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Alleviating Effects of Linalool Fumigation on *Botrytis cinerea* Infections in Postharvest Tomato Fruits

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Abstract: Gray mold is a disease that often occurs in postharvest tomato fruits, leading to a significant decline in the fruits' quality. In production, pesticides are mostly used to control gray mold, which negatively affect both the environment and food safety. The purpose of this article is to study the effects of linalool fumigation on controlling gray mold in tomato fruits and to further investigate the mechanism of linalool function, so as to provide technical support and a theoretical basis for the application of linalool in the green control of tomato gray mold. The results of the in vitro experiments showed that linalool fumigation had a strong inhibitory effect on the mycelial growth of *Botrytis cinerea* and that the fumigation of linalool inhibited the expansion of pathogens on tomato fruits. The disease index of tomato fruits in the linalool treatment was always lower than that of the control within 72 h after inoculation with *Botrytis cinerea*; at the end of experiment, the disease index of the control reached 100.0, which was only 8.0 with the 30 μL/L linalool treatment. Linalool fumigation increased the antioxidant capacity of the tomato fruits under *Botrytis cinerea* infection through regulating activities of SOD, POD and CAT as well as the ascorbic acid (AsA) content, which could be responsible for the lower malondialdehyde (MDA) accumulation. Linalool fumigation increased the activities of polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) in tomato fruits, indicating that secondary metabolism was involved in the function of linalool in the regulation of tomato fruit resistance to pathogens. As the main enzymes related to cell structure, polygalacturonase (PG), cellulase (CL) and β-galactosidase (β-GAL) were inhibited by linalool, which could protect cell wall structures from damage, and strengthened the mechanical barrier against pathogen access to fruit flesh.

Keywords: tomato fruit; *Botrytis cinerea*; linalool; anti-fungal activity; postharvest

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetable crops worldwide, among which, the cherry tomato is the most economically valuable cultivar owing to its favorable flavor and richer nutrition [1,2]. However, tomatoes are typical respiratory climacteric fruits with a soft texture and thin skin, undergoing vigorous life activity after harvesting. As a result, tomato fruits are susceptible to mechanical damage and spoilage microorganisms during transportation [3,4], on which diseases such as gray mold, soft rot and umbilical rot frequently occur, resulting in a significant decline in fruit quality [5–7]. Among these diseases, gray mold caused by *Botrytis cinerea* (*B. cinerea*) has been listed as the most serious disease in tomatoes, which occurs not only in the growing period but also in the postharvest storage and transportation period.

Although synthetic chemical fungicides are commonly used to control the gray mold caused by *B. cinerea*, several restrictions have been imposed on their application due to the harmful effects on environmental and human health caused by their residues in edible organs. In addition, many synthetic fungicides have side effects on non-target organisms,

and the exclusive use of them induces the development of fungicide-resistant strains in pathogens. Consequently, it has been considered a sustainable strategy to explore green, safe and efficient alternatives to chemical fungicides for the control of postharvest disease in horticultural crops [8–10]. Recent studies have revealed the important roles of plant volatile organic compounds (VOCs) in controlling postharvest pathogens in fruits [11]; for example, thymol and carvacrol can effectively suppress gray mold caused by *B. cinerea* [12].

Linalool, a colorless and fragrant acyclic monoterpene alcohol compound, is a typical volatile organic compound that plays an important role in the inhibition of a variety of pathogenic bacteria. A previous study showed that linalool has high level of antibacterial activity against *Escherichia coli*, *Staphylococcus*, *Streptococcus albus* and *Aspergillus niger*, etc. [13–15]. The addition of linalool to plant essential oils could significantly enhance their anti-bacterial effects [16]. Linalool can effectively inhibit the activity of *Pseudomonas aeruginosa* and *Pseudomonas fragi* by breaking their structure [17,18]. Emissions from VOCs are affected by infections with pathogenic microorganisms [19]; for example, after infection with *Fusarium* (*F. avenaceum* (Fr.) Sacc., *F. culmorum* (W. G. Smith) Sacc. and *F. graminearum* Schwabe), the main VOCs, i.e., linalool, linalool oxide and β-farnesene are altered in wheat [20]. Furthermore, the release of VOCs in strawberries also changes after infection with *B. cinerea*, the linalool content significantly increases especially, accounting for about 14% of the total VOCs [21]. Exogenous linalool has a significant inhibitory effect on *B. cinerea* in strawberry fruits, and its function of linalool could be due to pathogen cell-membrane structure destruction [21].

Although linalool can inhibit the growth of *B. cinerea* in artificial media [22], its effects and underlying mechanisms in the control of tomato fruit gray mold have not been well investigated. In this study, we found that linalool fumigation clearly inhibited gray mold infection caused by *B. cinerea* in tomato fruits. To study its possible mechanisms, the activities of antioxidant enzymes, ascorbic acid (AsA) and malondialdehyde (MDA) content, as well as pathogenesis-related and cell wall-related enzymes activities were detected. The main goal of this study is to provide technical support and a theoretical basis for linalool application as a new approach for the control of postharvest disease in tomato fruits.

2. Materials and Methods

2.1. Fruit Materials

Cherry tomato fruits (*Solanum lycopersicum* L. cv. "Busan 88th") were harvested at the ripe stage, after flowering for 40 days, from a greenhouse at Shandong Agricultural University and were immediately transported to the laboratory. Fruits used as experimental materials were similar in size and without visual defects and were picked from the same second inflorescence of tomato seedings.

2.2. Pathogen Preparation

B. cinerea (B05. 10) was provided by Professor Chuanyou Li and Jiuhai Zhao and maintained on Potato Dextrose Agar (PDA; potato 200 g/L, glucose 20 g/L, agar 18 g/L) at 4 °C [23]. After 14 days cultivation in an incubator at 25 °C, spores were scraped from the cultures on the agar surface with sterile distilled water containing 0.05% (v/v) Tween 80 and then filtered through sterile cheesecloth to remove any adhering mycelia. The spore suspension, whose number was calculated using a hemocytometer, was diluted to 1×10^4 spores/mL with sterile distilled water [24].

