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Determination of Qualitative and Quantitative Features of Sage (*Salvia officinalis* L.) Essential Oils

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Abstract: This study was carried out in the experimental area of Field Crops Department, Faculty of Agriculture, University of Cukurova, in 1992, to find out the effects of different N doses (0, 5, 10 and 15 kg N/da) on essential oil and quality of sage (*Salvia officinalis* L.), grown in Cukurova region. As a result of qualitative analysis carried out by using TASS-OVEN thin layer chromotography (TLC), it was observed that essential oils belonging to plants with varied morphological features possessed the same components. In addition, through GC Chromotography analysis, the rates of cineol, bornylasetat, thujon, campher and borneol, which are important components of essential oils, were determined. And it was observed that these components were considerably affected at different N dose applications and had also negative effects on the thujon content, which has an important place in industrial usage.

Key words: Sage, essential oil, TLC, nitrogen fertilization

Introduction

Sage is used in pharmacology, food, beverages, spirits and cosmetics and in the production of relaxing and curiative herbal tea. The introduction of new utilization areas in the industrially developed countries and the advances in the pharmacology have increased the need of herbal raw material 15 to 20 times and have led the prices to increase as well (Ceylan, 1983).

The sage is not cultured in Turkey. The *Salvia* species, which is consumed at a great amount particularly in the Aegean Region as tea and which is collected from the flora and exported is *Salvia triloba.* The collection of tons of this species every year has badly destroyed the present flora, causing the extinction of that plant. Furthermore, there is not wide need for *Salvia triloba* in foreign countries; in fact, it is only used to increase the amount of *Salvia officinalis* in drug sales.

Salvia species which are known with their curiative characteristics have had an important role in human health since the ancient times. Upon the development of information and new techniques in volatile oil chemistry and cosmetics, various elements constituting the core of the oil have been isolated and the plant's utilization areas have been widened by recognizing the characteristics of the plant (Dogan, 1972).

Salvia species are the plants grown in the Mediterranean Region and have been known to provide drugs since older times. The importance of *Salvia* species comes from the substances found in their volatile oil which have a strong antiseptic effect, that is monoterpens and their oxygen derivatives (Tanker *et al*., 1976).

Salvia officinalis is used in the preparation of 12 medicines in the form of folium *Salvia* and of 15 medicines in the form of oleum *Salvia* in Western Germany (Sezik *et al*., 1982).

This research was carried out to investigate the effect of different nitrogen applications on the drug efficiency, volatile oil amount and quality of sage, and to establish whether the morphological characteristics of the plants in the population are dependent on different chemotypes or not.

It is reported that the rate of volatile oil in the dry leaves of sage is between 0.75 to 2.04%, in the stem varies between 0.15 to 0.60% and that the volatile oil contains 42.5% thujon, 14% cineol (Ceylan, 1976).

In a study conducted under the ecological conditions of Bornova, it was found that the rate of volatile oil in the sage was between 0.75 to 2.50%, that nitrogen fertilization had a great effect on the composition of the volatile oil, and that, at the dose of 0, 5 and 10 kg N/da, they obtained 1.70, 1.40 and 1.42% of volatile oil, respectively at the first harvest, and 2.50, 2.48 and 2.24% of volatile oil in the second harvest, They also reported that they obtained 13.15, 14, 15, 12.19% of cineol, 47.95, 45.48, 43.54% of thujon, 21.37, 22.58, 25.46% of campher, 3.39, 3.60, 3.84% of borneol and 1.68, 1.73, 1.73% bornylasetate components Ceylan *et al*. (1979).

It is stated that the rate of volatile oil in the leaves of the sage is between 1.0 to 2.5% and that volatile oil contains 30 to 50 thujon, 15 cineol and 10% borneol (Baytop, 1984). In another study, the volatile oil of the sage was reported to contain 35 to 60% thujon, 15% 1.8cineol, 5 to 10% borneol and 3 to 9% bornylasetate.

According to Bayrak and Akgul (1987), the volatile.oil sage contained 35 to 60% thujon, 15% 1.8-cineol, 5 to 10% borneol and 3 to 9% bornylasetate. In a study carried out on sage, it was reported that volatile oil was obtained at a rate of 1.40 to 1.68%, borneol at a rate of 0.10 to 9.33%, 2.17 to 34.00%, 1.8-cineol, 7.91 to 76.69% thujon and 5.25 to 19.80% campher (Bezzi *et al*., 1992). In addition, sage was determined to contain 6 to 24% 1.8-cineol, 14 to 35% thujon and 2 to 19% campher (Laenger *et al*., 1992). It was also identified, in a study performed in Hungary, that sage contained 6.1 to 14.8% 1.8-cineol, 16.2 to 35.5% thujon, 6.0 to 13.5% campher and 3.6 to 17.7% borneol (Mathe *et al*., 1992).

