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Chemical Composition, Seasonal Variability, and Antifungal Activity of *Lavandula stoechas* L. ssp. *stoechas* Essential Oils from Stem/Leaves and Flowers

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Essential oils from the stems/leaves (L) and flowers (F) of *Lavandula stoechas* L. ssp. *stoechas* growing wild in southern Sardinia (Italy) were extracted by hydrodistillation and analyzed by gas chromatography coupled with flame ionization detector and ion trap mass spectrometry. The major compound was fenchone, accounting for, on average, 52.60% in L and 66.20% in F, followed by camphor (13.13% versus 27.08%, in L and F, respectively). F essential oil yields (volume per dry weight) decreased from the beginning to the end of the flowering stage, whereas L yields remained constant during the year. The nine main compounds derived from two different subpathways, A and B. The compounds that belong to the same subpathway showed a similar behavior during the year. The essential oils were tested for their antifungal activity using the paper disk diffusion method. The essential oils tested were effective on the inactivation of *Rhizoctonia solani* and *Fusarium oxysporum* and less effective against *Aspergillus flavus*. Among the single compounds tested, fenchone, limonene, and myrtenal appeared to be the more effective on the inhibition of *R. solani* growth.

KEYWORDS: ***Lavandula stoechas* L. ssp. *stoechas*; essential oil; harvesting time; fenchone; biosynthetic pathways; antifungal activity**

INTRODUCTION

The *Lavandula* genus is distributed in all Mediterranean regions. It belongs to the family of Lamiacee and consists of about 20 species of small evergreen shrubs having aromatic foliage and flowers (*1*). In Italy, five species are diffused, but mainly two, *stoechas* and *angustifolia*, grow wild or are cultivated (*2*). In Sardinia, only the ssp. *stoechas* grows spontaneously (*3*). *Lavandula stoechas* L. ssp. *stoechas* is used in perfumery and cosmetics (*4*, *5*). Recently, Gilani et al. (*6*) reported anticonvulsant, sedative, and antispasmodic activities. Dadalioğlu et al. (*7*) confirmed that the essential oil (EO) of *L. stoechas* possesses weak antibacterial activity. In Sardinia, it is used in folk medicine as an antispasmodic, a sedative, and a diuretic and for rheumatic diseases (*5*).

The most widely spread chemotype of *L. stoechas* L. ssp. *stoechas* is the camphor−fenchone (*1*). Many papers deal with the EO chemical composition of *L. stoechas* L. ssp. *stoechas* from different Mediterranean regions; the Moroccan (*9*), Corsican (*10*, *11*), Grecian (*12*, *13*) and Turkish (*7*) EO are of the camphor−fenchone chemotype with an important contribution of 1,8-cineole. Among these Skoula et al. (*13*) reported also the chemical differences between leaves and inflorescence EO, and Goren et al. (*14*) reported a peculiar Turkish chemotype. Several researchers studied EO composition and yield modifications due to the season, the plant parts, and the site of harvest (*15*−*19*), but no paper was found concerning the seasonal and plant part variability of the EO of *L. stoechas*. Essential oils are the fragrant volatile product of the secondary metabolism of aromatic plants, although isoprenoids are universally synthesized through condensation of the five-carbon compound isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP) (*20*). Two distinct and independent biosynthetic pathways from these precursors exist in the plants, the mevalonate pathway and the mevalonate independent pathway (*21*, *22*). **Figure 1** reports the biosynthetic pathway of *L. stoechas* ssp. *stoechas* essential oils main compounds, starting from the α-terpinyl intermediate. Knowledge of how volatiles change over the year is essential in choosing the most appropriate organ type and the period of harvest.

Continuing our investigations on aromatic plant volatile composition (*23*−*26*), we studied in this paper (a) the essential oil yields and compositions from stems/leaves and flowers of *L. stoechas* L. ssp. *stoechas* growing wild in southern Sardinia (Italy); (b) the chemical variation of EO profiles during one year of harvesting, and during the flowering stage over two

