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Chemical composition and yield of essential oil from two sweet basil species (*Ocimum ciliatum* L. and *O. basilicum* L.) under different fertilizers

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ABSTRACT

Background & Aim: Iranian sweet basil (*Ocimum ciliatum* L.) and great sweet basil (*O. basilicum* L.) belonging to the family Lamiaceae are the most important aromatic, culinary and medicinal herbs, which widely cultivated in many countries. The areal parts especially, stem and leaves of sweet basil before flowering are widely used to enhance the flavor of foods such as salads, pasta, tomato products, pizza, meat , soups, marine foods, confectioneries and other products.

Experimental: Phytochemical response of two basil species to different fertilizers including C (control), CM (cow manure 20 ton ha^{-1}), CM + CF (cow manure 20 ton ha^{-1} + chemical fertilizers N.P.K), CM + CF (cow manure 10 ton ha^{-1}+ chemical fertilizers N.P.K), CF (chemical fertilizers, N.P.K), and VC (vermicompost) was evaluated in a factorial experimental based RCBD at the field, Saman, Chaharmahal va Bakhtiari Province. The hydro-distillated essential oils were analyzed using GC-FID and GC/MS.

Results: Results indicated that interaction effects of fertilizers and basil species had significant influences on some main components such as methyl chavicol (estragole), neral, and geranial. The maximum percentage of methyl chavicol was achieved from the applied CM in green basil. The highest value for essential oil yield was observed from the applied CM + CF.

Recommended applications/industries: The application of organic fertilizer can be a promising strategy in achieving sustainable production of medicinal and aromatic plants such as Iranian basil.

1. Introduction

The major challenge for agriculture is to enhance crop production in a manner that is sustainable for present and future as well. The agricultural production and sustainable intensification goals in commercially important medicinal and horticultural crops over both short and long terms demand proper plant nutrient and soil management**.** In arid and semi-arid areas, organic matter level of the soil is often very low. Thus, conservation and improvement of the soil organic matter is crucial for maintaining soil health and sustainability of farming in these regions (Yadav, 2003). Chemical fertilizers are one of the most important supplements for plant nutrition and in recent years, the fastest way to compensate of nutrient deficiency and soil fertility (Gyaneshwar *et al.,* 2002). However, as a consequence of the continually increasing demand for environmental protection and production of healthy food, it is necessary to increase the use of eco-friendly and environmentally safe natural and organic fertilizers. Soil organic amendments therefore are considered as sustainable alternative sources of nutrients compared with synthesized fertilizers (Sharafzadeh and Ordookhani, 2011). In sustainable agriculture, the applications of biological and organic fertilizers to increase soil fertility are considered as the alternative methods for chemical fertilizers (Wu *et al*., 2005). A lot of studies have been conducted on the use of bio-fertilizers instead of chemical fertilizers(Zheljazkov and Warman, 2004; Naguib, 2011; Bistgani *et al*., 2018). Generally, the use of biological and organic fertilizers such as manure and vermicompost in sustainable agriculture, in addition to increasing the support and activity of useful soil microorganisms, serves the nutrient requirements of plants such as nitrogen, phosphorus and potassium, and improves the growth, development, and yield of crops (Arancon *et al*., 2004).

An increasing use of medicinal and aromatic plants and their derivatives has highlighted the role of these plants in the global economic cycle, so that their consumption is not limited to developing countries and they have also become widespread in advanced countries (Van Wyk and Wink, 2017). The medicinal and aromatic plants are compatible with organic cultivation practices, which also have a tendency for producers and consumers (Khalil *et al*., 2007). In the production of the medicinal and aromatic plants, in addition to climatic conditions and soil factors (Moghaddam *et al*., 2018; Bajalan *et al*., 2018), the type of nutrients is of great importance, because these elements, by affecting the growth of plants, change the ratio of reproductive organs to vegetative, therefore, the quality and quantity of essential oils are affected (Ravindra, 2004; Naguib, 2011; Bistgani *et al*., 2018).

