

## Identification of the Most Common Disease Agents in Edible Culture Mushroom (*Agaricus bisporus*) Production Areas in Korkuteli District, Antalya Province, and Evaluation of the Effectiveness of Different Essential Oil Treatments Against These Pathogens

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### Abstract

In this study, surveys were conducted between 2023 and 2025 in cultivated mushroom production areas of the Korkuteli district in Antalya province, and disease agents were isolated from these areas. The prevalence rates of these agents were determined. Among the isolated disease agents, the most common were *Mycogone perniciosa*, *Verticillium fungicola*, *Trichoderma* spp. and *Pseudomonas tolaasii*. The antifungal and antibacterial activities of different doses of essential oils from rosemary, cumin, basil, fennel and black thyme were tested *in vitro* on PDA and NA. Doses of 1, 10, 50, 100, 250, 500, and 1000 ppm of these essential oils were tested against these pathogens. Sterile deionized water served as a control. The lowest doses of thyme oil (50 ppm) were found to inhibit wet bubble disease, while 100 ppm thyme oil was associated with dry bubble and green mold pathogens. Additionally, 1000 ppm thyme oil exhibited antibacterial activity against *P. tolaasii*. Cumin, basil, and rosemary essential oils showed different effects against various pathogens. On the other hand, fennel oil, was effective at 500 ppm against *Trichoderma* spp., *M. perniciosa*, and *P. tolaasii*, while at 1000 ppm it was effective against *V. fungicola*. The antifungal and antibacterial effects of rosemary, basil, cumin, thyme, and fennel oils are believed to stem from their distinct active ingredients. A detailed study of these active compounds could lead to promising results for pathogen control.

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## Antalya İli Korkuteli İlçesi Yemeklik Kültür Mantarı (*Agaricus bisporus*) Üretim Alanlarında En Yaygın Olarak Görülen Hastalık Etmenlerinin Belirlenmesi ve Bu Hastalık Etmenlerine Karşı Farklı Uçucu Yağ Uygulamalarının Etkinliklerinin Değerlendirilmesi

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### Öz

Bu çalışmada, Antalya ili Korkuteli ilçesi yemeklik kültür mantarı üretim alanlarında 2023 ile 2025 yılları arasında sürveyler yapılmış ve bu üretim alanlarından hastalık etmenleri izole edilmiştir. Hastalık etmenlerinin yaygınlık oranları belirlenmiştir. Bu izole edilen hastalık etmenleri arasında en yaygın olanlarının *Mycogone perniciosa*, *Verticillium fungicola*, *Trichoderma* spp. ve *Pseudomonas tolaasii* olduğu belirlenmiştir. İzole edilen hastalık etmenlerinin mücadelesi için farklı baharat olarak tüketilen biberiye, kimyon, fesleğen, rezene ile karabaş kekiği uçucu yağlarının farklı dozlarının antifungal ve antibakteriyel etkileri PDA ve NA besi yerlerinde *in vitro* koşullarda test edilmiştir. Biberiye, kimyon, fesleğen, rezene ve karabaş kekiği uçucu yağlarının 1,10, 50, 100, 250, 500,1000 ppm dozları patojenlere karşı test edilmiştir. Kontrol olarak steril deiyonize su kullanılmıştır. Kekik yağının en düşük dozlarının (50 ppm) ıslak kabarcık hastalığına, 100 ppm kekik yağının ise kuru kabarcık ve yeşil küf patojenlerine karşı antifungal etki gösterdiği, 1000 ppm kekik yağı dozunun ise *P. tolaasii* üzerinde antibakteriyel etkiye sahip olduğu belirlenmiştir. Diğer yandan rezene yağının,

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*Trichoderma* spp., *M. perniciosa* ve *P. tolaasii* üzerinde 500 ppm dozunda, *V. fungicola*'ya ise 1000 ppm dozunda etkili olduğu bulunmuştur. Biberiye, fesleğen, kimyon, kekik ve rezene yağlarının hem antifungal hem de antibakteriyel etkilerinin her bir yağın içeriğinde bulunan farklı aktif bileşenlerden kaynaklandığı düşünülmektedir. Bu etken maddelerin patojenler üzerine olan etkilerinin detaylı olarak çalışılması, patojenlere karşı mücadelede ümit verici sonuçların elde edilmesine katkı sağlayabilir.

