Journal of Medicinal Plants and By-products (2021) 2: 141-148

Original Article

The Changes in Yield and Chemical Profile of Essential Oil and Leaf Minerals of *Satureja macrantha* C.A.Mey. Under Combined Manure and NPK Fertilizer

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Article History: Received: 13 October 2020/Accepted in revised form: 04 November 2020

Abstract

The use of environmentally friendly fertilizers is a new strategy to subside the undesirable effect of chemical fertilizers. The field experiment was conducted to investigate the effect of cow manure and NPK fertilizers on physiochemical characteristics and nutrients accumulation of *Satureja macrantha* C.A.Mey. in a randomized complete block design (RCBD) in three replications. Cow manure and NPK (20, 20, 20) were used in four levels as cow manure (60 t/ha), NPK fertilizer (50: 25: 25 kg/ha), combined cow manure (30 t/ha) and NPK fertilizer, and untreated treatment (control) in Karaj, Iran, during 2017 and 2018. The results showed increased dry weight yield under manure or the combined NPK and manure fertilizers in second year. The relative water content (RWC) in second-year plants treated with manure was higher compared to other experimental treatments. Essential oil percentage increased by manure application and its combination with NPK fertilizer, ranging from 1.06 to 1.86%. Essential oil yield of aerial parts of *S. macrantha* under organic manure in the second year was significantly higher in comparison with control and NPK-treated plants. The GC/MS analysis showed that the main essential oil compounds of *S. macrantha* were p-cymene (22.14%-33.78%), γ-terpinene (21.7%-35.22%), and thymol (13.1%-37.9%). The minerals in aerial parts of *S. macrantha* were changed under fertilizers and year. Manure and its combination with NPK fertilizer significantly improved phosphorus (P), and potassium (K). Both NPK fertilizer and cow manure significantly led to increased calcium (Ca) concentration in second-year plants. NPK fertilizer had negative effect on iron (Fe) in second-year plants, but the enhanced Fe was observed in the second year when plants treated with cow manure. The present study recommended the combined use of manure and NPK fertilizers to reach the optimum essential oil of *S. macrantha*.

Keywords: Essential oil profile, Medicinal plant, Nutrients, Organic manure

Introduction

Satureja is well-known genus of Lamiaceae family, which consists of over 200 species, mainly distributed in eastern part of the Mediterranean area [1]. The species of *Satureja* are annual or perennial semi-bushy aromatic plants that grow in arid and mountainous regions [2, 3]. This genus is characterized by highly aromatic and medicinal features. In folk medicine, the species of *Satureja* are widely used for treating the various diseases [4]. *S. macrantha* with up to 50 cm in height, widely grows in north-west of Iran as well as east parts of Iraq [4]. The leaves are coated with a large number of glands, which are corresponded to essential oil (EO) production [5]. *S. macrantha* has certainly biological properties such as antimicrobial activity [6].

Organic and chemical fertilizers have both advantages and breakdowns on soil and plant development. Chemical fertilizers are relatively inexpensive, and have high nutrient contents. However, the excess use of chemical fertilizers makes great problems such as nutrient loss, surface water and groundwater contamination, and soil acidification [7]. Organic fertilizers have multiple benefits due to the balanced supply of nutrients, including micronutrients, increased soil nutrient availability due to increased soil microbial activity, the decomposition of harmful elements, soil structure improvements and root development, and increased soil water availability. However, these soil amendments have some defects such

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as low nutrient content, slow decomposition, and different nutrient compositions depending on its organic materials, compared to chemical fertilizers [5,7].

To maintain consistently high yield in plants particularly in medicinal plants, soil nutrient management is essential, and fertilization is the only way to supply soil nutrients within a short period of time [5,7]. The effects of the mixed use of chemical fertilizer and organic manure on the quality and quantity of medicinal plants and soil fertility vary substantially according to the fertilizer amounts and characteristics. Organic manure, produced from animal by products, has been utilized to overcome environmental contamination and declines of plant yield resulted from the constant utilization of chemical fertilizers [7,8]. Recycling waste from the livestock industry prevents environmental contamination and reduces treatment costs. At the same time, it promotes soil improvements and agricultural productivity. However, the simultaneous use of chemical fertilizer and organic manure has revealed diverse results relative to the plant types and soil characteristics [8]. Organic and chemical fertilizers in combination or separately can change plant yield. Bakhtiari *et al*. [5] showed increased secondary metabolites such as phenol and flavonoid contents and also essential oil content of *S. macrantha* under bio-fertilizers compared to chemical fertilizers. Han *et al*. [8] reported increased plant yield in yellow poplar (*Liriodendron tulipifera* Lin.) under organic manure in comparison with chemical fertilizers. Most studies in agricultural fields have reported that the simultaneous application of chemical and organic fertilizer decreases the damages induced by excess use of chemical materials [7, 8]. Increased EO content and yield of *S. mutica* were reported by Saki et al. [3] when plants were treated with cattle manure.

