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Gray Mold Control and Quality of 'Albion' Strawberries with Postharvest Application of Tea Tree Essential Oil

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Abstract

The objective of this study was to evaluate the effect of tea tree essential oil (*Melaleuca alternifolia*) on postharvest rots caused by *Botrytis cinerea* and on the sensory quality of 'Albion' strawberries. The experiment was conducted with fruits from a production area in Lages, Santa Catarina, Brazil. The treatments consisted of a control (without essential oil) and tea tree essential oil at 50 and 250 μL L^{-1}, applied by fumigation. After treatment, the fruits were stored for three days. Rot incidence and severity, respiration and ethylene production, flesh penetration force, soluble solids, titratable acidity, skin color, total phenolic compounds, peroxidase activity, and volatile profile were evaluated. Tea tree oil reduced the development of *B. cinerea* at both concentrations. It also delayed red color development and reduced total phenolic content. At 250 μL L^{-1}, the oil increased respiration rate and reduced peroxidase activity. Ethylene production, titratable acidity, soluble solids, and flesh penetration force were not affected. The volatile profile was altered, with lower levels of characteristic strawberry volatiles and greater presence of terpene compounds typical of tea tree oil, especially at the higher concentration. Thus, tea tree oil reduced disease development but caused undesirable changes in fruit color and aroma profile.

Keywords: decay; *Fragaria* x *ananassa*; postharvest

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1. Introduction

Gray mold, caused by the fungus Botrytis cinerea, is the most significant disease affecting strawberries. Although it can occur in the field, symptoms are more commonly observed during the postharvest period, leading to substantial losses. Controlling this disease is challenging, and the use of fungicides before harvest is the main strategy adopted. In addition, fruit refrigeration after harvest is recommended to slow the development of gray mold [1]. However, in practice, strawberries are usually marketed at ambient temperature on supermarket shelves. For this reason, it is important to investigate alternative control methods that can be applied under these conditions.

Among alternative approaches, the use of essential oils (EOs) stands out in postharvest management. EOs are complex mixtures of aromatic compounds synthesized by plants through secondary metabolism and extracted from different plant parts. Their composition, rich in terpenoids, alkaloids, and phenolic substances [2], provides antimicrobial properties that have attracted increasing interest in the field of food preservation. In addition to their antifungal potential, EOs may also positively influence postharvest quality attributes, such as the maintenance of firmness, color, and levels of acids and sugars [3–5].

The essential oil of *Melaleuca alternifolia* (tea tree essential oil, TTEO) has well-documented antifungal activity, mainly attributed to the presence of terpinen-4-ol [6]. Studies such as that of [7] have demonstrated the effectiveness of TTEO vapor application in reducing the incidence of rots

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caused by B. cinerea in strawberries. Moreover, the response to TTEO may vary among cultivars of the same species. These variations are associated with genetic, physiological, and morphological traits specific to each cultivar. For instance, in a study with peaches, the cultivar 'Florida King 6-A' exhibited greater resistance to gray mold, whereas 'Early Gold' was highly susceptible, requiring different EO concentrations for effective control [8]. Characteristics such as epidermis thickness, fruit chemical composition, and natural defense mechanisms influence the absorption, distribution, and efficacy of EOs. Therefore, considering the cultivar used is essential to optimize the desired effects of the treatment while preserving fruit quality. Nevertheless, there is a scarcity of studies evaluating the effects of TTEO under non-refrigerated conditions on the cultivar 'Albion', which is widely accepted commercially due to its firmness, sweetness, and resistance to transportation.

The objective of this study was to evaluate the effect of postharvest application of TTEO on the control of *Botrytis cinerea*, as well as on the physicochemical attributes and volatile compound profile of 'Albion' strawberries kept under ambient conditions.

