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ORIGINAL PAPER

Essential oils combat *Botrytis cinerea* in apples: unveiling sugar, vitamin, and antioxidant dynamics

Sinem Karakus[1,2] · Okkes Atıcı[3] · Metin Turan[4] · Ozkan Kaya[5,6]

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Abstract

This study investigated the effects of individual and combination applications of EOCs against *Botrytis Cinerea* in apple fruits, with a focus on sugar, vitamin, and antioxidant enzyme dynamics. In protective and curative treatments, sucrose content varied between 18.9 and 87.9 $g.L^{-1}$, with the "CT" group exhibiting significantly higher sucrose levels (87.9 $g.L^{-1}$). Glucose content ranged from 6.2 $g.L^{-1}$ ("CT" and "C + F") to 35.5 $g.L^{-1}$ ("T + C + E + F"). Fructose content varied significantly, with the "F + T + C + E" treatment showing the highest (11.4 $g.L^{-1}$) and the "CT" group the lowest (2.5 $g.L^{-1}$). Rhamnose content in the curative treatment reached 14.9 $g.L^{-1}$ ("F + T") and in the protective treatment, the "C + E + F" group had the highest (3.3 $g.L^{-1}$). Galactose content ranged from 0.1 to 13.0 $g.L^{-1}$. Xylose content in curative treatment ranged from 12.1 to 13.6 $g.L^{-1}$, with the combination "F + T" displaying the highest. In preventive treatment, arabinose content ranged from 0.7 to 3.0 $g.L^{-1}$, with the "T + C + F" group having the highest. In terms of vitamins, the combination "T + F" showed the highest B1 (25.4 mg.100 g^{-1}) and B2 (24.6 mg.100 g^{-1}) content. Vitamin A, B1, and B2 increased in the "F + T" group, and vitamin C increased in the "F + T + C" group (33.4 mg.100 g^{-1}). The quantification of oxidative stress markers and antioxidant enzymes revealed significant reductions in H_2O_2, MDA, and proline levels in EOC applications. In preventive treatment, the combination "T + C + E + F" exhibited the most effective improvement in SOD activity (556.5 EU mg^{-1}), CAT activity increased significantly in the "T + C + F" group (189.3 EU mg^{-1}), and APX activity saw the highest increase in the "T + C + E + F" group (185.6 EU mg^{-1}). Contrastingly, curative treatments showed higher H_2O_2, MDA, and proline levels. The most significant decrease in H_2O_2 level occurred in the "T + C + E + F" group (36.7 $nmol.g^{-1}$), and a significant decrease in MDA level was noted in the same group. Notable changes were observed in the activities of antioxidant enzymes, with the "F + T + C + E" group displaying the highest SOD activity (440.7 EU mg^{-1}), and the "F + T" group showing the highest GST activity (542.0 EU mg^{-1}). This research provided comprehensive insights into the biochemical responses of apple fruits to EOC treatments, offering potential strategies for effective fungal management in agriculture.

Keywords Antioxidants · Apple · Biocontrol · *Botrytis cinerea* · Sugars · Vitamins

✉ Ozkan Kaya
ozkan.kaya@ndsu.edu; kayaozkan25@hotmail.com

[1] Çölemerik Vocational School, Hakkari University, Hakkari 30000, Türkiye

[2] Department of Biology, Faculty of Science and Art, Erzincan Binali Yıldırım University, Erzincan 24002, Türkiye

[3] Department of Biology, Science Faculty, Atatürk University, Erzurum 25240, Türkiye

[4] Faculty of Economy and Administrative Science, Yeditepe University, Istanbul 34755, Türkiye

[5] Republic of Türkiye Ministry of Agriculture and Forestry, Erzincan Horticultural Research Institute, Erzincan 24060, Türkiye

[6] Department of Plant Sciences, North Dakota State University, Fargo, ND 58102, USA

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Introduction

Malus domestica (apple) is known as the third most consumed fruit in the world; it is trendy among people due to its high nutritional value and delicious taste [1]. Apples grow widely in temperate regions and come in many different varieties. According to a study conducted by Muder et al. [2], the most preferred order among apple cultivars is as follows: Golden Delicious, Red Delicious, Grany Smith, and Amasya. In this ranking, Golden Delicious apples are preferred by consumers more than other cultivars. That is due to the characteristics of Golden Delicious apples as light crunch, intense taste, sweetness, firmness, and color. The entire apple fruit, except the seeds, is edible. Apples are consumed fresh and used to make many different products such as jam, juice, cider, dried apples, wine, or tea. Apples are also used to enhance the flavor of desserts, stews, and various dishes. They are a healthy fruit and contain polyphenols, minerals, antioxidants, fiber, and vitamins [3]. Globally, 5–25% of apples are lost during storage due to fungal diseases [4]. The increase in plant pathogenic fungal invasions of apples is believed to result from globalization, environmental damage, and climate change. Factors such as microbial infection, temperature, insect attacks, and humidity affect shelf life and preservation of fruits after harvest [5]. Generally, fruits become sensitive during this period as they enter the ripening process from the moment they bloom. During the flowering stage, fruits undergo cellular and chemical changes that cause them to ripen. This ripening process makes the fruits more susceptible to fungal rot and other diseases. The skin and tissue structure of the fruits changes, creating a suitable environment for the evolution of fungi [6, 7]. *Botrytis cinerea* Pers., among these pathogens, is the predominant pathogen of apples stored in the post-harvest period in many countries [8]. It causes gray mold-like spots on the skin surface of apples, causing the skin to rot. That spoils the appearance of the fruits and reduces the product quality [9]. Additionally, rot and spoilage occur in the infected fruit, negatively affecting their marketability and shelf life [10]. Therefore, it is crucial to maintain the maturity level and shelf life of fruits during post-harvest storage and marketing. Recently, fungicide use has been restricted due to environmental and health concerns. This situation has indicated the importance of post-harvest preservation of fruits and vegetables, prompting the exploration of alternative methods and techniques [6, 7].

Methods such as biological control agents, natural biocides, and stimulation of fruit defense systems are seen as substitute approaches to fungicides. Natural products have a wide range of applications in cosmetics, agricultural entomology, pest control, and more. Essential oils (EOs), in particular, are volatile compounds obtained from aromatic plants and are abundant in bioactive phytochemical components [11]. Studies have shown that EOs have antifungal, antioxidant, insecticidal properties, and antibacterial [12, 13]. It has been stated that EOs reduce dependence on chemical fungicides and promote environmental sustainability. EOs are valuable for producers who want to maintain product quality and practice more environmentally friendly agriculture, and for sectors that want to meet consumer demands. EOs promote a more sustainable approach to fruit production by replacing conventional chemical control methods [1]. Treating plants with bioagents such as EOs stimulates defense mechanisms against pathogens, resulting in them developing local and systemic resistance [14]. Post-harvest treatments increase antioxidant mechanisms against stress induced by pathogens. These treatments can increase the enzymatic and non-enzymatic antioxidant activity of plants, thereby helping the plant deal with reactive oxygen species (ROS) more effectively. These mechanisms play a crucial role in combating plant diseases in product storage and preservation [15].

In this context, we have also stated in our previous research that the protective and curative effects of essential oil components (EOCs) (such as thymol, 1,8-cineole, and eugenol) may have positive effects on apple fruits against *B. cinerea*, improving fruit quality and extending the storage period [16, 17]. However, there is no data in the existing literature about the effects of these EOCs on quality parameters such as antioxidant enzymes, sugars, and vitamins of post-harvest apple fruits. For this reason, this research aimed to examine the dissimilarity in antioxidants, vitamins, and sugars in the protective-curative effect of EOCs (individual and combination) applied against *B. cinerea* in the Golden Delicious apple cultivar. The main goal of the investigation is to determine in more detail the ability of these EOCs to improve fruit quality and their effects on fruit.

Materials and methods

Fruit materials, pathogen isolates, and essential oils components

The experiment was realized using "Golden Delicious" apples that were harvested from orchards located at 39°75′E, 39°36′N, and 1309 m above sea level in Erzincan in September. The temperature during the investigation was 25 °C with 30.0% humidity. Only commercially ripe fruits were used, which were free of any physical damage, uniform in size, and pathogen-free.

Botrytis cinerea, known for its highly polyphagous nature, exhibited a consistent pathogenicity mechanism across a diverse array of host species. The vineyard isolates selected

for this study had been previously verified for its virulence on apples through preliminary evaluations, affirming its suitability for the objectives of our research. The employment of this particular isolate ensured continuity with our preceding studies [16], thereby enabling comparative and longitudinal analyses that were critical for advancing our understanding of its pathogenic behavior. To identify the fungus, we utilized primer sets 515f and 806r, which contain regions V3-V4 of the 18 S rRNA gene. These primer sets were analyzed by RefGen Biotechnology Company, which is located (https://www.refgen.com) in Ankara, Turkey. The molecular diagnoses were performed by comparing the 18 S rRNA gene region sequences detected in our previous studies with the 18 S rRNA gene regions identified in previous studies. We utilized *B. cinerea* isolates numbered MF7413141, MH997908, MK562062, and MH782039, obtained from the Genbank database, for molecular identification. We built a tree using the UPGMA method, utilization of the MEGA v.5 program using the Jukes-Counter model. Before conducting the experiments, *B. cinerea* was incubated in PDA (Potato dextrose agar) medium for seven days at 25 °C in a constant temperature incubator. The study utilized 1,8-cineole (Aldrich 183,164), eugenol (Fluka 45,980), and thymol (Aldrich C121452) purchased from Sigma-Aldrich (Shanghai, China) and stored long-term in the dark at 4 °C. A 10% stock solution (10% EOCs was added to the water consisting of 2% (v/v) Tween-20 and 88% sterile pure water) was prepared for each EOCs. Then, 5 mL of this stock solution was taken and added to 400 mL of water to create a new solution with a lower concentration.

