Nano-Thymol Emulsion Inhibits *Botrytis cinerea* to Control Postharvest Gray Mold on Tomato Fruit

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Abstract: Thymol is a plant-derived natural compound with antimicrobial activity. However, we have little knowledge about the application of thymol in agriculture. One of the limitations is the high volatility and low aqueous solubility of thymol. Tomato gray mold caused by *Botrytis cinerea* is one of the most devastating postharvest diseases. In this study, we prepared a nano-emulsion of thymol (named as Nano-Thy) to form a stable O/W (oil in water) microemulsion. In vitro experiments showed that Nano-Thy had antifungal activity against *B. cinerea* by inhibiting mycelial growth and spore germination. Nano-Thy induced ROS accumulation in mycelia, further leading to lipid peroxidation, cell membrane damage, and subsequent cell death. Nano-Thy significantly prevented the infection of *B. cinerea* on fresh tomato fruits. Finally, we discussed the mechanisms and their significance in controlling postharvest disease of fruit crops.

Keywords: *Botrytis cinerea*; gray mold; nano emulsion; postharvest disease; thymol

1. Introduction

Tomato is an important vegetable with the highest annual yield among vegetable crops [1]. The loss of fruit quality affects tomato storage and transportation. Mechanical damage and respiratory metabolism easily cause microbial invasion, resulting in postharvest tomato disease with fruit softening and shortened shelf life [2]. The gray mold disease caused by fungi *Botrytis cinerea* is one of the most damaging postharvest diseases of tomato fruit [3]. Postharvest diseases should be carefully managed because the fruits are usually ready to be consumed. Chemical fungicides can effectively control gray mold in field, but are not applicable for postharvest treatment of tomato fruit. First, chemical fungicide residues may threaten human health and the environment. Second, *B. cinerea* can easily develop resistance to chemical fungicides. Therefore, safe and efficient bio-approaches are needed to control the development of postharvest gray mold on tomato [4]. In addition to the application of microbial antagonists for the control of postharvest diseases of fruit crops [5,6], plant essential oils are also sustainable alternatives for the postharvest treatment of fruits. Natural phytochemicals (e.g., plant essential oils, phenolics, and flavonoids) have been widely used as food preservatives [7,8]. Exploring the ability of phytochemicals against agricultural pathogens is important for the development of approaches for the biocontrol of postharvest disease of fruit crops.

Thymol is a kind of environmental-friendly monoterpenoids phenol, the main component of essential oil from thyme species. In a medical study, the antimicrobial properties of thymol have been associated with its activity of disrupting and permeabilizing the cellular...
membrane of pathogenic microorganisms [9]. Thymol shows an inhibitory effect on some plant pathogenic fungi in seeds or leaves [10]. We also found that thymol had fungicidal activity against crop pathogens in vitro [11]. We still have little knowledge about the antifungal effect of thymol against B. cinerea. Thymol has fungicidal activity, but its application in agriculture is still limited. The antifungal activity of thymol may decrease in natural environment because of its photosensitivity, volatility, and instability. In addition, many essential oils (including thymol) have strong hydrophobicity, restricting their disperse in water for the application at field conditions [12,13]. The direct exposure of thymol may hurt plants as well [14]. Therefore, various formulations have been developing in order to improve the efficiency of using essential oils as antimicrobial agents.

Micro-encapsulation is one of the effective formulations to increase the efficiency of essential oils and to extend their application [15]. Micro-encapsulation of essential oils in the form of oil-in-water (O/W) can increase their solubility in water effectively [16]. Nanotechnology can help minimize the size of micro-encapsulation, further enhancing the bioavailability of essential oils [17]. Nano-emulsion is a microemulsion with emulsion particle size at 20–200 nm, which has high stability and good biocompatibility [18]. Spontaneous emulsification is conducive to scale-up production as it does not need high shear, pressure homogenization, ultrasonic and other processes [19]. It is a practical method for transferring hydrophobic components into aqueous phases [20]. In a nano-emulsion system, surfactants improve the stability of emulsion and reduce the particle size, which could increase the dispersibility of essential oils. It can also effectively control the release of essential oils in a microemulsion, providing a long-term control of pathogenic fungal growth. Until now, little has been known about the formulation of thymol nano-emulsion for the control of fungi causing postharvest disease in agriculture. In this study, we formulated and characterized a stable nano-emulsion of thymol (Nano-Thy). Then we investigated the antifungal activity of Nano-Thy against B. cinerea in vitro, followed by evaluating the control effect of Nano-Thy on the protection of tomato fruit from the infection of B. cinerea.