2. Materials and Methods

The experiment was conducted using strawberries (*Fragaria* × *ananassa*) of the Albion cultivar, obtained from a commercial production area located in Lages, Santa Catarina, Brazil (27°46′ W; 50°12′ S; 930 m altitude). The fruits were harvested at commercial maturity for consumption, corresponding to approximately 75% of bright red color on the fruit surface.

To obtain Botrytis cinerea spores, isolates B. cinerea 222 and 223 from the Erlei Mello Reis Mycotheca of the Plant Pathology Laboratory, Centro de Ciências Agroveterinárias, Universidade de Santa Catarina (CAV/UDESC) were used. The isolates were subcultured onto Petri dishes containing potato dextrose agar (PDA) medium and incubated in a growth chamber under controlled temperature and photoperiod conditions (23 ± 2 °C and a 12-hour photoperiod) for seven days. Subsequently, 7 mm diameter discs from these cultures were transferred to fresh PDA plates. The plates were kept in the growth chamber under the same conditions for an additional seven days, after which the spores were obtained for subsequent fruit inoculation.

The fruits were initially sanitized with 70% ethanol for 5 minutes and air-dried for one hour. After drying, a *Botrytis cinerea* spore suspension (10^5 spores mL^{-1}) was sprayed on one side of the fruit to inoculate them. The inoculated fruit were then exposed to three concentrations of TTEO: 0 $\mu L\ L^{-1}$, 50 $\mu L\ L^{-1}$, and 250 $\mu L\ L^{-1}$. Each concentration consisted of five replications, with each experimental unit containing 15 fruit inoculated with B. cinerea for the evaluation of incidence and severity. For the analysis of physicochemical attributes and volatile compound profile, non-inoculated fruit were used. These fruits were treated with the same TTEO concentrations as previously described. Each treatment had five replications, and each experimental unit consisted of 20 fruit. The TTEO used was obtained from Ferquima Indústria e Comércio de Óleos Essenciais (Vargem Grande Paulista, Brazil).

The treatments were applied by fumigation. The fruit were placed in 30 L drums with hermetic sealing. A closed system was established in which the essential oil was volatilized using a nebulizer (G-TECH, model NEBCOM V, Brazil) for 12 hours, which was the time required for complete volatilization of the oil.

After treatment application, the fruit were kept under ambient conditions (23 ± 3 °C and 80 ± 2% relative humidity) for 3 days. During this period, the inoculated fruit were evaluated every 24 hours for rot incidence and severity.

Non-inoculated fruit were evaluated for a range of attributes. These included rot incidence and severity, flesh penetration force, soluble solids content (SSC), titratable acidity (TA), and skin color (hº, L*, and C*). Additional analyses comprised respiration and ethylene production rates, total phenolic compound (TPC) content, peroxidase (POD) enzyme activity, and volatile compound profile. SS, TA, skin color, and TPC were analyzed according to the method described by [9]. Flesh penetration force (N), respiration rate ($\mu mol\ CO_2\ kg^{-1}\ s^{-1}$), ethylene production rate ($\mu mol\ C_2H_4\ kg^{-1}\ s^{-1}$), POD activity, and volatile compounds were determined following the methodology described by [3].

The incidence (%) of rot caused by B. cinerea was determined as the proportion of fruit showing symptoms of rot caused by the fungus relative to the total number of fruit evaluated. Disease severity was assessed using an index based on a rating scale, where: 1 = up to 25% of the fruit surface with rot; 2 = 26–50%; 3 = 51–75%; and 4 = more than 75% of the fruit surface with rot. The index was determined for fruits inoculated with B. cinerea at 0, 12, 24, and 36 h after treatment application. The area under the disease progress curve for incidence (AUDPCi) and severity (AUDPCs) was calculated according to the formula described by [10].

The experiment followed a completely randomized design. Data for the area under the disease progress curve for severity and incidence, as well as quality parameters, were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test ($p<0.05$) with the SAS statistical software (SAS Institute Inc., Cary, NC, USA). Rot incidence data were arcsine square-root transformed using the equation arcsin(x+0.5)/100(x+0.5)/100 before being subjected to statistical analysis..

