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Volatile Metabolites from *Salvia fruticosa* as Antifungal Agents in Soilborne Pathogens

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The volatile metabolites of *Salvia fruticosa* plants, growing wild in 15 localities scattered across Greece, were analyzed by means of GC and GC-MS. The essential oil content ranged from 0.69 to 4.68%, and the results of the analyses showed a noticeable variation in the amounts of the five main components [1,8-cineole, α-thujone, β-thujone, camphor, and (*E*)-caryophyllene]. The antifungal activities of the essential oils from two localities, belonging in two different groups of cluster and principal component analysis, and their main components (1,8-cineole and camphor) were evaluated in vitro against five phytopathogenic fungi. Both oils were slightly effective against *Fusarium oxysporum* f. sp. *dianthi* and *Fusarium proliferatum*, whereas against *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Fusarium solani* f. sp. *cucurbitae* the oils exhibited high antifungal activities.

KEYWORDS: ***Salvia fruticosa*; essential oil composition; GC-MS; 1,8-cineole; camphor; α-thujone; β-thujone; antifungal activity; soilborne pathogens**

INTRODUCTION

Salvia fruticosa Miller, formerly known as *Salvia triloba* L., is a native species of the eastern Mediterranean basin. In Greece *S. fruticosa* occurs in maquis and phrygana ecosystems of the mainland as well as on the islands. *S. fruticosa*, known as "faskomilia", "lelisfakia", and "alisfakia" (*1*) is renowned since ancient times for its pharmaceutical properties as well as a culinary herb. Local people use a leaf infusion as an antiphlogistic, especially for inflammation of the mouth and throat (*2*).

S. fruticosa, commonly known as Greek sage, has already been the object of several studies due to its economic importance (see **Table 1**). In the majority of the studied plants, wild or cultivated or even in commercial samples, 1,8-cineole was found to be the dominant compound (**Table 1**).

Recently there has been a tendency to use alternative methods for pest, disease, and weed control in agriculture, methods that are less harmful to human health and the environment. Research on natural products including plant extracts and essential oils, which might contribute to the development of new agents for pest control, is very important. Studies on the antifungal activity of essential oils and their components have been reported by numerous investigators (*3*−*7*). Several essential oils derived from many aromatic plants are known to possess insecticidal and antimicrobial activities (*8*−*11*). Previous reports on the genus *Salvia* have indicated antimicrobial, cytotoxic, and antiviral activities, but they used either organic extracts or they were performed on different fungal species from those assayed herein (*12*−*14*).

In continuation of our investigation toward the evaluation of the antimycotic potential against phytopathogenic fungi and the chemical variation in the genus *Salvia* (*15*−*18*), we assess the essential oil content, the qualitative and quantitative compositions of 15 populations of *S. fruticosa* scattered across Greece. The in vitro efficacy of the essential oils of two of the examined populations of *S. fruticosa*, belonging in two separate groups of cluster and principal component analyses, and that of their main components on certain fungi, which cause serious diseases in cultivated plants, are reported.

MATERIALS AND METHODS

Materials. Aerial parts of wild-growing *S. fruticosa* plants were collected at the flowering stage from 15 localities scattered across Greece: (1) Kira Panagia, altitude 60 m, North Sporades (April 1999); (2) Mt. Pilio, Agios Ioannis, altitude 10 m, Pilio, County Magnesia (July 1998); (3) Argostoli, altitude 10 m, Cephalonia island (April 1999); (4) Mt. Parnitha, altitude 400 m, County Attiki (June 1997); (5) between the villages of Kamares and Apollonia, altitude 0 m, Sifnos island (May 1996); (6) Katavati, altitude 500 m, Sifnos island (May 1998); (7) Archanes, altitude 750 m, County Heraclio, Crete island (June 1998); (8) Agies Paraskies, altitude 600 m, Perfecture of Heraclio, Crete island (June 1998); (9) Karnari, altitude 650 m, Perfecture of Heraclio, Crete island (June 1998); (10) Episkopi, altitude 0 m, Perfecture of Lasithi, Crete island (June 1997); (11) Chios, altitude 0 m, Chios island (May 1998); (12) Scalia, altitude 50 m, Kalymnos island (July 1998); (3) Pili, altitude 100 m, Kos island (April 1999); (14) Mt. Diceos, altitude 400 m, Kos island (April 1999); and (15) between the villages of Soroni and Kamiros, altitude 0 m, Rhodes island (May 1999). Voucher specimens are kept at the Laboratory of the Pharmacognosy Department, University of Athens. Pure commercial oil components were purchased from the Sigma-Aldrich Co.

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Table 1. Main Previously Published Compounds of Oils of *S. fruticosa*

