

Essential Oil Content and Composition of Sweet Basil (*Ocimum basilicum*.L) Under Integrated Nutrient Management

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Abstract: Field experiments were conducted at ICAR - Indian Institute of Horticultural Research, Bengaluru during Kharif season of 2015 and 2016 with nine treatments and three replications in a randomized block design to find out the effects of integrated nutrient management on Quantity and Quality of essential oil of basil (*Ocimum basilicum*). The results revealed that combined application of recommended FYM (10 t ha⁻¹) and NPK (160:80:80 kg ha⁻¹) registered the highest oil yield in the main crop (211.94, 187.46 l ha⁻¹) and in ratoon (144.36, 70.81 l ha⁻¹) during 2015, 2016, respectively. For oil quality, the main constituents of basil essential oil have been reported and the result showed that chemical compositions of the essential oil for sweet basil were affected by fertilizers in the maincrop and ratoon during two years of the experiment. In general, application of NPK (160:80:80 kg ha⁻¹) + FYM (10 t ha⁻¹) i.e., T9 recorded the best quality. The results obtained from this study demonstrated that integrated nutrient management can maximize nutrient absorption as a result of increased soil fertility which reflected on oil yield and quality.

Keywords: Farm yard manure, Chemical fertilizers, Microbial consortia, NPK uptake, NPK availability.

INTRODUCTION

Basil belongs to *Lamiaceae* family, grows in many countries of the world as a medicinal, and aromatic plant. Its extracts are also used as a food flavoring, in perfumery, and as dental applications [1]. Phenolic compounds in basil, justify its importance, in modern medicine as antioxidant, and antitumor [2]. Furthermore, its strong aroma helps to prevent problems caused by insects [3]. There are several chemo types of basil as methyl cinnamate; methyl chavicol; eugenol and linalool types [4], so the importance of basil oil is due to the presence of these phenyl propanoids, with their derivatives and terpenoids [5]. Which determine the specific aroma of plants and the flavor of the condiment.

Nutrient application affects the quality of basil essential oil which rely on the percentage of its constituents [6], that differ depending on the type of fertilizer [7]. Therefore, recently it has been focused on developing strategies to increase phenolic compounds [8]. Integrated nutrient management through combined applications of organic and inorganic fertilizers becoming an essential aspect of sustainable agriculture,

specifically in cultivation of medicinal and aromatic plants, since that the real importance of this cultivation is given to the quality rather than quantity. Sustainable agricultural is the best way to achieve high yield without harming the nature. Application of organic, bio fertilizers is considered as an alternative strategies, often based on cumulative positive that lead to increase yields and best quality [9].

Physical, chemical and biological properties of the soil determine its type [10]. Application of organic manure has positive affect on soil properties that facilitate the availability of nutrient to the plant [11]. Organic manure gave significant effect on essential oil quality of some medicinal and aromatic plants such as lemon grass [12] lavender [13]; and basil [14].

Also, biological fertilizers improve the availability of nitrogen and phosphor, and play important role in sustainability [15] beside to, Some studies indicated that bio-fertilizer has positive effect on some medicinal plants such as davana [16]; lemon grass [17] and basil [18].

So that , the main goal of this study was to investigate the quantity and quality of essential oil of basil (*Ocimum basilicum*) under integrated nutrient management, to choose the best combination which lead to increase its oil yield and gain the best quality.

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MATERIAL AND METHODS

Experimental Location and Treatment Details

Field experiments were conducted in a randomized complete block design with three replications in the experimental field of ICAR-Indian Institute of Horticultural Research (IIHR), Bangalore during the *kharif* season of 2015 and 2016. The experimental station is located at an altitude of 890 m above mean sea level, 13°58" North latitude and 77°29" East longitudes and the climate is semi-arid tropical. The treatments of the experiments consisted of nine combinations of organic manure (FYM), bio-fertilizer and inorganic fertilizers (NPK): T₁ - (FYM (10 t/ha) + 100% recommended N through FYM), T₂ - (FYM (10 t/ha) + 100% recommended N through FYM + bio-fertilizer), T₃ - (FYM (10 t/ha) + 75% recommended N through FYM), T₄ - (FYM (10 t/ha) + 75% recommended N through FYM + bio-fertilizer), T₅ - (FYM (10 t/ha) + 50% recommended N through FYM), T₆ - (FYM (10 t/ha) + 50% recommended N through FYM + bio-fertilizer), T₇ (recommended FYM (10 t/ha) only), T₈ - (recommended NPK (160:80:80 kg/ha) only), and T₉ - (recommended FYM (10 t/ha) + recommended NPK (160:80:80 kg/ha).

