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Evaluation of Chemical Composition, Essential Oil and Morphological Traits in Wild Populations of *Lavandula stoechas* L. in the Mediterranean Environment

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The morphological traits, essential oil compositions and the relationship among 25 populations of *Lavandula stoechas* L. from different locations in the west part of Turkey were determined. Twenty-five *Lavandula stoechas* L. populations varied between 16.71 to 32.90 mm for flower spike length, 6.70 to 11.04 mm for flower spike width, 0.84 to 2.83 g for flower spike weight and 0.39 to 2.04% essential oil contents. Results indicated that the variability of the morphological characters, essential oil content and essential oil composition in different populations of the *Lavandula stoechas* L. would be attributed to genetic diversity. Comparing the essential oil content among different populations, there is a negative correlation between morphological characters (flower spike weight) and essential oil content and a positive correlation between altitudes of population growing location and essential oil. Eleven essential oil components were identified in *Lavandula stoechas* L. populations. All populations were characterized as 1,8-cineole/camphor chemo type.

Key Words: ***Lavandula stoechas*** **L., Morphological traits, Essential oil composition, 1,8-cineole/camphor chemo type.**

INTRODUCTION

The genus *Lavandula* plants, a member of the Lamiaceae family, grow naturally in Atlantic Island in West, over the Mediterranean Basin, North Africa, Arabian Peninsula and as far as Central and Southern India in East[1]. Two species of this genus, *Lavandula stoechas* L. *and L. canariensis* are wild growing in Anatolia[2–5].

Lavandula stoechas L., locally known as "karabasotu, gargan and kesisotu", is a perennial plant growing up to 50 cm, hairy, strong odorous, shrub, corolla blackish-purple[4, 5]. The distinguishing characteristic of its flower is that the flower head topped by infertile bracts of varying plants hybridizes readily[1]. Essential oils are obtained from the flower spikes of the plant. It is registered in many pharmacopoeias due to its medicinal properties[6–8]. This plant has been traditionally used as carminative, antispasmodic, expectorant and wound healing[5].

L. stoechas L. has been extensively studied by many researchers. Essential oil content of *L. stoechas* L. varies from 0.77% to 1.2%[3, 9]. Its aerial parts contain

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ursalic acid, vergatic acid, oleanolic acid, β-sitosterol, α-amyrin, α-amyrin acetate, lupeol, erythrodiol and flavonoids, luteolin, acecetin and vitexin[10] and two longipinane derivatives, longipin-2-ene, 7β,9α-diol-1-one and longipin-2-ene, monoacetate[11]. Its essential oil has been used as a remedy against colic and chest affections and to relieve nervous headache, biliousness and for cleaning wounds[5, 12, 13]. *L. stoechas* L. has been studied by many researchers in Mediterranean countries[3, 14–18].

In the present study, the relationship among 25 populations of *L. stoechas* L. collected from different locations in Aydin province in Turkey, based on their chemical composition of essential oils, essential oil content and morphological traits is reported.

EXPERIMENTAL

L. stoechas L. flowering plant samples were collected from 25 different locations in Kocarli-Aydin, between May and June 2004.

Plant material was identified according to flora of Turkey and East Aegean Islands[4]. Locations and altitudes in which the plant materials were collected are presented in Table-1. Plant materials were collected from 10 to 15 plants for each population in the wild.

Aydin, located in the west part of Turkey, has typical Mediterranean climate conditions (latitude 37°44′–37°49′ N, longitude 27°44′–27°50′ E). The most characteristic trait of Mediterranean ecosystem is the summer drought[19]. According to data obtained for long-term years, the annual average temperature is 17.5°C, the minimum average temperature is 8°C in January, the maximum average temperature is 28.1°C in July and the annual total rainfall is 657.7 mm.

During the full flowering stage, each individual plant was harvested for each locality from May to June. All samples were air-dried in shade at room temperature for 10 days. Flower spikes were separated from stalk, then the width and length of flower spikes were measured from 10 to 15 plants for each population and these flower spikes were weighed to calculate flower spike weight. The plant materials were stored in paper bags in dark conditions until the analysis.

Essential oil analysis

The essential oil was extracted from drug flores spikes by hydro-distillation for 3 h under continuous steam using Clevenger apparatus according to standard procedure described in European Pharmacopoeia[20]; the essential oil content was measured as percentage (v/w).

