

Antifungal effects of extracts from *Origanum compactum* Benth and *Eucalyptus grandis* on gray mold from Strawberry

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ARTICLE HISTORY

Received: Apr. 11, 2025

Accepted: Nov. 9, 2025

KEYWORDS

Botrytis cinerea,
Plant extracts,
Eucalyptus grandis,
Origanum compactum
Strawberries.

Abstract: *Botrytis cinerea*, responsible for gray mold, is a highly destructive disease of strawberries in Morocco. Fungicides are commonly used to control this pathogen, but they present considerable dangers to the environment and human health. Thus, it is necessary to look for new efficient non-polluting alternatives such as medicinal plant extracts. The goal of this research was to evaluate the antifungal potency of aqueous extracts and essential oil (EO) of *Origanum compactum* and *Eucalyptus grandis* against *B. cinerea* *in vitro*. The chemical composition of EO was also examined through gas chromatography-mass spectrometry (GC-MS). Different concentrations of extracts were tested on the Malt medium inoculated with the pathogen. The findings revealed that the Minimum Inhibitory Concentrations (MICs) of aqueous extracts of all medicinal plants are the same for mycelial growth and sporulation of *B. cinerea*. MICs of EO from *O. compactum* are 0.5 µL/mL against *B. cinerea*, while the MICs of EO of *E. grandis* are 0.6 µL/mL. The different compounds of EOs were identified. Thymol and carvacrol are the major chemical compounds of *O. compactum*, while Eucalyptol and Gamma-Terpinene are the major compounds for *E. grandis*. Extracts of two medicinal plants induced a total irreversible inhibition on the mycelial growth of *B. cinerea*.

INTRODUCTION

Strawberry (*Fragaria ananassa*), one of the most popular fruits, produces more than 9 million tons annually worldwide, cultivated on 389,665 hectares (FAOSTAT, 2023). In Morocco, the area occupied by strawberries during the 2022/23 campaign is 3,800 ha (Agriculture du Maghreb., 2023). Unfortunately, strawberries are vulnerable to a range of pathogens, like

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e-ISSN: 2148-6905

viruses, nematode, bacteria, and fungi. The latter can infect all parts of the plant, causing severe damage or death, and are the most economically damaging (Garrido *et al.*, 2011).

The necrotrophic ascomycete fungus *Botrytis cinerea* Pers (perfect stage as *Botryotinia fuckeliana*) is considered a major pathogen of harvested strawberries worldwide, causing gray mold and significant economic damage in the strawberry industry. More than 80% of strawberry fruit and flowers can be lost if plants are not treated with fungicides under wet conditions (Petrasch *et al.*, 2019). In Tunisia, Essghaier *et al.* (2009), observed a 25% yield loss if no spraying is applied. In the United States (State of Florida), losses due to fruit rot caused by *B. cinerea* can exceed 50% under favorable conditions such as humidity and temperature, even in well-managed fields (Vorotnikova *et al.*, 2012).

The main approach used to control strawberry pathogenic fungi is to use synthetic chemical fungicides. Unfortunately, it causes an increase in fungicide resistance, and new legal restrictions have raised concerns about their use (Feliziani *et al.*, 2016). Moreover, the presence of fungicide residues on fruits and vegetables poses potential risks to human health and the environment (Paster & Bullerman, 1988). Therefore, it has increased the interest in the use of biodegradable, natural alternatives to replace synthetic fungicides (Tripathi & Dubey, 2004). Eco-friendly plant extracts have emerged as effective alternatives to synthetic fungicides. They offer potential in controlling diseases and maintaining the quality of fruit and vegetables. The goals of our research are to evaluate *in vitro* effects of aqueous extracts of *Origanum compactum* Benth. and *Eucalyptus grandis* as well as their EOs on mycelial growth and sporulation of *B. cinerea*, and identify and analyze phytochemical components of EOs using gas chromatography-mass spectrometry (GC/MS) analysis.

MATERIAL and METHODS

Pathogen

Our collection consists of the most virulent fungi conserved in the National Institute of Agronomic Research (INRA) Phytopathology laboratory's mycothec. The isolate of *B. cinerea* was previously obtained from strawberry fruits with gray mold symptoms.

Plant samples

O. compactum Benth leaves were collected from the unit of aromatic and medicinal plants of INRA, while the leaves of *E. grandis* were collected from Bouknadel (Sale, Morocco). Leaves were dried in the shade, after which they were crushed and stored at 4°C for use in this study.

