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Enhancing Post-Harvest Resilience: Investigating the Synergistic Effects of Essential Oil Combinations on Biochemical Profiles in *Botrytis cinerea*-Infected Apples

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Abstract: This study explored the impact of essential oil combinations on *Botrytis cinerea*-infected apples, focusing on how these treatments affect the levels of amino acids, anthocyanins, organic acids, phenolic compounds, and hormones. We discovered significant differences in amino acid concentrations, particularly asparagine, serine, histidine, glycine, and arginine, between control and fungus-treated apples. Preventive measures notably increased anthocyanins like delphinidin-3-glycoside and peonidin-3-glycoside, while combinations such as thymol + fungus balanced anthocyanin profiles effectively. Organic acid and phenolic compound analyses showed that curative strategies generally increased concentrations, with the thymol + cineole + fungus treatment being especially effective. Hormonal analysis highlighted the benefits of preventive measures in raising indole-3-acetic acid and gibberellic acid levels, whereas curative treatments increased abscisic acid and salicylic acid concentrations. The combination of cineole and thymol with fungicide emerged as a potent strategy for enhancing phenolic content. These findings underscore the potential of specific essential oil combinations in improving the biochemical composition of *B. cinerea*-infected apples, offering new avenues for enhancing fruit quality and sustainability in the agriculture sector.

Keywords: amino acids; anthocyanins; organic acids; phenolic compounds; hormones; preventive and curative applications

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1. Introduction

Apples, renowned for their rich heritage and pivotal role globally, stand as a cornerstone in the culinary, cultural, and economic realms. Known scientifically as *Malus domestica*, they are an integral part of a myriad of cuisines, enhancing both sweet and savory dishes with their distinct flavors [1]. Rich in dietary fiber, antioxidants, and vitamins, apples contribute significantly to health and wellness [2]. The apple sector plays a critical role in global agriculture, supporting millions of livelihoods and generating considerable economic value [3]. With a spectrum from sweet to tart, the diversity of apple varieties meets the broad preferences of consumers around the globe, solidifying their status as an essential fruit [4]. In Turkey, apples are deeply embedded in the nation's cultural and economic fabric, bolstering its agricultural legacy and prosperity. The country's diverse terrain fosters the cultivation of apples, especially in renowned regions such as Amasya and Erzincan, known for their premium-quality produce [5]. Apples are a staple in Turkish cuisine, featured in a variety of dishes, from crisp salads to traditional sweets, highlighting their culinary flexibility [6]. They also carry cultural significance, symbolizing prosperity and fortune in celebrations and festivals. The Turkish apple industry is vital for local economies, providing jobs and boosting agricultural productivity [7]. The availability of various apple types ensures a steady supply for both the domestic market and international exports, affirming the fruit's key role in Turkey's agriculture and cultural heritage.

Amino acids, anthocyanins, organic acids, phenolic compounds, and hormones found in apples play crucial roles in human health [8]. Essential amino acids like tryptophan, lysine, and leucine are integral for protein synthesis, influencing muscle development, immune response, and neurotransmitter production [9]. Apples, rich in anthocyanins, offer potent antioxidants associated with anti-inflammatory effects and cardiovascular health benefits [10]. Organic acids, including citric acid and malic acid, contribute to the tart flavor of apples and may participate in metabolic processes, aiding digestion and potentially providing anti-inflammatory effects [11]. Phenolic compounds, such as flavonoids and polyphenols present in apples, are linked to reduced oxidative stress and a lowered risk of chronic diseases, promoting overall health. Additionally, plant hormones such as cytokinins and auxins in apples regulate development and growth and may contribute to health benefits, including enhanced cell regeneration and anti-aging effects [12]. EOs, on the other hand, derived from a variety of plant sources [13], have been under investigation for their potential role in preventing diseases, including those caused by pathogens like *B. cinerea* in plants [14]. Some EOs have shown antifungal properties and are being explored as potential natural alternatives for disease control [15]. *B. cinerea*, a notorious fungal pathogen, is responsible for causing grey mold disease in various crops [16]. Although there is promising evidence supporting the efficacy of EOs against other plant pathogens and *B. cinerea*, the body of research in this field is still relatively limited. Existing studies often highlight the potential of essential oils in inhibiting fungal disease development [17], but further comprehensive study is needed to understand the mechanisms of action, optimize application methods, and assess the broader implications of incorporating essential oils into agricultural disease management strategies. There is limited scientific evidence supporting the assertion that applying EOCs can effectively reduce *B. cinerea* disease in apples.

The scarcity of existing studies underscores the ongoing need for research to assess the practicality and effectiveness of EOCs in preventing diseases caused by pathogens like *B. cinerea*. Despite the well-established antifungal and antimicrobial traits of EOs, their specific efficacy against *B. cinerea* in apple orchards remains uncertain. It is crucial to understand the impact of EOCs on various components, unraveling the intricate mechanisms behind their effectiveness in mitigating *B. cinerea* disease. This investigation is pivotal for refining application methods and gaining insights into the practicality and effectiveness of EOs in agricultural disease management strategies. In this context, our study aims to examine how the application of EOCs may induce alterations in the levels of crucial compounds, including phenolic compounds, anthocyanins, organic acids, amino acids, and hormones, as we explore the potential benefits of EOCs in preventing diseases caused by pathogens like *B. cinerea* in Golden Delicious apples.

2. Materials and Methods

2.1. Fruit Materials, Pathogen, Chemicals

The experiment used apples, namely Golden Delicious, harvested from Erzincan orchards in Sept., with environmental conditions maintained at a temperature of 25 °C and humidity of 30.0%. Only commercially ripe fruits, devoid of uniformity in size and physical damage and free from pathogen infections, were selected for the study.

B. cinerea was isolated, molecularly identified, and employed in this study based on previous work [18]. Prior to the experiments, *B. cinerea* was incubated in potato dextrose agar (PDA) medium for seven days at a constant temperature of 25 °C.

Thymol, eugenol, and 1,8-cineole were procured from Sigma-Aldrich, Shanghai, China and stored at 4 °C in a dark environment.

2.2. Inoculation and Storage of Fruits

To prepare the solution for inoculation, 5 mL of the stock solution was combined with 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. Using a sterile puncture needle, two wounds measuring 3 mm in width and 3 mm in depth were created at the equator of disinfected apple fruits [19]. For protective purposes, the following applications were established in the trial: 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. For curative purposes, the following applications were set up: 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; F+C, 1.25 µL; 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. Dosage combinations were determined based on preliminary results to prevent potential fruit peel deformation when combining individual concentrations. The experiment included both preservative and curative treatments (22 in total), with each treatment replicated three times, using three apples per replication, in a completely randomized design. For the curative effect, the fruits were submerged in essential oil solutions and incubated for 30 min. Afterward, they were air-dried at room temperature for 24 h. Subsequently, the wound sites were inoculated with 125 µL of a conidial suspension of *B. cinerea* at a concentration of 1×10^5 spores/mL. For the preservative effect, the wound sites on the fruits were initially inoculated with 125 µL of a conidial suspension of *B. cinerea* at a concentration of 1×10^5 spores/mL. Following a 24 h incubation period at room temperature, the fruits were submerged in essential oil solutions for 30 min. Infected fruits were then stored in transparent plastic boxes in the dark for one week in a specially designed room to keep temperature and humidity constant (4 °C, 90 ± 5% humidity). The inspection of the infected fruits took place after seven days of incubation [20].

2.3. Identification of Amino Acid Profiling in Apple by HPLC

Apple samples from the Golden Delicious cultivar, collected at harvest, underwent amino acid profiling using a modified method inspired by Barrado et al. [21]. The apples were homogenized, and the resulting pulp was freeze-dried to produce a fine powder. Amino acids were extracted from 1 g of the freeze-dried apple powder by suspending it in 10 mL of 0.1 M HCl. The suspension was thoroughly mixed and sonicated for 15 min to ensure efficient extraction. Subsequently, the supernatant was obtained following centrifugation. Amino acids in the supernatant were derived using the o-phthaldialdehyde (OPA) reagent. For this, 20 µL of the extracted sample was mixed with 80 µL of OPA reagent (10 mg OPA dissolved in 1 mL methanol) and incubated for 2 min at room temperature. This derivatization process enhanced the detectability of amino acids during HPLC analysis. The derivatized amino acids were separated and quantified using a high-performance liquid chromatograph (HPLC) equipped with a C18 column. The mobile phase typically consisted of two components: buffer A (0.1 M sodium acetate, pH 7.2) and buffer B (acetonitrile). An isocratic elution at a suitable flow rate was employed for the separation of amino acids. Detection was carried out using a fluorescence detector set at specific excitation and emission wavelengths for OPA-derivatized amino acids. Amino acids were quantified by comparing their peak areas or retention times with those of known amino acid standards. The concentration of each amino acid in the samples was determined based on standard calibration curves.

2.4. Identification of Anthocyanins Profiling in Apple by HPLC

Anthocyanins were analyzed with modifications to the method developed by Yousef et al. [22] using a high-performance liquid chromatography (HPLC) system, specifically the Agilent 1100 Series system (Agilent, Waldbronn, Germany). This system was equipped with a diode array detector (DAD) (G1315B) and coupled to an LC/MSD Trap VL (G2445C VL) ESI-MS/MS system. Data processing was conducted using an Agilent ChemStation (version B.01.03) data processing station, and mass spectral data were further

processed using the Agilent LC/MS Trap software (version 5.3). Anthocyanin extracts, in volumes of 10 µL, were carefully injected onto a reversed-phase column, specifically the Zorbax Eclipse XDB-C18 (2.1 mm × 150 mm; 3.5 µm particle; Agilent, Germany). The column temperature was precisely maintained at 40 °C. The separation method was based on a previously established technique, with slight adjustments to improve the separation of the detected anthocyanins. The mobile phase for separation consisted of a mixture of water, acetonitrile, and formic acid, with two solvent compositions: 88.5% water, 3% acetonitrile, and 8.5% formic acid (solvent A); and 41.5% water, 50% acetonitrile, and 8.5% formic acid (solvent B). The separation gradient employed was as follows: 97% A and 3% B for 8 min; 70% A and 30% B over 20 min; 50% A and 50% B over 6 min; 0% A and 100% B over 4 min, maintained for 2 min, followed by a return to initial conditions in 6 min. A conditioning period of 8 min between injections was incorporated. The flow rate was set at 0.19 mL/min.