The chemical components of the oil samples were identified by using a Carlo-Erba Fractovap series 2350 gas chromatograph (GC) equipped with a flame ionization detector at the Central Laboratory of the Ege University. A glass column (3 m long, 3.18 mm internal diameter), packed with 3% OV-150 Chromosorb 80/100-mesh, was used to identify the essential oil content. Each GC run lasted for 20 min. The chromatographic conditions were: column temperature 110°C, injector and detector temperature 250°C, flow rates of carrier gases: nitrogen 25 mL/min, hydrogen 1.5 kg/cm^2, dry weather 1.5 kg/cm^2, printer

Beckman, integrator Spectra Physic, paper speed 0.5 cm/min, injection volume 0.5 μ, solvent used chloroform.

Peaks taken from GC were identified using the retention times obtained for reference standards of α-pinene, β-prinene, 1,8-cineole, fenchon, camphor, mentol, borneol, α-terpineole, pulegon, piperiton and bornyl acetate. Relative content (%) of individual constituents of the oil was calculated proportionally on the basis of the peak area corresponding to each component.

Statistical analysis

The data collected in all populations were analyzed separately by using the SPSS statistical software package[21].

RESULTS AND DISCUSSION

Locations and altitudes of twenty-five *Lavandula stoechas* L. population collected from different areas of Aydin are presented in Table-1. These altitudes were between 70–630 m. Davis[4] reported that they also occur at higher altitudes (up to 700 m).

TABLE-1
COLLECTED LOCATIONS OF *LAVANDULA STOECHAS* L.

Population No.	Locations	Altitude
1.	Kocarli-Karatas	120
2.	Buyukdere-Cinli cuma	75
3.	Sobuca-Harim	75
4.	Cerkez-Kale	85
5.	Tekeli-Taslidere	210
6.	Kizilkaya	200
7.	Kizilkaya-Top sahasi	150
8.	Guduslu-Ibrahimaga tepesi	100
9.	Haydarli-Akmar tepesi (Kargan tepesi)	100
10.	Cincin-Develi	70
11.	Boydere-Evsekler yolu	100
12.	Evsekler-Cesme	200
13.	Evsekler-Gozkayasi	250
14.	Evsekler-Alemdag	290
15.	Karacaoren-Harman beleni	490
16.	Karacaoren-Mezarlik	510
17.	Zeytin-Baglar ustu	350
18.	Timinciler-Boyak	500
19.	Satilar-Topderesi	630
20.	Akmescit-Kavakli	490
21.	Dere-Aci bahce	375
22.	Yagcideresi-Arap menderes kuyu	350
23.	Cesme-Kostepesi	550
24.	Cesme-Demirciftligi	400
25.	Sapalan-Kale alti	530

Variance, standard deviation, standard error and variation coefficient of investigated characteristics in *Lavandula stoechas* L. populations are given in Table-2. Coefficient of variation for flower spike weight and essential oil content was higher than other characters. It shows large variability in populations for these characters.

TABLE-2
VARIANCE, STANDARD DEVIATION, MEAN AND VARIATION COEFFICIENT OF INVESTIGATED CHARACTERS IN WILD GROWING POPULATIONS

Characters	Variance	Standard deviation	$S\bar{x}$	CV
Flower spike length (mm)	7.6381	2.7637	0.5527	11.5887
Flower spike width (mm)	0.8889	0.9428	0.1886	9.3471
Flower spike weight (g)	0.1980	0.4450	0.0890	23.0500
Essential oil content (%)	0.1880	0.4336	0.0867	33.6881

Populations of *Lavandula stoechas* L. varied between 16.71–32.90 mm for flower spike length, 6.70–11.04 mm for flower spike width, 0.84–2.83 g for flower spike weight, 0.39–2.04% for essential oil content and the average values were 23.85 mm, 10.09 mm, 1.93 mm and 1.29% for flower spike length, width, weight and essential oil content, respectively (Table-3). The highest values were recorded in populations 3 (for flower spike length and weight), 24 (for flower spike width) and 16 (for essential oil content) (Table-3).

