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Antifungal activity of plant essential oils against gray mold (*Botrytis cinerea*) under *In Vitro* conditions

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Abstract

Plant essential oils (EOs) have gained attention for their antifungal properties, effectively preventing fungal contamination and extending the shelf life of agricultural products, with their efficacy varying based on concentration and type. This study investigates the antifungal effects of 13 medicinal EOs on the growth of gray mold (*Botrytis cinerea*) under in vitro conditions. An isolate of *B. cinerea* and Potato Dextrose Agar (PDA) was utilized as the culture medium for fungal growth. The antifungal activity was assessed by applying EOs at 500, 1000, and 2000 ppm concentrations alongside the fungicide carbendazim at 1500 and 2000 ppm. Key growth parameters, such as colony diameter, colony density, height, mean colony diameter growth rate (DR), mycelia growth coefficient (MGC), and mycelia growth inhibition (MGI), were evaluated 24 hours post-treatment. Results indicated that variance analysis revealed significant differences ($P<0.01$) across treatments. EOs notably reduced colony diameter and density compared to the control group, with higher concentrations typically exhibiting increased effectiveness. Specifically, lemongrass, garden thyme, Shirazi thyme, and cinnamon EOs more effectively inhibited colony growth than other EOs and carbendazim. At all concentrations, these EOs resulted in zero colony growth, contrasting with higher colony heights observed in untreated controls (2.33 mm). MGI analysis indicated significant inhibition of mycelial growth, particularly with EOs, which consistently demonstrated superior results over the fungicide, especially at 1000 and 2000 ppm. Remarkably, 100% MGI was achieved with lemongrass, garden thyme, Shirazi thyme, and cinnamon EOs at higher concentrations throughout the incubation period. Carbendazim was less effective, demonstrating a lag phase in its antifungal activity. While the control exhibited the highest mycelial growth coefficient (67.11), all treatments significantly reduced growth relative to controls. The results underscore the potential of specific EOs as viable alternatives to chemical fungicides in managing *B. cinerea*, with implications for sustainable agricultural practices. Further studies should explore the mechanisms underlying these antifungal properties and their applicability in field conditions.

Keywords – Colony diameter – density and height – fungi – medicinal plants – mycelium growth

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Introduction

One of the problems the agricultural industry is currently facing is plant diseases, which cause significant damage to farmers yearly. Fungi, bacteria, and viruses are plant pathogens that cause considerable harm to agricultural products (Jones et al. 2021). Plant diseases account for substantial losses, costing the global economy over $220 billion annually. Additionally, managing post-harvest decay is essential, as it is estimated that nearly half of all fruits and vegetables produced worldwide are either lost or not consumed (FAO 2021). *B. cinerea*, commonly known as gray mold, is one of the most destructive pathogens affecting a wide range of plants and leading to the decay of fruits and vegetables post-harvest. This fungus can grow at low temperatures, further deteriorating product storage and marketing phases (Roca-Couso et al. 2021).

B. cinerea is a significant postharvest disease affecting fresh fruit. Its management involves a combination of preharvest and postharvest strategies, predominantly using fungicides in conventional agriculture (Adaskaveg et al. 2021). However, the excessive and inappropriate use of fungicides has led to resistant genotypes and reduced their effectiveness (Yin et al. 2023). In addition, the negative impact of fungicide residues on human health and the environment has raised public concerns (Kalyabina et al. 2021).

There is a growing interest in developing alternative treatments to reduce losses through integrated management approaches. Therefore, the importance of using safe and healthy alternatives to fungicides has recently received significant attention (Burandt et al. 2024). One of the resources for controlling fungi is plant essential oils (EOs) or extracts, which have gained considerable attention due to their ability to prevent the spread of fungal contamination and extend the shelf life of agricultural products (Gupta et al. 2023). Essential oils are complex and diverse mixtures of aromatic compounds, many of which have volatile properties that can be extracted from plant organs and tissues using various methods, including water distillation (El Khetabi et al. 2022). They usually consist of 20 to 60 components, however, may include over 100 individual components at varying concentrations. Among these components, two or three are typically the primary components present in relatively high concentrations (20–70%). These primary components contribute to the antifungal activity, while the rest are found only in trace amounts (Burt et al. 2004).

So far, some EOs have been reported to control plant diseases caused by various fungal pathogens, such as *Botrytis*, *Rhizopus*, *Penicillium*, *Alternaria*, and *Monilinia*, in fresh fruits and vegetables (Kawhena et al. 2021). Moreover, recently, antifungal effects of EOs from different medicinal plant species on *B. cinerea* have been reported and, the antimicrobial activity of EOs depends on the concentration of the EO and the type of EO (Zhao et al. 2021, Álvarez-García et al. 2023, Fincheira et al. 2023, Hong et al. 2023, Kgang et al. 2023, Leesutthiphonchai et al. 2024, Luo, 2024, Sharifzadeh et al. 2024).

In certain studies, it was found that concentrations of various plant essential oils exceeding 500 ppm are optimal for completely halting the growth and reproduction of *B. cinerea* fungus (Oliveira Filho et al. 2021, Zhao et al. 2021, Akhtari et al. 2022, Almasaudi et al. 2022, Fincheira et al. 2023, Tančinová et al. 2022, Zinno et al. 2023). The observed effectiveness of higher concentrations of EOs in reducing fungal growth is consistent with the dose-dependent nature of phytochemicals, where increased concentrations often correlate with enhanced biological activity (Álvarez-García et al. 2023, Lopes et al. 2023).

Numerous studies have been conducted on the positive effects of plant essential oils in suppressing or completely inhibiting the growth of plant pathogenic fungi, including *B. cinerea*, in vitro and in vivo environments by limiting mycelial growth and spore germination, altering the morphology of fungus hyphae and damage the plasma membrane of the cells, leading to the leakage of intracellular components such as nucleic acids, soluble proteins, and soluble sugars. (Yan et al. 2021,

Almasaudi et al. 2022, Ranjbar et al. 2022, Fincheira et al. 2023, He et al. 2023, Leesutthiphonchai et al. 2024, Luo et al. 2024).

Benzimidazoles have been significant in the history of plant disease control, as they were the first systemic single-site fungicides utilized in agriculture, introduced during the 1970s (Walker et al. 2013). Among the benzimidazoles, such as carbendazim, benomyl, and thiabendazole, carbendazim is the most commonly used fungicide. This compound disrupts nuclear division by binding to β-tubulin (Leroux et al. 2002), effectively managing several phytopathogenic fungi, including *B. cinerea* (Liu et al. 2014, Zhu et al. 2016). However, resistant strains of *B. cinerea* fungus have been recently documented (He et al. 2020).

