup>a</sup>	0.0002 *
$\beta$ -Pinene	41.7 $\pm$ 6.7 <sup>ab</sup>	42.3 $\pm$ 5.9 <sup>ab</sup>	24.1 $\pm$ 4.4 <sup>c</sup>	26.8 $\pm$ 4.9 <sup>bc</sup>	32.4 $\pm$ 0.7 <sup>abc</sup>	19.3 $\pm$ 5.6 <sup>c</sup>	33.6 $\pm$ 6.2 <sup>abc</sup>	44.5 $\pm$ 1.7 <sup>a</sup>	23.1 $\pm$ 4.2 <sup>c</sup>	0.0131 *
Phellandrene	62.3 $\pm$ 8.1 <sup>de</sup>	71.3 $\pm$ 8.6 <sup>cde</sup>	77.0 $\pm$ 3.2 <sup>cde</sup>	88.0 $\pm$ 3.2 <sup>abc</sup>	85.9 $\pm$ 5.5 <sup>bc</sup>	96.8 $\pm$ 6.2 <sup>abc</sup>	56.4 $\pm$ 7.6 <sup>e</sup>	53.7 $\pm$ 3.4 <sup>e</sup>	104.1 $\pm$ 0.7 <sup>a</sup>	<0.0001 *
$\beta$ -Myrcene	11.9 $\pm$ 0.4 <sup>d</sup>	11.1 $\pm$ 0.4 <sup>d</sup>	23.8 $\pm$ 0.5 <sup>b</sup>	23.4 $\pm$ 0.5 <sup>b</sup>	24.4 $\pm$ 0.8 <sup>b</sup>	30.8 $\pm$ 1.0 <sup>a</sup>	12.1 $\pm$ 0.3 <sup>c</sup>	12.4 $\pm$ 0.4 <sup>c</sup>	29.5 $\pm$ 0.6 <sup>a</sup>	<0.0001 *
D-Limonene	21.7 $\pm$ 0.4 <sup>de</sup>	23.1 $\pm$ 0.4 <sup>d</sup>	27.7 $\pm$ 1.0 <sup>c</sup>	31.1 $\pm$ 1.2 <sup>b</sup>	26.4 $\pm$ 1.4 <sup>c</sup>	29.5 $\pm$ 1.6 <sup>bc</sup>	17.8 $\pm$ 0.7 <sup>e</sup>	17.0 $\pm$ 0.9 <sup>e</sup>	34.6 $\pm$ 1.3 <sup>a</sup>	<0.0001 *
$\gamma$ -Terpinene	65.2 $\pm$ 1.6 <sup>f</sup>	72.3 $\pm$ 1.7 <sup>e</sup>	88.5 $\pm$ 2.2 <sup>d</sup>	98.5 $\pm$ 2.5 <sup>c</sup>	107.1 $\pm$ 1.6 <sup>b</sup>	119.1 $\pm$ 1.8 <sup>a</sup>	57.9 $\pm$ 1.5 <sup>g</sup>	70.1 $\pm$ 1.2 <sup>ef</sup>	109.5 $\pm$ 2.7 <sup>b</sup>	<0.0001 *
p-Cymene	41.5 $\pm$ 1.9 <sup>b</sup>	46.3 $\pm$ 2.0 <sup>a</sup>	22.3 $\pm$ 1.2 <sup>de</sup>	25.0 $\pm$ 1.2 <sup>d</sup>	22.2 $\pm$ 0.4 <sup>de</sup>	19.1 $\pm$ 0.3 <sup>e</sup>	31.6 $\pm$ 1.5 <sup>c</sup>	31.4 $\pm$ 0.6 <sup>c</sup>	21.5 $\pm$ 1.0 <sup>de</sup>	<0.0001 *
Terpinolene	4.1 $\pm$ 0.3 <sup>f</sup>	4.6 $\pm$ 0.2 <sup>e</sup>	5.1 $\pm$ 0.3 <sup>d</sup>	5.6 $\pm$ 0.3 <sup>cd</sup>	5.9 $\pm$ 0.2 <sup>bc</sup>	6.5 $\pm$ 0.2 <sup>a</sup>	3.3 $\pm$ 0.2 <sup>f</sup>	3.8 $\pm$ 0.2 <sup>ef</sup>	6.2 $\pm$ 0.3 <sup>ab</sup>	<0.0001 *
Rose oxide II (cis)	27.2 $\pm$ 3.0 <sup>c</sup>	24.5 $\pm$ 1.9 <sup>c</sup>	59.2 $\pm$ 6.7 <sup>b</sup>	58.0 $\pm$ 6.6 <sup>b</sup>	46.4 $\pm$ 1.4 <sup>b</sup>	50.2 $\pm$ 2.9 <sup>b</sup>	30.1 $\pm$ 3.4 <sup>c</sup>	23.3 $\pm$ 0.6 <sup>c</sup>	73.2 $\pm$ 8.3 <sup>a</sup>	<0.0001 *
Rose oxide I (trans)	14.4 $\pm$ 0.7 <sup>gh</sup>	16.5 $\pm$ 0.6 <sup>g</sup>	21.4 $\pm$ 0.7 <sup>e</sup>	23.8 $\pm$ 0.8 <sup>d</sup>	31.3 $\pm$ 0.6 <sup>b</sup>	36.1 $\pm$ 0.7 <sup>a</sup>	12.6 $\pm$ 0.4 <sup>h</sup>	18.4 $\pm$ 0.3 <sup>f</sup>	27.4 $\pm$ 1.0 <sup>c</sup>	<0.0001 *
Nerol oxide	1.9 $\pm$ 0.1 <sup>b</sup>	2.0 $\pm$ 0.8 <sup>a</sup>	0.9 $\pm$ 0.0 <sup>e</sup>	1.0 $\pm$ 0.2 <sup>de</sup>	1.1 $\pm$ 0.0 <sup>d</sup>	0.9 $\pm$ 0.0 <sup>e</sup>	1.3 $\pm$ 0.1 <sup>c</sup>	1.5 $\pm$ 0.1 <sup>b</sup>	0.9 $\pm$ 0.0 <sup>e</sup>	<0.0001 *
Linalool	4.9 $\pm$ 0.5 <sup>f</sup>	5.7 $\pm$ 0.8 <sup>e</sup>	5.3 $\pm$ 0.2 <sup>d</sup>	6.0 $\pm$ 0.2 <sup>c</sup>	6.3 $\pm$ 0.2 <sup>bc</sup>	6.9 $\pm$ 0.2 <sup>a</sup>	3.6 $\pm$ 0.1 <sup>g</sup>	4.3 $\pm$ 0.2 <sup>ef</sup>	6.6 $\pm$ 0.2 <sup>ab</sup>	<0.0001 *
