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RESEARCH ARTICLE

Soil test based nourishment to enhance yield attributes, nutrient-uptake, pigment and economics of beetroot grown in Western Ghats of Tamil Nadu

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Abstract

Beetroot (*Beta vulgaris* L.) is a widely cultivated root vegetable globally known for its significant health benefits. This study was conducted on a farmer's field in the Nilgiris district of Tamil Nadu's Western Ghats, using the improved crystal hybrid beetroot, to evaluate the effects of varying dosages of nitrogen (N), phosphorus (P), potassium (K) and integrated nutrient management systems on yield attributes, nutrient uptake, pigment content and cost-effectiveness. The experimental trials were carried out in Kadanad village during the winter of 2024 and in Emerald village during the summer of 2024. The treatments included untreated nutrient controls, a general fertilizer recommended dose (GFRD), GFRD combined with farm yard manure (FYM), soil test crop response (STCR) inorganic-based nutrient doses targeting yields of 350, 400 and 450 q ha^{-1}, STCR integrated-based nutrient doses with the same yield targets and the farmer's standard fertilizer application. The findings showed that the treatment with 1.80 N + 2.33 P_2O_5 + 1.16 K_2O q ha^{-1} combined with FYM @125 q ha^{-1} (T3) achieved the largest root diameter (8.76 cm), root perimeter (28.67 cm), total root length (20.40 cm), edible root length (8.1 cm), root fresh weight (264.51g per plant), root dry weight (23.53g per plant), root yield (442.10 q ha^{-1}), nutrient uptake of N (181.65 kg ha^{-1}), P (35.26 kg ha^{-1}), K (195.98 kg ha^{-1}) and pigment content (total betalain 138.02 mg 100g^{-1} FW, betacyanins 93.34 mg 100g^{-1} FW, betaxanthins 44.68 mg 100g^{-1} FW). This treatment also achieved the highest gross revenue (₹1,326,300), net income (₹1,100,300) and benefit-cost ratio (4.87:1).

Keywords

Beta vulgaris; economics; NPK uptake; pigment; STCR- integrated; yield attributes

Introduction

Since independence, achieving food security has been a major national concern. Population growth and rising incomes drive increased demand for food and processed goods. Beetroot (*Beta vulgaris* L.), a prominent root vegetable commonly referred to as garden or table beet, is a member of the Chenopodiaceae family . This plant produces green leaves and a swollen root, widely used as vegetables and salads. Beetroot is a rich source of nutrients, including carbohydrates, fiber, vitamins A and C, niacin and antioxidants. The leading beetroot-growing states in India are Maharashtra, West Bengal, Uttar Pradesh, Himachal Pradesh and Haryana. Nationwide, beetroot is cultivated on over 0.079 lakh hectares, yielding an annual production of 1.51 lakh tonnes (1).

In temperate regions, beetroot is cultivated as a biennial plant. Its vivid red hue is attributed to betalains, which include yellow pigments (betaxanthins) and red-violet pigments (betacyanins) that give the root its colour. Betacyanin, a naturally occurring pigment, is utilized to create artificial colorants and functions as a natural food coloring in products such as beverages, dairy products, soups, and others (2). Vegetables serve a crucial part in human nutrition and in recent years, interest in boosting vegetable production has markedly expanded due to heightened knowledge of their nutritional worth and significance in fulfilling the nation's food requirements .

As a primary source of dietary nitrates, vegetables raise concerns regarding nitrate accumulation in fresh produce. Beetroot provides numerous health benefits, such as maintaining cholesterol levels, lowering the risk of heart disease and offering protection against congenital disabilities and certain types of cancer (3). Moreover, beetroot functions as a comprehensive tonic, advantageous for pregnant women and assists in cleansing the liver, kidneys and gallbladder. However, excessive nitrate buildup in beetroot can pose health risks, as nitrates may convert into nitrites within the body, potentially causing methemoglobinemia (4).

