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Research article

Role of organic and inorganic fertilizers in enhancing biomass yield and eugenol content of ornamental basil (*Ocimum gratissimum* L.)

Sunandani Chandel [a,*,1], Bimal S. Desai [b], Suman Kumar Jha [c], Satish Kumar Sinha [a], Dhiraji P. Patel [d], Nilay Kumar [e]

[a] *Department of Forest Products and Utilization, College of Forestry, Navsari Agricultural University, Navsari, India*
[b] *Department of Basic Sciences and Humanities, College of Forestry, Navsari Agricultural University, Navsari, India*
[c] *Department of Forest Biology & Tree Improvement, College of Forestry, Navsari Agricultural University, Navsari, India*
[d] *Department of Natural Resource Management, College of Forestry, Navsari Agricultural University, Navsari, India*
[e] *Department of Forest Products and Utilization, College of Horticulture and Forestry, Central Agricultural University (I), Pasighat, Arunachal Pradesh, India*

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Ornamental basil is an important essential oil yielding species in the Lamiaceae family is an extensive source of eugenol content and exhibits potential pharmacological properties. In the present scenario the environment conditions are constantly changing thus present study was under taken to study the influence of different growing condition, open and natural ventilated polyhouse, and organic and inorganic fertilizers and their interactive effect to optimise the best environment for higher biomass and improved essential oil quality under high rainfall conditions of South Gujarat, India. The eugenol content in leaf essential oil was characterized using Gas Chromatography – Flame Ionization Detection (GC-FID). The results showed that plants grown under polyhouse conditions had higher growth rates and biomass yields than those grown in open conditions due to the change in microclimatic conditions. Among the different growing environments, plant height (12.98 %), number of branches (67.73 %), number of leaves (131.49 %), leaf area (151.09 %), fresh biomass (131.90 %), dry biomass (108.27 %), leaf essential oil yield (206.09 %) and eugenol (20.95 %) per plant higher in comparison to open condition. Similarly, the 25 % NPK and 75 % N through vermicompost (F5) showed significantly better results in number of branches (6.25), number of leaves (135.30), leaf area (2936.49 cm^2), total fresh biomass (89.71 g), dry biomass (17.76 g) and leaf essential oil yield (0.189 g) per plant. The interactive effect of growing conditions and fertilizer treatments reported that maximum plant height (85.15 cm), number of branches per plant (7.73), number of leaves per plant (191.07), leaf area per plant

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* Corresponding author.

E-mail address: sunandanichandel26@gmail.com (S. Chandel).

[1] Corresponding author present address: Department of Forest Products and Utilization, College of Forestry, Kerala Agricultural University, Thrissur, India.

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(4455.77 cm^2), total fresh biomass per plant (124.92 g), total dry biomass per plant (23.93 g), leaf essential oil yield per plant (0.284 g) and eugenol content (54.117 %) was observed in plants under polyhouse conditions treated with 25 % NPK + 75 % N through vermicompost, respectively. Thus, the results from the present study provides significant insights towards the best growth condition and fertilizer dosage for optimising the package of practices for better growth, yield and essential oil quality in ornamental basil for the farmers to meet the demands of market.

1. Introduction

Ocimum gratissimum L., commonly known as ornamental basil [1], is a highly polymorphic species that belongs to the family Lamiaceae [2]. This versatile plant can be found in a wide range of geographic locations, including peninsular India, Bangladesh, African countries, Thailand, Haiti, Egypt, and Indonesia [3]. In India, it can be found in the states of Tamil Nadu, Bengal, Orissa, Karnataka, Kerala, and J&K, and can grow at altitudes of up to 1800 m. It can be cultivated on a variety of soils, but is best suited for moderately fertile, well-drained loamy or sandy loam soils [4].

In traditional Indian medicine, the entire plant of *O. gratissimum* is widely used to treat influenza and headaches. The herbage and essential oil of this plant possess a range of medicinal properties, including antifungal, antioxidant, anti-inflammatory, insecticidal, antimicrobial, and anaesthetic properties [5–7]. The essential oil is particularly rich in eugenol (70–75 %), which is the main constituent of clove oil, and also contains 10–15 % myrcene. Eugenol is used in a variety of industries, including perfumes, cosmetics, pharmaceuticals, confectionery, and even the bakery and ice cream industries [8]. In addition to this essential oil demand has tremendously increased in various industries, including food & beverages, aromatherapy, cosmetics, toiletries, and homecare products in the last few years. At the global level market, essential oil has recorded significantly higher growth and can reach up to US$ 3226.2 million by 2025. It contributes up to 70 % of the market share. U.S., China, and India are among the leading producers, consumers as well as exporters of essential oil throughout the world [9]. India exported the essential oil contributing to 172 million US$ in the financial year 2019. A steady growth has been observed in exports since 2015. Among the South Asian countries, India contributes up to 0.05 % of essential oils exports [10].

In medicinal and aromatic plants different growing conditions such as open and playhouse significantly alter the plant growth, yield, and essential oil quality owing to variations in microclimatic conditions. Variations in temperature, light intensity, and relative humidity are the main key factors that lead to the distinctions in growing environment conditions [11,12]. Several literatures reported that the plants grown under low light intensity in closed environments exhibited better growth, yield, and enhancement of the secondary metabolites such as essential oils because of the reduction in the photosynthetic activity [13]. The enhanced essential oil content under low intensities was reported in yarrow (*Achillea millefolium* L.) [14] and oregano (*Origanum vulgare* L.) [15]. Thus, cultivation of medicinal and aromatic plants under controlled environments such as greenhouses, growth chambers, and playhouses provides a sustainable opportunity to enhance the principal chief phytochemicals, maximize the biomass yield, and in addition help to prevent the loss through insects and pests, and regulate both productivity and its production [16]. However, these factors are both site and specie specific due to variations in genotype in response to different growing conditions [17].

