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ORIGINAL PAPER

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ANTIFUNGAL EFFECTS OF SOME ESSENTIAL OILS ON SELECTED ALLERGENIC FUNGI *IN VITRO*

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ABSTRACT

The aim of the study was to determine the effect of four different essential oils on the sensitivity of allergenic fungi i.e. *Alternaria alternata*, *Botrytis cinerea* and *Cladosporium cladosporioides*. The studied fungi were isolated from infected fennel. The tested oils were added to Potato Dextrose Agar medium at the concentrations of 0.1 mg/mL, 0.25 mg/mL and 0.5 mg/mL. The activity of the oils on the inhibition of the linear growth of mycelium was evaluated by measuring of fungal colonies, while the fungistatic activity was evaluated on the basis of the percentage growth inhibition of fungal colony and calculated according to Abbot's formula. The sensitivity of the tested isolates of fungi was variable and depended on the type and concentration of the studied essential oils. The most effective antifungal effect on all tested fungi was in essential oil of oregano even at the concentration of 0.1 mg/mL, while the weakest effect was in essential oil of grapefruit at all studied concentrations. Moreover, oregano essential oil caused degradation and decay of mycelium and spores. Essential oils are potential and promising antifungal agents used as bio fungicides in plant protection and indoor air disinfection. Therefore, further in vivo studies should be carried out.

Key words: *Alternaria alternata*, *Cladosporium cladosporioides*, *Botrytis cinerea*, essential oil, antifungal properties of essential oils

INTRODUCTION

Good health is one of the most important values in human life. Recently, there has been an increase in reports on the incidence of respiratory diseases. Often these are publications on allergies occurring due to contact with fungal allergens living both outside and inside the human body. Of the nearly 100,000 genus of fungi known and described, about 100 have been identified as species that can cause human and animal mycoses [De Hoog et al. 2000]. Particularly sensitive persons are at risk of developing allergies. About 80 genus of fungi have been shown to cause allergies, and allergy-causing proteins have been identified in 23 genera [Simon-Nobbe et al. 2008]. The cause of allergies may also be species of fungi pathogenic to plants i.e. *Alternaria* spp. and *Cladosporium* spp. which are strongly allergenic fungi known for a long time as well as that have not been considered harmful to humans so far, e.g., from the genus *Bipolaris*, *Botrytis*, *Candida*, and *Culvularia*. Moreover allergenic properties have fungi inhabiting buildings and waste paper for example from the *Aspergillus* and *Penicillium* genera [Skyberg et al. 2003, Jurgensen and Madsen 2009, Weryszko-

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-Chmielewska et al. 2018, Kilic et al. 2020, Abbas et al. 2021, Sio et al. 2021]. Mold fungi spores pollute the air, and their concentration is exceptionally high during the development of crop diseases as well as it is correlated with weather-related variables [Sabariego et al. 2012]. Thus, the source of these fungi will be diseased, vegetables, flowers, and plant materials intended for the production of cosmetics and air, especially in areas where intensive agricultural production is carried out [Sabariego et al. 2012, Weryszko-Chmielewska et al. 2018]. Allergenic fungi, through physical contact with human skin, as well as after getting into the respiratory system, as inhaled allergens (when it comes to the production of toxic products of metabolism, the so-called mycotoxins) can pose a severe threat to human health and life [Simon-Nobbe et al. 2008, Sabariego et al. 2012, Weryszko-Chmielewska et al. 2018]. Due to the frequent occurrence of problems related to the contamination of plant raw materials and the toxicity of chemical substances used in plant protection, there is a growing demand for non-toxic, non-hazardous natural alternatives. Plant extracts have been used since ancient times, and now more and more attention are focused on their role in health promotion, healing properties, and possible use in limiting microbial growth and preventing various diseases [Halberstein et al. 2005, Mendes de Toledo et al. 2015, Kaur et al. 2021]. Apart from plant extracts, essential oils are crucial compounds commonly used in human and animal therapy. Their antiseptic properties have been known and used for centuries. The term „Essential Oils" (EOs) was first used by the Swedish medical reformer Paracelsus von Hohenheim [Nazzaro et al. 2017]. According to The International Organization for Standardization – ISO (ISO/D1S9235.2), essential oils are defined as products resulting from the process of distillation with water or steam, as well as dry distillation of various plant raw materials and natural materials [Tongnuanchan and Benjakul 2014, Babar et al. 2015, Fengfeng 2017, Nazzaro et al. 2017, Singh 2019].

Essential oils are highly complex mixtures of volatile compounds. They may contain about 20 to 60 individual substances, although some may contain more than 400 different ingredients, such as jasmine, lemon, and cinnamon oils. As the latest research shows, essential oils are among the more and more frequently used agents in the control of pests, i.e., fungi-plant pathogens, bacteria, and insects [Behnam et al. 2006, Peighami-Ashnaei 2009, Raveau et al. 2020, Alonso-Gato 2021]. The rich chemical composition of essential oils influences their pharmacological activity and their applicability in aromatherapy [Bakkali et al. 2008, Babar et al. 2015, Abers et al. 2021, Alonso-Gato et al. 2021, Soares-Castro et al. 2021]. The diversity of chemical compounds in essential oils determines their properties and a wide range of action. The active substances of the oils show anti-inflammatory, antiseptic, bactericidal, antifungal, antiviral, chemoprotective, spasmolytic, diuretic and analgesic properties. They positively affect the nervous system and emotional and mental state [Zhou et al. 2014, Bennike and Johansen 2018, Abd-El-Gawad et al. 2019, Assaeed et al. 2020, Elshamy et al. 2020, Malik et al. 2021].

Numerous studies and growing interest in the effects of essential oils indicate that they have the potential to be used as natural, innovative agents in many industries [Turek and Stintzing 2013, Salvatore et al. 2022]. Currently, plant essential oils are gaining more and more popularity, mainly due to their safety and various pharmacological properties, including bacteriostatic, free radical scavenging, anti-inflammatory and anti-proliferation of cancer cells [Bhalla et al. 2013, Elgamal et al. 2021]. In addition, essential oils have a high potential for indoor use as an alternative to traditional indoor air disinfectants [Whiley et al. 2018].

The study aimed to assess the impact of selected essential oils on chosen three different species of allergenic fungi. The research determined the effect of oils on colony growth and sporulation of the studied species of fungi. In addition, oils with the most vigorous fungicidal activity were selected, which can be recommended for use in plant protection, aromatherapy and for room disinfection.

MATERIAL AND METHODS

The research fungal and essential oils. The study of the influence of EOs on the growth and sporulation of allergenic mold fungi was carried out at the Department of Plant Protection, University of Life Sciences in Lublin. Three isolates of the following species were selected for the study: *Alternaria alternata* Keissler – isolate F22, *Botrytis cinerea* Pers.

