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Article

Antifungal activity of lemongrass and thyme essential oils and effect on gray mold control and postharvest quality of 'Italia' grape

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ABSTRACT: The aim of this study was to test the hypothesis that lemongrass and thyme essential oils (EOs) reduce *Botrytis cinerea* development and control gray mold in 'Italia' grapes. The fungitoxicity evaluation was performed by EOs direct contact with the pathogen, in culture medium, and by exposure to the EOs volatile phase. Individualized rachis berries were inoculated by subcuticular injection of a conidia suspension and after 4 hours sprayed with the EOs of lemongrass and thyme (100 to 1,000 mg·L^{-1}), or oils blend (500 mg·L^{-1} thyme + 100 mg·L^{-1} lemongrass), and then stored at 25 °C / 75% relative humidity. To verify the possibility that the EOs protect the fruit against *B. cinerea*, the berries were sprayed with 400 mg·L^{-1} of each EOs or with the blend (200 mg·L^{-1} thyme + 200 mg·L^{-1} lemongrass), and after 24 hours inoculated with *B. cinerea*. On clusters, thyme (800 mg·L^{-1}) and the blend (500 mg·L^{-1} thyme + 100 mg·L^{-1} lemongrass) were sprayed 4 hours after inoculation and then stored at 25 and 1 °C. The disease incidence and severity were analyzed, as well as the fruit quality attributes. EOs had antimicrobial effect, *in-vitro*, incorporated into the culture medium or by volatilization against *B. cinerea*. On detached berries, the thyme and oil blend reduced the gray mold severity when inoculated 4 hours before spraying. In clusters, thyme at 800 mg·L^{-1} significantly reduced the gray mold development only in fruits kept at 1 °C, without impairing the clusters quality, which could be an alternative for the disease management in postharvest.

Key words: *Vitis vinifera, Cymbopogon citratus, Thymus vulgaris, Botrytis cinerea*, cold storage.

INTRODUCTION

Gray mold, caused by *Botrytis cinerea* Pers., is one of the most important postharvest diseases of table grapes. In the postharvest, during clusters storage and transport, generating sulfur dioxide sachets (SO_2) are commonly used to control this rot. However, the use of synthetic fungicides and sulfur dioxide is not allowed on organic grapes (Rommanazzi et al. 2012). Considering the absence of phytosanitary products registered for disease postharvest control in table grapes in Brazil, as well as the restrictions on the use of sulfur dioxide, control alternatives have been sought, notably those that are safer for the consumer's health and with low environmental impact.

In this sense, it has been reported the constant search for alternative methods of disease control that can reduce or replace the use of fungicides and prolong the fruits storage period. Among these methods, the use of natural antimicrobial compounds such as thyme essential oil could be a promising strategy (Ardakani and Mostofi 2019).

Essential oils are volatile aromatic compounds extracted from plant materials by hydrodistillation or pressing processes and have been used as alternative to fungicides on the control of postharvest diseases. Considered as generally recognized as safe (GRAS) compounds, essential oils show an advantage when compared to other GRAS, such as sodium bicarbonate and metabisulphite, *i.e.*, ease of handling and use, low cost, and wide availability (Kalupahana et al. 2020).

Positive results have been reported in studies that evaluated the effects of essential oils on the development of postharvest pathogens. Combrinck et al. (2011) investigated the *in-vitro* use of essential oils against several fungi, isolated from various postharvest fruits, and found that thyme essential oil at 1,000 $\mu L \cdot L^{-1}$ totally inhibited *Lasiodiplodia theobromae, Colletotrichum gloeosporioides, Alternaria citri*, and *B. cinerea*. Anaruma et al. (2010) determined the activity of 28 essential oils against *C. gloeosporioides*, and found that the *Cymbopogon citratus* essential oil was efficient in controlling the pathogen and anthracnose on passion fruit postharvest.

Sellamuthu et al. (2013) evaluated the effects of the volatile phase of thyme essential oil against *C. gloeosporioides* and observed anthracnose reduction in avocados. The authors suggested that the effects of thyme oil are due to the elicitation of biochemical defense responses in the fruit. Yan et al. (2021) reported that essential oils from lemongrass, thyme, and oregano inhibited the mycelial growth of *B. cinerea*. It was also found that exposing strawberries to essential oil vapors significantly reduced gray mold.

Thus, the aim of this study was to test the hypothesis that lemongrass and thyme essential oils reduce *B. cinerea* development and exert a positive effect on the control of gray mold in 'Italia' grapes.

MATERIALS AND METHODS

Pathogen and essential oils

Botrytis cinerea isolate used in this study (CNPUV 58) belongs to a collection maintained by the Brazilian Agricultural Research Corporation (EMBRAPA) and was isolated from 'Cabernet Sauvignon' vine, from Bento Gonçalves/RS, Brazil.

The evaluated essential oils (EOs) were lemongrass (*C. citratus*: 44.4% geranial and 33.5% neral) and white thyme (*Thymus vulgaris*; 46.6% thymol and 38.9% ρ-cymene), purchased from Laszlo (Belo Horizonte, Brazil).

In-vitro antifungal activity of essential oils by contact

Essential oils were initially weighed on a precision scale and diluted in sterile distilled water with Tween80 surfactant agent (0.05 mL) and then incorporated into the potato dextrose agar (PDA) culture medium at 45 °C, in order to obtain final concentrations of 100, 200, 400, and 800 $mg \cdot L^{-1}$. As a control, sterile distilled water with Tween80 was used.

After culture medium solidification, a mycelium disc (7 mm in diameter), removed from the edge of seven days old *B. cinerea* colony, was placed in the center of each Petri dish (90 × 15 mm). The plates were kept in an incubator at 25 °C for five days.

Two measurements diametrically opposite were performed to evaluate the colony diameter. Assessments were performed daily, with the aid of a digital caliper, until one treatment reached the plate edge. The obtained data was used to calculate the mycelial growth inhibition by Eq. 1:

$$MGI\ (\%) = ((dc - dt)/dc) \times 100 \quad (1)$$

where: MGI: mycelial growth inhibition; dc and dt: mycelial growth diameter on control and treated Petri dishes, respectively, according to Soylu et al. (2010).

