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RESEARCH ARTICLE

Augmenting brinjal productivity through foliar biostimulant application of symbiotic co-culture of acetic acid bacteria and yeast fermented cashew apple juice: A sustainable approach

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Abstract

The study evaluated the efficacy of Symbiotic Co-culture of Bacteria and Yeast (SCOBY), primarily *Komagataeibacter rhaeticus* and *Brettanomyces bruxellensis* as a biostimulant to enhance brinjal (*Solanum melongena*) growth and productivity. In India, ~90 % of cashew apples are discarded during cashew nut production. This study utilized SCOBY cultured in cashew apple waste as biostimulant foliar spray in a field experiment conducted in Semberi village, Cuddalore district (2023 - 2024), using the traditional Semberi brinjal variety in a Randomized Block Design (RBD) with three replications. Eight different foliar treatment combinations, involving cell-free extracts of SCOBY cultured in cashew apple juice were applied at 30, 45 and 60 Days After Transplanting (DAT). Growth and yield parameters were recorded throughout the season. Among the treatments, a 50:50 ratio of 1 % cashew apple juice-fermented SCOBY and 1 % Pink Pigmented Facultative Methylotrophs (PPFM) (T_8) produced a significant amount of growth-promoting phytohormones, with 26.81 µg/mL of Indole-3-Acetic Acid (IAA) and 87.4 µg/mL of Gibberellic Acid (GA_3), which enhanced root development, stem elongation and flowering in brinjal. T_8-treated plants recorded a maximum height (163.02 cm), leaf area (88.27 cm^2) and the highest chlorophyll content (35.90). These plants recorded earlier flowering (50 % flowering in 41 days), produced more flowers (47 flowers/ plant) and yielded more and larger fruits (14 fruits/ plant, with a length of 20 cm and girth of 7.1 cm), resulting in a maximum yield of 3.1 kg/ plant, compared to the control (1.8 kg/ plant). Additionally, nutrient uptake was higher in T_8-treated plants (95.81 kg/ha N, 25.23 kg/ha P and 99.56 kg/ha K). Principal Component Analysis (PCA) confirmed a strong correlation between T_8 treatment and improved yield parameters. This study highlights cashew apple juice-fermented SCOBY as a sustainable, eco-friendly biostimulant that enhances brinjal productivity, recycles agricultural waste and supports regenerative agriculture.

Keywords: brinjal; cashew apple juice; fermented SCOBY; nutrients; sustainability; yield

Introduction

Symbiotic co-cultures of cellulose-producing acetic acid bacteria and fermentative yeast, commonly referred to as SCOBY, are responsible for the production of kombucha - a tangy, tea-like beverage that has become increasingly popular worldwide. Kombucha, made through fermentation using SCOBY is known by several names including tea fungus, haipao, teakwass, comboutea, Manchurian mushroom and kambotscha (1). The drink is believed to have originated in northeastern China (Manchuria) during the Tsin Dynasty (Ling Chi) around 220 BC, with purported health benefits (2). Acetic acid bacteria (AAB), specifically *Acetobacter, Gluconobacter and Komagataeibacter* species (3) are the key microorganisms that form the SCOBY and facilitate kombucha production. A functional kombucha was being developed by fermenting sugared black tea blended with 15 % roselle (*Hibiscus sabdariffa*) calyx extract using *K. rhaeticus* and *B. bruxellensis* cultures for enhancing the organoleptic properties of kombucha beverage (4). As consumer demand for kombucha has risen, its industrial production has expanded significantly (5). This increased production results in a larger quantity of SCOBY, which forms at the water-air interface during fermentation. Bacterial cellulose produced by SCOBY presents innovative opportunities for advancing food production, promoting sustainable regenerative ecosystems and improving waste management (6). It also offers numerous advantages including excellent mechanical properties, high chemical versatility and the ability to form nanostructures (7). Additionally, kombucha fermented with *Acetobacter xylinum* and *Saccharomyces cerevisiae* in sweetened black tea was analyzed using Gas Chromatography Mass Spectrometry (GC-MS). The analysis identified 18 bioactive metabolites including antibacterial agents like dodecane and flavour compounds such as neopentyl-2-oxobutanoate. Acetic acid and a cellulosic mat with potential food applications were also produced, highlighting kombucha's antibacterial properties and multifunctionality (8). Investigations were done on the production of bacterial cellulose by the *Novacetimonas hansenii* P3 strain, which was freshly isolated from rotten pomegranate fruit waste (9).

One promising use for SCOBY waste lies in its potential as a biostimulant. The biostimulants are made from effective microorganisms and are known to enhance agricultural productivity. SCOBY microbes found in kombucha waste have proven to be effective plant biostimulants. These biostimulants can improve plant growth, development and resilience to stress (10). In modern farming, fermented organic formulations containing beneficial microorganisms are commonly used as foliar sprays to produce safe, high-quality food free from harmful residues (11). Biostimulants, which contain active or dormant cells of microorganisms such as phosphorus solubilizers and nitrogen fixers, can mobilize soil nutrients, making them more available to plants. Additionally, biostimulants help produce plant hormones like auxins, gibberellins and cytokinins which regulate growth (10).

Biostimulants have become a critical and cost-effective input for both organic farming and integrated nutrient management (INM). However, many farmers view the application of biostimulants as a labour-intensive process that increases production costs. Still, research has demonstrated that biostimulants derived from kombucha fermentation waste including those made from *Clitoria* flower extracts, have beneficial effects on crops like tomatoes (12), chilli (13), mustard greens (14) and brinjal (15). SCOBY-based biostimulants are particularly effective in balancing carbon (C) and nitrogen (N), which is vital for successful biostimulant production.

