

A study on antifungal activity of thymol, eugenol, and 1,8-cineole against *Botrytis cinerea* Persoon isolated from grapevine (*Vitis vinifera* Linné)

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ABSTRACT

This study investigated the effects of essential oils thymol, eugenol, and 1,8-cineol for the prevention of losses caused by gray mold (*Botrytis cinerea* Persoon) in grapevine (*Vitis vinifera* Linné, cv. Karaerik) under *in vitro* and *in vivo* conditions. *B. cinerea* isolated from diseased grapevines was identified by 18S rRNA gene sequence analysis. The effects of 2.5, 5, and 10 µL doses of essential oils (EO) on mycelial growth, germ tube elongation, and spore germination were investigated *in vitro*, and the lesion formation on the leaves was examined *in vivo*. Compared to the control, both thymol and eugenol suppressed mycelial growth, depending on the dose increase, and caused 100% inhibition at the 10 µL dose, whereas 1,8-cineole had no effect on the same parameter at either dose. Germ tube elongation was 100% inhibited by 5 and 10 µL doses of thymol and eugenol, while the same doses of 1,8-cineole inhibited it by 49% and 85%, respectively. Conidiospore germination was inhibited by 22, 45, and 72% at 2.5, 5, and 10 µL doses of thymol, respectively. Under *in vivo* conditions, the three EOs inhibited lesion formation on leaves, depending on the concentration increase, and inhibition ended with 100% at their 100 µL dose. In addition, thymol was found to be the most effective EO under *in vivo* conditions. Our results show that these EOs, which are of biological origin and non-toxic to environmental health, have the potential to be used in the control of gray mold.

Keywords: antifungal effect, biocontrol, essential oil, gray mold

INTRODUCTION

Grapevine, which is grown in widespread geography in the World and Turkey, is a significant plant (Semerci et al., 2015). However, various fungal pathogens such as *Botrytis cinerea* Persoon, *Phomopsis viticola* Saccardo, *Elinoë ampelina* Shear, *Erysiphe necator* Schwein, and *Plasmopara viticola* Berlese et de Toni in grape varieties could cause yield losses in the vineyards (Babalik et al., 2020; Karakuş et al., 2021a). *B. cinerea* a polyphagous fungus, damages hundreds of plant species, including many fruits worldwide. *B. cinerea* infects flowers, stems, leaves, and fruits, causing outbreaks of gray mold (Wang et al., 2010; Zhao et al., 2021). In addition, the damage caused to vineyards at different development stages, such as flowering, veraison, harvesting and storage has

been reputable over the past decades (Rosslenbroich and Stuebler 2000; Burggraf and Rienth 2020; Zhao et al., 2021). The application of synthetic fungicides is the most widely used control method against phytopathogenic fungi. The proliferation of these synthetic compounds is a serious problem leading to increased resistance to pathogens, and detrimental impacts on ecosystems and human health. In the last decades, the use of natural products has been increasingly preferred in order to control biodegradation and for longer storage. For example, nowadays, natural pesticides produced from plant essential oils (EO) find more use in the fight against disease factors (Isman, 2000; Drobny et al., 2009; Nazzaro et al., 2017; Karakuş et al., 2021b; Zhao et al., 2021).

Various applications like essential oils both eco-friendly and safer choices has, therefore, been one of the hottest topics in recent years (Astani and Schnitzler, 2014; Böhme et al., 2014; Civitelli et al., 2014; Gilling et al., 2014; Raut and Karuppayil, 2014; Teixeira et al., 2014).

Thymol (2-isopropyl-5-methyl phenol), is a natural phenolic compound found in essential oils extracted from various plants belonging to the *Lamiaceae* family (Rajput et al., 2018; Jannati et al., 2021). Eugenol (4-allyl-2-methoxy phenol), used in fragrance factor and food flavoring, is a phenol. It is the main component of clove oil, and also found in a wide range of other herbs, namely basil, nutmeg, cinnamon, and lemon balm (Zari et al., 2021). The 1,8-cineole also familiar as eucalyptol is a terpene oxide and the key ingredient in most *Eucalyptus* oils, *Salvia lavandulifolia* Vahl, *Melaleuca quinquenervia* (Cavanilles) S.T. Blake, and many other essential oils (Juergens et al., 2020; Cai et al., 2021). These compounds have proven anticarcinogenic, insecticidal, antifungal, antibacterial and antioxidant properties (Cai et al., 2021; Jannati et al., 2021; Zari et al., 2021).

