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RESEARCH ARTICLE

Influence of biostimulants and organic mulch on physiological, biochemical and yield attributes of Strawberry (*F. × ananassa* Dutch.) cv. Katrain Sweet

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Abstract

The aim of this research was to access the influence of bio-stimulants and organic mulch on physiological, biochemical and yield attributes of Strawberry (*F. × ananassa* Dutch.) cv. Katrain Sweet. The research was conducted over 2 consecutive years (2022-23 and 2023-24) at the Garden, Department of Fruit Science, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh. The experiment employed a randomized block design (RBD) with 13 treatments, each replicated 3 times. The treatments consisted of combinations of *Azotobacter*, PSB (Phosphate Solubilizing Bacteria), *Trichoderma* and organic mulches such as paddy straw and dried leaves. Among the treatments, T_{12}, consisted of a combination of *Azotobacter* (8 g per plant), PSB (8 g per plant), *Trichoderma* (6 g per plant) and dried leaves, significantly enhanced various fruit characteristics such as length, weight, diameter, volume, specific gravity, juice content, pulp content and TSS (Total Soluble Solids). The finding highlighted that optimal nutrient availability, coupled with the beneficial effects of bio-fertilizers and organic mulch, led to superior fruit quality and higher yields. This study underscored the value of integrating bio-fertilizers and organic mulch in strawberry cultivation for sustainable and high-quality fruit production.

Keywords: biochemical attributes; bio-stimulants; organic mulching; physiological; strawberry yield

Introduction

Strawberries (*Fragaria × ananassa* Dutch.) were among the most widely cultivated soft fruits globally, celebrated for their exquisite taste and nutritional value. They were grown in over 75 countries and represented an economically potential important crop, particularly in regions such as Maharashtra in India, where their cultivation yielded high returns per unit area compared to other fruits (1). Strawberry fruit is predominantly composed of water (approximately 90 %) and is rich in essential nutrients like vitamins A and C, fiber and pectin, making it a low-calorie, highly nutritious food (2). Moreover, strawberries were known for their high content of ellagic acid, a plant phenol that demonstrated potential in inhibiting the development of cancer and asthma when consumed regularly (1). The cultivated strawberry was a hybrid of 2 distinct species, *Fragaria chilonensis* and *Fragaria virginiana* and belonged to the Rosaceae family with a chromosomal number of 2n = 56, indicating its octaploid nature (3). The cultivation of strawberries was concentrated in regions such as Maharashtra, Haryana, Punjab, Uttar Pradesh, Jammu & Kashmir, Uttarakhand and the lower slopes of Himachal Pradesh in India (4). Beyond fresh consumption, strawberry pulp was also utilized in the production of alcoholic beverages and fruit wines, with its succulent flesh contributing to its popularity in the beverage industry (5).

In recent years, there had been increasing interest in sustainable agricultural practices, mainly in the use of biofertilizers and organic mulches as viable alternatives to chemical fertilizers. Biofertilizers, which were natural products containing living microorganisms, enhanced plant growth and soil fertility without the environmental drawbacks associated with chemical fertilizers (6, 7). These microorganisms, either free-living in the soil or forming symbiotic relationships with plants, played a vital role in nutrient cycling by improving the availability of essential nutrients such as nitrogen and phosphorus.

The use of biofertilizers had been shown to significantly enhance the growth and productivity of horticultural crops. For example, a study reported a substantial increase in crop yield-ranging from 15 - 30 %, following biofertilizers application (7). This improvement was attributed to the production of hormones, vitamins and other growth-promoting substances by these microorganisms, which were essential for healthy plant development (8). Despite these benefits, conventional agricultural practices often relied on costly chemical fertilizers, which, although effective in the short term, pose several long-term challenges. The excessive use led to environmental pollution, soil degradation and reduced soil biodiversity (9–11). To mitigate these adverse effects, there was a growing emphasis on the use of biofertilizers and organic mulches, which not only reduced the reliance on chemical inputs but also enhanced the sustainability of crop production.

Bio-stimulants and organic mulches were particularly significant for their ability to enhance the physical, physico-chemical and yield attributes of strawberry plants. Bio-stimulants, comprising of a variety of organic materials, beneficial microbes and natural compounds, improved plant growth by enhancing nutrient uptake, stress tolerance and soil health. Meanwhile, organic mulches helped conserve soil moisture, suppress weeds and improve soil structure, thereby contributing to increased crop yields.

This study aimed to evaluate the effect of bio-stimulants and organic mulch on the physical, physico-chemical and yield parameters of the strawberry cultivar 'Katrain Sweet'. By considering previous research and integrating new findings, this study sought to provide a comprehensive understanding of how these sustainable practices could benefit strawberry cultivation and contribute to the broader field of horticultural science and sustainable agriculture.

Materials and Methods

The experiment was conducted in the garden of the Department of Fruit Science, Chandra Shekhar Azad University of Agriculture and Techology (CSAUA & T), Kanpur, Uttar Pradesh. Kanpur district, located at an elevation of 135 m above sea level, lay within a subtropical zone between 25.26° and 26.58° North latitude and 79.31° and 80.34° East longitude. The climate during winter was moderate -neither too extreme nor too mild. Between October and April, the temperature raged from17 - 34 ° C during day and 20 - 27 °C at night. The soil was characterized by medium level of phosphorous and potassium and low levels of nitrogen. The experiment followed a randomized block design (RBD) with 13 treatment combinations, each replicated 3 times to ensure statistical reliability. The treatments involved the use of 3 types of biofertilizers: *Azotobacter*, Phosphate Solubilizing Bacteria (PSB) and *Trichoderma*, which were applied in varying quantities. Organic mulches such as paddy straw and dried leaves were incorporated into the planting beds, along with 5 kg of farmyard manure (FYM) per bed. The rationale for selecting these specific treatments was based on their relevance to improving soil health and nutrient availability in cashew cultivation, with an emphasis on promoting sustainable agricultural practices.

