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Poljoprivreda / Agriculture

ISSN: 1848-8080 (Online)
ISSN: 1330-7142 (Print)

https://doi.org/10.18047/poljo.32.1.6

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ISSN 1330-7142
UDK=632.4'952
https://doi.org/10.18047/poljo.32.1.6

VOLATILE ANTIFUNGAL ACTIVITY OF ESSENTIAL OILS AGAINST *Fusarium culmorum*: THE EFFECTS OF CONCENTRATION AND TEMPERATURE

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Original scientific paper
Izvorni znanstveni članak

SUMMARY

The phytopathogenic fungus Fusarium culmorum represents a serious threat to wheat production due to its aggressiveness and ability to survive in soil and plant residues, and as a significant producer of toxicologically important mycotoxins that pose a risk to food safety and human and animal health. In this regard, essential oils are considered potential natural substitutes because of their volatile properties. This study aimed to assess the volatile antifungal activity of five essential oils (anise, thyme, cinnamon, sweet orange, and mountain pine) against the mycelial growth of F. culmorum isolate under different concentrations, temperatures, and light regimes. The experiment was carried out in Petri dishes using essential oils at 20 and 100 µL at three temperatures (10, 20, and 30 °C) and under different light regimes. The mycelial growth was measured at 72 and 168 hours of incubation, and antifungal activity was expressed as the percentage growth of inhibition. Thyme, anise, and cinnamon essential oils fully and stably inhibited mycelial growth at all concentrations and temperatures. On the other hand, sweet orange and mountain pine essential oils showed weaker temperature sensitivity with higher dependence on concentration. The effects of concentration and temperature were more conspicuous at the early stage of incubation but decreased over time. These results suggest that volatile antifungal activity is primarily determined by the type of essential oil and indicate their potential to develop natural strategies to control F. culmorum.

Keywords: phytopathogenic fungi, antifungal activity, mycelial inhibition, natural antifungals, essential oil vapours

INTRODUCTION

Key issues in agricultural production are keeping crops safe from plant diseases that can greatly endanger both yield and quality. Phytopathogenic fungi are one of the main causes of plant diseases, causing a significant threat to global agriculture. It is estimated that fungal diseases are responsible for 70 to 80% of all microbial diseases in agricultural systems (Peng et al., 2021), causing yield losses of 20 to 40% (Cenobio-Galinido et al., 2024). Species of the genus *Fusarium* are notable among these fungi for their widespread distribution, broad host range, and ability to infect economically significant crops (Bentley et al., 2006; Ferrigo et al., 2016). Their management is particularly challenging due to their high adaptability and ability to survive in soil and plant residues. *Fusarium culmorum* (W.G. Sm.) Sacc. is a highly violent pathogen of wheat (*Triticum aestivum* L.) and causes seedling blight, root and crown rot, and Fusarium head blight. These infections frequently result in reduced germination, necrotic lesions, and decreased yield and grain quality (Scherm et al., 2013; Karlsson et al., 2021). Among the species of the genus *Fusarium*, *F. culmorum* is one of the most important producers of toxicologically significant mycotoxins, such as zearalenone and trichothecene (including deoxynivalenol, nivalenol, T-2 toxin), which contaminate cereals, threaten food safety, and consequently have a harmful effect on human

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and animal health (Nguyen et al., 2017; Pastuszak et al., 2021).

The increasing interest in natural plant protection products is linked to efforts to diminish the negative environmental impact of synthetic pesticides and to raise concerns about human health and food safety.

Based on this, essential oils have been recognized as environmentally friendly alternatives for controlling cereal diseases (Raveau et al., 2020). Plants produce many compounds that help defend against adverse environmental conditions and pathogenic organisms. Among these, plant secondary metabolites, otherwise known as phytochemicals, represent a diverse group of bioactive substances that contribute significantly to plant responses to biotic and abiotic stresses (Soleimani et al., 2022).

