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Antifungal Activity of Thymus Fontanesii Boiss. & Reut. Essential Oil Against the Main Phytopathogenic Fungi of Tomato (Solanum Lycopersicum L.): Study of Effects on Alternaria Spp., Fusarium Oxysporum F. P. Lycopersici and Aspergillus Niger

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ABSTRACT

This study investigated the chemical composition and the antifungal, antioxidant, and anti-inflammatory properties of the essential oil obtained from Thymus fontanesii Boiss. & Reut., an endemic Algerian aromatic species with promising value in sustainable crop protection. The essential oil was extracted from the aerial parts by hydrodistillation using a Clevenger-type apparatus, giving a yield of 1.36 ± 0.01%, and was subsequently characterized by gas chromatography-mass spectrometry (GC-MS). The analysis led to the identification of twenty-eight compounds, with p-cymene (11.68%), a terpinen-related compound (6.02%), β-caryophyllene (5.72%), and myrcene (1.93%) as the main detected constituents. The biological activity of the oil was evaluated in vitro against three important tomato phytopathogens, namely Fusarium oxysporum, Alternaria spp., and Aspergillus niger. Antioxidant activity was determined using the DPPH radical-scavenging assay, whereas anti-inflammatory potential was assessed through inhibition of protein denaturation. The essential oil exhibited a clear dose-dependent antifungal effect against all tested fungi. Complete inhibition of mycelial growth was observed at 5 µL/50 mL for F. oxysporum, 10 µL/50 mL for A. niger, and 25 µL/50 mL for Alternaria spp. In the antioxidant assay, the oil reached 83.5 ± 2.1% inhibition at 200 µg/mL, compared with 89.1 ± 2.2% for vitamin C. Anti-inflammatory activity also increased with concentration and reached 81.5 ± 2.4% inhibition at 400 µg/mL, showing values comparable to the reference drugs under the same conditions. These findings indicate that T. fontanesii essential oil is a valuable natural source of bioactive compounds and support its potential use in eco-friendly formulations for plant protection and related biological applications.

Keywords: *Thymus fontanesii, essential oil, GC-MS; antifungal activity, antioxidant activity, anti-inflammatory activity, tomato phytopathogens.*

INTRODUCTION

Tomato (Solanum lycopersicum L.) is one of the most important vegetable crops worldwide and remains a major component of food supply and market gardening systems throughout the Mediterranean region. Its agronomic and commercial importance is, however, seriously limited by fungal diseases that affect both plant development in the field and fruit quality during storage and marketing. Among the most important fungal threats reported in tomato production are Fusarium oxysporum, responsible for vascular wilt, as well as species of Alternaria and Aspergillus, which are associated with foliar damage, fruit deterioration, and postharvest spoilage (McGovern, 2015; Rodrigues and Furlong, 2022).

Management of these pathogens still relies largely on synthetic fungicides. While these products can provide rapid and efficient disease suppression, their repeated use has raised several concerns, including the development of resistant fungal populations, persistence of chemical residues, and increasing environmental pressure. These limitations have encouraged a progressive shift toward safer and more sustainable disease-

management strategies, particularly those based on natural products with lower ecological impact (Dorman and Deans, 2000; Burt, 2004; Raveau et al., 2020).

Among the most promising plant-derived alternatives, essential oils have attracted considerable attention because they are rich in volatile secondary metabolites with recognized biological activity. Their antimicrobial effects are commonly linked to the presence of monoterpenes, sesquiterpenes, and oxygenated derivatives able to disrupt membrane integrity, modify permeability, and interfere with microbial metabolism. Because essential oils act as complex mixtures rather than single-target compounds, they are often viewed as biologically versatile agents with interesting potential in crop protection. In addition to their antimicrobial effects, many essential oils also exhibit antioxidant and anti-inflammatory properties, which may broaden their practical value in agriculture, food preservation, and natural-health applications (Burt, 2004; Miguel, 2010; Raveau et al., 2020).

The family Lamiaceae occupies a prominent place in this field, and the genus Thymus is particularly recognized as a rich source of biologically active essential oils. Early studies on aromatic and medicinal plants showed that the activity of volatile oils is closely related to their chemical composition, and that even oils belonging to the same botanical group may differ markedly in effectiveness according to their major and minor constituents (Dorman and Deans, 2000; Cimanga et al., 2002). This point is especially relevant for thyme species, whose essential oils are known for their remarkable chemical variability. Recent work has confirmed that thyme oils may vary substantially according to genotype, ecological conditions, phenological stage, harvesting period, extraction procedure, and storage conditions, giving rise to different chemotypes with distinct biological properties (Etri and Pluhár, 2024). As a result, the evaluation of bioactivity should be based on the actual chemical profile of the investigated oil rather than on assumptions derived from previous reports on other populations.

Thymus fontanesii Boiss. & Reut. is a North African aromatic species occurring in Algeria and Tunisia and traditionally valued for its medicinal uses. Previous studies conducted on Algerian material have shown that this species can produce essential oils with relevant antimicrobial and antioxidant properties, although the reported dominant compounds are not always identical from one study to another (Dob et al., 2006; Sid Ali et al., 2020). Such differences are not trivial, because chemical composition directly affects the interpretation of biological assays and the reproducibility of any practical application. For this reason, the characterization of each oil sample remains an essential prerequisite for any sound discussion of biological activity.

The interest in T. fontanesii is further supported by the growing need to identify locally available plant resources that may contribute to more ecological crop-protection strategies. Reviews dedicated to essential oils as alternative biocontrol agents have underlined their potential against a wide range of plant pathogens affecting crops before and after harvest, while also emphasizing the importance of species-specific evaluation and formulation development (Raveau et al., 2020). In tomato production, where fungal pressure remains a persistent constraint and environmentally safer approaches are increasingly sought, endemic or regionally adapted aromatic plants deserve closer scientific attention.

Although T. fontanesii has already been reported for some antimicrobial or antioxidant effects, information remains limited when its essential oil is specifically considered against tomato-associated fungal pathogens and when its antifungal activity is examined together with other biologically relevant properties. A combined evaluation of antifungal, antioxidant, and anti-inflammatory potential may therefore provide a broader and more useful view of the value of this species, especially in a context where multifunctional plant-derived products are increasingly sought for agricultural and related applications.

Accordingly, the present study was undertaken to investigate the essential oil of Thymus fontanesii Boiss. & Reut. with three main objectives: first, to characterize its chemical composition; second, to evaluate its in vitro antifungal activity against major fungal pathogens associated with tomato; and third, to assess its antioxidant and anti-inflammatory potential. Through this integrated approach, the study aims to clarify the biological interest of this endemic species and to examine its possible relevance as a natural source of compounds for eco-friendly crop protection and other value-added applications.