Many studies have investigated not only the antifungal activity of different compounds isolated from plants but also the prevention of fungal infection by essential oils *in vitro* (Wang et al., 2010; Nazzaro et al., 2017; Burggraf and Rienth, 2020; Cai et al., 2021; Karakuş et al., 2021a; Karakuş et al., 2021b). The effects of thymol, eugenol, and 1,8-cineol EO on the obstruction of losses caused by gray mold in the Karaerik grape cultivar have not been investigated *in vitro* and *in vivo*, and related knowledge is also very limited. Therefore, the purpose of the present study was to investigate the *in vitro* and *in vivo* antifungal activities of thymol, eugenol, and 1,8-cineole EOs against *Botrytis cinerea*.

MATERIALS AND METHODS

Isolation, purification and identification of Botrytis cinerea

B. cinerea isolate was obtained from the vineyard of Üzümlü District of Erzincan (Turkey) and isolation from multiple spores, and purification were conducted

using potato dextrose agar (PDA) (Benjilali et al., 1984). For the identification of the fungus in 18S rRNA gene-based PCR test, primer sets 515f and 806r containing regions V3-V4 were analyzed by RefGen Biotechnology Company, Ankara (Turkiye). Molecular diagnoses were performed by comparing the 18S rRNA gene region sequences detected in the study with the 18S rRNA gene regions identified in previous studies. *B. cinerea* isolates numbered MF7413141, MH997908, MK562062, and MH782039 obtained from the Genbank database were used for molecular identification. The tree was built using the UPGMA method, created with the MEGA v.5 program using the Jukes-Counter model. The fungal strain was stored at 4 °C in agar and -20 °C in silica gel.

Plant material

In the study, *Vitis vinifera* Linné cv Karaerik was the standard grape cultivar. Cuttings were taken from vineyards during dormancy in March. The collected cuttings were rooted in the greenhouse at the Hakkâri University Horticulture Department (± 23 °C, L:D 16:8). Cuttings were grown to the 5-8 leaf stage, and the leaves were used for experimental studies.

Chemicals

Thymol (Aldrich C121452), eugenol (Fluka 45980), and 1,8-cineole (Aldrich 183164) were purchased from Sigma-Aldrich (Shanghai, China), and they were stored in the dark at 4 °C.

Determination of the antifungal effects of essential oils on mycelial growth of the pathogen in vitro

Thymol, eugenol, and 1,8-cineol essential oils (EO) were dissolved in Tween-20. For this, 10% essential oil was added to 88% sterile purified water containing 2% (v/v) Tween-20 and mixed until well dissolved (Lopez-Reyes et al., 2010). Then, 25 μ L, 50 μ L, and 100 μ L of EO solution were mixed in for each EO from the stock solutions prepared in Petri dishes containing 20 mL of PDA. The final concentrations were calculated to be 2.5, 5, and 10 μ L. Tween-20 (0.1%) and sterile distilled water were added to the control Petri dishes. After the prepared

Petri dishes were incubated at room temperature for 24 h, 4 mm discs in diameter were taken from 7-10 days old colonies and inoculated. Then, the Petri dishes were incubated at 25 °C in the dark for seven days. Each treatment was performed in five repetitions. From the second day of incubation, the diameters of the fungal mycelium were measured and recorded daily. Mycelium diameters were measured according to the Benjlali et al., (1984) and Yahyazadeh et al., (2008) method. Percentages of inhibition were calculated according to the equation used by Deans and Svoboda (1990) below.

$$E = [(K - M)] / K \times 100$$

where: *E* - inhibition (%), *K* - mycelium diameter in the control Petri dish (mm), *M* - mycelium diameter in the experimental Petri dish (mm).

Essential oils effects on spore germination of *B. cinerea* and germ tube elongation

The effects of thymol, eugenol, and 1,8-cineol essential oils on spore germination and germ tube elongation of *B. cinerea* were investigated. Spores of 7-10 days were scraped with a sterile glass swab into 5 mL sterilized pure water. The suspension was passed through cheesecloth to deport micelle particles. 10 µL of spore suspension was added to Petri dishes containing essential oil (2.5, 5, and 10 µL) prepared at 1×10^5 conidial/mL using a hemocytometer. The Petri dishes were incubated at 25 °C in the dark. After 24 h, evaluation was conducted by counting germinated and non-germinated spores from 100 randomly selected spores under a light microscope. The spores were counted with a 40x magnifying glass in three different areas then the germination rate (%) was determined by comparing the total number of spores with the number of germinated spores (Qin et al., 2010):

$$\text{Germination (\%)} = [a / (a + b)] \times 100$$

where: *a* - number of germinated spores; *b* - number of non-germinated spores.

