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Research note

Use of citral incorporated in postharvest wax of citrus fruit as a botanical fungicide against *Penicillium digitatum*

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ABSTRACT

The antifungal activity of citral against *Penicillium digitatum*, the causal agent of citrus green mold, was tested by *in vitro* and *in vivo* experiments. *In vitro* assays showed that the minimum inhibitory concentration and the minimum fungicidal concentration (MFC) were both 4000 μL L^{-1}. Results of *in vivo* tests demonstrated that wax + citral (1× MFC) treatment did not effectively inhibit the growth of *P. digitatum* in Ponkan mandarin fruit, whereas wax + citral (10× MFC) treatment significantly decreased the incidence of green mold after 6 days of storage at 25 ± 2 °C. Wax + citral (10× MFC) treatment remarkably increased the content of vitamin C and antioxidant enzyme activities such as catalase, superoxidase dismutase, and peroxidase but decreased the activities of phenylalanine ammonia lyase, polyphenol oxidase, and malonaldehyde. The treatment had minor effects on the pH, coloration index, and total soluble solids. This study provided theoretical data for the practical application of citral on citrus fruit quality during postharvest storage.

1. Introduction

Citrus is susceptible to a wide variety of postharvest fungal diseases and green mold caused by *Penicillium digitatum* is one of the most damaging postharvest citrus diseases (Droby et al., 2008). Infection occurs through injuries during picking or handling, resulting in serious postharvest losses (Smilanick et al., 2006). To prevent fruit decay, a number of fungicides such as benzimidazoles, aromatic hydrocarbons, and sterol biosynthesis inhibitors are used as postharvest treatments (Yahyazadeh et al., 2008). However, consumer demand for chemical-free products and development of pathogen resistance as a result of excessive use of fungicides, has necessitated the search for alternative control measures (Sukorini et al., 2013). Indeed, the need for the development of new safe and biodegradable alternatives as natural fungicides has increased.

In the past decades, the incorporation of essential oils or their volatile compounds has been widely used for the control of postharvest diseases in mango, avocado, and citrus fruit (Regnier et al., 2008, 2010; Pérez-Alfonso et al., 2012). Du Plooy et al. (2009) found that modified commercial coatings (Carnauba Tropical®) supplied with *Lippia scaberrima* essential oils (2500 μL L^{-1}) could achieve 100% disease control (preventive treatment) against green mold of citrus fruit. Yahyazadeh et al. (2009) also reported that thyme or clove oil at 800 μL L^{-1} applied to the outer surface of oranges in polyethylene films effectively reduced green mold in vapor-phase experiments at 25 °C. In another report, excellent disease control (90%) was also achieved using carnauba wax supplemented with *Cinnamomum zeylanicum* essential oil (0.5%, v/v) as preventive treatment to control postharvest blue and green molds of citrus (Kouassi et al., 2012). Citral, a naturally occurring isoprenoid compound with two isomers (geranial and neral), reportedly exerted antifungal activity against *P. digitatum* (Wolken et al., 2002; Wuryatmo et al., 2003; Droby et al., 2008). However, information on the effects of postharvest applied wax coatings that are enriched with citral on postharvest diseases and the fruit quality of citrus is limited.

This study aimed to evaluate the effects of citral on mycelial growth of *P. digitatum* and its effects on reducing green mold in Ponkan fruit *in vivo*. The effects of wax and citral on fruit quality parameters such as pH, coloration index, as well as the total soluble solids (TSS), vitamin C (Vc), antioxidant enzymes, defense related enzymes, and malonaldehyde (MDA), were also analyzed.

2. Materials and methods

Ponkan (*Citrus reticulata* Blanco) fruit free of defects and uniform in size were collected from a local orchard near Xiangtan University. The pathogen *P. digitatum* was isolated from infected citrus fruit and maintained on potato dextrose agar for a 7 day incubation period at 25 ± 2 °C. Citral (95%) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Commercial wax-coatings (SP-1) used in *in vivo* trials were provided by Bo Cheng Chemical Co., Ltd., Guangzhou, China.

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Table 1
Decay incidence in inoculated fruit treated with wax + citral (0 μL L^{-1}; 1× and 10× MFC) during storage for 6 days (25 ± 2 °C; 85–90% RH).

Inoculation period (days)	Diseased incidence (%)		
	Treatment		
	Wax	Wax + citral (1× MFC)	Wax + citral (10× MFC)
1	0a	0a	0a
2	0a	0a	0a
3	0a	0a	0a
4	57a	43b	0c
5	87b	93a	0c
6	93a	93a	50b

Data presented are the means of pooled data (n = 10). Rows with different letters at each time point indicate significant differences according to LSD test (P < 0.05).

