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Determination of chemical component of essential oil of *Origanum dubium* plants grown at different altitudes and antifungal activity against *Sclerotinia sclerotiorum*

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Abstract

The aim of this study was to determine effect of altitude on the yields, composition, and antifungal activities of the essential oil isolated from *Origanum dubium* plant populations collected at four different altitudes. The essential oil yield, the content of four compounds and antifungal activities against *Sclerotinia sclerotiorum* were found to vary in relation to the different altitudes examined. According to the GC-MS analysis, carvacrol (46.51%–85.65%), linalool (0.24–48.37), *p*-cymene (1.89%–7.89%), and γ-terpinene (0.28%–6.31%) were determined as major compounds of essential oils of the plants collected from different altitudes. Altitude seems one of the factors that affected the quantity and quality of the essential oil of *O. dubium*. A significant correlation between the amount of carvacrol, *p*-cymene, and antifungal activities was determined. The essential oil of plant having the highest content of carvacrol (85.65%) showed the strongest antifungal activity against *S. sclerotiorum* at relatively very low concentration used (0.75 μl petri^{-1}).

Practical applications

The essential oil yields, the chemical compositions and antifungal activities against *Sclerotinia sclerotiorum* were found to vary in relation to the altitudes where plants grown. Since the yield, chemical composition, and biological activities was affected, altitude seems to be an important factor that should be considered. To the best of our knowledge, this is the first study reporting antifungal activities of essential oil of *Origanum dubium* against devastating plant fungal disease agent *S. sclerotiorum*.

1 | INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary, as soil borne and post-harvest fungal disease agent, causes white mold disease on more than 400 plant species including important vegetable plants and fruit grown worldwide (Boland & Hall, 1994; Bolton et al., 2006; de Aguiar et al., 2014). Disease agent causes significant yield loses on various economically important crops including tomato, which is one the most extensively produced and consumed vegetables in all over the world (Lu, 2003). Typical disease symptoms on tomato plants growing in field conditions occur on stems, crowns, and leaves as water-soaked lesions that expand and become necrotic lesions with white cottony fungal mycelium. Pathogen produce irregular, black overwintering structures called sclerotia inside the stems, crowns and soil around the infected plant roots (Bolton et al., 2006; Smolinska & Kowalska, 2018).

Since the pathogen has broad host range infects and great capacity to survive under different environmental conditions, disease management of white rot is very difficult (Bolton et al., 2006; Ferreira et al., 2019). The major methods of controlling white mold disease are applying fungicides and crop rotation in some host plants. Extensive chemical fungicides, which are expensive and not environmentally safe (Lu, 2003), has led to adverse effects to humans and animals, contamination to the environment (Damalas

& Eleftherohorinos, 2011) and the development of resistant isolates of *S. sclerotiorum* (Firoz et al., 2016; Lehner et al., 2015; Liu et al., 2018). Resistant tomato hybrids to disease agent are also not available to growers (Bolton et al., 2006; de Aguiar et al., 2014). The limited effectiveness of fungicides used to control disease agent, the development of resistant isolates against the fungicides used, the side effects of fungicides on nontarget beneficial microorganisms reveal the necessity of researching alternative methods to chemical control with this disease. Recent studies have focused on natural plant-derived essential oils and their possible applications for controlling crop pests and diseases (Bozkurt et al., 2020; Sertkaya et al., 2010; Soylu et al., 2010). The antifungal activities of essential oils, extracts, and main components from a wide range of aromatics have been investigated against plant disease agents (Bakkali et al., 2008).