Germ tube length was evaluated by measuring the germ tube elongation (µm) of 3x40 spores.

In vivo* efficiency of the essential oils on grape leaves infected with *Botrytis cinerea

Thymol, eugenol, and 1,8-cineol essential oils (EO) were dissolved in water containing: 10% essential oil, 88% sterile purified water, and 2% (v/v) Tween-20 (Lopez-Reyez et al., 2010). The prepared stock solution was diluted by 10% and sprayed on the leaves at 25, 50, and 100 µL. Concentrations applied to leaves from the stock solution include 0.25, 0.5, and 1 µL of oil. Before application, all leaves were sterilized in 5% aqueous sodium hypochlorite for a few minutes and rinsed in sterile water. The stems of the leaves were wrapped with moist cotton and placed in Petri dishes. EO solutions were sprayed on the applied leaves at the determined concentrations. Rectangulars of 10 mm diameter were taken from the edges of 4-day-old *B. cinerea* colonies and placed on the slightly injured needle tip upper part of the leaf. These Petri dishes were incubated in the climate cabinet at 23 °C for seven days (12 h day length). After that, the lesion diameters were then measured on the leaves (Deliere et al., 2010). The EOs solutions were applied to inoculated and uninoculated (control) leaves in the following experimental style: Control (C): treated with distilled water containing Tween-20 (0.1%); EO: treated with the EO only; (F): treated with the fungus and Tween-20 (0.1%); (EO+F): treated with the fungus and the EO at the same time.

Statistical analysis

One-way ANOVA of comparison and variance of means were carried out utilizing the SPSS statistical software package (SPSS; version 22). Means were compared using the Duncan Multiple Range Test test at $P < 0.05$.

RESULTS

Molecular diagnosis of *B. cinerea* isolates on agar plates

18S rRNA gene region sequence (amplification yielded a 294 bp product) from *B. cinerea* isolate shown in Figure 1 was 100%, compared to *B. cinerea* isolates from Genbank.

