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# Cinnamaldehyde inhibits the mycelial growth of *Geotrichum citri-aurantii* and induces defense responses against sour rot in citrus fruit

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ABSTRACT

Sour rot is caused by *Geotrichum citri-aurantii* and is one of the devastating diseases in citrus fruit. This disease is difficult to control because of the lack of effective fungicides. In this study, cinnamaldehyde, a common food preservative, was evaluated to control postharvest sour rot in citrus fruit through in vivo and in vitro experiments. Results showed that cinnamaldehyde with a minimum inhibitory concentration and fungicidal concentration of 0.50 mL $L^{-1}$ dose-dependently inhibited the mycelial growth of *G. citri-aurantii*. The application of wax with cinnamaldehyde (WCA; 0.50, 1.0, and 2.0 mL $L^{-1}$) significantly reduced the incidence of sour rot on citrus fruit inoculated with *G. citri-aurantii* during storage. After 8 d of storage, the decay incidences in the fruit treated with WCA (0.50, 1.0, and 2.0 mL $L^{-1}$) were only 80%, 77% and 50%, respectively. By contrast, the decay incidence in the control fruit was 100%. In addition, WCA treatment increased the activities of superoxide dismutase (SOD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL), but did not affect the activities of catalase (CAT) and peroxidase (POD), and the total phenol content. Our results suggested that WCA might induce defense responses against sour rot in citrus fruit.



## 1. Introduction

Postharvest diseases cause significant losses in citrus industry. Green mold, blue mold and sour rot, which are respectively caused by the filamentous fungi *Penicillium digitatum*, *P. italicum* and *Geotrichum citri-aurantii*, are the most common postharvest fungal diseases affecting citrus fruit worldwide (Wuryatmo et al., 2014). These pathogenic fungi usually infect their hosts through wounds sustained during harvest, handling and processing (Karim et al., 2016). Although sour rot is less common than green and blue molds, has spread in some areas of China, especially during wet and rainfall seasons (Liu et al., 2009). This disease cannot be efficiently controlled by registered fungicides commonly used for controlling green or blue molds (Zhou et al., 2014a). Sodium *o*-phenylphenate (SOPP) and propiconazole are commercial fungicides that can partially control sour rot; however, the use of SOPP is limited because of the risk of fruit damage (Feng et al., 2011; McKay et al., 2012). Consequently, new compounds and additives that can help control sour rot should be applied during postharvest handling of citrus fruit.

Naturally occurring substances, such as essential oil or their volatile compounds, have emerged as effective and safe strategies to control postharvest citrus disease due to their notable antifungal activities (Liu et al., 2009; Regnier et al., 2014; Talibi et al., 2012a,b; Wuryatmo et al., 2014; Zhou et al., 2014a). Liu et al. (2009) found that thyme oil applied to 'Satsuma' mandarin oranges artificially wounded and inoculated with *G. citri-aurantii* reduced sour rot from 78.1% in untreated control fruit to 14.1% after 5 d at 26 °C. They also observed that thyme oil applied to intact fruit reduced the decay incidence from 76% in the untreated control fruit to 35% after 30 d at 20 °C. Thyme oil did not also harm 'Satsuma' mandarin oranges examined after treatment and storage at 20 °C for 30 d. Regnier et al. (2014) investigated the antifungal activity of 59 commercially available essential oils or their major components against *G. citri-aurantii*, and found that nine of these oils completely inhibited the mycelial growth of the pathogen at 1000 μL $L^{-1}$. In vivo results showed that essential oils of *Cymbopogon citratus*, *Cymbopogon martinii*, *Origanum vulgare*, and *Geranium graveolens* roseum incorporated into coating or

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applied as a curative dip could drastically reduce the sour rot decay of 'Valencia' oranges without an evident physiological breakdown of the fruit during storage. Wuryatmo et al. (2014) observed that the fumigation of oranges with citral (20, 60, or 150 mL $L^{-1}$ in absorbent pads) in a closed system, followed by the application of conidia (20 μL of $10^6$ conidia $mL^{-1}$) to puncture wounds, delayed the onset of sour rot at room temperature for 7–10 d and at 5 °C for 13–30 d. The application of 60 mL $L^{-1}$ volatile citral potentially controled the sour rot, although phytotoxicity was associated with high volatile citral concentrations.

*trans*-Cinnamaldehyde is the main component of cinnamon oil and is a safe food additive widely used in food industry (Taguchi et al., 2013; Xing et al., 2014). The encapsulation of cinnamaldehyde into a multilayered edible coating composed of chitosan and pectin effectively inhibited the growth of aerobic and psychrophilic bacteria, yeast and mold in fresh-cut papaya, and had no negative effects on fruit flavor (Brasil et al., 2012). Sipahi et al. (2013) found that an improved multilayered antimicrobial alginate-based edible coating with cinnamaldehyde was effectively increased the shelf life of fresh-cut watermelon without affecting its quality attributes. Fumigation treatment with 5 μL $L^{-1}$ cinnamaldehyde decreased the browning index, delayed cap opening, reduced microbial counts, and promoted the accumulation of phenolics and ascorbic acid in button mushroom. In addition, cinnamaldehyde treatment inhibited the activities of polyphenol oxidase (PPO) and peroxidase (POD), but increased phenylalanine ammonia lyase (PAL) activity during storage (Gao et al., 2014). The growth of microorganisms in fresh-cut apples was also significantly inhibited by a poly (lactide)/cinnamaldehyde composite film (Wu et al., 2014). In another study, cinnamaldehyde inhibited the spore germination and mycelial growth of *Botrytis cinema* in tomato fruit, and suppressed the development of postharvest blue gray lesions in tomato fruit; these fruit also maintained low weight loss and high hardness, total soluble solids, titratable acid, and ascorbic acid contents (Zhang et al., 2014). Hong et al. (2015) reported that *trans*-cinnamaldehyde not only drastically inhibited the spore germination and mycelial growth of *Colletotrichum gloeosporioides*, which is the causal agent of anthracnose in pepper fruit, but also reduced the lesion diameter on *C. gloeosporioides*-inoculated immature green pepper fruit. Thus, the application of cinnamaldehyde may be used for eco-friendly disease management of diseases in citrus fruit.