Further amendment of both organic and inorganic fertilizers *viz.* vermicompost and NPK in medicinal and aromatic plants are known to enhance productivity and quality essential oil production is a common practice throughout the ages [18–20]. The optimum dosage management helps to improve the soil fertility status as well as improve the physicochemical characteristics, such as the cations exchange capacity, pH, water, and microbial community [21,22]. However, a lot of work has been carried out to evaluate the effect of NPK and vermicompost for maximizing production, yield, and essential oil quality of medicinal and aromatic plants. In addition to this previous work have reported the effect of different growing conditions on the growth and yield of various medicinal and aromatic crops [23,24].

Previous literature reveals the effect of growth conditions and organic and inorganic fertilization individually but to optimise better growth, biomass, and essential oil yield deeper understanding of the different dynamics is needed to enhance the productivity of medicinal and aromatic plants. However, the specific knowledge on the interactive effect of different growing conditions, and organic and inorganic fertilizers is lacking and has not been evaluated previously in the current geographical region. Thus, the present study was laid with the hypothesis that the open ventilated polyhouse cultivation in conjunction with specific doses of NPK and vermicompost will improve the growth, biomass yield, and essential oil quality of *O. gratissimum* in the high rainfall zone of South Gujarat to derive the valuable insights and develop a package of practices for farmers to meet the cost effectiveness along with enhanced productivity.

2. Materials and methods

2.1. Experimental site

The study was conducted at the Model Nursery on Medicinal and Aromatic Plants at the ASPEE College of Horticulture and Forestry, NAU, Navsari, Gujarat (AES III-Heavy Rainfall Zone) located at 20°55′20"N to 20°55′21"N latitude, 72°54′17"E to 72°54′21"E longitude, and has an altitude of 32 m above mean sea level. The study period was from September to December 2020, during which the maximum rainfall received was 15.71 mm. The average relative humidity in open conditions was 87.56 % in the morning and

61.44 % in the evening. Inside the polyhouse, the relative humidity was 5.71 % and 10.77 % higher in the morning and evening hours, respectively (as shown in Fig. 1). The average maximum and minimum temperatures in open conditions were 33.03 °C and 19.48 °C, respectively. Inside the polyhouse, the average temperature was 2 °C higher compared with open conditions (as shown in Fig. 2). The average light intensity in open conditions during the morning and evening hours was 3978.63 lux and 52509.13 lux, respectively. Inside the polyhouse, the average light intensity was 2307.31 lux and 16189.93 lux in the morning and evening hours, respectively (as shown in Figs. 3 and 4).

2.2. *Experimental details*

The study aimed to investigate the effect of growing conditions and fertilizer dose on the growth, biomass yield, and essential oil quality of *O. gratissimum.* The planting material was sourced from one year old seedlings propagated through seeds from the Directorate of Medicinal and Aromatic Plants, Anand, Gujarat, and a botanist verified the plants (Dr. Bimal S. Desai). One-year-old stem cuttings with 6–8 nodes were planted in black polythene bags (1 plant per bag and 4 pots per m^2) filled with a 2:1 ratio of red soil and sand. The soil in the experimental area was sandy clay loam in texture, neutral in reaction (pH = 7.12), and had low organic carbon content (0.43 %) but moderate levels of available nitrogen (98 mg/kg), phosphorus (29 mg/kg), and potassium (178 mg/kg).

The study employed a Completely Randomized Design (CRD) with factorial layout and included two growing conditions (open condition, G1 (as shown in Fig. 5A); and naturally ventilated polyhouse, G2 (as shown in Fig. 5B) and six fertilizer doses comprising of NPK and vermicompost (VC) (control, F1; 100 % NPK, F2; 75 % NPK fertilizer + 25 % N through VC, F3; 50 % NPK fertilizer + 50 % N through VC, F4; 25 % NPK fertilizer + 75 % N through VC, F5; and 100 % N through VC, F6) that were replicated thrice. The recommended dose of NPK (0.132:0.066:0.066 g per pot) was applied uniformly in each polybag on a volume basis. The full dose of P, K and vermicompost along with 50 % N was applied as a basal dose at the time of polybag filling, and the remaining dose of N was applied in two equal splits at 30 and 60 days after planting (DAP). Nitrogen was supplied through Urea (N = 46 %), phosphorus through SSP (P = 16 %), and potassium through MOP (K = 60 %). The NPK content of vermicompost used in the experiment was 0.5 %, 0.3 %, and 0.18 %, respectively.

2.3. *Soil analysis*

To analyze the initial soil characteristics, three composite samples were collected and analyzed for textural and chemical properties using the international pipette method [25] and various chemical analysis techniques. At the end of the experiment, soil samples were collected from five randomly selected plants per replication, and a composite representative sample was taken to evaluate the soil fertility status. The soil samples were analyzed for pH [26], electrical conductivity [26], organic carbon content [27] using the Walkley-Black method, available nitrogen [28], available phosphorus [29], and available potassium [30].

2.4. *Morphometric measurements*

In order to assess morphometric measurements, five plants were randomly selected per treatment per replication for biometric observations, such as plant height (cm; measured from the base to the tip of the highest flowering stem), number of branches per plant, number of leaves per plant, stem diameter (cm; using a digital vernier calliper), and leaf area per plant (cm^2; using a portable leaf area meter). The leaf area per plant was calculated by multiplying the mean leaf area of big and small leaves by the number of big and small leaves per plant, and reported as the mean leaf area per plant at harvest. Biomass yield parameters, including fresh and dry biomass per plant, were also recorded at the time of harvest. The dry weight was determined by oven-drying the plant at 70 °C until a constant weight was achieved (typically within 48 h).