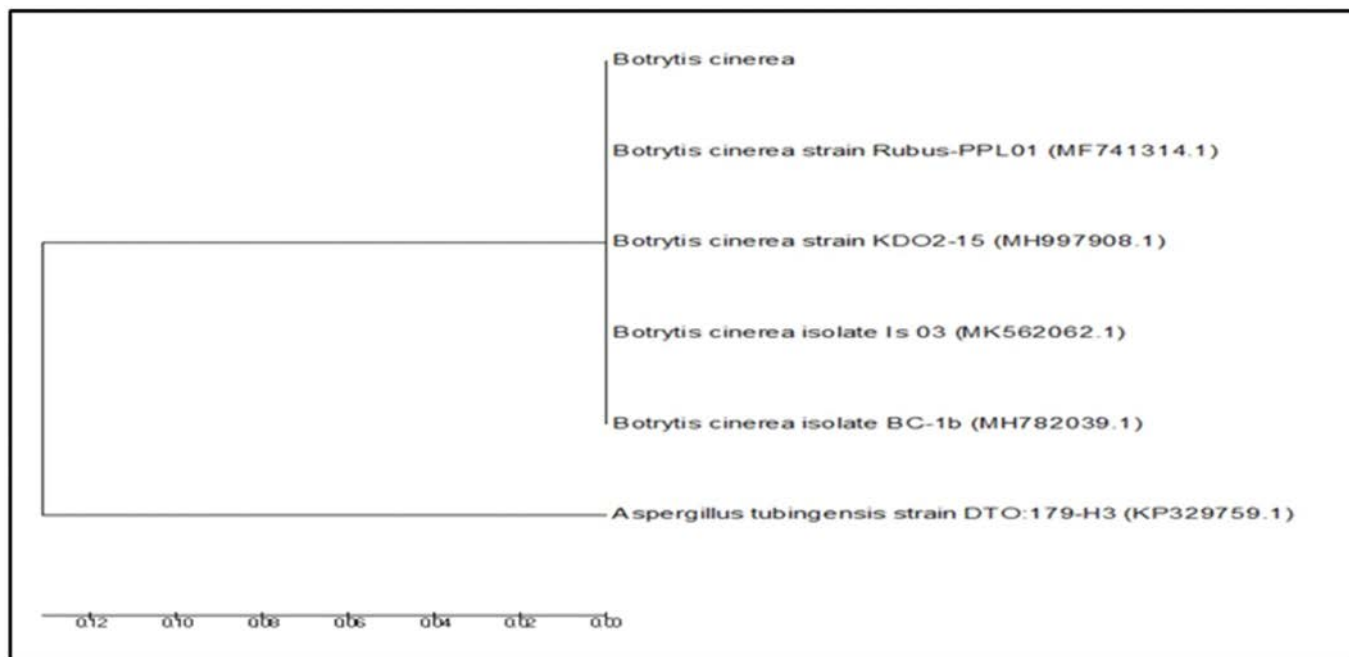


Figure 1. UPGMA distance tree of *B. cinerea* isolates

Effect of essential oils on mycelial growth

When the effect of thymol, eugenol, and 1,8-cineol on the mycelial growth of *B. cinerea* *in vitro* was appraised, thymol and eugenol, in addition to inhibiting mycelial growth in connection with the increase in concentration, 10 and 5 μL doses of thymol, and 10 μL of eugenol inhibited mycelial growth by 100% (Table 1 and Figure 2). At 5 μL concentration, showed 100% inhibition, whereas eugenol only 77.29% and 1,8-cineol showed 0% inhibition. However, all concentrations of 1,8-cineol (2.5, 5, and 10 μL) showed no inhibitory effect on mycelial growth (Figure 2).

Table 1. Effects of different concentrations of essential oils (EO) on mycelium inhibition rate (MIR)

EO concentration (μL /Petri dish)	2.5 μL	5 μL	10 μL
Thymol	84.78 \pm 1.8 ^a	100 \pm 0.0 ^b	100 \pm 0.0 ^c
Eugenol	65.00 \pm 0.57 ^a	77.29 \pm 1.58 ^b	100 \pm 0.0 ^c
1,8-Cineol	0.0 \pm 0	0.0 \pm 0	0.0 \pm 0

*According to Duncan's multiple range test, the difference between group means is significant at the $P < 0.05$ level

Effect of essential oils on spore germination and germ tube elongation

The inhibitory effects of the EOs on spore germination and germ tube elongation were consistent with those on mycelial growth (Table 2-4). Similarly, thymol and eugenol were more successful in the inhibition of spore germination and germ tube elongation of *B. cinera*, compared to those of 1,8-cineol. For example, the control group germinated 100%, while the spores treated with 10 μL of thymol germinated 18% (Table 2).

Table 2. Inhibition rate of different concentrations thymol on the germination and the germ tube elongation

EO concentration (μL /Petri dish)	Germ tube elongation (μm)	Spore germination (%)
Control	74.55 \pm 4.17	100 \pm 0.0
2.5 μL	5.55 \pm 0.74 ^{a*}	78 \pm 1.58 ^{a*}
5 μL	0 \pm 0 ^b	55 \pm 1.37 ^b
10 μL	0 \pm 0 ^c	18 \pm 0.7 ^c

* According to Duncan's multiple range test, the difference between group means is significant at the $P < 0.05$ level

Eugenol induced 100% germination of conidiospores at the concentrations of 2.5, 5, and 10 μL (Table 3).