Iranian sweet basil (*Ocimum ciliatum* L.) and great sweet bail (*O. basilicum* L.) belonging to the family Lamiaceae are ornamental, culinary, and medicinal and aromatic plants (Putievsky and Galambosi, 1999; Makri and Kintzios, 2007). Both bail as native plants of Iran are grown in home and vegetable gardens by the indigenous people in every parts of Iran and herbaceous parts of stem with many properties are used as vegetable, medicinal, and culinary herbs (Moghaddam *et al*., 2015; Ghasemi Pirbalouti *et al*., 2017). Sweet basil is widely used in traditional medicine as a digestive tonic and for curing ailments such as warts, inflammations, colds, and headaches (Ghasemi Pirbalouti *et al*., 2013; Ghasemi Pirbalouti, 2014; Moghaddam *et al*., 2015; Ghasemi Pirbalouti *et al*., 2017). Basil extract has known sedative with anticonvulsant, anti-carcinogenic properties, as well as antiseptic (Suppakul *et al*., 2003; Carovic-Stanko *et al*., 2010). Sweet basil and other herbs are used not only for cooking but also in commercial fragrances, flavorings, and for increasing the shelf life of food products (Suppakul *et al*., 2003). Recent findings indicated that growth and quality and quantity of essential oils of the medicinal plants such as basil can be affected by genetic and ecological factors, including precipitation, temperature, plant competition, fertilization, and nitrogen content in the soil (Talebi *et al*., 2018). From the available literature, few studies have evaluated the influence of fertilization on growth and yield of basil (*O. ciliatum* and *O. basilicum*). For this reason, the aim of this study was to investigate the effect of different fertilizers on growth and the quality and quantity of essential oils from two basil species.

2. Materials and Methods

2.1. Site description

A experiment was done at the Research Farm of Oman-e-Saman, Chaharmahal va Bakhtiari Province (latitude 32° 29´ N, longitude 32° 43´ E, altitude 2112 m above sea level), southwestern Iran. Type of study area climate by Emberger’s climatology method is cold and semiarid and semi humid with temperate summer and very cold winter by Karimi’s climatology method (IRIMO, 2012).

2.2. Experimental details

The experiment was in a factorial experimental based randomized complete block design (RCBD) with three replicates. Treatments consisted of two species of basil (*O. ciliatum* and *O. basilicum*) and different fertilizers including C (control, without any fertilization), CM (cow manure 20 ton ha^{-1}), CM + CF (cow manure 20 ton ha^{-1} + chemical fertilizers N.P.K), CM + CF (cow manure 10 ton ha^{-1}+ chemical fertilizers N.P.K), CF (chemical fertilizers, N.P.K), and VC (vermicompost).

2.3. Soil amendments

Prior to sowing, soil samples were taken from 0 to 30 cm depth and were analyzed for selected physical and chemical characteristics. The soil was classified as silt clay, pH=8.23, O.C=28.19%, EC=10.34 dS m^{-1}, total N = 200 mg kg^{-1}, Mn = 48.39 mg kg^{-1}, Cu= 18.91 mg kg

$^{-1}$, Fe= 258 mg kg $^{-1}$, Zn= 48.02 mg kg $^{-1}$, Mg = 305.71 mg kg $^{-1}$, and Ca = 896.03 mg kg $^{-1}$ (Table 1). In addition, physicochemical characteristics of organic fertilizers were analyzed (Table 2).

Table 1. Soil analysis at 0-30 cm depth.

Cu	Fe	Mn	Zn	K ava.	P ava	pH	E.C	N	T.N.V	O.C
(mg.kg^{-1})						of past	(ds.m^{-1})	(%)		
0.76	1.96	6.21	0.42	195	8.6	7.65	0.891	0.049	32.5	0.346

Table 2. Some characteristics of animal manure and compost.

	Cu	Mn	Fe	Zn	Mg	Ca	E.C	pH	Na	Moisture	T.N.V	O.C	K	P_2O_5	N_2O
	(mg.kg^{-1})						(ds.m^{-1})		(%)						
animal manure	18.91	48.39	258	48.02	305.7	896.0	10.34	8.23	2.86	8.36	39.06	28.91	1.244	0.262	0.841
compost	19.71	247.2	4581.2	106.1	0.72	1.91	3.428	7.7	0.63	11.28	18.5	16.11	1.44	0.421	1.62

2.4. Plant material

O. ciliatum and *O. basilicum* seeds were purchased from the seed company (Esfahan, Iran). In April 2013, the transplants were produced in a green house in the pot (20 × 35 cm), and in June 2014, the seedlings were transplanted in the farm system. Experiment conducted based on randomized complete block design with three replications, during 2013 and 2014. The distance between plants in each row was 30 cm; each experimental plot size was 2.5 × 2 m.

