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Volatile organic compounds produced by some synthetic essential oils as biological fumigants against *Botrytis cinerea* on apples

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Abstract

Background Gray mold, attributed to *Botrytis cinerea*, poses a substantial threat to food security in fruit-growing regions impacted by global climate change. Addressing this disease requires the utilization of either resilient plant varieties or advanced technological interventions. In this study, the research focused on examining the volatile organic compounds (VOCs) emitted by synthetic essential oils, namely thymol, eugenol, 1,8-cineol, and their combination, as potential biological fumigants against *B. cinerea* on Golden Delicious apples.

Results In this study, a total of 53 compounds were identified and categorized into six distinct classes, which included (1) terpenes, (2) esters, (3) C6 compounds, (4) alcohols, (5) acids, and (6) aldehydes. The results we obtained revealed significant variations in the volatile compounds present in apples after harvest when treated with different essential oils to combat *B. cinerea*. Among the VOCs found in the fruits, the most abundant ones were pentanal, nerol, and ethyl octanoate. The essential oil combination of thymol, eugenol, and 1,8-cineol (Thy + Eug + Fun) had the most significant impact on the volatile compound content in the fruits. Conversely, both *B. cinerea* and the essential oils were observed to increase the volatile organic compound content in the fruits after harvest.

Conclusion The findings from this study underscore the significance of essential oils as effective biological fumigants for countering *Botrytis cinerea* on apples. Furthermore, the study suggests that these essential oils have the potential to influence the composition of volatile organic compounds in postharvest apples. This research offers valuable insights into the intricate interplay between volatile organic compounds and essential oils in apples, emphasizing the critical role of essential oils in preserving fruit quality during the post-harvest period.

Keywords Apple, Gray mold biocontrol, Volatile organic compounds, Thymol, Eugenol, 1,8-cineol

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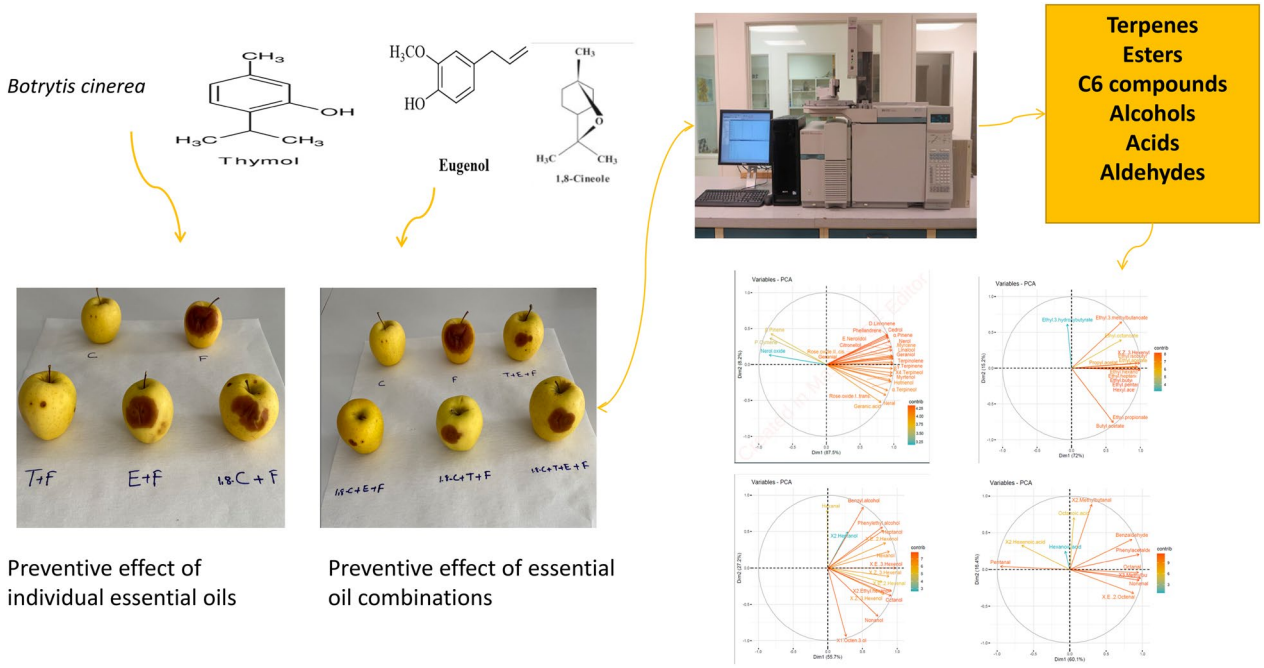
Graphical abstract



Introduction

Malus x domestica Borkhausen, commonly known as apples, holds a distinguished position among the most widely consumed fruits globally. Apples are prized for their rich mineral and vitamin content, as well as their abundance of polyphenols and dietary fiber [1]. They exhibit the advantage of being available throughout the year and can be stored for several months following harvest [2]. However, the storage phase exposes them to various pathogens, with particular attention directed towards fungal species such as *Penicillium expansum* (causing blue mold), *Botrytis cinerea* (responsible for gray mold), and *Alternaria alternata (*associated with brown spot disease) [3]. *B. cinerea* poses a significant threat by compromising the natural defense mechanisms of vegetables and fruits, particularly apples post-harvest, leading to substantial product losses [1, 5]. This pathogen is notoriously challenging to control due to its wide range of host plants and its remarkable resilience in adverse environmental conditions [6]. To mitigate the risk of disease and spoilage in apples following harvest, fungicides and controlled environmental practices are commonly advocated methods.

In light of the adverse effects of fungicides on human health and the environment, there is a growing quest for alternative approaches. Essential oils (EO) have emerged as a viable alternative for both pre- and post-harvest disease control [7, 8]. EOs have gained prominence due to their biodegradability, eco-friendly nature, and the preference of consumers for safer food production [9, 10]. Several studies have reported positive outcomes from employing EOs and their constituent components, derived from plants, in combatting *B. cinerea* [8, 10–13]. Specific EO components such as thymol [14, 15], eugenol [16, 17], and 1,8-cineol [18] have demonstrated antifungal activity against *B. cinerea*. Moreover, our previous research revealed that the components of thymol, eugenol, and 1,8-cineol EO exert protective and curative effects that enhance apple quality in the context of *B. cinerea* infection [19, 20].

B. cinerea is known to produce distinct volatile organic compounds (VOCs), including 1-octen-3-ol, which imparts an earthy, mushroom-like aroma [21]. The analysis of VOCs assumes significance as it can serve as an indicator of the level of fruit infection by *B. cinerea* and its susceptibility to the fungus. Additionally, volatile compounds play a pivotal role in determining fruit flavor and overall quality characteristics [22]. VOCs are low-molecular-weight carbon-based compounds characterized by weak polarities and high vapor pressure, encompassing alcohols, terpenes, fatty acids, aldehydes, esters, C_6 compounds, and C13-nor isoprenoids [23, 24].

These compounds play crucial roles in plant development and act as signaling molecules in response to both biotic and abiotic stresses. However, the biosynthesis of VOCs can be influenced by environmental factors, including agricultural practices, biotic interactions, and seasonal variations [25]. It has been observed that certain metabolites are involved in defense mechanisms by increasing VOC production during the growth of post-harvest pathogens [26]. Recently, research interest has grown around the emission of VOCs by post-harvest biocontrol agents, which could serve not only as biofumigants [27], but also as an alternative approach to disease control in post-harvest storage. Several studies have demonstrated that VOCs with fungistatic properties inhibit *B. cinerea* infection [28–31]. VOCs may exert antifungal effects by disrupting the morphological structure of pathogens, affecting cell membranes and metabolism, akin to the mechanisms of action of EOs [31]. Given that EOs are known to disrupt the plasma membrane structure, integrity, and lipid structures of fungi [14, 32], it is conceivable that VOCs might operate through similar antifungal mechanisms.

In recent years, there has been extensive research into the application of EOs for biological control against *B. cinerea* infection [9, 10, 14]. Nevertheless, while the positive impact of EO components on *B. cinerea* control is acknowledged, there is limited information available regarding their effect on the VOCs contributing to the aroma of the fruit. This study aims to evaluate the potential of three synthetic EOs (thymol, eugenol, and 1,8-cineole) as bio-fungicides against *B. cinerea* and to provide insights into the characterization of VOC composition in treated apples following harvest.

Materials and methods

Fruit materials

The experiment was conducted with "Golden Delicious" apples harvested from orchard (39°75′E, 39°36′N. 1309 m asl). in Erzincan in September (temperature 25 °C and 30.0% humidity). Commercial ripe fruits, free from physical damage, uniform size, and pathogen infection were used for the experiments.