– isolate F34, and *Cladosporium cladosporioides* (Fresen.) G.A. de Vries – isolate F25. The mentioned fungal single spore isolates were obtained as a result of the research on the health of fennel *Foenicum vulgare* var. *vulgare*, conducted at the Department of Vegetable and Herb Crops University of Life Sciences in Lublin in 2021.

Among the commercially available essential oils with antimicrobial properties, four different oils were used in the experiments, i.e., grapefruit oil/*Citrus Grandis oil* (CGO) and oregano essential oil (OEO) from Etja, tea tree oil (TTO) and lavender oil (LO) from Avicenna-Oil company.

According to Etja company CGO is in 100% a natural oil obtained from grapefruit tree fruits *Citrus grandis* during hydro-distillation process. CGO is a yellow liquid with a fresh, citrusy, bittersweet, characteristic aroma, with a hint of bitter orange. In general the major components of the volatile part the essential oil contains 80–95% limonene, myrcene (0.8–2%), α-pinene (0.2–1.6%), γ-terpinene (0.1–0.8%). The character of the aroma is mainly influenced by the aerobic constituents, whose total content is 1–1.5%. These include aliphatic aldehydes (nonanal, decanal, undecanal, dodecanal), oxygen derivatives of the monoterpenes (citronellal, neral, geranial, perilla aldehyde) and α- and β-sinensal, particularly notcatone, occurring in an amount of 0.1–0.3%. CGO is rich in antioxidants, especially vitamin C [Góra and Lis 2019].

Natural oregano oil produced by Etja company are extracted by steam distillation of the leaves of the common oregano plant (*Origanum vulgare* L.). An essential oil of 0.12–1.2% is present in the oregano leaves, which has a high antimicrobial activity. It contains a number of chemical compounds, but the most important of which are carvacrol and thymol whose content in the raw plant material is quite high. As indicated in the literature, the content and composition of essential oil of oregano populations are independent of cultivation conditions. In research of Azizi et al. [2009] it was shown that carvacrol was the dominant compound (70.0–77.4%) in all studied essential oil samples, followed by γ-terpinene (8.1–9.5%) and *p*-cymene (4.5–5.3%).

TTO (*Melaleucae aetheroleum*) produced by Avicenna-Oil company is a 100% natural product obtained from the leaves and twigs of the tea tree *Melaleuca alternifolia* (Maiden & Bethe) Cheel by steam hydro-distillation. TTO contains mainly monoterpenes, which account for almost 90%, including 40–50% monoterpene hydrocarbons and 40–60% monoterpene oxygen derivatives. The predominant constituent is terpinen-4-ol (30–60%). This is the a monoterpene alcohol with a warm, peppery, slightly earthy aroma with citrus and green apple notes. Moreover, TTO contains γ-terpinene (10–30%) and α-terpinene (5–15%). Other constituents with several percentages include 1,8-cyneol (1–15%), *p*-cymene (1–10%), α-terpineol (1–8%), terpinolene (1–5%), α-pinene (1–5%) and limonene (1–5%) [Lis 2019].

The oil of lavender produced by Avicenna-Oil company is a 100% natural product extracted from the narrow-leafed lavender flowers (*Lavandula angustifolia* Mill.), a member of the lavender family (Lamiaceae). LO is a colourless or pale-yellow liquid with a fresh, characteristic lavender fragrance. The constituents of LO are: linalool, linalool acetate, lavendulol, β-caryophyllene, terpinen-4-ol, cineol, ocymene and camphor. The most important ingredients of oil are linalool (20–40%) and linalyl acetatate (15–45%) [Góra and Lis 2019].

In vitro antifungal assay of essential oils. Laboratory tests were carried out using Potato Dextrose Agar (PDA) special culture medium for fungi – a ready-made product from Difco™, and the substrate poisoning method described by Palfi et al. [2019]. The tested essential oils were added to a sterile, cooled to 50–65°C PDA medium containing 1% (v/v) Tween-80 immediately before pouring the medium into 90 mm Petri dishes. Tween-80 is a polysorbate surfactant which helps in stabilizing proteins. The oils were tested in three concentrations, i.e., 0.1 mg/mL, 0.25 mg/mL and 0.5 mg/mL. The fungi inoculum was placed on the nutrient medium with the specified EO content and after that the Petri dishes were secured with parafilm. The inoculum was made of mycelium discs of the three species of molds tested with a diameter of 3 mm, excised with cork borer from the grown, two-week-old mother colonies. After inoculation, the Petri dishes were put into an incubator, at the temperature of 24°C and under the light regime 12 h of light/12 h of dark.

The mother colonies of the tested fungi were grown on a PDA medium at 24°C. Four replicates

were considered for each preparation at the tested concentration, treated as a target, and for the fungus isolate, considering the plate as a replicate. The controls were colonies of the mentioned species of fungi growing on a PDA medium containing the same amount of Tween-80 preparation without adding oils.

The measure of the fungicidal activity of the studied essential oils on the growth of 4, and 8-day-old colonies of *A. alternata*, *B. cinerea* and *C. cladosporioides* was made and expressed in millimetres. Then the percentage of inhibition of growth of the fungus colony on the medium with the addition of the oil, concerning the growth of the colony on the control medium were calculated according to the Abbott formula:

$$I = C - T / C \times 100\%$$

where:
I – inhibition process;
C – control colony diameter;
T – diameter of the colony on the plate with the addition of the given essential oil [Burgieł and Smagłowski 2019]. Experiment were performed in two series.

In vitro tests of the toxic effect of EOs and microscoping observations. The toxic effects of essential oils on *A. alternata*, *B. cinerea* and *C. cladosporioides* have been determined. Lacking colony growth of the fungus on medium with an addition of essential oil in Petri dishes, the mycelial disk was transferred to a PDA medium containing no added oil. After 3 days of cultivation of so prepared dishes at a temperature of 24°C, the type of toxic activity was determined on the basis on the growth or death of the colony as well as on microscopic examination. When the fungus undertook growth, the activity of the essential oil was determined as fungistatic, while in the absence of fungal growth it was determined as fungicidal.

Microscopic examinations of 8-day-old colonies of the studied species of fungi growing on the medium with oils were also carried out to find changes in the structural appearance of the hyphae and spores of the studied fungi [Machowicz-Stefaniak and Zalewska 2011]. Mycelium and spores of studied species of fungi, taken from colonies and plugs (in the case of no fungal growth) were observed with light microscope (Nikon Microscope ECLIPSE E200, LED, trinocular, PH, infinity, e-plan, 40×–1000×) at a 400× magnification. The described activities were performed to determine the viability of the fungus.

Statistical analyses. The obtained data were statistically analyzed using Fisher's LSD (Last Significant Difference) test with a significance level of $p \leq 0.05$ (Statistica ver. 13.3).

RESULTS

Research on the impact of grapefruit oil (CGO). As a result of the observations of the growth and development of fungi on the PDA medium with the addition of grapefruit essential oil at the concentrations of 0.1 mg/mL, 0.25 mg/mL and 0.5 mg/mL, it was found that after four days, the average size of the colonies of the studied culture isolates was at the compared size like their control colonies. As demonstrated by the statistical analysis, these values did not differ significantly between each other and in comparison with the size of the control colony diameter (Tab. 1). For this combination of experiments, there was no fungistatic effect of the tested oil after four days of cultivation.