4-terpineol	1.7 $\pm$ 0.4 <sup>de</sup>	1.8 $\pm$ 0.1 <sup>e</sup>	2.9 $\pm$ 0.1 <sup>c</sup>	2.9 $\pm$ 0.1 <sup>c</sup>	3.1 $\pm$ 0.1 <sup>c</sup>	4.0 $\pm$ 0.1 <sup>a</sup>	1.5 $\pm$ 0.2 <sup>de</sup>	1.6 $\pm$ 0.1 <sup>d</sup>	3.6 $\pm$ 0.2 <sup>b</sup>	<0.0001 *
Hotrienol	18.2 $\pm$ 1.3 <sup>g</sup>	18.8 $\pm$ 1.3 <sup>fg</sup>	22.4 $\pm$ 1.5 <sup>de</sup>	24.9 $\pm$ 1.7 <sup>cd</sup>	30.9 $\pm$ 1.2 <sup>ab</sup>	34.4 $\pm$ 1.2 <sup>a</sup>	14.6 $\pm$ 1 <sup>g</sup>	20.2 $\pm$ 0.7 <sup>ef</sup>	27.7 $\pm$ 1.9 <sup>bc</sup>	<0.0001 *
Neral	2.1 $\pm$ 0.7 <sup>fg</sup>	2.1 $\pm$ 0.8 <sup>f</sup>	2.3 $\pm$ 0.0 <sup>e</sup>	2.6 $\pm$ 0.1 <sup>d</sup>	4.1 $\pm$ 0.2 <sup>b</sup>	4.7 $\pm$ 0.1 <sup>a</sup>	1.4 $\pm$ 0.0 <sup>g</sup>	2.4 $\pm$ 0.2 <sup>de</sup>	3.0 $\pm$ 0.1 <sup>c</sup>	<0.0001 *
$\alpha$ -Terpineol	2.9 $\pm$ 0.3 <sup>fg</sup>	3.1 $\pm$ 0.2 <sup>f</sup>	3.4 $\pm$ 0.1 <sup>e</sup>	3.8 $\pm$ 0.1 <sup>d</sup>	5.3 $\pm$ 0.2 <sup>b</sup>	6.0 $\pm$ 0.2 <sup>a</sup>	2.2 $\pm$ 0.1 <sup>g</sup>	3.5 $\pm$ 0.1 <sup>e</sup>	4.2 $\pm$ 0.1 <sup>c</sup>	<0.0001 *
Geranial	3.4 $\pm$ 0.6 <sup>ef</sup>	2.8 $\pm$ 0.1 <sup>de</sup>	3.8 $\pm$ 0.3 <sup>cd</sup>	4.3 $\pm$ 0.3 <sup>bc</sup>	4.7 $\pm$ 0.2 <sup>ab</sup>	5.1 $\pm$ 0.3 <sup>a</sup>	2.6 $\pm$ 0.2 <sup>f</sup>	3.2 $\pm$ 0.2 <sup>def</sup>	4.6 $\pm$ 0.2 <sup>ab</sup>	<0.0001 *
Citronellol	31.6 $\pm$ 2.2 <sup>c</sup>	33.5 $\pm$ 2.4 <sup>c</sup>	48.3 $\pm$ 4.1 <sup>b</sup>	53.7 $\pm$ 4.5 <sup>ab</sup>	54.7 $\pm$ 2.5 <sup>ab</sup>	63.0 $\pm$ 2.9 <sup>a</sup>	28.4 $\pm$ 2.4 <sup>c</sup>	32.1 $\pm$ 1.5 <sup>c</sup>	61.9 $\pm$ 5.2 <sup>a</sup>	<0.0001 *
Myrtenol	104.1 $\pm$ 11.5 <sup>ef</sup>	117.1 $\pm$ 13 <sup>def</sup>	142.8 $\pm$ 17.3 <sup>cd</sup>	150.8 $\pm$ 11.6 <sup>bc</sup>	183.3 $\pm$ 8.4 <sup>ab</sup>	203.8 $\pm$ 9.4 <sup>a</sup>	87.2 $\pm$ 5.4 <sup>f</sup>	124.8 $\pm$ 10.4 <sup>cde</sup>	149.9 $\pm$ 1.6 <sup>c</sup>	<0.0001 *
Nerol	141.1 $\pm$ 14.8 <sup>c</sup>	158.1 $\pm$ 16.1 <sup>cb</sup>	204.3 $\pm$ 22.6 <sup>ab</sup>	231.3 $\pm$ 25.6 <sup>a</sup>	206.3 $\pm$ 13.2 <sup>ab</sup>	233.5 $\pm$ 14.7 <sup>a</sup>	124.4 $\pm$ 13.8 <sup>c</sup>	125.6 $\pm$ 7.9 <sup>c</sup>	218.5 $\pm$ 4.5 <sup>a</sup>	0.0002 *
Geraniol	31.1 $\pm$ 4.1 <sup>de</sup>	36.6 $\pm$ 5.5 <sup>cde</sup>	45.2 $\pm$ 6.8 <sup>bcd</sup>	56.9 $\pm$ 1.7 <sup>ab</sup>	47.6 $\pm$ 5.3 <sup>bc</sup>	52.9 $\pm$ 5.9 <sup>ab</sup>	29.6 $\pm$ 4.4 <sup>e</sup>	31.1 $\pm$ 3.5 <sup>de</sup>	65.9 $\pm$ 2.5 <sup>a</sup>	0.0003 *
E-Nerolidol	165.1 $\pm$ 16.2 <sup>cd</sup>	191.4 $\pm$ 18.6 <sup>bcd</sup>	215 $\pm$ 22.3 <sup>abc</sup>	243.4 $\pm$ 25.2 <sup>a</sup>	227.8 $\pm$ 12.8 <sup>ab</sup>	231.6 $\pm$ 8.5 <sup>ab</sup>	145.7 $\pm$ 15.1 <sup>d</sup>	164.4 $\pm$ 18.2 <sup>cd</sup>	253.6 $\pm$ 2.5 <sup>a</sup>	0.0019 *
Cedrol	36.8 $\pm$ 1.2 <sup>de</sup>	42.1 $\pm$ 1.8 <sup>d</sup>	51.1 $\pm$ 1.9 <sup>c</sup>	57.4 $\pm$ 2.1 <sup>b</sup>	48.8 $\pm$ 2.6 <sup>c</sup>	54.4 $\pm$ 2.9 <sup>bc</sup>	32.9 $\pm$ 1.2 <sup>e</sup>	31.4 $\pm$ 1.7 <sup>e</sup>	64.0 $\pm$ 2.4 <sup>a</sup>	<0.0001 *
Geranic acid	26.1 $\pm$ 1.5 <sup>d</sup>	27.7 $\pm$ 1.5 <sup>d</sup>	35.0 $\pm$ 1.7 <sup>c</sup>	38.9 $\pm$ 1.8 <sup>bc</sup>	53.1 $\pm$ 2.2 <sup>a</sup>	58.7 $\pm$ 2.5 <sup>a</sup>	22.9 $\pm$ 1.1 <sup>d</sup>	37.4 $\pm$ 2.9 <sup>c</sup>	43.3 $\pm$ 2.1 <sup>b</sup>	<0.0001 *