Pathogenic strain

In our previous study (unpublished data), *B. cinerea* isolate was obtained from the vineyard of Üzümlü District of Erzincan. For the identification of the fungus in 18S rRNA gene-based PCR test, primer sets 515f and 806r containing regions V3-V4 were analyzed by RefGen Biotechnology Company, (https://www.refgen.com) Ankara (Turkiye). Molecular diagnose were performed by comparing the 18S rRNA gene region sequences detected in the study with the 18S rRNA gene regions identified in previous studies. *B. cinerea* isolates numbered MF7413141, MH997908, MK562062, and MH782039 obtained from the Genbank database were used for molecular identification. The tree was built using the UPGMA method, created with the MEGA v.5 program using the Jukes-Counter model. Before the experiments, *B. cinerea* was incubated in PDA (Potato dextrose agar) medium for 7 days at 25 °C in a constant temperature incubator.

Chemicals

The chemicals used in this study, i.e., 1,8-cineole (Aldrich 183164), eugenol (Fluka 45980) and thymol (Aldrich C121452) were purchased from Sigma-Aldrich (Shanghai, China), and long term stored in the dark at 4 °C.

Fruit inoculation and storage

A mixture of 2% (v/v) Tween-20 and 88% sterile pure water was prepared in a beaker, and then 10% essential oil was added to this mixture to dissolve each essential oil. 10% stock solution was prepared, and then, this solution was added to 400 mL water after 5 mL of the stock solution was taken. Firstly, apples were washed in 10 mL sodium hypochlorite solution for 5 min, rinsed with tap water, and dried at room temperature. Two wounds at the equator of disinfected apple fruits (3 mm wide and 3 mm deep) were made by using a sterile puncture needle [33]. The following applications were set up in trial for protective; control (distilled water), *B. cinerea* (spore suspension of the pathogen; 1×10^5 conidia mL^{-1}); 1.25 µL, thymol; 1.25 µL, eugenol; 1.25 µL, 1,8-cineole; 1.25 µL, Thymol + Fungus; 1.25 µL, Cineole + Fungus; 1.25 µL, Eugenol + Fungus; 1.25 µL, Cineol + Eugenol + Fungus; 2.5 µL, Thymol + Eugenol + Fungus; 2.5 µL, Thymol + Cineole + Fungus; 2.5 µL Thymol + Cineole + Eugenol + Fungus; 3.75 µL. The most appropriate dose for combinations in the trial was determined by considering our previous preliminary study results due to individually applied concentrations causing deformation in the fruit peel when applied in combination (unpublished data). The experiment consisted of 11 protective treatments. Each treatment was replicated three times with three apples per repetition in a completely randomized design. As a protective effect, the fruits were dipped in EO solutions and incubated for 30 min. Then, it dried at room temperature for 24 h. Spores of 7–10 days were scraped with a sterile glass swab into 5 mL sterilized pure water. After that, the spore suspension was passed through a sterile muslin sheet. The concentration of the conidial suspension was adjusted to 1×10^5 conidia/ mL using a hemocytometer. Subsequently, wound sites were inoculated using 125 µL of a conidial suspension of *B. cinerea* at 1×10^5 spores/ mL. The fruits were placed in a storage room, inside

transparent plastic boxes incubated at + 4 °C with high humidity (90 ± 5%). The fruits were incubated in the dark for 1 week at 4 °C in 90 ± 5% humidity. Fruits infected were checked 7 days after incubation [34].

Chemicals and reagents

Sodium chloride (NaCl), glucose, citric acid, tartaric acid, sodium hydroxide (NaOH) and sodium dihydrogen phosphate were obtained from Sigma-Aldrich (Millipore, Bedford, MA, USA). Ethanol, methanol, dichloromethane (Millipore, Bedford, MA, USA), and the following chemical standards (in quantification and identification) for analysis were obtained from Sigma-Aldrich (St. Louis, MO): 1-octanol (99.0%), 3-methyl-1-butanol (99.0%), 1-octen-3-ol (98.0%), benzyl alcohol (98.0%), octanoic acid (99.0%), 2-methylpropanoic acid (99.5%), 2-ethyl-1-hexanol (99.0%), benzaldehyde (99.0%), heptanoic acid (99.0%), 1-nonanol (99.0%), benzeneacetaldehyde (90.0%), (E)-2-hexenal (98.0%), hexanal (98.0%), methyl salicylate (99.0%), nonanal (95.0%), hexanoic acid (99.5%), ethyl acetate (99.8%), diethyl succinate (99.0%), limonene (97.0%), ethyl phenylacetate (99.0%), neral (95.0%), octanal (99.0%), *p*-cymene (98.0%), 6-methyl-5-hepten-2-one (99.0%), ethyl nonanoate (98.0%), acetoin (96.0%), *α*-terpineol (90.0%), linalool (97.0%), geranylacetone (containing 35% nerylacetone), geraniol (99.5%), terpinolene (97.0%), *β*-citronellol (95.0%), hotrienol (97.0%), geranic acid (85.0%), *β*-damascenone (> 90.0%), 3-ethyl-2,5-dimethyl pyrazine (98.0%), 2-ethyl-6-methylpyrazine (99.5%), 4-methyl-2-pentanol (98.0%, internal standard) phenol (99.9%), furfural (99.5%), naphthalene (99.0%), 2,3-diethylpyrazine (98.0%), and 5-methyl-2-furfural (99.0%). Additionally, glycosidase AR2000 (Rapidase) was obtained from cleanert PEP-SPE (200 mg 6 mL^{-1}) (Millipore, Bedford, MA, USA), and DSM Food Specialties (France).

Sample pretreatment

In the present work, each treatment was prepared in triplicate and 3 apples were kept in water at 4 °C. It was homogenized in a blender and softened for 240 min. Until all the supernatant was obtained, the apple pulps were centrifuged three times for 10 min at 8.000 rpm at 4 °C.

Preparation of free—and bound—form volatiles

VOCs of apple was extracted by HS-SPME (headspace solid phase micro-extraction) and detected by GC–MS (gas chromatography–mass spectrometry). The extraction of VOCs was carried out utilizing the previously optimized method by Wen et al. [35] and Wang et al. [36]. The Cleanert PED-SEP column was activated by 10 mL water and 10 mL methanol separately, before 1 mL supernatant was added, and the samples were tested in triplicate. Then, most of the free-form volatiles for samples were washed out with 5 mL dichloromethane, and acid and sugar were removed by 5 mL water. Finally, it was collected in a 50 mL flask using 20 mL of methanol to elute the glycosidically bound volatiles. The solvent for samples was then removed by decreased pressure distillation in 30 °C water bath to obtain the bound-form VOCs. Afterward, 5 mL of phosphate/citrate (2 M) buffer solution at pH 5 was added into the flask, the bound-form VOCs were enzymatically hydrolyzed with the action of 100 mg L^{-1} in 2 M citrate/phosphate buffer at pH 5.0 (100 µL AR2000) for 16 h at 40 °C in an incubator [35, 37].

SPME conditions

The extraction of the free and bound-form VOCs in apples was detected with the following conditions: 5 mL of sample and 10 µL 4-methyl-2-pentanol (1.0018 mg L^{-1}) were blended in a 15 mL vial containing a magnetic stirrer. Then, 1.3 g NaCl was added, and the vial was capped. Samples were then equilibrated at 60 °C for 40 min on agitation and a heating platform. The extraction coating fiber of CAR/PDMS/DVB was then placed in the headspace for 40 min with continuous agitation and heating to remove volatiles in the samples. The extraction fiber was then sucked directly into the GC injection port for 8 min.

GC–MS analysis

For identification and separation in samples was determined with a 0.25 mm × 60 m id. HP-INNOWAX capillary column with a 0.25 mm film thickness in an Agilent 7890 GC equipped with an Agilent 5975 MS (Scientific, Folsom, CA). The GC–MS temperature conditions were used from the procedure published by Wu et al. [38]. In the unpartitioned GC inlet mode, helium was utilized as the carrier gas at a flow rate of 1 mL min^{-1}. Then it was raised to 220 °C at 3 °C min^{-1} after the oven temperature was set up at 50 °C (for 1 min), and it held at 220 °C for 5 min, and after, temperature of oven was raised from 220 to 250 °C at 5 °C min^{-1} and after it was held at 250 °C for 5 min. Mass spectra were determined in the electron impact (EI) mode source temperature (230 °C and ionisation energy, 70 eV). The acquisition was in full-scan mode (mass range m/z 20–450) and in selective ion mode under auto tune conditions. RI (the retention indices) were calculated by the retention time (RT) of a C7–C24 n-alkane series (Supelco, Bellefonte, PA) under the same chromatographic conditions. Mass spectra defined according to the RI of the current standards after it were compared with the NIST08 library. Tentative identifications were

Table 1 Terpene contents (μg L^{-1}) of harvested apples of protective applications of individuals and combinations of EOs against *B. Cinerea* (X ± SE)

