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Chemical Profile and Bioactivity of Essential Oils from Five Turkish Thyme Species Against White Mold Fungal Disease Agent Sclerotinia sclerotiorum

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Abstract

This research examined the phytochemical profile, antioxidant properties, and antifungal efficacy of essential oils (EOs) derived from five thyme species growing wild in Türkiye: *Origanum saccatum* P.H. Davis, *Origanum vulgare* subsp. *hirtum* (link) Ietswaart, *Satureja cilicica* P.H. Davis, *Thymbra spicata* L. var. *spicata* L, and *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus*. The extraction of essential oils was performed using the hydro distillation technique. To identify the volatile compounds, present in the oils, gas chromatography–mass spectrometry (GC–MS) was employed. The antioxidant potential of each sample was determined based on their ability to scavenge DPPH free radicals, while the total phenolic content was quantified utilizing the Folin–Ciocalteu assay. The antifungal potential of the oils against *Sclerotinia sclerotiorum*, a soil-borne plant pathogen, was evaluated through vapour-phase exposure at varying concentrations. GC-MS results showed that the predominant constituents of the essential oils were phenolic monoterpenes, particularly carvacrol and thymol. Among the analyzed species, *O. vulgare* subsp. *hirtum* exhibited the highest carvacrol content, reaching 88.12%. Among the tested oils, *T. spicata* var. *spicata* showed the highest antioxidant capacity, as indicated by its low IC_{50} value of 10.698 mg/L, and was also rich in phenolic compounds, containing 4.771 µg/mg. Furthermore, the oils from *S. cilicica* and *T. spicata* var. *spicata* completely inhibited fungal mycelial growth at a dose of 1.0 µL/Petri dish, indicating strong antifungal efficacy. EC_{50} values were found to range between 0.14 and 1.90 µL/Petri dish, with *T. spicata* var. *spicata* demonstrating the greatest effectiveness. In summary, the results emphasize the potential of thyme-derived EOs especially those rich in carvacrol as environmentally friendly and natural substitutes for chemical fungicides in controlling fungal infections in plants.

Introduction

The Lamiaceae family is one of the most diverse plant families in the world and is particularly well represented in Türkiye due to the country's favorable Mediterranean climate and unique geographical location. Türkiye is considered a major center of endemism for the family, which comprises about 45 genera and over 740 taxa, almost half of which are endemic[1,2]. Many members of this family are characterized by their rich composition of EOs and secondary metabolites, leading to their widespread use in traditional healing practices and a variety of pharmacological functions, including antimicrobial, antioxidant and antifungal properties[3,4].

The term 'thyme' generally refers to aromatic plants belonging to various genera of the Lamiaceae, in particular *Origanum, Thymus, Thymbra* and *Satureja*. These species contain high levels of monoterpene compounds, particularly thymol and carvacrol. These plants are frequently used in culinary, cosmetic and ethnopharmacological contexts in Türkiye[5,6]. In addition to their aromatic properties, they are traditionally used to treat respiratory diseases, digestive problems and inflammation[7,8].

Of the species analyzed, *O. saccatum* is endemic to the Alanya region, while *O. vulgare* subsp. *hirtum* occurs throughout Marmara and Thrace. Both *S. cilicica* and *T. spicata* var. *spicata* are used locally as spices and medicinal plants, while *T. sipyleus* subsp. *sipyleus* var. *sipyleus* is widely used in herbal teas and is valued for its antioxidant and antimicrobial properties[9,10].

S. sclerotiorum is a soil-borne fungal pathogen known to infect more than 450 plant species, including key agricultural crops like sunflower and cucumber. This necrotrophic fungus is difficult to control due to its persistent survival structures (sclerotia) and its ability to develop resistance to chemical fungicides, the use of

which also poses risks to the environment[11]. Consequently, attention has shifted to plant-based alternatives such as essential oils as they have the potential to act as biodegradable, environmentally friendly bio fungicides.

While the antifungal effects of EOs from certain thyme species have been previously studied, limited information is available regarding the activity of oils derived specifically from *O. saccatum, S. cilicica, T. spicata* var. *spicata, T. sipyleus* subsp. *sipyleus* var. *sipyleus* and *O. vulgare* subsp. *hirtum* against *S. sclerotiorum*.

The purpose of this work was to evaluate the chemical constituents of essential oils derived from five distinct thyme species, two of which are endemic to Türkiye, and to evaluate their antioxidant properties as well as them *in vitro* antifungal activity against *S. sclerotiorum*, the causative agent of cucumber stem rot. These results could contribute to the development of environmentally friendly bio fungicides for use in sustainable agriculture.

Results and discussions

Essential Oil Content and Phytochemical Profile. The chemical profiles of the EOs from *O. saccatum*, *O. vulgare* subsp. *hirtum*, *S. cilicica*, *T. spicata* var. *spicata*, and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* were ascertained using GC-MS. The EO content ranged from 0.80–4.12% (v/w), with the highest amount provided by *O. vulgare* subsp. *hirtum*. The analysis revealed different chemotypes among the species, which were dominated by monoterpenes such as carvacrol, p-cymene and γ-terpinene, and oxygenated monoterpenes such as α-terpineol and linalool. Table 2 displays the EO components' comprehensive chemical makeup and classification.

The main ingredients identified in the EOs are listed below: *O. saccatum* EO: p-cymene (38.28%), carvacrol (37.63%), γ-terpinene (8.22%); *O. vulgare* subsp. *hirtum* EO: carvacrol (88.%, p-cymene (4.37%) and γ-terpinene (1.71%); and *S. cilicica* EO consisted of carvacrol (42.91%), p-cymene (18.3%), thymol (14.11%) and γ-terpinene (12.14%); *T. spicata* var. *spicata* EO contained carvacrol (50.9%), thymol (20.59%), γ-terpinene (14.98%) and p-cymene (7%).89%); *T. sipyleus* subsp. *sipyleus* var. *sipyleus* EO contained α-terpineol (24.89%), linalool (19.27%), 1,8-cineole (10.60%) and camphor (6.80%) (Fig. 1).

