Effect of Thymol and Linalool Fumigation on Postharvest Diseases of Table Grapes

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Abstract  Several postharvest diseases of table grapes (Vitis vinifera) occur during storage, and gray mold rot is a particularly severe disease because the causal agent, Botrytis cinerea, grows at temperatures as low as 0°C. Other postharvest diseases, such as those caused by Penicillium spp. and Aspergillus spp., also often lead to deterioration in the quality of table grapes after harvest. The use of plant essential oils such as thymol and linalool, to reduce postharvest diseases in several kinds of fruits, including table grapes and oranges, has received much attention in European countries. However, to the best of our knowledge there has been no report of the use of thymol fumigation to control gray mold in table grapes in Korea. Thymol (30 µg/mL) and linalool (120 µg/mL) significantly inhibited mycelial growth and conidia germination of B. cinerea. The occurrence rate of gray mold rot of B. cinerea and other unknown fungi was significantly reduced by fumigation with 30 µg/mL thymol in several table grape cultivars, such as Campbell early, Muscat Bailey A, Sheridan, and Geobong. In this study, fumigation with 30 µg/mL thymol, had no influence on the sugar content and hardness of grapes, but reduced fungal infection significantly. This suggests that 30 µg/mL thymol could be utilized to reduce deterioration of grapes due to gray mold and other fungal infections during long-term storage.

Keywords  Botrytis cinerea, Fumigation, Linalool, Postharvest diseases, Table grapes, Thymol

Table grapes (Vitis vinifera) are harvested from late July to the end of October in Korea. During the harvesting season, the price of table grapes often decreases because of over production. However, the market price may increase during the off-harvest season. Specifically, the demand for high-quality grapes such as Geobong (“Kyoho” in Japan) rapidly increases the price. Therefore, the Farmer’s income may increase with appropriate control of postharvest disease. The storage properties of table grapes vary depending on the cultivar. Table grapes are non-climacteric fruits that are highly sensitive to the conditions during postharvest handling, storage, transport, and marketing. Postharvest diseases in table grapes result in severe economic losses to farmers. The decrease in quality is mainly shown by weight loss, color change, and accelerated softening of the fruits. Additionally, table grapes also deteriorate due to rachis browning and the high incidence of berry decay [1-4].

The presence and long survival of conidia of Botrytis cinerea and Penicillium expansum have been reported in grapes and other fruits [5]. These well-known necrotrophic fungi affect several kinds of horticultural crops. The gray mold caused by B. cinerea is known to be the most important disease of table grapes, and uncontrolled infections result in the growth of aerial mycelium spreading rapidly to adjacent berries resulting in a severe loss of quality [6]. In order to overcome this problem, several methods have been developed such as controlled atmosphere storage [2, 7-10], modified atmosphere storage [11], sulfur dioxide (SO2) treatment, polyethylene film storage, and the use of O2 during storage [3, 12, 13]. For many years, SO2 fumigation has been recommended in combination with rapid pre-cooling, followed by storage and transportation at 0°C [14-18]. Other synthetic fungicides such as anilinopyridines were also found to be effective against B. cinerea, although fungal populations resistant to these chemicals have been reported [19]. However, storage methods are not used widely because of the need for expensive equipment and maintenance. The use of modified atmosphere packaging has been shown to maintain berry quality and to reduce decay in combination with acetic acid, chlorine gas, or a SO2-commercial generator [20-23]. However, in recent years, table grape consumers have raised concerns about possible SO2 residues that may elicit an allergic response.
The attempt to characterize the bioactive properties of essential oils has recently gained much attention in many pharmaceutical and food-processing applications [24]. In foods, the use of essential oils or their individual components, as potential natural preservatives has been reported in cheese [25], bakery products [26], and meat [27], although there is little evidence of a role for them in the control of fruit decay. Essential oils containing high amounts of thymol and carvacrol were reported to possess the highest antioxidant activity [28-32]. In addition, these compounds exhibit other bioactivities, for example, thymol has antiseptic, antibacterial, antifungal, antioxidative, and food preservative properties [33], while carvacrol possesses antifungal properties [34]. Thymol is the major phenolic constituent of thyme oils [35]. Thymol, as a plant essential oil, is generally regarded as a safe compound by the U.S. Food and Drug Administration (FDA) and Environmental Protection Agency (EPA), and was exempt from the requirement of a tolerance by the EPA for application on edible agricultural products (40 CFR Part 180, 6 June 2003) [36]. The mechanism of the antifungal activity of linalool is not known. It is speculated that the enantioselectivity [37] of the compound may regulate the inhibition of 7-1-3 glucan or chitin synthesis in the fungal cell wall.