In modern agriculture, chemical fertilizers have become the primary method for supplying essential nutrients to plants to maximize production. However, their extensive use has resulted in negative environmental impacts and rising production costs. Issues like declining soil fertility, food contamination and environmental degradation stem directly from the overuse of synthetic fertilizers on farmland. Therefore, exploring alternative solutions that enhance profitability, improve quality and are environmentally sustainable is crucial. Combining synthetic and organic fertilizers provides a balanced approach, boosting crop production while mitigating adverse effects on soil and the environment (5).

Integrated nutrient management (INM) offers a sustainable approach to crop production by reducing dependence on inorganic fertilizers. By combining organic manure with synthetic fertilizers, INM enhances soil health, minimizes pollution, increases productivity and supports food security. This approach also promotes better crop growth, higher economic yields, improved pigment content and greater profitability in beetroot farming. The present study aims to identify the optimal combination of chemical and organic fertilizers to enhance beetroot yield, quality, nutrient uptake, pigment concentration and profitability, considering all these key factors.

Materials and Methods

Soil samples were analysed for organic carbon using the Walkley-Black method (6), potassium permanganate oxidizable nitrogen (7), Bray phosphorus (8), ammonium acetate-extractable potassium (9), cation exchange capacity (CEC) with neutral normal ammonium acetate (10) and DTPA-extractable micronutrients (11). The trial plots were set up in a farmer's field in Kadanad Village (11°49' N, 76°73' E) during the winter of 2024 and in Emerald Village (11°32' N, 76°64' E) during the summer of 2024. Both sites are located in the Nilgiris region of the "Western Ghats" of Tamil Nadu.

Initial soil samples were collected and analysed for various parameters. The soil was non-calcareous, red and clay loam, with an acidic pH of 4.45 (pH meter) and low salinity, with an EC of 0.35 mS cm^{-1} (EC meter). The soil's CEC was 18 centimoles per kg. Pre-sowing soil fertility assessments showed an organic carbon content of 30.27 g kg^{-1}, with potassium permanganate-nitrogen ($KMnO_4$-N), Bray phosphorus and ammonium acetate-potassium (NH_4OAc-K) levels at 424, 191 and 522 kg ha^{-1}, respectively. The diethylenetriaminepentaacetic acid (DTPA)-extractable micronutrients (mg kg^{-1}) were in the optimal range: Iron (Fe) (43.06), manganese (Mn) (11.23), zinc (Zn) (2.16) and copper (Cu) (2.74).

The field trial included ten treatment combinations, as outlined in Table 1. The quantities of $KMnO_4$-N, Bray-P, NH_4OAc -K needed to achieve the desired beetroot yield were determined using fertilizer recommendation equations.

Table 1. Nutrient prescription for beetroot

STCR-Inorganic nutrient	Soil Test Crop Response (STCR)-Integrated nutrient
$FN = 0.69TY-0.37STV_N$	$FN = 0.69TY-0.37STV_N-0.62FYM_N$
$FP_2O_5 = 0.61TY-0.54STV_P$	$FP_2O_5 = 0.61TY-0.54STV_P-0.86FYM_P$
$FK_2O = 0.82TY-0.42STV_K$	$FK_2O = 0.82TY-0.42STV_K-0.63FYM_K$

TY= target yield (q ha^{-1}), FN= nitrogen fertilizer (kg ha^{-1}), FP_2O_5= phosphorus fertilizer (kg ha^{-1}), FK_2O= potassium fertilizer (kg ha^{-1}), STV_N= soil-test value nitrogen (kg ha^{-1}), STV_P= soil- test value phosphorus (kg ha^{-1}), STV_K=soil-test value potassium (kg ha^{-1}), FYM_N= farmyard manure nitrogen (kg ha^{-1}), FYM_P= farmyard manure phosphorus (kg ha^{-1}), FYM_K= farmyard manure potassium (kg ha^{-1}).

