

**VARIATIONS IN ESSENTIAL OIL MAIN COMPONENTS OF NATIVE GROWN *Salvia aramiensis* RECH FIL. GENOTYPES DEPENDING ON YEARS**

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The essential oils and components of sage, which is mostly consumed as a tea and spice plant, have different uses in the field of medicine and cosmetics. The high camphor and thujone contained in many sage species limit the use of the plant as tea and spice in the food sector due to its toxic and carcinogenic effects. In such cases, which directly concern human health, new species and varieties containing low camphor and thujone should be introduced to the market, and the consumer should be provided with safe food. Studies in the *Salvia aramiensis* species revealed that the camphor rate in this plant is very low and there is no thujone. Antakya sage (*Salvia aramiensis* Rech. fil.), which is only found in the flora of Hatay in Türkiye, is important for the herbal tea market due to its chemical content. For this reason, this study was carried out in seventy-nine genotypes growing naturally in different locations for two consecutive years, in order to reveal the variation of essential oil compositions of *S. aramiensis* genotypes. In general terms, it was determined that the ratio of essential oil components of genotypes did not change much over the years. In the study, first- and second-year cineole averages of the genotypes were found as 44.40% and 45.69%, camphor averages 12.74% and 12.58%, -pinene averages 4.70% and 5.07%, borneol averages 6.53% and 6.22%, respectively.

Keywords: Essential oil, camphor, cineole, -pinene, borneol GC-MS, *Salvia aramiensis*

## INTRODUCTION

The return to nature is gradually accelerating in the world and the use of herbal preparations as nutritional supplements, tea and spices is increasing day by day. In this context, one of the most consumed plant species is sage (*Salvia* spp.). It is known that the genus *Salvia*, which can spread in many different ecosystems, has about 1000 species in the world (Kintzios, 2000; Walker and Sytsma, 2007; nce and Karaca, 2015).

Sage has ninety-seven species, 4 subspecies and 8 varieties in Türkiye. Fifty-five of these species are endemic (Hedge, 1982; Davis *et al.*, 1988; pek and Gürbüz, 2010; Vural and Adıgüzel, 1996; Dönmez, 2001; Hamzao lu *et al.*, 2005; Celep and Do an, 2010; Celep *et al.*, 2009; Behçet and Avlamaz, 2009; Kahraman *et al.*, 2009). Various studies have shown that there are around twenty *Salvia* species in the flora of Hatay (Davis *et al.*, 1988; Türkmen and Düzenli, 1998; Düzenli and Çakan, 2001; Celep and Do an, 2010; Ayano lu *et al.*, 2012). Among these species, *S. aramiensis* Rech. Fill. It is included in VU: Vulnerable species group (Ekim *et al.*, 2000; Celep *et al.*, 2010).

Among the sage species, *S. officinalis* is the most used species as tea and spice. 1.8 cineole, camphor, thujone, pinene and borneol, which are generally monoterpene in the essential oil of *Salvia* species used as tea and spice, are the most common components (Lamaison *et al.*, 1991; Cuvelier *et al.*, 1994; Lawrence, 1998). Although each of these components has many uses in the field of medicine and cosmetics, the toxic effects of camphor and thujone components are the most important factors limiting the use of sage as tea and spice. Although the components of essential oils vary depending on the source of the plants, it is generally assumed that thujone and camphor components are common in *S. officinalis* essential oil (Lawrence, 1983; Tucker and Maciarello, 1990; Lawrence, 1998).