3. Results and Discussion

The fumigation treatment with TTEO significantly reduced the AUDPC for gray mold, both for disease incidence and severity, with no statistical differences between the 50 and 250 μL L^{-1} concentrations (Table 1). The reduction in AUDPC for incidence was 45% and 72%, and for severity 42% and 34% at 50 and 250 μL L^{-1}, respectively, compared with the control. These results indicate a direct effect of TTEO on the pathogen. Previous studies support these findings. [11] reported that application of 10 μL L^{-1} TTEO reduced B. cinerea incidence by 50% in cherries, with a 12 mm decrease in lesion diameter. [3] observed reductions of 95% in AUDPC for incidence and 45% for severity of B. cinerea in 'San Andreas' strawberries using fumigation with 600 μL L^{-1} TTEO. [12] found that applying 1,400 μL L^{-1} TTEO to peaches reduced *Monilinia fructicola* incidence by about 20% and lesion diameter by 40%. In bananas, [13] reported that TTEO application reduced the incidence of *Colletotrichum musae* and slowed lesion development over eight days. Taken together, these results indicate that TTEO has fungicidal activity against several phytopathogens. Its main components, terpinen-4-ol and terpineol, inhibit mycelial growth and spore germination, and when evaluated individually they caused more destructive morphological effects on hyphae, spores, and the fungal plasma membrane than the whole oil itself [14].

Table 1. Area under the disease progress curve for incidence (AUDPCi) and severity (AUDPCs) of Botrytis cinerea in strawberries treated with tea tree essential oil.

Treatments (μL L^{-1})	AUDPCi	AUDPCs
0	2130 A	22.9 A
50	1170 B	13.1 B
250	600 B	8.0 B
CV (%)	28.4	22.5

Means followed by the same letter in the column do not differ according to Tukey's test ($p<0.05$). CV: coefficient of variation. AUDPC for incidence: area under the disease progress curve for incidence. AUDPC for severity: area under the disease progress curve for severity.

The respiratory rate of the fruits increased significantly with the application of 250 μL L^{-1} of TTEO, differing from the values observed in the control and at 50 μL L^{-1} (Table 2). This result contrasts with the findings of [15], who reported a reduction in the respiration rate of kiwifruit treated with TTEO. Similarly, [3] also observed a decrease in respiration in strawberries subjected to TTEO application. With respect to ethylene production rate, no significant differences were detected among treatments (Table 2). This outcome was expected, since strawberry is a non-climacteric fruit and therefore has a limited ethylene production rate [16]. However, the literature presents contradictory results. [17] reported increased ethylene production in strawberries treated with sweet orange essential oil, whereas [3] found a reduction in ethylene production following TTEO application.

Given these discrepancies, the results observed in the present study may be related to possible damage to fruit cell structures, which could have stimulated respiration as a stress response.

Table 2. Respiration and ethylene production rates, soluble solids content (SSC), titratable acidity (TA), and flesh penetration force of strawberries treated with tea tree essential oil.

Treatments (μL L^{-1})	Respiration (ηmol de CO_2 kg^{-1} s^{-1})	Ethylene prodction (pmol C_2H_4 kg^{-1} s^{-1})	SSC (ºBrix)	TA (% citric acid)	Flesh penetration force (N)
0	414.7 B	1.7^{ns}	8.3^{ns}	1.2^{ns}	1.7^{ns}
50	432.7 B	2.2	8.4	1.2	1.7
250	522.2 A	1.9	8.2	1.2	1.5
CV (%)	7.7	18.2	3.7	3.5	6.6

Means followed by the same letter in the column do not differ according to Tukey's test ($p<0.05$). CV: coefficient of variation. ns: not significant.