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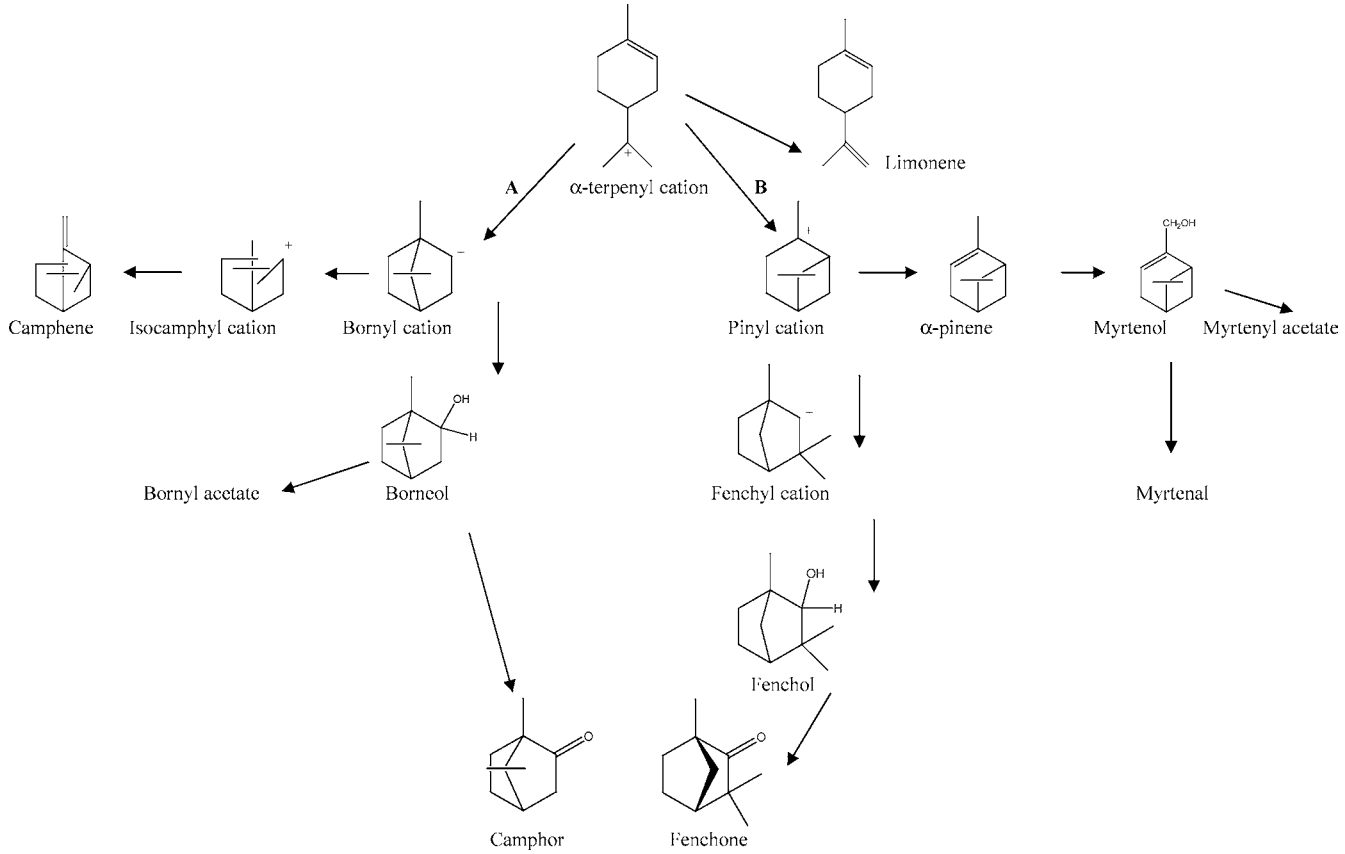

Figure 1. Biosynthetic pathway of the major compounds of *L. stoechas* L. ssp. *stoechas* essential oils [modified from Dewick (*20*)].

Table 1. Temperature and Rainfall Registered during the Experiment

month	temperature (°C) max	min	mean	rainfall (mm)
March 2004	17.9	7.1	12.0	59.0
April 2004	21.1	9.7	14.7	127.8
May 2004	24.6	11.3	17.7	44.0
June 2004	33.0	15.7	24.4	0.2
July 2004	34.7	19.4	26.9	0.0
Sept 2004	31.9	17.5	24.1	54.8
Oct 2004	28.9	15.4	21.2	30.8
Nov 2004	20.2	9.7	14.0	154.4
Dec 2004	18.0	9.2	12.9	137.6
Jan 2005	15.9	3.8	8.9	22.8
Feb 2005	14.2	4.0	8.6	109.6
March 2005	20.0	5.9	11.9	22.8
April 2005	22.5	9.3	15.5	92.2
May 2005	30.2	12.6	21.1	3.0
June 2005	34.4	17.7	25.8	0.6

years; and (c) the antifungal activity of the essential oils against three common plant pests, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Aspergillus flavus*.

MATERIALS AND METHODS

Plant Material. Samples of *L. stoechas* L. ssp. *stoechas* were collected early in the morning from a natural station of almost 5000 m^2 (Uta, Cagliari, Italy) at sea level. Three random plant samples (RPS) of ≈2.5 kg from 20 plants were harvested between March 2004 and June 2005 (**Table 1**). After harvesting, the samples were carried in jute bags at ≈20 °C to the laboratory for processing and analysis. The fresh flowering tops, when present, were separated from the stems/leaves. Ten grams of each sample was left to dry at 100 °C for 1 h and then placed in desiccators to rich ambient temperature and weighed to verify their water content. The specimens were identified and deposited in the Herbarium of the Department of Toxicology of the University of Cagliari.

Table 2. Harvesting Periods, Moisture, and Yields from Different Parts of *L. stoechas* L. ssp. *stoechas*[a§]

no.	sampling	moisture (%) stem/leaves	flowers	yield[b] (%) stem/leaves	flowers
1	March 22, 2004	65.4fgh		1.6abc	
2	April 5, 2004	72.7bcd	78.9b#[c]	1.2abc	2.7fg#
3	April 19, 2004	76.5ab	78.6b	1.8abc	1.9ef
4	May 3, 2004	72.2bcde	75.3b	1.3abc	0.9abcd
5	May 17, 2004	72.0bcd	73.3b	1.4abc	1.0abc
6	May 31, 2004	65.5fgh	62.6c	1.7abc	0.7ab#
7	June 14, 2004	65.7fgh	59.4c#	2.1bc	0.5ab#
8	June 27, 2004	27.7n	25.8e	1.2abc	0.2a#
9	July 29, 2004	45.7l		1.5abc	
10	Sept 27, 2004	53.2i		0.8ab	
11	Oct 18, 2004	53.3i		2.1bc	
12	Nov 17, 2004	63.1gh		1.2abc	
13	Dec 6, 2004	66.9efg		1.6abc	
14	Jan 13, 2005	68.1defg		1.8abc	
15	Feb 10, 2005	68.2defg		1.3abc	
16	March 6, 2005	73.4abcd		2.4c	
17	April 5, 2005	78.6a	87.8a#	1.1abc	3.1g#
18	April 19, 2005	68.1defg	76.2b	1.4abc	1.8def
19	May 4, 2005	70.5cdef	78.9b#	1.3abc	1.4bcde
20	May 18, 2005	74.2abc	75.7b	0.9ab	1.7cdef#
21	June 8, 2005	60.4h	59.7c	1.9abc	0.7abc#

[a] Values within a column for each harvesting period having different letters are significantly different from each other, using Tukey's LSD test ($P < 0.05$). [b] Expressed on 100 g of dried weight; [c] #, indicates significant differences between plant parts.