2.5. Essential oil isolation

One hundred grams of the powdered tissue was distilled for 3 h using a Clevenger-type apparatus. The essential oils were dried with anhydrous sodium sulphate and kept in amber vials at 4 °C prior to analysis.

2.6. GC-FID and GC/MS analysis

Chemical compositions of the essential oils were identified by GC-FID and GC/MS. GC analysis was done on an Agilent Technologies 7890 GC equipped with a single injector and a flame ionization detector (FID) using a HP-5MS 5% capillary column (30.00 m × 0.25 mm, 0.25 µm film thicknesses). The carrier gas was helium (99.999% pure) at a flow of 0.8 ml/min. Initial column temperature was 60 °C and programmed to increase at 4 °C/min to 280 °C. The split ratio was 1:100 and samples (0.1 µL) were injected manually in the split mode. The injector temperature was set at 300 °C.

GC-MS analyses of volatile oil samples were performed on an Agilent Technologies 7890 gas chromatograph coupled to Agilent 5975 C mass selective detector (MSD) and quadrupole EI mass analyser (Agilent Technologies, Palo Alto, CA, USA). A HP–5MS 5% column (coated with methyl silicone) (30 m × 0.25 mm, 0.25 µm film thicknesses) was used as the stationary phase. Helium was used as the carrier gas at 0.8 mL/min flow rate. The temperature was programmed from 60 to 280 °C at 4 °C/min ramp rate. The injector and the GC-MS interface temperatures were maintained at 290 and 300 °C, respectively. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 50–550. The ion source and the detector temperatures were maintained at 250 and 150 °C, respectively. The samples (0.1 µL) were injected neat with 1:100 split ratio.

Constituents were identified by comparison of their KI (Kovats index) relative to C_5-C_{24} *n*-alkanes obtained on a nonpolar HP-5MS column by comparison of the KI, provided in the literature, by comparison of the mass spectra with those recorded by the NIST 08 (National Institute of Standards and Technology) and Willey (ChemStation data system). The individual constituents were identified by retention indices and compared with constituents known from the literature (Adams, 2007). The peak area percentages were computed from HP–5MS column without the use of FID response factors.

2.7. Statistical analysis

For statistical analysis an ANOVA method was done using SAS software (version 9.1). Mean comparisons were applied using least significant difference (LSD) tests at 5% level of probability.

3. Results and discussion

3.1. Essential oil yield

Data presented in Table 4 indicated a significant effect ($p \leq 0.01$) of treatments on essential oil yield of basil. The highest essential oil yield was found in plants under mixed fertilizer application i.e. F3= CM+ CF (cow manure 20 ton ha^{-1} + chemical fertilizers) with 1.5%. Other fertilizers treatments [F4= CM+CF (cow manure 10 ton ha^{-1} + chemical fertilizers) and F5=CF (chemical fertilizers, N.P.K)], also indicated significant difference compared to the control (Fig. 1). There are no significant differences among F2 = CM (cow manure 20 ton ha^{-1}) and F6= VC (vermicompost) to the control. The enhance in essential oil yield by using of integrated fertilizer (CF + CM) may be due the higher supply of N from cow manure and chemical fertilizer, which could lead to higher essential oil yield and dry matter (Pandey and Patra, 2015).

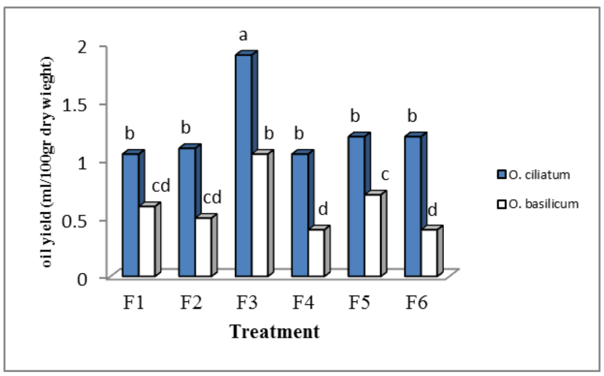

Fig. 1. Mean comparison effect of different fertilizers on oil yield of basil via LSD test at 5% probability level. F1= C (Control, without any fertilization), F2= CM (Cow manure 20 t.ha^{-1}), F3= CM+CF (Cow manure 20 t.ha^{-1} + chemical fertilizers), F4= CM+CF (Cow manure 10 t.ha^{-1}+ chemical fertilizers), F5= CF (Chemical fertilizers, N.P.K), F6= VC (Vermicompost)