Monthly meteorological data gathered during the experimental duration (October to April)

Source: Department of Agronomy, C.S.A.U.A.T., Kanpur, Uttar Pradesh

Month	Temperature (°C)				Relative Humidity (%)				Evaporation rate (mm/day)		Rainfall (mm)	
	Max.		Min.		Max.		Min.					
	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
October	30.8	32.9	19.3	18.2	90	82	58	44	83	104	221.6	19.1
November	28.5	28.1	12.9	13.1	88	93	44	53	71.2	37.9	0	2.8
December	23.5	23	8.8	9.7	92	93	51	58	69.6	37.2	0	15.6
January	18.9	16.6	7	6.6	94	95	65	72	43.8	34.9	11.2	11.2
February	28.2	24.5	11.3	10.4	89	87	50	48	50.2	38.8	0	27.4
March	30.7	29.8	15.7	14.5	88	76	56	40	76.2	70	24	32.2
April	35.2	38.8	19.1	21.4	57	47	30	22	102.4	111.8	47.8	2

Treatments Details

T_1- *Azotobacter* (5 g/ plant) + *Trichoderma* (5 g/ plant) + paddy straw,

T_2- *Azotobacter* (8 g/ plant) + *Trichoderma* (6 g/ plant) + paddy straw,

T_3- *Azotobacter* (5 g/ plant) + *Trichoderma* (5 g/ plant) + dried leaves,

T_4- *Azotobacter* (8 g/ plant) + *Trichoderma* (6 g/ plant) + dried leaves,

T_5- *Azotobacter* (5 g/ plant) + PSB (5 g/ plant) + paddy straw,

T_6- *Azotobacter* (8 g/ plant) + PSB (8 g/ plant) + paddy straw,

T_7- *Azotobacter* (5 g/ plant) + PSB (5 g/ plant) + dried leaves,

T_8- *Azotobacter* (8 g/ plant) + PSB (8 g/ plant) + dried leaves,

T_9- *Azotobacter* (5 g/ plant) + PSB (5 g/ plant) + *Trichoderma* (5 g/ plant) + paddy straw,

T_{10}- *Azotobacter* (8 g/ plant) + PSB (8 g/ plant) + *Trichoderma* (6 g/ plant) + paddy straw,

T_{11}- *Azotobacter* (5 g/ plant) + PSB (5 g/ plant) + *Trichoderma* (5 g/ plant) + dried leaves,

T_{12}- *Azotobacter* (8 g/ plant) + PSB (8 g/ plant) + *Trichoderma* (6 g/ plant) + dried leaves,

T_{13}- control group using 5 kg FYM per bed.

Each plant was provided with precise quantities of biofertilizers and organic mulches, ensuring uniformity across all treatment groups.

Morphological observation was recorded on various traits fruit length (cm), weight (g), diameter (mm), volume (cc) and specific gravity (g /cm^3), to assess the physical impact of the treatments. Quality attributes such as juice content (%), TSS content (°Brix), ascorbic acid levels (mg per 100 g), titratable acidity (%), pulp content (%), TSS: acid ratio, juice: pulp ratio, fruit pH, total sugars (%) and shelf life (days) at 25 °C were systematically evaluated. A composite soil sample was collected from the open field at a depth of 10 – 15 cm before the application of any treatments. This sample underwent mechanical, physical and chemical analysis to establish baseline soil conditions.

Runer plants of the 'Katrain Sweet' strawberry cultivar, sourced from the ICAR-NBPGR Regional Station in Bhowali, Nainital, Uttarakhand, India, were carefully planted in beds measuring 90 x 60 cm, with a spacing of 45 x 20 cm between plants. Fruit harvesting was performed when over 75 % of fruit surface exhibited coloration. Statistical analysis was conducted to ensure the reliability of the results, with appropriate procedures used to validate the significance of the treatment effects.

The data obtained during the study were subjected to through statistical evaluation. Treatment effects were analysed using the 'F' statistic to determine variance ratios, allowing us to distinguish between significant and non-significant results. Mean differences were tested against the critical difference at a 5 % significance level. To identify significant differences among the attributes, analysis of variance (ANOVA) was performed using XLSTAT 2014.5.03 Means comparison was conducted using Duncan's Multiple Range Test (DMRT) at $p < 0.05$ and principal component analysis (PCA) with correlation was also carried out (12). This statistical analysis ensured the accuracy and reliability of the results, with proper validation used to determine the significance of differences among treatments.

Results and Discussion

Morphological characters

In the present investigation the pooled data indicated that the highest fruit length of 3.61 cm was recorded in the treatment T_{12}. In contrast the lowest fruit length of 1.33 cm was observed in control treatment (T_{13}) as shown in Table 1. Fruit length was positively influenced by growth rate, which in turn was affected by nutrient availability and application of bio-fertilizer across different treatment combinations. Adequate nutrient supply boosted plant metabolism and yield, leading to increased protein and glucose production. The use of biofertilizer such as *Azotobacter* contributed to efficient allocation of photosynthate toward the sink, there by extending fruit life (13, 14).

It is observed that application of T_{12} resulted in the highest berry weight of 17.92 g, whereas, the control (T_{13}) recorded lowest berry weight of 8.62 g (15, 16). The highest berry weight throughout the experiment may have been due to the improved photosynthetic capacity induced by *Azotobacter* and other bio-stimulants. This likely accelerated the built up of dry matter. Berry weight was dependent on dry matter accumulation and hormone balance. Nitrogen fixers are noted for effectively transporting dry materials. Additionally, they promoted the synthesis of various growth regulators, as demonstrated (2).