2. Materials and Methods

2.1. Preparation of Nano-Thy

Nano-Thy was prepared by spontaneous emulsification (Figure 1). Briefly, we added thymol (2% w/w), tween-80 (27% w/w), ethyl acetate (9% w/w), ethanol (2% w/w) and ultrapure water (60% w/w) under continuous stirring [21].

![Figure 1. Schematic diagram for the preparation of Nano-Thy.](image)

2.2. Characterization of Nano-Thy

2.2.1. Morphological Analysis

We dropped the diluted fresh Nano-Thy (×50) onto a copper grid for 2 min and stained with one drop of 2% phosphotungstic acid [22]. The coated grid was allowed to dry under ventilation and observed under Transmission Electron Microscope (TEM) (HT7700,
HITACHI, Tokyo, Japan). The particle size of Nano-Thy was measured with nanoparticle size potentiometer (PSS Nicomp 380 Z3000 Standard, Particulate Sensors Ltd., Newcastle, UK) [23].

2.2.2. Determination of Structure

Methylene blue and Sudan III were simultaneously dropped into fresh Nano-Thy to observe the diffusion. The O/W emulsion could be verified when the diffusion rate of Nano-Thy in methylene blue is higher than that in Sudan III, on the contrary, it would be the type of water-in-oil (W/O) [24].

2.2.3. Determination of Stability

The fresh Nano-Thy was centrifuged at 1000 rpm for 30 min to observe lamination and demulsification. It was considered to be stable if phase separation and turbidity did not occur under 100 rpm centrifugal force. The fresh Nano-Thy was incubated for cyclic alternating storage for 24 h (4 °C and 40 °C) with five cycles to observe the occurrence of separation, lamination, turbidity, and demulsification [25].

2.3. Determination of the Antifungal Activity of Nano-Thy

2.3.1. Test of B. cinerea Growth and Spore Germination

B. cinerea was cultured in PDA medium contained Nano-Thy at final concentrations of 0, 0.1, 0.2, 0.4, and 0.8 mM, respectively. Then the colony diameter was measured after cultivation at 25 °C for 5 days. Each treatment was replicated three times. The fungicidal effect of Nano-Thy was evaluated based on the inhibition of radical growth of mycelia [26].

Nano-Thy was added to B. cinerea spore suspension (1 × 10⁵ spores mL⁻¹) at final concentrations of 0.1, 0.2, 0.4, and 0.8 mM, respectively. Then, we determined the number of germinated spores and germ tube elongation at 2, 4, 6, 8, and 10 h, respectively [11].

2.3.2. Morphological Observation of the Mycelia of B. cinerea

The mycelia of B. cinerea after Nano-Thy treatment were observed using scanning electron microscopy (SEM). The mycelia were collected and washed with phosphate buffer solution (PBS, 0.2 mol L⁻¹, pH = 7.0). Then, the mycelia were fixed according to our previous publication [11], followed by observation under SEM (EVO-LS10, ZEISS, Jena, Germany).

2.3.3. Fluorescent Detection of Reactive Oxygen Species (ROS), Cell Death, and Lipid Peroxidation in Mycelia

Nano-Thy-treated mycelia of B. cinerea were collected and washed with distilled water for three times, followed by incubation in 1 µM 2',7’-dichlorofluorescein diacetate (DCFH-DA) for 30 min for the detection of endogenous ROS [27]. The mycelia were incubated at in 5 µM (propidium iodide) PI for 30 min for the detection of cell death [28]. The mycelia were incubated in 10 µM C11 BODIPY for 20 min for the detection of lipid peroxidation [29]. Then, the mycelia were washed with deionized water three times to remove additional fluorescent probes on surface, followed by photographing under a fluorescence microscope (ECLIPSE Series, TE2000-S, Nikon, Tokyo, Japan).

