Drug yield and essential oil and carvacrol contents of two species of *Satureja* (*S. khuzistanica* Jamzad and *S. rechingeri* Jamzad) cultivated in two different locations

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Abstract

Satureja khuzistanica and *Satureja rechingeri* are two valuable medicinal perennial species endemic to Iran. They grow wild in southwest parts of Iran. These species are rich in carvacrol, with considerable biological activities. In order to study the effects of climate and region conditions on plant height, leaves + flowering shoots yield (drug yield), essential oil content and yield, and carvacrol content, two years field experiments were conducted in two different locations (Andimeshk and Kashkan) in Iran during 2012 and 2013. The result showed that (average of both years) drug yield of either species in Kashkan (2.6 t ha⁻¹) was higher than in Andimeshk (1.05 t ha⁻¹). Drug yield of *S. khuzistanica* (1.9 t ha⁻¹) in either region was higher than that of *S. rechingeri* (1.7 t ha⁻¹). *S. rechingeri* (37.6 ± 3.5 cm) was shorter than *S. khuzistanica* (40.5 cm) in either location. In Kashkan, the species grew taller (40.9 cm) than in Andimeshk (37.3 cm). In addition, essential oil content of *S. rechingeri* (4.5 %) in both regions was more than that of *S. khuzistanica* (1.9%). Essential oil content of both species in Andimeshk (3.3%) was more than in Kashkan (3.1%). Essential oil yield obtained from *S. rechingeri* (77.3 kg ha⁻¹) was higher than that from *S. khuzistanica* due to its higher essential oil content. However, neither location nor species significantly affected carvacrol content. Carvacrol was the main component of the essential oil (95.9-96.7%). Overall, the results showed that both species performed better in the cooler region (Kashkan) but with a higher essential oil content in the warmer region (Andimeshk).

Keywords: Carvacrol, Essential oil, Drug yield, *Satureja khuzistanica*, *Satureja rechingeri*

1. Introduction

The genus *Satureja*, belonging to the Lamiaceae family and the Nepetoideae subfamily, consists of 30 species. It is mainly distributed in the Mediterranean areas. 14 species of *Satureja* are growing wild in northern, northwestern, western, southwestern, and central parts of Iran (Jamzad, 1994), nine of which (*S. khuzistanica* Jamzad, *S. rechingeri* Jamzad, *S. bakhtiarica* bunge, *S. atropatana*, *S. edmondi*, *S. isophylla*, *S. kallarica* jamzad, *S. sahendica*, and *S. macrosiphonia*) are endemic to Iran. *S. khuzistanica* and *S. rechingeri*, as perennial plants, are widely distributed in the southwestern parts of Iran (Jamzad, 2009). High similarities have been observed between *S. rechingeri* and *S. khuzistanica* regarding morphological characteristics and essential oil composition. *S. khuzistanica* and *S. rechingeri* are popular herbs for the local

population, being used as herbal tea and for their therapeutic virtues as analgesic and antiseptic medicines in the traditional medicine. Recently, many diverse activities (e.g. antifungal, antibacterial, antinociceptive, antioxidant, antidiabetic, antihyperlipidemic, antibiofilm, antiinflammatory, and triglyceride-lowering activities) have also been observed in *S. khuzistanica* and *S. reshingeri* (Hadian et al., 2011). The composition of *S. khuzistanica* and *S. reshingeri* essential oils have been reported previously. The main components of the essential oil of *S. khuzistanica* were carvacrol (48%), *p*-cymene (18.5%), and γ -terpinene (11%) (Farzaneh et al., 2015), and carvacrol (80–95.4%) (Farsam et al., 2004; Hadian et al., 2011). Similarly, carvacrol (86.6%) and *p*-cymene (2.4%) were the main components of the essential oil of *S. reshingeri* (Sefidkon et al., 2007). Carvacrol has also been reported as the major component of the essential oils of other *Satureja* species (*S. boissieri*, *S. mutica*, and *S. hortensis*), but its content was lower than 40% (Sefidkon, 2007). The comparison of the essential oils of *S. khuzistanica* and *S. reshingeri* with those of other *Satureja* species indicates that the highest percentage of carvacrol exists in these two species. Carvacrol seems to be responsible for the biological activities of these species.