MATERIALS AND METHODS

Plant material

Flowering aerial parts of Thymus fontanesii Boiss. & Reut. were collected in the Ain Defla region of Algeria during January and February 2025. Only healthy plant material was selected. After harvesting, the material was cleaned manually to remove dust and foreign particles, then dried in the shade in a well-ventilated room for 20 days until constant weight was reached. Shade drying was preferred in order to reduce the loss of volatile constituents and to avoid thermal degradation of sensitive compounds. The dried material was stored in clean paper bags at room temperature until extraction.

Extraction of essential oil

The essential oil was obtained by hydrodistillation using a Clevenger-type apparatus. Fifty grams of dried plant material were immersed in 500 mL of distilled water and distilled for 1 h 30 min. After extraction, the oily phase was separated from the aqueous phase, dried over anhydrous sodium sulfate, and transferred to dark glass vials. The oil was then stored at 4°C until use. This extraction procedure follows the classical Clevenger method widely used for essential oils, while storage in dark containers minimizes oxidation and volatilization losses (Clevenger, 1928; ISO, 2023b). The extraction yield was calculated as the ratio between the mass of essential oil recovered and the dry mass of plant material used for distillation, and the result was expressed as percentage (w/w).

Physicochemical characterization of the essential oil

The physicochemical properties of the essential oil were evaluated using standard procedures commonly applied to essential oils. Relative density was determined at 20°C according to ISO 279. Refractive index was measured according to ISO 280 using a refractometer maintained at the appropriate temperature. Acid value was determined by titration with 0.1 N potassium hydroxide according to ISO 1242. These parameters were used to provide a basic quality profile of the oil and to facilitate comparison with published data for aromatic species of the family Lamiaceae (ISO, 1998a; ISO, 1998b; ISO, 2023a).

GC-MS analysis of volatile constituents

The chemical composition of the essential oil was determined by gas chromatography coupled to mass spectrometry (GC-MS) using a Shimadzu GCMS-TQ8030 / GC-2010 Plus system equipped with an AOC-20i auto-injector. The essential oil sample was analyzed under the operating conditions of the instrument, and compound identification was based on retention time data and comparison of the mass spectra with reference spectra from the NIST/EPA/NIH mass spectral library. The relative percentage of each constituent was calculated from peak areas in the total ion chromatogram. The use of the NIST electron-ionization library is standard practice for GC-MS identification of volatile compounds and provides a recognized reference framework for essential-oil analysis (NIST, 2023).

Fungal material and culture conditions

Three phytopathogenic fungi associated with diseased tomato tissues were used in this study: Alternaria sp., Fusarium oxysporum f. sp. lycopersici, and Aspergillus niger. The isolates were obtained from symptomatic tomato material collected in the study region and were maintained on potato dextrose agar (PDA). Preliminary identification was based on colony morphology and microscopic features after subculturing on fresh PDA plates. All cultures were incubated at 25°C and maintained under the same laboratory conditions throughout the experiments. (figure.1)

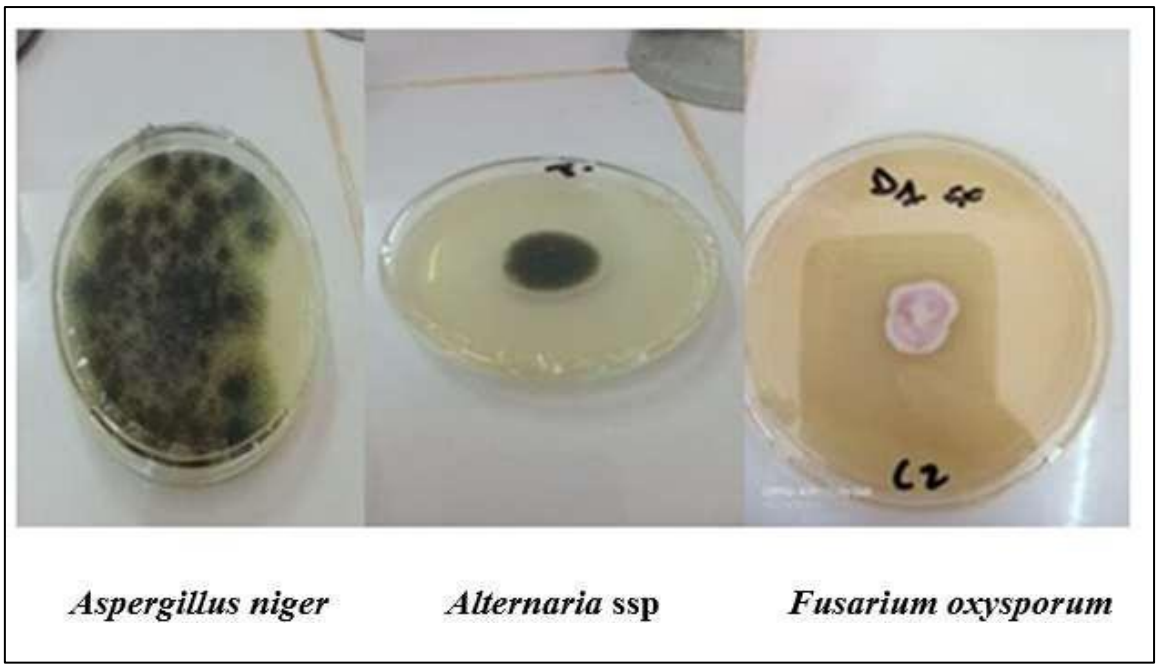

Figure 1: Phytopathogenic fungi in tomato cultivation.