The objective of this study was to investigate the effects of separate and mixed application of chemical fertilizer (NPK) and organic manure on the growth, EO quality and quantity, and nutrients of aerial parts of *S. macrantha* We hypothesized that organic manure would be comparable to that of chemical fertilizer on plant yield because of its indirect effect on soil property improvement. By evaluating the possibility of the utilization of organic manure produced as animal byproducts, which can be continually utilized in soil for a long time, the findings of this study help to suggest ways to increase plant productivity.

Material and Methods

Experimental Site

The study was carried out at the experimental farm in Alborz Research Station, Research Institute of Forests and Rangelands, Karaj (1312 m above sea level, 35°48′45” N, 51°01′30” E), Iran during 2017–2018. Soil properties in the experimental site are presented in Table 1. The maximum and minimum temperature during the study period fluctuating from January to July as coldest and hottest month, respectively. The highest precipitation occurred in March (43.2 mm) and April (39.1 mm).

NPK and Cow Manure

The recommended rate of nitrogen regarding to the soil texture was used at a rate of 50 kg N ha^{-1} applying ammonium nitrate; phosphorus was used at a rate of 25 kg P ha^{-1} using calcium super phosphate; and potassium was used at a rate of 25 kg K ha^{-1} using potassium sulphate. Cow manure was prepared in a dairy farm closed to Tehran (Table 1). It was used as 60 t/ha separately and 30 t/ha in combination with NPK fertilizer. Manure was incorporated into the top 20 cm soil manually before each crop was planted.

Experimental Details

The experiment consisted of four treatments including NPK (50:25:25 kg/ha), manure (30 t/ha), the combination of NPK and manure (30 t/ha), and control (untreated plants). The study was carried out in a randomized complete block design (RCBD) with three replications. Foam trays filled with vermiculite and peat moss (1:1 Volume) were used for cultivating *S. macrantha* seeds. Two-month seedlings were transplanted in the open field at a spacing of 50 cm (row to row) × 50 cm (plant to plant) during March 2017 and 2018. Inorganic fertilizer (N, P, and K) were applied utilizing urea (N 50%). During non-rainy season, the plants were irrigated at 7-day intervals with 50 mm irrigation water.

Table 1 The soil and manure characteristics of the experimental field

Year	pH	EC (dS/m)	OC (%)	N (%)	P (mg/kg)	K (mg/kg)	Sand (%)	Silt (%)	Clay (%)
Soil	7.8	0.85	1.29	0.97	19	312	22	44	32
Manure	8.2	16.4	0.39	2.1	1.1	1299	-	-	-

Shoot Dry Weight (SDW)

The plants were cut from the bottom of stem and sent to the paper bags and dried at 40 ºC in the dark conditions till got a constant weight to measure SDW [9].

Relative Water Content (RWC) Measurement

The RWC of leaves was calculated as a percentage according to the method of Dhopte and Manuel [10] as follows:

$$RWC = \frac{(FW - DW)}{(SW - DW)} \times 100$$

Where, FW is fresh weight, SW is leaf weight after soaking for 24 hours at room temperature and DW is leaf dry weight after drying for 24 h at 75 °C.

Essential Oil Extraction

In order to measure the EO content, 100 g of dried aerial parts from each treatment were hydro distilled in the Clevenger type apparatus for 3 h, and reported as v/w percentage. The EO yield (kg ha^{-1}) was measured with multiplying the EO content with the plant yield of the experimental treatments. All the EO samples were stored at 4 °C for analyzing by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

Gas Chromatography (GC) Analysis

Thermo-UFM ultrafast gas chromatograph equipment with a ph-5 fused silica column (10m length × 0.1 mm id., film thickness 0.4 µm) was used to identify EOs. Oven temperature was maintained at 60 ºC for 5 min and then programmed to 285 ºC at a rate of 5 ºC/min; flame ionization detector (FID) and injector temperature were 290 ºC and 280 ºC, respectively; helium was applied as carrier gas with an inlet *pressure* of 0.5 kg cm^{-2}.

