



RESEARCH ARTICLE

Impact of bioformulations on growth, yield and quality parameters of field-grown lettuce (*Lactuca sativa* L.)

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Received: 27 January 2026; Accepted: 20 May 2026; Available online: Version 1.0: 14 July 2026

Cite this article: Dinesh C, Shweta, Varun A. Impact of bioformulations on growth, yield and quality parameters of field-grown lettuce (*Lactuca sativa* L.). Plant Science Today. 2026;13(sp5):01-07. <https://doi.org/10.14719/pst.13855>

Abstract

The heavy reliance on fertilisers in crop cultivation has led to soil degradation and human health concerns, particularly in the cultivation of salad crops, where the leaves are consumed fresh. This necessitates the adoption of organic and bio-based sustainable bioformulations. The field experiment was conducted during the rabi 2024–2025 at the Department of Horticulture, Lovely Professional University, Punjab, to investigate the effects of bioformulations on growth, yield and quality parameters of lettuce (*Lactuca sativa* L.) cv. Green Wave. The trial was conducted using a randomised complete block design (RCBD) with 16 different treatments, each replicated 3 times. Statistical significance was assessed using analysis of variance (ANOVA) and means were compared using critical difference (CD) at $p \leq 0.05$. The investigation revealed that T₆ (Arka microbial consortium at 5 kg ha⁻¹+ local microbial consortium at 5 kg ha⁻¹, each mixed at 20 g L⁻¹ of water) recorded the highest values for growth and yield parameters i.e., plant height, number of leaves, leaf area, leaf length, leaf width, leaf fresh weight, leaf dry weight, days taken to maturity, yield per pot, yield per hectare followed by T₁₁ (Arka vegetable special at 5 kg ha⁻¹, mixed at 20 g L⁻¹+ seaweed extract 1.5 L ha⁻¹) whereas T₁₃ (Arka microbial consortium at 5 kg ha⁻¹+ neem seed kernel extract 15 L ha⁻¹) recorded higher values for quality parameters such as calcium (Ca), iron (Fe), beta-carotene and vitamin C, followed by T₆.

Keywords: biofertilisers; bioformulations; lettuce; sustainable agriculture

Introduction

Lettuce (*Lactuca sativa* L.) is an annual of the Asteraceae family, which is commonly grown for use as a salad green. It is a vital salad vegetable in terms of vitamin content, minerals and other attributes. It exhibits antioxidants and anti-inflammatory properties and is also associated with neuroprotective effects. The top three countries with the highest lettuce production are China, producing 15.2 million tonnes (Mt), the USA, with 4.1 Mt and followed by India, with 1.3 Mt (1). The commercial cultivation of salad crops in India is currently in an emerging phase, unlike the United States and other European countries, where there is a large number of growers. The cultivation of the crop in Punjab occurs on a very limited scale. However, its cultivation has seen an ongoing rise, as the winter conditions of Punjab are favourable for its growth. The quantity and quality of this crop are influenced by various factors, with fertilisers being the most significant element. To achieve optimal plant growth, nutrients must be present in adequate and balanced amounts. Given the rising population, it is essential to significantly enhance crop production. Consequently, growers extensively utilise agrochemicals, often neglecting the long-term impacts on soil and human health.

The increasing application of chemicals, namely synthetic fertilisers in intensive farming, has disrupted the balance between the soil microbial and plant populations, as well as contaminating surface and ground water (2–4). Numerous techniques, including crop rotation, genetic improvement, organic farming, biofortification

and the use of bioinoculants, have been implemented to meet these new issues in the agricultural sector. By promoting sustainable agricultural methods, microbial bioinoculants are advantageous microorganisms in an appropriate carrier that assist plant development, disease prevention and soil health enhancement.

Bioformulations are biologically active materials derived either from microorganisms or their metabolites that enhance nutrient availability, biocontrol and plant growth promotion in an environmentally sustainable manner (5–7). These formulations represent an eco-friendly strategy to improve agricultural productivity and environmental sustainability (8). Bioformulation is a noteworthy development that scientists have achieved after carefully examining the rhizosphere's micro-ecology and processes to increase protection and productivity (9). The various combinations of microorganisms improve macro and micronutrients by solubilisation or mobilisation, which raises the soil's nutritional value and increases soil fertility. Additionally, they serve as biocontrol agents by offering defence mechanisms against pests, plant diseases, etc. Extensive research is being conducted to identify and screen a variety of microorganisms that promote plant development in order to use them to create efficient bioinoculants. With an environmentally friendly approach of reducing poverty and hunger, research and development on bioformulations can assist in achieving sustainable goal development (10, 11).

