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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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This study investigated the phytochemical profile, antioxidant properties, and antifungal activity of essential oils (EOs) from five thyme species growing wild in Turkey: *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 essential oils (EOs) were extracted using the hydro distillation technique. Gas chromatography–mass spectrometry (GC–MS) was used to identify the volatile compounds contained in the oils. The antioxidant potential of each sample was determined by its ability to scavenge DPPH free radicals, while the total phenolic content was quantified using the Folin-Ciocalteu assay. The antifungal potential of the oils against *Sclerotinia sclerotiorum* (Lib.) de Bary, a soil-borne plant pathogen, was evaluated through vapour-phase exposure at different concentrations. GC–MS results showed that the predominant constituents of the essential oils were phenolic monoterpenes, in particular carvacrol and thymol. Among the species analyzed, *O. vulgare* subsp. *hirtum* had the highest carvacrol content, reaching 88.12%. Among the oils tested, *T. spicata* L. var. *spicata* showed the highest antioxidant capacity, as shown by its low IC_{50} value of 10.698 mg L^{-1}, and was also rich in phenolic compounds, containing 4.771 µg mg^{-1}. In addition, the EOs of *S. cilicica* and *T. spicata* L. var. *spicata* completely inhibited the growth of fungal mycelia at a dose of 1.0 µL/Petri dish, indicating strong antifungal activity. The EC_{50} values were between 0.14 and 1.90 µL/Petri dish, with *T. spicata* L. var. *spicata* showing the greatest efficacy. In summary, the results emphasise the potential of thyme-derived EOs especially those rich in carvacrol as an environmentally friendly and natural substitute for chemical fungicides in the control of fungal infections in plants.

Keywords Antifungal activity, Antioxidant activity, Essential oils, GC–MS analysis, *Sclerotinia sclerotiorum*

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 favourable Mediterranean climate and unique geographical location. Türkiye is considered a major centre of endemism for the family, which comprises around 45 genera and over 740 taxa, almost half of which are endemic[1,2]. Many members of this family are characterised by their rich composition of EOs and secondary metabolites, leading to their widespread use in traditional healing practises 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, especially 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 studied, *O. saccatum* P.H. Davis is endemic to the Alanya region, while *O. vulgare* subsp. *hirtum* (Link) Ietswaart occurs throughout Marmara and Thrace. Both *S. cilicica* P.H. Davis and *T. spicata* L. var.

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spicata L 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 important agricultural crops such as 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]. The problem is exacerbated by the increasing resistance to fungal diseases worldwide, which reduces the effectiveness of conventional fungicides and poses a serious challenge for plant protection. This increasing resistance emphasises the urgent need for innovative, environmentally friendly alternatives that enable effective disease control without contributing to the development of resistance. Consequently, attention has shifted to plant-based alternatives such as essential oils as these have the potential to act as biodegradable, environmentally friendly bio fungicides.

While the antifungal activity of EOs from certain thyme species has already been investigated, only limited information is available on the activity of EOs obtained specifically from *O. saccatum, S. cilicica, T. spicata* L. var. *spicata, T. sipyleus* subsp. *sipyleus* var. *sipyleus* and *O. vulgare* subsp. *hirtum* against *S. sclerotiorum*.

The aim of this work was to investigate the chemical constituents of the EOs of five different thyme species, two of which are endemic to Türkiye, and to evaluate their antioxidant properties and antifungal activity against *S. sclerotiorum*, the causative agent of cucumber stem rot, in vitro. 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 of *O. saccatum*, *O. vulgare* subsp. *hirtum*, *S. cilicica*, *T. spicata* L. var. *spicata*, and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* were determined by GC–MS. The EO contents varied significantly between the species and ranged from 0.80 to 4.12% (v w^{-1}). The highest yield was obtained from *O. vulgare* subsp. *hirtum* (4.12%), while the lowest yield was recorded for the endemic species *O. saccatum* (0.80%). *T. spicata* L. var. *spicata* (3.04%) and *S. cilicica* (2.44%) also had relatively high EO contents, while *T. sipyleus* subsp. *sipyleus* var. *sipyleus* had a moderate yield of 1.24%. These differences in EO yield may be attributed to genetic differences, ecological factors such as altitude and microclimate, and differences in glandular trichome density, which have been reported to strongly influence essential oil productivity in Lamiaceae species. The analysis revealed different chemotypes among the species, which were dominated by monoterpenes such as carvacrol, p-cymene and γ-terpinene as well as oxygenated monoterpenes such as α-terpineol and linalool. Table 1 shows the comprehensive chemical composition and classification the EO components.