2.3. In Vitro Effects of Linalool on B. cinerea Growth

Linalool (98%), geraniol (98%) and nerol (98%) were extracted from natural essential oils purchased from Aladdin, Shanghai, China. Linalool, geraniol and nerol with similar structures were used to select effective substances in the in vitro experiment with *B. cinerea*. A 9 mm sterilized petri dish, which was evenly divided into two equal parts, was used for the experiment on linalool inhibition of *B. cinerea*. The sterilized PDA medium was poured

to one part of the bisected plate and a piece of sterile filter paper was soaked in linalool (fumigation concentrations, 15, 30 and 45 μL/L) was placed into the other part. Then, 6 mm *B. cinerea* agar plugs separated from a PDA plate in which the evenly distributed mycelium had cultured for 5–6 days was inoculated on the surface of the bisected agar. In the control, moderate amount of sterile water was used instead of linalool with different fumigation concentrations [22]. The treated media of 3 replicates were incubated at 25 °C and the diameter of the mycelium was measured by the cross method after 24 h, 48 h and 72 h. The inhibition rate of mycelium growth was calculated according to the following formula [25], where dc (mm) was the average colony diameter of the control and dt (mm) was the average colony diameter of the treatment.

$$\text{MGI (\%)} = [(\text{dc-dt})/\text{dc}] \times 100$$

2.4. In Vivo Effects of Linalool on B. cinerea Growth

Cherry tomato fruits were surface-disinfected with 1% (*v*/*v*) sodium hypochlorite for 3 min, rinsed with distilled water and air-dried before treatment [26]. These tomatoes were randomly divided into 4 groups: Control, without *B. cinerea* and linalool treatment; L, fumigation with 30 μL/L of linalool; B, inoculation with *B. cinerea*; and L + B, inoculation with *B. cinerea* followed by fumigation with 30 μL/L of linalool. A syringe was inserted into the pericarp at the midline of the tomato fruit to create a 3 -mm wound and a total of 5 μL of *B. cinerea* spore suspension was inoculated into fruit of the B and L + B groups. Additionally, 5 μL of sterilized distilled water was added to the control and L groups [27]. All tomato fruits were uniformly placed into a 1 L crisper, which was immediately sealed with a lid taped to filter paper with 30 μL linalool (L and L + B groups) or sterilized water (Control and B groups) for 1 day. Then, the filter papers with linalool or water were removed and the crisper with fruits was incubated at 25 °C at 85% humidity for another 3 days (marked as 0–72 h). The lesion diameter was recorded at 0 h, 24 h, 48 h and 72 h. Each treatment was replicated three times and 15 tomato fruits were used as one replicate.

2.5. Determination of the Disease Index of Tomato Fruits Gray Mold

The disease index was consulted during the study to quantify the incidence and severity of the disease in tomatoes [28]. The degree of gray mold incidence in tomato fruits was classified into six classes: Level 0, without disease spots; Level 1, disease spot diameter < 0.2 cm; Level 2, disease spot diameter 0.2–0.4 cm; Level 3, disease spot diameter 0.4–0.6 cm; Level 4, disease spot diameter 0.6–0.8 cm; Level 5: disease spot diameter >0.8 cm. The disease index of tomato fruits was calculated to reflect the alleviating effects of the linalool according to the following formula:

$$\text{Disease Index} = \frac{\sum(\text{Disease level} \times \text{Number of fruits at this level})}{\text{Highest level of disease} \times \text{Total number of fruits}} \times 100$$

$$\text{Control Effect (\%)} = \frac{\text{Fruit disease of control} - \text{Fruit disease of treatment}}{\text{Fruit disease of control}} \times 100\%$$

2.6. Determination of Antioxidant Enzymes, Phenylalanine Ammonia-Lyase (PAL) and Polyphenol Oxidase Enzyme (PPO) Activity

A punch with a diameter of 1.5 cm was used to take the pericarp of the tomatoes, with a thickness of 0.3 cm. Each treatment was repeated three times, and five tomatoes were used in each repeat. Tomato fruit samples were chopped with a knife and put in liquid nitrogen immediately and were then stored in −80 °C for the following experiment. Superoxide dismutase (SOD) activity was measured by the nitroblue tetrazolium (NBT) reduction method [29]. Catalase (CAT) activity was measured by the consumption of H_2O_2, determined as a decline in absorbance at 240 nm (A240) [30]. One unit of Peroxidase (POD) activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01/min following H_2O_2 induced guaiacol oxidation, determined by absorbance changes at A470 [31].

PAL enzyme activity was determined following the method of Assis et al. [32]. Tomato fruits were ground with 50 mM borate buffer containing 1% mercaptoethanol (pH = 8.8). After centrifugation at 5000× *g* for 20 min at 4 °C, 1.9 mL L-phenylalanine was added in 0.1 mL supernatant. PAL activity was detected at A290 after the addition of the stop solution. PPO enzyme activity was determined at A525 according to the method of Maehly et al. [33]. A total of 0.5 g of tomato fruits were ground with 5 mL phosphate buffer solution (0.1 M, PH = 6.0). After centrifugation at 5000× *g* for 15 min at 4 °C, 0.1 mL of supernatant was mixed with 3.9 mL phosphate buffer solution and 1 ml pyrocatechol (1 mM). The PPO activity was detected at A525 after the addition of the stop solution.

2.7. Determination of AsA and MDA Content

For the AsA assay, 0.5 g of tomato fruits were ground with a 4 mL 5% metaphosphate ice bath and centrifugated at 12,000× *g* for 15 min. Then 100 µL of supernatant was mixed with 3 mL solution of phosphoric acid buffer (pH = 6.8, 100 mM) and ascorbate oxidase (AAO; 1.25 U/mL). The total AsA content was detected at 265 nm by spectrophotometry [34].

For the MDA assay, fruits sample were homogenized in 5 mL extraction solution (5% trichloroacetic acid (TCA) as the extraction solution). After centrifugation at 5000× *g* for 15 min at 4 °C, 1 mL of 10% TCA containing 0.67% thiobarbituric acid was fixed in 0.5 mL supernatant. The mixture was incubated at 90 °C in a water bath for 0.5 h and then cooled in ice for 2 min. After centrifuging at 5000× *g* for 20 min, the supernatant was detected at A450, A532 and A600. The concentration of MDA was determined using the formula: C_{MDA} (µmol g^{-1} FW) = 6.45 × (OD_{532}-OD_{600})-0.56 × OD_{450} [35,36].

2.8. Determination of Cell Wall-Related Enzymes

Ploygalacturonase (PG), cellulase (CL) and *β*-Galactosidase (*β*-GAL) activity were quantified using an assay kit (Suzhou Kemin Biotechnology Co., Ltd., Suzhou, China). A total 0.2 g of tomato fruits were ground in 1 mL extracting solution and the supernatant was obtained after centrifugation. The PG, CL and *β*-GAL activities were determined, respectively, at absorbances of 540 nm, 650 nm and 400 nm.

2.9. Preparation of Paraffin Slices of Tomato Pericarp

Fruit tissue was fixed in FAA (absolute alcohol/formaldehyde/glacial acetic acid 16:1:1) for 24 h. The fixed samples were dehydrated in a graded series of ethanol (70%, 80%, 90%, 95% and 100%). Then, the fixed samples were added to a mixture of 1:1 absolute ethanol/xylene for 2 h followed by dehydration in, xylene for three times, each time for 1.5 h. The wax was poured into the xylene solution containing the fruit tissue and sliced and then dried in an oven at 45 °C for 2 d. Paraffin sections were stained with Safranine O-Fast Green for 15 min. The samples were observed and photographed under a light microscope (ECLIPSE Ni-U, Nikon, Japan) [37].