In Korea, postharvest treatment is not yet in the case of table grapes. There are some storage containers used for delayed distribution during the postharvest season, where the table grapes are maintained at 0.5 ± 1°C. If table grapes are maintained without any significant loss by fungal decay with postharvest treatments, farmers could get better profit through delaying the market period by cold chamber storage during the overproduction season. The objectives of the present study were to determine the inhibitory concentration of thymol on mycelial growth and conidia germination of postharvest pathogens. This study was extended to investigate the effect of thymol on the development of postharvest disease in table grapes.

**MATERIALS AND METHODS**

The effect of different concentrations of thymol and linalool on inhibition of *Botrytis cinerea*. Mycelial plugs (3 mm in diameter) of *B. cinerea* KB and GB were transferred onto PDA. The plates were stored upside down after thymol (30 μg/mL), linalool (120 μg/mL) (Sigma-Aldrich) were dropped onto paper disks on the cover plate. The plates were incubated at 24°C until the mycelium completely covered the control plate, as determined by the diameter of the mycelium.

Comparison of the effects of thymol and linalool on fungal growth inhibition. Mycelial plugs (3 mm in diameter) of *B. cinerea* KB and GB were transferred onto PDA. The plates were stored upside down after thymol (30 μg/mL), linalool (120 μg/mL) (Sigma-Aldrich) were dropped onto paper disks on the cover plate. The plates were incubated at 24°C until the mycelium completely covered the control plate, as determined by the diameter of the mycelium.

Inoculum preparation of *Botrytis cinerea*. *B. cinerea* (GB and KB) were grown under darkness for 3 days and UV (200~290 nm) for 1 day, followed by 4 days of incubation on V-8 juice agar at room temperature (20 ± 2°C). Next, conidia obtained from the plates were suspended in 0.5% tween 20 at the concentration of 1.4 × 10³ conidia/mL.

Inoculation of table grape cultivars. The grape berries were cut from the branch using sterilized experimental scissors, without any wound to the surface of berries. The cut berries were inoculated with conidia suspension (10⁶ conidia/mL) by immersing the base of the berry, where the stalk was attached, in conidial suspensions.

Thymol fumigation of *B. cinerea*-inoculated and naturally infested table grapes inside plastic containers. Plastic 2.1 L containers (large size ziploc; Korea Johnson Co. Ltd., Seoul, Korea) were rinsed with 70% ethanol. Campbell early, Muscat Bailey A (MBA), Sheridan, and Geobong (Kyoho in Japanese) varieties were used. Thirty berries of cv. Geobong and 40 berries of each of the remaining cultivars were used to fill the plastic containers. The edges of the plastic containers were greased before the lids were replaced to make sure each was airtight after thymol (30 μg/mL) was dropped onto filler papers (Whatman 42 cat. No. 1055; Whatman International Ltd., Kent, UK). Grapes were stored for 7 days inside a plastic container. The thymol disks were taken out 7 days after fumigation and the plastic containers of grapes were stored for 30 days in a refrigerator set at 1 ± 1°C. The berries of Campbell early and Geobong cv. in the plastic containers were further incubated at 24°C for 7-30 days after storage to determine fungal infection.