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## Introduction

*Agaricus bisporus* (J.E. Lange) Imbach (*A. bisporus*), or button mushroom, is the most commonly cultivated mushroom species in the world, accounting for about one-sixth of total global mushroom production (Duan et al., 2024). It has significant market potential and research prospects due to its rich nutritional value and increasing sales volume (Faraj and Nouri, 2024). Mushrooms can be used as functional foods or as valuable sources of nutraceuticals to enhance health and quality of life. They are available fresh, canned, dried, frozen, and even powdered (Singh et al., 2016).

A variety of harmful organisms can affect the yield and quality of mushrooms. Diseases of various origins are common during different stages of mushroom cultivation. The production of *A. bisporus* is vulnerable to invasion by pathogens, including fungi, bacteria, and viruses, which can cause severe economic losses. *A. bisporus* cultivation faces significant challenges from various fungal diseases such as bacterial blotch (*Pseudomonas tolaasii*) (Lincoln et al., 1999), dry bubble (*Verticillium fungicola*) (Largeteau and Savoie, 2008), cobweb (*Cladobotryum mycophilum*) (McKay et al., 1999), and wet bubble (*Mycogone perniciosa*) (Novikova and Titova, 2023). *P. tolaasii* is considered the most dominant or destructive pathogen in mushroom production, leading to brown blotch disease (Tajalipour et al., 2014). *P. tolaasii* produces a lipopeptide consisting of 18 amino acids called tolaasin, which is the main pathogenic toxin and causes lesions with symptoms similar to those from direct inoculation with *P. tolaasii* suspensions on *A. bisporus* (Huang et al., 2024). *V. fungicola* (Preuss) Hassebrauk, is the etiological agent of dry bubble in commercial mushroom (*A. bisporus*) production and results in major economic losses (Berendsen et al., 2010). Symptoms range from small brown necrotic lesions on the basidiocarp to stem peeling, bending, or splitting of the stipe, and shapeless, undifferentiated non-necrotic masses in the mushroom tissue (Largeteau and Savoie, 2008). Control of the disease relies on thorough sanitation and hygiene, as well as the use of fungicides. Fungicide resistance is common (Mehrpour et al., 2013; Staub, 1991). If the disease gets out of control, it can cause crop losses of 20% or more, but 1-5% losses are typical (Berendsen et al., 2010). *Trichoderma aggressivum* is the agent that causes green mold in *A. bisporus*. It is not a pathogen but competes with *Agaricus* (Seaby, 1996). Mushroom compost infected with the mold often appears normal even two weeks after inoculation with *A. bisporus*. However, afterward, the compost turns dark green because of *Trichoderma* sporulation (Rinker, 1996), which may be linked to brown spots on mushroom caps and significant crop losses (Kosanovic et al., 2020). Green mold disease affecting mushrooms can reduce yields by 30% to 100% (Samuels et al., 2002). In managing pathogens of button mushrooms, few chemicals are registered because *A. bisporus* mycelium is sensitive to various substances, including chemicals (Šantrić et al., 2018). Disinfectants like chlorine (household bleach) and selected fungicides are generally used in mushroom cultivation, but these involve substantial costs. Additionally, using chemicals poses a high risk of pesticide residues on mushrooms, some of which have been banned from use. Most chemicals still permitted have failed to control major mushroom diseases because resistance is easily induced. Although synthetic fungicides are effective, continuous spraying has disrupted biological control by natural enemies and led to disease outbreaks, along with the extensive development of resistance to various fungicide mixtures (Elad et al., 1992). For these reasons, the button mushroom sector faces

pressure regarding human and environmental health and food safety (Savoie et al., 2016). Synthetic fungicides also have unfavorable environmental effects, prompting the development of new types of selective controls, such as broadly accepted plant-based products or botanical fungicides. These contain bioactive chemicals, including essential oils (EOs), making them suitable for botanical control as substitutes for currently used disease control agents (Burt, 2004). Therefore, effective alternative management strategies should be developed for mushroom cultivation. Several *in vitro* studies utilizing essential oils have been conducted as potential alternative practices (Stanojević et al., 2016).

