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Original Article

Chemical Variation in the Essential Oil of *Salvia leriifolia* Benth. in Response to Organic and Biological Fertilizers

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Abstract

In order to study the effect of application of organic and biological fertilizers on leaf dry weight (LDW), essential oils content (EO), and composition of *Salvia leriifolia* Benth., a factorial experiment was carried out based on randomized complete block design with three replications and 10 treatments. The experiment was performed at Agricultural and Natural Resources Research and Education Center, Mashhad- Iran, during two growing seasons (2014-2015 and 2015-2016). The treatments consisted of five levels of organic fertilizers [control (C_0), compost 10 ton/ha (C_{10}) compost 20 ton/ ha(C_{20}), vermicompost three ton/ ha(V_3) and vermicompost six ton/ ha(V_6)] and *Pseudomonas putida* biofertilizer in two levels [Inoculation (S_1) and without inoculation (S_0)]. The results indicated that the highest LDW (1470 kg/ha) in the second year was obtained in $C_{20}S_1$, which increased by 47%, compared to C_0S_0, respectively. The EO in both growing seasons was not affected by the treatments. However, the application of 20 ton/ haof compost caused the EO yield was significantly higher than other treatments, except V_6. A significant increase in LDW caused the highest EO yield of 5.0 and 5.9 kg/hawas obtained in $C_{20}S_0$ treatment in first and second growing seasons, respectively, which increased by 60% compared to the control (C_0S_0). The results showed that out of 99.9% of EO compounds, 95.6% including 33 identified compounds and only 4.2% contained other compounds. The valuable compounds of β-pinene (26.3%), 1,8-cineole (14.8%), α-pinene (13.8%) and Borneol (4.8%) have the highest percentage of EO compounds in the studied treatments. Organic fertilizer treatments had no significant effect on the four main compounds of *S. leriifolia* EO but increased Guaiol, Epi-α-eudesmol and δ-cadinol compared with control. The highest percent of Guaiol (2.3) and epi-α- eudesmol (3.2) were obtained in V_6S_1, respectively, which increased by 68% and 178% compared with control. Also, bacterial inoculation showed a significant difference with non-inoculation in Guaiol and epi-α- eudesmol using V_6S_1 treatment. Despite the increase in the percentage of these compounds, some of the EO compounds significantly decreased with the application of vermicompost treatments. Accordingly, Sabinene, epi-α-bisabolol compounds in V_6S_0 were decreased by 116% and 52%, respectively, and Terpine-4-ol and *cis*-sabinol compounds in V_3S_0 decreased by 21% and 19%, respectively, compared with control. Finally, the application of organic fertilizers had different effects on the *S. leriifolia* EO components, but the percent and amounts of all four main components were not affected by treatments.

Keyword: *Salvia leriifolia*, Compost, Vermicompost, *Pseudomonas putida*, Essential oil components

Introduction

The genus Salvia is the largest genus of plants in the Lamiaceae family, includes approximately 900 species spread throughout the world, some of which are economically important, and they have been used as spices in food industry and flavoring agent in cosmetic industry and aromatherapy [1,2].

Salvia leriifolia Benth. (with vernacular name of Noroozak) is an endemic and perennial herbaceous plant

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species of Iran, growing in rocky mountains and desert rangelands in cold-semi arid to cold-arid climate, exclusively in south regions of Khorasan razavi and Semnan provinces in Iran, and also small parts of Afghanistan [3]. Various medicinal properties of *S. leriifolia* such as antibacterial, anti-inflammatory, antioxidant, anti-ischemia, anti-convulsion, anti-ulcer and hypoglycemic were evaluated [4]. The early onset of vegetative and reproductive growth and drought and cold tolerance of *S. leriifolia* indicated that this plant could be adapted, considering its medicinal and oil qualities, especially in arid and cold zones. On the other hand, *S. leriifolia* is an endangered species, facing a very high risk of extinction, so it seems that sustainable exploitation along with domestication are important ways of conserving *S. leriifolia* habitats [5].

Nutrition is an important factor that increases both plant productivity and the concentrations of bioactive compounds, thereby improving the quality of medicinal and aromatic plants [6]. Application of mineral fertilizers during large period has been leaded to the environmental pollution particularly in water and soil that threaten human society. Sustainable agriculture based on using organic and biological fertilizers is an effective solution for overcoming these problems [7]. The production of compost can be considered as an appropriate management method for making municipal solid waste (MSW) into valuable organic fertilizer, which is an appropriate tool for reducing the use of mineral fertilizers and more absorption of nutrient, especially micro elements by plants under calcareous soils conditions.