Essential oils make up a specific group of plant secondary metabolites and consist of complex mixtures of volatile organic compounds, mainly terpenes and their derivatives, as well as aromatic and aliphatic components. They are extracted from different plant organs, including roots, stems, leaves, flowers, fruits, and seeds. Also, their biological activities, including antimicrobial and antioxidant properties, have promoted their widespread use (Ebadollahi et al., 2020; Catani et al., 2022; Zhang et al., 2022). There are more than 3,000 known essential oils, of which 300 are of commercial importance. Their biological properties include bactericidal, fungicidal, insecticidal, nematicidal, herbicidal, virucidal, and antiparasitic effects (Zuzarte & Salgueiro, 2015; Nazzaro et al., 2017; Mutlu-İnğok et al., 2020). The antifungal property of essential oils depends on their chemical composition, applied concentration, and the sensitivity of the targeted fungus. Many studies have proved their effectiveness and identified additional benefits, including reduced mycotoxin synthesis. As a result, essential oils are increasingly recognised as sustainable and eco-friendly options to conventional fungicides (Ćosić et al., 2014; Perczak et al., 2019; Bocate et al., 2021; Feng et al., 2023). Their mode of action involves disrupting the fungal cell membrane and wall, inhibiting the synthesis of ergosterol, proteins, and nucleic acids, as well as impairing mitochondrial function, which ultimately decreases their ability to produce mycotoxins (Feng et al., 2023).

In spite of their many advantages, the use of essential oils is restricted by their volatility, sensitivity to light, temperature, and oxygen, as well as their variable chemical composition, which varies with origin, growing conditions, and extraction methods. These components can lower biological activity and complicate reproducibility, whereas higher concentrations of certain oils may cause phytotoxic effects (Bakkali et al., 2008; Pavela & Benelli, 2016). To defeat these limitations, modern formulations such as nanoemulsions, microcapsules, and biofilms are increasingly used to enhance stability, prolong efficacy, and facilitate their application in agriculture (Feng et al., 2023). All things considered, it is important to evaluate how different incubation conditions affect the volatile antifungal activity of essential oils. This study intended to evaluate the volatile antifungal activity of five essential oils against the phytopathogenic fungus *F. culmorum* under different incubation conditions, with special emphasis on the effects of temperature and concentration. The hypothesis was that the volatile antifungal activity of essential oils against the tested isolate *F. culmorum* mainly depends on the type of essential oils. Simultaneously, incubation conditions may modulate the intensity of the observed effect.

MATERIALS AND METHODS

The research was conducted at the Faculty of Agrobiotechnical Sciences Osijek, at the Central Agrobiotechnical Analytical Unit, in the Laboratory of Phytopathology. The study aimed to examine the effects of different temperatures (10, 20, and 30°C) and light regimes (24 h darkness, 24 h light, and 12 h light/12 h darkness) on the volatile antifungal activity of essential oils against the pathogen *F. culmorum*.

Fungal isolate and culture conditions

A single isolate of *F. culmorum* was used in this study, isolated from wheat grains with disease symptoms in 2022 at the Karanac site (Croatia). The same isolate was previously used in *in vivo* experiments on wheat, where its pathogenicity was confirmed (Siber et al., 2025). The isolate was identified based on morphological and molecular features. Morphological identification was performed according to standard keys (Leslie & Summerell, 2006; Lević, 2012), after cultivation on PDA medium and observation of mycelia and conidia. DNA was isolated from mycelia using OmniPrep™ for Fungi PCR Kit (G-Biosciences, USA). Molecular identification was performed by amplification and sequencing of the gene for translation elongation factor 1-alpha (TEF-1α), using primers EF1 (5′-ATG GGT AAG GAR GAC AAG AC-3′) and EF2 (5′-GGA RGT ACC AGT SAT CAT G-3′). The obtained sequence showed 99.97% similarity to the reference sequences of the species *F. culmorum* in the NCBI GenBank database, thus confirming the identity of the isolate. For the purposes of the experiment, the isolate was cultured on potato dextrose agar (PDA, Biolife, Italy). To prevent bacterial contamination, streptomycin (0.5 g dissolved in 2 mL of sterile distilled water) was added to the cooled medium (60 °C). Each sterile Petri dish (Ø 90 mm, height 15 mm) was poured with 10 mL of the prepared medium. A seven-day-old pure culture of *F. culmorum*, grown at 25 ± 1 °C, was used for inoculation (Figure 1). Fungal inoculation was performed by transferring 4-mm mycelial discs from the actively growing colony using a sterile metal cutter. The disc was transferred to the center of each Petri dish containing the nutrient medium using a sterile needle.