Essential Oils Extraction

The EOs were extracted by the hydrodistillation method in an apparatus of the Clevenger type (Clevenger, 1928). For each plant, we took 130 g of dried leaves, immersed them in 1 liter of water in the flask at 100°C. After 2 h, EO was collected by decantation and stored at 4°C. Yield of EO was calculated based on the dry weight of leaves according to the following formula:

$$R (\%) = \text{Mass of EO} / \text{Dry leaves mass} \times 100$$

GC-MS Analysis and Identification of Components

Origanum compactum Benth. and *Eucalyptus grandis* EO components were identified using GC-MS (Hewlett-Packard HP6890/HP5973), based on retention indices and mass spectra, which were compared with those reported in literature (Adams, 2001). For percentage determination, a Perkin Elmer Clarus® 580 gas chromatograph with a DB-5 fused silica column and FID detector was used. Oven temperature was initially set to 60°C (3 min), then ramped to 280°C at 5°C/min. Injector and detector temperatures were 260°C and 280°C, respectively, with a helium flow rate of 1 mL/min and a 20:1 split ratio. Essential oil samples were diluted with hexane (1:9), and 1 µL was injected for analysis (Adams, 2007).

Antifungal Activity of *O. compactum* and *E. grandis* Against *B. cinerea* in vitro

Effects on mycelial growth

Leaves of two medicinal plants, *O. compactum* and *E. grandis*, were used to prepare both aqueous extracts and essential oils (EOs). For aqueous extracts, the decoction method was used. Different quantities of leaf powder were introduced into the culture medium to obtain the final concentrations shown in Table 1. The powder was placed in a sterile pouch, securely tied, and immersed in the Malt Extract Agar (MEA) medium (Biokar Diagnostics, Ref: A1101HA, France). The mixture was then autoclaved at 121°C for 15 minutes, allowing both the sterilization of the medium and the extraction of water-soluble compounds from the plant material. After cooling to room temperature, the medium was poured into sterile petri dishes and left to solidify. These plates were then used for antifungal testing. The concentrations used were selected based on preliminary tests.

Antifungal activity of EOs was evaluated by the direct contact method, as described by Terzi et al. (2014). To improve miscibility, EOs were first emulsified with 0.5% (v/v) Tween 80 (Merck, Allemagne). The emulsions were then mixed with sterile Malt Extract Agar (MEA) medium (Biokar Diagnostics, Ref: A1101HA, France) maintained at 40–45°C to achieve the final concentrations shown in Table 1. The prepared media were poured into sterile petri dishes and left to solidify at room temperature. These plates were subsequently used for testing the antifungal effect of the EOs.

A 5 mm *B. cinerea* mycelial disc was positioned in the middle of each Petri dish. The experiment was performed twice with 3 replicates for each aqueous extract and EO concentration, with a control for each replicate. Mycelial growth was assessed after 4–7 days of incubation (at 24°C) by measuring the average diameter of the mycelium. The formula used to calculate the percentage of inhibition (I%) is (Leroux & Gredet, 1978):

$$I (\%) = (T - A) / T \times 100$$

A: growth zone diameter in experimental dish (cm); T: growth zone diameter in control dish (cm).

Table 1. Range of concentrations of aqueous extracts (AE) and essential oils (EO) used in this study.

Medicinal plant	Range of AE concentrations in mg/mL	Range of EO concentrations in µL/mL
<i>O. compactum</i>	1-2-3-4-8	0.2-0.4-0.5-0.6
<i>E. grandis</i>	1-50-100-200-220-230	0.2-0.4-0.5-0.6

Effects on sporulation

The test is performed according to the method of Leroux et al. (1999). After assessing the effect of aqueous extracts and EOs on mycelial growth, the petri dishes were reused to evaluate sporulation after 12 days of incubation (at 24°C). A negative control (without extracts) was included in all assays. To count the spores, sterile distilled water (1 mL) was added to each dish, and the surface was gently scraped to release the spores into the water. The resulting suspension was transferred into Eppendorf tubes. Then, 17 µl of this spore suspension was placed into a Neubauer counting chamber, and spores were counted under a microscope using a 40x objective. The number of spores obtained was used to determine the inhibitory effect of the treatments in comparison to the control. Percentages of sporulation inhibition were calculated, in comparison to the negative control, using the following formulas (Leroux & Gredet, 1978):

$$I (\%) = (NT - NA) / NT \times 100$$

I (%): Percentage of sporulation inhibition, NT: Number of spores in absence of the extract, NA: Number of spores in the presence of the extract

Determination of IC₅₀ and MIC Values

The half maximal inhibitory concentration (IC₅₀) value was calculated by the linear regression equation between the decimal log of the studied extract concentrations (x-axis) and the percent growth inhibition transformed into probit values (y-axis), Probit = f (Log₁₀ concentration). The concentrations that reduce mycelial growth by 50% (IC₅₀) are determined from the equation of the line $y = ax + b$.

The minimum inhibitory concentration (MIC) was examined by the method reported by Yen & Chang (2008). It was identified as the lowest concentration of aqueous extracts or EOs where no mycelial growth was detected when mycelium reached the edges of the control dishes.