Temperature and Precipitation. Environmental conditions were continuously recorded with an AD-2 automatic weather station (Silimet, Modena, Italy). Average monthly temperature and rainfall are reported in **Table 2**.

Distillation. One hundred grams of leaves (L) or flowers (F), when present, was selected from the homogeneous RPS. The samples were

hydrodistilled simultaneously, for 1 h in a Clevenger-type apparatus according to the Italian Official Pharmacopoeia XI (2002) (*27*). The essential oils were recovered directly using a micropipet from the upper part of the distillate without adding any solvent and were stored with anhydrous sodium sulfate in dark vials at 4 °C. Solutions of 1% (v/v) oil were prepared in *n*-hexane before gas chromatographic analysis.

Gas Chromatography−Flame Ionization Detection (GC-FID) Analysis. A gas chromatograph Trace (Thermo Finningan, Rodano, Milan, Italy) equipped with a FID, an AS 800 autosampler, and a split−splitless injector was used.

The capillary column was a fused silica DB5 (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d.; 0.25 μm film thickness) (J&W Scientific, Folsom, CA). The injector and the detector were operated at 150 and 280 °C, respectively. One microliter of sample was injected in the split mode (1:20). The oven was programmed as follows: 60 °C, raised to 180 °C (3 °C/min), and isothermally held for 15 min. Helium (purity = 99.999%) was used as carrier gas and nitrogen as makeup gas at 120 kPa (1.8 mL/min) and 80 kPa, respectively. H_2 and air were at 150 and 100 kPa, respectively.

Gas Chromatography−Ion Trap Mass Spectrometry (GC-ITMS) Analysis. A Varian CP 3800 gas chromatograph (Varian, Inc., Palo Alto, CA) coupled with a Saturn 2000 ion trap mass selective detector, a Varian CP 7800 autosampler, a split−splitless injector, and a MS ChemStation was used.

The column was a fused silica capillary DB-5MS (5% phenylmethylpolysiloxane, 30 m × 0.25 mm; film thickness = 0.25 μm) (J&W Scientific). Injector, trap, manifold, and transfer line temperatures were set at 150, 170, 100, and 200 °C, respectively. The mass spectrometer was calibrated weekly, following the autotune test of the software (Saturn GC/MS workstation 5.41). The oven temperature was programmed as follows: from 60 to 180 °C at 3 °C/min and isothermally held for 15 min. Helium was used as carrier gas at 1 mL/min; 1 μL of sample was injected in split mode (1:20). MS conditions were as follows: ionization mode EI from 50 to 450 amu, multiplier voltage (1×10^5 gain) of 1400 V, and multiplier offset of +100. The oil components were identified by comparison of their relative retention times with those of authentic standard references, computer matching against commercial library (*28*, *29*) and homemade library mass spectra made from pure substances and components of known oils. Mass spectrometry literature data were also used for the identification, which was confirmed by comparison of the GC retention indices (RI) on an apolar column (determined from the retention times of a series of *n*-alkanes mixture). The Kovats indices (KI) calculated were in agreement with those reported by Adams (*28*). A quantitative analysis of each oil component (expressed as percentages) was carried out by peak area normalization measurement.

Chemicals. α-Pinene, camphene, β-pinene, myrcene, α-terpinene, *p*-cymene, 1,8-cineole, γ-terpinene, terpinolene, borneol, terpinen-4-ol, α-terpineol, geraniol, verbenone, carvacrol, thymol, bornyl acetate, α-copaene, β-caryophyllene, fenchone, thujone, *trans*-pinocarveol, *trans*-verbenol, lavandulol, myrtenal, *trans*-carveol, carvone, aromadendrene, *allo*-aromadendrene, γ-gurjunene, and *cis*-ocimene were obtained from Aldrich, Acros, and Fluka (Milan, Italy); α-thujene, sabinene, δ-3-carene, limonene, linalool, α-humulene, *cis*-pinane, α-phellandrene, *p*-cymenene, myrtenyl acetate, longifolene, and valencene were purchased from Extrasynthese (Genay, France); camphor was from Carlo Erba (Milan, Italy). All compounds were of analytical standard grade. *n*-Hexane was an analytical grade solvent purchased from Carlo Erba, and Na_2SO_4 was of analytical reagent grade from Carlo Erba.