compound	Brieskorn and Wenger (16)	Dalferth (17)	Igolen (18)	Buil et al. (19)	Rhyu (20)	Tucker et al. (21)	Katsiotis and Iconomou (22)	Putviesky et al. (23)	Bayrak and Akgul (24)	Harvala et al. (25)	Bellomaria et al. (26)	Langer et al. (27)	Karousou et al. (14)	Kanias et al. (28)
α-pinene	3.3–3.5	3.2	+	6.3	4.1–6.0	3.3–3.6	3.8–6.7	18.6–37.3	3.2	2.6	0.3–5.7	3.6–21.8	1.8–5.2	0.22–3.87
camphene	3.7	2.4	+	5.5	2.2–4.0	0.8–0.9	0.4–7.5	2.1–3.1	0.7	2.7	1.4–7.3	0.4–8.1	0.2–9.9	0.08–1.61
β-pinene	2.8–5.6	7.9	+	7.4	2.3–5.3	6.8–8.7	3.0–10.7	5.0–6.6	4.3	2.75	2.2–13.9	1.2–11.6	3.5–9.4	0.91–8.79
myrcene	0.3	–		5.6	1.5–3.0	2.1–1.7	0.6–3.5	0.1–3.4	3.1	1.95	0.9–2.9	-	1.5–5.3	1.14–5.17
1,8-cineole	14–14.8	64.0	+	+ limonene	40.7–54.1	38.3–44.6	38.8–66.2	30.8–44.0	55.5	+ limonene	4.0–67.5	42.0–74.4	22.7–64.2	28.2–63.7
				42.4						38.5				
thujone				1.0			1.4–12.1							
				(probably α-)										
α-thujone	28.0	2.3			1.0–2.8	+ α-*p*-dimethyl-styrene		–	0.2	+ 1-octen-3-ol	0–0.6	0–12.0	1.0–19.2	1.96–34.1
						0.1–12.8				2.3				
β-thujone	14.5	2.8			–	0.9–6.7		–	2.0	4.1	0–1.1	0–6.3	0.9–25.6	0.94–8.59
camphor	8.2–18	8.2	+	9.1	1.5–2.6	+ β-bourbonene	1.9–23.8	3.3–6.8	8.4	15.2	5.7–44.5	0.9–25.8	0.8–30.3	0.41–10.2
						2.0–2.1								
borneol	22–6.8	+ bornyl acetate	+	4.1	-	+ α-terpinyl acetate		1.0–1.3	4.6	+ α-terpineol 7.3	1.4–7.6	0–5.2	+ α-terpineol 1.2–7.5	+ α-terpineol 4.37–14.65
		1.9				0.8–2.2								
caryophyllene	1.7	2.0		8.2	5.0–7.6	3.1–18.7	1.2–7.3	7.6–11.3	5.2	4.1	1.4–23.0	1.3–13.2	+ terpinen-4-ol 0.2–6.9	1.42–5.66

Figure 1. Principal component analysis of the volatile metabolites of the 15 populations of *S. fruticosa*.

Isolation of the Essential Oils. The plants were air-dried and stored under the same conditions. Semicrushed leaves of each of the 15 wild-growing populations of *S. fruticosa* were subjected to hydrodistillation for 2 h, using a modified Clevenger-type apparatus with a water-cooled receiver, which lowers the temperature in the oil receiver, thus reducing hydrodistillation−overheating artifacts. The essential oils were dried over anhydrous sodium sulfate and were stored under an N_2 atmosphere in amber vials at 4 °C until they were analyzed or used in bioassays. All oils were analyzed within 24 h from their production. On the basis of the obtained volume of essential oil, the corresponding amount of capillary GC grade *n*-pentane was added to afford an appropriate concentration of 10 μL/mL.

Gas Chromatography—Mass Spectrometry (GC-MS). The chemical composition of the oils was analyzed using GC and GC-MS. GC-MS analyses were carried out using a Hewlett-Packard 5973-6890 GC-MS system operating in the EI mode at 70 eV, equipped with an HP-5 MS capillary silica column (30 m × 0.25 mm; film thickness = 0.25 μm). The initial temperature of the column was 60 °C and was raised to 280 °C at a 3 °C/min rate. Carrier gas was He, flow rate = 1 mL/min. Split ratio was 1:10. The injection volume of each sample was 1 μL. *n*-Alkanes were used as reference points in the calculation of the Kovats indices (KI). Identification of the chemical constituents was based on comparisons of their relative retention times and mass spectra with those obtained from authentic samples and/or the NIST/NBS and Wiley libraries spectra as well as literature data (*19*).

Gas Chromatography. GC analyses were carried out using a SRI 8610C GC-FID system, equipped with a DB-5 capillary column (30 m × 0.32 mm, film thickness = 0.25 μm) and connected to an FID detector. The injector and detector temperatures were 280 °C. Carrier gas was He at 1.2 mL/min. The thermal program was the same as that used for the GC-MS analysis. Relative percentage amounts were calculated on the basis of peak areas.

Fungal Strains and Media. Strains of the phytopathogenic fungi *Fusarium oxysporum* f. sp. *dianthi* (Fo), *Fusarium solani* f. sp. *cucurbitae* (Fs), *Fusarium proliferatum* (Fp), *Sclerotinia sclerotiorum* (Ss), and *Rhizoctonia solani* (Rs) were supplied by the Benaki Phytopathological Institute. Cultures of the phytopathogenic organisms were maintained on potato dextrose agar (PDA) medium.

Antifungal Assays. The antifungal tests were carried out in vitro, in Petri dishes, 9 cm in diameter, containing PDA. The essential oils were dispersed as an emulsion in water using ethanol and Tween 20 and added to PDA immediately before it was emptied into the Petri dishes at a temperature of 45−50 °C. The concentrations tested were 50, 100, 250, 500, 1000, and 2000 μL/L. The controls received the same quantity of ethanol and Tween 20 mixed with PDA. The phytopathogenic fungi were inoculated immediately after preparation of the Petri dishes by placing in the center of each plate a 6 mm diameter disk of the test species, cut with a sterile cork borer from the periphery

Table 2. Yield and Physical Properties of the Oils of the 15 Populations of *S. fruticosa*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
yield (% v/dry wt)	4.68	2.58	0.69	2.40	2.53	1.92	3.36	4.43	3.00	3.10	1.24	3.75	4.25	3.44	3.63
n_D^{25}	1.46	1.46	1.47	1.47	1.46	1.46	1.46	1.46	1.46	1.46	1.46	1.47	1.46	1.46	1.46
$[\alpha]_D^{20}$	−5.82	−7.35	+3.57	−0.98	−5.9	−2.49	+1.16	−14.87	+17.05	+4.98	−8.70	+0.05	+4.04	−6.02	+4.04
d^{25}	0.87	0.90	0.99	0.90	0.96	0.88	0.89	0.90	0.88	0.90	0.92	0.94	0.73	0.87	0.73