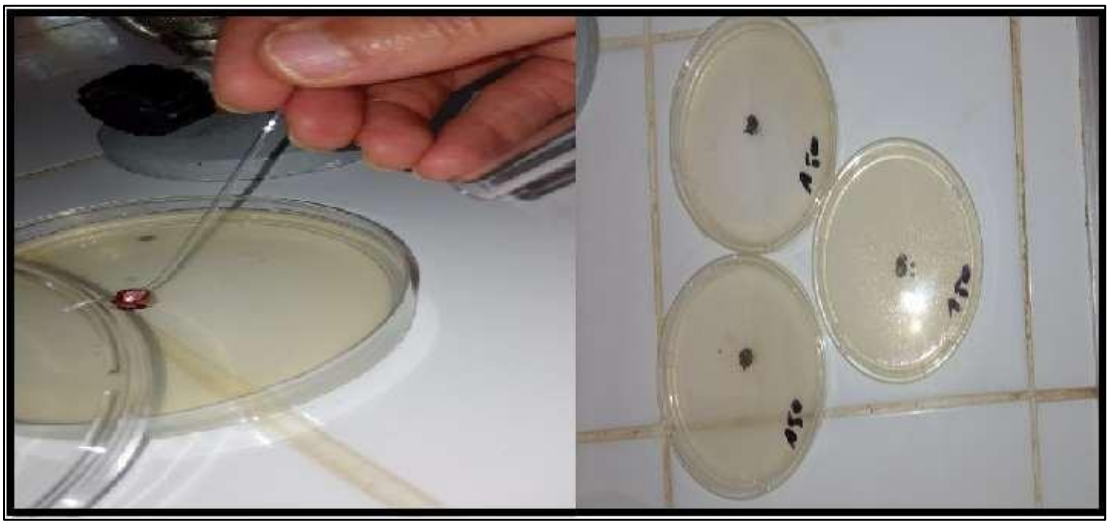

Figure 2: Direct contact method

In vitro antifungal assay

The antifungal activity of the essential oil was evaluated using the poisoned-food or direct-contact method (figure.3), which is commonly used to assess the effect of essential oils on mycelial growth. Different concentrations of the essential oil were incorporated into molten PDA medium immediately before pouring into sterile Petri dishes. The oil was dispersed using DMSO, and control plates were prepared under the same conditions but without essential oil. After solidification, each plate was inoculated at the center with a 6-mm-diameter mycelial disc cut from the margin of a 4-day-old actively growing fungal colony. Plates were incubated at 25°C for 7 days. Each concentration was tested in triplicate for each fungal strain. Radial fungal growth was recorded daily by measuring two perpendicular colony diameters and calculating the mean value for each plate. This method is widely used for the evaluation of plant essential oils against phytopathogenic fungi (Tripathi et al., 2009; Sharma et al., 2017). The concentrations tested are shown in Table 1.

Table 1. Concentrations used in the antifungal assay

Essential oil concentration (µL/50 mL PDA)	Equivalent concentration (%)
1	0.002
2	0.004
5	0.010
10	0.020
25	0.050

Antifungal activity was expressed as antifungal index (AI), calculated as:

$AI\ (\%) = [(Dt - De) / Dt] \times 100$

where **Dt** is the mean colony diameter in the control and **De** is the mean colony diameter in the treated plates. The minimum inhibitory concentration (MIC) was defined as the lowest concentration at which no visible mycelial growth was observed after incubation. Mycelial growth rate (MGR) was calculated as:

$MGR = (Df - Di) / (tf - ti)$

where **Df** and **Di** are the final and initial colony diameters, respectively, and **tf** and **ti** are the corresponding times of measurement.

Antioxidant activity

Antioxidant activity was assessed using the DPPH radical-scavenging assay. Essential-oil samples were tested at different concentrations, and vitamin C was used as a reference antioxidant (figure3). After reaction with the DPPH solution and incubation in the dark, absorbance was measured spectrophotometrically, and radical-scavenging activity was expressed as percentage inhibition relative to the control. Each concentration was analyzed in triplicate. The inhibition percentage was calculated using the following equation:

$DPPH\ scavenging\ activity\ (\%) = [(A0 - A1) / A0] \times 100$

where **A0** is the absorbance of the control and **A1** is the absorbance of the sample or standard (Brand-Williams et al., 1995).

Anti-inflammatory activity

The anti-inflammatory activity of the essential oil was evaluated by inhibition of protein denaturation using bovine serum albumin as the reaction substrate. Essential-oil samples at different concentrations were incubated with the protein solution under controlled conditions, and the degree of denaturation was then estimated spectrophotometrically. Diclofenac and Aspégic® were used as reference drugs. Each treatment was carried out in triplicate. This assay is based on the classical observation that anti-inflammatory agents can reduce heat-induced protein denaturation and is still widely used as a simple in vitro screening method (Mizushima and Kobayashi, 1968).

The inhibition percentage was calculated as:

$Inhibition\ of\ protein\ denaturation\ (\%) = [(Ac - As) / Ac] \times 100$

where **Ac** is the absorbance of the control and **As** is the absorbance of the tested sample.

Statistical analysis

All experimental results were expressed as mean ± standard deviation (SD). For the biological assays, each treatment was performed in triplicate. Data were subjected to one-way analysis of variance (ANOVA), and differences between means were compared using Tukey's test at a significance level of $p < 0.05$. This design was adopted to improve the reliability of comparisons among treatments and to support interpretation of dose-dependent responses.

RESULTS

Table 2. Chemical composition of Thymus fontanesii essential oil by GC–MS

No.	Compound name	Chemical formula	Molecular weight (MW)	Retention time (RT, min)	Relative percentage (%)	Peak area	Peak height
1	**α-Pinene**	C10H16	136	7.791	1.17	480,698	156,325

2	Bicyclo[2.2.1] heptane, 2,2-dimethyl-3-methylene-	—	—	8.215	0.24	96,577	37,185
3	Vinyl amyl carbinol	C7H14O	114	9.088	0.18	72,873	24,067
4	Myrcene	C10H16	136	9.451	1.93	791,870	241,371
5	Benzene, methyl(1-methylethyl)-	—	—	9.777	0.43	177,345	70,001
6	1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-	—	—	9.858	0.29	120,232	42,838
7	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	—	—	10.244	2.54	1,043,612	262,034
8	**p-Cymene**	C10H14	134	10.562	11.68	4,798,374	741,789
9	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	C12H20O2	196	10.650	0.92	377,687	127,172
10	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	—	—	11.018	0.15	62,381	24,530

GC–MS analysis revealed a chemically diverse essential oil profile for Thymus fontanesii Boiss. & Reut., with the identification of several volatile constituents belonging mainly to the monoterpene and sesquiterpene groups. As shown in **Table 2**, the most abundant detected compound in the validated profile was **p-cymene (11.68%)**, followed by a **terpinen-derived compound (6.02%)**, **β-caryophyllene (5.72%)**, and **myrcene (1.93%)**. Minor constituents such as **α-pinene (1.17%)**, oxygenated monoterpenes, and other terpene-related compounds were also detected.

Overall, the chromatographic profile suggests that the biological properties of the oil may result from the combined contribution of hydrocarbon monoterpenes, oxygenated derivatives, and sesquiterpenes rather than from a single dominant constituent. This type of chemical composition is consistent with the variability commonly reported in essential oils of the genus Thymus, in which the relative abundance of volatile compounds may change according to the plant origin, environmental conditions, and extraction parameters. Therefore, the antifungal, antioxidant, and anti-inflammatory activities observed in the present study are more likely associated with the **synergistic action of the identified constituents** than with the effect of one isolated molecule (**Table 2**).

