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# Effects of Different Organic Manure Applications on the Essential Oil Components of Turkish Sage (*Salvia fruticosa Mill.*)

ISIN KOCABAS*, MUSTAFA KAPLAN, MINE KURKCUOGLU† and KEMAL HUSNU CAN BASER†
*Department of Soil Science, Faculty of Agriculture, Akdeniz University, Antalya-07059, Turkey*
*E-mail: isinkocabas@akdeniz.edu.tr*

The effects of different organic manure applications on chemical composition of essential oils of sage (*Salvia fruticosa Mill.*) were investigated. Cattle, sheep and poultry manure were used. The essential oil yields increased with organic manure applications and the highest values were found with poultry manure + sheep manure (2.9 %) applications. Percentage values of 1,8 cineole, the main component of the oil, ranged from 44.0 to 50.7 % with the application of organic manures and the highest values were found in poultry manure + sheep manure (50.7 %). As a result, the essential oil content of Turkish sage increased with organic manure applications and had positive effect on plant growth.

**Key Words: Turkish sage, Essential oil components, Organic manures.**

## INTRODUCTION

The genus *Salvia L.* comprises *ca.* 900 species in the family *Lamiaceae*[1]. *Salvia* species are attributed both medical and economic significance and have a rich potential in Turkey in terms of their natural distribution[2]. *S. fruticosa Mill.* is commercially one of the most important species, which is also known as its synonym *Salvia triloba L.*[3]. *Salvia fruticosa* is collected from nature and exported. It is an oil-rich species[4,5].

*Salvia fruticosa* is also important for its antimicrobial[6], antihypertensive, antihyperglycaemic and spasmolytic effects[7]. Furthermore, *Salvia fruticosa* was reported in many studies to have a higher antioxidant activity than many other aromatic plants[8,9] and considered as a source of natural antioxidant in pharmacy and food sectors[10].

Many studies have shown that correct irrigation and fertilization would increase the production output of medical and aromatic plants[11] and that especially chemical nitrogen fertilization would increase the herbage output, essential oil content and would make an effect in the essential oil components of *Salvia* species[5,12-14].

This study examines the effects of different organic fertilizers and their combinations on the essential oil components of *Salvia fruticosa*.

†Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470-Eskisehir, Turkey.

## EXPERIMENTAL

Turkish sage (*Salvia fruticosa Mill.*) was used as the plant material in this study. Sage cuttings were rooted in substrate. The effects of 3 different manures and their combinations, essential oil content and oil quantity of sage were investigated.

Experimental randomized parcels were planned in 4 replicates according to the experimental design and 7 manure applications cattle manure (CM), sheep manure (SM), poultry manure (PM), sheep manure + cattle manure (SM + CM), poultry manure + cattle manure (PM + CM), sheep manure + poultry manure (SM + PM) and sheep manure + poultry manure + cattle manure (CM + SM + CM) were compared with the control. Nitrogen content of manures was determined according to 15 kg/da N and the mixtures were applied to pots.

The results of pH, EC (dS $m^{-1}$), organic matter and the results of chemical analyses (%) of manure are given in Table-1. Calculated ratios of manures were added to experimental soil and plants were planted in this soil after a month. There were 4 plants in each pot.

TABLE-1
CHEMICAL AND PHYSICAL ANALYSIS RESULTS OF MANURES

| Organic Manure | pH | EC | Organic Matter | N | P | K | Ca | Mg | Fe | Zn | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | dS $m^{-1}$ | | % | | | | ppm | | | | |
| Cattle Manure | 8.84 | 8.84 | 78 | 1.64 | 0.79 | 1.76 | 2.82 | 0.76 | 1426 | 134.8 | 164 | 22 |
| Sheep Manure | 9.17 | 9.17 | 29 | 1.55 | 0.34 | 0.32 | 1.87 | 0.27 | 1218 | 93.83 | 120 | 30 |
| Poultry Manure | 4.91 | 4.91 | 47 | 1.72 | 1.49 | 1.48 | 10.41 | 0.80 | 1538 | 14.80 | 334 | 45 |

For this purpose, soil samples were taken from the study area at the beginning of the experiment. Soil samples were analyzed for physical and chemical properties. Experimental soil had sandy-clay-loam textured (clay 20 %, silt 16 %, sand 64 %), pH 8.28 (1:2.5 soil: water ratio), electrical conductivity (EC) 0.1 dS $m^{-1}$, organic matter 2.01 %, $CaCO_3$ 21.75 %, total N 0.34 %, extractable P 14.6 mg $kg^{-1}$, extractable K 106.5 mg $kg^{-1}$, extractable Ca 332 mg $kg^{-1}$, extractable Mg 18 mg $kg^{-1}$, DTPA-extractable Fe 9.21 mg $kg^{-1}$, DTPA-extractable Mn 10.71 mg $kg^{-1}$, DTPA-extractable Zn 2.59 mg $kg^{-1}$ and DTPA-extractable Cu 1.44 mg $kg^{-1}$.