Inoculation and storage of fruits

Five mL of prepared stock solution was attached to 400 mL of water. Initially, apples were washed in a 10 mL/L sodium hypochlorite solution for 5 min., rinsed with water, and air-dried at room temperature. Two 3 mm wide and 3 mm deep wounds were produced at the equator of the disinfected apple fruits using a sterile puncture needle [17]. The following applications were established in the trial for protective purposes: CT (distilled water); F (spore suspension of the pathogen; 1×10^5 conidia mL^{-1}); T; 1.25 µL; E; 1.25 µL; C, 1.25 µL; T + F; 1.25 µL; C + F; 1.25 µL; E + F; 1.25 µL; C + E + F; 1.25 µL; T + E + F; 2.5 µL; T + C + F; 2.5 µL; T + C + E + F; 3.75 µL. The following applications were set up in the trial for curative purposes: control (distilled water), fungus (spore suspension of the pathogen; 1×10^5 conidia mL^{-1}); 1.25 µL, T; 1.25 µL, E; 1.25 µL, C; 1.25 µL, F + T; 1.25 µL, C + F; 1.25 mm, F + E; 1.25 µL, F + C + E; 2.5 µL, F + T + E; 2.5 µL, F + T + C; 2.5 µL, F + T + C + E; 3.75 µL. The most suitable dosage combinations for the trial were determined based on preliminary work results. This was conducted to avoid possible deformation in the fruit peel when combining individual concentrations (unpublished data). The experiment involved treatments both preservative and curative (in total; 22 treatments) and each treatment was replicated three times, with three apples per replication, in a completely randomized design.

For the curative effect, the fruits were submerged in EOCs solutions and incubated for 30 min. After, they were air-dried at room temperature for 24 h. Following that, the wound sites were inoculated with 125 µL of a conidial suspension of *B. cinerea* at a concentration of 1×10^5 spores/mL. On the other hand, for the protective effect, three distinct wound tissues were then created on the equatorial regions of the fruits, and the wound areas were inoculated with a 125 µL solution of *B. cinerea* spores at a concentration of 1×10^5 spores/mL. The inoculation was carried out using a conidial suspension of *B. cinerea*. Subsequently, the inoculated fruits were incubated under controlled conditions of temperature and humidity to facilitate the development of infection. Following inoculation, the fruits were removed from the chambers and incubated for 24 h in oil emulsions for 30 min. The fruits were incubated for a week at 4 °C and 90 ± 5% humidity. Infected fruits were evaluated seven days after the incubation period [18].

Analyzing sugar content in treated apples

Sugars, including galactose, rhamnose, maltose, glucose, xylose, sucrose, and fructose used in the apple analysis were acquired from Sigma in Steinheim, Germany. Sugar extraction followed the method reported by Nikolidaki et al. [19]. Two grams of mechanically homogenized apples were weighed and extracted using 80% v/v aqueous ethanol (20 mL). The process required sonication and overnight agitation for 2.5 h. For the sugar analysis in the apples, an HPLC system (Agilent Technologies, 1100 series, USA) was recruited, coupled with a refractive index detector (RID, 1260 series). This system comprised an auto-sampler, an isocratic pump, and data analysis software. Isocratic elution was implemented using a water/acetonitrile mixture (30:70) on a Purospher® star NH2 (250×4.6 mm, 5 µm) column from Merck-Millipore in Darmstadt, Germany, with a flow rate of 1 mL/min. The injection volume was 10 µL. Both the RID and oven were maintained at 40 °C. The determination of samples was achieved using calibration standards of HPLC grade sugars obtained from Sigma-Aldrich in Shanghai, China.

Analyzing vitamins content in treated apples

The analysis of vitamins in apple samples was conducted following the procedure defined in Keskin et al. [20].

Initially, apple samples were sliced and instantly frozen with liquid nitrogen, then stored at -80 °C till the vitamin C analysis. For the vitamin C analysis, the frozen apple samples were weighed, and combined with 2.5 mL of an extraction solution created of 3% metaphosphoric acid (MPA), 0.1% oxalic acid, and 8% acetic acid. The resulting sample mixture was titrated with an indophenol solution containing 21% sodium bicarbonate ($NaHCO_3$) and 25% 2,6-dichloroindophenol in water. The titration continued till an evident rose-pink color emerged. In vitamin E analysis, 0.5 g of apple samples were submerged in 20 mL of ethanol for 30 min in a water bath maintained at 85 °C. Hereafter, the solution was chilled, filtered, and transferred into a separatory funnel. To the solution, 10 mL of heptane was added, and the mixture was shaken for 5 min. Afterward, 20 mL of a 1.25% sodium sulfate solution was added to easier the disconnection of layers. Determination of vitamin E was achieved through complexation with 2,2'-biquinoline and a reaction with cupric ions, based on the method reported by Samydurai et al. [21]. The samples were transferred to the conical flask for vitamin B complex analysis, and 25 mL of extraction solution was added. Their samples were transferred to a conical flask to perform vitamin B complex analysis. Then, 25 mL of extraction solution was added. Afterward, the mixture was sonicated for 40 min at 70 °C. The samples were cooled and filtered after sonication. Then, it was combined with the extraction solution to obtain a 50 mL mixture. This mixture was later filtered once more using a 0.45 µm filter. 20 µL of the filtrate was injected into the HPLC (High-Performance Liquid Chromatography) system via an autosampler. The B complex vitamins were separated utilizing an analytical reversed-phase C-18 column (STR ODS-M, 150 mm × 4.6 mm I.D., 5 µm, Shimadzu Corporation, Japan). The mobile phase consisted of a mixture of containing 0.8 mM sodium-1-octane sulfonate, and 100 mM sodium phosphate buffer with a pH of 2.2, acetonitrile in a 9:1 ratio (v/v) at 40 °C. The flow rate was set to 0.8 mL/min and monitored using a photodiode array (PDA) at 270 nm. The quantification of vitamin B was semi-quantified using the standard method reported by Mozumder et al. [22].

Analyzing antioxidant enzymes content in treated apples

The analysis of apple samples involved several preparatory steps, following the procedure outlined by Keskin et al. [20]. Initially, the samples were subjected to three washes utilizing a solution composed of 50 mM Tris-HCl and 0.1 M Na_2SO_4 at pH 8.0. Subsequently, the samples were homogenized with liquid nitrogen to make ready them for further analysis. These homogenized samples were then transferred to a buffer solution of 10 mM NaN_3, 0.1 M Na_2SO_4, 100 mM PVP, and 50 mM Tris-HCl at pH 8.0. After the sample preparation steps, the next stage included centrifugation at 15,000 rpm for 60 min at a temperature of 4 °C. This centrifugation step intended to separate the specific components of interest, which were necessary for the subsequent enzyme activity analysis. The GSH activity in the samples was carried out following the method reported by Minucci et al. [23]. This method enabled the quantification of GSH activity in the apple samples. Furthermore, the activities of GR (EC 1.8.1.7) and GST (EC 2.5.1.18) were determined using the methodology published by Chikezie et al. [24]. Enzymatic activities were measured spectrophotometrically at 25 °C, employing a Shimadzu 1208 UV spectrophotometer from Kyoto, Japan. The enzyme solution was introduced to initiate the enzymatic reactions. The activities of SOD (EC 1.15.1.1), POD (EC 1.11.1.7), and CAT (EC 1.11.1.6), in the apple samples were reported based on the methods outlined by Abedi and Pakniyat [25] and Angelini et al. [26]. The spectrophotometric assessment of enzymatic activities was assessed at a temperature of 25 °C.