2.5. Identification of Organic Acids from Apple by HPLC

The extraction of organic acids followed the method established by Keskin et al. [23]. For extraction, a mixture was created by combining 5 mL of apple must with 20 mL of a 0.009 M NH_2SO_4 solution. After thorough homogenization, the mixture underwent 1 h of agitation on a shaker and was subsequently centrifuged at 15,000 rpm for 15 min. The resulting supernatants were subjected to a filtration process, initially passing through filter paper to eliminate larger particles, followed by two additional filtrations using a 0.45 µm membrane filter to remove finer particulate matter. Further purification was achieved by passing the filtered solutions through a SEP-PAK C18 cartridge. The subsequent analysis of the extracted organic acids was conducted using high-performance liquid chromatography (HPLC). An Aminex column (HPX-87 H, 300 mm × 7.8 mm) served as the chromatographic medium for the separation and quantification of organic acids in the samples. Throughout the study, chemicals with high analytical purity were employed. Standards, specifically tartaric, malic, citric, succinic, and fumaric acids, were sourced from Sigma-Aldrich in St. Louis, MO, USA.

2.6. Identification of Phenolic Compounds from Apple by HPLC

Apple samples from the Golden Delicious cultivar, collected at harvest, underwent phenolic compound analysis. Whole apple samples from clusters were triturated with a conventional beater until a homogeneous apple sample was obtained for analysis. The analyzed phenolic compounds included gallic acid, vanillic acid, trans-caffeic acid, trans-p-coumaric acid, ferulic acid, kaftaric acid, catechin, epicatechin, quercetin, rutin, myricetin, and tyrosol. Phenolic compounds were extracted from the apple samples using a modified version of the method described by Barrado et al. [21], with three replications. The triturated apple samples were mixed with distilled water at a 1:1 ratio and then centrifuged at 15,000 rpm for 15 min. The upper part of the samples was filtered using 0.45 µm MF-Millipore filters. The filtered samples were injected into an HPLC device for phenolic compound analysis. Chromatography assays were conducted using an Agilent 1100 HPLC device equipped with a diode-array detector (Agilent, Santa Clara, CA, USA) and a 4 µm octadecyl–silica column (4.6 × 250 mm, Hichrom, Reading, UK). The mobile phase was prepared using two components: A—methanol–water–acetic acid (10:28:2, v/v) and B—methanol–water–acetic acid (90:8:2, v/v). Phenolic compounds were detected at 254 nm and 280 nm. The injection volume was 20 µL, and the flow rate was set at 1 mL/min.

2.7. Identification of Hormones from Apple by HPLC

The identification of hormones from fruit samples involved a series of steps. Initially, the fruits were homogenized and subjected to triple filtration into a solution composed of 80% ethanol (based on 5 g of fresh weight). Subsequently, 200 pmol of 13C6-IAA and d6-ABA were added as internal standards. The resulting solution underwent concentration

using a rotary evaporator, pH adjustment to 2.8 with dilute hydrochloric acid, and filtration through a 0.22 µm membrane filter. A partition extraction step was then performed with diethyl ether, followed by concentration and filtration with a 0.22 µm membrane filter. The extracts were subjected to fractionation using an Agilent 1200 Series HPLC system equipped with an ultraviolet detector. A Zorbax Eclipse-AAA C-18 column was utilized, isocratically eluted with a solution consisting of 40% ethanol and 0.1% acetic acid. The eluates corresponding to the retention times of indole-3-acetic acid (IAA) and abscisic acid (ABA) were collected separately. These IAA and ABA fractions were dried under reduced pressure and further purified using the same HPLC system under isocratic elution conditions. The chromatographic parameters for the identification and quantification of these plant hormones were in accordance with the methodology reported by Kojima et al. [24]. For the analysis of gibberellin (GA_3), a solution comprising 80% ethanol (based on 9 g of fresh weight) was prepared, and 200 pmol of GA_3 was introduced. The solution underwent concentration, pH adjustment to 3.5 with dilute hydrochloric acid, and filtration through a 0.22 µm membrane filter. Partition extraction with ethyl acetate was conducted, and anhydrous sodium sulfate was added for dehydration. The ethyl acetate layer was decanted, concentrated, dissolved in 1 mL of ethanol, and filtered through a 0.22 µm membrane filter. The further extraction, separation, and purification of GA_3 followed the methodologies outlined by Kojima et al. [24]. The analysis of salicylic acid (SA) was based on the method described by Kaya et al. [25] with modifications. Samples were reduced to a fine powder using a mortar and pestle in liquid nitrogen, and 100 mg of the sample was combined with extraction solvents. SA was separated and quantified using an Agilent 1200 Series HPLC system equipped with a photodiode array detector. The mobile phases consisted of 0.3% phosphoric acid in water (solvent A) and 100% methanol (solvent B). The flow rate was set at 0.8 mL min^{-1}, and the solvent system was programmed through several stages. Data acquisition and analysis were performed using YL-clarity 4.0 software, with SA contents calculated using an external standard.

2.8. Data Analysis

In this study, we employed the statistical package integrated within R Studio for all descriptive and inferential analyses. To thoroughly examine the influence of applications and treatments, as well as their potential interactions, on the concentrations of amino acids, anthocyanins, organic acids, phenolic compounds, and hormones, we conducted a comprehensive analysis of variance (ANOVA) utilizing the statistical capabilities within R Studio. The statistical model encompassed both main effects and interaction effects, with subsequent assessments for adherence to normality assumptions. Four distinct models were meticulously crafted to assess the primary effects of both applications and treatments on the concentrations of amino acids, anthocyanins, organic acids, phenolic compounds, and hormones. In instances where statistical significance was established through ANOVA, post hoc analysis was undertaken employing Tukey's test, a widely recognized method for thoroughly exploring differences among multiple groups. For a more nuanced understanding and to visualize the interrelationships among various variables, principal component analyses (PCAs) were carried out. This analytical technique, applied to amino acids, anthocyanins, organic acids, phenolic compounds, and hormones, was executed using ggplot2 within R Studio. The use of PCA helps reduce multidimensional data into a more interpretable form, enabling the identification of underlying patterns and trends within complex datasets.

3. Results

In the analysis of the preventive and curative applications (A), no statistically significant distinctions emerged in the concentrations of aspartate ($p = 0.446$) and glutamate ($p = 0.85$). However, considerable variations were noted in asparagine ($p < 0.001$ ***), serine

(p = 0.011 *), histidine (p = 0.001 ***), glycine (p = 0.001 ***), and arginine (p = 0.001 ***), indicating distinct impacts on these amino acids. Thionine (p = 0.1810) and alanine (p = 0.497) did not exhibit statistically significant differences. Upon scrutinizing the effects of different treatments (T), highly significant variations were apparent across all amino acids (p < 0.001 ***), underscoring the efficacy of the treatments in influencing amino acid levels. Treatment C, serving as the control, exhibited concentrations of amino acids such as tyrosine (189.8 ± 4.1), cystine (96.7 ± 8.3), and valine (98.8 ± 10.1), among others. Treatment F, representing a specific essential oil application, demonstrated distinct amino acid profiles with elevated concentrations of phenylalanine (643.8 ± 3.5), isoleucine (515.1 ± 11.1), and leucine (503.2 ± 20.1), suggesting a notable impact on these components. The combination of treatments (T+F) showcased a unique amalgamation of effects, with amino acid concentrations such as cystine (318.2 ± 5.2), tryptophan (426 ± 17.7), and proline (377.4 ± 14.9) standing out. Treatment combinations involving the control (C) and essential oil application (C+F, T+C+F, T+C+E+F) displayed varying amino acid concentrations, illustrating the intricate interactions between the control and essential oil treatments. Notably, the combination of all treatments (C+E+F) demonstrated substantial changes in amino acid concentrations, particularly for valine (690.0 ± 9.7), methionine (208 ± 17.7), and lysine (371.1 ± 10.5). Similarly, the combination of treatments T+E+F showed distinct effects on amino acids, with elevated concentrations of phenylalanine (104.3 ± 3.5) and lysine (381.6 ± 13.1). Treatment T+C+E+F, incorporating all components, displayed unique effects on the amino acid content, with particularly high concentrations observed for sarcosine (163 ± 17.7) and hydroxyproline (231.5 ± 26.3). Analyzing the preventive and curative applications (A), no significant differences were observed in the concentrations of tyrosine (p = 0.628), leucine (p = 0.293), and proline (p = 0.507). However, statistically significant differences were identified for cystine (p = 0.001 ***), valine (p = 0.001 ***), methionine (p = 0.003 **), tryptophan (p = 0.001 ***), phenylalanine (p = 0.001 ***), isoleucine (p = 0.047 *), lysine (p = 0.001 ***), hydroxyproline (p = 0.179), and sarcosine (p = 0.001 ***). Moving to the effects of different treatments (T), highly significant differences were evident across all amino acids (p-values < 0.001 ***). The examination of the effects of treatments (T) on the amino acid composition of *B. cinerea*-infected apples revealed distinctive patterns in various treatments. Treatment C, serving as the control, displayed concentrations of amino acids such as tyrosine (162.2 ± 9.6), cystine (186.6 ± 7.6), and valine (121.4 ± 5.6). In contrast, Treatment F, representing a specific essential oil application, exhibited higher concentrations in amino acids like phenylalanine (643.8 ± 3.5), isoleucine (515.7 ± 21.1), and leucine (503.2 ± 20.1). The combined effects of treatments (T+F) demonstrated unique alterations in amino acid concentrations, with notable increases in cystine (210.5 ± 7.6), tryptophan (266.3 ± 12.1), and proline (291.2 ± 6.3). Treatment combinations involving the control (C) and essential oil application (C+F, T+C+F) exhibited diverse amino acid profiles, underscoring the complex interactions between the control and essential oil treatments. Particularly, the combination of all treatments (C+E+F) showcased significant changes in amino acid concentrations, especially for tyrosine (280.3 ± 8.6), valine (28.0 ± 3.6), and methionine (58.9 ± 11.0). Similarly, the combination of treatments T+E+F showed distinct effects on amino acids, with elevated concentrations of phenylalanine (89.1 ± 8.4) and lysine (106.0 ± 5.1). Treatment T+C+E+F, encompassing all components, displayed unique effects on the amino acid content, with particularly high concentrations observed for sarcosine (163 ± 17.7) and hydroxyproline (231.5 ± 26.3) (Table 1).