The mycelial growth velocity index was obtained using Eq. 2:

$$MGVI\ (mm \cdot day^{-1}) = \sum (D - Da) \cdot N^{-1} \quad (2)$$

where: MGVI: mycelial growth velocity index; D: diameter current average of the colony; Da: average diameter of the colony from the previous day; N: number of days after inoculation, according to Lorenzetti et al. (2011).

The experimental design was completely randomized with six repetitions and one plate per plot. In order to evaluate the fungicidal or fungistatic effect promoted by the EOs, after the end of the previous test, the pathogen mycelium discs, which did not grow after the controls reached the edge of the plates, were transferred to a new potato dextrose agar (PDA) culture medium and evaluated for mycelial growth for four days.

In-vitro antifungal activity of essential oils volatile compounds

Bipartite Petri dishes (90 × 15 mm) were used to evaluate the antifungal activity of EO volatiles. An aliquot (10 and 20 µL) of lemongrass and thyme oils, and a blend with 10 µL of each oil were placed on a disc of sterilized filter paper (19 mm in diameter), disposed on one side of the Petri dish. In the other compartment, a *B. cinerea* mycelium disc (7 mm in diameter) was deposited on the PDA culture medium. Sterile distilled water on sterile filter paper was used as a control.

The concentration in the volatile phase of the OEs was calculated by the free area occupied by them, using the plate dimensions and the volume of the aliquot (10 and 20 µL) for each oil, as to approximately 180 and 360 $\mu L \cdot L^{-1}$ of air, respectively (Sellamuthu et al. 2013).

The plates were sealed and placed in incubator at 25 °C for three days. Daily, the *B. cinerea* mycelium growth was measured with the aid of a digital caliper, until the control colony reached the plate edge. The experimental design was completely randomized, with six repetitions per treatment, with one plate per plot. The data were used to calculate the MGI (%) and the MGVI ($mm \cdot day^{-1}$), as described previously.

Curative and protective effect of essential oils on *Botrytis cinerea* control in 'Italia' grape berries

The 'Italia' grape clusters, from Pilar do Sul and Jales regions, SP, Brazil, were purchased in Campinas Wholesale Market (CEASA), SP, Brazil. At the laboratory, clusters were selected by the absence of defects and rots. The berries obtained from the clusters equatorial region were individualized from the rachis and superficially disinfected with sodium hypochlorite (200 $\mu g \cdot mL^{-1}$ chlorine) for 1 min and then rinsed in distilled water and air dried on filter paper. The inoculum was prepared by the addition of sterile distilled water on the pathogen colony, and the conidia were dislodged. The obtained suspension was filtered through gauze, and the spore number adjusted to 10^5 $conidia \cdot mL^{-1}$ in a hemocytometer.

The inoculation sites were previously identified with a pen, and inoculation occurred by the deposition of 10 µL of the pathogen conidia suspension on a small wound (2 mm depth) performed with the aid of a chromatography syringe (Hamilton, 100 µL).

Essential oils curative effect on *B. cinerea* development was evaluated by spraying individualized berries with thyme and lemongrass at 100, 200, and 400 $mg \cdot L^{-1}$, 4 hours after pathogen inoculation. As a control, untreated berries (blank control) or water and Tween80 sprayed berries were used. The oil solutions were applied to the run-off point. After air dried, the berries were placed in acrylic boxes (11.50 × 11.50 × 3.50 cm), sealed with a tape, and kept in an incubator at 25 °C and 75% relative humidity (RH). Berries were daily assessed for incidence (number of berries presenting rot symptoms) and severity (lesion diameter), for nine days.

Considering the obtained results, another evaluation was carried out to assess higher concentrations of thyme EO (600, 800, and 1,000 $mg \cdot L^{-1}$) and a blend (500 $mg \cdot L^{-1}$ thyme + 100 $mg \cdot L^{-1}$ lemongrass) effect. Individualized grape berries were inoculated and treated as described previously.

After air drying, the berries were placed in acrylic boxes, and kept in an incubator at 25 °C and 75% RH, for six days. Assessments were performed daily, regarding rot incidence and severity. The obtained data were used to calculate the area under the disease progress curve (AUDPC), according to Shaner and Finney (1977) (Eq. 3):

$$AUDPC = \Sigma \left[(y_i + y_{i+1})/2 \times (t_{i+1} - t_i) \right] \quad (3)$$

where: y_i: the lesion diameter or incidence at time t_i, in days; y_{i+1}: the lesion diameter or incidence at time t_{i+1}.

The experimental design was completely randomized, and four repetitions and nine berries as an experimental unit were included.

To evaluate the possibility of a protective effect promoted by the EOs, the berries were inoculated 24 hours after the treatment application. Individualized berries were sprayed with thyme at 400 mg·L^{-1}, lemongrass at 400 mg·L^{-1} and the blend (thyme at 200 mg·L^{-1} + lemongrass at 200 mg·L^{-1}). As a control, untreated berries (blank control) or water and Tween80 sprayed berries were used. The prepared solutions were applied to the run-off point.

After air drying, the berries were placed in acrylic boxes, and kept in an incubator at 25 °C and 75% RH, for six days. Assessments were performed daily, regarding the rot incidence and severity. The obtained data were used to calculate AUDPC, as previously described. The experimental design was completely randomized, and four repetitions and nine berries as an experimental unit were included.

Essential oils on the gray mold control and on the quality of 'Italia' grape clusters

'Italia' grape clusters, from Jales, were transported to the laboratory, where they were selected by its uniformity, absence of defects and rots. The clusters were first inoculated with *B. cinerea*. For the inoculation, in each grape cluster, ten randomly chosen berries were marked and then wounded with the aid of a chromatography syringe. After micro-wounding, the clusters were sprayed with the spore suspension (10^5 conidia·mL^{-1}) and 4 hours after incubation at 25 °C / 80% RH treated with thyme at 800 mg·L^{-1} and the blend (thyme at 500 mg·L^{-1} + lemongrass at 100 mg·L^{-1}). As a control, grape clusters were sprayed with water plus Tween80.