Soil Samples Collection and Treatments Imposition

Initial experimental soil samples (0-30 cm depth) were taken for the nutrient analysis prior to land preparation and analyzed using standard procedures [19-21]. Physical and chemical properties of the initial experimental soil are presented in Table 1. The nutrients were supplied in the form of straight fertilizers like urea (160 kg N/ha), single super phosphate (80 kg P₂O₅/ha) and nitrate of potash (80 kg K₂O /ha). Fifty per cent of nitrogen and full dose of phosphorus and potash were applied as basal and the remaining fifty per cent of N was applied after 45 days of transplanting in T₈ and T₉ treatments. For bio-fertilizers, Arka Microbial Consortium (AMC) developed by ICAR-IIHR was used in the experiment and it contains N fixing, P and Zn solubilizing and plant growth promoting microbes in a single carrier. After 15 days of transplanting, recommended dose of AMC @ 5 kg/ha was applied at 2 cm depth to individual plants and immediately covered by soil. Similar method of application was followed for ratoon crop after harvest of main crop in T₂, T₄, and T₆ treatments.

Field Preparation, Planting and Data Collection

The land was brought to a fine tilth by ploughing and harrowing. The experimental site was divided into

plots having dimensions of 4.8 m long and 4.0 m wide with the spacing of 40 cm between the plants and 60 cm between the rows. There was a space of 0.5 meter between plots and 0.5 meter between replications. Seeds of basil cultivar *Cim-Saumya* were sown in two nursery beds of 6.0 m in length with 0.1 m in width and 10 cm height. Forty days old (40) healthy and uniformly rooted seedlings of sweet basil were transplanted to the main field. Weeding was done manually and drip irrigation was given daily for half an hour during the early stages of the crop and subsequently irrigation was given depending on the soil moisture condition. Totally, two main crop and two ratoons were taken at full bloom during 2015-2016. After the first ratoon, the crop was removed and again 40 days old seedlings were planted on the same plot with the same treatments. In this study, quantity and quality of basil essential oil consisted of essential oil content and chemical constitute in essential oil were evaluated.

Table 1: Physical and Chemical Proprieties of Initial Experimental Soil

Physical Properties	
Bulk density (Mg m ⁻³)	1.32
Particle Density (Mg m ⁻³)	2.65
Pore space (%)	42
Chemical Properties	
pH (1:2.5)	7.75
Electrical Conductivity (dSm ⁻¹)	0.36
Organic Carbon (g kg ⁻¹)	5.0
Available N (kg ha ⁻¹)	185
Available P (kg ha ⁻¹)	28
Available K (kg ha ⁻¹)	200
Exchangeable Ca (cmol(p ⁺)kg ⁻¹)	5.25
Exchangeable Mg (cmol(p ⁺)kg ⁻¹)	0.84
DTPA Fe (mg kg ⁻¹)	7.5
DTPA Mn (mg kg ⁻¹)	5.8
DTPA Cu (mg kg ⁻¹)	1.33
DTPA Zn (mg kg ⁻¹)	1.22

Extraction of Essential Oil

In order to determine the essential oil content (%), a sample of 100 g of basil fresh herb from each plot was collected and mixed with 500 ml distilled water and then was subjected to hydro-distillation for 3 h using a Clevenger-type apparatus [22].

Determination of the Essential Oil Yield (l/ha)

Oil volume was recorded and oil yield was calculated as the volume (liter) of oil per weight (t) of fresh herbage yield.

Identification of Essential oil Components

The quality of basil oil samples was analyzed by gas chromatography (Varian 3800 series) using VH-5 column for GC and VH-5 MS column for GCMS 30 m x 0.2 mm with 0.2 mm film thickness, oven temperature programmed at 60 °C for 5 min then 210 °C hold for 1 min then 240 °C hold it for 1 min and helium gas as a carrier at 1 ml.min⁻¹. Injector and detector temperature were 270 °C and 240 °C, respectively. Methyl chavicol and Linalool constituents of the oil were identified based on their retention time by comparing with the peak retentions times of those authentic standards obtained from Sigma, Aldrich, Bengaluru and run under identical conditions, then they was estimated in respect to total components and expressed as percentage [23].

