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A combination of cinnamaldehyde and citral greatly alleviates postharvest occurrence of sour rot in citrus fruits without compromising the fruit quality

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Abstract One of the most troublesome postharvest diseases of citrus fruits is sour rot, caused by *Geotrichum citri-aurantii*. Sour rot reduces the shelf life of the fruits leading to massive economic losses. This study investigated the potential for a combination of cinnamaldehyde and citral (CC; 1: 2, v/v) at reducing the incidence of sour rot postharvest and its possible effect on fruit quality. Our findings show that CC could totally inhibit germination of *G. citri-aurantii* spores, with the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) both being 0.80 mL L^{-1}. The combination (CC) acted against *G. citri-aurantii* by targeting the chitin content of the cell wall. Wax + CC (WCC; 1 × MFC) treatment also showed high efficiency in reducing the incidence of sour rot, which was 40% lower than in the control group by day 8 when all the fruits in the latter were rotten. Apart from vitamin c (Vc) content which was higher in the test group than in the control group, WCC treatment did not have any significant effect on the quality of the citrus fruits, the examined fruit quality parameters being weight loss rate, coloration index, firmness, pH, total soluble solid (TSS) content, Vc content, as well as solid acid ratio. These results indicate that the combination of cinnamaldehyde and citral (CC, 1: 2, v/v) can be used as a natural preservative to alleviate the progress of sour rot in citrus fruits postharvest.

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Introduction

Fruit spoilage by fungi is a major concern during storage as it affects the fruit quality and shortens their shelf life (Liu et al. 2017; Suwanamornlert et al. 2018). Citrus fruits in particular are susceptible to several postharvest diseases which cause extensive losses. Among these diseases include sour rot caused by *Geotrichum citri-aurantii*, which greatly deteriorates the fruit quality, leading to massive economic losses in citrus fruit industry (Deng et al. 2018; Singh and Sharma 2018). Developing any potential control measures is thus a significant part in maintaining quality and extending the shelf-life of citrus fruits.

Currently, the management of sour rot postharvest is generally through use of synthetic chemicals or their mixtures (Li et al. 2019; Zhou et al. 2014). However, the effect of chemical fungicides to control sour rot is limited, and their prolonged use may lead to the development of fungicide-resistant pathogens (Bazioli et al. 2019; Mckay et al. 2012). Moreover, fungicide residues left on fruit surfaces may seriously affect the health of the consumers and pollute the environment (Chen et al. 2019; Liu et al. 2017). This has prompted efforts to develop non-hazardous and highly bioactive approaches to control sour rot disease in postharvest citrus fruits (Bazioli et al. 2019; Duan et al. 2018; Wuryatmo et al. 2014), among which include the use of plant essential oils.

Essential oils (EOs) are recognized by consumers as natural components of plants, making their application in the control of certain postharvest diseases of fruits and

vegetables highly favored as they are reported to have broad fungicidal properties and are safer to both the consumers and to the environment than the synthetic fungicides (Boubaker et al. 2016; Shao et al. 2015). For example, cinnamaldehyde and citral are generally accepted as safe (GRAS) for human health and environment and have been registered by the Food and Drug Administration (FDA) as flavoring agents in foods (Wang et al. 2016, 2018a, b). Moreover, studies by Camele et al. (2012) and Manso et al. (2015) revealed that cinnamaldehyde and citral have strong inhibitory activities against postharvest *Aspergillus*, *Fusarium*, *Penicillium* and *G. citri-aurantii* in in vitro, being able to significantly reduce their postharvest diseases (Wu et al. 2017; Duan et al. 2018; Suwanamornlert et al. 2018; Tejeswini et al. 2014), thus can potentially be used in the control of sour rot. However, essential oils have low solubility in water and a very strong aroma which might be unpleasant to the consumers, hence limiting their application in postharvest fruit preservation (Ali et al. 2014; Fan et al. 2014). A combination of EOs and wax is preferred as it not only does not have a strong aroma but also effectively prevents the occurrence of postharvest disease, extending the shelf life of fruits while retaining and/or improving their natural appearance as well as improving the nutritional quality (Fan et al. 2014; Wu et al. 2017; Bazioli et al. 2019; Kong et al. 2019).