Compounds	CT	Fun	Thy + Fun	Cin + Fun	Eug + Fun	Cin + Eug + Fun	Thy + Eug + Fun	Thy + Cin + Fun	Thy + Cin + Eug + Fun	*p*-value
Terpenes										
α-Pinene	15.6 ± 1.2^{h}	17.3 ± 1.3^{f}	14.7 ± 4.7^{j}	17.1 ± 5.5^{g}	27.3 ± 8.8^{d}	32.2 ± 2.4^{b}	30.4 ± 9.8^{c}	35.8 ± 2.7^{a}	19.4 ± 6.3^{e}	0.0007
β-Pinene	42.4 ± 13.3	47.1 ± 14.8	39.4 ± 19.7	23.3 ± 11.6	18.9 ± 9.4	22.6 ± 7.1	21.0 ± 10.5	25.2 ± 7.9	20.1 ± 10.0	0.0599
Phellandrene	63.1 ± 14.8^{h}	70.9 ± 16.6^{f}	60.1 ± 6.6^{j}	68.0 ± 7.5^{g}	108.8 ± 12.0^{d}	128.3 ± 30.1^{b}	122.0 ± 13.4^{c}	143.9 ± 33.7^{a}	76.6 ± 8.4^{e}	< 0.0001
β-Myrcene	10.4 ± 0.4^{h}	10.2 ± 0.4^{i}	10.7 ± 0.6^{g}	19.7 ± 1.1^{f}	34.2 ± 1.9^{a}	32.8 ± 1.1^{c}	33.6 ± 1.8^{b}	32.1 ± 1.1^{d}	24.9 ± 1.4^{e}	< 0.00001
D-Limonene	20.1 ± 1.2^{h}	22.6 ± 1.4^{f}	19.2 ± 1.7^{i}	21.1 ± 1.9^{g}	32.9 ± 3.0^{d}	38.7 ± 2.4^{b}	37.0 ± 3.4^{c}	43.6 ± 2.8^{a}	23.5 ± 2.1^{e}	< 0.00001
γ-Terpinene	64.4 ± 2.8^{i}	71.6 ± 3.1^{h}	77.9 ± 2.0^{g}	86.6 ± 2.2^{f}	132.5 ± 3.5^{c}	121.7 ± 5.3^{d}	147.3 ± 3.8^{a}	135.4 ± 5.8^{b}	96.3 ± 2.5^{e}	< 0.00001
P-Cymene	40.3 ± 3.2^{b}	45.1 ± 3.6^{a}	40.1 ± 1.2^{c}	23.1 ± 0.7^{e}	18.6 ± 0.5i	21.0 ± 1.6^{f}	20.9 ± 0.6^{g}	23.5 ± 0.6^{d}	19.8 ± 1.9^{h}	< 0.00001
Terpinolene	3.6 ± 0.3^{i}	4.0 ± 0.3^{h}	4.2 ± 0.1^{g}	4.7 ± 0.2^{f}	7.2c ± 0.3	6.9 ± 0.6^{d}	8.0 ± 0.3^{a}	7.7 ± 0.6^{b}	5.2 ± 0.2^{e}	< 0.00001
Rose oxide II (cis)	25.1 ± 5.0^{g}	25.4 ± 4.9^{h}	23.2 ± 13.6^{i}	42.9 ± 2.5^{f}	74.4 ± 43.5^{c}	81.4 ± 15.9^{a}	72.9 ± 42.6^{d}	79.8 ± 15.6^{b}	54.1 ± 31.6^{e}	0.03830
Rose oxide I (trans)	13.7 ± 0.8^{i}	15.2 ± 0.1^{h}	20.1 ± 0.7^{g}	24.4 ± 0.6^{f}	40.8 ± 0.8^{b}	31.0 ± 1.2^{d}	45.4 ± 1.8^{a}	34.5 ± 1.4^{c}	28.1 ± 2.0^{e}	< 0.00001
Nerol oxide	1.6 ± 0.1^{c}	1.7 ± 0.1^{b}	1.9 ± 0.1^{a}	1.1 ± 0.0^{d}	0.1 ± 0.0^{h}	0.9 ± 0.0^{i}	1.0 ± 0.0^{e}	0.1 ± 0.0^{g}	0.1 ± 0.0^{f}	< 0.00001
Linalool	4.1 ± 0.2^{i}	4.7 ± 0.2^{h}	4.9 ± 0.2^{g}	5.0 ± 0.2^{f}	7.6 ± 0.4^{c}	7.3 ± 0.4^{d}	8.6 ± 0.4^{a}	8.3 ± 0.4^{b}	5.5 ± 0.2^{e}	< 0.00001
4-Terpineol	1.2 ± 0.1^{h}	1.2 ± 0.1^{i}	1.3 ± 0.0^{g}	2.5 ± 0.1^{f}	4.4 ± 0.2^{a}	3.9 ± 0.3^{c}	4.3 ± 0.2^{b}	3.9 ± 0.3^{d}	3.2 ± 0.1^{e}	< 0.00001
Hotrienol	16.2 ± 1.8^{i}	18.0 ± 2.1^{h}	22.5 ± 1.3^{g}	25.0 ± 1.5^{f}	38.2 ± 2.3^{b}	30.7 ± 3.5^{d}	42.5 ± 2.5^{a}	34.2 ± 3.9^{c}	27.8 ± 1.6^{e}	< 0.00001
Neral	1.5 ± 0.0^{i}	1.6 ± 0.0^{h}	2.6 ± 0.1^{g}	3.2 ± 0.2^{f}	5.4 ± 0.3^{b}	3.4 ± 0.1^{e}	6.0 ± 0.4^{a}	3.8 ± 0.1^{c}	3.7 ± 0.2^{d}	< 0.00001
α-Terpineol	2.5 ± 0.1^{i}	2.8 ± 0.1^{h}	3.9 ± 0.2^{g}	4.3 ± 0.2^{f}	6.6 ± 0.3^{b}	4.7 ± 0.2^{e}	7.3 ± 0.4^{a}	5.2 ± 0.2^{c}	4.8 ± 0.2^{d}	< 0.00001
Geranial	2.9 ± 0.3^{i}	3.3 ± 0.4^{h}	3.6 ± 0.3f	3.8 ± 0.3^{f}	5.7 ± 0.5^{c}	5.2 ± 0.6^{d}	6.4 ± 0.5^{a}	5.9 ± 0.7^{b}	4.1 ± 0.3^{e}	< 0.00001
Citronellol	31.2 ± 4.5^{i}	34.5 ± 5.0^{h}	35.1 ± 2.7g	42.6 ± 3.3^{f}	71.3 ± 5.6^{c}	70.1 ± 10.2^{d}	79.3 ± 6.2^{a}	77.9 ± 11.3^{b}	49.1 ± 3.8^{e}	< 0.00001
Myrtenol	103.8 ± 21.8^{i}	115.4 ± 24.2^{h}	138.7 ± 19.9^{g}	154.3 ± 22.2^{f}	235.9 ± 33.9^{b}	196.3 ± 41.2^{d}	262.3 ± 37.7^{a}	218.3 ± 45.8^{c}	171.5 ± 24.7^{e}	< 0.0001
Nerol	140.9 ± 26.9^{i}	159.5 ± 30.5^{g}	142.2 ± 15.5^{h}	161.0 ± 17.6^{f}	264.3 ± 28.9^{d}	296.4 ± 56.7^{c}	299.2 ± 32.7^{b}	335.5 ± 64.2^{a}	182.2 ± 19.9^{e}	< 0.00001
Geraniol	32.8 ± 8.5^{i}	36.6 ± 9.5^{g}	34.6 ± 6.7^{h}	38.5 ± 7.4^{f}	58.8 ± 11.4^{d}	62.1 ± 16.1^{c}	65.5 ± 12.6^{b}	69.1 ± 17.9^{a}	42.8 ± 8.5^{e}	0.0027
E-Nerolidol	167.9 ± 27.8^{i}	190.1 ± 28.6^{g}	189.4 ± 30.1^{h}	196.2 ± 34.1^{f}	294.6 ± 36.1^{d}	295.6 ± 37.4^{c}	333.5 ± 56.1^{b}	334.6 ± 53.0^{a}	214.3 ± 63.3^{e}	0.0007
Cedrol	37.1 ± 2.3^{h}	41.7 ± 2.6^{f}	35.5 ± 3.2^{j}	38.9 ± 3.6^{g}	60.8 ± 5.6^{d}	71.6 ± 6.3^{b}	68.4 ± 5.1^{c}	80.5 ± 3.1^{a}	43.4 ± 2.5^{e}	< 0.00001
Geranic acid	25.4 ± 2.1^{i}	28.3 ± 2.3^{h}	47.1 ± 4.4^{g}	52.4 ± 14.7^{e}	80.0 ± 16.4^{b}	48.1 ± 25.0^{f}	89.0 ± 3.9^{a}	53.5 ± 27.9^{d}	58.2 ± 18.2^{c}	0.0018

Different letters in the same column indicate statistically significant differences ($p \leq 0.01$)

Table 2 Esters contents (μg L^{-1}) of harvested apples of protective applications of individuals and combinations of EOs *against B. Cinerea* (X ± SE)