2.10. Statistical Analysis

Data were analyzed via DPS V9.01 (Qiyi Tang, Hangzhou, China) and graphed with Microsoft Excel 2013 (Redmond, Washington, DC, USA). Duncan's new complex polarization method was used for significance testing ($p < 0.05$).

3. Results

3.1. Inhibition of B. cinerea by Linalool

3.1.1. Linalool Was Selected as the Substance for *B. cinerea* Inhibition

Although the linalool, geraniol and nerol were similar in structure, their fumigation showed different degrees of inhibition on the growth of *B. cinerea* mycelium. The colony diameter of the control was 44.1 mm after 3 days of incubation, and the colony diameters after fumigation with three concentrations of linalool, geraniol and nerolidol were smaller

than that of the control, with the inhibition effect of the linalool being the most significant (Figure S1). Therefore, linalool was chosen as the inhibitor for the following experiments.

3.1.2. Inhibitory Effect of Linalool on *B. cinerea* Colonies

As shown in Figure 1, the inhibitory effect of linalool on the growth of *B. cinerea* was dependent on the concentration used. The average colony diameter of the control was 44.1 mm after 3-days cultivation, and the *B. cinerea* colony diameters were 38.4 mm, 36.6 mm, 24.6 mm, 15.7 mm and 9.9 mm in treatments with 5 µL/L, 10 µL/L, 15 µL/L, 30 µL/L and 45 µL/L linalool, respectively (Figure 1), indicating that linalool showed more obvious control effects on the growth of *B. cinerea* mycelia with increasing concentrations. However, to ensure a positive effect of linalool on both *B. cinerea* control and fruit quality, the appropriate concentration of linalool treatment for tomato fruits requires further experiments.

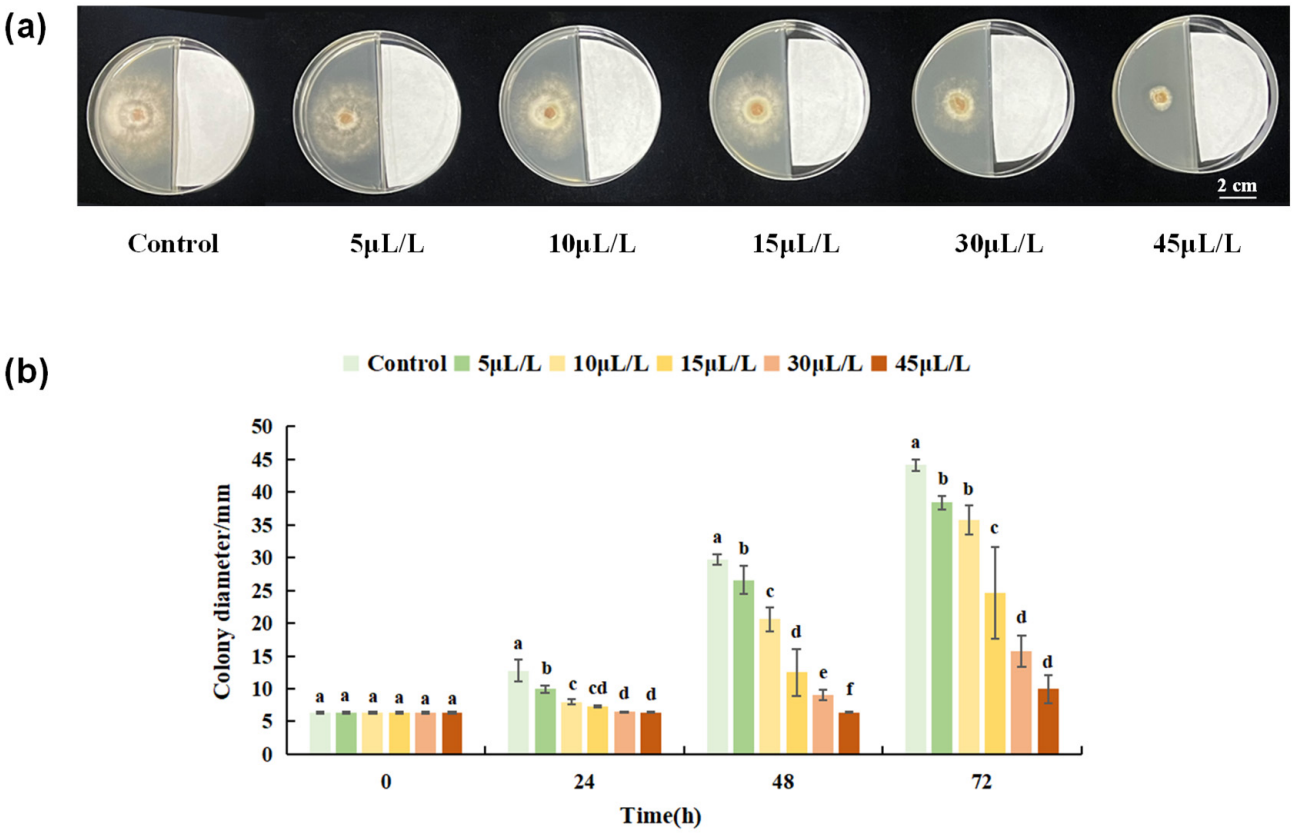

Figure 1. Effects of different concentrations of linalool on the in vitro mycelial growth of *Botrytis cinerea* (*B. cinerea*) colonies. (**a**) Mycelium growth phenotype of *B. cinerea* after 72 h of linalool fumigation; (**b**) Colony diameter of *B. cinerea* after 24 h, 48 h and 72 h of linalool fumigation. Data show the means of three replicates ± standard deviation (SD). The different letters indicate significant difference at $p < 0.05$.

3.2. Effects of Linalool Fumigation on the Gray Mold Control of Tomato Fruits

3.2.1. Screening of Linalool Fumigation Concentration in Tomato Fruits

Damages to the tomato fruits were evaluated in in vivo experiments, and it was found that the fruits were undamaged at a fumigation concentration of 30 µL/L but were damaged at 45 µL/L (Figure 2a). Cross-sections of tomato fruit pericarp at the midline were cut off and then paraffin sections were made and photographed. In the fumigation treatment with 30 µL/L of linalool, the cells of the tomato pericarp showed similar structures to the control (Figure 2b,c), while 45 µL/L linalool fumigation treatment clearly damaged the cell

structure of the tomato pericarp (Figure 2d). Therefore, 30 µL/L linalool was chosen as the fumigation treatment of tomato fruits for the following experiments.

