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The combined effects of tea tree oil and hot air treatment on the quality and sensory characteristics and decay of strawberry

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A B S T R A C T

In this study, the antifungal activities of tea tree oil (TTO) combined with hot air (HA) treatment against *Botrytis cinerea*, the causal agent of strawberry gray mold, was tested *in vitro* and *in vivo*. Results showed that both TTO and HA treatment inhibit mycelial growth and destroy the hyphal ultrastructure of *B. cinerea*. The combined treatment (TTO + HA) results in a significantly synergistic effects in *in vitro* tests. Compared with control fruit, each treatment (TTO, HA, TTO + HA) reduced the gray mold in strawberry fruit inoculated with *B. cinerea*, the combined treatment resulted in lowest decay incidence and lesion diameter. The combined treatment also induced higher activities of chitinase (CHI) and β-1, 3-glucanase (GLU) and these strawberries subjected to this combined treatment maintained a better commercial quality as measured by firmness, TSS, a* value and sensory evolution, than those treated with TTO or HA alone. It was concluded that combined TTO and HA treatment of strawberry fruit is more effective at controlling decay and maintaining fruit quality than single treatment, that may be a potential strategy for commercial application in strawberries.

1. Introduction

Strawberries (*Fragaria* × *ananassa*) are a fast growing, non-climacteric fruit, classified within the family *Rosaceae*. Strawberries enjoy worldwide popularity because of their delicious flavor and aroma, exceptionally rich nutrient content, they offer potential health benefits. Unfortunately strawberries are highly susceptible to mechanical injury, water loss, and fungal decay during storage (Wei et al., 2016). Gray mold spoilage by *Botrytis cinerea* is one of the most destructive postharvest diseases of strawberries (Zhang et al., 2013; Liu et al., 2016). Chemical control is essential to prevent pre and postharvest fruit decay; in conventional agriculture, anilinopyrimidine, cyprodinil, phenylpyrrole and fludioxonil had been widely applied for gray mold control (Ugolini et al., 2014). However, these typical fungicides have serious shortcomings, including the development of multi-resistant fungal strains, environmental contamination, and potential harm to human health. For these reasons, the identification of alternative antifungal agents to extend postharvest quality is of great interest.

Because multiple investigations have demonstrated that many essential oils from plants have antimicrobial activities, they have been increasing use as natural antimicrobial agents to control postharvest disease in strawberry, pomegranate, and citrus among others (Boubaker et al., 2016; Liu et al., 2016; Lombardo et al., 2016; Thomidis and Filotheou, 2016; Wu et al., 2017). Tea tree oil (TTO) from the Australian native plant *Melaleuca alternifolia*, is a complex mixture of terpene hydrocarbons (Sánchez-González et al., 2010). It has gained the attention of scientists, physicians and consumers due to its broad-spectrum antimicrobial activities against a variety of microbes, including *B. cinerea*, *Staphylococcus aureus*, *Escherichia coli*, *Pythium sulcatum* and *Rhizopus stolonifer* (Shao et al., 2013; Mantil et al., 2015; Shi et al., 2016).

Hot air (HA) treatment, an environmentally friendly technology, has been widely used to reduce postharvest decay and maintain quality in various fruits, including loquat, Chinese bayberry, sweet cherry and cherry tomato (Liu et al., 2010; Wang et al., 2010, 2015; Wei et al., 2016). The application of heat (45 °C, 3 h) to strawberry fruit delays softening and causes a temporary reduction in the enzymes involved in cell wall disassembly (Dotto et al., 2011; Martínez and Civello, 2008; Vicente et al., 2005).