TABLE-3
MINIMUM, MAXIMUM AND MEAN VALUES OF INVESTIGATED CHARACTERS FOR 25 POPULATIONS

Populations	Sample number	Flower spike length (mm)	Flower spike width (mm)	Flower spike weight (g)	Essential oil content (%)	Altitudes of populations (m)
1.	100	16.71	6.70	0.84	1.28	120
2.	110	26.19	10.05	1.64	0.39	75
3.	120	32.90	10.71	2.83	0.90	75
4.	130	25.54	10.39	2.06	0.91	85
5.	120	23.55	10.85	1.78	1.20	210
6.	130	23.03	10.01	1.83	1.07	200
7.	150	23.32	9.83	1.74	1.39	150
8.	120	26.73	10.36	2.24	1.03	100
9.	120	21.55	10.44	2.18	0.78	100
10.	120	22.48	10.72	2.35	1.38	70
11.	130	24.37	10.65	2.47	0.85	100
12.	130	23.65	11.03	2.51	0.92	200
13.	130	25.20	10.54	2.25	1.11	250
14.	130	23.37	10.43	2.21	0.94	290

Populations	Sample number	Flower spike length (mm)	Flower spike width (mm)	Flower spike weight (g)	Essential oil content (%)	Altitudes of populations (m)
15.	130	24.65	10.62	2.13	1.52	490
16.	130	22.28	8.40	1.22	2.04	510
17.	130	22.69	9.97	1.85	1.83	350
18.	130	24.69	9.95	1.93	0.89	500
19.	140	23.71	9.30	1.36	1.69	630
20.	130	21.69	8.89	1.64	1.81	490
21.	130	22.15	9.94	1.48	1.92	375
22.	130	24.77	10.64	1.94	1.92	350
23.	130	24.93	10.31	2.11	1.53	550
24.	130	24.92	11.04	2.22	1.21	400
25.	130	21.14	10.40	1.49	1.70	530
Min.		16.71	6.70	0.84	0.39	70
Max.		32.90	11.04	2.83	2.04	630
Mean		23.848	10.087	1.930	1.287	288

With respect to flower spike length, 80% flower spikes in populations varied between 21.57–26.42 mm. When the values of frequency on flower spike width were examined, these varied between 9.74–11.04 mm on 84% of interval values (Table-4). The distribution of frequency for flower spike weight and essential oil content were found 75% and varied between 1.64–2.63 and 0.89–1.87 of interval values, respectively (Table-5). These results indicate that the variability of the morphological characters and essential oil content in different populations of the same plant species might be attributed to genetic diversity.

TABLE-4
FREQUENCY OF *LAVANDULA STOECHAS* L. FOR FLOWER SPIKE LENGTH AND FLOWER SPIKE WIDTH

Flower spike length (mm)			Flower spike width (mm)		
Interval values	Number	%	Interval values	Number	%
16.710–18.328	1	4	6.700–7.133	1	4
18.329–19.947	0	0	7.134–7.567	0	0
19.948–21.566	2	8	7.568–8.001	0	0
21.567–23.185	6	24	8.002–8.435	1	4
23.186–24.804	9	36	8.436–8.869	0	0
24.805–26.423	5	20	8.870–9.303	2	8
26.424–28.042	1	4	9.304–9.737	0	0
28.043–29.661	0	0	9.738–10.171	6	24
29.662–31.280	0	0	10.172–10.605	7	28
31.281–32.899	1	4	10.606–11.039	8	32

TABLE-5
FREQUENCY OF *LAVANDULA STOECHAS* L. FOR FLOWER SPIKE WEIGHT AND ESSENTIAL OIL CONTENT

Flower spike weight (g)			Essential oil content (%)		
Interval values	Number	%	Interval values	Number	%
0.840–1.038	1	4	0.390–0.554	1	4
1.039–1.237	1	4	0.555–0.719	0	0
1.238–1.436	1	4	0.720–0.884	2	8
1.437–1.635	2	8	0.885–1.049	6	24
1.636–1.834	5	20	1.050–1.214	4	16
1.835–2.033	3	12	1.215–1.379	2	8
2.034–2.232	6	24	1.380–1.544	3	12
2.233–2.431	3	12	1.545–1.709	2	8
2.432–2.630	2	8	1.710–1.874	2	8
2.631–2.829	1	4	1.875–2.039	3	12

Correlation coefficients of investigated characters are given in Table-6. Flower spike length had a high positive correlation with flower spike width and flower spike weight ($r = 0.594$ and 0.721, respectively). Flower spike width had also a positive correlation with flower spike weight ($r = 0.739$). On the other hand, flower spike weight showed significant negative correlations with essential oil content ($r = -0.426$). Also, essential oil content showed significant positive correlations with altitudes of population. These results indicate that it would be important to select plant species for higher content of essential oil before bringing it from the wild to cultivation.