Materials and Methods

Material: Sage (*Salvia officinalis* L.) provided from Borntrager seed firm located in Germany was used in the research.

Method: The research was carried out in the research and application area of Cukurova University Faculty of

Table 1: Some morfological features of sage the used in the research

Flower cluster		Sepal	Leaf		Petal	Leaf	
Colour	Height (cm)	Colour	Largeness	Height (cm)	Colour	Largeness	Heights (cm)
Light lilac colored	17.5	Light lilac colored	Big	1.26	Light lilac colored	Big	1.75
Purple	17.5	Purple	Small	1.06	Purple	Small	1.50
Purple pink	13.5	Purple pink	Big	1.23	Purple pink	Big	2.33
Pink	17.0	Pink	Big	0.93	Pink	Small	1.23

Table 2: hRf values of volatile oil standarts and obtained colour changes in the sage

Standards	hRf	Indicator	Colour
1.8-cineol	52.9	etanolic H_2SO_4+etanolic vanillin	Gray blue
bornylasetat	70.5	etanolic H_2SO_4+etanolic vanillin	Navy blue
campher	32.3	etanolic H_2SO_4+etanolic vanillin	Gray
borneol	35.2	etanolic H_2SO_4+etanolic vanillin	Light blue
thujon	45.8	etanolic H_2SO_4+etanolic vanillin	Dark blue

Table 3: The components of the sage volatile oil obtained from different nitrogen applications

Nitrogen fertilizer (kg/de)	Volatile oil components (%)					
	1.8-cineol	Bornylasetat	Campher	Thujon	Borneol	Average
N_o	10.31 c	2.19	12.59 b	35.23 a	3.89 a	64.21
N_5	14.48 a	2.28	14.43 ab	20.52 c	3.28 ab	54.99
N10	11.83 hc	2.11	16.39 a	23.21 b	2.81 b	56.35
N_{15}	12.44 ab	1.79	14.13 ab	24.12 h	3.74 a	56.22
L.S.D. (%)	2.06	N.S.	2.42	1.49	0.89	

Department of Field Crops, in a four replications according to the randomised blocks test pattern. Before plantation, 0, 5, 10 and 15 kg of N/da were applied to the plots. The plants were marked according to flowers with different colours and then harvested. The volatile oil rate was identified by water-vapor distillation device. The effect of different nitrogen applications on the elements of volatile oil was identified through Gas Chromatography analysis. Furthermore, in order to identify the chemotypes with high content of a-thujon, which is preferred in industrial use, TASS-OVEN (thin layer chromatography) was employed to diagnose the volatile oil components of plants with different morphological characteristics. The components and the standards of the leaf samples obtained from sage with different flower colours and sizes were run on TLC plates and the components were identified. Borneol, 1.8-cineol, bornylasetate, a-thujon and campher were taken as standards. The conditions of TLC and gas chromatography were as follows in the experiment.

Conditions of thin layer chromatography:

Layer	: Slica gel 60 merck 5721, 20x20 dimension aliminium thin layer chromatography
Solution	: Toluol+ethylasetat (93+7)
Injection	: Hamilton microliter injector (701-SNR)
Spreading time	: 45 minute/17 cm
Indicator	: Etanolic H_2SO_4+etanolic vanillin

Condition of gas chromatography

Device	: Tracor 560 model, GC. (FI Detector)
Column	: % 5 OV-1 chromosorb W.H.P. 80/100 meshb*174 SSGC (steel)
Carrying gas	: 30 ml/minute N_2
First heat of column	: 50°C
Waiting time at the First heating	: 1 minute
Last heat	: 220°C
Waiting time at the Last heating	: 6 minute
Increase of heat	: 6°C/minute
Heat of detector	: 220°C
Acceleration of Integrator paper	: 0.2 mm

Results and Discussion

Quantitative identification of volatile oil components in dose folia in the sage: The diagnosis of volatile oil components was realized by (TLC) thin layer chromatography method. During the study, it was investigated whether the leaf samples which were taken from sage populations and different morphological characteristics of which are stated below, revealed any chemotaxonomic differences or not.