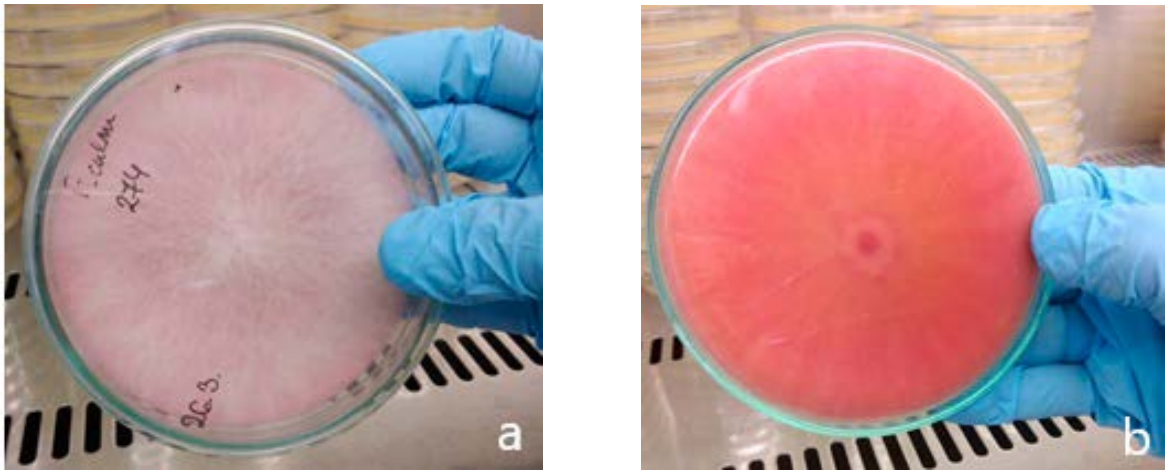

Figure 1. *F. culmorum* colony on PDA medium after 7 days of incubation: a) front view; b) reverse side of the Petri dish (Source: Lekić, M.).

Slika 1. Kolonija gljive F. culmorum na KDA podlozi nakon 7 dana inkubacije: a) prednja strana; b) stražnja strana Petrijeve zdjelice (Izvor: Lekić, M.).

Experimental design

For the volatile antifungal test, the experiment was conducted according to the method described by Edris and Farrag (2003). A strip of double-sided adhesive tape was attached to the inside of each Petri dish lid. A sterile filter paper was attached to the tape with sterilized forceps, and a specified volume of essential oil (20 μL or 100 μL) was applied to the center of the paper. Five essential oils were tested: anise (*Illicium verum* L.), thyme (*Thymus vulgaris* L.), sweet orange (*Aurantium dulcis* L. Osbeck), cinnamon bark (*Cinnamomum verum* J. Presl), and mountain pine (*Pinus pumilio* Haenke). All essential oils used in this study were purchased from Fagron Hrvatska d. o. o. (a Croatian LLC, Donjozelinska ulica 114, 10380 Donja Zelina, Croatia). The oils were supplied in their original amber glass bottles and stored at 4°C in the dark until use. All essential oils were applied in their pure form (100%), and the working volumes (20 μL and 100 μL) were prepared immediately before each treatment.

The volumes of 20 μL and 100 μL corresponded to vapor concentrations of approximately 250 μL/L and 1250 μL/L air, respectively, calculated for an air volume of 0.080 L inside the Petri dish (Ø 90 mm, height 15 mm, 15 mL agar). The control treatment was prepared in the same way, using sterile distilled water instead of essential oil. All treatments, including the control, were performed in triplicate. Petri dishes were sealed with parafilm and incubated in an Aralab climate chamber at 10, 20, and 30 °C with 70% relative humidity. Three light regimes were tested: continuous darkness (24 hours of darkness), constant light (24 hours of light), and alternating light/darkness (12 hours of light / 12 hours of darkness). Mycelial growth of the *F. culmorum* isolate was measured on the third (72 hours) and seventh (168 hours) days after the start of the treatment. Results are expressed as percentage inhibition of mycelial growth compared to the control.