The most important 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* L. var. *spicata* EO contained carvacrol (50.9%), thymol (20.59%), γ-terpinene (14.98%) and p-cymene (7%)0.89%); *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 abundant substances in thyme EOs are oxygenated monoterpenes (47.89–90.42%) (Table 1). 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* L. var. *spicata* (74.15%) and *S. cilicica* (63.48%) EOs. The lowest content of oxygenated monoterpenes (47.89%) was found in *O. saccatum* EO. 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* (49.38%) (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 found 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 related to various factors such as the geographical origin of the plant, soil characteristics, age and the specific part of the plant used for extraction[12]. These results suggest that both genetic make-up and environmental conditions significantly influences the composition of the EOs of the species studied, possibly affecting their antifungal activities.

Antioxidant activity

The EOs extracted from five thyme species were analysed for their antioxidant activity using the DPPH test and for their TPC values using the Folin–Ciocalteu method. The detailed results can be found in Table 2. Of the analyzed species, the highest antioxidant potential was observed in *T. spicata* L. var. *spicata* (IC_{50} = 10.698 mg L^{-1}), 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 EOs corresponded to their ability to scavenge free radicals, confirming that a higher phenolic concentration generally leads to a stronger antioxidant activity.

Total phenolic content

Significant interspecies was observed in the TPC of EOs, with values ranging from 2.235 to 4.771 µg mg^{-1}. *T. spicata* L. var. *spicata* had the highest phenol concentration (4.771 µg mg^{-1}), closely followed by *O. saccatum*

No	RT	RI	RI_L*	Compounds	Os	Ov	Sc	Tss	Tspy
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
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					47.89	90.53	63.48	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 (%)					98.80	99.53	98.96	99.56	95.45
Essential oil content (%)					0.80	4.12	2.44	3.04	1.24

Table 1. Main constituents (%) and chemical groupings of the EOs obtained from five thyme species. RT, Retention time; RI, Retention index; RIL, Retention indices that were calculated in different literatures; nd, Not detected; *, Babushok et al.[23]; Os, O. saccatum P.H. Davis; Ov: O. vulgare subsp. hirtum (link) Ietswaart; Sc, S. cilicica P.H. Davis; Tss, T. spicata L. var. spicata L; Tspy, Thymus sipyleus subsp. sipyleus var. sipyleus

(4.692 μg mg^{-1}), while *T. sipyleus* subsp. *sipyleus* var. *sipyleus* had the lowest (2.235 μg mg^{-1}). These results are consistent with the results of antioxidant activity, which demonstrate a positive relationship between phenolic content and free radical scavenging ability. Phenolic compounds such as carvacrol and thymol probably contribute most to this activity due to their known redox properties and their ability to donate hydrogen.

These results emphasize the importance of certain phenolic compounds, particularly thymol and carvacrol, which exhibit strong antioxidant activity through mechanisms such as hydrogen atom donation and electron transfer. The significantly higher activity observed in *T. spicata* L. var. *spicata* L and *O. saccatum* P.H. Davis 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 ecological, geographical and genetic factors that influence the biosynthesis of EOs[26]. In this context, the role of terpenoid structures,