EO components extracted from aromatic plants have attracted considerable attention due to their diverse biological activities, particularly their notable antibacterial properties (Meenu et al., 2023). These components are complex mixtures of bioactive compounds, with phenolic monoterpenoids such as carvacrol, thymol, eugenol, and cinnamaldehyde standing out as key contributors to their antimicrobial effects (Souza et al., 2022; Bolouri et al., 2022). These EO components have proven pharmacological activities, including anti-arthritis (Marrelli et al., 2020), antiviral (Asif et al., 2020), antibacterial (Alvarez-Martinez et al., 2021), anticancer (Sharma et al., 2022), anti-inflammatory (Yang et al., 2021), antifungal, and antioxidant activities (Kumar et al., 2025). Many plant species are not only alternatives to synthetic medicines but also serve as spices, flavoring agents, and additions to cosmetics. *Ocimum basilicum* L. is an example of such a plant. Essential oil can be extracted from these plants and used as antibacterial and antifungal agents. Species of the *Ocimum* genus in the Lamiaceae family are commonly called basil or sweet basil in Türkiye (Gunay and Telci, 2017). The basil plant (*O. basilicum* L.) is a medicinal and aromatic plant widely used in aromatherapy, cosmetics, perfumes, and food products. It has also been reported that basil exhibits pharmacological activities such as antibacterial, antifungal, anticancer, anti-inflammatory, antioxidant, antiulcer, antiviral, insecticidal, anthelmintic, hypoglycemic, hypolipidemic, cardiac stimulant, and wound healing effects (Hikmawenti et al., 2019). The volatile components of basil (*O. basilicum*) are as important as its oil, exhibiting antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*, as well as antifungal activities against *Aspergillus niger* and *Rhizoctonia bataticola*, and are also used for flavoring and cosmetics (López et al., 2005).

Fennel is a common name for the plant species *Foeniculum vulgare* Mill, which belongs to the Apiaceae (carrot) family. It is a widely cultivated, edible, and popular plant traditionally used in medicine (Malhotra, 2012). The scent of fennel essential oil results from the high content of trans-anethole (over 90%) and estragole in its composition; both compounds have a sweet, phenolic, anise-like odor (Grzyb et al., 2025). Its biological activity can be attributed to the presence of active compounds in the seeds (*Foeniculi fructus*), including essential oils containing trans-anethole, fenchone, and estragole (Mimica-Dukic, 2003). Zellagui et al. (2011) and Dua et al. (2013) described the high antimicrobial potential of crude fennel extracts against Gram-positive and Gram-negative bacteria, as well as fungi such as *Aspergillus versicolor*, *Aspergillus fumigatus*, and *Penicillium camemberti*.

*Rosmarinus officinalis* (Labiatae) is an ancient plant recognized as medicinal in the European Pharmacopoeia. Rosemary (*R. officinalis* L.) is a common aromatic and medicinal plant used at home, belonging to the Labiatae family. It is native to Europe, Asia, and Africa, mainly around the Mediterranean Sea (Pintore et al., 2002). It is used to flavor food and in cosmetics and traditional medicine for its choleric, hepatoprotective, antimutagenic, antifungal, and antibacterial properties (Lalitha et al., 2011).

Cumin seeds, scientifically called *Cuminum cyminum*, are a popular spice used in many cuisines around the world. Cumin seeds have been found to have anti-inflammatory and antioxidant effects that may help lower the risk of chronic diseases such as diabetes, heart disease, and Alzheimer's disease (Panickar,

2015). They are also known for their antimicrobial properties, which may help protect against various pathogens (Packiavathy et al., 2012).

*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. The flower colors of *Thymbra* can be purple, pink, or blue (Bräuchler, 2018). The *T. capitata* species is used in tea, spices, breakfast dishes, salads, soups, pastries, and ice cream. It has been reported to be preferred for conditions such as antiseptic, antibacterial, antioxidant, anticholinesterase, antidiabetic, antihypercholesterolemic, antisteatotic, antitumor, antiviral, and antifungal uses (Prasanth et al., 2014). The main components in the essential oil of *T. spicata* include carvacrol (34.9-78.53%),  $\gamma$ -terpinene (6.87-25.6%), p-cymene (0.85-22.11%), trans caryophyllene (5.1-10.41%),  $\beta$ -myrcene (4.8%),  $\alpha$ -terpinene (6.9%), thujene (5.2%), and thymol (11.98%) (Khalil et al., 2019).

This study aims to examine the essential oils of rosemary (*R. officinalis* L.), cumin (*C. cyminum* L.), basil (*O. basilicum* L.), fennel (*F. vulgare* Mill.), and black thyme (*T. spicata* L.), which are consumed as spices, for their antifungal and antibacterial effects against *M. perniciosus*, the agent of wet bubble disease; *V. fungicola*, the agent of dry bubble disease; *Trichoderma* spp., the agent of green mold; and *P. tolaasii*, the agent of bacterial brown blotch diseases in cultivated mushrooms (*A. bisporus*).