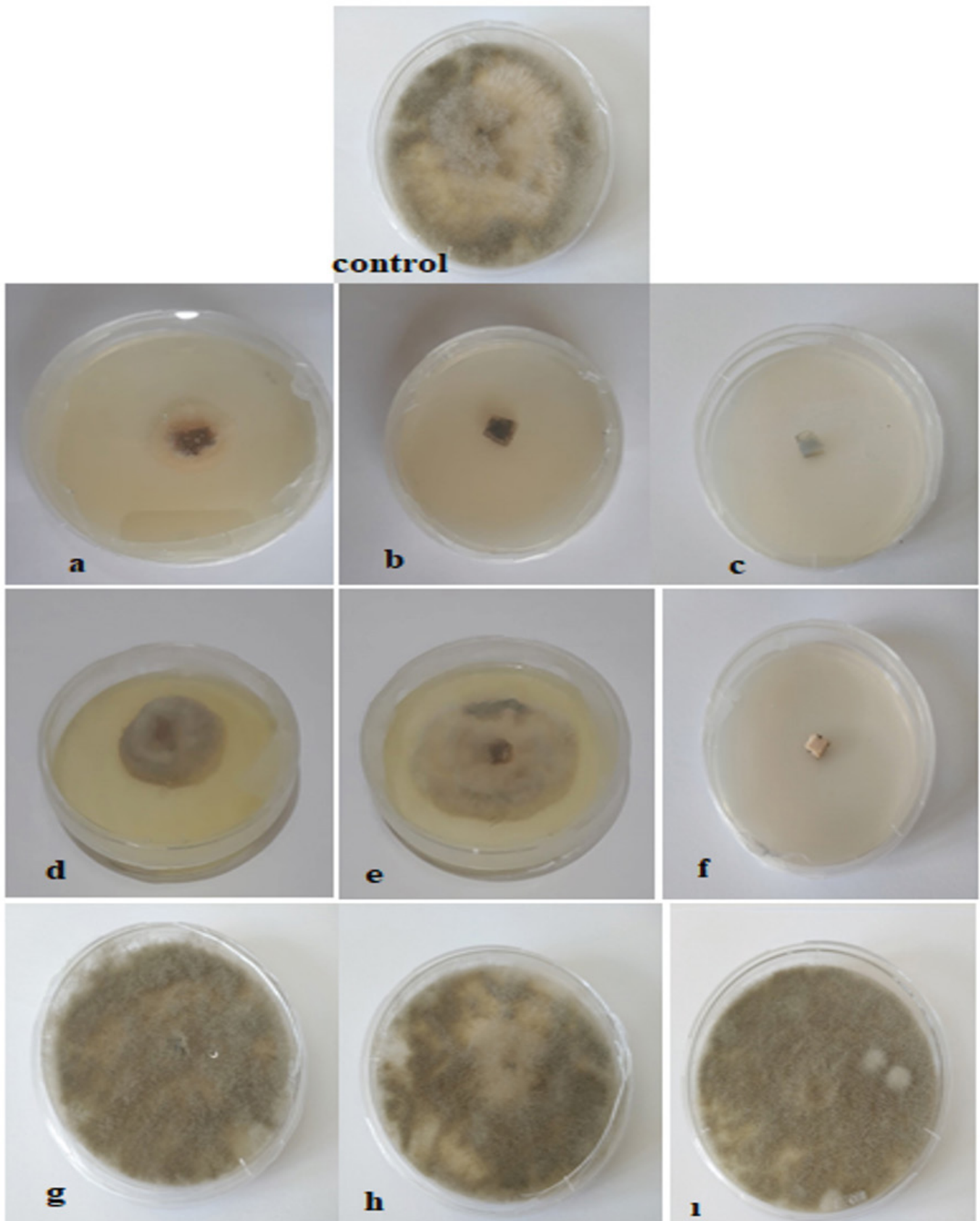


Figure 2. Control and effects of essential oils on the mycelial growth of *B. cinerea*. a. Thymol 2.5 µL; b. Thymol 5 µL; c. Thymol 10 µL; d. Eugenol 2.5 µL; e. Eugenol 5 µL; f. Eugenol 10 µL; g. 1,8- Cineol 2.5 µL; h. 1,8- Cineol 5 µL; i. 1,8- Cineol 10 µL

Table 3. Inhibition rate of different concentrations of eugenol on the germination and the germ tube elongation

EO concentration (μL/Petri dish)	Germ tube elongation (μm)	Spore germination (%)
Control	74.55 ± 4.17	100±0.0
2.5 μL	11.11 ± 1.34 ^a	100±0.0
5 μL	0 ± 0 ^b	100±0.0
10μL	0 ± 0 ^c	100±0.0

* According to Duncan's multiple range test, the difference between group means is significant at the $P < 0.05$ level

Besides, conidiospores germinated 100% at all applied doses of 1,8-cineol (Table 4). The germ tube elongation was also determined to be 74.55 μm in the control group, while for thymol and eugenol, there was no elongation at 5, 10 μL (Table 2,3). At the concentration of 2.5 μL, the germ tube elongation of thymol, eugenol, and 1,8- cineol EOs was 5.55, 11.11, and 72.33 μm, respectively. Thymol had the highest antifungal effects, compared with the other EOs, (Table 2-4).

