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 μL 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.
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 ± 2°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 1×10⁷ 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. Prodim 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, 70 and 80 μL/L. 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 ± 2°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 ± 2°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 ± 2°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 μL/L. Fungal growth was estimated gravimetrically by weighting the biomass 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×MFC) for 2 h at 28 ± 2°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 prodim iodide (PI, 1 μg/ml, final concentration) for 30 min at 28 ± 2°C in the dark [28]. After centrifugation at 8000×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:

\[ \text{Membrane leakage(\%)} = \frac{\text{number of stained spores}}{\text{number of total spores}} \times 100 \]

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 μL of a spore suspension of Botrytis cinerea (10⁷ spores L⁻¹) for 4 h, and left to air-dry. After inoculation, the fruits were coated with wax amended with nonanal at 1×MFC, 4×MFC or 10×MFC, respectively. Tomato fruits coated with wax alone served as control. After treatment, the inoculated fruit were stored in sealed incubators at 28 ± 2°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:
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 weighing 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-dichlorophenolindophenol 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 1 cm into the fruit at a speed of 1 mm/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 μL/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 μL/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 μL/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±2°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, grey 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<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 ± 2°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 ± 2°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 ± 2°C for 8 d and 85-90% RH

| Treatment   | 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±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±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 ± 2°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, 1×, 4×, 10×MFC) 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, 1×, 4×, 10×MFC) 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) |
|--------------------------|--------------------|---------------------------|
| pH                       | Wax                | 4.12±0.02a                |
|                          | Wax + nonanal (1×MFC) | 4.12±0.02a                |
|                          | Wax + nonanal (4×MFC) | 4.12±0.02a                |
|                          | Wax + nonanal (10×MFC) | 4.12±0.02a               |
| TSS (%)                  | Wax                | 3.28±0.18a                |
|                          | Wax + nonanal (1×MFC) | 3.28±0.18a                |
|                          | Wax + nonanal (4×MFC) | 3.28±0.18a                |
|                          | Wax + nonanal (10×MFC) | 3.28±0.18a               |
| Firmness                 | Wax                | 1.93±0.05a                |
|                          | Wax + nonanal (1×MFC) | 1.93±0.07a                |
|                          | Wax + nonanal (4×MFC) | 1.93±0.05a                |
|                          | Wax + nonanal (10×MFC) | 1.93±0.05a               |
| Coloration index         | Wax                | 11.78±3.50a               |
|                          | Wax + nonanal (1×MFC) | 11.78±3.50a               |
|                          | Wax + nonanal (4×MFC) | 11.78±3.50a               |
|                          | Wax + nonanal (10×MFC) | 11.78±3.50a               |

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 10×MFC-treated fruits (*P*≥0.05). 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 10×MFC-treated fruits occurred. The SOD activities in 4× or 10× 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 1×MFC treatment group and control at 4 d of storage. After that, the SOD activities decreased slowly, only the SOD activity in 10×MFC- 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, 1×, 4× or 10× 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 10×MFC-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 1×, 4×MFC-treated fruits after 8 d of storage.
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 μL/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 μL/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 μl/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).