Fungitoxic Activity of EO

After MIC determination, fragments from the colony on MIC plates were transferred to fresh Malt Extract Agar (MEA) medium and incubated at 24°C for 5 days. The minimum fungicidal concentrations (MFC) were determined by the lowest concentration of the aqueous extracts or EOs at which no fungal regrowth was observed (Yen & Chang, 2008).

RESULTS

Antifungal Activity of Aqueous Extracts Against *B. cinerea* in vitro

Aqueous extract of *O. compactum* exhibited strong antifungal activity against *B. cinerea*, with clear, dose-dependent inhibition of mycelial growth and sporulation. At 0 mg/mL, mycelial growth measured 54.0 ± 0.8 mm, and sporulation reached 174.00 ± 1.00 spores/mL (Table 2). At just 1 mg/mL, the extract inhibited mycelial growth by 69.69% and sporulation by 46.36% (Figure 1), indicating high efficacy at low concentrations. In fact, complete inhibition of mycelial growth and sporulation (0.00 ± 0.00) was observed at 4 mg/mL, confirming the strong antifungal activity of this extract.

Aqueous extract of *E. grandis* also demonstrated antifungal activity against *B. cinerea*, although higher concentrations were required to achieve complete inhibition compared to *O. compactum*. In the absence of extract (0 mg/mL), mycelial growth reached 58.8 ± 0.21 mm and sporulation 176.67 ± 1.15 spores/mL (Table 2). As shown in Figure 2, the extract caused a gradual, concentration-dependent reduction in mycelial growth and sporulation. At 100 mg/mL, the inhibition rate of mycelial growth was approximately 50%, while sporulation inhibition exceeded 50% at 50 mg/mL. Moreover, a concentration of 230 mg/mL (0.00 ± 0.00) stopped mycelial growth and sporulation of *B. cinerea*.

Table 2. Effect of aqueous extracts of *O. compactum* and *E. grandis* on mycelial growth and sporulation (mean $\pm$ SD).

<i>O. compactum</i>			<i>E. grandis</i>		
Concentration (mg/mL)	Mycelial growth (mm) $\pm$ SD	Sporulation (spores/mL $\pm$ SD)	Concentration (mg/mL)	Mycelial growth (mm) $\pm$ SD	Sporulation (spore/mL) $\pm$ SD
0	54.0 ± 0.8	174.00 ± 1.00	0	58.8 ± 0.21	176.67 ± 1.15
1	16.3 ± 0.2	90.33 ± 0.58	1	44.5 ± 0.43	112.67 ± 1.15
2	15.9 ± 0.1	55.33 ± 0.58	50	35.4 ± 0.18	75.00 ± 1.00
3	01.0 ± 0.7	14.67 ± 0.58	100	30.8 ± 0.08	30.33 ± 0.57
4	00.0 ± 0.0	00.00 ± 0.00	200	21.4 ± 0.09	20.67 ± 1.15
8	00.0 ± 0.0	00.00 ± 0.00	220	02.9 ± 0.58	11.33 ± 1.15
			230	00.0 ± 0.00	00.00 ± 0.00

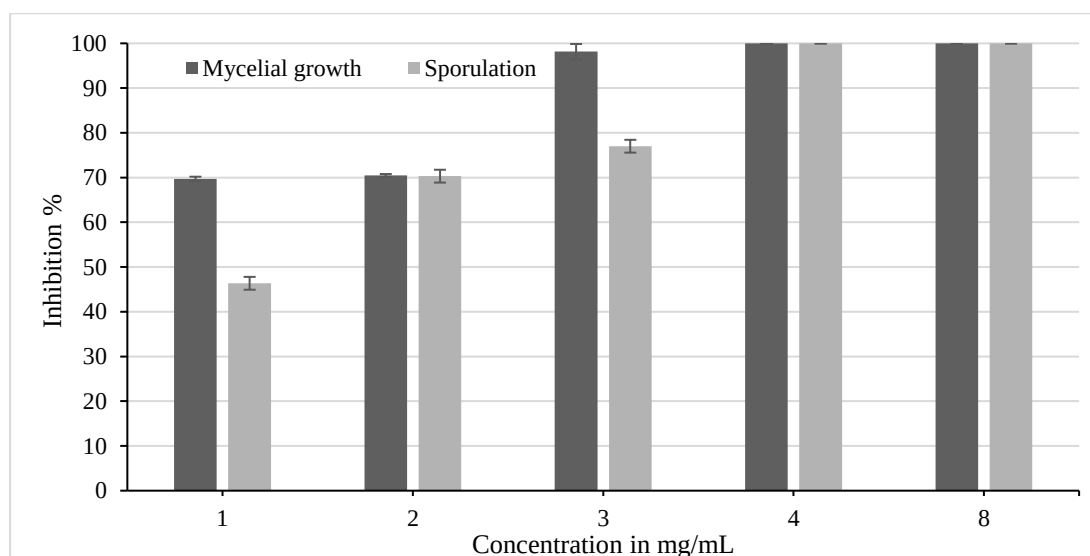


Figure 1. Aqueous extract effect of *O. compactum* on mycelial growth and sporulation of *B. cinerea*. Data were presented as mean $\pm$ SD of three replicates. Significant differences ($p < 0.05$) between mean values are indicated by the letters above the bars in the histogram.