Gas chromatography—mass spectrometry (GC-MS)

GC-MS analyses were accomplished by Varian 3400 GC-MS system equipment with AOC-5000 auto injector and DB-5 fused silica capillary column (30 m × 0.25 mm i.d.; film thicknesses 0.25 µm). Temperature was programmed from 60 °C to 250 °C with 3 °C/min; Injector and interface temperature were 260 °C and 270 °C, respectively; acquisition mass range of 40–340 amu; ionization voltage of 70 eV; the carrier gas was helium at a velocity of 45 cm/sec.

Component Identification

Homologous series of n-alkanes (C7–C25) determined the retention index for all volatile constituents. According to Adams, the components of oil were identified by matching their retention indices (RI) and mass spectra. EO components were identified by GC/MS spectroscopy [3].

Leaf Minerals

The dry ash method was applied to assay the minerals in leaf black cumin. The samples were dried in an oven at 70 °C. To obtain the white ash, 1g dried leaf was transferred into ceramic vessels and slowly subjected to 500 °C in the oven. The obtained ash was cooled in room temperature, which each sample was subsequently changed with 20 mL 1N HCl, and put in the sand bath for 30 min. The samples were elutriated in a 100 mL volumetric balloon. Potassium (K) and calcium (Ca) concentrations were measure by flame photometer (Model 410, Corning, Halstead, UK) in the volumetric balloons [11]. Phosphorous (P) was measured by yellow method with vanadate-molybdate [12]. P concentration was determined at 430 nm using the spectrophotometer (Shimadzu, UV3100). Iron (Fe) was analyzed by atomic absorption spectrophotometer, model-2380 [13].

Statistical Analysis

The data were subjected to two-way analysis of variance (ANOVA) and using the SAS software package for Windows (SAS, version 9.3, SAS Institute, Cary, NC). The mean values subjected to Duncan's multiple range tests after detection of statistical significance ($p < 0.05$).

Results

Dry Weight Yield

Dry weight yield of aerial parts of *S. macrantha* was significantly changed under fertilizer application and year ($P \leq 0.01$, Table 2). Plants treated with cow manure or the combined use of NPK and cow manure in the second year was higher than other treatments. In second-year plants, cow manure and combined application of manure and NPK fertilizer increased the yield of dry weight by 22% and 18%, respectively, compared to control (Fig. 1a).

RWC

The interaction effect of fertilizer and year significantly affected the RWC of leaves in *S. macrantha* ($P \leq 0.05$, Table 2). Fertilizers particularly cow manure improved RWC. It ranged from 75% in control plants in the first year to 89.3% in cow manure treatment in the second year. Manure had no significant deference for RWC with NPK fertilizer in first-year plants. However, the remarkable progress of RWC due to manure application was observed in the second year (Fig. 1b).

EO Content and EO Yield

EO content and yield were significantly changed under the interaction of year and fertilizer ($P \leq 0.01$, Table 2). The EO percentage increased by manure application and its combination with NPK fertilizer. EO ranged from 1.06 to 1.86%. Plants treated with manure and NPK fertilizers showed the higher EO content compared to untreated plants (control) or those treated with only NPK fertilizer (Fig. 2a). EO yield of aerial parts of *S. macrantha* under organic manure in the second year (44.9 kg/ha) was significantly higher in comparison with control and NPK-treated plants. In addition, we found a significant increase in EO yield for second-year plants treated with combined manure and NPK fertilizer (Fig. 2b).

EO Composition

The GC/MS analysis showed that the main EO compounds of *S. macrantha* were monoterpenes (Table 3). The main constitutes were p-cymene (22.14%-33.78%), γ-terpinene (21.7%-35.22%), and thymol (13.1%-37.9%), were of *S. macrantha* EO (Table 3 and Fig. 3). p-cymene was different in two years and fertilizer treatments. p-cymene was changed by the use of organic and inorganic fertilizers and harvesting time. The minimum and maximum amount of p-cymene was observed in control and manure-treated plants, respectively, in the first year (Table 3). γ-Terpinene also ranged from control (21.7%) to manure application in first-year plants (35.22%). However, the behavior of thymol was different as ranging from manure application (13.1%) to control plants application (37.9%).

