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Determination of Chemical Components in Different Essential Oils and Their *In Vitro* Antifungal Effects on Wet Bubble Disease (*Mycogone perniciosa*) in Cultivated Mushroom (*Agaricus bisporus*)

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Abstract

The chemical components of the essential oils (EOs) from cumin (*Cuminum cyminum* L.), laurel (*Laurus nobilis* L.), oregano (*Oreganum vulgare* L.), and thyme (*Thymbra spicata* L.), which are used as spices, were determined through GC-MS analysis. These EOs were tested in vitro for their antifungal effects against *Mycogone perniciosa*, the causal agent of wet bubble disease in cultivated mushrooms (*Agaricus bisporus* (Lange) Sing.). The antifungal effects of various doses (1, 5, 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, 1000 ppm) of the essential oils from cumin, laurel, oregano, and thyme were examined *in vitro* to assess their inhibition of *M. perniciosa* mycelial growth. Sterile deionized water served as the control. A 5 mm diameter disc, taken with a cork borer from the colony edge of the 7-day-old pathogen fungus, was placed on Potato Dextrose Agar (PDA) medium. Different doses of the essential oils were added to the center of the petri dish lid. The dishes were wrapped with parafilm, inverted, and incubated at 27°C. Notably, the lower doses (100 ppm of thyme oil and 200 ppm of oregano oil) resulted in 100% inhibition of *M. perniciosa* mycelial growth. The antifungal effects of cumin and laurel oils varied depending on the doses used. Overall, the essential oils of cumin, laurel, oregano, and thyme show potential as natural control agents against the wet bubble disease pathogen, *M. perniciosa*. Further research on the practical application of these oils in direct cultivation of mushrooms is needed to explore their full potential.

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Farklı Eterik Yağların Kimyasal Bileşenlerinin ve Kültür Mantarında (*Agaricus bisporus*) Islak Kabarcık Hastalığı (*Mycogone perniciosa)* Üzerindeki *In Vitro* Antifungal Etkilerinin Belirlenmesi

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Öz

Baharat olarak tüketilen kimyon (*Cuminum cyminum* L.), defne (*Laurus nobilis* L.), kekik (*Oreganum vulgare* L.) ve karabaş kekiği (*Thymbra spicata* L.) uçucu yağlarının kimyasal bileşenleri GC-MS'de belirlenmiş ve bu uçucu yağların antifungal etkileri, kültür mantarında (*Agaricus bisporus* (Lange) Sing.) ıslak kabarcık hastalığı etmeni olan *Mycogone perniciosa*'ya karşı *in vitro* olarak test edilmiştir. Kimyon, defne, kekik ve karabaş kekiği uçucu yağlarının farklı dozlarının (1, 5, 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, 1000 ppm) antifungal etkileri, *M. pernisiosa*'nın miselyal büyümesi üzerindeki inhibe edici etkisi açısından *in vitro* olarak araştırılmıştır. Kontrol olarak steril deiyonize su kullanılmıştır. 7 günlük patojen fungusa ait 5 mm çapındaki diskler mantar delici ile alınarak Patates Dekstroz Agar (PDA) besiyerine konulmuştur. Uçucu yağların farklı dozları, petri kabı kapağının ortasına pipetle konularak hemen petri kutuları parafilm ile sarılmış ve ayrıca petri kutuları ters çevrilerek 27^{0}C'de inkübe edilmiştir. Kekik yağının daha düşük dozları bile *M. perniciosa*'nın miselyal gelişimi üzerinde %100 antifungal etki göstermiştir. Kimyon ve defnenin *M. perniciosa* üzerindeki antifungal etkileri, kullanılan uçucu yağların dozlarına göre farklılık göstermiştir. Kimyon, defne, kekik ve karabaş kekiği uçucu yağları, ıslak kabarcık hastalığı patojeni *M. perniciosa*'yı kontrol etmek

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için doğal ajanlar olarak kullanılma potansiyeline sahiptir. Uçucu yağların pratik kullanımı, doğrudan kültür mantarları üzerinde gelecekte yapılacak detaylı çalışmalarla tamamlanmalıdır.