In case of naturally infested table grapes in the experiment, only two cultivars of Campbell early and Geobong cv. in the plastic containers were used due to the availability of grape cultivars produced, and the table grapes were stored for 60 days after fumigation. However, all other treatments were consistent with those used to inoculate table grapes with *B. cinerea*.

Linalool fumigation of naturally infested grapes inside plastic containers. Thirty berries taken from cultivars Campbell early, Sheridan, and MBA, were placed inside the plastic containers as described previously. The grapes were treated with 120 μg/mL linalool fumigation for 7 days and stored at 1 ± 1°C for 30 days. Each treatment was replicated.
four times. Linalool fumigation of grapes inoculated with *B. cinerea* (KB and GB) could not be conducted because the table grapes were out of season during the experimental period. Furthermore, 120 μg/mL linalool resulted in a strong odor during the experiment, so was not chosen for use in the further experiment investigating the storage of table grapes for 60 days.

**Statistical analysis.** All data were analyzed using one-way analysis of variance (ANOVA), and *t*-test and Fisher’s least significant difference method were applied to determine differences among the means with significance set at *p* ≤ 0.05. Data were analyzed using Sigma Stat ver. 2.0 (Jandel Engineering Ltd., Beds, UK). Data were arcsine transformed when percentages were analyzed [38].

**RESULTS**

The effect of different concentrations of thymol and linalool on fungal inhibition. *B. cinerea* (GB), 30 μg/mL thymol and 120 μg/mL linalool were able to completely inhibit mycelial growth. Mycelial growth was significantly reduced by fumigation with 30 μg/mL thymol and 120 μg/mL linalool, compared with 9 cm of growth (in diameter) and 71.4% of conidial germination of the control plate (*t*-test, *p* = 0.05). Ethanol had a small inhibitory effect on mycelial growth and 71.4% of conidial germination of the control plate (*t*-test, *p* = 0.05). Data were analyzed using Sigma Stat ver. 2.0 (Jandel Engineering Ltd., Beds, UK). Data were arcsine transformed when percentages were analyzed [38].

The effects of thymol and linalool on mycelial growth and conidia germination in *B. cinerea* (KB), showed almost the same trend as that observed in *B. cinerea* (GB) in terms of fungal inhibition (Tables 1 and 2).

**Comparison of the inhibitory effects of thymol and linalool on inhibition of fungal growth.** Linalool at 120 μg/mL showed better control against mycelial growth and conidia germination, but this concentration resulted in a strong odor. Due to this, thymol at 30 μg/mL was subsequently used to further control the Gray mold rot of table grapes in further experiments. This is because it was evident that 30 μg/mL thymol and 120 μg/mL linalool provided good protection against mycelial growth and conidia germination. The effect of 30 μg/mL thymol on mycelial growth was determined to be optimal following PDA analysis. *B. cinerea* KB and GB exhibited some growth at 20 μg/mL thymol, but growth was inhibited at 30 μg/mL (Table 2).

**Thymol fumigation of the *B. cinerea*-inoculated table grapes inside plastic containers.** Fungal infection of *B. cinerea* from inoculated table grapes stored without thymol treatment for 30 days at 1 ± 1°C were 4.4%, 13.8%, 1.9%, and 2.5% for Campbell early, MBA, Sheridan, and Geobong, respectively. In contrast, thymol treatment at a final concentration of 30 μg/mL resulted in a significant reduction of fungal infection of *B. cinerea*, which were 1.3%, 5.6%, 0%, and 1.7% for Campbell early, MBA, Sheridan, and Geobong cvs., respectively. An unknown fungal infection that showed only white hyphae was also identified during storage. The Geobong cultivar exhibited severe infection by unknown species in the absence of thymol treatment, which was 64.2%, followed by 39.4% of MBA, and 10.5% of Campbell early. However, thymol significantly reduced fungal infection to 29.2%, 39.4%, and 10.5% for Geobong, MBA, and Campbell early, respectively. However, the unknown fungal infection in 19.2% of Sheridan grapes treated with thymol, was not reduced compared to that (19.4%) of the control. *Penicillium* infections of the