Minerals

The minerals in aerial parts of *S. macrantha* were changed under fertilizers and year. The results showed that P was significantly changed by the interaction of fertilizer and year ($P≤0.01$, Table 2). Increased P content was observed in the combination of NPK and manure compared to control. In first-year plants, NPK had a significant role in increasing the P concentration. However, in the second year plants, the combined organic and inorganic fertilizers resulted in increased P content (Fig. 4 a). Leaf K content significantly influenced under year and fertilizer ($P≤0.01$, Table 2). K in second year was significantly higher compared to first year in treated plants. Both NPK fertilizer and cow manure significantly led to increased K concentration in second-year plants (Fig. 4 b). The interaction of year and fertilizer was significant on Ca concentration ($P≤0.01$, Table 2). The highest Ca concentration was observed under manure application to be 24.1 mg/g DW.

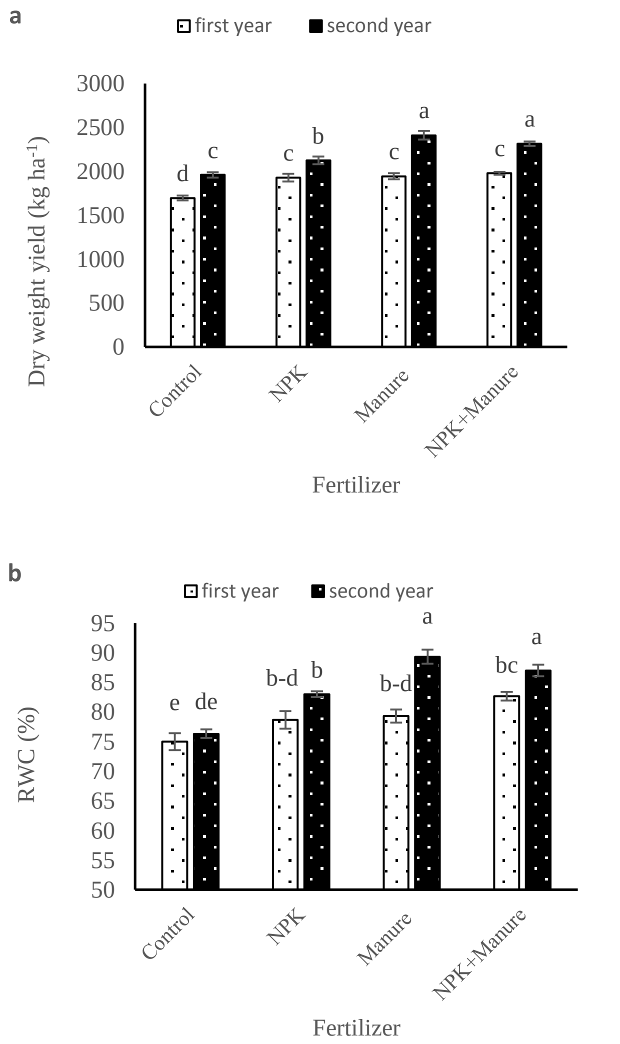


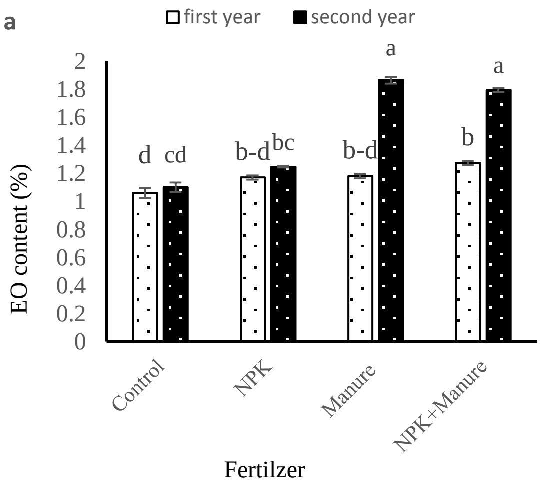
Fig. 1 The dry weight yield and relative water content (RWC) of *S. macrantha* under cow manure and NPK fertilizer. Values are means ± standard error of the mean (SEM) of three replications (n= 3). Different letters show statistically significant differences among treatments at $P≤0.05$.