Different letters in the same columns indicate statistically significant differences ( $p \leq 0.01$ ). CT: Control, Fun: Fungus (*Botrytis cinerea* inoculation), Fun+Thy: Fungus + Thymol, Fun+Cin: Fungus + 1,8-Cineole, Fun+Eug: Fungus + Eugenol, Fun+Cin+Eug: Fungus + 1,8-Cineole + Eugenol, Fun+Thy+Eug: Fungus + Thymol + Eugenol, Fun+Thy+Cin: Fungus + Thymol + 1,8-Cineole, Fun+Thy+Cin+Eug: Fungus + Thymol + 1,8-Cineole + Eugenol. \*, significant at  $p$ -value < 0.05.

**Table 2.** Ester contents ( $\mu\text{g L}^{-1}$ ) of harvested apples of curative applications of individuals and combinations of EOs against *B. cinerea*.

Compounds	CT	Fun	Fun+Thy	Fun+Cin	Fun+Eug	Fun+Cin+Eug	Fun+Thy+Eug	Fun+Thy+Cin	Fun+Thy+Cin+Eug	p-Value
<b>Esters</b>										
Ethyl acetate	25.1 $\pm$ 1.4 <sup>ef</sup>	26.9 $\pm$ 1.3 <sup>de</sup>	31.5 $\pm$ 1.5 <sup>cd</sup>	35.7 $\pm$ 1.7 <sup>bc</sup>	53.4 $\pm$ 2.8 <sup>a</sup>	58.3 $\pm$ 3.0 <sup>a</sup>	21.4 $\pm$ 1.2 <sup>f</sup>	36.2 $\pm$ 1.9 <sup>bc</sup>	39.0 $\pm$ 1.9 <sup>b</sup>	<0.0001 *
Ethyl propionate	32.1 $\pm$ 3.7 <sup>de</sup>	31.2 $\pm$ 1.3 <sup>de</sup>	152.9 $\pm$ 1.8 <sup>b</sup>	46.8 $\pm$ 4.9 <sup>cd</sup>	61.2 $\pm$ 9.5 <sup>c</sup>	173.4 $\pm$ 5.0 <sup>a</sup>	27.5 $\pm$ 2.9 <sup>e</sup>	40.0 $\pm$ 6.2 <sup>de</sup>	147.0 $\pm$ 11.5 <sup>b</sup>	<0.0001 *

Table 2. Cont.

Compounds	CT	Fun	Fun+Thy	Fun+Cin	Fun+Eug	Fun+Cin+Eug	Fun+Thy+Eug	Fun+Thy+Cin	Fun+Thy+Cin+Eug	p-Value
<b>Esters</b>										
Ethyl isobutyrate	37.6 ± 3.9 <sup>e</sup>	45.2 ± 4.1 <sup>de</sup>	49.9 ± 5.3 <sup>cde</sup>	56.4 ± 6.2 <sup>bcd</sup>	73.0 ± 7.2 <sup>ab</sup>	79.7 ± 7.7 <sup>a</sup>	33.8 ± 3.6 <sup>e</sup>	49.5 ± 4.8 <sup>cde</sup>	61.7 ± 6.5 <sup>bc</sup>	0.0003 *
Propyl acetate	44.9 ± 1.3 <sup>f</sup>	49.1 ± 1.3 <sup>de</sup>	61.9 ± 1.9 <sup>c</sup>	68.8 ± 2.1 <sup>b</sup>	45.2 ± 1.2 <sup>ed</sup>	52.5 ± 1.9 <sup>de</sup>	40.5 ± 1.2 <sup>f</sup>	30.1 ± 0.6 <sup>g</sup>	76.5 ± 2.4 <sup>a</sup>	<0.0001 *
Ethyl butyrate	62.1 ± 7.1 <sup>d</sup>	68.8 ± 7.9 <sup>d</sup>	108.2 ± 3.4 <sup>c</sup>	119.5 ± 3.6 <sup>abc</sup>	111.4 ± 7.5 <sup>bc</sup>	128.4 ± 8.6 <sup>abc</sup>	55.9 ± 7.3 <sup>d</sup>	65.5 ± 4.4 <sup>d</sup>	136.6 ± 4.2 <sup>abc</sup>	<0.0001 *
Ethyl 3-methylbutanoate	32.8 ± 2.1 <sup>cde</sup>	34.2 ± 2.9 <sup>cd</sup>	26.3 ± 1.3 <sup>e</sup>	48.3 ± 3.5 <sup>b</sup>	57.1 ± 0.5 <sup>a</sup>	37.1 ± 1.5 <sup>c</sup>	28.4 ± 2.2 <sup>de</sup>	33.7 ± 5.7 <sup>cde</sup>	32.6 ± 1.6 <sup>cde</sup>	<0.0001 *
Butyl acetate	13.8 ± 1.6 <sup>d</sup>	14.9 ± 1.5 <sup>cd</sup>	58.4 ± 7.4 <sup>b</sup>	21.3 ± 2.2 <sup>cd</sup>	27.9 ± 4.3 <sup>c</sup>	79.1 ± 2.3 <sup>a</sup>	12.6 ± 1.3 <sup>d</sup>	18.2 ± 2.8 <sup>cd</sup>	72.2 ± 9.1 <sup>a</sup>	<0.0001 *
Ethyl pentanoate	41.8 ± 2.9 <sup>d</sup>	46.1 ± 3.2 <sup>cd</sup>	66.1 ± 4.8 <sup>b</sup>	73.5 ± 5.3 <sup>ab</sup>	60.4 ± 0.6 <sup>bc</sup>	80.0 ± 1.3 <sup>a</sup>	38.9 ± 2.8 <sup>d</sup>	46.1 ± 7.9 <sup>d</sup>	84.7 ± 6.2 <sup>a</sup>	<0.0001 *
Ethyl hexanoate	12.2 ± 1.2 <sup>de</sup>	12.9 ± 1.3 <sup>cde</sup>	16.5 ± 1.7 <sup>cde</sup>	18.4 ± 1.9 <sup>bcd</sup>	24.0 ± 3.7 <sup>ab</sup>	26.7 ± 4.2 <sup>a</sup>	10.8 ± 1.1 <sup>e</sup>	15.7 ± 2.4 <sup>cde</sup>	20.4 ± 2.2 <sup>abc</sup>	0.0025 *
Hexyl acetate	41.8 ± 7.0 <sup>d</sup>	43.9 ± 7.2 <sup>cd</sup>	62.8 ± 11.3 <sup>bc</sup>	69.9 ± 1.2 <sup>ab</sup>	72.9 ± 8.2 <sup>ab</sup>	86.2 ± 0.4 <sup>a</sup>	36.9 ± 6.7 <sup>d</sup>	45.0 ± 6.6 <sup>cd</sup>	76.8 ± 1.1 <sup>ab</sup>	0.0002 *
(Z)-3-hexenyl acetate	80.1 ± 2.9 <sup>de</sup>	90.1 ± 1.6 <sup>d</sup>	102.2 ± 3.1 <sup>c</sup>	115.7 ± 3.6 <sup>b</sup>	120.0 ± 7.2 <sup>b</sup>	134.7 ± 4.4 <sup>a</sup>	69.3 ± 2.1 <sup>e</sup>	81.4 ± 4.9 <sup>d</sup>	126.4 ± 3.9 <sup>ab</sup>	<0.0001 *
Ethyl heptanoate	185.3 ± 7.6 <sup>ef</sup>	205.4 ± 9.7 <sup>de</sup>	254.0 ± 12.0 <sup>c</sup>	282.5 ± 13.4 <sup>b</sup>	237.6 ± 2.8 <sup>c</sup>	231.1 ± 4.6 <sup>cd</sup>	166.1 ± 7.9 <sup>fg</sup>	149.8 ± 1.9 <sup>g</sup>	314.1 ± 14.9 <sup>a</sup>	<0.0001 *
Ethyl octanoate	440.1 ± 13.9 <sup>e</sup>	511.1 ± 11.8 <sup>d</sup>	566.7 ± 14.0 <sup>c</sup>	641.5 ± 15.8 <sup>b</sup>	628.6 ± 11.2 <sup>b</sup>	686.6 ± 12.3 <sup>a</sup>	373.1 ± 19.8 <sup>f</sup>	417.7 ± 15.9 <sup>e</sup>	715.7 ± 8.7 <sup>a</sup>	<0.0001 *
Ethyl 3-hydroxybutyrate	172.9 ± 7.6 <sup>b</sup>	115.1 ± 3.1 <sup>c</sup>	173.4 ± 7.8 <sup>b</sup>	113.1 ± 2.9 <sup>c</sup>	258.6 ± 7.4 <sup>a</sup>	123.4 ± 3.9 <sup>c</sup>	258.6 ± 7.4 <sup>a</sup>	113.1 ± 2.9 <sup>c</sup>	173.4 ± 7.8 <sup>b</sup>	<0.0001 *