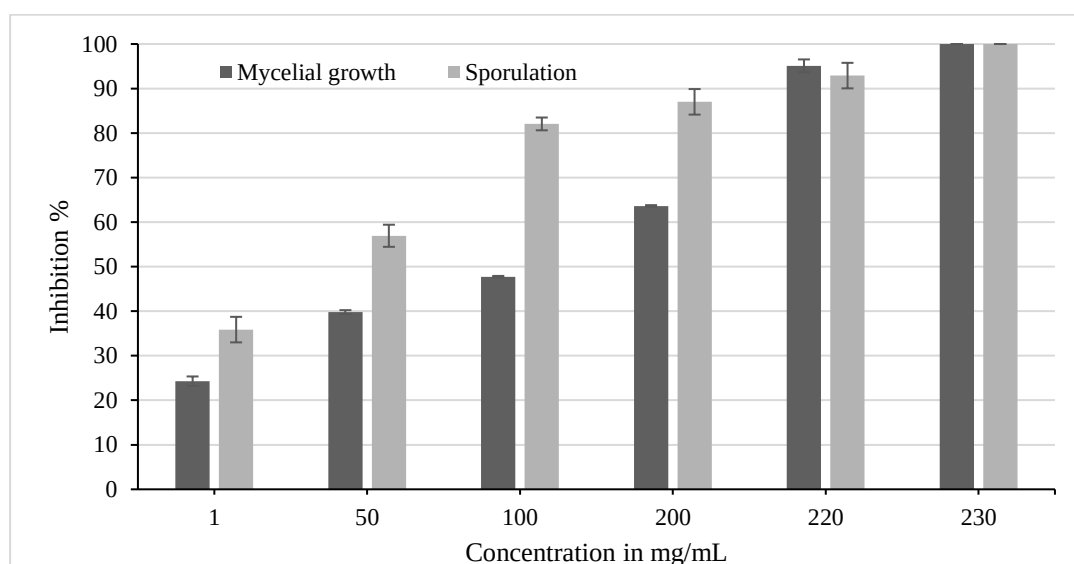


Figure 2. Aqueous extract effect of *E. grandis* on mycelial growth and sporulation of *B. cinerea*.

Data were presented as mean $\pm$ SD of three replicates. Significant differences ($p < 0.05$) between mean values are indicated by the letters above the bars in the histogram.

Chemical analysis of EO from *O. compactum* and *E. grandis*

The EOs showed variable colors, ranging from light yellow for *E. grandis* to dark yellow for *O. compactum*, along with strong odors. Their yields were 3.05% for *O. compactum* Benth. and 0.54% for *E. grandis*. GC-MS analyses of *O. compactum* (Figure 3A) and *E. grandis* (Figure 3B). EOs allow for the identification of different compounds. These EOs contain mainly oxygenated monoterpenes and sesquiterpenes, as well as monoterpene and sesquiterpene hydrocarbons. For EO of *O. compactum*, 97% were identified (Table 3); its main constituents are carvacrol (42.84%) and thymol (17.76%). A total of 96.37% of compounds were identified in EO of *E. grandis* (Table 4). Eucalyptol (1,8-cineole) was the main compound, which made up 76.08%, followed by gamma-terpinene (10.64%).