After air drying, the clusters were placed in open plastic packages and stored at 25 ± 2 °C / 80 ± 5% RH for six days. Another batch was placed into polystyrene tray, wrapped up with low density polyethylene (LDPE) film (30 µm), and stored at 1 ± 1 °C / 90 ± 5% RH for 14 days, followed by another three days at 25 ± 2 °C / 80 ± 5% RH in open plastic packages.

The clusters were evaluated for incidence (number of previously inoculated berries showing rot symptoms) and severity (lesion diameter) according to the method described in Camili et al. (2010). Berries inoculated without injury and those not inoculated were also evaluated for the gray mold incidence, using Eq. 4:

$$\text{Mass of rotten berries / total mass of the cluster} \times 100\ (\%) \quad (4)$$

The experimental design was completely randomized, with nine repetitions per treatment and one cluster as an experimental unit. The results were submitted to analysis of variance, and the means were compared by the Tukey's test ($p < 0.05$).

For the evaluation of quality attributes, uninoculated clusters of 'Italia' grapes were treated as described before and assessed at the beginning of the experiment and after each storage period, according to the methods described ahead:

- Skin color: determined using a Minolta colorimeter, CIELab system, where L* is the luminosity (0 = black and 100 = white), a* and b* the chromaticity (-a* = green and +a* = red; -b* = blue and +b* = yellow). Readings were performed on six berries per cluster (two from the top, two from the median, and two from the bottom of the cluster). The results were expressed in: luminosity (L*), color angle [hue = tan (b*/a*)] and chroma [$C^* = (\sqrt{a^{*2}+b^{*2}})$];
- Rachis appearance: grades from 1 to 5 were assigned, being: 1 = green, fresh, and turgid, 2 = green opaque, 3 = green to brown, 4 = predominantly brown, and 5 = brown to dry brown (Cia et al. 2009);
- Weight loss (%): clusters were weighed at the beginning and after the storage periods, and the weight loss was obtained using Eq. 5:

$$\text{Weight loss \%} = (\text{initial weight} - \text{final weight / initial weight}) \times 100 \quad (5);$$

- Detached berries (%): determined by weighing the entire cluster and the naturally detached berries, after slight shaking, using Eq. 6:

$$\text{D \%} = (\text{mass of detached berries / mass of the entire cluster}) \times 100 \quad (6);$$

- Soluble solids (°Brix): 10 berries per cluster were removed, three from the upper region, four from the median region, and three from the lower region, and their juice extracted to determine the soluble solids content in an Atago digital refractometer;

- Titratable acidity (g tartaric acid/100 g juice): determined from grape juice (1:9) and titration using NaOH (0.1 N), until reaching pH = 8.1;
- Ratio: rate between soluble solids and titratable acidity.

Data analysis

The obtained data were subjected to analysis of variance, and the means were compared using the Tukey's test ($p < 0.05$), with the aid of the R Bio statistical program (Bhering 2017). Regression analysis was performed when required.

RESULTS AND DISCUSSION

In-vitro antifungal activity of essential oils by contact

Thyme and lemongrass EOs significantly reduced the *in-vitro* development of *B. cinerea* (Fig. 1). The thyme essential oil, applied by contact at 400 and 800 mg·L^{-1}, showed the highest percentages of MGI and the lowest MGVI (Fig. 2a). Lemongrass oil significantly reduced MGVI at 400 and 800 mg·L^{-1}, completely inhibiting the development of *B. cinerea* (Fig. 2b). Thus, thyme essential oil, with major components of thymol and ρ-cymene, performed well against the development of the pathogen.

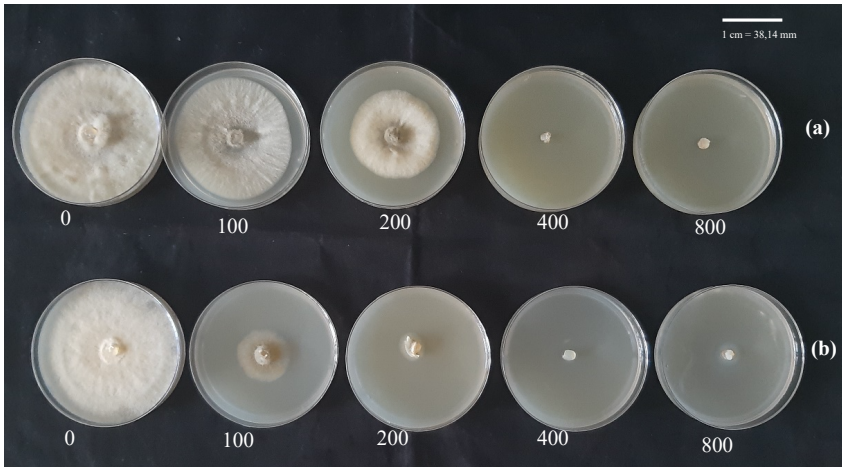
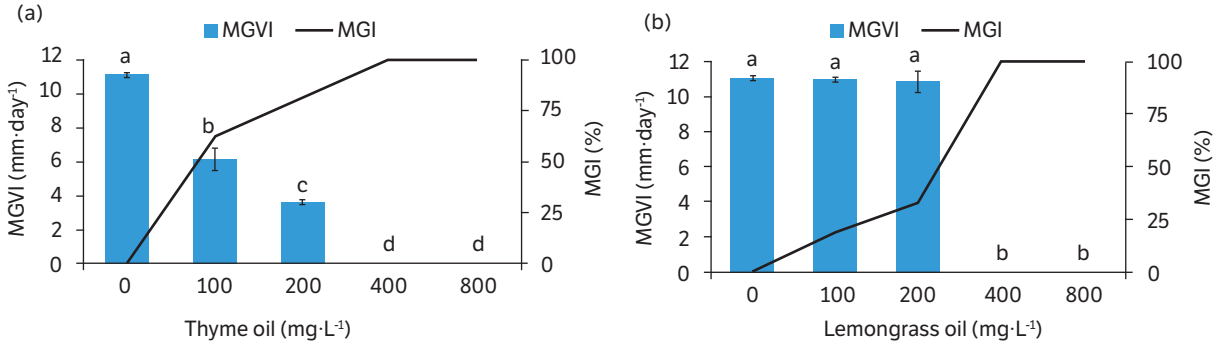


Figure 1. *Botrytis cinerea*, cultivated in potato dextrose agar medium incorporated with (a) lemongrass and (b) thyme essential oils (mg·L^{-1}).