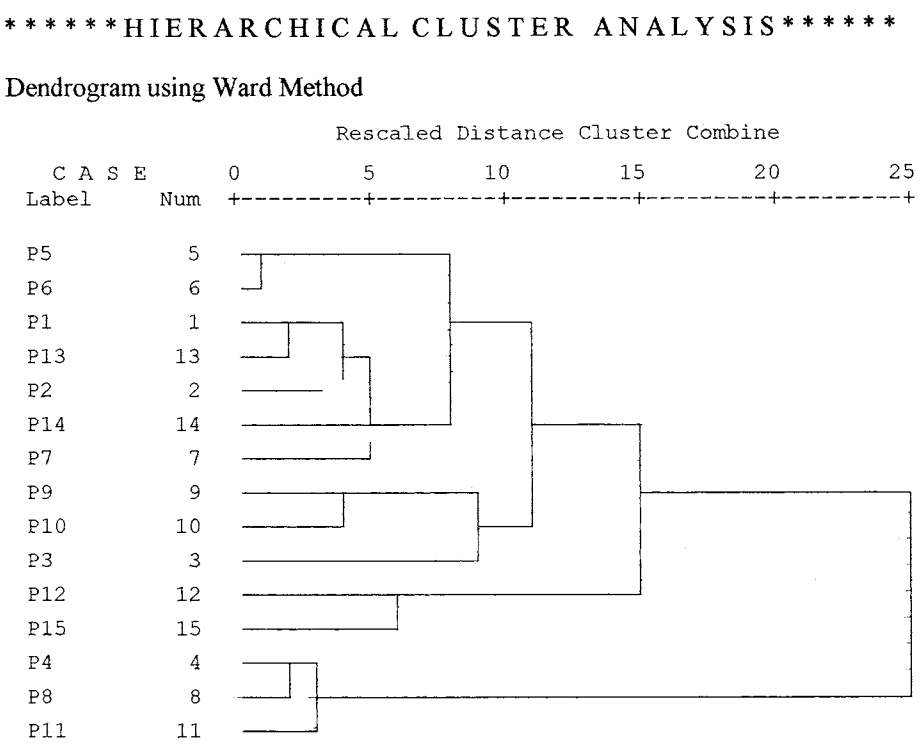

Figure 2. Dendrogram obtained by application of Ward's linkage method to the Euclidean distances.

of actively growing cultures on PDA plates. The Petri dishes were incubated in the dark at a temperature of 23 ± 1 °C. Mean growth measurements were calculated from six replicates of each fungal species every 24 h for 12 days. The measurement of the fourth day was used to determine the minimum inhibitory concentration (MIC) and the EC_{50} values (concentration causing 50% inhibition of mycelial growth on control media). Fungitoxicity was expressed in terms of percentage of mycelial growth inhibition. EC_{50} values were calculated from the data subjected to probit analysis (statistical software SPSS 7.0 Inc., Chicago, IL). To ascertain if the essential oils showed a fungicidal or fungistatic activity, parts of media from plates without mycotic growth were transferred into new PDA plates; no fungal growth after an incubation of 10 days was indicative of fungicidal activity. The two main components of the oils of *S. fruticosa*, 1,8-cineole and camphor, were also screened for their antifungal activity using the method described above. The concentrations tested were 0, 20, 50, 100, 200, and 500 μL/L.

Data Analyses. All data were statistically processed with SPSS 7.0 software. Analyses included (a) cluster analysis (Ward's method and Euclidean distance measure) to establish the different groups/chemotypes within the individual essential oils and (b) principal component analysis (PCA, based on correlation matrix), which was applied to examine the interrelationships between the *S. fruticosa* populations of different geographic areas.

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oils. The yields and physical properties of the 15 populations of *S. fruticosa* are presented in **Table 2**. A total of 145 components were identified, constituting 94.5−100% of the oils (**Table 3**); 107 components are reported for the first time in this species, and most of them belong to the group of sesquiterpene hydrocarbons. Even though the qualitative oil compositions of the different populations are similar, there are noticeable quantitative differences. The highest fluctuations were found in the amount of the following compounds: 1,8-cineole, ranging from 16.9 to 54.4%; α-thujone, ranging from traces to 14.5%; β-thujone, ranging from 0.6 to 9.0%; camphor, ranging from 0.6 to 15.9%; and (*E*)-caryophyllene, ranging from traces to 15.6%. The sum of the five compounds ranges from 45.1% (population 4) to 69.6% (population 12) of the oil. The oxygenated monoterpene hydrocarbons, which represented 38.9−75.2% of the oils, comprised the main portion in all studied populations. Besides the quantitative oil composition, the total essential oil content varies noticeably, from 0.69% (population 3) to 4.68% (population 1) based on dry weight.

As it was expected in all analyzed oils 1,8-cineole was the most abundant component (16.9−48.3% of total oil). Also, high contents of α- and β-thujone were observed, amounting to 23.0% (population 10). The present findings are in accordance with published data, which suggest that *S. fruticosa* essential oils, besides a high cineole and/or camphor content, may also be characterized by high α- and β-thujone amounts (*20*). *Salvia* species are known to contain biologically active diterpenoids, mainly with an abietane or clerodane skeleton. To the best of our knowledge, this is the first time that manool and isopimar-9(11),15-diene have been detected in the essential oil of *S. fruticosa*, whereas manool has been referred to in another member (*S. officinalis*) of the section *Salvia*.