Table 2 illustrates the impact of preventive and curative applications of individual and combinations of essential oils on the anthocyanin content (%) of harvested apples infected with *B. cinerea*. The findings reveal significant variations in anthocyanin concentrations across different treatments. Curative measures, on the other hand, showed lower concentrations across various anthocyanins compared to the preventive approach,

with delphinidin-3-glycoside (2.5 ± 0.0) and cyanidin-3-glycoside (1.6 ± 0.1) being notably affected. The application of preventive measures significantly increased the concentrations of delphinidin-3-glycoside (4.8 ± 0.1) and peonidin-3-glycoside (45.6 ± 0.5), among others. In terms of individual applications, both preventive and curative measures demonstrated substantial effects on all types of anthocyanins. Treatment C, as the control, showed moderate concentrations across different anthocyanins. Notably, the concentrations of delphinidin-3-glycoside (3.8 ± 0.1), cyanidin-3-glycoside (2.2 ± 0.0), and peonidin-3-glycoside (37.9 ± 1.2) were observed. The combined treatment T+F showcased a combination of the effects observed in individual treatments C and F. Treatment F demonstrated a distinct effect, significantly increasing the concentrations of malvidin-3-glycoside-acetyl (40.2 ± 2.3) and malvidin-3-glycoside-p-coumaryl (20.5 ± 0.6) compared to other anthocyanins. Treatment E+F displayed an interesting impact, with increased concentrations in delphinidin-3-glycoside (3.5 ± 0.2) and peonidin-3-glycoside (38.6 ± 1.1). Noteworthy increases were observed in malvidin-3-glycoside_acetyl (38.6 ± 2.2) and malvidin-3-glycoside-p-coumaryl (5.6 ± 0.5). The combined treatments C+E+F and T+E+F demonstrated a synergistic effect on anthocyanin concentrations, showing higher values across various anthocyanins compared to individual treatments. Treatment T+C+E+F displayed a moderate increase in anthocyanin concentrations, with significant increments observed in malvidin-3-glycoside-acetyl (29.6 ± 2.0) and malvidin-3-glycoside-p-coumaryl (2.9 ± 0.6). Treatment C+F exhibited a significant increase in various anthocyanin concentrations, particularly in peonidin-3-glycoside (46.8 ± 1.3) and malvidin-3-glycoside (47.8 ± 2.2). Treatment T+C+F exhibited lower concentrations, especially in delphinidin-3-glycoside (2.2 ± 0.3) and peonidin-3-glycoside (16.9 ± 1.2).

The applications (A) of preventive and curative measures exhibited notable differences in the concentrations of various organic acids. Specifically, oxalic acid (p = 0.01053), propionic acid (p = 0.01054), tartaric acid (p = 0.01031), malic acid (p = 0.00374), and citric acid (p = 0.00167) displayed significant variations between preventive and curative treatments. Curative applications generally led to higher concentrations of organic acids compared to preventive measures. Regarding individual treatments (T), all organic acids showed significant differences ($p < 0.001$) between C and F applications. For the C, the first column recorded an average value of 3.9, while subsequent columns displayed values ranging from 2.1 to 7.5. In the F, the values ranged from 3.1 to 9.9 across different parameters, with the highest observed in the fifth column. The combined treatment of temperature and T+F yielded the most substantial outcomes, with values ranging from 8.7 to 169.2 across the parameters. Upon comparing the combined treatments, it is evident that the T+F treatment consistently exhibited superior growth outcomes, showcasing significantly higher values in all parameters compared to individual or other combined treatments. For instance, in the fourth column, the T+F treatment displayed a remarkable growth rate of 94.15, while the closest competitor, C+E+F, recorded a value of 72.2 (Table 3). On the other hand, statistical analyses underscored the significance of the observed differences across treatments. Treatment (T) wielded a substantial impact on almost all measured phenolic compounds, as evidenced by p-values < 0.05. This underscored the overall efficacy of the various treatments. The preventive and curative applications also demonstrated statistically significant effects on the concentrations of phenolic compounds. C demonstrated moderate concentrations of key phenolic compounds, including 2.50 g/L of gallic acid, 3.82 g/L of vanillic acid, and 4.67 g/L of caftaric acid. Conversely, lower concentrations were observed for trans-caffeic-acid (2.70 g/L), trans-p-coumaric-acid (4.02 g/L), and myricetin (5.69 g/L). In contrast, treatment with F exhibited slightly higher concentrations in several phenolic compounds compared to the control. The combined treatment of essential oils with curative application (T+F) demonstrated marked increases in multiple phenolic compounds, such as gallic acid (3.33 g/L), trans-caffeic-acid (3.28 g/L), and rutin

(2.64 g/L). Notable increases were observed in ferulic acid (1.75 g/L), catechin (4.82 g/L), and epicatechin (6.01 g/L), along with elevated levels of caftaric acid (5.21 g/L) and routine (1.69 g/L). The incorporation of essential oils into the control treatment (C+F) resulted in higher concentrations, particularly for gallic acid (3.52 g/L), vanillic acid (5.11 g/L), and rutin (2.76 g/L), suggesting an enhanced preservation effect. Treatment with essential oils and preventive application (E+F) revealed increased concentrations in several phenolic compounds, including 6.30 g/L of catechin, 6.48 g/L of epicatechin, and 7.47 g/L of rutin. In the comprehensive treatment involving control, essential oils, and preventive application (C+E+F), significantly elevated concentrations were observed across various phenolic compounds, such as 3.89 g/L of gallic acid, 5.71 g/L of vanillic acid, and 2.95 g/L of rutin. The preventive application of EOCs contributed to higher levels of phenolic compounds. The combined impact of incorporating EOCs with both preventive and curative applications was evident in the elevated levels of phenolic compounds. In addition, treatment with essential oils, curative, and preventive application (T+E+F) exhibited notable concentrations, particularly in 5.38 g/L of catechin, 6.39 g/L of routine, and 1.81 g/L of tyrosol. On the other hand, treatment with essential oils, control, and curative application (T+C+F) yielded relatively lower concentrations across various phenolic compounds. In the most comprehensive treatment, involving essential oils, control, preventive, and curative application (T+C+E+F), the highest concentrations were observed for most phenolic compounds, including 2.17 g/L of gallic acid, 3.16 g/L of vanillic acid, and 5.40 g/L of routine (Table 4).

Table 5 presents the impact of both preventive and curative applications of individual and combined essential oils against *B. cinerea* on the concentrations of hormones in harvested apples. In the preventive application strategy, significant differences were observed in the concentrations of hormones compared to the curative approach. Specifically, preventive treatment resulted in higher levels of IAA (ng/mg) and GA_3 (4.6 ng/mg) compared to the curative treatment (2.2 ng/mg and 12 ng/mg, respectively). Conversely, curative treatment exhibited higher concentrations of ABA (230.2 ng/mg) and SA (1.8 ng/mg) than preventive treatment (196.0 and 2.1 ng/mg, respectively). Examining the various treatments, Treatment F showed elevated concentrations in several hormones, including ABA (232.3 ng/mg), GA_3 (4.5 ng/mg), and JA (ng/mg), compared to the control (C). Treatment T+F exhibited significant increases in hormones such as GA_3 (9.8 ng/mg) and zeatin (1.6 ng/mg). Treatment C+F demonstrated a substantial increase in ABA concentration (362.4 ng/mg) compared to other treatments, suggesting a significant influence of essential oils in combination with the control strategy. Treatment E+F contributed to elevated levels of ABA (483.0 ng/mg) and zeatin (ng/mg). The comprehensive Treatment C+E+F displayed varied hormone concentrations, with notable increases in cytokinin (1.9 ng/mg) and JA (0.6 ng/mg). Treatment T+E+F exhibited significant increases in GA_3 (12.1 ng/mg) and zeatin (1.6 ng/mg). On the other hand, Figure 1 depicted a set of PCA biplots designed to visually differentiate an array of treatments through color coding, offering insights into the multivariate relationships among amino acids, anthocyanins, organic acids, phenolic compounds, and hormones in the context of diverse treatments and applications. Each data point represented the centroid of quadruplicate measurements for the respective parameter, ensuring a robust and representative dataset. Panels A–B (Dim1: 52.9%, Dim2: 28.2%) and subsequent panels C through M elucidated the distribution of variables along the first two principal components, cumulatively explaining a substantial proportion of the total observed variance. The contribution of each variable to the principal components was indicated by cos2 values, with a color gradient denoting their relative influence. In Panels C–D (Dim1: 80.2%, Dim2: 19.8%), variables associated with anthocyanins showcased a predominant influence of the first principal component, emphasizing their strong differentiation among the cultivars. Similarly, Panels L–M (Dim1: 84.2%, Dim2: 15.8%) highlighted the impact of the first principal component on variables linked to organic acids, indicating pronounced differences among the cultivars in this biochemical category. Contrastingly,

Panels E–F (Dim1: 81.7%, Dim2: 18.3%) shed light on phenolic compounds such as ferulic acid and catechin, displaying a more balanced distribution across both dimensions. This suggested a more intricate interaction pattern within this group of compounds. The proximity of data points to each other in the biplots denoted similarities in the composition profile, while the angles between vectors provided insights into the correlations between variables. The comprehensive overview offered by the biplots illuminated the metabolic landscape of the treatments and parameters, revealing both distinct and shared biochemical features. Figure 2 presented a comprehensive heatmap analysis, elucidating the abundance of diverse metabolites, spanning amino acids, anthocyanins, organic acids, phenolic compounds, and hormones, across distinct treatments and applications. Each row corresponded to a unique metabolite, while columns denoted samples or treatment conditions, annotated as P-T+F and C-C+T, among others. Clustering at the margins, both top and left, signified hierarchical groupings based on similarities in metabolite profiles or treatment conditions. The color gradient, transitioning from green to orange, signified relative concentration levels of metabolites across samples, where green indicated lower concentrations and orange represented higher concentrations. The color intensity was proportionate to the abundance of the corresponding metabolite, detailed in the legend on the right, providing a scale for expression levels. Distinct metabolite signatures for each treatment condition emerged, with blocks of color indicating uniform expression patterns within specific compound groups. This observed expression pattern reflected underlying biological processes and responses in diverse apple samples. The heatmap facilitated the swift visualization of intricate datasets, emphasizing specific modulations in metabolites under different conditions. Notably, discernible trends in the abundance of amino acids and phenolic compounds suggested a potential influence on specific metabolic pathways.