References

[1] Schuch, W., “Improving tomato quality through biotechnology,” Food Technology, 48(11): 78-83. Nov. 1994.
[2] Krinsky, N.I., Johnson, E., “Carotenoid actions and their relation to health and disease,” Molecular Aspects of Medicine, 26(6): 459-516. Nov. 2005.
[3] Marquenie, D., Geeraerd, A.H., Lammertyn, J., Soontjens, C., Van Impe, J.F., Michiels, C.W., Nicolai, B.M, “Combinations of pulsed white light and UV-C or mild heat treatment to inactivate conidia of Botrytis cinerea and Monilia fructiceti,” International Journal of Food Microbiology, 85(1-2):185-196. Aug. 2003.
[4] Spadaro, D., Garibaldi, A., Martines, G.F, “Control of Penicillium expansum and Botrytis cinerea on apple combining a biocontrol agent with hot water dipping and acibenzolar-S- methyl, baking soda, or ethanol application,” Postharvest Biology & Technology, 33(2):141-151. Aug. 2004.
[5] Cantu, D., Blanco-Ulate, B., Yang, L., Labavitch, J.M., Bennett, A.B., Powell, A.L, “Ripening- regulated susceptibility of tomato fruit to Botrytis cinerea requires NOR but not RIN or ethylene,” Plant Physiology, 150(3):1434-1449. May. 2009.
[6] Choquer, M., Fournier, E., Kunz, C., Levis, C., Pradier, J.M., Simon, A., Viala, M., “Botrytis cinerea virulence factors: new insights into a necrotrophic and polyphagous pathogen,” Fems Microbiology Letters, 277(1):1-10. Dec. 2007.
[7] Fang, X.L., Li, Z.Z., Wang, Y.H., Zhang, X, “In vitro and in vivo antimicrobial activity of Xenorhabdus bovienii YL002 against Phytophthora capsici and Botrytis cinerea,” Journal of Applied Microbiology, 111(1):145-154. Jul. 2011.
Blacharski, R.W., Bartz, J.A., Xiao, C.L., Legard, D.E., “Control of post-harvest Botrytis fruit rot with pre-harvest fungicide applications in annual strawberry,” Plant Disease, 85(6): 597-602. Jun. 2001.

Mertely, J.C., MacKenzie, S.J., Legard, D.E., “Timing of fungicide applications for Botrytis cinerea based on development stage of strawberry flowers and fruit,” Plant Disease, 86(9): 1019-1024. Sep. 2002.

Karabulut, O.A., Romanazzi, G., Smilancic, J.L., Lichter, A., “Postharvest ethanol and hot water treatments of grape table grapes to control gray mold,” Postharvest Biology & Technology, 37(2): 129-134. Aug. 2005.

Navarro, D., Diaz-Mula, H.M., Guifil, F., Zapata, P.J., Castillo, S., Serrano, M., Valero, D., Martinez-Romero, D., “Reduction of nectarine decay caused by Bzhizapos stolonifer, Botrytis cinerea and Penicillium digitatum with Aloe vera gel alone or with the addition of thymol,” International Journal of Food Microbiology, 151(2): 241-246. Sep. 2011.

Xu, W., Huang, K., Guo, F., Wu, W., Yang, J., Liang, Z., Luo, Y., “Postharvest grapefruit seed extract and chitosan treatments of table grapes to control Botrytis cinerea,” Postharvest Biology & Technology, 46(1): 86-94. Oct. 2007.

Neria, F., Cappellini, L., Spadoni, A., Cameli, I., Algora Alarcon, A., Aprea, E., Romano, A., Gasperi, F. and Biasiolli, F., “Role of strawberry volatile organic compounds in the development of Botrytis cinerea infection,” Plant Pathology, 63(3): 709-717. Sep. 2015.

Rattanapritporn, P., Arakawa, M. & Tsuro, M., “Vanillin enhances the antifungal effect of plant essential oils against Botrytis cinerea,” International Journal of Aromatherapy, 16(3-4): 193-198. Nov. 2006.

Peretto, G., Du, W.X., Avena-Bustillos, R.J., Sarreal, S.B.L., Hua, S.S.T. & Sambo, P., “Increasing Strawberry shelf-life by carvacrol and methyl cinnamate antimicrobial vapors released from edible films,” Postharvest Biology & Technology, 89: 1-18. Mar. 2014.

Palik, E., Archbold, D.D., Hamilton-Kemp, T.R., Clements, A.M., Collins, W., Barth, M.M., “(E)-2-hexenal can stimulate Botrytis cinerea growth in vitro and on strawberries in vivo during storage,” Journal of the American Society for Horticultural Science American Society for Horticultural Science, 123(5): 875-881. Sep. 1998.

Myung, K., Hamilton-Kemp, T.R., Archbold, D.D., “Interaction with and effects on the profile of products of Botrytis cinerea by C6 aldehydes,” Journal of Agricultural & Food Chemistry, 55(6): 2182-2188. Mar. 2007.

Neri, F., Mari, M., Menitti, A.M., Brigati, S., “Activity of trans-2-hexenal against Penicillium expansum in ‘Conference’ pears,” Journal of Applied Microbiology, 109(6): 1186-1193. Jun. 2006.

