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Four-Year (2018-2021) Morphological Observation of Winter Savory (*Satureja montana* Spp.) with Determination of Essential Oil Content and Composition

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Abstract

Because the morphology of winter savory has been poorly studied in the literature, the morphological characteristics (determined by identification descriptors) of six different *Satureja montana* L. accessions (BFL 28-002/06, BFL 13-001/05, BFL 35-001/06, BFL 46-003/ 06, BFL 39-002/06/2, BFL 31-001/06) were compared in a four-year trial (2018-2021). In addition, a thorough analysis of the content and composition of the essential oil, extracted from the above-mentioned accessions was conducted using GC-MS. The extracted essential oils were analyzed for their content of biologically active compounds with health-promoting effects by comparing the results with the literature data. Extremely low variability in morphological characteristics between populations and within species was found. The most favorable years for shoot and leaf development were 2018 and 2019, and the highest percentage of flowering was recorded in 2021, followed by 2018. The results showed that the main essential oil components in most accessions were thymol, carvacrol, *p*-cymene and γ-terpinene. In general, the accession BFL 39-002/06/2 was the richest in essential oil composition over the years. The least number of compounds, explaining the relative peak area of 64.03% of total essential oil, were found in accession BFL 35-001/06 in 2018. Comparing the results of the research with the literature, we found that the analyzed samples are a rich source of bioactive substances (*p*-cymene, thymol, and geraniol) and could potentially be used for medicinal purposes (antioxidant, anti-inflammatory, antiparasitic, antidiabetic, antiviral, antitumor, antibacterial, and antifungal activities…).

Keywords: Aromatic Plants; Characteristics; Descriptors; Essential Oil; Morphology; GC-MS; Hydrodistillation; *Satureja montana*; Winter Savory

Introduction

The genus *Satureja* L. (family Lamiaceae) includes about 7000 species distributed all over the world. Most of them are shrubs or fragrant plants with interesting biological properties. About 30 species are known under the name savory, of which garden savory (*Satureja* hortensis L.) and winter savory (*Satureja montana* L.) are the most widespread [1]. Winter savory is common in the Mediterranean region and Central Asia. It grows on rocky and bushy slopes and in meadows [2]. The leaves are green and lanceolate with glandular, dotted and crisscrossed bristles on the margin. The stem is woody and round at the base. The flowers are white and bloom from July to September.

S. montana has been used for over 2000 years. Records state that it appeared in European monastery gardens as early as in the 9th century. It is native to southern Europe, Turkey and North Africa. It is characterized by an extremely strong aroma that justifies its use in cooking as a spice, for preparing dishes from dry legumes and for preparing various meat dishes [1,2]. Due to the content of bioactive secondary metabolites S. montana is known for its numerous medicinal and skin care effects and plays an important role in the pharmaceutical and cosmetic industry, as well as in traditional medicine. The dried herbs are used in teas, syrups, tinctures, mouthwashes and essential oils. *S. montana* has antimicrobial, antispasmodic, carminative, diuretic, and anticancer effects, as well as beneficial effects on digestion, colds, diarrhea, and injuries [3-6].

S. montana is known for its antioxidant activity, which is mainly due to the carvacrol and thymol contents [7]. It hunts down free radicals (peroxide, hydroperoxide and others) and limits the harmful effects of oxidative stress, which is an imbalance between the effects of oxidants and antioxidants. Oxidation of proteins, lipids, and nucleic acids causes degenerative diseases, cancer, and aging [2]. The antimicrobial, antibacterial, antifungal, and antiviral activities attributed to carvacrol and geraniol have also been well studied [7].

The most abundant constituents of *S. montana* essential oil are carvacrol, thymol, *p*-cymene, α- and γ-terpineol, 1,8-cineole, and borneol [7]. The predominant essential oil constituent is carvacrol [5,9], of which the largest proportion was detected at the time of flowering. The other two most abundant constituents are eugenol and thymol. The highest content of the latter has been detected after flowering, while eugenol is known to be the predominant constituent in young plants. Other sources have also indicated the content of *p*-cymene, γ-terpinene, linalool, and limonene in the essential oil of *S. montana* [5].

A lot of research has already been done on the topic of winter savory essential oil, but very little is known about its morphological characteristics and about changes in the content and composition of the essential oil over the years. Therefore, the main purpose of the research was to compare the morphological characteristics and determine the essential oil content and composition of six different accessions of winter cherry (*Satureja montana* L.) over a four-year period (2018-2021). Since the descriptors for determining the morphological characteristics of savory have not yet been established, we determined them ourselves, thus setting guidelines for subsequent research. Considering the content of biologically active compounds with beneficial effects on health, the possibility of using the obtained essential oils in medicinal preparations was also discussed. Differences in morphological characteristics such as bush width and bush height, number of shoots, inflorescence length, leaf and stem color, presence of glands on the upper and lower leaf surface, plant habitus and flowering percentage between the studied populations and within the species were expected. Statistically significant differences in the essential oil content between the observed years and between the studied accessions were also expected.

Materials and Methods

Plant Material

A four-year trial (2018-2021) was conducted with six different *S. montana* accessions (BFL 28-002/06, BFL 13-001/05, BFL 35-001/06, BFL 46-003/06, BFL 39-002/06/2, BFL 31-001/06). Seeds were obtained from the collection of medicinal and aromatic plants in the public service of the

Plant Genebank (JSRGB) and are of Slovenian origin. Populations were generatively propagated and planted in the laboratory field of the Biotehnical Faculty, University of Ljubljana. No fertilisers or pesticides were used in the experiment. Soil cultivation was done exclusively manually.

Morphological Analyses

Morphological characteristics were evaluated by macroscopic observation (using a ruler and a magnifying glass) of 10 randomly selected plants of each winter savory accession. The assessment took place in August, during full bloom. Since official plant specific descriptors have not been known, these were subjectively determined. Emphasis was placed on growth habit, width and height of the shrub, number of shoots per shrub, color of leaves and stems, pubescence, presence and density of leaf lobes and percentage of flowering. Stems were harvested each year at the stage of full flowering period in late August/early September and then dried in a dryer at 38 with an accuracy of ± 2°C for three days. The drying rates varied between 0.0002%/s (accession BFL 28-002/06) and 0.0.0003%/s (accession BFL 35-001/06).

Hydrodistillation

Since *S. montana* is not included in the European Pharmacopoeia, the procedure was adapted to the recommendation ISO (7928-1), which prescribes an essential oil content of not less than 5 mL/kg as a quality criterion for *S. montana* (ISO, 1991). Essential oil yields were determined by hydrodistillation for 3 h using a Clevenger apparatus (Council of Europe, 2019) in three replications for each individual accession. After distillation, the essential oil and water fractions were collected in dark vials and stored in a refrigerator at 4°C until further GC-MS analysis.

GC-MS Analyses

GC-MS was performed using a QP -2010 Ultra (Shimadzu, Kyoto, Japan), Rxi-5Sil MS column, 30 m × 0.25 mm i.d. layer thickness 0.25 μm (Restek, Bellefonte, PA, USA) under the following conditions: temperature program 40°C, 3°C/min to 220°C, 220°C (15 min); injector temperature 250°C; ion source temperature 200°C; interface temperature 300°C; injection volume 1μl; pitch 1: 100; carrier gas He; carrier gas flow 1 mL/min. The conditions for mass spectrometry were electron impact mode, total ion current consumption, 1 kV detector voltage. Electron mass spectra were used to record at 70 eV. Ionization energy in full scan model was in the ranges of 20-350 atomic mass units (amu) with 5scans/s. The mass spectra of the compounds were compared with spectra from the mass spectra libraries of NIST14 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and FFNSC 3 (Shimadzu, Kyoto, Japan). Concentrations were calculated as relative peak areas in % [11]. Samples were prepared as 1% essential oil diluted in hexane.

Statistical Analysis

Results were analyzed with the statistical program R Commander using one-way analysis of variance (ANOVA). Tukey's test was used to compare treatments when ANOVA showed significant differences between values. Results are presented as mean with standard deviation (SD). When p-values were less than 0.05, differences between treatments were considered statistically significant.