The soil used in the experiments was chemically analyzed after being air-dried and passed through a 2 mm sieve. Total carbonates were determined according to the calcimeter method of Nelson[15]. Soil texture was determined by hydrometer method[16] and organic matter by the method described by Walkley-Black[17]. Eelectrical conductivity was determined according to Olsen and Sommers[18]; pH (1/2.5 soil/ water) was determined according to Jackson[19]. Total N was determined by modified Kjehldahl procedure[20]. Extractable P content was extracted by $NaHCO_3$[15] and determined by a molybdate colorimetric method[19]; extractable K, Ca and Mg were extracted with ammonium acetate and determined by atomic absorption spectrophotometer (AAS)[20]. Soil Fe, Mn, Zn and Cu were extracted with DTPA[21] and then determined in the obtained extract by AAS. Also, soil samples were taken from each application plot in the beginning of spring experiment and then analyzed for some physical and chemical properties.

For essential oil analysis, samples of fresh herbs were taken from each treatment variable during each harvest and dried. Dried herbal parts of the samples were subjected to hydrodistillation for 1 h at 100 ºC in a modified Clevenger apparatus for the distillation of essential oil. Percentage yields of essential oil was calculated on dry weight basis[22].

In organic manure, organic material, N, P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu contents were measured. In addition, electrical conductivity[23] (EC) and pH[24] were determined.

**GC and GC/MS conditions:** The oils were analyzed by capillary GC and GC/MS using an Agilent GC-MSD system.

**GC/MS:** The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m × 0.25 mm, 0.25 m film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 ºC for 10 min and programmed to 220 ºC at a rate of 4 ºC/min and kept constant at 220 ºC for 10 min and then programmed to 240 ºC at a rate of 1 ºC/min. Split ratio was adjusted 40:1. The injector temperature was at 250 ºC. MS were taken at 70 eV. Mass range was from m/z 35 to 450.

**GC:** The GC analysis was carried out using an Agilent 6890N GC system. In order to obtain same elution order with GC/MS, simultaneous injection was done by using same column and appropriate operational conditions. FID temperature was 300 ºC.

The components of essential oils were identified by comparison of their mass spectra with those in House Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, Mass Finder Library and confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of analysis are shown in Table-2.

## RESULTS AND DISCUSSION

The effects of manure applications on essential oil content of sage were found statistically important ($p < 0.001$) and the highest value was obtained from the poultry manure + sheep manure application (2.9 %) (Fig. 1). Organic applications led to the increase of essential oil content of study plants as compared with the control plants[25].

The effects of different organic manure applications on the essential oil components of sage plants are shown in Table-2. A total of 48 components were characterized in the essential oil analysis of the above ground parts of *S. fruticosa*. These components add up to 95, 6-98, 9 % of the total oil.