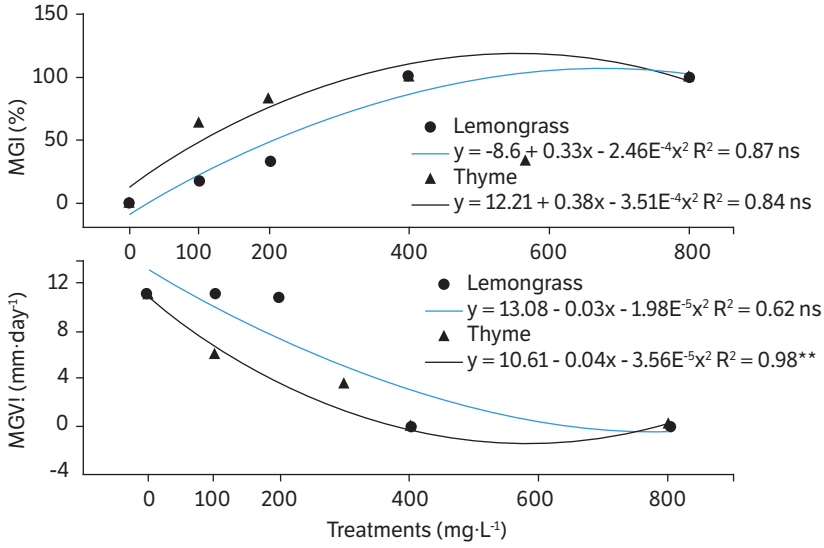
MGVI: mycelial growth velocity index; MGI: mycelial growth inhibition; *mean of six repetitions and standard error bar. Means followed by the same letter do not differ significantly (Tukey's test, $p < 0.05$).

Figure 2. MGVI and MGI (%) of *Botrytis cinerea*, cultivated in potato dextrose agar medium incorporated with the (a) thyme and (b) lemongrass essential oils*.

This finding corroborates with other studies. Combrinck et al. (2011) found that thyme oil at concentrations at 1,000 μL·L^{-1} (or 910 mg·L^{-1}) and lower inhibited the *B. cinerea* development. Lambert et al. (2001) demonstrated that the mixture of carvacrol and thymol inhibited *Pseudomonas aeruginosa* and *Staphylococcus aureus* and stated that the inhibition is due to damage to membrane integrity, affecting pH homeostasis and the balance of inorganic ions.

Yan et al. (2021) reported that *C. citratus, T. vulgaris* and *Origanum heracleoticum* EOs inhibited *B. cinerea* mycelial growth and explained that EOs can modify the fungus hyphae morphology, damage the cell plasma membrane, resulting in the leakage of intercellular contents, such as nucleic acids, soluble proteins and soluble sugars, mainly due to its lipophilic characteristics reacting with cell's phospholipid bilayer.

In this experiment, a regression test was also applied. Dose dependence was observed when *B. cinerea* was grown on PDA incorporated with thyme EO, and a significant reduction in the mycelial growth rate of the pathogen was observed as the oil concentration increased. The reduction was significant at 1%, and the fit curve had an $R^2 = 0.98$. No differences were observed in the pathogen's growth rate, so no other correlation was significant at 1%, regardless of the oils used in the treatments (Fig. 3).



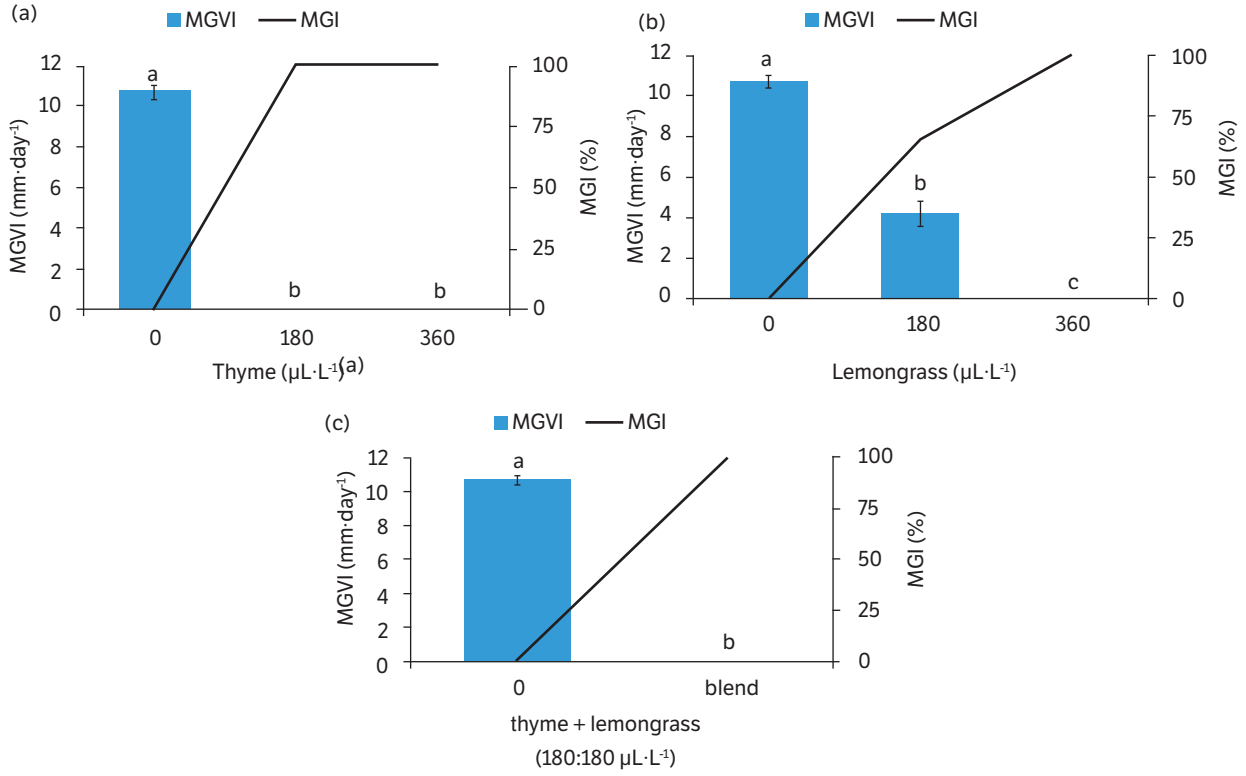
MGVI: mycelial growth velocity index; MGI: mycelial growth inhibition; *data represent the mean of six repetitions; **significant at 1% probability; ns: not significant.
Figure 3. Influence of different doses of essential oils on the MGVI (mm·day^{-1}) and MGI (%) of *Botrytis cinerea*, cultivated in potato dextrose agar medium incorporated with the thyme and lemongrass essential oils*.