TABLE-6
CORRELATION COEFFICIENT BETWEEN INVESTIGATED CHARACTERS IN *LAVANDULA STOECHAS* L.

	Flower spike width (mm)	Flower spike weight (g)	Essential oil content (%)
Flower spike length (mm)	0.594†	0.721†	–0.287 ns
Flower spike width (mm)		0.739†	–0.191 ns
Flower spike weight (g)			–0.426*
Altitudes of populations (m)			0.667†

* Significant at $p \leq 0.01$ † Significant at $p \leq 0.05$

Regarding the morphological characters, relationship among characters and essential oil content in *Lavandula stoechas* L., there is no published report for Aydin, Turkey. Our study is the first data for 25 populations of *Lavandula stoechas* L.

In previous studies, the essential oil contents of *Lavandula stoechas* L. were reported by Tanker *et al.*[3], Baytop[5] and Sharma *et al.*[9] as 0.86, 0.5 and 0.77–1.2% respectively. In this study, essential oil content varied from 0.39 to 2.04. The present results values were higher than those of previous researchers' results. Environmental factors such as light, nutrient and season have effect on quantity and content of essential oils in *Lamiaceae*[22–24].

The essential oil compositions of *L. stoechas* L. are shown in Table-7 and Fig. 1. Totally, eleven essential oil components (α-pinene, β-pinene, 1,8-cineole, fenchon, camphor, menthol, borneol, α-terpineole, pulegon, piperiton and bornyl acetate) were identified. These eleven components generally constituted more than 97% of essential oil. In some populations[2, 7, 16, 19, 20, 22], 100% of essential oil consited of the eleven components mentioned above.

TABLE-7

THE ESSENTIAL OIL COMPOSITION OF *LAVANDULA STOECHAS* L

Pop.No.	α-pinene	β-pinene	1,8-cineole	Fenchon	Camphor	Menthol	Borneole	α-terpineole	Pulegon	Piperiton	Bornyl acetate	Others
1.	8.27	1.84	34.56	1.17	45.37	0.65	0.83	0.98	0.90	0.86	3.71	0.86
2.	0	0	31.94	2.72	57.77	0	0	0	2.54	0	5.03	0
3.	4.03	1.85	51.43	1.23	36.25	0	0	0.57	1.11	0	2.77	0.76
4.	2.48	1.43	43.31	0.80	44.93	0.33	0.83	0.64	0.75	0.59	3.12	0.79
5.	4.61	1.91	46.88	1.34	39.87	0.41	0	0.89	0.91	0.37	2.18	0.63
6.	5.09	1.61	45.73	0.73	42.82	0	0	0.38	0.84	0	2.30	0.50
7.	3.23	1.66	36.24	1.21	52.28	0	0	1.66	0	0.76	2.96	0
8.	3.34	1.56	47.64	1.21	39.71	0.47	0.84	0.76	0.95	0.48	2.50	0.54
9.	2.95	1.27	35.39	0.69	55.10	0.45	0	0.73	0	0.40	2.57	0.45
10.	3.61	1.48	41.48	0.74	48.15	0	0.88	0.71	0.73	0.85	0.96	0.41
11.	3.04	1.19	45.32	0.71	45.67	0	0.38	0.51	0.84	0	1.85	0.49
12.	2.24	0.96	47.08	0.52	44.40	0	0.78	0.39	0.63	0	1.89	1.11
13.	2.48	1.24	48.38	0.84	41.83	0.31	0	0.50	0.73	0.98	1.95	0.76
14.	3.82	1.44	47.15	0.76	39.60	0.43	0.84	0.51	0.87	0.73	2.61	1.24
15.	2.01	1.48	44.42	0.55	45.25	0	0.87	0	0.77	0	2.27	2.38
16.	1.45	1.51	42.17	1.15	49.42	0	1.30	0.81	1.19	1.00	0	0
17.	1.58	1.38	42.50	1.08	46.46	0	0	0.82	0.94	1.39	2.60	1.25
18.	2.23	1.40	47.84	0.79	42.23	0	0.41	0.53	0.87	0.93	1.88	0.89
19.	1.45	0	40.06	1.71	51.46	0	1.47	1.56	0	0	2.29	0
20.	1.42	1.19	49.51	1.19	44.16	0	0	0.84	0	0	1.69	0
21.	1.14	1.25	49.39	1.28	41.88	0	1.35	0.86	0.98	0.86	1.00	0.01
22.	3.41	1.60	45.90	1.04	44.72	0	0	1.32	0	0.80	1.21	0
23.	0	1.21	45.46	1.46	43.93	0	0.70	1.49	0	2.53	2.44	0.78
24.	0.85	1.51	41.00	1.03	48.54	0	0	0	1.08	0.83	2.64	2.52
25.	1.54	1.31	42.94	1.14	47.62	0	0	1.39	0.86	0.79	1.80	0.61