Table 3. Chemical Composition of the Oils of *S. fruticosa* Populations Growing in Greece

compound	KI[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
(*Z*)-salvene		–	0.1	–	–	–	–	–	–	–	0.1	–	0.1	–	tr[b]	–
tricyclene	905	0.2	0.3	–	0.2	0.4	0.5	–	0.4	–	tr	1.3	0.1	tr	tr	tr
α-thujene	909	0.4	0.5	tr	tr	0.3	1.3	0.4	0.5	0.6	0.6	tr	0.4	0.6	0.8	0.5
α-pinene	918	5.5	6.2	1.5	3.1	6.2	7.4	4.2	5.0	3.4	2.7	5.7	5.1	4.4	4.2	4.1
α-fenchene	924	–	5.8	–	–	–	1.1	–	–	–	–	–	2.1	–	–	–
camphene	934	5.0	–	tr	3.2	6.4	7.0	0.4	6.1	2.6	0.8	6.9	–	1.6	5.5	0.6
sabinene	955	tr	–	tr	–	–	0.6	–	–	tr	–	tr	8.6	tr	tr	tr
β-pinene	962	7.9	7.6	3.8	1.9	6.4	3.6	7.3	4.2	5.5	5.3	5.3	–	9.0	5.5	9.0
3-octanone	966	–	–	tr	tr	tr		–	tr	–	–	–	–	–	–	–
myrcene	976	5.2	5.0	1.8	2.1	3.4	3.9	4.9	2.8	2.9	3.8	4.8	3.3	4.3	2.3	1.6
3-octenol	988	–	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–
α-phellandrene	990	tr	–	tr	tr	0.1	0.2	–	tr	tr	–	tr	–	–	–	–
δ-3-carene	991	–	–	–	–	–	–	–	–	–	–	–	–	–	tr	–
α-terpinene	1005	0.4	tr	0.3	–	0.5	0.6	tr	0.1	tr	tr	0.6	–	tr	tr	tr
o-cymene	1006	–	–	–	–	–	0.2	–	–	–	–	–	–	–	–	–
p-cymene	1010	tr	tr	tr	tr	tr	–	–	–	–	–	–	–	tr	tr	tr
1,8-cineole	1024	35.5	34.9	29.9	16.9	34.7	32.1	37.7	21.3	28.8	27.3	17.5	48.3	38.4	42.5	54.4
benzene acetaldehyde	1029	–	–	–	–	–	–	tr	tr	tr	tr	tr	–	tr	–	tr
(*E*)-β-ocimene	1032	–	–	–	–	tr	–	0.1	tr	tr	–	tr	tr	tr	tr	tr
γ-terpinene	1043	0.6	0.7	0.7	0.4	0.6	0.8	1.1	0.8	1.2	1.0	1.0	0.9	0.7	0.7	0.6
cis-sabinene hydrate	1051	0.3	0.3	tr	–	0.4	–	0.75	0.6	0.8	0.1	tr	0.2	0.4	0.6	0.8
cis-linalool oxide	1056	–	–	tr	–	tr	–	–	–	–	–	–	0.1	–	tr	–
terpinolene	1072	0.2	0.2	tr	0.3	0.3	0.4	0.4	0.5	0.9	0.3	0.6	0.3	tr	tr	tr
trans-sabinene hydrate	1086	tr	–		–	–	–	–	–	–	–	–	–	tr	–	0.28
linalool	1088	tr	–	0.5	0.5	1.3	0.3	–	tr	tr	–	tr	–	–	tr	tr
α-thujone	1091	1.1	2.9	1.6	1.3	2.3	1.3	8.1	3.3	4.2	14.5	1.04	5.6	2.0	3.4	tr
1-octen-3-yl-acetate	1097	–	tr	–	–	0.2	–	–	tr	–	–	–	–	–	–	–
1,3,8-*p*-menthatriene	1098	–	–	–	–	–	–	–	–	–	–	–	–	tr	–	–
β-thujone	1102	0.8	1.3	1.1	0.9	3.0	0.9	1.3	2.6	9.0	8.5	0.6	7.2	2.5	1.5	1.9
exo-fenchol	1105	–	–	–	–	–	–	–	–	–	–	tr	–	–	–	–
cis-*p*-2-menthen-1-ol	1110	tr	–	–	–	–	–	tr	tr	tr	–	–	–	tr	–	tr
α-campholenal	1111	tr	tr	–	tr	–	–		–	–	tr	tr	–	tr	tr	tr
trans-pinocarveol	1123	–	–	–	–	–	–	–	–	–	–	–	–	–	–	tr