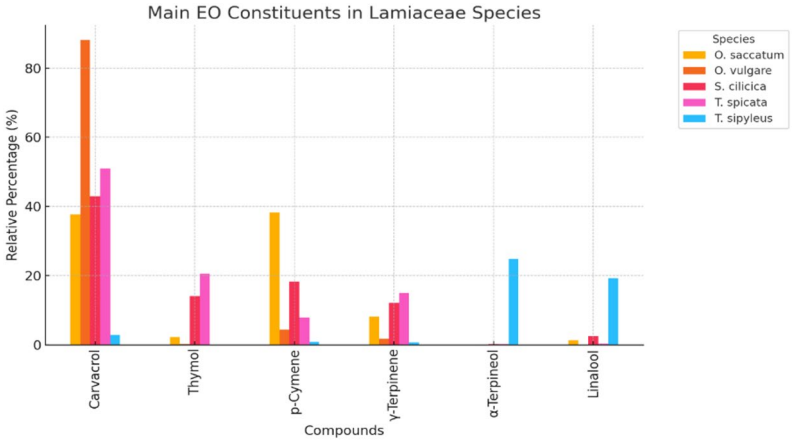

Fig. 1. Distribution of the main components identified in the EOs of the analysed thyme species.

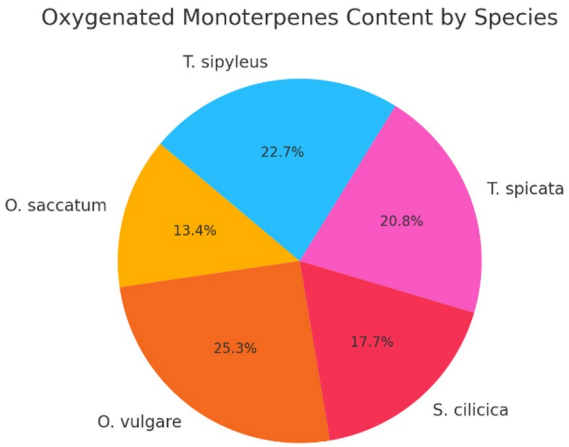

Fig. 2. Comparative representation of the most important compound groups in EOs of five thyme species.

Essential oils	Radical scavenging activity ($IC_{50,}$ mg L^{-1})	Phenolic contents (µg mg^{-1})
O. saccatum P.H. Davis	17.350	4.692
O. vulgare subsp. *hirtum* (link) Ietswaart	24.682	3.760
S. cilicica P.H. Davis	39.884	3.512
T. spicata L. var. *spicata* L	10.698	4.771
Thymus sipyleus subsp. *sipyleus* var. *sipyleus*	46.220	2.235

Table 2. DPPH radical scavenging capacity and TPC of thyme EOsimproving oxidative stability in both biological systems and foods.

including monoterpenes and phenolic monoterpenoids, should not be overlooked as these structures play an important role in improving oxidative stability in both biological systems and foods.

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 performed according to the protocol described 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 molecular identity. For antifungal testing, *S. sclerotiorum* was subcultured by transferring 8 mm mycelial plugs taken from the edges of actively growing colonies to PDA plates, incubated at 24 °C for five days. After this period, the isolate exhibited the characteristic white, cotton-like 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 EOs against 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* P.H. Davis, *T. spicata* L. var. *spicata* L and *T. sipyleus* subsp. *sipyleus* var. *sipyleus*) had fungistatic properties that allowed the pathogen to recover and grow again when transferred to a fresh medium.

Antifungal activities of essential oils on mycelial growth of fungal disease agent

The antifungal activities of the EOs against *S. sclerotiorum* were evaluated in the vapour phase. All five EOs showed an inhibitory effect on mycelial growth on PDA medium (Fig. 3). In particular, the EOs of *S. cilicica* and *T. spicata* L. var. *spicata* showed the strongest antifungal effect, with minimum inhibitory concentrations (MICs) of 1.0 µL per Petri dish (Fig. 3A). In addition, the EOs of *O. vulgare* subsp. *hirtum* at 2.0 µL per Petri dish (Fig. 3B), *O. saccatum* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* at 6.0 µL per Petri dish (Fig. 3C) completely suppressed mycelial growth of the pathogen. The statistical analysis showed significant differences between different concentrations of the same essential oil as well as between different EOs at identical concentrations (Table 3).

