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RESEARCH ARTICLE

Effects of Various Nutrient Sources on Growth and Essential Oil Characteristics of *Salvia Officinalis* L. in Greenhouse

Amir Soltanbeigi[1*] • Mustafa Yildiz[2] • Erhan Sakartepe[3]

[1*]*Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey. E-mail: amir.soltanbeigi@afsu.edu.tr*

[2]*Department of Molecular Biology and Genetics, Afyon Kocatepe University, Afyonkarahisar/Turkey.*

E-mail: mustafa_yildizus@yahoo.com, ORCID: https://orcid.org/0000-0002-6819-9891

[3]*Department of Molecular Biology and Genetics, Afyon Kocatepe University, Afyonkarahisar/Turkey.*

E-mail: sakartepe.erhan@hotmail.com, ORCID: https://orcid.org/0000-0002-7079-0007

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ABSTRACT

The agronomic and chemical characteristics of aromatic plants are affected by nutritional sources. *Salvia officinalis* (common sage) is an aromatic plant extensively used in food, popular medicine, and many pharmacological research studies. In this study, the effects of NPK, vermicompost and two types of microbiological fertilizer were investigated on the agronomic parameters and volatile oil characteristics of *S. officinalis* grown in the greenhouse. The plants were harvested two times (1st and 2nd cuttings) during the growing season. The consumption of NPK has the highest effect on stem number (54 no plant^{-1}), fresh and dry herb weight (96.8 and 27.2 g p^{-1}), fresh and dry leaf weight (74.6 and 19.6 g p^{-1}), and volatile oil percentage (1.64%). While the herb weight at the 1st cutting (74.3 g p^{-1}) was higher than the 2nd cutting (70.2 g p^{-1}), the fresh (58.2 g p^{-1}) and dry (16.6 g p^{-1}) leaf weights and the leaf ratio (80.5 %) at the 2nd cutting were higher than the 1st cutting. Moreover, the volatile oil contents at the 1st cutting (1.44%) were higher than the 2nd cutting (1.18%). In total, 31 compounds were identified in the volatile oils by using GC/FID-MS. The percentages of α-Thujone (22.4-31.4%) and Camphor (21.0-25.4%) were found higher than other compounds. The content of α-Thujone was higher in NPK and vermicompost treatments. Based on the results, the application of different nutritional sources improved the yield and chemical properties of *S. officinalis*. Among the non-chemical nutrient sources, vermicompost had high efficiency.

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Introduction

The genus *Salvia* belongs to the Lamiaceae family represented by 87 species, of which 44 are endemic in Turkey (Nakipoğlu, 1993; Delamare et al., 2007). *Salvia officinalis* L. (common sage), as the most economic species of genus *Salvia*, is a perennial woody sub-shrub with blue to purple flowers. This plant does not grow naturally in Turkey. But in recent years, due to the economic values of *Salvia* species, it has attracted the interest of producers (Bahtiyarca Bağdat et al., 2017; Sönmez and Bayram, 2017).

Common sage is widely used as the raw material of Pharmaceutical, perfumery, and the food industries (Nadjafi et al., 2014). Most phytochemical research of this species has focused on its volatile oil. α-Pinene (0.6-6.4%), Camphene (0.6-5.5%), 1,8-Cineole (5.3-14.6%), α-Thujone (15.2-26.6%), β-Thujone (5.2-12.9%), Camphor (16.4-20.0%), Borneol (1.8-4.9%), Bornyl acetate (2.1-2.2%), (E)-b-Caryophyllene (2.4-4.5%), α-Humulene (5.3-8.5%), Viridiflorol (4.0-8.5%) were identified as the main compounds of *S. officinalis* (Raal et al., 2007). The anti-cancer, anti-mutagenic, hypoglycemic, antioxidant, antimicrobial, antidementia, antinociceptive, and anti-

* Corresponding author: amir.soltanbeigi@afsu.edu.tr

inflammatory activities of *S. officinalis* were reported according to various studies (Jakovljevic et al., 2019).