## Materials and Methods

Samples of cultivated *A. bisporus* exhibiting symptoms of brown blotch, dry bubbles, wet bubbles, and green mold diseases were collected from various mushroom farms in Datk y (10), İmrahor (10), K     y (10), B     y (10), Yazır (10), Yelten (10), Ye ilyayla (10), S lekler (10), and the center of Korkuteli (20), all within the Korkuteli district, during surveys conducted between 2023-2024 and 2024-2025. Diseased samples were labeled and transported to the laboratory in ice bags.

### Isolation and Identification of Bacteria

Bacterial disease samples were collected from infected mushroom fruiting bodies. The caps were cut, surface sterilized in 70% ethanol, and then rinsed three times with sterilized distilled water. The outer contaminated layers of the caps were scraped off, and the internal necrotic tissues were carefully cut and placed into an Eppendorf tube containing 800  $\mu$ L of sterilized distilled water. The tissues were ground with sterilized plastic pestles until a uniform mixture was formed. A loopful of the contents was streaked onto Nutrient Agar (NA) (Merck) and King's B (KB) (Merck) media (Wong and Preece, 1979; Basim and Basim, 2018). The bacterial colonies were further purified by re-streaking single desired colonies onto fresh KB media. The purified colonies were stored at -80 C in storage media (Nutrient Broth with 30% glycerol) for later use. The identification of *P. tolaasii* was made according to Basim and Basim (2018).

### Isolation and Identification of Fungi

Pure cultures of *M. perniciosus* and *V. fungicola* were isolated from diseased *A. bisporus* in Korkuteli edible mushroom production areas. Using routine pathological techniques, these pathogens were obtained from mushroom sporophores showing typical symptoms of wet bubble and dry bubble disease (Holliday, 1980). For fungal isolation, 5 mm flesh disc segments were taken from infected sporophores with a sterilized cork borer, surface sterilized with 2% sodium hypochlorite (NaClO) solution for 60 seconds, then rinsed three times with sterilized distilled water, blotted dry, and inoculated onto PDA (Potato Dextrose Agar) (Condalab, Spain) medium in sterilized 90 mm Petri dishes. The plates were

incubated for 3 days at 25°C. Disease compost samples from edible mushroom substrate bags and three additional samples from mushroom yards were used to isolate *Trichoderma* spp., with several spores obtained by the dilution plate method. Initial identification of the pathogenic fungi was performed by examining cultures grown on PDA (Hermosa et al., 1999). *Trichoderma* spp. isolates were cultured on PDA at 25°C for 8 days. Under these conditions, colonies initially appeared whitish, then turned green after 4 to 5 days of sporulation. The fungal isolates were aseptically subcultured and purified through serial transfers on PDA (Difco). Cultures were purified by hyphal tip (Pathak, 1972) and single spore isolation (George, 1947). The pure stock cultures were obtained and stored at -80°C in storage media (potato dextrose with 30% glycerol) for future use. Macroscopic identification was based on the characteristics of the fungus grown on the PDA plate, while microscopic identification was based on spores and hyphae.

### Essential Oils

EOs of basil, thyme, cumin, fennel, and rosemary used in this study are listed in Table 1. EO of the spices was obtained by the Clevenger hydrodistillation method (Basim et al., 2000; Basim and Basim, 2003; Basim and Basim, 2004). Cumin and fennel oils were extracted from dried fruits, while thyme, basil, and rosemary oils were obtained from fresh leaves. The oils were used as homogeneous emulsions at concentrations of 1, 10, 50, 100, 250, 500, and 1000 ppm. The EOs were stored at 4°C in the refrigerator before use.

**Table 1.** List of EOs used in this study

Scientific name	Family	English name	Brand name
<i>Cuminum cyminum</i> L.	Apiaceae	Cumin	Cumin Oil
<i>Foeniculum vulgare</i> Mill.	Apiaceae	Fennel	Fennel Oil
<i>Rosmarinus officinalis</i> L.	Lamiaceae	Rosemary	Rosemary Oil
<i>Thymbra spicata</i> L.	Lamiaceae	Thyme	Thyme Oil
<i>Ocimum basilicum</i> L.	Lamiaceae	Basil	Basil Oil

