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Influence of organic manures and bio fertilizers on growth, yield and quality of holy basil

Nilanjan Biswas[1*], Trinayanee Sharma[1], Soumik Samanta[1], Narendra Nath Hansda[2] and Narayan Chattopadhyay[1]

[1]Department of Plantation, Spices, Medicinal and Aromatic Crops, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741252, India

ABSTRACT

Organic nutrient inputs combined with beneficial microbes offer sustainable alternatives to chemical fertilizers in Tulsi (*Ocimum* spp.) cultivation, yet field evidence from the eastern Indian alluvial zone remains limited. A two-season trial (early summer 2022–2024) was conducted at the Horticultural Research Station, Mondouri, BCKV, Nadia, West Bengal, on loamy sand soil (pH 6.8) with moderate NPK levels. Seven combinations of vermicompost, farmyard manure (FYM), and mustard cake (MC) with *Azotobacter* and phosphate-solubilising bacteria (*PSB*) were evaluated in a randomized block design with three replications. At 180 and 360 days after planting, treatments significantly outperformed the control in growth, herbage yield, essential oil content, and phytochemicals. T_3 (MC 1.5 t ha^{-1} + *Azotobacter*) recorded the highest plant height (86.47 cm), while T_4 (vermicompost 5 t ha^{-1} + PSB) yielded the maximum fresh herbage (23.59 t ha^{-1}) and eugenol content (8.04%). T_2 (FYM 15 t ha^{-1} + *Azotobacter*) produced the highest essential oil yield (75.58 kg ha^{-1}). T_6 (MC + PSB) enhanced methyl eugenol (7.21%) and β-elemene (0.74%). Strong correlations (r = 0.98–1.00) between early-season traits and final output highlight predictive value. Results suggest integrating moderate-to-high organic inputs with biofertilizers can enhance Tulsi biomass and tailor essential oil quality.

Key words*:* *Azotobacter,* essential oil, farm yard manure, holy basil*,* vermicompost.

INTRODUCTION

Ocimum sanctum L., belongs to family Lamiaceae widely known as Tulsi, holds a sacred status in Indian traditions and is esteemed for its medicinal value. It is also known as Holy Basil, Sacred Basil and the Queen of Herbs. The term "Basil" is derived from the Greek word Basilica, meaning "Royal plant." In ancient Greece, royal individuals utilized an *Ocimum* species, specifically *Ocimum basilicum* var. glabratam, to flavour their distinctive culinary preparations (Nikbakht *et al*., 13). In India, this plant has been cultivated for centuries due to its diverse therapeutic, cultural and industrial uses. Essential oils like eugenol, linalool, camphor, citral, thymol and geraniol are abundant in Tulsi and are of high commercial importance in perfumery and cosmetic industries. Notably, eugenol, abundantly present in its leaves and flowers, is known for its analgesic and hypoglycaemic properties (Gupta *et al*., 10). In addition to these volatile constituents, the plant harbours a wide range of bioactive compounds like coumestans, triterpenes and their glycosides, which contribute to its pharmacological significance and justify its reputation as a medicinally potent herb (Dakshayani *et al*., 5). With growing consumer preference for herbal remedies, there has been a notable surge in the cultivation of medicinal and aromatic plants. This shift has intensified the need for sustainable production systems that are environmentally safe and capable of enhancing both yield and quality. Organic manures now serve as environmentally sound substitutes for synthetic fertilizers in sustainable cropping systems. These inputs contribute key macro and micronutrients, improve soil moisture holding ability and enhance nutrient exchange dynamics.