Recent research has focused on the use of liquid

formulations as an alternative to overcome the inconvenience that comes with using solid bioformulations. Since liquid bio fusion has a number of benefits over solid bioformulation, it can be utilised as a substitute for carrier-based bioformulation. Liquid formulations offer several advantages, including longer shelf life of one to two years, reduced contamination risk, higher production efficiency and improved effectiveness. They do not require binding agents, ensure ease of use and handling with modern equipment, exhibit resistance to high temperatures, enhance microbial growth, simplify seed and soil application and improve resilience to environmental stress (12–14). Consumers prefer liquid formulations because they prevent the clumping of desired microorganisms and provide homogeneity that solid formulations are unable to maintain (8).

It is feasible to give the soil structure, stop its deterioration and promote microbial mineralisation processes by employing bioformulations. These advantages reduce the usage of fossil fuel energy and ecosystem degradation while increasing crop yield and soil fertility in comparatively short amounts of time (15, 16).

While the general advantages of bioformulations on leafy greens are proven, there remains limited information regarding the interaction between various Arka microbial consortia and local formulations under the agro-climatic conditions of Punjab.

This study was conducted to evaluate a novel framework on the influence of bioformulations (Arka microbial consortium, Arka vegetable special, seaweed extract, neem seed kernel extract and local microbial consortium) on various attributes related to growth, yield and quality of field-grown lettuce. This study addresses the existing research gap on the application of bio-based formulations in lettuce, particularly given the agroclimatic conditions in India.

Materials and Methods

General description

The experimental instruments and methods are described under the ensuing subsections.

Location and climate

The field study was conducted during the winter season of 2024–2025, from the month of October to January at the Department of Horticulture, Lovely Professional University, Phagwara, Punjab (31°22′31.83″ N, 75°23′03.02″ E) (Fig. 1).

The region falls under a subtropical climate. The average annual temperature during the trial period was 23.52 °C, dipping to 9.92 °C in January. The soil in the experimental site was sandy loam, slightly alkaline, rich in organic matter and well-drained, suitable for optimal lettuce growth. The soil characteristics were tested before transplantation and revealed a pH of 6.78, an electrical conductivity of 0.224, an organic carbon content of 0.725 and the nitrogen, phosphorus and potassium (NPK) composition of the soil was 204:24.3:176 kg/ha.

Experimental design

The field trial was conducted on lettuce (*Lactuca sativa* L.) using the cultivar, Green Wave (green leafy), with seeds procured online from the seed-selling platform, supplied by The Indian Beej Agricultural Company based in Geyzing, Sikkim. The crop was sown in October 2024 and planted in a total area of 450 m² at a spacing of 45 cm × 45 cm (row × plant), arranged into 48 plots, each measuring 3.75 m × 2.5 m (9.37 m²), with 5 rows per plot, 8 plants per row and a total of 40 plants per plot. The study aimed to evaluate the effects of bioformulation on growth, yield and quality of field-grown lettuce.



Fig. 1. Field view of the research trial.

Treatment details

The experiment was laid out in a randomised complete block design (RCBD) in which sixteen treatments were evaluated, consisting of 5 distinct types of bioformulations, applied individually and in combinations of Arka microbial consortium (AMC), Arka vegetable special (AVS), neem seed kernel extract (NSKE), seaweed extract (biostimulant) and local microbial formulation (LMF). The treatment combinations are detailed in Table 1.

Neem seed kernel extract was manufactured by Virinchi Organics Private Ltd, Warangal, Telangana, India. Arka microbial consortium and AVS developed by ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru, India, were manufactured by Peak Laboratories Pvt. Ltd., Kolhapur, Maharashtra, India and Greenovate Agrotech Pvt. Ltd., Akola, Maharashtra, India respectively. The seaweed extract (Sagarika) was manufactured by Indian Farmers Fertiliser Cooperative Ltd., India and the microbial bioformulation was prepared locally from vegetable waste using a standardised fermentation process.