Results

Morphological Characteristics

Color of Leaves and Shoots

The color of the leaves of the first accession (BFL 28-002/06) was light green, and the shoots were dark brown. In the second accession (BFL 13-001/05), the same color of shoots and leaves was observed, i.e. yellow-green. In the third accession (BFL 35-001/06), the leaves were yellow-green and the shoots were yellow-brown. The fourth accession (BFL 46-003/06) had light green leaves and yellow-brown shoots. The fifth (BFL 39-002/06/2) and sixth accessions (BFL 31-001/06) did not differ from each other in the color of the shoots and leaves. The leaves were light green and the shoots were yellow-brown.

Bush Width

The growth habit was erect in all accessions. There were no statistically significant differences between accessions and years ($p > 0.05$). In 2018, the average width of the bush was 48 cm, while in other years it averaged 49 cm. In the second accession (BFL 13-001/05), the width of the bush was narrowest in 2021, averaging 33 cm, and widest in 2018, averaging 48 cm. In the third accession (BFL 35-001/06), the width of the bush was narrowest in 2020 (25 cm) and widest in 2018 (43 cm). In the fourth accession (BFL 46-003/06), there was no difference in bush width between 2020 and 2021, when bush width averaged 32 cm in both cases. In this accession the bush was widest in 2018 (average 36 cm) and narrowest in 2019 (average 31 cm). The widest bush in the fifth accession (BFL 39-002/06/2) was in 2018 (average 39 cm) and the narrowest in 2021 (average 27 cm). In the last observed accession (BFL 31-001/06) bush width varied on average from 31 cm (2021) to 45 cm (2018).

Table 1: The average width (cm) of the bushes of six accessions (BFL 28-002/06, BFL 13-001/05, BFL 35-001/06, BFL 46-003/06, BFL 39-002/06/2, BFL 31-001/06) of winter savory (*S. montana*) in the analyzed period of four years (2018-2021). Different lowercase letters indicate statistical differences ($p < 0.05$) between years, within the population. Different capital letters indicate statistically significant differences ($p < 0.05$) between populations in a given year

Year	BFL 28-002/06 (cm)	BFL 13-001/05 (cm)	BFL 35-001/06 (cm)	BFL 46-003/06 (cm)	BFL 39-002/06/2 (cm)	BFL 31-001/06 (cm)
2018	48 ± 24 aA	48 ± 10 aA	43 ± 16 aA	36 ± 14 aA	39 ± 14 aA	45 ± 9 aA
2019	49 ± 13 aA	39 ± 17 aA	27 ± 14 aA	31 ± 12 aA	31 ± 12 aA	32 ± 11 aA
2020	49 ± 19 aA	34 ± 9 aA	25 ± 7 aA	32 ± 10 aA	30 ± 6 aA	32 ± 18 aA
2021	49 ± 26 aA	33 ± 18 aA	26 ± 7 aA	32 ± 20 aA	27 ± 11 aA	31 ± 9 aA

Looking at the individual accessions in 2018 (Table 1), it was found that on average, the widest bushes were observed in the first (BFL 28-002/06) and the second accession (BFL 13-001/05) (48 cm), while the narrowest bush (36 cm on average) in the fourth accession (BFL 46-003/06). In the period 2019-2021 the widest bush was that of the first accession (BFL 28-002/06) (measuring 49 cm on average) and the narrowest that of the third accession (measuring 25 cm on average).

Shoot Length

When considering shoot length, there where no statistically significant differences between studied accessions nor between years of observation ($p > 0.05$). The average shoot length (Table 2) ranged from 25 cm (BFL 35-001/06 and BFL 31-001/06, observation year 2021) to 36 cm (BFL 35-001/06, observation year 2019).

Table 2: The average shoot length (cm) of the bushes of six accessions (BFL 28-002/06, BFL 13-001/05, BFL 35-001/06, BFL 46-003/06, BFL 39-002/06/2, BFL 31-001/06) of winter savory (*S. montana*) during the analyzed period of four years (2018-2021). Different lowercase letters indicate statistical differences ($p < 0.05$) between years, within the population. Different capital letters indicate statistically significant differences ($p < 0.05$) between populations in a given year.

Year	BFL 28-002/06 (cm)	BFL 13-001/05 (cm)	BFL 35-001/06 (cm)	BFL 46-003/06 (cm)	BFL 39-002/06/2 (cm)	BFL 31-001/06 (cm)
2018	29 ± 8 aA	31 ± 4 aA	31 ± 9 aA	30 ± 9 aA	32 ± 13 aA	26 ± 7 aA
2019	28 ± 4 aA	30 ± 3 aA	36 ± 7 aA	32 ± 11 aA	34 ± 7 aA	27 ± 5 aA
2020	30 ± 4 aA	28 ± 4 aA	34 ± 10 aA	31 ± 11 aA	30 ± 11 aA	27 ± 7 aA
2021	28 ± 6 aA	26 ± 5 aA	25 ± 6 aA	29 ± 5 aA	28 ± 8 aA	25 ± 6 aA

Number of Shoots

Statistically significant differencies have been detected in 2018 between accessions BFL 28-002/06 (9 shoots per bush), BFL 13-001/05 (24 shoots per bush) and BFL 35-001/06 (28 shoots per bush) ($p < 0.05$) (Table 3). Taking into consideration differences in individual accessions during different observation years, statistical significancy has been registered in accession BFL 35-001/06 between 2018 (28 shoots per bush) and 2020 (14 shoots per bush) as well as 2021 (7 shoots per bush) and in accession BFL 39-002/06/2 between 2019 (27 shoots per bush) and other observation years (2018, 2020, 2021) (from 10 to 14 shoots per bush).

Table 3: The average number of shoots per bush of six accessions (BFL 28-002/06, BFL 13-001/05, BFL 35-001/06, BFL 46-003/06, BFL 39-002/06/2, BFL 31-001/06) of winter savory (*S. montana*) in the analyzed period of four years (2018-2021). Different lowercase letters indicate statistical differences ($p < 0.05$) between years, within the population. Different capital letters indicate statistically significant differences ($p < 0.05$) between populations in a given year.

Year	BFL 28-002/06 (cm)	BFL 13-001/05 (cm)	BFL 35-001/06 (cm)	BFL 46-003/06 (cm)	BFL 39-002/06/2 (cm)	BFL 31-001/06 (cm)
2018	9 ± 4 aA	24 ± 5 aBC	28 ± 4 bC	18 ± 4 aAC	13 ± 4 aAB	19 ± 8 aAC
2019	14 ± 5 aA	22 ± 4 aA	19 ± 8 abA	23 ± 4 aA	27 ± 4 bA	18 ± 6 aA
2020	11 ± 6 aA	18 ± 6 aA	14 ± 5 aA	18 ± 5 aA	14 ± 5 aA	14 ± 5 aA
2021	12 ± 5 aA	13 ± 6 aA	7 ± 4 aA	12 ± 7 aA	10 ± 4 aA	15 ± 4 aA

Inflorescence Length

In 2018 inflorescences of accession BFL 35-001/06 (1.1 cm) were significantly longer when compared to the accessions BFL 28-002/06 (0.5 cm), BFL 13-001/05 (0.4 cm) and BFL 39-002/06/2 (0.5 cm). ($p < 0.05$) (Table 4). Similar result was noticed in 2019, when accessions BFL 46-003/06 and BFL 31-001/06 (1.1 cm and 1.3 cm, respectively) had longer inflorescences than other observed accessions. In 2020 the longest inflorescences were detected in accession BFL 39-002/06/2 (1 cm). Taking into consideration differences in individual accessions during different observation years, statistical significancy in inflorescence length has been registered in following accessions: in BFL 35-001/06 between 2018 (1.1 cm) and 2020 (0.4 cm), in BFL 46-003/06 between 2019 (1.1 cm) and 2020 (0.4 cm) , in BFL 39-002/06/2 between 2018 (0.5 cm) and 2020 (1.0 cm) and in accession BFL 31-001/06 between 2019 (1.3 cm) and 2020 (0.6 cm) as well as 2021 (0.7 cm).