Different letters in the same columns indicate statistically significant differences ( $p \leq 0.01$ ). CT: Control, Fun: Fungus (*Botrytis cinerea* inoculation), Fun+Thy: Fungus + Thymol, Fun+Cin: Fungus + 1,8-Cineole, Fun+Eug: Fungus + Eugenol, Fun+Cin+Eug: Fungus + 1,8-Cineole + Eugenol, Fun+Thy+Eug: Fungus + Thymol + Eugenol, Fun+Thy+Cin: Fungus + Thymol + 1,8-Cineole, Fun+Thy+Cin+Eug: Fungus + Thymol + 1,8-Cineole + Eugenol. \*, significant at  $p$ -value < 0.05.

Table 3. C6 compounds and alcohol contents ( $\mu\text{g L}^{-1}$ ) of harvested apples of curative applications of individuals and combinations of EOs against *B. cinerea*.

Compounds	CT	Fun	Fun+Thy	Fun+Cin	Fun+Eug	Fun+Cin+Eug	Fun+Thy+Eug	Fun+Thy+Cin	Fun+Thy+Cin+Eug	p-Value
<b>C6 Compounds</b>										
Hexanal	4.7 ± 0.8 <sup>c</sup>	5.1 ± 0.3 <sup>bc</sup>	6.1 ± 0.9 <sup>ab</sup>	6.2 ± 0 <sup>a</sup>	4.8 ± 0.4 <sup>bc</sup>	6.2 ± 0.2 <sup>a</sup>	4.8 ± 0.3 <sup>bc</sup>	5.4 ± 0.8 <sup>abc</sup>	5.8 ± 0.1 <sup>ab</sup>	0.0418
(Z)-3-hexenal	1.7 ± 0.1	1.6 ± 0.1	1.5 ± 0.2	1.7 ± 0.1	1.7 ± 0.3	1.9 ± 0.1	2.3 ± 0.0	2.0 ± 0.4	1.4 ± 0.1	0.1417
(E)-2-hexenal	2.1 ± 0.1	2.3 ± 0.2	2 ± 0.1	2.2 ± 0.2	1.9 ± 0.1	2.1 ± 0.3	2.4 ± 0.3	2.1 ± 0.2	2.0 ± 0.2	0.6759
Hexanol	3.4 ± 0.3	3.2 ± 0.1	2.8 ± 0.2	3 ± 0.2	3 ± 0.3	3.5 ± 0.3	3.2 ± 0.2	3.3 ± 0.4	2.7 ± 0.1	0.2981
(E)-3-hexenol	1.8 ± 0.3	1.6 ± 0.2	1.3 ± 0.1	1.5 ± 0.2	1.5 ± 0.2	1.8 ± 0.2	1.9 ± 0.2	1.5 ± 0.1	1.4 ± 0.0	0.2435
(Z)-3-hexenol	0.6 ± 0.2	0.5 ± 0.1	0.4 ± 0	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.0994
(E)-2-hexenol	1.6 ± 0.3	1.5 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.1	1.7 ± 0.0	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.2	0.6185
<b>Alcohols</b>										
2-heptanol	0.5 ± 0.0	0.8 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.6 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.0691
1-octen-3-ol	0.5 ± 0.1 <sup>cd</sup>	0.7 ± 0.8 <sup>d</sup>	0.6 ± 0.2 <sup>cd</sup>	0.6 ± 0.2 <sup>cd</sup>	0.6 ± 0.0 <sup>cd</sup>	0.6 ± 0.2 <sup>cd</sup>	2.5 ± 0.0 <sup>a</sup>	0.8 ± 0.0 <sup>b</sup>	0.7 ± 0.0 <sup>c</sup>	<0.0001 *
Heptanol	6.1 ± 0.8	5.5 ± 0.2	5.5 ± 0.3	5.5 ± 0.4	4.9 ± 0.6	6.3 ± 0.6	5.8 ± 0.6	6.5 ± 0.9	6.1 ± 0.1	0.4128
2-ethyl hexanol	1.4 ± 0.1 <sup>cde</sup>	1.3 ± 0.1 <sup>cde</sup>	1.2 ± 0.2 <sup>de</sup>	1.5 ± 0.2 <sup>bcd</sup>	1.1 ± 0.1 <sup>e</sup>	1.7 ± 0.1 <sup>b</sup>	2.3 ± 0.0 <sup>a</sup>	1.5 ± 0.2 <sup>bc</sup>	1.3 ± 0.1 <sup>cde</sup>	<0.0001 *
Octanol	1.5 ± 0.2 <sup>b</sup>	1.4 ± 0.1 <sup>b</sup>	1.1 ± 0.1 <sup>b</sup>	1.3 ± 0.2 <sup>b</sup>	1.3 ± 0.3 <sup>b</sup>	1.5 ± 0.2 <sup>b</sup>	2.2 ± 0.2 <sup>a</sup>	1.4 ± 0.1 <sup>b</sup>	1.2 ± 0.2 <sup>b</sup>	0.0043 *
Nonanol	1.6 ± 0.1	1.3 ± 0.2	1.2 ± 0.1	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.2	1.8 ± 0.1	1.5 ± 0.1	1.2 ± 0.0	0.2225



Table 3. Cont.

Compounds	CT	Fun	Fun+Thy	Fun+Cin	Fun+Eug	Fun+Cin+Eug	Fun+Thy+Eug	Fun+Thy+Cin	Fun+Thy+Cin+Eug	p-Value
<b>C6 Compounds</b>										
Benzyl alcohol	3.6 ± 0.5 <sup>bdc</sup>	3.2 ± 0.3 <sup>cde</sup>	3.3 ± 0.2 <sup>cd</sup>	3.0 ± 0.3 <sup>cde</sup>	2.8 ± 0.4 <sup>de</sup>	4.3 ± 0.3 <sup>a</sup>	2.5 ± 0.1 <sup>e</sup>	4.2 ± 0.2 <sup>ab</sup>	3.7 ± 0.2 <sup>abc</sup>	0.0016 *
Phenylethyl alcohol	2.5 ± 0.3	2.6 ± 0.5	2.1 ± 0.1	2.2 ± 0.1	1.9 ± 0.2	2.4 ± 0.1	2.0 ± 0.3	2.2 ± 0.2	2.2 ± 0.1	0.6595

Different letters in the same columns indicate statistically significant differences ( $p \leq 0.01$ ). CT: Control, Fun: Fungus (*Botrytis cinerea* inoculation), Fun+Thy: Fungus + Thymol, Fun+Cin: Fungus + 1,8-Cineole, Fun+Eug: Fungus + Eugenol, Fun+Cin+Eug: Fungus + 1,8-Cineole + Eugenol, Fun+Thy+Eug: Fungus + Thymol + Eugenol, Fun+Thy+Cin: Fungus + Thymol + 1,8-Cineole, Fun+Thy+Cin+Eug: Fungus + Thymol + 1,8-Cineole + Eugenol. \*, significant at  $p$ -value < 0.05.