S. khuzistanica and *S. reshingeri* are valuable herbs used in pharmaceutical and food industries due to their pharmacological and biological properties. Herbal drops of the species are used as dental anesthetic and oral antiseptic drugs (Hadian et al., 2011). Nowadays, herbal drugs of these endemic plants are collected from wild niches to meet the demands of the pharmaceutical and food industries. The degradation of genetic diversity, the extinction of ecotypes, and the unreliable supply of herbaceous materials are the main challenges facing wild collection. To solve these problems, the emphasis is placed on domestication and cultivation, which contribute to germplasm conservation and an ensured supply of herbal raw material.

Growth and essential oil composition of plants in natural conditions or agro-ecosystems can be affected by many factors, categorized into internal (genetic constitution and age or development stage) and external factors (soil properties, moisture, temperature, day length, light quality, nutrient levels, and wind patterns) (Carrubba and Catalano, 2009). Both categories have extremely variable effects on the plant growth, yield, quantity, and quality of the essential oil. The effects of ecological conditions on (1) *Cunila galioides* in Brazil (Echeverrigaray et al., 2003), (2) *Thymus sp.* in southeast Spain (Horwath et al., 2008), (3) *Lavandula latifolia* in Spain (Munoz et al., 2007), (4) *Salvia officinalis* L. in south-central Italy (Russo et al., 2013), (5)

Thymus daenensis Celak and *Thymus vulgaris* L. in central Iran (Ghasemi Pirbalouti et al., 2013), and (6) *Mentha piperita* L. in Turkey (Telci et al., 2011) have been studied and the results indicate that the yield, quantity, and quality of the essential oils were affected by ecological factors (due to the different soil and climatic conditions). Therefore, determining the effects of different ecological conditions on cultivated plants is important to produce a high quality crop. The objective of the present study was to investigate the effects of different ecological conditions on yield, essential oil production, and carvacrol content of two valuable endemic medicinal species: *S. khuzistanica* and *S. rechingeri*. For this purpose, *S. khuzistanica* and *S. rechingeri* were cultivated in two different regions in Iran (Andimeshk and Kashakn).

2. Material and methods

2.1. Plant material and field experiments

Two two-year field trials, arranged in randomized complete block designs, each with six replications per treatment, were conducted in two locations in Iran: (1) Edrisi sofla village, Andimeshk, Khuzestan (32.45° N, 48.35° E) and (2) Kashkan bridge, Lorestan (33.98° N, 51.41° E) during 2012–2013. The geographical positions, soil properties, and some climatic data of the locations are presented in Tables 1 and 2, respectively.

"insert Table 1 and Table 2"

The two species (*S. reshingeri* and *S. khuzistanica*) were identified by the Department of Botany of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran. Voucher specimens of the two species (No. 58416 for *S. khuzestanica* and No. 75587 for *S. reshingeri*) have been deposited at the Herbarium of TARI. Healthy cuttings of the two species were used as propagules. 8-10 cm cuttings were taken from a mother plantation of *S. khuzistanica* grown at the Khorraman Pharmaceutical Company field (Khorramabad, Lorestan) on 27 December 2011. For *S. reshingeri*, cuttings were taken from a natural habitat (Mehran region in Ilam province) on 31 December 2011. Cuttings were planted in small pots and grown in a greenhouse for 135 days before being transplanted in the experimental field. Small plants of the two species were transplanted 50 cm × 50 cm apart in the field on 4 May 2012. Each plot was sized 2 m × 4 m and contained 4 rows. The average density was 4 plants per m². At the early growth stage, the plants were watered once a week for better plant establishment, and then at 15-day intervals. Weeds were controlled by hand-hoeing when necessary. No fertilizer (organic or inorganic) or pesticide