TABLE-2
CHEMICAL COMPOSITION OF ESSENTIAL OILS OF *Salvia Fruticosa Mill.*

| Compound | RRI | Control | CM | SM | PM | SM + CM | PM + CM | PM + SM | SM + PM + CM |
|---|---|---|---|---|---|---|---|---|---|
| Tricyclene | 1014 | 0.1 | tr | 0.1 | 0.1 | tr | 0.1 | tr | 0.1 |
| α-Pinene | 1032 | 5.0 | 4.0 | 3.5 | 4.6 | 4.4 | 4.3 | 4.2 | 4.3 |
| α-Thujene | 1035 | 0.1 | 0.2 | 0.3 | 0.5 | 0.3 | 0.2 | 0.2 | 0.2 |
| α-Fenchene | 1072 | tr | tr | tr | tr | tr | Tr | tr | tr |
| Camphene | 1076 | 3.0 | 1.7 | 2.9 | 2.4 | 2.8 | 1.8 | 1.6 | 2.4 |
| **β-Pinene** | 1118 | **5.3** | **6.6** | **6.9** | **5.7** | **6.0** | **6.0** | **6.5** | **6.2** |
| Sabinene | 1132 | 0.1 | 0.6 | 0.8 | 0.4 | 0.5 | 0.3 | 0.3 | 0.4 |
| Myrcene | 1174 | 2.1 | 2.4 | 2.5 | 2.3 | 2.3 | 2.4 | 2.2 | 2.2 |
| α-Terpinene | 1188 | 0.3 | 0.4 | 0.4 | 0.5 | 0.5 | 0.4 | 0.3 | 0.4 |
| Limonene | 1203 | 1.4 | 1.6 | 2.3 | 1.7 | 1.7 | 1.5 | 1.4 | 1.6 |
| **1,8-Cineole** | 1213 | **45.0** | **49.2** | **44.0** | **47.1** | **45.8** | **49.7** | **50.7** | **46.6** |
| γ-Terpinene | 1255 | 0.4 | 0.6 | 0.7 | 0.7 | 0.7 | 0.5 | 0.5 | 0.5 |
| *p*-Cymene | 1280 | 0.9 | 0.9 | 0.6 | 1.2 | 0.9 | 1.0 | 0.8 | 0.8 |
| Terpinolene | 1290 | 0.1 | 0.2 | 0.4 | 0.2 | 0.3 | 0.2 | 0.2 | 0.2 |
| Octenyl acetate | 1386 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | tr | tr | 0.1 |
| α-Thujone | 1437 | 1.4 | 2.6 | 0.1 | 3.4 | 2.8 | 3.0 | 3.3 | 1.7 |
| *p*-Cymenene (= α,*p*-Dimethylstyrene) | 1452 | tr | tr | tr | tr | tr | tr | tr | tr |
| β-Thujone | 1451 | 5.1 | 3.4 | 2.6 | 5.0 | 4.5 | 4.5 | 4.9 | 4.5 |
| *trans*-Sabinene hydrate | 1474 | 0.3 | 0.6 | 0.8 | 0.4 | 0.5 | 0.3 | 0.5 | 0.5 |
| α-Copaene | 1497 | 0.5 | 0.8 | 0.6 | 0.5 | 0.5 | 0.5 | 0.4 | 0.6 |
| **Camphor** | 1532 | **7.0** | **5.9** | **13.1** | **6.9** | **9.9** | **5.4** | **4.4** | **8.2** |
| Linalool | 1553 | 0.7 | 0.4 | 0.5 | 0.4 | 0.7 | 0.5 | 0.8 | 0.5 |
| *cis*-Sabinene hydrate | 1556 | 0.1 | 0.2 | 0.3 | 0.2 | 0.3 | 0.1 | 0.2 | 0.2 |
| Linalyl acetate | 1565 | 0.6 | 0.6 | 0.6 | 0.5 | 0.7 | 0.4 | 0.4 | 0.4 |
| *trans*-*p*-Menth-2-en-1-ol | 1571 | tr | tr | tr | tr | tr | tr | tr | tr |
| Bornyl acetate | 1590 | 0.6 | 0.3 | 0.9 | 0.7 | 0.9 | 0.4 | 0.4 | 0.5 |
| Terpinen-4-ol | 1611 | 0.4 | 0.5 | 0.4 | 0.4 | 0.4 | 0.5 | 0.4 | 0.3 |
| β-Caryophyllene | 1612 | 5.7 | 4.2 | 2.9 | 3.1 | 3.8 | 4.3 | 4.2 | 4.3 |
| Aromadendrene | 1628 | 0.8 | 0.2 | tr | tr | 0.1 | 0.2 | 0.3 | 0.5 |
| Sabinyl acetate | 1658 | 0.2 | 0.1 | 0.4 | 0.3 | 0.1 | 0.2 | 0.1 | 0.2 |
| α-Humulene | 1687 | 1.3 | 1.7 | 1.1 | 1.6 | 1.2 | 1.5 | 1.4 | 1.3 |
| α-Terpineol | 1706 | 0.6 | 0.3 | 0.9 | 0.4 | 0.6 | 0.3 | 1.0 | 0.6 |
| Borneol | 1719 | 1.5 | 1.3 | 2.9 | 1.5 | 1.4 | 1.3 | 1.0 | 1.4 |
| *trans*-Sabinol | 1720 | 0.7 | 0.7 | 1.5 | 0.8 | 0.7 | 0.7 | 0.5 | 0.7 |
| Germacrene D | 1726 | tr | 0.1 | 0.1 | tr | 0.1 | tr | tr | 0.1 |
| Neryl acetate | 1733 | tr | tr | tr | tr | tr | tr | tr | tr |
| α-Muurolene | 1740 | tr | tr | tr | tr | tr | tr | tr | tr |
| β-Selinene | 1742 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| α-Selinene | 1744 | tr | 0.1 | tr | tr | tr | tr | tr | tr |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Geranyl acetate | 1765 | tr | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | tr |
| δ-Cadinene | 1773 | 0.3 | 0.4 | 0.2 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| γ-Cadinene | 1776 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| *cis*-calamenene | 1853 | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 |
| Epicubebol | 1904 | tr | tr | 0.1 | tr | tr | tr | tr | tr |
| Caryophyllene oxide | 2008 | 0.9 | 0.7 | 0.6 | 0.5 | 0.6 | 1.0 | 0.9 | 0.8 |
| Humulene epoxide-II | 2071 | 0.3 | 0.4 | 0.2 | 0.5 | 0.3 | 0.5 | 0.4 | 0.3 |
| Viridiflorol | 2104 | 1.8 | 1.3 | 1.4 | 1.2 | 1.2 | 1.2 | 1.1 | 1.6 |
| Spathulenol | 2130 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 |
| Caryophylladienol I (= *Caryophylla-2(12),6(13)-dien-5*β*-ol*) | 2316 | 0.2 | 0.2 | 0.3 | 0.2 | 0.3 | 0.2 | 0.2 | 0.2 |
| 14-Hydroxy-α-muurolene | 2565 | 0.1 | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.1 | 0.2 |
| % | – | 95.6 | 96.3 | 98.7 | 97 | 98.9 | 96.5 | 96.7 | 96.5 |