Statistical analysis

The variables were tested in triplicate, and the results presented show the means along with their respective standard deviations. A statistical analysis of the data was carried out through analysis of variance (ANOVA). Tukey's test was used to differentiate means with a significance level of $p < 0.05$. SPSS program version 13.0 was used for statistical analysis (SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows the effects of sucrose, glucose, fructose, rhamnose, galactose, xylose, and arabinose content in apples treated with EOCs (individual and combination) in protective-curative treatment against *B. cinerea*. Sucrose content in the application groups varies between 18.9 and 87.9 $g.L^{-1}$. The results show that the sucrose content of the "CT" group was significantly higher than the other groups (87.9 $g.L^{-1}$). On the other hand, the "F + C + E" group has the lowest value in terms of sucrose content (18.9 $g.L^{-1}$). Differences between other groups are not statistically significant. While "CT" (6.2 $g.L^{-1}$) and "C + F" (6.5 $g.L^{-1}$) had the lowest glucose content among all treatment groups, "T + C + E + F" (35.5 $g.L^{-1}$) stood out as the group with the highest glucose content. Fructose content shows different effects on the sugar content of apples between treatments. The "F + T + C + E" treatment was noted to have the highest fructose content (11.4 $g.L^{-1}$), while the "CT" group had the lowest fructose content (2.5 $g.L^{-1}$). Rhamnose

Table 1 Effect of EOCs (individual and combination) on the sugar content ($g.L^{-1}$) for the protective-therapeutic treatment against *B. cinerea* in the apple

Treatments	Sucrose	Glucose	Fructose	Rhamnose	Galactose	Xylose	Arabinose
CT	87.9 ± 3.3^{a}	6.2 ± 2.2^{e}	2.5 ± 0.7^{e}	1.7 ± 0.6^{def}	0.2 ± 0.0^{g}	0.1 ± 0.2^{f}	1.4 ± 0.2^{e}
F	80.9 ± 3.9^{a}	9.8 ± 2.1^{e}	4.3 ± 0.9^{d}	3.4 ± 1.1^{d}	0.1 ± 0.0^{g}	0.1 ± 0.1^{f}	1.3 ± 0.1^{e}
T + F	86.6 ± 0.5^{a}	6.4 ± 0.2^{e}	2.8 ± 0.2^{de}	1.7 ± 0.1^{def}	0.1 ± 0.2^{g}	0.2 ± 0.1^{f}	2.0 ± 0.1^{d}
E + F	80.7 ± 2.9^{a}	9.6 ± 1.9^{e}	4.0 ± 0.3^{d}	3.0 ± 0.4^{de}	0.1 ± 0.1^{g}	0.2 ± 0.1^{f}	2.4 ± 0.2^{c}
C + F	86.2 ± 1.4^{a}	6.5 ± 1.0^{e}	2.8 ± 0.3^{de}	1.7 ± 0.1^{def}	0.3 ± 0.2^{g}	0.2 ± 0.1^{f}	2.6 ± 0.1^{bc}
T + E + F	56.0 ± 6.8^{b}	5.8 ± 0.1^{e}	2.3 ± 0.1^{e}	1.3 ± 0.3^{def}	0.5 ± 0.3^{fg}	0.3 ± 0.1^{f}	2.8 ± 0.1^{ab}
T + C + F	50.3 ± 1.9^{bc}	31.4 ± 1.1^{a}	6.3 ± 0.2^{c}	0.6 ± 0.0^{f}	0.1 ± 0.1^{f}	9.8 ± 0.6^{e}	0.7 ± 0.1^{g}
C + E + F	79.1 ± 3.4^{a}	9.8 ± 1.6^{e}	4.2 ± 0.8^{d}	3.3 ± 0.9^{d}	0.3 ± 0.9^{fg}	0.2 ± 0.1^{f}	3.0 ± 0.1^{ab}
T + C + E + F	46.5 ± 2.1^{cd}	35.5 ± 1.7^{a}	7.4 ± 0.5^{bc}	0.7 ± 0.0^{ef}	1.1 ± 0.1^{f}	11.4 ± 0.6^{cd}	0.8 ± 0.1^{fg}
F + T	25.8 ± 2.6^{fg}	26.0 ± 1.3^{bcd}	8.7 ± 0.6^{b}	14.9 ± 1.2^{bc}	10.3 ± 0.3^{b}	13.6 ± 1.1^{a}	0.9 ± 0.1^{f}
F + C	34.5 ± 3.5^{ef}	20.6 ± 1.4^{d}	8.1 ± 0.2^{b}	13.8 ± 0.8^{bc}	9.3 ± 0.3^{cd}	12.6 ± 0.8^{abc}	0.9 ± 0.1^{fg}
F + E	39.0 ± 1.2^{de}	21.6 ± 1.5^{d}	7.3 ± 0.6^{bc}	12.3 ± 0.6^{c}	7.8 ± 0.5^{e}	12.1 ± 0.4^{bcd}	0.8 ± 0.1^{fg}
F + C + E	18.9 ± 2.5^{g}	34.6 ± 4.5^{a}	8.9 ± 0.6^{b}	14.5 ± 0.7^{bc}	8.9 ± 0.3^{d}	13.3 ± 0.6^{ab}	0.9 ± 0.1^{fg}
F + T + C	23.8 ± 5.4^{g}	29.9 ± 5.4^{a}	8.6 ± 0.2^{b}	13.4 ± 1.8^{bc}	9.8 ± 0.3^{bc}	13.1 ± 0.5^{ab}	0.9 ± 0.1^{f}
F + T + E	24.0 ± 2.7^{g}	32.2 ± 4.6^{a}	8.1 ± 0.6^{b}	13.6 ± 0.6^{bc}	8.7 ± 0.5^{d}	12.5 ± 0.6^{abc}	0.8 ± 0.1^{fg}
F + T + C + E	23.9 ± 1.4^{g}	23.5 ± 2.7^{cd}	11.4 ± 0.5^{a}	17.3 ± 1.3^{a}	13.0 ± 0.6^{a}	11.0 ± 0.5^{de}	1.3 ± 0.1^{e}

Data are expressed as mean of the data. Significance (p-value) of application method (AM), treatment (T). For a given factor and significance ($p < 0.01$), different letters within a column represent significant differences (Duncan test, $p < 0.01$)

content varied significantly between treatments. In curative effect, the "F + T" group had the highest content of 14.9 g. L^{-1} and the lowest CT content of 1.7 $g.L^{-1}$. Regarding the protective effect, the "C + E + F" treatment had the highest rhamnose content (3.3 $g.L^{-1}$), while the "T + C + F" group had the lowest rhamnose content (0.6 $g.L^{-1}$). Galactose content ranged from 0.1 $g.L^{-1}$ (F, T + F, E + F, C + F, and T + C + F) to a maximum of 13.0 $g.L^{-1}$ (F + T + C + E). In the protective treatment group, the xylose content of apple fruits ranged from 0.1 to 11.4 $g.L^{-1}$. Especially, the highest xylose content was noticed in the treatment combination T + C + E + F. In contrast, CT, T, E, and C groups exhibited relatively low xylose content. The xylose content for the curative treatment group was found to be between 12.1 and 13.6 $g.L^{-1}$. Out of all the curative treatments used, the combination of F + T showed the highest amount of xylose content. In addition, it showed a significant improvement in the F + T + C + E group with a xylose content of 11.0 $g.L^{-1}$. In the preventive treatment group, arabinose content was recorded between 0.7 and 3.0 $g.L^{-1}$. The highest arabinose content was determined in the T + C + F group. On the other hand, arabinose content was low in groups CT, T, E, and C. Arabinose content is between 0.8 and 3.0 g^{-1} in curative treatment application groups. The highest arabinose content was detected in the F + T (0.9 $g.L^{-1}$), F + T + C (0.9 $g.L^{-1}$), and F + T + C + E (1.3 $g.L^{-1}$) groups.

The effect of individual and combination applications of EOCs against *B. cinerea* on the vitamin contents (Vitamin A, B1, B2, B6, and C) of apple fruits are given in Table 2. It was determined that vitamins B1 and B2 had the highest content in the T + F group (25.4 and 24.6 mg.100 g^{-1}). An increase in Vitamins A, B1, and B2 was observed in the F + T group (13.2, 24.1 and 25.1 mg.100 g^{-1}, respectively). An increase in vitamin C was detected in the F + T + C group (33.4 mg.100 g^{-1}). It was determined that applications such as F + T, T + F, and F + T + C had a positive effect on the vitamins determined in apples. The activity of H_2O_2 MDA, proline, CAT, POD, SOD, GR, GST, G6PD, 6GPD, and APX were quantified in the apples studied (Table 3). It was determined that EOCs used alone significantly reduced H_2O_2, MDA, and proline levels, which indicate oxidative stress and cell damage. In preventive treatment, it showed promising effects in reducing H_2O_2, MDA, and proline levels in T and E application groups. EOC applications affected CAT, SOD, and POD levels. While a significant effect was determined in increasing the CAT activity of the T + C + E + F application group, the highest effect was determined in the POD activity of the C + E + F application group. In addition, the T + C + E + F application group exhibited the most effective improvement in SOD activity against the fungus. When GST and GR activities were examined in preventive treatment; it has been determined that T increases GR and GST activities when applied in combination with other EOCs. The most effective combination was found to be the T + C + E + F application (95.6 and 556.5 EU mg^{-1}). G6PD and 6GPD enzymes were also affected by the application groups. APX activity increased in EOC combinations, and the highest increase was determined in the T + C + E + F (185.6 EU mg^{-1}) group.