### Antifungal Activity Assay

Plugs of *M. perniciosa*, *V. fungicola*, and *Trichoderma* spp., each 5 mm in diameter, were cut from the edge of a 4-day-old culture grown on PDA using a cork borer and placed in the center of a Petri dish (90 mm) containing PDA medium. The effect of EOs was tested *in vitro* against the mycelial growth of these pathogens on PDA. Various concentrations of EOs, 1, 10, 50, 100, 250, 500, and 1000 ppm, were added to the lid of each Petri dish. The plates were sealed with Parafilm and incubated at 27°C for 3 days. Colony diameters were measured at multiple points, and the percentage inhibition of mycelial growth was calculated by comparing with the control using the formula (Deans and Svoboda, 1990):  $I = (C - T) / C \times 100$ , where I is the inhibition percentage, C is the colony diameter in the control plate (mm), and T is the colony diameter in the test plate (mm). Measurements were taken until the control mycelium reached the edges of the plate. Three replicates per treatment were conducted, and the data were averaged. Control sets were also prepared without essential oils, using sterile distilled water as the control. The experiment was carried out with a completely randomized plot design.

### Antibacterial Activity Assay

Stock cultures of *P. tolaasii* were grown in Nutrient Broth (NB, Acumedia Manufacturers, Inc., Maryland, USA) at 26-27°C for 24 hours on a shaker. The bacteria cultivated in NB were enumerated using a serial dilution method. Final cell concentrations reached 10<sup>8</sup> CFU/ml. The bacterial suspensions (100 µl) were added to NA petri dishes (90 mm). The antibacterial activity of different EOs was determined using a volatile effect method. Various doses of EOs (1, 10, 50, 100, 250, 500, 1000 ppm) were placed on the lids of petri dishes. The dishes were sealed with parafilm and incubated at 27°C for 3 days. Inhibition of bacterial growth was assessed by comparing test plates with control plates. Sterilized distilled water served as a control (Basim and Basim, 2003). Inhibition zones were measured with a ruler (mm). All tests were performed in triplicate. The experiment followed a completely randomized plot design. Treatments included essential oils from each plant and application rates for each oil.

### Statistical Analysis

Data were analyzed using two-way analysis of variance (ANOVA) to test for interactions between essential oil and its concentration. Differences among essential oils were examined with one-way ANOVA. Standard ANOVA procedures were performed using SPSS 10.0 software. Significance was determined by Duncan's multiple range test ( $p \leq 0.01$ ).

### Results and Discussion

In this study, disease agents were isolated from the production areas of edible cultivated mushrooms in the Korkuteli district of Antalya province during 2023-2024 and 2024-2025, and the most common disease agents in these areas were identified. Diseased mushroom samples were collected from 100 mushroom production sites in the Korkuteli district of Antalya, and various disease pathogens were isolated from these samples. The most frequently identified pathogens included *Trichoderma* spp., the green mold agent; *P. tolaasii*, the bacterial brown blotch agent; and *M. perniciosa* and *V. fungicola*, responsible for wet bubble and dry bubble diseases, respectively. Accordingly, *Trichoderma* spp. was present at a rate of 78% between 2023-2024, but this decreased to 45% in 2024-2025 (Table 2).

**Table 2.** Disease ratio (%) and disease agents isolated from various edible culture mushroom production areas in Korkuteli district, Antalya

Sample areas	2023-2024	Disease ratio (%) (Total sample/disease sample)- <i>M. perniciosa</i>	2024-2025	Disease ratio (%)
Center	20/12	60	20/6	30
Datköy	10/7	70	10/5	50
Imrahor	10/6	60	10/4	40
Yelten	10/8	80	10/5	50
Yeşilyayla	10/9	90	10/6	60
Küçükköy	10/6	60	10/3	30
Sülekler	10/7	70	10/4	40
Yazır	10/8	80	10/4	40
Büyükköy	10/5	50	10/4	40
Total :100/68		68 %	Total: 100/41	41%

(Total sample/disease sample)- <i>P. tolaasii</i>				
Center	20/18	90	20/10	50
Datköy	10/8	80	10/6	60
Imrahor	10/6	60	10/4	40
Yelten	10/5	50	10/3	30
Yeşilyayla	10/6	60	10/4	40
Küçükköy	10/5	50	10/3	30
Sülekler	10/6	60	10/4	40
Yazır	10/9	90	10/4	40
Büyükköy	10/8	80	10/6	60
Total :100/71		71%	Total: 100/44	44%
(Total sample/disease sample)- <i>V. fungicola</i>				
Center	20/10	50	20/5	25
Datköy	10/8	80	10/4	40
Imrahor	10/8	80	10/5	50
Yelten	10/5	50	10/2	20
Yeşilyayla	10/7	70	10/4	40
Küçükköy	10/6	60	10/2	20
Sülekler	10/6	60	10/3	30
Yazır	10/5	50	10/1	10
Büyükköy	10/5	50	10/1	10
Total :100/60		60 %	Total: 100/27	27%