The EC_{50} value, which indicates the concentration required to inhibit 50% of the fungal mycelium growth, was calculated using probit analysis (Table 3). The EO of *T. spicata* L. var. *spicata* showed the lowest EC_{50} value

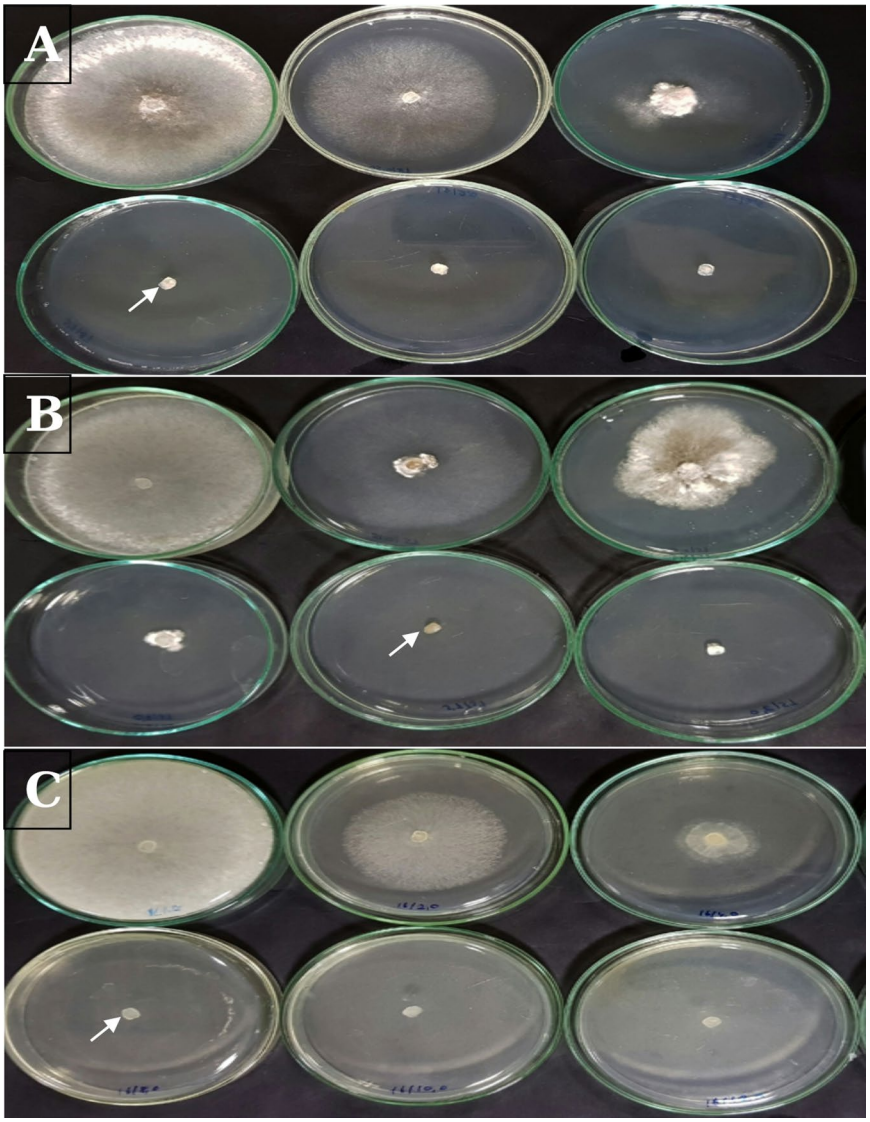

Fig. 3. Inhibitory effect of different concentrations of plant EOs on mycelial growth of *S. sclerotiorum* in the vapour phase. Arrows indicate the complete inhibition of mycelial growth in Petri dishes treated with different doses of (**A**) *S. cilicica* (0.0, 0.50, 0.75, 1.0, 2.0, 4.0 µL), (**B**) *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).