The application of bio-fertilizers and mulching over the course of both experimental years significantly increased berry diameter. The data in Table 1 clearly showed that the plants under treatment T_{12} produced the largest berries, with diameter of 24.47 mm, consistently across both years. Conversely, the control group exhibited the smallest berry diameter of 15.25 mm. This increase in fruit diameter maybe related to improved photosynthetic efficiency, which likely led to increased dry matter accumulation and reduced weed competition (17, 18).

Table 1 also illustrated significant variations in fruit volume observed upon application of various types and combinations of biofertilizer to strawberry cv. Katrain sweet. The T_{12} treatment yielded the highest fruit volume at 21.96 cc. This treatment was statistically similar to T_{11} (*Azotobacter* (5 g per plant) + PSB (5 g per plant) + *Trichoderma* (5 g per plant) + dried leaves) which was recorded a fruit volume of 20.74 cc. In contrast, the control group (T_{13}) recorded the lowest values at 16.61 cc. Multi-inoculation may have enhanced nitrogen and phosphorus availability, resulting in larger berries. This effect may be attributed to nitrogen-fixing bacteria improving nitrogen fixation and phosphate-solubilizing bacteria increasing phosphorus availability (13). The combination of PSB and *Azotobacter* likely resulted in larger berries compared to their individual inoculation (17, 19, 20).

Data in Table 1 also indicated that T_{12} treatment resulted in highest specific gravity at 0.81 g per cc, followed by T_{10} with a values of 0.74 g per cc. In contrast, the untreated control plants (T_{13}) recorded the lowest specific gravity of 0.52 g per cc during both years of the experiment. Previous reports shows that *Trichoderma*, *Azotobacter* and Phosphate Solubilizing Bacteria (PSB) improved nutrient uptake and availability, enhancing photosynthesis and production of carbohydrate and other essential compound needed for fruit development and density

Table 1. Influence of bio-stimulants and organic mulch on the physical characters of strawberry fruit: Pooled data from 2022-23 and 2023-24

Treatment	Fruit length (cm)	Fruit weight (gram)	Fruit diameter (mm)	Fruit volume (cc)	Specific gravity (g/cc)
T_1	1.62 ± 0.02j	11.20 ± 067d	16.80 ± 0.09i	18.47 ± 0.14h	0.61 ± 0.03ef
T_2	1.69 ± 0.02j	11.39 ± 0.45d	17.63 ± 0.09h	19.23 ± 0.14ef	0.59 ± 0.02f
T_3	2.07 ± 0.02i	11.57 ± 0.50d	18.49 ± 0.09g	19.62 ± 0.15de	0.59 ± 0.03f
T_4	2.44 ± 0.02g	11.99 ± 0.39d	19.19 ± 0.09ef	19.02 ± 0.12fg	0.63 ± 0.02def
T_5	2.26 ± 0.02h	12.27 ± 0.35d	19.10 ± 0.09f	18.66 ± 0.09gh	0.66 ± 0.01cde
T_6	2.61 ± 0.02f	12.15 ± 0.27d	19.49 ± 0.09e	18.29 ± 0.12h	0.66 ± 0.01cde
T_7	2.72 ± 0.02e	11.44 ± 0.02d	19.29 ± 0.09ef	18.60 ± 0.17gh	0.61 ± 0.007ef
T_8	3.09 ± 0.02d	13.61 ± 0.18c	19.42 ± 0.09e	19.88 ± 0.14d	0.68 ± 0.009bcd
T_9	3.22 ± 0.02c	14.38 ± 0.02bc	20.87 ± 0.09d	20.36 ± 0.14c	0.70 ± 0.005bc
T_{10}	3.55 ± 0.02a	15.43 ± 0.45b	22.86 ± 0.09b	20.87 ± 0.14b	0.74 ± 0.02b
T_{11}	3.39 ± 0.02b	14.90 ± 0.30b	22.0 ± 0.09c	20.74 ± 0.14bc	0.72 ± 0.01bc
T_{12}	3.61 ± 0.02a	17.92 ± 0.10a	24.47 ± 0.09a	21.96 ± 0.14a	0.81 ± 0.005a
T_{13}	1.33 ± 0.04k	8.62 ± 0.29e	15.25 ± 0.13j	16.61 ± 0.27i	0.52 ± 0.01g

Where,

T_1 - *Azotobacter* (5 g/plant) + *Trichoderma* (5 g/plant) + paddy straw, T_2 - *Azotobacter* (8 g/plant) + *Trichoderma* (6 g/plant) + paddy straw, T_3 - *Azotobacter* (5 g/plant) + *Trichoderma* (5 g/plant) + dried leaves, T_4 - *Azotobacter* (8 g/plant) + *Trichoderma* (6 g/plant) + dried leaves, T_5 - *Azotobacter* (5 g/plant) + PSB (5 g/plant) + paddy straw, T_6 - *Azotobacter* (8 g/plant) + PSB (8 g/plant) + paddy straw, T_7 - *Azotobacter* (5 g/plant) + PSB (5 g/plant) + dried leaves, T_8 - *Azotobacter* (8 g/plant) + PSB (8 g/plant) + dried leaves, T_9 - *Azotobacter* (5 g/plant) + PSB (5 g/plant) + *Trichoderma* (5 g/plant) + paddy straw, T_{10} - *Azotobacter* (8 g/plant) + PSB (8 g/ plant) + *Trichoderma* (6 g/plant) + paddy straw, T_{11} - *Azotobacter* (5 g/plant) + PSB(5 g/plant) + *Trichoderma* (5 g/plant) + dried leaves, T_{12} - *Azotobacter* (8 g/plant) + PSB (8 g/plant) + *Trichoderma* (6 g/plant) + dried leaves, T_{13} - control.

(21). *Trichoderma* and PSB also enhance root development, which promote improved water and nutrient absorption. This helped fruit grow and may have increased their density and specific gravity (22, 23). Plants require *Azotobacter* for nitrogen fixation. Nitrogen is utilized for protein synthesis, enzyme activity and overall plant growth. The increased physiological activity enhanced plant development (3).