RESULTS AND DISCUSSION

Oil Yield (l ha⁻¹)

The most important goal in basil cultivation is to increase oil yield; different levels of N through FYM, bio-fertilizers and inorganic fertilizer had different reflection on oil yield of main crop and ratoon during the two years of experiment (Figure 1). Treatment T₉ with NPK (160:80:80 kg ha⁻¹) + FYM (10 t ha⁻¹) gave highest oil yield in the main crop (211.94, 187.46 l ha⁻¹) and in ratoon (144.36, 70.81 l ha⁻¹) during 2015, 2016, respectively. The lowest oil yield per hectare was recorded with recommended dose of FYM alone in T₇ in the main crop (95.13, 52.40 l ha⁻¹) and in ratoon (38.52, 15.36 l ha⁻¹) during first and second year, respectively. Organic manure increases nutrient absorption reflecting on plant foliage growth ultimately, leading to increase plant oil yield [24]. These observations are in harmony with finding of El-naggar *et al.*; Tahami *et al.*; Zeinab (2005) and Samsunahar (2006) [25-28].

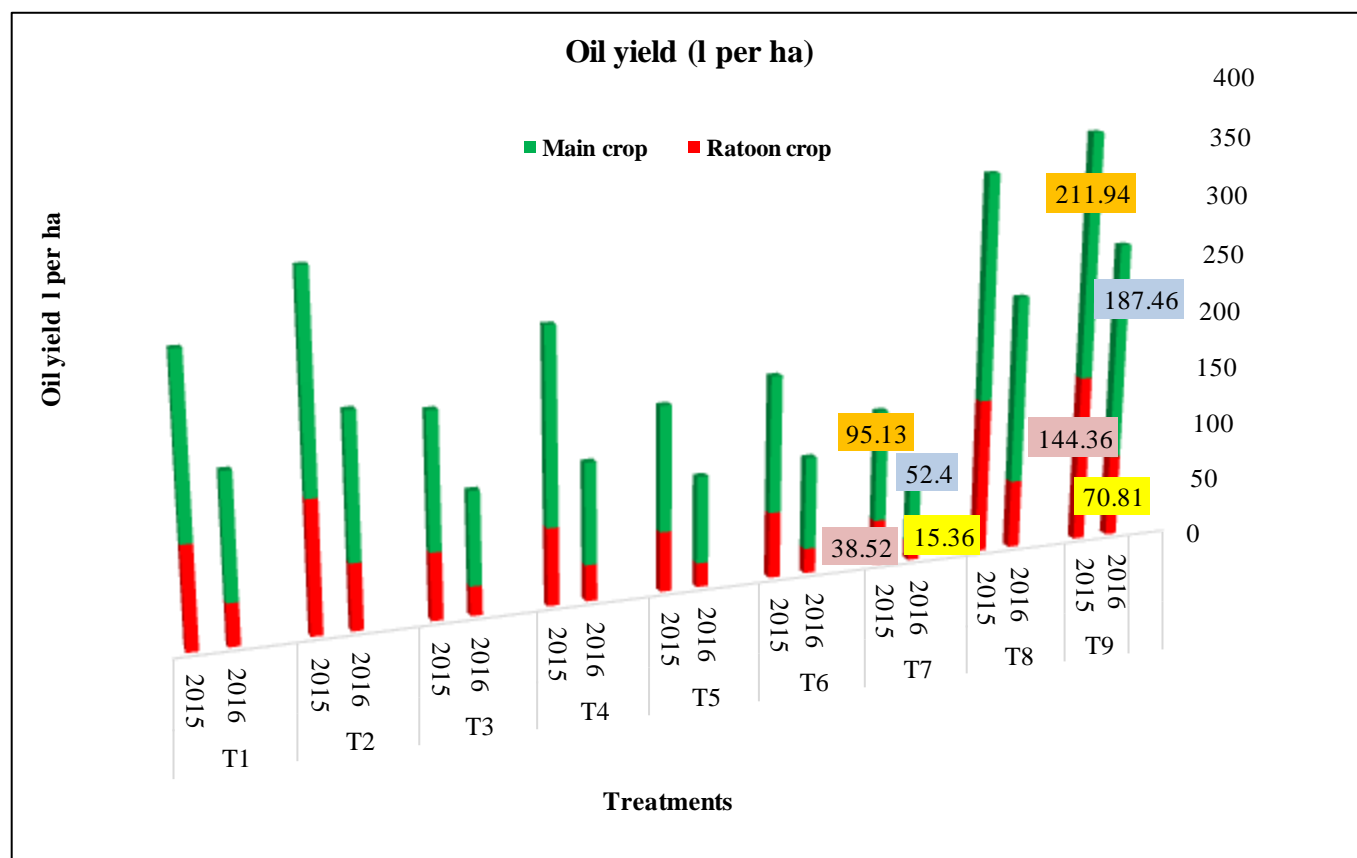


Figure 1. Cumulative oil yield per hectare (l/ha) of basil as influenced by organic manure, bio-fertilizer and inorganic fertilizer.

Quality Parameters

The chemical compositions of the essential oil for sweet basil differed under different types of fertilizers in the maincrop and ratoon during two years of the experiment, as shown from Table (Table 2-3A,B). Forteen constituents were identified in *O.basilicum* by GLC. The high economic value of basil oil is due to the presence of phenyl propanoids, like eugenol, Methyl chavicol and their derivatives or terpenoids like monoterpen linalool, and limonene [5]. Therefore, there has been growing interest in developing simple methodologies to increase polyphenols concentration and essential oil content in basil to further enhance their overall nutritional value [8]. Factors which may potentially affect essential oil composition may differ, in the same genotype, depending on the light regime [29] or the type of fertilizer [7].

Methyl Chavicol

Methyl chavicol as phenolic compound has been reported as main constituents of basil essential oil [23]. Different treatments had significant difference in methyl chavicol percentage. The results (Table 2A,B) showed that application of NPK (160:80:80 kg ha⁻¹) + FYM (10 t ha⁻¹) i.e., T₉ recorded the highest percentage of methyl chavicol in the main crop of 2015 (52.3%) while, in the main crop of 2016 application of FYM (10 t ha⁻¹) +100%