Since *B. cinerea* is a major pathogen that decreases the quantity and quality of agricultural and horticultural products globally, including in Iran, this research aims to investigate the effect of applying EOs from various medicinal plants in controlling this pathogen in laboratory conditions. Since resistant strains of *B. cinerea* fungus have recently been reported, replacing chemical control agents with biological control factors, particularly plant EOs, could provide a sustainable solution for managing this fungus in agriculture. The results of this study may be significant for healthy agricultural products.

Materials & Methods

Preparation of *B. cinerea* and culture conditions

This research was conducted to study the effect of various EOs on the growth of *B. cinerea* under laboratory conditions at the Faculty of Agriculture and Natural Resources University of Hormozgan in 2024. To investigate the effect of different medicinal plant EOs (Table 1) on *B. cinerea*, a fungal isolate (collection number: IRAN4283C) was obtained from the Iranian Research Institute of Plant Protection (IRIPP), Tehran, Iran. Potato Dextrose Agar (PDA, 200 g extract of boiled potatoes, 20 g dextrose, and 20 g agar powder in 1,000 mL distilled water for mass propagation) was used as the culture medium. Subsequently, for the pure culture of the fungus, under a laminar flow hood in sterile conditions and in the dark at 24C˚, a piece of the margin of the actively growing culture from the culture medium containing the fungus was transferred to a Petri dish containing the culture medium (Hou et al. 2020). In the next step, to prepare these media, the required amount of PDA was mixed with distilled water, and after the culture medium was completely dissolved in water, it was autoclaved at 121C˚ and 1.5 atmospheric pressure for 20 minutes to sterilize. After autoclaving, the culture medium was taken under the laminar flow hood to cool to 45-40C˚. Once the medium solidified, a five-millimeter mycelial disk from young (3-day-old) fungal cultures were placed in the sterilized Petri dishes (diameter = 7 centimeters) for the study of the effect of the EOs (Wang et al. 2010). While the sterile culture medium was still warm (about 45 degrees Celsius), the EOs were added in three concentrations of 500, 1000, and 2000 ppm, along with the fungicides carbendazim at two concentrations of 1500 and 2000 ppm, all uniformly added in a volume of 15 mL. To mix with the culture medium, they were gently shaken and finally, each mixture was added to the corresponding Petri dishes. The PDA culture medium combined with 2 mL of distilled water, devoid of EOs and fungicides, was considered for control. To prevent the entry of other fungi into the culture medium, the edges of the Petri dish were covered with Parafilm and then kept in an incubator at 25°C (Fincheira et al. 2023). Each concentration was triplicated three times to confirm the reproducibility of the results.

EOs

Thirteen commercially available EOs (Table 1) were utilized. EO extraction was performed using the Clevenger apparatus method (British Pharmacopoeia 2007). The manufacturers confirmed the plant materials and the purity (100%) of the EOs. For result analysis, the chemical compounds present in the superior EOs were separated and identified using GC and GC-MS techniques.

Table 1 List of essential oils of medicinal plants under study and their production locations.

Common name	Scientific name	Family	EO producer
English lavender	*Lavandula angustifolia* Mill.	Lamiaceae	University of Hormozgan
Peppermint	*Mentha piperita* L.	Lamiaceae	University of Hormozgan
Common sage	*Salvia officinalis* L.	Lamiaceae	University of Hormozgan
Garden thyme	*Thymus Vulgaris* L.	Lamiaceae	University of Hormozgan
Shirazi thyme*	*Zataria multiflora* Boiss.	Lamiaceae	University of Hormozgan
Moor-e-Talkh*	*Salvia mirzayanii* Rech. f. and Esfand.	Lamiaceae	University of Hormozgan
Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	Giah Essence & Phytopharm Co
Fennel	*Foeniculum vulgare* Mill	Apiaceae	Giah Essence & Phytopharm Co
Persian cumin	*Carum carvi* L.	Apiaceae	Giah Essence & Phytopharm Co
Lemon grass	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Giah Essence & Phytopharm Co
Chamomile	*Matricaria chamomilla* L.	Asteraceae	Giah Essence & Phytopharm Co
Carnation	*Dianthus caryophyllus* L.	Caryophyllaceae	Barij Essence Co
Cinnamon	*Cinnamomum verum* J.Presl	Lauraceae	Barij Essence Co

* Since they are native to Iran, a common name prevalent in Iran has been mentioned.

Evaluation of the antifungal activity of EOs

Twenty-four hours after the application of treatments, fungal growth indices including colony diameter (d), density and height, percentage of mycelia growth inhibition (MGI), mean colony diameter growth rate (DR), and mycelia growth coefficient (GC) were evaluated as criteria for examining the antifungal activity of the EOs.

Colony diameter (d)

The radial growth of mycelium as a colony diameter of the fungus was measured daily using a caliper. The colony diameter growth assessment continued until the fungal mycelium completely covered the culture medium's surface in the control treatment (Duan et al. 2012). These two experiments assessed this trait daily from the fourth to the seventh day.

Colony Density or CFU

The Colony Forming Unit (CFU) method measured the fungal colony density. For this purpose, 30-day-old fungal cultures were utilized in the incubator at 25C˚ and exposed to each treatment separately. Distilled water was added to a petri dish to cover the surface, and then one milliliter of Tween 80 was added. The petri dish was shaken vigorously to separate the conidia. Next, a drop of the suspension was placed under a sterilized hemocytometer. Finally, conidia were counted under the microscope per unit area (Basso et al. 2023).

Colony Height (h)

Thirty days after fungal cultivation, to determine the colony height (mm), a caliper was used to measure the vertical distance from the surface of the growth medium to the top of the fungus colony (Lewinsohn et al. 2000).

Mycelia growth coefficient (MGC)

To measure the mycelial growth coefficient (MGC), the daily growth of the mycelium diameter (mm) was measured using a caliper until the Petri dish was filled by the fungus in the control sample, along with the height of the fungal colony on the seventh day. Additionally, the density of the fungus (g) evaluated as thickness degree on the seventh day was measured, and finally, MGC was calculated using Buchalo's (1988) equation:

$MGC = (d - 5) hg / t$

d - 5: colony diameter (mm) minus 5 (diameter of the disk) mm; h: colony height (mm); g: density (numbers between 1 and 3 were considered, with 1 being the lowest density and three the highest density); t: number of days.