In-vitro antifungal activity of essential oils volatile compounds

The volatile constituents of lemongrass and thyme EOs, as well as the blend, significantly reduced the MGVI for *B. cinerea* (Figs. 4a–4c). The EOs volatiles showed a fungistatic effect on the pathogen growth. Thyme was found to be more effective than lemongrass at 180 μL·L^{-1} of air in reducing the mycelial growth.

Soylu et al. (2006) showed that volatile phase of thyme oil at 0.3 μg·mL^{-1} (or 0.3 mg·L^{-1}) inhibited *Phytophthora infestans* development. The authors observed, with an optical and a scanning electron microscope, hyphae morphological changes, such as cytoplasmic aggregation, wrinkling and protoplast leakage. Yan et al. (2021) found that volatile phase of EOs from lemongrass, thyme and oregano inhibited *B. cinerea* mycelial growth.

Although the blend of thyme and lemongrass was found to act positively in reducing the development of *B. cinerea* (Fig. 4c), this could only be due to thyme oil (Fig. 4a). There are different modes of interaction between EOs, the best known being the synergistic effect, which generally occurs with a reduction in the concentration of the mixture when compared to concentrations evaluated separately (López-Malo et al. 2006). The application of volatile treatments during postharvest of fruit consists in a good option for delicate products, such as berries, avoiding excessive handling and consequent injuries. Besides that, it could be applied during transportation or inside packages.



MGVI: mycelial growth velocity index; MGI: mycelial growth inhibition; *mean of six repetitions and standard error bar. Means followed by the same letter do not differ significantly (Tukey's test, $p < 0.05$).

Figure 4. MGVI (mm.day-1) and MGI (%) of *Botrytis cinerea* exposed to the essential oils volatiles of (a) thyme, (b) lemongrass and (c) to the blend (10 µL thyme + 10 µL lemongrass). 10 µL and 20 µL are equivalent to approximately 180 µL·L^{-1} and 360 µL·L^{-1} of free air, respectively*.

Curative and protective effect of essential oils on *Botrytis cinerea* control in 'Italia' grape berries

Based on the results obtained in the *in-vitro* assays, the thyme and lemongrass EOs at 100 to 400 mg·L^{-1} were evaluated in 'Italia' grape berries. Despite the reduction in the *B. cinerea* development found in the *in-vitro* assays (Figs. 2, 3 and 4), these concentrations did not significantly reduce the incidence and/or severity of gray mold in berries kept at 25 °C (data not shown).

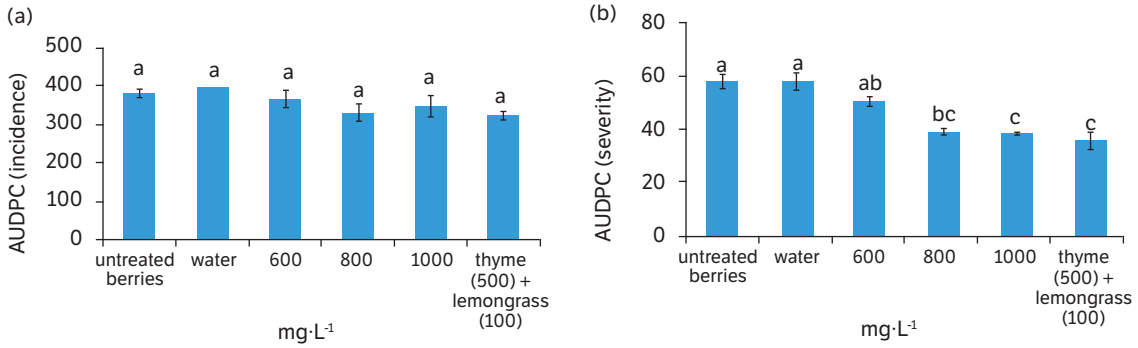
Similarly, Martínez-Romero et al. (2007) demonstrated that carvacrol EO was highly efficient in reducing the *B. cinerea* growth in culture medium and on berries. However, the growth of the fungus in the culture medium was observed for all carvacrol oil concentrations evaluated, while in berries inhibition of 97% was obtained only for the highest oil concentration.

One of the possible causes for a more evident effect *in-vitro* than *in-vivo* may be related to a difference in composition, *i.e.*, the content of the culture medium is different from the environment found inside fruit cells. Possibly, there is a greater availability of nutrients inside grape cells, allowing the fungus to grow and repair its damaged parts, despite the antimicrobial action of EOs, which one could not find when in culture medium.

Gill et al. (2002) investigated that microbial growth in a complex and nutrient-rich environment promote its maximum replication rate, and yet it is able to have plentiful nutrients available for repair or increase renewal of its cellular components. So, despite the stress, there is a greater resistance by the microorganism.

Furthermore, the difference among EOs composition, the cultivar and its response might have influenced the tests outcome. Carvacrol can be found in thyme EO (Martínez-Romero et al. 2007), however in the present work the thyme EO contained mainly thymol and ρ-cymene. Even when the same components are found, it might not be in similar amounts, or could be also blended with other components. Yan et al. (2021) used thyme EO to control *B. cinerea*, with different compositions of thymol (22.71%) and ρ-cymene (20.43%), and obtained a significant reduction of fungus growth and development.