The use of various fertilizers to increase crop yields is inevitable today. Due to the increasing use of chemical fertilizers, agricultural lands' pollution has also become a principal challenge facing sustainable agriculture. It is imperative to turn to organic and microbiological fertilizers to prevent pollution of agricultural soils (Savci, 2012). Even long-term use of chemical fertilizers can reduce the yield of crops due to damage to soil's physical and chemical structures (Liu et al., 2010). Because medicinal plants are directly related to human health, soil fertility management is one of the main determinants of growth, quantity, and quality (Omidbeigi, 2018). In the region of low phytoavailability, needed mineral elements are supplied to crops as fertilizers to produce optimal yields (White and Brown, 2010). In recent years, the application of microbiological and organic fertilizers raised extensively as an eco-friendly standpoint to minimize chemical fertilizers. These nutrient sources enhance soil fertility status and improve crop production by their biological activity rhizosphere (Ram Rao et al., 2007).

As an indispensable component of organic farming, microbiological fertilizers contain living organisms that are effective nitrogen-fixing and phosphate-dissolving species. Microbiological fertilizers are used for seed, soil, or compost-containing sites to increase these microorganisms and accelerate microbial processes. Thus, the microbial processes provide the nutrients for proper assimilation (Venkatashwarlu, 2008). The effectiveness of biofertilization is correlated with host cultivar and agricultural practices (Scagel, 2005). Vermicompost is a type of organic fertilizer produced due to the activity of earthworms on municipal, industrial and agricultural wastes (Sangwan et al., 2008). Vermicompost is a rich source of high and low-consumption nutrients, vitamins, enzymes, and plant growth hormones (Prabha et al., 2007; Padmavathiamma et al., 2008). Nitrogen, vermicompost, and nitrogen-fixing bacteria (*Azotobacter* + *Azospirillum* + *Pseudomonas*) improved the yield, quantity, and quality of *S. officinalis* essential oil (Govahi et al., 2015).

The present study aimed to determine the effects of various nutrient sources on agronomic yield, content, and characteristics of *Salvia officinalis* L. volatile oil under greenhouse conditions.

Materials and Methods

Location and Plant Material

This study was conducted in the greenhouse conditions located at Afyonkarahisar Medicinal and Aromatic Plants Centre/Turkey (38°46' N, 30°30' E) in 2017. The greenhouse temperature was controlled using a central heating system (average 22/32 °C for night/day). *Salvia officinalis* L. (common sage) seeds were used for seedlings obtaining. The seeds were imbibed in distilled water for 4 hours. Then, the seeds were sown into the holes of plug trays at a depth of 7±2 mm on April 17, 2017. The seedbed was a mix of disinfected peat and soil in equal amounts.

Experimental Treatments

The five experimental treatments were control (without fertilizer), chemical fertilizer (N: 120, P: 60, and K: 60 kg ha^{-1}), vermicompost (60 ton ha^{-1}), and two types of commercial microbiological fertilizers. The experiment was carried out in a completely randomized design with three replications. Some properties of vermicompost and microbiological fertilizers are given in Tables 1 and 2. The well-aerated (ten days) and sifted forest soil was used to fill the pots. For sterilizing the soil, it was exposed to 180 degrees for 30 minutes in two stages. The physico-chemical properties of the sterilized soil are given in Table 3. Five pots (diameter: 27, depth: 28.5 cm) were filled with 15 kg of disinfected forest soil for each treatment.

Table 1. Some chemical characteristics of the vermicompost

pH	E.C. Dc ms^{-1}	Total N (%)	O.C. (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)
7.4	2.25	2.30	21	0.85	1.2	8.3	8.3	225	1580	30.2	168.4

Table 2. Some chemical characteristics of the microbiological fertilizers

	M1	M2
Live microorganisms name	*Bacillus megaterium* *Pantoea agglomerans* *Paenibacillus polymyxa* *Bacillus subtilis*	*Bacillus megaterium* *Pantoea agglomerans* *Pseudomonas fluorescens*
Total live microorganisms count (CFU ml^{-1}) *	1.7×10^7	2.1×10^8
pH	6.1-8.1	6-8
CFU: Colony Forming Unit		

Table 3. The values of some physico-chemical properties of the soil

Properties	(%)	Elements	(ppm)
Organic matter content	0.97	Ca	2157
Total N	0.09	Mg	514
Sand	52.92	K	316
Clay	26.61	Na	92
Dust	20.47	Fe	1.08
Lime	0.23	P	91
Field capacity	22.12	Cu	2.2
Wilting point	14.95	Zn	0.23
Available moisture	7.17	Mn	8.54
Soil class: Sandy clay; EC (mS cm^{-1}): 0.12; pH: 7.20			