After eight days of cultivation, the average size of *A. alternata* colonies was, respectively: 61.5 mm, 56.0 mm and 55.5 mm, on the medium containing essential oil at the following concentrations: 0.1 mg/mL, 0.25 mg/mLand 0.5 mg/mL (Tab. 1). *Botrytis cinerea* colonies reached a diameter of 90.0 mm on all tested media (Tab. 1). On the other hand, the diameter of the *C. cladosporioides* colony on the medium with the addition of essential oil at the concentration of 0.1 mg/mL was slightly smaller compared to the control colony. With a higher content of essential oil in the medium, it was found that the diameter of the fungus colony was greater than that of the control colony (Tab. 1). Statistical analysis of the obtained results showed no significant differences between the colony's size and the percentage of growth inhibition of the studied fungi species (Tab. 2). The results showed that CGO was fungistatic concerning *A. alternata* in all tested concentrations and to *C. cladosporioides* at the concentration of 0.1 mg/mL (Tab. 3). However, with the oil content of 0.25 mg/mL and 0.5 mg/mL in the medium, the oil stimulated the growth of *C. cladosporioides*. Regarding *B. cinerea*, there was no effect of the essential oil on the growth and size of the fungus colony (Tab. 3). Macroscopic observations of *A. alternata* and *C. cladosporioides* colonies showed no differences in

Table 1. Growth of colonies of studied fungal species on PDA medium with an addition of essential oils

Species and isolate of fungi	The diameter of the colony of the fungus after 4 days of growth Essential oil content [mg/mL]				The diameter of the colony of the fungus after 8 days of growth Essential oil content [mg/mL]			
	0.1	0.25	0.5	Control	0.1	0.25	0.5	Control
Grapefruit oil (CGO)								
A. alternata F22	32.7 def	31.0 de	34.2 def	38.0 efg	61.5 i	56.0 hi	55.5 hi	69.0 ij
B. cinerea F34	87.0 k	82.0 jk	77.0 jk	90.0 k	90.0 k	90.0 k	90.0 k	90.0 k
C. cladosporioides F25	18.5 bc	19.2 bcd	19.0 bcd	19.0 bcd	35.2 defg	36.3 defg	36.7 defg	35.75 defg
Tea tree oil (TTO)								
A. alternata F22	19.7 cd	21.0 cd	0.0 a	38.0 efg	57.7 hi	58.2 hi	28.6 c	69.0 ij
B. cinerea F34	30.7 de	6.0 a	0.0 a	90.0 k	90.0 k	30.7 de	2.5 a	90.0 k
C. cladosporioides F25	11.2 b	5.0 a	5.0 a	19.0 bcd	26.2 cd	11.0 b	14.0 bc	35.75 defg
Oregano oil (OEO)								
A. alternata F22	0.0 a	0.0 a	0.0 a	38.0 efg	0.0 a	0.0 a	0.0 a	69.0 ij
B. cinerea F34	0.0 a	0.0 a	0.0 a	90.0 k	0.0 a	0.0 a	0.0 a	90.0 k
C. cladosporioides F25	0.0 a	0.0 a	0.0 a	19.0 bcd	0.0 a	0.0 a	0.0 a	35.75 defg
Lavender oil (LO)								
A. alternata F22	33.0 def	22.2 cd	33.5 def	38.0 efg	61.2 i	59.5 i	56.7 hi	69.0 ij
B. cinerea F34	85.5 k	68.0 i	20.5 cd	90.0 k	90.0 k	90.0 k	73.0 k	90.0 k
C. cladosporioides F25	19.2 bcd	19.5 bcd	13.5 bc	19.0 bcd	35.1 defg	36.0 defg	27.8 cd	35.75 defg

Values marked with the same letter do not differ significantly (significance level – $p \leq 0.05$).

colony colour and structure of fungal hyphae grown on the medium with the addition of essential oil. On the other hand, in *B. cinerea*, the presence of sclerotia, i.e. surviving forms of the fungus, was found on the surface of colonies growing on the medium with the content of essential oil in the tested concentrations. These structures were not found in the control colony.

Microscopic examination of the mycelium growing on the medium with essential oil revealed the presence of conidial spores of *A. alternata*, *B. cinerea* and *C. cladosporioides* species, with diagnostic features corresponding to the spores obtained in the control combination in all experimental combinations.

Research on the impact of tea tree oil (TTO). On the PDA medium with the addition of TTO at the concentrations of 0.1 mg/mL and 0.25 mg/mL, the diameter of the *A. alternata* colony was significantly lower than the diameter of control colony. On the other hand, the fungus did not grow on the medium containing the essential oil at the concentration of 0.5 mg/mL (Tab. 1). After eight days of cultivation, the diameter of the *A. alternata* colony was 57.7 mm and 58.2 mm, respectively, in the medium with the addition of essential oil at the concentrations of 0.1 mg/mL and 0.25 mg/mL (Tab. 1). Moreover, a slight fungus growth was obtained on the medium in which the oil concentration was 0.5 mg/mL (Tab. 1). As shown by the statistical analysis, both the diameter of the colony and the percentage of growth inhibition of the fungus colonies after eight days of cultivation in the medium containing 0.1 mg/mL and 0.25 mg/mL of essential oil did not differ significantly from the size of the diameter and the per cent inhibition of growth of the control colony (Tabs 1 and 2). On the other hand, the diameter of the *A. alternata* colony in the medium with an addition of essential oil in the amount of 0.5 mg/mL was significantly smaller than that of the control colony (Tab. 1).

Table 2. Percentage of inhibition of colony growth of the studied fungal species on PDA with the addition of essential oils