Although the use of EOs had no effect on the pathogen incidence (Fig. 5a), when evaluating higher concentrations of thyme and lemongrass oils (600 to 1,000 mg·L^{-1}), it was found that thyme oil and the blend (thyme + lemongrass) reduced by more than 30% the severity of gray mold lesions (Fig. 5b) in grape berries inoculated 4 hours before spraying (Fig. 6).

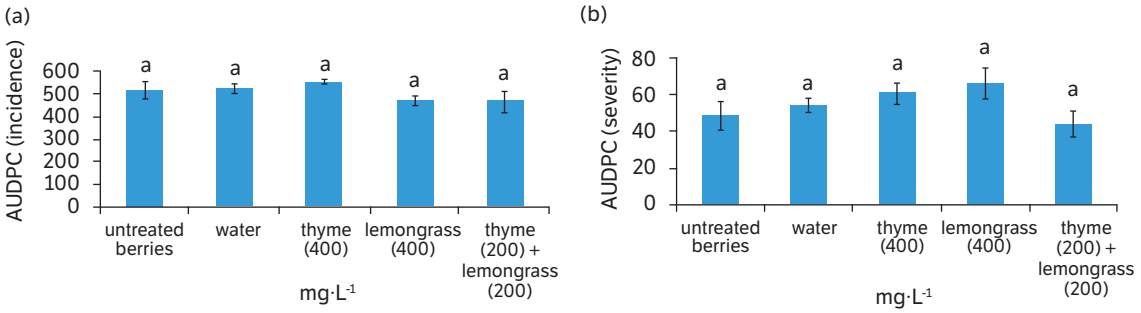


AUDPC: area under the disease progress curve; *data represent the average of four repetitions, with nine berries per plot and standard error bar. Means followed by the same letter do not differ significantly (Tukey's test, $p < 0.05$).

Figure 5. AUDPC for (a) incidence and (b) severity of gray mold in 'Italia' grape berries sprayed with thyme essential oil at 600, 800 and 1,000 mg·L^{-1} or with the blend (500 mg·L^{-1} thyme + 100 mg·L^{-1} lemongrass) 4 hours after pathogen inoculation. Berries were stored at 25 ± 2 °C / 75 ± 5% relative humidity, for six days*.

Figure 6. Symptoms of gray mold in 'Italia' grape berries sprayed with thyme essential oil at 600, 800 and 1,000 mg·L^{-1} or with the blend (500 mg·L^{-1} thyme + 100 mg·L^{-1} lemongrass) 4 hours after pathogen inoculation. Berries were stored at 25 ± 2 °C / 75 ± 5% relative humidity, for six days.

To assess the possibility of the protective effect promoted by the oils, the inoculation was carried out 24 hours after berries spraying with the EOs of thyme, lemongrass or the blend; there was no reduction in the incidence and/or severity of *B. cinerea* (Figs. 7a and 7b).

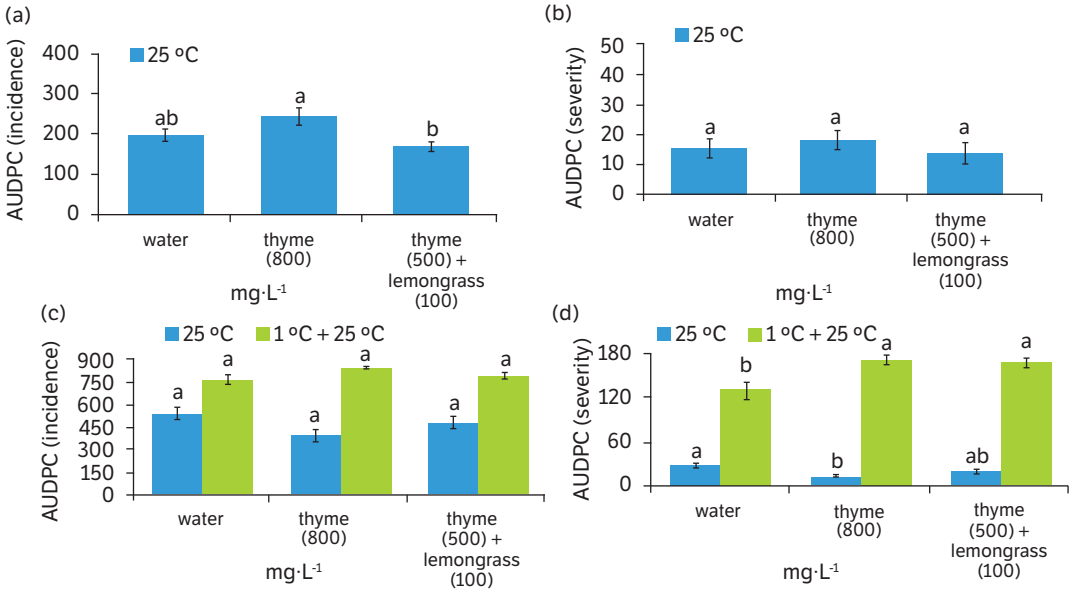


AUDPC: area under the disease progress curve; *data represent the average of five repetitions, with nine berries per plot and standard error bar. Means followed by the same letter do not differ significantly (Tukey's test, $p < 0.05$).

Figure 7. AUDPC for (a) incidence and (b) severity of gray mold in 'Italia' grape berries sprayed with thyme or lemongrass essential oil at 400 mg·L^{-1} or with the blend (200 mg·L^{-1} thyme + 200 mg·L^{-1} lemongrass) 24 hours before pathogen inoculation. Berries were stored at 25 ± 2 °C / 75 ± 5% relative humidity, for eight days*.

Essential oils on the gray mold control and on the quality of 'Italia' grape clusters

Thyme essential oil at 800 mg·L^{-1} and blend (500 mg·L^{-1} thyme + 100 mg·L^{-1} lemongrass) sprayed on clusters of 'Italia' grapes, previously inoculated with *B. cinerea*, did not significantly reduce the incidence and severity of gray mold in fruits kept at 25 °C for six days (Figs. 8a and 8b).