recommended N through FYM + bio fertilizers i.e., T₂ recorded maximum methyl chavicol percentage (63.78%). In ratoon, application of FYM (10 t ha⁻¹) +100% recommended N through FYM + bio fertilizers recorded the highest methyl chavicol percentage (59.39 and 59.67%) shown in Table (3A,B) during first and second year, respectively. The lowest percentage of methyl chavicol was recorded with T₇ (40.05 and 46.2%) in main crop and in ratoon (40.22 and 41.49%) of 2015 and 2016, respectively. This result is on harmony with Anwar *et al.*, [14] who found that using organic manure on basil (*O. basilicum*) increased methyl chavicol content which improved the quality of essential oil.

Eugenol

Eugenol content was also detected (Table 2,3). Although its lower concentration, but the highest percentage was recorded with application of NPK (160:80:80 kg /ha) i.e., T₈ in the main crop (2.48 and 1.34 %) and ratoon (1 and 3.20%) during 2015 and 2016, respectively. While, the lowest percentage was recorded in T₁ in the main crop (0.01 and 0.02%) and ratoon (0.02 and 0.01%) respectively.

Linalool

Different applications showed different Linalool concentration (Table 2,3). The treatment T₉ recorded

Table 2A: Influence of Different Levels of N Through Essential Oil Components of Basil Main Crop 2015

Essential Oil Components	Percentage of Components (%)								
	T1	T2	T3	T4	T5	T6	T7	T8	T9
α-Pinene	0.40	1.74	0.28	0.30	0.29	0.31	0.13	0.10	0.20
β-Ocimene	0.98	0.59	0.78	0.42	1.10	0.45	0.62	0.89	0.21
Linalol	20.01	22.3	20.7	21.13	19.56	20.49	19.20	23.44	25.29
Eugenol	0.01	0.02	0.03	0.04	0.10	0.13	0.21	2.48	0.73
Camphor	0.16	0.22	0.20	0.02	0.20	0.55	2.02	0.30	0.88
α-Terpinen	0.35	5.75	0.38	6.76	2.00	5.24	3.09	0.05	0.12
Limonene	0.42	0.48	0.39	0.40	0.37	0.40	0.37	0.9	1.24
Methyl chavicol	45.49	49.39	41.42	50.83	40.39	46.06	40.05	42.5	52.31
β-Elementene	0.88	0.65	0.64	2.20	1.93	0.31	0.49	0.23	1.87
Cubanol	2.99	1.01	1.92	0.22	0.76	1.19	1.88	1.35	1.22
Camphene	1.34	1.31	1.33	0.41	0.51	0.74	1.05	0.71	1.12
α-Humulene	1.04	0.03	1.30	0.40	0.48	0.43	0.64	0.29	0.49
Germacrene D	1.48	0.76	2.56	1.07	0.60	0.38	0.42	0.21	1.28
β-Cadinene	3.32	3.24	3.45	6.16	0.34	1.41	1.90	1.26	1.47