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Introduction

Essential oils (EOs) have been utilized in various fields such as food, pharmacy, perfume, and cosmetics, and have been studied since the 1980s due to their antimicrobial effects (Deans and Svoboda, 1990). EOs are natural, volatile compounds extracted from different plants. They are widely used in many industries, especially in food and pharmaceuticals. In the pharmaceutical industry, EOs are incorporated into topical medications, used in aromatherapy, and employed in alternative medicine because of their antibacterial, anti-inflammatory, antioxidant, and therapeutic qualities (Kumar et al., 2025). EOs listed as "Generally Recognized As Safe" (GRAS) have become attractive antimicrobial agents with great potential for use in bioactive food packaging (Kayıran et al., 2025). Recently, essential oils have been extensively used in agriculture and the food industry. These oils possess bactericidal, fungicidal, antiparasitic, and insecticidal properties and are also used in medical and cosmetic applications (Sokovic et al., 2010; Aćimović et al., 2022; Bolouri et al., 2022).

Essential oils are considered to be more advantageous than synthetic pesticides because they do not pollute the environment and lack residual or phytotoxic properties (Badei et al., 1996; Bishop and Thorton, 1997). The cultivation of mushrooms is susceptible to many competitive organisms and weed fungi, which cause substantial economic losses to growers due to decreased yields. *Mycogone perniciosa* Magn. is the most common fungus causing severe yield losses in *A. bisporus* across the country. Wet bubble disease of white button mushroom, also called La mole, white mold, bubble, or *Mycogone* disease, has been reported as one of the serious diseases in almost all major mushroom-growing countries worldwide (Sharma and Kumar, 2000; Bhatt and Singh, 2000; Sharma and Singh, 2003; Kouser and Shah, 2013). The pathogen, *M. perniciosa*, causes pathological changes in the fruit bodies of *A. bisporus*, leading to the formation of undifferentiated primordia. These primordia are characterized by being highly hyperplastic and tumorous. They produce large, irregular, confluent lumps of *A. bisporus* with no signs of differentiation or organ formation (Umar et al., 2000). *M. perniciosa* can easily spread from the initial source of infection through air currents, water splashes, and phorid and sciarid flies. Symptoms typically appear between 3 and 12 days in most cases (Glamo'clija et al., 2008). Wet bubble disease causes significant economic losses in button mushroom production (Novikova and Titova, 2023). Several fungicides are used to control this disease; however, recent studies report the development of resistance to these available chemicals (Novikova and Titova, 2023). The number of fungicides approved for use in mushroom cultivation is very limited, and it is challenging to find safe disease sprays for use on mushrooms close to harvest. Fungicides based on plant products have various beneficial properties, such as lower resistance and being environmentally friendly (Gea et al., 2010). One option could be using herbal sprays. Medical and aromatic plants have been the focus of extensive research. Cumin fruits contain 2.5-6% essential oil, 10-23% fixed oils, 15-25% protein, tannins, flavonoids, oleic acid, and linoleic acid. The protein content includes 18 amino acids, primarily glutamic acid, aspartic acid, and glycine. Cumin is used in the food, pharmacy, and perfumery industries (Akgül, 1993).

