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Stachys lavandulifolia Populations: Volatile Oil Profile and Morphological Diversity

Mohammad Bagher Hassanpouraghdam [1,*], Yadeghar Salimi [1], Mohammad Reza Morshedloo [1], Mohammad Asadi [2], Farzad Rasouli [1], Sezai Ercisli [3], Hafize Fidan [4], Crina Carmen Muresan [5] and Romina Alina Marc [5,*]

1 Department of Horticultural Science, Faculty of Agriculture, University of Maragheh, Maragheh 55181-83111, Iran; yadgarsalimy1373@gmail.com (Y.S.); morshedlooreza@gmail.com (M.R.M.); farrasoli@gmail.com (F.R.)
2 Department of Plant Production and Genetics, Faculty of Agriculture, University of Maragheh, Maragheh 55181-83111, Iran; mohammadasadi704@gmail.com
3 Department of Horticulture, Faculty of Agriculture, Ataturk University, Erzurum 25240, Turkey; sercisli@gmail.com
4 Department of Tourism and Culinary Management, Faculty of Economics, University of Food Technologies, 4000 Plovdiv, Bulgaria; hfidan@abv.bg
5 Food Engineering Department, Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine, 400372 Cluj-Napoca, Romania; crina.muresan@usamvcluj.ro
* Correspondence: hassanpouraghdam@gmail.com (M.B.H.); romina.vlaic@usamvcluj.ro (R.A.M.); Tel.: +98-91-4502-7100 (M.B.H.)

Citation: Hassanpouraghdam, M.B.; Salimi, Y.; Morshedloo, M.R.; Asadi, M.; Rasouli, F.; Ercisli, S.; Fidan, H.; Muresan, C.C.; Marc, R.A. *Stachys lavandulifolia* Populations: Volatile Oil Profile and Morphological Diversity. *Agronomy* **2022**, *12*, 1430. https://doi.org/10.3390/agronomy12061430

Academic Editors: Renata Nurzyńska-Wierdak, Agnieszka Najda and Anita Biesiada

Received: 26 April 2022
Accepted: 13 June 2022
Published: 14 June 2022

Abstract: The morphological and essential oil diversity of *Stachys lavandulifolia* populations from the west and northwest of Iran were evaluated. The results showed a significant difference between the populations for nearly all the evaluated traits. The broadest variation ranges were recorded for the auxiliary shoot length, leaf length in the main branch, and the number of flowers in the inflorescences. Furthermore, cluster analysis divided 13 populations into four separate groups. GC/MS analysis verified the presence of 28 components comprising up to 94/4% of the oils. The dominant constituents were α-pinene (1.07–34.87%), (*E*)-caryophyllene (0.45–25.99%), germacrene D (3.36–20.61%), Δ-cadinene (2.82–19.90%), bicyclogermacrene (1.72–12.08%) α-terpineol (0–11.86%), α-muurolol (0.31–11.50%), *p*-cymene (0.67–9.67%), β-elemene (0.63–9.31%), and sabinene (0.32–6.29%). The results revealed that natural habitats and the related geo-climatological cues influenced morphological traits and oil composition. Considering the substantial environmental variations and the broad diversity, there would be a rich selection pool for the traits of interest. The populations are a step forward in the breeding programs for the highlighted essential oil constituents needed by the pharmaceutical and related industries. Furthermore, with the future comparative study of the populations from all Iranian territories and the neighboring countries, we will have a realistic idea of the coming conservational and exploitation programs.

Keywords: morphological diversity; *Stachys lavandulifolia*; essential oil; Iran

1. Introduction

The increasing demand for medicinal plants and their products has highlighted the role of these plants in the global economic market. Millions of people worldwide use herbs and essential oils for various therapeutic purposes [1]. Essential oils are a complex mixture of volatile low molecular weight and hydrophobic compounds found in different parts of aromatic plants, including leaves, flowers, seeds, buds, and branches [2].

The genus *Stachys* includes 300 species of annual and perennial plants. One of the species of this genus is *Stachys lavandulifolia* Vahal, which grows in different parts of Iran [3]. *Stachys lavandulifolia* Vahl, also known as lamb's ears or wood betony, an herbaceous perennial plant of the family Lamiaceae, is native to Iran, Iraq, Transcaucasus, Turkey, and

Turkmenistan. The species is important as a source of natural products used in traditional Iranian medicine. It grows in a variety of habitats including at different altitudes and is phenotypically highly variable, thus presenting itself as a rich potential source for the breeding programs [4]. This plant is used as an herbal tea and medicinal plant in traditional Iranian medicine [5]. *S. lavandulifolia* is a perennial plant that is woody at the base, hairy with numerous stems, and reaches 15–25 cm high with simple branches. The cluster inflorescences are pinkish-purple, rarely white or yellowish [6]. The aerial parts extract of this plant is used in traditional Iranian medicine to treat infections, asthma, inflammatory diseases, and rheumatism [7]. In addition to antimicrobial effects, this plant has analgesic properties and is commonly used for joint pain, rheumatism, and headache, dizziness, and nerve pain treatment [8]. This plant's major essential oil constituents are α-bisabolol, bicyclogermacrene, δ-cadinene, and spathulenol [9]. In a study, the significant compounds reported in the essential oils of *S. lavandulifolia* from Tehran, Iran were α-pinene, β-pinene, and spathulenol [10]. Evaluation of morphological diversity and essential oil yield in *S. lavandulifolia* populations collected from West Azarbaijan (Demarji and Qara Zagh), Alborz (Gachsar), Qazvin (Alamut), Isfahan (Sayad Strait), and Lorestan (Nurabad Delfan) provinces showed that the plants were significantly different in terms of plant height, leaf length and width, stem diameter, the ratio of leaf length to width, the number of leaves per plant, and the plants' fresh and dry weight [11].

Stachys species are commonly used in traditional Iranian medicine, so studying the diversity among different populations, using the morphological and essential oil criteria, is necessary to find the desired traits for future breeding programs. There have not been studies to evaluate *S. lavandulifolia* populations from their significant habitats in the western parts of Iran. There is a need to survey the distribution areas and environmental requirements to understand the impact of different climates on this species' growth traits and essential oil components. As a result, the current study aimed to assay the morphological and essential oil compositional diversity of 13 *S. lavandulifolia* populations from the west and northwest of Iran in hopes that the data would be beneficial for germplasm preservation, genetic pool studies, domestication, and breeding programs of this valuable medicinal species for the final goal of drug discovery.

2. Materials and Methods

2.1. Plant Material

The different habitats of *Stachys lavandulifolia* were identified using Flora Iranica, available literature, and local information [12]. Then, according to the populations' distribution from different areas of the natural habitats of west and northwest Iran (Figure 1), including six provinces of East Azarbaijan, West Azarbaijan, Kermanshah, Zanjan, Hamedan, and Kordestan, 13 populations *S. lavandulifolia* were collected. Samplings were done at flowering from early June to early July 2018 (Table 1). The geo-climatological information was obtained from the nearest synoptic station in the habitats.

Figure 1. Geographic map (marked location) of sampling sites for *S. lavandulifolia* plants from west and northwest Iran.

Table 1. The collection areas (habitats) geographical data of *S. lavandulifolia* plants from west and northwest Iran.