Vermicomposts have distinctive features of their own in comparison to typical compost. They contain considerably much finer and favorable structure and contain nutrients in easily access forms by plants [8]. The inoculation of biological fertilizers has been reported to improve the quality and quantity of several medicinal herbs, including Black cumin [9], Thyme and Marjoram [10]. *P. putida* bacteria is one of the most important phosphate solubilizing microorganisms (PSM) that increase the overall performance of plants by providing mainly soluble P to plants in different production systems [11]. Fatma *et al*. [12] reported that the use of biofertilizers including PSM had a significant effect on growth indices and EO content of Marjoram (*Majorana hortensis*). The biosynthesis of secondary metabolites in medicinal and aromatic plants are strongly influenced by environmental factors, harvest time and genetic characteristics [13, 14]. Several reports have been published on the study of the diversity of chemical compositions of EO of different species of sage in Iran [15, 16]. Some researchers showed that the application of organic fertilizers can increase the EO yield and main components of *Ocimum basilicum* L. [17], *Anethum graveolens* L. [18] and *Mentha piperita* L. [19]. Alizadeh *et al*. [20] showed that using different amounts of complete chemical fertilizer (macro and micro elements) in *Satureja hortensis* caused a very slight and non-significant change in EO composition, but the amount of some component such as carvacrol, δ-terpinene and α-terpinene changed.

Since the application of organic and biological fertilizers proved to be very important and essential in reducing the use of chemical fertilizers and help agricultural sustainability and environmental preservation, this study was conducted to find the effects of appropriate levels of MSW compost and vermicompost on EO content and composition of *S. leriifolia* in the presence of two levels of *P. putida* strain.

Material and Methods

A field experiment was carried out for two growing seasons (2014-2015 and 2015-2016) at Khorasan–e-Razavi Agricultural and Natural Resources Research and Education Center, Mashhad-Iran. The experimental farm located at latitude 36º 13´N, longitude 59 º40´ E, and at an altitude of 980 m above the sea level with semi-arid climate, mean annual precipitation of 254.2 mm, mean annual temperature of 14.5 °C and the average temperature in the cold (winter) and warm (summer) seasons of -1.8 °C and 33 °C, respectively. Preparing the seedbed was done by plowing, two perpendicular discs, and leveling in November 2014. Experimental plots had not received any fertilizer treatments in previous years. Soil samples were collected from the depth of 0-60 cm. Organic fertilizers treatments including MSW compost and vermicompost were produced in the Municipality Recycling Factory of Mashhad. The analysis of soil chemical and physical properties as well as organic fertilizers are presented in Table 1 and 2.

P. putida bacteria with population density of 1×10^9 cells per gram of inoculum obtained from Tehran Institute of Water and Soil Research. Two months before seed sowing, the organic fertilizers were completely mixed into soil. The mature seeds of *S. leriifolia* were cultivated in early March 2015 at 25x50 cm and immediately irrigated. At the end of the first and second growing seasons, one square meter of each experimental plot was harvested and whole plants transferred to the lab for measuring quantitative and qualitative traits. The treatments consisted of five levels of organic fertilizers [control (C_0), compost 10 ton/ha (C_{10}) compost 20 ton/ha (C_{20}), vermicompost three ton/ha (V_3), vermicompost six ton/ha(V_6)] and *P. putida* biofertilizer in two levels [Inoculation (S_1) and no inoculation (S_0)]. A factorial field experiment was conducted based on a randomized complete block design (RCBD) with three replications. Data were analyzed using SAS 9.0 and MSTAT-C statistical software and the means were compared by LSD at 0.05% significant level method.

Essential Oil Isolation

Air-dried leaves of the *S. leriifolia* were ground in a grinder and 100 g of powder were subjected to hydro-distillation for 3 hours using a Clevenger-type apparatus based on British Pharmacopoeia [21]. The EO yield was calculated relative to the dry matter (w/w). The obtained EO were dried over anhydrous sodium sulfate and stored in dark vials at low temperature (4 °C) until tested and analyzed.

Gas Chromatography (GC)

GC analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m X 0.25 mm i.d., film thickness 0.25 µm). Oven temperature was started at 60 °C and then programmed to 210 °C at a rate of 3 °C/min. and finally the temperature was increased from 210 °C to 240 °C at a rate of 20 °C/ min and was held at this temperature for 8.5 min. Detector (FID) temperature was 280 °C and injector temperature 300°C helium was used as carrier gas . GC-MS analyses were carried out in a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m X 0.25 mm i.d.). Oven temperature was 60–250 °C at a rate of 3 °C, transfer line temperature 270 °C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, ionization energy 70 eV; scan time 1 s and mass range of 40–340 amu.

The compounds percent were calculated by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices (RIs), either with those of authentic compounds, or with data published in the literature [22,23].