Table 4. The effect of different concentrations of 1,8- cineol on the germination and the germ tube elongation

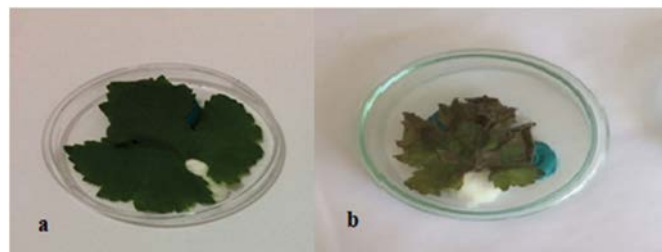
EO concentration (μL/Petri dish)	Germ tube elongation (μm)	Spore germination (%)
Control	74.55 ± 4.17	100±0.0
2.5 μL	72.33 ± 4.69 ^a	100±0.0
5 μL	50.55 ± 6.16 ^b	100±0.0
10μL	14.77 ± 1.52 ^c	100±0.0

* According to Duncan's multiple range test, the difference between group means is significant at the $P < 0.05$ level

Effects of *in vivo* antifungal activity of EOs

The antifungal effects of essential oil concentrations were detected by *in vivo* experiments on detached leaves. Essential oil concentrations were used diluted to 10% due to the EO doses used in *in vitro* experiments causing leaf rot when tested on live leaves. When the trials were compared with the control group and within itself, no lesion formation was observed at the concentration

of 100 μL of all essential oils, and the same effect was shown with the control group (Figures 3-6).

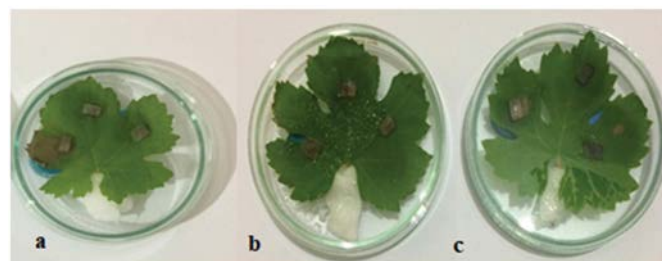
**Figure 3.** a) control; b) fungus application alone

Already using lesions formation at 25 μL concentrations an inhibitory effect of all essential oils was observed. The lesion formation of EOs on leaves was on average 8.22 mm in the thymol, 12.56 mm in eugenol, 14.12 mm in 1,8-cineol treatment (Table 5), as compared to 20.17 mm on leaves. Furthermore, the lesion formation was completely inhibited at thymol 50 and 100 μL concentrations (Table 5). On the other hand, all EO applications have importantly reduced the width of the lesion as per the fungus application alone. As a result of these evaluations, thymol EO had the highest effect on the mycelium growth rate (Table 5, Figure 4).

Table 5. Inhibition rate of different concentrations of essential oils (EO) doses on leaves mycelium inhibition rate (MIR)

EO concentration (μL/Petri dish)	25 μL	50 μL	100 μL
Fungus	20.17 ± 0.74	20.17 ± 0.74	20.17 ± 0.74
Thymol	8.22 ± 1.48 ^a	0.0 ± 0.0 ^b	0.0 ± 0.0 ^c
Eugenol	12.56 ± 1.1 ^a	7.65 ± 0.91 ^b	0.0 ± 0.0 ^c
1,8-Cineole	14.12 ± 2.05 ^a	8.75 ± 1.93 ^b	0.0 ± 0.0 ^c

* According to Duncan's multiple range test, the difference between group means is significant at the $P < 0.05$ level

**Figure 4.** Effect of thymol at different concentrations on *B. cinerea* (a: 25, b: 50, c: 100 μL)

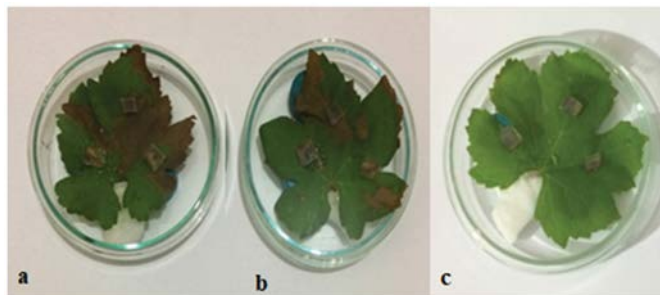


Figure 5. Effect of eugenol at different concentrations on *B. cinerea* (a: 25, b: 50, c: 100 μ L)

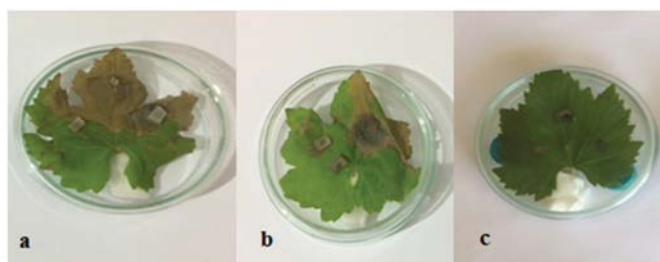


Figure 6. Effect of 1,8-cineol at different concentrations on *B. cinerea* (a: 25, b: 50, c: 100 μ L)