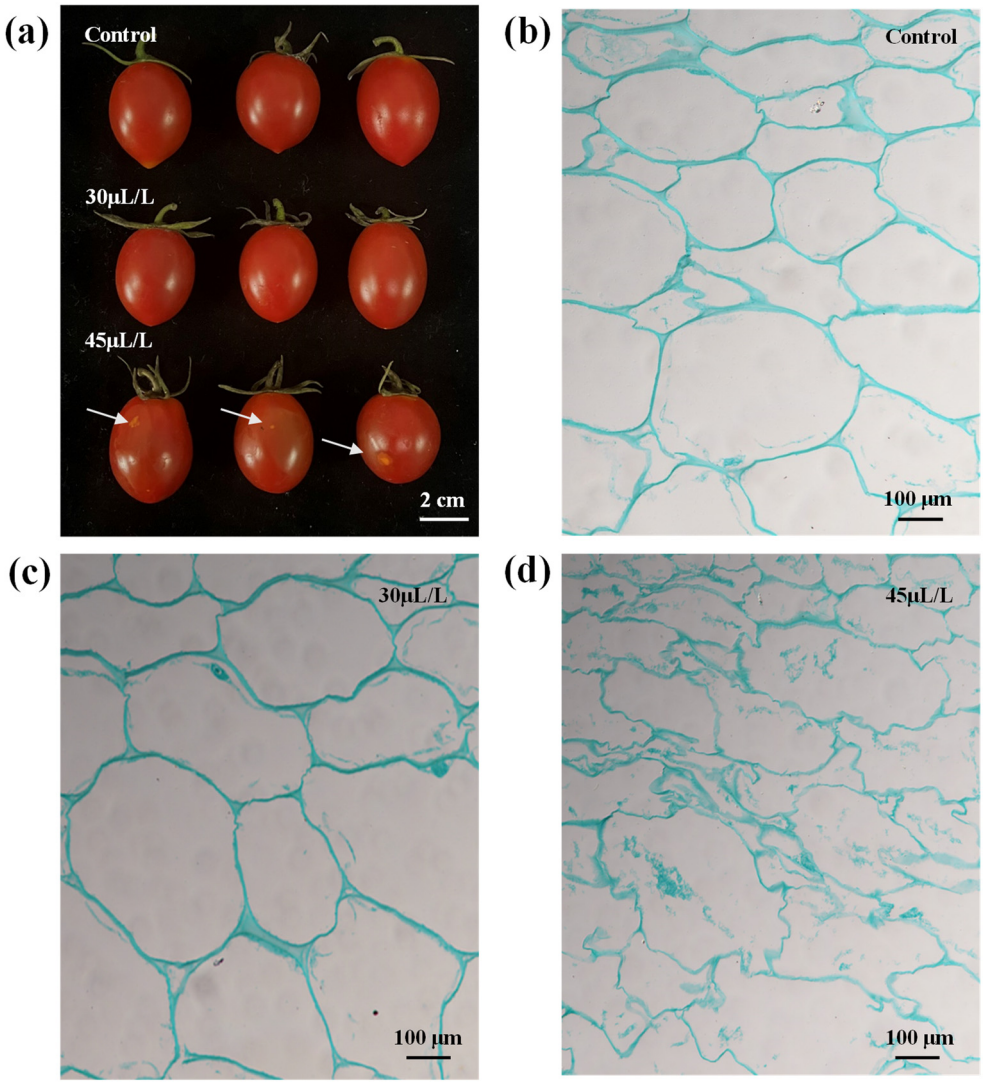

Figure 2. Screening of linalool fumigation concentrations in tomato fruits. (**a**) Phenotype of tomato fruits after fumigation with different linalool concentrations. White arrows indicate damage with 45 µL/L linalool in tomato fruits; (**b**–**d**) Cell morphology of tomato pericarp in control; (**b**) fumigated with linalool at 30 µL/L (**c**) and 45 µL/L (**d**).

3.2.2. Effects of Linalool Fumigation on the Expansion of Tomato Fruit Disease Spots

From Figure 3, it could be observed that tomato fruits showed disease symptom from 24 h after inoculation, and that the disease spot diameter grew from 1.8 mm to 18.2 mm between 0 and 72 h (Figure 3a,b). Fumigation with linalool showed obvious inhibition of disease spot diameter, which was obvious after 3 d of treatment with the L + B treatment. The disease index of the B treatment at 24 h was 44.0, while the disease index of the L + B treatment was 5.3 and the control effect was 87.9%; the disease index of the B treatment at 48 h was 84.0, while the disease index of the L + B treatment was 5.3, and the control effect was 93.7%; the disease index of the B treatment at 72 h reached 100.0, while the disease index of the L + B treatment was 8 and the control effect was 92.0% (Figure 3c). Therefore, linalool fumigation displays alleviating effects in controlling gray mold caused by *B. cinerea* in postharvest tomato fruits.

