Morphogenetic Variation for Essential Oil Content and Composition of Sage (*Salvia officinalis* L.) In Çukurova Condition

Tuncay ÇALIŞKAN[a], Hasan MARAL[b*], Cemre PALA[a], Ebru KAFKAS[c], Saliha KIRICI[a]

[a]Department of Field Crops, Faculty of Agriculture, University of Cukurova, Turkey
[b]Karamanoğlu Mehmetbey University, Ermenek Vocational School, Karaman, Turkey
[c]Department of Horticulture , Faculty of Agriculture, University of Cukurova, Turkey
[*]Corresponding author e*mail address*: hasmaral@kmu.edu.tr
Accepted: Marsh 11th, 2019

Abstract

This study was carried out in 2016 in the Research Area of the Cukurova University Faculty of Agriculture Field Crops Research to determine the morphogenetic variation in yield and essential oil content and composition in *Salvia officinalis* L... Field trial was arranged randomized complete block design, with three replications. Seed of sage was sowing at December 17, 2015 in the greenhouse. Seedling was transferred to field at March 31, 2016. The plants were harvested on November 29, 2016. After harvest, imminently all plant material were separated into three part, lower (1/3), middle (1/3) and upper (1/3) for morphogenetic variation and fresh herb weight were determined for each part. The following results were obtained: plant height (37.28 – 52.84 cm), fresh herbage yield (608-822 kg da−1), drug herbage yield (236-259 kg da−1) and essential oil content (1.65 – 2.32 %). The highest essential oil content was obtained as 2.32 % from upper part of plant. The lowest one (1.65%) was obtained from lower parts.

Keywords: Sage; *Salvia officinalis* L.; morphogenetic variation; essential oil

Introduction:

Salvia, belongs to the family *Lamiaceae (Labiatae)*, is found in both halves of the world, especially in the tropics and subtropics, with over 700 species distributed in the Mediterranean and Central Europe (Karaaslan, 1994) Of these species, 92 are naturally distributed in our country, of which 44 are endemic. Turkey is ranked 13th in the world in terms of species richness of Salvia species (Arslan et al, 1995). Medicinal sage (Salvia officinalis L.) is a typical Mediterranean plant that contains essential. The sage (Salvia officinalis L.), which has been formally approved for medicinal use in Europe, is not found naturally in Turkey. Sage naturally grows on rocky and barren slopes in dwarf bushes in the Adriatic Sea and Dalmatia (Karaaslan, 1994; Koç, 2006).

As a drug leaves of Sage (*Folia salviae*) and essential oil from leaves (*Oleum salviae*) are used (Baytop, 1963). Sage leaves contain 0.5-2.5% essential oil (Ekren et al, 2007). Codex requires at least 1.5% of essential oil (Ekren et al, 2007). The medically acceptable fat content is α, β Thujonee, 1, 8 Cineol, Camphor, Borneol and Bornylacetate. Some essential oils have been reported to contain Thymol and Carvacrol (Zeybek and Zeybek, 2002). 30-50% thujonee, 15% cineol and 10% borneol have been reported in sage essential oil (Baytop, 1999; Ekren et al, 2007).

The main component in the essential oil of *salvia officinalis* is thujone. Thujone It is an additive for drugs made for throat infections, inflammation of the teeth and mouth sores due to its strong antiseptic and antibiotic effect (Baydar, 2005). Thujone should not be taken with an overdose due to its toxic and carcinogenic effect (Zeybek and Zeybek, 2002).

Essential oils, are the genetic structure of the plant, climate, environmental factors, cultural practices, the region where the plant is grown, the different parts of the plant (morphogenetic variability), period of development of the plant (Ontogenetic variability) and temperature changes during the day (diurnal variability) varies depending on many factors. Therefore, the exchange of substances that are synthesized by medicinal plants has been and continues to be the subject of research in many economically important plants (Kırıcı et al, 1996).