When the seedlings reached 6-8 leaves, they were transplanted to pots (on May 30, 2017). For the application of chemical fertilizers treatments (NPK) in pots, soil bulk density was calculated:

$$\text{Bulk Density (g cm}^{-3}\text{)} = \text{Dry soil weight (g)} / \text{Soil volume (cm}^{3}\text{)}$$

The weight of one hectare of farm soil was calculated at a depth of 30 cm based on the bulk density. Thus, the amounts of fertilizers were determined. Half of N and whole of PK supplied from the 15:15:15 chemical fertilizer granules (1.5 g pot^{-1}), and all of the vermicompost (220 g pot^{-1}) were added at the same time of transplanting. The rest of the N was applied to the related pots after 1st cutting using 46% urea (0.48 g pot^{-1}). The microbiological fertilizers were applied in three stages (1st: at transplanting time, 2nd: 15 days later, and 3rd: after 1st cutting). For preparing the microbiological fertilizers, equal volumes (30 mL) of the liquid microbiological fertilizers and supplemental solution were dissolved in 600 mL of distilled water. The obtained solution was incubated at 37 °C for 24 hours. Then 2340 mL of distilled water was added to the incubated solution, and this preparation was applied to the pots. The plants were irrigated (2 L pot^{-1}) after each 70±5 mm evaporation from class A evaporation pan placed in the greenhouse.

Harvesting and Records

The cuttings were done twice above 5-8 cm of the soil surface when the first flowers were observed (Table 4). The plant height was calculated from the average of 5 plants. All plants of each treatment were weighted to determine fresh herb weight. After the leaves and stems were separated, the fresh weights were determined. The samples were dried at 37 °C for 72 h and were determined dry weights.

Table 4. Date of plant cuttings during the experiment

Cuttings	Date	Growth Period
1st	3 August	65 days
2nd	25 September	53 days

Volatile oil Isolation

A certain amount of dried leaves and distilled water (1:10) were subjected to hydro-distillation using a neo-Clevenger apparatus for 3 hours with three replications. The obtained volatile oil samples were dried over anhydrous sodium sulphate and stored at 4 °C in ambered vials until the chromatographic analysis.

Chemical Analyses

A gas chromatography (GC) system (Agilent Technologies, 7890B) equipped with a flame ionization detector (FID) and coupled to a mass spectrometry detector (MSD) (Agilent Technologies, 5977A) was used for identifying the chemical components of the essential oils. The column for separating the compounds was HP-Innowax (Agilent 19091N-116: 60 m × 0.320 mm internal diameter and 0.25 µm film thickness). The carrier gas was Helium (99.999%) with 1.3 mL min^{-1} flow rate. Injection volume was set at 1 µl (20 µL essential oil dissolved in 1 mL n-Hexane). The solvent delay time was 8.20 min. The injection was performed in split mode (40:1). The samples were analyzed with the column held initially at 70 °C after injecting with 5 min hold time. Then, the temperature raised to 160 °C with 3°C min^{-1} heating ramp. Eventually, the temperature reached 250 °C with 6 °C min^{-1} heating ramp with 5 min hold time. The detector, injector, and ion source temperatures were 270 °C, 250 °C, and 230 °C, respectively. MS scan range was (m z^{-1}): 50-550 atomic mass units (AMU) under electron impact (EI) ionization of 70 eV.

The retention indices (RI) were determined by injecting C7-C30 n-alkanes (Sigma-Aldrich) to (GC/FID) system (Agilent Technologies, 7890B) under the same conditions of the analyses of the essential oils. Identifying the essential oil components was determined by comparing retention indices, mass spectra by the computer library database of the US National Institute of Standards and Technology (NIST), Wiley libraries, and other published mass spectra data (Adams, 2017) and our database. Relative abundance (% area) was calculated based on the ratio between the peak area of each compound and the sum of areas of all compounds. No response factors were calculated.

Statistical Analyses

The collected data were analyzed by using the MSTAT-C computer software program. The means of treatments were compared using the Least Significant Difference (LSD) method at a 0.05 probability level. The analysis of variance was conducted on the samples to determine variations of parameters between the planting season and various cuttings.