Table 4: The average inflorescence length (cm) of six accessions (BFL 28-002/06, BFL 13-001/05, BFL 35-001/06, BFL 46-003/06, BFL 39-002/06/2, BFL 31-001/06) of winter savory (*S. montana*) in the analyzed period of four years (2018-2021). Different lowercase letters indicate statistical differences ($p < 0.05$) between years, within the population. Different capital letters indicate statistically significant differences ($p < 0.05$) between populations in a given year.

Year	BFL 28-002/06 (cm)	BFL 13-001/05 (cm)	BFL 35-001/06 (cm)	BFL 46-003/06 (cm)	BFL 39-002/06/2 (cm)	BFL 31-001/06 (cm)
2018	0.5 ± 0.1 aA	0.4 ± 0.2 aA	1.1 ± 0.2 bB	0.8 ± 0.2 abAB	0.5 ± 0.1 aA	0.8 ± 0.2 abAB
2019	0.7 ± 0.3 aA	0.7 ± 0.2 aA	0.7 ± 0.3 abA	1.1 ± 0.2 bA	0.7 ± 0.2 abA	1.3 ± 0.3 bA
2020	0.5 ± 0.4 aA	0.5 ± 0.4 aA	0.4 ± 0.2 aA	0.4 ± 0.2 aA	1.0 ± 0.3 bA	0.6 ± 0.2 aA

2021	0.3 ± 0.2 aA	0.7 ± 0.2 aA	0.7 ± 0.3 abA	0.8 ± 0.2 abA	0.8 ± 0.1 abA	0.7 ± 0.2 aA

Glands

Both glandular and non-glandular trichomes were observed in accessions BFL 28-002/06 and BFL 13-001/05. The density of glandular trichomes was higher on the lower side of the leaves. In contrast to this, only non-glandular trichomes were present on the lower and upper side of the leaves of accessions BFL 35-001/06, BFL 46-003/06, BFL 39-002/06/2 and BFL 31-001/06. The density of non-glandular trichomes was sparse on the upper side of the leaves of accession BFL 35-001/06, but dense in all other accessions.

Flowering

A high variability was observed in the percentage of flowering of winter savory (Figure 1) (Table 5). It varies between 13 % (accession BFL 39-002/06/2 in 2018) and 69 % (accession BFL 13-001/05 in 2021). Statistically significant differencies have not been detected between studied accessions in any of observation years ($p > 0.05$). Taking into consideration differences in individual accessions during different observation years, statistical significancy ($p < 0.05$) has been registered only in accession BFL 39-002/06/2 between 2018 (13 %) and 2021 (56 %).

Figure 1: The flower od winter savory

Table 5: Average flowering percentage (%) of the bushes of six accessions (BFL 28-002/06, BFL 13-001/05, BFL 35-001/06, BFL 46-003/06, BFL 39-002/06/2, BFL 31-001/06) of winter savory (*S. montana*) in the analyzed period of four years (2018-2021). Different lowercase letters indicate statistical differences ($p < 0.05$) between years, within the population. Different capital letters indicate statistically significant differences ($p < 0.05$) between populations in a given year.

Year	BFL 28-002/06 (%)	BFL 13-001/05 (%)	BFL 35-001/06 (%)	BFL 46-003/06 (%)	BFL 39-002/06/2 (%)	BFL 31-001/06 (%)
2018	31 ± 4 aA	31 ± 8 aA	24 ± 10 aA	38 ± 12 aA	13 ± 16 aA	36 ± 23 aA
2019	50 ± 18 aA	59 ± 18 aA	53 ± 19 aA	64 ± 18 aA	36 ± 11 abA	32 ± 12 aA
2020	38 ± 14 aA	60 ± 24 aA	38 ± 6 aA	55 ± 22 aA	40 ± 11 abA	40 ± 28 aA
2021	42 ± 21 aA	69 ± 18 aA	30 ± 15 aA	62 ± 24 aA	56 ± 22 bA	52 ± 34 aA

Essential Oil

Essential Oil Yield

When comparing essential oil yields (Table 6) between populations in all observed years, the results of the study showed that the yields varried between 2.2 mL/kg (accession BFL 13-001/05 in 2019) and 8.5 mL/kg (accession BFL 39-002/06/2 in 2021). Statistically significant difference ($p < 0.05$) has been detected between accessions BFL 13-001/05 (2.20 mL/kg) and BFL 46-003/06 (4.66 mL/kg) in 2019. Comparing the differences in individual accessions during different observation years, statistical significancy ($p < 0.05$) has been registered only in accession BFL 13-001/05, between years 2019 (2.2 mL/kg) and 2021 (5.45 mL/kg).

Table 6: Average yield (mL/kg) of *S. montana* essential oil ± standard deviation (SD) for individual accessions (BFL 28-002/06, BFL 13-001/05, BFL 35-001/06, BFL 46-003/06, BFL 39-002/06/2, BFL 31-001/06) during the study period (2018-2021). Different lowercase letters indicate statistical differences between years, within each accession. Different capital letters indicate statistically significant differences between accessions, within each year.

Year	BFL 28-002/06 (mL/kg)	BFL 13-001/05 (mL/kg)	BFL 35-001/06 (mL/kg)	BFL 46-003/06 (mL/kg)	BFL 39-002/06/2 (mL/kg)	BFL 31-001/06 (mL/kg)
2018	6.46 ± 1.23 aA	3.96 ± 0.95 abA	7.49 ± 2.25 aA	6.40 ± 1.25 aA	6.23 ± 1.51 aA	6.42 ± 1.48 aA
2019	4.51 ± 1 aAB	2.20 ± 0.84 aA	6.10 ± 1.69 aAB	6.53 ± 1.53 aB	4.66 ± 1.73 aAB	5.42 ± 1.91 aAB
2020	5.32 ± 1.31 aA	3.62 ± 0.66 abA	6.84 ± 1.72 aA	6.45 ± 2.06 aA	5.97 ± 154 aA	5.25 ± 0.98 aA
2021	6.98 ± 1.31 aA	5.45 ± 0.74 bA	7.62 ± 1.39 aA	6.03 ± 1.59 aA	8.50 ± 1.79 aA	5.31 ± 0.58 aA

Essential Oil Composition

The chemical composition of the essential oil of *S. montana* from the first accession (BFL 28-002/06), determined by GC-MS, is shown in Table 7. The essential oil constituents that account for at least 1% of the total relative peak area in at least one of the years studied are presented.

Table 7: Average relative peak area (%) ± standard deviation (SD) of each compound in essential oils from the first accession (BFL 28-002/06) of *S. montana*. Retention times are given in minutes. Different letters indicate statistical differences between years.

Compound	2018 (%)	2019 (%)	2020 (%)	2021 (%)
α-thujene (ret. time 10.169)	1.23 ± 0.24 a	-	-	1.02 ± 0.25 a
α-pinene (ret. time 10.540)	1.22 ± 0.76	+	-	+
δ-elemene (ret. time 14.331)	-	1.32 ± 0.33	-	-
p-cymene (ret. time 14.605)	27.41 ± 2.65 bc	19.07 ± 0.55 b	4.1 ± 0.51 a	33.64 ± 6.83 c
γ-terpinene (ret. time 16.219)	4.69 ± 1.04 b	2.45 ± 1.34 a	-	3.87 ± 1.18 b
borneol (ret. time 21.680)	1.83 ± 0.48 bc	1.38 ± 0.36 c	2.78 ± 0.45 ab	3.41 ± 0.35 a

terpinen-4-ol (ret. time 22.082)	1.81 ± 0.17 a	1.04 ± 0.59 a	1.77 ± 0.76 a	1.31 ± 0.19 a
thymol methyl ether (ret. time 24.822)	9.05 ± 1.39 ab	4.23 ± 1.14 a	13.09 ± 2.94 b	13.27 ± 2.56 b
carvacryl methyl ether (ret. time 24.827)	-	3.12 ± 1.50	-	-
thymol (ret. time 27.740)	11.59 ± 1.06 a	33.57 ± 2.62 b	61.12 ± 15.88 c	2.05 ± 0.73 a
carvacrol (ret. time 27.845)	+	28.37 ± 2.35 a	+	26.43 ± 2.87 a
β-caryophyllene (ret. time 32.891)	1.37 ± 0.50 a	1.6 ± 0.36 a	1.84 ± 0.49 a	+
2-methylene-4,8,8-trimethyl-4-vinyl- (ret. time 32.958)	-	-	-	1.72 ± 0.57
unknown (ret. time 35.049)	28.76 ± 5.72	-	-	-
unknown (ret. time 35.137)	7.90 ± 1.11 a	1.57 ± 1.03 b		
TOTAL (of compounds with a relative peak area of at least 1%)	98.16 ± 15.43 b	98.73 ± 12.83 b	84.70 ± 21.03 a	88.11 ± 16.64 a

x (-): Compound was not detected.