The most prevalent substances in thyme EOs are oxygenated monoterpenes (47.91–90.42%) (Table 2). The highest content of oxygenated monoterpenes (90.42%) was observed in *O. vulgare* subsp. *hirtum* EO, followed by *T. sipyleus* subsp. *sipyleus* var. *sipyleus* (80.98%), *T. spicata* var. *spicata* (74.15%) and *S. cilicica* (63.31%). The lowest content of oxygenated sesquiterpenes (47.91%) was found in *O. saccatum* oil. Monoterpenic hydrocarbons were the next most abundant category of compounds (7.26–49.38%). High amounts of these compounds were observed in the EO of *O. saccatum* (Fig. 2).

Various endogenous and environmental factors play a role in shaping the chemical profile of EOs. The production and quality of secondary metabolites are directly related to genetic traits, climatic conditions (e.g. light, temperature, precipitation, irrigation, soil, altitude and location), environmental organisms, agricultural techniques and post-production processing[12,13].

The main constituents of the EOs used in this study and the proportions of these constituents were largely consistent with previously reported results for the same plant species. However, some differences in the total number of chemicals and their relative concentrations were observed in other studies. In our study as well as in the studies of others[6–8,14–22], high levels of p-cymene, carvacrol, camphor, γ-terpinene, 1,8-cineole, linalool and thymol were found in the EOs of plants from the Lamiaceae family. Variations in the chemical profiles of

essential oils are linked to several factors such as the plant's geographical origin, soil characteristics, age, and the specific plant part utilized for extraction[12]. These findings indicate that both genetic makeup and environmental conditions substantially affect the essential oil composition of the species studied, potentially influencing their antifungal activities.

Table 2
Principal constituents (%) and chemical groupings of EOs obtained from five Lamiaceae species

No	RT	RI	RI_L	Compounds	1	2	3	4	5
1	6.52	930	930*	α-Thujene	1.18	0.69	1.15	1.02	3.27
2	7.60	952	952*	Camphene	1.36	0.09	0.15	0.09	2.88
3	8.70	1030	1030*	β-Phellandrene	nd	nd	nd	0.04	1.57
4	8.98	1091	1161*	Myrcene	0.34	0.53	0.75	0.64	0.41
5	9.41	1167	1167*	α-Phellandrene	0.09	0.06	0.12	0.10	0.05
6	9.83	1178	1180*	α-Terpinene	0.77	0.36	1.16	1.26	0.07
7	10.04	1198	1198*	Limonene	0.10	0.03	0.37	0.02	3.95
8	10.62	1211	1211*	1,8-Cineole	nd	nd	0.32	0.16	10.60
9	11.12	1250	1250*	cis-Ocimene	0.22	0.11	0.96	nd	nd
10	12.03	1266	1255*	γ-Terpinene	8.22	1.71	12.14	14.98	0.63
11	12.64	1272	1272*	p-Cymene	38.28	4.37	18.30	7.89	0.81
12	17.11	1301	1300*	1-Octen-3-ol	0.20	0.03	0.15	0.09	0.15
13	19.63	1460	1463*	cis-sabinene hydrate	0.13	0.18	0.20	0.08	1.25
14	1984	1518	1518*	Camphor	nd	nd	nd	nd	6.80
15	20.64	1547	1547*	Linalool	1.33	0.03	2.47	0.28	19.27
16	22.89	1573	1556*	trans-sabinene hydrate	0.12	0.09	0.09	0.07	0.75
17	23.39	1598	1589*	Thymol methyl ether	nd	1.66	0.26	nd	nd
18	23.41	1601	1601*	Carvacrol methyl ether	0.12	nd	nd	nd	nd
19	24.17	1611	1602*	Terpinen-4-ol	0.95	0.57	0.81	0.63	3.72
20	25.16	1660	1664*	trans-Pinocarveol	nd	nd	nd	nd	0.47
21	25.82	1695	1697*	α-Terpineol	0.13	0.11	0.18	0.21	24.89
22	25.94	1698	1701*	Borneol	3.94	0.28	0.42	0.15	5.44
23	26.19	1727	1727*	β-Bisabolene	0.50	0.16	0.42	nd	nd
24	26.98	1734	1735*	Verbenone	0.07	nd	0.07	0.01	1.20

No	RT	RI	RI_L	Compounds	1	2	3	4	5
25	28.39	1740	1738*	Carvone	nd	0.03	0.17	0.03	0.25
26	28.65	1780	1782*	Dihydrocarvone	0.21	0.07	0.18	0.02	nd
27	30.30	1790	1790*	Myrtenol	nd	nd	nd	nd	0.26
28	31.62	1845	1845*	Geraniol	nd	nd	0.40	nd	nd
29	38.78	1986	1987*	Caryophyllene oxide	0.36	nd	0.45	0.24	3.71
30	39.86	2133	2133*	Spathulenol	0.33	nd	0.25	0.06	0.24
31	40.15	2164	2164*	Thymol	2.22	0.25	14.11	20.59	nd
32	40.38	2223	2223*	Carvacrol	37.63	88.12	42.91	50.90	2.81
Chemical grouped compounds (%)									
Oxygenated monoterpenes					47.91	90.42	63.31	74.15	80.98
Monoterpene hydrocarbons					49.38	7.26	33.95	25.02	10.37
Oxygenated sesquiterpenes					0.66	nd	0.90	0.30	3.95
Sesquiterpene hydrocarbon					0.50	0.16	0.42	nd	nd
Ether					0.12	1.66	0.26	nd	nd
Ketone					0.20	0.03	0.15	0.09	0.15
Number of identified compounds					24	22	27	24	25
Total (%)					98.77	99.53	98.99	99.56	95.45
Essential oil content (%)					0.80	4.12	2.44	3.04	1.24