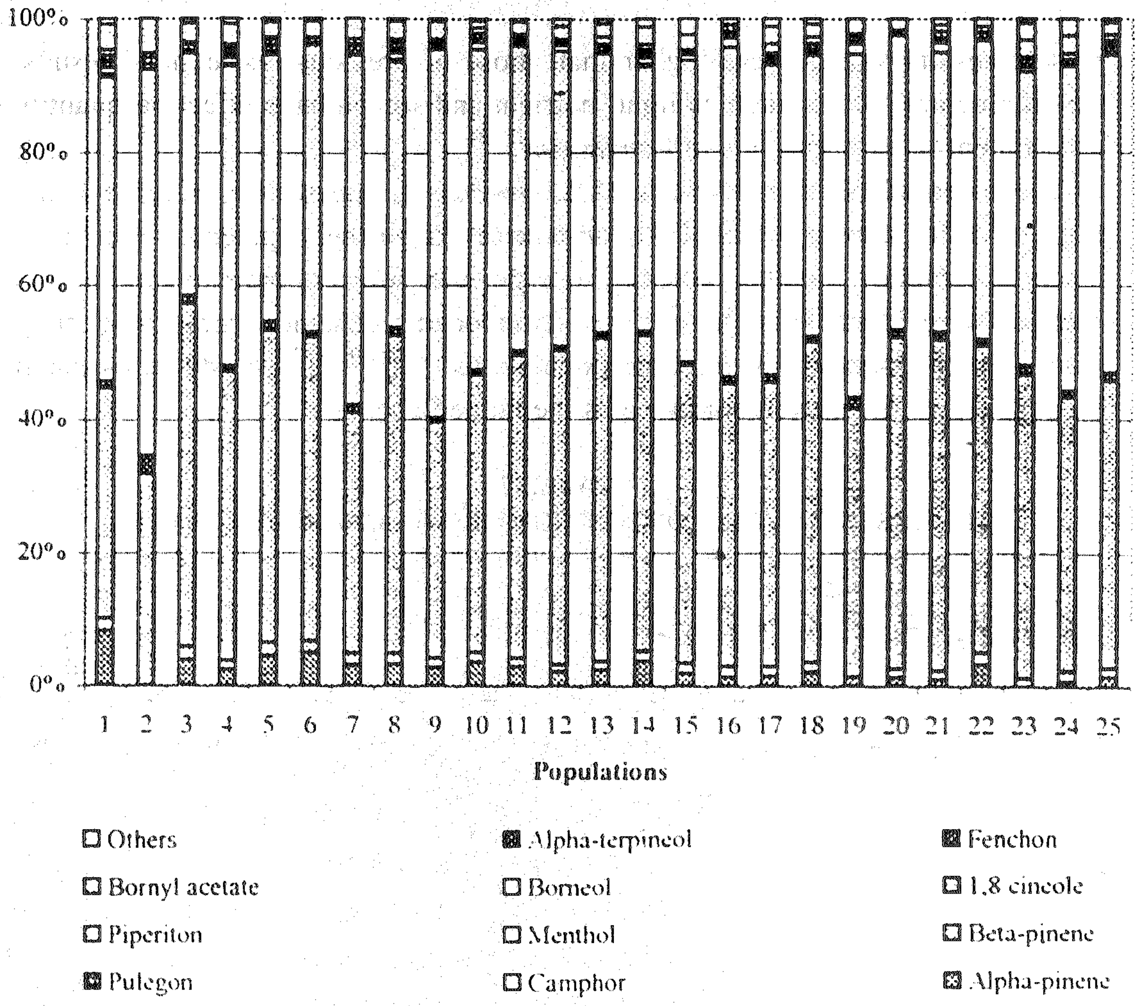

Fig. 1. The essential oil composition of *Lavandula stoechas* L

The main components of essential oil of populations, which are numerically 3, 8, 12, 13, 14, 18, 20, 21 were 1,8-cineole. 1,8-cineole varied between 31.94 and 51.43% in these populations. The highest percentage of 1,8-cineole was recorded in population 3 and the lowest one was recorded in population 2. The main components of essential oil of populations, which are numerically 2, 7, 9, 10, 16, 19, 24, 25 were camphor. Camphor varied between 36.25 and 57.77% in these populations. The highest camphor percentage was determined in population 2 and the lowest one was in population 3. The negative relationship was observed between 1,8-cineole and camphor in 3, 8, 12, 13, 14, 18, 20, 21, 2, 7, 9, 10, 16, 19, 24, 25 populations. In other words, while 1,8-cineole increased in populations, camphor decreased or *vice-versa*. The percentage of fenchon in populations varied from 0.55 to 2.72. The highest percentage was obtained in population 2 and the lowest in population 15. Generally, fenchon content in all populations was very low. Essential oil of *Lavandula stoechas* L. populations is characterized by high level contents of 1,8-cineole/camphor and lower amounts of fenchon. Thus, populations were determined for 1,8-cineole/camphor chemotype.

Skoula *et al.*[15] reported that fenchone (48.8%) and 1,8-cineole (16.7%) of *L. stoechas* L. ssp. *stoechas* growing wild in Crete were the principal constituents of leaf and flower oils, repsectively. Ristocelli *et al.*[16] indicated that the essential oil of *L. stoechas* L. ssp. *stoechas* in France were found to be fenchone (14.9–75.5%), camphor (2.6–56.2%) and 1,8-cineole (3.0–14.5%). According to Valentini *et al.*[17] fenchone and camphor contents in essential oil were in amounts higher than 10%. Our results show that specimens in populations were significantly different according to previous studies. These findings reveal that the *L. stoechas* L. growing wild in Aydin can be distinguished as having different genetic sources from those of previous studies.