Brinjal is primarily grown in subtropical Asia, where it accounts for 94 % of global production. It is a highly popular vegetable often referred to as the "king of vegetables" (16). The growth of brinjal can benefit from microbial biostimulants made from fermented kombucha, improving plant height, leaf count as well as both wet and dry weight. Furthermore, cashew apple juice fermented with SCOBY has shown great promise as a biostimulant to enhance brinjal productivity. By improving nutrient uptake, promoting growth, increasing stress tolerance and enhancing soil health, cashew apple juice fermented with SCOBY has the potential to contribute to sustainable agriculture. However, further research is needed to standardize SCOBY-based products, explore their mechanisms of action and conduct large-scale field trials while addressing regulatory and safety concerns.

Cashew apples (*Anacardium occidentale* L.), which is a pseudo-fruit is a low-cost agricultural by-product commonly discarded in commercial cashew nut production. Cashew is a significant commercial crop in India and is often referred to as a "gold mine" for wasteland development. Cashew apples are highly nutritious. They are rich in sugars and have a distinct flavour, yet a significant portion of cashew apples go to waste in India (19). India annually discards over 4.1 Mt of cashew apples, a considerable national waste (20). Cashew apple juice has valuable properties, including the ability to produce ethanol and tannins that inhibit certain pathogens (21).

Existing literature provides encouraging evidence for the potential of cashew apple-fermented SCOBY as a biostimulant. However, its use in agriculture especially as a foliar treatment on the phyllosphere or as a plant growth promoter has received limited research attention. Studies exploring this novel product in circular regenerative agriculture remain scarce, with only theoretical insights into SCOBY's potential. The symbiotic bacteria and yeast in SCOBY are anticipated to play key roles in improving plant growth, health and performance. In the cashew-growing regions of Cuddalore, cashew apples left in the fields after nut extraction represent an underutilized resource. These nutrient-dense and sugar-rich apples can be repurposed into an organic crop stimulant particularly for vegetables through fermentation with SCOBY. This study investigates the effectiveness of the foliar application of cashew apple juice fermented with SCOBY derived from plant growth-promoting phyllosphere microbes, focusing on its impact on growth and yield parameters in brinjal cultivation.

Materials and Methods

Collection of cashew apple samples

Two varieties of cashew apples, orange (VRI-2) and red (VRI-3), were aseptically collected from TNAU, ICAR-KVK, Vriddhachalam and brought to the laboratory in clean polythene bags.

Preparation of Cashew Apple Juice (CAJ) Medium

The cashew apples were washed with distilled water, sterilized by dipping in 70 % (w/v) ethanol for 5 sec and rinsed with sterile water. The apples were then crushed using a sterile mortar and pestle and the juice was filtered. Gelatin was added to clarify the juice by removing tannins. The clarified juice was centrifuged and the supernatant was stored in the refrigerator for further analysis.

Determination of mineral content of clarified cashew apple juice

All glassware used for sample preparation was cleaned with neutral detergent, rinsed with deionized water and soaked in a deionized water bath with 10 % HCl for 24 hr. The cashew apple juice (5 mL) was mineralized using a solution of HNO_3 and $HClO_4$ (3:1 ratio) in a dry block digester at 200 °C for 3 - 4 hr until its volume reduced to 1.5 mL. The solution was transferred to a 50 mL volumetric flask and diluted with deionized water. A blank sample was prepared in the same manner. Iron (Fe), Calcium (Ca), Magnesium (Mg), Mangenese (Mn), Zinc (Zn) and Copper (Cu) concentrations were measured using atomic absorption spectrometry (Perkin-Elmer, model A-Analyst 300). Potassium (K) and sodium (Na) were quantified using flame photometry (DIGIMED model DM-61).

Fermentation of cashew apple juice using SCOBY

A SCOBY containing *Acetobacter* (*K. rhaeticus*) and *B. bruxellensis* was sourced from TNAU, ICAR-KVK, Vriddhachalam. A loopful of SCOBY culture was inoculated into 100 mL of diluted cashew apple juice supplemented with basal nutrients. The inoculated media were incubated at 30 °C for 2 - 3 days and used as inoculum for subsequent experiments. The pH of the media was adjusted to 6.5 using H_3PO_4, sterilized at 121 °C for 15 min and added to the cashew apple juice medium (10 % volume). Fermentation proceeded for 5 - 7 days and cells were removed by centrifugation at 15000 rpm for 10 min.

Preparation of biostimulant from cashew apple juice fermented with SCOBY

A 50-L fermentor (Scigenics India Pvt. Ltd.) was filled with 40 L of clarified cashew apple juice supplemented media. It was sterilized at 121 °C for 20 min and then cooled. One percent of the SCOBY culture was added to the fermentor. During incubation, the pH was maintained at 6.7±0.2 and air was sparged using a ring sparger. The media was separated from the fermentation mixture using tangential flow filtration (TFF). After fermentation, the concentrate was collected in a 5 L flask and processed through a TFF column.

Preparation of Cell-Free Supernatant (CFS) from fermented cashew apple juice

The culture was incubated in cashew apple juice supplemented media at 37 °C for 24 hr. A CFS was obtained by centrifuging the culture at 9500 g for 10 min at 4 °C and filtering through a 0.2 µm cellulose acetate filter. The supernatant was adjusted to pH 6.5 or dialyzed for 24 hr against glucose-free cashew apple juice supplemented media (pH 6.5) at 4 °C. Catalase (5 mg/mL) was added to remove hydrogen peroxide. In some experiments, the supernatant was concentrated tenfold using a rotary evaporator.

Microbial load and biostimulant quality assessment

The microbial load of the final product was measured at 10^{12} CFU/mL and the TFF sample showed 10^{18} CFU/mL. The biostimulant's quality was evaluated over 60 days by monitoring microbial population, pH and contamination.