Compounds	CT	Fun	Thy + Fun	Cin + Fun	Eug + Fun	Cin + Eug + Fun	Thy + Eug + Fun	Thy + Cin + Fun	Thy + Cin + Eug + Fun	*p*-value
Esters										
Ethyl acetate	24.6 ± 2.0^{i}	27.9 ± 2.3^{h}	41.7 ± 4.1^{g}	43.2 ± 3.7^{f}	64.9 ± 3.8^{b}	43.3 ± 6.5^{e}	73.4 ± 5.7^{a}	49.0 ± 3.6^{c}	47.2 ± 4.2^{d}	< 0.00001
Ethyl propionate	30.6 ± 5.5^{i}	34.0 ± 6.2^{h}	44.5 ± 11.9^{g}	49.5 ± 13.3^{f}	192.9 ± 9.5^{a}	176.3 ± 38.5^{b}	84.1 ± 22.6^{d}	64.4 ± 11.7^{e}	140.3 ± 6.9^{c}	< 0.00001
Ethyl isobutyrate	38.9 ± 7.1^{i}	44.1 ± 8.1^{h}	57.0 ± 9.5^{g}	59.0 ± 9.9^{f}	88.6 ± 14.8^{b}	68.6 ± 12.6^{d}	100.3 ± 16.8^{a}	77.6 ± 14.2^{c}	64.5 ± 10.8^{e}	< 0.0001
Propyl acetate	45.0 ± 2.4^{h}	50.1 ± 2.6^{f}	43.1 ± 16.9^{i}	47.9 ± 18.8^{g}	73.3 ± 28.8^{d}	85.1 ± 4.5^{b}	81.5 ± 3.2^{c}	94.7 ± 5.0^{a}	53.3 ± 21.0^{e}	0.0125
Ethyl butyrate acetate	61.0 ± 13.8^{i}	67.9 ± 15.4^{h}	71.5 ± 8.3^{g}	86.9 ± 10.1^{f}	145.3 ± 16.8^{c}	138.0 ± 31.2^{d}	161.6 ± 18.7^{a}	153.5 ± 34.7^{b}	100.2 ± 11.6^{e}	< 0.00001
Ethyl 3-methylbutanoate	31.5 ± 3.9^{h}	35.1 ± 4.4^{g}	37.4 ± 1.1^{e}	41.6 ± 12.2^{c}	41.2 ± 2.9^{d}	36.2 ± 3.0^{f}	70.8 ± 20.8^{a}	66.4 ± 8.3^{b}	30.0 ± 2.1^{i}	0.0003
Butyl acetate	13.9 ± 2.5^{i}	15.5 ± 2.8^{h}	20.3 ± 5.4^{g}	22.6 ± 6.0^{f}	87.9 ± 4.3^{a}	80.3 ± 17.5^{b}	38.3 ± 10.3^{d}	29.3 ± 5.3^{e}	63.9 ± 3.1^{c}	< 0.00001
Ethyl pentanoate	42.4 ± 5.3^{i}	47.2 ± 5.9^{h}	50.3 ± 14.8^{g}	61.2 ± 1.8^{f}	102.3 ± 30.2^{c}	95.9 ± 12.1^{d}	113.7 ± 33.5^{a}	106.6 ± 13.4^{b}	70.5 ± 20.8^{e}	0.0006
Ethyl hexanoate	12.0 ± 2.2^{i}	13.4 ± 2.4^{h}	17.5 ± 4.7^{g}	19.4 ± 5.2^{f}	29.7 ± 8.0^{b}	22.7 ± 4.1^{d}	33.0 ± 8.9^{a}	25.3 ± 4.6^{c}	21.6 ± 5.8^{e}	0.0027
Hexyl acetate	40.3 ± 12.6^{i}	44.8 ± 1.4^{h}	49.1 ± 12.5^{g}	59.7 ± 15.2^{f}	99.9 ± 25.4^{c}	91.1 ± 28.2^{d}	111.0 ± 31.6^{a}	101.3 ± 17.4^{b}	68.8 ± 28.4^{e}	0.0032
(Z)-3-Hexenyl acetate	79.8 ± 4.2^{i}	90.3 ± 4.8^{h}	93.7 ± 9.8^{g}	97.0 ± 10.1^{f}	145.8 ± 15.2^{c}	140.5 ± 7.5^{d}	165.0 ± 17.2^{a}	159.0 ± 8.5^{b}	106.0 ± 11.1^{e}	< 0.00001
Ethyl heptanoate	184.7 ± 15.1^{i}	205.4 ± 16.8^{g}	192.5 ± 85.4^{h}	214.1 ± 130.7^{f}	327.4 ± 28.6^{d}	349.3 ± 145.3^{c}	364.1 ± 31.8^{b}	388.4 ± 95^{a}	238.1 ± 76.9^{e}	0.029
Ethyl octanoate	429.9 ± 39.4^{i}	486.7 ± 44.6^{g}	481.3 ± 31.7^{h}	498.4 ± 32.7^{f}	748.6 ± 49.2^{d}	757.0 ± 69.4^{c}	847.4 ± 55.7^{b}	856.9 ± 78.6^{a}	544.4 ± 35.8^{e}	< 0.00001
Ethyl 3-hydroxybutyrate	173.4 ± 13.5^{b}	113.1 ± 5.0^{d}	265.9 ± 25.6^{a}	265.9 ± 25.6^{a}	123.4 ± 6.7^{c}	173.4 ± 13.5^{b}	265.9 ± 25.6^{a}	113.1 ± 5.0^{d}	123.4 ± 6.7^{c}	< 0.00001

Different letters in the same column indicate statistically significant differences ($p \leq 0.01$). ns; not significant

Table 3 C6 compounds and alcohols contents (μg L^{-1}) of harvested apples of protective applications of individuals and combinations of EOs *against B. Cinerea* (X ± SE)

Compounds	CT	Fun	Thy + Fun	Cin + Fun	Eug + Fun	Cin + Eug + Fun	Thy + Eug + Fun	Thy + Cin + Fun	Thy + Cin + Eug + Fun	*p*-value
C6 compounds										
Hexanal	4.3 ± 0.6^{ns}	4.8 ± 0.1	4.9 ± 0.1	5.3 ± 1.0	5.0 ± 0.3	4.2 ± 0.3	5.2 ± 0.3	5.2 ± 0.5	5.4 ± 0.7	0.1018
(Z)-3-Hexenal	1.8 ± 0.4^{ns}	1.7 ± 0.1	1.7 ± 0.0	1.8 ± 0.1	1.5 ± 0.0	1.8 ± 0.4	1.7 ± 0.3	1.6 ± 0.2	2.0 ± 0.0	0.1837
(E)-2-Hexenal	2.4 ± 0.4^{ns}	2.2 ± 0.2	2.0 ± 0.3	2.1 ± 0.4	1.8 ± 0.1	2.2 ± 0.2	1.9 ± 0.1	2.2 ± 0.5	2.2 ± 0.4	0.4204
Hexanol	3.5 ± 0.3^{ab}	3.2 ± 0.6^{c}	3.0 ± 0.0^{c}	3.3 ± 0.3^{bc}	2.7 ± 0.4^{d}	3.0 ± 0.1^{c}	3.0 ± 0.1^{c}	3.0 ± 0.3^{c}	3.8 ± 0.1^{a}	0.0284
(E)-3-Hexenol	1.6 ± 0.3^{ns}	1.4 ± 0.1	1.5 ± 0.0	1.5 ± 0.1	1.3 ± 0.0	1.6 ± 0.3	1.5 ± 0.3	1.6 ± 0.2	1.8 ± 0.3	0.4260
(Z)-3-Hexenol	0.4 ± 0.1^{ns}	0.4 ± 0.0	0.4 ± 0.0	0.3 ± 0.3	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.5968
(E)-2-Hexenol	1.3 ± 0.3^{ns}	1.2 ± 0.2	1.3 ± 0.0	1.4 ± 0.0	1.1 ± 0.1	1.2 ± 0.2	1.3 ± 0.3	1.4 ± 0.3	1.4 ± 0.1	0.5365
Alcohols										
2-Heptanol	0.4 ± 0.0^{ns}	0.56 ± 0.16	0.5 ± 0.0	0.4 ± 0.2	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.2	0.5 ± 0.0	0.7 ± 0.2	0.1627
1-Octen-3-ol	0.6 ± 0.1^{bc}	0.56 ± 0.02^{bc}	0.5 ± 0.0^{bc}	0.6 ± 0.0^{bc}	0.5 ± 0.0^{c}	1.8 ± 1.0^{a}	0.5 ± 0.0^{bc}	0.6 ± 0.0^{bc}	0.6 ± 0.1^{b}	0.0106
Heptanol	5.9 ± 1.2^{b}	5.07 ± 0.07^{cd}	4.9 ± 0.4^{de}	5.3 ± 0.6^{c}	4.4 ± 0.2^{f}	4.4 ± 0.2^{f}	4.7 ± 0.0^{e}	5.6 ± 0.5^{b}	6.2 ± 1.1^{a}	0.0246
2-Ethyl hexanol	1.4 ± 0.2^{abc}	1.24 ± 0.01^{abc}	1.2 ± 0.1^{bc}	1.3 ± 0.2^{abc}	1.0 ± 0.0^{c}	1.5 ± 0.3^{ab}	1.3 ± 0.2^{abc}	1.3 ± 0.0^{abc}	1.6 ± 0.1^{a}	0.0479
Octanol	1.4 ± 0.2^{ns}	1.28 ± 0.02	1.2 ± 0.1	1.3 ± 0.2	1.0 ± 0.0	1.5 ± 0.3	1.3 ± 0.1	1.3 ± 0.1	1.5 ± 0.3	0.1952
Nonanol	1.3 ± 0.3^{ns}	1.17 ± 0.14	1.2 ± 0.0	1.3 ± 0.0	1.0 ± 0.0	1.6 ± 0.5	1.2 ± 0.1	1.3 ± 0.2	1.4 ± 0.2	0.3462
Benzyl alcohol	3.4 ± 0.7^{c}	3.07 ± 0.24^{e}	3.1 ± 0.0^{e}	3.3 ± 0.2^{d}	2.8 ± 0.1^{f}	2.2 ± 0.5^{g}	3.1 ± 0.5^{e}	3.6 ± 0.3^{b}	3.8 ± 0.2^{a}	0.0021
Phenylethyl alcohol	2.2 ± 0.3^{ns}	2.05 ± 0.02	2.0 ± 0.1	2.1 ± 0.3	1.8 ± 0.0	1.8 ± 0.1	1.9 ± 0.0	2.1 ± 0.4	2.3 ± 0.3	0.0836