The rates of other components of *L. stoechas* L. were as follows: α-pinene varied from 0.85% to 8.27% and the highest in population 1. Contents of β-pinene were found in 0.96–1.91 intervals and the highest in population 5. Tanker *et al.*[3] reported that α-pinene and β-pinene were obtained 3.7% and 1.11%, respectively. The percentages of α-pinene and β-pinene in the present results were slightly higher than of Tanker *et al.*[3] On the other hand, percentage of bornyl acetate in different populations was found between 0.96–5.03% and the highest in population 2.

The percentages of menthol, borneol, α-terpineole, pulegon and piperiton values were close to each other and varied between 0.31–0.65, 0.38–1.47, 0.38–1.66, 0.63–2.54 and 0.37–2.53, respectively (Table-7). The changes in the essential oil compositions might have arisen from genetic, geographic localization and climatic factors.

In conclusion, twenty-five populations of *L. stoechas* L. within the Mediterranean phytogeographical region were determined with significant variation in morphological and chemical parameters. Comparing the essential oil content among different populations, there is a negative correlation between morphological characters (flower spike weight) and essential oil content and a positive correlation between altitudes and essential oil content. All populations were characterized as 1,8-cineole/camphor chemotype. Variations in populations for morphological and chemical characters help us in selecting individuals with high yield and quality. Preserving local varieties in Aydin Province, these plants can be used in high-yielding population breeding and selection. Also, plants in these populations would be significant gene resources for aromatic and medicinal effect in further studies.

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