The number of taxa in the flora of Turkey is 11,707 according to recent studies. Turkey is an important gene center of the plant species belonging to *Lamiaceae* family. *Origanum*, *Thymus*, *Thymbra*, *Satureja*, *Coridothymus*, which are collectively known as thyme, are the most commonly known and widespread genera among the *Lamiaceae* species found in Turkey (Maral & Kırıcı, 2018). The *Origanum* genus comprises about 38 different species indigenous to the Mediterranean countries (Karioti et al., 2006). Recently, a new basis for taxonomic uncertainties related to the *Majorana* section was reported (Lukas et al., 2013). According to molecular, morphological, and phytochemical data, the taxonomic status of *O. onites*, *O. syriacum*, *O. dubium*, and *O. majorana* were evaluated and evolutionary relationships were discussed in the *Majorana* section (Lukas et al., 2010). It was concluded that "cymyl" chemotype of *Origanum majarona* L. was renamed as *O. dubium* Boiss (Kaplan et al., 2019; Lukas et al., 2013). *O. dubium* is known as one of the economically important wild grown oregano species in Turkey. The most important feature of this oregano species is its high essential oil yield content (6%–8%). There are several genotypes and chemotypes such as high carvacrol and high linalool types within the wild populations of *O. dubium*. Since, natural populations have decreased with each passing year cultivation of *O. dubium* appears to be the most suitable way for the preservation of wild populations and the production of stable product such as essential oil (Kaplan et al., 2019; Turgut et al., 2017). In previously published literatures, yield (ratio), chemical composition, and biological activities of essential oils were reported to be affected by several factors including genotype, growing conditions, environmental conditions such as altitude, climate, topography, soil structure, and harvesting time (Demasi et al., 2018). Therefore, it is interesting to study the chemical composition and biological activities of essential oils obtained from plants grown at different geographical areas in order to choose the optimum source of possible antimicrobial substance. Although, chemical composition of the essential oils of this plant species has already been reported (Kaplan et al., 2019), no information is available about environmental factor impact on the quantity, quality and antifungal activities of this plant.

The objectives of this study were to compare the essential oil yield, chemical compositions and antifungal activities of *O. dubium* plants collected from four different altitudes against fungal disease agent *S. sclerotiorum*.

2 | MATERIALS AND METHODS

2.1 | Plants

O. dubium plant samples used in the study were collected from four different Natural Flora (at least 30 plants from each altitude) during summer 2020 in Ermenek District of Karaman province of Turkey. The locations, date, altitudes, and coordinates of the plants are given in Table 1. The identification of the plants was confirmed by Prof. Dr. Saliha Kırıcı (Çukurova University, Adana, Turkey). Voucher specimens were deposited at the BİSAK Herbarium Center of Hatay Mustafa Kemal University (MKUBK-H10-H13).

2.2 | Isolation of essential oils

Plant leaves, shoots, and flowers from three different air-dried plant material lots were used separately for essential oil isolation. For each lot, 100 g of air-dried and powdered flowers and leaves of plants from each locations were separately placed in a 2,000 ml round-bottom distillation flask and 1,000 ml of distilled water added (Taraj et al., 2019). The essential oils were then obtained by water-distillation for 3 hr by using a Clevenger type apparatus (Ildam, Ankara, Turkey) according to European Pharmacopoeia method (1997). Percentage essential oil yield was calculated according to dry weight of plant materials and amount of essential oils obtained. The obtained essential oil samples (n = 3) were dried over anhydrous sodium sulfate and stored in amber vials at +4°C (Türkmen & Mert, 2020).

TABLE 1 The location, collection date, altitudes, and coordinates of *Origanum dubium* plants used in this study

Type Id	Location	Collection date	Altitudes (m)	Coordinates
Od10	Murande	14.07.2020	467	36.597525, 33.029669
Od11	Kayaönü	14.07.2020	1,096	36.620039, 33.031999
Od12	Kazancı	16.07.2020	1,331	36.485255, 32.861702
Od13	Sarı Vadi	16.07.2020	1,478	36.546102, 32.842137

2.3 | GC-MS analyses

The analysis of the main components of the essential oils of each samples were performed using Thermo Scientific ISQ Single Quadrupole Gas Chromatography-Mass Spectrometry device (Milan, Italy) fitted with TR-FAME capillary column (5% Phenyl Polysilphenylene-siloxane, 60 m × 0.25 mm i.d. × 0.25 μm film thickness). Helium (99.9%) was used as the carrier gas at a flow rate of 1 ml/min. Mass spectra were recorded at 70 eV, the mass range was from 1.2 to 1,200 *m/z*. Scan Mode was used for data collection. MS transfer line, ionization source and the injection port temperatures were 250, 230, and 220°C, respectively. The samples were injected with a 150 split ratio. The injection volume was 1 μl. The oven temperature was initially 50°C, increased to 220°C at a rate of 3°C min^{-1}. The major components of essential oils were tentatively identified on the basis of comparison of their GC Kovats retention indices (RI). The RI were calculated for all volatile constituents using a homologous series of *n*-alkanes C_8-C_{40} injected under the same chromatographic conditions (Babushok et al., 2011). Identification was confirmed by comparison of their mass spectral fragmentation patterns with those stored in the MS database (National Institute of Standards and Technology and Wiley 9 Registry of Mass Spectral Data) and with published mass spectra literature data (Adams, 2007; McLafferty & Staufer, 1989;). The data were handled using the Xcalibur software program (version 2.1.0 SP1, Thermo Fisher Scientific, Milan, Italy).