Neither essential oil nor HA treatment alone has yet to provide the decay control offered by synthetic fungicide. However, the combination of heat and essential oils has a synergistic antimicrobial effect on *Escherichia coli* O157: H7, *Leuconostoc fallax* 74, and *Saccharomyces cerevisiae* in apple juice (Ait-Ouazzou et al., 2012; Chueca et al., 2016). Combined TTO and HA treatments have not yet been evaluated as a potential control measure for postharvest decay and quality decline in strawberries. The objectives of this study were to (1) investigate the antifungal effects of TTO in combination with HA treatment on mycelial

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growth and the effects on *B. cinerea* ultrastructure; (2) study the effects of TTO, in combination with HA treatment on gray mold development and defense-related enzymes in artificially inoculated strawberries; (3) to verify the effects of the combined treatment on the quality of fresh strawberries.

2. Materials and methods

2.1. Fruit, TTO, and pathogens

Strawberries were hand-harvested at commercial maturity from an orchard in Ningbo, Zhejiang Province, China, and transported within 1 h to the laboratory. Uniform fruit, free from blemishes were selected by size, color, ripeness, and absence of visually apparent infections.

TTO was obtained from Fuzhou Melalyn Biotechnology Co., Ltd. (Fujian, China). The TTO contained 37.11% terpinen-4-ol and 4.97% 1,8-cineole, which were adjusted to comply with the ISO 4730 and European Pharmacopoeia standards.

*B*otrytis *cinerea* was isolated from the surfaces of infected strawberry fruits in a greenhouse and identified by morphological and molecular biological methods. Cultures were maintained on potato dextrose agar medium containing 200 g boiled potato extract, 20 g glucose, and 20 g agar in 1 L of the medium. Spores were removed from the surface of cultures incubated for 5 d on plates at 25 °C, and then suspended in 5 mL of sterile saline water. Spore concentrations were determined using a hemocytometer and adjusted to 10^5 conidia/mL.

2.2. Effects of TTO and HA treatments on mycelial growth in vitro

Glass Petri dishes (9 cm diameter) were filled with 20 mL of potato dextrose agar medium, and then 80 mL air space remains. One disc (5 mm diameter) of a mycelial plug was taken from the edge of a 5-day-old fungal culture, placed onto the agar, and subjected to one of three treatments. (1) Hot air treatment (HA): plates was incubated for 1, 2, or 3 h at 45 °C. (2) Various tea tree oil concentrations (TTO): sterile filter paper discs (3 cm diameter) were attached to the inner surface of each Petri dish lid. An appropriate volume of TTO was pipetted onto the filter paper to obtain final concentrations of 0.3, 0.6, 0.9 g L^{-1} air, and the dishes were quickly covered, then sealed with Parafilm M (Bemis Company, Inc.) along the rim to inhibit the escape of volatile components. The compounds were allowed to volatilize inside the Petri dishes at 25 °C for 3 h. (3) Combined treatment (HA + TTO): Petri dishes, prepared as described above for the TTO treatment, were incubated at 45 °C for 1, 2, or 3 h. (4) Control: The Petri dishes were incubated at 25 °C without TTO. After 3 h of TTO or/and HA treatment, the filter paper discs were removed and the dishes were incubated at 25 °C for 5 d when the diameter of the mycelial growth was recorded. The percentage inhibition of mycelial growth was calculated according to the following formula:

$$\text{Inhibition of mycelial growth (\%)} = [(d_c - d_t)/d_c]*100\%$$

where d_c (cm) is the mean colony diameter for the control sets, and d_t (cm) is the mean colony diameter for the treatment sets. Each treatment was performed in quintuplicate.

In order to reveal the synergic interaction of combined treatment, Limpel's formula, as described by Sanzani et al. (2009), was used to determine the presence of synergic interactions between TTO and HA in controlling mycelial growth of *B. cinerea*.

$$Ee\ (\%) = (X + Y) - (X \times Y)/100$$

where *E*e is the expected effect from additive responses of two treated methods used in combination, and X and Y are the found percentages of inhibition rate of mycelial growth relative to TTO and HA used alone. If the inhibition rate of combined treatment was greater than *E*e, synergism exists.