The synthesis of essential oils is dependent on photosynthetic activity. Providing of photosynthetic nutrient boost and metabolic processes correlated to cell division and elongation (Hatwar *et al.,* 2003). According to Pandey and Patra (2015) nitrogen plays a key role in the division, growth and development of cells that stimulate essential oil accumulation via higher density of oil glands due to the improvement in biomass yield. Thus, it is useful to combine organic fertilizer with chemical fertilizer for optimum basil productivity and essential oil yield.

Kandeel *et al.* (2002) focused on the effect of inorganic and organic nitrogen fertilizers and their combinations on yield and oil composition of basil. They showed that when combined, nitrogen supply increased oil yield (mainly composed by terpenoid-like compounds) compared to plants fertilized with inorganic nitrogen alone. However, fertilizers can significantly affect the content of essential oil. These results can be explained in the light of facts that, using organic manure, led to increase organic matter, availability of nutrients, nitrogen fixation and rizosphere microorganisms that release phytohormones, and substances which lead to increased growth and dry matter accumulation and in turn increases the concentration of oil (Edris *et al.,* 2003). Results of other studies indicate increasing the essential oil yield and improving the quality of essential oils in chamomile (Liuc and Pank, 2005) following organic fertilizer application. These results were in agreement with those obtained by Naga (2004) on *Foeniculum vulgare* and *Carium carvi* L. and Louise *et al*. (2009) on *Plectranthus neochilus* plants.

3.2. Chemical composition of the essential oil

The essential oil compositions obtained from the aerial parts of basil are presented in Table 4. The main components determining the quality of basil essential oil are 1,8-cineole, linalool, methyl chavicol, neral, Geranial, ß-caryophyllene, Z-bergamotene, α-humulene, germaceren-D, cis-α-bisabolene and caryophyllene oxide. Our results demonstrated that the essential oils obtained from basil contained phenylpropanoids (methyl chavicol), oxygenated monoterpenes (linalool, gernial, and neral) and sesquiterpenes hydrocarbons (β-caryophyllene) confirming earlier reports that major chemical groups obtained from the aerial parts of basil were phenylpropanoids and oxygenated monoterpenes (Sajjadi, 2006; Ghasemi Pirbalouti, 2014). Table 4 shows species treatment had significant effect on the amount of 1,8-cineole, linalool, methyl chavicol, neral, geranial, Z-bergamotene, germaceren-D and cis-α-bisabolene. The analysis of the essential oils obtained from aerial parts of the experimentally cultivated plants showed that the percentage of Z-bergamotene was affected by the fertilizer treatments (*p*-value <0.05). The most of yield component of basil significantly responded to species. The mean comparison effect of different treatments on the main compounds such as linalool, methyl chavicol, Neral and geranial are presented in Fig. 2.

Previous studies showed that different fertilizers such as chemical and organic manure can influence the aroma profile of medicinal and aromatic plants (Singh *et al.*, 2007; Tanu *et al*., 2004). However, the rest of the compounds except Z-bergamotene were not affected by fertilizer treatments. In addition, interaction between species and fertilizer had significant effect on methyl chavicol content, as the most important compound of basil. It seems that the combined fertilizers have positive effects on methyl chavicol as the major compound, due to the fact these combinations contain different minerals which affect the secondary metabolism of plants (Pandey and Patra, 2015; Pandey *et al.*, 2015). These minerals will positively affect cellular metabolism and biomass production. As a consequence, an enhanced vegetative growth along with an increase of glandular trichomes is obtained (Pandey and Patra, 2015; Pandey *et al.*, 2015). As a matter of fact, several authors (Jha *et al.*, 2011; Pandey *et al.*, 2015) showed that application of fertilizers changed significantly the aroma profile of the essential oils. Thus, organic manure and mineral fertilizers play a fundamental role in determining the quali-quantative production of secondary metabolites.

Table 4. Analysis of variance of studied traits.