Results and Discussion

Leaf Dry Weight (LDW)

The results of variance analysis indicated that LDW was significantly (P <0.01) affected by fertilizer treatments in both growing seasons. MSW compost application at 20 ton/ha (C_{20}) increased LDW but other fertilizer treatments did not show any significant difference with control. Seed inoculation with *P. putida* increased significantly (P≤ 0.05) LDW in the second year by 12 percent compared with non-inoculated plants but its interaction with other fertilizer treatments was not significant in both growing seasons (Table 3). Results also indicated that LDW in the first year (1393 kg/ha) increased by 69.5 percent in $C_{20}S_0$ comparing with C_0S_0 treatment. The highest LDW in the second growing season (1470 kg/ha) was obtained in $C_{20}S_1$ which showed 47 percent increase compared to C_0S_0, but there was not significant difference with $C_{10}S_1$ and V_6S_1 (Table 3). These results were in correspondence with those concluded by Darzi and Sadeghi Nikoo [24] on Hyssop, indicating that the highest plant dry weight was obtained in 10 ton/ haof compost with 6 ton/ haof vermicompost and biofertilizer. Zariri *et al*. [25] also concluded that application of 10 t/ha of compost or vermicompost increased the dry matter yield of the peppermint plants by 28 percent compared with chemical fertilizers.

It seems that the insignificant effect of *P. putida* inoculation on plant growth characteristics in the first year is due to the lesser chance of phosphate decomposition by *pseudomonas* bacteria and, consequently, the small amount of soluble phosphorus in the soil. The release of nutrients and their use by bacteria, in the second growing season, increased the rate of phosphorus uptake by the plants, and consequently higher plant leaf area and leaf dry weight [26].

Table 1 Physical and chemical properties of soil before cultivation

Soil depth (Cm)	Ec (dS/m)	pH v	Sand (%)	Silt (%)	Clay (%)	Organic carbon (%)	N (%)	P (mg/ kg)	K (mg/ kg)	Fe (mg/ kg)	Mn (mg/ kg)	Zn (mg/ kg)	Cu (mg/ kg)	Textural class
0-30	1.6	7.8	41	36	23	0.48	0.09	10	219	3.8	5.4	0.56	0.96	Loam
30-60	1.4	8.0	43	34	23	0.19	0.04	4.4	69	5.9	3.3	0.12	0.56	Loam

Table 2 Chemical analysis of organic fertilizers used in this experiment

Organic Fertilizers	EC (dS/m)	pH	N (%)	Organic Carbon (%)	Ash (%)	P (mg/ kg)	K (mg/ kg)	Fe (mg/ kg)	Mn (mg/ kg)	Zn (mg/ kg)	Cu (mg/ kg)
MSW Compost	7.7	7.8	1.4	14.2	75	4300	4500	155	195	159	225
Vermicompost	5.0	7.6	1.7	22	62	3400	2900	155	210	55	

Table 3 Effects of organic fertilizers and *P. putida* on leaves dry weight, Essential oil content and essential oil yield of *S. leriifolia* Benth. in two growing seasons.

Treatments	leaves dry weight (Kg/ha)		Essential oil content (%)		Essential oil yield (Kg/ha)	
	First Season	Second Season	First Season	Second Season	First Season	Second Season
Organic fertilizers						
Control	742 ± 72	1050 ± 41	0.38 ± 0.03	0.37 ± 0.03	2.84 ± 0.42	3.86 ± 0.40
C_{10}	898 ± 83	1200 ± 43	0.40 ± 0.02	0.37 ± 0.01	3.54 ± 0.32	4.47 ± 0.16
C_{20}	1284 ± 96	1434 ± 82	0.37 ± 0.02	0.41 ± 0.01	4.72 ± 0.36	5.92 ± 0.29
V_3	868 ± 75	1078 ± 60	0.39 ± 0.03	0.39 ± 0.03	3.40 ± 0.44	4.29 ± 0.52
V_6	889 ± 51	1218 ± 102	0.42 ± 0.03	0.40 ± 0.02	3.75 ± 0.39	4.96 ± 0.61
LSD (0.05)	200	191	0.08	0.08	1.1	1.3
P. putida						
S_0	969 ± 74	1127±58	0.39 ± 0.02	0.39 ± 0.02	3.81 ± 0.32	4.40 ± 0.32
S_1	903 ± 59	1264±45	0.39 ± 0.01	0.39 ± 0.01	3.49 ± 0.24	4.99 ± 0.29
LSD (0.05)	127	121	0.05	0.05	0.7	0.08
Interactions						
C_0S_0	822 ± 124	1005 ± 67	0.36 ± 0.06	0.37 ± 0.07	3.04 ± 0.86	3.77 ± 0.87
C_0S_1	661 ± 65	1096 ± 43	0.40 ± 0.02	0.36 ± 0.02	2.64 ± 0.31	3.95 ± 0.14
$C_{10}S_0$	961 ± 158	1165 ± 32	0.39 ± 0.02	0.37 ± 0.01	3.77 ± 0.65	4.25 ± 0.16
$C_{10}S_1$	836 ± 73	1233 ± 42	0.40 ± 0.03	0.38 ± 0.02	3.32 ± 0.21	4.69 ± 0.13
$C_{20}S_0$	1393 ± 109	1400 ± 34	0.36 ± 0.05	0.42 ± 0.03	4.98 ± 0.52	5.89 ± 0.48
$C_{20}S_1$	1175 ± 148	1470 ± 101	0.38 ± 0.01	0.40 ± 0.01	4.46 ± 0.57	5.95 ± 0.42
V_3S_0	782 ± 109	1010 ± 172	0.39 ± 0.06	0.39 ± 0.05	3.03 ± 0.56	3.98 ± 1.11
V_3S_1	955 ± 94	1150 ± 108	0.39 ± 0.03	0.40 ± 0.03	3.77 ± 0.72	4.60 ± 0.80
V_6S_0	887 ± 60	1060 ± 87	0.47 ± 0.04	0.39 ± 0.02	4.24 ± 0.62	4.14 ± 0.27
V_6S_1	890 ± 98	1377 ± 26	0.37 ± 0.03	0.41 ± 0.02	3.26 ± 0.35	5.78 ± 0.15
LSD (0.05)	283	270	0.11	0.11	1.6	1.9