2.4 | Isolation of fungal disease agent

The pathogen was isolated from stem tissues taken from the infected tomato plants. Symptomatic stem tissues were surface disinfested in ethanol 70% for 1 min, sodium hypochlorite (1%) for 3 min, washed with distilled water for 1 min, and placed on potato sucrose agar (PDA, Merck, Darmstadt, Germany) plates amended with 100 μg/ml of streptomycin sulfate. The plates were incubated in the dark for 5 days at 24°C, and individual colonies were transferred to new plates of PDA to obtain pure cultures. The colonies developed white cottony mycelium and a ring of large black sclerotia at the periphery of the plates. The identity of the fungal isolate was confirmed using MALDI-TOF MS and sequencing of the ITS rDNA region, using ITS4/ITS5 primers (Kurt et al., 2017). Fungal isolate was transferred and stored on PDA plates at 4°C for using in vitro studies.

2.5 | Determination of antifungal activities of essential oils on fungal mycelial growth

In vitro antifungal effects of essential oils of *O. dubium* grown in different altitudes were investigated toward the mycelial growth of fungal disease agents, as described earlier (Soylu et al., 2007). Fungal isolate was grown on PDA medium for 3–5 days at 25°C. Sterile PDA was poured into sterile 90 mm glass Petri plates (20 ml $plate^{-1}$). Mycelial disk (6 mm), cut from the margin of a four days-old fresh fungal culture, were placed at the center of each PDA plate amended with the different concentrations of essential oil which were dropped onto the inner surface of each petri lids. Treated petri plates were then immediately sealed with parafilm to prevent loss of essential oils from petri plates. Petri plates were incubated at 25°C for 5 days. In order to determine the inhibitory effect of essential oils, fungal colony diameters were measured. PDA plate without essential oil was used as control. The mean mycelial growth values were obtained and then converted in to the inhibition percentage of fungal mycelial growth depending on the control treatment by using the formula, MGI (%) = ([dc−dt]/dc) × 100, where dc and dt represent fungal mycelial growth diameter in control and treated Petri plates, respectively (Kara et al., 2020).

2.6 | Statistical analysis

In vitro antifungal experiments were performed twice with at least three replications of each oil concentrations. SPSS statistic program (version 17, USA) was performed for all calculations and statistical analysis. Analysis of variance was used to assess treatment effects. The significant differences between concentrations were determined by means of Duncan's Multiple Range Test ($p < .05$). The efficient concentration (EC_{50}) values for each essential oil were estimated by using Probit analysis.

3 | RESULTS AND DISCUSSIONS

3.1 | Essential oil content and composition

Since the type and amount of sunlight, water and the nutrients that plants receive differ at different altitudes, elevation plays an important role in the health and growth of plants. The essential oil yield and components of *O. dubium* collected at four different altitudes are given in Table 2. The EO yield of *O. dubium* was varied between 8.35% and 9.07%. The highest EO yield was obtained from plant population (Od10) collected at 467 m with 9.07% while the lowest yield was obtained from plant population (Od13) collected at 1,478 m with 8.35% (Table 2). In medicinal and aromatic plants, the most important criterion determining the quality of the product is the yield of essential oil contained in the plant part to be used (Katar et al., 2020). In order to determine the economic value of the product, the essential oil yield also plays an important role for determining the income to be earned by the producers of aromatic plants (Karık et al., 2017; Katar et al., 2020). Previously conducted studies reported that the essential oil yields of *O. dubium* varied 5%–14% depending on the environment in which the plant grows (Baser et al., 1993; Kaplan et al., 2019; Sarer et al., 1982; Turgut et al., 2017). When all these are taken into consideration, it is very important to know the factor(s) that cause changes in the rate of essential oil yield (Katar et al., 2018; Tursun & Telci, 2020).