In contrast, H_2O_2, MDA, and proline levels were higher in curative treatment applications than in preventive treatments. The most significant decrease in H_2O_2 level was

Table 2 Effect of EOCs (individual and combination) on the vitamin contents (mg.100 g^{-1}) for the protective-therapeutic treatment against *B. cinerea* in the apple

Treatments	Vitamin A	Vitamin B1	Vitamin B2	Vitamin B6	Vitamin C
CT	13.7 ± 0.1^{bf}	18.9 ± 0.6^{cde}	15.1 ± 2.2	12.8 ± 3.8	19.4 ± 0.9^{cde}
F	17.5 ± 2.5^{abc}	24.3 ± 1.6^{ae}	21.9 ± 2.6	10.4 ± 3.0	21.5 ± 1.7^{be}
T + F	16.7 ± 1.7^{ad}	25.4 ± 1.6^{ae}	24.6 ± 1.8	13.3 ± 1.4	26.2 ± 2.2^{ad}
E + F	12.3 ± 1.6^{def}	21.6 ± 7.2^{be}	20.4 ± 1.2	8.3 ± 1.6	17.4 ± 4.7^{de}
C + F	12.1 ± 2.6^{ef}	16.4 ± 3.1^{e}	20.1 ± 1.4	9.0 ± 2.1	18.4 ± 4.4^{cde}
T + E + F	16.6 ± 0.8^{ae}	22.8 ± 1.1^{ae}	19.1 ± 2.6	16.5 ± 5.1	25.9 ± 2.6^{ad}
T + C + F	17.2 ± 1.8^{abc}	25.7 ± 2.7^{ad}	22.3 ± 2.5	9.1 ± 2.7	22.5 ± 1.4^{be}
C + E + F	18.2 ± 2.6^{ab}	29.9 ± 3.7^{ab}	21.9 ± 1.7	7.3 ± 0.5	28.0 ± 4.3^{abc}
T + C + E + F	14.5 ± 0.8^{bf}	26.8 ± 0.1^{ac}	19.9 ± 0.8	10.9 ± 1.6	22.5 ± 5.5^{be}
F + T	$13.2 \pm 2.3^{cf.}$	24.1 ± 5.9^{ae}	25.1 ± 1.6	7.7 ± 0.8	24.8 ± 5.1^{ae}
F + C	10.0 ± 1.0^{f}	15.3 ± 1.7^{e}	21.5 ± 1.1	8.6 ± 1.9	14.6 ± 1.1^{e}
F + E	11.6 ± 0.4^{f}	15.7 ± 1.8^{de}	22.0 ± 1.2	8.9 ± 2.1	16.8 ± 1.3^{de}
F + C + E	$13.5 \pm 1.7^{cf.}$	24.6 ± 2.5^{ae}	19.7 ± 2.8	16.7 ± 3.9	21.4 ± 2.2^{be}
F + T + C	19.6 ± 1.1^{a}	30.8 ± 3.8^{ab}	20.0 ± 1.4	11.5 ± 3.8	33.4 ± 3.6^{a}
F + T + E	14.5 ± 0.3^{bf}	32.5 ± 6.3^{a}	24.1 ± 1.9	7.5 ± 1.0	28.7 ± 4.9^{abc}
F + T + C + E	16.9 ± 0.5^{abc}	31.6 ± 2.7^{ab}	22.1 ± 1.3	8.8 ± 0.1	30.2 ± 5.7^{ab}

Data are expressed as mean of the data. Significance (p-value) of application method (AM), treatment (T). For a given factor and significance ($p < 0.01$), different letters within a column represent significant differences (Duncan test, $p < 0.01$)

observed in the T + C + E + F group (36.7 nmol.g^{-1}). At the same time, a significant decrease in MDA level was noted in the T + C + E + F group. Significant changes were recorded in the activities of antioxidant enzymes in the curative treatment application groups. CAT activity increased significantly in the T + C + F group (189.3 EU mg^{-1}). SOD activity was observed to be generally high in the curative treatment groups. The most significant increase was found to be in the F + T + C + E group (440.7 EU mg^{-1}). It caused a significant increase in GR activity in the F + T + C + E group (62.6 EU mg^{-1}), while the F + T group (542.0 EU mg^{-1}) showed the highest GST activity. G6PD and 6GPD enzymes were affected by the pathogen, and various EOC treatments alleviated the observed differences (Table 3).

On the other hand, the heatmap visualization (Fig. 1) presented a comprehensive overview of the variation in biochemical constituents among different treatment groups. Each row indicated a specific treatment, encompassing control, various combinations of Cineole, Eugenol, Thymol, and Fungus, and their interactions. Columns represented different measured variables, ranging from sugars and vitamins to antioxidant enzymes. Distinct patterns surfaced in the heatmap, where color intensities reflected concentration levels of components across treatments. Red hues signified higher concentrations, while blue hues indicated lower concentrations. This color gradation unveiled the divergent accumulation of biochemical constituents under each treatment condition. Treatments involving 'T + E + F' exhibited a significant increase in specific variables, suggesting a potential synergistic effect on the biochemical pathways. Accompanying the heatmap was a dendrogram (Fig. 2) illustrating the hierarchical clustering of treatments based on their biochemical profiles. Vertical lines and interconnections represented similarity between treatment groups, with shorter lines indicating closer relationships. The color-coded dendrogram facilitated the identification of major clusters, revealing distinct biochemical signatures in response to various substance combinations. The largest cluster (green) comprised treatments showing enhanced antioxidant enzymes, hinting at a potential strategy for mitigating oxidative stress. Conversely, a smaller red cluster separated treatments characterized by lower levels of sugars and vitamins, implying a depletion or inhibitory effect on these components. Further supporting the clustering, the constellation plot analysis (Fig. 2) visually represented the proximity between different treatment groups. The plot's spatial arrangement mirrored the hierarchical structure of the dendrogram, reinforcing the classification of treatment groups into distinct biochemical profiles. Based on result of constellation plot analysis, each node was labeled with a combination of substances such as "T," "C," "E," and "F," or descriptions of interactions between them. The lines (or edges) connecting the nodes indicated relationships or interactions, potentially in a biological or chemical context. The pattern of connections suggested how these substances or elements interacted with each other. The radial layout hinted at a central point of significance, with branches radiating outwards. The central node appeared to be a primary substance or condition, while the outer nodes represented the results or effects when combined with other substances. Different colors on the nodes and edges were used to denote various types or strengths of interactions (Fig. 2).

Table 3 Effect of EOCs (individual and combination) on the antioxidant enzyme contents for the protective-therapeutic treatment against *B. cinerea* in the apple