The application of TTEO did not affect SSC or TA (Table 2). These results indicate that, under the conditions evaluated, TTEO treatment did not compromise taste-related attributes associated with the sensory acceptance of the fruit. [18] also found no effect of basil essential oil application on SSC in strawberries. In contrast, [5] reported reductions in both TSS and TA in 'Albion' strawberries treated with *Aloysia citrodora* essential oil, suggesting that fruit responses may vary according to the type of essential oil and the cultivar used. The SSC/TA ratio is widely used as an indicator of ripening stage and is one of the main determinants of taste and consumer acceptance of fruits [19]. In this context, the stability of these parameters after TTEO application can be considered a positive outcome, as it contributes to maintaining the sensory quality of strawberries.

Flesh penetration force was not affected by TTEO application (Table 2). [20] also reported no changes in strawberry firmness following treatment with peppermint essential oil. Conversely, [4] found increased firmness in strawberries coated with zein fiber loaded with thyme essential oil. These discrepancies may be related to factors such as the specific chemical composition of each oil, the concentration applied, exposure time, and the physiological characteristics of the cultivars studied. In addition, inherent fruit-related factors, such as harvest maturity stage, cultivation history, and uniformity of size and texture, may have contributed to the variability in results. Fruit firmness is a key parameter for commercialization and consumer acceptance [21], especially in strawberries, which exhibit a rapid loss of firmness during postharvest storage due to degradation of the cell wall, mainly pectin solubilization and hemicellulose depletion. The absence of any effect of TTEO on firmness may indicate that the treatment did not significantly interfere with the structural processes associated with cell wall degradation in the fruit.

Regarding color attributes, fruits treated with TTEO showed a significant increase in L*, C*, and hº values compared with the control (Table 3), indicating a brighter color and a less intense red tone. These changes suggest that the treatment promoted visually lighter fruit with a hue shifted away from deep red. Similar results were reported by [17], who observed less red strawberries after the application of sweet orange essential oil. [3] likewise found a reduction in red coloration in 'San Andreas' strawberries treated with TTEO, and [20] also reported brighter coloration in strawberries treated with nanoemulsions of thyme and peppermint essential oils, comparable to the effect observed here with TTEO. The intensification of red color in strawberries after harvest is associated with anthocyanin biosynthesis during ripening; however, the maintenance of higher lightness and lower red saturation in treated fruits may reflect a partial slowdown of this process.

TTEO treated-fruit showed lower total phenolic compound (TPC) content (Table 3). Phenolic compounds play a crucial role in the antioxidant defense of plant tissues by neutralizing oxygen free radicals, decomposing peroxides, and inhibiting lipid autooxidation [22]. Similar results were reported by [3], who observed a reduction in TPC in strawberries treated with TTEO, as well as by [23] in tomatoes treated with sage essential oil. In contrast, [5] found an increase in TPC in 'Albion'

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strawberries treated with *Aloysia citrodora* essential oil, attributing this effect to the phenolic composition of the oil itself. The divergence among studies may be related both to the specific chemical composition of the essential oils used and to their interactions with the fruits' secondary metabolites. In the present study, the reduction in TPC likely indicates mild oxidative stress or the degradation of phenolic compounds during exposure to TTEO, without negatively affecting other quality attributes.

Table 3. Skin color, total phenolic content, and guaiacol peroxidase activity in strawberries treated with tea tree essential oil.

Treatments	Skin color			Total phenolic content	Guaiacol peroxidase
(μL L^{-1})	L*	C*	hº	(mg EAG 100 g^{-1})	(DO mg^{-1} protein min^{-1})
0	37.0 C	41.4 B	35.1 B	127.5 A	0.83 A
50	38.1 B	42.1 AB	36.6 A	110.7 B	0.86 A
250	39.0 A	43.2 A	37.3 A	97.6 B	0.43 B
CV (%)	1.5	2.0	1.7	5.9	12.0

Means followed by the same letter in the column do not differ according to Tukey's test ($p<0.05$). CV: coefficient of variation.