TABLE 2 Chemical compositions[a] of essential oils of *Origanum dubium* collected from different altitudes

					Altitudes (m)			
No	RT	RI	RI_L	Compounds	Od10 (467)	Od11 (1,096)	Od12 (1,331)	Od13 (1,478)
1	5.95	1,023	1,025	α-Pinene	1.23	0.28	0.8	0.43
2	7.95	1,160	1,160	Myrcene	1.25	0.19	0.57	0.37
3	8.35	1,186	1,186	α-Phellandrene	0.15	0.04	0.08	0.06
4	8.54	1,197	1,195	α-Terpinene	1.13	0.19	0.62	0.37
5	9.75	1,267	1,245	γ-Terpinene	6.31[d]	0.28[a]	3.03[c]	2.03[b]
6	10.25	1,293	1,211	Eucalyptol	0.17	0.14	0.02	0.07
7	10.57	1,310	1,310	*p*-Cymene	6.18[c]	1.89[a]	7.89[d]	2.01[b]
8	13.40	1,445	1,444	1-Octen-3-ol	0.06	0.03	0.04	0.04
9	15.12	1,527	1,564	*cis*-Sabinenehydrate	0.59	0.13	0.22	0.22
10	17.25	1,663	1,630	Terpinen-4-ol	0.74	0.31	0.36	0.26
11	18.25	1,689	1,694	α-Terpineol	0.20	0.32	0.04	0.14
12	19.01	1,731	1,716	Linalooloxide	0.03	0.15	0.04	0.05
13	19.35	1,749	1,728	Borneol	0.21	0.18	0.11	0.08
14	19.50	1,757	1,751	Carvone	0.04	0.06	0.01	0.03
15	19.85	1,775	1,770	Linalool	0.80[b]	48.37[d]	0.24[a]	24.04[c]
16	20.40	1,802	1,800	*trans* Sabinenehydrate	0.14	0.06	0.01	0.05
17	22.01	1,879	1,867	Methy lthymylether	0.39	0.04	0.02	0.01
18	29.85	2,193	2,198	Thymol	0.22	0.21	0.21	0.25
19	30.90	2,228	2,210	Carvacrol	79.22[c]	46.51[a]	85.65[d]	69.14[b]
Chemical compounds (%)								
Oxygenatedmonoterpenes					82.58	96.22	87.75	94.27
Monoterpenehydrocarbons					16.42	3.13	12.06	5.34
Ester					0.06	0.03	0.15	0.04
Total (%)					99.06	99.38	99.96	99.65
Essential oil yields (%)[b]					9.07[c]	8.97[c]	8.58[b]	8.35[a]

Abbreviations: RI, retention indices calculated against *n*-alkanes (C_8-C_{40}) on TR-FAME column; RI_L, retention indices were calculated according to Babushok et al. (2011); RT, retention time.

[a]Percent amount of each compounds given at each column are the mean values ($n = 3$) obtained from different essential oil lots. Mean values of selected compounds followed by different letters within same row indicate significant difference according to Duncan's multiple range test ($p < .05$).

[b]Mean values followed by different letters within same row indicate significant difference according to Duncan's Multiple range test ($p < .05$).

The chemical components of essential oils ($n = 3$) from plants growing at different altitudes were separately identified by GC-MS analysis (Figure 1). Since no significant difference was observed in the % amounts of the detected components, the average values obtained from three different GC-MS analysis determined for each altitudes are given in Table 2. Oxygenated monoterpenes (82.58%–96.22%) and monoterpene hydrocarbons (3.13%–16.42%) were the major class of volatile compounds distributed in *O. dubium* essential oil. The highest oxygenated monoterpene ratio was obtained from plant population Od13 collected from the altitude of 1,478 m, while the lowest yield was obtained from plant population Od10 collected at 467 m (Table 2). Following GC-MS analysis, 19 different components were identified in essential oils, representing 99.06%–99.96% of the essential oils of the plants collected at different altitudes (Figure 1). Among the components detected, carvacrol (46.51%–85.65%), linalool (0.24–48.37), *p*-cymene (1.89%–7.89%), and γ-Terpinene (0.28%–6.31%) were determined as major compounds in each essential oils investigated (Figure 1 and Table 2). The presence of these major components in each essential oils were found to vary in relation to the altitudes where plant population grown. The highest carvacrol (85.65%) content was detected in essential oils of plant population (Od12) collected from 1,331 m with, while the lowest carvacrol (49.51%) content was detected in plant population (Od11) growing at 1,096 m. Linalool is another important major compounds, which also varied greatly depending on the altitudes where plant population collected. The highest linalool ratio (48.37%) was detected in plant population (Od11) collected from 1,096 m, while the lowest linalool ratio (0.24%) was detected in plant population (Od12) growing at 1,331 m. The essential oil yield and major components of thyme can vary greatly between species