2.3.4. Determination of Glycerol Contents in Mycelia

The content of glycerol in mycelia was determined by copper glycerin colorimetry [30]. The mycelia (0.1 g) were ground in a mortar and resuspended with distilled water in a centrifuge tube. Then, the suspension was incubated in water bath at 80 °C for 15 min, followed with centrifuge at 8500 rpm for 10 min. The supernatant was collected and mixed with 0.05 g mL⁻¹ CuSO₄, shaking at 150 rpm for 3 min. Then, the mixture was filtered with filter paper for the measurement of absorbance at 630 nm with a spectrophotometer (UV/Vis-4802, Unico, Shanghai, China). A standard curve of glycerol (0–0.008 g mL⁻¹) was prepared to calibrate the concentration of glycerin in mycelia.
2.3.5. Determination of Relative Conductivity of Mycelia

Nano-Thy-treated mycelia (0.5 g) were washed and resuspended with 20 mL distilled water. Then the conductivity was measured at 0, 5, 10, 20, 40, 60, 80, 100, 120, 140, 160, and 180 min, respectively, with a conductivity meter (DDSJ-308F, Rex Electric Chemical, China). After 180 min, the mycelia were incubated in boiling water for 5 min and cooled to room temperature for measuring the final conductivity. The mycelial relative conductivity was calculated as follows,

\[
\text{Relative conductivity (\%)} = \left( \frac{\text{Conductivity at different times}}{\text{Final conductivity}} \right) \times 100
\]

2.3.6. Measurement of TBARS Content in Mycelia

The mycelia of \textit{B. cinerea} after treatment were ground in a mortar with 10% trichloroacetic acid, followed by centrifugation at 10,000 rpm for 30 min. Then, the supernatant was collected and mixed with 0.8% 2-thiobarbituric acid. The mixture was incubated in boiled water for 30 min, followed by centrifugation at 10,000 rpm for 5 min. Then, the mixture was measured for the absorbance at 532 nm, 600 nm, and 450 nm, respectively. The TBARS content was calculated as follows [11],

\[
\text{TBARS (\mu mol g}^{-1} \text{FW)} = [6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}] \times \frac{V}{W}
\]

2.4. Evaluation of Gray Mold in Cherry Tomato upon Nano-Thy Treatment

Fresh cherry tomatoes were surface-sterilized with 2% NaClO for 2 min. Then tomatoes were washed with sterilized water and surface dried. A total of 10 \mu L Nano-Thy was injected on the surface of each fruit. Two hours later, 5 \mu L of spore suspension of \textit{B. cinerea} (\(1 \times 10^6\) CFU mL\(^{-1}\)) were inoculated at the same spot of Nano-Thy injection in fruit. Then all the tomatoes were placed in a plastic box at 25 \degree C for 5 days to allow the infection of \textit{B. cinerea}. The final infection was evaluated by measuring the disease lesions on tomato [31].

2.5. Statistical Analysis

Each result was presented as the mean of three replicates with standard deviation (SD). The significant difference between two treatments was evaluated by SD and one-way analysis of variance at \(p < 0.05\) by using SPSS Statistics 24.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Nano-Thy Was a Stable Nano Emulsion

TEM observation showed spherical Nano-Thy without aggregations or coalescence (Figure 2a). Nano-Thy could be evenly distributed in water. The dispersing agent (Tween-80) surrounded the particles of thymol to prevent them from agglomeration. The droplet diameter of the prepared Nano-Thy was 33.45 nm (Figure 2b). The smaller emulsion droplets tend to be more resistant to destabilization.
Nano-Thy was a light yellow clear and transparent liquid. Water soluble methylene blue and oil soluble Sudan III were used to determine the structure of Nano-Thy. The time-course observation (5 s, 15 s, and 5 min) indicated that the diffusion of methylene blue in Nano-Thy was faster than that of Sudan III (Figure 3). Methylene blue, but not Sudan III, was compatible with Nano-Thy, suggesting an O/W type of Nano-Thy.

The thermodynamic stability of Nano-Thy was detected by alternant hot-cold storage, and high-speed centrifugation. Precipitation and lamination were not observed after alternant storage of Nano-Thy at 4 °C and 40 °C for 24 h. Centrifuge at 1000 rpm for 30 min failed to change the appearance of Nano-Thy. These results suggested that Nano-Thy was a stable nano emulsion.

3.2. Nano-Thy Inhibited the Growth and Spore Germination of B. cinerea

Nano-Thy significantly inhibited the mycelial growth of B. cinerea in a dose-dependent manner (Figure 4a). Compared to the control, the colony diameter significantly decreased by 7.78%, 37.1%, 59.2%, and 74.9% at 0.1, 0.2, 0.4, and 0.8 mM Nano-Thy, respectively (Figure 4b). B. cinerea growth was not affected by the solvent for the preparation of nano-emulsion. These results indicated that Nano-Thy was able to inhibit the growth of B. cinerea.