Different letters in the same column indicate statistically significant differences ($p \leq 0.01$). ns; not significant

Table 4 Acids and aldehydes contents (μg L^{-1}) of harvested apples of protective applications of individuals and combinations of EOs *against B. Cinerea* (X ± SE)

Compounds	CT	Fun	Thy + Fun	Cin + Fun	Eug + Fun	Cin + Eug + Fun	Thy + Eug + Fun	Thy + Cin + Fun	Thy + Cin + Eug + Fun	*p*-value
Acids										
Hexanoic acid	0.1 ± 0.0^{ns}	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3015
2-Hexenoic acid	0.2 ± 0.0^{ns}	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.4658
Octanoic acid	0.9 ± 0.1^{b}	0.8 ± 0.0^{bc}	0.7 ± 0.1^{bc}	0.8 ± 0.1^{bc}	0.7 ± 0.1^{c}	2.3 ± 1.3^{a}	0.8 ± 0.0^{bc}	0.8 ± 0.2^{bc}	0.8 ± 0.1^{b}	0.0115
Aldehydes										
2-Methylbutanal	0.1 ± 0.0^{ns}	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2574
3-Methylbutanal	6.0 ± 0.2^{a}	5.3 ± 0.2^{b}	3.7 ± 0.1^{f}	5.0 ± 0.1^{c}	4.3 ± 0.1^{d}	3.7 ± 0.0^{e}	3.3 ± 0.0^{g}	2.0 ± 0.1^{h}	1.4 ± 0.3^{i}	< 00001
Pentanal	395.0 ± 22.6^{i}	548.9 ± 19.8^{h}	857.1 ± 35.9^{g}	1550.0 ± 38.9^{f}	1974.0 ± 121.4^{e}	2202.0 ± 19.4^{d}	2720.0 ± 95.0^{c}	3056.0 ± 61.6^{b}	3242.0 ± 154.9^{a}	< 00001
Octanal	7.0 ± 0.2^{a}	6.9 ± 0.1^{b}	6.5 ± 0.1^{c}	5.7 ± 0.1^{d}	5.3 ± 0.2^{e}	4.8 ± 0.1^{f}	4.2 ± 0.1^{g}	4.0 ± 0.2^{h}	3.8 ± 0.3^{i}	< 00001
Nonanal	8.7 ± 0.0^{a}	8.2 ± 0.1^{b}	7.9 ± 0.1^{c}	7.0 ± 0.1^{d}	6.8 ± 0.4^{e}	6.5 ± 0.3^{f}	5.2 ± 0.3^{g}	4.6 ± 0.1^{h}	4.5 ± 0.2^{i}	< 00001
(E)-2-Octenal	10.1 ± 0.1^{a}	9.8 ± 0.1^{b}	9.6 ± 0.2^{c}	9.0 ± 0.4^{d}	8.7 ± 0.2^{e}	8.2 ± 0.3^{f}	5.8 ± 0.2^{g}	5.8 ± 0.1^{h}	5.7 ± 0.1^{i}	< 00001
Benzaldehyde	2.3 ± 0.1^{a}	2.0 ± 0.0^{c}	1.9 ± 0.0^{d}	2.1 ± 0.6^{b}	1.9 ± 0.1^{e}	1.9 ± 0.1^{f}	2.1 ± 0.1^{b}	1.8 ± 0.0^{g}	1.7 ± 0.1^{h}	0.0002
Phenylacetaldehyde	26.8 ± 0.6^{a}	24.7 ± 1.4^{b}	20.5 ± 1.7^{c}	17.8 ± 1.6^{d}	16.8 ± 1.7^{f}	15.5 ± 0.1^{g}	16.8 ± 0.6^{e}	13.0 ± 1.0^{h}	12.4 ± 0.5^{i}	< 00001

Different letters in the same column indicate statistically significant differences ($p \leq 0.01$). ns; not significant

performed by comparison of mass spectra with those in RI reported in the literature and the NIST08 library when reference standards were unavailable.

Quantification and odour activity values (OAVs) calculation

The preparation of the simulated raisin solution was carried out based on the average concentration of sugar and acid in the raisin supernatant. The solution of samples was prepared with distilled water, including 400 g L^{-1} glucose and 5 g L^{-1} tartaric acid. The pH of solution was adjusted to 4.2 with a 1 M NaOH solution. In HPLC grade ethanol, the known concentrations of standard VOCs were individually dissolved. Then, it was added to the solution and the mixed flavor standard solution was diluted 15 times using a simulated apple solution. The aroma standard solution was analyzed in the same way as the sample supernatant after it was extracted. Estimates of VOC concentrations in apples without standards were obtained by considering standards with the same functional group and a similar number of C atoms as VOCs. The VOCs were measured from the characteristic ion peak areas in relation to the internal standard of 4-methyl-2-pentanol (1.0018 mg L^{-1}) [38].

Data analysis

Analyses for the obtained values were carried out utilizing SPSS software (SPSS Version 23, IBM, VA, USA). After the variables of the data were subjected to analysis of variance (ANOVA), the separation of the averages was performed utilizing the Duncan test ($p \leq 0.01$). PCA was performed to determine relationships among variables based on treatments, utilizing the average data in each case.

Results

This study provides information on the effect of applying some synthetic essential oils on the change of VOCs for the prevention of fungal infection under post-harvest storage conditions. A total of 53 compounds were determined in the study and were classified into six classes (Tables 1, 2, 3, 4), namely, terpenes, esters, C6 compounds, alcohols, acids, and aldehydes. There are significant differences in the contents of other terpenes, whereas there is no significant difference in the contents of α-Pinene, β-Pinene, Rose oxide II (cis), Geraniol, E-Nerolidol, and Geranic acid terpenes between applications. It was determined that Thy + Eug + Fun was the most effective on terpene contents (γ-Terpinene, Terpinolene, Rose oxide I (trans), Hotrienol, Linalool, α-Terpineol, Neral, Geranic acid, Geranial, Myrtenol, and Citronellol) among the application groups. The lowest effect on terpene contents was observed in group CT. Among the Nerol application methods, terpene was the most abundant, followed by E-Nerolidol, Myrtenol, and Phellandrene in general. The terpens content ranged from 0.1 (in Nerol oxide for Eug + Fun, Thy + Cin + Fun, Thy + Cin + Eug + Fun) to 335.5 μg L^{-1} (in Nerol for Thy + Cin + Fun). Nerol oxide and 4-Terpineol had the lowest content among terpens in application methods.

Table 2 shows the ester content of the protective application of individuals and combinations of EOs against *B. cinerea* on the harvested apples. All application groups significantly affected the content of esters Ethyl acetate, Ethyl propionate, Ethyl isobutyrate, Ethyl octanoate, Ethyl butyrate acetate, (Z)-3-Hexenyl acetate, Butyl acetate, Ethyl 3-hydroxybutyrate. Propyl acetate, Ethyl 3-methyl butanoate, Ethyl heptanoate, Ethyl pentanoate, Ethyl hexanoate, and Hexyl acetate esters were not affected by the application groups. It was determined that Thy + Eug + Fun was the most effective on ester contents (Ethyl 3-hydroxybutyrate, Ethyl butyrate acetate, Ethyl pentanoate, Ethyl hexanoate, Ethyl acetate Ethyl 3-methyl butanoate, (Z)-3-Hexenyl acetate, Hexyl acetate, and Ethyl isobutyrate) among the application groups. The most abundant esters found in application groups were Ethyl heptanoate, Ethyl 3-hydroxybutyrate, and Ethyl octanoate. In addition, the lowest ester contents were observed in the CT group (Table 2).

The C6 compounds and alcohol contents of individual and combination protective applications of EOs against *B. cinerea* on harvested apples are shown in Table 3. None of the C6 compounds and alcohols were affected by the application group. While the application groups affected only Hexanol from C6 compounds, it affected 1-Octen-3-ol, Benzyl alcohol, 2-Ethyl hexanol, and Heptanol contents from alcohols. Among the application groups, Thy + Cin + Eug + Fun was determined to be the best practice affecting C6 compounds and alcohol contents.