(+): Compound was detected in trace amounts, <1%.

Based on the information provided in Table 8, it appears that the essential oils obtained from the first accession BFL 28-002/06 in 2018 and in 2019, had the highest number of compounds detected (12 compounds, occupying at least 1% of the total relative peak surface area) and accounting for 98.16% and 98.71% of the total essential oil, respectively. In 2020 and in 2021, only 6 and 10 compounds were identified, respectively.

The major components of the essential oil in 2018 were an unknown compound with a retention time of 35.049 min (28.76%), *p*-cymene (27.41%), thymol (11.59%), thymol methyl ether (9.05%), and another unknown compound with a retention time of 35.137 min (7.90%). In 2019, the main components were thymol (33.57%), carvacrol (28.37%), and *p*-cymene (19.07%). In 2020, only thymol (61.12%) and thymol methyl ether (13.09%) were among the main components, while in 2021, the major constituents were *p*-cymene (33.64%), carvacrol (26.43%), and thymol methyl ether (13.27%).

It is worth noting that five compounds (*p*-cymene, thymol methyl ether, thymol, terpinen-4-ol, and borneol) were present in the essential oils of all production years.

Statistically significant differences ($p < 0.05$) have been detected between year 2020 and 2018, 2019 as well between 2021 and 2018, 2019.

Table 8: Average relative peak area (%) ± standard deviation (SD) of each compound in essential oils from the second accession (BFL 13-001/05) of *S. montana*. Retention times are given in minutes. Different letters indicate statistical differences ($p < 0.05$) between years.

Compound	2018 (%)	2019 (%)	2020 (%)	2021 (%)
heptane (ret. time 3.682)	-	-	2.15 ± 0.55	-
1-octen-3-ol (ret. time 12.582)	1.71 ± 0.69	-	-	-

α-terpinene (ret. time 14.247)	2.57 ± 0.75	+	-	-
p-cymene (ret. time 14.605)	40.5 ± 5.09 a	33.38 ± 2.12 a	-	33.68 ± 3.37 a
γ-terpinene (ret. time 16.219)	8.53 ± 1.02 a	+	-	3.09 ± 0.42 b
cis-sabinene hydrate (ret. time 16.782)	1.21 ± 0.38	-	-	+
borneol (ret. time 21.680)	1.79 ± 0.86 a	2.08 ± 0.38 a	3.07 ± .37 a	2.09 ± 0.24 a
terpinen-4-ol (ret. time 22.082)	1.64 ± 0.51 a	1.47 ± 0.47 a	-	1.22 ± 0.42 a
thymol methyl ether (ret. time 24.822)	7.47 ± 1.06 a	9.42 ± 0.39 ab	8.44 ± 1.02 ab	10.78 ± 1.63 b
thymol (ret. time 27.740)	21.63 ± 3.15 c	22.16 ± 1.14 c	69.56 ± 13.99 a	39.19 ± 1.35 b
unknown (ret. time 27.887)	-	3.08 ± 0.66	-	-
unknown (ret. time 27.973)	-	2.78 ± 1.56	-	-
trans-geranyl acetate (ret. time 31.137)	-	2.10 ± 0.21	-	-
β-caryophyllene (ret. time 32.891)	+	1.18 ± 0.65 a	-	1.22 ± 0.39 a
unknown (ret. time 35.113)	-	10.25 ± 1.23	-	-
caryophyllene oxide (ret. time 39.564)	+	1.45 ± 0.49 a	-	1.89 ± 0.71 a
thujopsan-2-alpha-ol (ret. time 39.567)	-	1.29 ± 0.34	-	-
unknown (ret. time 41.539)	-	4.05 ± 0.54	-	-
TOTAL (of compounds with a relative peak area of at least 1%)	87.11 ± 13.51 a	94.68 ± 10.18 a	83.22 ± 6.68 a	1.06 a

[x] (-): Compound was not detected.

(+): Compound was detected in trace amounts, <1%.

The results of chemical analyses showed that the essential oil of the accession BFL 13-001/05 from production year 2019 contained 13 compounds, occupying at least 1% of the total relative surface area (%) and representing 94.68% of total essential oil (Table 8). 9 compounds (occupying at least 1% of the total relative surface area (%) and representing 87.11% of the total essential oil) were identified in the essential oil from 2018, followed by the essential oil from the production year 2021 (93.17% of the total essential oil was identified with 8 compounds) and the essential oil from the production year 2020 (83.22% of the total essential oil was identified by only 4 compounds, thymol being the leading compound). The most represented compounds in the analyzed essential oils were *p*-cymene (40.5%), thymol (21.63%), γ-terpinene (8.53%) and thymol methyl ether (7.47%) from the production year 2018 and from production year 2019 *p*-cymene (33.38% of the total essential oil), thymol (22.16%), an unknown compound with a retention time of 35.113 (10.25%) and thymol methyl ether (9.42%). The main components of the essential oil in 2020 were thymol (69.56%) and thymol methyl ether (8.44%). Also in 2021, the main components were thymol (39.19%), *p*-cymene (33.68%) and thymol methyl ether (10.7%). The compounds present in the essential oil in all observation years were thymol, thymol methyl ether and borneol. Statistically significant differences ($p > 0.05$) have not been detected between observed years.

The composition of the essential oil of the accession BFL

35-001/06 is shown in Table 9. This accession had the lowest percentage (64.03%) of detected compounds of all the samples analyzed in the first year. This percentage was represented by 10 compounds, including geraniol (20.48%), *p*-cymene (18.75%), cis-geranyl acetate (7.98%), and γ-terpinene (7.39%). This value increased significantly in 2019, when 17 compounds, occupying at least 1% of the total relative surface area (%) and accounting for 94.76% of the essential oil. This was also the highest percentage in terms of clarification of the content of essential oil in all four years of the experiment. In 2019, the essential oil composition was the most diverse in terms of the number of compounds detected, with 17 compounds, including geraniol (26.89%), *p*-cymene (12.99%), thymol (11.38%), geranyl butyrate (11.14%), and γ-terpinene (7.82%). In 2020, 11 identified compounds accounted for 86.42% of the essential oil, among which thymol (30.32%) and *p*-cymene (18.30%) dominated. However, in year 2021 the percentage increased again to 93.69%. In 2020 and 2021, the essential oil consisted of 11 compounds with a percentage of at least 1%, corresponding to 86.42% and 93.69% of the total essential oil, respectively. In 2020, thymol (30.32%), *p*-cymene (18.30%), geraniol (14.59%), cis-geranyl acetate (7.73%), and γ-terpinene (6.39%) dominated. Also in the last year of experimentation, the most important essential oil compound was thymol with 33.9%, followed by geraniol (16.03%), cis-geranyl acetate (11.89%), trans-geranyl acetate (9.81%) and *p*-cymene (5.59%). The compounds present in all four years were α-terpinene, *p*-cymene, γ-terpinene, geraniol, geranial, β-caryophyllene, and caryophyllene oxide. Statistically significant difference ($p < 0.05$) has been detected between year 2018 and other observed years.

Table 9: Average relative peak area (%) ± standard deviation (SD) of individual compounds in essential oils from the third accession (BFL 35-001/06) of *S. montana*. Retention times are given in minutes. Different letters indicate statistical differences ($p < 0.05$) between years.