This study focused on (1) determining the antifungal effects of cinnamaldehyde, citral, and a combination of cinnamaldehyde and citral (CC) against mycelial growth and spores germination of *G. citri-aurantii* in vitro, (2) estimating the capacity of CC to control the occurrence of sour rot in vivo, and the effect of combination of wax + CC (WCC) on the quality of the citrus fruits, and (3) evaluating the effects of CC, and cinnamaldehyde and citral individually on the cell wall and cell membrane integrity of *G. citri-aurantii*.

Materials and methods

Pathogen

Geotrichum citri-aurantii used in this study was isolated from infected citrus fruits and identified by morphological and molecular biology methods. They were incubated on potato dextrose agar (PDA) at 28 ± 2 °C and the spore concentration adjusted to 5×10^6 spores mL^{-1} using a haemocytometer.

Fruit

Mature Satsuma mandarin fruits (*Citrus unshiu* Marc. cv. Miyagawa Wase) were harvested on 21th October, 2017,

from a local orchard (latitude 27° 87' N, longitude 112° 9' E, altitude 44 m above sea level) near Xiangtan University, Xiangtan, China. Healthy fruits of uniform size and without scars were chosen for the experiments.

Chemicals

Cinnamaldehyde (99%) was obtained from Darui Fine Chemicals Co., Ltd, Shanghai, China, and citral (95%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Cinnamaldehyde, citral and CC solutions were prepared by dissolving the requisite amount in Tween-80 (0.05%, v: v) and topped up to the final volume using distilled water. All the chemicals used were of analytic grade.

Effect of cinnamaldehyde, citral and CC on mycelial growth

This was tested in vitro by agar dilution method (Wu et al. 2017). Briefly, CC stock solution was added into sterilized PDA medium to generate final concentrations of 0, 0.20, 0.40, and 0.80 mL L^{-1}. Similarly, final concentrations of cinnamaldehyde (0, 0.125, 0.25, 0.50, and 1.00 mL L^{-1}) or citral (0, 0.125, 0.25, 0.50, and 1.00 mL L^{-1}) were respectively added into PDA media. The mended media (20 mL) were poured into sterilized petri dishes (90 mm diameter). A 6-mm diameter disc of inoculum was cut from the edge of an actively growing culture on PDA plates with a sterile cork borer, and placed at the center of each new petri dish. Each treatment was performed in triplicate and the culture plates incubated at 28 ± 2 °C for 2 days. The lowest concentration that completely inhibited the growth of the pathogen after the 2 d of incubation was considered as the MIC. The lowest concentration that prevented 99.5% growth of the pathogen after 4 d of incubation was regarded as the MFC.

Effect of cinnamaldehyde, citral and CC on spore germination

For the potato dextrose broth (PDB) incubation method, cinnamaldehyde (0.27 mL L^{-1}), citral (0.53 mL L^{-1}) and CC (0.80 mL L^{-1}) were separately added into PDB, each with a final concentration of 10^6 spores mL^{-1}. The mixtures were each incubated for 3, 6 and 9 h in triplicate at 28 ± 2 °C, in a shaking incubator at 160 r min^{-1}. Spores in PDB without essential oils served as the control group. The spore germination at 3, 6 and 9 h of incubation was monitored under the microscope (Wu et al. 2017), and the percentage germination of spores calculated according by the formula;

$$Spore\ germination = \frac{Number\ of\ germinated\ spores}{Total\ number\ of\ spores} \times 100\%$$