Following the treatment guidelines, the total quantities of K and P and 50 % of N were applied as a basal dose before sowing. The remaining N dosage was applied 30 days after sowing (DAS). Farm yard manure (FYM) was incorporated into the plots before sowing, in line with the treatment specifications. N, P and K were supplied through urea, single superphosphate (SSP) and potassium chloride, respectively. Agronomic practices and crop protection measures were followed according to standard protocols.

Data collected included root diameter (cm), root perimeter (cm), total root length (cm), edible root length (cm), root fresh weight per plant (g), root dry weight per plant (g), root yield (q ha^{-1}) and nutrient uptake (kg ha^{-1}) for N, P and K. Pigment content was measured as total betalain (TB), betaxanthins (BX) and betacyanins (BC) (mg $100g^{-1}$ FW). Economic parameters such as total revenue (Rs), net revenue (Rs) and benefit-cost ratio were also recorded. All plant parameters were assessed at harvest (110 DAS).

Nutrient uptake

N content was measured using the micro Kjeldahl method with diacid extraction. Phosphorus was quantified using the vanado molybdophosphoric yellow color method (triacid extraction) and K was measured with a flame photometer (triacid extraction) (12). Nutrient uptake was calculated by multiplying the nutrient content values by the corresponding dry mass yield of beetroot.

Nitrogen

A measured 1 g sample of the powdered material was treated with a di-acid mixture to oxidize the organic matter and dissolve the mineral elements. The digested sample was then distilled with an alkali and the resulting ammonia was absorbed in a standard acid solution. The excess acid was back-titrated and the N content was subsequently calculated.

Phosphorus

The total P content in the plant samples was extracted using a triple-acid method. A heteropoly compound formed by the reaction between vanadomolybdate and phosphate radicals in a nitric acid medium produced a yellow color. The intensity of this color was measured at 470 nm using a spectrophotometer and the P content was determined based on a standard calibration curve.

Potassium

The total K content in the plant samples was extracted using a triple-acid method. When liquid samples containing K are ignited in a flame, K emits photons at characteristic wavelengths. The intensity of this emission, which is directly proportional to the K content, was measured with a flame photometer to determine the K concentration in the plant samples.

Pigment analysis

Pigment analysis using chromatographic techniques typically employs a 'Betalain' standard. However, this study used a spectrophotometric method to measure absorbance values for three color compounds: total betalain at 600 nm, betacyanin at 537 nm and betaxanthin at 478 nm. Based on the optical density (OD) values, pigment concentrations were calculated as follows (13):

$$\text{Betalain pigments (mg L}^{-1}) = \frac{A^{*}DF^{*}MW^{*}1000}{\varepsilon^{*} L} \quad \text{(Eqn. 1)}$$

Where A is the absorption value, DF is the dilution factor, MW is the molecular weight, L is the path length of the cuvette and ε the molar extinction coefficients, DF is the dilution factor, MW is the molecular weight, L is the path length of the cuvette and ε is the molar extinction coefficient. The results obtained in mg L^{-1} were then converted to mg $100g^{-1}$.

Economics

Economic analysis was conducted based on current market costs for inputs and outputs. Data collected over two consecutive years were statistically assessed (14). Statistical analysis of the data was carried out using analysis of variance (ANOVA) for a randomized block design (RBD):

$$\text{B:C ratio} = \frac{\text{Net revenue}}{\text{Cost of Cultivation}} \quad \text{(Eqn. 2)}$$

Results and Discussion

Yield attributes of beetroot

***Root diameter*:** Among the various organic and inorganic nutrient management treatments, the highest root diameter (8.76 cm) was observed under the treatment of 1.80 q N, 2.33 q phosphorus pentoxide and 1.16 q potassium oxide ha^{-1}, combined with FYM at 125 q ha^{-1} (T3). This treatment out performed all other treatments, while the smallest root diameter (4.81 cm) was recorded in the control treatment without any nutrient application (T10).