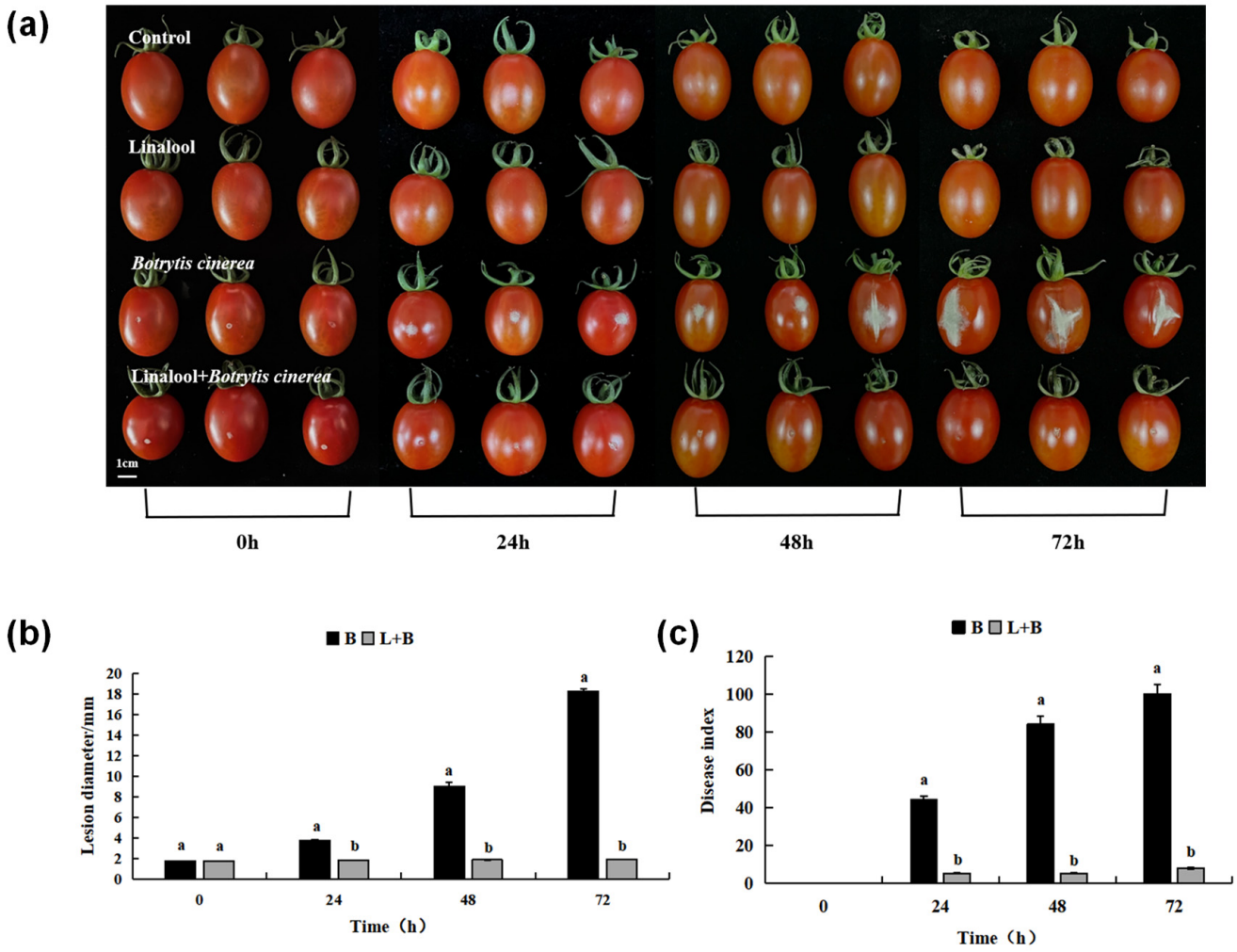

Figure 3. Effects of different concentrations of linalool on the expansion of *B. cinerea* on tomato fruits. (**a**) The expansion phenotype of tomato fruit lesions from 0 h to 72 h after linalool fumigation; (**b**,**c**) Changes in lesion diameter; (**b**) and disease index; (**c**) of tomato fruit from 0 h to 72 h after linalool fumigation. Control, without *B. cinerea* and linalool treatment; Linalool, fumigation with 30 μL/L of linalool; *B. cinerea* (B), inoculation with *B. cinerea*; Linalool + *B. cinerea* (L + B), inoculation with *B. cinerea* followed by fumigation with 30 μL/L of linalool. Data show the means of three replicates ± standard deviation (SD). The different letters indicate significant difference at $p < 0.05$.

3.3. Effects of Linalool Fumigation on Antioxidant Systems

3.3.1. Effects of Linalool Fumigation on Defense Enzymes Activities and AsA Content in Tomato Fruits

From Figure 4, it can be seen that SOD activity did not show significant differences between the control and the L treatment and that *B. cinerea* inoculation significantly inhibited SOD activity in the later period of treatment. Compared with the *B. cinerea* inoculation treatment, SOD activity was increased with the L + B treatment especially at 72 h (Figure 4a). In the process of treatment, the change trends of the CAT and POD activities were not good in agreement with that of the SOD; however, at the end of experiment, higher activities of CAT and POD were also observed with the L + B treatment (Figure 4b,c), indicating that linalool increased the antioxidant capacity of tomato fruits infected with *B. cinerea.*

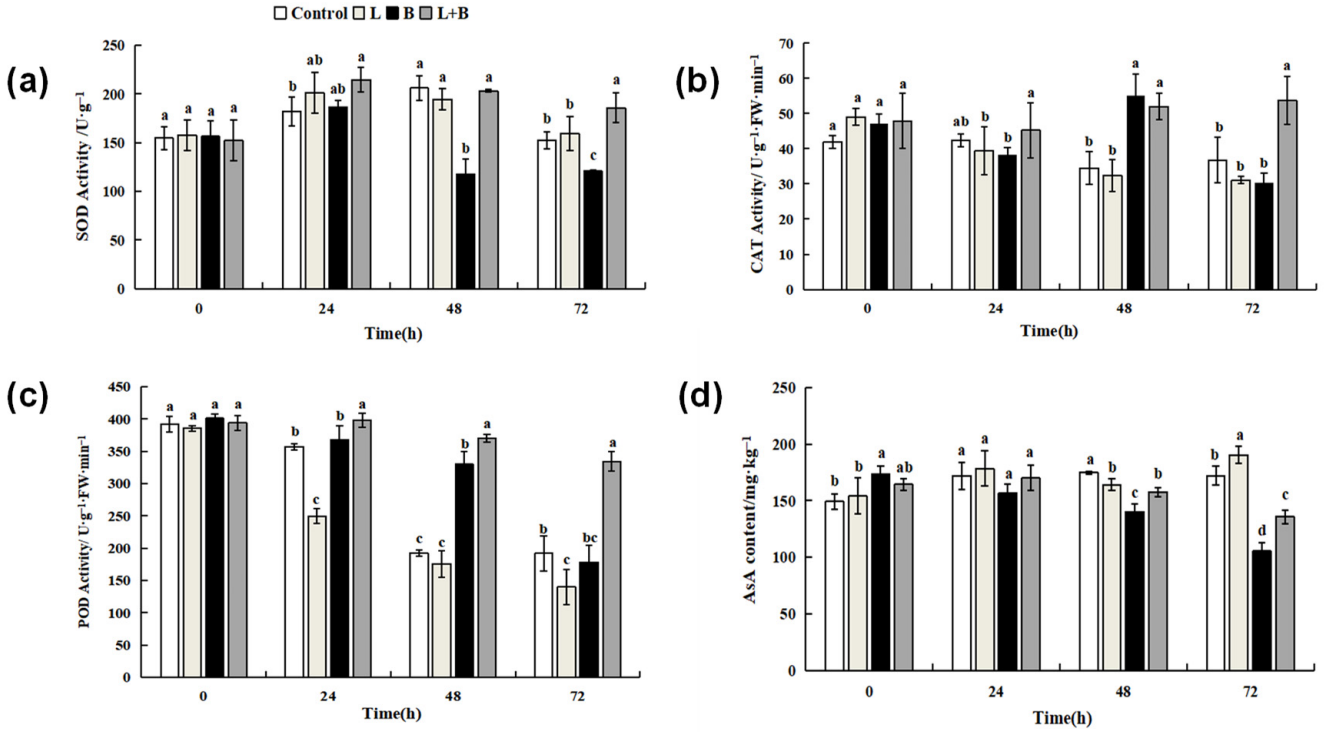

Figure 4. Effects of linalool fumigation on the activities of SOD (**a**), POD (**b**)and CAT (**c**), as well as on ASA content (**d**). Control, without *B. cinerea* and linalool treatment; L, fumigation with 30 μL/L linalool; B, inoculation with *B. cinerea*; L + B, inoculation with *B. cinerea* followed by fumigation with 30 μL/L linalool. Data show the means of three replicates ± standard deviation (SD). The different letters indicate significant difference at $p < 0.05$.