was used during the experiment. Since *S. khuzistanica* and *S. rechingeri* are perennial plants, the data of the second year were obtained from the same plants, which regrew. After full flowering stage, five plants were selected from the middle of each plot, and plant heights were measured. The aerial parts of the species (four plants in 1 m²) were harvested in each region at full flowering stage in September and October either in 2012 or in 2013 (The harvest was done at the same date in both years) by cutting five plants approximately 10 cm above the ground (Table 3). The herbage was air-dried in the shade at room temperature (Karamanos and Sotiropoulou, 2013; Teles et al., 2013). The leaves and flowering shoots (oil bearing parts of the plant) were separated from the dried woody stems, weighed, and recorded as the drug yield.

"insert Table 3"

2.2. Essential oil isolation

After the leaves and flowering shoots were ground into a fine powder, the essential oil was isolated via hydro-distillation, using a Clevenger-type apparatus (30 g sample were mixed into 300 mL distilled water), with a distillation time being approximately 3 h at boiling point. The oils extracted were dried with anhydrous sodium sulfate (Na₂SO₄), and then stored in tightly closed, dark vials at 4 °C until analysis. The volume of the essential oil produced was measured and expressed as g 100g⁻¹ dry weight (Farzaneh et al., 2015). Three extraction replicates were prepared for each treatment.

2.3. Gas chromatography and compound identification

The GC-FID analysis of the oils was conducted using a *Thermoquest-Finnigan* apparatus equipped with a flame ionization detector (FID) and a DB-5 fused-silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 mm). The oven temperature was programmed from 60 to 250 °C at 4 °C min⁻¹ and held isothermal at 250 °C for 10 min; injector temperature 250 °C; detector temperature 300 °C; carrier gas N₂ (1.1 ml min⁻¹); split ratio 1:50. Quantitative data was obtained from GC-FID area percentages without the use of correction factors (Hadian et al., 2011). Each sample and standard was injected three times.

The GC/MS analysis was conducted with a *Thermoquest-Finnigan* Trace apparatus equipped with a DB-5 fused-silica capillary column (60 m×0.25 mm i.d., film thickness 0.25 mm). The oven temperature was programmed from 60 to 250 °C at 4 °C min⁻¹ and held isothermal at 250

°C for 10 min; ion source and transfer-line temperature 250 °C; carrier gas He (1.1 ml min⁻¹). The mass spectrum was acquired over the mass range of 35-465 amu with an ionization voltage of 70 eV (Hadian et al., 2011).

The identification of carvacrol was performed by comparing its mass with those of the internal reference mass spectra library (Adams and Wiley 7.0) or with the authentic compound and confirmed by comparing its retention indices (RI) with the authentic compound or with those reported in the literature (Adams, 2007). Quantification of the relative percentage of carvacrol in the essential oil was performed according to the area percentage method.

2.4. Statistical analysis

The mean of data for each variable (plant height, drug yield, essential oil content and yield, and carvacrol content) collected in two years were statistically analyzed by using two-way analysis of variance (ANOVA) based on the GLM procedure of the SAS statistical package (SAS; version 9.1). Significance of the differences between location (L), species (S), and their interactions (L×S) for the variables were tested using LSD ($p < 0.05$). The data of all variables were also shown as mean $\pm$ standard deviation calculated from replications.