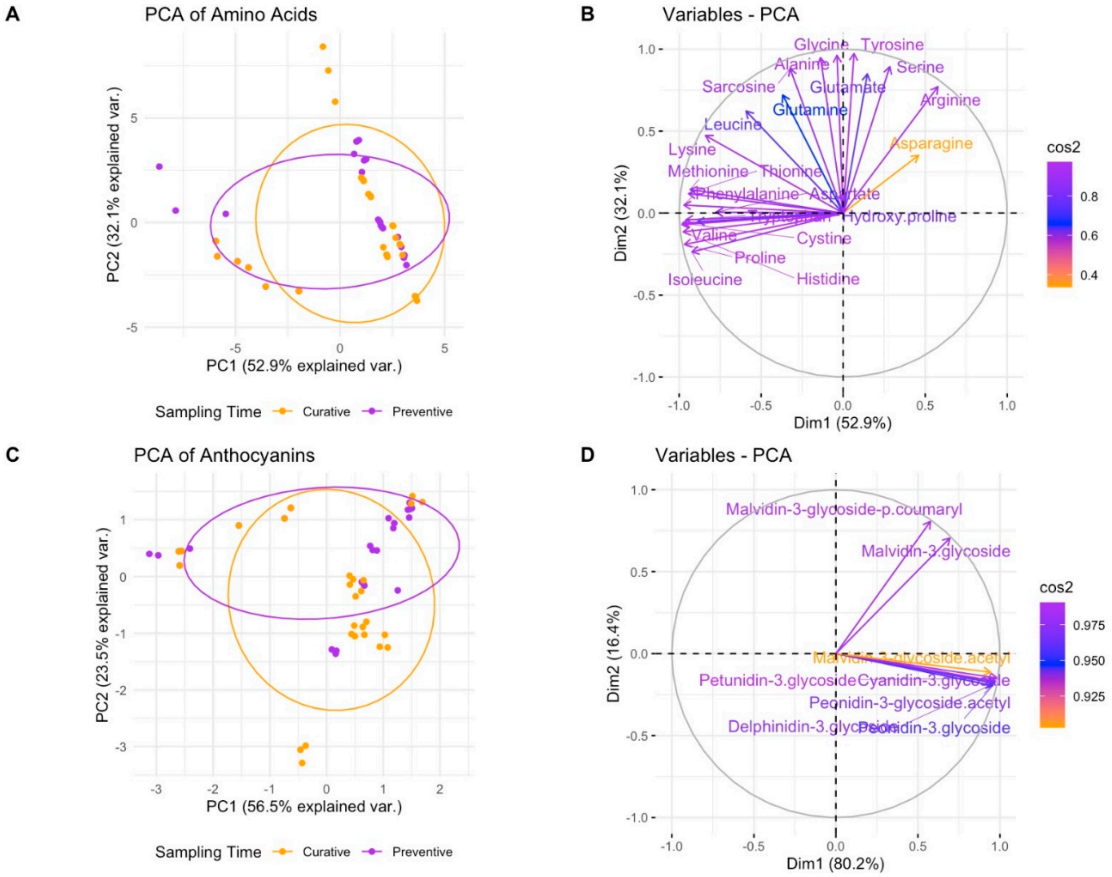


Figure 1. *Cont.*

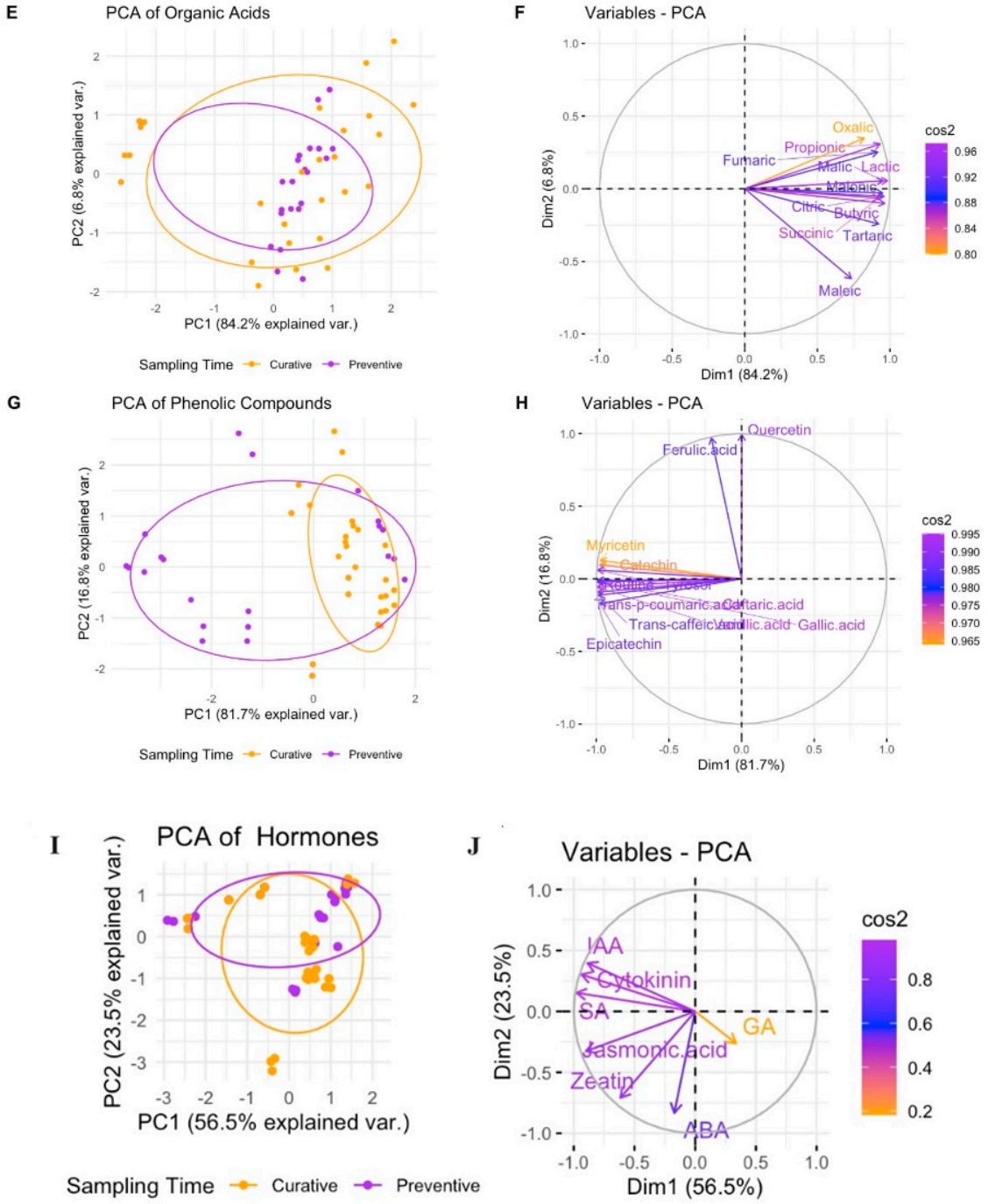


Figure 1. PCA biplot of berries colored by cultivars. All amino acids (**A**,**B**), anthocyanins (**C**,**D**), organic acids (**E**,**F**), phenolic compounds (**G**,**H**), and hormones (**I**,**J**) are visualized. Each point is the average of quaternary replicates of each parameter.

Table 1. The effect of preventive and curative applications of individual and combinations of essential oils against *B. cinerea* on the amino acid content (pmol μL^{-1}) of harvested apples.

Applications (A) [X]	Aspartate	Glutamate	Asparagine	Serine	Glutamine	Histidine	Glycine	Thionine	Arginine	Alanine
Preventive	109 ± 1.1	200 ± 3.2	322 ± 5.3 a	381 ± 8.8 b	292 ± 6.3 b	271 ± 11.3 b	116 ± 2.2 b	328 ± 10.9	275 ± 6.0 a	274 ± 3.4
Curative	111 ± 2.1	201 ± 4.1	535 ± 4.5 b	352 ± 6.8 a	251 ± 8.3 a	188 ± 10.3 a	97 ± 2.9 a	305 ± 11.8	327 ± 7.1 b	267 ± 6.1
Treatments (T) [Y]										
C	189.8 ± 4.1 d	96.7 ± 8.3 a	98.8 ± 10.1 a	189.8 ± 3.5 d	260 ± 17.7 bc	424.3 ± 4.1 b	56.2 ± 6.2 a	427.1 ± 22.3 b	53.3 ± 11.9 a	204.0 ± 9.5 a
F	185.2 ± 3.5 d	147.2 ± 7.8 b	133.3 ± 9.7 a	185.2 ± 4.1 d	285 ± 17.7 bc	643.8 ± 3.5 a	70.4 ± 5.1 ab	503.2 ± 20.1 b	67.1 ± 13.3 a	227.8 ± 12.9 a
T+F	128.6 ± 4.4 c	318.2 ± 5.2 f	515.1 ± 11.1 d	128.6 ± 4.2 c	426 ± 17.7 d	471.6 ± 4.3 b	201.7 ± 6.1 e	479.8 ± 19.3 b	377.4 ± 14.9 bc	423.1 ± 6.9 c
C+F	79.3 ± 2.2 ab	209.1 ± 8.3 cde	351.0 ± 11.4 bc	79.3 ± 5.1 ab	270 ± 17.7 bc	98.2 ± 4.2 a	102.0 ± 7.2 c	230.0 ± 17.1 a	353.0 ± 15.0 bc	256.3 ± 4.1 ab
E+F	95.2 ± 4.3 b	240.3 ± 5.5 e	529.0 ± 10.7 d	95.2 ± 3.5 b	307 ± 17.7 c	111.9 ± 3.5 a	162.5 ± 3.3 d	276.2 ± 25.3 a	458.5 ± 11.9 d	316.8 ± 9.9 b
C+E+F	82.8 ± 4.1 ab	192.6 ± 6.8 cd	690.0 ± 9.7 e	82.8 ± 2.9 ab	208 ± 17.7 ab	66.3 ± 4.1 a	109.2 ± 3.1 c	243.0 ± 23.1 a	371.1 ± 10.5 bc	273.5 ± 7.3 ab
T+E+F	83.5 ± 2.5 ab	228.2 ± 7.7 de	397.8 ± 11.7 c	83.5 ± 4.6 ab	272 ± 17.7 bc	104.3 ± 3.5 a	108.8 ± 5.1 c	238.1 ± 13.3 a	381.6 ± 13.1 c	263.2 ± 5.9 ab
T+C+F	75.4 ± 3.5 ab	197.5 ± 8.4 cd	342.7 ± 13.1 b	75.4 ± 3.2 ab	256 ± 17.7 bc	94.0 ± 4.2 a	98.7 ± 5.2 bc	220.2 ± 21.1 a	340.2 ± 12.4 bc	244.3 ± 6.8 ab
T+C+E+F	69.8 ± 5.1 a	178.6 ± 5.8 bc	798.3 ± 12.7 f	69.8 ± 6.5 a	163 ± 17.7 a	52.1 ± 4.0 a	50.9 ± 6.1 a	231.5 ± 26.3 a	308.5 ± 13.2 b	230.4 ± 11.5 a
Significance										
A	0.446	0.855	0.001 ***	0.011 *	0.001 **	0.001 ***	0.001 ***	0.181	0.001 ***	0.497
T	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***
A × T	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.0287 *	0.001 ***	0.0287 *