S. O. V	df	Yield oil	1,8-cineole	Linalool	Methyl chavicol	Neral	Geranial	Z-Bergamotene	Germacren-D	*Cis-α-*Bisabolene
Block	2	0.01^{ns}	0.87^{ns}	6.06^{ns}	73.43^{ns}	5.17^{ns}	6.56^{ns}	1.06^{ns}	1.67^{ns}	0.69^{ns}
Species	1	3.86**	3.72**	1870.9**	288.49**	503.78**	604.7**	15.01**	3.39*	4.10**
Fertilizer	5	0.44**	0.36^{ns}	4.8^{ns}	34.83^{ns}	2.13^{ns}	2.37^{ns}	0.93*	0.68^{ns}	0.85^{ns}
Species × Fertilizer	5	0.28^{ns}	0.24^{ns}	3.48^{ns}	60.73**	5.73^{ns}	7.19^{ns}	0.93*	1.16^{ns}	0.24^{ns}
Error		279951	0.26	7.66	12.18	3.50	4.52	0.23	0.68	0.38
CV	-	14.91	0.30	33	6.53	30.41	30.87	34.13	49.24	53

ns**, *** and ******: non-significant, significant at 5% and 1% probability levels, respectively.

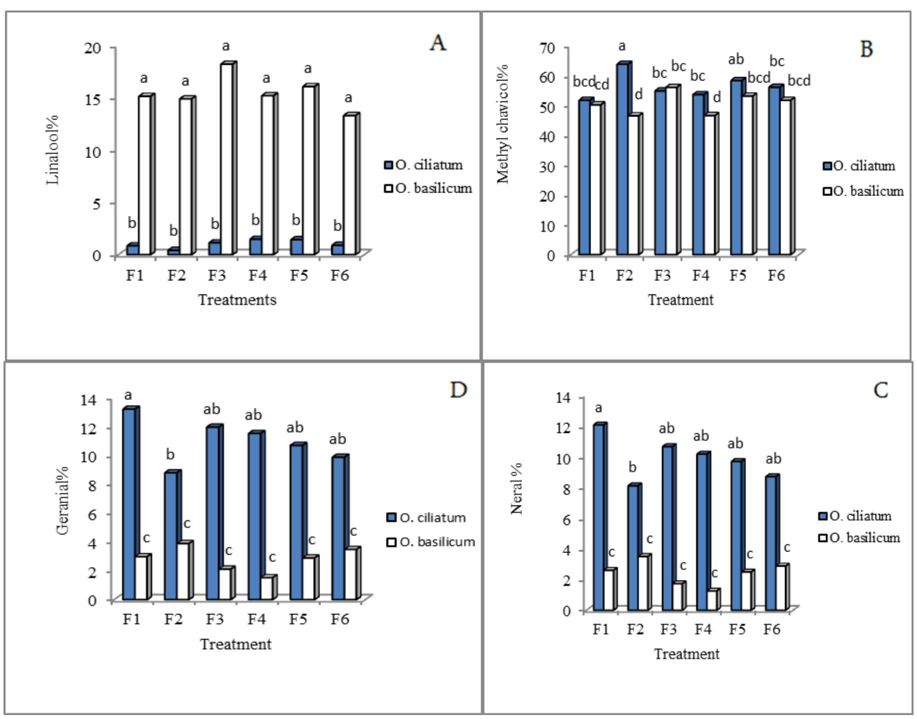

Fig. 2. Mean comparison effect of different fertilizers on linalool (A), methyl chavicol (B), Neral (C) and geranial (D) of basil via LSD test at 5% probability level. F1= C (Control, without any fertilization), F2= CM (Cow manure 20 t.ha−1), F3= CM+CF (Cow manure 20 t.ha−1 + chemical fertilizers), F4= CM+CF (Cow manure 10 t.ha−1+ chemical fertilizers), F5= CF (Chemical fertilizers, N.P.K), F6= VC (Vermicompost)

4. Conclusion

The results recommended that combination of organic manure and chemical fertilizer gave higher essential oil in basil. Combination of organic manure and chemical fertilizers was the most suited combination for improving the crop productivity, oil yield and overall profitability and economics of cultivation of the basil. In general, this improvement will greatly help in development of organic farming techniques and considerably reduce the cost of production and environmental hazards due to dependence on synthetic fertilizers.

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