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

Table 3. Impact of different EO concentrations on the mycelial development of *S. sclerotiorum*. MG, Mycelial Growth (mm); MGI, Inhibition of Mycelial Growth (%); Os: *O. saccatum*; Ov: *O. vulgare* subsp. *hirtum* (link) Ietswaart; Sc: *S. cilicica* P.H. Davis; Tss: *T. spicata* L. var. *spicata* L; Tspy: *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* Darkcolored 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.

at 0.14 µL per Petri dish, while *T. sipyleus* subsp. *sipyleus* var. *sipyleus* showed the highest EC_{50} value at 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* L. var. *spicata, S. cilicica, O. vulgare* subsp. *hirtum, O. saccatum* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* exhibited fungicidal activity as they completely inhibited mycelial growth at their respective MICs.

As a necrotrophic pathogen, *S. sclerotiorum* is usually controlled with 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 for the control of plant diseases[28]. The antifungal potential of EOs against *S. sclerotiorum* has been the focus of various research studies[29,30]. While EOs from various *Origanum*, *Satureja*, *Thymbra*, and *Thymus* species have been shown to inhibit mycelial growth of *Sclerotinia sclerotiorum*[17,31,32], the antifungal activity of EOs from *S. cilicica, O. vulgare* subsp. *hirtum, O. saccatum* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* was not investigated. This study is the first to report on the activity of these EOs against S. sclerotiorum and provides new insights into environmentally friendly biocontrol strategies for the management of soilborne fungal pathogens in agriculture. Given the challenges associated with conventional control methods, including pesticide residues and limited efficacy[17,33], research into plant-based alternatives such as essential oils becoming increasingly important.

Carvacrol is a monoterpenoid phenol known for its broad antimicrobial spectrum, especially for its strong antifungal activity against fungal pathogens[34,35]. The high carvacrol content of the EOs of *S. cilicica* P.H. Davis (42.91%) and *T. spicata* L. var. *spicata* L (50.90%) is probably the main reason for their strong antifungal properties. The EO of citrus fruits also showed inhibition of mycelial growth of *S. sclerotiorum* by up to 82.91%, with limonene recognised as the major bioactive compound[36]. Consistent with these results, our study also detected significant amounts of monoterpenes, including p-cymene and γ-terpinene, addition to carvacrol. These findings are consistent with our results and suggest that EOs with high carvacrol content could be promising candidates for the development of bio fungicide. In addition, the EOs of *O. vulgare* subsp. *hirtum*, *O. saccatum* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* showed antifungal activity at certain concentrations. In particular, *O. vulgare* subsp. *Hirtum* inhibited mycelial growth completely (100%) at a dose of 2.00 µL per Petri dish. The main component of this EO, carvacrol (88.12%), is known for its strong antifungal properties[6]. Morever, the synergistic interactions between γ-terpinene and p-cymene, also present in this essential oil, may have enhanced its antifungal activity[37]. These results support the potential application of essential oils as natural bio-fungicides in agricultural.

Previous research has shown that the antimicrobial efficacy of EOs depends on the proportion of their primary constituents[38,39]. In line with these findings, 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, leading to the leakage of intracellular contents-an effective mechanism that inhibits the growth of fungal spores and mycelia[40,41]. However, our study showed that the effects of some EOs on mycelial growth varied depending on the concentration. In particular,

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

Table 4. Geographical origin, collection times and altitudes for the five thyme species used in the study.

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[42].

To summarise, this study highlights the promising antifungal properties of EOs from *S. cilicica* P.H. Davis and *T. spicata* L. var. *spicata* L against the economically important pathogen *S. sclerotiorum*. To further establish their suitability as biocontrol agents, comprehensive toxicity evaluations and field trials are required. In addition, the results suggest that carvacrol-rich EOs may have broader application in the control of other fungal pathogens.

Conclusions

This study has shown that the EOs of the five thyme species *O. saccatum* , *O. vulgare* subsp. *hirtum, S. cilicica, T. spicata* L. var. *spicata* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* exhibit 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.