Antifungal Assay. The agar disk diffusion method was used. *Fusarium oxysporum*, *Rhizoctonia solani*, and *Aspergillus flavus* were the fungi used. Fungi were isolated and identified from feeds, in the Laboratory of Hygiene of the University of Cagliari. Petri dishes containing potato dextrose agar (PDA) were inseminated with mycelia fragments of 6 mm in diameter (10-day hold). Three paper disks (6-mm diameter, Oxioid, Milan, Italy) for each experiment were placed on the PDA medium, in each Petri disk. Three different experiments were carried out: (a) pure essential oil; (b) diluted (ethanol 80%) essential oil (1.8 and 0.9 mL/L); (c) single compounds (α-pinene, camphene, limonene, fenchone, camphor, borneol, myrtenal, bornyl acetate, and myrtenyl acetate), The plates were sealed with Parafilm M to limit air dispersion of the essential oils and incubated in the dark at 22 °C. Samples with the mycelia in PDA and paper disk impregnated of distilled water instead the essential oil were used as control. The effectiveness of the treatments was evaluated by measuring the average diametric growth of the colonies at 4, 8, and 12 days after the inoculation. The percentage of inhibition was calculated according to the equation of Zygadlo et al. (*30*)

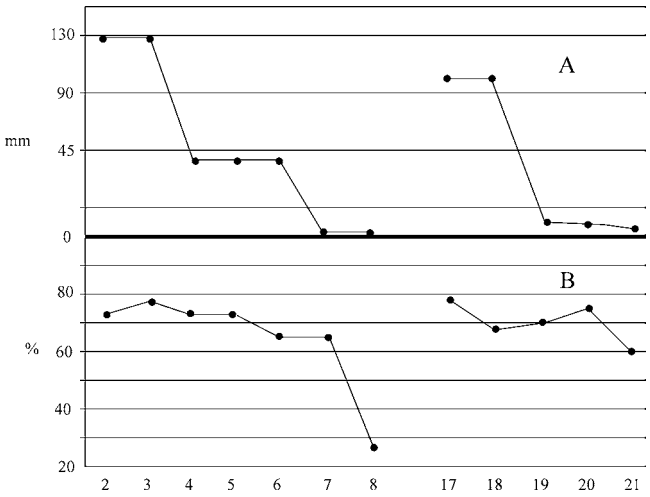

Figure 2. (**A**) Rainfall (mm) and (**B**) moisture (%) during 1-year experiment.

$$I = 100(C - T)^{C-1}$$

where I = inhibition, C = average diameter of fungal grown in PDA + water, and T = average diameter of fungi cultivated in PDA + essential oil.

All of the experiments were replicated three times, and the standard error was calculated.

Statistical Analysis. Analysis of variance (ANOVA) was carried out, and the average values have been compared with Tukey's LSD test at $P < 0.05$ using GenStat v. 7.1 software (VSN International Ltd., Herts, U.K.).

RESULTS AND DISCUSSION

Moisture Content and Essential Oil Yields. Table 2 reports monthly rainfall (mm) and maximum and minimum temperatures (°C) registered during the year of the study. The total rainfall in the period March−June 2004 was higher than in the same period of 2005, accounting for 231 and 119 mm, respectively. The maximum and minimum temperatures during the experiment ranged between 14.2 and 34.7 °C and between 3.8 and 17.7 °C, respectively. Samples of L were harvested from March 2004 to June 2005 as reported in **Table 1**; the F were collected only in springtime (between April and June) during the flowering stage. During the flowering stage harvest was carried out every 15 days, whereas during autumn and winter, harvest was performed once a month. The data reported in **Tables 1** and **2** show that the modification in the moisture content during the year did not affect significantly L yields and that the moisture content was not correlated to the rainfall amount, with an average value of 64.8% (RSD 12%). In the two blooming periods of 2004 and 2005 F showed average moisture contents of 71.3% (RSD 8%) and 75.7% (RSD 10%), respectively. However, as reported in **Figure 2**, when the rainfall dropped to its minimum, the moisture content also decreased consistently.

The highest yields in F were registered at the beginning of April (2.7 vs 3.1% in 2004 and 2005, respectively), when blooming starts, but they decreased rapidly.

Table 3. Chemical Composition (Area Percent ± SD) of the Stems/Leaves and Flowers of *L. stoechas* L. ssp. *stoechas*[a]