trans-sabinol	1127	–	–	–	–	–	–	0.2	–	–	tr	–	–	–	–	–
thujyl alcohol[c]	1130	–	–	–	–	–	–	–	–	–	0.4	–	–	–	–	–
camphor	1136	8.7	13.4	2.4	15.6	15.9	16.4	0.6	15.4	11.5	3.1	14.6	6.2	5.8	10.6	2.8
neo-3-thujanol	1130	–	–	–	–	–	–	–	–	–	tr	–	–	–	–	–
camphene hydrate	1135	–	–	–	–	–	–	–	–	–	–	–	–	tr	–	–
trans-pinocamphone	1144	–	0.2	–	0.8	0.1	0.6	–	–	0.2	1.1	–	–	1.0	0.8	tr
pinocarvone	1143	–	3.2	tr	–	–	–	–	–	–	–	–	tr	–	–	–
borneol	1152	2.6	tr	tr	8.0	3.4	4.7	–	5.6	tr	tr	2.4	–	tr	3.5	tr
borneol + δ-terpineol		–	–	1.0	–	–	–	–	–	–	–	–	–	–	–	–
3-thujanol + *p*-mentha-1,5-dien-8-ol	1161	–	–	–	–	–	–	–	–	2.7	–	–	1.2	0.8	–	–
p-mentha-1,5-dien-8-ol	1161	–	tr	–	–	–	–	–	–	–	–	–	–	–	–	–
cis-pinocamphone	1162	–	tr	–	0.3	0.1	0.2	–	–	1.2	0.3	–	0.1	tr	0.2	tr
terpin-4-ol	1162	0.4	0.8	1.0	1.2	0.9	0.8	0.8	1.1	0.9	1.6	0.9	1.3	0.6	0.7	0.5
m-cymen-8-ol	1169	–	–	–	–	–	–	–	–	–	–	tr	–	–	–	–
α-terpineol	1174	1.0	2.6	3.2	5.2	1.7	2.7	5.6	4.7	6.7	4.8	0.3	2.4	2.2	2.7	tr
myrtenal	1176	–	–	–	–	–	–	–	–	–	–	–	–	–	tr	–
cis-piperitol + myrtenol	1178	–	–	–	–	–	–	–	–	–	–	–	0.1	–	–	–
myrtenol	1179	tr	–	–	0.7	tr	0.4	–	–	0.4	0.5	tr	–	tr	0.5	tr
trans-piperitol	1195	–	tr	–	–	–	–	–	–	tr	tr	–	–	tr	tr	tr
trans-carveol	1203	–	–	–	–	–	–	tr	–	tr	tr	–		tr	tr	tr
nerol	1204	–	–	–	–	–	–	tr	–		tr	–	tr	–	–	–
cis-carveol	1205	–	tr	–	–	–	–	–	–	–	–	–	–	–	–	–
(*Z*)-ocimenone	1216	–	–	–	–	–	–	–	–	tr	–	–	–	–	–	–
cumin aldehyde	1221	–	–	–	–	–	–	–	–	–	–	–	–	–	tr	–
carvone	1229	–	–	–	–	–	–	–	–	–	tr	–	–	–	tr	–
carvotanacetone	1230	–	tr	–	–	–	–	–	–	tr	tr	–	tr	tr	–	–
cis-myrtanol	1234	–	–	–	–	–	–	–	–	–	–	–	–	–	tr	–
linalool acetate	1238	tr	0.1	0.5	–	1.1	–	3.4	0.4	–	1.0	–	0.6	tr	0.6	tr
trans-myrtanol	1239	–	–	–	–	–	–	–	–	–	–	–	–	tr	–	–
(*E*)-2-decenal	1242	–	–	–	–	–	–	–	–	–	–	–	–	tr	–	–
perilla aldehyde	1251	–	–	–	–	–	–	–	–	–	–	–	–	tr	–	–
neo-3-thujyl acetate	1252	–	tr	–	–	tr	–	–	tr	–	tr	–	0.1	–	–	–
bornyl acetate	1269	1.3	0.5	tr	4.4	2.2	2.6	0.2	6.8	1.2	0.3	1.6	0.2	tr	0.4	–
p-cymen-7-ol	1270	–	–	–	–	–	–	–	–	–	–	–	–	tr	–	–
thymol	1272	tr	tr	tr	–	–	–	–	–	–	–	tr	–	tr	0.2	tr
trans-sabinyl acetate	1275	–	tr	–	–	0.2	–	0.4	tr	0.3	0.6	–	0.1	–	–	–
carvacrol	1287	tr	tr	tr	0.3	tr	–	tr	tr	tr	tr	tr	–	tr	0.3	tr
iso-3-thujyl acetate	1298	–	tr	–	–	–	–	–	–	–	–	–	–	–	–	–
cis-pinocarvyl acetate	1299	–	–	–	–	0.1	–		–	0.2	–	–	–	–	–	–