2.3. Effects of TTO and HA treatments on B. cinerea ultrastructure

Samples were obtained from control mycelium, TTO-treated mycelium (0.9 g L^{-1} TTO vapor at 25 °C for 3 h), HA-treated mycelium (45 °C for 3 h), and HA + TTO-treated mycelium (0.9 g L^{-1} TTO at 45 °C for 3 h). Samples were fixed with 2.5% glutaraldehyde for 2 h, 1% osmic acid for 1 h, and then washed three times with cold phosphate buffer solution (PBS, 0.1 M, pH = 7.2). Three replicates were prepared for the treated and control groups. Cell ultrastructure was observed by transmission electron micrographs (Model JEM-1230, Hitachi), using the method of Yu et al. (2015).

2.4. Effects of TTO and HA treatments on disease development and defense-related enzyme activities in strawberry fruits artificially inoculated with B. cinerea

Strawberry fruits were surface-cleared with 75% ethanol, then punctured with the tip of a sterile puncture needle to generate wounds 3 mm deep and 3 mm wide at the equator of each fruit. Aliquots (15 μL) of a *B. cinerea* spore suspension (10^5 spores mL^{-1}) were inoculated into wounds. Fruits were air-dried at room temperature for 1 h and randomly divided into four groups, that were treated as follows. (I) Control: fruits did not receive any TTO or HA treatment. (II) HA: fruits were treated at 45 °C air for 3 h in a 500-L heating chamber (HWS-500, Ningbo Jiangnan Instrument Factory, Ningbo, People's Republic of China), and the relative humid was 85 ± 2%. (III) TTO: fruit was exposed to TTO vapor (0.9 g L^{-1}) at 25 °C for 3 h. (4) HA + TTO: fruit was exposed to TTO vapor (0.9 g L^{-1}) at 45 °C for 3 h in the heating chamber. Following treatment, fruit in all groups were stored at 20 °C. Each group involved three replications, and 80 fruit were used in each replicate. The whole experiments were repeated twice.

Three days after treatment, 10 fruits from each group of each replicate were observed for development of decay. Decay incidence was the percentage of fruits showing decay symptoms around the artificial wound, relative to the total number of fruit in each treatment group.

To evaluate the active defense responses elicited by treatment, tissue samples surrounding each wound were collected from each group at 0, 12, 24, 36, 48, 60 and 72 h post treatment. At each time point, 10 fruits were randomly selected from each group of each replicate, and tissue samples surrounding wounds were ground into powder with liquid nitrogen. The defense-related enzymes chitinase (CHI, EC 3.2.1.14) and β-1,3-glucanase (GLU, EC 3.2.1.58) were extracted from 1 g of tissue sample with 5 mL of 50 mM sodium acetate buffer (pH 5.0). The homogenate was centrifuged at 12,000 × *g* for 20 min at 4 °C and the supernatant was used to determine CHI and GLU activities (Ippolito et al., 2000). CHI activity was measured by the release of N-acetyl-D-glucosamine from colloidal chitin using the 4-dimethylamino-benzaldehyde method. One unit of CHI activity is defined as the amount of enzyme required to catalyze the production of 1 g N-acetyl-D-glucosamine per hour at 37 °C. For GLU activity, laminarin were used as the substrates, one glucanase unit catalyzes the creation of 1 mg of glucose per hour.

2.5. Effects of TTO and HA treatments on quality changes and sensory evolution in fresh strawberry fruits

Strawberry fruits were randomly divided into 4 groups (80 fruit per group x 3 replicates): control, HA, TTO, and HA + TTO treatment conditions were as described above. Following treatment, fruit in all groups were stored at 20 °C. Three days after storage, 10 fruits from each group of each replicate were selected for evaluation of disease incidence and quality parameters.

Firmness was determined on opposite sides of each fruit tested, using a hand penetrometer (GY-1, Hangzhou Top Instrument Co., LTD, China) equipped with a 3 mm diameter head and conical-shaped spear. The results were expressed in newtons (N). Total soluble solid content

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