Yield and organoleptic characteristics

The essential oil obtained from Thymus fontanesii Boiss. & Reut. showed a yield of **1.36 ± 0.01%**, which is within the range commonly reported for species of the genus Thymus. As presented in **Table 3**, the oil was characterized by a **very light-yellow colour**, a **clear appearance**, and a **strong, distinctive odour**. These organoleptic features indicate good preservation of the volatile fraction during extraction and suggest satisfactory essential-oil quality. The combination of acceptable extraction yield and favorable sensory characteristics supports the suitability of this oil for subsequent physicochemical and biological evaluation (**Table 3**).

Table 3. Yield and organoleptic characteristics of Thymus fontanesii essential oil

Plant	Yield (%)	Colour	Appearance	Smell
Thymus fontanesii Boiss. & Reut.	1.36 ± 0.01	Very light yellow	Clear	Strong and distinctive

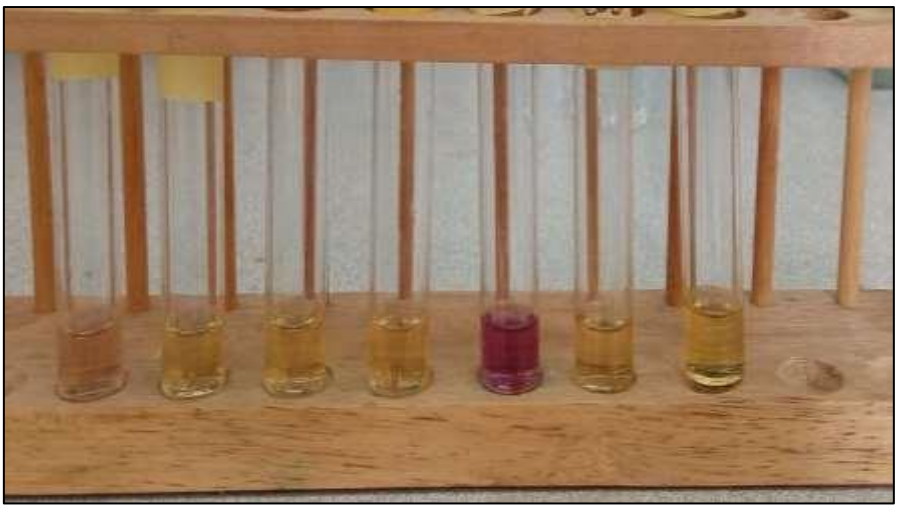

Figure 3: Antioxidant power of Thymus fantanesii essential oil.

Physicochemical properties

The physicochemical parameters of Thymus fontanesii essential oil are summarized in **Table 4**. The oil showed a **refractive index of 1.4985 ± 0.0000**, an **acid index of 4.122 ± 0.000**, and a **density of 0.9542 ± 0.0000**. The refractive index and acid index were within the reference ranges considered in this study, whereas the density was slightly higher than the AFNOR standard range. Overall, these data indicate that the oil possesses acceptable physicochemical quality and satisfactory stability under proper storage conditions. The slight variation observed for density may reflect differences in the relative abundance of some volatile constituents in the analyzed sample (**Table 4**)

Table 4. Physicochemical properties of Thymus fontanesii essential oil compared with AFNOR standards

Essential oil	Refractive index	Acid index	Density
Thymus fontanesii essential oil	1.4985 ± 0.0000	4.122 ± 0.000	0.9542 ± 0.0000
AFNOR standards	1.495 – 1.505	4.1 – 5.2	0.910 – 0.935

Antioxidant activity

The antioxidant activity of the essential oil, evaluated by the DPPH radical-scavenging assay, increased progressively with concentration, indicating a clear dose-dependent effect. As shown in **Table 5**, the inhibition percentage rose from **24.6 ± 1.2%** at **20 µg/mL** to **83.5 ± 2.1%** at **200 µg/mL**. Although vitamin C exhibited slightly higher activity at all tested concentrations, the difference became narrower at the highest concentration, where vitamin C reached **89.1 ± 2.2%** inhibition. These results indicate that Thymus fontanesii essential oil has substantial antioxidant potential and may serve as a natural source of radical-scavenging compounds (**Table 5**).

Table 5. DPPH radical-scavenging activity of Thymus fontanesii essential oil

Concentration (µg/mL)	% inhibition HE	% inhibition Vitamin C
20	24.6 ± 1.2 (a)	31.2 ± 1.4 (a)
40	54.4 ± 2.3 (b)	60.4 ± 1.5 (b)
100	78.3 ± 2.0 (c)	82.2 ± 2.1 (c)
200	83.5 ± 2.1 (c)	89.1 ± 2.2 (c)

Anti-inflammatory activity

The anti-inflammatory activity of the essential oil was assessed through inhibition of protein denaturation (figure.4). The results showed a concentration-dependent increase in activity, from **16.4 ± 1.1%** at **50 µg/mL** to **81.5 ± 2.4%** at **400 µg/mL**, as shown in **Table 6**. At low concentrations, the oil exhibited lower inhibition values than the reference drugs diclofenac and Aspégic®. However, at higher concentrations, the activity of the essential oil became very close to that of both standards. At **400 µg/mL**, the inhibition values recorded for the essential oil, diclofenac, and Aspégic® were highly comparable, indicating that the oil possesses marked anti-inflammatory potential under the experimental conditions used in this study (**Table 6**).

Table 6. Inhibition of protein denaturation by Thymus fontanesii essential oil and reference drugs

Concentration (µg/mL)	Thymus fontanesii essential oil (%)	Diclofenac (%)	Aspégic (%)
50	16.4 ± 1.1 (a)	22.7 ± 1.2 (a)	25.4 ± 1.3 (a)
100	38.2 ± 2.0 (b)	40.4 ± 1.4 (b)	41.2 ± 1.7 (b)
200	68.8 ± 2.4 (c)	68.3 ± 2.5 (c)	74.6 ± 2.5 (c)
300	73.9 ± 2.8 (c)	75.3 ± 2.6 (c)	76.7 ± 2.7 (c)
400	81.5 ± 2.4 (d)	80.8 ± 2.3 (d)	80.5 ± 2.1 (d)