Table 2 Analysis of variance for the studied traits of *S. macrantha* C.A.Mey. under different fertilizers

Source	df	MS							
		Dry weight	RWC	EO	EO yield	P	K	Ca	Fe
Year	1	597241^{**}	150^{**}	0.56^{**}	789.3^{**}	2.4^{**}	41.1^{**}	22.9^{**}	11.3^{**}
Year (block)	4	3580^{ns}	5.8^{ns}	0.003^{ns}	0.23^{**}	0.16^{**}	3.1^{ns}	5.8^{**}	0.63^{ns}
Fertilizer	3	150010^{**}	22.9^{**}	0.31^{**}	288.4^{**}	2.72^{**}	74.9^{**}	58.4^{**}	8.25^{**}
Year*fertilizer	3	20109^{*}	4.2^{*}	0.15^{**}	127.2^{**}	0.36^{**}	0.9^{ns}	13.9^{**}	0.44^{ns}
Error	12	6120.5	4.6	0.006	5.2	0.05	2.2	0.83	0.71
CV	-	3.82	2.1	6.1	8.1	7.9	5.1	4.9	6.3

**, *, and ns show the statistically significant at 1%, 5% level, and not statistically significant.

Ca in the second year was higher compared to the first year. Under manure application, a 23% increase of Ca was obtained at second-year plants compared to first-year plants (Fig. 4c). Leaf Fe significantly influenced under year and fertilizer (*P*≤0.01, Table 2). NPK fertilizer had negative effect on Fe in second-year plants. The enhanced Fe was observed in the second year when plants treated with cow manure (Fig. 4 d).



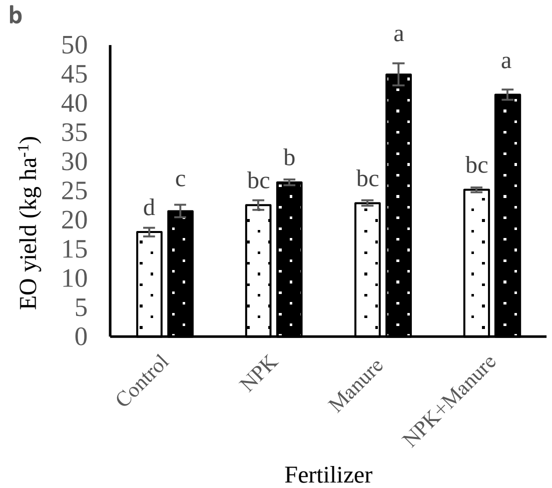


Fig. 2 The dry weight yield and RWC in aerial parts of *S. macrantha* under cow manure and NPK fertilizer. Values are means ± standard error of the mean (SEM) of three replications (n= 3). Different letters show statistically significant differences among treatments at *P*≤0.05.

Table 3 Essential oil composition under different treatments during 2017-2018. C: control, M: manure of *S. macrantha* C.A.Mey.

