Use of Nonanal-wax as Postharvest Fungicide of Tomato against *Botrytis cinerea*

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Abstract The antifungal activity of nonanal against *Botrytis cinerea*, one of the most important postharvest diseases of tomato gray mold, was tested by *in vitro* and *in vivo* experiments. Results of *in vivo* tests demonstrated that wax + nonanal treatment significantly decreased the incidence of gray mold during the entire storage period. After 8 d of storage, the disease incidences in Wax + nonanal (1×, 4× or 10× MFC)-treated fruits were 46.7%, 56.7%, 73.3%, respectively, in contrast to 100% of the control fruits. Loss of membrane integrity was examined and quantified under 10×MFC nonanal condition by the method of propidium iodide fluorescent staining. Wax + nonanal (10×MFC) treatment remarkably increased antioxidant enzyme activities, such as catalase (CAT), superoxidase dismutase (SOD), peroxidase (POD) and phenylalanine ammonia lyase (PAL). Meanwhile, this treatment (10×MFC) evidently exhibited a delayed decline in antioxidant enzyme activities. Furthermore, nonanal treatment retained the fruit quality of tomatoes because it reduced the coloration index and weight loss and retained fruit firmness. No significant differences were found between the pH, Firmness and total soluble solid (TSS) content for all treatment under the same storage time. Our results suggest that nonanal can be considered as a good alternative to conventional fungicides in controlling the decay of tomato fruits.

Keywords: nonanal, tomato, fruit quality, enzymes activity, postharvest

Cite This Article: Jihong ZHANG, Li ZENG, Helong SUN, Shaoyang CHEN, Taotao WANG, and Shuang MA, "Use of Nonanal-wax as Postharvest Fungicide of Tomato against *Botrytis cinerea*." *Journal of Food and Nutrition Research*, vol. 5, no. 7 (2017): 458-466. doi: 10.12691/jfnr-5-7-2.

1. Introduction

Tomatoes (*Solanum lycopersicum*) are one of the most important vegetables in the world because of its contribution to human nutrition [1] and health [2]. Storage life of fresh tomato often terminates early due to fungal pathogens. Growth of fungal pathogens is the main cause of considerable economic loss during postharvest handling of fruits and vegetables [3,4]. *Botrytis cinerea* causes economic losses on a wide range of cultivated plants, stored fruits and vegetables, such as grapes, stone-fruit, berries, and vegetables [5]. Previous studies showed that *Botrytis cinerea* infects plant tissues through multiple mechanisms, including secretion of cell wall-degrading enzymes and phytotoxic metabolites [6], which causes fruit rot and renders fruits unmarketable [7]. Currently control of gray mold disease is primarily dependent on the use of synthetic fungicides [8,9]. Although the synthetic fungicides are effective, their continued or repeated application has disrupted biological control by natural enemies and led to disease outbreaks, widespread development of resistance to various types of fungicides [10,11,12]. For some VOCs, the exposure to the higher concentration used in the experiments (12.3 $\mu\text{L L}^{-1}$) reduced the development of *Botrytis cinerea*. In particular, the aldehydes trans-2-hexenal and nonanal completely

inhibited conidial germination and nonanal showed the best reduction of mycelial growth (-66.8%) at this concentration [13]. Nowadays, application of plant volatiles as inhibitory compounds towards fungal pathogens could be another interesting alternative.

Lavender essential oil could also effectively inhibit *Botrytis cinerea* [14]. Peretto et al.[15] found significant reduction in visible decay in berries packed in clamshells containing edible film incorporating carvacrol and methyl cinnamate during storage at 10°C for 10 days. Trans-2-hexenal with 1 μM or higher concentration was more effective in inhibiting hyphal growth of *B. cinerea* [16,17]. Trans-2-hexenal effectively reduced blue mold, gray mould infections and patulin content in 'Conference' pears and apples [18,19], and the shelf life of apple slices is extended by inhibiting *Pichia subpelliculosa* infection [20].