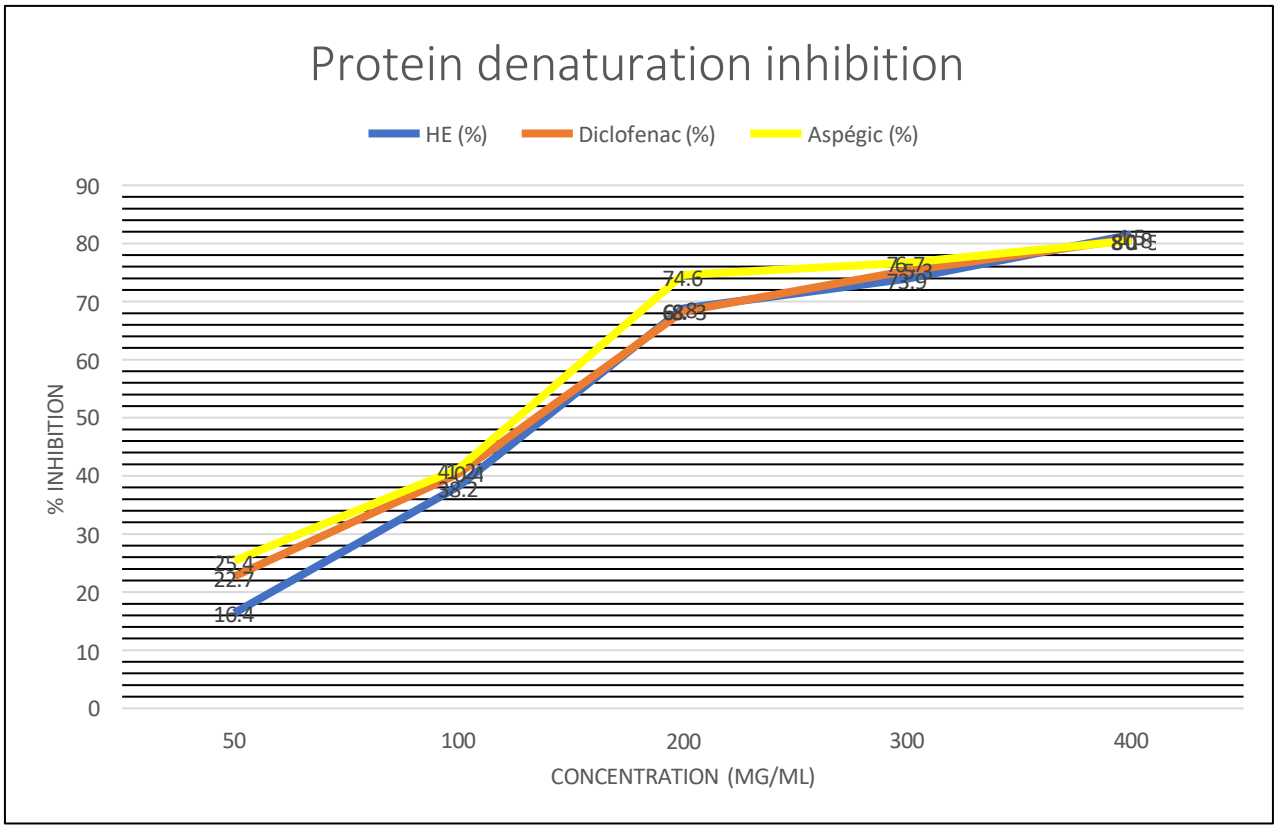

Figure 4. Inhibition of protein denaturation by Thymus fontanesii essential oil according to concentration.

Antifungal activity

The essential oil exerted a marked inhibitory effect on the mycelial growth of the three phytopathogenic fungi tested, namely Fusarium oxysporum, Alternaria sp., and Aspergillus niger. As illustrated in Figure 5, the inhibitory effect increased with concentration for all fungal strains, confirming a clear dose-dependent antifungal response. Among the tested fungi, Fusarium oxysporum appeared to be the most sensitive, as complete inhibition of mycelial growth was observed from 5 µL/50 mL onward. In contrast, Aspergillus niger required a concentration of 10 µL/50 mL for complete inhibition, whereas Alternaria sp. showed total inhibition only at 25 µL/50 mL. These results indicate that the essential oil possesses strong antifungal activity, although the sensitivity of the fungal strains varied according to species (Figure 5).

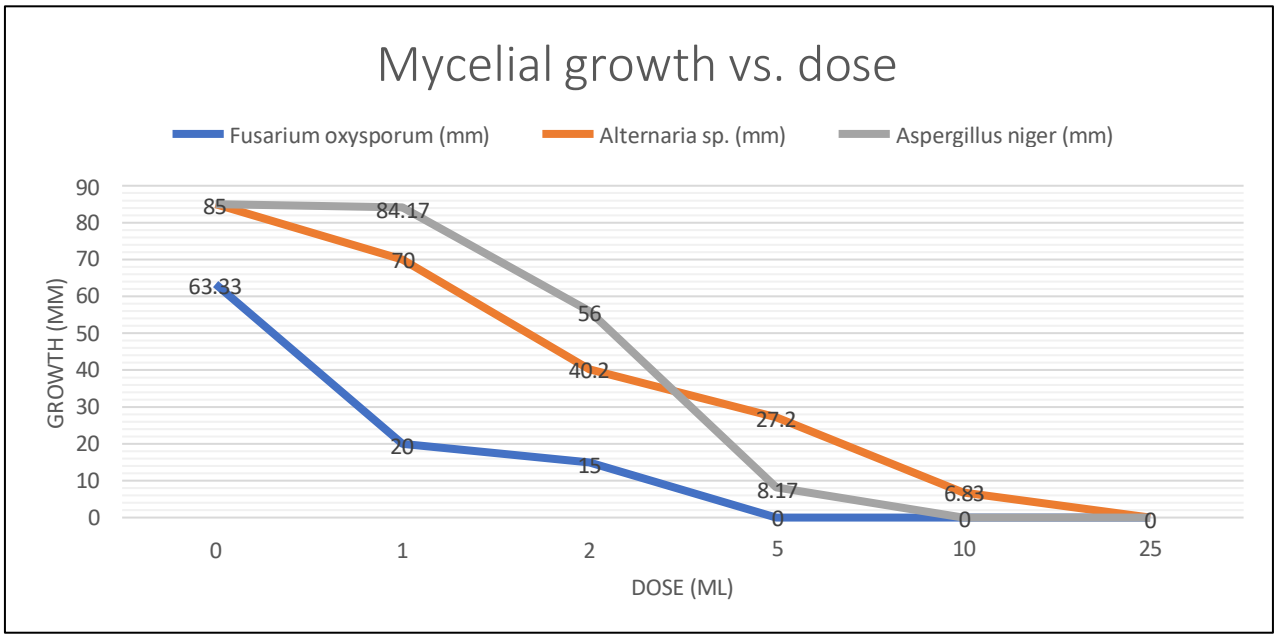

Figure 5. Dose-dependent effect of Thymus fontanesii essential oil on the mycelial growth of the phytopathogenic fungi Fusarium oxysporum, Alternaria sp., and Aspergillus niger.