Laurel belongs to the Lauraceae family, which includes 32 genera and about 2000 to 2500 species. It is one of the most valued aromatic herbs, known for its multiple properties (Barla et al., 2007). Laurel leaves, called bay leaves, are a major spice traditionally used to enhance food flavor and taste. Naturally occurring biologically active compounds in the leaves include terpenes, terpene derivatives, polyphenols,alkaloids, minerals, and vitamins (Caputo et al., 2017; Chahal et al., 2017; Stefanova et al.,

2020). They have antibacterial and antimicrobial properties (Knobloch et al., 1989; Özcan and Erkmen, 2001).
Among the most studied essential oils (EOs) with antifungal activity is *Origanum* vulgare. Oregano is one of the most widely used aromatic species, both as a spice and medicinal plant, mainly due to its high EO content (Lombrea et al., 2020). This oil can inhibit the growth of different fungal pathogens such as *Botrytis cinerea* (Zhao et al., 2021) and *Candida* spp. (Cid-Chevecich et al., 2022). The strong antifungal potential of the oil is mainly due to components like thymol and carvacrol (Elshafie et al., 2015). *Origanum* species from the Lamiaceae family, which is an important culinary herb in world trade, are widely distributed in the fields of China and some central Asian countries (Hudaberdi, 2004). The essential oil of oregano has proven antimicrobial, fungicidal, and antioxidant properties (Busatta et al., 2007; Bouhdid et al., 2008; Gong et al., 2014). *Origanum vulgare* L. has been a valuable source of natural products for maintaining human health for a long time and has been the subject of extensive analysis for natural therapies (Force et al., 2000). The dried herb and essential oil of *O. vulgare* are used in medicine (Hummer et al., 1999). *Thymbra,* known as Mediterranean thyme, is a genus of plants in the Lamiaceae family. It is commonly native to the Mediterranean region of Southern Europe, North Africa, and the Middle East (Uysal et al., 2023). The biological activity of *T. spicata* EO is recognized for its rich phenolic content and strong bioactivity but is limited by volatility and poor water solubility (Özoğul et al., 2020; Kayıran et al., 2025). The essential oils of this plant have wide industrial applications, including flavoring foods, liqueur production, perfumery, and use as an antiseptic antimicrobial agent. Additionally, dried oregano, softened in boiled water, has been traditionally applied to wounds as a remedy (Akin et al., 2010; Saraç et al., 2009).

The aim of this study is to test the essential oils of cumin (*Cuminum cyminum* L.), laurel (*Laurus nobilis* L.), oregano (*Origanum vulgare* L.), and thyme (*Thymbra spicata* L.), which are used as spices, for their antifungal effects against *M. perniciosa*, a causal agent of wet bubble disease in cultivated mushrooms (*A. bisporus*).

Material and Method

Fungal isolates

Pure culture of *M. perniciosa* was isolated from diseased *A. bisporus* in Korkuteli mushroom production areas. Fungal isolates were aseptically subcultured and purified through serial transfers on Potato Dextrose Agar (PDA-Difco). The cultures were incubated in the dark at 26°C for 7 days and stored at 4°C.

Plant Essential Oils

Essential oils (EOs) used in this study included cumin, laurel, oregano, and thyme (Table 1). EO of the spices was obtained by Clevenger hydrodistillation method (Basım and Basım, 2003; Basım and Basım, 2004; Basım et al., 2000). Cumin oil was derived from dried fruits, while oregano, laurel, and thyme oils were extracted from fresh leaves. The oils were used as homogeneous emulsions at concentrations of 1, 5, 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, and 1000 ppm. 200 grams of plant material were hydrodistilled with 2 liters of double-distilled water for four hours. The oils were stored in a refrigerator at 4°C before use.

Table 1. List of Essential Oils (EOs) used in this study

Scientific name	Family	English name	Brand name
Cuminum cyminum L.	Apiaceae	Cumin	Cumin Oil
Laurus nobilis L.	Lauraceae	Laurel	Laurel Oil
Origanum vulgare L.	Lamiaceae	Oregano	Oregano Oil
Thymbra spicata L.	Lamiaceae	Thyme	Thyme oil

Determination of EO Contents by GC-MSD (Gas Chromatography-Mass Selective Detector) Analysis

EOs were analyzed using an HP 7980-5975 GC-MSD instrument equipped with an 19091N116 capillary column (60 m / 0.32 mm, 0.25 mm thickness). Helium served as the carrier gas at a flow rate of 0.7 mL/min. The injection volume was 1 µL with a split ratio of 1:50. The column oven was temperature programmed starting from 50°C to 250°C at a rate of 7°C/min. After a 10-minute hold at 250°C, the temperature was increased at 10°C/min to 260°C and held for 15 minutes. The analysis conditions followed those described by Basım and Basım (2018). Essential oil components were analyzed using the ChemStation program and NIST 0.5 AL (Basım and Basım, 2012; Basım and Basım, 2018).