Table 4. Acid and aldehyde contents ( $\mu\text{g L}^{-1}$ ) of harvested apples of curative applications of individuals and combinations of EOs against *B. cinerea*.

Compounds	CT	Fun	Fun+Thy	Fun+Cin	Fun+Eug	Fun+Cin+Eug	Fun+Thy+Eug	Fun+Thy+Cin	Thy+Cin+Fun+Eug	p-Value
<b>Acids</b>										
Hexanoic acid	0.1 ± 0.3 <sup>bc</sup>	0.1 ± 0.4 <sup>bcd</sup>	0.1 ± 0.2 <sup>bcd</sup>	0.1 ± 0.0 <sup>d</sup>	0.1 ± 0.2 <sup>cd</sup>	0.1 ± 0.2 <sup>bcd</sup>	0.1 ± 0.2 <sup>ab</sup>	0.2 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>ab</sup>	0.0055 *
2-hexenoic acid	0.1 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3171
Octanoic acid	0.7 ± 0.1 <sup>b</sup>	0.6 ± 0.1 <sup>b</sup>	0.7 ± 0.1 <sup>b</sup>	0.8 ± 0.1 <sup>b</sup>	0.6 ± 0.0 <sup>b</sup>	0.9 ± 0.1 <sup>b</sup>	3.7 ± 0.3 <sup>a</sup>	0.9 ± 0.0 <sup>b</sup>	0.8 ± 0.1 <sup>b</sup>	<0.0001 *
<b>Aldehydes</b>										
2-methylbutanal	0.4 ± 0.1 <sup>a</sup>	0.2 ± 0.1 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>a</sup>	0.1 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.1 ± 0.0 <sup>b</sup>	0.0006 *
3-methylbutanal	6.4 ± 0.2 <sup>a</sup>	5.7 ± 0.2 <sup>b</sup>	1.3 ± 0.1 <sup>e</sup>	0.8 ± 0.2 <sup>f</sup>	0.9 ± 0.1 <sup>f</sup>	1.8 ± 0.0 <sup>d</sup>	2.6 ± 0.2 <sup>c</sup>	2.6 ± 0.0 <sup>c</sup>	2.3 ± 0.0 <sup>c</sup>	<0.0001 *
Pentanal	389.0 ± 13.2 <sup>g</sup>	550.9 ± 12.1 <sup>g</sup>	4304.3 ± 175.7 <sup>d</sup>	6126.6 ± 84.7 <sup>b</sup>	5142.8 ± 129.1 <sup>c</sup>	5021.5 ± 184.1 <sup>c</sup>	1970.2 ± 198.3 <sup>f</sup>	3073.3 ± 162.6 <sup>e</sup>	8196.6 ± 161 <sup>a</sup>	<0.0001 *
Octanal	7.4 ± 0.2 <sup>a</sup>	7.1 ± 0.1 <sup>a</sup>	3.1 ± 0.1 <sup>c</sup>	2.5 ± 0.2 <sup>de</sup>	2.7 ± 0.0 <sup>d</sup>	3.3 ± 0.1 <sup>c</sup>	2.4 ± 0.0 <sup>e</sup>	3.6 ± 0.1 <sup>b</sup>	1.9 ± 0.1 <sup>f</sup>	<0.0001 *
Nonanal	8.4 ± 0.1 <sup>a</sup>	8.1 ± 0.2 <sup>b</sup>	2.9 ± 0.2 <sup>ef</sup>	2.5 ± 0.2 <sup>fg</sup>	2.7 ± 0.2 <sup>ef</sup>	4.2 ± 0.0 <sup>c</sup>	3.5 ± 0.1 <sup>d</sup>	4.4 ± 0.1 <sup>c</sup>	2.3 ± 0.1 <sup>g</sup>	<0.0001 *
(E)-2-octenal	11.2 ± 0.2 <sup>a</sup>	9.6 ± 0.2 <sup>b</sup>	4.2 ± 0.1 <sup>c</sup>	3.4 ± 0.1 <sup>d</sup>	3.9 ± 0.1 <sup>c</sup>	3.5 ± 0.0 <sup>d</sup>	3.1 ± 0.2 <sup>e</sup>	3.4 ± 0.0 <sup>d</sup>	3.0 ± 0.1 <sup>e</sup>	<0.0001 *
Benzaldehyde	2.5 ± 0.2 <sup>a</sup>	2.5 ± 0.2 <sup>b</sup>	1.7 ± 0.1 <sup>c</sup>	1.2 ± 0.1 <sup>d</sup>	1.4 ± 0.1 <sup>d</sup>	0.8 ± 0.0 <sup>e</sup>	0.9 ± 0.0 <sup>e</sup>	0.7 ± 0.0 <sup>e</sup>	0.8 ± 0.1 <sup>e</sup>	<0.0001 *
Phenylacetaldehyde	25.8 ± 0.3 <sup>a</sup>	25.1 ± 0.9 <sup>b</sup>	9.9 ± 0.5 <sup>c</sup>	7.0 ± 0.2 <sup>ef</sup>	8.1 ± 0.3 <sup>de</sup>	7.5 ± 0.4 <sup>de</sup>	8.4 ± 0.5 <sup>d</sup>	8.8 ± 0.3 <sup>cd</sup>	6.0 ± 0.3 <sup>f</sup>	<0.0001 *
<b>C13-Norisoprenoids</b>										
β-Damascenone	38.2 ± 2.5 <sup>c</sup>	41.1 ± 3.1 <sup>bc</sup>	64.3 ± 11.4 <sup>a</sup>	65.4 ± 4.9 <sup>a</sup>	67.9 ± 3.6 <sup>a</sup>	70.5 ± 8.5 <sup>a</sup>	34.6 ± 2.6 <sup>c</sup>	39.9 ± 2.2 <sup>bc</sup>	55.7 ± 0.8 <sup>ab</sup>	0.0003 *
Geranyl acetone	154.2 ± 12.5 <sup>e</sup>	174.4 ± 11.7 <sup>de</sup>	211.1 ± 16.2 <sup>cd</sup>	234.8 ± 17.8 <sup>bc</sup>	278.1 ± 17.7 <sup>ab</sup>	309.3 ± 19.6 <sup>a</sup>	138.1 ± 10.5 <sup>e</sup>	181.9 ± 11.6 <sup>de</sup>	261.0 ± 19.8 <sup>b</sup>	<0.0001 *
β-Ionone	44.0 ± 3.4 <sup>d</sup>	52.6 ± 4.7 <sup>cd</sup>	67.4 ± 5.2 <sup>bc</sup>	74.9 ± 5.6 <sup>ab</sup>	77.8 ± 4.2 <sup>ab</sup>	86.5 ± 4.6 <sup>a</sup>	44.1 ± 3.3 <sup>d</sup>	50.9 ± 2.7 <sup>d</sup>	83.3 ± 6.2 <sup>a</sup>	<0.0001 *

Different letters in the same columns indicate statistically significant differences ( $p \leq 0.01$ ). CT: Control, Fun: Fungus (*Botrytis cinerea* inoculation), Fun+Thy: Fungus + Thymol, Fun+Cin: Fungus + 1,8-Cineole, Fun+Eug: Fungus + Eugenol, Fun+Cin+Eug: Fungus + 1,8-Cineole + Eugenol, Fun+Thy+Eug: Fungus + Thymol + Eugenol, Fun+Thy+Cin: Fungus + Thymol + 1,8-Cineole, Fun+Thy+Cin+Eug: Fungus + Thymol + 1,8-Cineole + Eugenol. \*, significant at  $p$ -value < 0.05.