Nonanal reported to exhibit antimicrobial activity against gram-positive and gram-negative bacteria in the range concentration of 100 to more than 800 mg/kg [21]. Nonanal as a component of essential oils, are present in large quantities of species, such as Citrus [22]. Nonanal, citral, γ -terpinene, linalool, and α -terpineol exhibited moderate or weak anti-fungal activity against *Penicillium italicum* and *Penicillium digitatum* [22,23]. The anti-fungal activity of *Citrus reticulata* Blanco essential oils against *P. italicum* and *P. digitatum* can be primarily attributed to octanal, citral, decanal, nonanal, linalool, and γ -terpinene

[22]. However, it is rarely reported that nonanal acted as a single bacteriostatic agent about the efficacy of nonanal against *B. cinerea* on tomatoes.

Since tomatoes, after harvest, are particularly perishable fruit, highly susceptible to *Botrytis cinerea* infection. The study aimed to evaluate the effects of nonanal, a natural antimicrobial compound, on reducing mycelial growth of *B. cinerea* in tomato fruits in vivo. The effects of wax and nonanal treatment on fruit quality parameters such as pH, coloration index, as well as total soluble solids (TSS), vitamin C, firmness, and defense related enzymes, were also analyzed.

2. Materials and Methods

2.1. Fruits

The mature tomato fruits were harvested from a local plantation near Xiangtan University, Xiangtan, China, at July 10, 2016. Tomato fruits (*Solanum lycopersicum* cv. Zhongshu No.4) with uniform size, maturity (with red surface color), and free of physical injuries and fungal infections were selected for the experiments. The fresh tomato fruits (*Solanum lycopersicum*) were surface-sterilized by dipping in 1% sodium hypochlorite solution (v/v) for 2 min, followed by washing with distilled water, and then allowed to dry.

2.2. Pathogen Inoculum

The fungal pathogen *B. cinerea* was grown on potato dextrose agar (PDA) at $28 \pm 2^\circ\text{C}$ for 6 days. The conidial suspension was prepared by washing the colonies of pathogen with 5 mL of sterile distilled water containing 0.05% (v/v) Tween-80. The concentration of suspension was quantified using a hemacytometer and diluted to a final concentration of 1×10^7 spore/L with sterile distilled water.

2.3. Chemicals

Nonanal (>96%) was purchased from Dieckmann Reagent Corporation (Shenzhen, China). Commercial wax coatings (SP-1) were provided by Bo Cheng Chemical Co., Ltd., Guangzhou, China. Proidium iodide (PI) was obtained from Solarbio science & technology Co., Ltd., Beijing, China.

2.4. In Vitro Experiments

The effect of nonanal on the mycelial growth of *Botrytis cinerea* was tested in vitro by agar dilution method [24]. PDB (20 ml) was poured into sterilized Petri dishes (90 mm diameter) and specific amounts of nonanal were added to PDB mediums (plus with 0.05% Tween-80) to achieve the desired concentrations of 0, 20, 40, 60, 80, 100, 120, 140, 160, 180 and $200 \mu\text{L}^{-1}$. A 6 mm diameter disc of inocula was cut from the periphery of a 6-day-old *B. cinerea* growing culture on PDA plates with a puncher, and then was placed at the center of each new Petri plate. Petri dishes were sealed with parafilm and incubated for 4 d at $28 \pm 2^\circ\text{C}$. The diameter (in mm) of colony zone was

measured with a caliper. All of the tests were performed in triplicate.

The lowest concentration that completely inhibited the growth of the fungus after 48 h of incubation at $28 \pm 2^\circ\text{C}$ was considered as the minimum inhibitory concentration (MIC). The minimum fungicidal concentration (MFC) was regarded as the lowest concentration that prevented pathogen growth after 96 h of incubation at $28 \pm 2^\circ\text{C}$ on a fresh PDA plate, thereby indicating fungicidal activity >99.5% of the original inocula [25].