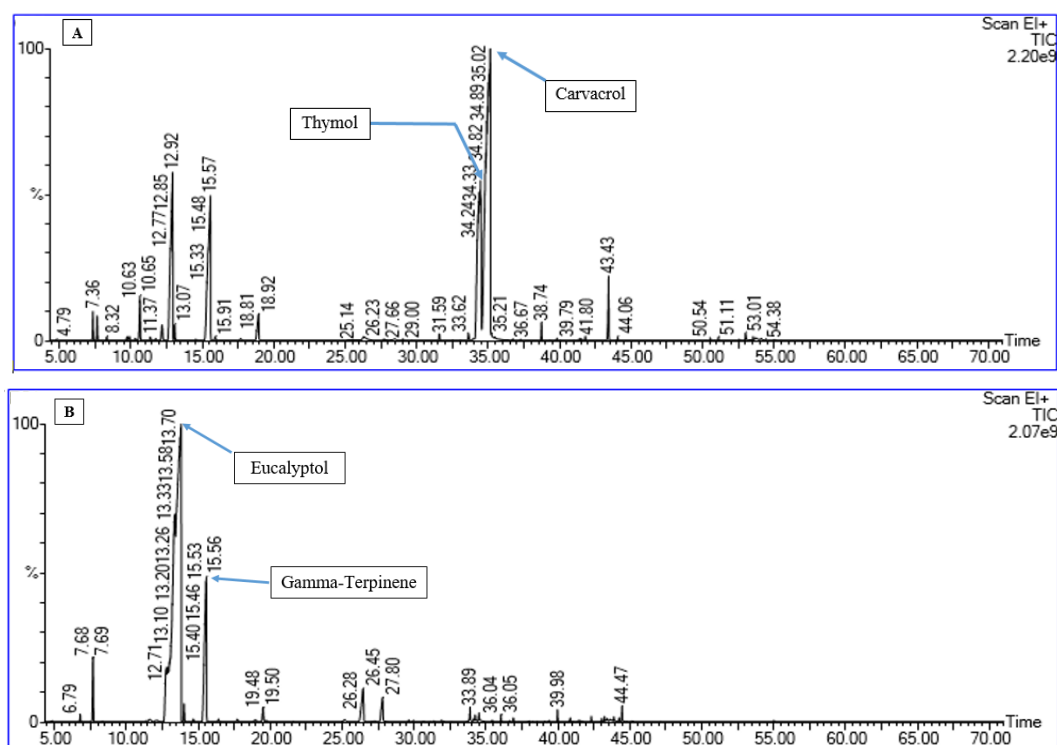


Figure 3. Essential oils GC-MS Chromatogram from *O. compactum* (A) and *E. grandis* (B).

Table 3. Chemical components and class of EO from *O. compactum* determined by GC/MS.

Name of compound	Retention time (min)	Area %	Formula	Compound class
Carvacrol	35.149	42.843	C ₁₀ H ₁₄ O	Oxygenated monoterpenes
Thymol	34.473	17.769	C ₁₀ H ₁₄ O	Oxygenated monoterpenes
Trans-3-Carene-2-Ol	15.572	13.934	C ₁₀ H ₁₆ O	Other
1-Methyl-2-Propan-2-Ylbenzene	12.920	13.242	C ₁₀ H ₁₃ ⁺	Other
Caryophyllene Oxide	43.432	1.722	C ₁₅ H ₂₄ O	Oxygenated Sesquiterpene
Linalool	18.940	1.542	C ₁₀ H ₁₈ O	Oxygenated monoterpenes
Myrcene	10.648	1.447	C ₁₀ H ₁₆	Monoterpene Hydrocarbons
Alpha-Pinene	7.355	0.723	C ₁₀ H ₁₆	Monoterpene Hydrocarbons
4-Carene	12.191	0.714	C ₁₀ H ₁₆	Other
Alpha-Pinene	7.647	0.628	C ₁₀ H ₁₆	Monoterpene Hydrocarbons
Linalool	26.307	0.577	C ₁₀ H ₁₈ O	Oxygenated monoterpenes
Camphene	13.070	0.421	C ₁₀ H ₁₆	Monoterpene Hydrocarbons
Beta-Caryophyllene	38.742	0.400	C ₁₅ H ₂₄	Sesquiterpene hydrocarbons
Anethole	33.627	0.254	C ₁₀ H ₁₂ O	Other
Ethyl Tetramethyl Cyclopentadiene	53.008	0.210	C ₁₁ H ₁₈	Other
Isothymol Methyl Ether	31.589	0.207	C ₁₁ H ₁₆ O	Other
Alpha-Terpineol	28.462	0.157	C ₁₀ H ₁₈ O	Oxygenated monoterpenes
1-Octen-3-Ol	9.898	0.156	C ₈ H ₁₆ O	Other
Pseudocarveol	25.214	0.150	C ₁₀ H ₁₆ O	Oxygenated monoterpenes

Table 4. Chemical components and class of EO from *E. grandis* determined by GC/MS.

Name of compound	Retention time (min)	Area %	Formula	Compound class
Eucalyptol	13.813	76.085	C ₁₀ H ₁₈ O	Oxygenated monoterpenes
Terpinen-4-Ol	26.448	2.656	C ₁₀ H ₁₈ O	Oxygenated monoterpenes
Alpha-Pinene	7.689	2.033	C ₁₀ H ₁₆	Monoterpene hydrocarbons
Gamma-Terpinene	15.555	10.642	C ₁₀ H ₁₆	Monoterpene hydrocarbons
Alpha-Terpineol	27.807	1.479	C ₁₀ H ₁₈ O	Oxygenated monoterpenes
Isoamyl Isovalerate	19.516	0.628	C ₁₀ H ₂₀ O ₂	Other
Beta-Ocimene	14.025	0.533	C ₁₀ H ₁₆	Monoterpene Hydrocarbons
Thymol	33.894	0.439	C ₁₀ H ₁₄ O	Oxygenated monoterpenes
2,5-Furandione	44.466	0.362	C ₁₆ H ₂₆ O ₃	Other
Widdrol Epoxide	39.976	0.280	C ₁₅ H ₂₆ O ₂	Other
Carvacrol	34.524	0.266	C ₁₀ H ₁₄ O	Oxygenated monoterpenes
Isobutyl Isovalerate	11.599	0.216	C ₉ H ₁₈ O ₂	Other
Isobutyl Isobutyrate	6.796	0.189	C ₈ H ₁₆ O ₂	Other
Santolina Alcohol	25.169	0.181	C ₁₀ H ₁₈ O	Other
Cis-Linalool Oxide	17.719	0.132	C ₁₀ H ₁₈ O ₂	Oxygenated monoterpenes
Isoamyl Butyrate	12.116	0.120	C ₉ H ₁₈ O ₂	Other