Mean colony diameter growth rate (DR)

To measure DR (mm d^{-1}), the colony diameter of the fungus was measured for all treatments four days after incubation. From the fourth day to the seventh day (between days 5-7), the colony diameter of the fungus was measured every day, and DR was calculated using the following equation (Semerdzieva & Cejp 1966):

$DR = (D_n - D_4 / t_n - t_4)$

Dn: the diameter of the colony (millimeter) on, for example, days 5-7; D_4: the diameter of the colony (millimeter) on day 4; t_n: incubation time, i.e. (5-7); t^4: incubation time (in this study; 4).

Mycelial growth inhibition (MGI)

The fungal mycelial growth inhibition was done daily until the fungal mycelium completely covered the control Petri dish. The following formula was used to calculate mycelial growth inhibition (Duan et al. 2012):

$$MGI = \frac{dc - dt}{dc} \times 100$$

dc: the average diameter of mycelium in the control treatment, dt: the average diameter of mycelia treated with different concentrations of EOs. If dc ≤ dt, then MGI was considered to be zero.

Statistical Analysis

This experiment was conducted in a completely randomized design with three replications. Finally, the obtained data were compared using SAS software and Duncan's multiple range test at a 5% probability level ($P<0.05$).

Results

Colony diameter (d)

Based on the results of variance analysis (Table 2), it was observed that the effect of treatments on colony diameter was significant ($P\leq0.01$). The application of EOs significantly reduced colony diameter compared to the control without fungicide, and in most cases, higher concentrations were more effective in this regard (Table 3). The examination of colony diameter changes over time showed that although the fungicide carbendazim significantly reduced colony diameter compared to the control in most cases, it could not completely inhibit the growth of the fungus. Some EOs halt colony growth during the four sampling periods. On the other hand, when comparing the effect of EOs with the fungicide carbendazim, lemongrass, garden thyme, Shirazi thyme, and cinnamon EOs were more effective than other EOs; specifically, the application of lemongrass, garden thyme, and Shirazi thyme oils at all three concentrations, and cinnamon EO at a concentration of 2000 ppm showed the highest

effectiveness, while the lowest was observed with fennel EO (8.5 mm) at all concentrations and with control (8.5 mm) at the end of the seventh day (Table 3).

Colony Density (CFU)

The highest CFU in the control culture media (without fungicide) was obtained at 4.92×10^5, which did not show a significant difference from carbendazim at a concentration of 1500 ppm. Eos significantly reduced CFU compared to the untreated control. In most cases, higher concentrations of EOs were found to be more effective. In this regard lemongrass, garden thyme, Shirazi thyme, and cinnamon EOs were more effective than others, with the lowest CFU (0.0) recorded with the application of these EOs of 500, 1000, and 2000 ppm. A concentration of 500 ppm of some EOs could not reduce CFU compared to the untreated control and carbendazim at a concentration of 1500 ppm, resulting in CFU values of 4.80×10^5, 4.61×10^5, and 3.22×10^5 for common sage, rosemary, and Moor-e-Talkh EOs, respectively (Table 3).

Colony Height

The results of ANOVA in Table 2 showed that the effect of treatments on the colony height was significant ($P \leq 0.01$). The colony height significantly decreased when exposed to various concentrations of EOs and the fungicide carbendazim, compared to the untreated control group. Furthermore, in most instances, higher concentrations were more effective. Additionally, when comparing the impact of EOs with the fungicide carbendazim, lemongrass, garden thyme, Shirazi thyme, and cinnamon EOs showed higher effectiveness compared to other EOs, with the lowest colony height (0 mm) observed with the application of lemongrass and garden thyme at all concentrations. In contrast, the highest colony height (2.33 mm) was observed in untreated control and cumin EO at concentrations of 1000 and 2000 ppm (Table 3).

Table 2 Analysis of variance of the effect of the fungicide carbendazim and plant essences on the growth characteristics of the *B. cinerea*.

S.O.V	df	Mean of squares													
		d4	d5	d6	d7	CFU	Colony height	MGC	DR1	DR2	DR3	DRm	MGI1	MGI2(%)	MGI3	MGI4
Treatment	39	14.98**	22.92**	23.9**	32.3**	529116.4**	1.00**	862.5**	0.08**	0.027**	0.014**	0.020**	2926.4**	3247.3**	3933.0**	4172.3**
Error	86	0.27	0.25	0.62	0.6	121318.1	0.14	47.8	0.004	0.0012	0.00022	0.0018	56.9	32.8	115.5	218.4
CV	-	22.0	18.0	23.8	20.8	22.6	14.5	14.2	18.2	17.0	23.4	22.2	9.8	8.6	17.2	26.3

** Significant (P≤0.01).

Table 3 Effect of the fungicide carbendazim and EOs on the *B. cinerea* colony diameter(d), density (CFU), and height.