Effect of CC on the incidence of sour rot

This was determined in vivo as previously described (Duan et al. 2018; Fan et al. 2014) but with minor modifications. All fresh citrus fruits were surface-sterilized by immersing in sodium hypochlorite solution (2%, v/v) for 2 min, then washed with distilled water, wounded (depth of 3 mm and width of 3 mm) with a sterile needle, inoculated with 20 μL of *G. citri-aurantii* spore suspension (10^5 spores mL^{-1}), and left to air-dry. After being inoculated with fungi, the fruits were sprayed with wax amended with CC at 0 × and 1 × MFC. Fruits with wax and pathogen inoculation were used as a control. The inoculated fruits were kept in sealed incubators at 25 ± 2 °C to ensure high relative humidity (85–90% relative humidity). Twenty Satsuma mandarin fruits constituted a single replicate, and each treatment was performed in triplicate. The incidence rate of disease (measured by counting the number of green mold-infected wounds) was calculated as follows:

$$Disease\ germination = \frac{Number\ of\ rotten\ wounds}{Total\ number\ of\ wounds} \times 100\%$$

Fruit quality parameters

Fruits were randomly chosen from each of the test and control groups (similar to those described in Sect. 2.6) after storage at an interval of 2 d. Physiological fruit quality indicators, including weight loss rate, coloration index, firmness, pH, total soluble solid content (TSS), titratable acidity (TA), and vitamin C (Vc) content were tested. The methods used were included in previous papers (Fan et al. 2014; Wilkerson et al. 2013).

Effects of cinnamaldehyde, citral and CC on the cell wall integrity of G. citri-aurantii

The distribution of chitin in the cell wall of *G. citri-aurantii* was analyzed by calcofluor white (Sigma, St. Louis, MO, USA) staining coupled with fluorescence microscopy (Nikon ECLIPSE TS100, Japan) (OuYang et al. 2019). Cinnamaldehyde (0.27 mL L^{-1}), citral (0.53 mL L^{-1}) and CC (0.80 mL L^{-1}) were added into PDB with a final concentration of 10^6 spores mL^{-1}. The mixtures were incubated for 3, 6 and 9 h in triplicate at 28 ± 2 °C in a shaking incubator at 160 r min^{-1}, then centrifuged at 4000 g min^{-1} for 10 min. The collected spores were stained with 10 μL of calcofluor white (CFW) stain and 10 μL KOH (10%), following the manufacturer's instructions. Fungal cultures in PDB without essential oils were used as a control, and the samples were observed under a fluorescence microscope.

Effects of cinnamaldehyde, citral and CC on plasma membrane integrity

Membrane integrity (MI) was examined by the method of Wu et al. (2017). Cinnamaldehyde (0.27 mL L^{-1}), citral (0.53 mL L^{-1}) and CC (0.80 mL L^{-1}) were added into PDB with a final concentration of 10^6 spores mL^{-1}. The mixtures were incubated for 3, 6 and 9 h in triplicate at 28 ± 2 °C in a shaking incubator at 160 r min^{-1}, then centrifuged at 4000 g min^{-1} for 10 min, washed with distilled water and suspended in PBS (0.05 mol L^{-1}, pH = 7.0). The suspension was then stained with propidium iodide (PI) (0.01 g L^{-1}) in a 37 °C water bath for 5 min and washed twice using PBS. Fungal culture in PDB without EOs was used as a control. The number of spores in bright-field constituted the 'total number of spores'. The stained spores that could be observed under the fluorescent microscope (Nikon ECLIPSE TS100, Japan) were counted and MI calculated according to the formula:

$$MI(\%) = \left(1 - \frac{Number\ of\ stained\ spores}{Total\ number\ of\ spores}\right) \times 100\%$$

Statistical analysis

Each assay was performed in triplicate, and the data analyzed using SPSS 19.0 Software. Significance in differences between mean values of the data sets were determined using Duncan's Multiple Range test ($P < 0.05$) following one-way ANOVA.

Results

Mycelial growth in vitro

The mycelial growth of *G. citri-aurantii* in vitro considerably decreased with increase in concentrations of cinnamaldehyde, citral and CC (Table 1). The MIC values of cinnamaldehyde, citral and CC were equal to their MFC values, which were estimated to be 0.50, 1.0 and 0.80 mL L^{-1} respectively. Basing on the ratio of the MIC values of cinnamaldehyde and citral (0.5: 1.0), a combination ratio of 1: 2 was chosen for the formation of CC. When the CC treatment completely inhibited the growth of *G. citri-aurantii*, the concentrations of cinnamaldehyde and citral in the mixture were only 0.27 and 0.53 mL L^{-1}, respectively.