Number	Region	Latitude (E)	Latitude (N)	Altitude (Meter above Sea Level)	Average Annual Rainfall (mm)
1	Kordestan-Sanandaj Abidar	46°57′61.6″	35°18′59.01″	2029	499
2	Kordestan-Marivan Wisser	46°44′21.8″	35°20′14.6″	1863	800
3	Kordestan-Saqez Arav	46°42′19.8″	36°04′03.6″	1936	498
4	Kordestan-Divandareh Qezel Bolagh	46°45′20.3″	35°48′17.8″	2329	455
5	Kordestan-Baneh Gardaneh Khan	45°58′41.3″	36°36′37.8″	2107	800
6	Kordestan-Qorveh Sangin Abad	47°46′12.9″	35°08′16.3″	1400	418
7	Hamedan-Asadabad	48°08′20″	34°48′15.3″	1923	363
8	Kermanshah-Paveh Shaho	46°24′12.8″	35°00′18″	2000	439
9	Zanjan Province	48°35′23.2″	36°42′11.8″	2206	311
10	West Azarbaijan-Shahin Dej	46°43′22.7″	36°25′14.6″	1617	323
11	West Azarbaijan-Mahabad	45°39′18.4″	36°47′10.8″	1856	390
12	West Azarbaijan-Oshnavieh	45°48′13″	37°55′16.5″	2287	500
13	East Azarbaijan-Maragheh	45°31′52.2″	37°23′31.23″	2255	322

2.2. Morphological Traits Analysis

To evaluate the morphological traits diversity among populations, up to 15 plant samples were taken from each habitat depending on the size of the population while considering the distribution area and at a suitable distance to have a homogenous realistic sample of the habitat. Attempts were taken to prevent the harvesting of close relatives. Morphological assays were performed on plants at the full-bloom stage. Plant samples were revisited and verified by a taxonomist and herbarium specimens were stored in the Horticulture Department of the University of Maragheh, Iran.

The evaluated traits were: petal length, stem diameter, collar diameter, sepal width, sepal length, leaf width, leaf length in the sub-branches, leaf length of the main branch, the distance between internodes, petiole diameter, petiole length, inflorescence length, branch length, plant height, the number of flowering branches, and the number of flowers per inflorescence, which were measured using a digital caliper and ruler. Aerial parts were dried under shade conditions at the ambient laboratory temperature to reach the constant weight.

2.3. Phytochemical Analysis

2.3.1. Essential Oil (EO) Extraction

To extract the *Stachys lavandulifolia* essential oils, 50 g of the shade-dried aerial parts was ground and subjected to hydro-distillation for three hours using a Clevenger-type apparatus (British Pharmacopoeia model). To remove the possible water drops in the essential oils, the samples were dehydrated over anhydrous sodium sulfate (0.5−1 g) and then kept at 4 °C until gas chromatography–mass spectrometry. The essential oil content (EO %) was calculated considering dry mass.

2.3.2. GS–MS Analysis

The essential oils were analyzed using GC–FID and GC–MS. Briefly, the analysis was conducted using an Agilent 7990 B gas chromatograph equipped with a 5988A mass spectrometer and an HP-5MS (0.25 mm i.d., 30 m l., 0.25 µm f.t., 5% phenyl methylpolysiloxane). The following oven temperature was used: 5 min at 60 °C, then up to 240 °C with the rate of 3 °C/min, held for 10 min. Helium (carrier gas) flow rate was 1 mL/min; the injector split ratio was 1:30; and the mass range and electron impact (EI) were 40–400 m/z and 70 eV, respectively. The identification of constituents was performed using the procedure explained by Morshedloo et al. [13], which is based on the interactive combination of linear retention indices (RIs), calculated against a homologous series of n-alkanes (C8–C40, Supelco, Bellefonte, CA, USA), and the mass spectrum (MS) matching with commercial libraries (ADAMS, WILEY 275, and NIST 17). GC–FID analysis was performed using an Agilent 7990 B gas chromatograph equipped with a flame ionization detector (FID) and capillary column VF 5MS (30 m l., 0.25 mm i.d., 0.50 µm f.t., 5% phenyl methylpolysiloxane). The same oven temperature reported for GC–MS was used. The injection volume was 1 µL of essential oil sample in n-hexane (1:100). Quantification of the constituents was performed by peak area normalization without using correction factors [14].

2.4. Statistical Analysis

ANOVA was performed using MSTAT-C ver. 2.1 software. The mean comparisons of the data were analyzed using the least significant difference (LSD) test at a 1 and 5% probability level. Pearson's correlation coefficient and cluster analysis dendrograms were drawn by XLSTAT software using the Ward method and Euclidean square distance.

3. Results

3.1. Morphological Traits Diversity

The results from Table 2 show that the morphological traits (with the exclusion of sepal and petal length) had significant differences in the studied ecotypes of *S. lavandulifolia* ($p < 0.01$).

Table 2. ANOVA for the morphological traits of S. *lavandulifolia* populations from the west and northwest of Iran.

Trait	Average	Ecotypes #	Error	Standard Deviation	CV%
Length of the inflorescence	6.78	19.24 **	5	0.35	23.49
Flower stalk length	3.58	4.40 **	0.59	0.53	21.23
Flower stalk diameter	2.16	1.92 **	0.15	0.66	18.17
Distance between nodes	2.74	4.71 **	1.01	0.41	26.48
Leaf length in the main branches	3.13	2.45 **	0.40	0.49	21.25
Leaf length in the sub-branches	2.03	19.14 **	1.29	0.70	21.21
Leaf width	5.43	34.86 **	4.09	0.56	17.38
Sepal length	11.54	10.72 ns	9.69	0.17	31.53
Sepal width	3.71	1.96 **	0.42	0.42	24.91
Collar diameter	2.56	0.33 **	0.12	0.35	22.41
Stem diameter	1.62	73.79 **	9.56	0.53	23.56
Plant height	13.79	113.6 **	1.73	0.52	29.31
Number of flowers in the inflorescence	14.17	20.63 **	1.30	0.70	20.93
Petal length	5.23	1.16 ns	0.72	0.21	32.48
Number of flowering branches	1.62	11.4 **	2.43	0.42	29.05
Length of branches	3.19	111.56 **	0.055	0.90	23.38

ns and ** indicate no significant difference and significant at 1% probability level, respectively; # describes the statistical differences of ecotypes for the trait.

Table 3 reports the mean values of the morphological traits for 13 *S. lavandulifolia* populations from different localities. Indeed, there was no harmonized pattern for the variations. Still, due to diverse geo-climatological and edaphic differences, the traits measured showed correspondent diversity in their range between the various locations. These variations could be ascribed to the relevant environmental cues and as an epigenetic signaling response.

Table 3. Mean value for the morphological traits of 13 *S. lavandulifolia* populations from the west and northwest of Iran.