Data represent the mean ± SE of three replicates

Table 4 Analysis of variance and mean squares of essential oil components (%) affected by organic fertilizers and *P. putida* in *S. leriifolia* Benth. in second growing season

S.O.V	df.	α-pinene	β -pinene	1,8-Cineole	Borneol	Epi-α-eudesmol	Guaiol	Bulnesol	α-bisabolol	Epi-α-bisabolol	Terpine4-ol
Block	2	0.034 ^{ns}	0.01 ^{ns}	7.06 ^{ns}	0.003 ^{ns}	0.31 ^{ns}	0.27 ^{ns}	0.08 ^{ns}	0.07 ^{ns}	0.05 ^{ns}	0.001 ^{ns}
Fertilizers (F)	4	2.20 ^{ns}	7.30 ^{ns}	4.08 ^{ns}	0.48 ^{ns}	3.11 ***	1.01 **	0.25 ^{ns}	0.30 ^{ns}	0.92 **	0.07 ***
P. putida (P)	1	0.92 ^{ns}	6.59 ^{ns}	0.003 ^{ns}	0.02 ^{ns}	1.06 ^{ns}	0.20 ^{ns}	0.55 *	0.52 *	0.16 ^{ns}	0.01 ^{ns}
(F*P)	4	2.27 ^{ns}	5.40 ^{ns}	3.79 ^{ns}	0.28 ^{ns}	0.55 ^{ns}	0.58 *	0.12 ^{ns}	0.16 ^{ns}	0.27 ^{ns}	0.05 **
Error	18	0.60	2.97	2.38	0.30	0.37	0.17	0.12	0.11	0.14	0.008
CV(%)		5.6	6.5	10.4	11.4	21.3	28.9	23.3	21.3	25.2	5.2

ns , *, ** , *** non-significant and significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 4 Continued

S.O.V	df.	Camphene	Juniper camphor	Myrtenol	*cis*-sabinol	δ-cadinene	Sabinene	δ-cadinol	p-cymene	α-gurjunene
Block	2	0.006 ^{ns}	0.14 ^{ns}	0.001 ^{ns}	0.001 ^{ns}	0.001 ^{ns}	0.007 ^{ns}	0.002 ^{ns}	0.02 ^{ns}	0.03 ^{ns}
Fertilizers (F)	4	0.14 **	0.31 ^{ns}	0.093 *	0.007 **	0.52 **	0.08 **	0.58 **	0.08 ^{ns}	0.04 *
P. putida (P)	1	0.04 ^{ns}	0.94 *	0.001 ^{ns}	0.001 ^{ns}	0.02 ^{ns}	0.16 **	0.006 ^{ns}	0.20 *	0.02 ^{ns}
(F*P)	4	0.15 **	0.18 ^{ns}	0.052 ^{ns}	0.006 **	0.26 *	0.04 ^{ns}	0.17 ^{ns}	0.09 ^{ns}	0.02 ^{ns}
Error	18	0.02	0.18	0.03	0.001	0.08	0.01	0.09	0.04	0.01
CV(%)		7.3	17.0	29.4	5.2	25.0	11.9	29.0	18.0	8.5

ns , *, ** , *** non-significant and significant at 0.05, 0.01 and 0.001 probability levels, respectively

Some investigators reported that by inoculation of PSM, the rate of absorption of nutrients was faster than that of non-inoculated plants, and also the accumulation of N, P, and K elements in leaves, increased the growth and yield of medicinal plants [27, 28]. Similar results have been reported on four-year-old *S. leriifolia* [29], *Nigella sativa* [9] and *Foeniculum vulgare* [30].