Table 3. (Continued)

compound	KI[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
δ-elemene	1331	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–	–
verbanol acetate	1332	–	–	–	–	–	–	–	–	–	0.2	–	–	–	–	–
α-terpinyl acetate	1340	–	1.8	–	0.7	1.8	–	4.1	2.3	–	2.7	–	1.4	1.0	1.2	0.3
α-cubebene	1341	–	–	–	–	–	–	–	–	–	–	tr	–	–	–	–
eugenol	1342	–	–	–	–	–	–	tr	–	–	tr	tr	–	tr	–	tr
neryl acetate	1345	–	tr	tr	–	0.1	–	0.2	–	–	tr	–	0.1	–	tr	–
α-ylangene	1351	–	tr	tr	0.5	–	0.2	tr	–	tr	tr	–	–	tr	–	–
isoledene	1353	tr	tr	tr	–	–	–	tr	–	–	–	–	–	tr	tr	–
α-copaene	1357	tr	tr	tr	0.2	–	–	0.2	0.1	tr	tr	0.4	0.1	tr	tr	tr
geranyl acetate	1365	–	tr	–	–	tr	–		–	–	0.2	–	0.1	–	–	–
geranyl acetate + β-bourbonene		–	–	–	–	–	–	0.5	–	–	–	–	–	–	–	–
β-bourbonene	1365	–	0.1	tr	tr	–	–		0.1	–	–	tr	–	tr	–	–
β-cubebene	1371	–	tr	–	–	–	–	tr	–	–	–	tr	–	tr	tr	tr
1,7-di-*epi*-α-cedrene	1381	–	–	–	–	–	–	–	–	–	–	–	–	tr	–	tr
(*Z*)-caryophyllene	1387	–	tr	tr	tr	–	–	–	–	–	–	tr	tr	tr	–	tr
α-gurjunene	1390	tr	tr	tr	–	–	–	tr	–	–	–	tr	tr	tr	tr	–
(*E*)-caryophyllene	1406	10.1	4.2	15.6	10.4	2.8	5.0	5.5	8.1	3.7	3.0	11.5	2.3	7.9	tr	8.7
β-gurjunene	1409	tr	tr	tr	tr	–	–	0.3	0.1	tr	tr	tr	tr	tr	tr	tr
α-*trans*-bergamotene	1415	–	–	–	–	–	–	–	–	–	tr	–	tr	–	–	–
aromadendrene	1420	1.1	0.6	2.1	1.7	0.1	0.6	1.1	0.2	0.7	0.2	1.1	0.1	1.2	0.9	–
α-guaiene	1423	tr	–	–	0.3	–	–	–	–	–	–	tr	–	tr	–	–
α-humulene	1435	0.6	1.2	2.0	2.8	0.8	1.0	3.0	2.3	3.0	2.1	2.1	0.4	2.9	0.9	2.0
dehydroaromadendrene	1444	–	–	–	tr	–	–	tr	–	–	–	–	tr	–	tr	–
dehydroaromadendrene + *cis*-muurola-4(14,)5-diene		–	–	1.1	–	–	–	–	–	0.4	–	–	–	–	–	–
cis-muurola-4(14),5-diene	1445	tr	–	–	0.4	–	–	tr	–	–	tr	–	tr	0.7	tr	0.6
α-acoradiene	1445	–	–	–	–	–	–	–	–	tr	–	–	–	–	–	tr
β-acoradiene	1446	–	–	tr	tr	–	–	–	–	–	–	tr	–	tr	–	–
γ-gurjunene	1451	–	–	tr	tr	–	–	–	–	–	–	–	–	tr	tr	–
γ-muurolene	1457	tr	0.2	tr	0.5	tr	tr	0.5	0.2	0.2	0.2	0.3	tr	0.5	tr	tr
germacrene D	1461	–	tr	–	–	–	–	0.3	0.1	tr	tr	tr	tr	tr	tr	tr
ar-curcumene	1461	–	–	tr	tr	–	–	–	–	–	0.6	–	–	tr	–	tr
β-selinene	1466	–	tr	tr	tr	–	–	tr	–	–	tr	tr	tr	tr	tr	tr
cis-β-guaiene	1467	–	tr	tr	tr	–	–	–	–	–	–	–	–	tr	–	–
viridiflorene	1471	0.5	0.2	1.7	0.7	tr	0.2	tr	0.2	0.3	0.2	0.5	tr	0.5	tr	–
epi-cubebol	1471	–	–	–	–	–	–	tr	–	–	–	–	–	–	–	tr
α-selinene	1476	–	–	–	–	–	–	0.7	–	–	–	–	–	–	–	–
bicyclogermacrene	1476	–	–	–	–	–	–	–	–	–	tr	–	–	–	0.6	tr
epizonarene	1478	–	–	tr	0.4	–	–	–	–	0.2	–	–	–	tr	–	tr
epizonarene + α-muurolene	1479	–	–	–		–	–	–	–	–	–	0.4	–	–	–	–
α-muurolene	1480	–	tr	–	–	–	–	tr	tr	–	tr	–	tr	tr	tr	–
(*E*,*E*)-α-farnesene	1485	–	–	–	–	–	–	–	–	–	–	–	–	–	–	tr
β-bisabolene	1490	–	–	–	–	–	–	–	–	–	tr	–	–	–	–	–
β-curcumene	1492	–	–	–	–	–	–	–	–	–	0.3	–	–	–	–	–
γ-cadinene	1494	tr	tr	tr	0.5	tr	tr	0.2	0.1	0.3	tr	0.8	tr	0.6	tr	tr
cubebol	1495	–	–	–	–	–	–	–	–	–	–	–	–	–	–	tr
cis-calamenene	1501	–	–	2.7	1.2		–	–	–	–	–	–	–	1.5	tr	0.7
cis-calamenene + δ-cadinene		–	–	–	–	0.1	0.3	–	–	0.4	–	–	–	–	–	–
δ-cadinene	1503	0.3	0.2	–	tr	–	–	0.7	0.3	–	0.3	1.5	0.1	tr	tr	–
trans-calamenene		–	–	tr	–	–	–	–	–	–	–	–	–	–	–	–
trans-calamene + cadina-1,4-diene		–	–	–	–	–	–	–	–	–	–	–	–	tr	–	–
cadina-1,4-diene	1512	–	tr	–	–	–	–	tr	–	tr	tr	tr	–	–	–	–
α-cadinene	1517	–	tr	tr	tr	–	–	tr	–	tr	tr	tr	–	tr	tr	–
α-calacorene	1521	–	tr	tr	0.4	–	tr	tr	–	tr	tr	tr	–	tr	tr	–
β-calacorene	1542	–	tr		–	–	–	tr	–	–	–	–	–	tr	tr	–
(*E*)-nerolidol	1544	–	tr		–	–	–	–	–	tr	–	–	–	–	–	–
caryophyllene alcohol	1551	–	–		–	–	–	–	–	–	–	tr	–	–	–	–
(*Z*)-3-hexenyl benzoate	1552	–	–		–	–	–	tr	–	–	–	tr	–	–	–	–
spathulenol	1557	tr	–	–	0.3	–	–	tr	–	0.3	–	–	tr	1.1	1.7	tr
spathulenol + caryophyllene oxide		–	–	4.1	–	–	–	–	–	–	–	–	–	–	–	–
caryophyllene oxide	1565	1.7	1.8	–	2.1	0.1	0.4	0.3	0.7	0.4	2.4	3.5	0.5	2.4	tr	3.9
viridiflorol	1573	2.5	1.4	8.4	–	0.1	0.8	1.2	1.1	1.2	2.6	2.4	0. 1	2.6	5.9	2.5
guaiol	1574	0.3	–	–	4.0	–	–	–	–	–	–	–	–	–	–	–
humulene epoxide II	1589	tr	0.4	–	0.7	–	tr	0.2	0.1	0.4	1.5	0.7	0.1	1.0	1.0	0.7
1,10-di-*epi*-cubenol	1592	–	–	–	–	–	–	–	–	–	–	–	–	tr	–	tr
1-*epi*-cubenol	1611	–	–	–	0.4	–	–	–		–	–	–	–	–	–	–
caryophylladienol[c]	1611	0.2		–										tr	–	0.4
epi-α-cadinol	1623	–	–	–	–	–	–	tr	–	tr	–	–	–	–	–	tr
cubenol	1625	–	–	–	–	–	–	tr	–	tr	–	tr	–	tr	–	–
cubenol + cedr-8(15)-en-9-α-ol		0.6		–	0.4									–	–	–
cedr-8(15)-en-9-α-ol	1626	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.4