Table 4 also shows the contents of acids and aldehydes. While acids (Hexanoic acid, 2-Hexenoic acid, and Octanoic acid) were insignificant in all application groups, Benzaldehyde and 2-Methylbutanal were found to be trivial in aldehydes. While there is no difference between the application groups in the contents of Hexanoic acid and 2-Hexenoic acid, there is a difference between the application groups in the contents of Octanoic acid. Octanoic acid content was most effective in the Cin + Eug + Fun group (Table 4). 3-Methylbutanal, Octanal, Nonanal, (E)-2-Octenal, Phenylacetaldehyde, and Benzaldehyde aldehyde contents were found to have the highest content in the CT group. The most abundant aldehyde found in application groups were Pentanal.

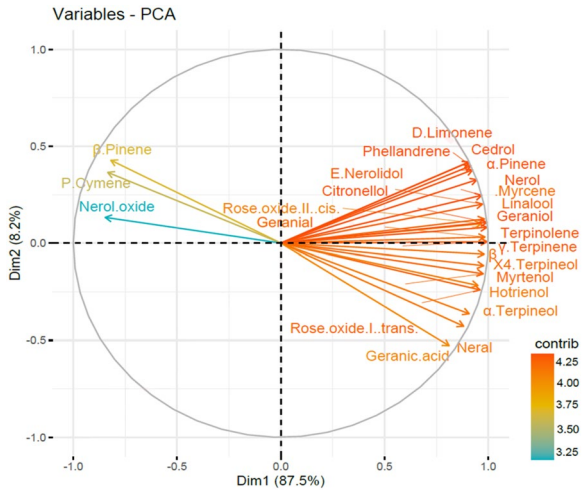


Fig. 1 P.C.A. biplot (score and loadings plots) of terpene contents berries colored by varieties

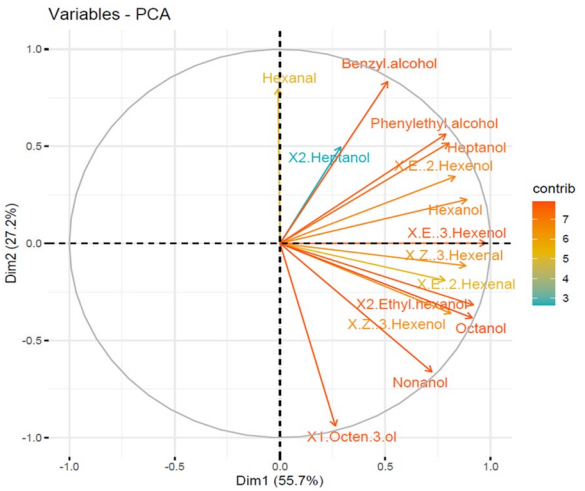


Fig. 3 PCA biplot (score and loadings plots) of C6 compounds and alcohols contents berries colored by varieties

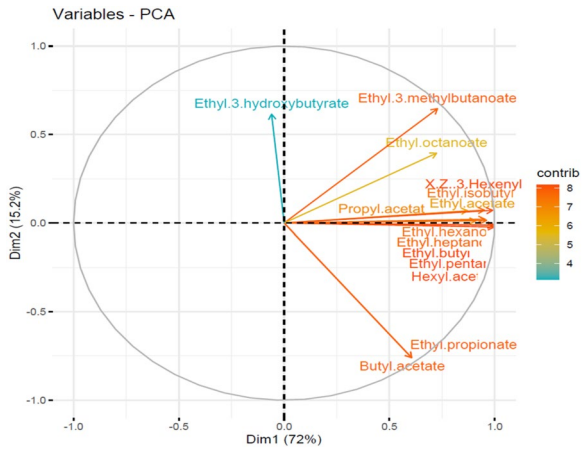


Fig. 2 PCA biplot (score and loadings plots) of esters contents berries colored by varieties

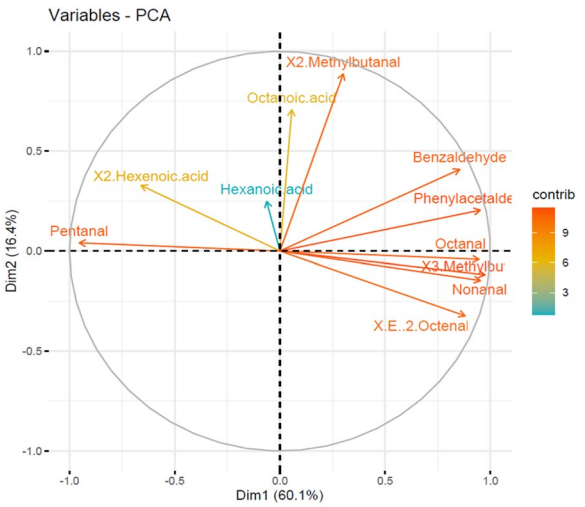


Fig. 4 PCA biplot (score and loadings plots) of acids and aldehydes contents berries colored by varieties

Pentanal was the most abundant aldehyde, and 2-Methylbutanal was the least found aldehyde in the application groups (Table 4).

Tables 1, 2, 3, 4 shows that the Thy + Eug + Fun group was the group that significantly affected the content of terpenes and esters. The most abundant VOCs found in the treated samples were terpene, ester, and aldehyde, such as Pentanal, Ethyl octanoate, and Nerol. Although EOs treatments increased terpene and ester contents compared to CT and Fun treatment, EOs treatments did not significantly affect C6 compounds, alcohols, acids, and aldehydes content compared to the CT group (Tables 1, 2, 3, 4).

Pearson correlation analyses for the VOCs contents (i.e., terpenes, esters, C6 compounds, alcohols, acids, and aldehydes) appear in Figs. 1, 2, 3, 4 for all data sets. Based on Fig. 1, it appears that there is a clear positive correlation between varieties of terpenes. However, it's worth noting that β-Pinene, P-Cymene, and Nerol oxide do not seem to follow this trend. β-Pinene and P-Cymene have a high correlation but Nerol oxide has a low correlation with β-Pinene and P-Cymene (Fig. 1). Looking at the relationship between esters, there is a noticeable negative correlation between Ethyl-3-hydroxybutyrate and all other esters. Ethyl 3-methyl butanoate, Butyl acetate, and Ethyl propionate esters have positive but weak interactions with other esters (Fig. 2). Besides, the positive correlation between C6 components and alcohols is shown

in Fig. 3. Hexanal has a positive but weak interaction with C6 components and alcohols. Similarly, 1-Octen-3-ol, Benzyl alcohol, and 2-Heptanol have a positive but weak interaction with C6 components and alcohols (Fig. 3). Figure 4 shows the correlation of acids and aldehydes. Pentanal correlates negatively with all aldehydes, but Octanal, 3-Methylbutanal, Nonanal, (E)-2-Octenal, Benzaldehyde, and there is a strong correlation between phenylacetaldehyde aldehydes. Acids, on the other hand, show a positive and weak correlation among themselves (Fig. 4).

Discussion

Essential oils (EOs) have demonstrated their potential as biofungicides in the preservation of postharvest apples, particularly in the prevention of diseases [11]. In line with this approach, thymol, eugenol, and 1,8-cineol EOs were applied to apples following harvest, effectively curtailing the growth of *B. cinerea* and mitigating fruit rot. A similar study conducted by Ou-Ani et al. [39] explored the use of the essential oil from *Teucrium luteum* subsp. *flavovirens* against *B. cinerea* in postharvest apples, highlighting it as a promising alternative to synthetic fungicides for gray rot control and the preservation of apple quality. Consistent with our prior findings, the application of individual and combined EOs, namely thymol, eugenol, and 1,8-cineol, resulted in a 100% inhibition of *B. cinerea* growth and an enhancement of quality parameters in postharvest apples [19, 20]. Notably, while studies have evaluated the concentration of volatile compounds in postharvest Golden Delicious apple cultivars treated with EOs (thymol, eugenol, and 1,8-cineol) against *B. cinerea*, this particular research appears to be beyond our knowledge.