*S. aramiensis* is a species that grows only in Hatay in Türkiye, is used as a tea and spice, has a low camphor content and does not contain thujone in some genotypes. Demirci *et al.* (2002), in the study in which they examined the essential oil contents of *S. Aramiensis* samples taken from three different locations of Hatay, they found that the camphor content is between 7.5-10.1%. Karaman *et al.* (2007) found that the camphor ratio in the essential oil of *S. aramiensis* was 2.1%. Askun *et al.* (2010) examined the *S. aramiensis* plant from the Hatay flora in terms of essential oil components and the camphor content was determined as 5.8% in the study. Kelen and Tepe (2008), in their study investigating the chemical contents and antimicrobial properties of *S. aramiensis* essential oil obtained from the flora of Hatay, determined that the essential oil contains 1.8 cineole (46.0%) camphor (8.7%) and no thujone. Ayanoglu *et al.* (2012), in a study of *S. aramiensis* species in Hatay, reported that 1.8 cineole was determined as 34.94%-51.53% and camphor was around 1%, while thujone was not found. As can be seen from these studies, the camphor ratio of *S. aramiensis* essential oil varies between 2.1-11.1%, and the thujone ratio varies between 0-1.1%.

It has the potential to replace *S. officinalis* species, especially in the food sector, by giving priority to the food safety problem, which directly concerns human health, to reduce the damage to biodiversity caused by the trade made only with the collections made from natural flora, and to increase the level of competition by using high quality genotypes in growing these increasingly cultivated plants. A clonal selection study (TÜB TAK, 119O331) conducted based on chemotype for the development of cultivars bearing the same name was conducted for two years in order to monitor the change of essential oil components over the years.

## MATERIALS AND METHODS

The material of the experiment consists of *Salvia aramiensis* plants growing in Hatay natural flora. Alien pollinator, *Salvia aramiensis* Rech. Elephant. is a perennial shrub, upright plant whose trunk can grow up to 1.3 m. The body is quadrangular, with dense eglandular-tomentose (glandless-soft-hairy) and sessile glandular (glandular) hairs below, slightly pilose (soft-hairy) or subglabrous (almost glabrous) above. Leaves simple, narrowly oblong, elliptic to obovate (ellipse to obovate), 1.4-6 x 0.4-3 cm, crenulate (small-carved); Petiole 0.5-3 cm. Verticillasters have 2-10 flowers and are concentrated at the tip. Bract ovate, 5-8 x 4-8 mm, shed. Bracteoles are present. Petioles 1.5-4 mm, sepals ± tubular, 9-15 mm, in fruit elongated to 10-17 mm, violet colored, striped, short-stalked glandular hairy, few eglandular pilose hairs sometimes present or absent, upper lip triangular and suddenly narrowed and elongated at the top. Petals mauve to pink, ca. 22-30 mm long; corolla tube straight, 15-20 mm; upper lip is straight. Stamens are Type A. Anther about 3 mm, inferior theca about 2 mm, filaments about 4-5 mm. Flowering and habitat: April-May. It is found at 150-600 m in red pine forest clearing and rocky slopes. This species, which spreads only in the Amanos Mountains in Türkiye, is consumed as tea by the people in the region (Do an *et al.* 2008).

In the study, essential oil components of seventy-nine genotypes grown in 16 different locations in Hatay region were investigated for two years. Locations and their code numbers are 1 (Alan), 21 (Belen), 24 (Çabala), 31 (Fırız), 5 (Gökdere), 27

(Gümürük), 25 (Güveççi), 17 (Harlısu), 11 (Haymaseki), 13 (Höyük), 14 (kinehir), 22 (Kömürçukuru), 4 (Kuyuluk), 2 (Kuzuculu), 32 (Üçgedik) and 26 (Yukarı pulluyazı).

The study was carried out in the autumn of 2019 and 2020. Plants in predetermined locations where *S. aramiensis* grows naturally are marked with weatherproof labels. Coordinates and altitudes of genotypes were determined. Leaf samples were taken from these marked plants for essential oil analysis. The reason essential oil analyzes are carried out in autumn is that even though the plants are in different ecologies, they experience the same physiological period, that is, the period when the plants go to rest. Thus, the analyzes made in this period when the plants are more stable reveal the situation that they are more comparable in terms of chemical properties.