The increased beetroot diameter observed in T3 may be attributed to improved overall plant growth, which enhanced photosynthetic activity from the leaves (source) to the roots (sink), leading to a larger diameter. This larger diameter likely resulted from increased cell division and rapid cell proliferation vegetative growth likely contributed directly to root size, with more carbohydrate storage promoting an increase in diameter as storage organs became nutrient-rich (15, 16). The combination of NPK fertilizer and FYM favoured plant growth regulator activities, ultimately contributing to the increase in root diameter (17).

***Root perimeter*:** The impact of various treatments on root perimeter was found to be statistically significant. The largest root perimeter (28.67 cm) was recorded in treatment T3, which involved the application of 1.80 q N, 2.33 q P_2O_5 and 1.16 K_2O q ha^{-1} along with FYM at 125 q ha^{-1}. This treatment significantly outperformed all others at harvest. In contrast, the smallest root perimeter (15.24 cm) was observed in T10 (untreated nutrients).

The increased root perimeter in T3 is likely due to improved nutrient availability and absorption, contributing to a larger root circumference and higher root yield. Similar findings have also been reported (18). The increase in root perimeter may also be attributed to enhanced nutrient uptake and balanced nutrition. Additionally, the interaction between NPK and FYM prompted the synthesis of growth regulators, amino acids and vitamins, thereby enhancing photosynthate production (19).

***Total root length and edible root length*:** The longest total root length (20.40 cm) was recorded in T3, significantly greater than all other treatments. Conversely, the shortest total root length (8.96 cm) was observed in T10.

The data regarding edible beetroot length, as shown in Table 2, indicate a statistically significant effect of the treatments on edible beetroot length. The largest edible root length (8.10 cm) was significantly observed in T3, while the shortest (3.50 cm) was found in T10.

The increased root length in T3 may be attributed to the consistent supply of P, which stimulated root growth and facilitated greater nutrient uptake. Applying organic manure also improved soil structure, promoting beetroot growth by enhancing soil microbial activity and the production of polysaccharides (20).

Phosphorus plays a crucial role in root development by enhancing nutrient absorption and translocation and is involved in various enzyme functions and ATP synthesis, ultimately leading to increased root growth (21). Incorporating FYM into the soil enhances its physical condition by improving

Table 2. Treatment details for a field trial on chemical and combined nutrient management in beetroot

Treatments	FYM (q ha^{-1})	Fertilizer nutrient doses (q ha^{-1})		
		FN	FP_2O_5	FK_2O
T1-STCR-Integrated-TY_1 350 q ha^{-1}	125	0.91	1.14	0.50
T2-STCR-Integrated-TY_2 400 q ha^{-1}	125	1.40	1.74	0.77
T3-STCR-Integrated-TY_3 450 q ha^{-1}	125	1.80	2.33	1.16
T4-STCR-Inorganic-TY_1 350 q ha^{-1}	-	1.23	1.41	0.89
T5-STCR-Inorganic-TY_2 400 q ha^{-1}	-	1.72	2.01	1.28
T6-STCR-Inorganic-TY_3 450 q ha^{-1}	-	1.80	2.40	1.50
T7-General fertilizer recommended dose (100 % GFRD alone)	-	1.20	1.60	1.00
T8-General fertilizer recommended dose (100 % GFRD) + FYM @ 125 q ha^{-1}	125	1.20	1.60	1.00
T9-Farmer's fertilizer Practice (FFP)	-	0.80	0.85	0.76
10-Absolute control (untreated nutrients)	-	0	0	0

soil particle aggregation, which further contributes to increased root length (22). These aggregates positively affect soil fertility and influence water retention, gas diffusion and root growth and development, all contributing to overall plant growth (23). The addition of organic manures enhances the suitability of the physical environment by optimizing soil bulk density, hence promoting root elongation, tuber development and nutrient uptake from soil and nutrient sources (24).

***Root fresh mass per plant*:** There were significant differences in root fresh mass per plant among the treatments. The highest root fresh mass per plant (264.51 g) was observed in treatment T3, while the lowest root fresh weight (144.21 g) was recorded in T10 (the control treatment).