Compound	RI	C 2017	C 2018	NPK 2017	NPK 2018	M 2017	M 2018	NPK+M 2017	NPK+M 2018
α-Thujene	937.56	0.78	1.32	1.24	1.87	1.24	1.43	0.55	1.73
α-Pinene	949.29	1.23	1.15	1.86	2.12	1.67	2.08	0.85	3.12
Camphene	966.22	*	1.25	0.21	0.28	0.24	0.54	*	0.23
β-Pinene	980.65	*	3.12	1.27	2.14	0.85	2.12	0.66	1.26
3-Octanone	1007.94	*	1.5	0.18	0.59	0.2	1.21	0.14	1.76
Myrcene	1033.65	1.84	0.26	2.73	0.34	3.2	0.15	2.15	0.29
α-Phellandrene	1043.61	*	2.17	*	3.12	*	2.12	*	2.45
α-Terpinene	1048.53	1.34	1.54	2.14	0.19	1.42	0.72	1.04	0.51
p-cymene	1055.83	24.8	25.7	25.52	25.7	33.78	25.4	22.14	27.28
Limonene	1058.24	*	0.26	*	0.37	*	0.23	*	0.22
(Z)-β-Ocimene	1060.64	*	2.16	2.77	0.45	1.71	0.93	1.16	0.65
(E)-β-Ocimene	1063.04	0.18	0.22	0.19	0.32	0.19	0.17	0.17	0.14
γ-Terpinene	1086.42	21.7	28.3	34.33	31.55	35.22	26.12	25.88	27.66
cis-Sabinene hydrate	1100	0.37	0.15	0.18	0.21	0.14	0.22	0.21	0.26
Borneol	1222.64	0.18	0.45	*	0.26	*	0.4	0.17	0.54
Thymol	1325.1	37.9	23.2	18.44	27.12	13.1	28.12	37.15	28.9
Carvacrol	1332.44	1.22	0.82	1.04	0.36	1.03	0.57	1.12	0.39
Thymol acetate	1363.79	0.69	0.12	1.02	*	0.42	0.27	0.76	0.34
(E)-Caryophyllene	1474.38	1.22	1.01	1.7	0.57	1.2	0.84	1.41	0.13
Germacrene D	1530.53	0.2	1.16	0.14	0.21	*	0.78	0.14	0.43
β-Bisabolene	1544.18	0.14	0.76	0.54	0.14	*	0.24	0.34	0.3
Spathulenol	1647.52	0.23	0.66	0.15	*	*	1.24	0.13	0.54
Caryophyllene oxide	1657.48	*	0.85	*	0.18	*	0.87	0.12	0.34
Sabinene	*	1.54	*	2.47	*	1.52	0.78	*	*
β-Phellandrene	*	1.76	*	*	*	0.45	*	0.2	0.12
Terpinene-4-ol	*	0.25	0.14	0.16	*	0.21	0.45	0.17	*
Aromadendrene	*	0.14	*	0.16	*	*	0.13	0.78	*
Bicyclogermacrene	*	1.25	*	1.01	*	0.64	*	0.88	*
σ -cadinene	*	0.32	*	0.41	*	0.45	*	0.67	*
Total	-	99.28	98.27	99.86	98.09	98.88	98.13	98.99	99.59

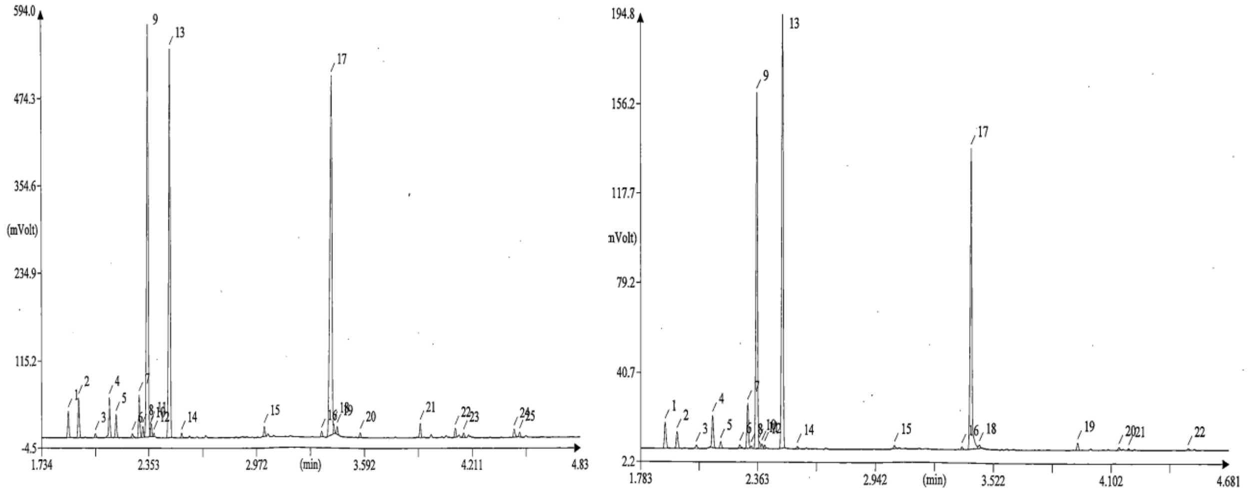


Fig. 3 The chromatograms from essential oil composition of *S. macrantha* C.A.Mey. for control (left) and integrated treatment of NPK and manure (right).