Quantitative estimation of IAA production in SCOBY

IAA production by SCOBY had been estimated (22). After three days of incubation at 28 ± 2 °C, the cultures were cultivated in Hestrin-Schramm broth with 0.1 % tryptophan added. The cultures were incubated for a total of 30 min. Following this, 2 mL of supernatant was taken to which 2 to 3 drops of 0.1 mM orthophosphoric acid and 4 mL of Salkowski reagent (one mL of 0.5 M $FeCl_3$ in 50 mL of 35 % perchloric acid) were added. Using a spectrophotometer (UV-160 A, Shimadzu, Japan), the development of the pink colour was noted and an optical density reading was taken at 530 nm. Using the IAA standard graph, IAA production was estimated.

Quantification of GA_3 in SCOBY

GA_3 estimation was carried out (23). The isolates were incubated for seven days in Hestrin-Schramm broth. The cultures were centrifuged for 15 min at 10000 rpm after incubation. After adding 2 mL of ethyl acetate to 2 mL of supernatant, the shaker was left for 15 min. The ethyl acetate layer evaporated at room temperature for 15 min and the residue was dissolved in 1 mL of alcohol. A 2 mL suspension was taken from the above material and 1 mL of DNPH reagent was added before being incubated for 5 min. 5 mL of 10 % KOH was added after 5 min and the mixture was then incubated to develop the colour. In a spectrophotometer, the intensity of the red wine colour developed was measured at 430 nm. GA_3 was utilized as standard and the production amount was expressed in µg/mL.

Experimental design and details

The field experiment was conducted in Semberi village, Cuddalore district, Tamil Nadu. The study was conducted on brinjal, specifically using a local traditional variety known as Semberi. A RBD was employed for the experiment, featuring eight different treatments with three replications each. The total experimental area spanned 10 cents, allowing for the systematic evaluation of growth and yield parameters across the treatments (Fig. 1). The details of foliar treatments applied in the field experiment is described in Table 1.

Brinjal field preparation and crop management

The brinjal field was prepared using thorough ploughing to achieve fine tilth and 35-day-old healthy seedlings were transplanted at a spacing of 90 x 90 cm, followed by immediate irrigation for establishment. Gap filling was done 10 days after planting to ensure uniformity. Manures and inorganic fertilizers (urea, superphosphate, muriate of potash) were applied as per treatments; with nitrogen applied in two split doses and phosphorus and potassium fully applied at transplanting. Foliar sprays of 19:19:19 NPK were conducted every 10 days, starting 30 DAT. Irrigation was provided at 3-4-day intervals based on soil moisture, with additional practices like weeding, earthing up and plant protection implemented as required.

Plant growth and nutrient analysis of brinjal

Plant growth observations were taken from five randomly selected plants per plot 60 DAT. Key growth parameters including plant height, number of primary and secondary branches, stem girth, leaf area, chlorophyll content index (CCI) and total dry matter were measured. Flowering attributes such as days to 50 % flowering and the number of flowers per plant were also recorded. Yield characteristics including the number of fruits per plant, fruit length, fruit girth, fruit yield and root length were observed. Plant nutrient analysis included nitrogen, phosphorus and potassium content, measured using standard methods, with uptake calculated based on dry matter. All measurements were conducted to assess the effects of different treatments on brinjal growth and yield.

Influence of treatments on brinjal yield parameters using PCA

PCA was adopted to analyse the data and identify the primary factors impacting yield parameters. The analysis was conducted using GRAPES software, version 1.1.0 and the results were represented through biplots.

Statistical analysis

The experimental data were analyzed statistically (24). Significant treatment differences were determined using the F-test and critical differences were calculated at a 5 % significance level. Non -significant differences between treatments are marked as 'NS'.

Table 1. Details of foliar treatments applied in the field experiment

Treatment Code	Treatment Details
T_1	Absolute control + RDF
T_2	Foliar spray of 5 % Uninoculated control of cashew apple juice alone @ 30, 45 and 60 DAT + RDF
T_3	Foliar spray of 2 % cashew apple juice-fermented SCOBY @ 30, 45 and 60 DAT + RDF
T_4	Foliar spray of 4 % cashew apple juice-fermented SCOBY @ 30, 45 and 60 DAT + RDF
T_5	Foliar spray of 6 % cashew apple juice-fermented SCOBY @ 30, 45 and 60 DAT + RDF
T_6	Foliar spray of 8 % cashew apple juice-fermented SCOBY @ 30, 45 and 60 DAT + RDF
T_7	Foliar spray of 1 % PPFM @ 30, 45 and 60 DAT + RDF
T_8	Foliar spray of 50:50 ratio of 1 % cashew apple juice-fermented SCOBY and 1 % PPFM @ 30, 45 and 60 DAT + RDF