This increase in root fresh mass may be attributed to the nutrient-solubilizing effects of FYM, which enhances the uptake of nitrogen, phosphorus and potassium (NPK). FYM supplies essential macronutrients during mineralization, directly supporting plant growth by improving the soil's physical properties. Similar findings have been reported in previous studies (25-27). As plants absorb nutrients throughout their growth cycle, there is a corresponding increase in root fresh mass (28).

***Root dry mass per plant*:** The higher root dry mass per plant (23.53 g) was recorded in treatment T3, while the lowest dry mass (12.85 g) was observed in T10 (the untreated control). The observed increase in dry mass in T3 is likely due to the elevated dry matter content of beetroot, which varies from 9 % to 13 % . A similar outcome was reported in previous studies (29).

***Root yield*:** Data on beetroot yield per hectare indicated significant differences between treatments (Table 3). Treatment T3 exhibited the highest root yield per hectare (442.10 q ha^{-1}), demonstrating statistical superiority over all other treatments. In contrast, the lowest root yield was observed in T10 (251.90 q ha^{-1}).

The increased root production observed in T3, associated with the STCR-integrated treatment, may be attributed to improved nutrient accessibility, which enhanced the growth and yield factors of beetroot, ultimately resulting in a higher yield. The accumulation of humus substances likely mobilized stored nutrients to the roots, facilitated by hydrolysing and oxidizing enzymes. The higher yield can also be linked to increased plant height, leaf number and fresh root weight (30).

The combination of NPK and FYM significantly improved growth traits, contributing to the higher yield. Similar findings have been reported in previous studies (31, 32). Nitrogen is one of the most yield-limiting nutrients for crops like potatoes, where the greatest reduction in production is often attributed to N deficiency, along with deficiencies in K and P (33). The effectiveness of organic manure in enhancing crop yield stems from its ability to provide adequate quantities of essential plant nutrients throughout the growth period, especially during critical growth stages, which leads to improved nutrient uptake, plant vigour and superior yield attributes (34).

Table 3. Impact of chemical and combined nutrient management on yield characteristics of beetroot (average data from two seasons)

Treatments	Root diameter (cm)	Root perimeter (cm)	Total root length (cm)	Edible root length (cm)	Root fresh mass per plant (g)	Root dry mass per plant (g)	Yield (q ha^{-1})
T1	6.61	20.93	13.76	5.50	206.34	18.51	354.00
T2	7.76	26.31	16.23	6.90	240.61	21.34	403.50
T3	8.76	28.67	20.40	8.10	264.51	23.53	442.10
T4	6.10	19.24	12.73	5.10	173.95	17.46	291.90
T5	7.58	24.56	15.28	6.10	230.61	20.23	389.00
T6	7.95	27.51	18.52	7.70	253.08	22.16	423.30
T7	5.65	18.02	10.71	4.70	164.28	15.21	283.40
T8	6.78	22.16	14.85	5.80	215.62	19.24	360.60
T9	5.38	17.12	9.82	4.30	154.26	13.52	259.60
T10	4.81	15.24	8.96	3.50	144.21	12.85	251.90
SEd	**0.21**	**0.45**	**0.16**	**0.07**	**3.60**	**0.18**	**7.21**
CD (0.05)	**0.45**	**0.96**	**0.34**	**0.16**	**7.56**	**0.38**	**15.16**
Cv	**2.01**	**2.55**	**1.42**	**1.65**	**2.15**	**1.72**	**2.55**

Nutrient uptake

Nitrogen uptake: Nitrogen uptake in beetroot plants was highest in T3, with an N uptake of 181.65 kg ha^{-1}, significantly higher than all the other treatments. The lowest N uptake was recorded in T10, with a value of 102.75 kg ha^{-1} (Fig. 1).