Results and Discussion

Plant Height

In this study that cuttings were conducted twice, a significant difference was found between cuttings. The plant heights of 1st cutting were higher than 2nd cutting (Table 5). The reason for decreasing plant height in the 2nd cuttings is shorter plant growth periods (Table 2) and less access to nutrient resources. It can be suggested the growth period of plants in the 1st cutting, from April 17 to August 3, was influential on plant height. Abd-El-Azim and Badawy (2015) and Govahi et al. (2015) also support the idea of cutting number effect on the height. Based on the other research, the height of *S. officinalis* was higher in applying N fixing bacteria and a combination of Phosphate solubilizing bacteria and N fixing bacteria compared to control treatment, but the differences were not significant (Nadjafi et al., 2014).

Table 5. Agronomic parameters and volatile oil content of common sage affected by various nutrient sources in greenhouse

Treatment	Height (cm)	Stem No (no p^{-1})	Fresh Herb Weight (g p^{-1})	Dry Herb Weight (g p^{-1})	Fresh Leaf Weight (g p^{-1})	Dry Leaf Weight (g p^{-1})	Leaf Ratio (%)	V.O. Content (%)
Fertilizer (F)								
Control (F_1)	40.23	36.33 b	58.59 d	18.34 d	45.42 d	13.38 c	77.3	1.15 c
NPK (F_2)	43.15	54.83 a	96.82 a	27.29 a	74.68 a	19.66 a	71.6	1.64 a
Vermicompost (F_3)	43.15	35.50 b	75.450 b	22.11 b	58.83 b	15.94 b	72.8	1.32 b
Microbiological I (F_4)	42.88	30.17 b	65.76 c	18.52 cd	51.31 c	13.87 c	74.6	1.24 bc
Microbiological II (F_5)	40.03	32.00 b	64.91 c	19.84 c	51.12 c	14.60 bc	73.9	1.20 bc
LSD $_{(5\%)}$	-	8.630	4.301	1.445	4.489	1.475	-	0.1575
Probability level	-	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	-	$P \leq 0.01$
Cutting No (C)								
1st Cutting (C_1)	56.47 a	18.200 b	74.36 a	21.61	54.29 b	14.33 b	67.6 b	1.44 a
2nd Cutting (C_2)	27.307 b	57.333 a	70.25 b	20.82	58.25 a	16.65 a	80.5 a	1.18 b
Probability level	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.05$	-	$P \leq 0.05$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$
Fertilizer × Cutting								
$F_1 \times C_1$	55.30	18.0 c	68.80 c	20.73 c	50.27 ef	13.73 cd	72.9	1.17 cd
$F_1 \times C_2$	25.17	54.7 b	48.38 d	15.94 d	40.57 g	13.03 d	81.8	1.14 cd
$F_2 \times C_1$	54.30	19.7 c	87.67 b	24.53 b	64.87 b	16.60 b	67.6	1.68 a
$F_2 \times C_2$	32.00	90.0 a	105.97 a	30.04 a	84.49 a	22.72 a	75.6	1.60 ab
$F_3 \times C_1$	57.70	19.3 c	82.13 b	24.20 b	59.93 bc	15.87 bc	65.7	1.44 b
$F_3 \times C_2$	28.60	51.7 b	68.77 c	20.02 c	57.73 cd	16.02 b	79.9	1.20 c
$F_4 \times C_1$	59.77	16.7 c	64.47 c	17.80 cd	46.00 fg	11.67 d	65.5	1.44 b
$F_4 \times C_2$	26.00	43.7 b	67.04 c	19.23 c	56.61 ce	16.07 b	83.6	1.03 de
$F_5 \times C_1$	55.30	17.3 c	68.73 c	20.80 c	50.40 ef	13.80 cd	66.4	1.44 b
$F_5 \times C_2$	24.77	46.7 b	61.08 c	18.87 cd	51.85 df	15.41 bc	81.6	0.95 e
LSD $_{(5\%)}$	-	11.98	8.803	3.016	6.730	2.145	-	0.1627
Probability level	-	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	$P \leq 0.01$	-	$P \leq 0.01$
CV (%)	8.88	17.43	6.69	7.81	6.57	7.61	5.0	6.91
The means which have no letters are statistically non-significant at 5% probability level								