3. Results and discussion

3.1. Yield of leaves + flowering shoots (drug yield)

The results indicated that the effects of species, location, and species×location on drug yield ($p < 0.01$) were significant (Table 4). The drug yield of *S. khuzistanica* (1.95 ± 0.93 t ha⁻¹) was higher in either region, compared to that of *S. reshigeri*. The highest drug yield (2.63 ± 0.4 t ha⁻¹) was obtained from the plants grown in Kashkan (Table 4). *S. khuzistanica* cultivated in Kashkan produced the highest drug yield (2.84 ± 0.31 t ha⁻¹), while *S. reshigeri* in Andimeshk had the lowest yield (1.04 ± 0.15 t ha⁻¹). The differences observed in the two regions can be attributed to climatic differences between the locations, as reported in table 1 and 2. The mean annual temperature for Andimeshk and Kashkan was 27.2 °C and 15.9 °C, respectively (Table 1). In addition, the average temperature during the growth period was 32 °C in Andimeshk and 21 °C in Kashkan (Table 2). The lower temperature and soil EC, higher soil available P, and better soil texture in Kashkan compared with Andimeshk resulted in a better plant growth and performance. Therefore, the highest yield was obtained from the plants grown in Kashkan (Table 4). Similar

results were found for *Coriandrum sativum* L. in Turkey, where plants in an area with a longer growing period produced a higher biomass and seed yield (Telci et al., 2006). In contrast, some studies have reported that the highest biomass yield in *Mentha × piperita* L. (Telci et al., 2011) and *Thymus vulgaris* (Ghasemi Pirbalouti et al., 2013) were obtained in warmer regions. The results of the present study could also be partially related with the soil characteristics (Telci et al., 2011). The reason that the plants had the lowest drug yield in Andimeshk is probably the clay heavy soil of the region (soil texture) and the higher salinity of the soil (Table 1). In heavy and saline soils, plants have to consume more energy for the root growth and the absorption of water and nutrients. These conditions lead to a decrease in plant growth and yield.

3.2. Plant height

The results showed that the plant height was affected by both of the environmental conditions (location) and the species. Plant height of *S. reshingeri* (37.6 ± 3.5 cm) was lower than that of *S. khuzistanica* (40.5 ± 3.9 cm) in either location. The plant height in Kashkan (40.9 ± 4.2 cm) was higher than in Andimeshk (37.3 ± 2.7 cm) (Table 4). The difference between locations in terms of plant height can be attributed to the different temperature and soil characteristics: EC, texture, available P, etc. (Table 1, 2, 3).

3.3. Essential oil content

The production of essential oils in plants is an adaptation strategy to environmental factors. Statistical analysis showed that there were significant differences between the locations ($p < 0.05$) and species ($p < 0.01$) in terms of essential oil content. However, the interactive effect of species and location on essential oil content was not significant (Table 4). The essential oil content of *S. reshingeri* ($4.5 \pm 0.28\%$) was higher in either region. The differences observed between the species in terms of essential oil content can be attributed to their genetic differences. The highest essential oil content ($3.3 \pm 1.2\%$) was obtained from the plants grown in the warmer region (Andimeshk) (Table 4). In general, plants secrete more essential oil in warm conditions, a strategy that protects them against water stress and intense light (Horwath et al., 2008). Our results are in agreement with the results of studies on *Origanum vulgare* in Greece (Vokou et al., 1993), *Coriandrum sativum* L. in Turkey (Telci et al., 2011), and *Lavandula latifolia* in Spain (Munoz et al., 2007). The higher essential oil content in plants cultivated in Andimeshk might also be attributed to the lower altitude. The essential oil content usually increases when a plant is

grown at a low altitude. Similar results were found for *Origanum vulgare* (Vokou et al., 1993) and *Thymbra spicata* var. *spicata* (Kizil, 2010). On the contrary, Moghaddam and Farhadi (2015) reported that the essential oil content of *Ferula assa-foetida* L. reduced with increasing altitude and decreasing temperature.

3.4. Essential oil yield

The essential oil yield depends on the leaf yield and essential oil content. Species, locations, and species×location had significant effects ($p<0.01$) on the essential oil yield. The essential oil yield obtained from *S. reshigeri* ($77.3 \pm 33.7 \text{ kg ha}^{-1}$) was higher than that from *S. khuzistanica* due to its high essential oil content ($4.5 \pm 0.28\%$). The higher essential oil yield obtained from the cultivation in Kashkan region was due to the drug yield ($2.63 \pm 0.4 \text{ t ha}^{-1}$) (Table 4). This can be due to the reasons (the better plant growth in the colder region) mentioned above (section 3.1).