RT: Retention time; RI: Retention index; RI_L: Retention indices that were calculated in different literatures; nd: Not detected; *: Babushok et al ., 2011(23); 1: *Origanum saccatum; 2:Origanum vulgare* subsp. *hirtum*; 3: *Satureja cilicica;* 4: *Thymbra spicata* var. *spicata; 5: Thymus sipyleus* subsp. *sipyleus* var. *sipyleus*

Antioxidant activity. EOs extracted from five Lamiaceae species were evaluated for their antioxidant activity through the DPPH assay and for TPC using the Folin–Ciocalteu technique. The detailed results can be found in Table 3. The greatest antioxidant potential was observed in *T. spicata* var. *spicata* (IC_{50} = 10,698 mg L^{-1}) of the analyzed species, followed by *O. saccatum* (IC_{50} = 17,350 mg L^{-1}). *T. sipyleus* subsp. *sipyleus* var. *sipyleus* showed the weakest activity (IC_{50} = 46,220 mg L^{-1}). The TPC of the oils corresponded to their free radical scavenging capacity, confirming that a higher phenol concentration generally leads to stronger antioxidant activity.

Total phenolic content. Significant variation was observed in the TPC of the EOs among the species, with values ranging from 2,235 to 4,771 µg mg^{-1}. *T. spicata* var. *spicata* presented the greatest phenolic concentration (4.771 µg/mg), followed closely by *O. saccatum* (4.692 µg/mg), while *T. sipyleus* subsp. *sipyleus* var. *sipyleus* contained

the least (2.235 µg/mg). These findings align with the antioxidant activity results, demonstrating a positive relationship between phenolic content and free radical scavenging ability. Phenolic compounds such as carvacrol and thymol probably contribute the most to this activity due to their known redox properties and their ability to donate hydrogen.

These findings highlight the significance of particular phenolic compounds, notably thymol and carvacrol, which exhibit potent antioxidant activity through mechanisms such as hydrogen atom donation and electron transfer. The significantly higher activity observed in *T. spicata* var. *spicata* and *O. saccatum* is consistent with previous studies showing that species rich in carvacrol exhibit higher bioactivity in oxidative stress assays[24,25]. Furthermore, the differences between species can be attributed to environmental, geographical and genetic factors that influence essential oil biosynthesis[26]. In this context, the role of terpenoid structures, including monoterpenes and phenolic monoterpenoids, should not be overlooked as these structures play a significant role in improving oxidative stability in both biological and food systems.

Table 3
DPPH radical scavenging capacity and TPC of thyme Eos

Essential Oil	Radical Scavenging Activity ($IC_{50,}$ mg L^{-1})	Phenolic contents (µg mg^{-1})
Origanum saccatum	17.350	4.692
Origanum vulgare subsp. *hirtum*	24.682	3.760
Satureja cilicica	39.884	3.512
Thymbra spicata var. *spicata*	10.698	4.771
Thymus sipyleus subsp. *sipyleus* var. *sipyleus*	46.220	2.235

Isolation and identification of fungal disease agent. The *S. sclerotiorum* fungal isolate (SsH5) was obtained from infected cucumber stems grown in the Samandağ district of Hatay province. Morphological identification was carried out following the protocol outlined by Bolton et al. (27). Using the universal fungal primers ITS1 and ITS4, the internal transcribed spacer (ITS) region of ribosomal DNA was amplified by PCR to confirm the molecular identity. For antifungal assays, *S. sclerotiorum* was sub cultured by transferring 8 mm mycelial plugs taken from the edges of actively growing colonies onto PDA plates, which were incubated at 24°C for five days. After this period, the isolate exhibited the characteristic white, cottony mycelium and the formation of sclerotia consistent with the cultural and morphological characteristics of *S. sclerotiorum*.

Fungistatic and fungicidal effect of essential oils on mycelial growth. The lid replacement technique was used to evaluate the fungistatic and fungicidal properties of essential oils against the mycelial growth of *S. sclerotiorum* SsH5 at their minimum inhibitory concentrations (MICs). The results showed that some EOs (*O. saccatum* and *O. vulgare* subsp. *hirtum*) had fungicidal properties and completely inhibited mycelial regrowth. Others (*S. cilicica, T. spicata* var. *spicata* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus*) had fungistatic properties that allowed the pathogen to recover and regrow when transferred to a fresh medium.

Antifungal activities of essential oils on mycelial growth of fungal disease agent. The antifungal effects of the EOs against *S. sclerotiorum* were assessed in the vapor phase. All five oils demonstrated inhibitory activity on

mycelial growth on PDA medium. Notably, the EOs from *S. cilicica* and *T. spicata* var. *spicata* exhibited the strongest antifungal effect, with minimum inhibitory concentrations (MICs) of 1.0 µL per Petri dish. Additionally, *O. vulgare* subsp. *hirtum* oils at 2.0 µL per dish, as well as *O. saccatum* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* oils at 6.0 µL per dish, completely suppressed the pathogen's mycelial growth. Statistical evaluation showed significant differences both among various concentrations of the same essential oil and between different oils at identical concentrations (Table 4).

The EC_{50} value, representing the concentration required to inhibit 50% of the fungal mycelial growth, was calculated using probit analysis (Table 4). The EO of *T. spicata* var. *spicata* exhibited the lowest EC_{50} at 0.14 µL per Petri dish, whereas *T. sipyleus* subsp. *sipyleus* var. *sipyleus* showed the highest EC_{50} value of 1.9 µL per dish.