Species and isolate of fungi	Percentage of inhibition of fungus colony growth after 4 days of growth Essential oil content [mg/mL]				Percentage of inhibition of fungus colony growth after 8 days of growth Essential oil content [mg/mL]			
	0.1	0.25	0.5	Control	0.1	0.25	0.5	Control
Grapefruit oil (CGO)								
A. alternata F22	13.8 bc	18.4 bc	9.8 bc	0.0 ab	9.3 bc	18.2 bc	50.2 d	0,0 ab
B. cinerea F34	3.3 ab	7.7 ab	14.4 bc	0.0 ab	0.0 ab	0.0 ab	0.0 ab	0,0 ab
C. cladosporioides F25	2.63 ab	–0.69 a	0.0 ab	0.0 ab	1.4 ab	–1.7 a	–1.4 a	0.0 ab
Tea tree oil (TTO)								
A. alternata F22	48.0 d	44.7 d	100.0 g	0.0 ab	16.0 bc	15.6 bc	19.52 bc	0.0 ab
B. cinerea F34	65.8 e	93.3 f	100.0 g	0.0 ab	0.0 a	65.6 e	97.2 f	0.0 ab
C. cladosporioides F25	40.7 d	73.3 e	73.3 e	0.0 ab	26.5 c	69.2 e	60.8 e	0.0 ab
Oregano oil (OEO)								
A. alternata F22	100.0 g	100.0 g	100.0 g	0.0 ab	100.0 g	100.0 g	100.0 g	0.0 ab
B. cinerea F34	100.0 g	100.0 g	100.0 g	0.0 ab	100.0 g	100.0 g	100.0 g	0.0 ab
C. cladosporioides F25	100.0 g	100.0 g	100.0 g	0.0 ab	100.0 g	100.0 g	100.0 g	0.0 ab
Lavender oil (LO)								
A. alternata F22	19.7 bc	41.4 d	11.8 b	0.0 ab	10.8 bc	14.5 bc	17.2 bc	0.0 ab
B. cinerea F34	5.0 ab	24.4 cd	77.2 e	0.0 ab	0.0 ab	0.0 ab	68.8 e	0.0 ab
C. cladosporioides F25	–1.05 a	–2.63 a	28.9 c	0.0 ab	1.7 ab	–0.69 a	22.23 c	0.0 ab

Values marked with the same letter do not differ significantly (significance level – $p \leq 0.05$).

Table 3. Type of influence of essential oils on the growth of colonies of studied fungal species

Species and isolate of fungi	Type of influence and content of essential oil [mg/mL]		
	0.1	0.25	0.5
Grapefruit oil (CGO)			
A. alternata F22	+	+	+
B. cinerea F34	0	0	0
C. cladosporioides F25	+	++	++
Tea tree oil (TTO)			
A. alternata F22	+	+	+
B. cinerea F34	+	+	+
C. cladosporioides F25	+	+	+
Oregano oil (OEO)			
A. alternata F22	+	–	–
B. cinerea F34	+	–	–
C. cladosporioides F25	–	–	–
Lavender oil (LO)			
A. alternata F22	+	+	+
B. cinerea F34	+	+	+
C. cladosporioides F25	+	++	+

– – fungicidal influence
+ – fungistatic influence
++ – stimulating influence
0 – lack of influence

On a PDA medium containing essential oil at concentrations of 0.1 mg/mL and 0.25 mg/mL, the diameter of the *B. cinerea* colony was 30.7 mm and 6.0 mm, respectively. These values were significantly lower compared to the size of the control colony diameter (Tab. 1). In the highest concentration of essential oil tested, no growth of the pathogen mycelium was found. The percentage of fungal growth inhibition was 100 and was significantly higher compared to the control and the percentage of inhibition of colony growth on the medium containing this oil at a concentration of 0.1 mg/mL and 0.25 mg/mL (Tab. 2).

After eight days of cultivation on a PDA medium containing essential oil at a concentration of 0.1 mg/mL, 0.25 mg/mL and 0.5 mg/mL, the diameter of the *B. cinerea* colony was 90.0 mm, 30.7 mm and 2.5 mm, respectively (Tab. 1). These values differed significantly from one another. Moreover, the diameter of the fungus colony on the medium containing 0.25 mg/mL and 0.5 mg/mL of the essential oil was significantly smaller than the size of the control colony and the diameter of the colony growing on the medium containing 0.1 mg/mL of essential oil. Statistical analysis of the percentage of inhibition of *B. cinerea* colony growth on a medium containing TTO showed significant differences between the size of this parameter depending on the oil content in the medium and the fungus growth time (Tab. 2).

The performed studies showed an inhibitory effect of TTO against *C. cladosporioides*. The diameter of the fungus colony was significantly smaller, after four and eight days of cultivation, on the medium containing the tested oil at the concentration of 0.25 mg/mL and 0.5 mg/mL (Tab. 1). The percentage of inhibition of pathogen colony growth after four and eight days of cultivation differed significantly and was significantly higher than the value of the control colony (Tab. 2).

Microscopic examination of the mycelium of *A. alternata*, *B. cinerea* and *C. cladosporioides* showed no effect of the essential oil on the sporulation of the fungi mentioned above. The conidial spores of the tested fungi were found in the microscopic slides made for all combinations. The conducted studies showed the fungistatic effect of TTO concerning all three tested species of allergenic fungi (Tab. 3).

Research on the effects of oregano oil (OEO). As a result of the observations of the growth and development of fungi on the PDA medium with the addition of OEO at the concentrations of 0.1 mg/mL, 0.25 mg/mL, 0.5 mg/mL total inhibition of the growth and development of the studied species of fungi was found both after four and eight days of cultivation (Tab. 1). Statistical analysis of the obtained results, i.e., the size of the colony diameter and the percentage of colony inhibition, showed significant differences in the parameters studied for *A. alternata*, *B. cinerea* and *C. cladosporioides* compared to the value of the control parameters (Tabs 1 and 2).

Laboratory tests showed that after transferring the fungi inoculum from the medium with the addition of OEO to the PDA medium without the addition of this oil, *A. alternata*, *B. cinerea* inocula obtained from the medium with the addition of 0.1 mg/mL oil started to grow, which proves a fungistatic influence of the oil. However, the fungus growth was not achieved in the other combinations, which proves its fungicidal properties (Tab. 3).

Microscopic examination of the mycelium derived from the inoculum of *A. alternata*, *B. cinerea* and *C. cladosporioides*, in direct contact with the medium containing essential oil, showed deformation and disintegration of the hyphae and conidial spores of the studied species of fungi at a concentration at which they did not grow after transfer PDA inoculum with no oil added (Fig. 1).

Research on the effects of lavender oil (LO). On the PDA medium with the addition of LO in concentrations of 0.1 mg/mL, 0.25 mg/mL, 0.5 mg·cm^{-3}, colony diameter of *A. alternata* was 33.0 mm, 22.2 mm and 35.5 mm, respectively, after four days of growth, and 61.2 mm, 59.5 mm and 56.7 mm after eight days of culture (Tab. 1). The size of the fungus colony on the medium containing essential oil, at a concentration of 0.25 mg/mL, was significantly smaller than that of the control colony (Tab. 1). Statistical analysis of the obtained results showed no significant differences in the size of the fungus colony diameter and the percentage of growth inhibition of the fungus after eight days of cultivation (Tabs 1 and 2). Due to the restriction of fungus colony growth, the oil's effect was considered fungistatic (Tab. 3).