Biofertilizers such as *Azotobacter* and phosphate solubilizing bacteria (*PSB*) play a pivotal role in organic nutrient management. *Azotobacter*, a non-symbiotic nitrogen fixer, aids plant development by improving nitrogen availability and secreting phytohormones such as indole acetic acid (IAA) and cytokinin. On the other hand, *PSB* mobilizes insoluble phosphorus in the soil, making it available to plants and thereby facilitates better root development, flowering and essential oil biosynthesis. When applied alongside organic matter, *Azotobacter* and *PSB* work synergistically to improve nutrient availability and plant uptake efficiency, resulting in improved growth performance and metabolite accumulation in medicinal plants (Harishkumar *et al*., 11). The food and pharmaceutical sectors are now among the largest

*Corresponding Author Email: biswasnilanjan97@gmail.com

[2]Department of Vegetable Science, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, 741252, India

consumers of plant based essential oils (Gunda *et al.*, 9), prompting further interest in safe and standardized raw material production. As a short duration, cost effective medicinal and aromatic crop, sacred basil is particularly well suited for large scale organic cultivation within existing cropping systems (Ajjan *et al.*, 4). Given the rising industrial demand and the need to ensure consistent quality and productivity, there is a growing imperative to optimize input strategies for Tulsi cultivation using sustainable methods.

Therefore, this study aimed to develop a comprehensive organic production technology for holy basil using on farm organic residues in combination with biofertilizers (*Azotobacter* and *PSB*). The investigation focused on evaluating the impact of organic nutrient management on vegetative growth, economic yield and chemical composition, with the broader goal of promoting environmentally responsible and high-quality production of this important medicinal herb.

MATERIALS AND METHODS

The present investigation was conducted during the early summer seasons (February-March) over two consecutive years (2022-2023 and 2023-2024), at the Horticultural Research Station, Mondouri, under Bidhan Chandra Krishi Viswavidyalaya, located in Nadia district of West Bengal, India. The experimental site lies in the New Alluvial Zone of West Bengal, situated at 23.5° N latitude and 89° E longitude, with an altitude of 9.75 meters above mean sea level.

The physico-chemical properties of the soil (0-15 cm depth) revealed a sandy loam texture, with 56% sand, 27.7% silt and 14.8% clay. The soil exhibited a moderately acidic pH of 6.8, a good water holding capacity and the following available nutrient status: nitrogen (267.20 kg ha^{-1}), phosphorus (24.19 kg ha^{-1}) and potassium (220.36 kg ha^{-1}).

The experiment was laid out in a Randomized Block Design (RBD) with three replications to minimize environmental variability and enhance precision. Seven treatments (T_1-Vermicompost 7.5 tha^{-1} + *Azotobacter* 10 g kg^{-1} of seed, T_2- Farm yard manure 15 tha^{-1} + *Azotobacter* 10 g kg^{-1} of seed , T_3-Mustard cake 1.5 tha^{-1} + *Azotobacter* 10 g kg^{-1} of seed, T_4- Vermicompost 5 tha^{-1} + Phosphate solubilising bacteria 10 gkg^{-1} of seed, T_5- Farm yard manure 10 tha^{-1} + Phosphate solubilising bacteria 10 g kg^{-1} of seed, T_6- Mustard cake 1.5 tha^{-1} + Phosphate solubilising bacteria 10 g kg^{-1} of seed and T_7-Control (seeds and roots soaked in distilled water) were evaluated. The plot size was 3 m × 1.8 m and a spacing of 60 cm × 60 cm was maintained, accommodating 15 plants per plot. Seeds of *Ocimum sanctum* were procured from the germplasm block of Medicinal and Aromatic Crops, HRS, Mondouri. Sowing was done in nursery beds by mid-January and healthy seedlings (10-15cm in height, 4 weeks old) were transplanted to the main field during the morning hours in mid-February.