Treatments	H_2O_2(nmol g^{-1})	MDA (nmol g^{-1})	Proline (nmol g^{-1})	CAT (EUmg^{-1})	POX (EUmg^{-1})	SOD (EUmg^{-1})	GR (EUmg^{-1})	GST(EUmg^{-1})	G6PD(EUmg^{-1})	6GPD (EUmg^{-1})	APX (EUmg^{-1})
CT	8.7 ± 0.5^{hij}	16.1 ± 1.6^{cd}	0.1 ± 0.0^{h}	132.5 ± 10.1^{i}	920.8 ± 75.3^{ij}	99.3 ± 7.9^{g}	82.3 ± 15.2^{ne}	496.8 ± 4.6^{c}	328.2 ± 85.6	611.6 ± 209.5^{be}	129.5 ± 3.9^{h}
F	8.8 ± 0.1^{hij}	16.5 ± 1.0^{c}	0.1 ± 0.0^{h}	133.2 ± 5.1^{hi}	940.8 ± 37.4^{i}	99.8 ± 4.3^{g}	106.4 ± 5.2^{n}	668.4 ± 8.3^{b}	454.1 ± 141.0	1030.9 ± 362.3^{ab}	170.1 ± 3.6^{ef}
T+F	8.3 ± 0.3^{ij}	12.5 ± 0.9^{ef}	0.1 ± 0.0^{h}	108.0 ± 6.6^{j}	739.9 ± 44.6^{j}	77.9 ± 5.4^{h}	75.4 ± 0.7^{cde}	480.6 ± 52.0^{c}	311.4 ± 78.0	658.0 ± 208.5^{be}	161.3 ± 10.9^{fg}
E+F	17.8 ± 0.4^{f}	11.2 ± 1.7^{fg}	0.1 ± 0.0^{g}	154.6 ± 6.6^{gh}	2576.4 ± 71.5^{gh}	69.6 ± 2.6^{h}	85.9 ± 13.8^{ne}	154.9 ± 15.0^{e}	244.9 ± 28.5	332.0 ± 65.9^{de}	126.8 ± 0.2^{h}
C+F	13.9 ± 0.3^{g}	11.5 ± 0.5^{fg}	0.1 ± 0.0^{h}	44.2 ± 2.3^{k}	1038.6 ± 13.5^{i}	102.0 ± 8.4^{g}	89.5 ± 8.8^{nd}	221.7 ± 28.9^{de}	319.4 ± 43.6	422.0 ± 27.3^{cde}	199.3 ± 7.3^{bcd}
T+E+F	22.3 ± 1.3^{e}	8.3 ± 0.8^{hi}	0.2 ± 0.1^{e}	204.6 ± 7.9^{e}	2813.4 ± 43.9^{de}	361.0 ± 15.7^{b}	93.7 ± 2.3^{nbc}	160.9 ± 22.9^{e}	209.4 ± 43.6	329.0 ± 25.7^{de}	180.8 ± 6.6^{de}
T+C+F	27.5 ± 0.2^{d}	9.8 ± 0.2^{gh}	0.3 ± 0.0^{d}	312.3 ± 2.0^{c}	3289.1 ± 58.5^{b}	210.6 ± 6.6^{d}	92.3 ± 9.8^{nbc}	144.8 ± 12.9^{e}	185.3 ± 22.4	270.9 ± 5.03^{e}	199.1 ± 3.1^{bcd}
C+E+F	17.7 ± 0.8^{f}	13.7 ± 0.9^{def}	0.1 ± 0.0^{f}	185.7 ± 2.4^{ef}	2936.8 ± 66.1^{cd}	217.9 ± 7.4^{d}	90.6 ± 15.2^{nbc}	186.5 ± 41.2^{de}	214.0 ± 32.3	347.3 ± 59.5^{de}	143.7 ± 10.5^{gh}
T+C+E+F	36.7 ± 1.1^{b}	12.41 ± 0.2^{ef}	0.4 ± 0.0^{c}	452.7 ± 17.2^{b}	3234.0 ± 32.2^{b}	242.3 ± 1.8^{c}	95.6 ± 7.5^{nbh}	556.5 ± 63.5^{c}	381.1 ± 85.7	808.6 ± 266.9^{bcd}	185.6 ± 4.6^{cde}
F+T	52.9 ± 0.5^{a}	22.2 ± 0.9^{b}	0.6 ± 0.0^{a}	525.6 ± 16.1^{a}	3628.8 ± 130.7^{a}	72.3 ± 2.1^{h}	64.3 ± 7.7^{de}	542.0 ± 39.2^{c}	428.3 ± 48.5	876.4 ± 133.9^{abc}	159.5 ± 7.5^{fg}
F+C	9.9 ± 0.5^{h}	11.6 ± 1.1^{fg}	0.1 ± 0.0^{g}	140.5 ± 3.1^{hi}	2418.0 ± 49.8^{h}	219.9 ± 7.9^{d}	104.3 ± 9.9^{abc}	795.8 ± 41.2^{a}	544.6 ± 109.2	1368.2 ± 163.7^{a}	184.8 ± 3.4^{cde}
F+E	12.4 ± 0.6^{g}	16.0 ± 0.3^{cd}	0.1 ± 0.0^{gh}	37.3 ± 1.1^{k}	944.4 ± 16.7^{i}	152.7 ± 4.2^{e}	90.9 ± 9.1^{abc}	173.4 ± 6.1^{e}	335.5 ± 104.9	544.2 ± 214.5^{be}	184.0 ± 3.4^{cde}
F+C+E	8.7 ± 0.3^{hij}	20.7 ± 0.5^{b}	0.3 ± 0.0^{d}	245.8 ± 1.9^{d}	2776.7 ± 146.4^{def}	427.5 ± 7.0^{a}	72.0 ± 4.9^{cde}	147.8 ± 5.0^{e}	209.5 ± 20.7	308.6 ± 47.6^{e}	140.1 ± 3.0^{h}
F+T+C	7.7 ± 0.1^{ij}	6.8 ± 0.2^{i}	0.2 ± 0.1^{e}	189.3 ± 3.3^{e}	2610.7 ± 15.8^{fg}	247.3 ± 5.3^{c}	78.5 ± 2.9^{cde}	211.4 ± 22.6^{de}	310.5 ± 31.8	467.9 ± 26.9^{cde}	223.1 ± 3.9^{a}
F+T+E	10.4 ± 0.6^{h}	15.0 ± 0.2^{cde}	0.1 ± 0.1^{f}	164.2 ± 2.0^{fg}	2633.8 ± 48.4^{efg}	128.7 ± 1.2^{f}	78.9 ± 5.3^{be}	278.0 ± 51.9^{d}	409.6 ± 101.5	662.0 ± 276.6^{be}	215.9 ± 9.3^{ab}
F+T+C+E	32.6 ± 1.3^{c}	25.7 ± 0.9^{a}	0.5 ± 0.0^{b}	323.2 ± 6.7^{c}	3012.6 ± 30.8^{c}	440.7 ± 7.9^{a}	62.6 ± 7.2^{e}	232.9 ± 32.3^{de}	281.3 ± 29.8	400.1 ± 56.8^{cde}	201.4 ± 11.7^{bc}

Data are expressed as mean of the data. Significance (p-value) of application method (AM), treatment (T). For a given factor and significance ($p < 0.01$), different letters within a column represent significant differences (Duncan test, $p < 0.01$)

Discussion

In recent years, there has been considerable interest in researching natural fungicides such as essential oils as alternatives to synthetic chemicals in the fight against plant diseases in agriculture, and in sustainable and environmentally friendly post-harvest disease management [15]. That's why EOs have been the subject of various studies. The antifungal activity of EOs is tightly dependent on their type, chemical content, and concentration. However, this activity is thought to depend on which compounds exhibit synergistic or antagonistic effects [27]. Thymol, carvacrol, eugenol, and 1,8-cineole oils have been proven to have antifungal activity. Previous studies have proven that these EOs show the highest fungicidal activity against post-harvest pathogens [28–30]. Furthermore, we reported that individual and combinations of these EOCs (thymol, eugenol, and 1,8-cineole) increased quality parameters against *B. cinerea* in post-harvest apples [16, 17]. In this study, the protective-curative ability of EOCs (individual and combination) applied against *B. cinerea* in the Golden Delicious apple cultivar and their effects on the fruit were determined in more detail. Although advances have been made in *B. cinerea* infection in harvested produce, many unanswered questions remain regarding the impact of EOCs against *B. cinerea* infection on the sugar, vitamin, and antioxidant content in stored apples. The utilization of essential fatty acids as a novel strategy for fruit quality management offers a promising avenue for enhancing sustainability within agricultural practices. This approach, which leverages the natural antifungal properties of these compounds, not only contributes to reducing the reliance on synthetic pesticides but also aligns with the principles of integrated pest management, potentially lowering environmental impact. The findings from this study enrich the broader academic discussion on sustainable farming, highlighting the role of bio-based compounds in mitigating post-harvest losses and extending the shelf life of produce. As the agricultural sector continues to investigate environmentally friendly solutions to pest management, the application of essential fatty acids could mark a significant shift towards more sustainable and health-conscious farming practices. On the other hand, the foundational research detailed in our previous paper [31], on the impact of essential fatty acids on pathogenic fungi offers critical insights into their potential as a natural fungicide. By outlining the methodologies used to evaluate the effects of these fatty acids on fungal growth rates and their specific mechanisms of inhibition, this work not only advances our understanding of their biological activity but also paves the way for practical applications in agricultural pest management. The discussion around these findings raises important questions about the broader applicability of essential fatty