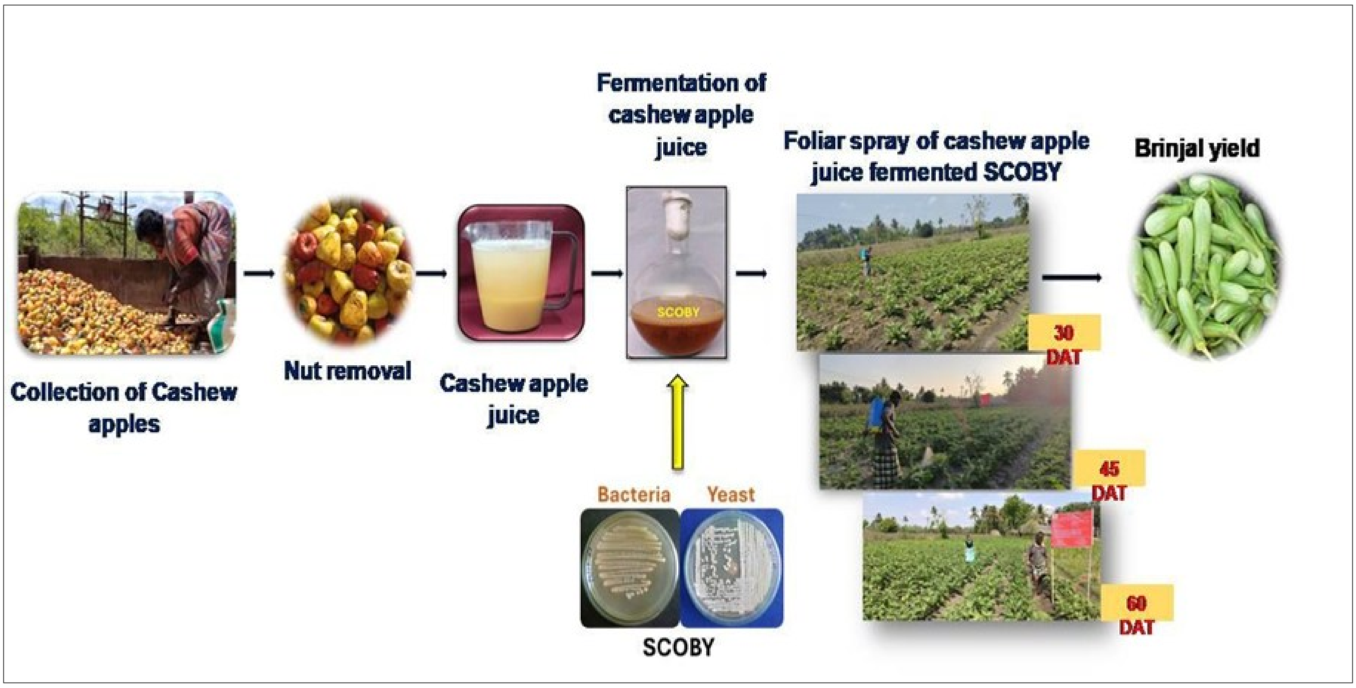

Fig. 1. Flow chart of the lab scale production of SCOBY and field application in brinjal.

Result and Discussion

Nutrient composition of clarified cashew juice for cashew culture medium

The clarified cashew juice, rich in potassium (12.00 ± 0.50 g/L), is an excellent nutrient source for plant growth, supporting water regulation, enzyme activation and plant vigour. Moderate levels of zinc (9.50 ± 3.00 g/L) and manganese (5.50 ± 0.20 g/L) enhance enzyme activity and photosynthesis, contributing to crop quality and growth. Magnesium (1.10 ± 0.04 g/L) aids chlorophyll synthesis, while phosphorus (1.10 ± 0.005 g/L) promotes root development and energy transfer. Sulphur (0.70 ± 0.01 g/L) is crucial for protein synthesis and its low sodium content (0.08 ± 0.0 g/L) reduces salinity risks. Trace ferrous levels ($6.00 \pm 1.50 \times 10^{-3}$ g/L) support chlorophyll production. However, the absence of calcium and copper may require supplementation for optimal biostimulant use. The nutrient composition was elaborated in Table 2 and Fig. 2. The mineral content of the cashew apple juice presented higher levels of magnesium and sodium than the synthetic medium (25). On the other hand, the levels of iron, manganese, potassium and phosphorous were lower in the cashew apple juice than in the synthetic medium.

Table 2. Estimation of the nutrient content of the clarified cashew apple juice

Component	Nutrient content in the clarified cashew juice (g/L)
Macronutrients	
Phosphorous	1.10 ± 0.005
Potassium	12.00 ± 0.50
Calcium	<DL
Magnesium	1.10 ± 0.04
Sodium	0.08 ± 0.0
Sulphur	0.70 ± 0.01
Micronutrients	
Copper	<DL
Ferrous	$6.00 \pm 1.50 \times 10^{-3}$
Zinc	9.50 ± 3.00
Manganese	5.50 ± 0.20

Values in each column are the mean of three replicates ± SE (Standard Error)

Quality assessment of the developed biostimulant

The viability of the developed crop biostimulant over a 60-day storage period, focusing on the microbial populations of SCOBY and PPFM as well as pH changes, is summarized in Table 3. Initially, the populations of SCOBY and PPFM were high, recorded at 9.2 ± 0.004 and 9.37 ± 0.004 log CFU/mL respectively, with a pH of 5.9 ± 0.003. The microbial populations in biostimulants are most effective shortly after production (26). Over the first 30 days microbial populations increased, peaking at 9.84 ± 0.008 log CFU/mL for SCOBY and 9.89 ± 0.005 log CFU/mL for PPFM, while the pH declined to 5.1 ± 0.005. After 30 days, the populations began to decline, with SCOBY reducing to 9.13 ±0.004 log CFU/mL and PPFM to 9.12 ± 0.004 log CFU/mL by Day 40, coinciding with a further drop in pH to 4.6 ± 0.002. By Day 60, the microbial populations had decreased to 8.74 ± 0.01 log CFU/mL for SCOBY and 8.78 ± 0.011 log CFU/mL for PPFM, accompanied by a slight rise in pH to 4.9 ± 0.007. These observations suggest that the biostimulant maintains optimal microbial activity during the first 30 days, after which increasing acidity reduces cell viability and diminishing its efficacy (Fig. 3).