**Table 2.** Disease ratio (%) and disease agents isolated from various edible culture mushroom production areas in Korkuteli district, Antalya

<u>Sample areas</u>	<u>2023-2024</u>	<u>Disease ratio</u> (%)	<u>2024-2025</u>	<u>Disease ratio</u> (%)
(Total sample/disease sample)- <i>Trichoderma</i> spp.				
Center	20/18	90	20/10	50
Datköy	10/8	80	10/5	50
Imrahor	10/9	90	10/5	50
Yelten	10/8	80	10/4	40
Yeşilyayla	10/6	60	10/3	30
Küçükköy	10/8	80	10/6	60
Sülekler	10/6	60	10/3	30
Yazır	10/8	80	10/5	50
Büyükköy	10/7	70	10/4	40
Total :100/78		78 %	Total: 100/45	45%

**Table 3.** Disease agents and disease ratios isolated from different edible culture mushroom production areas in the Korkuteli district between 2023-2024 and 2024-2025.

Disease Names	Isolated Pathogens	Disease ratios (%)	
		2023-2024	2024-2025
Green Mold	<i>Trichoderma</i> spp.	78%	45%
Brown Blotch	<i>P. tolaasii</i>	71%	44%
Wet Bubble	<i>M. perniciosa</i>	68%	42%
Dry Bubble	<i>V. fungicola</i>	60%	27%

While the bacterial pathogen *P. tolaasii* was observed at a rate of 71% between 2023 and 2024, its rate dropped to 44% between 2024 and 2025. Although both fungal and bacterial pathogens were present at high rates between 2023 and 2024, their prevalence significantly declined between 2024 and 2025. Notably, *V. fungicola*, the causal agent of dry bubble disease, was found at 60% during 2023-2024, and decreased to 27% in 2024-2025. *Trichoderma* spp. (78%) and *M. perniciosa* (68%) were the most common pathogens in 2023 and 2024. However, in 2024 and 2025, *Trichoderma* spp. became the leading pathogen with 45%, with *P. tolaasii*, the causal agent of brown bacterial blotch disease, ranking second at 44% (Table 3). Considering our own investigations into mushroom production areas regarding the reasons for the higher incidence of disease agents in edible mushroom production areas between 2023-2024 and the decrease in this rate between 2024-2025, it has been observed that producers pay more attention to hygiene and at the same time the use of pesticides has increased compared to previous years. Producers also believe that the high presence of green mold pathogens during production is due to the cover soil used and the environment's high humidity. Bacterial Blotch disease (BBD), traditionally a sporadic problem in mushroom cultivation, has been increasing globally and is now considered devastating in some producing countries. *P. tolaasii* is regarded as an endemic pathogen in casing soil and compost used for mushroom production, being part of its natural flora, where it was found to associate with mushroom hyphae (Basim and Basim, 2018). Samson et al. (1987) reported that the compost and casing soil are the main sources of inoculum for *P. tolaasii*, which causes brown blotch diseases in mushrooms. According to their results, thyme essential oil was identified as the most effective essential oil, followed by cumin oil, basil oil, and rosemary oil, respectively, against different mushroom pathogens (Table 4). Essential oils and their major components of thyme oil have been reported to induce rapid cell lysis of certain fungal and bacterial disease agents (Churklam et al., 2020). Because of essential oils and/or their main components such as carvacrol and thymol, these substances have been reported to possess fungicidal and bactericidal activities.

Treatment with thyme and cumin EOs significantly reduced the percentage of mushroom disease pathogens compared to the control. Thyme oil resulted in a higher reduction in the percentage of *Trichoderma* spp. (100 ppm), *M. perniciosa* (50 ppm), *V. fungicola* (100 ppm), and the bacterial pathogen *P. tolaasii* (100 ppm) compared to the control. Additionally, cumin oil showed the second highest reduction effect on *Trichoderma* spp. (100 ppm), *M. perniciosa* (50 ppm), *V. fungicola* (500 ppm), and *P. tolaasii* (250 ppm), respectively (Table 4 and Table 5). Rosemary oil and basil oil at 250 ppm dose also reduced green mold disease (Table 4). Thyme and cumin EOs were more effective against this fungal and bacterial disease than fennel and rosemary oils.