The highest essential oil yield ($107.97 \pm 16.19 \text{ kg ha}^{-1}$) was obtained from *S. reshigeri* cultivated in Kashkan location, whereas *S. khuzistanica* grown in Andimeshk produced the lowest essential oil yield ($23.03 \pm 5.4 \text{ kg ha}^{-1}$) (Table 4). Despite the higher drug yield of *S. khuzistanica* compared to *S. reshigeri*, the essential oil yield of *S. reshigeri* was about twice as that of *S. khuzistanica* in either location.

"insert Table 4"

3.5. Carvacrol content

The results revealed that carvacrol, as the main component of the essential oil at a high percentage (95.96-96.77%), was not significantly affected by the cultivated area in either species. There were no significant differences ($p<0.05$) between the two *Satureja* species cultivated in two regions in terms of carvacrol content (Table 5). Similar results have been reported by other researchers. Hadian et al. (2011) studied the essential oil composition of eight populations of *S. khuzistanica* from different locations of Iran. Their results indicated that all populations had carvacrol as the main component with a very high content (89.59–95.41%). The maximum and minimum carvacrol content was obtained in the Choveny (95.41%) and Takhteshan (89.59%) populations, respectively. In another study on *S. khuzistanica*, it was shown that geographic, climatology, and edaphic factors had no significant effects on carvacrol content. Furthermore, carvacrol was found as the principal component (94.8%) in the essential oil of all populations (Ghasemi Pirbalouti et al., 2011). Carvacrol has also been reported as the major component of

the essential oils of other *Satureja* species (*S. boissieri*, *S. mutica*, and *S. hortensis*), but its content was lower than 40% (Sefidkon et al., 2007). Carvacrol is a monoterpenoid phenol biosynthesized via aromatization of γ -terpinene to *p*-cymene and subsequent hydroxylation of *p*-cymene. It plays a significant role in the adaptation of oil-bearing plants to environmental conditions and has a wide range of activities including antimicrobial, antioxidant, anticandidal, and anti-inflammatory (Hadian et al., 2011).

"insert Table 5"

4. Conclusions

The drug yield, essential oil content, oil yield, and plant height of the two *Satureja* species are considerably affected by the geographical region. Neither location nor species had significant effects on the carvacrol content of the two *Satureja* species studied. Carvacrol was the main component of the essential oil present at a high percentage (95.96–96.77%) in both species and regions. Both species had higher plant height, yield of leaves + flowering shoots (drug yield), and essential oil yield in the cooler region (Kashkan), but higher essential oil contents in the warmer region (Andimeshk). These differences could be explained by different environmental parameters such as soil and climatic conditions.

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Table 1. Geographic positions, climatic data, and soil properties of the locations

Geographic positions	Andimeshk	Kashkan
Altitude (m)	477	1013
Latitude (N)	32°48′	33°35′
Longitude (E)	48°32′	47°53′
Climatic data (mean of 10 years 2004-2013)		
Rainfalls (mm)	371.4	376.7
Temperature (°C)	27.28	15.91
Relative humidity (%)	32	34.7
Evaporation (mm)	3636.6	1890
Sunny hours (h)	2756	2970
Soil properties		
Sand (%)	19	85
Silt (%)	52	8
Clay (%)	29	7
Texture	Silty Clay Loam	Loamy Sand
EC (ds/m ⁻¹)	4.24	0.95
pH	8.1	8.2
Organic carbon (%)	0.73	0.29
Total N (%)	0.07	0.03
Available P (mg kg ⁻¹)	5.1	13.25
Available K (mg kg ⁻¹)	214	157

Table 2. Weather conditions of the locations in the growing season (2012 – 2013)