Statistical analysis (ANOVA)

Analysis of variance followed by Tukey's post hoc test revealed statistically significant differences among the tested concentrations for all evaluated biological activities, with $p < 0.05$. As shown in Tables 7, 8, and 9, the different letters assigned to mean values indicate the existence of significant differences between treatments. This confirms that the observed dose-dependent responses were not random, but reflected a real effect of essential-oil concentration on fungal growth inhibition, antioxidant activity, and anti-inflammatory activity.

For the antifungal assay, the progressive reduction in mycelial growth with increasing essential-oil concentration was statistically significant for all three fungi, although the level of sensitivity differed among species (Table 7). Similarly, the DPPH assay showed a significant increase in radical-scavenging activity as concentration increased (Table 8). The same trend was observed for inhibition of protein denaturation, where the essential oil exhibited significantly higher activity at increasing concentrations (Table 9). Overall, the statistical analysis strengthens the reliability of the experimental data and supports the biological relevance of the dose-dependent effects observed in this study.

Table 7. ANOVA results for mycelial growth of fungal strains

Dose (µL/50 mL)	Fusarium oxysporum (mm)	Alternaria sp. (mm)	Aspergillus niger (mm)
0 (Control)	63.33 ± 0.58 (a)	85.00 ± 0.67 (a)	85.00 ± 1.40 (a)
1	20.00 ± 1.00 (b)	70.00 ± 1.53 (b)	84.17 ± 2.34 (a)
2	15.00 ± 1.00 (c)	40.20 ± 1.39 (c)	56.00 ± 1.53 (b)
5	0.00 ± 0.00 (d)	27.20 ± 2.10 (d)	8.17 ± 1.22 (c)
10	0.00 ± 0.00 (d)	6.83 ± 0.84 (e)	0.00 ± 0.00 (c)
25	0.00 ± 0.00 (d)	0.00 ± 0.00 (e)	0.00 ± 0.00 (c)

Table 8. ANOVA results for antioxidant activity

Concentration (µg/mL)	% inhibition HE	% inhibition Vitamin C
20	24.6 ± 1.2 (a)	31.2 ± 1.4 (a)
40	54.4 ± 2.3 (b)	60.4 ± 1.5 (b)
100	78.3 ± 2.0 (c)	82.2 ± 2.1 (c)
200	83.5 ± 2.1 (c)	89.1 ± 2.2 (c)

Table 9. ANOVA results for anti-inflammatory activity

Concentration (µg/mL)	Thymus fontanesii essential oil (%)	Diclofenac (%)	Aspégic (%)
50	16.4 ± 1.1 (a)	22.7 ± 1.2 (a)	25.4 ± 1.3 (a)
100	38.2 ± 2.0 (b)	40.4 ± 1.4 (b)	41.2 ± 1.7 (b)
200	68.8 ± 2.4 (c)	68.3 ± 2.5 (c)	74.6 ± 2.5 (c)
300	73.9 ± 2.8 (c)	75.3 ± 2.6 (c)	76.7 ± 2.7 (c)
400	81.5 ± 2.4 (d)	80.8 ± 2.3 (d)	80.5 ± 2.1 (d)

DISCUSSION

The present study confirms that the essential oil of Thymus fontanesii Boiss. & Reut. possesses a relevant biological profile, combining antifungal, antioxidant, and anti-inflammatory activities. However, the corrected interpretation of the GC–MS data changes the way these effects should be discussed. In the present sample, the oil was not dominated by carvacrol, as initially assumed, but was instead characterized mainly by p-cymene, a terpinen-related compound, β-caryophyllene, and myrcene. This point is important because the biological response observed here must be interpreted in relation to the actual validated composition of the oil rather than by analogy with previously published chemotypes of the same species (Ghannadi et al., 2004; Dob et al., 2006; Sid Ali et al., 2020; Selles et al., 2024).

This result is consistent with the well-known chemical variability of the genus Thymus. Essential oils of thyme species frequently differ according to geographic origin, harvest period, environmental conditions, plant developmental stage, and extraction method. Such variability has already been documented for several Thymus taxa and explains why oils from the same species may show distinct dominant constituents and, consequently, different biological properties (Dorman and Deans, 2000; Burt, 2004; Etri and Pluhár, 2024). In this regard, the present study usefully complements previous work on T. fontanesii, because it shows that this endemic species may remain biologically active even when the oil is not dominated by a phenolic monoterpene such as thymol or carvacrol (Ghannadi et al., 2004; Dob et al., 2006; Sid Ali et al., 2020; Selles et al., 2024). A critical comparison with earlier studies on Thymus fontanesii is especially relevant here. Ghannadi et al. reported a thymol-rich oil from Algerian material, with thymol, γ-terpinene and p-cymene as the major constituents, while Dob et al. also described an oil in which thymol-related compounds were predominant and associated with marked antimicrobial activity (Ghannadi et al., 2004; Dob et al., 2006).

By contrast, Sid Ali et al. and Selles et al. described T. fontanesii oils in which carvacrol was reported among the dominant constituents, together with appreciable antioxidant activity (Sid Ali et al., 2020; Selles et al., 2024). Compared with these studies, the present oil appears less phenolic and relatively richer in hydrocarbon monoterpenes and sesquiterpene-type constituents. This difference does not weaken the value of the current results; instead, it reinforces the idea that T. fontanesii is a chemically plastic species whose bioactivity cannot be reduced to a single fixed chemotype (Ghannadi et al., 2004; Dob et al., 2006; Etri and Pluhár, 2024).

The antifungal activity observed in the present study is one of the most significant findings. The essential oil produced a clear dose-dependent inhibition of mycelial growth in Fusarium oxysporum, Alternaria sp., and Aspergillus niger. F. oxysporum was the most sensitive strain, showing complete growth inhibition at a lower dose than the other fungi, whereas Alternaria sp. required a higher concentration for total inhibition. Such differences in susceptibility are not unusual, because fungal species differ in membrane composition, cell-wall architecture, stress-response systems, and detoxification capacity (Tripathi et al., 2009; Sharma et al., 2017; Mani-López et al., 2021; de Oliveira Filho et al., 2021).

The antifungal effect of essential oils is generally explained by a multi-target mechanism rather than a single-site mode of action. Volatile terpenoids may disturb membrane integrity, increase permeability, alter ion gradients, affect mitochondrial activity, and induce oxidative imbalance in fungal cells. Reviews dedicated to essential-oil antifungal mechanisms have repeatedly emphasized that this multicomponent and multitarget behavior may reduce the risk of rapid resistance development compared with some synthetic fungicides (Burt, 2004; Mani-López et al., 2021; de Oliveira Filho et al., 2021; de Oliveira et al., 2025). In the present case, the strong inhibition recorded against tomato-associated fungi may therefore result from the combined effect of p-cymene-associated compounds, terpinen-type constituents, β-caryophyllene, myrcene, and minor oxygenated terpenes acting together rather than from one isolated dominant molecule (Dob et al., 2006; Mani-López et al., 2021).