In this study, we investigated the impact of individual and combined EOs on gray mold (*B. cinerea*) in postharvest apples with a focus on the volatile organic compounds (VOCs) content in the fruits. A total of 53 VOCs were identified, spanning various categories such as terpenes, esters, C6 compounds, alcohols, acids, and aldehydes (Tables 1, 2, 3, 4). These compounds not only play a pivotal role in shaping the flavor profile of cider but have also been recognized for their potential involvement in *B. cinerea* control [40, 41]. It is worth noting that EO treatments resulted in an overall increase in terpenes, esters, C6 compounds, alcohols, acids, and aldehydes relative to the control group (Tables 1, 2, 3, 4). Conversely, *B. cinerea* infection generally led to an augmentation in the content of terpenes, esters, C6 compounds, alcohols, acids, and aldehydes. Terpenes, which are found in apple peels and contribute positively to the flavor of beverages, exhibited significant variation in abundance among the treatments, with the highest total terpene concentration observed in the Thy + Eug + Fun group apples (Table 1). Irrespective of the applied EO dose, individual or combined, the treated fruits exhibited an increase in terpene content compared to the control group. A study investigating the effect of tea tree EO (*Melaleuca alternifolia*; TTEO) on postharvest rot in strawberries caused by *B. cinerea* reported an increase in terpene content upon TTEO application [10]. Esters, known for their contribution to floral and fruity notes, were notably abundant in the Thy + Eug + Fun group, with compounds like Ethyl octanoate, Ethyl heptanoate, and Ethyl 3-hydroxybutyrate prevailing (Table 2). The highest concentration of esters was detected in the Thy + Cin + Fun application (856.9 µg L-1). In contrast, other studies have reported that decanoic acid ethyl ester, hexanoic acid ethyl ester, and octanoic acid ethyl ester were prevalent in postharvest grapes [42]. Furthermore, some studies have documented a decrease in ester content upon the application of TTEO (*M. alternifolia*) to strawberries [10]. However, our results indicate that the EOs used increased the ester content relative to the control group.

Alcohols, deriving from the degradation of carbohydrates, amino acids, and lipids, typically increase with fruit ripening and infection rate. In our study, no significant change was observed in alcohol content, which aligns with our previous findings demonstrating the effect of EOs on fruit quality [19, 20]. Specifically, the affected alcohols in all administration groups were 1-Octen-3-ol, Heptanol, and 2-Ethyl hexanol (Table 3). Regarding acids, their production is linked to the content of musts and fermentation conditions. In a study investigating the impact of *Eucalyptus staigeriana* EO on *B. cinerea* and *Colletotrichum acutatum* after grape harvest, octanoic acid was noted as significant in all samples [42]. In line with this finding, our results highlight the effectiveness of octanoic acid in all application groups (Table 4). Our study revealed that terpenes and esters were the most prominent families of volatile compounds in postharvest apples. Previous research has underscored the role of terpenes and C6 compounds in aroma properties [37, 38], and it has been shown that the presence of 1,8-cineol in EO-treated grapes impacts the aroma properties of the resulting wines [42]. It is plausible that the EOs we applied contribute to the aromatic profile of the fruits.

The Pearson correlation analyses conducted on the volatile organic compounds (VOCs) contents, which encompassed terpenes, esters, C6 compounds, alcohols, acids, and aldehydes, have provided valuable insights into the relationships among these compounds

(Figs. 1, 2, 3, 4). These findings help elucidate the complex interactions and dependencies within the volatile profile of postharvest apples. Figure 1 illustrates a clear positive correlation among various terpenes, suggesting that they tend to co-occur and positively influence each other's presence. However, it's noteworthy that β-Pinene, P-Cymene, and Nerol oxide do not entirely conform to this trend. β-Pinene and P-Cymene exhibit a strong positive correlation, indicating that they are often found together in the volatile profile. In contrast, Nerol oxide shows a lower correlation with β-Pinene and P-Cymene, suggesting that its presence might be influenced by different factors or pathways. The relationship among esters, as depicted in Fig. 2, reveals a noticeable negative correlation involving Ethyl-3-hydroxybutyrate and all other esters. This suggests that the presence of Ethyl-3-hydroxybutyrate is inversely related to the abundance of other esters in the VOC profile. On the other hand, Ethyl 3-methyl butanoate, Butyl acetate, and Ethyl propionate esters exhibit positive, albeit weak, correlations with other esters. This indicates that while they tend to co-occur with other esters, the relationship is not particularly strong. Figure 3 highlights a positive correlation between C6 components and alcohols. This implies that these two groups of compounds often appear together and positively influence each other's presence. However, it's important to note that Hexanal exhibits a weak positive correlation with C6 components and alcohols, suggesting that its presence is influenced by factors other than C6 compounds and alcohols. Similarly, 1-Octen-3-ol, Benzyl alcohol, and 2-Heptanol also show weak positive correlations with C6 components and alcohols, indicating that they may have additional factors affecting their presence. In Fig. 4, the correlation of acids and aldehydes is revealed. Pentanal exhibits a negative correlation with all aldehydes, indicating that its presence is generally inversely related to that of aldehydes. On the other hand, there is a strong positive correlation among Octanal, 3-Methylbutanal, Nonanal, (E)-2-Octenal, Benzaldehyde, and phenylacetaldehyde aldehydes. This suggests that these aldehydes are often found together and positively influence each other's presence. Among the acids, a positive but weak correlation is observed among themselves, indicating that they tend to co-occur, albeit with a relatively weaker interdependence compared to the aldehydes. In conclusion, the Pearson correlation analyses shed light on the complex interplay among various VOCs in postharvest apples. These insights into the relationships between different VOC classes provide a better understanding of the composition and dynamics of volatile compounds in apples, which can be valuable for both flavor characterization and the development of strategies for the preservation of fruit quality.

Conclusion

This study investigated the effects of essential oil treatments applied to Golden Delicious apples post-harvest, with a focus on their role as biological fumigants against *Botrytis cinerea*, a common fungal pathogen. The research was conducted in Erzincan, Turkey, and sought to assess the impact of these treatments on the VOC profiles of the fruit. The essential oils, namely thymol, eugenol, and 1,8-cineol EOs, exhibited a notable influence on *B. cinerea* growth, effectively reducing its development and preventing fruit rot. Furthermore, the application of these essential oils led to alterations in the composition of VOCs in the post-harvest apples. Fruits treated with these essential oils displayed an increased accumulation of specific VOCs, with pentanal, nerol, and ethyl octanoate being particularly prominent. This suggests that the post-harvest exposure to essential oils could have a significant impact on the composition of volatile compounds in the fruit. The abundance of certain terpenes in apples exhibited significant variations among the treatments, with the Thy + Eug + Fun group showing the highest total terpene concentration. Additionally, this group displayed the highest ester content, implying that the combination of these essential oils may have a more pronounced effect on the post-harvest fruit. In light of the observed inhibition of *B. cinerea* development in "Golden Delicious" apples during the post-harvest period, it is conceivable that thymol, eugenol, and 1,8-cineol EOs, both individually and in combination, hold promise as bio-fungicides. However, further studies are warranted to explore their potential as natural antifungal agents and assess their suitability for commercial applications in apple preservation.

Author contributions
SK and OA conceived and designed the experiments; OK performed the experiments; SA, HSH, OK and MT analyzed the data. SK and OK. wrote and proof the final paper. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
Correspondence and requests for materials should be addressed to H.S.H.

Declarations

Ethics approval and consent to participate
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The authors declare no competing interests.