Biochemical content

As shown in Table 2, the highest value of 11.71 °Brix was recorded in Treatment T_{12}, while the lowest TSS of 7.65 °Brix was observed in control (T_{13}). The improved content of TSS may be attributed to enhanced nutrient absorption, particularly phosphorus, which plays a vital role in photosynthesis and energy transfer. *Trichoderma* used in the treatment, is known to facilitate phosphorus uptake, thereby contributing to better fruit quality. Enhanced photosynthesis led to greater carbohydrate and sugar production in fruit, which in turn increased TSS content, *Trichoderma* also contributed to better plant growth and fruit quality by improving environmental tolerance (24). *Azactobacter* increased nitrogen availability, which supported plant growth and increased chlorophyll content. This helped to improve photosynthesis and sugar accumulation in fruit. PSB promoted phosphorous uptake, improving root growth and overall plant development. Improved nutrient absorption through the combined action of biofertilizers resulted in higher TSS levels in the fruit (6). Working synergistically, these microorganisms enhanced vital physiological processes in the strawberry plants, leading to increased TSS content and improved fruit quality (25 – 27).

The acidity levels of the berries varied depending on the treatment applied. The control T_{13} recorded the highest titratable acidity of 1.66 %, whereas the plants treated in T_{12} combination showed the lowest acidity of 0.79 % (Table 2). Strawberry fruits harvested from mulched plants showed variation in titratable acidity, which may have been influenced by photo-assimilate translocation (1, 18, 28, 29).

The TSS: acid ratio of strawberry cultivar Katrain Sweet exhibited significant variations across different treatments, as detailed in Table 1. The highest TSS: acid ratio of 15.21 was recorded in treatment T_{12}, followed by a ratio of 13.01 in treatment T_{10}. Conversely, treatment T_{13}, which served as the control, demonstrated the lowest ratio at 4.62. Inoculating strawberries with free-living nitrogen-fixing bacteria alone or in combination increased the TSS: acid ratio and lowered the reducing sugars I cultivar Katrain Sweet. This may have been due to bio-inoculants' enhanced nutritional availability throughout growth. Bio-inoculants strengthened strawberry plants and increased assimilate synthesis. Photosynthesis boosted assimilation. Increased photosynthetic product mobility from leaves to developing fruits may have increased mature fruit TSS and total sugars (4). The loss of TSS: acid ratio in the control treatment may have been due to increased vegetative growth consuming most of the metabolites, leaving little for the berries (30).

As it is evident that the rate of juice content gets gradually declined (Table 2). In the combination of *Azotobacter* (8 g per plant) + PSB (8 g per plant) + *Trichoderma* (6 g per plant) + dried leaves produced the highest juice content of fruits at 66.06 % (Table 2). Treatment T_{10} followed closely, with juice content at 64.73 % respectively. In contrast, the control treatment (T_{13}) had the lowest juice content, measuring at 56.40 %. The association between fruit juice content and specific gravity was significant. *Trichoderma*, *Azotobacter* and PSB (Phosphate Solubilizing Bacteria) enhanced strawberry juice via improving plant physiology. *Trichoderma* species increased root surface area and solubilized soil phosphates to optimise phosphorus uptake (31). This improved fruit sugar synthesis and storage metabolism and nutrient absorption. Comparable research found that *Azotobacter* increased nitrogen and chlorophyll levels and also enhanced photosynthesis and fruit sugar content (26, 32). Research showed that specific bacteria increased phosphorus availability in fruits, which was crucial for energy metabolism and carbohydrate synthesis. This greatly

Table 2. Influence of bio-stimulants and organic mulch on the bio-chemical characters of strawberry fruit: Pooled data from 2022-23 and 2023-24

Treatment	TSS (°Brix)	Titratable acidity (%)	TSS: acid ratio	Juice content (%)	Ascorbic acid (mg/100 g)
T_1	10.05 ± 0.11de	1.30 ± 0.005d	7.83 ± 0.06i	60.68 ± 0.32f	51.17 ± 0.31e
T_2	10.11 ± 0.10de	1.375 ± 0.005c	7.33 ± 0.05i	61.38 ± 0.32def	52.81 ± 0.41d
T_3	10.29 ± 0.12cde	1.54 ± 0.005b	6.74 ± 0.07j	61.63 ± 0.32def	52.67 ± 0.41d
T_4	10.19 ± 0.06de	1.39 ± 0.005c	7.37 ± 0.05i	61.99 ± 0.32cdef	53.83 ± 0.41d
T_5	10.21 ± 0.05de	0.99 ± 0.005h	10.67 ± 0.09e	61.04 ± 0.32ef	53.38 ± 0.82d
T_6	10.02 ± 0.20e	1.11 ± 0.005f	9.37 ± 0.24g	61.9 ± 0.32cdef	53.72 ± 0.41d
T_7	10.17 ± 0.12de	1.04 ± 0.005g	10.10 ± 0.20f	62.84 ± 0.32bcde	53.65 ± 0.41d
T_8	10.35 ± 0.05cde	1.2 ± 0.005e	8.67 ± 0.05h	63.07 ± 0.32bcd	50.62 ± 0.41ef
T_9	10.57 ± 0.27cd	0.96 ± 0.005h	11.39 ± 0.21d	63.75 ± 0.32bc	54.11 ± 0.41d
T_{10}	11.11 ± 0.13b	0.87 ± 0.005i	13.01 ± 0.16b	64.73 ± 0.32ab	57.28 ± 0.56b
T_{11}	10.76 ± 0.24bc	0.89 ± 0.005i	12.36 ± 0.08c	64.02 ± 0.32b	55.52 ± 0.26c
T_{12}	11.71 ± 0.22a	0.79 ± 0.005j	15.21 ± 0.41a	66.06 ± 0.47a	59.25 ± 0.35a
T_{13}	7.65 ± 0.17f	1.66 ± 0.04a	4.64 ± 0.04k	56.40 ± 1.80g	49.69 ± 0.41f