compound[b]	LRI[c]	RI[d]	ID method[e]	stem/leaves	flowers
α-thujene	930	924	MS, RI, std	0.01 ± 0.00	nd[g]
α-pinene	939	931	MS, RI, std	2.96 ± 0.24	0.69 ± 0.09
camphene	954	947	MS, RI, std	2.75 ± 0.28	2.02 ± 0.20
verbenene[f]	968	951	MS, RI	0.08 ± 0.01	0.02 ± 0.01
sabinene	975	969	MS, RI, std	0.03 ± 0.00	0.02 ± 0.01
β-pinene	979	975	MS, RI, std	0.07 ± 0.00	0.03 ± 0.00
cis-pinane	986	978	MS, RI, std	nd	0.01 ± 0.01
myrcene	991	988	MS, RI, std	0.02 ± 0.01	nd
β-phellandrene	1003	1007	MS, RI, std	0.09 ± 0.01	0.01 ± 0.01
δ-3-carene	1011	1009	MS, RI, std	0.06 ± 0.01	nd
α-terpinene	1017	1016	MS, RI, std	0.03 ± 0.00	0.04 ± 0.01
o-cymene[f]	1022	1018	MS, RI	0.10 ± 0.04	nd
p-cymene	1025	1023	MS, RI, std	0.41 ± 0.03	0.38 ± 0.01
limonene	1029	1028	MS, RI, std	1.10 ± 0.12	0.91 ± 0.04
1,8-cineole	1031	1031	MS, RI, std	0.22 ± 0.11	0.04 ± 0.01
β-phellandrene[f]	1031	1031	MS, RI	0.20 ± 0.02	0.17 ± 0.01
cis-ocimene	1037	1034	MS, RI, std	0.06 ± 0.01	0.01 ± 0.00
trans-ocimene[f]	1050	1044	MS, RI	0.01 ± 0.00	nd
γ-terpinene	1062	1056	MS, RI, std	0.06 ± 0.01	0.06 ± 0.01
α-terpinolene	1088	1083	MS, RI, std	0.14 ± 0.02	0.05 ± 0.01
fenchone	1087	1088	MS, RI, std	59.48 ± 0.86	72.97 ± 1.20
p-cymenene	1089	1090	MS, RI, std	0.02 ± 0.02	nd
linalool	1098	1101	MS, RI, std	0.41 ± 0.06	0.30 ± 0.08
trans-thujone	1114	1119	MS, RI, std	0.04 ± 0.04	0.01 ± 0.00
endo-fenchol [f]	1117	1119	MS, RI	0.51 ± 0.08	0.36 ± 0.02
chrysanthenone[f]	1123	1121	MS, RI	0.09 ± 0.01	0.03 ± 0.01
α-campholenal	1125	1126	MS, RI, std	0.15 ± 0.01	0.15 ± 0.01
trans-pinocarveol	1139	1139	MS, RI, std	0.04 ± 0.00	0.01 ± 0.01
cis-verbenol	1140	1141	MS, RI, std	0.04 ± 0.01	0.12 ± 0.17
camphor	1143	1146	MS, RI, std	15.36 ± 0.46	9.25 ± 0.20
p-mentha-1,5-dien-8-ol[f]	1166	1159	MS, RI	0.04 ± 0.01	0.02 ± 0.01
pinocarvone[f]	1162	1160	MS, RI	0.02 ± 0.01	0.01 ± 0.00
lavandulol	1166	1162	MS, RI, std	0.04 ± 0.06	nd
borneol	1165	1171	MS, RI, std	1.59 ± 0.29	0.68 ± 0.05
cis-pinocamphone[f]	1173	1180	MS, RI	0.06 ± 0.01	0.05 ± 0.02
terpinen-4-ol	1177	1180	MS, RI, std	0.14 ± 0.01	0.13 ± 0.01
p-cymen-8-ol[f]	1183	1187	MS, RI	0.31 ± 0.03	0.27 ± 0.02
myrtenal	1193	1195	MS, RI, std	1.42 ± 0.55	0.80 ± 0.06
verbenone	1204	1206	MS, RI, std	0.08 ± 0.04	0.05 ± 0.01
trans-carveol	1217	1219	MS, RI, std	0.02 ± 0.04	0.04 ± 0.06
carvone	1242	1243	MS, RI, std	0.08 ± 0.07	0.18 ± 0.01
bornyl acetate	1285	1283	MS, RI, std	2.42 ± 0.47	5.10 ± 0.44
lavandulyl acetate[f]	1289	1283	MS, RI	0.19 ± 0.05	0.40 ± 0.05
thymol	1290	1292	MS, RI, std	0.01 ± 0.00	0.01 ± 0.01
myrtenyl acetate	1327	1322	MS, RI, std	5.02 ± 1.22	3.69 ± 0.49
isoledene[f]	1373	1372	MS, RI	0.09 ± 0.04	0.02 ± 0.00
α-copaene	1376	1372	MS, RI, std	0.15 ± 0.04	0.03 ± 0.01
longifolene	1402	1403	MS, RI, std	0.03 ± 0.01	nd
β-caryophyllene	1418	1415	MS, RI, std	0.03 ± 0.01	0.01 ± 0.00
aromadendrene	1439	1436	MS, RI, std	nd	0.01 ± 0.01
α-humulene	1454	1451	MS, RI, std	0.02 ± 0.01	nd
allo-aromadendrene	1461	1455	MS, RI, std	0.05 ± 0.01	0.01 ± 0.00
γ-gurgjunene	1463	1469	MS, RI, std	0.01 ± 0.01	0.03 ± 0.01
germacrene D[f]	1480	1474	MS, RI	0.04 ± 0.01	nd
valencene	1491	1479	MS, RI, std	0.03 ± 0.05	nd
bicyclogermacrene[f]	1494	1488	MS, RI	0.02 ± 0.02	nd
cis-β-guaiene [f]	1490	1504	MS, RI	0.23 ± 0.03	0.18 ± 0.03
α-selinene[f]	1494	1505	MS, RI	0.40 ± 0.24	0.19 ± 0.11
rosifoliol[f]	1530	1537	MS, RI	0.01 ± 0.01	nd
viridiflorol[f]	1590	1593	MS, RI	0.01 ± 0.01	nd
cadinol[f]	1653	1660	MS, RI	0.04 ± 0.07	nd
identified compounds				97.17	99.6

[a] Essential oils of sample 4 at full flowering stage of the plant. [b] Compounds are listed in order of their elution from a DB-5MS column. [c] LRI, literature retention indices on DB-5MS column. [d] RI, retention indices as determined on DB-5MS column using homologous series of *n*-alkanes. [e] Methods of identification: MS, by comparison of the mass spectrum with those of the computer mass libraries; RI, by comparison of RI with those from the literature; std, by injection of an authentic sample. [f] Tentatively identified according to the mass spectrum (MS) and by comparison of RI with the literature (LRI). [g] nd, not detected (<0.01%).