Chemical composition of essential oils

The chemical composition of the essential oils was determined by gas chromatography (GC) and confirmed by analytical certificates issued by Fagron Hrvatska d. o. o. Cinnamon bark oil contained 90% cinnamaldehyde, while pine oil was dominated by monoterpenes such as δ-3-carene (38.25 %), β-pinene (25.11 %), and limonene (14.95 %). Anise oil was rich in trans-anethole (91.12 %), thyme (ct. thymol) contained high proportions of p-cymene (33.14 %) and thymol (25.32 %), and sweet orange oil consisted predominantly of limonene (95.7 %). The complete composition of all oils is shown in Table 1.

Table 1. Chemical composition of the essential oils used in the study, based on GC analytical certificates

Tablica 1. Kemijski sastav eteričnih ulja korištenih u studiji, na temelju analitičkih certifikata GC-a

Essential oil / *Eterično ulje*	**Main components (%)** / *Glavna komponenta*
Anise / Anis (Illicium verum)	trans-anethole (91.12), estragole (3.17), foeniculin (0.864), linalool (0.68)
Thyme / *Timijan* (*Thymus vulgaris*, ct. thymol)	p-cymene (33.14), thymol (25.32), carvacrol (2.85), linalool (1.83)
Sweet orange / *Slatka naranča* (*Aurantium dulcis* L.)	limonene (95.7), β-myrcene (1.9), α-pinene (0.5), linalool (0.5)
Cinnamon bark / *Cimet* (*Cinnamomum verum*)	cinnamaldehyde (90)
Pine / *Planinski bor* (*Pinus pumilio* Haenke)	δ-3-carene (38.25), β-pinene (25.11), limonene (14.95)

Values represent percentage composition based on the GC certificates provided by Fagron Hrvatska d. o. o. / *Vrijednosti predstavljaju pos-*

totni sastav na temelju GC certifikata koje je izdala tvrtka Fagron Hrvatska d. o. o.

Statistical analysis

Based on measured values, the percentage of mycelial growth inhibition (%) was calculated in Microsoft Excel using the following formula:

$$I(\%) = \frac{dc - dt}{dc - 4} \times 100,$$

where *dc* is the diameter of fungal growth in the control, and *dt* is the diameter of fungal growth on treated PDA.

Statistical analysis of the data was performed using analysis of variance (ANOVA) in SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Prior to performing the study, the assumptions of normality and homogeneity of variance were checked. Tukey's HSD test was used to compare means at the significance level $p < 0.05$. Results are presented as mean values ± standard deviation (n = 3).

RESULTS AND DISCUSSION

The results show the volatile effects of five essential oils on the mycelial growth of the tested isolate *F. culmorum* under different temperatures, concentrations, and light regimes. To determine the effects of individual factors and their interactions after 72 and 168 hours of incubation, ANOVA was performed. Table 2 presents the F values and significance levels (Pr > F). The main factors—essential oil, concentration, and temperature—had a strong, statistically significant effect on mycelial growth ($p < 0.0001$). In contrast, the light regime was significant only after 168 hours and had a minor biological effect. The highest F values were observed for the essential oil × concentration, essential oil × temperature, and concentration × temperature interactions; therefore, these interactions were included in subsequent interpretation. Although some interactions were statistically significant, their low F values indicate that the biological effect is insignificant (Sullivan & Feinn, 2012). Consequently, only interactions that demonstrated both statistical significance and biologically relevant effects were analysed in detail in the following sections.

Table 2. Significance of the effects of essential oil, concentration, temperature, regime of light, and their interactions on mycelial growth inhibition 72 and 168 h after incubation. ANOVA, F test.

Tablica 2. Značajnost učinaka eteričnoga ulja, koncentracije, temperature, režima i njihovih interakcija na inhibiciju rasta micelija nakon 72 i 168 h inkubacije. ANOVA, F test.