| Fungus         | Treatment (μg/mL) | Potato dextrose agar (mycelial growth in diameter) |
|---------------|------------------|--------------------------------------------------|
| Isolate from  |                  |                                                  |
| Campbell early | Control          | 9.0 ± 0.00a                                      |
|               | Thymol 20        | 0.3 ± 0.14b                                      |
|               | Thymol 30        | 0b                                               |
|               | Linalool 120     | 0b                                               |
| Geobong       | Control          | 9.0 ± 0.00a                                      |
|               | Thymol 20        | 0b                                               |
|               | Thymol 30        | 0b                                               |
|               | Linalool 120     | 0.3 ± 0.14b                                      |

*Values are presented as mean ± standard error of the mean.
'Means followed by same letters are not significantly different among treatments within columns of each isolate (least significant difference, *p* = 0.05).

Table 1. Effect of thymol and linalool on mycelial growth and conidia germination of *Botrytis cinerea* (KB) isolated from table grape, cv. Campbell early

| Essential oil | Conc. (μg/mL) | B. cinerea (KB) |
|---------------|--------------|----------------|
| Linalool      |              |                |
| Control       | 9.0 ± 0.00a  | 86.7a          |
| EtOH          | 9.0 ± 0.00a  | 53.3b          |
| 30            | 5.1 ± 0.07b  | 26.6c          |
| 60            | 4.2 ± 0.54c  | 0d             |
| 120           | 0d           | 0d             |
| 240           | 0d           | 0d             |
| 480           | 0d           | 0d             |
| Thymol        |              |                |
| Control       | 8.5 ± 0.55a  | 86.7a          |
| EtOH          | 7.1 ± 0.39b  | 26.7b          |
| 30            | 0c           | 0c             |
| 60            | 0c           | 0c             |
| 120           | 0c           | 0c             |
| 240           | 0c           | 0c             |
| 480           | 0c           | 0c             |

*The concentration of ethanol as a dilution reagent was 70%.
*Values are presented as mean ± standard error of the mean.
'Means followed by same letters are not significantly different among different concentrations within columns of each treatment (least significant difference, *p* = 0.05).
control were 0% of Campbell early, 0.6% of Sheridan, 4.4% of MBA, and 10.8% of Geobong. However, no disease developed following thymol treatment (Table 3).

Thymol significantly reduced postharvest fungal infection. However, overall, it had no significant effect on sugar content and hardness except for cv. Geobong (t-test, p = 0.05). The sugar content of untreated grapes ranged from 0.4~0.8 kg, and from 0.7~0.8 kg for non-treated grapes, respectively, depending on the cultivar.

**Thymol fumigation of naturally infested table grapes inside a plastic container.** Significant reductions of postharvest disease development were observed in the naturally infested thymol-treated grapes stored for 60 days. In this condition, 0% fungal infection occurred. However, the thymol-treatment control plates were infected with *B. cinerea* at 0.6%, unknown fungi 3.7%, and *Penicillium* spp. 3.0% from cv. Campbell early, respectively, and as *B. cinerea* at 18.3%, unknown fungi 26.7% and *Penicillium* spp. 0.8% from cv. Geobong, respectively. These table grapes were harvested in early September 2006 (Table 4).

Thymol treatment did not influence sugar content and hardness (t-test, p = 0.05). Sugar content and hardness of non-inoculated thymol-treated Geobong grapes stored for 60 days was each 13.1° Brix and 0.8 kg, respectively, and 13.4° Brix and 0.8 kg for non-treated grapes, respectively. The sugar content and hardness of non-inoculated Campbell early grapes was each 13.2° Brix and 0.7 kg, respectively, in non-treated control, and 13.0° Brix and 0.7 kg, respectively, following thymol treatment.