Stem Number

The results indicated that the differences between fertilizer treatments, cuttings, and interaction effects were significant (Table 5). Among fertilizer treatments, the application of chemical fertilizers (NPK) significantly affected the stem number. Other treatments were included in the same statistical group. The 2nd cuttings produced three times more than the 1st cuttings of the stems (Table 5). Investigating the interaction between fertilizer and cutting, while the 2nd cuttings with the chemical fertilizer application had the highest stem number, the 2nd cuttings with all chemical fertilizer treatments had more than the 1st cuttings. According to these results, the cutting effect is superior to fertilizer consumption. In 2016, According to Gerami et al. (2016), the number of stems augmented with increasing levels of cattle manure. In another study, the stem numbers increased with NPK application, but adding mycorrhiza did not affect the mentioned trait (Bahtiyarca Bagdat et al., 2017).

Fresh and Dry Herb Weight

The means of fresh herb weight revealed significant differences in the levels of fertilizers. The NPK application increased the fresh herb weight per plant. The vermicompost, microbiological I and II (jointly), and control treatments were ranked lower, statistically (Table 5). Fresh herb weights of 1st cuttings were higher than 2nd cuttings. Besides, in terms of interaction effects between treatments, NPK consumption in 2nd cuttings had higher plant weight. The lowest values were recorded in the 2nd cuttings and control group. The interaction effects indicated the efficiency of the NPK consumption on increasing herb weight in 2nd cuttings. These results showed the positive effects of various nutrient sources, especially the NPK, on the fresh herb weight. The NPK application had a higher amount of dry Herb weight. The vermicompost treatment was in second place after the NPK (Table 5). Although the effect of cuttings on dry herb weight was not significant, the interaction effects of the main treatments were found significant. Since dry yield is a relative function of the plant's fresh yield, the results are repeated with a slight change in dry herb weights. Various nutrient sources improve soil chemical and physical characteristics. Several studies supported that nitrogen and other nutrients sources application intensified on total fresh yield and aerial parts of *S. officinalis* (Ghoushchi et al., 2015; Gerami et al., 2016; Sönmez and Bayram, 2017; Hegab et al., 2018). It was reported that the application of cow manure, vermicompost, Azotobacter, and Azospirillum increased the final yield of *S. officinalis* compared to the control treatment (Radnezhad et al., 2015). Also, Hegab et al. (2018) reported that the 2nd cuttings produced more biomass than the 1st cuttings in *S. officinalis*. According to Nadjafi et al. (2014), the application of biofertilizers had no significant effect on the growth and yield of *S. officinalis*. The present results in terms of dry herb weight were largely in line with Bahtiyarca Bagdat et al. (2017) on *S. officinalis* in the greenhouse. Comparing the results of the present study and some similar studies on the use of biofertilizers showed that the application of these fertilizers alone is not so effective. Synergistic and positive effects between vermicompost and bacteria caused to increase soil bacteria activities (Govahi et al., 2015). These microorganisms increase root growth through mechanisms such as the secretion of growth hormones (Glick, 2012). Nitrogen and phosphorus are involved in many plant physiological processes. In particular, nitrogen is a structural component in amino acids, nucleic acids, enzymes and proteins, chlorophyll, and cell walls. Phosphorus also plays a vital role in energy transfer, cell membranes, and nucleic acids (Ghoushchi et al., 2015). Therefore, the lack of nitrogen and phosphorus sources in the growing season leads to defects in plant yield.

Fresh and Dry Leaf Weight

Sage leaves are the economic organ of the plant. Hence, the leaves yield of this plant is the most important factor in the production process. Based on Table 5 results, the application of NPK had a higher fresh leaf weight per plant. The vermicompost, microbiological I and II (jointly) and control treatments were placed in various statistical groups, respectively. Unlike what was seen in fresh herb weight, fresh leaf weight was higher in 2nd cuttings. It can be pointed out that the high yield of leaf weight in the 2nd harvest is related to the plant age, root system development, water, and nutrient uptake. Evaluation of interaction effects between cutting and fertilizer shows that except for vermicompost application and control group in both cuttings, other treatments have better leaf yield in the 2nd cuttings. It seems that the distribution of chemical and biological fertilizers in different growth stages has led to this result. The mean comparison of various nutrient sources indicated significant differences between all main treatments and interaction effects. NPK application had the highest dry leaf weight (Table 5). Like the leaf fresh weight, the 2nd cuttings were placed in a higher statistical group than the 1st cuttings. Ghoushchi et al. (2015) showed the leaf yield of *S. officinalis* increased with the application of NP and biofertilizers (Mycorrhiza and Pseudomonas). Increasing leaf fresh weight using chemical fertilizers (NP) has already been confirmed (Abaas, 2014). Bahtiyarca Bagdat et al. (2017) also recorded similar leaf fresh weight gain using different fertilizers in *S. officinalis*. However, in this study, the effect of biological fertilizers on leaf fresh weight gain was significant. The current study results were in line with various similar researches in dry leaf weight (Abaas, 2014; Bahtiyarca Bagdat et al., 2017). Since *S. officinalis* leaf weight is the most critical component of plant yield, any factor that increases the plant's final product also raises the leaf weight. As mentioned earlier, nitrogen and phosphorus increase cell division and, consequently, expand the leaf area.