Source of variation / *Izvor varijabilnosti*	F value (72 h)	Pr > F	F value (168 h)	Pr > F
Essential oil / *Eterično ulje*	376.74	< 0.0001	2423.17	< 0.0001
Concentration / *Koncentracija (µL)*	122.70	< 0.0001	129.76	< 0.0001
Temperature / *Temperatura*	149.18	< 0.0001	420.33	< 0.0001
Regime of light / *Režim osvjetljenja*	1.81	0.1125	5.72	< 0.0001
Essential oil × Concentration / *Eterično ulje × koncentracija*	58.84	< 0.0001	55.94	< 0.0001
Essential oil × Temperature / *Eterično ulje × temperature*	52.57	< 0.0001	153.30	< 0.0001
Essential oil × Regime / *Eterično ulje × režim osvjetljenja*	4.31	0.0010	7.10	< 0.0001
Concentration × Temperature / *Keoncentracija × temperature*	17.02	< 0.0001	23.01	< 0.0001
Concentration × Regime / *Koncentracija × režim osvjetljenja*	1.33	0.2520	3.19	0.0088
Temperature × Regime / *Temperatura × režim osvjetljenja*	8.33	< 0.0001	36.87	< 0.0001
Essential oil × Concentration × Temperature / *Eterično ulje × koncentracija × temperatura*	6.44	< 0.0001	15.45	< 0.0001
Essential oil × Concentration × Regime / *Eterično ulje × koncentracija × režim osvjetljenja*	1.56	0.1736	1.21	0.3077
Essential oil × Temperature × Regime / *Eterično ulje × temperature × režim*	7.46	< 0.0001	55.70	< 0.0001
Concentration × Temperature × Regime / *Koncentracija × temperature × režim osvjetljenja*	2.84	0.0027	1.49	0.1444
Essential oil × Concentration × Temperature × Regime / *Eterično ulje × koncentracija× temperature × režim*	5.01	< 0.0001	3.08	0.0012

The interaction between essential oil and concentration (Table 3) revealed apparent differences in volatile antifungal

activity against the *F. culmorum* isolate. Anise, thyme, and cinnamon essential oils showed the most stable and effective inhibitory activity (100 %) at both concentrations during both incubation periods. Their effectiveness can be related to their chemical composition (Table 1), particularly the presence of trans-anethole, thymol, and cinnamaldehyde (Faghih-Imani et al., 2020; Harčárová et al., 2021; Hong et al., 2021; Ateş, 2023). Since no statistically significant differences were observed between concentrations, the activity of these essential oils was already stable at the lower applied amount. Sweet orange essential oil showed the highest sensitivity to increasing concentration. A stronger inhibitory effect was observed at 100 μL after 72 hours (81.83 %), whereas after 168 hours the effect decreased significantly (40.47 %), particularly at 20 μL. This decrease in antifungal activity can be credited to limonene's high volatility, although some of the lost activity can be mitigated by using a higher oil concentration (Velázquez-Nuñez et al., 2013; Elgat et al., 2020). A weaker but more uniform effect was exhibited by the pine essential oil. The lower concentration did not result in statistically significant inhibition (57.06 %), whereas 100 μL showed moderate activity (77.60 %), as expected for oils rich in monoterpenes. These compounds are more volatile and generally exhibit lower antifungal activity than phenols and aldehydes (Mutlu-İnğok et al., 2020). Increasing the concentration may therefore improve the effect, although the overall efficacy remains limited. The interaction between essential oil and concentration clearly distinguishes oils with intense, persistent activity from those with weaker or more concentration-sensitive effects, consistent with differences in their chemical composition.

Table 3. Interaction between essential oils and concentrations on mycelial growth inhibition of *F. culmorum* isolate after 72 and 168 h of incubation

Tablica 3. Interakcija eteričnih ulja i koncentracije na inhibiciju rasta micelija izolata F. culmorum nakon 72 i 168 h inkubacije

Essential oil / *Eterično ulje*	**Concentration (μL)** / *Koncentracija (μL)*	**Inhibition (%) ± SD** / *Inhibicija (%) ± SD*	
		72 h	168 h
Anise / *Anis* (*Illicium verum*)	20	100.00 ± 0.00 a	97.92 ± 3.90 a
	100	100.00 ± 0.00 a	100.00 ± 0.00 a
Thyme / *Timijan* (*Thymus vulgaris*, ct. thymol)	20	100.00 ± 0.00 a	100.00 ± 0.00 a
	100	100.00 ± 0.00 a	100.00 ± 0.00 a
Sweet orange / *Slatka naranča* (*Aurantium dulcis* L.)	20	54.93 ± 24.97 c	20.43 ± 27.21 d
	100	81.83 ± 15.00 b	40.47 ± 36.70 bc
Cinnamon bark / *Cimet* (*Cinnamomum verum*)	20	100.00 ± 0.00 a	100.00 ± 0.00 a
	100	100.00 ± 0.00 a	100.00 ± 0.00 a
Pine / *Planinski bor* (*Pinus pumilio* Haenke)	20	57.06 ± 27.34 c	26.02 ± 25.63 cd
	100	77.60 ± 19.58 b	44.70 ± 32.55 b