RRI: Relative retention indices calculated against *n*-alkanes, % calculated from FID data, tr = Trace (< 0.1 %); CM = Cattle manure, SM = Sheep manure, PM = Poultry manure.

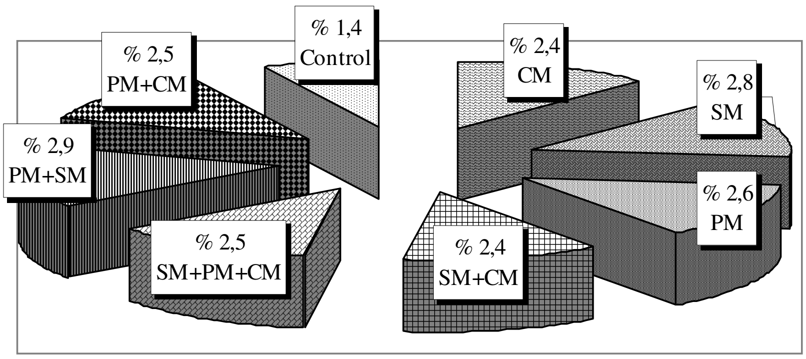


Fig. 1. Effects of different organic manure applications on the essential oil ratio (%) in the dry herb of sage[25]

1,8-Cineole was the main component of *Salvia fruticosa* as previously reported from Greece[26-28] and Turkey[4,7,29]. There is only one study conducted in Southern Brazil reporting the main component as α-thujone[30]. This should be treated as an exception[31]. Organic manure applications increased 1,8-cineole contents of sage plant when compared to control group and the highest 1,8-cineole contents were obtained from 50.7 % poultry manure and sheep manure combination.

Organic manure applications were observed to cause changes in the contents of camphor, β-pinene and borneol. For example, β-pinene is in the second order, camphor is in the third order after 1,8-cineole contents in cattle manure and poultry-cattle manure combination applications whereas camphor is in the second order and β-pinene is in the third order in other organic manure applications in control groups. Organic manure applications resulted in changes in the orders and amounts of components except for the main component (1,8-cineole).

The effects of organic manure applications on essential oil components are in parallel to the studies conducted by Catsiotis and Iconomou[26], Pitarokili[28] in terms of major components of sage plants growing in different parts of Greece not changing from region to region unlike other essential oil components.

Different organic manure applications caused quantitative changes in essential oil components. The highest increase was observed in the content of 1,8-cineole, from poultry-sheep combination whereas the increases in camphor and β-pinene were obtained from sheep manure applications. Fertilization practices resulted in changes in the orders of other components than1,8-cineole.

Turkish sage grows naturally but those which grow ecologically are preferred more. Using organic fertilizers in sage growth is important for its ecological production. This study concluded that organic fertilization in sage growing positively affects the growth rate and cause favorable changes in essential oil yield and its components.