Trait	Kordestan-Sanandaj Abidar	Kordestan-Saqez Arav	Kordestan-Baneh Gardaneh Khan	Kordestan-Divandareh Qezel Bolagh	Kordestan-Marivan Wisser	Hamadan-Asadabad	Kermanshah-Paveh Shaho	Zanjan Province	West Azarbaijan-Shahin Dej	East Azarbaijan-Maragheh	West Azarbaijan-Oshnavieh	West Azarbaijan-Mahabad	Kordestan-Qorveh Sangin Abad
Length of the inflorescence (cm)	7.53 ± 0.753	9.8 ± 0.628	8.87 ± 0.784	4.93 ± 0.924	4.20 ± 0.592	7.43 ± 0.328	8.03 ± 0.654	6.80 ± 0.763	4.63 ± 0.635	7.87 ± 0.468	6.67 ± 0.930	7.33 ± 0.509	5.20 ± 0.653
Flower stalk length (cm)	3.54 ± 0.412	3.68 ± 0.269	3.51 ± 0.228	3.31 ± 0.357	3.84 ± 0.184	2.55 ± 0.263	5.29 ± 0.607	3.56 ± 0.249	2.50 ± 0.199	4.59 ± 0.354	3.18 ± 0.535	3.25 ± 0.604	3.74 ± 0.345
Flower stalk diameter (mm)	2.18 ± 0.425	1.63 ± 0.358	2.74 ± 0.526	2.63 ± 0.612	2.50 ± 0.459	1.67 ± 0.489	2.80 ± 0.647	1.35 ± 0.368	1.41 ± 0.489	2.37 ± 0.445	1.93 ± 0.528	2.50 ± 0.706	2.35 ± 0.671
Distance between nodes (cm)	2.90 ± 0.365	4.30 ± 0.528	3.41 ± 0.658	1.53 ± 0.426	2.73 ± 0.552	2.33 ± 0.621	3.10 ± 0.762	2.73 ± 0.648	1.43 ± 0.587	3.53 ± 0.794	2.87 ± 0.456	2.33 ± 0.695	2.40 ± 0.716
Leaf length in the main branches (cm)	2.60 ± 0.365	2.10 ± 0.412	3.63 ± 0.521	3.03 ± 0.498	2.47 ± 0.542	1.83 ± 0.363	4.40 ± 0.621	2.73 ± 0.638	2.73 ± 0.723	4.87 ± 0.795	4.60 ± 0.845	2.53 ± 0.559	3.17 ± 0.648
Leaf length in the sub-branches (cm)	1.73 ± 0.295	1.87 ± 0.398	2.93 ± 0.426	1.90 ± 0.484	1.40 ± 0.584	1.20 ± 0.475	2.37 ± 0.562	2.43 ± 0.685	1.33 ± 0.725	2.63 ± 0.684	2.90 ± 0.658	1.53 ± 0.721	2.13 ± 0.575
Leaf width (cm)	6.82 ± 0.789	4.34 ± 0.569	4.46 ± 0.643	5.37 ± 0.754	8.76 ± 0.769	4.31 ± 0.684	5.91 ± 0.806	5.11 ± 0.921	2.42 ± 0.421	6.62 ± 0.824	5.53 ± 0.816	4.24 ± 0.789	6.84 ± 0.945
Sepal length (mm)	12.35 ± 1.02	10.08 ± 0.96	14.48 ± 0.89	11.48 ± 0.485	12.02 ± 1.52	15.72 ± 0.99	11.90 ± 0.658	11.29 ± 0.78	7.60 ± 0.845	12.30 ± 1.89	10.84 ± 1.12	10.11 ± 0.75	9.80 ± 0.843
Sepal width (mm)	3.17 ± 0.486	4.38 ± 0.621	3.44 ± 0.546	4.22 ± 0.716	3.53 ± 0.526	2.29 ± 0.359	5.53 ± 0.854	3.05 ± 0.635	1.65 ± 0.265	4.60 ± 0.754	6.43 ± 0.754	4.50 ± 0.852	3.69 ± 0.732
Collar diameter (cm)	2.54 ± 0.356	2.49 ± 0.465	2.45 ± 0.594	2.19 ± 0.462	3.13 ± 0.721	2.11 ± 0.642	2.93 ± 0.489	2.83 ± 0.628	1.44 ± 0.284	2.79 ± 0.745	2.88 ± 0.812	2.38 ± 0.561	3.13 ± 0.864
Stem diameter (cm)	1.60 ± 0.034	1.68 ± 0.041	1.77 ± 0.052	1.29 ± 0.39	1.78 ± 0.065	1.66 ± 0.057	1.91 ± 0.071	1.69 ± 0.074	1.15 ± 0.049	1.67 ± 0.068	1.71 ± 0.078	1.44 ± 0.036	1.73 ± 0.095
Plant height (cm)	13.70 ± 0.95	16.70 ± 1.21	20.19 ± 1.41	11.20 ± 0.89	14.13 ± 0.93	12.67 ± 0.86	14.00 ± 0.83	13.00 ± 1.03	7.53 ± 0.76	17.07 ± 0.76	18.07 ± 1.14	13.33 ± 1.23	8.93 ± 0.95
Number of flowers in the inflorescence	11.93 ± 0.81	19.87 ± 1.09	19.53 ± 0.87	11.07 ± 0.91	14.27 ± 0.76	15.93 ± 0.72	18.20 ± 0.83	15.53 ± 0.94	8.47 ± 0.72	14.00 ± 0.84	12.00 ± 0.68	15.73 ± 0.80	7.73 ± 0.84
Petal length (cm)	3.79 ± 0.521	7.07 ± 0.821	7.38 ± 0.920	2.59 ± 0.365	5.34 ± 0.751	5.68 ± 0.821	7.29 ± 0.841	5.81 ± 0.816	2.35 ± 0.456	6.29 ± 0.645	5.07 ± 0.724	4.50 ± 0.632	4.83 ± 0.723
Number of flowering branches	1.18 ± 0.021	3.00 ± 0.321	1.70 ± 0.041	1.13 ± 0.032	1.50 ± 0.052	1.25 ± 0.051	1.63 ± 0.062	1.80 ± 0.053	1.33 ± 0.072	1.73 ± 0.052	1.29 ± 0.064	1.67 ± 0.074	1.80 ± 0.071
Length of branches (cm)	2.43 ± 0.354	5.03 ± 0.631	3.77 ± 0.521	2.43 ± 0.423	3.30 ± 0.641	2.87 ± 0.561	3.43 ± 0.741	2.00 ± 0.452	1.42 ± 0.384	4.63 ± 0.754	2.31 ± 0.671	2.43 ± 0.685	5.40 ± 0.841

The results showed that the Oshnavieh, Azarbaijan population had the highest leaf width (64.3 mm), and the lowest value was related to the population of Shahin Dej (1.65 mm). Also, the plant height was variable in Baneh (20.19 cm), Oshnavieh (18.07 cm), and Shahin Dej (7.53 cm), respectively (Table 3).

3.2. Essential Oil Content

According to ANOVA, significant differences were observed in the essential oil content of *S. lavandulifolia* populations ($p < 0.05$). As depicted in Figure 2, the essential oil content of the studied population varied between 0.07 and 0.15%. The ecotypes related to Marivan Wisser, Kordestan and Shahin Dej, West Azarbaijan had lower oil content than other ecotypes. Otherwise, the highest essential oil content (0.15%) was obtained in the population of Oshnavieh, West Azarbaijan (0.06%).

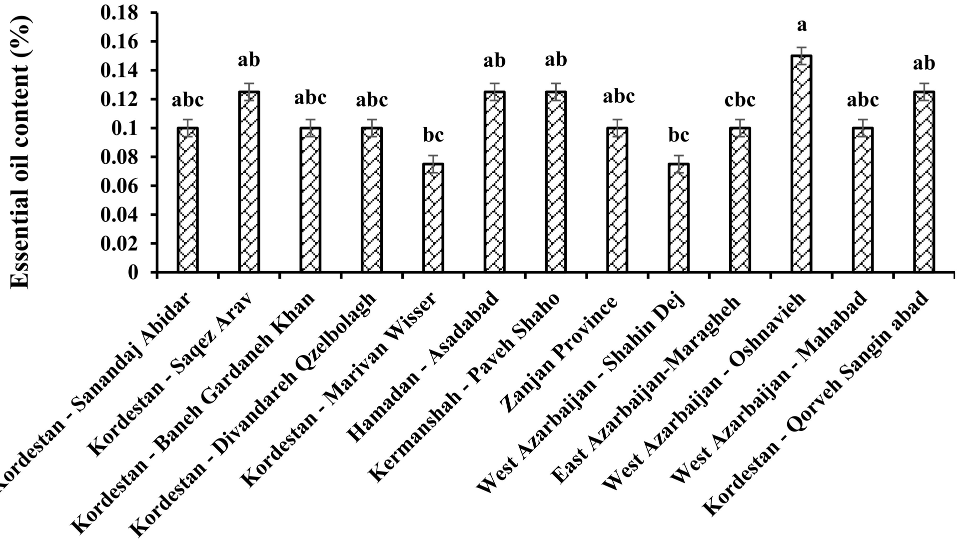

Figure 2. The essential oil content of *S. lavandulifolia* populations from the west and northwest of Iran. Different letters indicate a significant difference at $p < 0.05$.

The results clearly showed that the essential oil content was strongly affected by environmental conditions, such as morphological characteristics. Ecotypes at lower altitudes produced less essential oil than those at higher altitudes. In addition to altitude, factors such as light, temperature, and humidity also affect essential oil production. The ecotype of Marivan Wisser, Kordestan with 700–800 mm rainfall per year attained the least essential oil content. However, this ecotype had no significant difference with Shahin Dej, West Azarbaijan's ecotype with 323 mm rainfall. This similar essential oil content may be due to the effects of other environmental factors and diverse edaphic conditions besides different intrinsic genetic responses. As is previously known, secondary metabolites biosynthesis is stimulated in response to the variable environmental factors. In our populations, the various climatic and geographic criteria may induce the biosynthesis and accumulation of a series of defined constituents to guarantee the survival of plant species [15].