Chemical Analysis. Table 3 reports the chemical analysis of sample 4 (May 2004) during blooming and includes all compounds found in the EO from L and F. Sixty-one compounds were identified, which accounted for 97.2 and 99.6% of the total volatile fraction in the EO from L and F, respectively.

Fenchone was the main compound in both L and F, accounting, during the experiment, for average amounts 52.60 ± 6.77 and 66.20 ± 6.99%, respectively, followed by camphor (27.08 ± 8.80 vs 13.13 ± 4.82%). α-Pinene, camphene, limonene, borneol, myrtenal, bornyl acetate, and myrtenyl acetate were the most representative compounds in L and F. The *L. stoechas* L. ssp. *stoechas* EO samples analyzed could be considered, according to the thesis of Grayer et al. (*31*), a camphor−fenchone chemotype. The data here reported on the Sardinian *L. stoechas* are mostly in agreement with the data of Dadalioğlu et al. (*7*) and Zrira et al. (*9*) except for the lower content of 1,8-cineole. The former reported the chemical composition of the EO of the aerial parts of Turkish *L. stoechas*, wherein camphor was 18.2%, fenchone 55.6%, and 1,8-cineole 8%. The latter reported the EO chemical composition of Moroccan *L. stoechas*, finding camphor at 18.6%, fenchone at 30.5%, and 1,8-cineole at 8.6%. Nevertheless, the EO obtained from *L. stoechas*, harvested in a different Turkish region (*14*), appeared to be of a peculiar chemotype characterized by the presence of menthone (12.6%), menthol (18.1%), and pulegone (40,2%). Ristocelli et al. (*10*) reported the chemical composition of the essential oils from 50 samples of *L. stoechas* from different areas of Corsica during the flowering stage; they found significant differences in the major compounds: fenchone, 14.9−75.5%; camphor, 2.5−56.2%; and 1,8-cineole, 0.17−8%. Baldovini (*11*) discussed the chemical composition of Corsican *L. stoechas* essential oils and suggested the hypothesis that the longitude could have a role in its strong chemical variation. The Grecian *L. stoechas* EO obtained from the aerial parts of the plant (*12*) was characterized by camphor (9.58%), fenchone (30.85%), and 1,8-cineole (8.12%), and it was rich in pinocarvyl acetate (10.2%). The Cretan EO of *L. stoechas* from leaves and flowers (*13*) was characterized by the low amount of pinocarvyl acetate, but it was rich in α-cadinol (7.4 and 8% in L and F, respectively). Overall, the Sardinian *L. stoechas* EO analyzed in this experiment showed a low content of 1,8-cineole and the presence of bornyl acetate in quite high percentage with respect to literature data.

Seasonal Variability. Nine major compounds, listed in **Tables 4** and **5**, which accounted for >90% of the essential oils from both L and F, were selected to discuss the seasonal chemical variability.

All compounds considered belong to the α-terpinyl cation, as reported in **Figure 1**. Camphor, camphene, borneol, and bornyl acetate derive from the bornyl cation intermediate (subpathway A), whereas fenchone, α-pinene, myrtenal, and myrtenyl acetate derive from the pinyl cation intermediate (subpathway B). Fenchone and camphor are the most represented, accounting for 80% of the total EO compounds, over the entire year. The average values in L of fenchone and camphor during the 1-year experiment were 52.77 ± 5.94 and 26.90 ± 8.49%, respectively, but they showed an opposite behavior, which can be tentatively explained by the different biochemical pathways of formation. When fenchone increased, camphor decreased and vice versa in the essential oils from both L and F. The compounds, which belong to the same subpathway of fenchone and camphor, showed a similar behavior during the year (**Figures 3** and **4**).

Limonene, which derives directly from the α-terpinyl cation, showed a trend similar to that of camphor. In the two overlapping periods (April−June 2004 and 2005) the evolution

Table 4. Chemical Variability of the Major Compounds of *L. stoechas* L. ssp. *stoechas* Leaves Essential Oil during the Year[a]

	sampling										
compound	1	2	3	4	5	6	7	8	9	10	11
α-pinene	2.23abcdef	1.68abcde	1.54abcd	2.96cdefg	3.82efgh	6.13i	4.04fghi	2.44bcdef	3.11cdefgh	0.12a	1.23abc
camphene	2.95abc	2.43abc	3.23bcd	2.75abc	3.68bcd	3.43bcd	4.19cd	3.51bcd	3.94cd	0.37a	3.37bcd
limonene	0.78bcdef	0.92cdefg	1.30fghi	1.10efghi	1.43ghi	1.58i	1.58i	1.01defgh	0.73bcde	0.05a	0.03a
fenchone	44.12abc	51.64bcdefg	42.54ab	59.48efg	57.54defg	57.46defg	50.62abcdef	39.70a	54.10cdefg	59.24efg	55.30cdefg
camphor	33.02e	23.68cd	35.88e	15.36abc	15.06ab	12.72a	19.42abcd	33.87e	22.71bcd	33.67e	36.18e
borneol	0.39ab	0.74bc	1.07c	1.59d	1.53d	0.59b	0.69bc	0.46b	0.62b	nd	nd
myrtenal	0.92cdef	1.14ef	0.83bcdef	1.42f	0.81abcdef	0.61abcdef	0.64abcdef	1.01def	0.39abcde	nd	nd
bornyl acetate	1.81ab	2.40ab	3.86b	2.42ab	2.75ab	2.27ab	3.06ab	1.67a	3.24ab	3.09ab	2.02ab
myrtenyl acetate	5.87de	6.66e	4.62cde	5.02cde	4.30bcde	2.56abdc	4.23bcde	5.12cde	1.51abc	0.68ab	0.12a
total	93.13	91.47	95.25	92.32	91.01	88.86	89.65	89.90	93.01	98.05	98.25