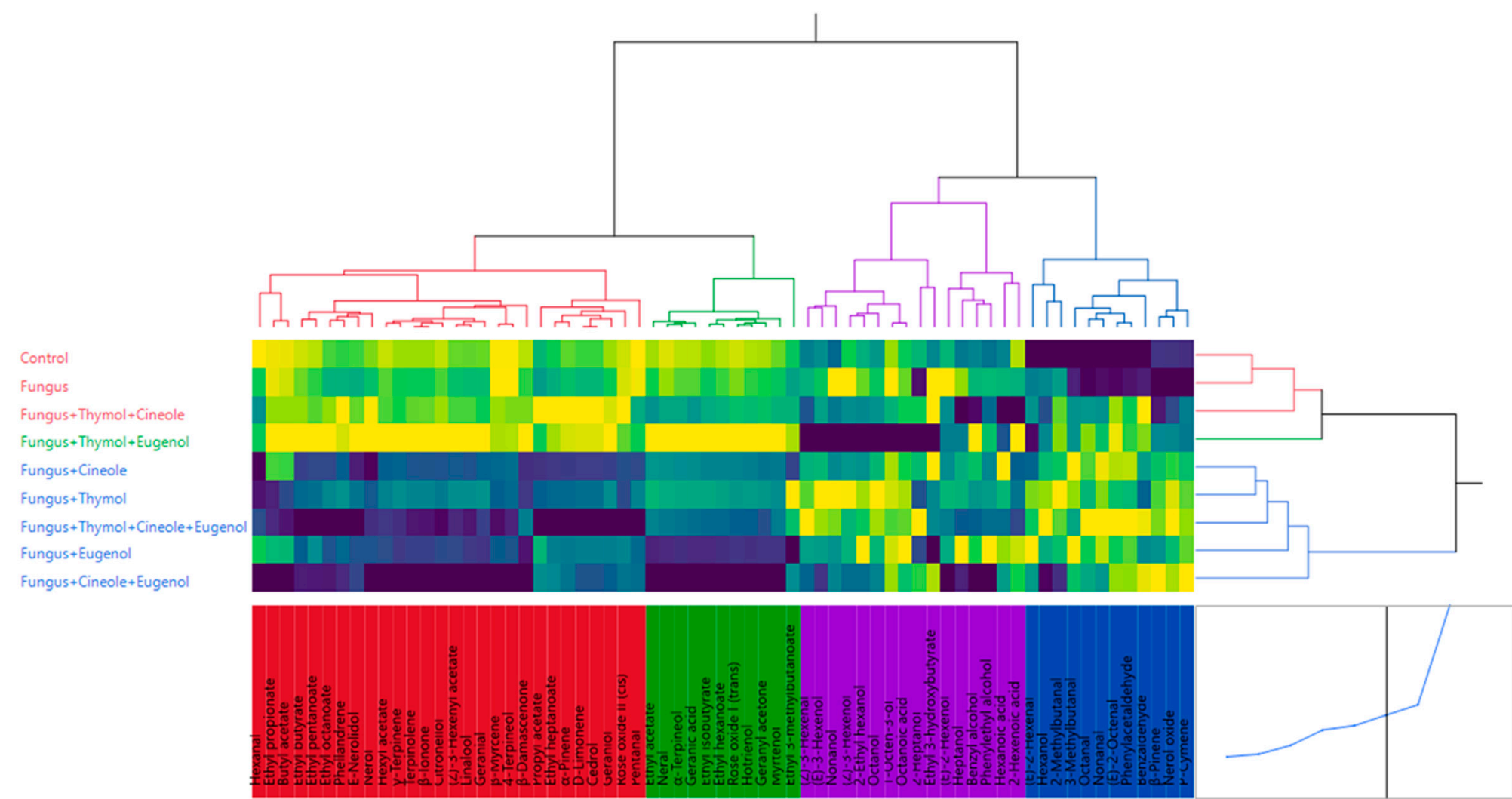
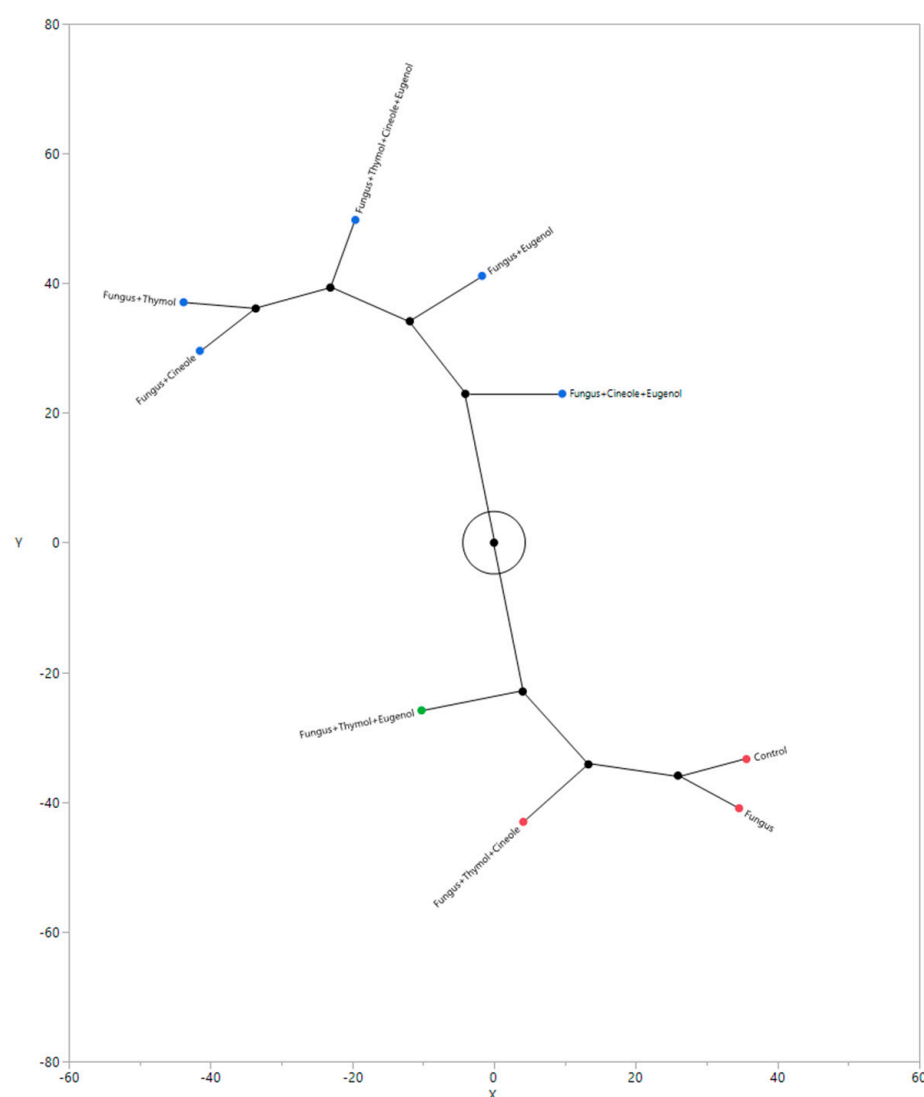


Figure 1. A heatmap analysis that scrutinizes numerous components from volatile organic compounds is demonstrated.



**Figure 2.** CDP 25, 50, 75 values ( $^{\circ}\text{C}$ ,  $X \pm \text{SE}$ ,  $n = 54$ ) detected in volatile organic compounds of essential oils applied to apples ( $m\text{CDP}$ ,  $p \leq 0.01$ ).