Leaf: Biomass Ratio

Except for cuttings, the effect of other treatments on the leaf to whole plant ratio was not significant (Table 5). The leaf ratio in the 2nd cuttings was higher than the 1st. The results in Table 5 show that the production of fresh and dry leaves was higher in the 2nd cuttings. However, fresh and dry herb weights were higher at the 1st cuttings. As a result, the superiority of the leaf ratio is justified in the 2nd cuttings.

Volatile Oil Content and Chemical Components

Differences between the volatile oil content of various nutrient sources application treatments were significant. The highest volatile oil content belonged to NPK application treatment. Vermicompost, microbiological fertilizer types (jointly), and control treatments followed NPK application, respectively. Also, the 1st cuttings produced more volatile oil compared to the 2nd cuttings. The prominence of the 1st cuttings on volatile oil content showed its dominance on the interaction effects of the treatments. Thus, the maximum and minimum amounts of volatile oil were obtained in the application of NPK at 1st cutting and the application of microbiological II at 2nd cutting (Table 5). The essential oils of medicinal plants are affected by many endogenous and exogenous factors. On the other hand, the essential oil is a terpenoid. Its constituent units, such as isopentenyl pyrophosphate and dimethylallyl pyrophosphate, are in dire need of NADPH and ATP. The presence of nitrogen is necessary for the formation of these compounds. Nutrient sources such as nitrogen, phosphorus, vermicompost, and nitrogen-fixing bacteria are needed to speed up biosynthesis and the volume of volatile oils (Govahi et al., 2015). As can be seen in the results, the consumption of these fertilizers increased the percentage of volatile oil (Table 5). Various studies had reported an increase in essential oils due to different nutrient sources application (Manukyan, 2011; Abaas, 2014; Ghoushchi et al., 2015; Govahi et al., 2015; Sönmez and Bayram, 2017; Hegab et al., 2018). Nevertheless, increasing the N dose, the production of essential oil decreased (Manukyan, 2011; Ghoushchi et al., 2015). In another study on *S. officinalis*, the effect of biofertilizer application on essential oil content and its components was not evaluated positively (Nadjafi et al., 2014).