The fungicidal or fungistatic activity of the MIC of the individual EOs was also determined. The lids of the petri dishes were replaced with new sterile petri lids and the dishes were incubated again to observe the growth of the fungal mycelium. The results showed that the EOs of T. *spicata* var. *spicata, S. cilicica, O. vulgare, O. saccatum* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* exhibited fungicidal activity as they completely inhibited mycelial growth at their respective MICs.

Table 4
Impact of different EO concentrations on the mycelial development of *S. sclerotiorum**

Mycelial growth of the fungus on petri dishes containing different plant essential oils										
	Tsp		**Sc**		**Ov**		**Os**		**Tspy**	
Doz µl/petri	**MG (mm)**	**MGI (%)**	**MG (mm)**	**MGI (%)**	**MG (mm)**	**MGI (%)**	**MG (mm)**	**MGI (%)**	**MG (mm)**	**MGI (%)**
0.00	90.0e	0.00	90.0e	0.00	90.0f	0.00	90.0g	0.0	90.0g	0.00
0.25	22.3dA	75.2	85.7dC	4.8	80.7eB	10.37	90.0gD	0.0	90.0gD	0.00
0.50	9.3cA	89.6	68.3cC	24.1	56.0dB	37.78	75.0fD	16.7	83.7fE	7.0
0.75	5.7bA	93.70	42.0bC	53.3	32.7cB	63.70	64.0eD	28.9	72.3eE	19.6
1.00	**0.0aA**	**100.0**	**0.0aA**	**100.0**	15.3bB	82.96	53.7dC	40.4	62.3dD	30.74
2.00	0.0aA	100.0	0.0aA	100.0	**0.0aA**	**100.0**	45.0cB	50.0	51.3cC	43.0
4.00	0.0aA	100.0	0.0aA	100.0	0.0aA	100.0	21.7bB	75.93	30.0bC	66.7
6.00	nt	-	nt		0.0aA	100.0	**0.0a**	**100.0**	**0.0a**	**100.0**
8.0	nt	-	nt		nt	-	0.0a	100.0	0.0a	100.0
10.0	nt	-	nt		nt	-	0.0a	100.0	0.0a	100.0
EC_{50}		**0.14**		**0.63**		**0.58**		**1.5**		**1.9**

MG: Mycelial Growth (mm); MGI: Inhibition of Mycelial Growth (%);Tsp: *T. spicata* var. *spicata*; Sc: *S. cilicica*; Ov: *O. vulgare*; Os: *O. saccatum*; Tspy: *T. sipyleus* subsp. *sipyleus* var. *sipyleus.* Dark-colored inhibition values indicate that the essential oil exhibited fungicidal activity at the tested dose.

* Different lowercase letters next to the mean values in the column indicate statistically significant differences between treatments according to Duncan's Multiple Comparison Test ($P < 0.05$). EC_{50} refers to the effective concentration values (µl/petri) that inhibited 50% of mycelial growth, respectively, as determined by Probit analysis.

As a necrotrophic pathogen, *S. sclerotiorum* is typically controlled using fungicidal treatments. However, due to pesticide residues and the limited efficacy of conventional methods, new strategies to control *S. sclerotiorum* are being researched. Plant extracts and essential oils are biodegradable, have low toxicity and are natural products. They are therefore being investigated as antifungal agents to control plant diseases[28]. The antifungal potential of EOs against *S. sclerotiorum* has been the focus of various research investigations[29,30]. While the antifungal activity of EOs from various *Origanum, Satureja, Thymbra* and *Thymus* species inhibits mycelial growth of the pathogen[17,31,32], the antifungal activity of EOs from *S. cilicica, O. vulgare, O. saccatum* or *T. sipyleus* subsp. *sipyleus* var. *sipyleus* against this fungal pathogen has not been investigated in any study. This study is the first to report on the antifungal activity of the essential oils (EO) of *S. cilicica, O. vulgare, O. saccatum and T. sipyleus* subsp. *sipyleus* var. *sipyleus* against *S. sclerotiorum*, providing new insights into potential biocontrol strategies for the control of soilborne fungal pathogens in agriculture.

Carvacrol is a monoterpenoid phenol known for its broad antimicrobial spectrum, especially for its strong antifungal activity against fungal pathogens[33,34]. The high carvacrol content of the EOs of *S. cilicica* (42.91%) and *T. spicata* var. *spicata* (50.90%) is probably the main reason for their strong antifungal properties. Likewise, citrus EOs demonstrated inhibition of *S. sclerotiorum* mycelial growth by up to 82.91%, with limonene recognized as the primary bioactive compound[35]. In line with these results, significant amounts of monoterpenes, including p-cymene and γ-terpinene, were also detected in our study in addition to carvacrol. These findings align with our results and indicate that EOs with high carvacrol content could be promising candidates for bio fungicide development. Moreover, the EOs from *O. vulgare* subsp. *hirtum*, *O. saccatum*, and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* exhibited antifungal activity at specific concentrations. Notably, *O. vulgare* subsp. *hirtum* completely inhibited mycelial growth (100%) at a dose of 2.00 µL per Petri dish. This oil's primary constituent, carvacrol (88.12%), is well-recognized for its potent antifungal properties[6]. Additionally, the synergistic interactions between γ-terpinene and p-cymene, also present in this essential oil, may have enhanced its antifungal efficacy[36]. These results reinforce the potential application of essential oils as natural bio fungicides in agricultural settings.

Earlier research has demonstrated that the antimicrobial efficacy of EOs depends on the proportion of their primary components[37,38]. In agreement with these results, our study showed that oils with a higher concentration of carvacrol exhibited significantly stronger antifungal properties. This suggests that the phenolic components play a central role in the antimicrobial activity of EOs. Indeed, phenolic compounds such as carvacrol, thymol, p-cymene and γ-terpinene are known to disrupt membrane permeability, resulting in the leakage of intracellular contents an effective mechanism that inhibits the growth of fungal spores and mycelia[39,40]. However, our study revealed that the effects of some EOs on mycelial growth varied depending on the concentration. In particular, *T. sipyleus* subsp. *sipyleus* var. *sipyleus* showed limited antifungal activity at low concentrations but completely inhibited mycelial growth at higher concentrations. This indicates that the antifungal activity of EOs depends on the ratio of their main components as well as on the dosage and the method of application[41].