PDB liquid medium was prepared and sterilized in conical flasks of 50 ml capacity, each containing 20 ml medium. Different amounts of nonanal were added to the PDB medium to give the following concentration: 0, 10, 20, 30, 40, 50, 60, 70 and $80 \mu\text{L/L}$. Fungal growth was estimated gravimetrically by weighting the biomasses by pumping filtration to a constant weight [26]. The inhibition percentage was calculated as follows:

$$\text{Percentage of inhibition (\%)} = \frac{(W_0 - W_t)}{W_0} \times 100$$

Where W_0 is the net fresh weight of control cells and W_t is the net fresh weight of nonanal-treated cells.

2.5. Plasma Membrane Integrity Assay

Membrane integrity was assayed following Liu et al. [27]. Spores of *Botrytis cinerea* were prepared in PDB, and the cells were incubated on a rotary shaker with various concentrations of nonanal (0, $10 \times \text{MFC}$) for 2 h at $28 \pm 2^\circ\text{C}$. PDB without nonanal served as the control. After incubation, the spores were washed and resuspended in PBS (pH 7.4). Subsequently, cells were stained with prodium iodide (PI, $1 \mu\text{g/ml}$, final concentration) for 30 min at $28 \pm 2^\circ\text{C}$ in the dark [28]. After centrifugation at $8000 \times g$ and washing twice with PBS (pH 7.4) to remove residual dye, the spores were observed on Nikon Eclipse Ni-U microscope (Nikon, Japan) equipped with individual fluorescein rhodamine filter set. Each treatment included three replicates. Images were collected using a Nikon DS-Fi1c high-definition cooled color camera (Nikon, Japan). Membrane leakage was calculated according to the formula:

$$\begin{aligned} \text{Membrane leakage (\%)} \\ = \frac{\text{number of stained spores}}{\text{number of total spores}} \times 100. \end{aligned}$$

2.6. Postharvest Treatment

Selected tomato fruits were distributed into four groups (80 fruits in each group). Thereafter, fruits were wounded (2 mm wide, and 1.5 mm deep) with a sterile needle, and inoculated with $10 \mu\text{L}$ of a spore suspension of *Botrytis cinerea* (10^7 spores L^{-1}) for 4 h, and left to air-dry. After inoculation, the fruits were coated with wax amended with nonanal at $1 \times \text{MFC}$, $4 \times \text{MFC}$ or $10 \times \text{MFC}$, respectively. Tomato fruits coated with wax alone served as control. After treatment, the inoculated fruit were stored in sealed incubators at $28 \pm 2^\circ\text{C}$ (85-90% RH) for 8 days. Finally, the percentage of infected fruit was recorded; each treatment was performed in triplicate. The incidence of disease was calculated as follows:

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

2.7. Fruit Quality Parameters

After storage at an interval of 2 d, fruit pulp samples were collected from three fruits randomly chosen from each group. The weight of the fruit was measured using electronic balance BL 320S (Shimadzu, Japan). Weight loss percentage of tomato fruits was determined by weighting the samples at specific time intervals compared to initial weight and reported as % weight loss. Vitamin C (ascorbic acid) content was determined by 2, 6-dichlorophenolindol -henol titration [29]. Total soluble solid (TSS) content was determined using a digital refractometer (Pocket PAL-1, Japan) equipped with 6 mm diameter flat probe. Firmness was measured around of the equatorial region using GY-J hand-held digital force measuring instrument (Zhejiang Top Instrument Company, China), which the fruits were cut into crisscross (2.0 mm dept and 2 mm width) with a sterile needle. The probe was penetrated 1cm into the fruit at a speed of 1mm/s, and the maximum force (N) was defined [30].

2.8. Color

The CIE $L^*a^*b^*$ (lightness, red/green and yellow/blue chromaticity coordinate) values were measured using a Minolta CR-400 portable colorimeter (Minolta Co. Ltd., Osaka, Japan) on three locations around the equatorial zone 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 tomato color index [TCI=100a/(Lb)] [31].