Antifungal Activity of EOs Against *B. cinerea* in vitro

O. compactum EO showed significant antifungal activity against *B. cinerea*. In the control (0 µL/mL), mycelial growth reached 50.1 ± 0.76 mm, and sporulation was recorded at 156.33 ± 1.52 spores/mL (Table 5). As the concentration increased, both parameters declined progressively, with complete inhibition (0.00 ± 0.00) observed at just 0.5 µL/mL (Figure 4). IC₅₀ values for mycelial growth and sporulation are 0.22 and 0.3 µL/mL, respectively, highlighting the high potency of this essential oil.

The results of the application of *E. grandis* EO at varying concentrations showed a growth inhibition in *B. cinerea*, which was dependent on the concentration of EO. In the control (0 µL/mL), mycelial growth reached 65.6 ± 0.26 mm, and sporulation was 125.60 ± 0.57 spores/mL (Table 5). Indeed, the concentration that generated a total inhibition is 0.6 µL/mL; other concentrations 0.5, 0.4, and 0.2 µL/mL induced an inhibitory effect of 71.1, 65.59, and 24.95%, respectively. The EO of *E. grandis* reduced the sporulation rate of *B. cinerea*. Indeed, at 0.2, 0.4, and 0.5 µL/mL, percentages of sporulation inhibition were 19.95, 65.14, and 78.16%, respectively. At 0.6 µL/mL, *E. grandis* EO completely inhibited sporulation of *B. cinerea* (Figure 5). The IC₅₀ values are the same for mycelial growth and sporulation (0.32 µL/mL). This suggests that although both essential oils are effective antifungal agents, *O. compactum* EO is slightly more potent, likely due to differences in the composition or concentration of active compounds.

Table 5. Effect of EOs of *O. compactum* and *E. grandis* on mycelial growth and sporulation (mean ± SD).

Concentration (µL/mL)	<i>O. compactum</i>		<i>E. grandis</i>	
	Mycelial growth (mm) ± SD	Sporulation (spores/mL ± SD)	Mycelial growth (mm) ± SD	Sporulation (spores/mL ± SD)
0	50.1 ± 0.76	156.33 ± 1.52	65.6 ± 0.26	125.60 ± 0.57
0.2	27.3 ± 0.40	137.33 ± 1.15	49.3 ± 0.18	100.67 ± 1.15
0.4	11.1 ± 0.18	37.33 ± 1.15	22.6 ± 0.25	44.33 ± 1.15
0.5	00.0 ± 0.00	00.00 ± 0.00	22.5 ± 0.05	30.33 ± 0.57
0.6	00.0 ± 0.00	00.00 ± 0.00	00.0 ± 0.00	00.00 ± 0.00

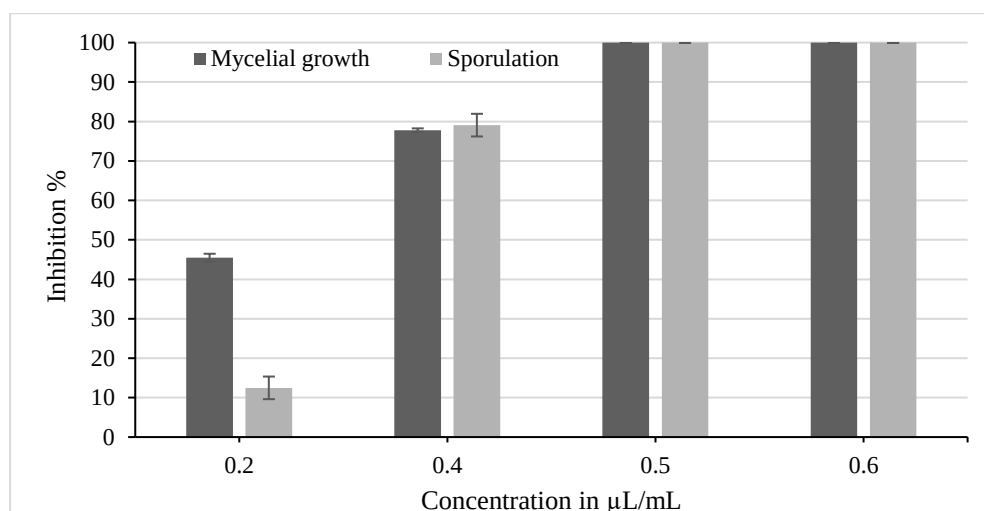


Figure 4. Effect of *O. compactum* EO on mycelial growth and sporulation of *Botrytis cinerea*.