Essential oils were obtained from 50 g dry leaf samples of each plant by hydrodistillation method for 3 hours in a Clevenger apparatus. While determining the essential oil components (%), essential oil samples of each plant were analyzed in GC-MS (Gas Chromatography-Mass Spectrometry) device. Analyzes were performed on a Thermo Scientific ISQ Single Quadrupole model gas chromatograph. Essential oil samples (5 µl oil diluted in 2 ml n-hexane) were determined using the TG-Wax MS model, (5% Phenyl Polysilphenylene-siloxane, 0.25 mm, 60 m long, 0.25 µm film thickness) column. The ionization energy was set to 70 eV and the mass range m/z 1.2-1200 amu. Scan mode was used for data collection. MS transfer line temperature was 250 °C, MS ionization temperature was 220 °C, column temperature was 50 °C at the beginning and increased to 220 °C with a temperature increase rate of 3 °C/min. The structure of each compound was defined with the Xcalibur program using mass spectra (Wiley 9). In addition, components have been validated using standards.

## RESULTS AND DISCUSSION

The main components (cineol, camphor,  $\alpha$ -pinene and borneol) ratios of essential oils obtained from the dry leaves of each *S. aramiensis* genotype in two consecutive studies were determined and shown in Table 1. As seen in the Table 1, there are quite large differences between genotypes in terms of the amounts of each major component. As can be seen in Table 1, there are quite enormous differences between genotypes in terms of the amounts of each major component. The cineole ratios of the genotypes varied between 15.93-76.30% in the first year of the trial and between 11.23-76.89% in the second year. According to genotypes, camphor rates were between 0,00-38,64% in the first year and between 0,009-36,64% in the second year. There were also great variations in  $\beta$ -pinene and borneol ratios between genotypes, and  $\alpha$ -pinene ratios ranged from 0,00-8,50% in the first year to 0,00-12,60% in the second year, while borneol ratios in the first year 0,00-20,07% and the second year varied between 0,00-24,09%. In some genotypes, camphor,  $\alpha$ -pinene and borneol were found to be negligible. This variation between genotypes reveals the importance of selection studies in the development of sage varieties required for the herbal tea industry.

Despite this variation between genotypes, there were no significant differences between the ratios of essential oil components of genotypes by years. It is understood that the rate of cineole increased by 2.91% compared to the previous year when considering the average cineole contents of the genotypes (Table 1). In other words, genotypes with high cineole content were high cineole content in the other year, while the cineole ratios of genotypes with low cineole content were found to be low. This is important in terms of pre-selection according to chemotypes. Considering the average camphor content of the genotypes, it was determined that the camphor rate changed by -

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1,26% compared to the previous year. In the mean  $\alpha$ -pinene values, there was a 7.87% difference over the years, while the average borneol ratios were found to vary by -4.74%. In the experiment, the standard deviation values of the components of the genotypes were 17.26 and 17.66 for cineole; 9.57 and 9.37 for camphor; 1.78 and 2.04 for  $\alpha$ -pinene; 5.05 and 5.29 for borneol, respectively.

Table 1. The main components of *S. aramiensis* Rech. Fil. genotypes for two years (%) and percentage of changes