1. Light-lily flower coloured early and big flowered
2. Dark-purple flower coloured, small flowered
3. Purple-pink flower coloured, flowers and glumes are big
4. Pink flower coloured, late and small flowers

1-4 leaf samples		Spot colours of leaf samples	
5. Campher	a. skalamen	b. yellow	c. gray
6. borneol	d. pink	e. dark blue	f. lead colour
7. cineol	g. skalamen	h. light blue	I. purple
8. thujon	I. purple	j. light colored lilac	k. pink
9. bornylasetat	I. gray	m. grey	n. yellow
o. pink	p. red purple	r. purple	

solution: toluol+ethylasetat (93+7)

The comparison of sage with different flower colours are indicated above in terms of several morphological characteristics is given in Table 1.
hrf values of the standards of volatile oil of the sage and colour changes occurring while using etanolic H_2SO_4 and etanolic vanillin as indicator (Table 2).

Volatile oil components in sage (%): The quantitative analysis of the volatile oil of sage was performed on gass chromatography. The rates of 1.8-cineol, bornylasetate, campher, α-thujon and borneol, which are the main components of sage, were calculated as the percentage of the total components within the volatile oil.

Cineol (%): Average values pertaining to the rate of 1.8 cineol observed at the end of gas chromatography analysis are indicated in Table 3.
As it is seen in Table 3, the highest cineol rate is 14.48% from the application of 5 kg/da nitrogen dose. However, among the values observed by 15 kg/da N application is not statistically significant. The lowest 1.8-cineol rate which was 10.31%, was observed at the control dose. 11.83% and 12.44% 1.8-cineol were observed from 10 kg/da and 15 kg/de nitrogen applications, respectively. As can be seen in the table, 1.8 cineol was affected by different nitrogen doses in various ways. The highest cineol rate was in parallel with the rate was reported. The findings have remained within the limits indicated by Ceylan (1976), Bezzi *et al*. (1992), Laenger *et al*. (1992) and Mathe *et al*. (1992). Ceylan *et al*. (1979) obtained the highest cineol rate from 5 kg/da N application. Baytop (1984) however, obtained the lowest values.

Bornylasetate (%): Mean values pertaining to bornylasetate rate observed as a result of gas chromatography analysis are indicated in Table 3. As can be observed from Table 3, the effect of different nitrogen doses on bornylasetate rate was not statistically significant. However, the highest rate (2.280) was obtained from 5 kg/da N application and the lowest rate (1.735) from the application of maximum N dose. In the light of this knowledge, it can be claimed that bornylasetate rate is adversely affected from plus N dose applications.

Camphor (%): Average values pertaining to campher rate are indicated in Table 3. As can be observed from Table 3, different nitrogen doses have an important effect on the campher rate in the sage. The highest campher rate was obtained from 190 kg/da N application. Furthermore, except for the control plot, the differences between the values obtained from nitrogen doses have not been statistically significant. The campher rate increased up to 10 kg/da in N application and then started declining. The data are within the limits set forth by Laenger *et al*. (1992) and are higher than those of Mathe *et al*. (1992).

Thujon (%): Average values pertaining to the thujon rate are indicated in Table 3. As can be observed from Table 3, different nitrogen doses affected the thujon rate negatively. In parallel with the increase of nitrogen doses, thujon rates declined. Thujon rate at 5, 10 and 15 kg/da nitrogen applications was lower than the thujon value in the control plot application. Findings are similar to those reported Leanger *et al*. (1992) and Mathe *et al*. (1992). However, the thujon rates obtained from control plots were at the same level with the lower limits indicated by Baytop (1984).

Borneol (%): Mean values pertaining to the borneol rate obtained from the volatile oil in the leaf samples of sage are indicated in Table 3. As can be observed from Table 3, the effect of different nitrogen doses on the borneol rate was found to be statistically significant. However, borneol rates obtained from 0, 5 and 15 kg/da N applications were the same in the latter group. Close results were obtained from 0, 15 kg/da N applications. The data were close to those obtained previously. Furthermore, they were compatible with the lower limits of the data reported by Mathe *et al*. (1992). However, they were lower than the upper limits of such data, particularly quite lower than 10% reported by Baytop (1984). The mean values of the volatile oil components of sage obtained from different nitrogen dose applications are indicated in Table 3.
In the study, as a result of the qualitative analysis performed by employing TASS-OVEN, thin layer chromatography (TLC), it was identified that volatile oil of plants with different morphological features possessed the same components. Furthermore, it was established that 10-15 kg/da nitrogen fertilization was suitable for the sage. On the other hand, as a result of the quantitative analysis of the volatile oil of the same plants in different fertilizer doses. It was determined that increasing dose of nitrogen decreases the amount of thujon, which is important in the industrial use of sage.

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