T_1 - *Azotobacter* (5 g/plant) + *Trichoderma* (5 g/plant) + paddy straw, T_2 - *Azotobacter* (8 g/plant) + *Trichoderma* (6 g/plant) + paddy straw, T_3 - *Azotobacter* (5 g/plant) + *Trichoderma* (5 g/plant) + dried leaves, T_4 - *Azotobacter* (8 g/plant) + *Trichoderma* (6 g/plant) + dried leaves, T_5 - *Azotobacter* (5 g/plant) + PSB (5 g/plant) + paddy straw, T_6 - *Azotobacter* (8 g/plant) + PSB (8 g/plant) + paddy straw, T_7 - *Azotobacter* (5 g/plant) + PSB (5 g/plant) + dried leaves, T_8 - *Azotobacter* (8 g/plant) + PSB (8 g/plant) + dried leaves, T_9 - *Azotobacter* (5 g/plant) + PSB (5 g/plant) + *Trichoderma* (5 g/plant) + paddy straw, T_{10} - *Azotobacter* (8 g/plant) + PSB (8 g/ plant) + *Trichoderma* (6 g/plant) + paddy straw, T_{11} - *Azotobacter* (5 g/plant) + PSB (5 g/plant) + *Trichoderma* (5 g/plant) + dried leaves, T_{12} - *Azotobacter* (8 g/plant) + PSB (8 g/plant) + *Trichoderma* (6 g/plant) + dried leaves, T_{13} - control.

affected the juice content of these fruits (33). Strawberry plants utilized these bacteria to improve nutrient consumption and metabolic processes, increasing fruit juice content (34).

The data presented in Table 2 regarding biochemical traits showed significant influence on the ascorbic acid content. Berries produced by plants treated with the T_{12} combination showed the highest levels of ascorbic acid at 59.25 mg. In contrast, the control plants had the lowest levels of ascorbic acid at 49.69 mg (Table 2). Microbial inoculants' enhanced nitrogen fixation, phosphorous availability, growth-promoting substances that accelerated physiological processes like carbohydrate synthesis and reduced weed competition may have increased ascorbic acid content (8, 28, 35).

The documentation showed that the untreated fruits were less effective in terms of pulp content (Table 3), whereas a significant effect was observed in treated plants with the combined application of biofertilizers and organic mulch. The minimum pulp content was recorded in T_{12} at 33.93 % which included a combination of *Azotobacter,* PSB, *Trichoderma* and dried leaves. Simultaneously, maximum content of 43.59 % was observed in T_{13} (control) over both years of experimentation (36, 37).

A significant intensive effect was observed with the combined application of biofertilizer and organic mulching. The maximum juice to pulp content was recorded in the T_{12} combination at 1.96, which was closely on par with treatment T_{10} at 1.85, whereas the minimum ratio was observed in T_{13} at 1.31, based on the analysis from both years. *Azotobacter* improved nitrogen uptake, which increases pulp (cellular mass) and juice (cytoplasmic content). PSB enhanced phosphorus uptake, which supports energy-intensive activities like sugar synthesis and transport, thereby increasing juice content (36, 38).

Table 3 clearly demonstrated that the used of biofertilizer had a significant impact on the pH of the fruit, as observed across the different treatments. T_{12}, which involved the application of *Azotobacter,* PSB, *Trichoderma* and dried leaves at specific quantities per plant, had the highest recorded fruit pH values at 3.53. The lowest pH was observed with T_{13} (control), with recorded value of 2.57. *Azotobacter*, PSB and other biofertilizers assisted the host plants in getting essential nutrients from the rhizosphere, thereby enhancing plant growth (36). PSB effectively created and released organic acids that helped dissolve tri-calcium, iron and aluminium phosphates, making them more readily available to plant (39, 40). Phosphorus was essential for rapid plant development and disease resistance. PSB represented as the most efficient phosphate-solubilizing bacteria for increasing soil P_2O_5 availability. They grew rapidly in soil, generating a vast rhizosphere population. They acquired nutrients from soil and root exudates fixed atmospheric nitrogen. The nitrogen fixed by *Azotobacter* cells underwent nitrification during decomposition.

It was observed that the combined use of *Azotobacter*, PSB and *Trichoderma* significantly increased total sugar content of berries (Table 3). Berries produced from T_{12} test combination plants recorded the highest total sugar contents at 10.92 %, whereas the plants serving as the control group (T_{13}) had the lowest contents at 6.19 %. The increase in TSS and total sugar might have resulted in from the combined bio-stimulants, which possibly enhance the rapid metabolic conversion of starch and pectin into soluble compounds and improved sugar transport from leaves to developing fruits (1, 29, 41).

The 2-year analysis of data from Table 3 revealed that the shelf life of strawberries varied by treatment. Fruits from the treatment involving *Azotobacter* and PSB (each at 8 g per plant) and *Trichoderma* (6 g per plant) along with dried leaves,

Table 3. Influence of bio-stimulants and organic mulch on the bio-chemical characters of strawberry fruit: Pooled data from 2022-23 and 2023-24