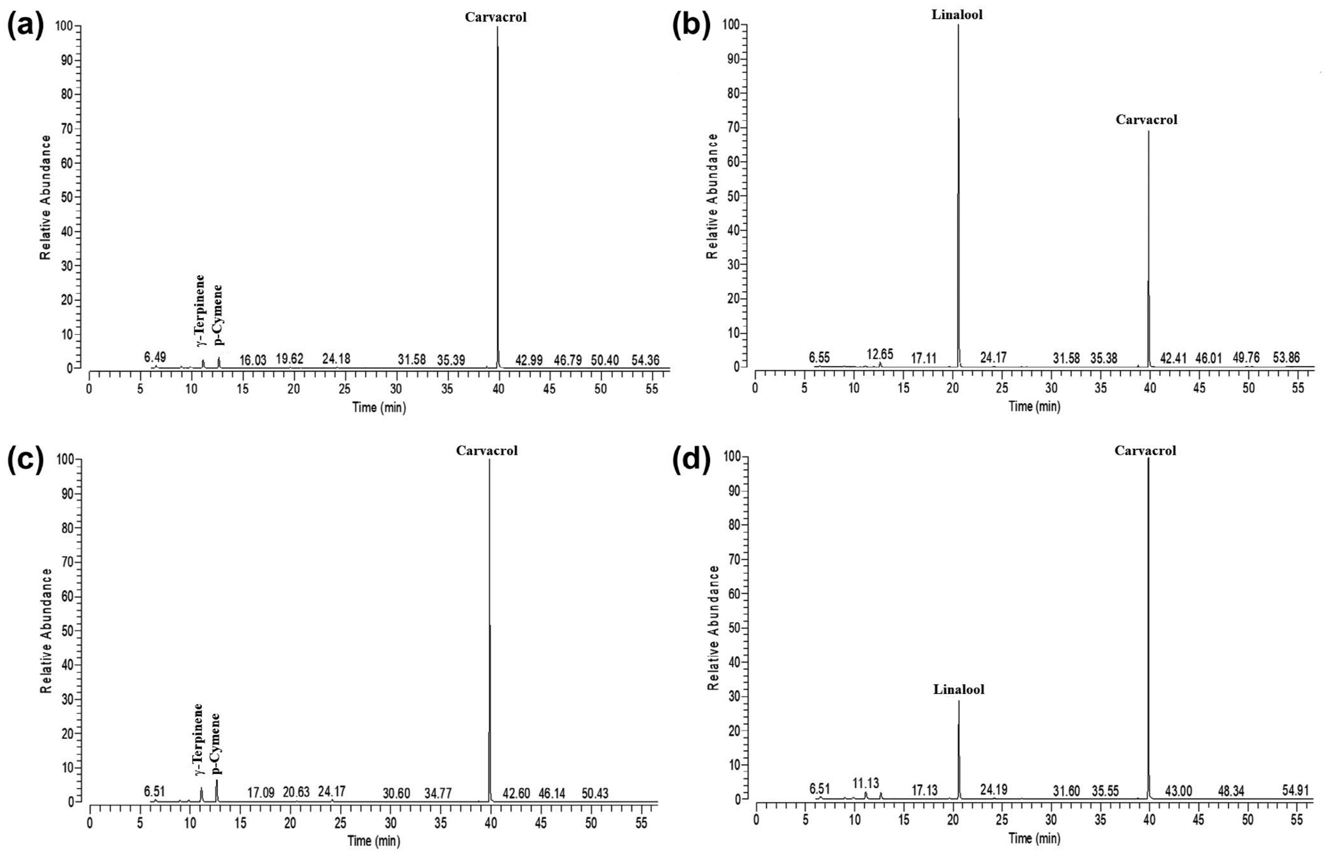

FIGURE 1 The chromatograms of the essential oils of *Origanum dubium* collected from (a) Od10 from 467 m, (b) Od11 from 1,096 m, (c) Od12 from 1,331 m, and (d) Od13 from 1,478 m altitudes