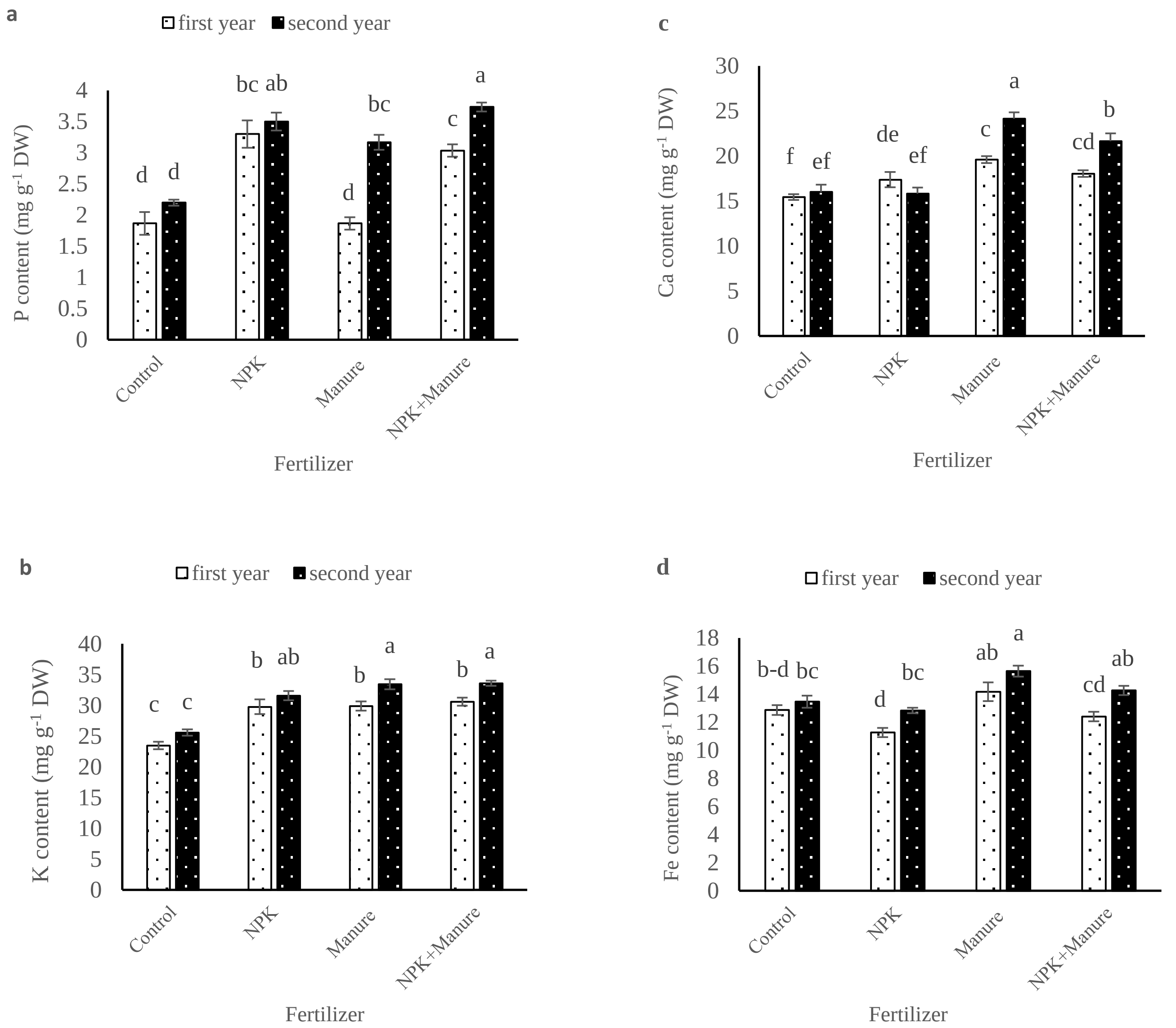


Fig. 4 Leaf minerals of *S. macrantha* C.A.Mey. under cow manure and NPK fertilizer. Values are means ± standard error of the mean (SEM) of three replications (n= 3). Different letters show statistically significant differences among treatments at $P \leq 0.05$.