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References

1. Schiavon G, Garello M, Prencipe S, Meloni GR, Buonsenso F, Spadaro D. Essential oils reduce grey mould rot of apples and modify the fruit microbiome during postharvest storage. J Fungi. 2022;9(1):22. https://doi.org/10.3390/jof9010022.
2. Spadaro D, Torres R, Errampalli D, Everett K, Ramos L, Mari M. Postharvest diseases of pome fruit. In: Palou L, Smilanick JL, editors. Postharvest pathology of fresh horticultural produce. Boca Raton: CRC Press; 2019. p. 55–109.
3. Arrarte E, Garmendia G, Rossini C, Wisniewski M, Vero S. Volatile organic compounds produced by Antarctic strains of Candida sake play a role in the control of postharvest pathogens of apples. Biol Control. 2017;109:14–20.
4. Williamson B, Tudzynski B, Tudzynski P, Van Kan JA. the cause of grey mould disease. Mol Plant Pathol. 2007;8(5):561–80. https://doi.org/10.1111/J.1364-3703.2007.00417.X.
5. Ramezani S, Rahemi M, Saharkhiz MJ. Biological control of postharvest disease caused by Penicillium digitutum and P italicum on stored citrus fruits by Shiraz Thyme essential oil. Adv Environ Biol. 2009;3(3):249–55.
6. Hua L, Yong C, Zhanquan Z, Boqiang L, Guozheng Q, Shiping T. Pathogenic mechanisms and control strategies of *Botrytis cinerea* causing post-harvest decay in fruits and vegetables. Food Quality Safety. 2018;2(3):111–9. https://doi.org/10.1093/fqsafe/fyy016.
7. Casals C, Teixidó N, Viñas I, Silvera E, Lamarca N, Usall J. Combination of hot water, *Bacillus subtilis* CPA-8 and sodium bicarbonate treatments to control postharvest brown rot on peaches and nectarines. Eur J Plant Pathol. 2010;128:51–63.
8. Banani H, Olivieri L, Santoro K, Garibaldi A, Gullino ML, Spadaro D. Thyme and savory essential oil efficacy and induction of resistance against *Botrytis cinerea* through priming of defense responses in apple. Foods. 2018;7(2):11.
9. Heredia N, García S. Animals as sources of food-borne pathogens: A review. Animal nutrition. 2018;4(3):250–5.
10. Alves JA, Steffens CA, da Silva JC, Pansera-Espíndola B, do Amarante CV, Moreira MA. Quality of 'San Andreas' strawberries and control of gray mold with essential melaleuca oil. J Food Process Preserv. 2022;46(1):e16130. https://doi.org/10.1111/jfpp.16130.
11. Mbili NC, Opara UL, Lennox CL, Vries FA. Citrus and lemongrass essential oils inhibit *Botrytis cinerea* on 'Golden Delicious', 'Pink Lady' and 'Granny Smith' apples. J Plant Dis Prot. 2017;124:499–511.
12. Šernaitė L, Rasiukevičiūtė N, Valiuškaitė A. Application of plant extracts to control postharvest gray mold and susceptibility of apple fruits to *B. cinerea* from different plant hosts. Foods. 2020;9(10):1430.
13. Tančinová D, Mašková Z, Mendelová A, Foltinová D, Barboráková Z, Medo J. Antifungal activities of essential oils in vapor phase against *Botrytis cinerea* and their potential to control postharvest strawberry gray mold. Foods. 2022;11(19):2945.
14. Zhang J, Ma S, Du S, Chen S, Sun H. Antifungal activity of thymol and carvacrol against postharvest pathogens *Botrytis cinerea*. J Food Sci Technol. 2019;1(56):2611–20. https://doi.org/10.1007/s13197-019-03747-0.
15. Hou H, Zhang X, Zhao T, Zhou L. Effects of Origanum vulgare essential oil and its two main components, carvacrol and thymol, on the plant pathogen *Botrytis cinerea*. Peer J. 2020;8: e9626.
16. Wang C, Zhang J, Chen H, Fan Y, Shi Z. Antifungal activity of eugenol against *Botrytis cinerea*. Tropical Plant Pathol. 2010;35:137–43.
17. Olea AF, Bravo A, Martínez R, Thomas M, Sedan C, Espinoza L, Zambrano E, Carvajal D, Silva-Moreno E, Carrasco H. Antifungal activity of eugenol derivatives against *Botrytis cinerea*. Molecules. 2019;24(7):1239.
18. Yılmaz A, Ermiş E, Boyraz N. Investigation of in vitro and in vivo anti-fungal activities of different plant essential oils against postharvest apple rot diseases-*Colletotrichum gloeosporioides*, *Botrytis cinerea* and *Penicillium expansum*. J Food Safety Food Quality-Archiv Fur Lebenmittelhygiene. 2016. https://doi.org/10.2376/0003-925X-67-122.
19. Karakuş S. Hasat Sonrası Uygulanan Bazı Uçucu Yağların Elmalarda *Botrytis cinerea* ve Meyve Besin İçeriğine Etkisi. Türk Tarım ve Doğa Bilimleri Dergisi. 2023;10(3):583–90.
20. Karakuş, S. Biocontrol of *Botrytis cinerea* in Postharvest Apples by Essential Oil Combinations. Avrasya 7th International Conference on Applied Sciences. Budapest. 10–12 March 2023
21. Morales-Valle H, Silva LC, Paterson RR, Oliveira JM, Venâncio A, Lima N. Microextraction and gas chromatography/mass spectrometry for improved analysis of geosmin and other fungal "off" volatiles in grape juice. J Microbiol Methods. 2010;83(1):48–52.
22. Yao H, Jin X, Feng M, Xu G, Zhang P, Fang Y, Xu T, Meng J. Evolution of volatile profile and aroma potential of table grape Hutai-8 during berry ripening. Food Res Int. 2021;143: 110330.
23. Dunlevy JD, Kalua CM, Keyzers RA, Boss PK. The production of flavour & aroma compounds in grape berries. Berlin: Sringer; 2009. p. 293–340.
24. Bennett JW, Hung R, Lee S, Padhi S. 18 Fungal and bacterial volatile organic compounds: an overview and their role as ecological signaling agents. Berlin: Springer; 2012. p. 373–93.
25. Lund ST, Bohlmann J. The molecular basis for wine grape quality-a volatile subject. Science. 2006;311(5762):804–5.
26. Spadoni A, Neri F, Mari M. Physical and chemical control of postharvest diseases. Adv Postharvest Fruit Veg Technol. 2015. https://doi.org/10.1201/b18489-6.
27. Usall J, Torres R, Teixido N. Biological control of postharvest diseases on fruit: a suitable alternative? Curr Opin Food Sci. 2016;11:51–5. https://doi.org/10.1016/j.cofs.2016.09.002.
28. Vandendriessche T, Keulemans J, Geeraerd A, Nicolai BM, Hertog ML. Evaluation of fast volatile analysis for detection of *Botrytis cinerea* infections in strawberry. Food Microbiol. 2012;32(2):406–14.
29. Neri F, Cappellin L, Aprea E, Biasioli F, Gasperi F, Spadoni A, Cameldi I, Folchi A, Baraldi E. Interplay of apple volatile organic compounds with *Neofabraea vagabunda* and other post-harvest pathogens. Plant Pathol. 2019;68(8):1508–24.
30. Junior WJ, Binati RL, Felis GE, Slaghenaufi D, Ugliano M, Torriani S. Volatile organic compounds from *Starmerella bacillaris* to control gray mold on apples and modulate cider aroma profile. Food Microbiol. 2020;89:103446.
31. Yue Y, Wang Z, Zhong T, Guo M, Huang L, Yang L, Kan J, Zalán Z, Hegyi F, Takács K, Du M. Antifungal mechanisms of volatile organic compounds produced by *Pseudomonas fluorescens* ZX as biological fumigants against *Botrytis cinerea*. Microbiol Res. 2023;267:127253. https://doi.org/10.1016/j.micres.2022.127253.
32. Prashar A, Hili P, Veness RG, Evans CS. Antimicrobial action of palmarosa oil (*Cymbopogon martinii*) on *Saccharomyces cerevisiae*. Phytochemistry. 2003;63(5):569–75.
33. Shao X, Wang H, Xu F, Cheng S. Effects and possible mechanisms of tea tree oil vapor treatment on the main disease in postharvest strawberry fruit. Postharvest Biol Technol. 2013;77:94–101.
34. Droby S, Wisniewski M, El Ghaouth A, Wilson C. Influence of food additives on the control of postharvest rots of apple and peach and efficacy of the yeast-based biocontrol product Aspire. Postharvest Biol Technol. 2003;27(2):127–35.
35. Wen YQ, He F, Zhu BQ, Lan YB, Pan QH, Li CY, Reeves MJ, Wang J. Free and glycosidically bound aroma compounds in cherry (*Prunus avium* L.). Food chemistry. 2014;152:29–36.
36. Wang D, Cai J, Zhu BQ, Wu GF, Duan CQ, Chen G, Shi Y. Study of free and glycosidically bound volatile compounds in air-dried raisins from

three seedless grape varieties using HS–SPME with GC–MS. Food Chem. 2015;177:346–53.
37. Ubeda C, San-Juan F, Concejero B, Callejon RM, Troncoso AM, Morales ML, Ferreira V, Hernandez-Orte P. Glycosidically bound aroma compounds and impact odorants of four strawberry varieties. J Agric Food Chem. 2012;60(24):6095–102.
38. Wu Y, Pan Q, Qu W, Duan C. Comparison of volatile profiles of nine litchi (*Litchi chinensis* Sonn.) cultivars from Southern China. J Agric Food Chem. 2009;57(20):9676–81.
39. Ou-Ani O, Moujane S, Oucheikh L, Youssefi Y, Ansari A, Oubair A, Chebabe D, Znini M. Essential oil from *Teucrium luteum* subsp. flavovirens: optimization of yield extraction using response surface methodology, molecular docking and biocontrol against apple gray mold caused by *Botrytis cinerea*. Arch Phytopathol Plant Prot. 2022;55(13):1504–29.
40. Lorenzini M, Simonato B, Slaghenaufi D, Ugliano M, Zapparoli G. Assessment of yeasts for apple juice fermentation and production of cider volatile compounds. Lwt. 2019;99:224–30.
41. Oro L, Feliziani E, Ciani M, Romanazzi G, Comitini F. Volatile organic compounds from *Wickerhamomyces anomalus*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* inhibit growth of decay causing fungi and control postharvest diseases of strawberries. Int J Food Microbiol. 2018;265:18–22.
42. Pedrotti C, Marcon ÂR, Sérgio EL, Ribeiro RT, Schwambach J. Essential oil as sustainable alternative for diseases management of grapes in postharvest and in vineyard and its influence on wine. J Environ Sci Health Part B. 2020;56(1):73–81.
43. Jiang B, Zhang Z. Volatile compounds of young wines from cabernet sauvignon, cabernet gernischet and chardonnay varieties grown in the loess plateau region of China. Molecules. 2010;15(12):9184–96.
44. Anami JM, Steffens CA, Moreira MA, Fernandes RC, Mosquera DJ, Amarante CV. Active modified atmosphere storage to preserve the quality of 'San Andreas' strawberries harvest at two ripening stages. Rev Bras Frutic. 2020;24:42.
45. Schreirer P. Flavor composition of wines: a review. Crit Rev Food Sci Nutr. 1979;12:59–111. https://doi.org/10.1080/10408397909527273.

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