Applications (A) [X]	Tyrosine	Cystine	Valine	Methionine	Tryptophan	Phenylalanine	Isoleucine	Leucine	Lysine	Hydroxy Proline	Sarcosine	Proline
Preventive	268.0 ± 5.2	120.6 ± 3.2 b	77.0 ± 2.3 b	118.0 ± 3.4 b	140.4 ± 7.2 b	153.1 ± 3.3 b	180 ± 311.3 b	116.4 ± 2.3 ns	186.4 ± 3.3 b	105.2 ± 2.4 ns	184.3 ± 4.1 ns	39.0 ± 1.3 a
Curative	272.1 ± 5.9	89.6 ± 3.5 a	54.8 ± 2.6 a	103.1 ± 3.3 a	99.8 ± 7.8 a	128.1 ± 3.9 a	146.2 ± 11.7 a	120.1 ± 2.6	162.1 ± 3.4 a	101.2 ± 2.4	180.1 ± 4.3	28.4 ± 1.1 b
Treatments (T) [Y]												
C	162.2 ± 9.6 a	186.6 ± 7.6 b	121.4 ± 5.6 b	139.2 ± 7.2 b	221.1 ± 11.6 b	220.1 ± 3.3 b	347.9 ± 21.8 b	110.1 ± 5.1 ab	204.0 ± 7.3 c	147.6 ± 4.1 c	141.8 ± 9.1 a	76.2 ± 2.2 bc
F	226.1 ± 2.3 b	290.2 ± 7.6 c	165.7 ± 2.2 c	196.9 ± 6.2 c	278.1 ± 13.3 b	243.9 ± 3.1 b	515.7 ± 21.1 c	142.7 ± 4.6 c	269.1 ± 3.3 d	194.5 ± 5.3 d	189.3 ± 6.2 bc	86.6 ± 1.8 f
T+F	393.3 ± 12.5 d	210.5 ± 7.6 b	144.4 ± 3.6 bc	197.2 ± 7.1 c	266.3 ± 12.1 b	250.3 ± 8.1 b	245.5 ± 23.8 b	177.2 ± 5.5 d	321.1 ± 5.1 e	146.2 ± 6.5 c	291.2 ± 6.3 d	66.9 ± 2.8 b
C+F	264.2 ± 11.6 b	35.5 ± 7.6 a	23.3 ± 3.1 a	74.3 ± 5.2 a	46.5 ± 14.6 a	88.1 ± 7.3 a	56.3 ± 22.4 a	110.1 ± 4.2 a	107.2 ± 5.6 a	69.3 ± 3.1 ab	152.0 ± 7.2 ab	11.8 ± 3.5 a
E+F	331.4 ± 9.1 c	61.4 ± 7.6 a	37.2 ± 5.6 a	93.8 ± 5.6 a	70.9 ± 14.5 a	108.0 ± 6.1 a	70.8 ± 22.2 a	130.3 ± 6.6 bc	187.0 ± 6.3 c	90.0 ± 1.4 b	226.8 ± 4.3 c	15.3 ± 2.5 a
C+E+F	280.3 ± 8.6 bc	53.6 ± 7.6 a	28.0 ± 3.6 a	75.3 ± 4.2 a	58.9 ± 11.0 a	101.0 ± 7.3 a	60.4 ± 21.3 a	104.9 ± 4.3 ab	142.3 ± 5.2 b	52.8 ± 3.1 a	181.1 ± 9.1 ab	10.8 ± 2.2 a
T+E+F	274.7 ± 11.2 bc	37.9 ± 7.6 a	25.6 ± 4.2 a	80.3 ± 7.4 a	49.4 ± 14.2 a	89.1 ± 8.4 a	58.7 ± 19.8 a	106.0 ± 5.1 ab	119.4 ± 7.3 ab	78.7 ± 5.1 b	164.3 ± 7.2 ab	12.8 ± 2.1 a
T+C+F	253.3 ± 12.6 b	34.4 ± 7.6 a	22.8 ± 7.1 a	71.6 ± 5.5 a	44.9 ± 6.6 a	83.6 ± 6.5 a	54.0 ± 17.5 a	92.6 ± 5.4 a	105.3 ± 2.3 a	68.7 ± 4.1 ab	147.3 ± 7.6 ab	11.5 ± 2.0 a
T+C+E+F	243.3 ± 12.2 b	35.2 ± 7.6 a	24.5 ± 3.6 a	68.3 ± 6.2 a	44.9 ± 12.5 a	80.7 ± 7.3 a	55.3 ± 24.4 a	101.6 ± 4.6 a	112.4 ± 4.4 ab	78.9 ± 5.3 b	148.2 ± 9.2 ab	11.5 ± 1.7 a
Significance												
A	0.628	0.001 ***	0.001 ***	0.003 **	0.001 ***	0.001 ***	0.047 *	0.293	0.001 ***	0.179	0.507	0.001 ***
T	0.001 ***	0.001 ***	0.01 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***
A × T	0.001 ***	0.001 ***	0.01 ***	0.001 ***	0.001 ***	0.001 ***	0.003 **	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***

X, mean separation in applications; Y, mean separation in treatments; ST, C × ST, interactions. For a given factor, different letters within a column represent significant differences (Tukey test; *, significant at p-value < 0.05; **, significant at p-value < 0.01; ***, significant at p-value < 0.001). Data are expressed as means of the data.

Table 2. The effect of preventive and curative applications of individual and combinations of essential oils against *B. cinerea* on the anthocyanins (%) of harvested apples.

Applications (A) [X]	Delphinidin-3-glycoside	Cyanidin-3-glycoside	Petunidin-3-glycoside	Peonidin-3-glycoside	Peonidin-3-glycoside_acetyl	Malvidin-3-glycoside_acetyl	Malvidin-3-glycoside	Malvidin-3-glycoside-p-coumaryl
Preventive	4.8 ± 0.1 b	2.6 ± 0.0 b	9.3 ± 0.2 b	45.6 ± 0.5 b	83.5 ± 1.0 b	55.6 ± 1.3 b	9.2 ± 0.1 b	9.4 ± 0.2 b
Curative	2.5 ± 0.0 a	1.6 ± 0.1 a	5.4 ± 0.3 a	19.7 ± 0.3 a	42.7 ± 1.1 a	31.5 ± 1.0 a	4.9 ± 0.2 a	4.9 ± 0.3 a
Treatments (T) [Y]								
C	3.8 ± 0.1 c	2.2 ± 0.0 b	7.9 ± 0.2 cd	37.9 ± 1.2 d	65.7 ± 2.3 bc	52.0 ± 2.6 de	6.1 ± 0.2 bc	5.2 ± 0.2 abc
F	2.6 ± 0.2 b	1.7 ± 0.4 a	6.1 ± 0.3 b	23.0 ± 1.1 bc	45.3 ± 2.1 a	40.2 ± 2.3 bc	17.2 ± 0.1 e	20.5 ± 0.6 e
T+F	3.7 ± 0.0 c	2.1 ± 0.0 b	7.3 ± 0.2 c	26.4 ± 1.1 c	63.3 ± 2.2 b	38.6 ± 2.2 bc	6.2 ± 0.2 bc	5.6 ± 0.5 bc
C+F	5.1 ± 0.3 e	2.6 ± 0.5 c	9.0 ± 0.4 e	46.8 ± 1.3 e	88.2 ± 2.1 d	47.8 ± 2.2 cd	5.8 ± 0.0 b	5.0 ± 0.6 abc
E+F	3.5 ± 0.2 c	2.1 ± 0.3 b	7.4 ± 0.3 c	38.6 ± 1.1 d	60.8 ± 2.41 b	47.8 ± 2.3 cd	7.1 ± 0.1 c	6.9 ± 0.6 c
C+E+F	5.3 ± 0.0 e	2.7 ± 0.0 c	9.6 ± 0.1 e	44.0 ± 1.4 e	90.6 ± 2.0 d	53.3 ± 2.4 de	9.6 ± 0.2 d	11.0 ± 0.4 d
T+E+F	4.3 ± 0.1 d	2.3 ± 0.1 bc	8.8 ± 0.2 de	42.5 ± 1.1 de	73.9 ± 2.1 c	59.8 ± 2.1 e	5.7 ± 0.3 b	4.4 ± 0.6 abc
T+C+F	2.2 ± 0.3 a	1.7 ± 0.0 a	4.5 ± 0.3 a	16.9 ± 1.2 a	37.9 ± 2.0 a	23.1 ± 2.2 a	2.7 ± 0.2 a	2.7 ± 0.3 a
T+C+E+F	2.4 ± 0.1 ab	1.6 ± 0.2 a	5.4 ± 0.2 ab	18.0 ± 1.1 ab	42.2 ± 2.1 a	29.6 ± 2.0 ab	3.3 ± 0.1 a	2.9 ± 0.6 ab
Significance								
A	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.293
T	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***
A × T	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***

X, mean separation in applications; Y, mean separation in treatments; ST, C × ST, interactions. For a given factor, different letters within a column represent significant differences (Tukey test; ***, significant at p-value < 0.001). Data are expressed as means of the data.

Table 3. The effect of preventive and curative applications of individual and combinations of essential oils against *B. cinerea* on the organic acid ($g \cdot L^{-1}$) content of harvested apples.

Applications (A) [X]	Oxalic	Propionic	Tartaric	Butyric	Malonic	Malic	Lactic	Citric	Maleic	Fumaric	Succinic
Preventive	6.3 ± 0.3 a	12.5 ± 0.1 a	20.5 ± 0.6 a	59.9 ± 2.3	55.4 ± 1.2 a	19.4 ± 0.3 a	79.8 ± 2.9 a	71.8 ± 1.4 a	20.5 ± 0.9 a	21.5 ± 1.1	94.2 ± 2.5
Curative	7.0 ± 0.1 b	14.2 ± 0.4 b	23.1 ± 0.3 b	64.6 ± 2.1	61.6 ± 1.4 a	22.8 ± 0.7 b	89.9 ± 2.4 b	83.0 ± 1.8 b	24.1 ± 0.4 b	24.3 ± 1.0	92.1 ± 2.6
Treatments (T) [Y]											
C	3.9 ± 0.0 a	5.3 ± 0.9 a	2.8 ± 1.3 a	4.3 ± 4.5 a	5.1 ± 2.8 a	2.9 ± 1.4 a	2.7 ± 6.1 a	7.5 ± 2.8 a	2.1 ± 1.9 a	4.6 ± 3.3 a	5.4 ± 6.0 a
F	5.8 ± 0.2 b	8.3 ± 0.9 a	3.4 ± 1.2 a	5.9 ± 3.3 b	6.8 ± 2.2 a	3.1 ± 1.3 a	2.9 ± 6.8 a	9.9 ± 3.1 a	2.6 ± 1.0 a	4.7 ± 2.1 a	8.8 ± 4.6 a
T+F	8.7 ± 0.3 c	17.4 ± 0.5 c	33.6 ± 1.4 c	94.15 ± 2.5 c	80.7 ± 2.1 c	26.3 ± 1.2 bc	127.7 ± 3.2 c	119.1 ± 4.2 c	25.9 ± 1.1 b	28.6 ± 1.1 b	169.2 ± 3.6 d
C+F	7.1 ± 0.4 bc	15.3 ± 0.4 bc	26.22 ± 1.4 b	77.1 ± 4.3 bc	74.3 ± 2.0 c	28.3 ± 1.1 c	108.3 ± 6.0 bc	89.9 ± 3.6 b	30.9 ± 1.9 b	29.4 ± 2.1 b	107.6 ± 5.6 bc
E+F	7.4 ± 0.3 bc	15.7 ± 0.6 bc	27.2 ± 1.2 bc	80.4 ± 4.4 bc	78.9 ± 2.9 c	26.4 ± 1.5 bc	112.7 ± 6.4 bc	102.0 ± 4.8 bc	22.7 ± 1.4 b	30.3 ± 2.4 b	125.3 ± 4.6 c
C+E+F	6.4 ± 0.3 b	12.9 ± 0.3 b	25.6 ± 1.3 b	72.2 ± 4.5 b	59.4 ± 2.2 b	20.8 ± 1.3 b	94.8 ± 3.2 b	89.9 ± 1.4 b	27.1 ± 1.1 b	21.7 ± 2.1 b	113.3 ± 5.6 bc
T+E+F	7.4 ± 0.1 bc	16.1 ± 0.9 bc	27.1 ± 1.3 bc	79.2 ± 4.0 bc	78.4 ± 2.3 c	29.7 ± 1.0 c	114.1 ± 3.4 bc	96.2 ± 2.8 b	30.0 ± 1.2 b	31.2 ± 2.5 b	109.8 ± 5.1 bc
T+C+F	6.7 ± 0.3 b	14.6 ± 0.2 bc	24.8 ± 1.7 b	73.1 ± 4.1 bc	71.6 ± 2.9 bc	27.0 ± 1.2 bc	103.4 ± 4.1 bc	86.3 ± 3.3 b	28.9 ± 1.9 b	28.4 ± 2.1 b	102.5 ± 5.6 bc
T+C+E+F	6.6 ± 0.2 b	14.4 ± 0.9 bc	25.3 ± 1.4 b	73.1 ± 4.5 bc	71.2 ± 2.4 bc	25.1 ± 1.5 bc	96.5 ± 6.4 b	95.3 ± 3.5 b	30.1 ± 1.4 b	26.7 ± 4.1 b	96.0 ± 5.3 b