Treatments	Concentration (ppm)	d4†	d5 (mm)	d6	d7	CFU×$10^5$$ml^{-1}$	Colony height (mm)
Control	0	cd 4.86	a 8.40	a 8.50	a 8.50	a 4.92	a 2.33
Carbendazim	1500	c 5.17	bc 7.37	ab 7.90	a 8.07	abc 3.72	c-f 1.13
	2000	cd 4.70	de 6.40	cd 6.93	bc 7.31	c-f 2.26	c-g 1.10
Lemongrass	500	i 0.00	o 0.00	m 0.00	k 0.00	e-h 0.84	j 0.00
	1000	i 0.00	o 0.00	m 0.00	k 0.00	h 0.00	j 0.00
	2000	i 0.00	o 0.00	m 0.00	k 0.00	e-h 1.03	j 0.00
Fennel	500	a 6.67	ab 8.03	a 8.40	a 8.50	1.69 d-h	d-h 1.03
	1000	a 7.17	bc 7.43	a 8.33	a 8.50	c-f 2.42	c-h 1.07
	2000	b 5.9	cd 6.97	a 8.27	a 8.50	d-h 1.28	0.53 i
Cumin	500	ef 2.87	ghi 4.90	def 6.00	de 4.73	c-h 1.81	c-f 1.20
	1000	i 0.00	o 0.14	m 0.10	gh 0.63	c-f 2.29	a 2.33
	2000	i 0.00	o 0.00	m 0.08	k 0.03	c-f 2.62	a 2.33
Chamomile	500	i 0.00	lmn 2.50	ijk 2.93	ef 3.83	gh 0.21	c-g 1.10
	1000	d 4.16	ef 5.73	abc 7.73	d 5.21	gf 0.21	d-h 1.03
	2000	i 0.00	kl 3.07	ef 4.33	d 5.33	b-e 2.78	cde 1.23
Common sage	500	cd 4.43	fgh 5.00	ef 5.37	5.63 d	a 4.80	b 1.83
	1000	fg 2.33	klm 2.70	hij 3.43	fgh 3.47	d-h 1.51	b 1.83
	2000	i 0.00	o 0.33	l 1.77	ij 2.20	c-f 2.64	c 1.43
Garden thyme	500	i 0.00	o 0.00	m 0.00	k 0.00	h 0.00	j 0.00
	1000	i 0.00	o 0.00	m 0.00	k 0.00	h 0.00	j 0.00
	2000	i 0.00	o 0.00	m 0.00	k 0.00	h 0.00	j 0.00
English lavender	500	i 0.00	jk 3.43	efg 4.20	d 5.17	c-f 2.58	c-h 1.07
	1000	e 3.33	ghi 4.60	fg 5.10	c 7.03	d-h 1.50	c-f 1.17
	2000	i 0.67	mn 2.03	2.33 kl	g-j 2.67	c-g 2.14	0.73 ghi
Shirazi Thyme	500	i 0.00	o 0.00	m 0.00	k 0.00	h 0.00	j 0.00
	1000	i 0.00	o 0.00	m 0.00	k 0.00	h 0.00	j 0.20
	2000	i 0.00	o 0.00	m 0.00	k 0.00	h 0.00	j 0.00
Peppermint	500	i 2.27	hi 4.43	ef 5.86	bc 7.37	b-e 2.76	cd 1.40
	1000	i 0.03	lmn 2.57	ijk 2.93	fgh 3.45	c-h 1.94	c-g 1.10
	2000	i 0.00	o 0.00	0.05 m	k 0.08	d-h 1.62	j 0.13
Moor-e-Talkh	500	i 0.17	o 0.45	kl 2.38	fg 3.50	a-d 3.22	d-h 1.03
	1000	i 0.33	n 1.76	2.50 jkl	f-i 2.93	d-h 1.40	c-f 1.20
	2000	0.20 i	o 0.48	m 0.50	k 0.50	d-h 1.63	c-f 1.13
Rosemary	500	fgh 2.20	hi 4.43	de 6.23	abc 7.60	ab 4.61	e-h 0.93
	1000	h 1.53	hi 4.37	fg 5.10	d 5.57	d-h 1.53	hi 0.70
	2000	gh 1.63	kl 3.03	hi 3.67	d 5.17	b-e 2.75	0.73 ghi
Carnation	500	d 4.13	fg 5.37	bc 7.20	a 8.50	b-e 2.77	c-f 1.17
	1000	ef 3.83	hi 4.27	fg 5.17	d 5.20	d-h 1.32	e-h 0.97
	2000	ef 2.80	ij 4.03	ef 5.43	d 5.73	1.68 d-h	fgh 0.83

Table 3 Continued

Treatments	Concentration (ppm)	d4†	d5 (mm)	d6	d7	CFU×105ml-1	Colony height (mm)
Cinnamon	500	^{i} 0.00	^{o} 0.00	^{m} 0.20	^{j} 1.77	^{fgh} 0.69	^{j} 0.10
	1000	^{i} 0.11	^{o} 0.33	^{m} 0.53	^{hij} 2.47	$^{b-e}$ 2.79	^{j} 0.10
	2000	^{i} 0.00	^{o} 0.00	^{m} 0.00	^{k} 0.00	^{h} 0.00	^{j} 0.00

† d4, d5, d6, and d7 represent the colony diameter on incubation's fourth, fifth, sixth, and seventh days respectively. In each column, the means with at least one same letter are not statistically significant ($P \leq 0.05$).

Mycelia growth coefficient (MGC)

Table 2 shows that the interaction effect of the treatments on mycelium growth coefficient was significant ($P<0.01$). The highest MGC (67.11) was estimated in the untreated control, significantly higher than other treatments (Table 4). EOs and the fungicide carbendazim resulted in a significant reduction in the MGC. In most cases, higher concentrations were more effective. When comparing the effect of the EOs with the fungicide carbendazim, in most cases, the Eos were more effective than the fungicide, especially the 2000 ppm concentration, in reducing MGC. The EOs of lemongrass, garden thyme, and Shirazi thyme were more effective compared to other EOs, such that at all three concentrations of these EOs, as well as cinnamon EO at 2000 ppm, the amount of MGC was calculated to be zero. The highest MGC observed after the untreated control, was at a concentration of 500 ppm of chamomile EO and 1500 ppm of the fungicide carbendazim, respectively, measuring 54.30 and 45.59 (Table 4).

Mean colony diameter growth rate (DR)

The results of the variance analysis of the data showed that the treatments had a significant effect ($P<0.01$) on colony growth (Table 2). Changes in DR over the incubation period indicated that, in most cases, the highest DR was obtained in the first measurement. However, in most treatments, the DR decreased over time. The changes in DR in the untreated control ranged from 1.21 to 3.54 mm day^{-1}, with an average of 2.2 mm day^{-1}. The highest DR in the untreated control (3.54 mm day^{-1}) was obtained in the first sampling. In all three samplings, the EOs, at most concentrations, along with the fungicide carbendazim, caused a reduction in DR compared to the untreated control, with many instances showing statistical significance. Generally, higher concentrations of EOs demonstrated greater effectiveness. On the other hand, in the culture media treated with lemongrass, garden thyme, and Shirazi thyme EOs at all concentrations and peppermint EO at a concentration of 2000 ppm, no fungal colony growth was observed until the end of sampling. Among the EOs, lavender at a concentration of 500 ppm had the lowest efficacy, resulting in a DR_m of 2.42 mm day^{-1}. The second least effective EO in controlling the growth was chamomile at a concentration of 2000 ppm, with DR_m of 2.34 mm day^{-1} (Table 4).

Table 4 Effect of the fungicide carbendazim and EOs on the *B. cinerea* mycelia growth coefficient (MGC) and mean colony diameter growth rate (DR).