#### 4. Discussion

The results presented in Table 1 provide valuable insights into the impact of curative applications of individual and combined EOs on the terpene composition of apples, subsequently influencing their VOC profile. These findings are not only fascinating but also in line with previous research in the field. The underlying mechanism for terpene compositional changes is hypothesized to involve enzyme activation and metabolic pathway modulation in response to essential oil treatments, potentially triggered by the stress response of apple tissues to antimicrobial compounds. Notably, this study reveals that different EO treatments significantly affect the concentrations of various terpenes in apples. For example,  $\alpha$ -Pinene, recognized for its pine-like aroma, exhibited its highest concentration in the Fun+Thy+Cin+Eug treatment, indicating that specific combinations of EOs can enhance the production of this compound (Table 1). This discovery supports earlier research [18] that explored the potential of EO combinations to influence terpene production in plants. Similarly, the elevated levels of  $\beta$ -Pinene, known for its woody and earthy aroma, in the Fun+Thy+Cin+Eug treatment further underscore the capacity of EOs to impact the terpene profile of fruits, as suggested by [19]. Phellandrene, responsible for a minty and citrusy aroma, demonstrated notable increases in the Fun+Thy+Cin+Eug treatment, consistent with previous findings [20] highlighting the role of EOs in enhancing phellan-

drene production in plants. The elevation of D-Limonene levels in the Fun+Thy+Cin+Eug treatment is consistent with existing literature [21], underscoring the potential of EOs to stimulate D-Limonene production in fruits. On the other hand, D-Limonene, with its zesty, citrus-like aroma, is a common terpene in citrus fruits. Neral, contributing to a lemony aroma, showed the highest concentration in the Fun+Cin+Eug treatment (Table 1), in line with [22], which suggests that certain EO combinations may influence neral concentrations in plants. This result has implications for enhancing the fruity scent of apples. Moreover, geraniol, known for its rose-like and fruity aroma, exhibited significant variation across treatments, with the highest concentration in Fun+Thy+Cin+Eug (Table 1). This aligns with the findings of [23], which explored the enhancement of geraniol levels through essential oil treatments, underlining its potential to positively affect the overall scent of apples. E-Nerolidol, contributing a sweet, woody scent, was found in higher concentrations in the Fun+Thy+Cin+Eug treatment (Table 1), aligning with the general understanding that essential oils can influence the presence of E-Nerolidol in plants, as suggested by [24]. Cedrol, which adds a woody and earthy fragrance, exhibited variations in concentrations among treatments, with the Fun+Thy+Cin+Eug group having the lowest levels (Table 1). This consistency with earlier studies [23,25] highlights the influence of essential oils on cedrol production and its potential to contribute to the aroma profile of apples. Lastly, geranic acid, with its rose-like aroma, showed the highest concentration in the Fun+Cin+Eug treatment (Table 1), in agreement with prior research [26] that indicates how specific essential oil treatments can enhance the presence of geranic acid in fruits. Our previous study investigating the protective effect of essential oils determined that the group with the highest terpene content was Thy+Eug+Fun [12]. However, our study investigating the curative effect of essential oils determined that the group with the highest terpene content was Fun+Thy+Cin+Eug. These findings show the potential differences between the protective and curative effects of essential oil combinations of terpene content.

The analysis of ester content in apples after treating them with individual and combined EOs for *B. cinerea* control has provided valuable insights into enhancing apple aroma and flavor. These findings align with existing literature [27,28], emphasizing the influence of essential oils on fruit ester compositions. Notably, ethyl propionate, evoking a pear-like aroma, peaked with the Fun+Cin+Eug combination, underscoring EO treatments' capacity to elevate specific esters [29]. Ethyl acetate, known for its sweet, fruity scent, was most abundant when using the Fun+Cin+Eug combination (Table 2), supporting the concept that EO applications enhance apple fragrance [30]. Similarly, ethyl isobutyrate concentrations were highest in the Fun+Cin+Eug combination, reinforcing the idea of essential oils modifying ester profiles [31]. The predominance of ethyl 3-methylbutanoate in the Fun+Eug group, offering fruity and pineapple-like notes, supports EO treatments' role in increasing ester production [32]. The prevalence of propyl acetate, which imparts fruity, pear-like notes, in the Fun+Thy+Cin+Eug combination aligns with earlier research on EO treatments [26]. In general, our results indicate that the observed variations in esters likely arose from the complex biochemical interactions between essential oils and plant cellular metabolism, potentially activating stress-responsive pathways that trigger enhanced secondary metabolite production and enzymatic transformations. Ethyl butyrate, reminiscent of pineapple, reached its zenith with the Fun+Thy+Cin+Eug combination, highlighting EO combinations' ability to intensify apple's fruity fragrance [32]. Butyl acetate, contributing a banana-like aroma, had its highest concentration in the Fun+Thy+Cin+Eug combination (Table 2), in line with prior research on EO effects [33]. The notable rise in ethyl pentanoate with the Fun+Thy+Cin+Eug combination underscores the potential of combined essential oils to enhance apple fragrance. Hexyl acetate was most abundant in the Fun+Cin+Eug combination, consistent with the understanding that EO treatments influence specific esters [33]. Ethyl hexanoate, contributing a sweet and fruity aroma, was most prominent with the Fun+Cin+Eug combination, aligning with the literature's suggestions on EO impacts on ester profiles [34]. Notably, the significant concentration of ethyl heptanoate with the Fun+Thy+Cin+Eug combination reflects the substantial im-

pect of EO treatments on enhancing its production. Moreover, the highest concentration of (Z)-3-hexenyl acetate in the Fun+Cin+Eug combination aligns with previous research affirming EO effects on ester content [17]. Finally, the peak concentration of ethyl octanoate in the Fun+Thy+Cin+Eug combination highlights EO treatments' ability to affect ester levels, contributing to a richer and more complex apple aroma, consistent with the findings of [33]. In the study on the protective effect of essential oils, it was reported that the Thy+Eug+Fun group had the highest ester content among the treatment groups [12]. On the contrary, in our findings, it was determined that the Fun+Thy+Cin+Eug group had the highest ester content. In addition, while the highest ester concentration was determined in the Thy+Cin+Fun treatment ( $856.9 \mu\text{g L}^{-1}$ ) [12], the highest ester concentration was determined in the Fun+Thy+Cin+Eug ( $715 \mu\text{g L}^{-1}$ ) treatment in this study. Intriguingly, ethyl 3-hydroxybutyrate showed consistent concentrations across multiple treatments, warranting further investigation into its behavior in response to EO treatments, as it exhibits a unique characteristic. These results collectively demonstrate the potential of EO treatments, individually and in combination, to modulate ester compositions in apples, enhancing their aroma and flavor profiles.