3.3. Essential Oil Constituents

The chemical composition of EO obtained from *S. lavandulifolia* populations was examined. As shown in Table 4, 28 constituents were identified, accounting for 94.4% of the total oil. The major essential oil constituent of *S. lavandulifolia* populations was α-pinene (1.07–34.87%). In addition, (*E*)-caryophyllene (0.45–25.99%), germacrene D (3.36–20.61%), Δ-cadinene (2.21–19.90%), bicyclogermacrene (1.72–12.08%), α-terpineol (0.38–11.86%), α-muurolol (0.31–11.50%), *p*-cymene (0.69–9.67%), β-elemene (0.63–9.31%), sabinene (0.32–6.29%), and α-Cadinol (0.77–3.42) were the other constituents with high amounts (Table 4).

Table 4. The essential oil composition (%) of 13 *S. lavandulifolia* populations from west and northwest Iran.

NO	Compound	RI	Kordestan-Sanandaj Abidar	Kordestan-Saqez Arav	Kordestan- Baneh Gardaneh Khan	Kordestan-Divandareh Qezel Bolagh	Kordestan-Marivan Wisser	Kordestan-Qorveh Sangin Abad	Hamadan-Asadabad	Kermanshah-Paveh Shaho	Zanjan Province	West Azarbaijan-Shahin Dej	East Azarbaijan-Maragheh	West Azarbaijan-Oshnavieh	West Azarbaijan-Mahabad
1	α-Thujene	930	0.35	1.26	0	1.11	0	0.88	1.29	0	0.05	0.63	0	2.07	0
2	**α-Pinene**	**932**	**12.38**	**34.87**	**9.41**	**19.98**	**1.07**	**10.32**	**25.98**	**1.49**	**1.48**	**2.33**	**2.3**	**16.76**	**2.53**
3	**Sabinene**	969	0.87	**2.32**	**1.2**	**2.88**	0	**2.51**	**1.87**	0	0.32	**2.39**	**6.29**	**1.19**	0.53
4	β-Pinene	974	0	2.32	0	0	0	0.10	0	0	0	0	3.44	0	0
5	Myrcene	988	1.77	6.47	8.89	10.19	0.65	**10.12**	9.71	1.66	1.12	21.11	10.27	15.05	2.34
6	α-Phellandrene	1002	0	4.67	1.74	0.78	0	0.29	2.11	0	0.17	0	10.27	1.11	0.33
7	Δ-3-Carene	1.008	0.35	0.97	1.56	1.25	0	1.17	2.55	0	0.14	2.44	0	1.95	0.53
8	*p*-Cymene	1020	0.69	3.92	2.48	2.85	0	1.80	4.05	0	0.51	1.27	9.67	2.19	0.47
9	**Limonene**	**1024**	**1.83**	**10.12**	**6.05**	**18.12**	**0**	**14.97**	**4.25**	**4.56**	**3.09**	**13.17**	**1.8**	**4.46**	**1.21**
10	***β*-Elemene**	**1389**	**2.85**	**3.98**	**4.14**	**3.05**	**4.36**	**0.63**	**1.17**	**4.30**	**9.31**	**3.46**	**3.36**	**3.44**	**1.62**
11	Dodecanal	1408	1.06	0.43	0.99	0.56	1.42	0.46	0.60	0.89	0.76	1.87	0.72	1.46	0.92
12	**(*E*)-Caryophyllene**	**1417**	**18.68**	**0.63**	**8.20**	**1.01**	**19.01**	**1.23**	**13.64**	**25.99**	**1.21**	0.63	0.98	0.45	**1.82**
13	α-Bergamotene	1432	0.24	0.28	0	0.17	0	0.58	0	4.88	1.30	0.23	1.46	0.45	0.94
14	Trans-*β*-Farnesene	1454	1.18	1.48	1.74	1.58	0	1.99	0.95	0	3.58	1.91	2.32	0.28	2.13
15	**Germacrene D**	**1484**	**13.58**	**3.36**	**10.20**	**6.65**	**20.61**	**6.35**	**7.94**	**8.41**	**9.23**	**18.08**	**5.07**	**14.21**	**4.68**
16	**Bicyclogermacrene**	**1500**	**1.72**	**6.88**	**8.99**	**8.97**	**9.54**	**11.48**	**4.19**	**2.74**	**12.08**	**6.48**	**2.95**	**3.11**	**4.09**
17	*β*-Bisabolene	1505	0	5.85	0	0	0	0.10	0	0	0.25	0	0	0	0
18	**Δ-Cadinene**	**1522**	**5.01**	**0**	**10.09**	**4.28**	**9.47**	**2.21**	**3.79**	**6.94**	**19.90**	**4.25**	**4.68**	**5.67**	**2.82**
19	**α-Terpineol**	1186	**1.83**	0.38	0	0.76	0	**2.15**	0	**2.11**	0.64	0	0	0	**11.86**
20	***p*-Cymene**	**1020**	0.69	**3.92**	**2.48**	**2.85**	0	**1.80**	**4.05**	0	0.51	**1.27**	**9.67**	**2.19**	0.47
21	Bisabolene <(E)-γ->	1529	0.61	0	0	0.15	0	0.42	0	0	0.63	0	0	0.25	0.35
22	Dodecanoic acid	1565	0	0	0	0.17	0	0.96	0	0	0.10	0	0	0.37	1.09
23	**Spathulenol**	**1577**	**2.44**	**2.11**	**4.46**	**3.13**	**3.68**	**4.59**	**2.08**	**1.03**	**2.76**	**5.69**	**1.99**	**1.19**	**4.33**
24	Caryophyllene oxide	1582	2.87	0	0	0.54	4.9	1.30	2.02	1.68	0.27	0	0	0	0.61
25	**α-Muurolol**	**1644**	**1.08**	0.62	**1.43**	0.31	**2.37**	0.59	0	**11.50**	0.46	**1.34**	0	0	0.45
26	**α-Cadinol**	**1652**	**2.12**	**0.9**	**2.42**	**0.92**	**3.46**	**1.07**	**1.43**	**2.44**	**2.43**	**1.69**	**2.43**	**1.06**	0.77
27	Bisabolol <epi-α->	1683	0	0.31	0	0.49	1.72	0.78	0	5.84	1.22	0	1.15	0.67	1.15
28	**Dibutyl phthalate**		**6.87**	**0.31**	**1.4**	**1.05**	**0.46**	**0.50**	**1.87**	**0**	**3.16**	**1.17**	**1.03**	**2.36**	**0.35**
	Total		**81.07**	**98.36**	**87.87**	**93.8**	**82.72**	**81.35**	**95.54**	**86.46**	**76.68**	**91.41**	**81.85**	**81.94**	**48.39**

3.4. Pearson's Correlations between Morphological Traits and Essential Oil Content

Pearson's correlations showed that the distance between nodes had a significant positive correlation with inflorescence length (r = 0.795, $p \leq 0.01$), stem diameter (r = 0.702, $p \leq 0.01$), plant height (r = 0.788, $p \leq 0.01$), the number of flowers in inflorescence (r = 0.698, $p \leq 0.01$), petal length (r = 0.840, $p \leq 0.01$), the number of flowering branches (r = 0.712, $p \leq 0.01$), branch length (r = 0.628, $p \leq 0.05$), and essential oil content (r = 0.598, $p \leq 0.05$). Moreover, leaf length in the sub-branches was significantly related to the leaf length in the main branch (r = 0.799, $p \leq 0.01$) and plant height (r = 0.633, $p \leq 0.05$). Sepal width was positively correlated with flower stalk length (r = 0.819, $p \leq 0.01$) and diameter (r = 0.665, $p \leq 0.05$). In addition, flower stalk length was significantly associated with sepal width (r = 0.819, $p \leq 0.01$), collar diameter (r = 0.668, $p \leq 0.05$), and stem diameter (r = 0.631, $p \leq 0.05$) (Table 5).