The marked sensitivity of F. oxysporum deserves particular attention because this pathogen is one of the most damaging fungi in tomato production systems. Previous reviews on tomato pathology have highlighted the importance of F. oxysporum in reducing plant vigor and yield, and have also stressed the need for alternatives to intensive fungicide use in disease management (McGovern, 2015; Rodrigues and Furlong, 2022). In that sense, the present results support the potential use of T. fontanesii essential oil as a candidate for eco-friendly crop-protection strategies, especially in integrated programs where natural compounds are intended to complement or reduce the use of conventional pesticides (Raveau et al., 2020; Rodrigues and Furlong, 2022).

The antioxidant activity recorded in the DPPH assay also deserves a more nuanced interpretation than the initial draft allowed. Since the corrected chromatographic profile no longer supports a carvacrol-dominant oil, the antioxidant capacity should not be attributed to one compound alone. Essential oils may express radical-scavenging activity through the cumulative action of several constituents, including monoterpenes, oxygenated derivatives, and certain sesquiterpenes, whose interactions may enhance or modulate overall antioxidant performance (Miguel, 2010; Sid Ali et al., 2020; Saad et al., 2013). The progressive increase in inhibition observed with increasing concentration indicates that the oil contains a sufficient pool of redox-active constituents to generate an effective antioxidant response, even though its activity remained slightly lower than vitamin C at equivalent concentrations.

This antioxidant effect is also relevant beyond the strictly biochemical perspective. Oxidative stress is closely related to tissue deterioration, postharvest spoilage, and stress responses in both plant and food systems. For this reason, an essential oil that combines antifungal and antioxidant properties may have an added value in practical applications, especially in postharvest protection or in the preservation of plant-derived materials. Reviews on essential oils in food and crop preservation have repeatedly underlined this multifunctional advantage, which may make them more attractive than compounds acting through only one biological pathway (Burt, 2004; Miguel, 2010; Raveau et al., 2020).

The anti-inflammatory activity observed through inhibition of protein denaturation adds a third dimension to the biological potential of the studied oil. Although the essential oil was less active than diclofenac or Aspégic® at lower concentrations, its inhibitory effect increased steadily and became very close to the reference drugs at the highest dose. This suggests that the oil contains constituents capable of interfering with denaturation-related processes and may possess moderate but genuine anti-inflammatory potential. Current reviews indicate that plant essential oils can modulate inflammatory response through several pathways, including inhibition of pro-inflammatory mediators, attenuation of oxidative stress, and regulation of signaling cascades such as NF-κB and MAPK pathways (Miguel, 2010; Li et al., 2023; Saad et al., 2013). Even if the present assay is only an in vitro screening model, the results remain valuable because they show that the oil is biologically active across more than one functional domain.

The physicochemical characteristics obtained in this work also support the overall quality of the extracted oil. The yield of 1.36% is realistic for a thyme essential oil and confirms that the species can provide a useful volatile fraction under conventional hydrodistillation. Although some previously published studies on *T. fontanesii* reported different yields, such differences are expected in aromatic species and may result from climatic conditions, local ecotype, phenological stage, and extraction variables (Dob et al., 2006; Sid Ali et al., 2020; Selles et al., 2024). The refractive index and acid value measured in the present study were within acceptable ranges, while the slightly elevated density may reflect compositional particularities of the sample rather than poor oil quality. Altogether, these data support the technological viability of the extraction and the suitability of the material for biological assays.

From a broader perspective, the present findings strengthen the argument that endemic North African aromatic plants deserve greater attention as sources of bioactive natural products. *Thymus fontanesii* appears particularly interesting because it combines local availability, essential-oil productivity, compositional diversity, and a broad range of biological activities. This is relevant not only for agricultural applications, but also for possible pharmaceutical, cosmetic, or nutraceutical uses, provided that future studies address toxicology, formulation, and application stability more thoroughly (Raveau et al., 2020; Li et al., 2023; Selles et al., 2024).

Nevertheless, some limitations should be recognized. First, the present work is based on in vitro assays, which are useful for screening but do not fully reflect the complexity of field conditions or host–pathogen interactions. Second, the species shows clear chemical variability, meaning that additional populations of T. fontanesii should be studied to determine how stable the observed activities are across regions and harvests. Third, the present study demonstrates overall bioactivity but does not distinguish the precise role of each individual constituent. For future research, it would therefore be valuable to combine in vivo assays, bio-guided fractionation, and more detailed mechanistic studies. Delivery systems such as nanoformulations may also improve oil stability, reduce volatility, and enhance biological efficiency, as already suggested for essential-oil-based products in recent studies (de Oliveira Filho et al., 2021; Raveau et al., 2020).

Overall, the present discussion supports three main points. First, the biological effects observed in this study are consistent with the corrected GC–MS profile and should be interpreted in terms of synergistic multicomponent activity. Second, comparison with earlier studies shows that Thymus fontanesii is chemically variable but repeatedly bioactive, which increases its scientific and practical interest. Third, the combination of antifungal, antioxidant, and anti-inflammatory properties reported here suggests that this endemic Algerian species could be considered a promising resource for eco-friendly crop protection and other natural-product applications (Ghannadi et al., 2004; Dob et al., 2006; Sid Ali et al., 2020; Selles et al., 2024; Raveau et al., 2020).

CONCLUSION

In conclusion, the present study highlights the biological relevance of the essential oil extracted from Thymus fontanesii Boiss. & Reut. as a promising natural source of bioactive compounds. The corrected GC–MS analysis showed that the oil was mainly characterized by p-cymene, terpinen-related compounds, β-caryophyllene, and myrcene, suggesting that its biological performance is linked to the combined action of a chemically diverse volatile fraction rather than to a single dominant constituent.

The oil exhibited substantial antifungal activity against major tomato-associated phytopathogenic fungi, with particularly strong inhibition of Fusarium oxysporum, confirming its potential value in plant disease management. It also demonstrated marked antioxidant activity and a significant anti-inflammatory effect, indicating that its biological interest extends beyond antifungal activity alone. This multifunctional profile increases the practical importance of T. fontanesii and supports its potential use in the development of natural products for agricultural and possibly pharmaceutical or cosmetic applications.

These findings contribute to a better understanding of the bioactive potential of this endemic Algerian species and reinforce the importance of studying local aromatic plants within the framework of sustainable valorization strategies. At the same time, the present work underlines the necessity of linking biological interpretation to the actual chemical composition of each analyzed oil, especially in chemically variable species such as Thymus fontanesii.