This study was conducted to determine the variation of yield and essential oil content and composition of different parts of *Salvia officinalis* (*Lamiaceae*) plant.

Materials and methods:

The experiment was carried out in 2016 at Çukurova University Field Crops Department Research Area. The soil of the study area is almost flat and flat near the topography and brought to the young alluvial soil of the creek along the Seyhan River. There are gravel deposits at various depths. The colors range from brown to pale brown. The amount of lime in the whole profile is very high and the amount of organic matter is low (Özbek et al, 1974; Maral et al, 2018). The values of precipitation, high temperature, low temperature and average temperature, relative humidity and wind speed 2016 of the trial site are shown in Table 1, the soil properties of the experiment area are given in Table 2.

The seeds of the Sage (Salvia officinalis) obtained from the Faculty of Agriculture at Ege University were planted on the hill on December 17, 2015 and the originals were planted on 31.03.2016.

Field experiment, Çukurova University Faculty of Agriculture Field crops Research field, seedlings were produced with 3 repetitions according to the Design of Random Blocks. The parcels size was 2.1m × 3.15m (6.3m2), 70 cm between rows and 50 cm above the row, each plot consisting of 3 rows and 15 plants in each row. Soil moisture was kept high until the plants were rooted. Because of the high temperature and drought in the summer months, the watering has been taken care of and irrigation has been done for as long as necessary. In order to determine the morphogenetic variability in the experiment, the harvested plants at 4-5 cm height from the soil surface on 29.11.2016 were dried in the shade and separated into 3 different parts as tip, middle and bottom.

Essential Oil Isolation

Air- dried plant material (25 g) was hydrodistilled for 3 h using a Clevenger type apparatus. The resulting oils were kept in sealed vials at 4 °C until analysis.

Gas chromatography mass spectrometry (GC/MS)

GC/MS analysis of the oils was carried out on a 7000 Series Triple Quad GC/MS apparatus [Agilent], equipped with split-split less injector and automatic liquid sampler, attachedto HP-5MS capiller column (30 m x 0.25 mm x 0.25 µm film thickness, %5 phenylmethylpolysiloxane). The carrier gas flow rate (He) was 1 ml/min, split ratio 1:30, oven temperature program was started at 50°C (held for 3 min.) while column temperature was linearly programmed from 50-240°C (at rate of 3°/min). The essential oil was diluted with dichloromethan; split ratio was 1:25, injected 1 µl. The constituents were identified by comparison of their mass spectrato those from Agilent Flavor libraries. The variance analysis of data was analyzed with MSTAT-C software by using LSD's test.

Results and discussion

The effect of essential oil on morphogenetic variance is important at 5% level (Table 3). The content of essential oil in the study was found between 1.65-2.32%. The highest essential oil content was obtained from the upper leaves. Kırıcı et al. (1996), investigated the effects of essential oil components on drug yield and essential oil content, according to different planting times in the sage (*Salvia officinalis* L.) under the Cukurova Region conditions,1.73-4.80% of essential oil rate were determined.

In previous studies, it was reported that the volatile oil ratio of sage ranged between 0.85-2.13% (Ceylan et al, 1979), 1.5-2.5% (Bernath et al, 1991), 0.83-1.63% [Yenikalaycı and Özgüven, 2001) and 1-3% and 1.5-1.7 (Otepka, 2007). According to the German pharmacopoeia (DAB), it is

desirable to have an essential oil content of 1-2.5% (average 1.5%). In our study, we have found that the essential oil ratios are in accordance with the literature values.

The composition of essential oil is indicated in Table 4 with according to the top, middle and lower leaves. Several major constituents have been observed in the chemical composition of the analysed essential oils: α-pinene 6.148-8.434 %, β-pinene 4.436-5.992%, thujone 11.77-24.91%, camphor 16.815-19.343%, eucalyptol 22.253-33.731% , and camphene 6.353-6.938%.