3.5. Principal Component Analysis (PCA)

PCA revealed the role of each trait in the variation between the studied *S. lavandulifolia* populations. Eigenvalues, the percentage of explained variation, and the cumulative percentage of variance are presented in Table 6. The analysis showed that the inflorescence length, number of flowers per inflorescence, internode distance, and petal length played the most critical role in the differentiation of ecotypes. Furthermore, eigenvalues, the percentage of explained variation, and the cumulative percentage of the variance of essential oil constituents are presented in Table 7. Four components described the variations between plant samples. α-Thujene, β-pinene, myrcene, dodecanal, (*E*)-caryophyllene, germacrene D, and α-cadinol were the most important oil constituents describing the variations.

3.6. Cluster Analysis

To group the *S. lavandulifolia* ecotypes, cluster analysis using the Ward method and Euclidean square distance was carried out on 16 morphological traits. Cluster analysis divided the ecotypes into four main groups (Figure 3). The populations in a given climatic condition were placed in the same group emphasizing the significant influence of the environment on the growth potential of plants. Moreover, to study the essential oil compositional diversity among the ecotypes, a cluster analysis dendrogram was drawn by XLSTAT software using Ward's method and Euclidean square distance. Accordingly, ecotypes were classified into three clusters (Figure 4). Only the East Azarbaijan ecotype of Maragheh was located in the first cluster. This ecotype was characterized by high amounts of (2*Z*, 6*E*)-farnesol, γ-terpinene, *p*-cymene, α-phellandrene, β-pinene, and sabinene. The second cluster contained a wide range of ecotypes, including West Azarbaijan-Mahabad, Kordestan-Saqez Arav, Kordestan-Qorveh Sangin Abad, Kordestan-Divandareh Qezel Bolagh, West Azarbaijan-Oshnavieh, and finally Hamadan-Asadabad. The major constituents were α-thujene, α-pinene, myrcene, 1.8-cineole, (*E*)-β-ocimene, and thymol. The third cluster included Kordestan-Marivan Wisser's ecotypes, Sanandaj-Abidar Kordestan, Zanjan, West Azarbaijan-Shahin Darreh Bayan, Kordestan-Baneh, and Paveh Shaho. This class had the lowest amounts of α-thujene and α-phellandrene, and the highest amount of β-elemene, Δ-cadinene, α-muurolol, and α-cadinol, which separated this cluster from others.

Table 5. Pearson's correlations (coefficients) between morphological traits and essential oil content *S. lavandulifolia* populations from Iran.

	The Length of the Inflorescence	Flower Stalk Length	Flower Stalk Diameter	Distance between Nodes	Leaf Length	Leaf Length in the Sub-branches	Leaf Width	Sepal Length	Sepal Width	Stem Diameter	Plant Height	Number of Flowers in the Inflorescence	Petal Length	Number of Flowering Branches	Length of Branches	Essential Oil Content	Dry Weight
The length of the inflorescence	1																
Flower stalk length	0.294	1															
Flower stalk diameter	0.01	0.558 *	1														
Distance between nodes	0.795 **	0.533	0.073	1													
Leaf length in the main branches	0.12	0.588 *	0.419	0.231	1												
Leaf length in the sub-branches	0.38	0.462	0.259	0.471	0.799 **	1											
Leaf width	−0.238	0.555 *	0.476	0.239	0.202	0.109	1										
Sepal length	0.374	0.085	0.278	0.267	0.001	0.14	0.228	1									
Sepal width	0.398	0.819 **	0.665 *	0.483	0.466	0.385	0.411	0.03	1								
Collar diameter	0.058	0.668 *	0.383	0.486	0.382	0.469	0.812 **	0.161	0.554 *	1							
Stem diameter	0.432	0.631 *	0.287	0.702 **	0.312	0.475	0.556 *	0.498	0.429	0.813 **							
Plant height	0.681 *	0.334	0.248	0.788 **	0.422	0.633 *	0.17	0.483	0.408	0.377	1						
Number of flowers in the inflorescence	0.786 **	0.335	0.14	0.698 **	−0.042	0.218	−0.106	0.495	0.381	0.139	0.683 *	1					
Petal length	0.722 **	0.570 *	0.178	0.840 **	0.27	0.497	0.165	0.474	0.421	0.514	0.700 **	0.813 **	1				
Number of flowering branches	0.564 *	0.284	−0.190	0.717 **	−0.143	0.141	−0.093	−0.226	0.318	0.211	0.3	0.526	0.582 *	1			
Length of branches	0.36	0.504	0.309	0.628 *	0.174	0.266	0.383	0.115	0.452	0.508	0.275	0.226	0.578 *	0.640 *	1		
Essential oil content	0.689 **	0.183	−0.128	0.598 *	0	0.232	−0.177	0.062	0.4	0.225	0.347	0.47	0.565 *	0.686 **	0.548	1	
Dry weight	0.415	0.548	0.296	0.27	0.505	0.373	0.06	0.272	0.561 *	0.35	0.401	0.472	0.49	−0.074	−0.108	0.268	1

Significant differences are indicated with * $p < 0.05$ and ** $p < 0.01$.

Table 6. Eigenvalues, percentage of explained variation, and cumulative percentage of variance for the morphological traits of 13 *S. lavandulifolia* populations from west and northwest Iran.

Factor	Eigenvalues	Percentage of Explained Variation	Cumulative Percentage of Explained Variation
1	7.251	45.322	45.322
2	2.870	17.935	63.256
3	1.656	10.349	73.606
4	1.494	9.338	82.943
5	1.057	6.605	89.549

Table 7. Eigenvalues, percentage of explained variation, and cumulative percentage of variance for the morphological traits of 13 *S. lavandulifolia* populations from west and northwest Iran.

Factor	Eigenvalues	Percentage of Explained Variation	Cumulative Percentage of Explained Variation
1	9.471	24.923	24.923
2	7.146	18.806	43.729
3	5.008	13.179	56.908
4	4.766	12.541	69.449

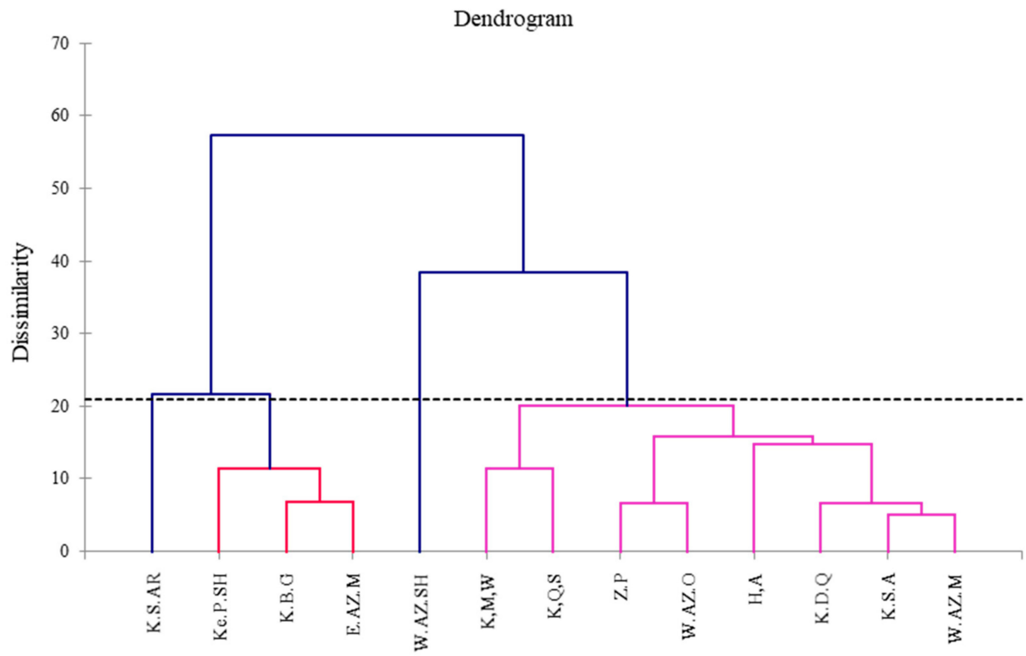

Figure 3. Dendrogram of cluster analysis for the morphological traits of thirteen *S. lavandulifolia* populations from west and northwest Iran. The populations studied were K.S.AR (Kordestan-Saqez Arav), Ke.P.SH (Kermanshah-Paveh Shaho), K.B.G (Kordestan-Baneh Gardaneh Khan), E.AZ. M (East Azarbaijan-Maragheh), W.AZ.SH (West Azarbaijan-Shahin Dej), K.M.W (Kordestan-Marivan Wisser), K.Q.S (Kordestan-Qorveh Sangin Abad), Z.P (Zanjan Province), W.AZ.O (West Azarbaijan-Oshnavieh), H.A (Hamadan-Asadabad), K.D.Q (Kordestan-Divandareh Qezel Bolagh), K.S.A (Kordestan-Sanandaj Abidar), and W.AZ.M (West Azarbaijan-Mahabad).