2.5. *Leaf essential oil content (%) and yield per plant (g)*

The plants were harvested 4 cm above the soil surface in polybags and then fresh leaves were separated, washed, and cleaned. The

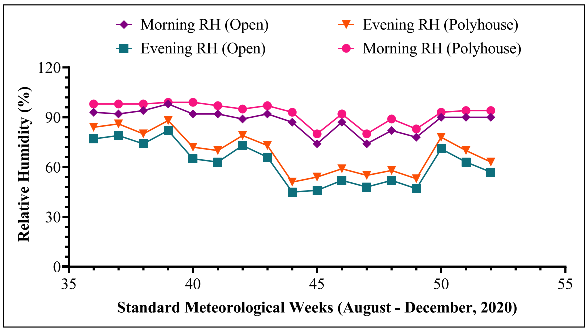

Fig. 1. Relative humidity (%) in the open and polyhouse condition.

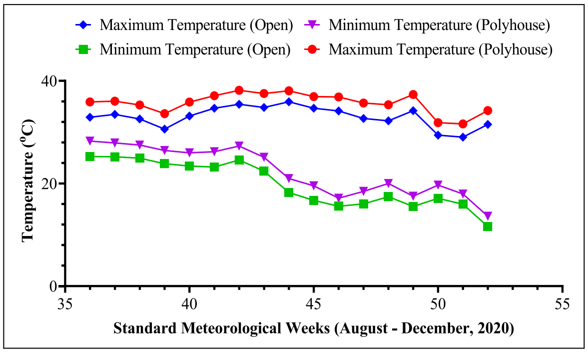

Fig. 2. Maximum and minimum temperature in the open and polyhouse condition.

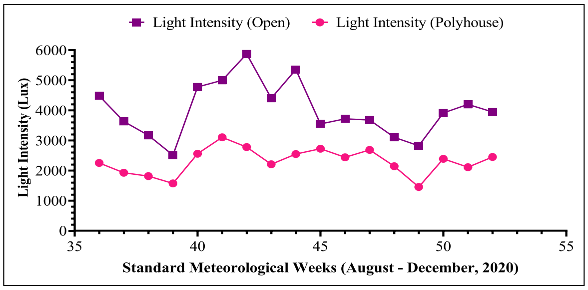

Fig. 3. Light intensity (lux) in open and polyhouse condition during morning hour.

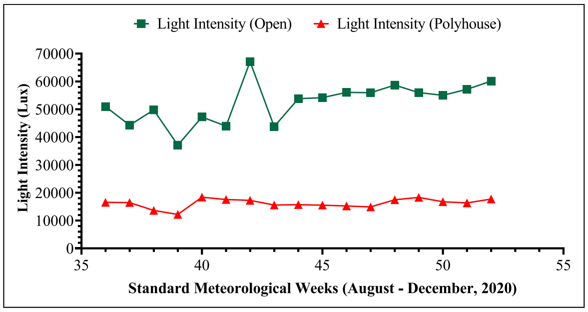

Fig. 4. Light intensity (lux) in open and polyhouse condition during evening hour.

essential oil from fresh leaves was extracted from randomly selected plants in each replication and subjected to hydro-distillation using clevenger type apparatus for 3 h. The leaf essential oil content (1) and leaf essential oil yield per plant (2) were expressed in the form of percentages and grams per plant were worked out by following formulas:

$$\text{Leaf essential oil content }(\%) = \left(\frac{\text{Essential oil extracted (ml)}}{\text{Weight of fresh leaves used for extraction (g)}}\right) \times 100 \tag{1}$$

$$\text{Leaf essential oil yield / plant (g)} = \text{Leaf fresh yield/ plant (g)} \times \text{Essential oil content }(\%) \tag{2}$$

2.6. *Eugenol content characterization in leaf essential oil using GC-FID*

The leaf essential oil was subjected to the GC – FID (Gas chromatography – Flame Ionization Detector) using Agilent 7890 A and DB - 5 ms fused silica (30 m × 0.25 mm id, 0.25 μm film thickness) capillary column. The maximum oven temperature was 425 °C programmed initially at 150 °C for 2 min and then 20 °C/min to 320 °C for 10 min. The front SS inlet He was in split mode 1:100 with a total flow of 51.317 mL/min. The injection volume was 1 μL and the column DB-5 ms with an initial temperature of 150 °C; a pressure of 10 Psi and a flow of 0. 49 mL/min. The front detector FID had a heater at 300 °C, an H2 flow of 40 mL/min, an air flow 400 mL/min,

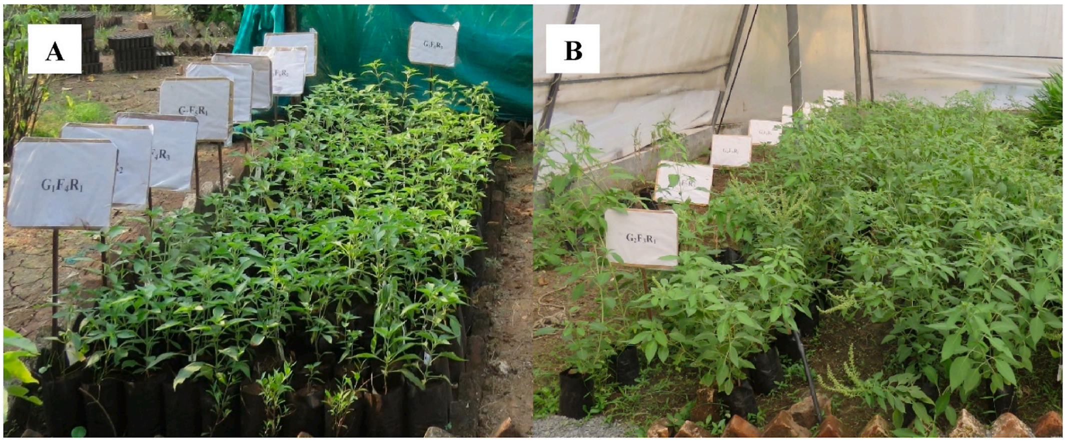

Fig. 5. Plants grown under open (A) and natural ventilated polyhouse (B) at different time intervals.