Treatment	Pulp content (%)	Juice: pulp ratio	Fruit pH	Total sugars (%)	Shelf life (Days)
T_1	39.31 ± 0.32b	1.54 ± 0.02f	3.28 ± 0.03bc	8.75 ± 0.11e	1.45 ± 0.05e
T_2	38.61 ± 0.32bcd	1.60 ± 0.02ef	3.33 ± 0.07abc	8.74 ± 0.10e	1.59 ± 0.08de
T_3	38.37 ± 0.32bcd	1.61 ± 0.02def	3.25 ± 0.02bc	8.75 ± 0.12e	1.46 ± 0.03e
T_4	38.01 ± 0.32bcde	1.64 ± 0.02def	3.30 ± 0.05abc	8.79 ± 0.06e	1.70 ± 0.07d
T_5	38.95 ± 0.32bc	1.57 ± 0.02f	3.21 ± 0.02bc	9.22 ± 0.04de	2.29 ± 0.14c
T_6	38.1 ± 0.32bcde	1.62 ± 0.02def	3.28 ± 0.10bc	8.90 ± 0.20e	2.4 ± 0.13c
T_7	37.15 ± 0.32cdef	1.70 ± 0.02cde	3.24 ± 0.01bc	9.13 ± 0.13de	2.45 ± 0.07c
T_8	36.92 ± 0.32def	1.72 ± 0.02cd	3.34 ± 0.09abc	9.15 ± 0.04de	2.50 ± 0.08bc
T_9	36.25 ± 0.32ef	1.77 ± 0.02bc	3.39 ± 0.08abc	9.6 ± 0.26cd	2.69 ± 0.02ab
T_{10}	35.27 ± 0.32fg	1.85 ± 0.02b	3.48 ± 0.07ab	10.24 ± 0.13b	2.83 ± 0.02a
T_{11}	35.98 ± 0.32f	1.79 ± 0.02bc	3.43 ± 0.05abc	9.87 ± 0.24bc	2.74 ± 0.01a
T_{12}	33.93 ± 0.47g	1.96 ± 0.41a	3.53 ± 0.03a	10.92 ± 0.22a	2.91 ± 0.02a
T_{13}	43.59 ± 1.80a	1.31 ± 0.09g	2.57 ± 0.14d	6.19 ± 0.05f	1.2 ± 0.03f

Where,

T1 - *Azotobacter* (5 g/plant) + *Trichoderma* (5 g/plant) + paddy straw, T2- *Azotobacter* (8 g/plant) + *Trichoderma* (6 g/plant) + paddy straw, T3 - *Azotobacter* (5 g/plant) + *Trichoderma* (5 g/plant) + dried leaves, T4 - *Azotobacter* (8 g/plant) + *Trichoderma* (6 g/plant) + dried leaves, T5 - *Azotobacter* (5 g/plant) + PSB (5 g/plant) + paddy straw, T6 - *Azotobacter* (8 g/plant) + PSB (8 g/plant) + paddy straw, T7 - *Azotobacter* (5 g/ plant) + PSB (5 g/plant) + dried leaves, T8 - *Azotobacter* (8 g/plant) + PSB (8 g/plant) + dried leaves, T9 - *Azotobacter* (5 g/plant) + PSB (5 g/plant) + *Trichoderma* (5 g/plant) + paddy straw, T10 - *Azotobacter* (8 g/plant) + PSB (8 g/ plant) + *Trichoderma* (6 g/plant) + paddy straw, T11 - *Azotobacter* (5 g/plant) + PSB(5 g/plant) + *Trichoderma* (5 g/plant) + dried leaves, T12 - *Azotobacter* (8 g/plant) + PSB (8 g/plant) + *Trichoderma* (6 g/plant) + dried leaves, T13 - control.

had the longest shelf life of 2.91 days. In contrast, untreated control fruits exhibited the highest respiration rate and the shortest shelf life, lasting only 1.2 days (3, 42).

Multivariate analysis of vegetative and reproductive parameters

The structure of a dataset was studied using principal components analysis (PCA), a statistical method for multivariate analysis, in an effort to identify the processes influencing the scores of the variables present in the data. A number of linear combinations of observable variables were created using PCA and these linear combinations were referred to as components or factors. The variation patterns of bio-stimulants and organic mulch on physiological, biochemical and yield attributes of strawberry (*Fragaria* × *ananassa* Dutch.) cv. Katrain Sweet were analyzed using principal components (PCs). These factors served to condense the correlations present in the observed correlation matrix and had the capacity to accurately replicate duplicate the observed matrix (43). Out of 5 PCs observed in the study, only 2 were reported as significant, having Eigenvalues greater than 1 and together they contributed 93.95 % to the total variations. The remaining 3 PCs had Eigenvalues less than 1 and were considered non-significant for the study (Table 3). Fifteen parameters were scattered throughout a range of ordinates and the length of the vectors indicated the contribution of each character to the primary component and the quality of their representation.

From PCA biplot analysis, traits were grouped into main and subgroups based on their homogeneity and dissimilarity (Table 4). Eight sets of traits were identified and were represented by PC1 and PC2. Most of the traits namely- fruit volume, total sugar, fruit weight, total soluble solid (TSS), juice-to -pulp ratio, fruit pH, juice content (group II), ascorbic acid, fruit length, TSS to acid ratio and shelf life, fell under cluster II. However, titratable acidity, fruit diameter and specific gravity was categorized under group III. Fruit diameters were also classified under cluster IV (Fig. 1.).

Notably, the PCA biplot indicated that group II, I and III, which contributed significantly to PC1, were strongly associated with T_{12} combination, Traits correlated with group I, II and III contributes more to PC2. The length and intensity of colour of the vector in the biplot expressed how well the traits were represented and how much they contributed to the PCs. Group II contributed the most to PC1, followed by group I and group III. The parameters of group I, II, III and IV appeared to be independent, as indicated by the angles between vectors emanating from the centre of the biplot (Fig. 1).

Based on the PCA, it was concluded that the effects of bio -stimulants and organic mulch on physiological, biochemical and yield attributes of strawberry characters aligned with PC1, were effective for enhancing crop growth and development, as most of the associated traits exhibited positive correlations. PC2 contributed more toward traits classified in group I.