2.9. Defensive Enzyme Assays

Fresh tomato pericarps were homogenized in a grinder and centrifuged for collecting the supernatant. The above supernatant was used for enzyme activity assay. All the enzyme activities were determined by photometric assay using a UV-2450 UV/Vis spectrophotometer (Shimadzu, Shanghai, China). CAT and peroxidase (POD) activities were estimated by the method of Lemoine et al. [32] whereas the superoxide dismutase (SOD) and PAL activities were assayed using the method described by Sellamuthu et al [33]. There were four samples per treatment. The specific activities of the enzymes were expressed in U/g fresh weight (FW).

2.10. Statistical Analysis

Each assay was performed in triplicate, and the data were processed by an analysis of variance (ANOVA). Daily analysis results of the treatments were compared at $P=0.05$ according to Duncan's multiple range tests.

3. Results

3.1. In Vitro Experiments

Nonanal at the tested concentrations showed the capacity

to reduce or inhibit the mycelial growth of *Botrytis cinerea* (Figure 1). The inhibitory effect increased in a dose-independent manner. Nonanal at low concentrations (10, 20, 30, 40, 50 and 60 $\mu\text{L/L}$) exhibited partially rather than totally inhibitory effects on the mycelial growth of *Botrytis cinerea* during the entire period. In contrast, after the addition of 100 and 160 $\mu\text{L/L}$ nonanal, no visible growth of *B. cinerea* was found until 2 d or 4 d of culture. Therefore, the MIC and MFC values of nonanal against *B. cinerea* were regarded as 100 and 160 $\mu\text{L/L}$, respectively, and the results were reported in Zhang et al. [34].

3.2. In Vivo Experiments

As shown in Table 1, a wax + nonanal combination treatment significantly ($P<0.05$) reduced disease incidence in tomato fruits inoculated with *Botrytis cinerea* during the first 4 d of incubation at $28 \pm 2^\circ\text{C}$. After 2 days of inoculation, gray mold incidence in wax-treated fruit (20.0%) was higher than those in wax + nonanal (1×MFC)-treated fruit (6.7%). In contrast, the fruits treated by wax + nonanal (4×MFC and 10×MFC) were not infected. The disease incidence of gray mould increased with prolonged time. At the 6th days of storage, gray mould incidence in wax + nonanal (1×MFC)-treated fruit (50.1%) was close to those in wax-treated fruit (73.3%), whereas the incidence in wax + nonanal (10×MFC)-treated fruit was only 33.3%. This reason that longer storage period more than 6 days increased disease incidence could be attributed to the high volatility of nonanal.

3.3. Nonanal Damages Plasma Membrane Integrity of *Botrytis cinerea* Spores

Propidium iodide (PI), a fluorescent molecule, is membrane impermeable and can bind to DNA by intercalating between the DNA bases, with little or no sequence preference [35] (Suzuki et al., 1997). As shown in Figure 2A, some nonanal-treated *Botrytis cinerea* spores released strong red fluorescence in fluorescence field, which indicated that the cell membranes of these spores were markedly damaged and the cells became permeable to the membrane-impermeant dye PI. During the 2 h of incubation, it was noted that, the most damage to the cell membrane of *B. cinerea* was with nonanal, and the membrane integrity rate of nonanal-treated spores declined to about 18% while that in control spores stayed at a high level (more than 95%) (Figure 2B). In general, damage increased during the incubation time in the treatment.