In conclusion, this research highlights the promising antifungal properties of EOs from *S. cilicica* and *T. spicata* var. *spicata* against the economically significant pathogen *S. sclerotiorum*. To further establish their suitability as biocontrol agents, comprehensive toxicity assessments and field evaluations are necessary. Moreover, the findings suggest that carvacrol-rich EOs may have broader applications in managing other fungal pathogens.

Conclusions

This study showed that the EOs from the five thyme species *O. saccatum, O. vulgare* subsp. *hirtum, S. cilicica, T. spicata* var. *spicata* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* have different degrees of antioxidant and antifungal activity, which is primarily influenced by their chemical composition. GC-MS analysis showed that the oils contain high levels of monoterpenes, especially carvacrol and thymol, which are closely related to their bioactive properties.

Among the species evaluated, *T. spicata* var. *spicata* and *S. cilicica* exhibited the most potent antifungal effects against *S. sclerotiorum*, completely inhibiting growth at relatively low concentrations. These oils also exhibited the greatest TPC and antioxidant capacity, underscoring the significance of phenolic monoterpenoids like carvacrol in their bioactivity.

Results point to the fact that essential oils rich in carvacrol could be promising candidates for the development of environmentally friendly bio fungicides. Their use could contribute to sustainable plant disease control strategies and reduce dependence on synthetic fungicides. It is recommended that further studies be conducted to confirm these findings through field trials and to evaluate the phytotoxic effects and long-term environmental consequences of the oils.

Materials and methods

Plant material and identification. All plant materials used in this study were collected in compliance with institutional, national, and international guidelines on the ethical and sustainable use of plant resources. No endangered or protected species were involved in the research. The plants were collected from the natural vegetation in the Ermenek district of Karaman province, Turkey, during their flowering period. The collected specimens were dried in the shade at room temperature. Detailed information on collection dates, altitudes, and geographic coordinates is provided in Table 1.

The taxonomic identification of plant specimens was carried out jointly by plant systematics experts Prof. Dr. Necattin Türkmen, Prof. Dr. Rüştü Hatipoğlu, and Prof. Dr. Saliha Kırıcı from Çukurova University (Adana, Turkey). Voucher specimens of all five thyme species are deposited in the Herbarium of the Biodiversity Research and Application Center at Karamanoğlu Mehmetbey University and are available for public access upon request. The voucher specimen numbers are as follows: *Origanum saccatum* (KMUB_8017), *Origanum vulgare* subsp. *hirtum* (KMUB_8018), *Satureja cilicica* (KMUB_8019), *Thymbra spicata* var. *spicata* (KMUB_8020) and *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* (KMUB_8021). As none of the species are classified as endangered, their collection was carried out solely for academic purposes, with permission from the university and in accordance with the necessary guidelines for plant collection, including the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

Table 1
Geographical origin, collection times and altitude data for the five thyme species used in the study

Id	Plants	Collection date	Altitudes (m)	Coordinates
1	*Origanum saccatum* P.H. Davis	03.07.2020	1196	36.615265, 32.785533
2	*Origanum vulgare* subsp. *hirtum* (link) letswaart	09.07.2020	672	36.589279, 33.111851
3	*Satureja cilicica* P.H. Davis	01.07.2020	1634	36.775613, 32.888750
4	*Thymbra spicata* L. var. *spicata* L.	26.06.2020	682	36.590448, 33.114877
5	*Thymus sipyleus* subsp. *sipyleus* var. *sipyleus*	02.07.2020	682	36.590448, 33.114877

Essential oil extraction. The essential oils were extracted from the aerial parts of each thyme species using the hydro distillation method. For each sample, 100 grams of freshly harvested plant material were placed in a 1-liter

distillation flask with 500 mL of distilled water. The mixture was subjected to hydro distillation for 3 hours using a Clevenger-type apparatus. After distillation, the oils were collected, dried over anhydrous sodium sulfate to remove residual moisture, and then stored in dark glass vials at 4°C until further chemical and biological analyses were performed[12].

GC-MS analysis of essential oils. A Thermo Scientific ISQ Single Quadrupole GC-MS system (Milan, Italy) fitted with a TR-FAME capillary column that was 60 meters long, 0.25 mm in internal diameter, and had a film thickness of 0.25 µm was used to ascertain the chemical makeup of the essential oils. Five percent phenylpolysilphenylene siloxane made up the stationary phase. The oven's temperature was set to begin at 50°C and rise by 3°C every minute until it reached 220°C. The carrier, which had a steady flow rate of 1 mL/min, was 99.9% pure helium gas. A 1:150 split ratio was used to inject 1 µL of essential oil that had been previously diluted in hexane for each analysis. The ion source and transfer line temperatures were kept at 230°C and 250°C, respectively, while the injector temperature was fixed at 220°C. Using electron ionization at 70 eV, mass spectra were captured in scan mode in the m/z range of 40 to 400. Retention indices and mass spectra were compared to information from the NIST and Wiley libraries, as well as pertinent literature sources[23,42], in order to identify the compounds. For data collecting and processing, Xcalibur software, version 2.1.0 SP1, was used.