Values represent mean ± SD (n = 3). Different letters within the same column indicate significant differences, with the letters ordered from highest to lowest mean value (one-way ANOVA followed by Tukey's HSD test, $p \leq 0.05$). / *Vrijednosti predstavljaju srednju vrijednost ± SD (n = 3). Različita slova unutar istoga stupca označuju statistički značajne razlike, pri čemu su slova poredana od najviše do najniže srednje vrijednosti (one-way ANOVA, Tukeyjev HSD test, $p \leq 0.05$).*

Antifungal activity against the *F. culmorum* isolate varied significantly with the essential oil used, temperature, and incubation time (Table 4). Significantly weaker, temperature-dependent antifungal activity was exhibited by sweet orange and mountain pine essential oils. Their inhibitory effects decrease with increasing temperature (20 °C and 30 °C), and the differences became more noticeable with longer incubation periods. This decrease in efficiency can be attributed to the volatility and degradation of bioactive compounds during longer incubation, compatible with the findings of Mutulu-inğok et al. (2020). Inhibitions by these oils were higher at the lower temperature (10 °C) and lowered as the temperature rose, which indicates lower stability of their antifungal effect at higher temperatures. On the other hand, anise, thyme, and cinnamon essential oils exhibited strong and stable antifungal activity with inhibition values ranging from 96.88 to 100 % across all tested temperatures and incubation times. This study showed that anise essential oil exhibited persistent activity against the *F. culmorum* isolate, with no statistically significant differences among temperature treatments.

The solid antifungal activity of anise essential oil seen in this study can be attributed to anethole, its dominant bioactive component. Corresponding findings were reported by Evangelista et al. (2024), who showed

that anethole completely inhibited the mycelial growth of *Colletotrichum* sp. Anise essential oils at a higher temperature (145 °C) showed a stronger antifungal effect. Absolute growth inhibition was achieved at a lower concentration. The stable antifungal activity of anise essential oil observed in this study can be attributed to anethole, its dominant bioactive component. Similar findings were reported by Evangelista et al. (2024), who showed that anethole completely inhibited the mycelial growth of *Colletotrichum* sp. Anise essential oil extracted at a higher temperature (145 °C) showed stronger antifungal activity. Complete growth inhibition was achieved at a lower concentration than the oil obtained at a lower extraction temperature (97 °C), suggesting a higher anethole content. Although the cited study focuses on the extraction process, and the present study on pathogen incubation, both studies indicate that the antifungal activity of anise essential oil remains preserved after exposure to elevated temperatures. Although a slight reduction in inhibition was observed at 30 °C after 168 h of incubation, this decrease was not statistically significant, indicating that the antifungal activity of anise essential oil remained stable across the tested temperature range.

The stable antifungal activity of anise essential oil has also been reported in the literature. However, short-term exposure to extremely high temperatures (100 °C) has been shown to affect its antifungal activity negatively (Matan et al., 2012). The differences between those findings and the results of the present study can be explained by the much milder temperature conditions used in this experiment.