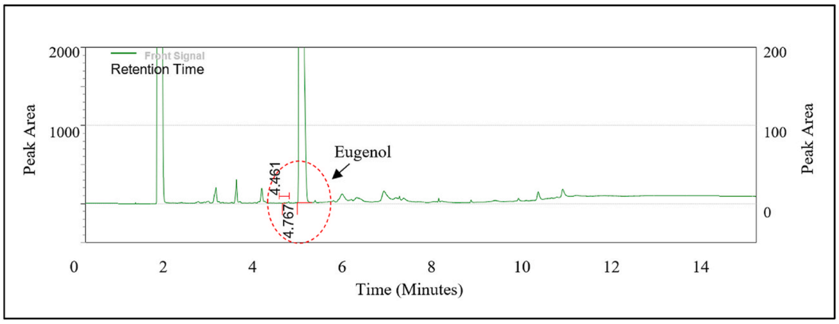

Fig. 6. GC - FID chromatogram of essential oil sample showing the presence of eugenol.

and a makeup flow of 25 mL/min. The eugenol was identified by comparing the retention time of sample (as shown in Fig. 6) with commercial standard of eugenol having 99 % purity purchased from Sigma-Aldrich (CAS no. 97-53-0). Quantification of constituents was carried out by integration of peak areas without using the correction factors. The essential oil samples were run in triplicates.

2.7. Statistical analysis

Statistical analysis was done according to the standard analysis of variance by software R. Variations among treatments were assessed by LSD values at 5 % probability ($p \leq 0.05$) level followed by letter grouping.

Table 1
Effect of different growing conditions and fertilizer doses on soil fertility status after harvesting of *O. gratissimum*.

Main effect	pH	EC (dS/m)	OC (%)	N (mg/kg)	P_2O_5 (mg/kg)	K_2O (mg/kg)
Growing Condition						
G1	7.20	0.59	0.47	91.56	35.67	176.68^a
G2	7.18	0.61	0.49	89.44	34.18	165.70^b
LSD	n.s.	n.s.	n.s.	n.s.	n.s.	*
Fertilizer Doses						
F1	7.24^a	0.57^c	0.42^e	85.75^c	24.48^e	162.41
F2	7.21^{ab}	0.57^c	0.43^{de}	86.79^{bc}	27.72^{de}	166.81
F3	7.21^{ab}	0.59^{bc}	0.45^d	88.35^{bc}	29.98^d	170.02
F4	7.19^{abc}	0.61^{abc}	0.49^c	91.54^{ab}	37.45^c	171.74
F5	7.16^{bc}	0.62^{ab}	0.53^b	93.82^a	42.79^b	176.60
F6	7.12^a	0.64^a	0.56^a	96.74^a	47.11^a	179.55
LSD	*	*	*	*	*	n.s.

(G1: Open condition; G2: Polyhouse condition; F1: Control, F2: 100 % NPK; F3: 75 % NPK + 25 % N through VC; F4: 50 % NPK + 50 % N through VC; F5: 25 % NPK + 75 % N through VC; F6: 100 % N through VC; VC - vermicompost; *- Significant at 5 % level of significance; n.s. - non significant). The same letters are not significantly different at the 5 % level of significance.

3. Results and discussion

3.1. Soil fertility status

The effect of different growing conditions on soil parameters was found to be insignificant, with the exception of K_2O at harvest in *O. gratissimum* as shown in Table 1. However, different doses of organic and inorganic fertilizers significantly affected the soil fertility (Table 3). The maximum pH (7.20), N (91.56 mg/kg), P_2O_5 (35.67 mg/kg) and K_2O (176.68 mg/kg) were reported in plants grown under open conditions (G1). In contrast, the highest EC (0.64 dS/m), organic carbon (0.56 %), N (96.74 mg/kg), P_2O_5 (47.11 mg/kg) and K_2O (179.55 mg/kg) were reported in plants treated with 100 % N through vermicompost (F6), followed by plants treated with 25 % NPK and 75 % N through vermicompost (F5). Similar findings were observed in patchouli (*Pogostemon cablin* Benth.) [18]. The interaction between the different growing conditions and fertilizer doses was found to be insignificant for all soil parameters. Among the different treatment combinations, the maximum N (98.6 mg/kg), P_2O_5 (47.3 mg/kg) and K_2O (179.55 mg/kg) were reported in plants grown under open conditions (G1) and treated with 25 % NPK + 75 % N through vermicompost (F5). The highest organic carbon (0.56 %) at harvest was reported in plants grown under polyhouse conditions (G2) and treated with 25 % NPK + 75 % N through vermicompost (F5).

3.2. Growth and biomass yield

3.2.1. Effect of growth condition

An analysis of the data indicates that plant height, number of branches, number of leaves, leaf area, fresh and dry biomass yield per plant were significantly affected by both open and polyhouse conditions, with the exception for stem diameter in *O. gratissimum*, as shown in Table 3. The plant height (12.98 %), stem diameter (1.69 %), number of branches (12.98 %), number of leaves (131.49 %), leaf area (151.09 %), fresh biomass (131.90 %), and dry biomass (108.27 %) per plant were higher under polyhouse conditions (G2) compared with open conditions (G1). Plants under polyhouse conditions displayed a significantly higher growth rate than those under open conditions due to changes in microclimatic conditions. The low intensity of light inside the polyhouse enhanced the metabolic activities(i.e. higher photosynthesis and respiration rate) which plays a major role in the growth and development of the plant. Furthermore, Fernandes et al. [24] revealed an inverse relationship between plant height and light intensity in *O. gratissimum*. The similar significant results are also reported by Panwar et al. in *Withania somnifera* and *Psoralea corylifolia*. Thus, plant height was significantly improved under polyhouse condition due to low light intensity. However, the variation in stem diameter may be due to variations in planting technique [32]. In addition to this, the congenial weather inside the polyhouse led to the development of a greater number of branches per plant. Deraman et al. [33] reported that low light intensity under polyhouse condition led to develop more number of primary branches per plant in *Mentha arevensis*. The results are in accordance with the findings of Amador et al. [11] in

Table 2
Growth and biomass productivity of *O. gratissimum*.