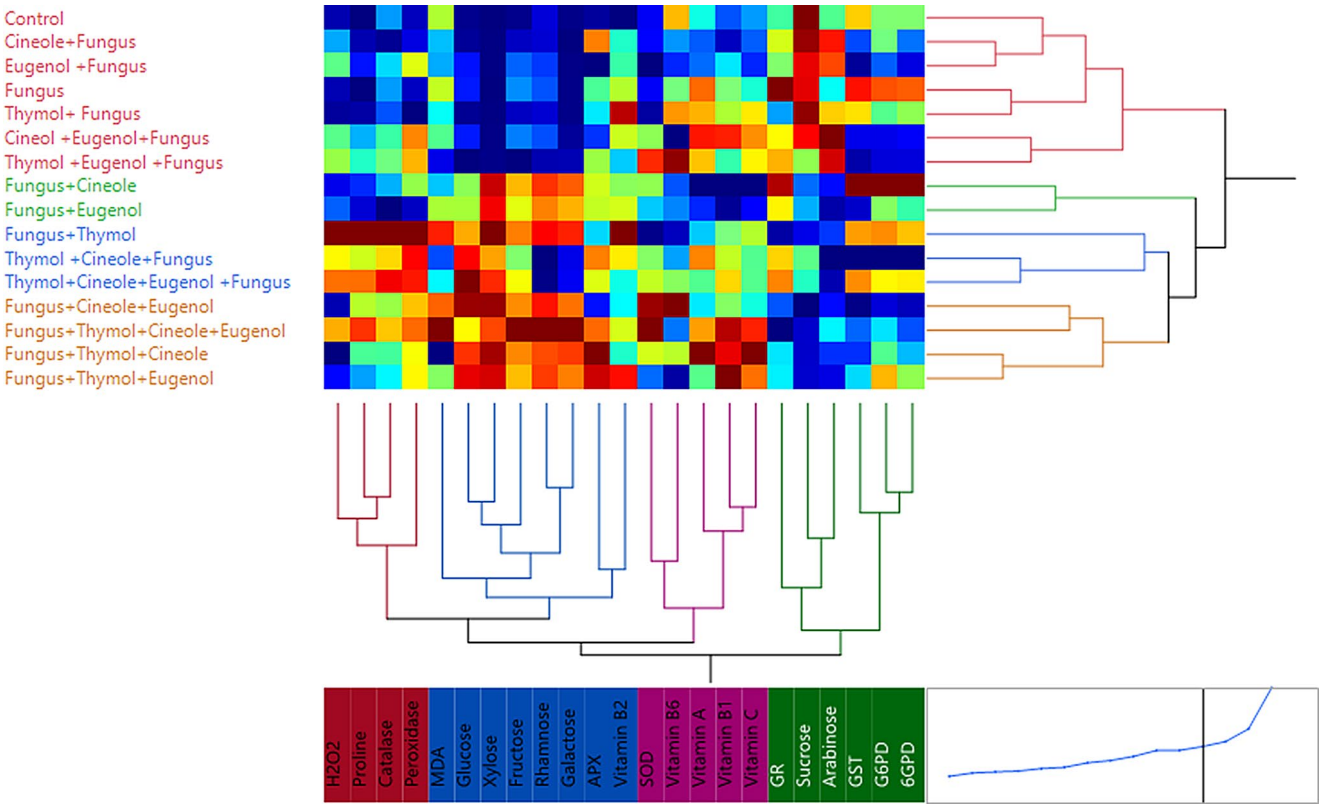


Fig. 1 Conducting a heatmap analysis that scrutinizes numerous components, sugar, vitamin and antioxidant enzyme contents from are demonstrated

acids in diverse agricultural settings and their integration into existing pest management strategies. It also highlights the necessity for further research to explore the efficacy and safety of these compounds, potentially leading to more sustainable and eco-friendly approaches to crop protection and post-harvest treatment. Present study conversation enriches the academic and practical discourse on leveraging natural compounds to enhance food security and environmental health.

Our findings in the study highlight the effect of EOC combinations on the sugar content of apples, especially sucrose, glucose, rhamnose, fructose, arabinose, galactose, and xylose. According to research findings, the most common sugars in treated fruits are sucrose, glucose, and fructose. The results are consistent with those of Aprea et al. [32]; they showed that sucrose, fructose, and glucose sugars were highest in apple fruits. Our findings contradict the previous report, which identified fructose as the highest sugar in fruit tissue [32]. Our results found that sucrose was the highest sugar (CT: 87.9 $g.L^{-1}$). On the other hand, xylose had the lowest level among the sugars whose content was determined. The amount of xylose ranged from 0.1 to 13.6 $g.L^{-1}$. Previous studies reported that xylose had the lowest sugar content in apples [32, 33]. It was observed that there was a significant difference in the amount of xylose present in the preventive and curative treatment groups. The content of xylose was higher in the curative treatment groups (F + T) compared to the preventive treatment groups (T + F, E + F, C + F, C + E + F). Xylose is a crucial component of xyloglucan, which is the second most abundant plant cell wall polysaccharide after cellulose. Xyloglucan plays a vital role in several processes such as, seed germination, cell growth, fruit ripening, and stress resistance [34]. Based on this information, the rise in xylose content in the curative treatment groups indicates the effectiveness of EOCs in combating pathogens in fruits. However, further research is necessary to improve our understanding. The differences in sugar content between different treatment groups in our findings indicate that EOCs and *B. cinerea* interact with apples. The interactions between EOCs, *B. cinerea*, and apples require a more comprehensive investigation of the specific biochemical pathways and metabolic processes responsible for sugar content changes.

Most EOC applications positively affected the vitamin A, B1, B2, and C content of fruits. Application groups such as F + T, T + F, and F + T + C showed a positive effect on the vitamin content of apples. On the other hand, a decrease in vitamin content was observed in other application groups. Our results were consistent with the findings of Salimi et al. [35] and Bagy et al. [36]. They stated that EOs preserved the vitamin C content of fruits during storage. They stated that this protection was due to the antioxidant properties of

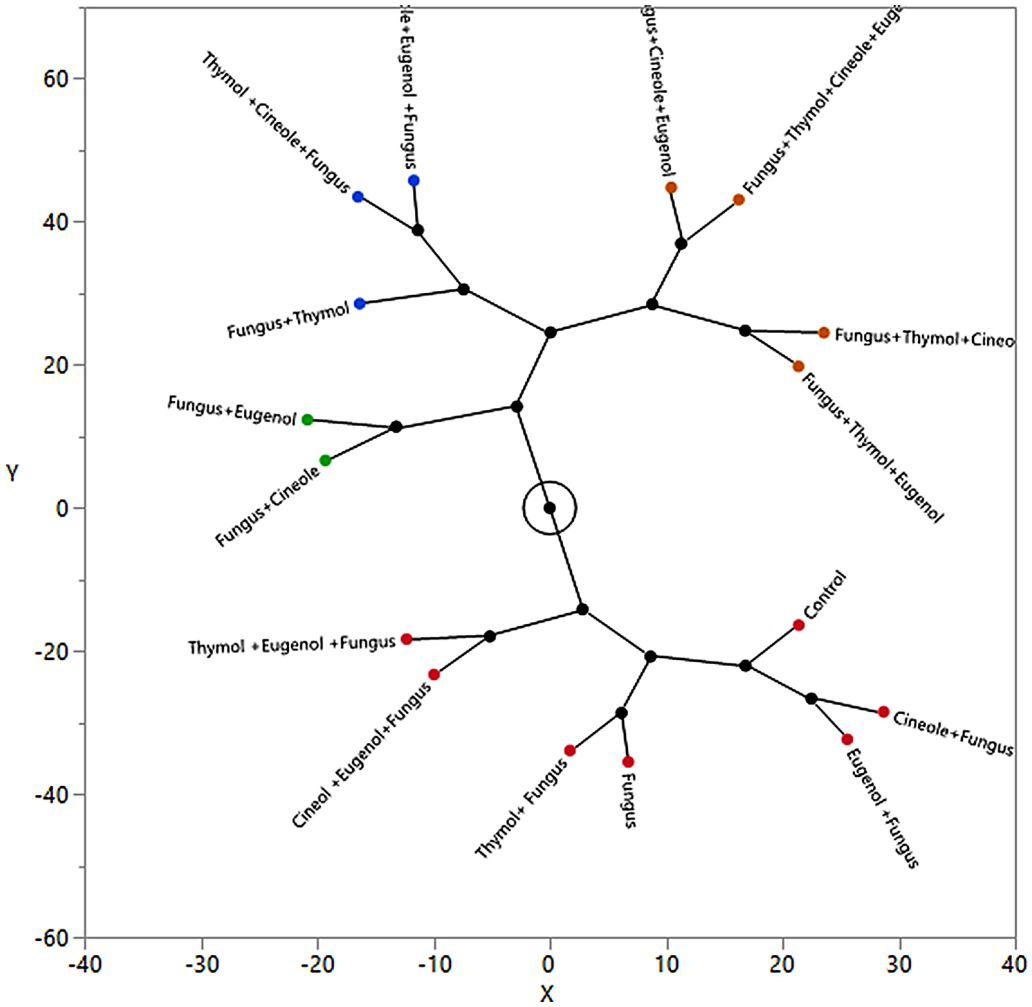


Fig. 2 Grouping of treatments as a result of constellation plot analysis

EOs, which delay ripening by reducing the respiration rate of fruits and reducing ascorbic acid oxidation and oxygen diffusion. It has also been determined that EOs affects the antioxidant activity in fruits by increasing flavonoids. As stated by Zhang et al. [37] vitamin content is important in preserving fruits after harvest. Since there are no studies on other vitamin contents, it isn't possible to make a comparison. Post-harvest storage conditions, light and temperature changes can lead to vitamin losses [38]. According to our findings, we can assume that the applied EOCs positively affect the contents of vitamins A, B1, and B2, which also influence preserving fruit quality.

Previous studies have revealed that antioxidant enzymes play a main role in post-harvest fruit preservation. These enzymes contribute to preserving the freshness and nutritional value of fruits by reducing oxidative stress, especially during the storage process of fruits. In addition, reducing post-harvest oxidative stress helps extend the shelf life and makes the fruits available for consumption healthier [39, 40]. SOD, POD, and CAT activities in cells maintain the balance of oxygen metabolism by rapidly removing reactive oxygen [41]. SOD prevents the formation of free radicals that damage cells by breaking down reactive oxygen species. POD and CAT break down H_2O_2 and transform this compound into less harmful substances [42, 43]. In the current study, EOCs significantly reduced stress parameters (H_2O_2, MDA, and proline levels); it has been proven to increase CAT, SOD, and POD enzyme activities. EOCs increased enzyme levels in both preventive and curative treatment. Similar results were observed in apples and tomatoes treated with *Cuminum cyminum* [40], in apples treated with essential oil [42], and in table grapes against *B. cinerea* in its control with thyme and cinnamon essential oils [15]. In addition, GR, GST, and G6PD enzyme activities affect the metabolism of fruits, helping them cope with oxidative stress and increase their resistance to toxins [20]. On the other hand, among essential fatty acids, linoleic acid has been found to be a potent inhibitor of *B. cinerea*. Inhibition of fungal growth by essential fatty acids, including linoleic acid, can be attributed to several mechanisms: Linoleic acid