Of the species tested, *T. spicata* L. var. *spicata* and *S. cilicica* Eos showed the strongest antifungal activity against *S. sclerotiorum* and completely inhibited growth at relatively low concentrations. These EOs also exhibited the highest TPC and antioxidant capacity, emphasizing the importance of phenolic monoterpenoids such as carvacrol for their bioactivity.

The results suggest that EOs 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. Further studies are recommended to confirm these results through field trials and to evaluate the phytotoxic effects and long-term environmental impact of the EOs.

Materials and methods

Plant material and identification

All plant materials used in this study were collected in accordance with institutional, national and international guidelines on the ethical and sustainable use of plant resources. No endangered or protected species were included in the research. The plants were collected during their flowering period in the natural vegetation in Ermenek district of Karaman province, Turkey. The collected specimens were dried in the shade at room temperature. Detailed information on the collection dates, altitudes and geographical coordinates can be found in Table 4.

The taxonomic identification of the plant specimens was carried out jointly by the plant systematists 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 of Karamanoğlu Mehmetbey University and are publicly accessible on request. The voucher specimen numbers are as follows: *O. saccatum* P.H. Davis (KMUB_8017), *O. vulgare* subsp. *hirtum* (link) Ietswaart (KMUB_8018), *S. cilicica* P.H. Davis (KMUB_8019), *T. spicata* L. var. *spicata* L (KMUB_8020) and *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* (KMUB_8021). As none of the species are classified as endangered, they were collected solely for academic purposes, with the permission of the University and in accordance with the necessary guidelines for collecting plant, including the IUCN Guidelines for Research on Endangered Species and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES).

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 grammes of freshly harvested plant material was placed in a 1 L distillation flask containing 500 mL of distilled water. The mixture was hydrodistilled in a Clevenger-type apparatus for 3 h. After distillation, the EOs were collected, dried over anhydrous sodium sulphate 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 EOs

A Thermo Scientific ISQ Single Quadrupole GC–MS system (Milan, Italy) equipped with a TR-FAME capillary column with a length of 60 m, an internal diameter of 0.25 mm, and a path length of 0.25 µm was used to determine the chemical composition of the EOs. The stationary phase consisted of five percent phenylpolysilphenylene siloxane. The temperature of the oven was set to start at 50 °C and increase by 3 °C every minute until it reached 220 °C. The carrier medium with constant flow rate of 1 mL min^{-1} was 99.9% pure helium gas. For each analysis, 1 µL of EO, previously diluted in hexane was injected at a ratio 1:150. The temperature of the ion source and transfer line was maintained at 230 °C and 250 °C respectively, while the temperature of the injector was fixed at

220 °C. Using electron ionisation at 70 eV, mass spectra were recorded in scan mode in the m z^{-1} range from 40 to 400. Retention indices and mass spectra were compared with information from the NIST and Wiley libraries and relevant literature sources[23,43], to identify the compounds. Xcalibur software, version 2.1.0 SP1, was used for data acquisition and processing.

Antioxidant activity assay (DPPH)

To evaluate the antioxidant capacity of the EOs, the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging experiment was performed. For this purposes 3.9 mL of a freshly prepared 0.1 mM DPPH solution in methanol was mixed with 0.1 mL of each EO sample. To avoid light-induced changes, the reaction mixtures were then incubated for 30 min at 25 °C in the dark. After incubation, the absorbance was measured at 517 nm using a UV–Visible spectrophotometer. The following formula was used to determine the scavenging effect:

$$\text{In the \% inhibition formula}: \left[\left(A_{control} - A_{test}\right)/A_{control}\right] \times 100$$

A control here refers to the absorbance of the DPPH solution without any sample, while A test refers to 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 the free radicals. To ensure precision and repeatability, each test was performed in triplicate.