Guo, M.R., Feng, J.Z., Zhang, P.Y., Jia, L.Y., Chen, K.S., “Postharvest treatment with trans-2-hexenal induced resistance against Botrytis cinerea in tomato fruit,” Australasian Plant Pathology, 44(1):121-128. Jan. 2015.

Corbo, M.R., Lanciotti, R., Gardini, F., Sinigaglia, M., Guerzoni, M.E., “Effects of hexanal, trans-2-hexenal, and storage temperature on shelf life of fresh sliced apples,” Journal of Agricultural & Food Chemistry, 48(6):2401-2408. Jun. 2000.

Muroi, H., Kubo, A., Kubo, I., “Antimicrobial activity of cashew apple flavor compounds,” Journal of Agricultural and Food Chemistry, 41 (7):1106-1109. Jul. 1993.

Tao, N.G., Jia, L., Zhou, H.E., “Anti-fungal activity of some Moroccan plants against Geotrichum candidum, the causal agent of post-harvest citrus sour rot,” Crop Protection, 35(3):41-46. May. 2012.

Helal, G.A., Sarhan, M.M., Abu Shahla, A.N.K. & Abou El-Khair, E.K., “Effects of Cymbopogon citratus L. essential oil on the growth, lipid content and morphogenesis of Aspergillus niger M2-strain,” Journal of Basic Microbiology, 46(6):456-469. Jan. 2006.

Liu, J., Tian, S.P., Meng, X.H., Xu, Y., “Effects of chitosan on control of postharvest diseases and physiological responses of tomato fruit,” Postharvest Biology & Technology, 44(3): 300-306. Jun. 2007.

Qin, G., Liu, J., Cao, B., Li, B., Tian, S., “Hydrogen peroxide acts on sensitive mitochondrial proteins to induce death of a fungal pathogen revealed by proteomic analysis,” PLoS One, 6(7): e21945. Jul. 2011.

Tao, N.G., Fan, F., Jia, L. & Zhang, M.L., “Octanal incorporated in postharvest wax of Satsuma mandarin fruit as a botanical fungicide against Penicillium digitatum,” Food Control, 45(3):56-61. Nov. 2014.

Castillo, S., Navarro, D., Zapata, P.J., Guillen, F., Valero, D., Serrano, M., Martinez-Romero, D., “Antifungal efficacy of Aloe vera in vitro and its use as a preharvest treatment to maintain postharvest table grape quality,” Postharvest Biology & Technology, 57(3):183-188. Sep. 2010.

Fan, F., Tao, N.G., Jia, L., He, X.L., “Use of citral incorporated in postharvest wax of citrus fruit as a botanical fungicide against Penicillium digitatum,” Postharvest Biology & Technology, 90(3):52-55. Apr. 2014.

Lemoine, M.L., Chaves, A.R., Martinez, G.A., “Influence of combined hot air UV-C treatment on the antioxidant system of minimally processed broccoli (Brassica oleracea L. var. Italica),” LWT-Food Science and Technology, 43(9):1313-1319. Nov. 2010.

Sasmuthu, P.S., Sivakumar, D., Soundy, P., Korsten, L., “Essential oil vapours suppress the development of anthracnose and enhance defense related and antioxidant enzyme activities in avocado fruit,” Postharvest Biology & Technology, 81(3): 66-72. Jul. 2013.

Zhang, J.H., Sun, H.L., Chen, S.Y., Zeng, L. and Wang, T.T., “Anti-fungal activity and mechanism studies on α-phellandrene and Nonanal against Penicillium cyclopium,” Botanical Studies, 58(13): 1-9. Mar. 2017.

Suzuki, T., Fujikura, K., Higashiyama, T., Takata, K., “DNA staining for fluorescence and laser confocal microscopy,” Journal of Histochemistry & Cytochemistry, 45(1): 49-53. Jan. 1997.

Combrink, S., Regnier, T., Kamatou, G.P.P., “In vitro activity of eighteen essential oils and some major components against common postharvest fungal pathogen fruit,” Industrial Crops & Products, 33(2): 344-349. Mar. 2011.