Main effect	Plant height (cm)	Stem diameter (cm)	Number of branches/plant	Number of leaves/plant	Leaf area/plant (cm^2)	Fresh biomass/ plant (g)	Dry biomass/ plant (g)
G1	69.53^{b}	0.60	4.06^{b}	72.71^{b}	1397.90^{b}	50.12^{b}	10.51^{b}
G2	78.56^{a}	0.59	6.81^{a}	168.32^{a}	3510.15^{a}	116.23^{a}	21.89^{a}
LSD	*	n.s.	*	*	*	*	*
F1	55.43^{c}	0.48^{e}	3.87^{d}	91.93^{d}	1734.64^{d}	69.26^{c}	13.66^{e}
F2	74.92^{b}	0.57^{d}	5.30^{c}	118.70^{c}	2627.21^{b}	82.71^{b}	15.40^{d}
F3	79.70^{a}	0.62^{bc}	5.63^{bc}	126.10^{b}	2841.08^{a}	86.40^{ab}	16.34^{cd}
F4	81.65^{a}	0.66^{a}	6.00^{ab}	132.83^{a}	2241.41^{c}	88.82^{a}	17.44^{ab}
F5	80.46^{a}	0.65^{ab}	6.25^{a}	135.30^{a}	2936.49^{a}	89.71^{a}	17.76^{a}
F6	72.13^{b}	0.61^{c}	5.57^{bc}	118.23^{c}	2343.31^{c}	82.18^{b}	16.60^{bc}
LSD	*	*	*	*	*	*	*
Interactions							
G1F1	47.48^{i}	0.47	2.93	58.07^{h}	1104.28^{i}	38.00	7.93
G1F2	68.83^{g}	0.56	3.67	67.60^{g}	1283.08^{hi}	48.74	9.85
G1F3	76.05^{de}	0.62	4.13	74.13^{fg}	1731.59^{f}	53.26	10.79
G1F4	78.57^{cd}	0.68	4.60	81.93^{f}	1609.50^{fg}	56.54	12.07
G1F5	75.77^{de}	0.66	4.77	79.53^{f}	1417.21^{gh}	54.49	11.58
G1F6	70.50^{fg}	0.62	4.27	75.00^{fg}	1241.71^{hi}	49.70	10.82
G2F1	63.37^{h}	0.49	4.80	125.80^{e}	2365.01^{e}	100.52	19.39
G2F2	81.02^{bc}	0.58	6.93	169.80^{cd}	3971.34^{b}	116.68	20.96
G2F3	83.34^{ab}	0.62	7.13	178.07^{bc}	3950.57^{b}	119.55	21.88
G2F4	84.74^{ab}	0.64	7.40	183.73^{ab}	2873.31^{d}	121.09	22.81
G2F5	85.15^{a}	0.64	7.73	191.07^{a}	4455.77^{a}	124.92	23.93
G2F6	73.77^{ef}	0.60	6.87	161.47^{d}	3444.91^{c}	114.66	22.39
LSD	*	n.s.	n.s.	*	*	n.s.	n.s.

(G1: Open condition; G2: Polyhouse condition; F1: Control, F2: 100 % NPK; F3: 75 % NPK + 25 % N through VC; F4: 50 % NPK + 50 % N through VC; F5: 25 % NPK + 75 % N through VC; F6: 100 % N through VC; VC – vermicompost; *- Significant at 5 % level of significance; n.s. – non significant). The same letters are not significantly different at the 5 % level of significance.

Table 3
Essential oil content and quality of *O. gratissimum*.

Main effect	Leaf essential oil content (%)	Leaf essential oil yield per plant (g)	Eugenol content (%)
G1	0.268	0.082^{b}	39.433^{b}
G2	0.264	0.251^{a}	47.694^{a}
LSD	n.s.	*	*
F1	0.245^{c}	0.133^{c}	37.023^{d}
F2	0.245^{c}	0.152^{b}	42.705^{c}
F3	0.254^{c}	0.163^{b}	43.345^{c}
F4	0.273^{b}	0.177^{a}	43.607^{bc}
F5	0.283^{ab}	0.189^{a}	47.202^{ab}
F6	0.298^{a}	0.184^{a}	47.500^{a}
LSD	*	*	*
Interaction			
G1F1	0.240	0.060^{g}	35.443^{d}
G1F2	0.246	0.073^{fg}	38.747^{bcd}
G1F3	0.256	0.081^{ef}	39.443^{bcd}
G1F4	0.281	0.095^{e}	39.793^{bcd}
G1F5	0.285	0.093^{e}	40.287^{bcd}
G1F6	0.299	0.087^{ef}	42.883^{bcd}
G2F1	0.249	0.206^{d}	38.603^{cd}
G2F2	0.243	0.231^{c}	46.663^{abc}
G2F3	0.252	0.245^{bc}	47.247^{abc}
G2F4	0.264	0.259^{b}	47.420^{ab}
G2F5	0.280	0.284^{a}	54.117^{a}
G2F6	0.297	0.280^{a}	52.117^{a}
LSD	n.s.	*	*