Table 3. *Cont.*

Applications (A) [X]	Oxalic	Propionic	Tartaric	Butyric	Malonic	Malic	Lactic	Citric	Maleic	Fumaric	Succinic
Significance											
A	0.010 *	0.010 *	0.010 *	0.125	0.003 **	0.001 **	0.019 *	0.001 ***	0.008 **	0.060	0.583
T	0.001 ***	0.001 ***	0.001	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***
A × T	0.001 ***	0.002 **	0.004 **	0.050	40.001 ***	0.002 **	0.001 **	0.001 ***	0.001 ***	0.001 ***	0.001 ***

X, mean separation in applications; Y, mean separation in treatments; ST, C × ST, interactions. For a given factor, different letters within a column represent significant differences (Tukey test; *, significant at *p*-value < 0.05; **, significant at *p*-value < 0.01; ***, significant at *p*-value < 0.001). Data are expressed as means of the data.

Table 4. The effect of preventive and curative applications of individual and combinations of essential oils against *B. cinerea* on the phenolic compounds (g/L) of harvested apples.

Applications (A) [X]	Gallic-Acid	Vanillic-Acid	Trans-Caffeic-Acid	Trans-p-Coumaric-Acid	Ferulic-Acid	Caftaric-Acid	Catechin	Epicatechin	Quercetin	Routine	Myricetin	Tyrosol
Preventive	3.57 ± 0.1 b	5.34 ± 0.3 b	3.73 ± 0.1 b	5.36 ± 0.1 b	1.71 ± 0.2	6.73 ± 0.3 b	7.10 ± 0.1 b	4.23 ± 0.1 b	2.57 ± 0.0	3.01 ± 0.1 b	2.61 ± 0.2 b	8.4 ± 0.3 b
Curative	2.12 ± 0.4 a	3.14 ± 0.2 a	2.04 ± 0.4 a	3.36 ± 0.2 a	1.66 ± 0.1	3.77 ± 0.2 a	4.39 ± 0.3 a	2.25 ± 0.3 a	2.65 ± 0.2	1.77 ± 0.2 a	1.45 ± 0.1 a	5.2 ± 0.2 a
Treatments (T) [Y]												
C	2.50 ± 0.3 abc	3.82 ± 0.3 abc	2.70 ± 0.1 abc	4.02 ± 0.3 abc	1.17 ± 0.1 a	4.57 ± 0.3 abc	4.67 ± 0.3 ab	3.15 ± 0.1 abc	1.79 ± 0.1 a	2.09 ± 0.2	1.59 ± 0.5 ab	5.69 ± 0.7 abc
F	2.48 ± 0.2 abc	3.89 ± 0.4 abc	2.57 ± 0.2 abc	4.04 ± 0.4 abc	1.75 ± 0.0 b	4.82 ± 0.6 abc	5.21 ± 0.7 ab	2.86 ± 0.2 abc	2.60 ± 0.3 b	2.11 ± 0.6	1.69 ± 0.2 abc	6.01 ± 0.3 abc
T+F	3.33 ± 1.3 bc	4.93 ± 0.4 bc	3.28 ± 0.3 bc	4.92 ± 0.4 bc	2.26 ± 0.0 c	6.30 ± 0.4 bc	7.08 ± 0.6 ab	3.54 ± 0.5 abc	3.44 ± 0.1 c	2.84 ± 0.7	2.64 ± 0.4 bc	8.25 ± 0.4 bc
C+F	3.52 ± 3.3 bc	5.11 ± 0.1 bc	3.58 ± 0.3 c	5.07 ± 0.3 bc	1.74 ± 0.1 b	6.46 ± 0.5 bc	6.98 ± 0.3 ab	3.97 ± 0.3 bc	2.70 ± 0.4 b	3.01 ± 0.3	2.76 ± 0.6 bc	8.42 ± 0.5 bc
E+F	3.22 ± 1.2 abc	4.74 ± 0.1 bc	3.17 ± 0.2 bc	5.04 ± 0.2 bc	1.54 ± 0.2 ab	5.80 ± 0.6 abc	6.48 ± 0.1 ab	3.72 ± 0.4 bc	2.38 ± 0.5 ab	2.58 ± 0.2	2.04 ± 0.3 abc	7.47 ± 0.7 abc
C+E+F	3.89 ± 0.4 c	5.71 ± 0.2 c	4.05 ± 0.3 c	5.66 ± 0.1 c	1.60 ± 0.1 ab	7.25 ± 0.1 c	7.65 ± 0.2 b	4.57 ± 0.2 c	2.43 ± 0.3 b	3.30 ± 0.1	2.95 ± 0.2 c	9.12 ± 0.6 c
T+E+F	2.75 ± 0.2 abc	4.23 ± 0.3 abc	2.96 ± 0.2 abc	4.39 ± 0.4 abc	1.56 ± 0.3 ab	5.19 ± 0.0 abc	5.38 ± 0.7 ab	3.42 ± 0.4 abc	2.40 ± 0.1 ab	2.27 ± 0.2	1.81 ± 0.1 abc	6.39 ± 0.2 abc
T+C+F	1.75 ± 0.5 a	2.56 ± 0.4 a	1.65 ± 0.1 a	2.69 ± 0.3 a	1.71 ± 0.1 b	3.10 ± 0.3 a	3.68 ± 0.5 a	1.74 ± 0.5 a	2.75 ± 0.6 b	1.50 ± 0.3	1.32 ± 0.2 a	4.44 ± 0.7 a
T+C+E+F	2.17 ± 0.3 ab	3.16 ± 0.3 ab	2.02 ± 0.3 ab	3.42 ± 0.2 ab	1.86 ± 0.1 bc	3.78 ± 0.6 ab	4.56 ± 0.7 ab	2.19 ± 0.3 ab	3.00 ± 0.1 bc	1.80 ± 0.5	1.51 ± 0.4 ab	5.40 ± 0.1 ab
Significance												
A	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.509	0.001 ***	0.001 ***	0.001 ***	0.430	0.001 ***	0.001 ***	0.001 ***
T	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.005 **	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***
A × T	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.898	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***

X, mean separation in applications; Y, mean separation in treatments; ST, C × ST, interactions. For a given factor, different letters within a column represent significant differences (Tukey test; **, significant at *p*-value < 0.01; ***, significant at *p*-value < 0.001). Data are expressed as means of the data.

Table 5. The effect of preventive and curative applications of individual and combinations of essential oils against *B. cinerea* on the hormones (ng/mg) of harvested apples.

Applications (A) [X]	IAA	ABA	GA	SA	Cytokinin	Zeatin	Jasmonic_Acid
Preventive	2.9 ± 0.1 b	196.0 ± 9.4 a	4.6 ± 0.1 a	2.1 ± 0.3 b	3.1 ± 0.0 b	1.8 ± 0.1 a	0.8 ± 0.0 a
Curative	2.2 ± 0.0 a	230.2 ± 10.4 b	12.3 ± 0.2 b	1.8 ± 0.0 a	2.0 ± 0.2 a	2.1 ± 0.0 b	1.0 ± 0.3 b
Treatments (T) [Y]							
C	3.6 ± 0.1 c	110.0 ± 21.2 a	2.9 ± 0.1 a	6.0 ± 0.1 d	7.2 ± 0.4 c	1.9 ± 0.1 b	0.9 ± 0.0 b
F	5.3 ± 0.2 d	232.3 ± 11.4 b	4.5 ± 0.2 b	4.0 ± 0.6 e	9.7 ± 0.1 d	2.5 ± 0.3 c	1.7 ± 0.1 c
T+F	3.9 ± 0.1 c	145.4 ± 15.8 ab	9.8 ± 0.3 c	3.3 ± 0.4 c	5.4 ± 0.5 b	1.6 ± 0.0 a	1.0 ± 0.0 b
C+F	1.7 ± 0.1 ab	362.4 ± 21.2 c	8.8 ± 0.6 c	1.1 ± 0.0 b	0.2 ± 0.1 a	2.3 ± 0.1 c	0.9 ± 0.0 b
E+F	1.8 ± 0.2 ab	483.0 ± 20.9 d	2.8 ± 0.3 a	1.1 ± 0.3 b	0.2 ± 0.5 a	2.6 ± 0.0 c	0.9 ± 0.2 b
C+E+F	1.8 ± 0.1 ab	141.2 ± 21.6 ab	4.0 ± 0.3 ab	0.6 ± 0.0 a	0.1 ± 0.2 a	1.9 ± 0.2 b	0.6 ± 0.0 a
T+E+F	1.6 ± 0.3 ab	168.3 ± 22.8 ab	12.1 ± 0.4 d	0.6 ± 0.1 a	0.1 ± 0.1 a	1.6 ± 0.0 a	0.7 ± 0.3 a
T+C+F	1.3 ± 0.1 a	118.4 ± 21.4 a	22.8 ± 0.3 e	0.6 ± 0.0 a	0.1 ± 0.7 a	1.6 ± 0.4 a	0.6 ± 0.0 a
T+C+E+F	2.1 ± 0.4 b	161.5 ± 23.1 ab	8.7 ± 0.1 c	0.5 ± 0.3 a	0.1 ± 0.1 a	1.5 ± 0.0 a	0.6 ± 0.1 a
Significance							
A	0.001 ***	0.026 *	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***
T	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***
A × T	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***	0.001 ***

X, mean separation in applications; Y, mean separation in treatments; ST, C × ST, interactions. For a given factor, different letters within a column represent significant differences (Tukey test; *, significant at p-value < 0.05; ***, significant at p-value < 0.001). Data are expressed as means of the data.