mixes with the fungal cell membrane, changing its fluidity and permeability [44]. This disruption leads to leakage of vital cell components and ions, impairing cell function and viability. Essential fatty acids can inhibit the activity of key fungal enzymes involved in cell wall synthesis and ergosterol production, which are essential for maintaining fungal cell membrane structure and function. Additionally, some fatty acids can produce ROS within fungal cells, overwhelming the cell's antioxidant defenses and leading to oxidative damage and cell death. In conclusion, although our study focused primarily on the effectiveness of EOCs against *B. cinerea* in apples, the observed antifungal activity may be partially attributed to the presence of essential fatty acids in these oils. However, the effect of these antioxidant enzymes and EOCs applied against *B. cinerea* in preventing oxidative damage in apples has not yet been examined. In our findings, it was determined that there was an increase and decrease in the GR, GST, and G6PD enzyme levels of EOCs. It has been determined that antioxidant levels are affected during post-harvest storage, and there are losses in antioxidant levels during shelf life. Zhao et al. [45] stated that antioxidants are compatible with each other. That was consistent with our results. APX is the enzyme that prevents vitamin C oxidation. Vitamin C effectively preserves the freshness and nutritional values of fruits [46]. The general trend observed in our findings is that APX enzyme and vitamin C were observed to be correlated in the application groups. The effective functioning of enzymes increases the resistance of fruits to cellular damage caused by oxidative stress and delays fruit aging [30].

On the other hand, the comprehensive analysis of biochemical constituents through heatmap visualization, dendrogram, and constellation plot shed light on the intricate relationships within different treatment groups. The heatmap revealed distinct concentration patterns, particularly in treatments involving the combination of Thymol, Eugenol, and Fungus ('T + E + F'), indicating a potential synergistic impact on biochemical pathways. The hierarchical clustering depicted by the dendrogram showcased major clusters, with the green cluster suggesting an enhancement in antioxidant enzymes, possibly serving as a strategy for mitigating oxidative stress. Conversely, the red cluster hinted at treatments characterized by lower levels of sugars and vitamins, implying a potential depletion effect. The constellation plot further confirmed these clusters, providing a visual representation of treatment proximity and reinforcing the hierarchical structure observed in the dendrogram. The radial layout and color-coded edges in the constellation plot added another layer of understanding, emphasizing the central significance of certain substances and highlighting the varying strengths and types of interactions. Overall, these visual analyses not only provided a nuanced comprehension of the biochemical responses but also paved the way for targeted investigations into the potential synergies and inhibitory effects observed, contributing to the broader understanding of biochemical pathways and treatment strategies.

Conclusions

This study unraveled the intricate dynamics of EOCs in mitigating the impact of *B. cinerea* on apple fruits, providing a nuanced understanding of their curative and preventive effects. The research delved into the modulation of sugar contents, including fructose, rhamnose, galactose, xylose, and arabinose, in response to various EOC treatments. These alterations in sugar profiles hinted at a subtle influence on the metabolic pathways governing plant defense mechanisms. Furthermore, the investigation extended to the realm of vitamins, revealing substantial increases in Vitamin A, B1, B2, B6, and C levels under specific EOC combinations. Notably, treatments like F + T, T + F, and F + T + C emerged as influential in enhancing the nutritional quality of apple fruits, potentially contributing to their overall health benefits. The assessment of oxidative stress markers and antioxidant enzyme activities provided crucial insights into cellular responses triggered by EOC applications. EOCs exhibited a remarkable capacity to reduce H_2O_2, MDA, and proline levels, indicating their potential in alleviating oxidative stress and cellular damage. The modulation of CAT, POD, SOD, GR, GST, G6PD, 6GPD, and APX underscored the multifaceted role of EOCs in the antioxidant defense system. Interestingly, differential responses between curative and preventive treatments were identified, with the latter showing promising effects in reducing H_2O_2, MDA, and proline levels. Enhanced activities of antioxidant enzymes in specific treatment combinations, such as T + C + E + F, highlighted the potential synergistic effects of EOCs in bolstering the plant's natural defense mechanisms. Looking ahead, future research endeavors could delve deeper into the molecular mechanisms underlying these observed effects, unraveling specific pathways and gene expressions modulated by EOCs. This deeper understanding could pave the way for the development of targeted and sustainable strategies for managing fungal infections in agriculture, optimizing EOC formulations for increased efficacy and minimal environmental impact.

Abbreviations

EOCs	Essential Oil Compounds
CT	Control
F	Fungus
E	Eugenol
C	1,8-Cineole

T+F	Thymol+Fungus
C+F	Cineole+Fungus
E+F	Eugenol+Fungus
C+E+F	Cineole+Eugenol+Fungus
T+E+F	Thymol+Eugenol+Fungus
T+C+F	Thymol+Cineole+Fungus
T+C+E+F	Thymol+Cineole+Eugenol+Fungus
µL	1.25
T	Thymol
F+T	Fungus+Thymol
F+E	Fungus+Eugenol
F+C+E	Fungus+Cineol+Eugenol
F+T+E	Fungus+Thymol+Eugenol
F+T+C	Fungus+Thymol+Cineole
F+T+C+E	Fungus+Thymol+Cineole+Eugenol
GSH	Glutathione
SOD	Superoxide Dismutase
POD	Peroxidase
CAT	Catalase
GR	Glutathione Reductase
GST	Glutathione S-Transferase
H_2O_2	Hydrogen Peroxide
MDA	Malondialdehyde

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Declarations

Conflict of interest The authors declare no conflict of interest.

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References

1. O. Ou-Ani, S. Moujane, L. Oucheikh, Y. Youssefi, A. Ansari, A. Oubair, D. Chebabe, M. Znini, 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. Pflanzenschutz. **55** (13), 1504–1529 (2022)
2. A. Muder, H. Garming, S. DreisiebnerLanz, K. Kerngast, F. Rosner, K. Klickova, G. Kurthy, K. Cimer, A. Bertazzoli, V. Altamura, G. De Ros, K.M.B.C. Zmarlicki Freitas, A. Duarte, E. Bravin, J. Kambor, D. Karamürsel, F.P. Öztürk, E. Kaçal,. Apple production and apple value chains in Europe. Eur. J. Hortic. Sci. 1611–4434 (2022)
3. J. Patocka, K. Bhardwaj, B. Klimova, E. Nepovimova, Q. Wu, Malus domestica: a review on nutritional features, chemical composition, traditional and medicinal value. Plants. **9**(11), 1408 (2020)
4. S. Konstantinou, G. Karaoglanidis, G. Bardas, I. Minas, E. Doukas, Postharvest fruit rots of apple in Greece: Pathogen incidence and relationships between fruit quality parameters, cultivar susceptibility, and patulin production Plant Dis. **95** (6), 666–672 (2011)
5. M. Manssouri, M. Znini, A. El Harrak, L. Majidi, Antifungal activity of essential oil from the fruits of *Ammodaucus Leucotrichus* Coss. & Dur., in liquid and vapour phase against postharvest phytopathogenic fungi in apples. J. Appl. Pharm. Sci. **6**(5), 131–136 (2016)
6. W.M. Jurick, O. Macarisin, V.L. Gaskins, E. Park, J. Yu, Characterization of postharvest fungicide-resistant *Botrytis cinerea* isolates from commercially stored apple fruit. Phytopathology. **107**(3), 362–336 (2017)
7. M. Sapper, L. Palou, M.B. Pérez-Gago, A. Chiralt, Antifungal starch–gellan edible coatings with thyme essential oil for the postharvest preservation of apple and persimmon. Coatings. **9**(5), 333 (2019)
8. C. Nunes, J. Usall, N. Teixido´, X.O. Eribe, I. Vin˜as, Control of post-harvest decay of apples by pre-harvest and post-harvest application of ammonium molybdate. Pest Manag Sci. **57**, 1093–1097 (2001)
9. A.T. Zatla, I. Mami, M.E. Dib, M.E. Sif, Efficacy of essential oil and hydrosol extract of *Marrubium vulgare* on fungi responsible for apples rot. Anti-Infective Agents. **18**(3), 285–293 (2020)
10. Y. Elouadi, H. Bendaif, H. Assaggaf, E.M. Abdallah, M. Mekkaoui, Efficacy of *Pelargonium graveolens* essential oils against some postharvest fungal diseases of apple. ALS. **9**(2), 195–201 (2022)
11. M. Moghaddam, L. Mehdizadeh, Chap. 13-Chemistry of essential oils and factors influencing their constituents. Soft Chem. Food Ferment. 379–419 (2017)
12. K. Jassal, S. Kaushal, R. Rani, Antifungal potential of guava (*Psidium guajava*) leaves essential oil, major compounds: beta-caryophyllene and caryophyllene oxide. Arch. Phytopathol. Pflanzenschutz. **54**(19–20), 2034–2050 (2021)
13. M.H. Nguyen, T.N.M. Tran, N.B.D. Vu, Antifungal activity of essential oil-encapsulated lipid nanoemulsions formulations against leaf spot disease on tomato caused by *Alternaria alternata*. Arch. Phytopathol. Pflanzenschutz. **55**(2), 235–257 (2022)
14. K.A.M. Abo-Elyousr, D.A. Adel, N.M. Almasoudi, Evaluation of the synergy between *Schwanniomyces vanrijiae* and propolis in the control of *Penicillium digitatum* on lemons. Egypt. J. Biol. Pest Control. **31**, 66 (2021)
15. N.M. Almasaudi, A.D. Al–Qurashi, M.I. Elsayed, K.A.M. Abo–Elyousr, Essential oils of oregano and cinnamon as an alternative method for control of gray mold disease of table grapes caused by *Botrytis Cinerea*. Plant. Pathol. J. **104**, 317–328 (2022)
16. B. Yelboğa, S. Karakuş, A study on antifungal activity of thymol, eugenol, and 1,8-cineole against Botrytis Cinerea Persoon isolated from grapevine (Vitis vinifera Linné). J. Cent. Eur. Agric. **24**(4), 888–898 (2023). https://doi.org/10.5513/JCEA01/24.4.3883