In Vitro Efficacy of EOs on Mycelial Growth of Mycogone perniciosa

The antifungal effect of essential oils (EOs) was tested against *M. perniciosa*, the causal agent of wet bubble disease. Potato Dextrose Agar (PDA) was used, with 20 ml poured into 90 mm sterile plastic petri dishes. A 5 mm diameter fungal disc, taken with a cork borer from the edge of a 7-day-old culture of *M. perniciosa*, was placed at the center of each dish containing PDA medium. The control consisted of PDA medium without EOs. Sterile deionized water served as a negative control. Different doses of essential oils (1, 5, 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, and 1000 ppm) were applied to the center of each petri dish cover. The dishes were wrapped with parafilm, inverted, and incubated at 27°C for 5 days. The antifungal activity of essential oils from cumin, laurel, oregano, and thyme was tested *in vitro* for their ability to inhibit *M. perniciosa*'s mycelial growth. The diameter of the mycelium was measured when it covered the plate in the control to assess inhibition. Mycelial growth inhibition (I) was calculated using the following formula:

I=(**C**-**T**)/**C** x 100 (Deans and Svoboda,1990)

Where I represents inhibition (%), C is the colony diameter of the mycelium on the control petri dish (mm), and T is the colony diameter of the mycelium on the test petri dish (mm). Measurements of diameters were taken when the control mycelium reached the edges of the plate. The experiment was conducted in a randomized plot design with three replications. Three replicates of each treatment were performed, and the data were averaged. Control sets were run simultaneously without the use of essential oils.

Statistical Analysis

Standard analysis of variance (ANOVA) was performed using the SPSS 10.0 software. Significance was assessed with Duncan's multiple range test ($p < 0.01$).

Results and Discussion

Determination of EO Content by GC-MSD

All EOs were analyzed using an HP 7980-5975 GC-MSD instrument. All identified compounds in the EOs are listed in Tables 2, 3, 4, and 5. The volatile oil extract of cumin exhibited the highest percentages of cuminaldehyde (28.6%), γ-terpinene (14.8%), β-pinene (12.2%), γ-terpinen-7-al (11.50%), o-cymene (10.15%), and β-phellandrene (9.91%), as shown in Table 2.
Current research on the chemical composition of cumin EOs mainly concentrates on its volatile oils, particularly on the high percentages of cuminaldehyde (28.6%) and γ-terpinene (14.8%). Cuminaldehyde, the primary component of cumin, shows anti-injury and anti-neuropathic effects on chronic compression nerve injury by stimulating opioid receptors, modulating the L-arginine /NO/ guanylyl cyclase /cGMP pathway, and inhibiting inflammatory cytokines (Kakarla et al., 2017; Bai et al., 2025). Additionally, cuminaldehyde has demonstrated antibacterial and anticancer activities according to some studies (Ebada, 2017; Bai et al., 2025). The literature indicates that the five most common compounds in *C. cyminum* are cuminaldehyde (19.9–64.31%), p-cymene (6.1%–47.08%), cuminal (36.31%), α–pinene (0.5%–29.2%), and α, β-dihydroxyethylbenzene (29.16%) (Beis et al., 2000; Ravi et al., 2013; Ebada, 2017; Bai et al., 2025).