Table 1 The effect of cinnamaldehyde, citral and CC on the mycelia of *G. citri-aurantii*

Treatment	Concentration (mL L^{-1})	Inhibitory rate (%)			
		1 d	2 d	3 d	4 d
cinnamaldehyde	0.125	23.07 ± 13.32c	32.79 ± 2.84c	16.07 ± 7.73c	4.54 ± 0.00c
	0.25	53.84 ± 0.00b	63.93 ± 2.84b	50.89 ± 4.09b	40.26 ± 5.62b
	0.50	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
	1.00	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
citral	0.125	12.90 ± 9.68c	11.24 ± 3.55d	7.17 ± 2.26d	3.62 ± 0.96d
	0.25	53.22 ± 7.39b	50.30 ± 6.15c	44.15 ± 6.92c	33.15 ± 6.69c
	0.50	100.00 ± 0.00a	73.37 ± 3.07b	68.30 ± 0.00b	63.23 ± 1.67b
	1.00	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a
CC	0.20	45.00 ± 9.35b	28.89 ± 3.14c	23.33 ± 1.89c	15.63 ± 0.00c
	0.40	100.00 ± 0.00a	66.67 ± 0.00b	54.67 ± 1.89b	42.71 ± 0.73b
	0.80	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a

Data presented are the means ± standard error of pooled data (n = 3). Columns with different letters at each time point of each treatment indicate significant differences according to Duncan's multiple range test ($P < 0.05$)

Inhibition of spore germination

The effects of cinnamaldehyde, citral and CC on the germination of *G. citri-aurantii* spores is shown in Fig. 1. After 3 h of incubation, all spores in the three treatment groups had not germinated, while those in the control group had germinated with a rate of 12.58 ± 1.72% ($P < 0.05$). 6 h after incubation, the germination rates in the cinnamaldehyde, citral and CC groups were 0.00 ± 0.00, 6.67 ± 1.36, and 0.00 ± 0.00%, respectively, which were significantly lower than that of the control group (49.82 ± 6.06%, $P < 0.05$). By 9 h of incubation, the germination rate in the control group increased to 90.47 ± 2.34%, while those of the test groups treated with cinnamaldehyde and citral individually were only 5.66 ± 7.81 and 10.81 ± 1.82%, respectively. Spores in the CC group had not germinated at all even after the 9 h incubation period.