Month	Kashkan location				Andimeshk location			
	Monthly rainfall (mm)		Mean monthly temp. (°C)		Monthly rainfall (mm)		Mean monthly temp. (°C)	
	2012	2013	2012	2013	2012	2013	2012	2013
April	9	9.9	12	13.5	47.5	3.3	21.7	24.5
May	11.8	91.6	18.7	15.9	5.3	143.8	30.8	25.9
June	0	0.5	24.8	23.26	0	0	36.7	35.2
July	0	0	27.9	28.3	0	0	39.5	39.6
August	0	0	29.3	26.8	0	0	40.8	37.7
September	0	0	25.05	24.02	0	0	36.3	35.9
October	2.6	0	20.4	17.4	1.8	0	31.4	30.2
Mean	3.34	14.5	22.59	21.31	7.8	21.01	33.88	32.7
Total	23.4	102	158.15	149.18	54.6	147.1	237.2	229

Table 3. Time of harvesting (time from transplanting to full flowering) of two species of *Satureja* (*S. khuzistanica* and *S. reshingeri*) in two locations (Andimeshk and Kashkan) in 2012.

	Andimeshk		Kashkan	
	<i>S. khuzestanica</i>	<i>S. reshingeri</i>	<i>S. khuzestanica</i>	<i>S. reshingeri</i>
Harvesting	16 September	11 October	12 October	27 October
TTF (day)	135	160	160	175

TTF: time from transplanting to full flowering

Table 4. The effects of species and locations on drug yield, essential oil content, essential oil yield, and plant height (mean of two years).

Locations	Drug yield (t ha ⁻¹)			Essential oil content (%)			Essential oil yield(kg ha ⁻¹)			Plant height(cm)		
	S ₁	S ₂	Mean _L	S ₁	S ₂	Mean _L	S ₁	S ₂	Mean _L	S ₁	S ₂	Mean _L
Andimeshk	1.07±0.13c	1.04±0.15c	1.05±0.14b	2.1±0.37b	4.5±0.29a	3.3±1.2a	23.03±5.4c	46.6±8.06b	34.8±13.8b	38±3.3b	36.6±1.7b	37.3±2.7b
Kashkan	2.8±0.31a	2.4±0.37b	2.6±0.4a	1.8±0.2b	4.4±0.27a	3.1±1.3b	57.07±12.04b	107.9±16.1a	82.5±29.5a	43.1±2.5a	38.6±4.5b	40.9±4.2a
Mean _S	1.9±0.93a	1.7±0.75b		1.9±0.3b	4.5±0.2a		40.05±19.6b	77.3±33.7a		40.5±3.9b	37.6±3.5b	
LSD _S			0.15 ^{**}			0.2 ^{**}			6.8 ^{**}			2.2 ^{**}
LSD _L			0.15 ^{**}			0.15 [*]			6.8 ^{**}			2.18 ^{**}
LSD _{L×S}			0.29 ^{**}			0.32 ^{ns}			12.31 ^{**}			3.5 ^{ns}

Drug yield: yield of leaves and flowering shoot; S₁:*S.khuzestanica*; S₂: *S. rechingeri*; S: Species; L: Location; L × S: Location ×Species; NS: not significant;

* p<0.05; ** p<0.01. Values within the same column followed by the same letter are not significantly different (p≤ 0.05).

Table 5. Carvacrol content of two species of *Satureja* (*S. khuzistanica* and *S. reshingeri*) in two regions

Carvacrol content (%)			
Locations	<i>S. khuzistanica</i>	<i>S. reshingeri</i>	Mean _L
Andimeshk	95.96±0.63a	95.67±0.12a	95.81±0.4a
Kashkan	96.24±0.06a	96.77±0.07a	96.5±0.3a
Mean _S	96.1±0.39a	96.22±0.64a	
LSD _L	1.15 ^{ns}		
LSD _S	1.1 ^{ns}		
LSD _{L×S}	0.89 ^{ns}		

L × S: Location ×Species; NS: not significant; * p<0.05; ** p<0.01. Values within the same column followed by the same letter are not significantly different at the P ≤ 0.05 level.

Satureja resingeri

Satureja Khuzestanica