	sampling									
compound	12	13	14	15	16	17	18	19	20	21
α-pinene	2.32bcdef	0.74ab	2.00abdcef	3.03cdefg	5.27hi	3.44defgh	2.32bcdef	3.02cdefg	5.03ghi	4.08fghi
camphene	3.80cd	0.94ab	2.41abc	3.91cd	5.01cd	4.95cd	4.83cd	5.16cd	4.20cd	6.05d
limonene	0.72bcde	0.29ab	0.47abcd	0.38abc	0.66bcde	0.76bcdef	0.50abcd	0.87cdef	1.54hi	1.51hi
fenchone	46.31abcd	53.89bcdefg	49.35abcde	52.98bcdefg	61.64fg	62.95g	50.32abcdef	54.30cdefg	52.07bcdefg	52.67bcdefg
camphor	37.76e	37.79e	37.35e	33.75e	18.69abcd	16.76abcd	32.84e	24.19d	20.84abcd	23.33bcd
borneol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
myrtenal	nd	nd	nd	nd	0.12abc	0.03ab	0.22abcd	0.50abcde	0.51abcde	0.11abc
bornyl acetate	1.96ab	2.80ab	2.50ab	2.36ab	2.45ab	2.98ab	1.67a	2.2ab	2.64ab	2.25ab
myrtenyl acetate	4.60cde	1.66abc	3.54abcde	1.65abc	4.51cde	2.21abcd	1.51abc	3.76abcde	3.39abcde	1.58abc
total	97.48	98.10	97.66	98.05	98.35	94.10	94.52	93.99	90.40	91.62

[a] Values within a row for each compound having different letters are significantly different from each other using Tukey's LSD test ($P < 0.05$). nd, not detected (<0.01%).

Table 5. Chemical Variability of the *L. stoechas* L. ssp. *stoechas* Flower Essential Oil during the Year [a]

	sampling											
compound	2	3	4	5	6	7	8	17	18	19	20	21
α–pinene	1.78ab	1.08ab	0.69a	0.70a	0.79a	0.73a	6.12c	8.28c	3.43b	1.79ab	1.66ab	2.12ab
camphene	2.18ab	2.06a	2.02a	1.93a	3.55c	1.98a	3.08c	3.73c	3.61c	3.16c	3.01bc	3.77c
limonene	1.85ef	1.72def	0.91abc	0.86ab	1.48cde	1.18bcd	1.74def	2.86g	2.20f	1.33bcde	0.90abc	0.44a
fenchone	65.42bcde	61.03bc	72.97e	72.57e	62.10bc	66.59cde	53.29a	64.30bcd	58.21ab	67.40cde	73.57e	67.52de
camphor	11.39ab	16.91bc	9.25a	10.70ab	16.23bc	11.97ab	14.86abc	9.42a	19.11c	12.45abc	10.35ab	15.61abc
borneol	0.41ab	0.52b	0.68b	1.25c	0.68b	0.81bc	0.66b	0.00a	0.03a	0.00a	0.02a	0.02a
myrtenal	1.45e	0.89d	0.80cd	0.82cd	0.84cd	0.79cd	0.63bcd	0.00a	0.79cd	0.92d	0.51bc	0.37b
bornyl acetate	3.85bc	5.24e	5.10de	4.82cde	6.55f	7.15f	6.91f	2.56a	3.29ab	4.49cde	4.55cde	4.00bcd
myrtenyl acetate	6.29de	5.00bcde	3.69abcd	2.54ab	2.18a	4.05abcd	4.42abcde	7.08e	4.46abcde	5.37cde	2.95abc	4.02abcd
total	94.81	94.62	96.16	96.23	94.44	95.51	94.25	98.22	95.18	96.96	97.55	97.92

[a] Values within a row for each compound having different letters are significantly different from each other using Tukey's LSD test ($P < 0.05$).

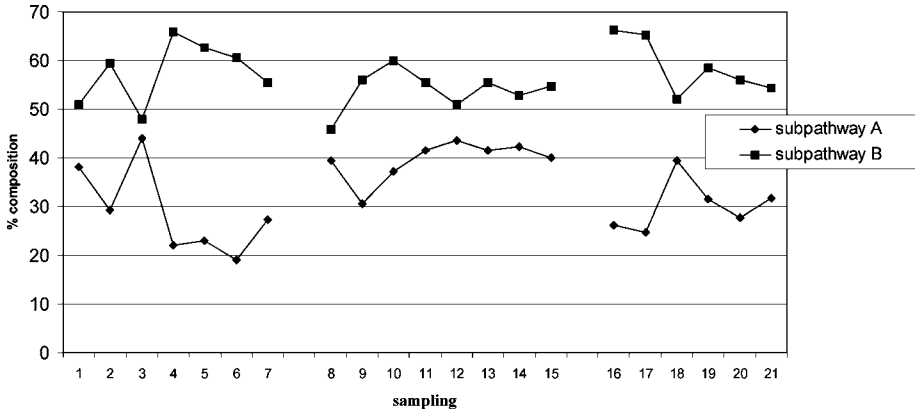

Figure 3. Variation in the percentage composition of the major compounds belonging to the two subpathways A and B in the stem/leaves essential oil during the year.

of the chemical compounds was similar for the L but it followed an opposite behavior in F.