Effect of CC treatment on the incidence of sour rot decay

WCC (1 × MFC) effectively reduced sour rot decay of citrus fruits (Fig. 2a), and alleviated the disease progression in inoculated citrus fruits treated with WCC (Fig. 2b).
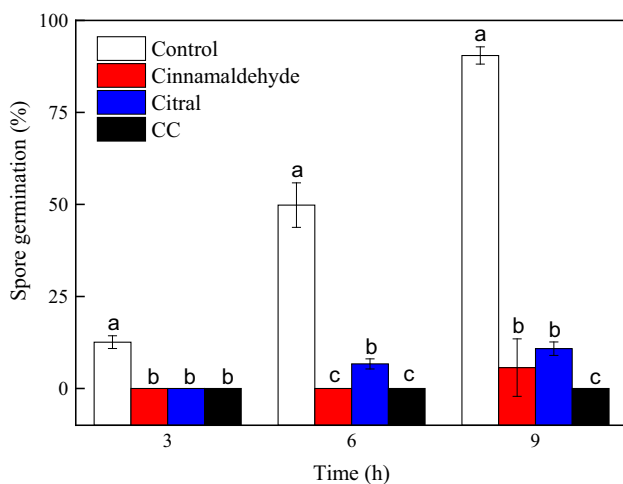
Fig. 1 The effects of cinnamaldehyde, citronellal and CC on the germination of *G. citri-aurantii* spores. The data presented are the means of pooled data. Error bars indicate the SDs of the means (*n* = 3)
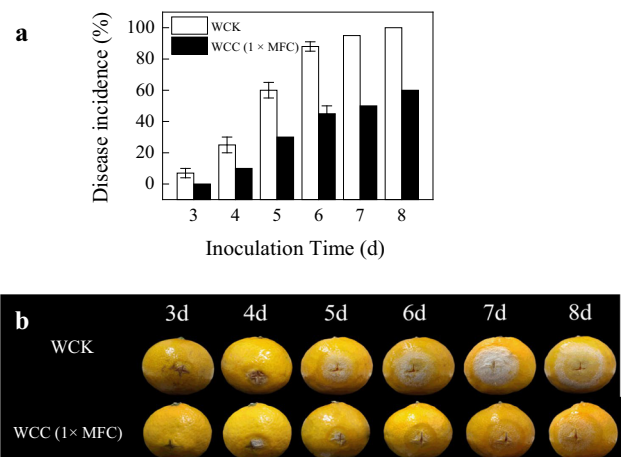
Fig. 2 a Decay incidence in inoculated fruit treated with WCC (0 × and 1 × MFC) during storage at 25 ± 2 °C for 5 d and 85%-90% relative humidity; **b** Disease progression in inoculated citrus fruit treated with CC (0 × and 1 × MFC) during storage at 25 ± 2 °C and 85%-90% RH. The data presented are the means of pooled data. Error bars indicate the SDs of the means (*n* = 3)

Fruits in the control group began to decay 3 d post inoculation, with $7 \pm 3\%$ decay rate, at which time, the CC treated fruits were not infected at all. Following 8 d of inoculation, the citrus fruits in the control group were all rotten and covered by a visible white mold layer, while the fruits in the $1 \times$ MFC WCC treatments had incurred a sour rot rate of only $60 \pm 0\%$, with merely watery lesions visible.

Effects of CC treatment on fruit quality parameters

WCC ($1 \times$ MFC) treatment did not significantly affect the quality of citrus fruits (Table 2). The weight loss rate, coloration index, firmness, pH, TSS content, as well as solid acid ratio were not influenced by the WCC treatments after storage at 25 ± 2 °C for 6 d. However, the Vc content in WCC treated fruits was about 39.33% and 21.54% higher than in WCK-treated fruits after 2 and 6 days, respectively.

Effects of cinnamaldehyde, citral and CC on the cell wall integrity

As shown in Fig. 3, cinnamaldehyde and CC treatments significantly affected the distribution of chitin on the cell walls of *G. citri-aurantii* spores. The cell walls of spores in the control group and citral treated group showed a normal deposition of chitin. The spores in the cinnamaldehyde and CC treated groups markedly showed weaker fluorescence
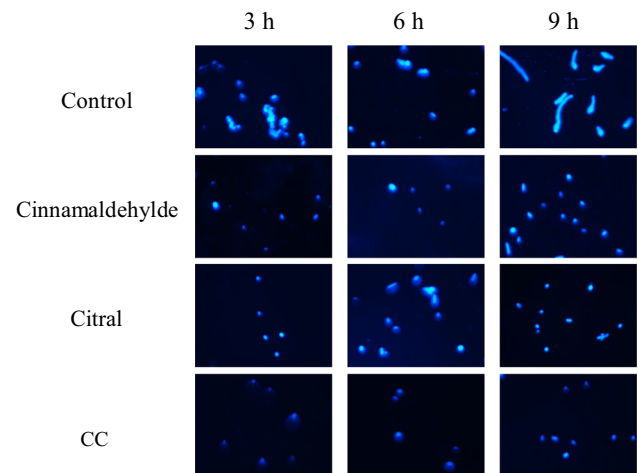

Fig. 3 The effects of cinnamaldehyde, citronellal and CC on the cell wall of *G. citri-aurantii* spores observed under a fluorescence microscopy after staining with calcofluor white (CFW) stain

than the other treatments after 3 h incubation. Similarly, the blue fluorescence of cells in the CC treated group was weaker than the corresponding single treatments and the control group as observed at 6 and 9 h incubation.