As the results of the chromatographic analysis of the chemical compounds of all *S. officinalis* samples, 31 compounds were identified in total (Table 6). Monoterpene hydrocarbons had the highest number of chemical compounds (12 compounds) in all treatments. Oxygenated monoterpenes (6 compounds), oxygenated sesquiterpenes (5 compounds), sesquiterpene hydrocarbons (4 compounds), esters (1 compound), and aldehydes (1 compound) also formed the next groups in terms of the number of chemical compounds, respectively. The amounts of monoterpene hydrocarbons had the highest percentage of compounds (67-74%). Apart from microbiological fertilizer II, other treatments of 2nd cutting were relatively superior in producing oxygenated monoterpenes. In both cuttings, the highest amounts of oxygenated monoterpenes were observed in vermicompost utilization. Monoterpene hydrocarbons, oxygenated sesquiterpenes, sesquiterpene hydrocarbons, and esters were higher in most of the 1st cutting treatments. Ketone compound was not observed in control, NPK, and vermicompost treatments of 2nd cutting. Nonanal was the only aldehyde compound presented in 2nd cuttings. α-Thujone (22.4-31.11%) and Camphor (21.04-25.40%) were the most major compounds. In both cuttings, α-Thujone content was higher in the NPK and vermicompost treatments than the others. Also, in the 2nd cuttings, the amounts of this constituent were somewhat higher. According to the ISO standards, 18-43% of α-thujone, 3-8.5% of β-thujone, and 4.5-24.5% of Camphor are allowed (ISO, 1997). Also, based on the German Drug Codex, the Thujones should be equal or more than 20.0%, and the Camphor should be about 4.5-24.5% (Bahtiyarca Bagdat et al., 2017). The amount of Camphor in NPK treatments of both cuttings was lower than the others. The other major chemical compounds were 1,8-Cineole, β-Thujone, Camphene, Viridiflorol, α-Pinene, α-Humulene, Borneol, (-)-bornyl acetate, Limonene, Caryophyllene, and β-Pinene. Among all the compounds identified, Nonanal was observed only in all treatments of the 2nd cuttings. α-thujone (13.25-40.37%), Camphor (13-21.15%), 1,8-cineole (7.54-19.48%), and β-thujone (5.27-8.12%) were reported as the main constituents of *S. officinalis* (Mossi et al., 2011). According to Zawiślak (2014), the main components of the *S. officinalis* essential oil were: 1,8-cineole (16.08-18.04%), α-Thujone (10.40-21.51%), and Camphor (5.24-18.08%). The researcher noted the variety of essential oil components depending on the harvest time. Current findings on the chemical constituents of *S. officinalis* volatile oil are in line with several previous investigations (Bettaieb et al., 2009; Mossi et al., 2011; Govahi et al., 2015; Sönmez and Bayram, 2017). Numerous factors affect the quality of the volatile oil, which are hard to be determined and distinct from each other. Production of secondary metabolites and their qualities in medicinal and aromatic plants are directly related to various factors such as genetic characteristics, climatic conditions (light, temperature, rainfall, irrigation, soil, height, location, etc.), environment organisms, applied agro-techniques and post-harvesting processing (Soltanbeigi and Sakartepe, 2020). Besides, the presence of water (hydrolysis), atmospheric gases (oxidation), storage time, ambient temperature, and light (reverse oxidation) affect the concentration of volatile oil components. These changes materialize due to conversion and/or evaporation of the compounds caused by their molecular weight, molecular structure, and boiling point (Soltanbeigi, 2020).

Table 6. Volatile oil compounds of common sage affected by various nutrient sources in greenhouse