Treatments	Concentration (ppm)	MGC	DR1†	DR2 mm	DR3	DRm
Control	0	^{a} 67.11	3.54^{a}	1.82^{ab}	1.21^{cde}	2.2^{ab}
Carbendazim	1500	^{c} 45.59	2.2^{c-g}	1.37^{cde}	0.97^{d-h}	1.51^{c-f}
	2000	^{jk} 18.57	1.7^{e-j}	1.12^{d-g}	0.87^{e-h}	1.23^{e-h}
Lemongrass	500	^{n} 0.00	0.00^{n}	0.00^{n}	0.00^{m}	0.00^{n}
	1000	^{n} 0.00	0.00^{n}	0.00^{n}	0.00^{m}	0.00^{n}
	2000	^{n} 0.00	0.00^{n}	0.00^{n}	0.00^{m}	0.00^{n}

Table 4 Continued

Treatments	Concentration (ppm)	MGC	DR1†	DR2 mm	DR3	DRm
Fennel	500	$^{g-l}$ 20.56	1.36^{h-k}	0.87^{f-j}	0.61^{h-k}	0.95^{g-j}
	1000	^{fg} 32.19	0.26^{n}	0.58^{ijk}	0.44^{i-l}	0.43^{klm}
	2000	^{hij} 23.03	1.07^{j-m}	1.19^{c-f}	0.87^{e-h}	1.04^{f-i}
Cumin	500	^{f} 33.10	2.03^{d-i}	1.57^{bc}	0.62^{hij}	1.41^{d-g}
	1000	^{n} 4.60	0.14^{n}	0.05^{n}	0.21^{klm}	0.13^{mn}
	2000	^{n} 2.04	0.00^{n}	0.04^{n}	0.01^{m}	0.02^{mn}
Chamomile	500	^{b} 54.30	2.5^{b-e}	1.47^{bcd}	1.28^{cd}	1.75^{bcd}
	1000	^{ef} 35.79	1.57^{f-j}	1.79^{ab}	0.35^{j-m}	1.24^{e-h}
	2000	^{hij} 23.23	3.07^{ab}	2.17^{a}	1.78^{a}	2.34^{a}
Common sage	500	^{cd} 41.5	0.57^{k-n}	0.47^{j-m}	0.4^{i-m}	0.48^{j-n}
	1000	^{lm} 13.25	0.37^{mn}	0.55^{i-l}	0.38^{j-m}	0.43^{klm}
	2000	^{lm} 12.69	0.33^{mn}	0.89^{f-i}	0.73^{g-j}	0.65^{i-l}
Garden thyme	500	^{n} 0.00	0.00^{n}	0.00^{n}	0.00^{m}	0.00^{n}
	1000	^{n} 0.00	0.00^{n}	0.00^{n}	0.00^{m}	0.00^{n}
	2000	^{n} 0.00	0.00^{n}	0.00^{n}	0.00^{m}	0.00^{n}
English lavender	500	^{f} 34.86	3.43^{a}	2.10^{a}	1.72^{ab}	2.42^{a}
	1000	^{c} 43.75	1.27^{ijk}	0.89^{f-i}	1.23^{cde}	1.13^{f-i}
	2000	^{gh} 27.14	1.36^{h-k}	0.78^{g-j}	0.67^{hij}	0.94^{g-j}
Shirazi Thyme	500	^{n} 0.00	0.00^{n}	0.00^{n}	0.00^{m}	0.00^{n}
	1000	^{n} 0.00	0.00^{n}	0.00^{n}	0.00^{m}	0.00^{n}
	2000	^{n} 0.00	0.00^{n}	0.00^{n}	0.00^{m}	0.00^{n}
Peppermint	500	^{cde} 41.14	2.16^{c-h}	1.80^{ab}	1.82^{a}	1.93^{abc}
	1000	24.22^{hij}	2.54^{bcd}	1.45^{bcd}	1.14^{c-f}	1.71^{b-e}
	2000	^{m} 10.47	0.00^{n}	0.03^{n}	0.03^{m}	0.02^{mn}
Moor-e-Talkh	500	^{jkl} 18.34	0.28^{mn}	1.11^{d-g}	1.11^{c-g}	0.83^{h-k}
	1000	^{klm} 13.58	1.43^{f-k}	1.09^{d-g}	0.87^{efg}	1.13^{f-i}
	2000	^{klm} 14.88	0.28^{mn}	0.15^{lmn}	0.15^{lm}	0.18^{lmn}
Rosemary	500	^{hi} 26.01	2.23^{c-f}	2.02^{a}	1.80^{a}	2.01^{abc}
	1000	^{ij} 21.08	2.84^{abc}	1.79^{ab}	1.35^{bcd}	1.99^{abc}
	2000	^{jk} 18.57	1.4^{g-k}	1.02^{e-h}	1.18^{c-f}	1.20^{fgh}
Carnation	500	^{ij} 20.88	1.24^{i-l}	1.54^{bc}	1.46^{abc}	1.41^{d-g}
	1000	36.68 ^{def}	0.44^{lmn}	0.67^{hij}	0.46^{i-l}	0.52^{j-m}
	2000	^{f} 32.63	1.23^{i-l}	1.32^{cde}	0.98^{d-h}	1.17^{fgh}
Cinnamon	500	^{klm} 14.22	0.00^{n}	0.10^{mn}	0.59^{h-k}	0.23^{lmn}
	1000	^{n} 3.45	0.22^{n}	0.21^{k-n}	0.79^{f-i}	0.41^{k-n}
	2000	^{n} 0.00	0.00^{n}	0.00^{n}	0.00^{m}	0.00^{n}

† DR1, DR2, DR3, and DRm represent the mean colony diameter growth rate on incubation's fifth, sixth, and seventh days and the mean of the incubation period respectively. In each column, the means with at least one same letter are not statistically significant (P≤0.05).

Mycelial growth inhibition (MGI)

Based on the ANOVA, the MGI of the *B. cinerea* was significantly (P≤0.01) affected by different EOs (Table 2). EOs significantly increased the MGI compared to the untreated control

(Table 5). In most cases, higher EO concentrations were more effective. On the other hand, in most EOs, the highest MGI was obtained in the first evaluation, followed by a decreasing trend. When the effect of EOs was compared with the fungicide carbendazim, lemon grass, garden thyme, Shirazi thyme, and cinnamon EOs were more effective compared to other EOs, such that the application of lemon grass, garden thyme, and Shirazi thyme EOs at all concentrations, as well as cinnamon EO at a concentration of 2000 ppm, showed 100% MGI until the end of incubation. Among the EOs, the lowest MGI throughout the experiment was observed in the fennel EO at all three concentrations and in the clove at 500 ppm (Table 5).

The fungicide carbendazim demonstrated its effect on MGI with a lag phase, such that on the first day, this index was zero in the presence of the fungicide. Nevertheless, on the second day, carbendazim at a concentration of 2000 ppm resulted in an MGI of 63.9%. In contrast, the effectiveness of carbendazim at a concentration of 1500 ppm was very low (0-12.3%) (Table 5).

Table 5 Effect of the fungicide carbendazim and EOs on the *B. cinerea* Mycelial growth inhibition (MGI).