Antioxidant activity assay (DPPH). In order to evaluate the essential oils' antioxidant capacity, the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging experiment was used. This process involved mixing 3.9 mL of a freshly made 0.1 mM DPPH solution in methanol with 0.1 mL of each essential oil sample. To avoid any changes brought on by light, the reaction mixtures were then incubated for 30 minutes at 25°C in the dark. After incubation, a UV-Visible spectrophotometer was used to measure the absorbance at 517 nm. The following formula was used to determine the scavenging effect:

% Inhibition = [(A_control – A_test) / A_control] × 100,

Here, Acontrol refers to the absorbance of the DPPH solution without any sample, while Atest denotes the absorbance measured in the presence of the essential oil. The dose-response curve was used to calculate the IC_{50} value, which represents the concentration of EO required to scavenge 50% of free radicals. To guarantee precision and repeatability, every assay was carried out in triplicate.

Quantification of total phenolic content. Using the Folin-Ciocalteu colorimetric test, the total phenolic content (TPC) of the essential oils was measured in accordance with Singleton and Rossi's (43) approach. In the experiment, 1 mL of Folin-Ciocalteu reagent, diluted ten times with distilled water, was mixed with 100 µL of each EO that had previously been diluted ten times with ethanol. 800 µL of sodium carbonate solution (75 g/L) was added to start the reaction after a 4-minute incubation. After that, the reaction mixtures were incubated for two hours at room temperature. A UV-Vis spectrophotometer was used to detect absorbance at 765 nm. A calibration curve was created using standards for gallic acid that ranged from 0 to 500 mg/L. Gallic acid equivalents (GAE) in micrograms per milligram of dry weight were used to express the TPC. To ensure precision and repeatability, every analysis was carried out in triplicate.

Isolation and identification of*Sclerotinia sclerotiorum*. The *S. sclerotiorum* fungal isolate (SsH5) was obtained from a single sclerom found in the stem of infected cucumber plants grown in the Samandağ district of Hatay province using potato dextrose agar (PDA). Streptomycin sulphate (100 µg/ml) was added to the PDA to prevent bacterial contamination. Morphological analysis was used to identify the fungal pathogen[27]. The DNeasy Plant

Mini Kit (Qiagen, GeneMark Technology Co., Valencia) was used to extract genomic DNA in accordance with the manufacturer's instructions in order to precisely identify the fungal isolate. Following the procedure described by White et al. (44), the identity was verified by amplifying the internal transcribed spacer (ITS) region of ribosomal DNA using universal fungal primers ITS1 and ITS4. For the subsequent experimental procedures, *S. sclerotiorum* was obtained by transferring 8 mm diameter mycelial plugs from the actively growing edges of young colonies to Petri dishes containing PDA and subsequent incubation at 24°C for five days.

Antifungal activities assay. The *in vitro* antifungal effect of the EOs of *O. saccatum, O. vulgare* subsp. *hirtum, S. cilicica, T. spicata* var. *spicata* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* on *S. sclerotiorum* was investigated using the mycelial growth inhibition method[45]. Initially, a 5 mm mycelial plug of the fungal isolate was placed onto the center of petri dishes containing potato dextrose agar (PDA). Various concentrations of the EOs (0.00, 0.25, 0.50, 0.75, 1.00, 2.00, 4.00, 6.00, 8.00, and 10.00 µL per dish) were applied in triplicate to the inner surface of each dish lid. As controls, PDA-containing Petri dishes devoid of EO were employed. For five days, every Petri dish was incubated at 24°C. The diameter of fungal colony growth was used to calculate the essential oil's rate of inhibition[30]. The percentage of growth inhibition was determined using the formula below:

$$I = \frac{C-T}{C} x100$$

where: I represent the percentage of mycelial growth inhibition; C is the colony diameter (in mm) on the control plate; and T denotes the colony diameter (in mm) on the treatment plate.

Evaluation of fungistatic and fungicidal effects. The fungicidal or fungistatic effect of EOs on the viability of mycelia at their minimum inhibitory concentrations (MICs) was evaluated by replacing the lids of Petri dishes treated with EOs with sterile lids and incubating them at 25°C for three to four days without sealing them with parafilm. Mycelial discs that showed no growth were then transferred to PDA medium and the antifungal effect was evaluated at the MIC. If no mycelial growth occurred after replacing the lids, the effect was categorized as fungicidal (completely inhibitory and lethal). Conversely, the effect was categorized as fungistatic (transient) if mycelial growth resumed after lid replacement[46].

Statistical analysis. Every treatment was duplicated at least three times in every experiment, which was conducted using a completely randomized design. Total phenolic content, antioxidant activity, and antifungal efficacy were statistically analyzed using one-way ANOVA in SPSS software (version 17.0, SPSS Inc., USA). At a significance level of $p < 0.05$, Duncan's Multiple Range Test was used to find significant differences between treatment means. Additionally, the effective concentration required to achieve 50% inhibit of fungal mycelial growth (EC_{50}) was determined through Probit regression analysis.

Declarations

Competing interests

The authors declare no competing interests.

Supplementary information

No supplementary material is associated with this study.

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Author Contribution

H.M. conceptualized the study and supervised the research. M.O. and S.S. conducted the antifungal activity assays. M.T. contributed to plant collection and essential oil extraction. H.M. and M.O. wrote the manuscript with input from all authors. All authors reviewed and approved the final manuscript.

Data Availability

The datasets generated and/or analyzed during the current study are available in the NCBI GenBank repository under the accession number PV652757 (https://www.ncbi.nlm.nih.gov/nucleotide/PV652757.1). Additional data are available from the corresponding author upon reasonable request.