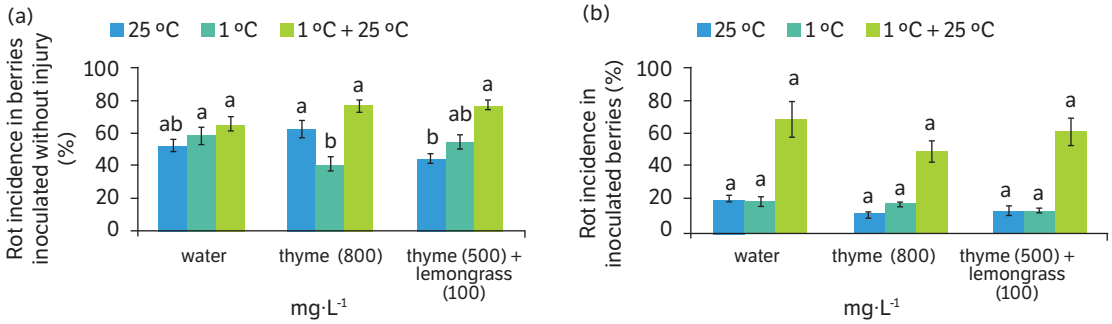
AUDPC: area under the disease progress curve; *average of nine repetitions with 10 berries inoculated per experimental unit and standard error bar. Means followed by the same letter do not differ statistically from each other (Tukey's test, $p < 0.05$).

Figure 8. AUDPC for (a and c) incidence and (b and d) severity of *Botrytis cinerea*, in 'Italia' grape clusters stored (a and b) at 25 °C / 80% relative humidity for six days, at 1 °C / 90% relative humidity for 14 days, and (c and d) at 1 °C / 90% relative humidity for 14 days, followed by another three days at 25 °C / 80% relative humidity. The treatments were carried out by spraying the clusters with thyme essential oil at 800 mg·L^{-1} and blend (500 mg·L^{-1} of thyme and 100 mg·L^{-1} of lemongrass), 4 hours after pathogen inoculation*.

When the fruits treated with the EOs were kept under refrigeration, there was reduction of approximately 50% in the severity of gray mold, but not in the number of clusters, showing disease symptoms. However, this reduction was not observed after transferring the clusters to 25 °C (Figs. 8c and 8d).

It was also found that, in grape clusters inoculated with *B. cinerea* without previous injury and kept under refrigeration for 14 days, thyme EO significantly reduced the incidence of gray mold (Fig. 9a), but not when stored at 25 °C. There was no reduction in the incidence of gray mold in uninoculated berries stored at 25 °C or under refrigeration (Fig. 9b). For Piljac-Žegarac and Šamec (2011), storage at 25 °C facilitated faster berries spoilage. When fruit were stored at 4 °C, they retained

marketable qualities nine days longer than fruit at 25 °C. There was maintenance of antioxidant capacity of the berries when the fruit were kept under 4 °C. Furthermore, the author explains that possibly there would have been an accumulation of anthocyanins in fruit stored at 25 °C similar to the one observed for those fruit at 4 °C, if the spoilage process did not interfere earlier in fruit stored at room temperature, proving refrigeration to be an efficient tool to postharvest conservation of fruit.



*Average of nine repetitions and standard error bar. Means followed by the same letter statistically compared in the same storage condition did not differ (Tukey's test, $p < 0.05$). For statistical analysis, the data were transformed into $\sqrt{(x)+0,5}$.

Figure 9. Incidence (%) of berries showing symptoms of gray mold in clusters of 'Italia' grapes (a) inoculated without injury and (b) non-inoculated, after storage at 25 °C / 80% relative humidity for six days, at 1 °C / 90% relative humidity for 14 days, and at 1 °C / 90% relative humidity for 14 days, followed by another three days at 25 °C / 80% relative humidity. The treatments were carried out by spraying the clusters with thyme essential oil at 800 mg·L^{-1} and blend (500 mg·L^{-1} of thyme and 100 mg·L^{-1} of lemongrass), 4 hours after pathogen inoculation*.

It is valid to mention that the causal agent of gray mold can produce quiescent infections, which can become active after the fruits are harvested, causing gray mold even at low temperatures. *B. cinerea* is a quiescent and necrotrophic lifestyle pathogen. Its pathogenicity consists in synthesizing a myriad of cell-wall-degrading enzymes, toxins, and low-molecular-weight compounds with evidence that the pathogen triggers the host to induce hypersensitivity reaction as an attack strategy (Williamson et al. 2007). As a quiescent pathogen, it establishes an infection on unripe fruit, but it does not develop until host tissue signals start to appear during ripening process (Petrasch et al. 2019). Other studies also demonstrate a positive effect of EOs combined with cold storage (Marandi et al. 2011, Santos et al. 2012).

Although individually EOs do not reduce gray mold, if integrated with other methods it can be an alternative for the disease management. Following the theory of multiple hurdles, when only one alternative method happens to cease its effectiveness, it still may become part of a group of methods of control, in which each one has a share in reducing the disease (Rommanazzi et al. 2012).

No significant changes were observed in grape clusters sprayed with EOs and stored at 25 °C or under refrigeration, in regard to soluble solids content, ratio, weight loss, incidence of natural rot, detached berries, and rachis appearance (Tables 1–3). Changes were observed only for titratable acidity and skin luminosity; clusters sprayed with the EOs were slightly more acid and brighter after six days of storage at 25 °C (Tables 1–3), but the berries darkening was not observed, which could be an indication of the product phytotoxicity. At 25 °C, although, the titratable acidity increased with the application of thyme EO and blend, and this reduced the ratio (soluble solids/titratable acidity); the recommended balance was maintained above 20 (Crisosto et al. 1998). Other authors have also found that the use of EOs does not affect the quality of table grapes (Geransayeh et al. 2012, Santos et al. 2012).

Table 1. SS, TA, and ratio in 'Italia' grapes clusters sprayed with thyme essential oil at 800 mg·L^{-1} or blend (500 mg·L^{-1} thyme + 100 mg·L^{-1} lemongrass), and stored under different conditions.