Antifungal Activity. Table 6 reports the effectiveness of experiment a (pure essential oils) against the three strains used in the assay, *F. oxysporum*, *A. flavus*, and *R. solani*. The essential oils used were number 3 F and L, numbers 11 and 14 L (year 2004), and number 18 F and L (year 2005), representing all harvesting periods, blooming 2004 and 2005, autumn, and winter. All essential oils were active against *F. oxysporum*, weakly effective against *A. flavus*, and highly active against *R.*

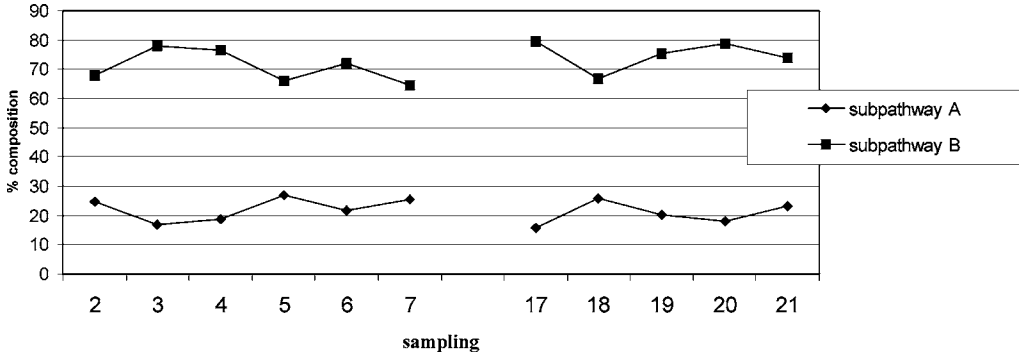

Figure 4. Variation in the percentage composition of the major compounds belonging to the two subpathways A and B in the flower essential oil during the flowering time.

Table 6. Inhibition of Mycelian Growth (Percent of Control) with 60 μL of Pure Essential Oil of *L. stoechas* L. ssp. *stoechas*, Experiment a[a]

sample tested	*F. oxysporum*	*A. flavus*	*R. solani*
flowers 3	57.2 ± 1.4	17.6 ± 0.6	100[b]
leaves 3	73.0 ± 2.3	22.0 ± 0.8	100
flowers 18	64.6 ± 3.6	15.4 ± 0.5	100
leaves 18	73.3 ± 4.1	22.2 ± 0.3	100
leaves 11	71.3 ± 4.6	31.3 ± 1.6	100
leaves 14	76.6 ± 3.4	23.1 ± 1.4	100
control	0[c]	0	0

[a] Growth of fungal species is given as mean ± standard error of three replicates. [b] 100 = no growth. [c] 0 = full growth.

Table 7. Inhibition of Mycelian Growth (Percent of Control) with Different Concentrations of the Major Compounds of *L. stoechas* L. ssp. *stoechas* Essential Oils against *R. solani,* Experiment c[a]

compound	20 μL	60 μL
α-pinene	0[c]	0
camphene	0	0
limonene	0	100[b]
fenchone	0	100
camphor	0	0
borneol	0	0
myrtenal	100	100
bornyl acetate	0	22.2 ± 1.3
myrtenyl acetate	0	33.3 ± 2.5

[a] Growth of fungal species is given as mean ± standard error of three replicates. [b] 100 = no growth. [c] 0 = full growth.

solani, which is the most sensitive fungus according to Pitarokili et al. (*32*). The oils from L were more active with respect to the oils obtained from F in 2004 as well in 2005. Experiment b with the diluted essential oils did not show any action on the fungi growth. In fact, after 12 days, the average diameters of the mycelia were similar in the control and in the sample. Experiment c was conducted against *R. solani*, and the data are reported in **Table 7**. Among the single compounds at the higher concentration (60 μL), fenchone, limonene, and myrtenal appeared to be the more effective on *R. solani*, with a total inhibition of fungi growth. Myrtenyl acetate and bornyl acetate were less active, whereas no activity was found for α-pinene, camphene, camphor, and borneol. Myrtenal was the only compound active when 20 μL of pure compound was used.

In conclusion, the yield in essential oils from L did not change during the year, whereas F essential oils evidenced a decrease of the yield to one-third in 2004 and to half in 2005 in almost 1 month, which cannot be ascribed to the moisture contents, which were nearly the same (78.9−73.3% in 2004 and 87.8−78.9% in 2005). The lesser yield can be explained only hypothesizing a progressive decrease in the production of volatiles in flowers during blooming. The chemical analysis has evidenced that the essential oils obtained from the samples investigated in this study belong to the camphor−fenchone chemotype.

The analysis of the biochemical pathways which lead to the synthesis of the major compounds found in the essential oil of *L. stoechas* L. ssp. *stoechas* showed that those belonging to sub-pathway A have an opposite behavior with respect to those belonging to pathway B, evidencing a fluctuation during the year of the biosynthetic pathways from the α-terpinyl cation intermediate.

The oils were effective on the inactivation of *R. solani* and *F. oxysporum* and less effective against *A. flavus*; nevertheless, the maximum inhibition level was observed for the essential oils obtained from the leaves. Among the major compounds tested, fenchone, limonene, and particularly myrtenal appeared to be the more effective on the inhibition of *R. solani* growth. The total EO showed a higher activity, even against the most sensitive fungi, compared to that of the single compounds; this could be explained by considering that the minor compounds could have antifungal activity or that the antifungal activity is the result of the synergism among the different compounds in the extract.

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