Table 2B: Influence of Different Levels of N Through Essential Oil Components of Basil Main Crop 2016

Essential Oil Components	Percentage of Components (%)								
	T1	T2	T3	T4	T5	T6	T7	T8	T9
α -Pinene	0.15	0.23	0.14	0.21	0.10	0.17	0.10	0.09	0.13
β -Ocimene	0.51	0.35	0.63	0.76	0.46	0.77	0.49	0.71	0.58
Linalol	21.46	21.37	20.76	21.11	18.43	17.59	15.20	21.74	22.88
Eugenol	0.02	0.07	0.05	0.06	0.04	0.06	0.03	1.34	1.10
Camphor	0.44	0.55	0.48	0.54	0.43	0.47	0.35	0.55	0.55
α -Terpinen	0.07	1.13	0.06	0.07	0.06	0.06	0.02	0.08	0.54
Limonene	0.46	0.40	0.89	0.76	0.57	0.72	0.24	0.73	1.02
Methyl chavicol	60.07	63.78	57.57	62.92	56.08	59.67	46.20	49.52	52.62
β -Elemene	0.16	0.14	0.16	1.04	2.06	2.05	2.94	1.42	2.32
Cubenol	0.58	0.46	0.59	0.37	0.82	0.75	0.66	0.57	0.74
Camphene	0.19	0.16	0.16	0.16	0.12	0.19	0.16	0.19	0.31
α -Humulene	0.15	0.84	0.50	0.82	0.42	1.09	0.41	0.24	0.45
Germacrene D	0.63	1.22	1.64	1.11	2.62	2.39	2.77	1.79	1.72
β -Cadinene	2.38	1.42	1.98	1.04	0.32	0.41	0.39	1.10	1.57

Table 3A: Influence of Different Levels of N through Essential Oil Components of Basil Ratoon Crop 2015

Essential Oil Components	Percentage of Components (%)								
	T1	T2	T3	T4	T5	T6	T7	T8	T9
α -Pinene	0.19	0.24	0.18	0.17	0.17	0.20	0.15	0.13	0.20
β -Ocimene	0.71	0.18	1.20	0.37	0.30	0.86	0.58	0.86	0.89
Linalol	23.06	20.07	19.08	20.14	19.81	21.16	15.28	22.22	26.59
Eugenol	0.02	0.03	0.04	0.18	0.90	0.10	0.37	1.00	0.33
Camphor	8.83	0.13	0.18	0.46	0.48	0.42	0.51	0.47	0.63
α -Terpinen	1.33	1.39	1.56	3.68	1.40	0.88	1.30	0.63	1.58
Limonene	0.37	1.75	0.23	0.33	0.27	0.38	0.20	0.27	0.49
Methyl chavicol	43.84	59.39	45.60	59.23	42.28	52.66	40.22	54.60	45.50
β -Elemene	2.07	0.80	2.75	3.75	2.48	1.56	3.01	2.01	2.90
Cubenol	0.83	0.72	0.99	2.20	0.86	0.68	0.89	0.63	0.82
Camphene	1.34	1.31	1.33	0.41	0.51	0.74	1.05	0.71	1.12
α -Humulene	0.35	0.33	0.51	0.80	0.40	0.33	0.40	0.43	0.47
Germacrene D	1.15	1.35	0.95	1.24	0.73	0.04	0.08	0.55	0.80
β -Cadinene	0.12	0.05	0.13	0.28	0.16	0.04	0.12	0.09	0.06

the highest percentage of Linalol in the main crop (25.29 and 22.88%) and ratoon (26.59 and 25.19%). While, the lowest Linalol percentage was recorded in T₇ of the main crop (19.20 and 15.20%) and ratoon (15.28 and 16.25%) during 2015 and 2016, respectively. Phenolic content increased with adaption organic cultivation, secondary metabolism increases with stress

caused by organic cultivation as reported by Sousa *et al.*, [30] Biofertilizers are beneficial in enriching the soil with those micro-organisms, which produce organic nutrients resulting in an increase in oil quality [31]. These findings are in accordance with the observations of Rashmi *et al.*, [18] in *Ocimum gratissimum*, and Moradi *et al.*, [32] in *Foeniculum vulgare*.