Table 2. Analysis of *Cuminum cyminum* L. components using GC-MS

Compounds	Molecular Formula	RT (min)	Composition (%)
Cuminaldehyde	$C_{10}H_{12}O$	19.20	28.6
y-Terpinene	$C_{10}H_{16}$	17.80	14.8
β-pinene	$C_{10}H_{16}$	16.90	12.2
y-terpinene-7-al	$C_{10}H_{14}O$	14.90	11.5
o-cymene	$C_{10}H_{14}$	12.00	10.15
β-phellandrene	$C_{10}H_{16}$	10.30	9.91
			Total: 87.16%
Other compounds (α-terpine, α–pinene, α-phellandrone, p-menthol 1,α-trans-anethole vb)			Total: 12.84%

RT: Retention Time

The volatile oil extract of laurel was found to contain the highest concentrations of 1,8-cineole (53.4%), α-terphyl acetate (11.5%), sabinene (9.9%), and α-terpinol (4.8%), as shown in Table 3. Current research on the chemical composition of laurel EOs mainly focuses on its volatile oils, with two compounds appearing at the highest percentages: 1,8-cineole (53.4%) and α-terphyl acetate (11.5%) (Table 3). 1,8-Cineole, sabinene, and terpinyl acetate are identified as major components in the EOs of many plants, including laurel (Sangun et al., 2007; Derwich et al., 2009; Chmit et al., 2014). Previous studies indicate that laurel leaves can contain up to 270 EO constituents, with the main ones being 1,8-cineole (22–56%), linalool (0.9–26.9%), α-terpinyl acetate (4.5–18.2%), α-pinene (2.2–15.9%), β-pinene (1.9–15.3%), sabinene (4.5–12.7%), α-terpineol (0.9–12.0%), and terpineol-4 (0.9–4.1%) (Bahmanzadegan et al., 2015; Chahal et al., 2017; El et al., 2014; Vasundhara et al., 2016). Laurel EOs have demonstrated antimicrobial (Fernandez-Andrade et al., 2016; Snuossi et al., 2016), antioxidant (Tanab et al., 2018; Snuossi et al., 2016; Goudjil et al., 2015), and pharmacological properties (Snuossi et al., 2016; Caputo

et al., 2017). Due to its biological activity, laurel leaf EO could be seen as a natural supplement or antioxidant in cosmetics (Vasundhara et al., 2016) and medicine (Zolfaghari et al., 2013).

Table 3. Analysis of Laurus nobilis L. components using GC-MS

Compounds	Molecular Formula	RT (min)	Composition (%)
1,8-cineole	$C_{10}H_{18}O$	15.70	53.4
α–terphyl acetate	$C_{12}H_{20}O_2$	33.10	11.5
Sabinene	$C_{10}H_{16}$	12	9.9
α-terpinol	$C_{10}H_{18}O$	26.20	4.8
α–pinene	$C_{10}H_{16}$	9.7	4.0
β-pinene	$C_{10}H_{16}$	13.30	3.0
			Total: 86.6%
Other compounds (Linalool, myrcene, terpinene-4-ol, methyl eugenol, pinacarvone vb)			Total:13.4%

RT: Retention Time

Akgul et al. (1989) reported that laurel leaves primarily contained 1,8-cineole, eugenol, acetyl eugenol, methyl eugenol, and terpineol. 1,8-cineole (51.8%), which is a phenolic compound, was identified as the main component (Yılmaz et al., 2013). Previous studies also present similar findings (Kovacevic et al., 2007; Politeo et al., 2007; Yılmaz et al., 2013). In our results, oregano EOs showed the highest percentages of carvacrol (49.99%), p-cymene (16.83%), γ-terpinene (7.99%), and thymol (6.92%), as detailed in Table 4.