Quantitative production of IAA and GA_3

The quantitative production of key phytohormones such as IAA (26.81 µg/mL) and GA_3 (87.4 µg/mL) as shown in Fig. 4 suggests that SCOBY significantly enhances root development and overall plant growth by promoting nutrient absorption and elongation. The production of GA_3 enhances the uptake of essential minerals such as potassium and calcium, which contributes to increased levels of chlorophyll, soluble sugars and protein in plants. The application of growth regulators and PPFM sprays leads to an improvement in the soluble protein content of tomato leaves (27). These hormones play a critical role in stimulating cell division, root hair formation and water uptake, which are vital for early plant establishment and growth.

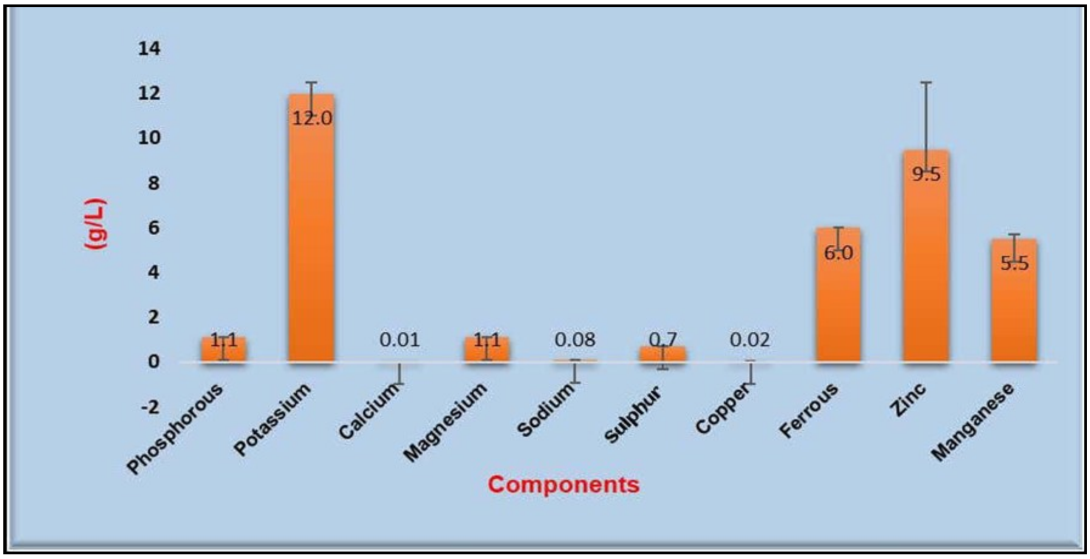

Fig. 2. The nutrient content of the clarified cashew apple juice.

Table 3. Assessing the quality of the developed SCOBY crop biostimulant

Days of storage	Viable cell population (log CFU/mL)		pH
	SCOBY	PPFM	
0	9.2±0.004	9.37±0.004	5.9±0.003
10	9.36±0.006	9.52±0.003	5.7±0.005
20	9.8±0.003	9.81±0.005	5.4±0.005
30	9.84±0.008	9.89±0.005	5.1±0.005
40	9.13±0.004	9.12±0.004	4.6±0.002
50	8.84±0.008	8.97±0.004	4.8±0.003
60	8.74±0.01	8.78±0.011	4.9±0.007
CD (P=0.05)	0.021	0.018	0.015
SEd	0.009	0.008	0.006

Values in each column are the mean of three replicates ± SE (Standard Error)

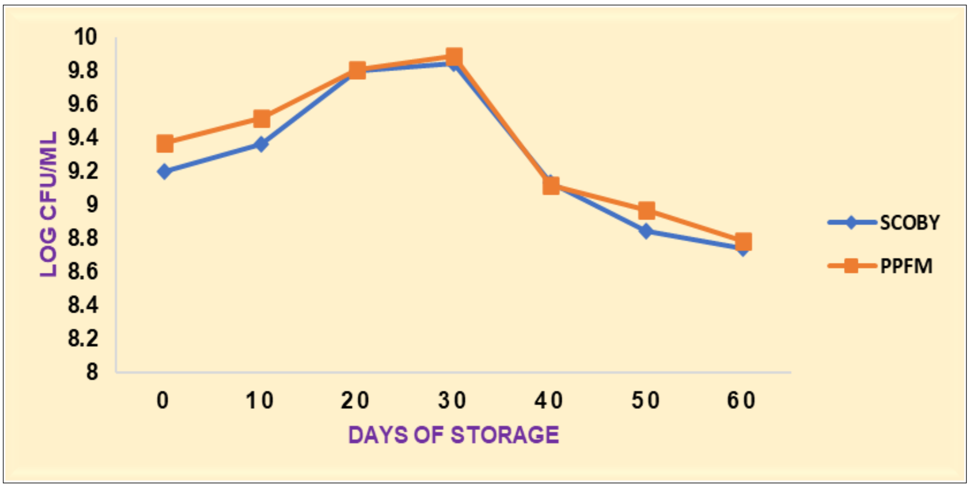

Fig. 3. Evaluation of the quality of the developed crop biostimulant.