Vermicompost (3.71 kg $plot^{-1}$), FYM (7.42 kg $plot^{-1}$) and mustard cake (0.74 kg $plot^{-1}$) were incorporated into the soil as per treatment allocation during bed preparation. Biofertilizers (*Azotobacter* and *PSB*) were applied through seed treatment, wherein seeds (200g in each treatment) were soaked for 3- 4 hours in a 2 g L^{-1} biofertilizer solution. In the control treatment, seeds were soaked only in distilled water. Standard agronomic practices recommended for Tulsi cultivation were followed uniformly across all plots throughout the experimental period. Five plants per plot were randomly tagged to record vegetative growth parameters like Plant height (cm), Number of primary branches, Number of secondary branches and Number of leaves per plant. For yield estimation, the following parameters were recorded: Fresh herbage yield (t ha^{-1}), Dry herbage yield (tha^{-1}) and Essential oil yield (kg ha^{-1}). The quality parameters included: Eugenol (%), β-elemene (%) and total phenolic content mg GAE g^{-1} DW (Ahamad *et al.,* 2). The collected data from both experimental years were subjected to analysis of variance (ANOVA) using Fisher's and Snedecor's F test at a 5% level of significance ($p \leq 0.05$) to evaluate the statistical significance of treatment effects on growth, yield and quality parameters of *Ocimum sanctum*. For the comparison of treatment means, Duncan's Multiple Range Test (DMRT) was employed as a post hoc analysis. DMRT was selected over other multiple comparison procedures such as the Least Significant Difference (LSD) or Tukey's HSD due to its greater sensitivity in detecting treatment differences, particularly when dealing with a moderate number of treatments and replications a common scenario in horticultural field research (Gomez and Gomez, 8). DMRT is particularly effective in distinguishing subtle yet biologically significant differences among treatments, while maintaining control over Type I error. The suitability of DMRT in crop production research has been well demonstrated in similar studies. For instance, Farooque *et al.* (6) applied DMRT to separate treatment means in a field experiment assessing the impact of organic and inorganic fertilizers on tomato (*Solanum lycopersicum*), reporting significant differences among treatments for growth and yield traits using this method. The appropriateness of DMRT in *Ocimum sanctum* studies has also been demonstrated notably by Gunda *et al.* (9), who used the test to distinguish among treatments influencing growth, dry herb yield, essential oil content and oil yield in holy basil when utilizing organic and microbial nutrient sources. In addition to mean comparisons, Pearson's correlation coefficient (r) was calculated to examine the relationships between vegetative growth traits, yield parameters and phytochemical quality attributes such as eugenol content, β-elemene and total phenolics. All statistical analyses were conducted using standard procedures as described by Gomez and Gomez (8).

RESULTS AND DISCUSSION

The data presented in table 1, 2 and 3 clearly showed that the organic manures and biofertilizer played significant role in directly affecting growth, yield and quality parameters.

At 180 days after planting (DAP), plant height was highest in T_3 (73.69 cm), closely followed by T_4 (73.10 cm) and T_2 (72.41 cm), while the lowest was recorded in T_7 (47.41 cm). At 360 DAP, T_3 observed the maximum height (86.47 cm), followed by T_4 (85.43 cm) and T_2 (82.87 cm), with T_7 again recording the minimum height (62.61 cm). Regarding the number of primary branches per plant at 180 DAP, the maximum was observed in T_6 (28.65), closely followed by T_5 (28.22), while T_7 had the minimum (20.32). At 360 DAP, T_6 again recorded the highest number of primary branches (37.43), followed closely by T_5 (35.42), whereas the minimum remained in T_7 (26.79). In terms of secondary branches at 180 DAP, T_6 led with 74.03 branches, followed by T_5 (73.02) and the lowest was in T_7 (45.76). At 360 DAP, T_5 recorded the highest number of secondary branches (87.60), marginally ahead of T_6 (86.44), while T_1 showed the minimum (67.82). For the number of leaves per plant at 180 DAP, T_3 had the maximum (967.34), followed by T_5 (958.36) and T_7 recorded the least (716.84). During 360 DAP, the leaf count peaked in T_3 (1450.66), followed by T_4 (1416.71), while the lowest count was again recorded in T_7 (907.13). This pattern clearly demonstrates that treatments T_3, T_4, T_5 and T_6 performed better than others across most vegetative parameters, while T_7 consistently recorded the poorest values across all observed traits and growth stages.