DISCUSSION

Finding biosafe antimicrobials has been one of the most highlighted topics in the last decade, both to cope with the resistance of traditional chemicals and to reduce environmental pollution (Hou et al., 2020). Considering the damage caused by *B. cinerea* to agricultural production, excessive use of fungicides suggests that the fungus has developed resistance to specific pathogens (Hou et al., 2020; Zhao et al., 2021). As such, the development of other natural substances, such as EO, plant extracts, and natural preservatives is urgently required for effective and sustainable control of gray mold (Zhao et al., 2021). Therefore, we prospected the effect of the *in vitro* and *in vivo* antifungal activities of EOs such as thymol, eugenol, and 1,8-cineole, along with their ability to control *B. cinerea* infections. The results were shown in Figure 2, where, the *in vitro* antifungal activity of thymol, eugenol, and 1,8-cineole EOs against *B. cinerea* at different concentrations. The mycelial growth of *B. cinerea* was inhibited depending on the EO dose, indicating that thymol and eugenol noticeably prevented mycelial growth of *B. cinerea* in a concentration-dependent manner. At 10 μ L, both thymol

and eugenol completely inhibited the mycelial growth of *B. cinerea*, whereas at 2.5 μ L thymol, the mycelial growth of *B. cinerea* was 20% lower than that of eugenol. (Table 1). It has been, as a matter of fact, reported that lipids on the cell membrane have an important role in regulating membrane liquidity, reducing the permeability of water-soluble substances and increasing membrane stability (Helal et al. 2007). In addition, volatile substances such as terpenes are also known to penetrate or disrupt these fungal lipid structures (Prashar et al. 2003). Based on our results, we may assume that treatment with thymol, eugenol, and 1,8-cineole obviously disrupted the integrity of the fungus as well as its total lipid content and plasma membrane structure. The findings in this work were similar to those of Camele et al. (2012), who reported that carvacrol at 250 mg/L and thymol at 150 mg/L stopped the growth of *B. cinerea*. Other studies have also noted that carvacrol and thymol are natural phenolic compounds with a number of pharmacological properties, including antifungal effects (López-Malo et al., 2002; Abbaszadeh et al., 2014). Besides, the findings in our study were similar to those of Wang et al. (2010), who stated that the mycelial growth of *B. cinerea* was the most sensitive to eugenol, with an EC₅₀ values of 38.6 μ g/mL. However, on the other hand, there was no inhibitory effect against *B. cinerea* at any concentration of 1,8-cineol (2.5, 5, and 10 μ L). We hypothesized that the main reason why 1,8-cineol was effective against *B. cinerea* could be due to the increase in the application dose, which is consistent with previous reports (Morcia et al. 2012; Juergens et al., 2020; Cai et al., 2021). It, indeed, reported that 1,8-cineol was toxic only at the highest concentration of all the fungal strains (*Fusarium subglutinans* Wollenweber & Reinking, *Fusarium cerealis* (Cooke) Saccardo, *Fusarium verticillioides* Nirenberg, *Fusarium proliferatum* Nirenberg ex Gerlach & Nirenberg, *Fusarium oxysporum* Schlechtendal, *Fusarium sporotrichioides* Sherbakoff, *Aspergillus tubingensis* Mosseray, *Aspergillus carbonarius* Thom, *Alternaria alternata* Keissler, and *Penicillium* spp.) (Morcia et al., 2012). Indeed, the fact that our results show that inhibition is directly proportional to the concentration of Eos, supporting our current hypothesis. Based on our results, when the spore germination and