Treatments	SS (%)[z]			TA (% tartaric acid)			Ratio (SS/TA)		
Starting day	25 °C[y]	1 °C	1 °C + 25 °C	25 °C	1 °C	1 °C + 25 °C	25 °C	1 °C	1 °C + 25 °C
		11.78			0.45			26.02	
Control	13.40 a	12.70 a	13.45 a	0.44 b	0.46 a	0.47 a	30.77 a	27.97 a	29.07 a
Thyme	12.35 a	12.08 a	13.30 a	0.51 a	0.53 a	0.49 a	24.04 b	22.93 a	27.45 a
Blend	12.10 a	12.73 a	12.13 a	0.51 a	0.52 a	0.54 a	23.97 b	24.71 a	22.75 a
CV (%)	6.52	7.33	7.97	6.54	8.61	7.44	11.49	14.33	14.81

SS: soluble solids; TA: titratable acidity; CV: coefficient of variation; [y]storage conditions: 25 °C / 80% relative humidity for six days, 1 °C / 90% relative humidity for 14 days, and 1 °C/90% relative humidity for 14 days followed by another three days at 25 °C / 80% relative humidity; [z]mean of four repetitions, with one cluster as the experimental unit. Means followed by the same letter, in the column, do not differ significantly from each other (Tukey's test, $p < 0.05$).

Table 2. Weight loss, incidence of natural rot, detached berries and rachis appearance in 'Italia' grapes sprayed with thyme essential oil at 800 mg·L^{-1} or blend (500 mg·L^{-1} thyme + 100 mg·L^{-1} lemongrass), and stored under different conditions.

Treatments	Weight loss (%)[Z]			Incidence of natural rot (%)		
	25 °C[y]	1 °C	1 °C + 25 °C	25 °C[y]	1 °C	1 °C + 25 °C
Control	1.26 a	0.19 a	2.04 a	5.09 a	4.27 a	19.28 a
Thyme	0.48 a	0.19 a	2.15 a	5.81 a	7.05 a	21.31 a
Blend	0.53 a	0.27 a	2.27 a	7.53 a	6.78 a	19.70 a
CV (%)	16.31	6.68	7.75	22.28	24.73	15.31
Treatments	**Detached berries (%)[Z]**			**Rachis appearance[X]**		
	25 °C[y]	**1 °C**	**1 °C + 25 °C**	**25 °C[y]**	**1 °C**	**1 °C + 25 °C**
Starting day		**0.99**			**1.5**	
Control	2.05 a	0.64 a	1.39 a	3.63 a	2.13 a	2.75 a
Thyme	2.35 a	1.13 a	1.89 a	4.0 a	2.13 a	2.75 a
Blend	1.02 a	2.83 a	4.26 a	3.5 a	2.13 a	2.63 a
CV (%)	32.04	37.71	37.59	23.25	22.53	13.41

CV: coefficient of variation; [X]Rachis score scale: 1 = green and turgid; 2 = opaque green; 3 = green to light brown; 4 = predominantly brown; 5 = brown to dry brown; [Y]storage conditions: 25 °C / 80% relative humidity for six days, 1 °C / 90% relative humidity for 14 days, and 1 °C / 90% relative humidity for 14 days followed by another three days at 25 °C / 80% relative humidity; [Z]mean of four repetitions, with one cluster as the experimental unit. Means followed by the same letter, in the column, do not differ significantly from each other (Tukey's test, $p < 0.05$). For statistical analysis, the data were transformed into $\sqrt{(x)+0,5}$.

Table 3. Luminosity, chroma and Hue angle in 'Italia' grapes sprayed with thyme essential oil at 800 mg·L^{-1} or blend (500 mg·L^{-1} thyme + 100 mg·L^{-1} lemongrass) and stored under different conditions.

Treatments	Skyn colour[Z]								
	Luminosity (L*)			Chroma (C*)			Hue angle		
Starting day	25 °C[y]	1 °C	1 °C + 25 °C	25 °C[y]	1 °C	1 °C + 25 °C	25 °C[y]	1 °C	1 °C + 25 °C
	46.5			**12.28**			**158.42**		
Control	42.96 b	42.42 a	44.08 a	10.71 a	10.52 a	10.94 a	161.91 a	159.67 a	163.94 a
Thyme	43.32 ab	41.18 a	44.21 a	11.57 a	10.56 a	11.43 a	158.74 a	158.07 a	164.00 a
Blend	44.70 a	41.50 a	43.58 a	11.54 a	10.82 a	12.01 a	161.22 a	158.55 a	162.70 a
CV (%)	1.81	1.93	3.97	9.3	6.79	8.28	1.61	0.92	1.83

CV: coefficient of variation; [Y]Storage conditions: 25 °C / 80% relative humidity for six days, 1 °C / 90% relative humidity for 14 days, and 1 °C / 90% relative humidity for 14 days followed by another three days at 25 °C / 80% relative humidity; [Z]mean of four repetitions, with one cluster as the experimental unit. Means followed by the same letter, in the column, do not differ significantly from each other (Tukey's test, $p < 0.05$).

One of the concerns about the use of EOs in postharvest fruits is associated with their possible effects on the alteration of sensory characteristics such as odor and flavor. Studies show that the use of EOs associated with other methods of disease control, applied by contact phase and by volatile compounds, was not perceptible in terms of sensory analysis of flavor (Santos et al. 2012, Liu et al. 2016).

CONCLUSION

Thyme EO has an antimicrobial effect on *B. cinerea* and reduces the severity of gray mold in clusters kept under refrigeration. The oil can contribute to the management of gray mold in Itália grapes postharvest.

AUTHORS' CONTRIBUTION

Conceptualization: Lopes, V. C., Benato, E. A. and Cia, P.; **Data Curation:** Lopes, V. C., Benato, E. A., Silva, B. M. P. and Veiga, J. C.; **Formal Analysis:** Lopes, V. C., Benato, E. A., Veiga, J. C. and Cia, P.; **Writing – Original Draft:** Lopes, V. C., Silva, B. M. P. and Cia, P.; **Writing – Review & Editing:** Silva, B. M. P., Bron, I. U. and Cia, P.

DATA AVAILABILITY STATEMENT

Data will be made available upon request.

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