3.4. Weight Loss and Ascorbic Acid Content

Tomatoes in all treatments gradually lost weight during storage. The effect of nonanal on the weight loss of tomato during storage is reported in Figure 3A. Nonanal treatment could delay weight loss of the fruit. The weight loss was almost the same within the initial 4 days of storage. On day 8, tomato treated with wax + nonanal (1×MFC, 4×MFC and 10×MFC) had significantly less weight loss than control ($p \leq 0.05$). The ascorbic acid contents in treated and untreated tomatoes were evaluated. During the initial four days storage, the ascorbic acid contents in fruit

treated with wax + nonanal were almost equal to that of wax-treated fruit (Figure 3B). The ascorbic acid content in fruit treated with wax + nonanal (10×MFC) was approximately 11.04% and 9.07% higher than in wax-treated fruit after 6 and 8 days, respectively, which was significantly higher than that of control group.

3.5. Effects of Nonanal Treatment on Fruit Quality

The effects of the wax and nonanal treatment on fruit quality was further evaluated, the results were presented in

Table 2. The firmness of tomatoes in all treatments tended to decrease during storage at $28 \pm 2^\circ\text{C}$. The increment in TSS of tomatoes treated with nonanal was a little higher than that in the control. The pH of tomatoes in all the treatments slightly increased with storage time. As storage time was prolonged, coloration index increased gradually. Whereas coloration index increased greatly in wax-treated fruit, this phenomenon indicated that nonanal treatment delayed fruit ripening (Table 2). Meanwhile, no significant differences were found between the pH, Firmness and TSS content for all treatment under the same storage time ($P>0.05$).

Figure 1. Effect of different concentrations of Nonanal on *B. cinerea* fungi mycelial growth at $28 \pm 2^\circ\text{C}$ for 4 days. Values were presented as mean±S.E. Different letters indicate significant differences ($p<0.05$) according to Duncan's new multiple range test

Table 1. Disease incidence in inoculated fruits with Nonanal (0×, 1×, 4× and 10×MFC) during storage at $28 \pm 2^\circ\text{C}$ for 8 d and 85-90% RH

Treatment	Disease incidence (%)							
	Inoculation period (d)							
	1	2	3	4	5	6	7	8
Control	0±0a	20.0±0.2a	23.3±0.5a	33.3±1.2a	53.3±3.2a	73.3±2.2a	86.7±3.5a	100.0±1.6a
1×MFC	0±0a	6.7±0.5b	13.3±0.6b	23.3±1.3b	36.7±2.1b	50.1±3.6b	60.0±1.8b	73.3±2.9b
4×MFC	0±0a	0±0c	3.3±0.1c	10.0±1.2c	23.3±0.6c	41.2±2.0c	46.7±0.7c	56.7±3.5c
10×MFC	0±0a	0±0c	0±0d	6.7±1.9d	16.7±0.2d	33.3±3.9d	36.7±0.9d	46.7±2.4d

Note: Data presented are means of pooled data (n=5). Columns with different letters each time point indicate significant differences according to Duncan's multiple range test at $p=0.05$.

Figure 2. Effect of 10×MFC Nonanal on the membrane integrity of *B. cinerea* spores. (A) Microscopy images of *B. cinerea* spores after 0-2 h incubation, which spores were cultured at $28 \pm 2^\circ\text{C}$ in PDB medium supplement with 0 or 10×MFC Nonanal. (B) The percentage of spores stained with PI were shown in the bar graph after incubation for the indicated times. Bars represent standard deviation of the treatment means of pooled data

Figure 3. Effect of nonanal treatment (0, 1x, 4x, 10xMFC) on weight loss rate (A) and ascorbic acid content (B) of postharvest tomato fruit inoculated with *B. cinerea* during storage at 28 ± 2°C. Values represent the means of the replicates, and error bars represent the standard error of the means (n=3)

Table 2. Effect of Nonanal (0, 1x, 4x, 10xMFC) treatment on postharvest qualities of tomato fruits inoculated with *B. cinerea* during storage at 28 ± 2°C for 8 d