## REFERENCES

1. R.M. Harley and T. Reynolds, Labiate Science, Botanic Garden Kew, p. 7 (1992).
2. M. Nakipoglu, *Tr. J. Botany*., **17**, 21 (1993).
3. P.H. Davis, Flora of Turkey and The East Aegean Islands, Edinburgh University Press, Edinburgh, p. 178 (1982).
4. A. Bayrak and A. Akgul, *Phytochem*., **26**, 846 (1987).
5. A. Ceylan, Ege University Agriculture Faculty Press, Izmir, Turkey, p. 225 (1997).
6. A. Sivropoulou, C. Nicolaou, E. Papanikolaou, S. Kokkini, T. Lanaras and M. Arsenakis, *J. Agric. Food Chem*., **45**, 3197 (1997).
7. E. Bayram, *Turk. J. Agric. For*., **25**, 351 (2001).
8. L. Pizzale, R. Bortolomeazzi, S. Vichi, E. Uberegger and L.S. Conte, *J. Sci. Food Agric*., **82**, 1645 (2002).
9. C. Saricoban and M. Ozcan, *Jeobp*, **7**, 91 (2004).
10. R.O. Urek, H.A. Kayali, M. Nakiboglu and L. Tarhan, *Asian J. Chem*., **20**, 2091 (2008).
11. C. Franz, *Acta Hort*., **132**, 203 (1983).
12. D. Karaaslan, Salvia Populasyonlarinda Farkli Azot Uygulamalarinda Drog Verimi ve Kemotaksonomik Arastirmalar (PhD. Thesis), Cukurova University Agriculture Faculty Press, Adana, Turkey (1994).
13. H. Koc, *J. Fac. Agric., GOU University*, **17**, 89 (2000).
14. J.P. Han, Z.S. Liang, Q. Sun, X.R. Wei, J.M. Wang and H.S. Deng, *Zhongguo Zhong Yao Za Zhi*., **29**, 3 (2004).
15. A.L. Page, R.H. Miller and D.R. Keeney, Methods of Soil Analysis, American Society of Agronomy press, Wisconsin, USA, p. 181 (1982).
16. G.J. Bouyoucos, *Agron. J*., **4**, 434 (1955).
17. C.A. Black, Methods of Soil Analysis Part 2, Amer. Society of Agronomy Inc., Publisher Madison, Wisconsin, USA, p. 1372 (1965).
18. A.L. Page, P.H. Miller and D.R. Keeney, Methods of Soil Analysis, American Society of Agronomy press, Wisconsin, USA, p. 404 (1982).
19. M.L. Jackson, Soil Chemical Analysis. Prentice Hall of India Private Limited, New Delhi (1967).
20. B. Kacar, Bitkive Topragin Kimyasal Analizleri: III. Toprak Analizleri, Ankara University Agriculture Faculty Press, Ankara, Turkey, p. 705 (1995).
21. W.L. Lindsay and W.A. Norwell, *Soil Sci. Am. J*., **42**, 421 (1978).
22. E. Guenther, The Essential Oils. Van Nostrand Comp., New York, p. 264 (1995).

23. B. Kacar, Gubre Analizleri, Ankara University Agriculture Faculty Press, Ankara, Turkey, p. 975 (1990).
24. DIN, 11542, Torf fur Gartenbau und Landwirtschaft (1978).
25. I. Kocabas, I. Sonmez, H. Kalkan and M. Kaplan, *J. Fac. Agric., Akdeniz Univ.*, **20**, 105 (2007).
26. S. Catsiotis and N.G. Iconomou, *Pharm. Acta Helv*., **59**, 29 (1984).
27. M. Skoula, J.E. Abes and C.B. Johnson, *Biochem. Systems Ecol*., **28**, 551 (2000).
28. D. Pitarokili, O. Tzakou, A. Loukis and C. Harvala, *J. Agric. Food Chem*. **51**, 3294 (2003).
29. P. Buil, J. Garnero, G. Guichard and Z. Konur, *Rivista Hal. EPPOS*, **59**, 379 (1977).
30. A.P.L. Delamera, I.T.M. Pistorello, L. Artico, L.A. Serafini and S. Echeverrigaray, *Food Chem*., **100**, 603 (2007).
31. N.B. Perry, R.E Anderson, N.J. Brennan, M.H. Douglas, A.J. Heaney, J.A McGrimpsey and B.M. Smallfield, *J. Agric. Food Chem*., **47**, 2048 (1999).

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