as well as even within the same species. Mastro (1996) reported that although carvacrol and thymol are the main components in the essential oil of the *Origanum* genus, linalool, terpinen-4-ol, and caryophyllene were also reported to present in some germplasms. Kaplan et al. (2019) reported that the essential oil of *O. dubium* contains 73.76%–88.21% carvacrol, 3.16%–9.10% *p*-cymene, and 1.14%–5.17% γ-Terpinene. Turgut et al. (2017) reported that the essential oil of *O. dubium* contains 81.85%–91.04% carvacrol, 1.77%–6.25% γ-Terpinene, and 2.40%–3.55% *p*-cymene. According to Karioti et al. (2006), carvacrol ratio varied between 69.5% and 71.3% in essential oil of *O. dubium* collected from Cyprus. Several study reported relationship between chemical composition, environmental factors, and chemotypes in *Origanum* spp (Aboukhalid et al., 2017; Economou et al., 2011; Fallah et al., 2020; Huaman-Castilla et al., 2020; Pirbalouti et al., 2011; Raina & Negi, 2014; Stefanaki et al., 2016).

Production, compositions, and qualities of secondary metabolites in essential oils of medicinal and aromatic plants are directly related to various external and internal factors such as genetic characteristics, climatic conditions, soil and environmental factors, cultural activities, plant phenological stages, and harvesting methods (Soltanbeigi & Sakartepe, 2020). Lamiaceae plants has been reported to be affected by environmental conditions such as local ecological conditions, geographical cultivation area, cultivation, and harvesting period. Altitude is one of the factors that affect plant metabolism. Quantity, quality, and compositions of essential oils of *Tanacetum polycephalum* Sch.-Bip. *polycephalum* (Asteraceae), *Thymus kotschyanus*, and *Lavandula angustifolia* (Lamiaceae) were reported to be affected by altitude (Demasi et al., 2018; Jamshidi et al., 2006; Mahdavi et al., 2013). In our study, major components of essential oil were also found to be affected by altitude differences. Recently, Khalil et al. (2020) also reported that altitude is an important factor that should be considered as it affected the yield, chemical composition and biological activities of *Satureja thymbra*. Lamiaceae species rich in phenol displayed great diversity in the content of thymol and carvacrol in their essential oils is also affected by climatic factors. Essential oil of *S. hortensis* contained a higher ratio of carvacrol at higher altitude (Katar et al., 2017). On the other hand, Bakhy et al., 2014) reported that there was no relationship between essential oil content, altitude and soil characteristics of plants collected from 36 different regions.

3.2 | Antifungal activities of the essential oils against mycelial growth of *S. sclerotiorum*

Antifungal activities of *O. dubium* has not previously determined against fungal disease agent *S. sclerotiorum*. The volatile inhibitory effects of different concentrations of essential oils obtained from different altitudes against *S. sclerotiorum* are given in Table 3. All

TABLE 3 The inhibitory effects (%) of different concentrations of volatile phase of essential oils of *Origanum dubium* collected from different altitudes (m) on the mycelial growth (mm)[a] of *Sclerotinia sclerotiorum*

Concentrations (μl petri^{-1})	Essential oils, mycelial growth, and mycelial growth inhibition							
	Od10 (467 m)		Od11 (1,096 m)		Od12 (1,331 m)		Od13 (1,478 m)	
	MG (mm)	MGI (%)	MG (mm)	MGI (%)	MG (mm)	MGI (%)	MG (mm)	MGI (%)
0.00	90.00d	0.0	90.0c	0.0	90.00c	0.0	90.00c	0.0
0.25	90.00dB	0.0	90.0cB	0.0	10.67bA	88.1	90.00cB	0.0
0.50	90.00dB	0.0	90.0cB	0.0	6.33aA	93.0	90.00cB	0.0
0.75	34.00cB	62.2	90.0cC	0.0	0.00aA	100.0	90.00cC	0.0
1.00	5.67bB	93.7	90.0cC	0.0	0.00aA	100.0	90.00cC	0.0
1.50	0.00aA	100.0	90.0cB	0.0	0.00aA	100.0	90.00cB	0.0
2.00	0.00aA	100.0	90.0cC	0.0	0.00aA	100.0	10.00bB	88.9
2.50	0.00aA	100.0	73.0bB	18.9	0.00aA	100.0	0.00aA	100.0
3.00	0.00a	100.0	0.0a	100.0	0.00a	100.0	0.00a	100.0
EC_{50}[b]	0.73		2.66		0.074		1.74	

Abbreviations: MG, mycelial growth; MGI, mycelial growth inhibition.

[a]The mean mycelial growth of fungal agent determined was based on the measurements of three replicate plates, recorded at 5 days after inoculation. Mean values followed by different small or capital letters within the column and row are significantly different according to Duncan multiple range test ($p < .05$).