Genotypes	<i>1.8 cineol</i>		<i>Camphor</i>		$\alpha$ - <i>pinene</i>		<i>Borneol</i>	
	2019	2020	2019	2020	2019	2020	2019	2020
1/3	30.96	30.16	18.13	18.52	6.58	12.60	0.00	3.41
1/6	30.67	42.28	18.85	23.04	6.74	11.07	4.40	4.74
1/7	15.93	76.89	19.34	0.35	7.91	8.27	10.09	0.27
1/12	28.06	25.99	11.70	24.12	7.08	9.34	13.95	7.94
1/13	26.68	23.45	30.42	36.64	4.95	4.75	5.90	5.74
1/15	32.64	34.67	6.66	6.73	7.65	6.57	8.20	8.41
21/6	26.87	69.53	0.00	1.73	0.00	2.30	5.30	1.10
21/8	58.61	54.79	11.09	8.47	3.69	4.81	2.42	3.17
21/10	47.97	52.51	14.56	13.43	5.22	4.87	6.86	5.94
24/7	16.38	16.9	20.65	19.13	6.19	8.18	19.74	7.49
24/10	40.94	37.27	15.09	13.37	5.08	0.39	5.13	6.42
31/4	41.52	45.66	4.50	3.95	4.34	4.87	5.12	9.04
31/12	20.12	18.35	22.09	25.34	7.45	6.14	10.18	7.83
31/13	22.85	27.43	9.69	18.16	5.35	3.06	11.78	11.55
31/14	6.88	33.52	3.87	24.71	0.00	4.37	3.71	15.80
5/5	31.36	36.42	22.86	15.70	6.20	5.30	8.51	9.75
5/7	38.65	53.79	13.66	17.93	6.27	3.48	6.52	6.02
5/8	36.51	34.62	12.33	15.41	4.52	3.93	18.67	19.81
5/13	23.39	30.66	26.09	14.32	5.55	7.08	13.80	13.67
5/17	24.37	19.02	20.73	27.95	0.00	6.23	9.31	13.20
5/18	29.66	23.78	23.67	34.05	5.66	5.90	14.48	12.31
5/19	27.49	16.87	19.18	24.34	8.50	7.33	9.53	13.39
5/20	33.90	38.03	23.97	12.07	5.16	6.57	4.83	6.73
27/4	39.57	42.52	29.08	12.48	3.53	6.85	4.19	6.56
27/20	26.41	11.23	23.57	21.96	5.51	8.31	8.59	16.71
27/23	23.90	13.71	38.52	27.38	3.47	5.73	6.24	3.70
25/3	38.50	35.81	9.52	12.05	6.22	6.36	15.00	14.86
25/4	50.12	39.58	8.34	11.03	4.30	5.58	6.32	7.28
25/6	48.37	50.20	8.51	5.84	3.25	4.19	0.00	2.67
25/8	58.67	39.58	5.19	14.75	4.63	4.15	1.81	3.41
25/16	43.86	28.05	18.91	17.77	5.80	7.01	2.75	3.77
25/20	16.41	26.33	22.78	22.44	7.47	6.65	6.47	18.62
17/1	22.85	37.94	21.16	14.15	5.44	5.17	16.00	16.76
17/5	21.66	64.35	25.16	10.05	6.20	5.95	17.60	14.06
17/8	43.99	67.83	13.76	0.85	4.97	3.34	9.23	5.39
17/9	64.73	54.77	0.55	10.68	3.13	3.66	0.75	0.32
17/10	56.86	57.99	9.80	8.28	4.74	5.47	4.51	5.16
17/12	54.77	66.27	8.52	10.16	5.02	4.35	6.44	6.99
17/19	52.37	50.36	13.82	16.02	5.62	4.51	3.94	1.92
17/20	45.25	47.37	15.21	21.37	4.98	3.88	7.81	6.23
11/8	45.93	55.16	20.53	13.62	2.83	3.12	8.69	4.26
11/19	58.03	71.68	12.76	3.26	2.83	4.14	5.76	5.03
13/1	70.09	69.95	4.04	1.03	3.50	4.07	2.21	1.54
13/4	57.59	75.11	0.96	0.09	4.91	3.73	5.24	1.92
13/5	73.89	71.00	0.44	2.06	3.09	2.67	0.84	0.15
13/6	51.21	75.20	5.78	0.78	4.56	4.05	9.77	1.86