Plant growth observations in the brinjal field

Plant growth parameters

The combined foliar application of 1% cashew apple juice fermented with SCOBY and PPFM (T_8) resulted in the most pronounced improvements in brinjal growth compared to the control (T_1) and other treatments (Table 4). Plants under T_8 treatment achieved a significantly greater plant height (163.02 ± 2.82[a] cm) than the control (120.4 ± 4.34[g] cm), likely attributed to enhanced nitrogen assimilation supporting overall vegetative development. In addition, T_8-treated plants exhibited a higher number of primary (6.01 ± 0.21[a]) and secondary branches (8.13 ± 0.29[a]) per plant, coupled with a maximum leaf area (88.27 ± 3.18[a] cm^2) and elevated chlorophyll content (35.90 ± 0.78[a]), reflecting improved photosynthetic capacity. The incorporation of PPFM in the foliar spray likely contributed to reduced chlorophyll degradation and delayed senescence under stress conditions (27). The highest total dry matter production recorded in T_8 further indicated superior plant vigour and metabolic activity (28). These observations are consistent with the findings of Saddam *et al.* (12), who demonstrated that liquid fertilizer derived from fermented butterfly pea flower (*Clitoria ternatea* L.) waste, produced via the kombucha method, significantly enhanced tomato (*Lycopersicum esculentum* L.) growth. In their study, a 5 mL/L application significantly improved plant height, leaf count and both fresh and dry biomass. Collectively, these results highlight the broader potential of kombucha fermentation waste as an effective and sustainable organic fertilizer for crop improvement.

Table 4. Effect of foliar application of SCOBY based crop biostimulant on the morphological characteristics of brinjal

Treatments	Plant height (cm)	Primary branches	Secondary branches	Stem girth (cm)	Leaf area (cm²)	Leaf chlorophyll content	TDMP (g)
T_1	120.4±4.34^{g}	3.13±0.05^{g}	5.69±0.10^{g}	2.71±0.05^{g}	63.34±1.10^{g}	23.29±0.42^{g}	192.19±6.92^{g}
T_2	126.96±3.36^{f}	3.59±0.07^{f}	6.05±0.12^{f}	3.02±0.06^{f}	67.23±1.34^{f}	26.89±0.79^{f}	202.72±5.46^{f}
T_3	154.17±1.54^{b}	5.39±0.05^{b}	7.69±0.08^{b}	3.99±0.04^{b}	82.45±0.82^{b}	33.63±1.13^{b}	244.94±2.18^{b}
T_4	140.75±4.3^{e}	4.05±0.06^{e}	6.44±0.10^{e}	3.27±0.05^{e}	71.27±1.09^{e}	29.96±0.84^{e}	213.35±5.88^{e}
T_5	147.2±3.89^{c}	4.97±0.15^{c}	7.28±0.22^{c}	3.76±0.12^{c}	78.72±2.40^{c}	31.95±0.84^{c}	234.41±6.28^{c}
T_6	133.81±2.04^{d}	4.51±0.12^{d}	6.87±0.18^{d}	3.54±0.10^{d}	75.01±1.98^{d}	30.69±0.73^{d}	223.88±3.17^{d}
T_7	156.03±3.12^{b}	5.45±0.14^{b}	7.72±0.21^{b}	4.05±0.11^{b}	84.61±2.24^{b}	34.14±0.90^{b}	245.47±4.30^{b}
T_8	163.02±2.82^{a}	6.01±0.21^{a}	8.13±0.29^{a}	4.27±0.15^{a}	88.27±3.18^{a}	35.90±0.78^{a}	256±4.38^{a}
CD (P=0.05)	5.434	0.232	0.32	0.169	3.458	0.897	8.653
SEd	2.533	0.11	0.149	0.079	1.612	0.414	4.034

Values in each column are the mean of three replicates ± SE (Standard Error)

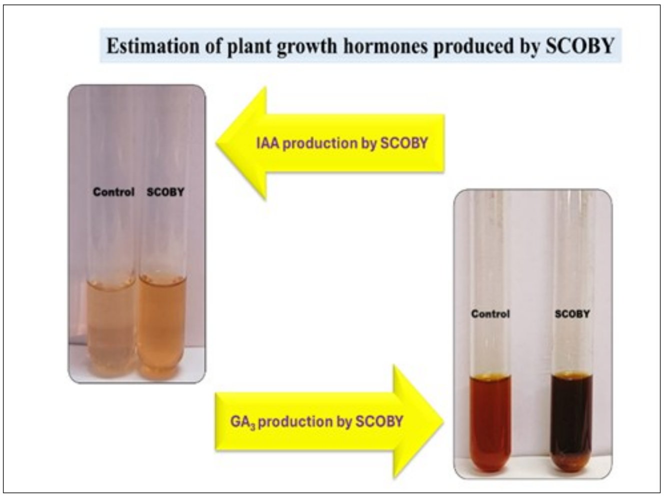

Fig. 4. Estimation of plant growth hormones produced by SCOBY.

Flowering attributes

The foliar application of SCOBY and PPFM (T_8) significantly accelerated flowering, with 50 % of plants flowering in 41 days compared to 60 days in the control. T_8 also produced the highest number of flowers per plant (47 ± 0.81^{a}), compared to only 29 ± 1.05^{g} in the control. Biostimulants play a role in enhancing flowering through improved hormonal regulation, particularly the stimulation of gibberellins and cytokinins (29), to which the present results are aligned with. Furthermore, the microbial inoculants in SCOBY likely enhanced nutrient uptake and supported reproductive development (30).

Yield attributes

T_8 exhibited the highest fruit yield, producing 14 ± 0.24^{a} fruits/plant with an average fruit length of 20 ± 0.35^{a} cm, compared to only 3 ± 0.11^{g} fruits/ plant in the control. The total fruit yield per plant in T_8 was 3.1 ± 0.731^{a} kg, nearly double the control (1.8 ± 0.453h kg). These results highlight the efficacy of biostimulants in improving both the yield and quality parameters of brinjal, aligning with the observations reported by Nasuelli *et al*. (31), who documented comparable enhancements using microbial and algae-based biostimulants.

Root Development

Root development was significantly enhanced in T_8, where root length reached 50 ± 0.89^{a} cm compared to 26 ± 0.88^{g} cm in the control. This improvement is likely due to the biostimulants ability to promote the production of auxins and cytokinins, which stimulate root elongation and branching (32). The presence of *K. rhaeticus* in SCOBY cultures may have increased the secretion of exopolysaccharides and improved root-soil interaction (33).