Discussion

The optimum plant yield was determined in plants treated with cattle manure in the second year. The essential micro and macro-nutrients in manure such as nitrogen, phosphorus, potassium, and zinc promote plant growth. Manure can fertilize the soil through increasing the organic matter and improving the physicochemical properties of soil such as soil structure, aeration, moisture, and pH [14,15]. Moreover, manure due to its potential plays a significant role in chlorophyll contents by improving the solubility of the main elements, which finally resulted in plant growth [3, 16]. The positive effects of organic fertilizer on plant weight yield was reported by Saki *et al*. [3] on *S. mutica*. Availability of nitrogen increases growth and leaf area index of plant which in turn increases absorption of light leading to more dry matter and yield [14,17]. Our results indicated that second-year plants had increased dry weight yield compared to first-year plant. Plants are capable to adapt the new environmental condition by time; thereby in second year they have more potential in nutrients uptake, resulting the improved plant weight. In addition, it could be noticed that the decomposition of manure happens at second year, resulting more nutrients viability for plants. There was no significant different between the combination of NPK and manure application and manure separately on plant yield. Accordingly, Han *et al*. [7] showed the increased plant biomass under the combination of organic manure and NPK fertilizer. Abumere *et al*. [18] showed that different rates of chicken manure and NPK fertilizer significantly have various responses to plant growth.

Water content in different parts of plants particularly in leaves are very important for chemical process. It can be affected by soil amendment treatments. [19]. RWC in present study increased with organic fertilizer especially cow manure (up to 17%) compared to control. Manure due to its high potential in improving nutrients uptake and water capacity has a significant role on plant water content [20]. Increased RWC results in an enhancement of photosynthesis and plant growth, where it was done by cow manure. Increased RWC under vermicompost and NPK has been reported by Bakhtiari *et al*. [5] on *S. macrantha*. In addition, Mendes *et al.* [20] reported improved RWC when plants were treated with cattle manure.

Cow manure and its combination with NPK fertilizer increased EO yield particularly at second year. The 70% and 63% increases of EO content were obtained when plants were treated with cow manure and its combination with NPK, respectively (Fig. 2a). The main significant increases of EO yield (44.9 kg/ha) with manure at second year is due to the fact that main elements in manure release slowly in the soil and its impact can be observed at second year [3]. Saki *et al.* [3] showed increased EO content and yield of *S. mutica* under cattle manure. EO compounds represented different amounts under fertilizers application (Table 3). The change of EO composition amount can be due to the biochemical pathways of producing the corresponded compounds that are strongly corresponded to external factors like soil and foliar application of organic and inorganic fertilizers [18]. Bakhtiari *et al.* [5] showed that the main constitutes of *S. macrantha* EO were p- cymene (16.30-34.64%), γ-terpinene (15.46-33.6%), and thymol (14.82-43.09%), which had different responses to sampling time and fertilizer treatments.

NPK fertilizer in combination with manure increased the K and P contents. NPK fertilizer increase the availability in rhizosphere and plant can uptake these essential elements properly [19]. Soils often lack these nutrients, either naturally, or as a result of over cultivation or other environmental factors. In cases where soils are lacking, nutrients must be put back into the soil in order to create the ideal environment for optimal plant growth [21]. Organic and inorganic fertilizers contain the essential elements for plant growth and development. Manure increases the availability of N and P for plants by N fixation and it dissolves P while giving off no unpleasant odor or stink in the process. Similarly, the increase of leaf minerals has been reported under manure [7, 22], NPK fertilizer [5,23], and combination of manure and NPK fertilizer [7,24].

Conclusion

Due to some environmental and economic constraints, the use of chemical fertilizers is not a good solution for the food shortage, especially in developing countries such as Iran. Nowadays, the strategy for nutrition improvement has been changed. Decline in the use of chemical fertilizers is considered as an important principle in agriculture. Organic fertilizer such as manure is an appropriate alternative of chemical fertilizers. The present study showed the reduction use of chemical feminizer with substituting by cow manure. There was no significant change of plant yield with manure and its combination with NPK. Hence, due to unavailability of cow manure in all fields, we recommended the combined use of NPK and cow manure to reach the optimum yield in essential oil of *S. macrantha* L. to guarantee the environment by reducing the use of chemicals.

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