The microscopic examination showed abundant sporulation of *A. alternata* on the essential oil medium at all tested concentrations. LO limited the growth of

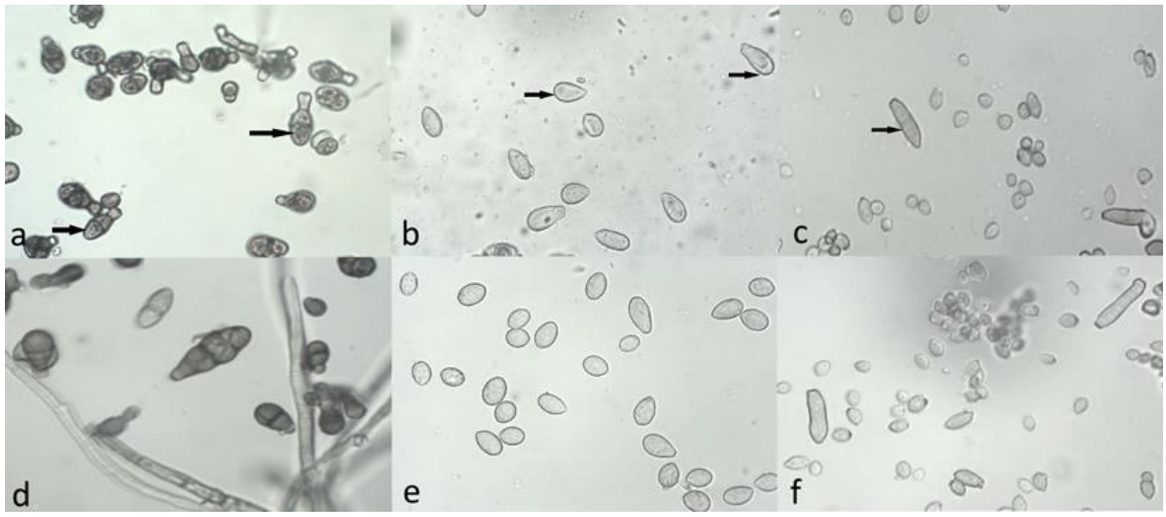

Fig. 1. Effects of oregano essential oil on conidia and hyphae of studied fungi by light microscope (Nicon ECLIPSE E200) with 400× magnification. First row: a) lysis of conidial spores of *A. alternata*, b) *B. cinerea* and c) *C. cladosporioides*, on PDA medium with the addition of oregano oil at the concentration of 0.5 mg/mL. Second row: Control – conidial spores of: d) *A. alternata*, e) *B. cinerea* and f) *C. cladosporioides* taken from colonies growing on PDA medium

B. cinerea colonies at the concentrations of 0.25 mg/mL and 0.5 mg/mL after four and at the concentration of 0.5 mg/mL after eight days of cultivation (Tab. 1). The diameter of the fungus colony on the medium containing essential oil at the concentration mentioned above was significantly smaller than the diameter of the control colony (Tab. 1). Statistical analysis showed that the per cent growth inhibition of fungal colonies grown on medium with low essential oil content did not differ significantly from the control value. On the other hand, it was significantly lower than the percentage of fungal colony growth inhibition on the medium containing the oil at the concentration of 0.5 mg/mL (Tab. 2).

Macroscopically, sclerotia were found on the surface of *B. cinerea* colonies growing on a medium with the addition of essential oil at the concentrations of 0.1 mg/mL and 0.25 mg/mL. Microscopic examination of the mycelium growing on a medium containing essential oil revealed the presence of *B. cinerea* conidia in all experiment combinations. The results indicated a slight fungistatic effect of LO on the growth and sporulation of *B. cinerea* (Tab. 3). In the medium with an addition of the essential oil, the effect on the growth of *C. cladosporioides* colonies was differentiated (Tab. 1). At the lowest concentration tested, the oil reduced the fungus growth after four and eight days of growth (Tab. 1). On the other hand, with lower essential oil content in the medium, the oil stimulated the growth of mycelium. The diameter of the fungus colony was more significantly higher than that of the control colony, and the percentage of colony growth inhibition was negative (Tabs 1 and 2). Microscopic examination of the mycelium revealed the presence of conidial spores of the fungus with macroscopic features characteristic of the species in all the tested concentrations.

DISCUSSION

The conducted research showed a high diversification of the influence of EOs on mold fungi with allergenic properties. It was shown that the most effective oil in limiting the growth and sporulation of the studied species of fungi was OEO, which, even at the lowest tested concentration, i.e., 0.1 mg/mL, completely inhibited the growth of the colony. Moreover, it was shown that this oil was fungicidal against *A. alternata* and *B. cinerea* at the higher tested concentrations and towards *C. cladosporides* at all tested concentrations. Similarly, the best inhibitory effect of *O. vulgare* essential oil (OVEO) was shown in a study undertaken by Hou et al. [2020]. OVEO was one of 17 plant EOs

tested that completely inhibited the mycelial growth of *B. cinerea* at the concentration of 0.5 mg/mL, i.e. in the same concertation like in ours study. Moreover, gas chromatography/mass spectrometry (GC/MS) analysis carried out by these researchers showed that the main components of OVEO were carvacrol, β-caryophyllene and thymol and the contain of these components constituted 89.9%, 3.34% and 2,39% respectively. The chemical composition of the individual oils was not investigated in the current study; however, as the general characteristics of OEO shows, it is these chemical compounds that exhibit antifungal properties. These properties were also demonstrated against pathogenic fungi and bacteria in the study by Wang et al. [2019] as well as against *B*. *cinera* in the research of Hou et al. [2020]. Furthermore, the authors of the last cited paper found that carvacrol and thymol tested as a single components showed higher fungicidal activity than the OVEO tested, both on fungal colony growth, spore germination as well as the therapeutic and protective effect on tomato gray mold caused by *B*. *cinerea*. They also indicated that the second main components of OVEO, i.e. β-caryophyllene, had no significant fungicidal properties according to *B*. *cinerea*. Based on these studies, we can conclude that carvacrol and thymol are the main components of OEO that exhibit antifungal properties, both as individual components and as constituents of the essential oil. Moreover, according to the literature data, the essential oil of oregano shows a broad spectrum of activity concerning pathogenic fungi. This oil, in the studies of Soylu et al. [2010], completely inhibited the growth of *B*. *cinerea* at a concentration of 12.8 µg/mL, while lavender oil showed such an effect, concerning *B*. *cinere*a, at a concentration twice as high, i.e., 25.6 µg/mL. The authors of the previous scientific work also indicated that oregano oil, as well as lavender and rosemary oil, inhibited the germination and elongation of the previously formed spore germination hypha of *B*. *cinerea*. Microscopic observations showed that these changes resulted from the progressive degeneration of fungal hyphae and spores through cytoplasmic coagulation and plasmolysis of hyphae cells. This study also showed similar results for all three tested species of fungi. The coagulation of spore cytoplasm, leakage of protoplasts and lysis of mycelial hyphae were observed. However, the effect of oil with oregano depends on the concentration because, concerning *Sclerotinia sclerotiorum*, the above effect was found at much higher concentrations [Soylu et al. 2007]. It was found, also based on in vivo tests, that oregano oil is the most effective essential oil in protecting tomatoes against grey mould in greenhouse conditions, even during the disease, after using the oil as a treatment [Palfi et al. 2019]. Similar research results were also obtained in Poland in the studies by Gwiazdowski et al. [2018] concerning several oilseed rape pathogens. It was shown that the marigold oil (oregano) was the most effective in limiting the colony growth of all the studied fungi, especially concerning *A*. *alternata* and other species of this genus, and *B*. *cinerea*, at the concentration of 0.04 mg/mL. Oregano oil is also highly effective in limiting the growth of fungi whose spores are found in bioaerosols, including *C*. *cladosporioides*, which was also shown in the studies by Zabka et al. [2014].