Treatments	**Concentration (ppm)**	**MGI1**[†]	**MGI2 (%)**	**MGI3**	**MGI4**
Control	0	0^{g}	0^{p}	0^{s}	0^{k}
Carbendazim	1500	0^{g}	12.3^{mn}	7.1^{q}	5.1^{jk}
	2000	0^{g}	63.9^{def}	18.5^{o}	14^{hi}
Lemongrass	500	100^{a}	100^{a}	100^{a}	100^{a}
	1000	100^{a}	100^{a}	100^{a}	100^{a}
	2000	100^{a}	100^{a}	100^{a}	100^{a}
Fennel	500	0^{g}	4.4^{op}	1.2^{rs}	0^{k}
	1000	0^{g}	11.5^{no}	2.0^{rs}	0^{k}
	2000	0^{g}	17.0^{mn}	2.7^{r}	0^{k}
Cumin	500	18.9^{f}	41.7^{ij}	29.4^{m}	44.4^{f}
	1000	100^{a}	98.3^{a}	98.8^{ab}	92.6^{a}
	2000	100^{a}	100^{a}	99.1^{a}	99.6^{a}
Chamomile	500	100^{a}	70.2^{b-e}	65.5^{g}	54.9^{e}
	1000	0^{g}	31.8^{kl}	9.1^{q}	38.7^{fg}
	2000	100^{a}	63.5^{ef}	49.1^{j}	37.3^{g}
Common sage	500	0^{g}	40.5^{i-k}	36.8^{l}	33.8^{g}
	1000	34.2^{e}	67.9^{c-f}	59.6^{h}	59.2^{de}
	2000	100^{a}	96.1^{a}	79.2^{d}	74.1^{b}
Garden thyme	500	100^{a}	100^{a}	100^{a}	100^{a}
	1000	100^{a}	100^{a}	100^{a}	100^{a}
	2000	100^{a}	100^{a}	100^{a}	100^{a}
English lavender	500	100^{a}	59.2^{fg}	50.6^{j}	39.2^{fg}
	1000	5.9^{g}	45.2^{hij}	40^{k}	17.3^{h}
	2000	81.1^{c}	75.8^{bc}	73.8^{e}	68.6^{c}
Shirazi Thyme	500	100^{a}	100^{a}	100^{a}	100^{a}
	1000	100^{a}	100^{a}	100^{a}	100^{a}
	2000	100^{a}	100^{a}	100^{a}	100^{a}
Peppermint	500	35.9^{e}	47.3^{hi}	31.1^{m}	9.1^{ij}
	1000	99.2^{a}	69.4^{b-e}	65.5^{g}	59.4^{de}
	2000	100^{a}	100^{a}	99.4^{ab}	99.1^{a}

Table 5 Continued

Treatments	Concentration (ppm)	MGI1†	MGI2 (%)	MGI3	MGI4
Moor-e-Talkh	500	95.2^{ab}	94.6^{a}	72.0^{ef}	58.8^{e}
	1000	90.7^{b}	79.0^{b}	70.6^{f}	65.5^{cd}
	2000	94.4^{ab}	94.3^{a}	94.1^{c}	94.1^{a}
Rosemary	500	37.9^{e}	47.3^{hi}	26.7^{n}	10.6^{hij}
	1000	56.8^{d}	48.0^{hi}	40.0^{k}	34.5^{g}
	2000	54.0^{d}	23.8^{no}	56.8^{i}	39.2^{fg}
Carnation	500	0^{g}	36.1^{jk}	15.3^{p}	0^{k}
	1000	0^{g}	49.2^{hi}	39.2^{k}	38.8^{fg}
	2000	20.9^{f}	52.0^{gh}	36.1^{l}	32.6^{g}
Cinnamon	500	100^{a}	100^{a}	97.6^{b}	79.2^{b}
	1000	96.9^{ab}	73.3^{bcd}	93.8^{c}	70.9^{c}
	2000	100^{a}	100^{a}	100^{a}	100^{a}

† MGI1, MGI2, MGI3, and MGI4 represent mycelial growth inhibition on incubation's first, third, fifth, and seventh days. In each column, the means with at least one same letter are not statistically significant ($P \leq 0.05$).

Discussion

Plant EOs are gaining popularity as sustainable alternatives to synthetic pesticides in agriculture due to their natural origins and effectiveness against pests and pathogens. Their use reduces reliance on chemical pesticides, protecting the environment and improving soil health, promoting sustainable practices, and enhancing food safety (Wińska et al. 2019). The results of this study demonstrate the significant antifungal properties of various EOs against *B. cinerea*, as evidenced by the observed reductions in colony diameter, colony density (CFU), colony height, mycelial growth coefficient (MGC), mean colony diameter growth rate (DR), and mycelial growth inhibition (MGI) (Tables 3-5).

The study found that EOs significantly affected colony diameter, density (CFU), height, and growth rate of *B. cinerea* (Tables 3 and 4). EOs, particularly lemongrass, garden thyme, Shirazi thyme, and cinnamon, and somewhat less cinnamon, effectively reduced colony diameter, CFU, and height compared to the untreated control, and carbendazim and higher concentrations of these EOs were generally more effective.

EOs and the fungicide significantly decreased the colony growth rate, with the EOs tested demonstrating superior efficacy over carbendazim and the untreated control. Lemongrass, garden thyme, and Shirazi thyme demonstrated powerful effects that resulted in no observable growth at various concentrations. However, some EOs, such as fennel and lavender, were less effective.

The results indicated a significant effect of treatments on mycelium density and growth rate ($P \leq 0.01$), with the untreated control showing the highest values. EOs and the fungicide carbendazim significantly reduced growth than the control, with higher concentrations being more effective. EOs outperformed carbendazim in most cases, especially at 2000 ppm. Lemongrass, garden thyme, Shirazi thyme, and cinnamon EOs were particularly effective, achieving a zero-mycelium growth count at specific concentrations.

The untreated control group exhibited the highest MGC, significantly greater than all other treatments (Table 4). Various concentrations of EOs and carbendazim led to a marked decrease in MGC compared to the control without fungicide. Generally, higher concentrations were more effective. When comparing EOs to carbendazim, the EOs typically outperformed the fungicide, in reducing MGC. Notably, lemongrass, garden thyme, Shirazi thyme, and cinnamon EOs showed superior effectiveness. The highest MGC recorded after the untreated control was found at 2000 ppm of chamomile EO and 1500 ppm of carbendazim.