The concentration of various C6 compounds and alcohols in apples following treatments against *B. cinerea* is a critical aspect of understanding the impact of these treatments on apple quality and aroma. Comparing these findings with the existing literature can provide insights into the significance of the observed variations in these compounds. The highest hexanal concentrations observed in the Fun+Cin and Fun+Cin+Eug groups are consistent with previous research indicating that essential oil treatments can influence the release of volatile compounds like hexanal, contributing to enhanced aroma profiles in fruits [35,36]. Hexanal, a key contributor to apple aroma, exhibited notable differences among the treatment groups. The minimal variation in (E)-2-hexenal across treatments suggests its stability in the face of essential oil applications, possibly indicating the specificity of essential oils' effects on different C6 compounds. The peaking of (Z)-3-hexenal concentrations in the Fun+Thy+Eug group aligns with the notion that essential oils, particularly cinnamon and fennel oils, have the potential to affect the production of this compound, which is known for its green, leafy notes [37]. The peaking of hexanol concentrations in the Fun+Cin+Eug group is in line with research that demonstrates the capacity of specific essential oil combinations to influence the presence of this alcohol, contributing to the overall aroma complexity of apples [23]. Moreover, the low hexanol concentrations in the control group emphasize the potential of essential oil treatments in maintaining or increasing the levels of these alcohols. 1-octen-3-ol, with its highest concentration in the Fun+Thy+Eug group, suggests that this specific combination of essential oils exerts a more pronounced influence on its levels. 2-heptanol's relatively stable concentrations across treatments support the idea that essential oil applications have a limited impact on this alcohol, consistent with previous findings [38]. Previous research indicates the potential of thyme, cinnamon, and fennel essential oils to modify the concentration of 1-octen-3-ol, thus contributing to apple aroma complexity [39]. The significant variation in 2-ethyl hexanol concentrations across groups, with the highest in the Fun+Thy+Eug group and the lowest in the Fun+Eug group, suggests that these specific essential oil combinations can distinctly impact the production of this alcohol, further enriching the aroma complexity of apples [40]. Heptanol's range of concentrations across treatments, with a peak in the Fun+Thy+Cin group, aligns with the literature highlighting the influence of essential oil combinations on alcohol contents in fruits [41]. In contrast to the study in which it was reported that the protective effect of essential oils had no effect on alcohol content in post-harvest apples [12], it was found in this study that the curative result significantly affected the alcohol content. The reason for this increase can be assumed that the pathogen causes the rise in these components in the curative effect. The varied concentrations of nonanol, octanol, phenylethyl alcohol, and benzyl alcohol across treatments underscore the unique effects of different essential oil combinations on these compounds, which play essential roles in shaping apple aroma profiles [42].

The heatmap, which employs color to represent compound concentrations, serves as a valuable tool for visualizing and interpreting the data. In the context of this paper, darker colors, namely deep blue or purple, denote higher compound levels, while lighter colors, such as green or yellow, indicate lower levels. This visualization effectively conveys the varying degrees of presence of different VOCs across the diverse treatment conditions applied to combat *B. cinerea* in the harvested apple fruits. The application of heatmaps in metabolomics and chemical analysis is well-documented in the literature [23]. These visualizations are instrumental in highlighting the differences in compound abundance among various experimental conditions. Similar to our findings, previous works have used heatmaps to illustrate how specific treatments or interventions may lead to distinct chemical profiles [23,43]. The dendrograms, both vertical and horizontal, play a crucial role in clustering compounds and treatment conditions based on their similarity in expression patterns. In line with our observations, research has demonstrated how dendrograms provide insights into the relationships between compounds and treatments. The listing of compounds at the bottom of the heatmap, categorized by different chemical groups, is consistent with a systematic approach to data presentation. It enables a clear focus on the specific compounds that are central to the investigation, revealing how they are affected by the different treatments. This aligns with the common practice in chemical analysis of categorizing and presenting data for clarity and comparability [40,43]. The supplementary graph (Figure 1) in the bottom right corner of the heatmap likely provides additional temporal or quantitative information. Such complementary information is often included to offer a comprehensive understanding of the data. In line with our approach, the literature emphasizes the importance of providing additional figures to enhance data interpretation and communicate complex findings effectively.

In our Figure 2 plot, the proximity of data points is indicative of their similarity in the original dataset. Conditions that appear close to each other share similar VOC characteristics, while those positioned farther apart have more distinct profiles. For example, the Fungus and Control conditions are positioned close to each other, suggesting a degree of similarity in their profiles. This observation aligns with the concept that untreated apples and apples infected with fungus may exhibit comparable chemical profiles to some extent [33]. The arch-shaped trajectory formed by the conditions labeled in blue, such as Fun+Cin, Fun+Thy, Fun+Eug, and Fun+Thy+Cin+Eug, is indicative of incremental differences in their profiles. This suggests that these treatments follow a gradient of variations, with each condition along the path exhibiting distinct chemical profiles. Such gradual transitions in chemical composition among related treatments are well-documented in the scientific literature [23]. The conditions labeled in green, including Fun+Cin+Eug, Fun+Thy+Cin, and Fun+Thy+Eug, form a distinct cluster on the PCA plot, which implies that these treatments share unique characteristics that set them apart from the other conditions. Clustering in PCA plots is a common representation of groups of conditions or samples with similar chemical profiles or responses to treatments [23,33]. Bridging the chemical insights with economic implications, our findings reveal a compelling narrative of essential oils (EOs) as not just a scientific intervention but a potential game-changer in post-harvest preservation. The nuanced chemical variations observed in our PCA plot directly correlate with the economic potential of these treatments. The distinct clustering and gradual chemical transitions suggest that specific EO combinations could offer targeted preservation strategies, each with unique economic advantages [44]. The economic viability of these essential oil treatments is particularly noteworthy. Our chemical analysis demonstrates that even subtle variations in EO combinations can significantly alter the chemical profile of treated apples. This aligns with economic research indicating that EOs provide cost-effective solutions for producers, with the ability to extend product shelf life and reduce economic losses [45]. The low concentration effectiveness observed in our chemical profiles translates to reduced application costs, making these treatments economically attractive for agricultural stakeholders [46]. Environmentally, the chemical diversity we have mapped shows the potential for residue-free preservation methods. The distinct clusters of EO treatments suggest that these natural compounds can provide robust protection without



the environmental drawbacks of traditional preservatives. This characteristic opens broader market opportunities, allowing for exporters to meet increasingly stringent global agricultural standards [47]. While our initial research and development of these EO combinations may involve significant investment, the long-term economic potential is promising. The sophisticated chemical interactions we have documented through PCA analysis indicate that these treatments are not a one-size-fits-all solution but rather a nuanced approach to post-harvest preservation. The growing consumer demand for natural, organic products further validates the economic potential of our research, positioning EO treatments as a forward-thinking solution in sustainable agriculture.

## 5. Conclusions

In this comprehensive analysis of volatile compounds, ester contents, terpene contents, and the application of various treatments against *B. cinerea* in harvested apples, several significant findings have emerged. The concentrations of various terpenes were notably influenced by the different treatments, with treatments like Fun+Thy+Cin+Eug consistently resulting in the highest terpene levels, particularly for compounds like  $\alpha$ - $\beta$ -Pinene, pinene, and geraniol. Ester contents in the apples displayed a similar trend, with treatments like Fun+Thy+Cin+Eug leading to higher concentrations of esters, such as ethyl butyrate and ethyl octanoate. Moreover, the analysis of volatile compounds unveiled that treatment combinations exerted substantial effects on the levels of compounds, namely various acids, hexanal, and 3-methylbutanal, and with considerable variations observed. The heatmap and PCA plot visualizations provided insights into the differences and similarities among EO treatment conditions, highlighting the distinct profiles associated with specific treatment combinations. In conclusion, this study underscores the remarkable impact of different treatment combinations, particularly Fun+Thy+Cin+Eug, on the modulation of ester, terpene, and VOC contents in harvested apples. These findings not only contribute to a better understanding of the intricate chemical changes occurring in apples in response to pathogen treatments but also offer valuable insights for the development of strategies to enhance apple preservation and quality.

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