**Linalool fumigation of naturally infested table grapes inside plastic containers.** The incidence of *B. cinerea*, *Penicillium* spp. and unknown spp. infections were 20.8%,

### Table 3. Effect of thymol on the postharvest disease incidence from the *Botrytis cinerea*-inoculated table grapes stored for 30 days after 7 days of thymol treatment

| Cultivar   | Treatment | Fungal infection (%) |       |       |       |
|------------|-----------|----------------------|-------|-------|-------|
|            |           | *B. cinerea*         | Penicillium spp. | Unknown spp. | Total  |
| Campbell early | Control   | 4.4 ± 1.18a          | 0a    | 10.5 ± 1.32a | 14.9a  |
|             | Thymol    | 1.3 ± 0.29b          | 0a    | 0b    | 1.3b  |
| MBA        | Control   | 13.8 ± 0.65a         | 4.4 ± 0.48a | 39.4 ± 2.90a | 57.6a  |
|            | Thymol    | 5.6 ± 0.63b          | 0b    | 4.2 ± 0.75b | 9.8b   |
| Sheridan   | Control   | 1.9 ± 0.48a          | 0.6 ± 0.25a | 19.4 ± 1.18a | 21.9a  |
|            | Thymol    | 0b                   | 0b    | 19.2 ± 1.68b | 19.2b  |
| Geobong    | Control   | 2.5 ± 0.48a          | 10.8 ± 1.03a | 64.2 ± 1.93a | 77.5a  |
|            | Thymol    | 1.7 ± 0.29b          | 0b    | 29.2 ± 1.75b | 30.9b  |

1Experiment was conducted using plastic containers, size of 2.1 L (Large size ziploc, KOREA JOHNSON Co. Ltd., Seoul). The berries were treated with 30 µg/mL of thymol. There were 5 replications per treatment and each replication had 40 berries for Campbell early and 30 berries for Geobong, respectively.

2Values are presented as mean ± standard error of the mean.

3Most of unknown fungi grown as mycelium only on berries turned out to be *B. cinerea* (approximately nine of 10 fungi) when the fungi were grown on potato dextrose agar at 25°C.

4Means followed by same letters are not significantly different between treatments within cultivars of each column (t-test, p = 0.05).

### Table 4. Postharvest disease incidence of naturally infested table grapes stored for 60 days after treated with 7 days of thymol fumigation

| Cultivar   | Treatment | Fungal infection (%) |       |       |       |
|------------|-----------|----------------------|-------|-------|-------|
|            |           | *Botrytis cinerea*   | *Penicillium* spp. | Unknown spp. | Total  |
| Campbell early | Control   | 0.6a                 | 3a    | 3.7a  | 7.3a  |
|             | Thymol    | 0a                   | 0b    | 0b    | 0b    |
| Geobong    | Control   | 18.3a                | 0.8a  | 26.7a | 45.8a |
|            | Thymol    | 0b                   | 0b    | 0b    | 0b    |

1Experiment was conducted using plastic containers, size of 2.1 L (Large size ziploc, KOREA JOHNSON Co. Ltd., Seoul). The grape was treated with 30 µg/mL of thymol. The disease incidence rate by different fungi after 2 months of storage in the refrigerator set at 2 ± 1°C. There were 5 replications per treatment and each replication had 40 berries for Campbell early and 30 berries for Geobong.

2When each berry is infected with different kinds of fungi, the infection rate for each fungi was determined.

3Most of unknown fungi grown as mycelium only on berries turned out to be *B. cinerea* (approximately nine of 10 fungi) when the fungi were grown on potato dextrose agar at 25°C.