germ tube elongation results were compared with the inhibitory effect of essential oil application on mycelial growth, it was noted that they supported each other (Table 2-4). Our results showed that thymol had the strongest antifungal activity against spore germination and germ tube elongation, which is consistent with previous findings. Indeed, it has been reported that its fungicidal effect concentration is 120 µL/L and 140 µL/L for carvacrol and 65 mg/L and 100 mg/L for thymol (Zhang et al., 2019). In addition, we found that thymol inhibits both spore germination and germ tube elongation, which is consistent with the findings of several authors, who have reported that thymol and carvacrol cause morphological changes by disrupting the mycelial and hyphae structure of *B. cinerea* (Wang et al., 2014; Abbaszadeh et al., 2014; Elshafie et al., 2015; Zhang et al., 2019; Hou et al., 2020). In addition, in our results, thymol had higher antifungal activity than eugenol and 1,8-cineol on the mycelial growth, spore germination, and germ tube elongation effects on *B. cinerea* (Fig 3). Although it is known that thymol and eugenol have antifungal activity against *B. cinerea* in many studies supporting our study results (Tsao and Zhou, 2000; Bouchra et al., 2003; Daferera et al., 2003; Valero et al., 2006; Campaniello et al., 2010; Wang et al., 2010; Morcia et al., 2012; Olea et al., 2019; Zhang et al., 2019), their mechanisms of action still remain unclear. Contrary to previous reports (Abu-Darwish et al., 2013; Giweli et al., 2013; Elgorban et al., 2015; Dammak et al., 2019), on the other hand, our results revealed that 1,8-cineole had no *in vitro* effect against *B. cinerea*. Indeed, Soković et al. (2009) reported that the fungicidal effects of *Salvia desoleana* Atzei and *Picci (Labiatae)* EO and linalool possessed higher antifungal potential, 1,8-cineol showed only medium antifungal effective. It also indicated that 1,8-cineole had antimicrobial activity against a wide range of pathogenic bacteria and fungi (Abu-Darwish et al., 2013; Giweli et al., 2013; Elgorban et al., 2015; Dammak et al., 2019). It may be likely the main reason for the differences in the inhibitory effect of 1,8-cineol against *B. cinerea* between these studies and our results is due to low concentration of 1,8-cineol.

Considering the phenological stages of the grape, the leaves on the shoot after bud burst are the primary source of inoculum for *B. cinerea*, and based on this fact, the study was carried out on the leaves. In fact, related work Balasubramaniam et al. (1997) suggested that the intensity of *B. cinerea* sporulation development from the phenological stage and overwintering sources from high to low: leaves>berries, rachis>tendrils and petioles>canes. Our results indicated that the protective efficacy of EOs against lesion formation on leaves increased with increasing concentrations of all treatments, and these results were also consistent with our experiments in Petri dishes. Thymol at 100 and 50 µL provided 100% protection, while thymol at 25 µL provided 40% protective, compared to the positive control. In addition, eugenol and 1,8-cineole at 100 µL provided 100% protective efficacy, compared to the positive control (Table 5). Although many studies have been reported on the inhibition of *B. cinerea* by EOs *in vitro*, as far as we know, there is no study in the literature on the *in vivo* efficacy of EOs on grapevine leaves. However, previous studies have highlighted that there is always a delay between the inhibition of germ tube elongation and onset of enzyme inhibition, depending on degradation rate, the sterol storage of the spores and the sterol synthesis rate (Pontzen and Scheinpflug, 1989). From this point of view, we assume in our results that essential oils prevent microbial spoilage as they preserve the freshness and quality of the leaf. Therefore, these could be the reasons why EOs exerted high activity against the germination of spores of *B. cinerea*. On the other hand, it is well known that there are studies on the effectiveness of essential oils against *B. cinerea* in grape berry. In fact, it has been previously reported that *Origanum vulgare* Linné (thyme) EO was effective against gray mold caused by *B. cinerea* on grape berries as an antifungal agent of EOs (Burggraf and Rienth, 2020). Studies have also indicated that fumigation of thymol and linalool has fungicidal effect against *B. cinerea* in table grapes using EOs obtained from *Eucalyptus staigeriana* F. Mueller and *E. globulus* Labillardière (Shin et al., 2014; Pedrotti et al., 2019). Our findings were not compared with those of previous research, as we did not

conduct a study on the effectiveness of EOs against *B. cinerea* in grape berries; therefore, further research on the efficacy of EOs on grapevine berries would be useful. Indeed, conducting experiments on berries to test the applicability of our EOs effect study to the grape varieties at large would be highly interesting.

CONCLUSION

This paper has revealed that thymol, eugenol, and 1,8-cineole can clearly repress the mycelial growth of *B. cinerea* in grape leaves, making them promising antifungal agents. These results indicated that essential oils have the potential to be used as biofungicides against the plant pathogen *B. cinerea*, due to their low toxicity and ready biodegradability. Thymol, eugenol, and 1,8-cineol could be used to control gray mold caused by *B. cinerea*, and are environmentally friendly and a potential alternative to synthetic pesticides. However, further studies are required before these EOs may be used as biofungicide agents in the treatment of wine grapes.

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