The effect of foliar application of crop biostimulant on the flowering along with the yield characteristics of brinjal was elucidated in Table 5.

Nutrient Uptake

T_8 achieved the highest nutrient uptake, with 95.81 ± 3.45^{a} kg/ha of nitrogen, 25.23 ± 0.91^{a} kg/ha of phosphorus and 49.38 ± 1.78^{a} kg/ha of potassium, significantly exceeding the control's nutrient absorption (Table 6). The enhanced nutrient uptake observed in T_8 likely to have contributed to improved plant growth and higher yield, which is consistent with findings (34) regarding the efficacy of foliar applications in boosting nutrient content in plants. PCA

A complex dataset was successfully simplified by the PCA used in this study, which identified important variables influencing variability across various treatments (Fig. 5). A substantial amount of the variance was explained by the two main dimensions that were the focus of the analysis: Dim1 (First Principal Component) and Dim2 (Second Principal Component). Days to 50 % Flowering (D50 %F) and Fruit Yield (FrY) were the main yield-related factors that affected Dim1, which accounted for 97.7 % of the total variance. Strong

Table 5. Effect of foliar application of SCOBY based crop biostimulant on the flowering and yield characteristics of brinjal

Treatments	Flowering attributes			Yield characteristics			
	Days to 50% flowering	Number of flowers/ plant	Number of fruits/ plant	Fruit length (cm)	Fruit girth (cm)	Fruit yield/ plant (kg)	Root length (cm)
T_1	60±0.16^{g}	29±1.05^{g}	3±0.11^{g}	9.1±0.33^{g}	4.5±0.16^{g}	1.8±0.453^{h}	26±0.88^{g}
T_2	56±0.09^{f}	34±0.9^{f}	4±0.11^{f}	10.4±0.28^{f}	5.1±0.13^{f}	2±0.528^{g}	32±0.84^{f}
T_3	44±0.09^{b}	44±0.44^{b}	13±0.13^{b}	16±0.18^{b}	6.3±0.06^{b}	2.8±0.812^{c}	41.9±0.40^{b}
T_4	50±0.07^{e}	37±1.13^{e}	7±0.11^{e}	11±0.34^{e}	5.4±0.16^{e}	2.2±0.588^{f}	35±0.98^{e}
T_5	46±0.10^{c}	42±1.11^{c}	11±0.29^{c}	15±0.40^{c}	6±0.16^{c}	2.6±0.681^{d}	40±1.01^{c}
T_6	47±0.47^{d}	39±0.6^{d}	9±0.28^{d}	12.7±0.19^{d}	5.7±0.09^{d}	2.5±0.689^{e}	39±0.55^{d}
T_7	43±0.53^{b}	45±0.9^{b}	13±0.26^{b}	18±0.37^{b}	6.49±0.13^{b}	2.9±0.186^{b}	42±0.74^{b}
T_8	41±0.25^{a}	47±0.81^{a}	14±0.24^{a}	20±0.35^{a}	7.1±0.12^{a}	3.1±0.731^{a}	50±0.89^{a}
CD (P=0.05)	0.738	1.445	0.378	0.489	0.211	1.549	1.368
SEd	0.341	0.674	0.176	0.228	0.098	0.715	0.638

Values in each column are the mean of three replicates ± SE (Standard Error)

Table 6. Effect of foliar application of SCOBY based crop biostimulant on the plant nutrient uptake in brinjal

Treatments	Nitrogen uptake (kg/ha)	Phosphorous uptake (kg/ha)	Potassium uptake (kg/ha)
T_1	67.20 ± 1.16^{h}	14.71 ± 0.25^{h}	35.59 ± 0.61^{h}
T_2	71.03 ± 1.42^{g}	15.75 ± 0.31^{g}	37.56 ± 0.75^{g}
T_3	86.65 ± 0.86^{c}	22.07 ± 0.22^{c}	45.44 ± 0.45^{c}
T_4	75.16 ± 1.14^{f}	17.33 ± 0.26^{f}	39.53 ± 0.60^{f}
T_5	82.82 ± 2.53^{d}	20.49 ± 0.62^{d}	43.47 ± 1.32^{d}
T_6	78.99 ± 2.09^{e}	18.91 ± 0.50^{e}	41.50 ± 1.09^{e}
T_7	90.48 ± 2.39^{b}	23.65 ± 0.62^{b}	47.41 ± 1.25^{b}
T_8	95.81 ± 3.45^{a}	25.23 ± 0.91^{a}	49.38 ± 1.78^{a}
CD (P=0.05)	3.74	0.978	1.945
SEd	1.731	0.452	0.898

Values in each column are the mean of three replicates ± SE (Standard Error)