Table 3B: Influence of Different Levels of N Through Essential Oil Components of Basil Ratoon Crop 2016

Essential Oil Components	Percentage of Components (%)								
	T1	T2	T3	T4	T5	T6	T7	T8	T9
α -Pinene	0.26	0.99	0.24	0.89	0.20	0.79	0.20	0.18	0.21
β -Ocimene	0.66	0.82	3.68	0.62	0.92	0.54	0.49	0.76	0.80
Linalol	20.19	23.48	19.60	22.71	17.67	20.25	16.25	24.22	25.19
Eugenol	0.01	0.03	0.06	0.05	0.10	0.13	0.19	3.20	0.53
Camphor	0.04	2.29	9.73	0.41	1.54	0.26	0.88	0.06	0.64
α -Terpinen	0.01	0.02	0.01	0.04	0.45	0.07	0.11	0.09	0.16
Limonene	0.33	2.70	0.01	0.45	0.32	0.36	0.12	1.06	1.60
Methyl chavicol	50.23	59.67	50.15	55.38	43.28	51.22	41.49	53.90	44.17
β -Elemene	2.01	7.13	2.61	4.66	1.28	0.26	0.29	0.42	2.68
Cubenol	0.02	0.03	0.13	0.04	0.86	0.44	0.67	0.27	0.91
Camphene	0.05	0.75	3.23	0.92	0.29	0.51	0.81	0.22	0.40
α -Humulene	0.35	1.15	5.85	0.97	0.20	0.47	0.59	0.04	0.47
Germacrene D	0.35	2.46	16.72	2.37	0.05	0.08	0.15	0.66	1.39
β -Cadinene	0.96	0.41	2.41	0.35	2.81	1.58	2.44	0.66	1.56

α -pinine

Among the monoterpenic hydrocarbons in basil oil, α -pinine is the most important. Results showed (Table 2,3) that application of FYM (10 t/ha) +100% Rec. N through FYM + BF i.e., T₂ recorded maximum α -Pinene percentage in the main crop (1.74 and 0.23%) and ratoon (0.24 and 0.99%) during 2015 and, 2016, respectively. While, a low percentage was recorded in T₈ (0.10 and 0.09%) of the main crop and in ratoon (0.13 and 0.18%) during 2015 and 2016, respectively.

Biostimulants application such as nitrogen biofertilizer and phosphatic biofertilizer through the improvement of biological activities of soil and nutrient elements absorption, caused more growth and biomass production which lead to improvement of the essential oil quality [33]. These findings are in accordance with the observations of Rashmi *et al.*, [18] in *Ocimum gratissimum* and Kumar *et al.*, [16] in *Artemisia pallens*.

Limonene

Limonene is used to determine the basil aromatic quality, and plays a role in traditional remedy. The results indicate impact of different fertilizers that release different rates of terpenoids in plant. The treatment T₉ recorded the highest percentage of Limonene in the main crop (1.24 and 1.02%), while application of FYM (10 t/ha) +100% Rec. N through

FYM + BF i.e., T₂ recorded maximum α - percentage in the ratoon (1.75 and 2.70%) during 2015 and, 2016, respectively. These results are in agreement with the investigations that have studied the impact of fertilization on essential oils (rich in terpenoid-like compounds) [34].

CONCLUSION

This study revealed that that organic manures with either chemical or biofertilizers increased the oil yield of basil compared with control. Since application of recommended FYM (10 t ha⁻¹) + recommended NPK (160:80:80 kg ha⁻¹) gave the highest yield and best quality of oil for both main as well as in ratoon basil crop. The addition of bio-fertilizers also was effective for increasing the content of principal constituents (methyl chavicol and linalool) of basil oil. Hence, integrated nutrient management through combined application of organic and inorganic fertilizers may be recommended for basil crop to obtain higher oil yield and better oil quality.

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