Effects of citral on mycelial growth of *P. digitatum* were evaluated by the poisoned food technique (Plaza et al., 2004; Yahyazadeh et al., 2008). For *in vivo* assays, fresh fruit were surface-sterilized by dipping in 1% sodium hypochlorite solution (v/v) for 2 min, and then washed with distilled water. Thereafter, fruit were wounded (1.5 mm deep and 2 mm wide) with a sterile needle, and inoculated with 10 μL of a spore suspension of *P. digitatum* (10^8 spores L^{-1}), and left to air-dry. After inoculation, the fruit were coated with wax amended with citral at MFC or 10× MFC. The inoculated fruit were kept in sealed incubators at 25 ± 2 °C (85–90% RH) for 6 days. Ten fruit constituted a single replicate, and each treatment was performed in triplicate. The incidence of disease was calculated as follows:

$$\text{disease incidence}\,(\%) = \frac{\text{number of rotten fruit}}{\text{number of total fruit}} \times 100$$

Fruit pulp at every 3 days of storage was used for the following analysis. The Vc content was determined by 2, 6-dichlorophenolindor-henol titration method (Lemoine et al., 2010). The pH value was determined by a Delta-320 pH-meter (Mettler-Toledo, Greifensee, Switzerland). TSS was analyzed using a LB 32T hand refractometer (Mingrui Electron Science-Technology Co., Ltd, Guangzhou, China) and the fruit peel color was measured using a Minolta CR-330 chromameter (Minolta Co. Ltd., Osaka, Japan) on three locations around the equatorial plane of each fruit. The mean values for lightness (L), red-green (a), and yellow-blue (b) Hunter parameters were calculated for each fruit and expressed as a citrus color index [CCI = 1000a/(Lb)].

CAT and peroxidase (POD) activities were estimated by the method of Lemoine et al. (2010) whereas the superoxide dismutase (SOD) and PAL activities were assayed using the method described by Sellamuthu et al. (2013). PPO was determined by the method of Jiang et al. (2010). MDA content in fruit peel was determined by MDA-thiobarbituric acid assay (Meng et al., 2012).

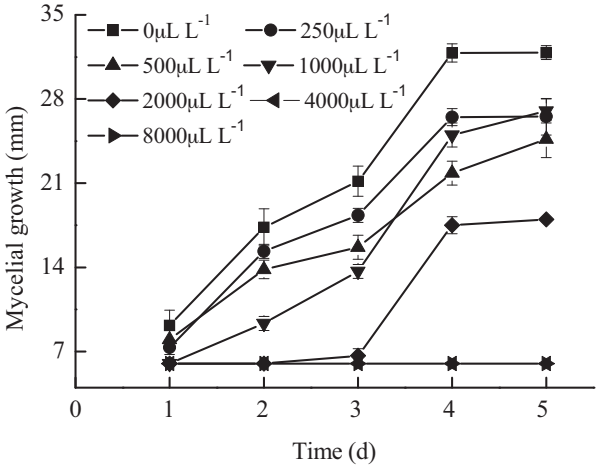

Fig. 1. The effect of different citral concentrations on the mycelial growth of *P. digitatum* incubated at 25 °C for 5 days.

Each assay was performed in triplicate, and the data were processed by an analysis of variance (ANOVA). The daily analyses of the treatments were compared at P = 0.05 according to the least significant difference (LSD) test.

3. Results and discussion

Citral had a good antifungal effect against *P. digitatum* (Fig. 1) and the mycelial growth of *P. digitatum* was influenced by citral in a dose-dependent manner (P < 0.05). Higher citral concentrations (≥4000 μL L^{-1}) completely inhibited the mycelial growth of *P. digitatum*; lower citral concentrations (<1000 μL L^{-1}) showed moderate antifungal activity against *P. digitatum* but inhibited nearly half of the mycelial growth. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of citral for *P. digitatum* were both determined to be 4000 μL L^{-1}. This result confirmed previous reports describing the antifungal activity of citral (Wolken et al., 2002; Wuryatmo et al., 2003; Droby et al., 2008).

The ability of a wax + citral combination treatment to inhibit the decay development of citrus fruit inoculated with *P. digitatum* is presented in Table 1. After 4 days of incubation, decay incidence in wax-treated fruit (57%) was higher than those in wax + citral (1× MFC)-treated fruit (43.3%). In contrast, the fruit treated by wax + citral (10× MFC) were not infected. The decay incidence of green mold increased with prolonged time. After 6 days of storage, green mold incidence in wax + citral (1× MFC)-treated fruit (93%) was equal to those in wax-treated fruit (93%), whereas the incidence in wax + citral (10× MFC)-treated fruit was only 50%. This phenomenon is probably due to the high volatility of essential oils under *in vivo* than *in vitro* conditions, as demonstrated by previous

Table 2
Effect of wax + citral (0 μL L^{-1} and 10× MFC) treatment on the postharvest qualities of citrus fruit during 6 days of storage time at 25 ± 2 °C.

Physiological indicators	Treatment	Inoculation period (days)		
		0	3	6
Vc (mg 100 g^{-1})	Wax	23.43a	21.37b	25.91b
	Wax + citral (10× MFC)	23.32a	25.91a	29.15a
pH	Wax	4.50a	4.15a	4.34a
	Wax + citral (10× MFC)	4.51a	4.14a	4.5a
TSS (%)	Wax	12.97a	12.01a	12.83a
	Wax + citral (10× MFC)	12.87a	11.01a	12.13a
Coloration index	Wax	7.33a	7.30a	7.96a
	Wax + citral (10× MFC)	7.32a	7.56a	7.56a

Data presented are the means of pooled data (n = 10). Rows with different letters in each physiological indicator at each time point between the two treatments indicate significant differences according to LSD test (P < 0.05).