At 360 days after planting (DAP), fresh herbage yield per plant was recorded as highest in T_4 (23.59 tha^{-1}), followed very closely by T_5 (23.56 tha^{-1}), while the lowest yield was observed in T_7 (11.36 tha^{-1}). Regarding dry herbage yield, the maximum was also recorded in T_5 (4.38 tha^{-1}), which slightly exceeded over T_4 (4.27 tha^{-1}), whereas the minimum dry yield was found in T_7 (2.55 tha^{-1}). For essential oil yield, the highest value was obtained in T_2 (75.58 $kgha^{-1}$), followed closely by T_1 (72.38 $kgha^{-1}$) and T_5 (72.12 $kgha^{-1}$). However, the

Table 2: Effect of organic manures and bio fertilizers on yield attributes of Holy Basil.

Treatment	Fresh herbage yield $plant^{-1}$ (t ha^{-1})	Dry herbage yield (t ha^{-1})	Essential oil yield (Kg ha^{-1})
T_1	16.61c	3.20bc	72.38ab
T_2	20.94ab	3.70b	75.58a
T_3	19.98abc	3.18bc	71.82ab
T_4	23.59a	4.27a	66.76bc
T_5	23.56a	4.38a	72.12ab
T_6	19.65bc	2.79cd	68.75abc
T_7	11.36d	2.55d	63.16c
S.Em(±)	1.13	0.18	2.47
C.D(P=0.05%)	3.42	0.54	7.50

(T_1- VC 7.5t ha^{-1}+ *Azoto*.10g kg^{-1} of seed, T_2 -F Y M 15t ha^{-1}+ *Azoto*.10g kg^{-1} of seed, T_3- MC 1.5t ha^{-1}+ *Azoto*.10g kg^{-1} of seed, T_4-VC 5t ha^{-1}+ *PSB*. 10g kg^{-1} of seed, T_5- FY M 10t ha^{-1}+ *PSB* 10g kg^{-1} of seed, T_6 - M C 1.5t ha^{-1}+ *PSB* 10g kg^{-1} of seed and T_7- Control (seed and root dipping in distilled water).

Table 1: Influence of organic manures and bio fertilizers on growth attributes of Holy Basil.

Treatment	Plant height (cm) Days after planting		No. of primary branches Days after planting		No. of secondary branches Days after planting		No. of leaves $plant^{-1}$ Days after planting	
	180	360	180	360	180	360	180	360
T_1	70.63a	80.67ab	22.98bc	31.85ab	55.30d	67.82b	819.24abc	1059.39c
T_2	72.41a	82.87ab	23.70bc	30.31ab	61.06cd	82.75ab	938.98ab	1057.94c
T_3	73.69a	86.47a	24.85b	29.84ab	59.82cd	66.71b	967.34a	1450.66a
T_4	73.10a	85.43ab	22.90bc	35.23a	66.73bc	80.50ab	787.85bc	1416.71ab
T_5	65.82ab	80.19ab	28.22a	35.42a	73.02ab	87.60a	958.36a	1328.87ab
T_6	58.53b	74.21abc	28.65a	37.43a	74.03a	86.44a	904.74ab	1250.63b
T_7	47.41c	62.61c	20.32c	26.79b	45.76e	70.29ab	716.84c	907.13c
S.Em(±)	2.50	4.57	1.07	2.27	2.27	5.56	49.19	58.04
C.D(P=0.05%)	7.59	13.86	3.25	6.88	6.89	16.85	149.21	176.05

(T_1- VC 7.5t ha^{-1}+ *Azoto*.10g kg^{-1} of seed, T_2 -F Y M 15t ha^{-1}+ *Azoto*.10g kg^{-1} of seed, T_3- MC 1.5t ha^{-1}+ *Azoto*.10g kg^{-1} of seed, T_4-VC 5t ha^{-1}+ *PSB*. 10g kg^{-1} of seed, T_5- FY M 10t ha^{-1}+ *PSB* 10g kg^{-1} of seed, T_6 - M C 1.5t ha^{-1}+ *PSB* 10g kg^{-1} of seed and T_7- Control (seed and root dipping in distilled water).

Table 3: Performance of organic manures and bio fertilizers on quality attributes of Holy Basil.