Figure 4. The effect of Nano-Thy on mycelial growth of B. cinerea. (a) The growth of B. cinerea in PDA with different concentrations of Nano-Thy for 72 h. (b) The colony diameter measured from (a). Different lowercase letters indicated significant difference among treatments (ANOVA, n = 3, p < 0.05). (c) The growth of B. cinerea in PDA with different concentrations of Nano solvent.

Then, we selected three concentrations (0.2, 0.4 and 0.8 mM) of Nano-Thy with low to high inhibitory effect of mycelial growth for the following experiments. Nano-Thy significantly hindered the germination of conidia. The conidia in control group almost finished complete germination at 6 h. We observed complete germination of conidia at 8, 10, and 10 h in the presence of 0.2, 0.4, and 0.8 mM of Nano-Thy, respectively (Figure 5a). Figure 5b showed microscopic images of conidial germination. The increase in Nano-Thy concentration led to short germinated tubes from conidia.
The effect of Nano-Thy on mycelial growth of *B. cinerea*. (a) The growth of *B. cinerea* in PDA with different concentrations of Nano-Thy for 72 h. (b) The colony diameter measured from (a). Different lowercase letters indicated significant difference among treatments (ANOVA, n = 3, p < 0.05). (c) The growth of *B. cinerea* in PDA with different concentrations of Nano solvent. Then, we selected three concentrations (0.2, 0.4 and 0.8 mM) of Nano-Thy with low to high inhibitory effect of mycelial growth for the following experiments. Nano-Thy significantly hindered the germination of conidia. The conidia in control group almost finished complete germination at 6 h. We observed complete germination of conidia at 8, 10, and 10 h in the presence of 0.2, 0.4, and 0.8 mM of Nano-Thy, respectively (Figure 5a).

The effect of Nano-Thy on conidial germination of *B. cinerea*. (a) Time-course monitoring of the germination rate of conidia in the presence of Nano-Thy at different concentrations. Different lowercase letters for each time point indicated significant difference among treatments (ANOVA, n = 3, p < 0.05). (b) Microscopic images of conidial germination at 6 h.

3.3. Nano-Thy Induced Morphological Alteration of *B. cinerea*

Observation from optical microscope showed morphological changes in the mycelia of *B. cinerea* upon Nano-Thy exposure. Compared to the control, Nano-Thy treatment resulted in smaller cells and more inclusions inside of cells (Figure 6a). Further observation with SEM showed that Nano-Thy treatment led to irregular shrinkage and loss of mycelial shape. Nano-Thy at high concentration resulted in break down and collapse of mycelial cells (Figure 6b).

The effect of Nano-Thy on the morphology of *B. cinerea* mycelia. (a) Observation of mycelium with optical microscope; (b) Observation of mycelia with SEM.
3.4. Nano-Thy Induced Oxidative Injury in B. cinerea

Total ROS was detected with specific probe DCFH-DA to emit green fluorescence. Nano-Thy induced increased fluorescence in mycelia in a dose-dependent manner, suggesting that Nano-Thy induced mycelial ROS accumulation (Figure 7a). Cell death mycelia was indicated with specific probe PI to emit red fluorescence. PI can only enter into dead cells with damaged cell membrane, but not healthy cells [32]. The mycelia showed extensive PI fluorescence with the increase in Nano-Thy concentration, indicating that Nano-Thy induced cell death in mycelia (Figure 7b).

Accumulated ROS always attack cell membrane, leading to membrane lipid peroxidation. Here we used specific fluorescent probe C11 BODIPY to detect mycelium lipid peroxidation. Nano-Thy induced extensive fluorescence in the mycelia of B. cinerea (Figure 8a). TBARS content is frequently used to indicate lipid peroxidation [33]. Nano-Thy treatment led to significant increase in mycelium TBARS content in a dose-dependent manner (Figure 8b). The membrane damage leads to the leakage of intracellular electrolyte that can be detected by relative conductivity. As expected, Nano-Thy treatment resulted in a remarkable increase in the relative conductivity of mycelia (Figure 8c). These results indicated that Nano-Thy induced lipid peroxidation and cell membrane damage in the mycelia of B. cinerea.
Nano-Thy remarkably inhibited the infection and disease lesion expansion of B. cinerea. As evidenced in Figure 9, the mycelial TBARS content significantly increased in a dose-dependent manner compared to the control group. This increase was evident at concentrations of 0.2, 0.4, and 0.8 mM Nano-Thy, with respective increases of 27.3%, 34.1%, and 47.7% in glycerol content. This result suggests that Nano-Thy exposure induced osmotic stress in the mycelia of B. cinerea.