4Means followed by same letters are not significantly different between treatments within cultivars (t-test, p = 0.05).
sulfur dioxide (SO₂) releasing pad is used commercially to reduce postharvest fungal infection of table grapes. A thymol fumigation could be a very successful treatment to The sugar contents of the untreated grapes were 12.1 to that observed at 120 \( \mu \text{g/mL} \) was 0.6 kg, 0.6 kg, and 0.9 kg for linalool Campbell early, Sheridan, respectively. Hardness of the untreated grapes was 0%, 0%, 4.2% in MBA, and 0%, 0%, 19.2% in Sheridan, respectively, at 30 days of storage after fumigation with 120 \( \mu \text{g/mL} \) linalool (Table 5).

The sugar content and hardness was not affected by treatment in this study resulted in residual odor much stronger than that from thymol (30 \( \mu \text{g/mL} \)). The antifungal activity of thymol against \( B. \text{cinerea} \) was observed in the present studies, both in vitro and in vivo. The growth of the isolates from both Campbell early and Geobong, that is, \( B. \text{cinerea} \) KB and GB, were significantly inhibited with the essential oils of 30 \( \mu \text{g/mL} \) thymol and 120 \( \mu \text{g/mL} \) linalool. It was obvious that thymol at 30 \( \mu \text{g/mL} \) was more effective at inhibiting fungal growth compared to linalool, and so thymol fumigation was further studied for the control of postharvest disease in our study, over a longer storage period of 60 days. The inhibitory effect of thymol on mycelial growth and conidial germination was significant on PDA treated with 250 \( \mu \text{g/mL} \) thymol dissolved in dimethyl sulfoxide, resulting in 48.2% reduction in the rate of mycelial growth and 2% of conidial germination of \( B. \text{cinerea} \) [40]. However, our study showed that 30 \( \mu \text{g/mL} \) thymol could completely inhibit mycelial growth and conidial germination. This difference might be because the method to treat \( B. \text{cinerea} \) with thymol in previous studies was different, such as the way in which thymol was incorporated into PDA [40]. In the present study, \( B. \text{cinerea} \) was exposed to thymol fumigation dissolved in 70% ethanol. Furthermore, the authors also reported that thymol was more effective in fungal inhibition than was linalool [40]; a result that was similar to that observed in the present study. In addition, thymol could inhibit mycelial growth and conidial germination of other fungi such as \( \text{Rhizoctonia solani, Alternaria mali, Phytophthora capsici} \) [40]. It was also reported that thymol could inhibit radial growth of \( \text{Penicillium digitatum} \) by 100% compared to the control at 200 \( \mu \text{g/mL} \) [41].

In this study, thymol fumigation significantly reduced \( B. \text{cinerea} \) infection on naturally infested or artificially inoculated grapes with conidia following 30 or 60 days of storage at 1 ± 1°C. Total fungal infection caused by \( B. \text{cinerea} \) and other fungi was often higher in cv. Geobong than that in cv. Campbell early, except in one experiment, implying that Campbell early might be more resistant to \( B. \text{cinerea} \).
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cineræa and other fungal infections. Liu et al. [42] reported that fogation with thymol at 30 μg/mL reduced the incidence of Botrytis rot from 35% in untreated cherry fruit to 0.5%. Furthermore, 50 μg/mL thymol in an aqueous solution significantly reduced the infection rate to 15.5% in strawberry fruits from 43.5% in the untreated control, when vaporized inside the container [32]. Considering that the postharvest fungal infection of MBA and Sheridan cultivars was higher than that of Campbell early, those table grape cultivars were speculated to be more susceptible to total fungal infection like cv. Geobong.

The data presented in this study suggest that the use of thymol inside storage containers can effectively reduce fungal decay caused by B. cinerea, Penicillium spp., and other fungi in table grapes during long-term cold storage. Thymol could be commercialized for the treatment of postharvest disease of table grapes, and possibly other agricultural products, considering that thymol can be purchased at just $14.00 per 1 kg [36].
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