Treatment	Eugenol (%)	Methyl eugenol (%)	ß-elemene (%)	Total phenolic compound (mg GAE g^{-1} DW)
	360 DAP			
T_1	7.39bc	5.40e	0.67b	19.43bc
T_2	7.73ab	5.92d	0.70ab	18.28d
T_3	7.29c	5.99cd	0.61c	20.49a
T_4	8.04a	6.21c	0.62c	19.77ab
T_5	7.96a	6.58b	0.69ab	20.18ab
T_6	7.87a	7.21a	0.74a	18.67cd
T_7	7.23c	5.17e	0.62c	17.99d
S.Em(±)	0.12	0.08	0.01	0.31
C.D(P=0.05%)	0.36	0.23	0.03	0.94

(T_1- VC 7.5t ha^{-1}+ *Azoto*.10g kg^{-1} of seed, T_2 -F Y M 15t ha^{-1}+ *Azoto*.10g kg^{-1} of seed, T_3- MC 1.5t ha^{-1}+ *Azoto*.10g kg^{-1} of seed, T_4-VC 5t ha^{-1}+ *PSB*. 10g kg^{-1} of seed, T_5- FY M 10t ha^{-1}+ *PSB* 10g kg^{-1} of seed, T_6 - M C 1.5t ha^{-1}+ *PSB* 10g kg^{-1} of seed and T_7- Control (seed and root dipping in distilled water).

lowest oil yield was recorded in T_7 (63.16 $kgha^{-1}$). These results indicate that treatments T_4 and T_5 were most effective in enhancing biomass accumulation, while T_2 was particularly superior in maximizing essential oil output. In contrast, T_7 consistently exhibited the lowest performance across all yield parameters, signifying minimal input effectiveness or control treatment conditions. Regarding quality parameters at 360 days after planting (DAP), eugenol content was recorded highest in T_4 (8.04%), followed closely by T_5 (7.96%) and T_6 (7.87%), while the lowest was observed in T_7 (7.23%). In terms of methyl eugenol, the maximum concentration was found in T_6 (7.21%) followed by T_5 (6.58%) and T_4 (6.21%), whereas minimum values was seen in T_7 (5.17%). Regarding β-elemene, the highest content was again recorded inT_6 (0.74%), followed by T_2 (0.70%) and T_5 (0.69%), while the lowest was noted in T_3 (0.61%). As for total phenolic compounds (TPC), T_3 exhibited the highest concentration (20.49 mg GAE g^{-1} DW), followed closely by T_5 (20.18 mg) and T_4 (19.77 mg), while the lowest phenolic content was observed in T_7 (17.99 mg). These findings clearly indicate that T_4, T_5 and T_6 were the top performing treatments for enhancing key biochemical constituents, with T_6 being particularly effective in increasing methyl eugenol and β-elemene. In contrast, T_7 consistently recorded the lowest concentrations across most phytochemical parameters, reflecting its suboptimal impact on secondary metabolite accumulation.

Fig. 1 and Fig. 2 display Pearson correlation coefficients among growth, yield and phytochemical traits averaged across two years and Pearson correlation between amendment levels (FYM or Vermicompost) and agronomic and biochemical traits, respectively. Strong correlations between growth metrics at 180 and 360 DAP (*e.g.*, plant height r = 0.98;

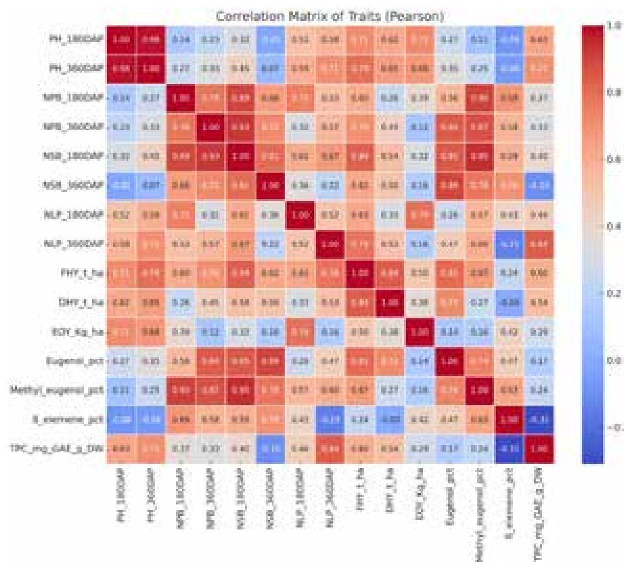

Fig. 1. Trait-to-trait correlation matrix in holy basil at 180 and 360 DAP.