Experimental methodology

The research trial was laid out in the experimental field of Lovely Professional University. To ensure the accuracy of the research, the trial was carried out in an organised manner. The research field was prepared by making ridges to ensure ease in carrying out various intercultural operations. Two water channels of 0.5 m each were prepared between two columns each. Bioformulations were applied at the rate of 5 kg ha⁻¹. It was prepared by mixing the formulation in water at 20 g L⁻¹ of water and applied as a soil drench near the root zone. In the month of October, nursery preparation was carried out by preparing three raised beds of 6 × 3 feet. Transplanting was done after 30 days of sowing on the ridges at a spacing of 45 × 45 (row × plant) cm. Foliar application was done twice. The first dose was applied at 15 days after transplanting, followed by a second dose at 30 days after transplanting. Irrigation was done at 10–12-day intervals. Weeds were controlled manually by pulling and hoeing. Harvesting started at 55 days after transplanting. Parameters were recorded from 5 plants randomly selected from each plot on 45 days after transplanting and at the time of harvest.

Table 1. Treatment specifications

Treatment notation	Treatment particulars
T ₁	AMC
T ₂	AVS
T ₃	NSKE
T ₄	Biostimulant
T ₅	LMF
T ₆	AMC + LMF
T ₇	AVS + LMF
T ₈	NSKE + LMF
T ₉	Biostimulant + LMF
T ₁₀	AMC + Biostimulant
T ₁₁	AVS + Biostimulant
T ₁₂	NSKE + Biostimulant
T ₁₃	AMC + NSKE
T ₁₄	AVS + NSKE
T ₁₅	AMC + AVS
T ₁₆	Control

AMC: Arka microbial consortium; AVS: Arka vegetable special; NSKE: neem seed kernel extract; LMF: local microbial consortium; Biostimulant: seaweed extract.

Observations recorded

As mentioned above, parameters were recorded at fixed time intervals of 45 days after transplanting (DAT) and at harvest. Growth parameters, namely plant height (cm) and plant spread (cm), leaf length (cm), were measured with a ruler and leaf area (cm²) was measured using a leaf area meter. The number of leaves was counted manually on sampled plants at each assessment date. Leaf fresh weight (gm) was recorded right after harvest on an electronic balance. Leaf dry weight (gm) was measured by putting the samples in paper envelopes and dried in a forced air oven at 105 °C for 24 hr until a constant weight was recorded. Days taken to maturity were calculated by counting the days taken to harvest from transplanting.

Yield parameters, i.e., yield per plot (kg), were the total harvested produce from one plot in kilograms and yield per hectare (ton) was calculated using the formula:

$$\text{Yield (t ha}^{-1}\text{)} = \frac{\text{Yield per plot (kg)} \times 10}{\text{Plot area (m}^2\text{)}}$$

Quality parameters, viz, chlorophyll content (mg/100 g) was estimated using acetone extraction method where fresh lettuce leaves (1 g) were homogenised in 80 % acetone using a chilled mortar and pestle and centrifuged at 5000 rpm for 10 min, after the supernatant was collected it was observed in a spectrophotometer at absorbance 645 and 663 nm for chlorophyll a and b respectively and total chlorophyll was calculated using standard equations.

$$\text{Total Chlorophyll (mg/L)} = 20.2 A_{645} + 8.02 A_{663}$$

Calcium (Ca) (mg/100 g) was measured by digesting ground oven-dried lettuce samples in nitric acid: perchloric acid mixture (HNO₃:HClO₄) (3:1) until a clear solution was obtained, filtered, diluted and observed under a flame photometer and was calculated using the formula,

$$\text{Ca (mg/100g)} = \frac{C \times V \times 100}{W \times 1000}$$

Where: C = Concentration from calibration curve (mg/L), V = Final volume of digest (mL), W = Sample weight (g)

Iron concentration (mg/100 g) was measured by observing the diluted solution under an atomic absorption spectrophotometer at a wavelength of 248.3 nm and calculated using the formula,

$$\text{Fe (mg/100g)} = \frac{C \times V \times 100}{W \times 1000}$$

Where, C = Concentration from calibration curve (mg/L), V = Final volume of digest (mL), W = Sample weight (g)

Beta-carotene (mg/100 g) was measured by homogenising 1 g of fresh lettuce in acetone, filtering, transferring to a separating funnel containing petroleum ether, the ether layer was then collected and washed thoroughly to remove the acetone and observed at 450 nm absorbance and was calculated using the formula,

$$\beta \text{ carotene (mg / 100 g)} = \frac{A \times V \times 10^4}{A_{1\text{ cm}}^{1\%} \times W \times 1000}$$

Where, A= Absorbance at 450 nm, V= Volume of extra (mL), A_{1 cm}^{1 %} = Extinction coefficient (≈ 2592 for β-carotene), W= Sample weight (g)