EOs showed significant increases in the MGI compared to the untreated control group that did not use fungicide, with higher concentrations proving more effective (Table 5). However, most EOs reached their peak MGI during the first assessment, followed by a decline over time. When comparing the EOs to the fungicide carbendazim, lemon grass, garden thyme, and Shirazi thyme at all concentrations, cinnamon EOs at 2000 ppm exhibited superior efficacy, achieving 100% MGI by the end of the incubation period. Conversely, carbendazim showed an ineffective effect on MGI. Among the EOs tested, fennel and clove EOs exhibited the lowest MGI (Table 5).

In confirmation of these results, essential oils from garden thyme (Fincheira et al. 2023, Tančinová et al 2022), Shirazi thyme (Akhtari et al. 2022), lemon grass (Tančinová et al. 2022, He et al. 2023), and cinnamon (Almasaudi et al. 2022, Ebrahimi et al. 2022, Tančinová et al. 2022, Álvarez-García et al. 2023, Fincheira et al. 2023, Hong et al. 2023), were found to be adequate in inhibiting conidial germination, colony density and height and mycelial growth of fungi including *B. cinerea* under both in vivo and in vitro conditions. In most of these studies, concentrations of 500 ppm and higher of plant essential oils inhibited fungal growth in both in vitro and in vivo environments. On the other hand, the effectiveness of higher concentrations of EOs in inhibiting fungal growth aligns with the established principle of dose-dependent responses found in phytochemicals. This phenomenon suggests that as the concentration of these natural compounds increases, there is often a corresponding enhancement in their biological activity (Lopes et al. 2023).

Conversely, carbendazim demonstrated only partial inhibition and could not completely inhibit the growth of the fungus, suggesting that it may not provide a long-lasting solution for managing *B. cinerea* infestations. The fungicide's effectiveness decreased over time, highlighting the potential for developing resistance among fungal populations a common issue with prolonged exposure to synthetic fungicides (He et al. 2020).

Our finding aligns with previous research showing that EOs possess strong antimicrobial activity against *B. cinerea* due to their complex chemical compositions (Rao et al. 2019, Oliveira Filho et al. 2021, Ebrahimi et al. 2022, Álvarez-García et al. 2023). The dominant compounds identified using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) in these EOs (Tables 7-9), geranial and neral in lemongrass; thymol and γ-terpinene in garden thyme and thymol and p-cymene in Shirazi thyme; and cinnamaldehyde and linalool in cinnamon are known for their antimicrobial properties (Golkar et al. 2020, Oliveira Filho et al. 2021, Álvarez-García et al. 2023).

EOs from plants can influence the metabolic pathways of microorganisms, with their antifungal effects attributed to the disruption of the fungal cell wall and cytoplasmic membrane. This disruption occurs due to the release of plant antimicrobial compounds and degrading enzymes targeting fungal cells (Masomi & Hassanshahian, 2016, Hu et al. 2017). Plant EOs can compromise the integrity of the fungal cell wall by causing the leakage of Ca^{2+}, K^{+}, and Mg^{2+} ions from the cell (Dwivedy et al. 2018, Da Rocha Neto et al. 2019).

In the present study, garden thyme and Shirazi thyme EOs were superior in controlling the *B. cinerea*. The primary compounds detected through GC and GC-MS include thymol (51.2%) and γ-terpinene (20.6%) in garden thyme (Table 6) and thymol (61.8%) and p-cymene (10.3%) in Shirazi thyme (Table 7). This result agreed with other researchers' findings, who also reported that the significant amounts of thymol in garden thyme (Pinto et al. 2020, Ranjbar et al. 2022, Álvarez-García et al. 2023, Buonsenso et al. 2023, Fincheira et al. 2023, Zinno et al. 2023) and the Shirazi thyme (Mahboubi et al. 2017, Farahpour et al. 2021, Akhtari et al. 2022, Karami-Osboo et al. 2023) EOs exhibit antifungal properties against different fungi including *B. cinerea* under both in vivo and in vitro conditions.

Thymol is a monoterpenoid phenol recognized for its antimicrobial and antioxidant properties (Lambert et al. 2001). When present in low concentrations, EO phenolic compounds can degrade proteins, while at high concentrations, they damage the enzymes responsible for energy production (Kedia et al. 2015). Thymol increases the permeability of target cells, disrupts mitochondrial membranes, and leads to the accumulation of reactive oxygen species (ROS) within *B. cinerea* cells

(Hou et al. 2020, Yan et al. 2021). This ultimately results in cell death due to oxidative damage to vital biological molecules or mediators involved in programmed cell death (Chen et al. 2013).

Thymol appears to interact with membrane ergosterol, enhancing ionic permeability and ultimately leading to cell death due to a lack of vital nutrients (Pavoni et al. 2019, Rao et al. 2019, Konuk & Erguden, 2020). Thymol causes surface wrinkles and bifurcation in the hyphal tips of fungi, with no spore production. Additionally, thymol inhibits fungal growth by reducing cellulase and pectinase activity (Ranjbar et al. 2022).

According to Table 8, lemongrass EO, containing 82.8% citral (geranial (50.8%) and neral (32.0%), exhibits strong antifungal properties. In line with our results, it has been reported that the isomeric mixture of geranial and neral, known as citral, is the primary source of lemongrass (65%–85%) (Muturi et al. 2020, He et al. 2023). Multiple in vitro and in vivo studies indicate the inhibitory effect of citral and its two main components, geranial, and neral, on the growth characteristics of various fungi, including *B. cinerea* (Wei et al. 2021, Yan 2021, Quintieri et al. 2022, He et al. 2023, Ju et al. 2023, Kgang et al. 2023, Luo 2024, Sharifzadeh et al. 2024).

Citral alters fungal hyphal morphology, leading to cellular content loss and mycelial deformities, increasing membrane permeability and conductivity (Wei et al. 2021, Yan et al. 2021). It significantly inhibits mycelial growth and spore germination by decreasing lipid content and dry weight of hyphae, while also increasing the leakage of nucleic acids and proteins (Luo et al. 2024). In addition, citral heightens the activities of oxidative stress-related enzymes, superoxide dismutase, and catalase, and promotes the accumulation of hydrogen peroxide and malondialdehyde (Kgang et al. 2023, Luo et al. 2024). Its effects disrupt mitochondrial structure and metabolism, inducing cellular apoptosis and oxidative stress in fungi like *B. cinerea*. Overall, citral acts as a potent elicitor of oxidative stress, damaging cellular components and enhancing levels of proteins associated with antioxidant activity (Kgang et al. 2023).