Table 4. Analysis of *Origanum vulgare* L. constituents using GC MS

Compounds	Molecular Formula	RT (min)	Composition (%)
Carvacrol	$C_{10}H_{14}O$	9.48	49.99
p-cymene	$C_{10}H_{14}$	7.01	16.83
y-terpinene	$C_{10}H_{16}$	8.33	7.99
Thymol	$C_{10}H_{14}O$	9.29	6.92
(+)-4 carene	$C_{10}H_{16}$	6.93	2.45
β-myrecene	$C_{10}H_{16}$	6.06	2.03
			Total: 86.21%
Other compounds (+ slyvestrene,linaool,caryophyllene, β–hisabole vb)			Total:13.79%

RT: Retention Time

Oregano is a popular spice in the food industry. It is mainly appreciated for its aromatic qualities, mainly to improve the flavor and scent of foods. Oregano contains high levels of oleanolic, ursolic, tannins, and phenolic glycosides, oregano (Kocic-Tanackov et al., 2012). Many studies have shown that the primary components in the essential oil of *T. spicata* species are carvacrol (34.9-78.53%), γ-terpinene (6.87-25.6%), p-cymene (0.85-22.11%), trans caryophyllene (5.1-10.41%), β-myrcene (4.8%), α-terpinene (6.9%), thujene (5.2%), and thymol (11.98%) (Akgül and Özcan, 1999; Kirkan et al., 2019; Koçer, 2021; Yücel-Sengun et al., 2021).

The main components in the essential oil content of *T. capitata* species are carvacrol (24.35-83%), γ-terpinene (2-26%), p-cymene (4.90-17%), thymol (9-49.33%), and β-caryophyllene (1-6.45%) (Merino et al., 2019; Moukhles et al., 2020; Kayıran et al., 2025). Additionally, EO compounds can be as effective as modern medicine in combating pathogenic microorganisms and serve as safe alternatives for treating infectious diseases. The presence of these compounds aligns with previous reports on the chemical compositions of these essential oils (Ninich et al., 2024). The identified compounds, such as carvacrol, thymol, and eucalyptol, have been linked to various biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects (Gupta et al., 2024).

The volatile oil extract of *T. spicata* was found to contain the highest percentages of thymol (36.2%), carvacrol (28.13%), γ-terpinene (10.1%), and p-cymene (6.9%) as shown in Table 5. Current research on thyme oil's chemical composition mainly focuses on its volatile oils, with thymol (36.2%) and carvacrol (28.13%) being the most prominent compounds (Table 5). Previous studies report similar results (Nath et al., 2023; Kocic-Tanackov et al., 2012). *T. spicata* oils obtained from Eskisehir showed that the major compounds were carvacrol (56.03%), p-cymene (9.61%), γ-terpinene (6.87%), and trans-caryophyllene (10.41%) (Şengün et al., 2021). Nath et al. (2023) studied the chemical composition of *T. spicata* EO from Kepsut, Balıkesir, and found that its main components were carvacrol (52.3%) and p-cymene (21.1%).

Table 5. Analysis of *Thymbra spicata* L. components using GC-MS.

Compounds	Molecular Formula	RT (min)	Composition (%)
Thymol	$C_{10}H_{14}O$	45	36.20
Carvacrol	$C_{10}H_{14}O$	50	28.13
y-terpinene	$C_{10}H_{16}$	20.3	10.1
p-cymene	$C_{10}H_{14}$	22.1	6.9
Caryophyllene	$C_{15}H_{24}$	33.4	3.0
β-myrecene	$C_{10}H_{16}$	15.2	2.0
α−terpenen	$C_{10}H_{16}$	18.1	1.8
			Total: 88.13%
Other compounds (Camphene,limonene,sabinene, p−cymene, linalool, eugenol vb)			Total:11.87%