Further investigations should focus on in vivo validation, field-scale evaluation, safety assessment, and formulation optimization in order to improve the stability and applicability of the oil under practical conditions. Overall, this study provides a useful scientific basis for the future exploitation of T. fontanesii essential oil as a natural alternative in eco-friendly crop protection and other high-value biological applications.

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REFERENCES

1. Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995) Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28(1), 25-30. doi: 10.1016/S0023-6438(95)80008-5.
2. Burt, S. (2004) Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology, 94(3), 223-253. doi: 10.1016/j.ijfoodmicro.2004.03.022.
3. Cimanga, K., Kambu, K., Tona, L., Apers, S., De Bruyne, T., Hermans, N., Totté, J., Pieters, L., Vlietinck, A.J. (2002) Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. Journal of Ethnopharmacology, 79(2), 213-220. doi: 10.1016/S0378-8741(01)00384-1.
4. Clevenger, J.F. (1928) Apparatus for the determination of volatile oil. Journal of the American Pharmaceutical Association, 17(4), 345-349. doi: 10.1002/jps.3080170407.
5. De Oliveira Filho, J.G., Santos, J.C.C., Rocha, J.M., et al. (2021) Antifungal activity and possible mechanisms of essential oils: a review. Food Bioscience, 41, 100936. doi: 10.1016/j.fbio.2021.100936.
6. De Oliveira, J.B.S., de Souza, A.R.F., Neta, M.T.S.L., et al. (2025) Antifungal mechanisms of plant essential oils: a comprehensive review. Agriculture, 15(21), 2303. doi: 10.3390/agriculture15212303.
7. Dob, T., Dahmane, D., Benabdelkader, T. and Chelghoum, C. (2006) Composition and antimicrobial activity of the essential oil of Thymus fontanesii. Pharmaceutical Biology, 44(8), 607–612. doi: 10.1080/13880200600897106.
8. Dorman, H.J.D., Deans, S.G. (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88(2), 308-316. doi: 10.1046/j.1365-2672.2000.00969. x.
9. Etri, K. and Pluhár, Z. (2024) Exploring chemical variability in the essential oils of the Thymus genus. Plants, 13(10), 1375. doi: 10.3390/plants13101375.
10. Ghannadi, A., Sajjadi, S.E., Kabouche, A. and Kabouche, Z. (2004) Thymus fontanesii Boiss. & Reut.—a potential source of thymol-rich essential oil in North Africa. Zeitschrift für Naturforschung C, 59(3–4), 187–189. doi: 10.1515/znc-2004-3-410.
11. ISO (1998a) ISO 279:1998. Essential oils — Determination of relative density at 20°C — Reference method. International Organization for Standardization, Geneva.
12. ISO (1998b) ISO 280:1998. Essential oils — Determination of refractive index. International Organization for Standardization, Geneva.
13. ISO (2023a) ISO 1242:2023. Essential oils — Determination of acid value by two titration methods, manual and automatic. International Organization for Standardization, Geneva.
14. ISO (2023b) ISO 210:2023. Essential oils — General requirements and guidelines for packaging, conditioning and storage. International Organization for Standardization, Geneva.
15. Li, Y., Wang, X., Yang, F. and Chen, J. (2023) Molecular mechanism of the anti-inflammatory effects of plant essential oils: a systematic review. Journal of Ethnopharmacology, 301, 115829. doi: 10.1016/j.jep.2022.115829.
16. Mani-López, E., Cortés-Zavaleta, O. and López-Malo, A. (2021) A review of the methods used to determine the target site or the mechanism of action of essential oils and their components against fungi. SN Applied Sciences, 3, 44. doi: 10.1007/s42452-020-04102-1.
17. McGovern, R.J. (2015) Management of tomato diseases caused by Fusarium oxysporum. Crop Protection, 73, 78-92. doi: 10.1016/j.cropro.2015.02.021.
18. Miguel, M.G. (2010) Antioxidant and anti-inflammatory activities of essential oils: a short review. Molecules, 15(12), 9252-9287. doi: 10.3390/molecules15129252.

19. Mizushima, Y. and Kobayashi, M. (1968) Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. Journal of Pharmacy and Pharmacology, 20(3), 169-173. doi: 10.1111/j.2042-7158.1968.tb09718.x.
20. NIST (2023) NIST Standard Reference Database 1A: NIST/EPA/NIH Mass Spectral Library, 2023 edition. National Institute of Standards and Technology, Gaithersburg, MD.
21. Raveau, R., Fontaine, J. and Lounès-Hadj Sahraoui, A. (2020) Essential oils as potential alternative biocontrol products against plant pathogens and weeds: a review. Foods, 9(3), 365. doi: 10.3390/foods9030365.
22. Rodrigues, M.H.P. and Furlong, E.B. (2022) Fungal diseases and natural defense mechanisms of tomatoes (Solanum lycopersicum): a review. Physiological and Molecular Plant Pathology, 120, 101906. doi: 10.1016/j.pmpp.2022.101906.
23. Saad, N.Y., Muller, C.D. and Lobstein, A. (2013) Major bioactivities and mechanism of action of essential oils and their components. Flavour and Fragrance Journal, 28(5), 269–279. doi: 10.1002/ffj.3165.
24. Selles, S.M.A., Belhamiti, B.T., Kouidri, M., Ait Amrane, A., Kadari, Y., Kaddour, Z. and Kabrit, S. (2024) Chemical compounds, antioxidant and scolicidal potencies of Thymus fontanesii essential oil. Experimental Parasitology, 257, 108699. doi: 10.1016/j.exppara.2024.108699.
25. Sharma, A., Rajendran, S., Srivastava, A., Sharma, S. and Kundu, B. (2017) Antifungal activities of selected essential oils against Fusarium oxysporum f. sp. lycopersici 1322, with emphasis on Syzygium aromaticum essential oil. Journal of Bioscience and Bioengineering, 123(3), 308-313. doi: 10.1016/j.jbiosc.2016.09.011.
26. Sid Ali, L., Brada, M., Fauconnier, M.-L. and Lognay, G. (2020) Chemical composition and antioxidant activity of Thymus fontanesii essential oil from Algeria. The Natural Products Journal, 10(3), 193–199. doi: 10.2174/2210315508666180427162542.
27. Tripathi, A., Sharma, N. and Sharma, V. (2009) In vitro efficacy of Hyptis suaveolens L. (Poit.) essential oil on growth and morphogenesis of Fusarium oxysporum f. sp. gladioli. World Journal of Microbiology and Biotechnology, 25, 503-512. doi: 10.1007/s11274-008-9916-y.