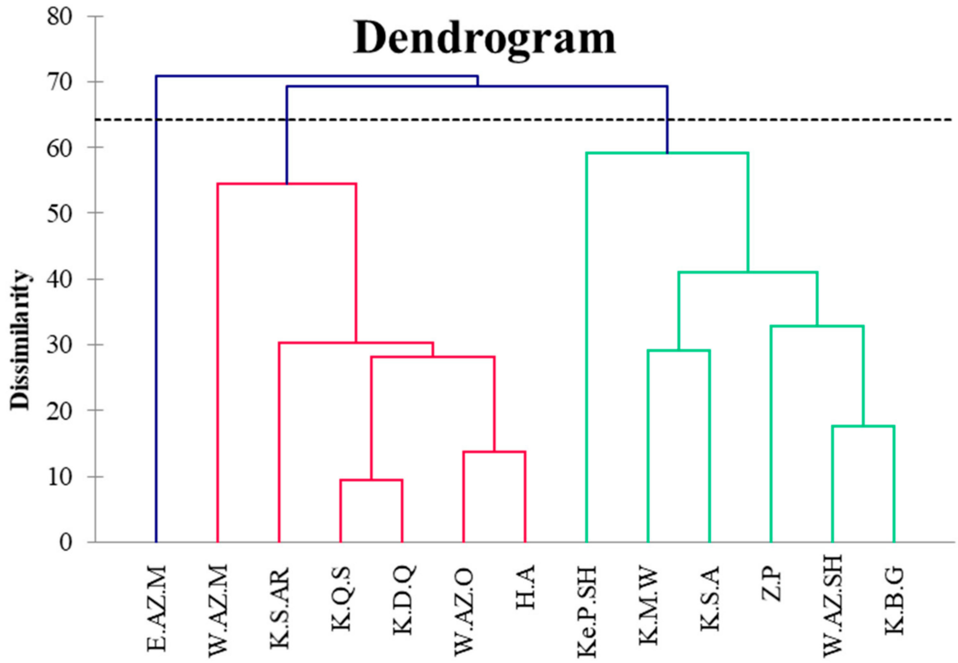

Figure 4. Dendrogram of cluster analysis for the essential oil compositional diversity of thirteen *S. lavandulifolia* populations from west and northwest Iran. The populations studied were K.S.AR (Kordestan-Saqez Arav), Ke.P.SH (Kermanshah-Paveh Shaho), K.B.G (Kordestan-Baneh Gardaneh Khan), E.AZ. M (East Azarbaijan-Maragheh), W.AZ.SH (West Azarbaijan-Shahin Dej), K.M.W (Kordestan-Marivan Wisser), K.Q.S (Kordestan-Qorveh Sangin Abad), Z.P (Zanjan Province), W.AZ.O (West Azarbaijan-Oshnavieh), H.A (Hamadan-Asadabad), K.D.Q (Kordestan-Divandareh Qezel Bolagh), K.S.A (Kordestan-Sanandaj Abidar), and W.AZ.M (West Azarbaijan-Mahabad).

4. Discussion

Localities drastically influenced the morphological and chemical diversity of populations. Compared to others, west Azarbaijan (Oshnavieh) is located in a semi-arid mountainous habitat, so the mentioned climatic and geographical conditions are suitable for producing high EO content. The high number of leaves and flowers is essential for producing the appropriate essential oil content in plants such as *S. lavandulifolia* [16]. In a study performed on different species of *Salvia*, significant differences were observed in the measured traits such as plant height and leaf width [17]. Also, in another research on the morphological characteristics of *Origanum vulgare* L., a highly significant difference was recorded between the evaluated characteristics [18]. Khadivi-Khub et al. [19] reported that the most variable morphological characteristics among the studied populations of *S. lavandulifolia* were inflorescence length, internode length, leaf shape, crown color, and flower bowl shape. Also, Mirzaie-Nodoushan [20] reported significant differences in morphological characteristics (plant height, stem diameter, number of branches, leaf length, and width) on different species of mint (*Mentha* spp.), quite similar to our findings. Yousefi et al. [21], in their study on *Salvia leriifolia* Benth, reported a high diversity of morphological traits and essential oil composition, which was consistent with the present study.

Traits with a high coefficient of variation have a broad selection potential for future programs. The range of deviation and the mean of traits measured for the ecotypes are shown in Table 2. The coefficient of variation varied depending on the trait. Among the studied features, the length of the sub-branch had the highest range (0.90) and then the number of flowers per inflorescence with 0.70 attained the highest range. Also, other traits such as petiole diameter (0.66) and leaf width had a high coefficient of variation.

In contrast, the least coefficient variation was related to sepal length (0.17) and petal length (0.21). In an earlier study on mint, the most critical traits such as flowering branch length, the distance between nodes, leaf length and width, the number of sub-stems, the number of leaves per sub-stem, and the height of the sub-branch had the highest coefficient of variation compared to other traits [22]. In contrast, other characteristics such as flower cup length and seed diameter had the lowest coefficients [23]. Hadian et al. [24] studied 30 populations of summer savory and reported that the highest coefficient of variation was related to the leaf width, petiole length, and leaf area. The broad coefficient variations in the phenotypic and morphological traits reflect the possible genetic diversity in a given plant species in different regions. Hosseini et al. [25], in their study on morphological traits of *Mentha longifolia* L. at different altitudes, reported that at high elevations, the higher light intensity and ultraviolet radiation resulted in the chlorophyll photo-oxidation and the reduced photosynthetically active radiation, and hence the limited plant growth potential. They have noted that climatic factors can be the predominant source of chemical and morphological variations by inducing massive biochemical, physiological, and metabolic changes.

The climatic and genetic parameters can influence the essential oil content of different species. In addition, local species show genetic diversities due to their adaptation to the environment, which may affect the biochemical composition, biological activity, and the secondary metabolite profile of plants [26]. The essential oil content of the studied populations varied from 0.07 to 0.15 % of dry weight. Essential oil production is an adaptation response to environmental cues. Cho et al. [27] showed that altitude was the most important environmental factor affecting essential oil content because higher essential oil contents were recorded at low altitudes. Hence, the higher essential oil content in the Saqez and Oshnavieh populations is possibly related to the lower altitude and less annual rainfall.

As is previously known, besides genetic nature, the environmental signals are the most influential criteria that influence the activity of genes involved in secondary metabolites biosynthesis in medicinal plants. Numerous factors such as altitude, water availability, temperature, and other geographical and seasonal conditions, plus plant growth stage, harvest time, postharvest handling, and extraction procedure, have a noticeable impact on essential oils' quantity and chemical composition [28]. Environmental and geographical indices determine the habitat characteristics of plants and play a crucial role in the distribution pattern of plants; so, by the assessment of the ecological conditions and requirements of a defined species, they will be able to characterize and manage the naturalization and agricultural large-scale production of high-valued species [29].