Essential Oil Content (%)

The percentage of EO in both growing seasons was not affected by organic fertilizers and *P. putida*. Application of organic fertilizers, especially in the second year, resulted in an increase in the EO content of *S. leriifolia*, but this difference was not significant (Table 3). Mean comparison among fertilizers treatments revealed that the highest EO content in the first year (0.47%) and second year (0.42%) of growth were obtained of V_6S_0 and $C_{20}S_0$ treatments, respectively (Table 3).

Table 5 Effects of organic fertilizers and *P. putida* on essential oil components (%) of *S. leriifolia* Benth. in second growing season

Treatments	α-pinene	β -pinene	1,8-Cineole	Borneol	Epi-α-eudesmol	Guaiol	Bulnesol	α-bisabolol	Epi-α-bisabolol	Terpine4-ol
Organic fertilizers:										
Control	14.20 ab	27.26 a	14.39 ab	5.15 a	1.09 b	1.34 b	1.53 ab	1.66 a	1.98 a	1.95 a
C10	14.63 a	27.60 a	14.92 ab	4.72 ab	1.22 b	1.42 ab	1.25 b	1.64 ab	1.76 a	1.70 c
C20	13.80 abc	26.08 ab	15.14 ab	4.73 ab	2.36 a	1.89 a	1.35 ab	1.52 ab	1.25 b	1.82 b
V3	13.43 bc	25.56 ab	13.87 b	5.02 ab	2.36 a	0.83 c	1.77 a	1.86 a	1.20 b	1.69 c
V6	13.10 c	25.00 b	16.06 a	4.43 b	2.23 a	1.73 ab	1.61 ab	1.25 b	1.09 b	1. 85 ab
LSD (0.05)	1.11	2.09	1.87	0.66	0.74	0.51	0.42	0.41	0.45	0.11
P. putida										
S0	13.66 a	25.83 a	14.87 a	4.78 a	1.74 a	1.36 a	1.64 a	1.72 a	1.53 a	1.82 a
S1	14.00 a	26.77 a	14.89 a	4.84 a	2.12 a	1.52 a	1.37 b	1.45 b	1.38 a	1.79 a
LSD (0.05)	0.59	1.32	1.19	0.42	0.47	0.32	0.27	0.26	0.28	0.07

Table 5 Continued

Treatments	Camphene	Juniper camphor	Myrtenol	*cis*-sabinol	δ-cadinene	Sabinene	δ-cadinol	p-cymene	α-gurjunene
Organic fertilizers:									
Control	2.18 a	2.21 b	0.23 a	0.53 a	0.99 a	1.16 a	0.68 c	1.24 a	1.33 ab
C10	2.32 a	2.38 ab	0.20 ab	0.48 b	1.05 a	0.99 bc	0.80 bc	1.09 ab	1.21 b
C20	2.13 a	2.48 ab	0.20 ab	0.52 a	0.93 a	1.09 ab	1.07 b	1.05 ab	1.38 a
V3	2.18 a	2.70 ab	0.35 a	0.48 b	0.32 b	0.89 c	1.48 a	0.94 b	1.43 a
V6	1.90 b	2.75 a	0.00 b	0.56 a	0.75 a	0.91 c	1.14 ab	0.99 b	1.36 a
LSD (0.05)	0.19	0.52	0.21	0.03	0.35	0.14	0.37	0.23	0.14
P. putida									
S0	2.10 a	2.68 a	0.19 a	0.51 a	0.84 a	0.93 b	1.05 a	0.98 b	1.37 b
S1	2.18 a	2.32 b	0.20 b	0.52 a	0.78 a	1.08 a	1.02 a	1.14 a	1.32 a
LSD (0.05)	0.12	0.33	0.13	0.02	0.22	0.09	0.23	0.15	0.09

Table 6 Analysis of variance and mean squares of some essential oil components (μg/gr leaf dry weight) affected by organic fertilizers and *P. putida* in *S. leriifolia* Benth. in second growing season