According to the German pharmacopoeia (DAB), the composition of essentialoil of sage should be 30-60% thujone, 20% monoterpenes (cineole, borynl acetate, champor, β-pinene). In a study conducted by Miladinovic and Miladinovic, (2000), *salvia officinalis* L. on the ratio of essential oil and composition on the effect of ecological factors such as climate, soil, and the main constituents of essential oil are α-thujone (24.88%), camphor (16.03%) and 1-8 cineole (9.79%). Salameh and Dordevic, (2000), found 29 compounds in the essential oil composition of *Salvia officinalis* Jordan. The main constituents were α-thujone (29.9%), β-thujone (13.68%), camphor (15.74%) and 1-8 cineole (12.31%). Sagareishvili et al, (2000), identified 11 different components in *Salvia officinalis* and identified α Thujone (31.56%), β Thujone (17.55%), Camphor (16.48%) and 1, 8 Cineol (17.53%) as main components. According to Kırıcı et al [10], the main constituents of essential oil of sage (*salvia officinalis*) were determined as camphor (16.69%), cineol (12.67%) and thujone (10.69%). Basyigit and Baydar (2017), Salvia officinalis essential oil ratio between 0.83-3.33% showed that the most important components of volatile oil composition 1.8 cineol (11.93% - 31.87%), α - tuyon (15.72 -26.26%), β - tuyon (4.51- 27.67%) and kamfor (3.65- 23.02%).

When the changes in the values of some important essential oil components are examined; it was determined that β-Pinene, Eucalyptol and Camphor were higher in the upper leaves, α-Pinene, α-Thujone and β-Thujone were higher in the lower leaves and Camphene was not effective in percentage change of the plant part (Table 4).

Conclusions:

The aim of this study was to determine the morphogenetic variability in sage genotypes. In order to determine the morphogenetic variability, the plant is divided into sub (1/3), medium (1/3), upper (1/3) leaves, stalks and flowers in each developmental period. According to the results obtained, the highest volatile oil content was obtained from the end parts of the plant. The lowest content was obtained from the plant material obtained from the lower part. Thujone, Camphor, Eucalyptol, Camphene, β-pinene and α-pinene are pharmacologically active components and have been found in every part of the plant. The results of the analysis revealed that the α-Pinene, Camphene, α-Thujone, β-Thujone ratios were higher in the lower parts, while the ratio of β-Pinene and Eucalyptol was higher in the lower parts and the Camphor ratio was higher in the middle.

Table 1. Adana Province Climate Data for 2016.

Months	Average monthly temperature(°C)			Monthly relative humidity Avg. (%)	Monthly Total RainfallAvg. (mm)
	Avg.	Max.	Min.		
March	13.9	19.8	7.9	61.1	2.3
April	18.1	24.1	12.1	72.0	1.4
May	22.7	29.4	16.3	72.3	1.9
June	25.3	31.4	19.1	65.7	0.0
July	28.2	33.9	22.2	65.2	0.0
August	28.6	35.1	22.2	69.0	0.0
September	25.3	32.0	28.5	63.1	0.0
October	22.2	29.6	16.4	54.1	40.2
November	15.9	22.0	11.8	66.3	119.4

Source: State Meteorological Service, Adana 2016.

Table 2. Soil Properties of Trial Area

Texture				Ph (1:2,5)	Salt (mmhos/cm)	Lime (%)	P_2O_5 (kg/da)	K_2o (kg/da)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)
Sand(%)	Plate (%)	Clay (%)	Structure									
46,0	25,5	28,5	CL	7,56	0,17	63,1	5,9	91,5	0,6	3,3	2,2	0,8

Source: Cukurova University Faculty of Agriculture Department of Soil Analysis Results 2016

Table 3. The Mean Essential Oil (%) Content Obtained in Sage (*Salvia officinalis* L.) Plant in Cukurova Conditions

	Essential Oil(% DM)
End Leaf	2.32**a**
Middle Leaf	1.78**b**
Bottom	1.65**b**
F-test	*
LSD(5 %)	0.0173
CV (%)	8.76

*Essential oil values P≤0.05: Statistical significance within error limits.