Quantification of total phenolic content

In addition to the detailed volatile composition determined by GC–MS, quantification of the total phenolic content (TPC) was included to assess the contribution of the non-volatile phenolic compounds to the antioxidant activity of the essential oils. Using the Folin-Ciocalteu colourimetric test, the total phenolic content (TPC) of the essential oils was measured according to the approach of Singleton and Rossi's[44]. In the experiment, 1 mL of Folin-Ciocalteu reagent, diluted ten times with distilled water was mixed with 100 µL of each EO previously diluted ten times with ethanol. A volume of 800 µL sodium carbonate solution (75 g L^{-1}) was added to start the reaction after incubation for 4 min. The reaction mixtures were then incubated for two hours at room temperature. A UV–Vis spectrophotometer was used to measure the absorbance at 765 nm. A calibration curve was established with standards for gallic acid ranging from 0 to 500 mg L^{-1}. Gallic acid equivalents (GAE) in micrograms per milligramme of weight were used to express the TPC. To ensure precision and repeatability, each analysis was performed in triplicate.

Isolation and identification of *Sclerotinia sclerotiorum*

The *S. sclerotiorum* fungal isolate (SsH5) was obtained from a single sclerotium 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^{-1}) was added to the PDA to prevent bacterial contamination. Morphological analysis was performed to identify the fungal pathogen[27]. The DNeasy Plant Mini Kit (Qiagen, GeneMark Technology Co., Valencia) was used to extract genomic DNA according to the manufacturer's instructions to accurately identify the fungal isolate. Following the procedure described by White et al.[45], identity was verified by amplification of the internal transcribed spacer (ITS) region of ribosomal DNA using the universal fungal primers ITS1 and ITS4. For the antifungal activity tests, a pure culture of *S. sclerotiorum* was obtained by transferring 8 mm diameter mycelial plugs from the actively growing edges of young colonies into Petri dishes containing PDA and then 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* L. var. *spicata* and *T. sipyleus* subsp. *sipyleus* var. *sipyleus* on *S. sclerotiorum* was investigated using the mycelial growth inhibition method[46]. First, a 5 mm mycelial plug of the fungal isolate was placed in the centre of Petri dishes containing potato dextrose agar (PDA). Different 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 dropped onto the sterile filter paper discs (5 mm diameter) and added to the inside of each Petri dish lid using a micropipette. The sides of the Petri dish were covered with Parafilm (2–3 layers), inverted (lid down) and incubated in an incubator (VELP, 220I) at 24 °C. Petri dishes containing PDA without EO were used as controls. Each Petri dish was incubated at 24 °C for five days. The diameter of growth of the fungal colonies was used to calculate the inhibition rate of the EO[30]. The percentage of growth inhibition was determined following the formula:

$$Inhibition\,(\%) = \frac{CFG - TFG}{TFG} \times 100$$

Where C_{FG} = Fungal Growth in Control Petri dishes, and T_{FG} = Fungal Growth in essential oil Treated Petri dishes.

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 previously treated with EOs with sterile lids without EOs and incubating them for four days at 24 °C without sealing them with parafilm. Mycelial discs that showed no growth were then transferred to PDA medium and the antifungal effect was evaluated by MIC. If no mycelial growth occurred after the lids were replaced, the effect was classified as fungicidal (completely inhibitory and

lethal). Conversely, the effect was classified as fungistatic (transient) if mycelial growth resumed after the lids were replaced[47].

Statistical analysis

Each treatment was duplicated at least three times in each experiment using a completely randomised design. Total phenolic content, antioxidant activity, and antifungal efficacy were statistically analysed using a 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 the treatment. In addition, the effective concentration required to achieve 50% inhibition of fungal mycelium growth (EC_{50}) was determined by Probit regression analysis.

Data availability

The data sets generated and/or analysed in the current study are available in the NCBI GenBank Repository under the access number PV652757 (https://www.ncbi.nlm.nih.gov/nucleotide/PV652757.1). Further data are available on request from the corresponding author.

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Author contributions

H.M. designed the study and supervised the research. M.O. and S.S. carried out the antifungal activity tests. M.T. contributed to the plant collection, essential oil extraction and chemical analysis. H.M. and S.S. wrote the manuscript with the collaboration of all authors. All authors reviewed and approved the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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