Physiological indicators	Treatment	Inoculation period (days)				
		0	2	4	6	8
pH	Wax	4.12±0.02a	4.25±0.02a	4.25±0.01a	4.27±0.01a	4.30±0.03a
	Wax + nonanal (1xMFC)	4.12±0.02a	4.23±0.01a	4.25±0.02a	4.27±0.01a	4.29±0.02a
	Wax + nonanal (4xMFC)	4.12±0.02a	4.26±0.02a	4.24±0.02a	4.26±0.02a	4.33±0.02a
	Wax + nonanal (10xMFC)	4.12±0.02a	4.25±0.02a	4.25±0.01a	4.27±0.02a	4.30±0.02a
TSS (%)	Wax	3.28±0.18a	3.23±0.18c	3.60±0.40b	4.00±0.01a	4.10±0.10a
	Wax + nonanal (1xMFC)	3.28±0.18a	3.50±0.40b	3.65±0.25b	4.00±0.01a	4.15±0.05a
	Wax + nonanal (4xMFC)	3.28±0.18a	3.50±0.40b	3.90±0.10a	4.05±0.05a	4.25±0.05a
	Wax + nonanal (10xMFC)	3.28±0.18a	3.90±0.01ba	4.00±0.01a	4.15±0.05a	4.25±0.10a
Firmness	Wax	1.93±0.05a	1.90±0.13a	1.65±0.05a	1.50±0.01a	1.43±0.07a
	Wax + nonanal (1xMFC)	1.93±0.07a	1.80±0.16a	1.60±0.01a	1.45±0.05a	1.43±0.04a
	Wax + nonanal (4xMFC)	1.93±0.05a	1.88±0.09a	1.70±0.02a	1.50±0.01a	1.45±0.05a
	Wax + nonanal (10xMFC)	1.93±0.05a	1.88±0.09a	1.70±0.01a	1.50±0.01a	1.48±0.06a
Coloration index	Wax	11.78±3.50a	14.09±3.55c	16.44±4.15b	19.62±5.18b	23.78±0.78a
	Wax + nonanal (1xMFC)	11.78±3.50a	15.06±3.30b	16.87±5.48a	18.56±3.63d	22.90±3.24c
	Wax + nonanal (4xMFC)	11.78±3.50a	14.98±3.24b	16.67±3.08b	18.85±3.31c	21.73±1.41d
	Wax + nonanal (10xMFC)	11.78±3.50a	15.55±3.88a	16.87±3.13a	21.72±4.14a	23.13±2.27b

Note: Data presented are means of pooled data (n=5). Different letters show significant difference at each treatment according to Duncan's multiple range test at $p=0.05$.

3.6. Activities of SOD, POD, CAT and PAL

As shown in Figure 4, four defense-related enzymes including SOD, POD, CAT and PAL were analyzed. The results of SOD activity are shown in Figure 4A. No significant changes were detected at day 2 except for 10xMFC-treated fruits ($P\geq0.05$). After 4 day of storage, a more rapid increase in SOD activity was observed in nonanal-treated fruits compared to the control fruit, and a peak value (1.47 ± 0.05 U/g FW) in 10x MFC-treated fruits occurred. The SOD activities in 4x or 10x MFC-treated fruits were 1.16 ± 0.08 and 1.47 ± 0.05 U/g FW, respectively, which were significantly higher ($P<0.05$) than that in control samples (0.76 ± 0.06 U/g FW). However, there was no significant difference between 1xMFC treatment group and control at 4 d of storage. After that, the SOD activities

decreased slowly, only the SOD activity in 10xMFC- treated fruits was always significantly higher than the control group at day 8.

The results in Figure 4B showed that POD activity increased continuously within 4 days of storage. After that, the POD activity declined rapidly. The nonanal could induce the POD activity which reached their peak values at day 4. The POD activities in control, 1x, 4x or 10x MFC-treated fruits were 5.52 ± 0.44 , 5.60 ± 0.24 , 5.91 ± 0.56 and 6.16 ± 0.48 U/g FW, respectively. The POD activity in 10xMFC-treated fruits remained a higher level as compared to control samples, whereas followed a same changing pattern with other nonanal-treated fruits at day 8. However, no obvious differences in PAL activity were observed between control and 1x, 4xMFC-treated fruits after 8 d of storage.