Table 4. Interaction between essential oils and temperatures on mycelial growth inhibition of *F. culmorum* isolate after 72 and 168 h of incubation

Tablica 4. Interakcija eteričnih ulja i temperatura na inhibiciju rasta micelija izolata F. culmorum nakon 72 i 168 h inkubacije

Essential oil / *Eterično ulje*	**Temperature (°C)** / *Temperatura (°C)*	**Inhibition (%) ± SD** / *Inhibicija (%) ± SD*	
		72 h	168 h
Anise / *Anis* (*Illicium verum*)	10	100.00 ± 0.00 a	100.00 ± 0.00 a
	20	100.00 ± 0.00 a	100.00 ± 0.00 a
	30	100.00 ± 0.00 a	96.88 ± 4.45 a
Thyme / *Timijan* (*Thymus vulgaris*, ct. thymol)	10	100.00 ± 0.00 a	100.00 ± 0.00 a
	20	100.00 ± 0.00 a	100.00 ± 0.00 a
	30	100.00 ± 0.00 a	100.00 ± 0.00 a
Sweet orange / *Slatka naranča* (*Aurantium dulcis* L.)	10	88.84 ± 19.67 a	60.28 ± 27.05 b
	20	53.31 ± 25.11 bc	0.65 ± 2.74 d
	30	63.00 ± 11.86 b	30.42 ± 29.81 c
Cinnamon bark / *Cimet* (*Cinnamomum verum*)	10	100.00 ± 0.00 a	100.00 ± 0.00 a
	20	100.00 ± 0.00 a	100.00 ± 0.00 a
	30	100.00 ± 0.00 a	100.00 ± 0.00 a
Pine / *Planinski bor* (*Pinus pumilio* Haenke)	10	93.70 ± 10.53 a	63.97 ± 18.07 b
	20	49.57 ± 22.72 c	4.39 ± 9.58 d
	30	58.73 ± 17.00 bc	37.72 ± 24.40 c

Values represent mean ± SD (n = 3). Different letters within the same column indicate significant differences, with the letters ordered from highest to lowest mean value (one-way ANOVA followed by Tukey's HSD test, $p \leq 0.05$). / *Vrijednosti predstavljaju srednju vrijednost ± SD (n = 3). Različita slova unutar istoga stupca označuju statistički značajne razlike pri čemu su slova poredana od najviše do najniže srednje vrijednosti (one-way ANOVA, Tukeyjev HSD test, $p \leq 0.05$).*

The interaction between concentration and temperature affected the inhibition of mycelial growth of the *F. culmorum* isolate, mainly during the early incubation period (Table 5). After 72 hours, higher inhibition values were generally observed at 100 μL compared to 20 μL, particularly at 10 °C, where complete growth inhibition was observed. Statistically significant differences among treatments after 72 hours indicate that concentration and temperature influenced antifungal activity during the initial phase of exposure.

In contrast, after 168 hours of incubation, no statistically significant differences were observed among the tested combinations of concentration and temperature. This loss of significance suggests a decrease in the effectiveness of volatile compounds over time, most likely due to their volatility or degradation during

prolonged incubation. This is consistent with previous studies reporting reduced antifungal activity of essential oils after prolonged exposure, especially at higher temperatures (Mutlu-İnğok et al., 2020). A significant reduction in the antifungal activity of mountain pine and sweet orange essential oils against the *F. culmorum* isolate was observed at 20 °C, particularly compared with lower temperatures was seen in this study. Since 20°C is close to the optimal temperature for *F. culmorum* growth, intensive mycelial development under these conditions may reduce the effectiveness of essential oils. Brennan et al. (2005) also reported that *F. culmorum* causes more severe disease symptoms at 20 °C than at 16 °C, indicating greater pathogen adaptation and infectivity at this temperature.

The results indicate that the combined effect of concentration and temperature plays a secondary role relative to the type of essential oil and that its influence is more pronounced during the early stage of incubation than during prolonged exposure. This study was conducted on a single isolate of *F. culmorum*, the results obtained relate to the isolate tested. Nevertheless, the results provide valuable insight into the antifungal activity of essential oils under controlled conditions. Future research should include a larger number of isolates of the same species to further confirm the obtained effects.

Table 5. Interaction between concentrations and temperatures on mycelial growth inhibition of *F. culmorum* isolate after 72 and 168 h incubation

Tablica 5. Interakcija koncentracija i temperatura na inhibiciju rasta micelija izolata F. culmorum nakon 72 i 168 h inkubacije

Concentration (µL) / *Koncentracija (µL)*	**Temperature (°C)** / *Temperatura (°C)*	**Inhibition (%) ± SD** / *Inhibicija (%) ± SD*	
		72 h	168 h
20	10	93.01 ± 14.48 ab	70.96 ± 38.86 a
20	20	72.66 ± 34.73 c	78.19 ± 33.79 a
20	30	81.52 ± 24.67 bc	68.18 ± 41.08 a
100	10	100.00 ± 0.00 a	75.72 ± 36.33 a
100	20	88.49 ± 16.41 ab	67.48 ± 43.63 a
100	30	87.17 ± 17.23 b	77.20 ± 37.71 a

Values represent mean ± SD (n = 3). Different letters within the same column indicate significant differences, with the letters ordered from highest to lowest mean value (one-way ANOVA followed by Tukey's HSD test, $p \leq 0.05$).