17. S. Karakuş, Hasat Sonrası Uygulanan Bazı Uçucu Yağların Elmalarda *Botrytis Cinerea* ve Meyve Besin İçeriğine Etkisi. Türk Tarım Ve Doğa. Bilimleri Dergisi. **10**(3), 583–590 (2023a Jul 7)
18. S. Droby, M. Wisniewski, A. El Ghaouth, C. Wilson, 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. **27**(2), 127–135 (2003)
19. E.K. Nikolidaki, A. Chiou, M. Christea, A.P. Gkegka, M. Karvelas, V.T. Karathanos, Sun dried corinthian currant (*Vitis Vinifera* L., var. *Apyrena*) simple Sugar Profile and Macronutrient characterization. Food Chem. **221**, 365–372 (2017)
20. N. Keskin, O. Kaya, F. Ates, M. Turan, G. Gutiérrez-Gamboa, Drying grapes after the application of different dipping solutions: effects on hormones, Minerals, vitamins, and antioxidant enzymes in Gök Üzüm (*Vitis Vinifera* L.) Raisins. Plants. **11**, 529 (2022)
21. P. Samydurai, R. Ramakrishnan, N. Nagarajan, V.-E. Polyphenols, Estimation and in Vitro antioxidant activity of Adiantum Capillus-Veneris. Int. J. Pharm. Sci. Res. **4**, 258–262 (2013)
22. N.H.M. Rubel Mozumder, M.J. Akhter, A.A. Khatun, M. Rokibuzzaman, M. Akhtaruzzaman, Estimation of water-soluble vitamin B-Complex in selected Leafy and Non-leafy vegetables by HPLC Method. Orient. J. Chem. **35**(4), 1344–1351 (2019)
23. A. Minucci, B. Giardina, C. Zuppi, E. Capoluongo, Glucose-6-Phosphate Dehydrogenase Laboratory Assay: How, When, and Why? IUBMB Life. **61**, 27–34 (2009)
24. P.C. Chikezie, C.M. Chikezie, A.A. Uwakwe, C.C. Monago, Comparative study of glutathione S-Transferase activity of three human erythrocyte genotypes infected with *Plasmodium Falciparum*. JASEM. **13**, 13–18 (2010)
25. T. Abedi, H. Pakniyat, Antioxidant enzymes changes in response to Drought stress in ten cultivars of Oilseed rape (*Brassica napus* L). Czech J. Genet. Plant. J. **46**, 27–34 (2010)
26. R. Angelini, F. Manes, R. Federico, Spatial and functional correlation between diamine-oxidase and peroxidase activities and their dependence upon de-etiolation and wounding in chick-pea stems. Planta. **182**, 89–96 (1990)
27. Y. El Ouadi, M. Manssouri, A. Bouyanzer, L. Majidi, H. Bendaif, H. Elmsellem, A. Shariati, A. Melhaoui, B. Hammouti, Essential oil composition and antifungal activity of *Melissa officinalis* originating from north-Est Morocco, against postharvest phytopathogenic fungi in apples. Microb. Pathog. **107**, 321e326 (2017)
28. D. Spadaro, M.L. Gullino, Use of essential oils to control postharvest rots on pome and stone fruit. *Post-Harvest Pathology*. Springer International Publishing, Cham, Switzerland, 101–110 (2014)
29. H.A. Abd El Aal, L.A. Ahmed, W.A. Hassan, H.M. Fawzy, H. Moawad, Combination of carvacrol with simvastatin improves the lipid-lowering efficacy and alleviates simvastatin side effects. J. Biochem. Mol. Toxicol. **31**, e21981 (2017)
30. M.I. Elsayed, A.D. Al-Qurashi, N.M. Almasaudi, K.A.M. Abo-Elyousr, Efficacy of essential oils against gray mold and effect on fruit quality during cold storage in table grapes. S Afr. J. Bot. **146**, 481–490 (2022)
31. S. Karakuş, O. Kaya, M. Şahin, Improving biogenic amines and combating *Botrytis cinerea* decay in 'Golden Delicious' apples with post-harvest essential oil treatments. App. Fruit Sci. https://doi.org/10.1007/s10341-024-01073-0 (2024)
32. E. Aprea, M. Charles, I. Endrizzi, M.L. Corollaro, E. Betta, F. Biasioli, F. Gasperi, Sweet taste in apple: the role of sorbitol, individual sugars, organic acids and volatile compounds. Sci. Rep. **7**, 44950 (2017)
33. G. Winisdorffer, M. Musse, S. Quelle, A. Barbacci, S.L. Gall, F. Mariette, Analysis of the dynamic mechanical properties of apple tissue and relationships with the intracellular water status, gas distribution, histological properties and chemical composition. Postharvest Biol. Technol. **104**, 1–16 (2015)
34. T. Hayashi, R. Kaida, Functions of Xyloglucan in Plant cells. Mol. Plant. **4**(1), 17–24 (2021)
35. L. Salimi, M. Arshad, A. Rahimi, A. Rokhzadi, S. Amini, M. Majid Azizi, Effect of some essential oils on post-harvest quality of grapevine (*Vitis vinifera* Cv *Rash*a (Siah-e-Sardasht)) during cold storageInt. J. Biosci. **3**, 72–85 (2013)
36. H.M.M.K. Bagy, F.M.I. Badawy, A.A.A. Eman, M.S. Badawy, N.M.A. Sallam, Control of green mold disease using chitosan and its effect on orange properties during cold storage. Arch. Phytopathol. Pflanzenschutz. **54**(11–12), 570–585 (2021)
37. C. Zhang, W. Li, B. Zhu, H. Chen, H. Chi, L. Li, Y. Qin, J. Xue, Thequality evaluation of postharvest strawberries stored in nano-ag packages at refrigeration temperature. Polymers. **10**, 894 (2018)
38. S.K. Lee, A.A. Kader, Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biol. Technol. **20**, 207–220 (2000)
39. E.A. Elsherbiny, M.A. Taher, Silicon induces resistance to postharvest rot of carrot caused by *Sclerotinia sclerotiorum* and the possible of defense mechanisms. Postharvest Biol. Technol. **140**, 11–17 (2018)
40. Y. Wang, Y. Qiao, M. Zhang, Z. Ma, Y. Xue, Q. Mi, A. Wang, J. Feng, Potential value of small-molecule organic acids for the control of postharvest gray mold caused by *Botrytis Cinerea*. Pestic Biochem. Phys. **177**, 104884 (2021)
41. P. Jin, S.Y. Wang, H. Gao, H. Chen, Y. Zheng, C.Y. Wang, Effect of cultural system and essential oil treatment on antioxidant capacity in raspberries. Food Chem. **132**(1), 399–405 (2012)
42. S.Y. Zhang, Y.W. Yu, C.L. Xiao, X.D. Wang, Y.F. Lei, Effect of ultraviolet irradiation combined with chitosan coating on preservation of jujube under ambient temperature. LWT-Food Sci. Technol. **57**, 749–754 (2014)
43. C.Y. Hua, K. Kai, W.L. Bi, W. Shi, Y.S. Liu, D.F. Zhang, Curcumin induces oxidative stress in *Botrytis Cinerea*, resulting in a reduction in gray mold decay in kiwifruit. J. Agric. Food Chem. **67**, 7968–7976 (2019)
44. M. Słaba, P. Bernat, S. Różalska, J. Nykiel, J. Długoński, (2013). Comparative study of metal induced phospholipid modifications in the heavy metal tolerant filamentous fungus Paecilomyces marquandii and implications for the fungal membrane integrity. Acta Biochim. Pol., **60**(4)
45. D.Y. Zhao, L. Shen, M.M. Yu, Y. Zheng, J.P. Sheng, Relationship between activities of antioxidant enzymes and cold tolerance of postharvest tomato fruits. Food Sci. **14**, 309–313 (2009)
46. M. Fenech, I. Amaya, V. Valpuesta, M.A. Botella, Vitamin C Content in Fruits: Biosynthesis and Regulation. Frontiers in Plant Science. **9**, 2006 (2018)

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