(G1: Open condition; G2: Polyhouse condition; F1: Control, F2: 100 % NPK; F3: 75 % NPK + 25 % N through VC; F4: 50 % NPK + 50 % N through VC; F5: 25 % NPK + 75 % N through VC; F6: 100 % N through VC; VC - vermicompost; *- Significant at 5 % level of significance; n.s. - non significant). The same letters are not significantly different at the 5 % level of significance.

thyme (*Thymus vulgaris* L.). Usually, the number of leaves per plant exhibits a linear relationship with increase in light intensity. But the findings of the present investigation were not in confirmation to previous study in *O. gratissimu m* [24]. The deflection obtained in results could be due to change in geographical location and environmental factors (such as temperature and relative humidity). Although, the congenial growth conditions inside the polyhouse led to higher plant height and number of branches per plant which ultimately led to the production of a higher number of leaves per plant. The leaf area is often affected by several factors such as temperature, light intensity, wind speed, the incidence of insects and pests as well as nutrient efficacy. Previous studies on basil (*O. bassilicum* L.) by Caliskan et al. [34] discovered higher leaf area per plant in plants grown at low light intensities signifying that specific leaf area linearly decreases with an increase in light intensity. Several other studies have reported that plants grown under high temperature environments usually have higher leaf area [35]. The results were in concurrence with those of Chang et al. [36] in *O. basillicum*; Ademilau et al. [23] in *O. gratissimum*; Amador et al. [11] in thyme and oregano.

In the present study the fresh and dry biomass yield was higher under polyhouse condition. The results from previous literature revealed that the variation in growing environment significantly affects the light intensity and temperature which play a major role in biomass production [24]. Similarly, Panwar et al. [31] and Hazarika and Saud [37] found higher fresh and dry biomass yield in plants grown under polyhouse condition in comparison to open condition. Several other studies have reported that dry matter accumulation linearly increases with the increase in light intensity but however inside polyhouse condition the due to reduction in the respiration rate to photosynthesis rate ratio the dry biomass yield is higher in comparison to open conditions [38]. The results regarding fresh and dry biomass yield are in agreement with those of Chang et al. [36] in *O. basillicum* and Ademilau et al. [23] in *O. gratissimum*. All the morphometric and biomass variables were significantly higher under the polyhouse condition due to the favourable growth conditions.

3.2.2. Effect of fertilizer doses

The different doses of organic and chemical fertilizer had a significant impact on all plant growth and biomass yield parameters, as shown in Table 2. The maximum plant height (47.30 %) and stem diameter (37.50 %) were observed among plants treated with a combination of 50 % NPK fertilizer and 50 % N through vermicompost (F4) compared with the control (F1). However, the highest number of branches (61.50 %), number of leaves (47.18 %), leaf area (69.29 %), fresh biomass (29.52 %), and dry biomass (30.01 %) per plant were recorded in plants treated with a combination of 25 % NPK fertilizer and 75 % N through vermicompost (F5) compared with the control (F1). The minimum growth was recorded on the control treatment (F1).

In the present study, the plant height and number of branches significantly enhanced under the combined application of NPK and vermicompost in comparison to the alone application. The increase in plant height may be due to the activity of the basic nutrients which are in readily available form for the plant as nitrate, phosphate, and potash in both organic and inorganic fertilizers during the whole growing period and that might have contributed in increasing higher number of axillary buds leading to development of lateral branches and height of the plant [39]. The results of the present study accord with Kalita et al. (2018) in *O. gratissimum* at different time intervals. Similarly, Singh et al. (2015) quantified that the combined application of both NPK and vermicompost significantly affected the number of branches per plant in patchouli and Zaman et al. (2018) also reported the same effect in *Stevia rebaudiana* under field condition.

In the present studies, it was found that the stem diameter is significantly affected by the combined application of both NPK and vermicompost might be due to the availability of both macro and micro nutrients throughout its growing period. Amin (2011) in maize (*Zea mays* L.) and Alhasan et al. (2020b) in *O. basilicum* cv. Dolly found the same effect on stem diameter by using different doses of NPK fertilizer alone. Whereas, Aslam et al. (2020) found the same results by using different treatments of vermicompost alone in *Solanum lycopersicum.* Thus, it is depicted from the above experiments that combined effect of both NPK and vermicompost can also show a significant effect on growth of stem diameter.

The number of leaves per plant and leaf area significantly increased with the combined application of both NPK and vermicompost as compared to applying alone. The increase in the number of leaves per plant is attributed to the improvement in the biological activities of soil and the availability of nutrients that may enhance the number of leaves. Similar results were also stated by Hasan et al. (2015) in *O. basilicum* with NPK; Ayyobi et al. (2017) in *M. piperita* and Dobhal et al. (2020) in *O. sanctum* with vermicompost.

The maximum fresh and dry biomass per plant was highest in F5. The similar results were found by Singh et al. (2015) in patchouli. Several other studies also revealed that the combined application of both NPK along with vermicompost increases the fresh biomass in *O. sanctum* (Raina et al., 2013), *M. officinalis* (Mafakheri et al., 2016), *M. canadensis* (Ardalani et al., 2017) and *O. gratissimum* (Kalita et al., 2018).

The addition of vermicompost along with NPK fertilizer resulted in improved plant growth and biomass yield per plant, likely due to the high microbial population and enhanced enzymatic activity in the soil, as well as increased accessibility of various macro and micronutrients. Except for pH all other soil parameters were enhanced under combined application of vermicompost and NPK in comparison to control (Table 1). However, the results were in lieu with the previous findings on *P. cablin* [18], *O. gratissimum* [40] and *S. rebaudiana* [41].