Peroxidase (POD) activity was significantly lower in fruits treated with 250 μL L^{-1} of TTEO (Table 3). POD is widely recognized as a key enzyme in the antioxidant defense system of plants, being involved in the detoxification of reactive oxygen species (ROS) and in the induction of resistance against pathogenic infections [22]. An increase in POD activity is generally interpreted as indicative of the activation of defense mechanisms in response to biotic and abiotic stresses. However, in the present study, TTEO application did not stimulate this enzymatic response, suggesting that, at the concentration used, the treatment may not have induced a sufficiently intense stress to activate this system or, alternatively, that POD activity was suppressed. In contrast, [5,24,25], and [26] reported increased POD activity in fruits treated with different essential oils, indicating that the response may vary according to the type and concentration of essential oil, the fruit species or cultivar, and storage conditions. This variability suggests that POD response to essential oils depends on several interconnected factors, including cultivar-specific traits, the type of pathogen involved, and pre-existing defense mechanisms in the fruit. The reduction observed in this study may also be related to possible interactions between TTEO components and the regulatory mechanisms of POD activity, which deserves further investigation.

The analysis of volatile compounds in 'Albion' strawberries showed that application of TTEO, regardless of dose, suppressed the presence of certain strawberry-characteristic volatiles (Table 4). In particular, there was a reduction or absence of several esters, a class of compounds strongly associated with the typical fruity aroma of strawberry. Among the main esters identified in control fruit, ethyl acetate, ethyl butanoate, and methyl hexanoate stood out, with ethyl acetate showing the highest relative area. In addition, the aldehyde (E)-2-hexenal, associated with green and fresh notes in strawberry aroma, was more abundant in fruit not treated with TTEO. By contrast, the aldehyde fenchone, characteristic of essential oils, was detected only in TTEO-treated fruit, indicating the incorporation of treatment-derived volatiles into the fruit profile. Among terpenes, linalool was identified in all treatments, although its relative area decreased with increasing TTEO dose. Typical TTEO terpenes, such as terpinen-4-ol, terpineol, eucalyptol, and m-cymene, became predominant in treated fruits, with terpinen-4-ol being the most abundant, consistent with its status as the major component of TTEO [27]. It is important to note that compounds such as linalool, nerolidol, and geraniol contribute substantially to strawberry aroma and flavor, providing floral and fruity notes appreciated by consumers [28]. The partial or total replacement of these natural strawberry volatiles by TTEO-typical compounds compromises the characteristic aromatic profile of the fruit. This interference was proportional to the applied dose, indicating that TTEO altered the volatile composition of the fruits in a dose-dependent manner. Moreover, the volatile profile may also have

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been influenced by environmental factors, such as temperature and humidity during treatment application, which affect both the diffusion of volatiles and the enzymatic activities involved in their biosynthesis.

Table 4. Volatile compound profile (%, area) in strawberries treated with tea tree essential oil.