S.O.V	df.	α-pinene	β -pinene	1,8-Cineole	Borneol	αcadinol	δ-cadinene	Sabinene	δ-cadinol	Epi-α-eudesmol	Guaiol
Block	2	2580^{ns}	9783^{ns}	9764^{ns}	443^{ns}	30.1^{ns}	11.0^{ns}	22.6^{ns}	32.1^{ns}	544^{ns}	491^{ns}
Fertilizers (F)	4	2652^{ns}	7046^{ns}	17398^{ns}	510^{ns}	138^{**}	864^{***}	109^{ns}	1127^{**}	5898^{***}	1805^{**}
P. putida (P)	1	2146^{ns}	13842^{ns}	268^{ns}	48^{ns}	36^{ns}	7.21^{ns}	201^{*}	2.23^{ns}	2059^{ns}	315^{ns}
(F*P)	4	1124^{ns}	1638^{ns}	8108^{ns}	459^{ns}	52^{ns}	382^{*}	87^{ns}	247^{ns}	1237^{ns}	992^{ns}
Error	18	9208	33497	13071	1573	28	118	54	184	710	328
CV(%)	17.8	17.8	17.9	19.7	21.2	21.9	24.9	18.5	28.2	27.2	-

ns , *, ** , *** non-significant and significant at 0.05, 0.01 and 0.001 probability levels, respectively

Table 7 Effects of organic fertilizers and *P. putida* on some essential oil components (µg/grleaf dry weight) of *S. leriifolia* Benth. in second growing season

Treatments	α-pinene	β - pinene	1,8-Cineole	Borneol	Epi-α-eudesmol	Guaiol	Bulnesol	α-bisabolol	Epi-α-bisabolol	Terpine4-ol
Organic fertilizers:										
Control	521 a	1000 a	526 a	188 a	19 b	36 a	42 ab	25 b	40 b	49 bc
C10	546 a	1020 a	557 a	177 a	20 b	39 a	39 ab	27 b	46 b	53 bc
C20	573 a	1082 a	629 a	196 a	28 a	39 a	45 a	44 a	98 a	78 a
V3	528 a	1003 a	545 a	197 a	24 ab	11 b	35 b	58 a	92 a	34 c
V6	527 a	1005 a	647 a	179 a	29 a	30 a	37 ab	46 a	108 a	70 ab
LSD (0.05)	116	222	139	48	6	13	9	16	32	22
P. putida										
S0	530 a	1003 a	578 a	186 a	25 a	31 a	37 b	40 a	68 a	54 a
S1	547 a	1046 a	583 a	189 a	22 a	30 a	42 a	40 a	85 a	60 a
LSD (0.05)	73	140	88	30	4	8	5	10	20	14

The findings are consistent with those of Aghhavani Shajari *et al*. [31] who found that application of organic fertilizers have no positive effect on the *Coriandrum sativum* L. EO content. However, increases in the EO content following the application of bio and organic fertilizer has been reported in *Ocimum basilicum* L. [32], *Dracocephalum moldavica* L. [33] and *Pimpinella anisum L.* [34] and *Majorana hortensis* L. [12].

Results also showed that *P. putida* inoculation and its interaction with organic fertilizers had no significant effect on EO content (Table 3). Our findings are in agreement with previous studies on Cumin plants [35] and Fennel [36]. However, increase of EO content by application of phosphate solubilizing bacteria has been reported in Basil [37] and Fennel [38].

Essential Oils Yield

EO yield as a function of EO percentage and LDW significantly affected by organic fertilizers in both growing seasons, but application of *P. putida* and its interaction with organic fertilizers were not significant. LDW increasing in $C_{20}S_0$ treatment in the first year of growth caused EO yield to increase by 64% compared to control (C_0S_0). Maximum LDW of C_20S_1 in the second growing season also resulted in the highest EO yield of 5.95 Kg/ha, which was 58% increase compared to no fertilizer treatment, but this increase was significant only with control and V_3S_0 treatments (Table 3). Kiani *et al*. [39] concluded that the highest percentage and yield of EO on peppermint (*Mentha spicata* L.) were observed in vermicompost treatment and had a significant difference compared to control. Khalesro *et al.* [40] also reported that EO yield in anise plants increased by application of 10 ton/ havermicompost. Moradi [36] found that the effects of compost and vermicompost fertilizers as well as *P. putida* on Fennel (*Foeniculum vulgare* L.) increased EO yield. Similar results was observed on Coriander [31] and Basil [17]. Essential Oil Composition

The compositions percentage and retention indices (RIs) of the leaf EO of *S. leriifolia* under different organic and biological fertilizer treatments in the first flowering year are summarized in Table 8. The results showed that from 99.9% of EO compounds, 95.6% including 33 identified compounds and only 4.3% of other compounds. B-pinene (26.3%), 1,8-cineole (14.9%), α-pinene (13.8%) and Borneol (4.8%) had the highest mean of EO composition.

The identified compounds were classified into four different groups so that the monoterpene hydrocarbons with 10 compounds comprised the highest mean EO percentage (47.7%). The other EO groups including the oxygenated monoterpenes with six compounds (22.5%), Sesquiterpene hydrocarbons with five compounds (6.3%) and oxygenated terpenes with 12 compounds (19.1%) (Table 8). Similar major EO compositions of *S. leriifolia* from natural habitats have also been reported by Yousefi *et al*. [2].