RT: Retention Times

The essential oils of *T. spicata* and *O. vulgare* primarily consisted of thymol and carvacrol, respectively. The main compound in *O. vulgare* was carvacrol (49.99%), while thymol (36.2%) was the major compound in *T. spicata* (Table 4 and Table 5). In this study, cuminaldehyde (28.60%) was found in *C. cyminum*, and 1,8-cineole (53.4%) was identified in *L. nobilis* (Table 2 and Table 3). The research demonstrated the antifungal activity of the essential oils from *C. cyminum, L. nobilis, O. vulgare*, and *T. spicata*. The results showed that *T. spicata* and *O. vulgare* exhibited strong and the most effective antifungal activity against *M. perniciosa* (Table 8 and Table 9). High doses of the essential oils from *L. nobilis* and *C. cyminum* were highly active against *M. perniciosa*, whereas lower concentrations showed moderate to less activity (Table 6 and Table 7).

Data presented are results from duplicate experiments on all samples. 200 ppm of oregano and 100 ppm of thyme oils showed the highest inhibitory effects against *M. perniciosa*. Conversely, 1 and 5 ppm of cumin and laurel oils exhibited low activity against *M. perniciosa* (Table 6, Table 7). However, thyme oil demonstrated strong antifungal activity against *M. perniciosa* (Table 9). This effect is believed to be due to the presence of major components thymol (36.2%) and carvacrol (28.13%) (Table 9).

Additionally, oregano oil showed the second highest antifungal activity against *M. perniciosa*, likely attributable to its high content of carvacrol (49.99%) and p-cymene (16.83%) (Table 8).

Table 6. Percentage inhibition of *M. perniciosa* mycelial growth by cumin oil*

	Doses (ppm)	Mycelial Growth Inhibition (%)
Cumin oil	1	0a
Cumin oil	5	11b
Cumin oil	10	33c
Cumin oil	20	46d
Cumin oil	30	49.5d
Cumin oil	40	53.5de
Cumin oil	50	60e
Cumin oil	100	66f
Cumin oil	200	72g
Cumin oil	300	78gh
Cumin oil	400	84.5h
Cumin oil	500	93i
Cumin oil	1000	100j
Control (sterile water)	-	0a

* Values expressed are mean of the three replicates. Values given separately for cumin oil within each row followed by different letters are significantly different at $p < 0.01$.

Table 7. Percentage inhibition of *M. perniciosa* mycelial growth by laurel oil*

Treatments	Doses (ppm)	Mycelial Growth Inhibition (%)
Laurel oil	1	0a
Laurel oil	5	10b
Laurel oil	10	23.5c
Laurel oil	20	38.1d
Laurel oil	30	43.4e
Laurel oil	40	51.6f
Laurel oil	50	59.65g
Laurel oil	100	67h
Laurel oil	200	72hi
Laurel oil	300	77i
Laurel oil	400	80j
Laurel oil	500	92k
Laurel oil	1000	100l
Control (steril water)	-	0a

* Values expressed are mean of the three replicates. Values given separately for laurel oil within each row followed by different letters are significantly different at $p < 0.01$.

Table 8. Percentage inhibition of *M. perniciosa* mycelial growth by oregano oil*

Treatments	Doses (ppm)	Mycelial Growth Inhibition (%)
Oregano oil	1	0a
Oregano oil	5	13b
Oregano oil	10	32c
Oregano oil	20	49.2d
Oregano oil	30	59.8e
Oregano oil	40	64f
Oregano oil	50	79.9g
Oregano oil	100	90h
Oregano oil	200	100i
Oregano oil	300	100i
Oregano oil	400	100i
Oregano oil	500	100i
Oregano oil	1000	100l
Control (steril water)	-	0a

* Values expressed are mean of the three replicates. Values given separately for oregano oil within each row followed by different letters are significantly different at $p < 0.01$

Table 9. Percentage inhibition of *M. perniciosa* mycelial growth by thyme oil*

Treatments	Doses (ppm)	Mycelial Growth Inhibition (%)
Thyme oil	1	0a
Thyme oil	5	25b
Thyme oil	10	39c
Thyme oil	20	57d
Thyme oil	30	69.4e
Thyme oil	40	81.7f
Thyme oil	50	90.9g
Thyme oil	100	100h
Thyme oil	200	100h
Thyme oil	300	100h
Thyme oil	400	100h
Thyme oil	500	100h
Thyme oil	1000	100h
Control (steril water)	-	0a

* Values expressed are mean of the three replicates. Values given separately for thyme oil within each row followed by different letters are significantly different at $p < 0.01$.