Effects of cinnamaldehyde, citral and CC on the plasma membrane integrity

The MI values of *G. citri-aurantii* spores treated with citral or CC greatly decreased with increase in incubation time,

Table 2 Effects of the WCK and WCC ($1 \times$ MFC) treatments on postharvest qualities of citrus fruit inoculated with *G. citri-aurantii* during storage at 25 ± 2 °C for 6 d

Quality index	Treatments	Inoculation period (d)			
		0	2	4	6
Weight loss rate (%)	WCK	0.00 ± 0.00a	2.04 ± 0.36a	3.66 ± 0.61a	6.19 ± 0.69a
	WCC ($1 \times$ MFC)	0.00 ± 0.00a	2.06 ± 0.30a	3.80 ± 0.49a	6.48 ± 0.71a
Coloration index	WCK	-1.18 ± 1.53a	-0.30 ± 1.20a	0.54 ± 1.26a	1.98 ± 1.38a
	WCC ($1 \times$ MFC)	-2.57 ± 1.71a	-1.05 ± 1.41a	0.20 ± 1.17a	1.84 ± 1.47a
Firmness (kg/cm^2)	WCK	0.83 ± 0.08a	1.01 ± 0.11ab	0.86 ± 0.09a	1.07 ± 0.17a
	WCC ($1 \times$ MFC)	0.83 ± 0.08a	0.98 ± 0.16b	0.83 ± 0.15a	1.03 ± 0.32a
pH	WCK	2.95 ± 0.12a	3.18 ± 0.07a	3.07 ± 0.08a	3.11 ± 0.04a
	WCC ($1 \times$ MFC)	2.95 ± 0.12a	3.04 ± 0.03a	3.13 ± 0.06a	3.12 ± 0.25a
TSS (%)	WCK	13.43 ± 0.40a	14.07 ± 0.23a	14.70 ± 0.26a	14.53 ± 0.31a
	WCC ($1 \times$ MFC)	13.43 ± 0.40a	14.47 ± 0.61a	14.73 ± 0.21a	14.40 ± 0.40a
Solid acid ratio	WCK	14.90 ± 0.78a	17.60 ± 0.64a	21.65 ± 0.91a	28.75 ± 5.90a
	WCC ($1 \times$ MFC)	14.90 ± 0.78a	18.76 ± 3.55a	22.89 ± 2.69a	43.11 ± 10.08a
Vc (mg/100 g)	WCK	14.23 ± 0.65a	22.42 ± 0.99b	25.23 ± 0.65a	25.02 ± 0.75b
	WCC ($1 \times$ MFC)	14.23 ± 0.65a	31.24 ± 2.12a	23.08 ± 1.08a	30.41 ± 2.82a

Data presented are the means $\pm$ standard error of pooled data (n = 3). Different capital letters and lowercase letters indicate significant differences of columns and rows at each time point according to Duncan's multiple range test ($P < 0.05$), respectively

as opposed to the relatively high levels observed in the control group and cinnamaldehyde treated group (100.00 ± 0.00%, $P < 0.05$) (Fig. 4). By 3 h of incubation, the MI values of all groups were 100.00 ± 0.00%, but as the exposure time increased to 9 h, treatment with CC resulted in a decreased MI value (33.38 ± 1.59%) compared to the corresponding single treatments of cinnamaldehyde (100.00 ± 0.00%) and citral (55.71 ± 5.15%), with the control group retaining an MI value of 100.00 ± 0.00%.