Analysis of traits associations for physiological, biochemical and yield attributes of strawberry

Pearson correlation coefficient analysis was performed for the pooled data to examine the association among the effect of bio-stimulants and organic mulch on physiological, biochemical and yield attributes of strawberry (Fig. 2). The result indicated that fruit length was positively and significantly correlated ($p < 0.01$) with fruit weight, fruit volume, TSS, juice content, TSS to acid ratio, ascorbic acid, juice to pulp ratio, total sugar and shelf life. Fruit pH also showed a positive correlation. Conversely, fruit diameter, specific gravity, titratable acidity and pulp content were negatively correlated ($p < 0.001$).

Fruit weight was positively correlated with fruit weight, fruit volume, TSS, juice content, TSS to acid ratio, ascorbic acid, juice: pulp ratio, total sugar and shelf life at $p < 0.01$ and negatively associated with fruit diameter, specific gravity, titratable acidity and pulp content at $p < 0.001$.

Fruit diameter was negatively correlated with most traits, except for specific gravity, titratable acidity and pulp content, which were positively correlated. In contrast, fruit weight, fruit volume, TSS, juice content, TSS: acid ratio, ascorbic acid, juice: pulp ratio, total sugar, shelf-life showed positive correlation ($p < 0.01$).

Table 4: The extracted eigenvalues and correlation (loading) values for physiological, biochemical and yield attributes of strawberry (*F. × ananassa* Dutch.) cv. Katrain Sweet, in response to bio-stimulants and organic mulch treatments, where analysed using Principal Component Analysis (PCA). The first 2 principal components account for 45 % of the total variability, effectively summarizing the major trends in the dataset

Variables	**Principles components**	
	PC1	**PC2**
Extracted Eigenvalues	13.189	0.903
Explained variance (%)	87.928	6.02
Cumulative variance (%)	87.93	93.95
Physiological, biochemical and yield attributes traits		
FL	0.926	-0.173
FW	0.979	0.093
FD	-0.918	0.109
FW.1	0.979	0.093
SG	-0.918	0.318
TSS	0.934	0.333
TA	-0.918	0.319
T. A	0.951	-0.242
JC	0.976	0.178
AA	0.874	-0.048
PC	-0.976	-0.177
JC.1	0.976	0.114
FP	0.879	0.424
TS	0.975	0.172
SL	0.874	-0.429

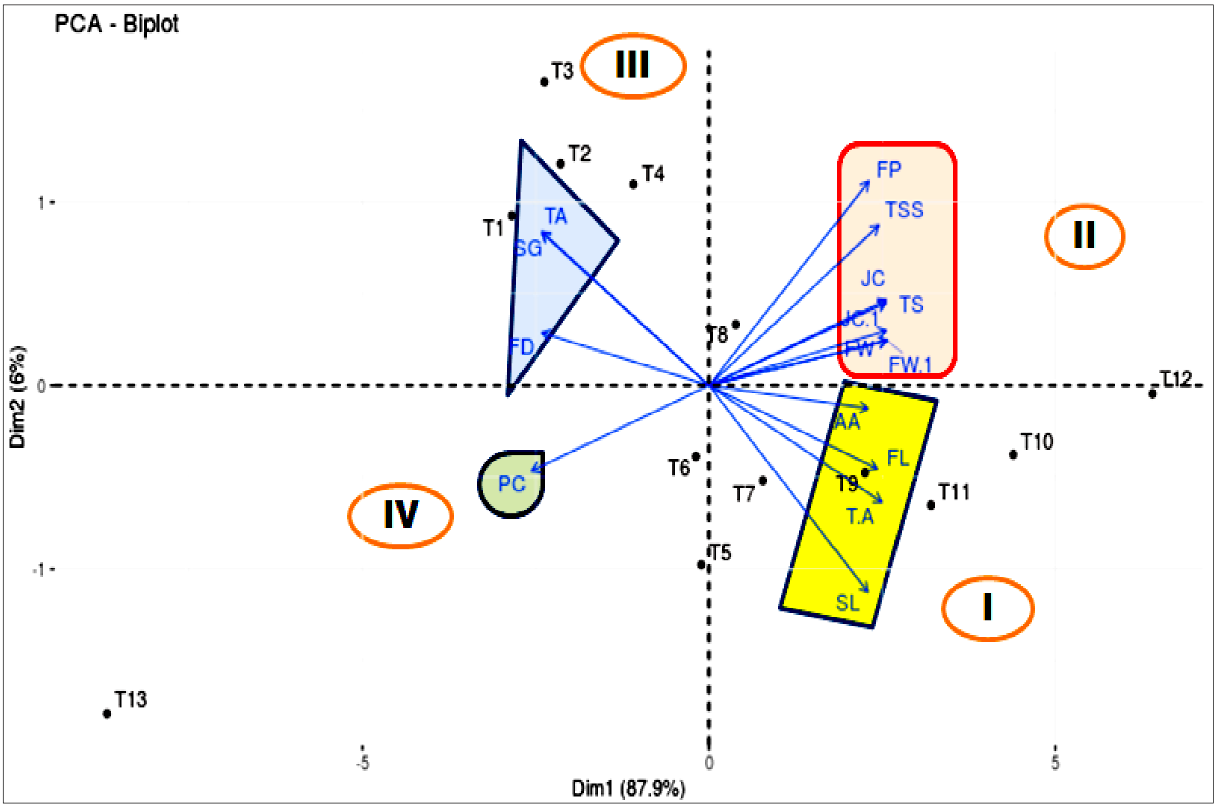

Fig. 1. Principal Component Analysis (PCA) of physiological, biochemical and yield attributes was conducted to assess the effect of bio-stimulants and organic mulch to strawberry (*F.* × *ananassa* Dutch.) cv. Katrain Sweet.