According to previous studies on essential oil compounds diversity among 10 wild populations of *S. lavandulifolia* Vahl from different regions of Iran, 49 compounds in the essential oils were identified. The main constituents were myrcene (26.2%), limonene (24.5%), germacrene D (19.2.4%), bicyclogermacrene (16.6%), Δ-cadinene (16%), (*E*) caryophyllene (12.9%), α-zingiberene (12.2%), and spathulenol (11.6%) [30]. In another study on *S. lavandulifolia* from the Fasham area near Tehran, Iran, the results of GC and GC/MS analysis showed that the main constituents were germacrene-D (13.2%), β-phellandrene (12.7%), β-pinene (10.2%), myrcene (9.4%), α-pinene (8.4%), and *Z*-β-ocimene (5.8%) [31]. Furthermore, another study on *S. lavandulifolia* depicted that the main components of essential oil were α-thujone (32.3%), α-pinene (up to 37.3%), myrcene (9.9%), β-phellandrene (37.9%), germacrene D (11.4%), and Δ-cadinene (11.6%). Those scientists concluded that the essential oil compounds' profile differences depended on the sample collection areas [32].

Skaltsa et al. [15] assessed the antimicrobial activity of EOs from eight Greek *Stachys* species in which all taxa displayed a relatively low content of aliphatics and phenylpropanoids. All evaluated EOs exhibited improved anti-bacterial behaviors. In another report from Turkey, the essential oil of 23 *Stachys* species contained germacrene D, β-caryophyllene, caryophyllene oxide, spathulenol, and α-cadinene as the main constituents of the oils [33]. In our study, a total of 28 constituents were identified, comprising 94.5% of the oil. Environmental and genetic factors control the essential oils' biosynthesis and the oils' proportional percentage. Plant populations and/or ecotypes from different ecological conditions inevitably have divergent essential oil compositions. It seems that the species' origin affects the content and composition of the essential oil. Environmental, genetic, and physiological factors, habitat management, grazing behavior, and agricultural practices hugely impress the secondary metabolites biosynthesis, accumulation, and profile. Geo-climatological factors such as climatic and edaphic conditions affect the genes expression and metabolic pathways, and hence the biosynthesis of active substances in the species. As a result, various secondary metabolites are synthesized under different environmental conditions [34]. The biological properties of essential oils from plants are strongly influenced by their constituents. The high diversity of essential oils of *S. lavandulifolia* observed in the current study might focus further studies on the bioassay evaluations. This makes possible the selection of local populations of *S. lavandulifolia* with special biological activities for use in the pharmaceutical and cosmetic industries and reveals the economic importance of this plant in advance.

Hadian et al. [23], via cluster analysis of the morphological diversity of Khuzestan savory populations, reported a high diversity for the studied traits. The populations were divided into three groups. In another morphological study, seven populations of thyme were divided into two separate groups. The populations from Kerman and Isfahan provinces were in the first, and the populations of Semnan province were in another group. The temperature variations have been defined as the predominant cause of this grouping [35].

Correlation estimation between the traits is another major procedure in cultivar improvement programs. In addition, estimating the relationships between morphological traits can provide information for the breeding studies which determine the most efficient design for genotypic evaluations [36]. In a study on *S. lavandulifolia*, the Pearson's correlation showed that flower length, the number of sub-stems, the number of leaves per sub-stem, the length of the sub-stem, stem diameter, plant height, collar diameter, leaf length, and leaf length-to-width ratio were positively correlated with the production and storage of essential oils. Thus, the biomass production of plants has a significant impact on the yield and productivity of essential oils, perhaps with the variations in the essential oil biosynthesis and accumulation potential [22]. In line with this, traits correlation studies are essential for plant breeding programs to produce the desired cultivar with an appropriate productivity and chemical profile [37]. The PCA analysis clarifies the main differences between the studied traits. The relative variance of each component indicates its percent importance in the variance of the studied traits. Moreover, PCA has been frequently employed to investigate the relationship between morphological traits and evaluate the different germplasms [18,38]. Furthermore, PCA is widely used to elucidate the morphological, genetic, and biochemical traits variations within and between species and populations [39].

5. Conclusions

When plants are exposed to different ecological conditions, the quantity and quality of their active ingredients change to adapt to these environments. As previously understood, the ecological diversity leads to differences in the range of the biological activities of plants. In our study, the different geographical areas influenced the morphological, physiological, and chemical characteristics of plants. As is known, for the exploitation, domestication, and/or breeding programs, we need to study the phenotypic and genetic diversity of plants from natural habitats. Eventually, it will be necessary to cultivate the selected ecotypes in agricultural systems for the mass production of the desired secondary metabolites.