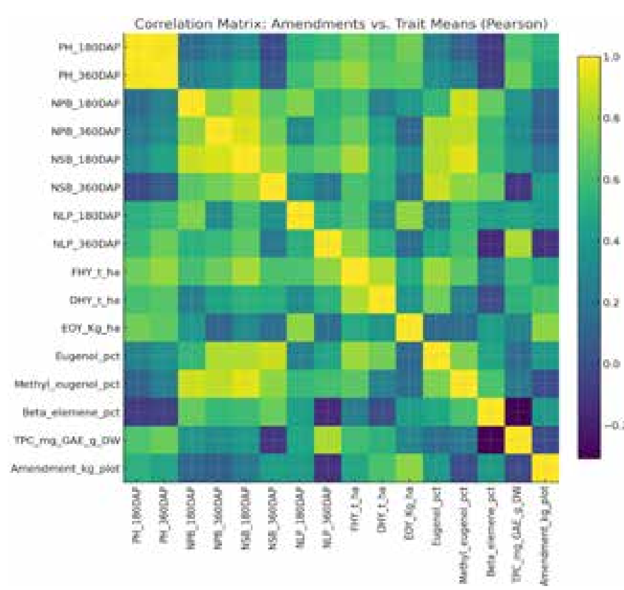

Fig. 2. Correlation matrix between organic amendment doses and mean trait responses in holy basil.

(PH=Plant Height (cm), NPB=No. of primary branches, NSB=No. of secondary branches, NLP=No. of leaves per plant, FHY= Fresh herbage yield (tha^{-1}), DHY= Dry herbage yield (tha^{-1}), EOY=Essential oil yield ($Kgha^{-1}$), TPC=Total Phenolic Compound (mg $GAEg^{-1}$ DW) leaf number r = 1.00) indicate that early vegetative traits in Tulsi largely determine final plant performance. Similar temporal continuities have been reported in sweet basil breeding panels, where plant height recorded at the 6 leaf stage explained >80 % of final biomass variance (Nurzynska *et al.*, 12). Practically, this means that a single, mid-February seedling census could substitute for far later and costlier measurements when forecasting yield or selecting parents. All classical growth traits (height, branching and leaf number) were tightly inter correlated ($0.59 \le r \le 0.79$) and converged on fresh and dry herbage yields (FHY and DHY). The pattern echoes agronomic trials in Krishna Tulsi, where vermicompost at 3 t/ha produced 14-18 % taller plants and a 22 % rise in leaf area, culminating in a 30 % oil bearing biomass gain relative to the unfertilised control. The dose response table in the present dataset further reinforces the dose yield relationship (FHY: r = 0.71).While, essential oil yield (EOY) still tracked height and leafiness (r up to 0.79), its link to FHY was weak (r = 0.16). This confirms that essential oil yield is more closely tied to trichome density and function than to total leaf biomass, alone. Nitrogen or sulphur factorials in basil have shown a similar pattern. Essential oil rose three fold between 0 and 60 kg N/ha even though dry matter increased only 1.7 fold, indicating additional biochemical controls beyond carbon capture (Zheljazkov *et al.*, 17). Eugenol and methyleugenol exhibited the strongest positive associations with the branching complex (0.85–0.95) and with total phenolics (0.63-0.84). This effect may stem from two factors: branch tips tend to have juvenile leaves with higher trichome density, thus increasing phenylpropanoid accumulation. Xie *et al*. (16) demonstrated that differential expression at the shikimate/phenyl propanoid branch point enzymes governs phenyl propene titres in basil trichomes. Nutrient primed allocation Higher organic matter loads boosted methyl eugenol most strongly (r = 0.78) while only marginally affecting eugenol (r = 0.34) and TPC (r =0.45). Comparable surges of methyl substituted phenylpropenes under rich nutrition were observed with organo mineral fertiliser in 'Cinnamon' basil, where methyl eugenol rose by 40 % over the unfertilised control (Oliveira *et al.,* 14). β-Elemene displayed weak or negative links to vegetative and phenolic traits and declined with heavier amendments (r = -0.23 with dose). Proteomic mapping in basil trichomes indicates that the terpenoid (MEP) and phenylpropanoid (shikimate) pathways compete for carbon precursors, up regulation of the latter under nutrient sufficiency can therefore suppress sesquiterpene flux. Similar inverse patterns were noted when high N fertilisation elevated methyl eugenol but reduced β-sesquiterpenes such as caryophyllene (Nurzynska *et al.*, 12).