Tea tree oil (TTO) was the second most effective in reducing growth. This oil at the lowest tested concentration slightly inhibited the growth of the studied species of fungi, while at the highest tested concentration, i.e., 0.5 mg/mL, it effectively limited their growth. Similar results were also obtained in Burgieł and Smagłowski [2008] studies, where TTO showed high fungicidal activity against *B*. *cinerea* at the concentration of 0.5 mg/mL, i.e., at the same concentration that was tested in the present study. However, as the results of the tests show, the oil did not cause hyphae lysis in any of the tested fungi because after the transfer of the inoculum of *A*. *alternata* and *B*. *cinerea* from the PDA medium containing 0.5 mg/mL of oil to the medium without oil, fungi have taken up growth. The fungistatic effect of the oil has also been demonstrated concerning, among other things, fungi of the genera *Alternaria* and *Cladosporium* [Garbusińska et al. 2010, 2011]. However, the studies of Yu et al. [2015] showed that this oil causes changes at the cellular level, both in the hyphae and spores of B. *cinerea*.

The study of antimicrobial activity of various essential oils was also the subject of earlier research conducted on *A*. *alternata*, *B*. *cinerea* and *Fusarium oxysporum* [Badawy and Abdelgaleil 2014]. However, the tests carried out showed that essential oils obtained from *Artemisia judaica* and *A*. *monosperma* had the highest fungicidal activity against the fungal species tested. The fungicidal activity of the above mentioned EOs

was significantly higher than that of the oil extracted from *Origanum vulgare* [Badawy and Abdelgaleil 2014]. In addition, Feng and Zeng [2007] showed that essential oil extracted from cassia plant inhibited the growth and development and spore germination of *A*. *alternata*. Moreover, in both *in vitro* and *in vitro* studies of cassia essential oil reduced the development of alternariosis in tomatoes stored at 25°C.

The conducted research showed the fungistatic effect of lavender essential oil concerning the studied species of fungi. According to the literature data, the activity of this oil varies and depends on the concentration of the oil in the medium and the tested microorganisms [Soylu et al. 2010, Özcan et al. 2018]. Therefore, it cannot be unequivocally stated that the oil's action is fungistatic. Grapefruit oil was characterized by the weakest growth-inhibiting properties of the tested species of fungi because, as it was calculated, the difference between the diameter of the fungal colony in the experimental combination and the control colony was negligible, and even as in the case of *B*. *cinerea*, this oil limited the fungus growth after four days of cultivation, and after eight days no effect on the growth of the fungus was found. The lack of influence of this oil on the growth and sporulation of fungi is puzzling, and it is advisable to conduct further research in this direction. Especially that preparations obtained from grapefruit pulp and seeds, i.e., Grevit 200 SL and Biosept 300 SL, for years have been known as effective agents of natural origin, used in plant protection [Sapieha-Waszkiewicz et al. 2011], and citrus oils are widely used as favoring agents in food, pharmaceutical industry, cosmetic and chemical [Salvatore et al. 2022]. The studies by Patkowska and Krawiec [2016] showed the high effectiveness of these preparations in limiting the development of soil pathogens, which increased soybean yield. Considering the results of the presented research as well as the guidelines contained in the work of Fidanza and Caneva [2019], we want to emphasize that conducting this research is justified and therefore further research using a standardized methodology is still needed.

CONCLUSIONS

The effect of essential oils on the growth of colonies of *A*. *alternata*, *B*. *cinerea* and *C*. *cladosporioides* varied and depended on the used oil and its concentration. The increase in the content of active substances in the oil in the medium resulted in a more significant limitation of the growth of the colonies of the tested fungi. The highest fungicidal activity with respect to *C*. *cladosporioides* regardless of the concentration, was shown by oregano essential oil. However, concerning to *A*. *alternate* and *B*. *cinerea*, such an effect was found at the concentrations of 0.25 mg/mL and 0,5 mg/mL. Only OEO caused degradation and lysis of hyphae and conidia of the tested fungal species. TTO limited the colony growth of the studied species of fungi but did not affect the process of spore formation and did not cause the death of the hyphae. CGO showed the weakest fungistatic effect concerning *A*. *alternata* and *C*. *cladosporioides*. However, regarding *B*. *cinerea*, no effect of the oil was found, and at the concentration of 0.25 mg/mL and 0.5 mg/mL, it stimulated the growth of *C*. *cladosporioides*. Considering the fungicidal activity of OEO as well as strong limitation of mycelial growth by TTO, these two essential oils can be recommended for use in aromatherapy and for disinfecting rooms. Therefore, further in vivo studies should be carried out.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abbas, N.B., Armin, S., Mahdieh, D., Ramin, N., Shahrokh, N., Rounak B. (2021). Pollution characteristics and non-carcinogenic risk assessment of fungal bioaerosol in different processing units of waste paper and cardboard recycling factory. Toxin Rev., 40(4), 752–763, https://doi.org/10.1080/15569543.2020.1769135

Abd-El-Gawad, A.M., Elshamy, A.I., Al-Rowaily, S.L., El-Amier, Y.A. (2019). Habitat affects the chemical profile, allelopathy, and antioxidant properties of essential oils and phenolic enriched extracts of the invasive plant *Heliotropium curassavicum*. Plants, 8(11), 482. https://doi.org/10.3390/plants8110482

Abers, M., Schroeder, S., Goelz, L., Sulser, A., St. Rose, T., Puchalski, K., Langland, J. (2021). Antimicrobial activity of the volatile substances from essential oils. BMC Complement. Med. Ther., 21, 1–14. https://doi.org/10.1186/s12906-021-03285-3

Alonso-Gato, M., Astray, G., Mejuto, J.C., Simal-Gandara, J. (2021). Essential oils as antimicrobials in crop protection. Antibiotics, 10(1), 34. https://doi.org/10.3390/antibiotics10010034

Assaeed, A., Elshamy, A., El Gendy, A.E.-N., Dar, B., Al-Rowaily, S., Abd El-Gawad, A. (2020). Sesquiterpenes-rich essential oil from above ground parts of *Pulicaria somalensis* exhibited antioxidant activity and allelopathy effect on weeds. Agronomy, 10(3), 399. https://doi.org/10.3390/agronomy10030399

Azizi, A., Yan, F., Honermeier, B. (2009). Herbage yield, essential oil content and composition of three oregano (*Origanum vulgare* L.) populations as affected by soil moisture regimes and nitrogen supply. Ind. Crops Prod., 29(2–3), 554–561. https://doi.org/10.1016/j.indcrop.2008.11.001