Table 3. (Continued)

compound	KI[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
α-muurolol	1627	–	–	–	–	–	–	–	–	–	tr	tr	–	tr	–	–
α-cadinol	1635	–	tr	–	–	–	–	–	–	–	tr	tr	–	tr	–	–
14-hydroxy-9-*epi*-(*E*)-caryophyllene	1651	0.6	0.3	–	0.2	–	–	–	0.1	tr	tr	1.7	–	0.7	–	0.5
cadalene	1656	–	–	–	tr	–	–	–		–		tr	–	–	–	–
cadalene + khusinol		–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.7
khusinol	1661	–	–	–	–	–	–	–		–		tr	–	tr	–	–
cis-14-muurol-5-en-4-one	1665	–	–	–	–	–	–	–	–	–	–	–	–	tr	–	–
14-hydroxy-α-humulene	1692	–	–	–	–	–	–	–	–	tr	tr	–	–	–	0.3	0.6
benzyl benzoate	1748	–	–	–	–	–	–	–	–	–	–	tr	–	–	–	–
isopimar-9(11),15-diene	1898	–	tr	–	0.2	–	–	tr	0.1	0.1	tr	tr	tr	tr	tr	–
manool	2052	4.0	0.6	11.2	2.0	1.9	0.2	0.4	0.9	1.5	0.3	2.5	tr	–	–	0.4
total (%)		99.6	99.6	98.2	98.3	100.0	99.3	97.5	99.3	98.5	95.5	94.5	99.8	98.9	100.0	99.48
monoterpenes (%)		25.4	26.4	8.1	11.2	24.8	27.6	18.8	20.4	17.1	14.6	26.2	20.9	20.6	19.0	16.4
oxygenated monoterpenes (%)		51.7	62.0	41.2	56.8	69.3	63.0	63.4	64.1	68.3	67.3	38.9	75.3	54.7	69.7	61.0
sesquiterpenes (%)		12.6	6.7	25.2	20.0	3.8	7.3	13.0	11.8	9.2	6.9	18.6	3.0	15.8	2.4	12.7
oxygenated sesquiterpenes (%)		5.9	3.9	12.5	8.1	0.2	1.2	1.7	2.0	2.3	6.5	8.3	0.6	7.8	8.9	9.0
diterpenes (%)		4.0	0.6	11.2	2.2	1.9	0.2	0.4	1.0	1.6	0.3	2.5	0	0	0	0.4

[a] Kovats retention index relative to C_9–C_{23} *n*-alkanes on the HP 5MS column. [b] Trace (<0.05%). [c] Correct isomer not identified.

Table 4. Inhibition of Mycelial Growth (Percent of control) at Different Concentrations of the Essential Oil of the Two Populations of *S. fruticosa* (1, Population 4, Mt. Parnes; 2, Population 5, Sifnos)

	50 μL/L		100 μL/L		250 μL/L		500 μL/L		1000 μL/L		2000 μL/L	
fungus	1	2	1	2	1	2	1	2	1	2	1	2
Ss	0	0	0	0	3.29	0	15.19	24.51	40.0	65.2	75.95	94.12
Rs	0	0	28.33	0	29.64	1.69	57.41	24.07	60.04	69.83	100	100
Fs	23.08	7.39	23.08	9.34	31.17	12.45	34.01	13.62	67.61	29.18	100	52.14
Fp	1.67	4.48	5.0	5.97	21.67	14.03	23.33	16.42	41.67	28.96	55.0	50.15
Fo	6.94	2.92	12.30	2.92	13.25	2.63	35.96	7.02	43.85	26.32	45.74	44.44

Statistical Analyses. To examine the relationship between the 15 populations of S. *fruticosa*, PCA was applied to the volatile oil constituent data, and the two eigenvalues correspond to 86.08 and 6.77 of the total variance. The spatial relationship of the 15 populations using the first two vectors from this analysis accounts for the 92.85% of the variation (**Figure 1**) and divides the populations into two groups. The first principal component (PC1) explains 86.08% of the variation and has an eigenvalue of 12.91. PC2 accounts for 6.77% of the variation, with an eigenvalue of 1.02. The PCAs separate the populations into two groups: (1) populations 4, 8, and 11; and (2) all of the other populations.

The dendrogram (**Figure 2**) suggests the existence of two clusters (clusters I and II). The essential oils of cluster I (populations 4, 8, and 11) are characterized by a high content of camphor (14.6−15.6%) and a low content of 1,8-cineole (16.9−21.3%) compared to the other populations.