Many studies have shown the inhibitory activity of oregano extracts and essential oils against the growth of many bacteria (Celikel and Kavas, 2008; Valero and Francés, 2006) and fungi (Özcan and Erkmen, 2001; Wogiatzi et al., 2009; Gümüş et al., 2010). Yotova and Ignatova-Ivanova (2015) demonstrated that oregano solutions have bactericidal activity against both pathogenic yeast and Fungi Imperfecta. Khan et al. (2017) report that carvacrol and thymol isolated from *O. vulgare* exhibit strong bactericidal

and antimicrobial effects against *Streptococcus* mutans and could be used as potential agents in controlling dental caries. Cumin, laurel, oregano, and thyme essential oils were investigated for their effects on bacterial and fungal plant diseases caused by various plant pathogens (Basım and Basım, 2004; Basım and Basım, 2012; Basım and Basım, 2018). *Origanum* and *Thymus* have antibacterial, antioxidant, anti-inflammatory, and anticancer properties (Basım et al., 2000; Koçak and Boyraz, 2006; Yotova and Ignatova-Ivanov, 2015; Martins and Bicas, 2024; Lukas et al., 2015; Medina and Rualez, 2025; Kayıran et al., 2025). The essential oils of cumin, laurel, oregano, and thyme can be an important part of Integrated Pest Management for controlling fungal diseases. They are considered safe for the environment and humans because they are commonly used in spice products. The results may aid in developing effective and environmentally friendly control agents for managing plant diseases. However, carvacrol and thymol, two key components of origanum and thyme oils, have notable effects on bacterial and fungal diseases. These compounds can be included in plant disease control programs since they can be synthetically produced. Cuminaldehyde and 1,8-cineole are the main compounds found in *C. cyminum* and *L. nobilis*, respectively. Additionally, *C. cyminum* and *L. nobilis* possess several pharmacological properties, including anti-HIV activity, antibacterial, antifungal, antioxidant effects, and a relaxing effect on tracheal muscles (Boskabady et al., 2011).

Conclusion

Thyme and oregano oils demonstrated strong antifungal effects against *M. perniciosa* in this study. The most effective doses were 100 ppm for thyme oil and 200 ppm for oregano oil. The antifungal activity of thyme and oregano oils was confirmed against *M. perniciosa*. The effectiveness of thyme and oregano oils is likely due to their main components, including Thymol (36.20%) and carvacrol (28.13%) in *T. spicata*, and carvacrol (49.99%) and p-cymene (16.83%) in *O. vulgare*. Future research on these oils may help develop alternative and safe methods to control the mushroom disease caused by *M. perniciosa*. Carvacrol and thymol are phenols with strong antimicrobial properties and have been extensively studied for their ability to inhibit the growth of various fungi.
Cumin oil and laurel oil have demonstrated antifungal activity at 1000 ppm against *M. perniciosa*. The main components of cumin oil are cuminaldehyde (28.6%), y-Terpinene (14.8%), and β-pinene (12.2%). Laurel oil primarily contains 1,8-cineole (53.4%) and α-terpineol acetate (11.5%).

Our work has also shown that four EOs exhibit antifungal activity against *M. perniciosa*. The results suggest that essential oils may offer an alternative treatment for eliminating certain fungi in mushroom cultivation. Further research is needed to determine the effects of different laurel and cumin EOs.

Author Contributions

Concept, design, data collection and/or processing, data analysis and/or interpretation, literature search, writing,critical review, submission and revision: Esin BASIM (100%).

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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