Results of analysis of variance showed that 11 EO components were affected by organic fertilizer treatments. Accordingly, Myrtenol and α-gurjunene at 5% statistical level (P≤0.05), Camphene, epi-α-Bisanolol, Guaiol, δ-cadinol, Sabinene, δ-cadinene, and *cis*-sabinol (P≤0.01), Terpine-4-ol and epi-α- eudesmol (P≤0.001) showed a significant difference in different levels of organic fertilizers (Table 4). Application of *P. putida* biofertilizer significantly (P≤0.05) affected five EO compositions including Juniper camphor, α-bisabolol, Bulnosol, p-cymene and Sabinene. The results also indicated that interaction of organic fertilizers and *P. putida* had significant difference on Camphene, Terpine-4-ol, Guaiol, δ-cadinene and *cis*-sabinol (Table 4). Means comparison showed that application 20 t/ha of compost significantly increased Guaiol, Epi-α-eudesmol and δ cadinol compared with control (Table 5).

Table 8 Means comparison of organic fertilizers and *P. putida* interaction on essential oil compounds (%) of *S. leriifolia* Benth. in second growing season

Composition	RI*	Control		Compost 10-ton/ha		Compost 20-ton/ha		Vermiompost 3- 3-ton/ha		Vermiompost 6-ton/ha		Mean	LSD (0.05)
		S_0	S_1	S_0	S_1	S_0	S_1	S_0	S_1	S_0	S_1		
α-thujene	933	0.36	0.38	0.39	0.30	0.39	0.37	0.34	0.34	0.33	0.34	0.36	0.05
α-pinene	**940**	**13.3**	**15.1**	**14.8**	**14.4**	**13.2**	**14.4**	**13.3**	**13.6**	**13.7**	**12.5**	**13.8**	**1.33**
Camphene	953	1.9	2.4	2.2	2.4	2.0	2.2	2.3	2.1	2.1	1.8	2.1	0.27
Sabinene	978	1.1	1.2	0.95	1.1	1.1	1.1	0.9	0.88	0.73	1.1	1.0	0.17
β –pinene	**982**	**25.7**	**28.8**	**27.7**	**27.5**	**24.8**	**27.4**	**25.2**	**25.9**	**25.8**	**24.2**	**26.3**	**2.96**
δ-3-carene	1013	1.2	1.0	1.1	1.5	1.1	1.0	1.1	1.0	0.95	0.97	1.1	0.27
p-cymene	1028	1.1	1.3	1.0	1.1	1.1	1.00	0.85	1.0	0.71	1.3	1.05	0.33
Limonene	1032	1.3	1.3	1.3	1.4	1.2	1.30	1.3	1.1	1.6	1.1	1.3	0.26
1,8- Cineole	**1035**	**14.9**	**13.9**	**13.5**	**16.3**	**15.3**	**15.00**	**14.4**	**13.3**	**16.2**	**15.9**	**14.9**	**2.65**
z-β-ocimene	1042	0.43	0.42	0.20	0.44	0.45	0.21	0.38	0.38	0.40	0.39	0.37	0.15
γ-terpinene	1063	0.18	0.21	0.19	0.17	0.37	0.40	0.17	0.33	0.32	0.33	0.27	0.24
cis- sabinol	1145	0.56	0.50	0.41	0.57	0.55	0.48	0.47	0.49	0.57	0.57	0.52	0.05
Borneol	**1166**	**4.9**	**5.4**	**4.5**	**5.0**	**4.8**	**4.7**	**5.2**	**4.9**	**4.6**	**4.3**	**4.81**	**0.94**
Terpine-4-ol	1180	2.0	1.9	1.6	1.8	1.9	1.7	1.7	1.7	1.8	1.9	1.80	0.16
α-terpineol	1190	0.38	0.17	0.00	0.54	0.18	0.33	0.23	0.38	0.64	0.15	0.30	0.25
Myrtenol	1195	0.22	0.23	0.16	0.35	0.23	0.16	0.45	0.24	0.00	0.00	0.20	0.30
α-gurjunene	1411	1.3	1.3	1.3	1.1	1.4	1.4	1.5	1.4	1.4	1.4	1.34	0.19
E-caryophyllene	1418	1.3	1.2	1.3	1.2	1.4	1.6	1.4	1.4	1.4	1.3	1.34	0.35
α-muurolene	1500	0.57	0.21	0.47	0.68	0.44	0.39	0.47	0.54	0.49	0.56	0.48	0.21
γ-cadinene	1515	2.6	2.1	2.4	2.1	2.2	2.1	2.6	2.6	2.4	2.4	2.34	0.44
δ-cadinene	1526	1.1	0.88	1.0	1.1	0.89	0.97	0.65	0.0	0.52	0.98	0.81	0.49
Guaiol	1598	1.3	1.3	1.6	1.2	2.1	1.7	0.55	1.1	1.2	2.3	1.44	0.72
δ-cadinol	1636	0.68	0.69	0.88	0.58	1.2	0.94	1.4	1.5	0.91	1.4	1.02	0.54
β-eudesmol	1652	1.9	1.6	1.9	1.4	2.0	1.5	1.7	2.0	1.4	3.0	1.73	0.49
α-eudesmol	1654	0.61	0.23	0.54	0.47	0.56	0.0	0.23	0.63	0.29	0.52	0.41	0.29
α-cadinol	1656	0.54	0.48	0.43	0.63	0.73	0.63	0.71	0.57	0.86	0.62	0.62	0.25
Epi-α-eudesmol	1661	1.1	1.0	0.77	1.7	2.50	2.2	2.2	2.5	2.1	3.2	1.93	1.04
Bulnesol	1668	1.5	1.5	1.6	0.92	1.4	1.3	2.0	1.5	1.7	1.5	1.50	0.60
β-bisabolol	1674	2.3	2.3	2.4	2.2	2.54	2.3	2.51	2.5	2.3	2.4	2.36	0.64
α – bisabolol	1684	1.7	1.7	1.8	1.5	1.5	1.5	2.0	1.8	1.6	0.86	1.59	0.58
Epi-α –bisabolol	1687	2.1	1.8	2.2	1.4	1.3	1.2	1.1	1.3	0.97	1.2	1.45	0.63
8-cedren-13-ol	1690	2.5	2.2	2.7	2.0	2.4	2.3	3.1	2.8	2.7	2.7	2.53	0.75
Juniper camphor	1694	2.4	2.0	2.6	2.1	2.6	2.3	2.6	2.8	3.1	2.4	2.50	0.73
Monoterpene hydrocarbons		46.7	52.2	49.9	50.4	45.7	49.4	45.8	46.7	46.5	44.0	47.7	4.45
Oxygenated monoterpenes		23.0	22.0	20.2	24.6	23.0	22.3	22.4	21.0	23.8	22.8	22.5	3.12
Sesquiterpene hydrocarbons		7.0	5.6	6.5	6.1	6.3	6.5	6.6	6.0	6.2	6.6	6.3	1.03
Oxygenated sesquiterpenes		18.6	16.9	19.4	16.0	20.8	17.9	20.0	21.0	19.1	21.0	19.1	4.65
Others		4.7	3.1	3.7	2.7	4.2	3.9	5.2	5.1	4.1	5.6	4.3	-
Sum		100	99.8	99.7	99.8	100	100	100	99.8	99.7	100	99.9	-