As is evident from both statistical analyses, the three populations of *S. fruticosa*, population 4 (Mt. Parnitha, central Greece), population 8 (Crete, southern Greece), and population 11 (Chios, eastern Greece), clearly stand out, forming a separate group in PCA or a dichotomy in CA. No relationship between the composition of the oils and the habitat can be established. Variability in quantity and composition of essential oils of *S. fruticosa* may be due to biodiversity/genetic differences or a mixture of reasons (*21*). Previous reports mention that originally existing differences are maintained even under glasshouse cultivation (*22*).

Antifungal Activity. The antifungal activities of the essential oils of two populations (4 and 5), belonging in the two different groups of CA and PCA analyses, and of their two main components (1,8-cineole and camphor) were studied against five phytopathogenic fungi. A dose-dependent inhibition of *F. oxysporum* f. sp. *dianthi*, *F. solani* f. sp. *cucurbitae*, *F. proliferatum*, *R. solani*, and *S. sclerotiorum* was caused by the oils of *S. fruticosa* (**Table 4**). The essential oil of the two populations presented noticeable activity on three of the fungi tested. In particular, the oil of population 4 inhibited the growth of *R. solani* (EC_{50} = 398.73 μL/L) and *F. solani* f. sp. *cucurbitae* (EC_{50} = 444.79 μL/L), and the oil of population 5 inhibited the growth of *R. solani* (EC_{50} = 706.27 μL/L) and *S. sclerotiorum* (EC_{50} = 773.20 μL/L) (**Table 7**). Mycelial growth of *R. solani*, which was found to be the most sensitive fungus, was completely inhibited by both oils at a concentration of 2000 μL/L (**Table 6**). In general, according to the EC_{50} values, the oil of population 4 was found to be more active than that of

Table 5. Inhibition of Mycelial Growth (Percent of Control) at Different Concentrations of Camphor (1) and 1,8-Cineole (2)

	20 μL/L		50 μL/L		100 μL/L		200 μL/L		500 μL/L	
fungus	1	2	1	2	1	2	1	2	1	2
Ss	0	0	5.93	1.57	5.93	2.01	16.48	3.82	51.65	15.73
Rs	0	0	0	0	0	0	9.72	0	64.86	4.41
Fs	1.16	0	1.94	1.96	5.81	1.96	11.63	3.92	31.01	9.80
Fp	0	0.96	3.05	6.39	7.01	7.90	12.20	9.58	35.06	19.17
Fo	0	0	0	0	0	0	4.84	0	37.10	21.02

Table 6. MIC Values (Microliters per Liter) of the Oils of Populations 4 and 5, 1,8-Cineole and Camphor Tested

	Fo	Fs	Fp	Ss	Rs
S. fruticosa oil (population 4)	>2000	2000	>2000	>2000	2000
S. fruticosa oil (population 5)	>2000	>2000	>2000	>2000	2000
1,8-cineole	>500	>500	>500	>500	>500
camphor	>500	>500	>500	>500	>500

Table 7. EC_{50} Values (Microliters per Liter) of the Oils of the Two Populations and Their Main Components on Fungal Species Tested

	Fo	Fs	Fp	Ss	Rs
S. fruticosa oil (population 4)	1934.11 (1458.49, 2796.39)[a]	444.79 (319.01, 627.34)	1566.73 (1169.33, 2342.80)	1164.74 (1070.52, 1276.57)	398.73 (321.24, 496.24)
S. fruticosa oil (population 5)	>2000	>2000	>2000	773.20 (683.29, 872.31)	706.27 (608.36, 816.11)
1,8-cineole	>500	>500	>500	>500	>500
camphor	>500	>500	>500	445.50 (388.99, 529.13)	403.74 (349.47, 483.22)

[a] Numbers in parentheses indicate 95% confidence limits determined by probit analysis.

population 5. Previous investigators reported that *S. fruticosa* essential oil was noninhibitory or slightly inhibitory to the mycelial growth of *Botrytis cinerea*, *Macrophomina phaseolina*, *F. oxysporum*, and *Exserohilum turcicum* (*3*) and *Fusarium moniliforme*, *R. solani*, *S. sclerotiorum*, and *Phytophthora capsici* (*4*). The results of this study indicate that the oils of both populations were slightly inhibitory to *F. oxysporum* f. sp. *dianthi* and *F. proliferatum*, whereas against *F. solani* f. sp. *cucurbitae* (population 4), *R. solani*, and *S. sclerotiorum* the oils exerted high antifungal activity. Differences between the previous and present results may be due to the fungal strain used or the chemical composition of the tested oil.

In an effort to evaluate the individual contributions of the main components of the oils to the antifungal property, pure commercial 1,8-cineole (16.9 and 34.7% in populations 4 and 5, respectively) and camphor (15.6 and 15.9%, respectively) were tested for their fungitoxicity to the radial growth of the five fungi at concentrations up to 500 μL/L (**Table 5**). Camphor was effective, reducing by 51.65 and 64.86%, the radial growth of *S. sclerotiorum* and *R. solani*, respectively, at the concentration of 500 μL/L. The three *Fusarium* species tested were less sensitive to the pure components. Pure commercial 1,8-cineole did not cause significant inhibition on the radial growth of all fungi species. Our results are in agreement with other researchers who have reported 1,8-cineole to have slight or noninhibitory effect on human and plant pathogens (*4*, *7*, *10*). Previous data have shown low to high activity of camphor on various human and soilborne fungi (*6*, *10*).

The differences in the activities of the two oils can be ascribed to their varying quantitative compositions. Their activity is not mainly attributable to the presence of camphor because the inhibitory effect of the concentration of 500 μL/L of the above pure component is moderate (51.65−64.86%) to the most sensitive fungi *R. solani* and *S. sclerotiorum*. The higher effectiveness of the oils leads us to believe that other components of the total oils can exert, if not a direct activity, at least a synergic effect on camphor action.

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