**Figure 8.** Effect of Nano-Thy on lipid peroxidation and relative conductivity in the mycelia of B. cinerea. (a) Fluorescent detection of lipid peroxidation using C11 BODIPY; different lowercase letters indicated significant difference among treatments (ANOVA, n = 3, p < 0.05); (b) mycelial TBARS content; (c) relative conductivity of mycelia.

### 3.5. Nano-Thy Induced the Accumulation of Glycerol in the Mycelia of B. cinerea

Fungal osmotic stress can be triggered by electrolyte leakage. The content of glycerol in fungi is a typical indicator of osmotic responses [34]. Nano-Thy treatment resulted in the accumulation of mycelial glycerol content in a dose-dependent manner. Compared to the control group, the content of glycerol significantly increased by 27.3%, 34.1%, and 47.7% in mycelia exposed to 0.2, 0.4, and 0.8 mM of Nano-Thy, respectively (Figure 9). This result suggested that Nano-Thy exposure induced osmotic stress in the mycelia of B. cinerea.

**Figure 9.** The effect of Nano-Thy on the glycerol content in the mycelia of B. cinerea. Different lowercase letters indicated significant difference among treatments (ANOVA, n = 3, p < 0.05).

### 3.6. Nano-Thy Inhibited the Infection of B. cinerea on Fresh Cherry Tomato Fruit

As the above results showed anti-fungal property of Nano-Thy against B. cinerea in vitro, we next investigated the effect of Nano-Thy on B. cinerea infection on tomato fruit. Nano-Thy remarkably inhibited the infection and disease lesion expansion of B. cinerea on fresh cherry tomatoes (Figure 10a). The disease lesion diameter significantly decreased by 22.2%, 55.6%, and 73.3% in tomatoes treated with 0.2, 0.4, and 0.8 mM Nano-Thy, respectively (Figure 10b). These results indicated that Nano-Thy effectively protected tomato fruits from the infection of B. cinerea.
Agronomy 2022, 12, x FOR PEER REVIEW 9 of 12

The self-assembly nano-emulsion system we applied was also an easy-to-go approach but not the solvent, inhibited the growth of B. cinerea in vitro, suggesting the antifungal activity of thymol against B. cinerea. We previously found that the direct contact of thymol with tomato easily caused fruit softening, limiting the utilization of thymol. Here we found that Nano-Thy effectively control the infection of B. cinerea on tomatoes without showing negative effect on fruits. This may due to the O/W characteristic of Nano-Thy. Incorporating thymol into a surfactant not only avoided the direct contact between thymol and tomato fruits but also promoted the solubility and homogeneous dispersion of thymol in water. In this study, we only tested the effect of Nano-Thy on the spot infection of B. cinerea on tomato fruits. Film packaging is a kind of frequently used approach for fruit preservation. Further studies maybe needed by incorporating Nano-Thy into edible or degradable film to develop an applicable coat covering fruits, achieving both the control of postharvest disease and the preservation of the freshness of the whole fruit.

The control of gray mold on tomatoes by Nano-Thy may resulted from the antifungal activity of Nano-Thy against B. cinerea. Nano-emulsion has been proposed as a novel drug delivery approach. In this study, Nano-Thy may enhance the bioavailability of

Figure 10. The effect of Nano-Thy on the infection of B. cinerea in tomato fruit. (a) Phenotype of tomatoes; (b) measurement of disease lesion diameter on tomatoes. Lowercase letters in (b) indicated significant difference among treatments (ANOVA, n = 3, p < 0.05).

4. Discussion

Plant-derived essential oils are important resources for developing environmental-friendly pesticides [35]. Thymol is a kind of essential oil with potential of killing pathogens. The application of thymol in agriculture is limited due to its volatility and hydrophobicity. In the present study, we developed a formulation of thymol with a stable nano-emulsion characteristics using ethyl acetate and tween-80 as solvent and surfactant, respectively. Nano-Thy had good solvability in water without gathering, belonging to O/W type [36].