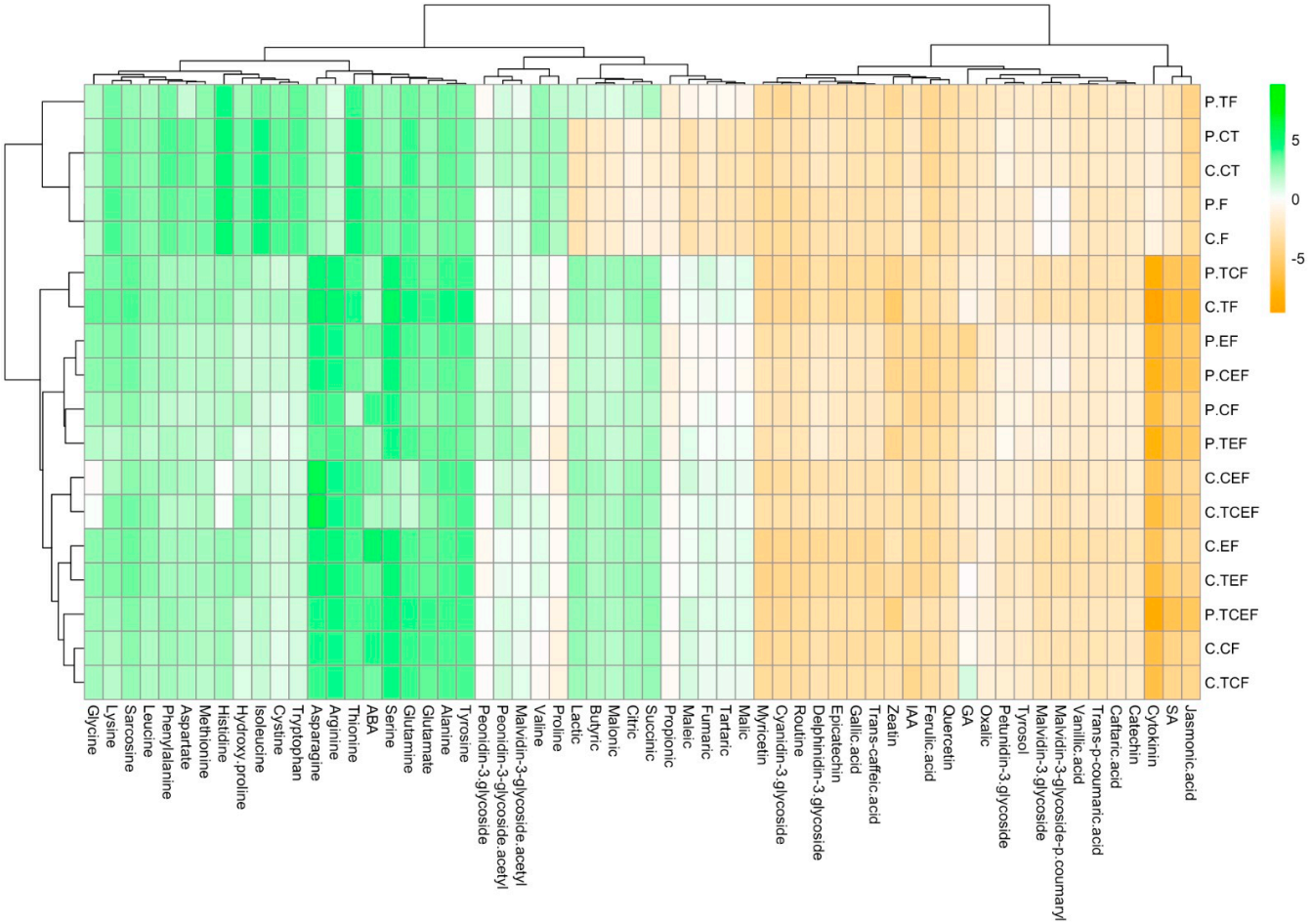


Figure 2. Heatmap analysis that scrutinizes numerous amino acids, anthocyanins, organic acid, phenolic compounds, and hormones.

4. Discussion

The meticulous analysis of preventive and curative applications in the context of *B. cinerea*-infected apples revealed intriguing insights into amino acid concentrations. Notably, aspartate and glutamate concentrations did not exhibit statistically significant distinctions, with *p*-values of 0.446 and 0.85, respectively, suggesting a consistent response regardless of the preventive or curative approach. However, asparagine, serine, histidine, glycine, and arginine showed substantial variations, indicating distinct impacts on these amino acids. These findings align with existing literature emphasizing the intricate relationship between pathogen response and amino acid metabolism in plants [26,27]. In scrutinizing the effects of different EOC treatments, highly significant variations were apparent across all amino acids. This underscores the efficacy of the treatments in influencing amino acid levels. Treatment C, serving as the control, displayed concentrations of amino acids such as tyrosine (189.8 $\pm$ 4.1), cystine (96.7 $\pm$ 8.3), and valine (98.8 $\pm$ 10.1). Conversely, Treatment F, representing a specific essential oil application, demonstrated distinct amino acid profiles with elevated concentrations of phenylalanine (643.8 $\pm$ 3.5), isoleucine (515.1 $\pm$ 11.1), and leucine (503.2 $\pm$ 20.1), suggesting a notable impact on these components. The T+F treatment showcased a unique amalgamation of effects, with cystine (318.2 $\pm$ 5.2), tryptophan (426 $\pm$ 17.7), and proline (377.4 $\pm$ 14.9) standing out. These results resonate with studies highlighting the modulatory effects of essential oils on plant metabolism, influencing the synthesis of specific amino acids and secondary metabolites [28,29]. Treatment combinations involving the C and C+F, T+C+F, and T+C+E+F displayed varying

amino acid concentrations, illustrating the intricate interactions between the control and essential oil treatments. Particularly, C+E+F demonstrated substantial changes in amino acid concentrations, especially for valine (690.0 ± 9.7), methionine (208 ± 17.7), and lysine (371.1 ± 10.5). Similarly, the combination of treatments T+E+F showed distinct effects on amino acids, with elevated concentrations of phenylalanine (104.3 ± 3.5) and lysine (381.6 ± 13.1). These findings align with research suggesting that the combination of different treatments can elicit synergistic effects on plant metabolism, leading to enhanced resistance against pathogens [30–32]. Certain components within essential oil blends can enhance the penetration of other bioactive molecules into the fungal cells. For instance, compounds with lipophilic properties can disrupt the fungal cell membrane, increasing its permeability and facilitating the entry of other antifungal agents into the cell. This synergistic interaction enhances the overall bioavailability and efficacy of the compounds against *B. cinerea* [33]. By modifying important metabolic pathways inside fungal cells, the combined impact of essential oils might further impede the growth and proliferation of the fungi. For instance, synergistic mixtures can prevent both the virulence factor and essential enzyme synthesis from occurring at the same time, impairing fungal metabolism, and decreasing pathogenicity [34].

Table 2 offers a detailed examination of the effects of the preventive and curative applications of individual and combinations of essential oils on the anthocyanin content (%) of *B. cinerea*-infected apples, shedding light on the effect of the intricate interplay of various treatments on secondary metabolite profiles. The observed significant variations in anthocyanin concentrations align with the existing literature, emphasizing the dynamic and nuanced nature of plant responses to different interventions [35,36]. This aligns with studies emphasizing the positive impact of preventive approaches on secondary metabolite accumulation as a part of the plant's defense response to potential stressors [28,37]. Within the realm of individual applications, both preventive and curative measures exerted substantial effects on various anthocyanins. Preventive measures led to significant increases in delphinidin-3-glycoside (4.8 ± 0.1) and peonidin-3-glycoside (45.6 ± 0.5), highlighting the efficacy of pre-emptive strategies in enhancing anthocyanin production. Conversely, curative measures demonstrated lower concentrations across several anthocyanins, such as delphinidin-3-glycoside (2.5 ± 0.0) and cyanidin-3-glycoside (1.6 ± 0.1), indicative of a differential impact on secondary metabolite accumulation. It is well-documented that curative applications may result in a less pronounced induction of secondary metabolites compared to preventive strategies, as the plant might already be in a stressed state [38]. These findings are consistent with research emphasizing the importance of timing and application methods in eliciting specific metabolic responses in plants [37]. Treatment C, acting as the control, displayed moderate concentrations of anthocyanins, including delphinidin-3-glycoside (3.8 ± 0.1), cyanidin-3-glycoside (2.2 ± 0.0), and peonidin-3-glycoside (37.9 ± 1.2). Treatment F exhibited a distinct effect, significantly increasing the concentrations of malvidin-3-glycoside-acetyl (40.2 ± 2.3) and malvidin-3-glycoside-p-coumaryl (20.5 ± 0.6). These results are in line with previous research highlighting the modulatory impact of essential oils on specific classes of secondary metabolites [38]. The combined treatment T+F demonstrated a balanced profile of anthocyanin concentrations, combining the effects observed in individual treatments C and F. Treatment C+F showed a significant increase in various anthocyanin concentrations, particularly in peonidin-3-glycoside (46.8 ± 1.3) and malvidin-3-glycoside (47.8 ± 2.2), suggesting a synergistic interaction between the control and essential oil application. These observations align with studies highlighting the potential synergy between conventional and alternative treatments in enhancing plant defense mechanisms [36]. Treatment E+F displayed an intriguing impact, with increased concentrations of delphinidin-3-glycoside (3.5 ± 0.2) and peonidin-3-glycoside (38.6 ± 1.1). The combined treatments T+E+F and C+E+F demonstrated a synergistic effect on anthocyanin concentrations, showing higher values across various anthocyanins compared to individual

EOs treatments. Conversely, treatment T+C+F exhibited lower concentrations, especially in delphinidin-3-glycoside (2.2 ± 0.3) and peonidin_3_glycoside (16.9 ± 1.2), indicating a potential antagonistic effect. Treatment T+C+E+F displayed a moderate increase in anthocyanin concentrations, with significant increments observed in malvidin-3-glycoside_acetyl (29.6 ± 2.0) and malvidin-3-glycoside-p-coumaryl (2.9 ± 0.6), highlighting the complex outcomes resulting from combined treatments. The intricacies of interactions among various treatments underscore the importance of conducting a case-specific assessment [39].