AsA is a potent reducing and antioxidant agent that functions in fighting bacterial infections and detoxifying reactions [38]. With the elongation of treatment time, *B. cinerea* significantly decreased the AsA content in tomato fruits, and linalool inhibited this decrease especially by 72 h of treatment ($p < 0.05$; Figure 4d).

3.3.2. Effect of Linalool Fumigation on Tomato Fruit MDA Content

Compared with the control, the MDA content was maintained at higher levels from 24 h to 72 h with the B treatment, and linalool fumigation dramatically decreased MDA accumulation in tomato fruits treated with *B. cinerea* (Figure 5), indicating that lipid peroxidation was decreased by linalool in tomato fruits infected with *B. cinerea*.

3.4. Effects of Linalool Fumigation on PAL and PPO Activity

The activities of PAL and PPO were not significantly different between the control and the linalool treatment over the whole period of experiment (Figure 6). The PAL enzyme activity was induced by *B. cinerea* by 24 h of treatment, and then significantly decreased by 48 h and 72 h of *B. cinerea* inoculation showing much lower levels than the control. PAL activity was increased at the later period of L + B treatment especially at 72 h, at which point PAL activity was increased by 67.7% compared to the B treatment ($p < 0.05$; Figure 6a). From 24 h to 72 h, *B. cinerea* inoculation obviously inhibited PPO activity, and linalool fumigation significantly alleviated this inhibitory effect ($p < 0.05$; Figure 6b).

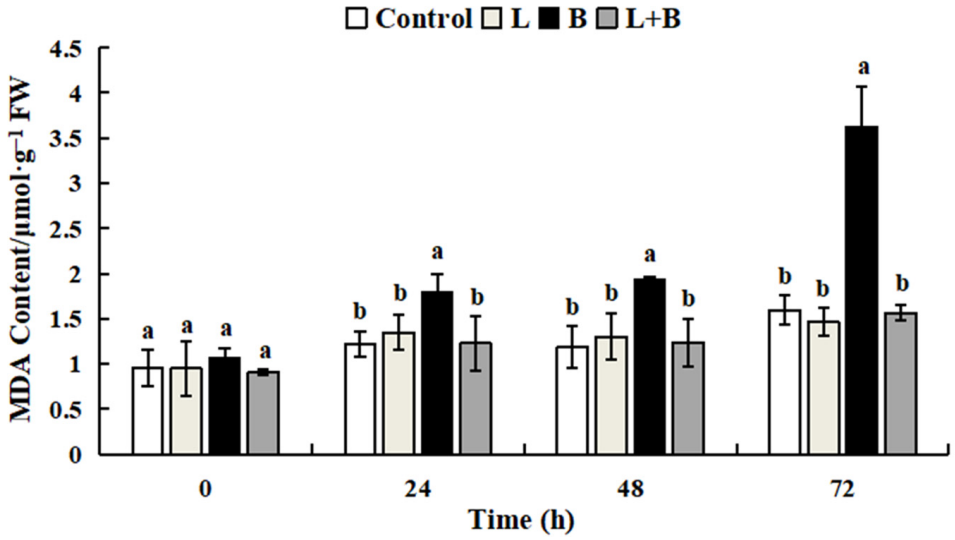

Figure 5. Effects of linalool fumigation on MDA content. Control, without *B. cinerea* and linalool treatment; L, fumigation with 30 μL/L linalool; B, inoculation with *B. cinerea*; L + B, inoculation with *B. cinerea* followed by fumigation with 30 μL/L linalool. Data show the means of three replicates ± standard deviation (SD). The different letters indicate significant difference at $p < 0.05$.

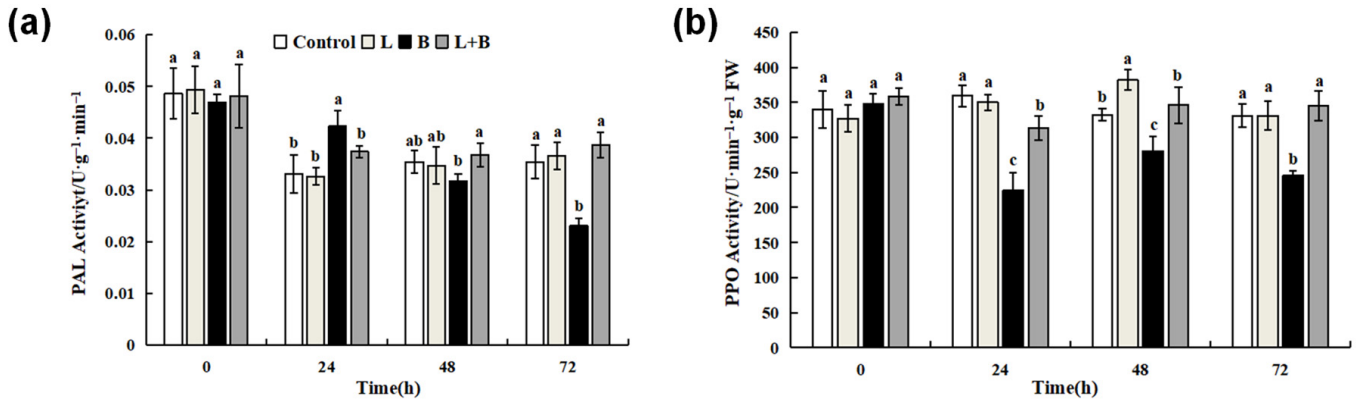

Figure 6. Effects of linalool fumigation on the activities of PAL (**a**) and PPO (**b**). Control, without *B. cinerea* and linalool treatment; L, fumigation with 30 μL/L linalool; B, inoculation with *B. cinerea*; L + B, inoculation with *B. cinerea* followed by fumigation with 30 μL/L linalool. Data show the means of three replicates ± standard deviation (SD). The different letters indicate significant difference at $p < 0.05$.

3.5. Effects of Linalool Fumigation on the Activity of Softening Related Enzymes in Tomato Fruits

In the process of tomato fruit storage, both maturation senescence and pathogen infection caused cell-wall structure destruction and softness. As shown in Figure 7, the activities of PG, CL and β-GAL related to fruit softness with both the B treatment and L + B treatment showed an increasing trend during 24–72 h after fumigation, and the fruit-softening enzyme activity with the B treatment was always higher than that of the L + B treatment. The differences between the two treatments were the greatest at 72 h, with an increase of 31.6%, 41.9%, and 12.2%, respectively (Figure 7). Keeping the cell structure intact was beneficial to the tomato fruit defense against diseases, and linalool fumigation inhibited the activity of fruit-softening enzymes which was beneficial to the suppression of postharvest tomato gray mold.