3.2.3. Interaction effect

The results of the data analysis indicate that the interaction effect between the open and polyhouse conditions, as well as the different doses of organic and chemical fertilizers, significantly affected the plant height (as shown in Fig. 7), number of leaves, and leaf area per plant (as shown in Fig. 8). However, there was no significant effect on the other growth and biomass yield parameters (Table 2). The treatment combination of 75 % NPK and 25 % N through vermicompost under polyhouse conditions (G2F5) resulted in the highest plant height, number of branches, number of leaves, leaf area, total fresh, and total dry biomass per plant. Conversely, the highest stem diameter was observed in the treatment combination of 50 % NPK +50 % N through vermicompost under open conditions (G1F4).

Fig. 7. Plant height at the harvesting stage under the influence of different growing conditions and fertilizer doses
(G1: Open Condition; G2: Polyhouse Condition; F1: Control, F2: 100 % NPK; F3: 75 % NPK + 25 % N through VC; F4: 50 % NPK + 50 % N through VC; F5: 25 % NPK + 75 % N through VC; F6: 100 % N through VC; VC - vermicompost).

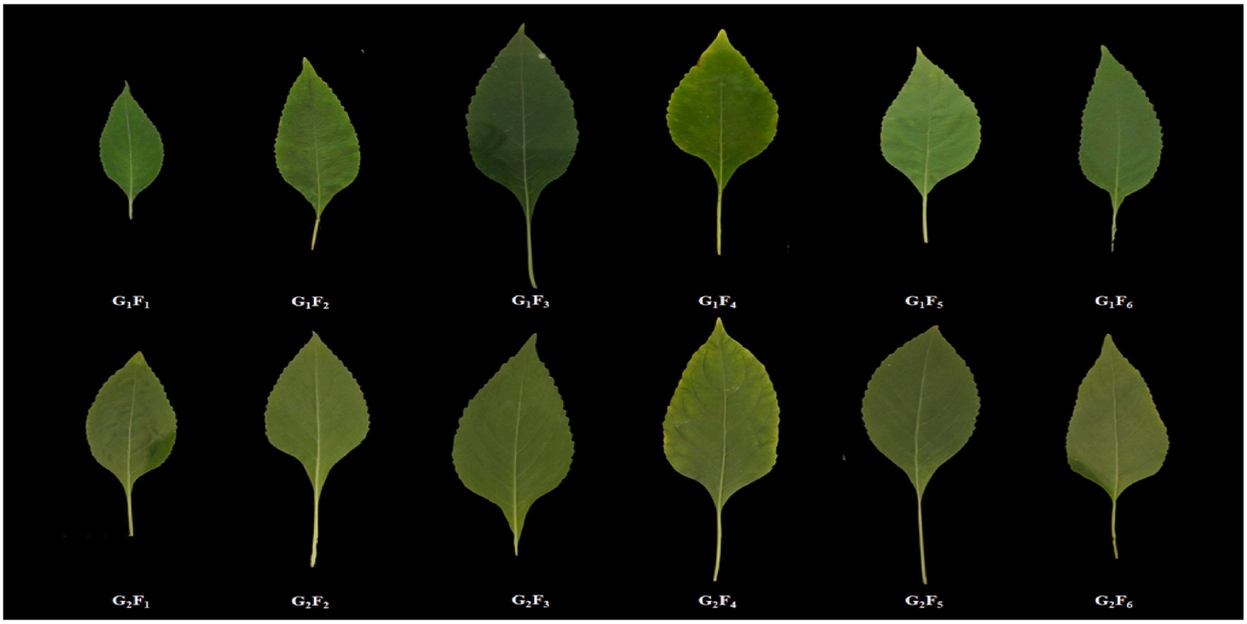

Fig. 8. Leaf area at the harvesting stage under the influence of different growing conditions and fertilizer doses
(G1: Open Condition; G2: Polyhouse Condition; F1: Control, F2: 100 % NPK; F3: 75 % NPK + 25 % N through VC; F4: 50 % NPK + 50 % N through VC; F5: 25 % NPK + 75 % N through VC; F6: 100 % N through VC; VC - vermicompost).

3.3. Essential oil and eugenol content

3.3.1. Effect of growth condition

In present study, different growing conditions showed a significant effect on leaf essential oil yield per plant and eugenol content, but not on leaf essential oil content as shown in Table 3. The maximum leaf essential oil content was found in plants grown under open conditions, while the highest leaf essential oil yield per plant and eugenol content were observed in plants grown under polyhouse conditions. The leaf essential oil content varied from 0.264 to 0.268 %. The leaf essential oil yield and eugenol production were 206.09 % and 20.95 % higher in the polyhouse (G2) compared with the open condition (G1), respectively.

Castronuovo et al. [42] reported that exposure of plants to different growing conditions leads to the development of stress in plants that leads to the production of higher secondary metabolites as compare to normal conditions. Nevertheless, several studies have reported that the abiotic stress under polyhouse condition due to higher temperature and reduced light intensity is an important attribute that significantly improves the metabolism and productivity of secondary metabolites [43]. Light intensity is an important factor that plays a significant role in plant growth, metabolism, and fixation of carbon essential for the synthesis of essential oils [36]. In addition to this the favourable growth conditions inside the polyhouse resulted in higher fresh leaf biomass which resulted in a higher amount of essential oil yield per plant [23]. Ademilau et al. [23] reported a higher yield of essential oil per plant under the reduced light conditions due to a congenial environment for growth exhibiting higher fresh leaf biomass in *O. gratissimum.* Similarly, Sabika [44] observed higher essential oil yield (0.61 mL per 100 g leaves) for plants grown under Low Density Polythene Film (LDPE) in comparison to open in *O. basilicum.* Whereas, in *Plectranthus vettiveroides* higher essential oil content was observed in plants grown under 50 % shade in comparison to open conditions.