Recently, there has been considerable interest in finding relatively safe bio-fungicides, such as EOs, to manage plant diseases in agriculture. In our study, we used various concentrations of thyme and cumin EOs (1, 10, 50, 100, 250, 500, and 1000 ppm) against fungal and bacterial pathogens causing mushroom disease. The most effective concentrations for inhibiting fungal pathogens, *Trichoderma* spp. and *M.*



*perniciosa*, were 100 ppm and 50 ppm, respectively. A dose of 100 ppm thyme oil and 500 ppm cumin oil were most effective against *V. fungicola* (Table 4). Both thyme (250 ppm) and cumin (250 ppm) EOs were most effective against *P. tolaasii*. Basil oil at 500 ppm was also effective against *P. tolaasii*. Rosemary at 1000 ppm and fennel at 500 ppm were effective against *P. tolaasii* (Table 5).

The study shows that the EOs of *Thymus* (thyme) species and *Cuminum cyminum* have strong antimicrobial effects against plant pathogenic fungi and bacteria. Cumin and thyme EOs were investigated for bacterial and fungal plant diseases caused by various plant pathogens (Basim and Basim, 2004; Kocak and Boyraz, 2006; Basim and Basim, 2012; Basim and Basim, 2018; Martins and Bicas, 2024; Medina and Ruales, 2025; Kayiran et al., 2025). Cumin and thyme EOs exhibited antibacterial, antioxidant, anti-inflammatory, and anticancer properties. This antimicrobial effect, as reported in relevant literature, is primarily due to the main active components of *Thymus* (thyme) EOs, thymol and carvacrol, which disrupt the cell membrane, increase bacterial membrane permeability, and lead to cell death. Similar results were observed in our study, aligning with previous findings, and suggesting that *Thymus* (thyme) EOs could be a promising resource for biological control of plant pathogens. The antifungal and antibacterial effectiveness of thyme, cumin, basil, rosemary, and fennel oils is likely attributed to their main components, including thymol, cuminaldehyde, camphor, 1,8-cineole, and trans-anethole, respectively.

The EOs of cumin, thyme, basil, rosemary, and fennel can be important components of Integrated Pest Management for controlling plant fungal and bacterial diseases. Many studies have reported that the main components in the essential oil content of *T. spicata* are carvacrol (34.9-78.53%) and thymol (11.98%). The identified compounds, such as carvacrol, thymol, and eucalyptol, have been associated with various biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects (Gupta et al., 2024). Cuminaldehyde and 1,8-cineole are the major compounds of *C. cyminum*. Additionally, *C. cyminum* has several pharmacological properties, including anti-HIV, antibacterial, antifungal, antioxidant, and a relaxing effect on tracheal chains (Boskabady et al., 2011). Major components of basil oil are eugenol and methyl chavicol, respectively. These components have been reported to possess antifungal activity (Kalagatur et al., 2015). Fennel EO content was found to be between 3-7%, consisting of 60-80% trans-anethole, 5-10% fenchone, limonene, methyl chavicol,  $\alpha$ -phellandrene, anisaldehyde, cis-anethole, anisic acid, anisketone, monoterpenes, and various alcohols (Choi and Hwang, 2004). The most abundant constituents in fennel are anethole (37.94%), estragole (35.56%), D-limonene (17.46%), trans- $\beta$ -ocimene (1.53%), and fenchone (1.49%) (Abd-El-Kareem et al., 2025). Rosemary EO contains camphor,  $\alpha$ -pinene, 1,8-cineole, heptane-2-one, trimethyl, eucalyptol, camphene,  $\beta$ -pinene, D-limonene,  $\alpha$ -phellandrene,  $\beta$ -caryophyllene, and other related components (Becer et al., 2023). Studies have shown that rosemary EO can inhibit the growth of *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* (Olivas-Méndez et al., 2022). Rosemary EO also has a strong inhibitory effect on some plant pathogens such as *Sclerotinia sclerotiorum*, *Sclerotinia nivalis*, *Alternaria panax*, *Cylindrocarpon destructans*, and *Fusarium oxysporum*. Rosemary EO has the potential to be used as a natural antimicrobial agent due to the presence of terpenes such as pinene, camphene, limonene, and eucalyptol (Hussein et al., 2020).

The available evidence suggests that all the medicinal herbs listed are safe for consumption within the recommended ranges. Aromatic herbs, as natural products and their EOs, provide a rich source of highly bioactive compounds, mainly polyphenols. The volatile components of fennel EO and phenolic compounds are believed to be the primary bioactive compounds responsible for most of its antifungal and antibacterial effects.