Figure 4. Changes in activities of SOD, POD, CAT and PAL in Nonanal (0×, 1×, 4×, 10×MFC)-treated tomato fruits at $28 \pm 2^\circ\text{C}$ for 8 d. Vertical bars represent standard deviations of the means. Different letters at each time point are statistically different according to Duncan's multiple range test at $p \leq 0.05$.

As for the CAT activity of tomatoes in all treatments (Figure 4C), its value increase slowly at the early stage of storage and then declined continuously after reaching the maximum value. The CAT activities in 1×, 4×MFC and 10× MFC-treated fruits reached the maximum value at day 4, which were significantly ($P < 0.05$) higher than those in control tomatoes. Throughout the whole storage period, the CAT activities of tomatoes treated by nonanal were significantly ($P < 0.05$) higher than those of control tomatoes.

The change in PAL activities is shown in Figure 4D, which the trend for PAL activities to change in treated fruits and the control were similar. The PAL activities increased in all the groups, reaching a peak value, and then declined. The PAL activities of tomatoes treated by nonanal (1×, 4× or 10× MFC) were statistically ($P < 0.05$) higher than those of the control after 2 d of storage. An increase tendency in the content of PAL activity in MFC-treated fruits and control until it reached its peak value within the first 4 days, and declined quickly thereafter. At day 4, PAL activity increased to 88.28 ± 1.58 U/g FW in 10×MFC-treated fruits, which was about 3.8 times of that in wax-treated samples (23.29 ± 1.22 U/g FW). The PAL activity in 4×MFC-treated fruits remained a similar level at 8 d of storage as compared to control samples, whereas followed a same changing pattern with other nonanal-treated fruits. Therefore, it is clear that nonanal induced stronger enzyme activities in tomato fruits upon challenged with the pathogen *Botrytis cinerea*.

4. Discussion

Studies have reported on the potential of using volatile compounds for storage fumigation, modified atmosphere storage and packaging and active packaging on a range of fruit and vegetable commodities [36,37]. Furthermore, exposure to VOCs such as trans-2-hexenal, cis-3-hexenal, or cis-3-hexenol enhanced resistance of *Arabidopsis thaliana* against the fungal pathogen *Botrytis cinerea* [38,39], which indicates that VOCs may also induce disease resistance. However, for 6-methyl-5-hepten-2-one, β -ionone, 2-methylbutyl acetate and nonanal, when their concentrations were close to $0.062 \mu\text{L/L}$, stimulated the growth of *Botrytis cinerea* [40,41]. In the present study, nonanal was found to inhibit mycelial growth of *Botrytis cinerea* in varying degrees, with the MIC and MFC values 100 and $160 \mu\text{L/L}$, respectively (Figure 1). These results confirmed the antifungal activity of nonanal previously found at relatively high concentrations [42]. Further, our results indicated that nonanal could damage the plasma membrane of spores (Figure 2). Sampathkumar et al. [43] observed that high pH may cause membrane damage and destruction of *Salmonella enterica* Serovar Enteritidis. Pinto et al. [44] found that PI penetrated over 95% of *Candida albicans* cells following a short incubation period with $2.5 \mu\text{L/ml}$ clove essential oil. Our study indicated that membrane integrity of *Botrytis cinerea* spores cultivated in PDB medium with 10×MFC nonanal (pH value was about 7.2) significantly reduced by PI staining experiment,

and 10×MFC nonanal caused cell membrane leakage by resulting in cell membranes disruption. Many observations reported that higher fungicide concentration is necessary to reduce fungal growth in vivo than in vitro [45,46]. This result confirmed previous reports describing the antifungal activity of nonanal [22,23].