* Retention Index † S_0 and S_1, no inoculation and inoculation with *P. pudita*, respectively

The highest percentages of Guaiol and Epi-α-eudesmol by 2.3% and 3.2% obtained in the six ton vermicompost treatment with P. putida (V6S1), which increased by 68% and 178% compared control, respectively. In both of compounds, bacterial inoculation showed a significant difference with no inoculation at V6S1. Results also indicated that application of three tons of vermicompost per hectare (V3S0 and V3S1) increased δ-cadinol by 120% compared to control (Table 8). The results of Darzi et al. [34] in Anis plant (Pimpinella anisum L.) indicated that the interaction effects of biofertilizer and vermicompost on gama-himachalene content and estragole content in EO were significant, and the lowest content were obtained at treatment of 5 ton/ havermicompost and two times application of phosphatic biofertilizer. Despite the increase in the percentages of the above EO composition, some compounds were significantly reduced by using different amounts of vermicompost. So that Sabinene, epi-α-bisanolol in the V_6S_0 treatment decreased by 51% and 116%, respectively. Also, Terpine-4-ol and *cis*-sabinol in the V_3S_0 decreased by 18% and 19%, respectively. Myrtenol was not found in the six-ton vermicompost (Table 8). The amounts of the above compounds per gram LDW were similar in fertilizer treatments (Table 6,7). Comparison of four groups of EO showed that organic fertilizers had only significant effect on the percentage of monoterpene hydrocarbons. However, the use of vermicompost fertilizers reduced this group compared to the control (Table 8). Bacterial inoculation in control treatment (C_0S_1) increased monoterpene hydrocarbons by 11.7% compared to non-inoculated treatment (C_0S_0) (Table 8). These results were in correspondence with those concluded by Banchio *et al*. [41] showed that inoculation of growth promoting bacteria such as *P. fluorescent* can significantly increase the production of monoterpenoids and sesquiterpenoids in Marjoram (*Origanum majorana* L.) by 10 and 24 percent, respectively. Results also revealed that the amounts of four groups of EO (µg/g LDW) were also not affected by fertilizer treatments. In addition, inoculation of *P. putida* and its interaction with organic fertilizers had no significant effect on the percentage and amount of four EO groups (Table 9).

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