Treatments (μL L-1)	CompoundTreatments (μL L-1)		
	0	50	250
Esters			
2-Methylbutyl acetate	0.15 ± 0.20	ND	ND
(Z2) Hexen-1-ol acetate	5.41 ± 3.01	3.18 ± 1.23	2.62 ± 2.05
Ethyl acetate	36.54 ± 13.80	29.29 ± 26.91	22.91 ± 16.53
Hexyl acetate	2.45 ± 1.46	1.60 ± 0.57	1.54 ± 1.65
Isobutyl acetate	0.14 ± 0.19	ND	ND
Methyl acetate	0.88 ± 0.31	ND	ND
Myrtenyl acetate	ND	0.76 ± 0.21	0.45 ± 0.42
Octyl acetate	0.64 ± 0.33	0.86 ± 0.45	0.35 ± 0.26
Ethyl benzoate	0.13 ± 0.15	ND	ND
Ethyl butanoate	14.34 ± 3.06	7.55 ± 1.85	8.67 ± 2.48
Methyl butanoate	0.26 ± 0.16	ND	ND
Ethyl hexanoate	12.06 ± 2.18	10.36 ± 2.70	3.79 ± 1.53
Methyl hexanoate	1.33 ± 0.90	ND	ND
Ethyl Iglate	0.38 ± 0.24	ND	ND
Alcoois			
1-Hexanol	0.74 ± 0.57	ND	ND
2-Ethyl Hexanol	4.27 ± 1.39	1.20 ± 1.23	ND
Aldeidos			
(E)-2-Hexenal	9.78 ± 8.24	2.35 ± 0.86	1.69 ± 0.99
Decanal	0.06 ± 0.05	ND	ND
Fenchone	ND	0.68 ± 0.49	1.43 ± 0.93
Nonanal	0.08 ± 0.08	ND	ND
Acids			
Acetic acid	ND	0.55 ± 0.63	ND
Ethyl 2-Acid β-hydroxy-β-methylbutyric acid	0.78 ± 0.28	ND	ND
Terpenes			
(E) Nerolidol	ND	0.25 ± 0.18	ND
Eucaliptol	ND	7.92 ± 1.72	13.15 ± 3.52
Isocineole	ND	0.33 ± 0.10	0.74 ± 0.51
Linalol	7.44 ± 2.30	2.83 ± 0.40	2.54 ± 0.79
m-Cimeno	ND	2.63 ± 0.87	4.06 ± 2.78
Piperitona	ND	1.29 ± 0.49	2.44 ± 1.23
Terpinen-4-ol	ND	18.54 ± 15.24	22.18 ± 19.16
Terpinene	ND	1.42 ± 0.42	3.32 ± 1.70
Terpineol	ND	4.97 ± 1.51	8.11 ± 3.32

Note: National Center for Biotechnology Information. PubChem Compound Summary. https://pubchem.ncbi.nlm.nih.gov/compound/. Acesso: Ago., 2020. ND: not detected.

Overall, the results demonstrate that TTEO application has potential for controlling gray mold in 'Albion' strawberries kept at ambient temperature, representing a promising alternative to synthetic fungicides in the postharvest stage. However, although a reduction in POD activity and rot incidence was observed, TTEO treatment also caused undesirable changes in fruit quality attributes. These changes included increased respiration rate, decreased total phenolic compound content, alterations in color parameters, and marked modifications in the volatile profile, with treatment-derived volatiles predominating over the compounds naturally present in strawberry aroma. Such negative effects may be particularly associated with the vapor application method and the exposure time of fruits to the oil. Therefore, further research is needed to optimize TTEO application conditions, including adjustments in concentration, exposure time, and controlled-release strategies, such as nanoencapsulated essential oils, in order to preserve the phytosanitary benefits of the treatment without compromising the sensory and nutritional quality of the fruit.

4. Conclusions

Postharvest fumigation with tea tree essential oil reduces the development of rots caused by Botrytis cinerea in 'Albion' strawberries. However, it impairs fruit color development, decreases total phenolic compounds, and alters the volatile compound profile, mainly due to the presence of terpenes characteristic of tea tree essential oil and to a reduced relative proportion of the esters ethyl butanoate and ethyl hexanoate, the alcohol 2-ethyl-1-hexanol, and the aldehyde 2-hexenal.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

EOs	Essential oils
TTEO	Tea tree essential oil
CAV	Centro de Ciências Agroveterinárias
UDESC	Universidade de Santa Catarina
PDA	Potato dextrose agar
SSC	Soluble solids content
TA	Titratable acidity
TPC	Total phenolic compound
POD	Peroxidase
AUDPCi	Disease progress curve for incidence
AUDPCs	Disease progress curve for severity
ROS	Reactive oxygen species

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