[b]The estimated efficient concentration (EC_{50}) values (μl petri^{-1}) for each essential oil were estimated by using Probit analysis.

essential oils inhibited mycelial growth of fungal disease agents in a dose dependent manner (Figure 2). As it was seen in Figure 2 and Table 3, the highest antifungal efficiency in preventing the mycelial growth of fungus was shown by the essential oil containing 85.65% carvacrol (Od12). The essential oil of this plant population, containing a high amount of carvacrol (85.65%), prevented the mycelial growth completely at relatively very low concentration used (0.75 μl petri^{-1}). The lowest antifungal activity was displayed by the essential oil containing low amount of carvacrol (46.51%) at a relatively higher concentration used (3.0 μl petri^{-1}). The estimated efficient concentration (EC_{50}) values for each essential oil were estimated by using Probit analysis. The efficient concentrations (EC_{50}) for each essential oil were estimated ranging from 0.074 to 2.66 μl/petri concentrations (Table 3).

Although, there are many reports on concentration-dependent antifungal activities of essential oils whereby the mycelial growth of fungal disease agents (Della Pepa et al., 2019; Elshafie et al., 2015; Hou et al., 2021; Stefanakis et al., 2013), very few report were available on *S. sclerotiorum* suppressed with increase in the concentration of essential oils from taxonomically different medicinal plants, including *Origanum* spp. (Soylu et al., 2007). There was no report available for antifungal activity of essential oils from *O. dubium*. To the best of our knowledge, this study was the first study to show that essential oils of *O. dubium* possess antifungal activities against devastating plant fungal disease agent *S. sclerotiorum*.

Results also clearly revealed that, the difference in antifungal activity between essential oils obtained from different altitudes against *S. sclerotiorum* was significant. The difference in the antifungal activity of the essential oils collected from different altitudes might be due to differences in the ratios of the main components in the essential oils of the plants grown at that altitude. Essential oils are plant derived several aromatic compounds, which have a varied composition of known bioactive substances, generally characterized by the presence of two or three major components (Chouhan et al., 2017; Costa et al., 2015). Depending upon different extraction methods, harvesting time, plant parts, or collected regions, different constituents were reported to reside in essential oils of *Origanum* spp., but most of them contain phenols, terpenes, and their derivatives, such as carvacrol, thymol, linalool, *p*-cymene, γ-terpinene, myrcene, terpinen-4-ol, and limonene (Karpinski, 2020; Khan et al., 2019; Mamadalieva et al., 2017). In our study, although *O. dubium* plant grown at low altitude yielded higher essential oil yield, higher carvacrol ratio was found in the essential oil of plants grown at higher altitudes, thus higher antifungal activity was observed against fungal disease agent *S. sclerotiorum*. Interestingly, although many of *Origanum* species shared the same ingredients, it showed significant differences to the same microorganism (Mamadalieva et al., 2017; De Martino et al., 2009; Moumni et al., 2020). This suggests that the antimicrobial effect of EOs may be related to the proportion of the main antimicrobial components or the synergistic with antimicrobial effects of different chemical components (Chouhan et al., 2017; Tepe et al., 2016; Wink, 2015). In our study, carvacrol, linalool, and *p*-cymene were determined as major components of *O. dubium* essential oils. Strong inhibitory activities of carvacrol, linalool, and *p*-cymene were previously reported on a variety of pathogenic fungi and bacteria (Abbaszadeh et al., 2014; Baj et al., 2020; Elshafie et al., 2015;