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Figures

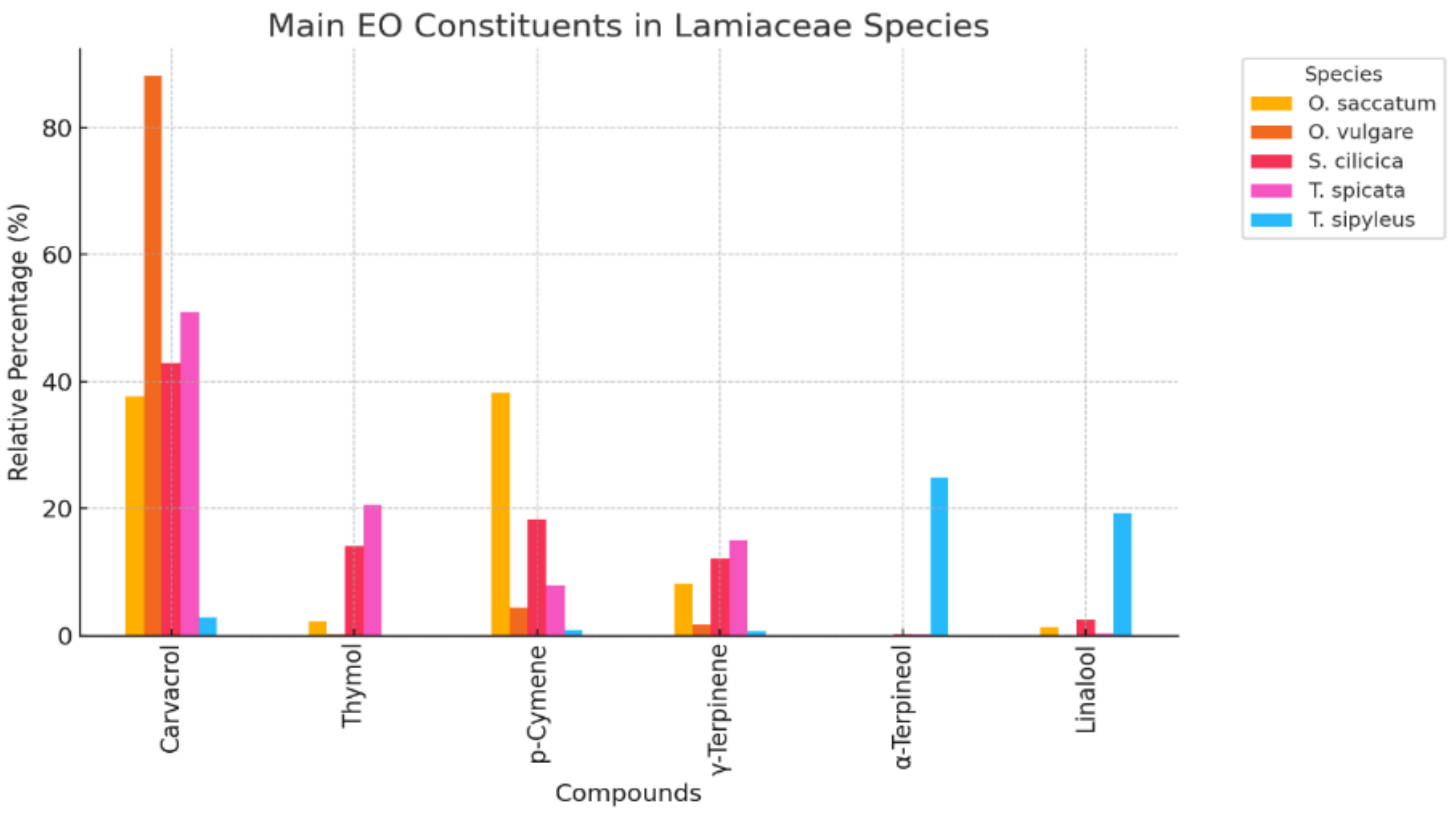


Figure 1

Distribution of main components identified in the EOs of the examined thyme species