To the best of our knowledge, limited information is available regarding the effect of cinnamaldehyde on sour rot affecting citrus. Therefore, our work aimed to evaluate the effectiveness of cinnamaldehyde in controlling postharvest citrus sour rot through in vitro and in vivo experiments. Cinnamaldehyde was incorporated with wax in in vivo assay to reduce the high volatility of essential oils. This study also determined the effects of wax with cinnamaldehyde treatment (WCA) on fruit quality, the activities of some antioxidant enzymes [e.g., catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD)] and defense-related enzymes [e.g., phenylalanine ammonia lyase (PAL) and polyphenol oxidase (PPO)], and the total contents of phenols and hydrogen peroxide ($H_2O_2$).

## 2. Materials and methods

### 2.1. Chemicals

Cinnamaldehyde (99%, CAS number 14371-10-9) was obtained from Darui Fine Chemicals Co., Ltd., Shanghai, China. Commercial wax-coatings (SP-1) used in in vivo trials were provided by Bo Cheng Chemical Co., Ltd, Guangzhou, China. SP-1 comprised food-grade shellac, resin, and fatty acid salt.

### 2.2. Fungal

The fungal pathogen *G. citri-aurantii* used in this study was isolated from infected citrus fruit (Zhou et al., 2014a) and preserved on potato dextrose agar (PDA) at 28 ± 2 °C.

### 2.3. Fruit

The mature fruit of Satsuma mandarin (*Citrus unshiu* Marc. cv. Miyagawa Wase) were harvested on November 4, 2014 from a local orchard near Xiangtan University, China. Defect-free fruit with uniform size were chosen for the experiments.

### 2.4. In vitro experiments

The effects of cinnamaldehyde on mycelial growth of *G. citri-aurantii* were evaluated by our previous method (Tao et al., 2014). An aliquot of 100 μL cinnamaldehyde stock solution was added to each sterilized PDA medium to generate a final concentration of 0, 0.13, 0.25, 0.50, 1.0, and 2.0 mL $L^{-1}$. These amended PDA media were immediately poured into sterilized Petri dishes (90 mm in diameter). Then a 6 mm diameter disc of inoculum, taken from the edge of 7 d-old cultures of *G. citri-aurantii*, was placed at the centre of each Petri dish. The plates were cultured for 5 d at 28 ± 2 °C. The diameter (mm) of colony zone was measured with a caliper. All of the tests were performed in triplicate. The lowest concentration that completely prevented the growth of the fungus after 48 h of incubation at 28 ± 2 °C was regarded as the minimum inhibitory concentration. The minimum fungicidal concentration was considered as the lowest concentration that inhibited pathogen growth after 96 h of incubation, which indicated that there was no visible mycelial growth after the period of incubation (Talibi et al., 2012a).

### 2.5. In vivo assays

The in vivo assays were performed according to the method described previously (Duan et al., 2016) with minor modifications. The fresh citrus fruit were surface-sterilized by immersing in 1% sodium hypochlorite solution (v/v) for 2 min, rinsing twice in sterile distilled water, and then drying in ambient air. Thereafter, two wounds (length of 3 mm and depth of 3 mm) were made evenly around each fruit equator using a sterilized scalpel. The spore suspension of *G. citri-aurantii* was prepared and adjusted to $1 \times 10^7$ spore $mL^{-1}$ by using a hemocytometer with sterile distilled water. Each incision was inoculated with 20 μL of conidial suspension. Fruit were then stored at room temperature for 4 h. After inoculation, the fruit were immersed in the wax supplied with cinnamaldehyde at various concentrations (0.50, 1.0, and 2.0 mL $L^{-1}$) for 10 s and stored in a sealed incubator at 25 ± 2 °C and 85–90% relative humidity (RH). The control fruit were waxed with the same wax. A single replicate comprised 30 fruit, and each treatment was performed in triplicate. Disease incidence during storage time was determined for each treatment by counting the number of fruit showing sour rot symptoms and applying the following formula:

Disease incidence (%) = (number of rotten fruit/number of total fruit) × 100.

### 2.6. Determination of CAT, SOD, POD, PPO, and PAL activities

The samples inoculated with the fungus but did not rot were used for the analyses of enzymatic activity. The fruit pericarp from three randomly selected fruit at each sampling time was collected, grounded to powers in liquid nitrogen, and used for further