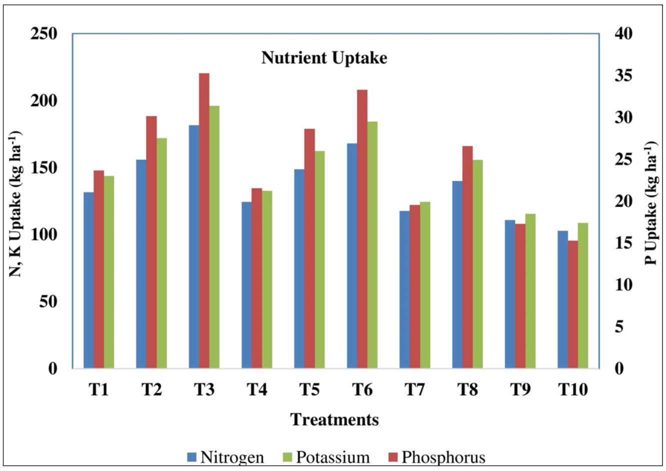

Fig. 1. Effect of treatments on nutrient uptake in beetroot (average data from two seasons).

The high amount of N uptake in T3 treatment could be attributed to the prolonged and enhanced availability of N in this treatment, along with an increase in dry matter yield (35-37). Additionally, the greater N uptake by beetroot tubers may be due to the increased mineralized N in the soil (38).

Phosphorus uptake: The highest P uptake (35.26 kg ha^{-1}) was recorded in T3, significantly surpassing all other treatments, while the lowest uptake (15.28 kg ha^{-1}) was observed in the treatment without any nutrient application (T10) (Table 4).

The increased P uptake in T3 can be attributed to the chelating effect of organic acids, which improve ion accessibility and the solubilizing action of FYM decomposition (39). The enhanced availability of P is likely due to organic acids produced during FYM breakdown, which solubilize P ions. Additionally, the superior organic matter content in FYM facilitates a gradual nutrient release into the soil system. This observation is consistent with previous findings (40).

Potassium uptake: The highest K uptake (195.98 kg ha^{-1}) was observed in treatment T3, while the lowest (108.67 kg ha^{-1}) was recorded in T10 (untreated nutrients).

The increased K uptake in T3 can be attributed to the retention of K in an exchangeable form in FYM-enriched soil, which prevents K fixation by clay particles (37, 41). The building up of organic matter complexes due to organic acids also contributes to the enhanced absorption of N, P and K following FYM application. The combined use of organic manure and chemical fertilizers significantly increased yield, improved soil chemical properties, boosted nutrient availability and thus led to higher nutrient uptake by beetroot (17). Enhanced K uptake promoted greater root growth, enabling the plant to access a broader area for nutrient absorption (42).

Effect of treatments on total betalains, betacyanins and betaxanthins

The highest concentration of total betalains (138.02 mg per 100 g FW) was observed in treatment T3, while the lowest concentration (97.36 mg per 100 g FW) was recorded in T10, which served as the control.

For betacyanins, the highest content (93.34 mg per 100 g FW) was achieved in treatment T3, significantly surpassing all other treatments. In contrast, the lowest betacyanins content (56.21 mg per 100 g FW) was observed in T10, where no nutrients were applied. Similarly, T3 also recorded the highest betaxanthins content (44.68 mg per 100 g FW), which was statistically superior to all other treatments. In comparison, the minimum betaxanthins content (41.15 mg per 100 g FW) was found in T10 (untreated nutrients).

The data indicate that the highest levels of total betalains, betacyanins and betaxanthins were found in treatment T3. This increase in pigment concentration in the roots could be attributed to the type and dosage of fertilization. Fertilizers rich in N have proven to be an effective strategy for enhancing betalains, betacyanins and betaxanthins content (43) (Fig. 2).

Economics

The highest total revenue (₹1,326,300), net revenue (₹1,100,300) and benefit-cost ratio (4.87:1) (Table 5) were recorded in treatment T3. This outcome is attributed to the greater root yield per hectare achieved with this treatment compared to others. These findings align with results of a previous study (44) .