The ability of a wax + nonanal combination treatment to inhibit the decay development of tomato fruit inoculated with *Botrytis cinerea* is presented in Table 1. After 3 days of incubation, gray mold incidence in wax-treated fruit (23.3%) was higher than those in wax + nonanal (1×MFC or 4×MFC)-treated fruit (13.3% and 3.3%). In contrast, the fruit treated by wax + nonanal (10×MFC) were not infected. This phenomenon is probably due to the high volatility of VOCs under in vivo than in vitro conditions, as demonstrated by previous reports [38,39]. However, the exact mechanism of action of nonanal against *B. cinerea* remained to be elucidated further.

During postharvest storage of fruits, changes related to quality, such as color, weight loss rate, firmness, TSS and Vc content, are generally observed [31,47]. There were no statistical differences in pH, TSS, firmness between treatments and control on a given day (Table 2). This result agrees with the finding of Lu et al. [48] who reported that thymol did not affect the texture of tomato during 16 days of storage at 4°C and 22°C.

The application of nonanal delayed the postharvest ripening of tomatoes. The delay was characterized by reducing the browning index and weight loss and retention of fruit firmness. Our results showed that all postharvest treatments prevented weight loss in comparison with the control (Figure 3), which are in agreement with the previous studies. Essential oil vapours have also been reported to be effective in reducing weight loss in cherry, grapes and strawberry [49,50,51]. Our study agrees with the findings of Peretto et al [15], who reported that release of carvacrol and methyl cinnamate from edible films in clamshell results in brighter colour of treated strawberry compared to the colour of untreated strawberry.

Antioxidant enzymes, such as CAT, SOD, and POD, serve an indispensable role in scavenging reactive oxygen species in plants. POD and PAL are commonly studied in the postharvest biocontrol area and known to be involved in plant disease resistance [52].

The balance among the activities of SOD, POD, and CAT in cells was crucial for determining the steady-state level of O₂⁻ and H₂O₂, whereas H₂O₂ is predominantly broken down by POD and CAT [29,53]. POD is involved in lignification of host plant cells and considered as key enzymes related to defense reaction against pathogen infections [54]. POD activity produces the oxidative power for cross-linking proteins and phenylpropanoid radicals leading to the reinforcement of cell walls for resisting fungal invasion [55]. In the current research, the SOD and POD activities was apparently induced by nonanal treatment, as evidenced by a higher values or the arrival of maximal values ahead of time.

PAL is responsible for the biosynthesis of *p*-coumaric acid derivatives, phytoalexin, and phenylpropanoid pathway that contribute to plant defense systems [56,57]. The induction of these defense related enzymes by different elicitors has been reported in various harvested fruits including apple, loquat, mango and tomato, which is

correlated to increased disease resistance and reduced disease severity [19,58,59,60]. In line with these results, our study showed that nonanal treatment evoked the activities of SOD, POD, CAT and PAL, which reached maximal values at 4 d (Figure 4) and reduced gray mold decay in tomatoes inoculated with *Botrytis cinerea* (Table 2). Thus, these results suggest that the induction of these defense related enzymes may be one part of the mechanism by which nonanal suppressed *B. cinerea* infection in tomato fruit.

5. Conclusion

Nonanal exhibited a pronounced antifungal activity against *Botrytis cinerea*, with MIC and MFC values being both 100 and 160 µL/L. Nonanal treatment decreased the incidence rate of postharvest *B. cinerea* fruits, and induced an increase in the activities of SOD, POD, CAT and PAL. In addition, it can only slightly affected TSS content, pH, firmness. Whereas nonanal treatment significantly reduced the rise in weight loss rate and coloration index, and kept a higher level of firmness and ascorbic acid content compared with the control group. Overall, nonanal treatment could delay fruit ripening, and maintain a high level of quality. These results confirmed that nonanal can be used as an alternative to traditional fungicides for the control of tomato *B. cinerea*.

Acknowledgements

This work was supported by National Natural Science Foundation of China (Nos. 31272181 and 31471887), Natural Science Foundation of Hunan Province (No.2015JJ6108) and PhD Research Fund of Xiangtan University (No. KZ08033).

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