Discussion

Cinnamaldehyde and citral have been reported to strongly inhibit growth of a wide range of fungal species (Utama et al. 2002; Duan et al. 2018; Gao et al. 2018; Wu et al. 2017). For example, at a concentration below 0.2%, cinnamaldehyde can completely inhibit growth of *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizopus* in vivo (Manso et al. 2015; Tejeswini et al. 2014), and citral exhibit significant antifungal efficiency against postharvest *Botrytis cinerea*, *P. expansum*, *P. digitatum*, and *P. italicum* (Camele et al. 2012; OuYang et al. 2019; Wang et al. 2018a, b). In this study, cinnamaldehyde and citral individually exhibited

Fig. 4 The effects of cinnamaldehyde, citronellal and CC on the plasma membrane of G. citri-aurantii spores, **a** stands for the percentage of plasma membrane integrity; **b** stands for the PI staining results of bright field and fluorescent images. The data presented are the means of pooled data. Error bars indicate the SDs of the means ($n = 3$)

very good fungicidal actions against *G. citri-aurantii* with MFC values of 0.50 and 1.00 mL L^{-1} respectively. In addition, cinnamaldehyde and citral treatments significantly reduced the germination rate of *G. citri-aurantii* spores, an observation that is consistent with findings by Thavong et al. (2011) and Wang et al. (2018b).

The combination of Cinnamaldehyde and citral (CC, v/v, 1:2) inhibited the growth of *G. citri-aurantii* with MFC of 0.80 mL L^{-1}, the concentrations of cinnamaldehyde and citral in the mixture being 0.27 and 0.53 mL L^{-1}, respectively. These values are almost half the MICs of each of the individual components, implying that within the combination, very low concentrations of each essential oil component is required to realize a significantly great inhibitory effect, hence pointing towards a kind of cooperative mechanism to their antifungal activity. Similarly, CC treatment totally inhibited germination of *G. citri-aurantii* spores during the 9 h incubation period yet separate treatments with cinnamaldehyde and citral during the same duration of time resulted in growth of some spores with rates of 5.66 ± 7.81% and 10.81 ± 1.82% respectively. These results imply that the combination of cinnamaldehyde and citral (CC) was highly effective at inhibiting overall growth of *G. citri-aurantii* than the individual components, an observation consistent with those reported by Hossain et al. (2016), Stević (2014), Sukatta et al. (2008), Suwanamornlert et al. (2018) and Zhou et al. (2019) that the combination of two or more individual components of essential oils might have higher antifungal potential than the individual components acting alone. In fact, Li et al. (2019) too alluded to the fact that the antimicrobial activities of fungicides can be enhanced by combining them with other antimicrobial agents or a variety of them.

A recent study by Ji et al. (2019) reported that the increase in antifungal activity of combinations of EOs was due to the cumulative effect of the mechanisms of the individual EOs in the mixture. Cinnamaldehyde is known to exert its antifungal activity by disrupting the cell wall (OuYang et al. 2019; Shreaz et al. 2016), while citral inhibits fungal growth by disrupting the cell membrane (Tao et al. 2014; Zhou et al. 2014). Basing on the report by Ji et al. (2019), we hypothesized that the effect of CC against *G. citri-aurantii* was relate to the destruction of both the cell wall and the cell membrane. Our results showed that CC treatment significantly weakened the blue fluorescence of *G. citri-aurantii* spores in the test samples than in the control group and the individual treatment groups, suggesting that CC damaged the cell wall integrity by reducing the chitin content. However, the cell membrane integrity of the spores in CC treatment did not lower compared to those of the control and the individual treatment groups, suggesting that the inhibitory mechanism of
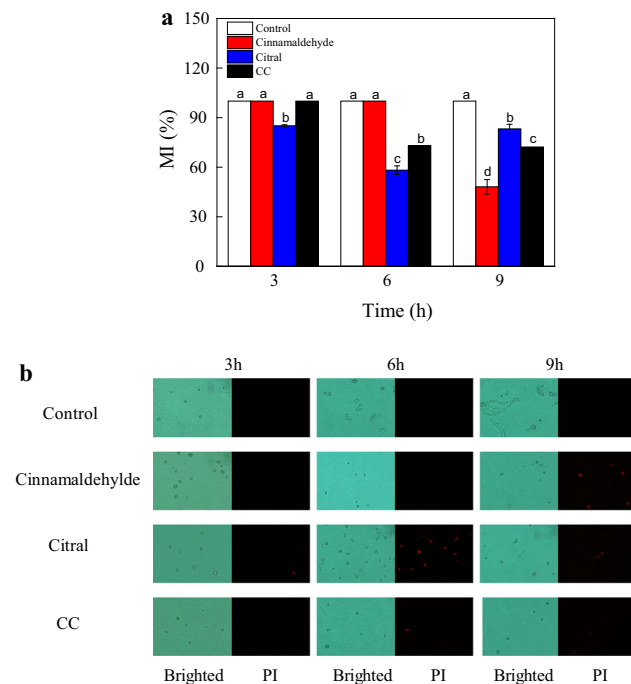
CC was not associated with damage of the cell membrane. On the contrary, while examining the antifungal mechanism of a combination of cinnamaldehyde (45 mg L^{-1}) and citral (70 mg L^{-1}) against *P. expansum,* Wang et al. (2018a, 2018b) found that the drug combination targeted the membrane structure but not the cell wall structure. This discrepancy may be caused by the difference in combination ratios of the individual EO components and the difference in strains used for the study. According to our current results, the inhibitory effect of CC evidently proceeded through destruction of cell wall integrity, with citral probably enhancing the ability of cinnamaldehyde to damage the cell wall. This suggests that the antifungal mechanism of a combination of EOs is not a simple superposition of individual mechanisms but rather an interaction between the two components resulting into a stronger, single mechanism, that might be very significant in reducing or eliminating the incidence of occurrence of resistant fungal pathogens.