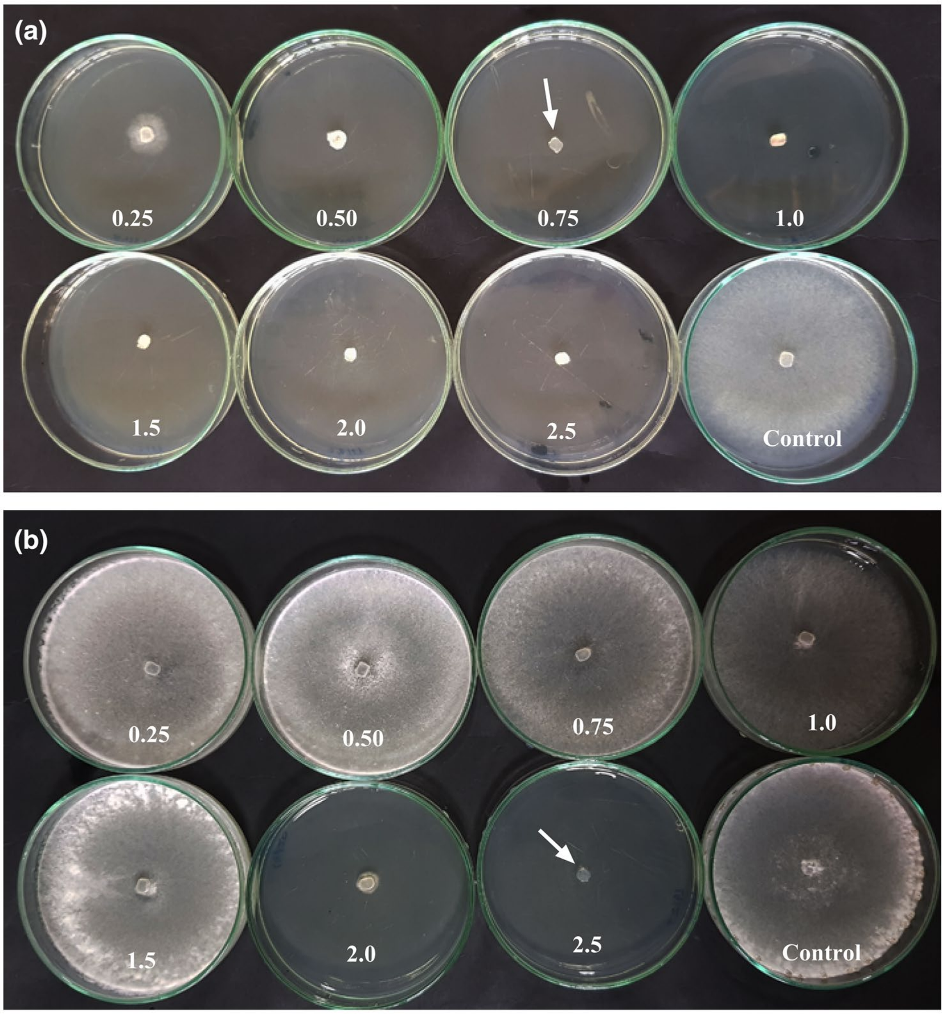

FIGURE 2 Effect of different concentrations of essential oils on inhibition of mycelial growth (arrows) of *Sclerotinia sclerotiorum*. Note complete inhibition of mycelial growth (arrow) at relatively low concentration (0.75 µl petri^{-1}) was caused by essential oils obtained from *Origanum dubium* Od12 population (a). (b) Shows inhibition of mycelial growth (arrow) by essential oil of *O. dubium* Od13 population at the relatively higher concentration (2.5 µl petri^{-1})

Hou et al., 2021; Rahmouni et al., 2019; Taleb et al., 2018; Wang et al., 2019). Previously conducted studies also reported that plant species belong to Lamiaceae family, which were rich in phenol content, have shown that the great diversity in the content of thymol and carvacrol in their essential oils is closely associated with climatic factors including altitude (Ghasemi et al., 2011; Karousou et al., 2005).

4 | CONCLUSIONS

In general, average temperatures are usually lower at higher altitudes, than those at the lower altitude of the mountains. Since the intensity and amount of sunlight, amount of water and nutrient availabilities that plants can absorb and receive significantly differ at different altitudes, elevation could play an important role in plant growth. In this study, the essential oil ratios, the chemical compositions, and antifungal activities against *S. sclerotiorum* were found to vary in relation to the altitudes plants collected. According to GC-MS analysis and antifungal experiments, altitude seems an important factor that might be considered as it affected the ratio, chemical composition, and antifungal activities of *O. dubium*. The main difference observed in the compositions of the essential oils was in the major identified compound such as carvacrol, linalool, and *p*-cymene. Since the essential oil containing a high amount of carvacrol prevented the mycelial growth completely at relatively very low concentration used, it may be said carvacrol possess a high potential in the control of white mold disease caused by *S. sclerotiorum*. This is the first study reporting antifungal activities of essential oil of *O. dubium* against plant pathogenic fungal disease agent.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Data curation; Investigation; Methodology: Musa Türkmen. *Data curation; Investigation; Methodology*: Merve Kara. *Data curation; Investigation; Methodology*: Hasan Maral. *Data curation; Investigation; Methodology; Writing-original draft; Writing-review & editing*: Soner Soylu.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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