Genotypes	<i>1.8 cineol</i>		<i>Camphor</i>		<i>-pinene</i>		<i>Borneol</i>	
	2019	2020	2019	2020	2019	2020	2019	2020
13/8	68.60	15.29	1.12	3.54	4.01	2.62	0.94	0.39
13/9	76.30	73.20	1.48	0.95	3.10	3.56	1.13	0.00
13/10	56.36	54.25	10.45	5.24	2.51	3.55	8.70	0.34
13/11	69.37	56.74	3.48	2.40	2.93	6.32	3.27	9.50
13/13	54.71	23.74	2.87	16.34	5.80	4.57	2.02	1.33
13/14	75.55	57.21	3.11	4.17	2.56	8.29	0.00	15.09
14/ 3	56.97	58.97	4.38	1.68	5.33	5.09	0.00	0.00
14/10	59.80	60.71	1.48	2.61	5.37	5.29	0.00	0.00
14/12	50.91	64.98	2.93	0.81	4.69	4.85	7.45	5.05
14/13	73.21	61.66	4.29	1.38	3.01	5.21	3.56	5.21
14/16	73.11	53.01	1.36	8.51	2.81	5.14	1.37	1.18
14/17	60.08	49.14	7.15	10.34	4.42	5.34	5.83	6.13
14/19	50.47	18.47	9.32	36.07	5.49	6.73	6.45	5.26
22/2	20.92	55.73	30.55	0.57	8.21	6.73	6.61	7.10
22/3	74.13	44.61	0.84	8.40	3.36	3.92	0.54	0.52
22/4	14.46	70.40	2.01	1.64	5.78	3.38	16.68	6.57
22/7	59.27	63.28	4.50	6.50	4.23	3.81	0.95	0.46
22/11	62.31	59.52	5.87	2.32	3.97	2.95	1.36	1.28
22/14	61.65	40.12	5.03	16.60	4.00	3.36	1.43	1.11
4/2	59.16	55.22	4.76	14.39	4.21	5.46	3.44	5.24
4/4	49.28	35.74	12.95	15.91	5.53	3.66	5.04	5.01
4/5	38.39	18.22	17.17	18.25	5.78	6.63	6.69	5.94
4/22	16.66	41.84	20.83	14.67	8.09	7.54	20.07	24.09
2/11	38.89	32.34	13.82	23.23	7.04	6.34	7.07	6.51
2/16	32.35	28.46	23.48	24.01	4.40	3.63	13.27	9.27
2/20	30.27	50.33	38.67	8.09	1.77	3.87	11.73	9.03
32/6	51.73	65.66	4.57	1.54	4.83	5.05	5.58	7.13
32/8	58.68	60.25	2.03	2.55	3.41	4.02	2.91	1.66
32/11	51.31	34.55	3.59	31.91	3.99	4.75	5.22	2.47
26/1	41.11	63.94	27.69	16.84	2.65	2.64	0.00	1.75
26/2	22.94	51.62	16.68	8.38	5.55	3.99	7.59	6.78
26/13	60.32	55.96	8.72	8.18	3.13	5.50	1.40	7.19
26/15	43.14	39.65	22.98	17.88	3.24	0.00	9.37	0.00
Min.	15.93	11.23	0.00	0.09	0.00	0.00	0.00	0.00
Max.	76.30	76.89	38.67	36.64	8.50	12.60	20.07	24.09
Mean	44.40	45.69	12.74	12.58	4.70	5.07	6.53	6.22
Percentage of change	2.91%		-1.26%		7.87%		-4.75%	
Standard deviation	17.26	17.66	9.57	9.37	1.78	2.04	5.05	5.29

## CONCLUSIONS

As a result of this study, which was carried out on naturally grown plants for two consecutive years, it was determined that *S. aramiensis* did not contain thujone, which is especially harmful to human health, and its camphor content was low. In addition, it was observed that there was not much change in the ratios of essential oil components in the analysis made on individual plants. These results revealed that promising genotypes for herbal tea production can be obtained by selection studies within *S. aramiensis* species.

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