Correlation coefficients against amendment dose highlight three agronomic takeaways :Linear biomass gains up to the highest tested dose (PH r = 0.67, FHY r = 0.71).Superior phenylpropanoid response in methyl eugenol (r = 0.78) versus eugenol (0.34) or total phenolic (0.45).Potential quality trade-off**,** as β-elemene falls and the eugenol, methyl eugenol ratio narrows important for markets that restrict methyl eugenol levels. Organic foliar feeds show the same "quality-first" nutrient effect: Fitokondi spray raised total phenolics by 27–29 % without altering photosynthetic efficiency (Onofrei *et al*., 15). Conversely, very high vermicompost rates (24 %) triggered a spike in phenolics but depressed height, illustrating the growth defence balance under nutrient or salt stress (Hepşen, 12).

As a key route for secondary metabolite biosynthesis, the phenylpropanoid pathway uses fixed carbon and nitrogen; its methylation reactions, involving S-adenosylmethionine, are influenced by nitrogen and sulphur availability (Ahamad *et al.,* 3; Gang *et al.,* 7). Nutrient rich treatments therefore intensify phenylpropanoid gene expression, as seen for CVOMT/EOMT transcripts under high N and even under abiotic cues such as drought (Abdollahi *et al.,*1). β-Elemene, by contrast, arises from the MEP or terpenoid route in plastids, drawing predominantly on pyruvate /glyceraldehyde-3-P, its down regulation under nutrient surplus suggests regulatory cross-talk rather than substrate limitation.

At 360 days after planting, treatments significantly outperformed the control in growth, herbage yield, essential oil content and phytochemicals. T_3 (Mustard cake 1.5 tha^{-1} + *Azotobacter* 10 g kg^{-1} of seed) noted the highest plant height (86.47 cm), while T_4 (Vermicompost 5 tha^{-1} + Phosphate solubilising bacteria 10 gkg^{-1} of seed) yielded the maximum fresh herbage yield (23.59 t ha^{-1}) and eugenol content (8.04%). T_2 (Farm yard manure 15 tha^{-1} + *Azotobacter* 10 g kg^{-1} of seed) produced the maximum essential oil (75.58 kg ha^{-1}) yield, while, T_6 (Mustard cake 1.5 tha^{-1} + Phosphate solubilising bacteria 10 g kg^{-1} of seed) enhanced methyl eugenol (7.21%) and β-elemene (0.74%). Strong correlations (r = 0.98–1.00) between early season traits and final output highlight predictive value. Results suggest integrating moderate-to-high organic inputs with biofertilizers can enhance Tulsi biomass and tailor essential oil quality in alluvial soils of West Bengal.

AUTHORS' CONTRIBUTION

Field experiments and laboratory analysis (NB, SS), Preparation of the manuscript (NB, SS, NNH), Conceptualization of the experiments (TS, NC, NB), Analysis of data (NB, SS), Editing of the manuscript (TS, SS, NC)

DECLARATION

Authors of this manuscript declare that they do not have any conflict of interest.

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