Further, several studies have revealed that light intensity and temperature play an important role in the composition of essential oils. Plants growing under daylight condition resulted in higher production of phenylpropene eugenol [45]. However, in the present study, a higher eugenol content was reported in plants grown under polyhouse conditions in comparison to open. The variation in essential oil content composition can be attributed due to the combined effect of genotype, environmental factors (such as light and temperature), and growing technique [46]. Additionally, eugenol production has a direct relationship with temperature, exhibiting higher production at higher temperatures. Since the average maximum temperature was higher under polyhouse conditions (G2), the results of the present study are in line with those of *O. basilicum* grown at high temperatures [47].

3.3.2. Effect of fertilizer doses

The different doses of organic and chemical fertilizers had a significant impact on essential oil quality, as shown in Table 3. In the present study the essential oil content varies from 0.245 to 0.298 % in *O. gratissimum.* The highest percentage leaf essential oil content and eugenol was observed in plants treated 100 % vermicompost (F5). While leaf essential oil yield per plant was recorded maximum in plants treated with 25 % NPK +75 % N through vermicompost (F5). The leaf essential oil yield per plant was 42.11 % higher among plants treated with 25 % NPK +75 % N through vermicompost (F5) compared with the control. Additionally, eugenol content increased by 28.29 % in plants treated with 100 % N through vermicompost compared with the control.

However, several other studies in medicinal and aromatic plant species clearly showed that the combined application of organic and inorganic fertilizer reveals enhanced essential oil content and yield in Lamiaceae family [40,48]. The nitrogen and phosphorous supplied by the different fertilizers play an important role in formation of ATP and NADPH that ultimately act as a key component in synthesis of several secondary metabolite compounds [49]. Raina et al. [50] demonstrated the effect of different nitrogen level and vermicompost in *O. sanctum.* The essential oil yield was significantly increased in plants under the combined application of nitrogen (60 kg ha-^{1}) and vermicompost (3 t ha^{-1}). Similarly, Ram et al. [51] emphasized that the combined application of synthetic nitrogen and vermicompost can significantly enhance the essential oil yield in *M. arvensis.* The maximum essential oil yield was obtained in plants treated with synthetic nitrogen (112.5 kg ha^{-1}) and vermicompost (9 t ha^{-1}). Furthermore, Anwar et al. [52] determined the effect of different organic (FYM - 10 t ha^{-1} and vermicompost - 10 t ha^{-1}) and inorganic fertilizers (NPK - 100:50:50 kg ha^{-1}) on *O. basilicum* L. cv. Vikas Sudha. The authors revealed that maximum essential oil yield (121.30 l ha^{-1}) was obtained in plants treated with vermicompost and NPK. In another study, Ardalani et al. [53] emphasized that the combined application of nitrogen (400 kg ha^{-1}) and vermicompost (15 t ha^{-1}) enhanced the essential oil content in Mentha. The results from the previous studies were in agreement with the finding of the present study and demonstrated that combined optimum dosage of organic and inorganic fertilizers is effective in improving the essential oil yield due to higher water retention capacity which plays a critical role in mineralization and uptake on nutrients by plants. In addition to this the enhanced biomass in plants treated with 25 % NPK +75 % N through VC (Table 2) lead to the maximum essential oil production.

However, the essential oil content and eugenol percentage was reported maximum in plants treated solely treated with the vermicompost attributed due to the improved the soil fertility status (Table 1) as reported in the present study. The organic fertilizers play a critical role in enhancing the soil structure as well as nutrient status by improving the secondary metabolite pathways than subsequently enhances their percentage in the essential oil [54,55]. The results are in congruence to findings on *O. sanctum* plants exhibiting higher eugenol content for those amended with organic fertilizers in comparison to inorganic fertilizers [56]. Similarly, higher Forskolin content in *Coleus forskohlii* was shown by plants treated with organic fertilizer *viz.* vermicompost [57]. Both the essential oil yield and enhancement of chief principal component under the combined application of organic and inorganic fertilizers can be effectively used to meet the market demands of eugenol from *O. gratissimum.*

3.3.3. *Interaction effect*

The interaction effect of different growing conditions, organic and chemical fertilizer was found significant for leaf essential oil yield per plant and Eugenol content. However, it has a non-significant effect on leaf essential oil content. The maximum leaf essential oil content was observed in treatment combination 100 % N through VC under open condition (G1F6). Whereas, the leaf essential oil yield per plant and Eugenol content was highest in treatment 25 % NPK +75 % N through VC under polyhouse condition (G2F5). The leaf essential oil yield, and Eugenol content increased up to 373.33 % and 52.68 % in comparison to control, respectively.

4. Conclusion

In conclusion, the study found that growing *O. gratissimum* under polyhouse conditions resulted in higher plant growth and biomass yield compared with open conditions. In terms of essential oil production, the highest leaf essential oil yield per plant and eugenol content were observed in plants grown under polyhouse conditions, while the highest leaf essential oil content was found in plants grown under open conditions. The use of a combination of 25 % NPK fertilizer and 75 % N through vermicompost also resulted in improved plant growth, biomass yield and essential oil quality as well as improved the soil fertility status at the end of experiment. Thus, present study indicates that the plants grown under polyhouse conditions supplemented with the 25 % NPK +75 % N through vermicompost is the optimum dosage for meeting the demands of eugenol production under the high rainfall areas of the South Gujarat. Overall, the results suggest that proper manipulation of environmental factors such as light intensity and temperature, and growing techniques can significantly enhance essential oil content and yield and benefit the farmers' economy.

Data availability statement

All the relevant data has been included in the manuscript.

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CRediT authorship contribution statement

Sunandani Chandel: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Bimal S. Desai:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Formal analysis, Conceptualization. **Suman Kumar Jha:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Formal analysis, Conceptualization. **Satish Kumar Sinha:** Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Dhiraji P. Patel:** Visualization, Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Nilay Kumar:** Writing – review &

editing, Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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