Data are expressed as mean $\pm$ SD of three replicates. Significant difference ($p < 0.05$) between mean values is indicated by the letters above the bars in the histogram.

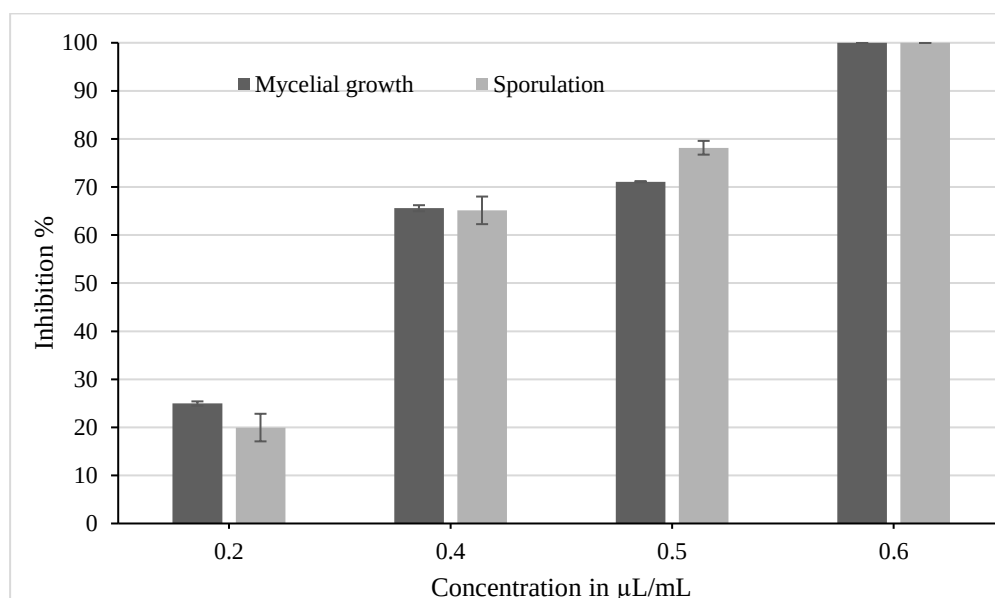


Figure 5. Effect of *E. grandis* EO on mycelial growth and sporulation of *B. cinerea*.

Data are expressed as mean $\pm$ SD of three replicates. Significant difference ($p < 0.05$) between mean values is indicated by the letters above the bars in the histogram.

The results show that in the case of *E. grandis* EO, MIC is about 0.6 $\mu\text{L/mL}$, both for mycelial growth and sporulation, while for *O. compactum* EO, it is 0.5 $\mu\text{L/mL}$. Moreover, the results of the transfer of explants of *B. cinerea* completely inhibited on a new culture medium did not revive after 5 days of incubation. These EOs caused the death of *B. cinerea* and are therefore fungicidal. The MIC of each product used alone corresponds to the minimum fungicidal concentration (MFC). EOs of two plants tested exhibited significant antifungal activity against *B. cinerea* at very low concentrations.

DISCUSSION

Antifungal activity of extracts from aromatic and medicinal plants, *O. compactum* and *E. grandis*, is effective against *B. cinerea* isolated from strawberry. The aqueous extracts demonstrated notable antifungal activity against *B. cinerea*, with clear differences in their minimum inhibitory concentrations (MIC). Notably, *O. compactum* was significantly more potent, requiring over 50 times lower concentration than *E. grandis* to achieve complete inhibition. The MIC for *O. compactum* was 4 mg/mL, at which complete inhibition of mycelial

growth and sporulation was observed. In contrast, *E. grandis* required a much higher concentration of 230 mg/mL to achieve the same inhibitory effect. Our results align with previous studies on Lamiaceae plants antifungal activity. Kasmi *et al.* (2017) reported that *Thymus vulgaris* aqueous extract inhibited *B. cinerea* mycelial growth by 40% and sporulation by 85% at 8 mg/mL. In comparison, *O. compactum* in our study showed stronger activity, achieving complete inhibition at just 4 mg/mL. The antifungal potential of *Eucalyptus* species has also been widely documented. Ramezani *et al.* (2002) and Bluma *et al.* (2008) showed that various *Eucalyptus* extracts inhibit fungal growth under both *in vitro* and *in vivo* conditions. Specifically, Oh *et al.* (2008) tested the antifungal activity of extracts from four *Eucalyptus* species and found that *E. unigera* was the most effective, with inhibition rates ranging from 50% to 80% against *B. cinerea* isolated from kiwi fruit. In our study, *E. grandis* extract achieved approximately 50% inhibition at 100 mg/mL, while complete inhibition was only observed at 230 mg/mL, indicating a moderate concentration-dependent antifungal activity. The differences in antifungal activity observed between the two extracts are likely due to variations in their chemical composition, particularly the presence and concentration of bioactive compounds such as polyphenols and triterpenoids (Bolou *et al.*, 2011). However, the aqueous extract of *O. compactum* appears especially promising as a natural antifungal agent for the management of postharvest diseases.