Table 4.Chemical composition of different plant parts in *Salvia officinalis*

RT	Compounds	Plant parts		
		Bottom	**Middle**	**Top**
4.7273	cis-6-Nonen-1-ol	0.243	0.186	0.170
6.8083	delta-3-carene	0.271	0.228	0.231
7.0274	α-phellandrene	0.416	0.324	0.320
7.2423	**α-Pinene**	**8.434**	**6.484**	**6.148**
7.7636	**Camphene**	**6.938**	**6.466**	**6.353**
8.7635	Sabinene	0.181	0.139	0.118
8.8432	**β-Pinene**	**4.436**	**5.358**	**5.992**
9.5403	Myrcene	1.560	1.894	2.495
10.5261	alpha-terpinene	0.241	0.181	0.205
10.8770	p-Cymene	0.663	0.524	0.352
11.1264	**Eucalyptol**	**22.253**	**29.217**	**33.731**
12.3799	gamma-Terpinene	0.519	0.392	0.458
13.6881	Terpinolene	0.251	0.262	0.318
14.3067	Linalol	0.119	0.197	0.252
14.4426	**α-Thujonee**	**16.433**	**10.694**	**7.870**
14.9294	**β-Thujonee**	**8.480**	**6.932**	**3.900**
16.1139	**Camphor**	**16.815**	**19.343**	**19.156**
17.0854	Isoborneol	2.476	2.291	2.304
17.6510	terpinene-4-ol	0.405	0.344	0.353
18.2740	α-Terpineol	0.876	1.807	2.507
28.0633	beta-Caryophyllene	1.527	1.542	2.022
29.4386	Bisabolene	1.606	1.360	1.089
34.4451	Farnesol (Z,E-)	0.678	0.505	0.442
34.7879	Guaiylacetate	1.631	0.834	0.548
	Sum of contents %	97.608	97.685	97.503

References

Arslan, N., Gürbüz, B. and Yılmaz, G., 1995. Adaçayı (*Salviaofficinalis* L.)'ında Tohum Tutma Oranı ve İndolButirikAsitin (IBA) Gövde Çeliklerinin Köklenmesine Etkileri Üzerinde Araştırmalar. Tr. J. of AgricultureandForestry, TÜBİTAK, 19: 83-87.

Başyiğit, M. and Baydar, H., 2017, The Effect of Different Harvest Times on Essential Oil and Phenolic Compounds and Antioxidant Activity in Medical Sage (Salvia officinalis L.), Süleyman Demirel University, Institute of Science and Technology, 21 (1), 131 -137.

Baydar, H., 2005. Tıbbi Aromatik ve Keyf Bitkileri Bilim ve Teknolojisi, Süleyman Demirel Üniv. Ziraat Fak., SDÜ Basımevi, No:51, Isparta, S.125.

Bayram, E., Sönmez, Ç., 2006. Adaçayı Yetiştiriciliği. E.Ü. Tar. Uyg. veAraş. Merkezi Yayım Bülteni. No:48. ISSN 1300-3518, Bornova/İzmir.

Baytop, T., 1963. Türkiye'nin Tıbbi ve Zehirli Bitkileri, İ.Ü. Yayınları, İstanbul No:1039, Tıp Fakültesi, No:59, S.351.

Baytop, T. 1999. Türkiye'de Bitkiler ile Tedavi. Nobel Tıp Kitabevleri. İstanbul.

Bernàth, J., B. Danos, E. Hethelyi. 1991. Variation of EssentialOilSpectrum of Salvia species Affected by Environment. In: Herba Hungarica. Vol. 30. No. 1- 2. pp. 35-48.