Vrijednosti predstavljaju srednju vrijednost ± SD (n = 3). Različita slova unutar istoga stupca označuju statistički značajne razlike pri čemu su slova poredana od najviše do najniže srednje vrijednosti (one-way ANOVA, Tukeyjev HSD test, $p \leq 0.05$).

CONCLUSION

Anise, thyme, and cinnamon essential oils showed strong, stable inhibitory activity throughout the incubation period, regardless of concentration or temperature, indicating high efficacy in the vapour phase. The interplay between essential oil type and concentration indicated that increasing concentration did not further increase the activity of anise, thyme, and cinnamon oils. In contrast, greater concentration dependence, with increased inhibition at higher applied amounts, was shown by sweet orange and mountain pine essential oils. These oils exhibited weaker and more temperature-sensitive antifungal activity that lowered with rising temperature and incubation time. Even though concentration and temperature affected the antifungal activity at the early stages of incubation, their effects decreased over time, while the intrinsic properties of the essential oils primarily determined long-term effectiveness. Found results verified that volatile antifungal activity is mainly determined by the type of essential oil and emphasise their potential as natural antifungal agents under controlled conditions, as well as the importance of carefully selecting essential oils in developing natural strategies to control *F. culmorum*.

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VOLATILNI ANTIFUNGALNI UČINAK ETERIČNIH ULJA NA *Fusarium culmorum*: UTJECAJ KONCENTRACIJE I TEMPERATURE

SAŽETAK

Fitopatogena gljiva Fusarium culmorum predstavlja ozbiljnu prijetnju proizvodnji pšenice zbog svoje agresivnosti i sposobnosti preživljavanja u tlu i biljnim ostatcima te kao značajan proizvođač toksikološki važnih mikotoksina, koji predstavljaju rizik za sigurnost hrane i zdravlje ljudi i životinja. U tome kontekstu eterična ulja smatraju se potencijalnim prirodnim alternativama zahvaljujući svojim hlapljivim svojstvima. Cilj ovoga istraživanja bio je procijeniti hlapljivo antifungalno djelovanje pet eteričnih ulja (anis, timijan, cimet, slatka naranča i planinski bor) na rast micelija izolata F. culmorum pri različitim koncentracijama, temperaturama i svjetlosnim režimima. Pokus je proveden u Petrijevim zdjelicama korištenjem eteričnih ulja u koncentracijama od 20 i 100 µL pri tri temperature (10, 20 i 30 °C) te pod različitim svjetlosnim režimima. Porast micelija mjeren je nakon 72 i 168 sati inkubacije, a antifungalno djelovanje izraženo je kao postotak inhibicije rasta. Eterična ulja anisa, timijana i cimeta potpuno su i stabilno inhibirala porast micelija pri svim koncentracijama i temperaturama. Suprotno tome, eterična ulja slatke naranče i planinskoga bora pokazala su slabije, temperaturno osjetljivo djelovanje, uz veću ovisnost o koncentraciji. Učinak koncentracije i temperature bili su izraženiji u ranoj fazi inkubacije, ali se s vremenom smanjivao. Ovi rezultati upućuju na to da je hlapljivo antifungalno djelovanje prvenstveno određeno vrstom eteričnoga ulja i ukazuju na njihov potencijal za razvoj prirodnih strategija za suzbijanje F. culmorum.

Ključne riječi: fitopatogene gljive, antifungalno djelovanje, inhibicija rasta micelija, prirodni antifungalni agensi, hlapive komponente eteričnih ulja

(**Received on December 31, 2025; accepted on May 12, 2026** – *Primljeno 31. prosinca 2025.; prihvaćeno 12. svibnja 2026.*)