The O/W type helped Nano-Thy disperse in water efficiently by raising the surface area of thymol, accelerating its dissolution into aqueous phase. The homodispersity of essential oils in water can be achieved by decreasing the size of micro-encapsulation as much as possible [37]. Previous researchers have prepared submicron emulsions of thymol with diameter more than 200 nm [38,39]. In the present study, we further lowered the diameter of Nano-Thy droplet to less than 50 nm (with an average of 33.45 nm). This formulation overcame the hydrophobicity of thymol, resulting in a better homodispersity. The self-assembly nano-emulsion system we applied was also an easy-to-go approach without requiring additional sonication or shearing equipment. Both the emulsion diameter and the surfactant affect the antimicrobial activity of encapsulated essential oil [40]. Further studies are needed to develop more formulations of Nano-Thy by screening different kind of surfactants in order to enhancing the fungicidal activity of thymol.

Thymol has great potential to be utilized as a food preservative [41]. We still have little knowledge about the effect of thymol on the control of vegetable postharvest diseases. Gray mold caused by B. cinerea is one of the most devastating postharvest diseases in vegetables and fruits. In addition to storage at low temperature or modified atmosphere, using preservatives is an effective approach to control gray mold [42]. Nano-Thy, but not the solvent, inhibited the growth of B. cinerea in vitro, suggesting the antifungal activity of thymol against B. cinerea. We previously found that the direct contact of thymol with tomato easily caused fruit softening, limiting the utilization of thymol. Here we found that Nano-Thy effectively control the infection of B. cinerea on tomatoes without showing negative effect on fruits. This may due to the O/W characteristic of Nano-Thy. Incorporating thymol into a surfactant not only avoided the direct contact between thymol and tomato fruits but also promoted the solubility and homogeneous dispersion of thymol in water. In this study, we only tested the effect of Nano-Thy on the spot infection of B. cinerea on tomato fruits. Film packaging is a kind of frequently used approach for fruit preservation. Further studies maybe needed by incorporating Nano-Thy into edible or degradable film to develop an applicable coat covering fruits, achieving both the control of postharvest disease and the preservation of the freshness of the whole fruit.

The control of gray mold on tomatoes by Nano-Thy may resulted from the antifungal activity of Nano-Thy against B. cinerea. Nano-emulsion has been proposed as a novel drug delivery approach [45]. In this study, Nano-Thy may enhance the bioavailability of
thymol by *B. cinerea* to achieve fungicidal activity. Nano-Thy inhibited *B. cinerea* growth by inducing oxidative injury and cell death. Nano-Thy caused mycelial cell membrane damage indicated by morphological observation and increased relative conductivity. The antimicrobial activity of essential oils has been closely associated with the disruption of membrane integrity causing cell death [46]. Little is known about the mechanism for essential oil-mediated membrane permeability in fungal cells. Nano-Thy induced ROS accumulation, further leading to mycelial lipid peroxidation. This was one of the possible reasons of membrane injury and consequent cell death of *B. cinerea* upon Nano-Thy treatment. Fungicide can induce ROS accumulation and common oxidative damage in fungi [47], but the sources of ROS generation maybe diverse. Fungi have basic level of endogenous ROS for the regulation of fungal development and pathogenicity. However, the overaccumulation of ROS is toxic to fungi. NOX, an important generator of ROS in fungi, can be activated by external stimuli [48,49]. In addition, the fungal cell wall stress is associated with endoplasmic reticulum ROS generated from protein folding-related oxidation of Ero1 [50]. Whether Nano-Thy induces ROS accumulation through the above mechanisms requires further study.

In sum, we prepared a stable O/W type nano-emulsion of thymol (Nano-Thy). It showed fungicidal effect against *B. cinerea*, which may result from the induction of ROS accumulation and oxidative injury in mycelia. Nano-Thy also effectively prevented gray mold on tomato fruits, which may be applicable for the control of postharvest disease of fruit crops. More studies are needed to further optimize the formulation of Nano-Thy in order to enhance its antifungal activity and to develop coating film for the preservation of tomato fruits.

**Author Contributions:** Conceptualization, H.M. and L.H.; methodology, J.Z. and Y.H.; investigation, J.Z., Y.H., H.L., P.L., J.C., Z.S. and Y.X.; writing—original draft preparation, J.Z. and Y.H.; writing—review and editing, H.M. and L.H.; funding acquisition, H.M. and L.H. All authors have read and agreed to the published version of the manuscript.

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