Vitamin C (mg/100 g) was measured by the 2,6-dichlorophenol indophenol dye technique, where 5 g fresh lettuce leaves were homogenised in 3 % metaphosphoric acid, filtered and titrated against standard 2,6-dichlorophenol indophenol (DCPIP) dye until a light pink endpoint was observed. The amount of dye consumed was used to calculate the ascorbic acid content using the formula,

$$\text{Ascorbic acid (mg/100g)} = \frac{V_1 \times C \times V_t \times 100}{V_2 \times W}$$

where: V_1 = Volume of dye used for sample titration (mL), C = Dye factor (mg ascorbic acid per mL dye), V_t = Total volume of extract (mL), V_2 = Volume of extract taken for titration (mL), W = Weight of sample (g).

Data analysis

Collected data from 3 replications were organised and subjected to ANOVA using the OP-STAT web tool (17). Degrees of freedom (df) for treatments and error were 15 and 30 respectively. Treatment means were tested for significance at 5 % probability level ($p \leq 0.05$) and compared using least significant difference (LSD).

Results and Discussion

Sixteen treatments (T_1 – T_{16}) significantly influenced growth, yield and quality parameters of lettuce, with distinct differences among bioformulation combinations.

Growth parameters

The analysis of variance on growth parameters of green leafy lettuce at harvest showed highly significant differences among 16 treatments (at $p \leq 0.05$) (Table 2 and 3). Treatment T_6 consistently gave the most favourable growth characteristics as it demonstrated superior performance in all measured morphological traits. Higher mean values were recorded for plant height (32.97 cm) and spread (34.43 cm), number of leaves per plant (33.87) and plant area (300 cm²) under T_6 . The enhanced vegetative growth observed under T_6 may be attributed to improved nutrient availability and stimulation of physiological and biological processes within the plant system. This is in line with the results of the most recent studies, according to which the use of certain types of biostimulants, protein hydrolysates and seaweed extracts in particular, significantly increases plant growth and development in lettuce (18–20). These substances contain amino acids and peptides that add to nitrogen assimilation and optimise hormonal signalling, which promotes rapid cell division and expansion necessary for high biomass accumulation (18).

Table 2. Effects of bioformulation on growth parameters of lettuce at 30 days after transplanting

Treatment	Plant height (cm)	Plant spread (cm)	No. of leaves per plant	Leaf area (cm ²)	Leaf length (cm)	Leaf width (cm)	Leaf fresh weight (g)	Leaf dry weight (g)
T ₁	13.17	15.83	13.87	151.17	21.23	5.59	4.83	1.20
T ₂	12.10	16.17	14.13	160.20	22.13	6.40	4.87	1.07
T ₃	12.33	16.60	13.67	152.87	21.47	6.33	5.63	1.47
T ₄	13.73	16.90	14.00	148.37	21.97	6.04	4.50	0.93
T ₅	12.47	16.60	13.40	163.23	22.07	5.57	5.60	1.30
T ₆	14.73	19.40	15.67	171.93	24.40	7.82	7.43	2.00
T ₇	12.77	16.77	13.13	140.00	20.77	5.16	4.80	1.10
T ₈	11.90	17.23	13.80	140.17	22.00	5.18	5.40	1.30
T ₉	11.77	16.43	13.07	153.60	21.23	6.41	4.63	1.00
T ₁₀	13.53	16.13	13.87	152.17	21.47	6.24	5.70	1.50
T ₁₁	14.40	20.70	14.67	165.57	23.20	7.27	6.60	1.70
T ₁₂	12.90	15.97	13.40	144.50	21.40	6.32	4.43	1.00
T ₁₃	13.17	18.17	13.27	152.60	21.60	5.16	5.23	1.30
T ₁₄	11.87	16.20	13.13	151.70	21.70	6.52	5.63	1.40
T ₁₅	13.33	15.97	13.13	158.63	22.07	6.41	4.70	1.00
T ₁₆	10.30	13.53	11.60	135.27	15.13	5.10	3.57	0.50
C.D.	1.41	0.47	0.50	4.62	0.84	0.37	0.23	0.04
SE(m)	0.48	0.16	0.17	1.59	0.29	0.13	0.08	0.02

T: treatment; SE(m): standard error of the mean; CD: critical difference.