Compound	2018 (%)	2019 (%)	2020 (%)	2021 (%)
vinyl amyl carbinol (ret. time 12.625)	+	1.15 ± 0.30 a	1.20 ± 0.40 a	+
myrcene (ret. time 13.028)	+	1.06 ± 0.19	+	+
α-terpinene (ret. time 14.247)	1.15 ± 0.19 b	1.35 ± 0.42 b	1.04 ± 0.36 b	9.49 ± 1.66 a
p-cymene (ret. time 14.605)	18.75 ± 2.30 b	12.99 ± 3.41 ab	18.30 ± 1.91 b	5.59 ± 1.99 a
γ-terpinene (ret. time 16.219)	7.39 ± 1.18 a	7.82 ± 3.03 a	6.39 ± 0.93 a	1.79 ± 0.25 b
borneol (ret. time 21.680)	+	1.09 ± 0.39	+	+
2,6-octadien-1-ol, 3,7-dimethyl (ret. time 24.220)	1.26 ± 0.40 a	1.03 ± 0.37 a	+	+
neral (ret. time 24.820)	-	1.51 ± 0.42 a	-	1.04 ± 0.38 a
thymol methyl ether (ret. time 24.822)	-	1.55 ± 0.61	1.99 ± 0.83	-
carvacryl methyl ether (ret. time 24.827)	2.39 ± 0.79 a	1.07 ± 0.57 b	-	-
geraniol (ret. time 25.418)	20.48 ± 1.22 ab	26.89 ± 6.88 b	14.59 ± 0.85 a	16.03 ± 5.44 ab
geranyl butyrate (ret. time 25.457)	-	11.14 ± 3.79	-	-
geranial (ret. time 26.157)	1.55 ± 0.69 a	1.16 ± 0.71 a	1.39 ± 0.54 a	1.28 ± 0.24 a
thymol (ret. time 27.740)	+	11.83 ± 3.80 a	30.32 ± 1.21 b	33.98 ± 5.99 b
cis-geranyl acetate (ret. time 30.236)	7.98 ± 0.53 a	-	7.73 ± 2.11 ab	11.89 ± 3.02 b

trans-geranyl acetate (ret. time 31.137)	-	10.25 ± 5.09 a	-	9.81 ± 2.11 a
β-caryophyllene (ret. time 32.891)	1.85 ± 0.17 a	1.46 ± 0.87 a	1.54 ± 0.51 a	1.67 ± 1.08 a
caryophyllene oxide (ret. time 39.521)	1.24 ± 0.27 a	1.34 ± 0.36 ab	1.91 ± 0.15 b	1.12 ± 0.13 a
TOTAL (of compounds with a relative peak area of at least 1%)	64.03 ± 7.76 a	94.76 ± 31.24 b	86.42 ± 9.81 b	93.69 ± 22.29 b

[x] (-): Compound was not detected.

(+): Compound was detected in trace amounts, <1%.

The fourth accession (BFL 46-003/06) had the most diverse essential oil composition in the last experimentation year (2021) (Table 10), when 13 compounds (occupying at least 1% of the total relative surface area (%) and representing 98.97% of the total essential oil) were identified. In 2018, 12 compounds were identified with at least 1% of the total relative surface area and representing 94.33% of the total essential oil). In production year 2019, 11 compounds accounted for 95.07% of the total essential oil); in the third production year (2020), 7 compounds (accounted for 96.42% of the total essential oil). The compounds present in all 4 years were α-terpinene, *p*-cymene, γ-terpinene, thymol methyl ether and thymol. The major essential oil constituents in the first year of the experiment were thymol (36.82%), *p*-cymene (22.78%), γ-terpinene (11.69%), and thymol methyl ether (7.97%). Also in 2019, the dominant compound was thymol (33.41%), followed by *p*-cymene (27.54%), an unknown compound with a retention time of 35.107 min (9.36%), and γ-terpinene (9.31%). In 2020, there were only two main compounds detected - thymol (47.51%) and *p*-cymene (40.07%). The largest share of essential oil in 2021was represented by *p*-cymene (30.64%), thymol (28.10%), carvacrol (18.04%) and γ-terpinene (7.86%) as the main compounds. Statistically significant differences ($p > 0.05$) have not been detected between observed years.

Table 10: Average relative peak area (%) ± standard deviation (SD) of each compound in essential oils from the fourth accession (BFL 46-003/06) of *S. montana*. Retention times are given in minutes. Different letters indicate statistical differences ($p < 0.05$) between years.

Compound	2018 (%)	2019 (%)	2020 (%)	2021 (%)
α-thujene (ret. time 10.169)	1.16 ± 0.15 a	+	-	1.27 ± 0.26 a
α-pinene (ret. time 10.540)	1.26 ± 0.09	+	-	+
2,2-dimethyl-5-methylene norbornane	1.28 ± 0.33 a	1.20 ± 0.19 a	-	1.12 ± 0.13 a
myrcene (ret. time 13.028)	1.39 ± 0.07 a	+	-	1.20 ± 0.13 a
α-terpinene (ret. time 14.247)	2.12 ± 0.25 a	1.54 ± 0.09 a	1.04 ± 0.05 a	1.83 ± 0.70 a
p-cymene (ret. time 14.605)	22.78 ± 0.76 a	27.54 ± 2.08 ab	40.07 ± 1.07 c	30.64 ± 5.25 b
ocimene (ret. time 15.279)	+	+	1.35 ± 0.54 a	1.25 ± 0.16 a
γ-terpinene (ret. time 16.219)	11.69 ± 0.07 b	9.31 ± 2.83 b	3.06 ± 0.17 a	7.86 ± 2.87 b
borneol (ret. time 21.680)	3.09 ± 0.19 ab	3.64 ± 0.08 b	-	2.19 ± 0.88 a
terpinen-4-ol (ret. time 22.082)	1.64 ± 0.21 a	1.56 ± 0.13 a	-	+

thymol methyl ether (ret. time 24.822)	7.97 ± 0.35 b	3.39 ± 0.33 a	1.67 ± 0.40 a	2.69 ± 1.19 a
thymol (ret. time 27.740)	36.82 ± 0.77 ab	34.41 ± 3.87 ab	47.51 ± 1.10 b	28.10 ± 8.09 a
carvacrol (ret. time 27.845)	-	-	+	18.04 ± 9.66
β-caryophyllene (ret. time 32.891)	3.14 ± 0.13 a	1.88 ± 0.20 b	-	1.31 ± 0.34 c
unknown (ret. time 35.107)	-	9.36 ± 0.34	-	-
caryophyllene oxide (ret. time 39.521)	+	1.22 ± 0.12 a	1.71 ± 0.35 a	1.46 ± 0.36 a
TOTAL (of compounds with a relative peak area of at least 1%)	94.33 ± 3.38 a	95.07 ± 10.27 a	96.42 ± 3.69 a	98.97 ± 30.03 a

[x] (-): Compound was not detected.

(+): Compound was detected in trace amounts, <1%.

The essential oil composition of the accession BFL 39-002/06/2 was similar in all experimental years. Table 11 lists the compounds with a content of at least 1% of the total relative surface area. In 2018 identified compounds accounted for 96.23% of the total essential oil; in 2019 this share was 98.25%; in 2020 94.22% and in the last experimental year (2021) 98.49%. In 2018, 13 compounds were detected, mainly thymol (41.28%), *p*-cymene (24.41%), and γ-terpinene (9.61%). In 2019, 15 compounds were identified. The most important were *p*-cymene (26.33%), thymol (16.73%), carvacol (15.69%), trans-geranyl acetate (15.8%), and an unknown compound with a retention time of 35.165 min (6.89%). In 2020, the fewest compounds (5) of the analyzed accession were identified: *p*-cymene (49.90%), thymol (18.50%), γ-terpinene (11.75%), and thymol methyl ether (11.56%). In 2021, 14 compounds were identified, among which the most represented were thymol (27.67%), carvacrol (24.44%), *p*-cymene (20.71%), and γ-terpinene (9.33%). The compounds identified in all four experimental years were *p*-cymene, γ-terpinene, thymol methyl ether and thymol. Statistically significant differences (p >0.05) have not been detected between observed years.