Ceylan, A., Yurtseven, M. ve Ozansoy, Y. 1979. *Salviaofficinalis* L.'inagronomik ve teknolojik özelliklerine azotlu gübrelemenin etkisi üzerinde araştırma. Ege Üniversitesi Ziraat Fakültesi Dergisi, 16:3, 83-96.

Ekren, S., Sönmez, Ç., Sancaktaroğlu, S., Bayram, E. 2007. Ege Üniv. Ziraat Fak. Derg., 44 (1): 55-70 ISSN 1018-8851

Habàn, P. Otepka. 2007. OrganicallyCultivatedMedicinalPlantsandTheirQuality Analysis. Proceeding of conference, OrganicFarming. 6-7 February.

Heeger, E.F. 1956. HandbuchdesArzneiundGewüzPflanzenbauesDentschen Ban Everlag.

Karaaslan, D. 1994. Salviapopulasyonlarında farklı azot uygulamalarında drog verimi ve kemotaksonomikarastırmalar. Çukurova Üniversitesi Fen Bilimleri Enstitüsü Tarla Bitkileri Anabilim Dalı, Yüksek Lisans Tezi, 58 s.

Kırıcı, S., Özgüven, M., Yenikalaycı, A., 1996. Çukurova Bölgesi'nde Tıbbi Adaçayı (*SalviaOfficinalis* L.) Üzerine Araştırmalar.Workshop Tıbbi Ve Aromatik Bitkiler, 25-26 Mayıs, Bildiri özetleri, Bornova-İzmir.39-40.

Maral, H., Çalışkan, T., Yıldırım, C.D, Akdoğan M.E, Kafkas, N.E, Kırıcı, S. 2018. The Influence of Harvesting Period and Cutting Hour on Yield and Quality in Thyme (Thymus vulgaris L.) in ÇukurovaCondition. ARCTIC Journal, 71(11).

Miladinovic, D.,Lj. Miladinovic, 2000. Animicrobial Activity of Essentialoil of SagefromSerbia. Physics, chemistryandTechnology. Vol. 2, n 2, 2000, pp. 97-100.

Oktay Koç, P. 2006. Azot ve kükürdün adaçayı (*Salviaofficinalis* L.) bitkisinin herba verimi ve bazı kalite parametreleri üzerine etkisi. Ankara Üniversitesi Fen Bilimleri Enstitüsü Yüksek Lisans Tezi, 48 s.

Özbek, H., Dinç, U., Kapur, S., 1974. Çukurova Üniversitesi Yerleşim Sahası Topraklarının Detaylı Temel Etüt ve Haritası, Ziraat Fakültesi Yayınları Bilimsel Araştırma ve İncelemeler.

Sagareishvili, T. G., B.L. Grigolava, N. E. Gelashvili, E.P. Kemertelidze. 2000. Composition of Essential Oil from *Salvia officinalis* L. Cultivated in Georgia. Chemistry of Natural Compounds. Vol: 36. N: 4. Pages: 360-361. New York.

Salameh A., S. Dordevic, 2000. TheInvestigation of theQuality of Sage (*Salviaofficinalis* L.) from Jordan, UDC 582.824, University of NišTheScientificJournalFactaUnversitatis, Series: WorkingandLivingEnvironmentalProtection Vol.1, No 5, pp.103-108.

Yenikalaycı, A., M., Özgüven, 2001. Çukurova Bölgesi'nde Farklı Ekolojilerde Tıbbi Adaçayı (*Salviaofficinalis* L.)'nın Verim ve Verim Komponentlerinin Araştırılması. Türkiye 4. Tarla Bitkileri Kongresi. 17-21 Eylül, Tekirdağ

Zeybek, U. ve Zeybek, N., 2002. Farmasötik Botanik (Kapalı Tohumlu Bitkiler (Angiospermae) Sistematiği ve Önemli Maddeleri), E.Ü. Eczacılık Fakültesi Yayınları, No:3, Bornova/İzmir, 380s.