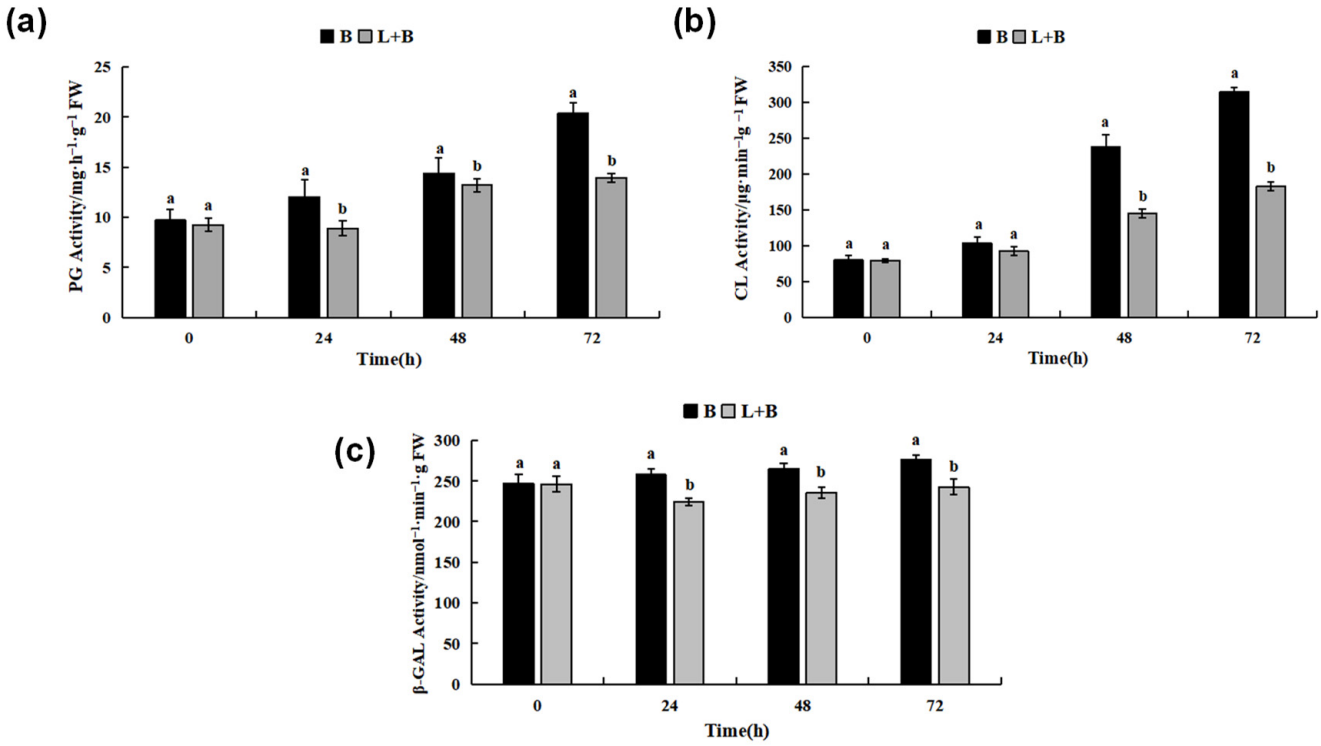

Figure 7. Effects of linalool fumigation on tomato fruit softening-related enzymes activity and lipoxygenase activity; (**a**) Changes in PG activity with two treatments after linalool fumigation for 24 h, 48 h and 72 h; (**b**) Changes in CL activity in two groups after linalool fumigation for or 24 h, 48 h and 72 h; (**c**) Changes in *β*-GAL activity with two treatments after linalool fumigation for or 24 h, 48 h and 72 h. B, inoculation with *B. cinerea*; L + B, inoculation with *B. cinerea* followed by fumigation with 30 μL/L linalool. Data show the means of three replicates ± standard deviation (SD). The different letters indicate significant difference at $p < 0.05$.

4. Discussion

In this study, it was first proved that linalool could effectively repress *B. cinerea* growth in vitro, and that this inhibitory effect had a concentration-dependent pattern. Similar results were also reported in this chemical against other pathogens [22]. However, the effective concentration was related to the pathogen species; for example, the minimum inhibitory concentration of linalool required against *L. monocytogenes* was 1.5 mL/L [39], while 45 μL/L linalool could cause *B. cinerea* to hardly grow in present study.

It has been reported that linalool could inhibit the growth of *B. cinerea* in artificial media [22]. Similar results were found in our study, where linalool showed obvious inhibitory effects on the growth of *B. cinerea* mycelia with increasing concentrations; 45 μL/L linalool had the best effect. Furthermore, the direct effect of linalool fumigation on tomato fruits was evaluated. Although 45 μL/L linalool fully inhibited the growth of *B. cinerea*, it obviously damaged the pericarp of tomato fruit. On the contrary, 30 μL/L linalool fumigation could not only control the *B. cinerea*, but also did not affect the tomato fruit pericarp structure; additionally, this concentration was suitable for the inhibition of gray mold in postharvest tomato fruits. In addition to the defense function in plants, linalool is also considered a major contributor to aroma, flavor and consumer retronasal perception in tomato and peach (*Prunus persica*) fruits [40,41], from which we speculated that appropriate linalool concentrations would have a positive effect on fruit flavor. Therefore, it is possible that linalool could be seen as a green chemical in the control of postharvest fruit gray mold in tomatoes.

However, the possible physiological mechanism of linalool decreasing the damage of tomato fruits by *B. cinerea* has not been well-investigated. It is well known that biotic stresses including *B. cinerea* infection will lead to ROS bursts and result in oxidative

stress [42]. To scavenge ROS, plants have evolved an antioxidant system including both enzymatic and non-enzymatic factors. Rahman et al. screened 81 grape genotypes for their responses to *B. cinerea*, and found that resistant genotype showed higher antioxidant enzyme activities, accompanied by reduced ROS accumulation with *B. cinerea* infection [43]. Our data showed that linalool significantly increases the activities of SOD, POD and CAT, which play important roles in scavenging O_2^- and H_2O_2 being responsible for lowering the accumulation of MDA in tomato fruits induced by *B. cinerea*. Thus, linalool might be effective in inducing disease resistant by activating antioxidant system in tomato fruit during postharvest.

As an important antioxidant substance, AsA is also involved in induced pathogen resistance in higher plants. The role of AsA in the induced-resistance process has been investigated using elicitors known for inducing the activity of the jasmonic acid (JA) signal pathway [44]. In previous researches works, it has also been observed that the exogenous application of JA up-regulates linalool synthase (LIS) gene expression and strongly promotes linalool accumulation in tomatoes and rice. Transgenic rice plants overexpressing *OsLIS* show significant antibacterial activity against *Xanthomonas oryzae pv. oryzae* (Xoo), and vapor treatment with linalool induces the expression of defense-related genes and enhances resistance to Xoo in rice [45]. Recently, Zhang et al. found that chitosan that increases the resistance of ripened fruits against *B. cinerea* also depends on its function of inducing JA production, as well as that of modulating oxidative stress [46]. Our study showed similar results, in that higher accumulation of AsA was observed in linalool-fumigated tomato fruits infected with *B. cinerea*.