Table 11: Average relative peak area (%) ± standard deviation (SD) of individual compounds in essential oils from the fifth accession (BFL 39-002/06/2) of *S. montana*. Retention times are given in minutes. Different letters indicate statistical differences (p < 0.05) between years.

Compound	2018 (%)	2019 (%)	2020 (%)	2021 (%)
2,2-dimethyl-5-methylene norbornane (ret. time 11.265)	1.33 ± 0.32	+	-	+
myrcene (ret. time 13.028)	1.37 ± 0.53 a	+	-	1.47 ± 0.52 a
1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)- (ret. time 14.267)	2.1 ± 0.21 a	-	-	1.46 ± 0.55 a
α-terpinene (ret. time 14.247)	2.28 ± 0.33 b	1.09 ± 0.38 a	-	1.25 ± 0.12 a
p-cymene (ret. time 14.605)	24.41 ± 2.7 a	26.33 ± 5.37 a	49.90 ± 0.23 b	20.71 ± 4.79 a
ocimene (ret. time 15.279)	-	+	-	2.07 ± 0.74

γ-terpinene (ret. time 16.219)	9.61 ± 1.82 b	2.88 ± 1.28 a	11.75 ± 0.56 b	9.33 ± 2.29 b
borneol (ret. time 21.680)	2.68 ± 1.66 b	0.94 ± 0.22 a	-	1.69 ± 0.93 a
terpinen-4-ol (ret. time 22.082)	1.75 ± 0.71 a	1.42 ± 0.54 b	-	1.20 ± 0.71 b
2,6-octadien-1-ol, 3,7-dimethyl- (ret. time 24.220)	-	1.09 ± 0.52	-	-
thymol methyl ether (ret. time 24.822)	4.65 ± 2.04 a	1.85 ± 1.03 a	11.56 ± 1.24 b	3.15 ± 1.79 a
carvacryl methyl ether (ret. time 24.827)	-	1.18 ± 0.34	-	-
geraniol (ret. time 25.418)	-	2.68 ± 1.09 a	-	1.29 ± 0.58 b
thymol (ret. time 27.740)	41.29 ± 2.79 c	16.73 ± 1.79a	18.50 ± 4.94 ab	27.67 ± 6.29 b
carvacrol (ret. time 27.845)	-	15.69 ± 3.49 a	-	24.44 ± 6.26 b
cis-geranyl acetate (ret. time 30.236)	1.29 ± 0.34	-	-	+
trans-geranyl acetate (ret. time 31.137)	-	15.8 ± 1.95 b	2.50 ± 1.12 a	1.42 ± 0.96 a
unknown (ret. time 35.165)	1.89 ± 0.82 b	6.89 ± 1.05 a	-	-
β-caryophyllene (ret. time 32.891)	1.67 ± 0.41 a	1.61 ± 1.07 a	-	1.34 ± 0.49 a
caryophyllene oxide (ret. time 39.564)	+	0.98 ± 0.06	-	+
TOTAL (of compounds with a relative peak area of at least 1%)	96.32 ± 14.67 a	98.25 ± 15.12 a	94.22 ± 8.08 a	98.49 ± 27.03 a

x (-): Compound was not detected.

(+): Compound was detected in trace amounts, <1%.

With regards the essential oil composition of the accession BFL 31-001/06, statistically significant differences ($p < 0.05$) have not been detected between observed years. In 2018, 97.26% of the total essential oil has been explained by 10 compounds, occupying at least 1% of the total relative surface area (Table 12). Among these, *p*-cymene (34.86%), thymol (31.94%), carvacrol (11.68%), and thymol methyl ether (6.60%) were the most abundant. In 2019, 12 compounds with a content of at least 1% were detected, including thymol (32.93%), *p*-cymene (27.03%), thymol methyl ether (11.37%), and γ-terpinene (8.21%). In 2020, 11 compounds were detected with a content of at least 1%, of which *p*-cymene (35.85%), carvacrol (26.37%), thymol methyl ether (14.42%), and γ-terpinene (6.75 %). As in 2019, *p*-cymene (38.31%), thymol (31.32%), thymol methyl ether (8.6%) and γ-terpinene (6.19%) dominated in 2021. In this year, 15 compounds were detected with a content of at least 1% of the total relative surface area.

Table 12: Average relative peak area (%) ± standard deviation (SD) of each compound in essential oils from the sixth accession (BFL 31-001/06) of *S. montana*. Retention times are given in minutes. Different letters indicate statistical differences ($p < 0.05$) between years.

Compound	2018 (%)	2019 (%)	2020 (%)	2021 (%)
α-thujene (ret. time 10.169)	1.32 ± 0.27 a	+	+	0.7 ± 0.65 b
α-pinene (ret. time 10.540)	+	1.15 ± 0.18 a	+	1.11 ± 0.19 b
2,2-dimethyl-5-methylene norbornane (ret. time 11.265)	+	1.18 ± 0.19 a	+	1.31 ± 0.24 a
vinyl amyl carbinol (ret. time 12.625)	-	-	+	1.34 ± 0.21
myrcene (ret. time 13.028)	+	1.31 ± 0.24 a	+	1.09 ± 0.28 a
ß-phellandrene (ret. time 13.820)	-	-	1.10 ± 0.19	-
α-terpinene (ret. time 14.247)	1.99 ± 0.03 b	1.67 ± 0.31 ab	1.67 ± 0.33 ab	1.02 ± 0.59 a
p-cymene (ret. time 14.605)	34.86 ± 3.11 ab	27.03 ± 1.47 a	35.85 ± 0.99 ab	38.31 ± 7.39 b
ocimene (ret. time 15.279)	+	-	-	1.26 ± 0.15
γ-terpinene (ret. time 16.219)	4.06 ± 0.45 a	8.21 ± 0.84 b	6.75 ± 1.34 ab	6.19 ± 2.34 ab
borneol (ret. time 21.680)	1.83 ± 0.38 a	2.23 ± 0.95 a	2.71 ± 0.24 a	2.18 ± 0.96 a
terpinen-4-ol (ret. time 22.082)	1.81 ± 0.33b	1.47 ± 0.10 ab	1.15 ± 0.24 a	1.35 ± 0.20 ab
thymol methyl ether (ret. time 24.822)	6.60 ± 1.82 a	11.37 ± 1.09 a	14.42 ± 2.04 a	8.6 ± 5.14 a
thymol (ret. time 27.740)	31.94 ± 2.11 b	32.93 ± 1.74 b	1.19 ± 0.18 a	31.32 ± 6.88 b
carvacrol (ret. time 27.845)	11.68 ± 0.89 a	-	26.37 ± 1.59 b	+
β-caryophyllene (ret. time 32.891)	1.17 ± 0.14 a	2.32 ± 0.10 b	1.33 ± 0.29 a	1.32 ± 0.24 a
caryophyllene oxide (ret. time 39.564)	+	1.60 ± 0.43	1.44 ± 0.74 a	1.68 ± 0.41 a
TOTAL (of compounds with a relative peak area of at least 1%)	97.26 ± 9.52 a	92.46 ± 7.63 a	93.98 ± 7.67 a	98.77 ± 25.88 a

^{x} (-): Compound was not detected.

(+): Compound was detected in trace amounts, <1%.

Discussion

As for morphology, we found that statistically significant differences have not been detected between observed accessions except in case of shoots number, inflorescence length and presence of different types of trichomes (both glandular and non-glandular trichomes were observed only in accessions BFL 28-002/06 and BFL 13-001/05). The results of the study showed that the variability of some of morphological characteristics (such as bush width, shoot length, percentage of flowering) within accessions of winter savory was very high and as a result, it was not possible to confirm statistically significant differences between individual accessions. [7] reported that the genus *Satureja* L. is known for its high variability due to polymorphism and differences in chemotypes within a single population. Otherwise, variability has been particularly pronounced in populations from remote habitats. Confirmed high metapopulation and intrapopulation variability in morphological characters of different populations of *S. bachtiar*.