Babar, A., Naser, A.Al-W., Saiba, S., Aftab, A., Shah, A.K., Firoz, A. (2015). Essential oils used in aromatherapy: a systemic review. Asian Pac. J. Trop. Biomed., 5(8), 601–611. https://doi.org/10.1016/j.apjtb.2015.05.007

Badawy, M.E., Abdelgaleil, S.A. (2014). Composition and antimicrobial activity of essential oils isolated from Egyptian plants against plant pathogenic bacteria and fungi. Ind. Crops Prod., 52, 776–782. https://doi.org/10.1016/j.indcrop.2013.12.003

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. (2008). Biological effects of essential oils – a review. Food Chem. Toxicol., 46(2), 446–475. https://doi.org/10.1016/j.fct.2007.09.106

Behnam, S., Farzaneh, M., Ahmadzadeh, M., Tehrani, A.S. (2006). Composition and antifungal activity of essential oils of *Mentha piperita* and *Lavendula angustifolia* on post-harvest phytopathogens. Commun. Agric. Appl. Biol. Sci., 71(3 Pt B), 1321–1326.

Bennike, N.H., Johansen, J.D. (2018). Aromatherapists. In: Kanerva's occupational dermatology, John, S., Johansen, J., Rustemeyer, T., Elsner, P., Maibach, H. (eds). Springer, Cham, 1–4.

Bhalla, Y., Gupta, V.K., Jaitak, V. (2013). Anticancer activity of essential oils: a review. J. Sci. Food Agric., 93(15), 3643–3653. https://doi.org/10.1002/jsfa.6267

Burgieł, Z.J., Smagłowski, M. (2008). Fungistic properties of tea tree oil. Probl. J. Agric. Sci., 529, 13–18.

De Hoog, G.S., Guarro, J., Gené, J., Figueras, M.J. (2000). Atlas of clinical fungi, 2nd ed. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Elgamal, A.M., Ahmed, R.F., Abd-ElGawad, A.M., El Gendy, A.E.-N.G., Elshamy, A.I., Nassar, M.I. (2021). Chemical profiles, anticancer, and anti-aging activities of essential oils of *Pluchea dioscoridis* (L.) DC. and *Erigeron bonariensis* L. plants, 10(4), 667. https://doi.org/10.3390/plants10040667

Elshamy, A.I., Ammar, N.M., Hassan, H.A., Al-Rowaily, S.L., Raga, T.R., El Gendy, A., Abd El-Gawad, A.M. (2020). Essential oil and its nanoemulsion of *Araucaria heterophylla* resin: chemical characterization, anti-inflammatory, and antipyretic activities. Ind. Crop. Prod., 148, 112272. https://doi.org/10.1016/j.indcrop.2020.112272

Feng, W., Zheng, X. (2007). Essential oils to control *Alternaria alternata* in vitro and in vivo. Food control, 18(9), 1126–1130. https://doi.org/10.1016/j.foodcont.2006.05.017

Fengfeng, W., Yamei, J., Xueming, X., Na, Y. (2017). Eletrofluidic pretreatment for enhancing essential oil extraction from citrus fruit peel waste. J. Clean. Prod., 159, 85–94. https://doi.org/10.1016/J.JCLEPRO.2017.05.010

Fidanza, M.R., Caneva, G. (2019). Natural biocides for the conservation of stone cultural heritage: a review. J. Cult. Herit., 38, 271–286. https://doi.org/10.1016/J.JCLEPRO.2017.05.01010.1016/j.culher.2019.01.005

Garbusińska, A., Mertas, A., Król, W. (2010). Przegląd badań in vitro oceniających aktywność przeciwdrobnoustrojową olejku z drzewa herbacianego (Tea Tree Oil). Cz. I [The antimicrobial activity of tea tree oil in the research in vitro. Part I]. Borgis – Post. Fitoter., 2, 85–96.

Garbusińska, A., Mertas, A., Szliszka, E., Król, W. (2011). Aktywność przeciwdrobnoustrojowa olejku z drzewa herbacianego (Tea Tree Oil) w badaniach in vitro. Cz. II [The antimicrobial activity of tea tree oil in the research in vitro. Part II], Borgis – Post Fitoter., 3, 175–184.

Góra, J., Lis, A. (2019). Najcenniejsze olejki eteryczne. Cz. 1 [The most valuable essential oils. Part 1]. Wydawnictwo Politechniki Łódzkiej [TUL Publishing House], 68–72, 165–170 [in Polish].

Gwiazdowski, R., Gwiazdowska, D., Marchwińska, K., Juś, K., Szutowska, J., Bednarek-Bartsch, A., Danielewicz, B. (2018). Właściwości fungistatyczne olejków eterycznych wobec wybranych patogenów rzepaku [Fungistatic activity of essential oils towards selected oilseed rape pathogens]. Progress Plant Protect., 58(4), 300–305 [in Polish]. https://doi.org/10.14199/ppp-2018-042

Halberstein, R. (2005). Medicinal plants: historical and cross-cultural usage patterns. Ann. Epidemiol., 15(9), 686–699. https://doi.org/10.1016/j.annepidem.2005.02.004

Hou, H., Zhang, X., Zhao, T., Zhou, L. (2020). Effects of *Origanum vulgare* essential oil and its two main components, carvacrol and thymol, on the plant pathogen *Botrytis cinerea*. Peer J., 8, e9626. https://doi.org/10.7717/peerj.9626

Jurgensen, C.W., Madsen, A. (2009). Exposure to the airborne mould *Botrytis* and its health effects. Ann. Agric. Environ. Med., 16(2), 183–196.

Kaur, N., Bains, A., Kaushik, R., Dhull, S.B., Melinda, F., Chawla, P. (2021). A review on antifungal efficiency of plant extracts entrenched polysaccharide-based nanohydrogels. Nutrients, 13(6), 2055. https://doi.org/10.3390/nu13062055

Kilic, M., Altunoglu, M.K., Akdogan, G.E., Akpınar, S., Taskın, E., Erkal, A.H. (2020). Airborne fungal spore relationships with meteorological parameters and skin prick test results in Elazig, Turkey. J. Environ. Health Sci. Engineer, 18, 1271–1280. https://doi.org/10.1007/s40201-020-00545-1

Lis, A. (2019). Najcenniejsze olejki eteryczne. Cz. 2 [The most valuable essential oils. Part 2]. Wydawnictwo Politechniki Łódzkiej [TUL Publishing House], 69–80.

Machowicz-Stefaniak, Z., Zalewska, E. (2011). Occurrence of *Colletotrichum dematium* on selected herbs species and preparations inhibiting pathogen's growth and development in vitro. Ecol. Chem. Eng., 18(4), 465–478.