The analysis includes the key traits such as : fruit length (FL), fruit weight (FW), fruit diameter (FD) fruit volume (FW.1), specific gravity (SG), total soluble solid (TSS), titratable acidity (TA), TSS: acid ratio (TS), juice content (JC), ascorbic acid (AA), pulp content (PC), fruit pH (FP), Juice: pulp ratio (JC-1), total sugars (TS).

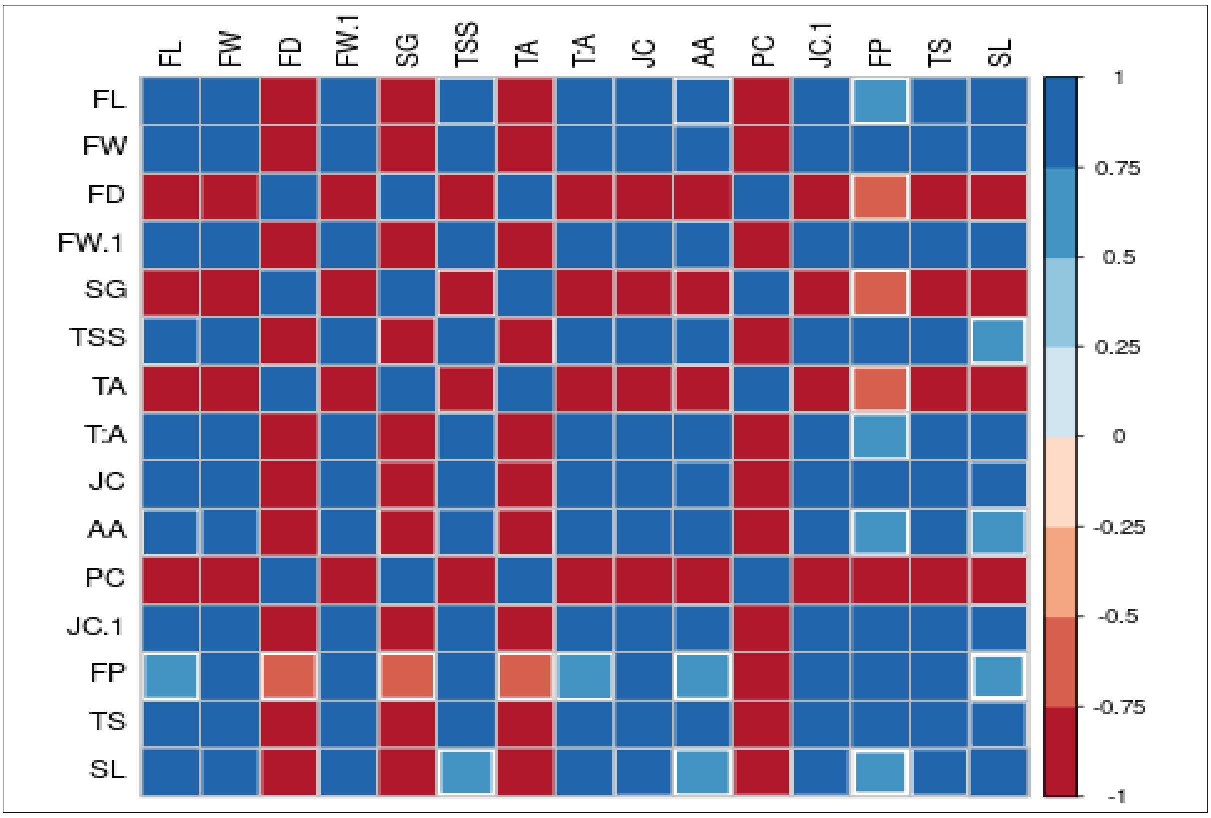

Fig. 2. Correlation analysis of biostimulants and organic mulch on physiological, biochemical and yield attributes of strawberry (*Fragaria x aanassa* Duch.) The analysis includes the key traits such as: fruit length (FL), fruit weight (FW), fruit diameter (FD) fruit volume (FW.1), specific gravity (SG), total soluble solid (TSS), titratable acidity (TA), TSS: acid ratio (TS), juice content (JC), ascorbic acid (AA), pulp content (PC), fruit pH (FP), Juice: pulp ratio (JC-1), total sugars (TS).

The manuscript includes an explicit data availability statement. The raw data are available upon request or have been deposited in a public repository at CSAUAT, Kanpur.

Fruit volume positively correlated with most parameters, except for fruit diameter, specific gravity, titratable acidity and pulp content, which showed significantly negative correlation ($p < 0.001$). Overall, the other traits considered in this study were positively and significantly correlated ($p < 0.001$).

Conclusion

This study confirmed that the combination of *Azotobacter* and PSB (each at 8 g per plant), *Trichoderma* (6 g per plant) and dried leaves (T_{12} treatment) significantly enhanced the physical qualities and nutritional value of strawberries. The application of these treatments resulted in increased fruit length, weight and diameter, reflecting improved growth. Additionally, it improved fruit quality, as indicated by a higher TSS to acid ratio, increased juice content and enhanced ascorbic acid levels, while maintaining appropriate pH levels. Although pulp content was lower, the higher juice-to-pulp ratio suggested an improvement in the fruit's juiciness.

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Authors' contributions

A conducted the experiment, curated the data, performed the formal analysis and wrote the original draft of the manuscript. VKT provided substantial guidance, supervised the research and facilitated the work by providing necessary laboratory facilities. He also contributed to data analysis, experimental guidance and manuscript drafting. Visualisation carried out by A and VKT. The writing review and editing were also done by A and VKT. Conceptualization, supervision, validation was also done by VKT, whereas data curation, formal analysis, methodology, writing the original draft was done by A. All authors reviewed and approved the final manuscript. The collaborative effort reflects the authors' commitment to producing a high- quality manuscript that accurately represents the research findings and contributes to the scientific community.

Compliance with ethical standards

Conflict of interest: All authors declare that they have no conflict of interest

Ethical issues: None

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