Based on the results of this study, it can be summarized that variability was observed in the color of shoots and leaves. In the first accession, it was light green, and the shoots were dark brown. In the second accession, the shoots and leaves were yellow-green, and in the third accession, the leaves were yellow-green, and the shoots were yellow-brown. In the fourth accession, the leaves were light green, and the shoots were yellow-brown. Only in the last two accessions were both the leaves and the shoots yellow-brown. The width of the bush itself was very similar in the first accession in all years studied; only in the first year were the plants on average 1 cm narrower. The first accession also had the widest bushes compared to the others. In the second accession, we observed a greater average variation from 33 cm to 48 cm. In the third accession, the shrubs had the narrowest diameter, as they measured between 25 cm and 27 cm, 43 cm only in the first year. In the fourth accession these values ranged from 31 cm to 36 cm, in the fifth from 27 cm to 39 cm and in the last from 31 to 45 cm. The first accession had the widest shrubs, but not the longest shoots. The longest shoots were found in the third accession with an average of up to 36 cm and the shortest in the last accession with 25 to 27 cm. The average number of shoots was also highest in the third accession, up to 28 shoots. The length of inflorescences generally ranged from 0.3 cm (first accession in 2021) to 1.3 cm in the last accession in 2019 reported that it is an evergreen shrub that reaches a height of 10 to 50 cm and has strongly branched shoots. The plant forms brown woody stems, glossy dark green leaves, and lavender/white flowers reported that the leaves of *S. hortensis* L. are green to gray-green, almost stalkless, with entire margins, linear-oblong to lanceolate when unfolded, glandular-punctate, acute to rounded at the apex, narrowed, and hairy at the base determined the effects of planting density on chestnut tree yield and morphology. The experiment was conducted in 2008 and 2009 in western Iran. The treatment of the experiment included three rows spacing (60, 70, and 80 cm) and three row spacing (25, 35, and 45 cm). At full flowering stage, traits such as plant height, crown diameter, number of main shoots, number of lateral branches, internode length, leaf length and width were measured. However, the aforementioned analyzes were not precisely defined in the article. It was simply stated that the results showed that the lowest yield of flowering shoots was obtained at the smallest row spacing (25 cm); the maximum crown diameter was obtained at the largest row spacing (45 cm) and the lowest planting density (45 × 80 cm). The maximum yield of flowering shoots was obtained at the smallest row spacing (65 cm) and the smallest row spacing (25 cm). The average comparison showed that the maximum leaf length (7.67 mm) was obtained at the maximum row spacing (35 cm) and the minimum leaf length (7.16 mm) was obtained at the minimum row spacing (25 cm).

Agrometeorological factors at the experimental site resulted in low variability. In 2018, the winter was extremely warm with temperatures around 5°C, and as high as 8°C in January and March. The lowest temperature was in February with -2°C. The percentage of average cloudiness was high, ranging from 70% to 86%. The average amount of precipitation was 163 mm. In spring, temperatures rose to 23.7°C. An average of 106 mm of rain fell. Cloud cover was present at a lower percentage than in winter (51% to 68%). In summer, during the flowering season, temperatures were up to 28°C. The average number of sunshine hours per month was 260 hours. The most precipitation fell in August, 223 mm. 2019 was also an extremely warm winter. Temperatures rose to 15.2°C in March. The lowest recorded temperature was in January, -2.1°C. Cloud cover was lower than in 2018, ranging from 47% to 52%. The most sunshine hours were recorded in March, 191.1 hours. The average amount of precipitation was 74 mm. In spring, temperatures ranged from 6.9°C to 17.6°C. The most precipitation was measured in May, 238.6 mm, when cloud cover was also 79%. In summer, temperatures rose to 29.4°C. The most precipitation fell in August, 141 mm. Cloud cover ranged from 39% to 62%. In 2020, there was a similar winter, which was again extremely warm, reaching 12.8°C. The lowest temperature was recorded in January, -1.8°C. The sunniest hours were in March, 174.2 hours. Cloud cover ranged from 49% to 60%, and only 13.9 mm of precipitation fell in January and 105.3 mm in March. In spring, temperatures rise to 20.8°C. On average, 131.0 mm of precipitation fell. The most hours of sunshine were recorded in April, 292.2 hours, and during the rest of the year the average cloudiness was 60%. In summer, temperatures were as high as 28.2°C. The lowest temperature was recorded in June, 14.5°C. The sunniest hours were in July, when there were 326.3 hours. In the last observed year, winter was generally colder than in previous years, as the average temperature in January was 1.2 °C, and the lowest measured temperature was -1.1 °C. Cloud cover averaged between 43% and 81%. The average amount of precipitation was 94 mm. In spring, temperatures rose to 19.3 °C. The most precipitation fell in May with 247.5 mm. The average number of sunshine hours was 196 h. In summer, temperatures rose up to 29°C. Also, this year, the sunniest hours were in the month of July, 310.9 hours. The cloudiness averaged between 36% and 41%. The average amount of precipitation during the summer months was 144 mm (ARSO, 2022).

According to the above findings, the highest essential oil content in 2021, based on the average of all accessions, should also be 6.6 mL/kg (0.66%). The average content in the first year was also sufficiently high at 6.15 mL/kg (0.615%). [12] reported an essential oil yield of 0.3% for *S. montana* from a natural habitat in the southern region of Bosnia and Herzegovina, and reported an essential oil yield in a Croatian population that ranged from 0.80 to 1.46%. [7] also studied Croatian *S. montana* and found that the yield of essential oil was 0.97%. [14] found that the essential oil yield of *S. montana* from Kosovo ranged from 0.09 to 0.70%. The yields obtained in our studies are comparable to the literature data. The highest yield of essential oil was obtained in 2021 in the fifth population (BFL 39-002/06/2) with 0.85% and the lowest in 2019 in the second population (BFL 13-001/05) with 0.22%. The second population had the lowest yield of essential oil in all years.

Differences in essential oil content occur not only when comparing different countries, but also over shorter distances (when fields are separated by a mountain ridge). The contents of the individual components of the essential oils of *Satureja* collected in three different countries (Kosovo, Albania and Montenegro) and at different locations were compared. The results show that there are differences in content, constituents and chemotype even at small distances. The two sites studied in Albania have the same climate, soil, and altitude, but different chemotypes and essential oil yields, despite being separated by only a short geographical barrier. Essential oil yield is positively correlated with low altitude and continental climate. Therefore, the differences are due to habitat, microclimate, and altitude [21].

In general, in all years, the fifth (BFL 39-002/06/2) accession of *S. montana* was the most diverse in terms of essential oil composition, then the fourth, followed by the sixth, the first, the second, and finally the third accession. The compounds present in all accessions were *p*-cymene, thymol methyl ether, thymol, β-caryophyllene, borneol, γ-terpinene, and α-terpinene. [22] reported that the main essential oil compounds of *S. montana* from Albania were thymol (28.99%), *p*-cymene (12.00%), linalool (11.00%), and carvacrol (10.71%). In contrast, linalool was not detected in our samples. Average thymol content of our samples was 29.8% of the essential oil, which agrees with the results of [22]. The value of *p*-cymene was on average more than twice of that of our study (27.21% of essential oil). The value closest to that determined by [22] was the third approach in 2019; *p*-cymene accounted for 12.99% of the essential oil. The carvacrol content in our samples averaged 21.39%, which was twice as high as in the above study. The 2018 value of carvacrol (11.68%) measured in the sixth accession was closest to them. In the above literature, the borneol content was reported to be 3.43%. The values in our study ranged from 0.94 to 3.64%. According to [22], a high content of β-caryophyllene (4.45%) was also found, but in our samples this value was lower, from 1.17 to 3.14%.