Table 3. Effects of bioformulations on growth parameters of lettuce at harvest

Treatment	Plant height (cm)	Plant spread (cm)	No. of leaves per plant	Leaf area (cm ²)	Leaf length (cm)	Leaf width (cm)	Leaf fresh weight (g)	Leaf dry weight (g)	Days taken to maturity
T ₁	28.50	30.90	30.87	272.70	31.77	10.30	10.70	1.83	65.80
T ₂	28.47	31.07	30.00	259.83	31.03	9.87	10.47	1.57	68.00
T ₃	29.33	31.77	29.73	252.57	31.47	11.20	11.53	2.10	64.00
T ₄	27.87	31.87	30.00	270.43	31.57	9.77	10.13	1.40	68.87
T ₅	27.13	32.00	30.80	261.97	32.20	11.23	11.37	2.10	62.53
T ₆	32.97	34.43	33.87	300.00	33.87	12.97	12.97	3.00	55.33
T ₇	28.97	32.50	30.00	246.90	30.97	10.10	10.40	1.70	66.33
T ₈	27.93	33.23	29.53	245.27	30.83	11.13	11.17	2.00	61.40
T ₉	28.90	32.43	31.00	275.53	31.13	10.00	10.23	1.60	70.00
T ₁₀	30.57	31.73	29.73	255.23	31.40	11.00	11.67	2.20	63.40
T ₁₁	31.47	35.33	32.20	287.83	33.00	12.00	12.07	2.70	57.47
T ₁₂	27.00	31.90	30.47	245.83	30.77	9.77	10.20	1.50	66.20
T ₁₃	27.10	32.20	30.13	253.53	31.63	10.77	11.10	1.93	58.40
T ₁₄	23.80	31.40	30.00	248.30	31.20	11.17	11.43	2.10	62.60
T ₁₅	23.97	30.47	29.47	274.30	32.33	9.97	10.20	1.40	69.53
T ₁₆	21.27	28.70	25.13	236.20	27.53	9.20	9.13	1.00	75.00
C.D.	5.30	0.57	1.12	4.01	0.77	0.09	0.21	0.04	0.56
SE(m)	1.83	0.20	0.39	1.38	0.27	0.03	0.07	0.02	0.19

T: treatment; SE(m): standard error of the mean; CD: critical difference.

In addition, T₆ accelerated the crop cycle, giving rise to the shortest time to reach maturity (55.33 days), an important commercial advantage usually related to treatments that improve nutrient use efficiency (NUE) and reduce stress factors, which are related to the improved rhizosphere due to the application of the bioformulations (21). In contrast, treatment T₁₆ had the lowest values for all growth parameters and the longest time to maturity (75 days), highlighting the importance of proper nutritional management to enhance crop potential. Higher leaf dry weight in T₆ (3.00 g) compared with T₁₆ (1.00 g) reflects enhanced assimilate accumulation and structural biomass accumulation. This superior performance of T₆ suggests that a synergy between the rhizosphere microbiome is optimised, which thereby enhances nutrient solubilisation and higher photosynthetic efficiency.

Yield parameters

The yield response of lettuce was very similar to the results derived from the growth analysis (Table 4). Yield per plant and, as a consequence, yield per hectare was highest under treatment T₆, which recorded 385.5 g per plant, corresponding to 21.42 tonnes per hectare (t/ha). This result was much higher compared to the lowest recorded yield for the treatment of T₁₆ (260.5 g or 14.47 t/ha). The second-ranked treatment, T₁₁ (382.6 g or 21.26 t/ha), also showed superior yield performance; these results indicate the strong influence of the applied bioformulations on its productivity.

Table 4. Effects of bioformulations on the yield parameters of lettuce

Treatment	Yield per plant (g)	Yield per hectare (tonnes)
T ₁	291.77	16.21
T ₂	323.83	17.99
T ₃	290.17	16.12
T ₄	338.10	18.78
T ₅	326.97	18.16
T ₆	385.50	21.42
T ₇	290.53	16.14
T ₈	335.53	18.64
T ₉	291.07	16.17
T ₁₀	327.17	18.17
T ₁₁	382.60	21.26
T ₁₂	336.83	18.71
T ₁₃	329.97	18.33
T ₁₄	331.63	18.42
T ₁₅	331.30	18.40
T ₁₆	260.50	14.47
C.D.	13.92	0.77
SE(m)	4.80	0.27

T: treatment; SE(m): standard error of the mean; CD: critical difference.