The EO yield of *O. compactum* (3.05%) in our study was richer than that of *O. compactum* from Bouyblan (0.82%) (Taza, Morocco) (Habbadi *et al.*, 2018), Boulemane (1.15%), and Taounate regions (0.97%) (Al-Mijalli *et al.*, 2022). This suggests that the *O. compactum* plants used in our study may have benefited from more favorable ecological or environmental conditions. Similarly, *E. grandis* from our study yielded 0.54% EO, which is significantly higher than that reported in Uruguay (0.06%) (Dellacassa *et al.*, 1990), but lower than the yield recorded from China (4.44%) (Lu *et al.*, 2014). Differences in results may be related to factors such as soil nature, tree age, collection area and time, extraction method, and climate. Furthermore, GC-MS analyses of EO revealed that *O. compactum* was composed of carvacrol and thymol, containing components at lower levels. These results are similar to those found by Habbadi *et al.* (2018), Al-Mijalli *et al.* (2022) in Boulmane, and Chroho *et al.* (2024) in the Middle Atlas of Morocco, all of which reported carvacrol as a main component. In contrast, EO of *E. grandis* is composed predominantly of eucalyptol (1,8-cineole) and gamma-terpinene, with other components at lower levels. Our results are similar to those obtained in China (Lu *et al.*, 2014); all had similar leaf essential oils with a predominance of 1,8-cineole. These comparisons suggest that variations in EO yield and composition are mainly influenced by environmental factors.

The EO of *O. compactum* showed significant antifungal activity against *B. cinerea* than that observed for *E. grandis*, with a lower MIC value of 0.5 $\mu\text{L/mL}$ compared to 0.6 $\mu\text{L/mL}$, respectively. This activity is probably due to the presence of two abundant components in this oil (carvacrol and thymol) and also possible synergistic effects with minor compounds (Table 3). Hou *et al.* (2020) found that carvacrol and thymol can destroy mycelial morphology, increase membrane permeability of target cells, induce mitochondrial membrane destruction, and reactive oxygen species (ROS) accumulation in *B. cinerea*. In contrast, the antifungal power of *E. grandis* EO could be attributed to its richness in eucalyptol or even synergy with minor compounds such as gamma-terpinene (Table 4). Vilela *et al.* (2009) showed that pure 1,8-cineole causes mycelial growth inhibition, but at higher concentrations than EOs as a whole, which may indicate that the main constituent of the oil is not the only component responsible for limiting mycelial growth. While both EOs were effective, the lower MIC of *O. compactum* EO indicates greater potency. Many studies have shown the antifungal activity of EOs of several *Eucalyptus* species (Ammad *et al.*, 2024; Hmiri *et al.*, 2011; Davari & Ezazi, 2017; Kenfaoui *et al.*, 2023). Davari & Ezazi (2017) showed that at 1500 $\mu\text{L/L}$ of *Eucalyptus* sp. EO reduced mycelial growth of *B. cinerea* up to 84.88%. These EOs offer a promising alternative to conventional anti-botrytis fungicides in the management of gray mold.

CONCLUSION

In conclusion, both *O. compactum* and *E. grandis* revealed significant antifungal activity against *B. cinerea* affecting strawberries, as evidenced by the effective inhibition of mycelial growth and sporulation in response to their aqueous extracts and EOs. In *O. compactum* EO, Thymol and Carvacrol are major compounds, while in *E. grandis* EO Eucalyptol and Gamma-Terpinene were major compounds. Both EOs are fungicidal in nature. These results are promising and allow us to conclude that extracts of the two medicinal plants studied represent an alternative to anti-botrytis fungicides.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Data Availability Statement

Research data is available and can be sent with e-mail upon request.

Authorship Contribution Statement

Soukaina Hammoumi: Conceptualization, Methodology, Investigation, Resources, Visualization, Software, Formal Analysis, Writing-original draft, and editing. **Fatiha Bentata, Aicha El Aissami and Mustapha Labhilili:** Methodology, Literature Review, Supervision, and Validation. **Sanae Karim, Houda Khalifi and Ilyass Maafa:** Investigation, Data Collection, Supervision. **Aouatif Benali:** Methodology, Analysis. **Najiba Brhadda and Rabea Ziri:** Review, Validation; Supervision.

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