From our current results, CC effectively inhibited *G. citri-aurantii* not only in vitro, but also in vivo. $1 \times$ MFC of CC proved highly effective at inhibiting the occurrence of sour rot in vivo, its effectiveness at controlling the incidence of sour rot being significantly higher than that of cinnamaldehyde alone. Similarly, the incidence of fruit decay in 0.80 mL L^{-1} CC treatment was 60%, at which time, all the fruits in the control group had decayed. This incidence rate is less than the 80% fruit decay rate for the 0.50 mL L^{-1} cinnamaldehyde treatments reported in our previous study (Wu et al., 2017). This confirms that combining cinnamaldehyde and citral enhances their in vivo antifungal ability against citrus fruit pathogens, which may be related to their good antifungal abilities plus their ability to induce defense responses in citrus fruits (Gao et al. 2018, Wang et al. 2016 and Fan et al. 2014). This results also show that a low concentration of the constituent essential oils is required in the combination (CC) to realize significant inhibitory effect which solves the concerns by Tejeswini et al. (2014) and Catherine et al. (2012) that a higher concentration of the antimicrobial compound was needed both in vivo than in vitro to realize significant inhibitory effect.

Apart from the Vc content which was higher in the test samples than in the control group, CC treatment had very minor effects on fruit quality parameters. Previous studies have similarly reported that cinnamaldehyde and citral reduced postharvest decay in fruits without exacerbating any fruit quality parameters (Duan et al. 2018; Fan et al. 2014; Gao et al. 2018; Wang et al. 2016). The observed increase in Vc content could actual be of nutritional advantage to the consumers and a testimony to reports that EOs do improve the nutritional quality of fruits (Fan et al. 2014; Wu et al. 2017; Bazioli et al. 2019; Kong et al.

2019). The combination of cinnamaldehyde and citral is therefore a potentially promising botanical fungicide to suppress *G. citri-aurantii* growth in the control of sour rot in citrus fruits.

In conclusion, the combination of cinnamaldehyde and citral (1:2, v/v) act by reducing the chitin content to destroy the integrity of fungal cell wall thus effectively inhibiting the growth of *G. citri-aurantii* both in vitro and in vivo. Very low concentrations of the individual components are required to form the combination, and a $1 \times$ MFC WCC treatment which effectively inhibits the fungal growth, does not impair the fruit quality indicating that it should be considered for use in the postharvest management of sour rot in citrus fruits.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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