Table 5. Effects of bioformulations on quality parameters of lettuce

Treatment	Chlorophyll (mg/100 g)	Iron (mg/100 g)	Calcium (mg/100 g)	Beta-carotene (mg/100 g)	Vitamin C (mg/100 g)
T ₁	24.28	2.07	27.47	2.07	8.20
T ₂	24.64	1.90	27.23	2.33	8.57
T ₃	24.29	1.90	27.00	2.00	8.33
T ₄	24.41	1.90	27.43	2.80	8.57
T ₅	24.44	1.87	26.57	1.73	8.33
T ₆	28.55	2.63	30.67	3.20	11.80
T ₇	24.37	1.80	27.10	2.37	8.20
T ₈	24.46	1.97	27.07	2.10	8.97
T ₉	24.44	1.87	26.53	2.70	7.87
T ₁₀	24.36	2.00	27.33	1.70	8.97
T ₁₁	27.34	2.33	30.37	3.10	11.60
T ₁₂	24.41	1.93	26.53	2.10	8.10
T ₁₃	27.59	3.20	33.97	3.30	11.90
T ₁₄	24.41	1.97	27.23	2.17	8.50
T ₁₅	24.34	1.90	26.87	1.60	8.13
T ₁₆	20.13	0.70	21.33	1.03	5.90
C.D.	0.18	0.16	0.87	0.13	0.51
SE(m)	0.06	0.06	0.30	0.05	0.18

T: treatment; SE(m): standard error of the mean; CD: critical difference.

The superior yields obtained with the treatments T₆ and T₁₁ were directly linked to the enhanced foliar development of the crop, which increases the photosynthetic surface area of the plant. Lettuce, being a leafy vegetable where the economic yield primarily depends on the biomass growth, improved plant growth directly enhances fresh weight accumulation. This relationship is well documented in the horticultural literature, where morphological parameters are positively associated with the final biomass production (22). The highly significant difference between treatments was recorded (13.92 critical difference for yield per plant), which shows the strong effect of the applied treatments.

Quality parameters

From the quality analysis, it was indicated that certain treatments had a notable effect in improving the nutritional profile of the lettuce, often independent of biomass yield (Table 5). Treatment T₆ recorded the highest chlorophyll content (28.553 mg/100 g), indicating enhanced nitrogen assimilation, improved chloroplast development and consequently higher photosynthetic activity and overall plant vigour. However, in general, rotational treatments were more effective in enhancing essential micronutrients and vitamins.

The T₁₃ had the highest contents of iron (3.2 mg/100 g), calcium (33.967 mg/100 g), vitamin C (11.9 mg/100 g) and beta-carotene (3.3 mg/100 g). This is attributed to enhanced nutrient uptake and translocation, which is due to the stimulated root activity and rhizosphere microbial populations as a consequence of applying bioformulations and organic amendments. This indicates that T₁₃ was particularly effective for improving nutrient density through biofortification mechanisms.

Preclinical evidence suggests that by modifying certain mineral composition (e.g., calcium), or the addition of some organic substances (e.g., nano fertilisers), micronutrient and antioxidant accumulation in leafy greens can be significantly enhanced (23). The fact that T₁₃ had higher quality parameters compared with T₆ and that T₆ had higher yield compared with T₁₃ reflects the well-known yield-quality trade-off in crop physiology (24). It was reported that conditions that promote rapid vegetative growth (high yield) can dilute nutritional compounds, while those that restrict growth slightly or an enrichment program can activate the enhanced synthesis of secondary metabolites such as β -carotene and vitamin C and optimise the final nutraceutical value.

Conclusion

The results identified T₆ and T₁₃ as the most effective treatments for yield and quality respectively. The T₆, followed by T₁₁, produced the highest vegetative growth and yield. On the contrary, T₁₃ was superior in terms of nutrient content, leading to the highest content of major micronutrients and vitamins, namely iron, calcium, beta-carotene and vitamin C. These results demonstrate the correlation of maximising biomass and maximising nutritional content, thereby suggesting that a single type of treatment cannot optimise both outcomes simultaneously. For commercial production, T₆ is recommended, whereas T₁₃ is recommended for biofortification. Future research should focus on a treatment combination to support both high yield and enhanced nutrient quality.

Acknowledgements

The authors sincerely thank Suresh for his assistance in preparing tables and formatting figures. The authors are also grateful to the Department of Horticulture, Lovely Professional University, Phagwara, for providing the amenities to carry out the research.

Authors' contributions

DC contributed to experimentation, investigation, data analysis, interpretation and drafting of the original manuscript. SH provided critical guidance in designing the experimental framework, supervised the overall execution of the research and contributed to the interpretation of results and refinement of the manuscript. VA assisted in data analysis and supported the experimental work. All authors read and approved the final 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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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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