Significant differences in the concentrations of various organic acids were observed in the analysis of preventive and curative applications. Oxalic acid (p = 0.01053), propionic acid (p = 0.01054), tartaric acid (p = 0.01031), malic acid (p = 0.00374), and citric acid (p = 0.00167) exhibited notable variations between preventive and curative approaches. This finding aligns with existing literature recognizing the differential regulation of organic acids under diverse stress conditions, emphasizing the nuanced responses of plants to preventive and curative measures [26,32]. Differences in organic acid concentrations were also observed under T treatments, where all organic acids showed significant differences ($p < 0.001$) between C and F. The control exhibited an average value of 3.9 in the first column, while subsequent columns displayed values ranging from 2.1 to 7.5. In contrast, treatment F showed values ranging from 3.1 to 9.9 across different parameters. The combined treatment of temperature and T+F yielded the most substantial outcomes, with values ranging from 8.7 to 169.2 across the parameters. Statistical analyses underscored the significance of the observed differences across treatments, emphasizing the overall efficacy of various treatments [40]. Upon comparing the combined treatments, it is evident that the T+F treatment consistently exhibited superior growth outcomes, showcasing significantly higher values in all parameters compared to individual or other combined treatments. For instance, in the fourth column, the T+F treatment displayed a remarkable growth rate of 94.15, while the closest competitor, C+E+F, recorded a value of 72.2. This aligns with the literature emphasizing the synergistic effects of combining temperature treatments with other interventions in promoting plant growth and metabolic responses [26,32]. Previous literature supports the notion that the combination of treatments can lead to synergistic effects, particularly in enhancing plant growth and metabolic responses. The observed higher values in the T+F treatment align with studies suggesting that temperature, when coupled with specific interventions, can amplify the positive effects on plant physiological processes [38]. Moving on to the concentrations of phenolic compounds, the control exhibited moderate levels, including 2.50 g/L of gallic acid, 3.82 g/L of vanillic acid, and 4.67 g/L of caftaric acid. In contrast, treatment with essential oils (F) showed slightly higher concentrations in several phenolic compounds, such as ferulic acid (1.75 g/L), catechin (4.82 g/L), and epicatechin (6.01 g/L), indicative of the potential impact of essential oils on phenolic compound synthesis. The combined treatment of essential oils with curative application (T+F) demonstrated marked increases in multiple phenolic compounds, such as gallic acid (3.33 g/L), trans-caffeic-acid (3.28 g/L), and routine (2.64 g/L), indicating a synergistic effect on the concentrations of essential phenolic compounds. This aligns with the literature suggesting that essential oil combinations, when combined with specific treatments, can enhance the production of phenolic compounds with potential antioxidant properties [26,27]. The comprehensive treatment involving control, essential oils, and preventive application (C+E+F) demonstrated the highest concentrations of phenolic compounds among the treatments, including 3.89 g/L of gallic acid, 5.71 g/L of vanillic acid, and 2.95 g/L of routine. This aligns with studies emphasizing the cumulative effects of combining multiple treatments on the enhancement of secondary metabolites in plants [28,37].

Striking differences were discerned between the preventive and curative strategies, underscoring the nuanced hormonal responses to these distinct approaches. The preventive treatment strategy notably resulted in higher levels of IAA at 4.6 ng/mg compared

to the curative treatment at 2.2 ng/mg. Similarly, GA_3 concentrations were higher in the preventive treatment (4.6 ng/mg) than in the curative treatment (2.2 ng/mg). Conversely, the curative treatment exhibited elevated concentrations of ABA at 230.2 ng/mg and SA at 1.8 ng/mg, surpassing the levels observed in the preventive treatment (ABA: 196.0 ng/mg, SA: 2.1 ng/mg). The observed higher levels of IAA and GA_3 under the preventive approach align with studies highlighting the role of these hormones in promoting plant growth and development, potentially enhancing resistance mechanisms [40–42]. Among the various treatments, treatment F demonstrated heightened concentrations in several hormones, notably ABA (232.3 ng/mg), GA_3 (4.5 ng/mg), and JAs, suggesting the potential of essential oils to modulate hormonal regulation. Treatment T+F exhibited significant increases in GA3 (9.8 ng/mg) and zeatin (1.6 ng/mg), showcasing a synergistic effect between temperature treatment and essential oils. Treatment C+F displayed a substantial increase in ABA concentration (362.4 ng/mg), indicating a significant influence of EOs when combined with the control strategy. Treatment E+F contributed to elevated levels of ABA (483.0 ng/mg) and zeatin, further highlighting the intricate interplay between EOs and preventive measures. The comprehensive treatment C+E+F showcased varied hormone concentrations, with notable increases in cytokinin (1.9 ng/mg) and JA (0.6 ng/mg), suggesting a multifaceted impact of combining essential oils, control, and preventive measures. Finally, Treatment T+E+F exhibited significant increases in GA_3 (12.1 ng/mg) and zeatin (1.6 ng/mg), demonstrating the complex interactions when combining temperature treatment, essential oils, and curative measures. The distinct hormonal profiles under preventive and curative approaches underscore the complex interplay between plant–pathogen interactions and the intricacies of hormonal regulation. This aligns with the literature emphasizing the role of ABA in stress response and SA in signaling pathways related to plant defense against pathogens, indicating the activation of defense mechanisms under curative measures [43]. Examining individual treatments, treatment F showed elevated concentrations in ABA, GA_3, and JA. This finding resonates with literature suggesting that essential oils can elicit plant responses through the modulation of hormonal signaling pathways, particularly those associated with stress and defense mechanisms [44]. The significant increases in GA_3 and zeatin under treatment T+F highlight the synergistic effects of combining temperature treatment with essential oils, potentially promoting growth and stress tolerance [45]. In addition to their direct antifungal properties, essential oil combinations can enhance apple tissues' natural defenses against *B. cinerea* by triggering induced systemic resistance (ISR). This entails modifying the hormonal signaling pathways inside the plant, which triggers the activation of genes linked to defense and the build-up of secondary metabolites possessing antifungal characteristics [46]. The synergistic effects of essential oil combinations on the biochemical profiles in apples infected with *B. cinerea* result from a multifaceted mechanism involving multi-target interactions, enhanced penetration and bioavailability, the modulation of biochemical pathways, the reduction in oxidative stress resistance, and the induction of systemic resistance [47]. On the other hand, the preventive application, where the pathogen's conidial suspension is first inoculated followed by treatment with EOCs, primarily leverages an inhibitory mechanism. This mechanism likely involves the direct antimicrobial properties of the essential oils, which may inhibit fungal growth or kill the pathogen on contact. The increase in specific secondary metabolites such as phenolic compounds, as observed in the treated apples, supports this notion. The preventive approach may also enhance the physical barrier of the fruit or preemptively activate certain defense pathways, making the environment less hospitable for the pathogen to establish and proliferate. On the other hand, the curative application, where the fruit is first treated with EOCs followed by inoculation with the pathogen's conidial suspension, relies on induced resistance within the apple. This suggests that EOCs prime the apple's defense mechanisms, making it more prepared to respond to pathogen attack. The observed increase in specific hormones, such as ABA and SA, in the curative treatment supports this

theory [42,43]. These hormones are known to play crucial roles in plant defense signaling, with ABA being associated with abiotic stress response and SA with systemic acquired resistance against pathogens. The modulation of these hormones indicates that EOCs may stimulate the apple's own defense systems, enhancing its ability to resist infection after the pathogen is introduced. Therefore, the difference in action modes between preventive and curative applications can be attributed to the timing and sequence of treatment and pathogen challenge. In the preventive approach, the focus is on the direct inhibition of the pathogen and possibly some early activation of the plant's defense mechanisms. In contrast, the curative approach primarily enhances the plant's inherent defense mechanisms, preparing it to more effectively combat the pathogen upon exposure. This dual mode of action underscores the versatility of EOCs as both a direct antimicrobial agent and an elicitor of plant defense responses, offering a comprehensive strategy for managing post-harvest diseases in apples.

The PCA biplots presented in Figure 1 offer valuable insights into the multivariate relationships among key biochemical components in the context of various treatments and applications. The clear differentiation among treatments, illustrated by color-coded data points, underscores the impact of different strategies on the metabolic profile of *B. cinerea*-infected apples. Panel A of Figure 1, with Dim1 and Dim2 explaining a substantial proportion of the observed variance, serves as a visual representation of the distinctiveness among treatments. The cos2 values and color gradients provide a nuanced understanding of the relative influence of each variable. Notably, Panels B and E highlight the strong differentiation among cultivars concerning anthocyanins and organic acids, respectively, emphasizing the significance of treatment strategies in influencing these biochemical categories. On the other hand, Panel C reveals a more balanced distribution of phenolic compounds, indicating a complex interaction pattern within this group. The heatmap analysis in Figure 2 complements these findings, offering a comprehensive view of the abundance of various metabolites across treatments. The distinct metabolite signatures for each treatment condition, revealed by blocks of color, suggest specific modulations in response to different strategies. The clustering of metabolites and treatments provides a clear visualization of the relationships and potential interactions between compounds and conditions. These findings collectively contribute to a deeper understanding of the metabolic landscape of *B. cinerea*-infected apples under different treatment scenarios. The observed trends in amino acids and phenolic compounds hint at potential metabolic pathways influenced by the applied strategies. This information is crucial for optimizing agricultural and post-harvest practices, with implications for enhancing apple quality and shelf life. The combination of PCA biplots and heatmap analysis proves to be a powerful approach for unraveling the intricate biochemical responses in this context.

5. Conclusions

Our study on essential oil combination applications for *B. cinerea*-infected apples uncovered significant insights into the fruit's biochemical composition. EOs, particularly in treatment F, notably influenced amino acid concentrations, emphasizing phenylalanine, isoleucine, and leucine. The complex interactions in combined treatments underscored the importance of comprehensive strategies in shaping amino acid levels. Significant differences in hormones were observed with EOCs treatments, particularly in combination treatments, suggesting a potential role in hormonal regulation. This could have implications for fruit maturation and development and resistance to *B. cinerea*. The anthocyanin content exhibited notable variations among treatments, with EOCs, especially in combinations, significantly impacting the concentrations of malvidin-3-glycoside-p-coumaryl and malvidin-3-glycoside-acetyl. This underscores the potential of EOCs to influence the composition of anthocyanins, which play a crucial role in determining the nutritional value and visual appeal of apples. In our findings, EOCs contributed to higher concentrations of phenolic compounds, with C+E+F demonstrating a synergistic effect, highlighting the potential of combining EOs with C and preventive measures to enhance antioxidant prop-

erties. Organic acid concentrations differed between preventive and curative applications, with EOCs, particularly in combination with curative, consistently yielding superior outcomes. This implies a potential role for essential oils in shaping the overall acidity of the fruit. These findings offered crucial insights into the multifaceted impact of essential oils on apple biochemistry. The study underscored the need for comprehensive treatment approaches to optimize fruit quality and preservation. Future research and practical applications guided by these findings hold promise for enhancing the resilience and market value of harvested apples.

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Abbreviations

EOCs: essential oil combinations; C: control; F: fungus; T+C+E+F: thymol + cineole + eugenol + fungus; T+E+F: thymol + eugenol + fungus; T+C+F: thymol + cineole + fungus; C+E+F: cineole + eugenol + fungus; T+F: thymol + fungus; E+F: eugenol + fungus; C+F: cineole + fungus; ABA: abscisic acid; IAA: indole-3-acetic acid; SA: salicylic acid; GA_3: gibberellic acid; JA: jasmonic acid.

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