Table 4. Antifungal effects of various EOs on pathogens isolated from edible mushroom cultivation areas\*

	<u>Doses (ppm)</u>						
	1	10	50	100	250	500	1000
<u>EOs</u>	<i>Trichoderma</i> spp. (Mycelial inhibition rate-%)						
<i>T. spicata</i>	15.0c	31.7g	90l	100n	100n	100n	100n
<i>C. cyminum</i>	16.9cd	38.9gh	91l	100n	100n	100n	100n
<i>R. officinalis</i>	10b	19.8e	62.8j	85k	100n	100n	100n
<i>O. basilicum</i>	12.3b	29.9f	58.6i	97.3m	100n	100n	100n
<i>F. vulgare</i>	0.0a	15.9c	45.9h	61.8j	87.5kl	100n	100n
Control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
<u>EOs</u>	<i>M. perniciosus</i> (Mycelial inhibition rate-%)						
<i>T. spicata</i>	18c	45f	100n	100n	100n	100n	100n
<i>C. cyminum</i>	25d	53g	100n	100n	100n	100n	100n
<i>R. officinalis</i>	15bc	28e	59.5h	80k	90l	100n	100n
<i>O. basilicum</i>	24d	51g	64i	85kl	100n	100n	100n
<i>F. vulgare</i>	12.5b	23.5d	42f	71j	95m	100n	100n
Control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a
<u>EOs</u>	<i>V. fungicola</i> (Mycelial inhibition rate-%)						
<i>T. spicata</i>	15c	35fg	85l	100p	100p	100p	100p
<i>C. cyminum</i>	13bc	29e	65i	86l	95n	100p	100p
<i>R. officinalis</i>	10b	21d	59.9h	72.5j	88.9m	100p	100p
<i>O. basilicum</i>	13bc	35fg	59.9h	85l	100p	100p	100p
<i>F. vulgare</i>	10b	30f	40.5g	55.8h	80.5k	97o	100p
Control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a

\*Values expressed are means of three replicates. Values given separately for EOs within each row, followed by different letters within the column, are significantly different at  $p \leq 0.01$ . Standard errors in the experiment ranged from 0.2 to 2.3.

Table 5. Antibacterial effects of various EOs against *P. tolaasii* isolated from edible mushroom cultivation areas\*

	<u>Doses (ppm)</u>						
	1	10	50	100	250	500	1000
<u>EOs</u>	<i>P. tolaasii</i> (Inhibition zone-mm)						
<i>T. spicata</i>	0.0a	5.8b	11.5e	32m	38n	43p	50r

<i>C. cyminum</i>	0.0a	9.9d	14.2f	25.5j	29l	32m	40o
<i>R. officinalis</i>	0.0a	5.0b	10.2e	13.5f	16.7g	19.5h	27.1
<i>O. basilicum</i>	0.0a	9.2c	13.8f	20.3hi	29l	34m	38.6n
<i>F. vulgare</i>	0.0a	9.8d	23.3i	25j	27.2k	30m3	3m
Control	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a	0.0a

\*Values expressed are means of three replicates. Values given separately for EOs within each row, followed by different letters within the column, are significantly different at  $p \leq 0.01$ . Standard errors in the experiment ranged from 0.3 to 2.5.

## Conclusion

The development of natural antimicrobials will help reduce the negative effects (residues, resistance, and environmental pollution) of synthetic drugs. In this respect, natural antimicrobials may also be effective, selective, biodegradable, and less toxic to the environment. Based on the results presented in this study, we suggest that the essential oils of these mushroom fungi and bacteria can be used as antimicrobial agents in managing other mushroom diseases. EOs and extracts from some medicinal herbs and their bioactive compounds have been available on the market as nutraceuticals, dietary supplements, or herbal products for decades, playing important roles in daily health routines. Current *in vitro* and *in vivo* studies indicate that the volatile properties of essential oils are promising, but their safety and toxicity still require further investigation. Natural essential oils have the potential to be powerful tools against many ailments, particularly bacteria, viruses, and fungi. EOs have gained global attention. Furthermore, EOs can also be used as disinfectants. In practice, minimizing pesticide use and the ability to use low, safe doses of these EOs, which are safe for human health and will be determined through further studies, may be vital for reducing pesticide residues, especially in edible cultivated mushrooms consumed raw.

## Author Contributions

Concept, design, data collection and/or processing, data analysis and/or interpretation, literature search, writing, critical review, submission, and revision: Esin BASIM (50%) and Hüseyin BASIM (50%).

## 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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