Author Contributions: Conceptualization, M.B.H.; data curation, M.B.H., Y.S. and M.R.M.; formal analysis, M.B.H. and F.R.; methodology, M.B.H., M.R.M. and M.A.; project administration, M.B.H.; visualization, M.B.H., S.E., H.F., C.C.M. and R.A.M.; writing—original draft, M.B.H. and M.A.; writing—review and editing, M.B.H., S.E. and R.A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded and carried out at the University of Maragheh, Iran.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All-new research data were presented in this contribution.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. He, J.; Yang, B.; Dong, M.; Wang, Y. Crossing the roof of the world: Trade in medicinal plants from Nepal to China. *J. Ethnopharmacol.* **2018**, *224*, 100–110. [CrossRef] [PubMed]
2. Mollova, S.; Fidan, H.; Antonova, D.; Bozhilov, D.; Stanev, S.; Kostova, I.; Stoyanova, A. Chemical composition and antimicrobial and antioxidant activity of *Helichrysum italicum* (Roth) G. Don subspecies essential oils. *Turk. J. Agric. For.* **2020**, *44*, 371–378. [CrossRef]
3. Rezakhanlo, A.; Talebi, S.M. Trichomes morphology of *Stachys lavandulifolia* vahl. (*Labiatae*) of Iran. *Procedia Soc. Behav. Sci.* **2010**, *2*, 3755–3763. [CrossRef]
4. POWO. Plants of the World Online. *Facilitated by the Royal Botanic Gardens, Kew.* 2022. Available online: http://www.plantsoftheworldonline.org/ (accessed on 31 May 2022).
5. Nabavizadeh, F.; Alizadeh, A.M.; Adeli, S.; Golestan, M.; Moloudian, H.; Kamalinejad, M. Gastroprotective effects of *Stachys Lavandulifolia* extract on experimental gastric ulcer. *Afr. J. Pharm. Pharmacol.* **2011**, *5*, 155–159. [CrossRef]
6. Bhattacharjee, R. Taxonomic studies in Stachys. II. A new infrageneric classification of *Stachys* L. *Notes R. Bot. Gard. Edinb.* **1980**, *38*, 65–96.
7. Rabbani, M.; Sajjadi, S.E.; Zarei, H.R. Anxiolytic effects of *Stachys lavandulifolia* Vahl on the elevated plus-maze model of anxiety in mice. *J. Ethnopharmacol.* **2003**, *8*, 271–276. [CrossRef]
8. Morteza-Semnani, K.; Akbarzadeh, M.; Changizi, S. Essential oils composition of *Stachys byzantina*, *S. inflata*, *S. lavandulifolia* and *S. laxa* from Iran. *Flavour Fragr. J.* **2006**, *21*, 300–303. [CrossRef]
9. Barreto, R.S.; Quintans, J.S.; Amarante, R.K.; Nascimento, T.S.; Amarante, R.S.; Barreto, A.S.; Quintans-Júnior, L.J. Evidence for the involvement of TNF-α and IL-1β in the antinociceptive and anti-inflammatory activity of *Stachys lavandulifolia* Vahl. (*Lamiaceae*) essential oil and (-)-α-bisabolol, its main compound, in mice. *J. Ethnopharmacol.* **2016**, *191*, 9–18. [CrossRef]
10. Ghasemi Pirbalouti, A.; Malekpoor, F.; Mohammadi, M.; Yousefi, M. Composition of the essential oil of *Stachys lavandulifolia* from central Zagros Mountains. In Proceedings of the I International Symposium on Medicinal, Aromatic and Nutraceutical Plants from Mountainous Areas (MAP-Mountain 2011), Saas-Fee, Switzerland, 6–9 July 2011; Volume 955, pp. 101–104. [CrossRef]
11. Keshavarzi, M.; Rezaei, M.; Miri, S. The comparison of morphological and phytochemical evaluation in some population of *Stachys lavandulifolia* Vahl. In different provinces under field conditions. *Eco-Phytochem. J. Med. Plan.* **2016**, *4*, 78–87.
12. Erzurumlu, G.S.; Sultana, N.; Vural, M.; Serce, S. Genetic and phenotypic variation among Turkish terrestrial orchid species as revealed by RAPD and morphological characteristics. *Turk. J. Agric. For.* **2018**, *42*, 227–236. [CrossRef]
13. Morshedloo, M.R.; Craker, L.E.; Salami, A.; Nazeri, V.; Sang, H.; Maggi, F. Effect of prolonged water stress on essential oil content, compositions and gene expression patterns of mono-and sesquiterpene synthesis in two oregano (*Origanum vulgare* L.) subspecies. *Plant Physiol. Biochem.* **2017**, *111*, 119–128. [CrossRef]
14. Morshedloo, M.R.; Maggi, F.; Neko, H.T.; Aghdam, M.S. Sumac (*Rhus coriaria* L.) fruit: Essential oil variability in Iranian populations. *Ind. Crops Prod.* **2018**, *111*, 1–7. [CrossRef]
15. Skaltsa, H.D.; Demetzos, C.; Lazari, D.; Sokovic, M. Essential oil analysis and antimicrobial activity of eight Stachys species from Greece. *Phytochemistry* **2003**, *64*, 743–752. [CrossRef]
16. Falciani, L.; Maleci, L.B.; Lippi, M.M. Morphology and distribution of trichomes in Italian species of the *Stachys germanica* group (*Labiatae*): A taxonomic evaluation. *Bot. J. Linn. Soc.* **1995**, *119*, 245–256. [CrossRef]
17. Mossi, A.J.; Cansian, R.L.; Paroul, N.; Toniazzo, G.; Oliveira, J.V.; Pierozan, M.K.; Serafini, L.A. Morphological characterization and agronomical parameters of different species of *Salvia* sp. (*Lamiaceae*). *Braz. J. Biol.* **2011**, *71*, 121–129. [CrossRef]
18. Azizi, A.; Hadian, J.; Gholami, M.; Friedt, W.; Honermeier, B. Correlations between genetic, morphological, and chemical diversities in a germplasm collection of the medicinal plant *Origanum vulgare* L. *Chem. Biodivers.* **2012**, *9*, 2784–2801. [CrossRef]
19. Khadivi-Khub, A.; Aghaei, Y.; Mirjalili, M.H. Phenotypic and phytochemical diversity among different populations of *Stachys lavandulifolia*. *Biochem. Syst. Ecol.* **2014**, *54*, 272–278. [CrossRef]
20. Mirzaie-Nodoushan, H.; Rezaie, M.B.; Jaimand, K. Path analysis of the essential oil-related characters in *Mentha* spp. *Flavour Fragr. J.* **2001**, *16*, 340–343. [CrossRef]
21. Yousefi, M.; Nazeri, V.; Mirza, M. Study on some ecological characteristics, morphological traits and essential oil yield of *Salvia leriifolia* Benth. *Iran. J. Med. Aromat. Plant Res.* **2013**, *29*, 157–175. [CrossRef]
22. Baqalian, K.; Naqdi Badi, H. *Essential Plants*; Andarz Publishing: Tehran, Iran, 2000.
23. Aghaei Noroozloo, Y.; Mirjalili, M.; Nazeri, V.; Moshrefi Araghi, A. Evaluation of some ecological factors, morphological traits and essential oil productivity of *Stachys lavandulifolia* Vahl. In four provinces of Iran. *Iran. J. Med. Aromat. Plant Res.* **2015**, *30*, 985–998. [CrossRef]
24. Hadian, J.; Ebrahimi, S.N.; Salehi, P. Variability of morphological and phytochemical characteristics among *Satureja hortensis* L. accessions of Iran. *Ind. Crops. Prod.* **2010**, *32*, 62–69. [CrossRef]

25. Hosseini, S.; Feizi, H.; Vatandoost Jertoodeh, S.; Alipanah, M. Evaluation of ecological and morphological traits and essential oil productivity of *Mentha longifolia* L. in Fars and Khorasan Razavi provinces. *J. Agroecol.* **2019**, *11*, 335–347. [CrossRef]
26. Heywood, V.H. The conservation of genetic and chemical diversity in medicinal and aromatic plants. In *Biodiversity*; Springer: Boston, MA, USA, 2002; pp. 13–22.
27. Cho, B.W.; Cha, C.N.; Lee, S.M.; Kim, M.J.; Park, J.Y.; Yoo, C.Y.; Lee, H.J. Therapeutic effect of oregano essential oil on subclinical bovine mastitis caused by *Staphylococcus aureus* and *Escherichia coli*. *Korean J. Vet. Res.* **2015**, *55*, 253–257. [CrossRef]
28. Shakeri, A.; Khakdan, F.; Soheili, V.; Sahebkar, A.; Shaddel, R.; Asili, J. Volatile composition, antimicrobial, cytotoxic and antioxidant evaluation of the essential oil from *Nepeta sintenisii* Bornm. *Ind. Crops Prod.* **2016**, *84*, 224–229. [CrossRef]
29. Ardakani, M.R. *Ecology*, 6th ed.; Tehran University Publisher: Tehran, Iran, 2006; p. 340.
30. Aghaei, Y.; Hossein Mirjalili, M.; Nazeri, V. Chemical diversity among the essential oils of wild populations of *Stachys lavandulifolia* VAHL (Lamiaceae) from Iran. *Chem. Biodivers.* **2013**, *10*, 262–273. [CrossRef]
31. Javidnia, K.; Mojab, F.; Mojahedi, S.A. Chemical constituents of the essential oil of *Stachys lavandulifolia* Vahl from Iran. *Iran. J. Pharm. Res.* **2010**, *1*, 61–63. [CrossRef]
32. Pirbalouti, A.G.; Mohammadi, M. Phytochemical composition of the essential oil of different populations of *Stachys lavandulifolia* Vahl. *Asian Pac. J. Trop. Biomed.* **2013**, *3*, 123–128. [CrossRef]
33. Gören, A.C.; Akcicek, E.; Dirmenci, T.; Kilic, T.; Mozioğlu, E.; Yilmaz, H. Fatty acid composition and chemotaxonomic evaluation of species of *Stachys*. *Nat. Prod. Res.* **2012**, *26*, 84–90. [CrossRef]
34. Moghtader, M. Comparative evaluation of the essential oil composition from the leaves and flowers of *Hyssopus officinalis* L. *J. Hortic. For.* **2014**, *6*, 1–5. [CrossRef]
35. Bigdelou, M. Evaluation of Morphological, Genetic and Phytochemical Diversity of Kermani Thyme. Master's Thesis, University of Tehran, Tehran, Iran, 2011; pp. 1–90.
36. Khadivi, A.; Anjam, R.; Anjam, K. Morphological and pomological characterization of edible fig (*Ficus carica* L.) to select the superior trees. *Sci. Hortic.* **2018**, *238*, 66–74. [CrossRef]
37. Arabsalehi, F.; Rahimmalek, M.; Ehtemam, M.H. Phytochemical and morphological variation of *Stachys lavandulifolia* Vahl. Populations as affected by genotype$\times$ year interaction. *Ind. Crops Prod.* **2018**, *112*, 342–352. [CrossRef]
38. Javidnia, K.; Miri, R.; Moein, M.R.; Kamalinejad, M.; Sarkarzadeh, H. Constituents of the essential oil of *Stachys pilifera* Benth. From Iran. *J. Essent. Oil Res.* **2006**, *18*, 275–277. [CrossRef]
39. Khadivi-Khub, A.; Ebrahimi, A.; Sheibani, F.; Esmaeili, A. Phenological and pomological characterization of Persian walnut to select promising trees. *Euphytica* **2015**, *205*, 557–567. [CrossRef]