RI	Compounds (%)	1st Cutting					2nd Cutting				
		C	NPK	Verm.	M_1	M_2	C	NPK	Verm.	M_1	M_2
1019	Tricyclene	0.18	0.14		0.16	0.16			0.16	0.15	0.17
1031	α-Pinene	4.09	3.87	2.74	2.95	3.25	3.67	2.83	3.37	2.8	2.92
1072	α-Thujene	0.17	0.2	0.18	0.19	0.19	0.19	0.18	0.17	0.18	0.14
1076	Camphene	4.36	4.7	4.71	4.46	5.2	4.73	4.06	5.1	5.14	5.36
1120	β-Pinene	1.91	2.04	1.98	2.11	2.04	1.9	2.3	2.07	2.01	1.89
1130	Sabinene		0.18		0.14						
1167	β-Myrcene	1.03	1	0.99	1.06	0.96	0.88	0.8	0.72	0.87	0.66
1190	α-Terpinene	0.18	0.22	0.19	0.21	0.24	0.21	0.2	0.19	0.22	0.17
1209	Limonene	2.25	2.1	2.15	2.43	2.17	1.95	1.54	1.57	1.98	1.71
1219	1,8-Cineole	11.57	11.62	11.56	11.85	11.76	11.85	12.95	13.34	10.73	10.99
1254	γ-Terpinene	0.39	0.45	0.38	0.43	0.48	0.41	0.41	0.34	0.42	0.33
1281	o-Cymene	0.25	0.24	0.25	0.28	0.25	0.39	0.38	0.38	0.41	0.35
1292	α-Terpinolen	0.59	0.58	0.57	0.64	0.57	0.38	0.31	0.27	0.37	0.3
1402	Nonanal						0.58	0.38	0.42	0.51	0.53
1435	α-Thujone	26.34	28.21	28.01	24.38	22.4	28.65	30.77	31.11	28.7	24.46
1454	β-Thujone	3.88	5.01	4.9	5.55	9.13	4.19	4.97	4.33	5.92	7.44
1469	cis-β-Terpineol	0.23	0.19	0.27	0.2			0.21			0.16
1530	Camphor	24.77	21.66	24.87	24.81	24.38	25.32	21.04	25.2	25.41	24.8
1550	Linalool	0.33	0.32	0.34	0.36	0.37	0.31	0.26	0.32	0.29	0.36
1553	cis-Sesquisabinene hydrate	0.2	0.17	0.23	0.18			0.18			
1590	(-)-bornyl acetate	2.7	2.45	2.69	2.92	2.83	1.85	1.72	1.78	1.94	1.92
1608	Caryophyllene	2.41	2.03	2.22	2.09	1.63	2.02	1.87	1.3	1.74	1.66
1659	Sabinyl acetate	0.23	0.24	0.23	0.17	0.23	0.21				
1681	α-Humulene	3.91	3.39	2.8	3.48	2.87	3.04	3.59	1.8	2.61	3.18
1698	γ-Muurolene	0.43	0.46	0.49	0.44	0.7	0.56	0.63	0.53	0.44	0.68
1703	α-Terpineol	0.27	0.23	0.28	0.26	0.27		0.22	0.26	0.24	0.27
1709	Borneol	2.49	2.5	2.73	3.15	3.04	2.42	2.44	3.09	2.92	3.65
1995	Caryophyllene oxide	0.17	0.16	0.17	0.17	0.17	0.34	0.31	0.19	0.27	0.36
2053	Humulene epoxide II	0.39	0.35	0.33	0.37	0.43	0.6	0.76	0.34	0.48	0.74
2076	Acetophloroglucine	0.28	0.18	0.26	0.28	0.23				0.17	0.13
2093	Viridiflorol	3.86	4.37	3.27	3.29	3.88	3.34	4.54	1.64	3.07	4.08
Number of identified compounds		29	30	28	30	27	25	27	26	27	28
Grouped compounds (%)											
Oxygenated monoterpenes (6 compounds)		67.11	67	69.95	67.15	68.03	70.33	70.2	74.31	71.04	68.21
Monoterpene hydrocarbons (12 compounds)		15.4	15.73	14.12	15.06	15.49	14.69	13.03	14.34	14.55	14
Oxygenated sesquiterpenes (5 compounds)		7.11	7.54	6.73	7.16	7.53	6.71	8.22	5.26	6.74	8.83
Sesquiterpene hydrocarbons (4 compounds)		7.02	6.12	5.8	6.27	5.47	5.64	6.31	3.9	5.05	5.79
Esters (2 compounds)		2.93	2.69	2.92	3.09	3.06	2.06	1.72	1.78	1.94	1.92
Ketones (1 compound)		0.28	0.18	0.26	0.28	0.23				0.17	0.13
Aldehydes (1 compound)							0.58	0.38	0.42	0.51	0.53

RI: Retention indices calculated against n-alkanes (C7-C30) on HP-Innowax column
C: Control; NPK: Chemical fertilizer; Verm: Vermicompost; M_1: Microbiological fertilizer I; M_2: Microbiological fertilizer II

Conclusion

The application of NPK, vermicompost and microbiological fertilizers improved the agronomic yield and volatile oil characteristics of *S. officinalis*. Based on the results, the application of NPK as chemical fertilizers was more effective than other nutrient sources. The vermicompost application partially compensated for the lack of nutrients in the soil for plant nutrition but could not compete with chemical fertilizers. The microbiological fertilizers appeared less than expected. The relative inefficiency of microbiological fertilizers may be due to the inadequacy of these microorganisms in soils with low organic matter content, like the soil used in this experiment. Another reason is probably related to species of microorganisms and plants that cannot coexist sustainability. It can be suggested that the exact selection of microbiological fertilizers types and the combination of vermicompost and microbiological fertilizers can fully supply the nutritional needs of plants.

Compliance with Ethical Standards

a) Authors' Contributions

AS: Study design, collection of data, statistical analysis and interpretation of results, performing laboratory analyzes and manuscript drafting;

MY: Interpretation of results and manuscript drafting;

ES: Study design, collection of data, performing laboratory analyzes and manuscript drafting.

b) Conflict of Interest

The authors declare that there is no conflict of interest.

c) Statement on the Welfare of Animals

Welfare of Animal is not limited in this study.

d) Statement of Human Rights

This study does not involve human participants.

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