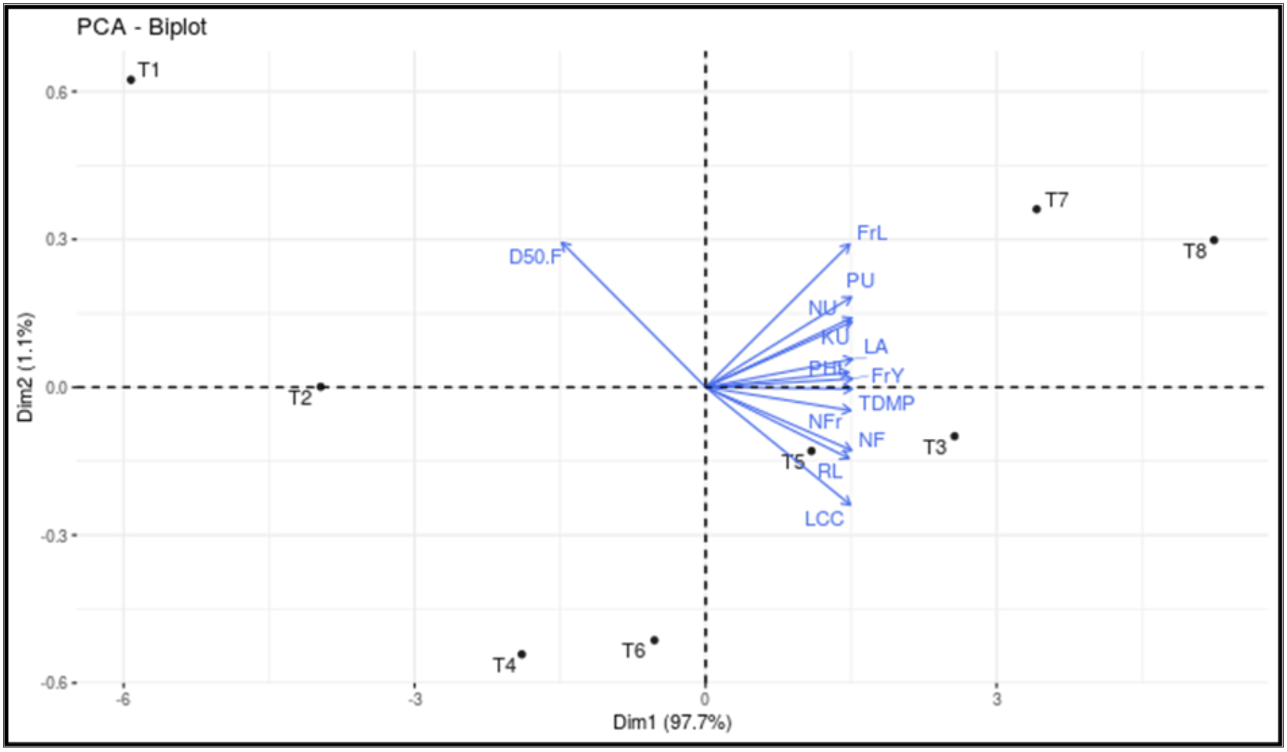

Fig. 5. Influence of treatments on brinjal yield parameters analyzed using PCA.

positive correlations with Dim1 were observed for treatments T_7 (1 % PPFM foliar spray) and T_8 (50:50 ratio of 1 % cashew apple juice-fermented SCOBY and 1 % PPFM), demonstrating their efficacy in increasing fruit weight and yield while hastening flowering. Notably, Dim1 revealed an inverse relationship between D50 %F and overall yield, implying that treatments that promoted earlier flowering were associated with higher fruit yields. Fruit produced by T_7 and T_8 was of higher quality and quantity, despite producing fewer flowers per plant. However, there was a negative correlation between Dim1 and treatments T_1 through T_4, particularly the control (T_1), indicating that delayed flowering resulted in lower yield performance. Growth-related factors like Plant Height (PHt), Leaf Area (LA) and Stem Girth (SG) had an impact on Dim2, which only explained 1.1 % of the total variance. Plant health was revealed by these growth variables, even though they were less important in explaining overall variation. Treatments T_5 and T_6 demonstrated modest increases in vegetative growth but less success in increasing yield, highlighting the fact that increased productivity is not always correlated with improved vegetative development. These associations were graphically depicted by the PCA biplot, which showed that the yield parameters fruit yield (FrY) and total dry matter production (TDMP) matched T_7 and T_8, indicating that they had a positive effect on yield. The number of flowers per plant (NF) and D50 % F on the other hand showed a negative connection with important yield parameters. This implies that T_7 and T_8 greatly increased total fruit yield and quality, even though they shortened the period to flowering and produced fewer flowers. These results highlight the need to optimize flowering time and fruit production efficiency in addition to improve vegetative development for achieving larger yields. The PCA results align with prior research (35, 36), which demonstrated the ability of microbial biostimulants to enhance plant growth, nutrient uptake and overall productivity through various mechanisms.

Conclusion

This study highlights the immense potential of cashew apple juice-fermented by SCOBY as an innovative and sustainable biostimulant for brinjal cultivation. By repurposing cashew apple juice, a typically underutilized by-product, the research addresses agricultural waste management while promoting circular regenerative practices. Field experiments on the Semberi brinjal variety revealed that the 50:50 combination of 1% cashew apple juice-fermented by SCOBY and 1 % PPFM significantly enhanced plant growth, flowering, yield and nutrient uptake compared to conventional practices. Treated plants exhibited superior vegetative growth, early flowering, increased fruit production and improved nutrient absorption, with nitrogen, phosphorus and potassium uptake significantly surpassing the control. Additionally, the robust root development and improved soil nutrient mobilization demonstrated the biostimulant's efficacy in fostering plant health. The dual microbial action of *K. rhaeticus* and *B. bruxellensis*, combined with PPFM offers a sustainable alternative to chemical fertilizers reducing environmental impact while enhancing crop productivity. By leveraging low-cost agricultural by-products and innovative microbial biotechnology, this research presents a scalable solution for eco-friendly farming. Future studies should focus on standardizing formulations, exploring broader applications across crops and regions and addressing regulatory aspects to ensure safe and effective commercialization. This breakthrough aligns with global efforts to promote sustainable agriculture, ensuring food security and environmental resilience.

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Authors' contributions

TJ and GG worked on conceptualization. TJ, GG and KK did the formal analysis. Funding acquisition was by TJ and GG. GG, TJ, NA and KK wrote the original draft. TJ, GG, SR, KK, NA and KGS carried out the writing, review and editing. All authors read and approved 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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