Cinnamon essential oil (EO), which contains cinnamaldehyde (61.6%) and linalool (19.3%) (Table 9), has also exhibited significant antifungal properties. It has been demonstrated that cinnamon EO affects various fungal stages, including vegetative structures, spores, growth, and germination, and has shown notable antifungal properties specifically against *B. cinerea* fungi (Kowalska et al. 2020, Ebrahimi et al. 2022, Leesutthiphonchai et al. 2024).

Studies indicate that the bioactive compounds found in cinnamon oil, particularly cinnamaldehyde, might interfere with the growth and development of pathogenic fungi, presenting a promising natural alternative for controlling *B. cinerea* infections. The mechanisms involved focus on disrupting membrane integrity and permeability, causing leakage of membrane and cellular components, generating reactive oxygen species, influencing protease activity, altering gene expression, and affecting sugar content (He et al. 2018, Lee et al. 2020, Carmello et al. 2022, Ebrahimi et al. 2022, Shahina et al. 2022, Zhang et al. 2021, Leesutthiphonchai et al. 2024).

Table 6 Constituents present in the essential oils of the garden thyme.

No	Constituent	RI[†]	%
1.	α-Thujene	930	1.5
2.	α-Pinene	939	0.9
3.	Camphene	957	0.5
4.	1-octen-3-ol	980	0.4
5.	β-Pinene	984	0.3
6.	Myrcene	988	2.1
7.	α-Terpinene	1022	2.1
8.	ρ-Cymene	1029	8.3
9.	Limonene	1033	0.4
10.	1,8-Cineole	1036	0.4
11.	γ-Terpinene	1063	20.6
12.	cis-Sabinene hydrate	1057	1.1

Table 6 Continued

No	Constituent	RI†	%
13.	Linalool	1100	1.7
14.	Borneol	1164	0.9
15.	Terpinen-4-ol	1175	0.4
16.	Thymol methyl ether	1232	1.0
17.	Carvacrol methyl ether	1242	0.6
18.	Thymol	1290	51.2
19.	Carvacrol	1294	2.2
20.	(ε)- Caryophyllene	1415	1.7
			98.3

†The calculated retention index (RI) in this study was determined from the homologous series of normal alkanes with 6 to 24 carbon atoms in a DB-5 column.

Table 7 Constituents present in the essential oils of the Shirazi thyme.

No	Constituent	RI†	%
1.	α- Thujene	932	0.2
2.	α– Pinene	944	1.1
3.	β– Pinene	971	0.1
4.	3- Octanone	976	1.5
5.	Myrcene	1005	0.2
6.	α- Terpinene	1038	1.5
7.	*p*- Cymene	1050	10.3
8.	Limonene	1053	0.2
9.	1,8- Cineole	1058	0.2
10.	γ- terpinene	1079	7.5
11.	Linalool	1108	1.1
12.	Terpinene-4-ol	1217	1.2
13.	α- Terpineol	1230	0.4
14.	Thymol	1317	61.8
15.	Carvacrol	1327	4.2
16.	Thymyol acetate	1359	3.5
17.	Carvacrol acetate	1370	0.1
18.	E- caryophyllene	1470	2.3
19.	Aromadendrene	1488	0.8
			98.2

†The calculated retention index (RI) in this study was determined from the homologous series of normal alkanes with 6 to 24 carbon atoms in a DB-5 column.

Table 8 Constituents present in the essential oils of the lemongrass.

No	Constituent	RI†	%
1.	myrcene	990	1.0
2.	*Z*-*β*-ocimene	1038	0.2
3.	linalool	1101	0.7
4.	exo-isocitral	1139	0.2
5.	citronellal	1153	0.4
6.	*Z*-isocitral	1167	1.1
7.	*E*-isocitral	1179	0.2
8.	citronellol	1229	0.5

Table 8 Continued

No	Constituent	RI†	%
9.	nerol	1236	0.2
10.	neral	1244	32.0
11.	geraniol	1254	2.9
12.	geranial	1273	50.8
13.	geranyl acetate	1363	0.1
14.	*E*-caryophyllene	1425	0.3
15.	-cadineneγ	1513	0.2
16.	δ-cadinene	1518	0.4
17.	germacrene D-4-ol	1567	0.5
18.	5-epi-7-epi-α-eudesmol	1610	0.2
19.	10-epi-γ-eudesmol	1628	4.1
20.	epi-α-cdinol	1639	0.2
21.	α-cadinol	1659	1.0
22.	selin-11-en-4-α-ol	1665	0.7
23.	eudesm-7(11)-en-4-ol	1701	0.7
			98.6

†The calculated retention index (RI) in this study was determined from the homologous series of normal alkanes with 6 to 24 carbon atoms in a DB-5 column.

Table 9 Constituents present in the essential oils of the cinnamon.

No	Constituent	RI†	%
1.	α-pinene	899	1.3
2.	Sabinene	972	0.2
3.	p-cymene	1027	0.9
4.	1,8-cineole	1030	0.3
5.	γ-terpinene	1055	4.4
6.	linalool	1105	19.3
7.	methyl eugenol	1389	3.7
8.	β-caryophyllene	1408	0.8
9.	(E)-Cinnamaldehyde	1421	61.6
10.	*trans*-Cinnamic acid	1462	4.1
11.	α- selinene	1494	1.3
			97.9

†The calculated retention index (RI) in this study was determined from the homologous series of normal alkanes with 6 to 24 carbon atoms in a DB-5 column.

Conclusion

In conclusion, this study provides compelling evidence that essential oils (EOs) derived from Shirazi thyme, garden thyme, lemongrass, and cinnamon exhibit antifungal properties against *B. cinerea*, significantly outperforming the synthetic fungicide carbendazim across various metrics. The pronounced antifungal effects observed, particularly at higher concentrations, can be attributed to the dominant bioactive compounds present in these EOs, such as geranial, neral, thymol, γ-terpinene, p-cymene, cinnamaldehyde, and linalool. Additionally, the statistical analysis confirmed that EOs significantly reduce the colony diameter, density, height, and growth rates, and fungal colony-forming units (CFU) along with the significant increase in MGI ($P \leq 0.01$), further reinforcing their potential as adequate replacements or complements to traditional fungicides.

These findings suggest that EOs can be eco-friendly alternatives to synthetic fungicides, minimizing harmful environmental and health effects. Promoting such natural alternatives aligns

with modern agricultural practices that aim to mitigate pesticide resistance, contamination, and health hazards associated with chemical use. This research is vital for developing bio-based pest management strategies, demonstrating that natural compounds can be effective in organic farming and integrated pest management (IPM). Future research is warranted to elucidate the specific mechanisms of action of these EOs, assess their efficacy under field conditions, and explore their impact across a broader range of fungal pathogens and crop health outcomes.

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