The values of the main constituents of the essential oils of *S. montana* present in all our samples were significantly different from those reported in the literature. [23] reported that the *p*-cymene content of *Satureja* from Albania ranged from 16.2 to 17.4%. [21] studied Albanian *Satureja*, and their *p*-cymene content ranged from 5.14 to 13.73%. [18] studied *Satureja* from Bosnia and Herzegovina and found values ranging from 19.5 to 47.7%. Similar results (6.7 to 57.7%) were also obtained by [24] in a study of *Satureja* growing in France. In our study, the *p*-cymene content ranged from 4.1 to 49.90%. [9] reported that the thymol content of *Satureja* grown in Croatia was 30.8%, which is within the range of our study (1.19 to 69.56%). Similar results were also obtained by in Croatia with contents ranging from 30.88 to 46.02%. Much lower contents (10.9 to 11.0%) were obtained by [25] and [22] (6.0 to 11.3%), who conducted a study in Spain. Thymol methyl ether and ß-caryophyllene were not dominant ingredients in the studies investigated. [26] found that the content of γ-terpinene in essential oils of *Satureja* from Albania ranged from 12.3 to 15.0%. We determined a content between 1.79 and 11.75% reported that the value of γ-terpinene in the essential oil of *Satureja* growing in the territory of Bosnia and Herzegovina was also slightly higher than ours (11.49 to 15.87%). The results of the present study are consistent with those of a study conducted in Albania by Ibraliu et al. (2011). Their values for the said compound ranged from 0.31 to 8.86%. [16], who studied the essential oil of *Satureja* from Croatia, also obtained results in our range (5.8%).

The most similar borneol contents in this study (0.94 to 3.64%) were found in the study conducted in Serbia by [28]; their content was 3.04 to 4.35%. Much lower values, 0.48 to 0.90%, were found in Portugal by [29]. The essential oil of *Satureja* from Serbia, studied by [18], was very rich in borneol. The content ranged from 2.6 to 28.8%.

The content of α-terpinene in our samples ranged from 1.02 to 9.49%; in the studies examined, this compound was not listed as a dominant compound in the essential oil of *Satureja*.

In the studies of Stoilova et al. (2008), who investigated *S. montana* from Slovenia, the major essential oil constituents were carvacrol (41.5%), *p*-cymene (11.0%), thymol (8.6%), α-terpinene (6.2%), and β-caryophyllene (4.1%). Our results differ from the values obtained by them. In particular, they obtained much lower concentrations of the mentioned compounds, except for carvacrol. This compound was the most abundant in their samples, while in our samples it appeared in the first accession 2021 (26.43%). In the fourth accession it also appeared in 2021 (18.04%) and in the fifth accession also in 2021 (24.44%). In the sixth accession in 2018, it reached 11.68% and in 2020, 26.37%. The content of β-caryophyllene was similar to that found by us. Thymol methyl ether, which was present in all studied accessions, was not mentioned in [30]. Borneol content was 2.4%, which is within the range of this study. Higher temperatures and drought have been shown to stimulate the biosynthesis of phenolic and terpenophenolic compounds. The maximum amount of carvacrol was reached in the phase of full flowering, which coincides with the highest air temperatures. [15] reported that *p*-cymenol content was highest after the end of flowering and γ-terpinene content was highest at the beginning of flowering. Geraniol content was constant during flowering [16]. The content of thymol, carvacrol, α- and γ-terpinene, and linalool decreases with maturity, while the content of *p*-cymene and borneol increases (up to fourfold). Analyzes have shown that a young plant contains the most eugenol [17]. However, [18] reported that the content of components (mainly carvacrol and thymol) depends on the origin of the plant. A sunny location has been shown to have a positive effect on leaf mass (2013). A high content of 1-methyl-3-benzene is an indicator of growth under stress conditions, according to [19]. The variability of the components also depends on the plant organ studied (flower, stem, leaf).

It can be concluded that the main components of the essential oil of winter savory were *p*-cymene, thymol, and geraniol [31] reported the pharmacological properties of *p*-cymene including antioxidant, anti-inflammatory, antiparasitic, antidiabetic, antiviral, antitumor, antibacterial, and antifungal activities. *p*-cymene also acts as an analgesic, antinociceptive, immunomodulator, vasorelaxant, and neuroprotectant. Its anticancer effects are due to specific mechanisms, such as inhibition of apoptosis and cell cycle arrest [32] confirmed the highly beneficial effects of *p*-cymene. The results of their study suggest that *p*-cymene has antioxidant potential in vivo and may act as a neuroprotective agent in the brain. This compound may represent a new strategy for developing treatments for numerous diseases in which oxidative stress plays an important pathophysiological role. The antibacterial activity of carvacrol and *p*-cymene (a precursor of carvacrol) was investigated against the food-borne microorganism V. cholerae to evaluate the potential use of these compounds as preservatives. The synergistic in vitro activity of *p*-cymene and carvacrol may indicate the potential use of the combination to inhibit V. cholerae and other foodborne pathogens in food [33,34] studied the growth of Candida lusitaniae in different concentrations of natural products such as carvacrol, thymol, and *p*-cymene to evaluate their potential use as food preservatives. The growth rate of C. lusitaniae decreased in the presence of these molecules, while the lag time increased with increasing concentration. All the molecules tested inhibited yeast growth. Thymol is a naturally occurring phenolic monoterpene derivative of cymol and an isomer of carvacrol. The interest in formulating drugs, dietary supplements and cosmetic products based on thymol is the result of numerous studies that have investigated the potential therapeutic benefits of this compound in the treatment of respiratory, nervous, and cardiovascular diseases. In addition, this compound also shows antimicrobial, antioxidant, anticarcinogenic, anti-inflammatory, and antispasmodic activities, as well as potential as a growth promoter and immunomodulator [35,36] reported that thymol has antibiofilm, antifungal, antileishmanial, antiviral, and anticancer properties. The large-scale use of thymol in health applications is very promising but requires further research and analysis. Geraniol is widely used in the food industry as a fragrance and flavouring agent. Other functional properties of geraniol have also been reported, such as antibacterial, antifungal, antioxidant, and anti-inflammatory [37,38] demonstrated that geraniol inhibited the proliferation of PC -3 human prostate cancer cells. A high-fat diet in obese humans and animals may increase endothelial dysfunction. It can cause many other serious cardiovascular diseases and metabolic disorders. [39] studied the effect of geraniol on endothelial function in mice fed a high-fat diet (HFD). Geraniol protected and ameliorated HFD-induced endothelial dysfunction in HFD-fed mice by decreasing the production of NADPH oxidase and ROS in the aorta. In the study, geraniol was administered orally for 7 weeks to STZ-induced obese rats. Geraniol significantly decreased diabetes-associated cardiac systolic function by reducing oxidative stress.

Conclusion

A four-year morphological comparison of six winter savory accessions and determination of the content and composition of their essential oil showed that inter- and intraspecific variability within the genus *Satureja* was low. In general, accession BFL 39-002/06/2 was the richest in terms of essential oil composition in all four years studied. The lowest amount of compounds, which represented 64.03% of the essential oil, was identified in 2018 in the accession BFL 35-001/06. From the presented results, it can be concluded that most of the accessions contain thymol or its isomer carvacrol or their biosynthetic precursors (*p*-cymol and γ-terpinene). In the third accession, geraniol, whose content varied from 14.59 to 26.89% (depending on the year of production), was one of the main components of the essential oil. As expected, the essential oil content was highest in 2021. The average content in the first year was also quite high at 6.15 mL/kg (0.615%). This is probably due to the fact that this year was the most favorable for the development of leaves and shoots, which produced larger amounts of essential oil, as it was distilled from flowering shoots with leaves.

The study has confirmed that the essential oils of all tested populations of *S. montana* grown in the laboratory area of the Biotehnical Faculty in Ljubljana are rich enough in bioactive compounds to have a positive effect on human health. The study represents a background tool for further research in the field of winter savory morphology and chemical composition allows for expansion by including winter savory from other parts of Slovenia and extending the experiment to other locations (greenhouse, other soil types). In this way, it will be possible to find out the population with a particular genetic predisposition fits best to specific cultivation conditions.

Author Contributions

Conceptualization, N.K., L.D. and D.B.; methodology, N.K., M.K.L., N.K.G., D.B. and L.D.; formal analysis, N.K., L.D., M.K.L., and N.K.G..; investigation, N.K.; data curation, N.K.; writing-original draft preparation, N.K., L.D. and D.B.; writing-review and editing, M.K.L., N.K.G., D.B.; visualization, N.K., L.D., D.B. and M.K.L.; supervision, M.K.L., N.K.G., B.T., D.B.; funding acquisition, D.B, M.K.L., N.K.G. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

All data are presented within the article.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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