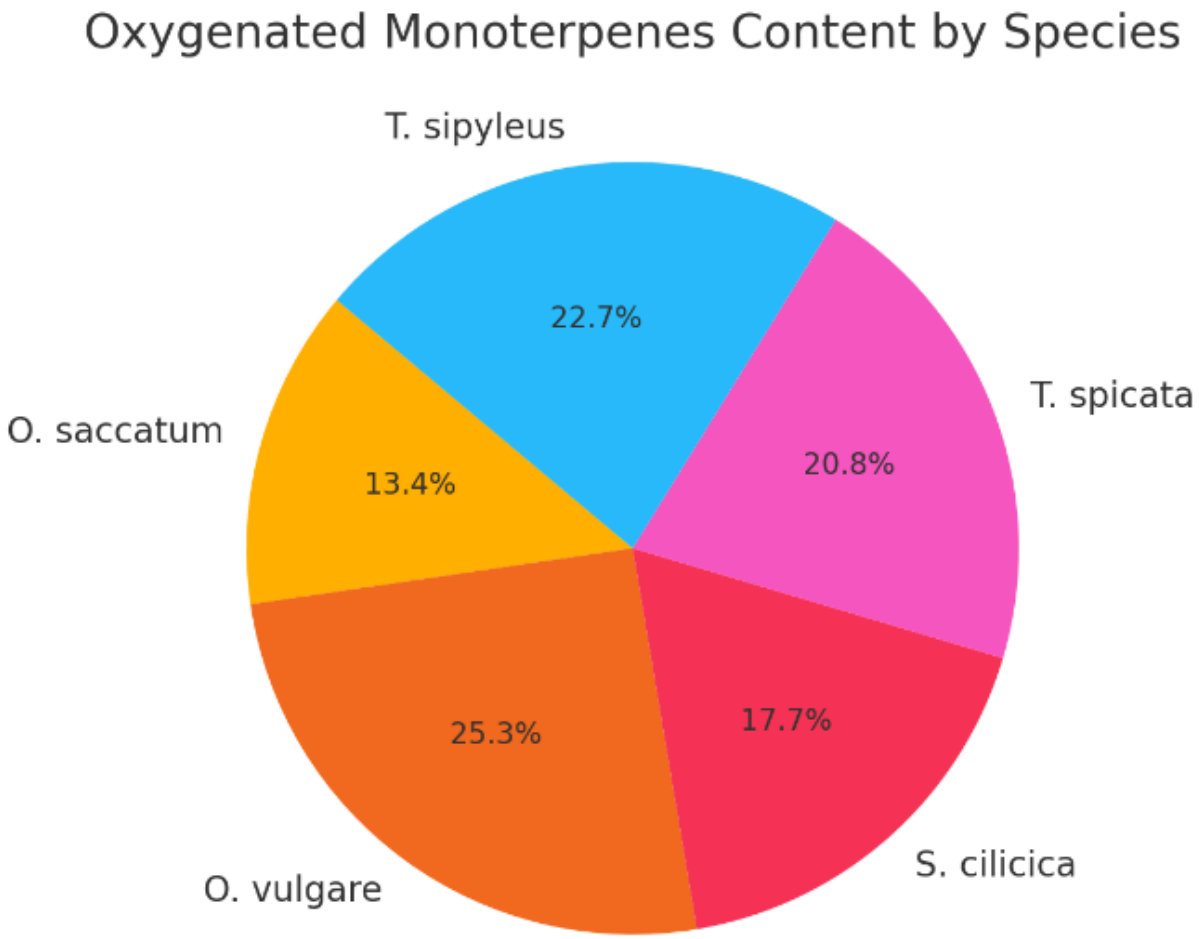


Figure 2

Comparative representation of major compound groups in EOs from five thyme species

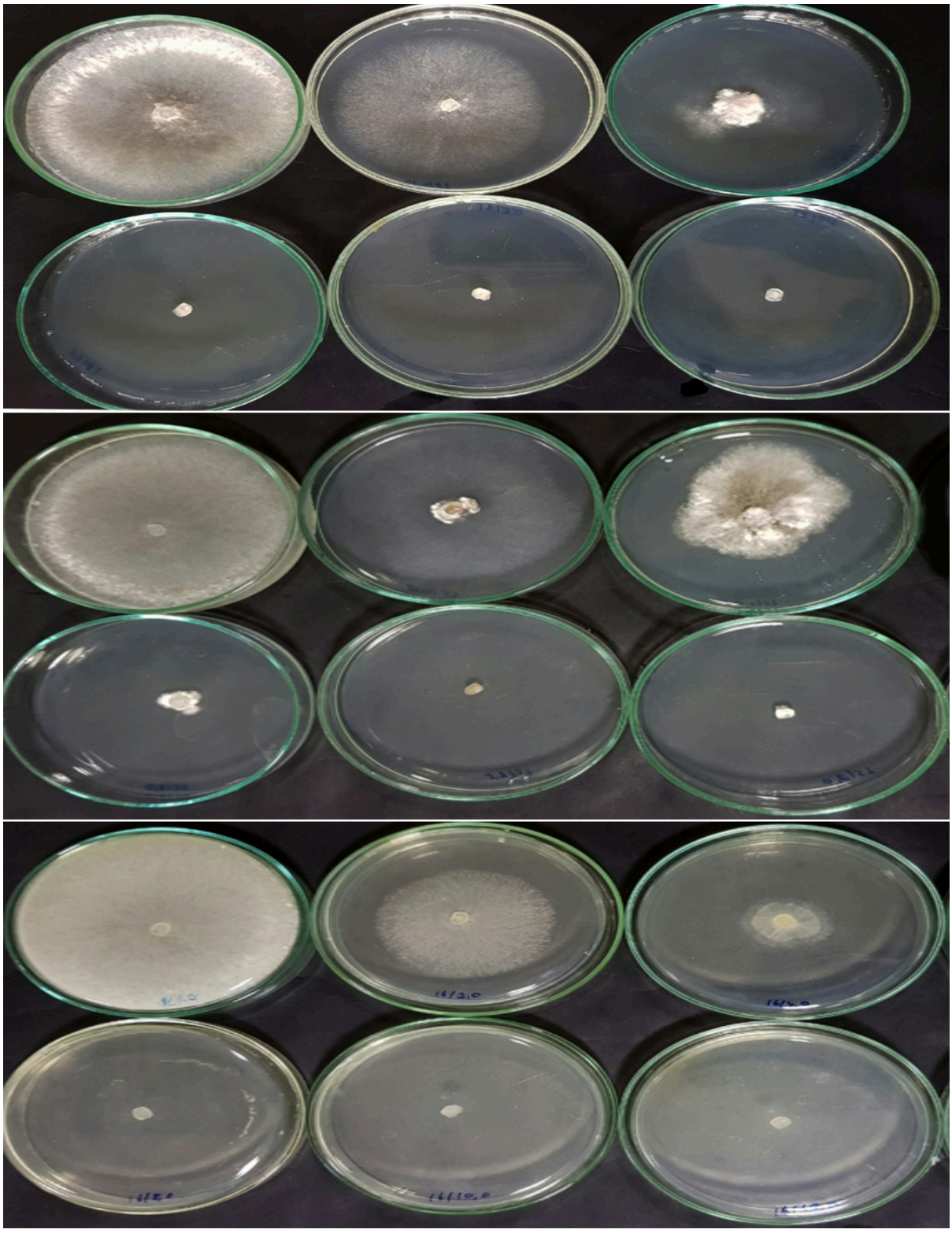


Figure 3

Inhibitory effects of varying concentrations of plant-derived EOs on the mycelial growth of *S. sclerotiorum* in the vapor phase. Mycelial growth inhibition is indicated by arrows in Petri dishes treated with different doses of (A) *S. cilicica*(0.0, 0.50, 0.75, 1.0, 2.0, 4.0 µL), (B) *O. vulgare* subsp. *hirtum* (0.0, 0.50, 0.75, 1.0, 2.0, 4.0 µL), and (C) *O. saccatum* EOs (0.0, 2.0, 4.0, 6.0, 8.0, 10.0 µL).