Imbalances in ROS metabolism are commonly observed with both abiotic and biotic stresses, although plants can activate some specific defense responses to slow down or halt infection by pathogens. These defense mechanisms include physical and chemical barriers that interfere with pathogen infection, in which PAL and PPO are two important enzymes involved in the response [47]. PAL is the primary enzyme in the phenylpropanoid pathway, which leads to the conversion of L-phenylalanine to trans-cinnamic acid with the elimination of ammonia. It is the key enzyme in the synthesis of several defense-related secondary compounds such as phenols and lignin [48]. PPO is a nuclear-encoded enzyme that catalyzes the oxygen-dependent oxidation of phenols to quinones, and its level was increased when a plant is wounded or infected [49]. In the present study, linalool fumigation significantly alleviated the inhibition of PAL and PPO activity by *B. cinerea*, indicating that secondary metabolism was involved in linalool's regulation of tomato fruits resistance to *B. cinerea*. Similarly, Jiang et al. reported that methyl jasmonate primes defense responses against *B. cinerea* and reduces disease development in harvested table grapes, which depended on the induction of PAL and PPO activity [50]. Sun et al. found that melatonin used to combat postharvest gray mold in apple fruits is also related to the increased activities of PAL and PPO [51]. Based on the above research works, it could be concluded that PAL and PPO are the key players in fruits resistance to *B. cinerea*.

As a mechanical barrier, the plant cell wall is a complex structure restricting most pathogens' access to the flesh of fruits. A previous study showed that *B. cinerea* accelerated cell-wall degradation and promoted spike stalk browning in Munage grape [52]. The importance of host cell-wall degradation during plant-pathogen interactions is highlighted by the significant expansion of cell wall degrading enzymes (CWDEs) including PG, CL and β-GAL during microbial evolution. Rasoul et al. reported that linalool showed a direct inhibitory effect on PG activity [53]. Besides this, in plenty of strategies against pathogens in fruits, inhibition of CWDEs was effective. As a modified atmosphere preservation technology, high-CO_2 treatment prolongs the Postharvest Shelf Life of strawberry fruits by reducing decay and cell-wall degradation. The antifungal activity of thymol against the main fungi that causes pomegranate fruit rot is also due to the suppression of the activity of cell wall degrading enzymes, which include cellulase and pectinase [54]. In the present study, the activities of PG, CL and β-GAL were inhibited by linalool after *B. cinerea* infection in tomato fruits. Therefore, it was presumed that linalool could maintain cell wall structure

integrity by inhibiting the activities of PG, CL and *β*-GAL to reduce *B. cinerea* infections in postharvest tomato fruits.

5. Conclusions

In conclusion, linalool fumigation was an effective method for protecting tomato fruits against *B. cinerea* infection. The mechanism for this can be summarized as the following aspects: firstly, in vitro experiments showed that linalool fumigation severely inhibited mycelial growth suggesting that linalool kills parts of *B. cinerea* directly, inhibiting pathogens from expanding on fruits. Secondly, the activities of antioxidant enzymes (SOD, POD and CAT) and the AsA content were increased and the MDA content was reduced, indicating that linalool activates the antioxidant system, playing important roles in lowering lipid peroxidation and regulating stress signal. Thirdly, the main enzymes related to cell structure, PG, CL and β-GAL, were inhibited by linalool, suggesting that linalool can maintain cell-wall structure integrity, strengthening the physical barrier against pathogens (Figure 8). As a result, the gray mold symptom of tomato fruits caused by *B. cinerea* is distinctly alleviated by linalool fumigation.

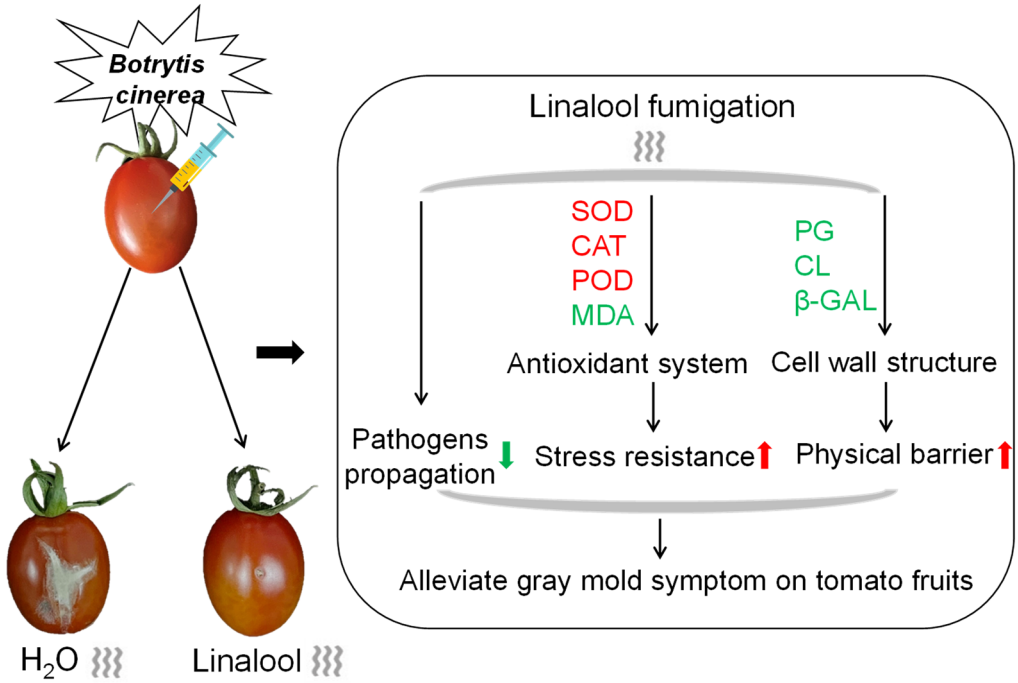

Figure 8. A working model depicting how linalool fumigation alleviates gray mold symptoms in tomato fruits. When tomato fruits suffer infection by *B. cinerea*, linalool fumigation (1) kills parts of *B. cinerea*, (2) activates the antioxidant system to improve stress resistance, and (3) inhibits the activity of softening-related enzymes to strengthen the physical barrier. As a results, a significant difference was showed in treatment with H_2O and linalool fumigation, which were named B and L + B group in this study, respectively. The gray curve represents fumigation; red letters and arrows indicate upregulated-effects; green letters and arrows indicate downregulated effects.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8111074/s1, Figure S1: Effects of different concentrations of linalool, geraniol and nerol on in vitro mycelial growth of *B. cinerea* colonies.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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