Malik, A., Najda, A., Bains, A., Nurzyńska-Wierdak, R., Chawła, P. (2021). Characterization of *Citrusnobilis* peel methanolic extract for antioxidant, antimicrobial, and anti-inflammatory activity. Molecules, 26(14), 4310. https://doi.org/10.3390/molecules26144310

Mendes de Toledo, C.E., Santos, P.R., Palazzo de Mello, J.C., Dias Filho, B.P., Nakamura, C.V., Ueda-Nakamura, T. (2015). Antifungal properties of crude extracts, fractions, and purified compounds from bark of *Curatella americana* L. (Dilleniaceae) against *Candida* species. Evid. Based Complement. Alternat. Med., 673962. https://doi.org/10.1155/2015/673962

Nazzaro, F., Fratianni, F., Coppola, R., Feo, V.D. (2017). Essential oils and antifungal activity. Pharmaceuticals, 10(4), 86. https://doi.org/10.3390/ph10040086

Özcan, M.M., Starovic, M., Aleksic, G., Figueredo, G., Juhaimi, F.A., Chalchat, J-C. (2018). Chemical composition and antifungal activity of lavender (*Lavandula stoechas*) oil. Nat. Prod. Commun. https://doi.org/10.1177/1934578X1801300728

Palfi, M., Konjevoda, P., Vrandečić, K. (2019). Antifungal activity of essential oils on mycelial growth of *Fusarium oxysporum* and *Bortytis cinerea*. Emir. J. Food Agric., 31(7), 544–554. https://doi.org/10.9755/ejfa.2019.v31.i7.1972.

Patkowska, E., Krawiec, M. (2016). Yielding and healthiness of pea cv. 'Sześciotygodniowy' after applying biotechnical preparations. Acta Sci. Pol. Hortorum Cultus, 15(2), 143–156.

Peighami-Ashnaei, S., Farzaneh, M., Sharifi-Tehrani, A., Behboudi, K. (2009). Effect of essential oils in control of plant diseases. Commun. Agric. Appl. Biol. Sci., 74(3), 843–847.

Raveau, R., Fontaine, J., Lounès-Hadj Sahraoui, A. (2020). Essential oils as potential alternative biocontrol products against plant pathogens and weeds: a review. Foods, 9(3), 365. https://doi.org/10.3390/foods9030365

Sabariego, S., Bouso, V., Pérez-Badia, R. (2012). Comparative study of airborne *Alternaria* conidia levels in two cities in Castilla-La Mancha (central Spain), and correlations with weather-related variables. Ann. Agric. Environ. Med., 19(2), 227–232.

Salvatore, M.M., Nicoletti, R., Andolfi, A. (2022). Essential oils in citrus fruit ripening and postharvest quality. Horticulturae, 8(5), 396. https://doi.org/10.3390/horticulturae8050396

Sapieha-Waszkiewicz, A., Marjańska-Cichoń, B., Miętkiewski, R. (2011). Effect of biopesticides on the growth and development of isolates of Botrytis cinerea Pers., in vitro obtained from raspberry plants. J. Plant Protect. Res., 51(2), 151–156. https://doi.org/10.2478/v10045-011-0026-8

Simon-Nobbe, B., Denk, U., Pöll, V., Rid, R., Breitenbach, M. (2008). The spectrum of fungal allergy. Int. Arch. Allergy Immunol., 145(1), 58–86. https://doi.org/10.1159/000107578

Singh, K. (2019). Keeping with tradition: essential oil history, use and production: a review. Cosmet. Toilet., 134(7), 61–70.

Sio, Y.Y., Pang, S.L., Say, YH., Teh, K.F., Wong, Y.R., Shah, S.M.R., Reginald, K., Chew F.T. (2021). Sensitization to airborne fungal allergens associates with asthma and allergic rhinitis presentation and severity in the Singaporean/Malaysian population. Mycopathologia, 186, 583–588. https://doi.org/10.1007/s11046-021-00532-6

Skyberg, K., Skulberg, K.R., Eduard, W. (2003). Symptoms prevalence among office employees and associations

to building characteristics. Indoor Air, 13(3), 246–252. https://doi.org/10.1034/j.1600-0668.2003.00190.x

Soares-Castro, P., Soares, F., Santos, P.M. (2021). Current advances in the bacterial toolbox for the biotechnological production of monoterpene-based aroma compounds. Molecules, 26(1), 91. https://doi.org/10.3390/molecules26010091

Soylu, E.M., Kurt, S., Soylu, S. (2010). In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mold disease agent *Botrytis cinerea*. Int. J. Food Microbiol., 143(3), 183–189, https://doi.org/10.1016/j.ijfoodmicro.2010.08.015

Soylu, S., Yigitbas, H., Soylu, E.M., Kurt, S. (2007). Effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum*. J. Appl. Microbiol., 103(4), 1021–1030. https://doi.org/10.1111/j.1365-2672.2007.03310.x

Tongnuanchan, P., Benjakul, S. (2014). Essential oils: extraction, bioactivities, and their uses for food preservation. J. Food Sci., 79(7), 1231–1249. https://doi.org/10.1111/1750-3841.12492

Turek C., Stintzing, C. (2013). Stability of essential oils: a review. Compr. Rev. Food Sci. Food Saf., 12(1), 40–53. https://doi.org/10.1111/1541-4337.12006

Wang, K., Jiang, S., Yang, Y., Fan, L., Su, F., Ye, M. (2019). Synthesis and antifungal activity of carvacrol and thymol esters with heteroaromatic carboxylic acids. Nat. Prod. Res., 33(13), 1924–1930. https://doi.org/10.1080/14786419.2018.1480618

Weryszko-Chmielewska, E., Kasprzyk, I., Nowak, M., Sulborska, A., Kaczmarek, J., Szymanska, A., Haratym, W., Gilski, M., Jedryczka, M. (2018). Health hazards related to conidia of *Cladosporium* – biological air pollutants in Poland, central Europe. J. Environ. Sci., 65, 271–281. https://doi.org/10.1016/j.jes.2017.02.018

Whiley, H., Gaskin, S., Schroder, T., Ross, K. (2018). Antifungal properties of essential oils for improvement of indoor air quality: a review. Rev. Environ. Health., 33(1), 63–76. https://doi.org/10.1515/reveh-2017-0023

Yu, D., Wang, J., Shao, X., Xu, F., Wang, H. (2015). Antifungal modes of action of tea tree oil and its two characteristic components against *Botrytis cinerea*. J. Appl. Microbiol., 119(5), 1253–1262. https://doi.org/10.1111/jam.12939

Zabka, M., Pavela, R., Prokinova, E. (2014). Antifungal activity and chemical composition of twenty essential oils against significant indoor and outdoor toxigenic and aeroallergenic fungi. Chemosphere, 112, 443–448. https://doi.org/10.1016/j.chemosphere.2014.05.014

Zhou, H.E., Tao, N.G., Jia, L. (2014). Antifungal activity of citral, octanal and α-terpineol against *Geotrichum citri-aurantii*. Food Control, 37, 277–283. https://doi.org/10.1016/j.foodcont.2013.09.057