Wood, E.M., Miles, T.D., Wharton, P.S., “The use of natural plant volatile com-pounds for the control of potato postharvest diseases, black dot, silver scurf and asco rot,” Biological Control, 64(2): 152-159. Feb. 2013.

Kishimoto, K., Matsui, K., Ozawa, R., Takabaysa, J., “Volatile C9-aldehydes and alio-ocimene activate defense genes and induce resistance against Botrytis cinerea in Arabidopsis thaliana,” Plant & Cell Physiology, 46(7):1093-1102. Jul. 2005.

Yi, H.S., Heil, M., Adame-Ivarez, R.M., Ballhorn, D.J., Ryu, C.M, “Airborne Induction and Priming of Plant Defenses against a Bacterial Pathogen,” Plant physiology, 151(4): 2152-2161. Dec. 2009.

Eduardo, I., Chietera, G., Bassi, D., Rossini, L., Vecchietti, A., “Identification of key odor volatile compounds in essential oil of nine peach accessions,” Journal of the Science of Food and Agriculture, 90(7): 1146-54. May. 2010.

Cebolla- Conrero, J., Rosello, S., Valcarcel, M., Serrano, E., Beltran, J., Nuez, F., “Evaluation of genotype and environment effects on taste and aroma flavor components of Spanish fresh tomato varieties,” Journal of Agricultural and Food Chemistry, 59(6):2440-50. Feb. 2011.

Scalia, A., Allmann, S., Mirabella, R., Haring, M.A., Scuarkin, R.C., “Green leaf volatiles: a plant’s multifunctional weapon against herbivores and pathogens,” International Journal of Molecular Sciences, 14(9): 17781-81. Sep. 2013.

Sampathkumar, B., Khachatourians, G.G., Korbner, D.R., “High pH during trisodium phosphate treatment causes membrane damage and destruction of Salmonella enterica serovar enteriditis,” Applied & Environmental Microbiology, 69(1):122-129. Jan. 2003.
Pinto, E., Vale-Silva, L., Cavaleiro, C., Salgueiro, L, “Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida, Aspergillus* and dermatophyte species,” *Journal of Medical Microbiology*, 58: 1454-1462. Nov. 2009.

Smilanick, J.L., Mansour , M.F., Gabler , F.M. & Sorenson , D, “Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides,” *Postharvest Biology & Technology*, 47(2): 226-238. Feb. 2008.

Pérez-Alfonso, C.O., Martínez-Romero, D., Zapata, P.J., Serrano, M., Valero, D. & Castillo, S, “The effects of essential oils carvacrol and thymol on growth of *Penicillium digitatum* and *P. italicum* involved in lemon decay,” *International Journal of Food Microbiology*, 158(2): 101-106. Aug. 2012.

Castillo, S., Pérez-Alfonso, C.O., Martínez-Romero, D., Guillén, F., Serrano, M. & Valero, D. “The essential oils thymol and carvacrol applied in the packing lines avoid lemon spoilage and maintain quality during storage,” *Food Control*, 35(1):132-136. Jan. 2014.

Lu, Y.J., Joerger, R. & Wu, C.Q, “Similar reduction of *Salmonella enterica* Typhimurium on grape tomatoes and its cross-contamination in wash water by washing with natural antimicrobials as compared with chlorine treatment,” *Food and Bioprocess Technology*, 7(3): 661-670. Mar. 2014.

Serrano, M., Martínez-Romero, D., Castillo, S., Guillen, F. & Valero, D, “The use of antifungal compounds improves the beneficial effect of map in sweet cherry storage,” *Innovative Food Science & Emerging Technologies*, 6(1):115-123. Mar. 2005.

Shao, X., Wang, H., Xu, F. & Cheng, S, “Effects and possible mechanisms of tea tree oil vapor treatment on the main disease in postharvest strawberry fruit,” *Postharvest Biology & Technology*, 77(3): 94-101. Mar. 2013.

Zhao, Y., Tu, K., Su, J., Hou, Y., Liu, F., Zou, X, “Heat treatment in combination with antagonistic yeast reduces diseases and elicits the active defense responses in harvested cherry tomato fruit,” *Journal of Agricultural & Food Chemistry*, 57(16): 7565-7570. Jul. 2009.