Table 4. Impact of chemical and combined nutrient management on nutrient uptake (kg ha^{-1}) and pigment content (mg 100g^{-1} FW) in beetroot (average data from two seasons)

Treatments	N uptake	P uptake	K uptake	Total Betalains	Betacyanins	Betaxanthins
T1	131.57	23.64	143.67	112.94	71.25	41.69
T2	155.82	30.14	171.94	118.62	81.56	37.06
T3	181.65	35.26	195.98	138.02	93.34	44.68
T4	124.38	21.54	132.67	108.72	67.64	41.08
T5	148.67	28.61	162.34	115.29	78.62	36.67
T6	167.94	33.28	184.29	125.47	86.52	38.95
T7	117.54	19.52	124.37	103.37	64.62	38.75
T8	139.85	26.57	155.72	114.57	75.21	39.36
T9	110.76	17.26	115.37	100.64	60.61	40.03
T10	102.75	15.28	108.67	97.36	56.21	41.15
SEd	**3.16**	**0.52**	**2.68**	**1.52**	**1.37**	**0.61**
CD (0.05)	**6.65**	**1.1**	**5.64**	**3.19**	**2.89**	**1.30**
Cv	**2.81**	**2.56**	**2.20**	**1.64**	**2.18**	**1.90**

FW=Fresh Weight

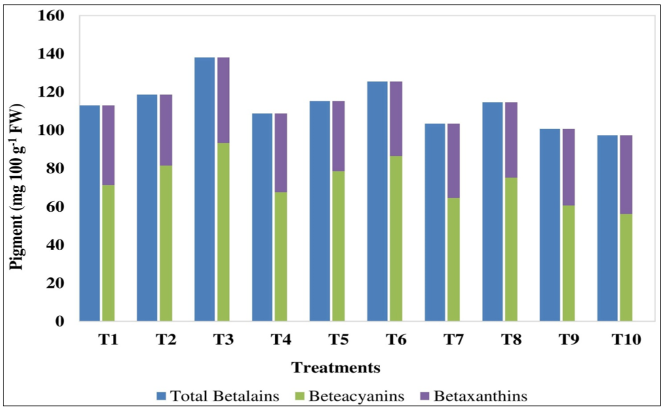

Fig. 2. Effect of treatments on total betalains, betacyanins and betaxanthins in beetroot (average data from two seasons).

Table 5. Economic analysis of the impact of chemical and combined nutrient management on beetroot (average data from two seasons)

Treatments	Cost of cultivation	Total revenue	Net revenue	B:C ratio
	(Rupees ha^{-1})			
T1	211658	1062000	850342	4.02
T2	218048	1210500	992452	4.55
T3	226000	1326300	1100300	4.87
T4	204590	875700	671110	3.28
T5	216050	1167000	950950	4.40
T6	225000	1269900	1044900	4.64
T7	210078	850200	640122	3.05
T8	255684	1081800	826116	3.23
T9	201640	778800	577160	2.86
T10	200140	755700	555560	2.78

Conclusion

Enhancing the pigment content of beetroot increases its economic value and broadens the potential for sustainable beetroot cultivation in hilly regions. The findings from the two field trials clearly demonstrate that the combined application of mineral nutrients (1.80 N + 2.33 P_2O_5 + 1.16 K_2O kg ha^{-1}) with 125 kg ha^{-1} of FYM (T3) resulted in the highest beetroot yield and superior growth parameters compared to other treatments. This combination not only increased yield characteristics, including root diameter, fresh and dry root weight, root perimeter and root length but also elevated nutrient absorption and pigment concentration in the plants.

The trial results indicate that using mineral nutrients with organic manure significantly improves beetroot yield, yield attributes, nutrient uptake, pigment content and overall economic benefits. This combination of inorganic and organic fertilizers offers a return of 4.87 rupees for every rupee invested. Therefore, integrating mineral fertilizers with available farmyard organic manures increases farmers' income and supports soil health sustainability.

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Authors' Contributions

RA conducted the experiment and drafted the manuscript. KMS designed the experiment and edited the manuscript. SM, PM and AS interpreted the study results. SPT and GS helped with the statistical analysis and edited the manuscript.

Compliance with Ethical Standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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