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RESEARCH ARTICLE

Influence on yield and quality traits of strawberry cv. Flamenco by nano chitosan, encapsulated PGPR bio capsules and *T. harzianum* bio capsules

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Abstract

The present investigation was conducted during 2023- 24 and 2024- 25 to evaluate the influence of nano chitosan, encapsulated Plant Growth- Promoting Rhizobacteria (PGPR) bio- capsules and *Trichoderma harzianum* bio- capsules on the quality and yield traits of strawberry (*Fragaria × ananassa* Duch.) cv. Flamenco, under subtropical conditions. Treatments comprising soil- drenching applications of Nano chitosan, PGPR and *T. harzianum* individually and in combinations demonstrated significant improvements in biochemical and yield parameters. The three- way combination treatment T_8- Nano chitosan 100 ppm + *T. harzianum* 200 ppm + PGPR 200 ppm recorded the highest enhancement in ascorbic acid (59.32 mg/100 g), total soluble solids (11.85°Brix), total sugar (9.64 %), TSS: acid ratio (13.31), total anthocyanin (31.10 mg/100 g), total flavonoids (33.92 mg/100 g) and total phenol content (77.04 mg GAE/100 g), accompanied by superior yield performance (18.19 fruits per plant, 199.99 g fruit yield per plant). PCA revealed that the first two components accounted for 98.88% of the total variation, with PC1 alone explaining 98.01%. PC1 was positively associated with total soluble solids (0.998), total sugars (0.997), sugar:acid ratio (0.999), total phenol content (0.979) and fruit yield per plant (0.998) and negatively associated with titratable acidity (-0.988) and ascorbic acid (-0.955). Treatment T8 exhibited the highest PC1 score, indicating its effectiveness in improving both biochemical quality and yield traits.

Keywords: bio- chemical attributes; *Fragaria × ananassa*; nano bio formulation; Principal Component Analysis; sustainable agriculture

Introduction

Strawberry (*Fragaria × ananassa* Duch.) is an economically significant and nutritionally rich fruit crop cultivated globally for its unique flavour, attractive colour and functional health properties. The genus *Fragaria*, under the family Rosaceae, comprises several species, with *F. × ananassa*, ($2n = 8x = 56$) were derived from *F. chiloensis* and *F. virginiana*, which is dominating in commercial cultivation worldwide (1). The fruit is a valuable source of minerals (0.49 g/1Wg), vitamin C (84.67 mg/100g), Carbohydrate (7.68 g/100g), organic acid (420 mg/100g), phenolics (*p*-coumaroyl hexose compound of Hydrooxycinnamic acids group) and anthocyanins (150- 600 mg/kg) and contributes significantly to human health and dietary diversity (2).

In India, despite its increasing popularity and diverse agro-climatic suitability, strawberry cultivation remains limited to niche pockets like Mahabaleshwar (Maharashtra), Nainital and Dehradun (Uttarakhand) and select regions of Himachal Pradesh, Karnataka and West Bengal (3). The crop's high perishability and challenges related to soil fertility, abiotic stress and disease susceptibility continue to constrain its widespread cultivation.

Enhancing fruit quality and yield by reducing chemical inputs has become a central goal in modern strawberry research. The integration of nano- biotechnological interventions, such as nano chitosan and encapsulated bio- inoculants like PGPR (Plant Growth- Promoting Rhizobacteria) and *Trichoderma harzianum*, offers an innovative solution to these challenges.

Chitin, a structural component of fungal cell walls and invertebrates exoskeleton, is recognized by the innate immune system of plants and animals. Chitosan, derived from the partial deacetylation of chitin, is a biodegradable, non- toxic biopolymer with antifungal and anti- bacterial properties. It disrupts the plasma membrane of pathogens, which is rich in saturated fatty acid and leads to oxidative damage. However, beneficial fungi like *Pochonia chlamydosporia* are resistant due to the saturated fatty acid- rich membrane. Chitosan elicits plant defence responses by inducing jasmonic acid (JA) and salicylic acid (SA) biosynthesis. Salicylic acid can be converted to methyl salicylate (MeSA), which enhances membrane permeability and activates systemic acquired resistance in distant and neighbouring plant tissues. Additionally, chitosan affect root membrane structure and function, influencing nutrient uptake and stress signalling. It also triggers membrane lipid modifications, leading to the production of defence- related signalling molecules like oxylipins and very long chain fatty acids, which are crucial for plant cuticle and sphingolipid biosynthesis involved in cell death regulation (4).

PGPR are beneficial soil microorganisms that colonize the rhizosphere and enhance plant growth through both direct and indirect mechanisms. They facilitate nutrient solubilization (e.g., phosphorus, potassium), nitrogen fixation and phytohormone (IAA, gibberellin) production, while also improving root development and stress tolerance. PGPR strains such as *Azotobacter*, Bacillus and Pseudomonas have been widely adopted in sustainable agriculture for their ability to reduce chemical input reliance and promote crop productivity (5-8).

Trichoderma is a genus of filamentous fungi widely recognized for its biocontrol properties and plant growth-promoting abilities. Predominantly soil- dwelling, *Trichoderma* species such as *T. harzianum*, *T. viride* and *T. asperellum* are effective antagonists against numerous plant pathogens through mechanisms including competition, mycoparasitism and antibiosis. In addition to suppressing fungal and nematode diseases, *Trichoderma* enhances nutrient uptake, stimulates systemic resistance and promotes root development, making it a key agent in sustainable agriculture (9).

Recent studies have demonstrated the nano chitosan helps plants defend themselves and take in nutrients, while microbial consortia improve soil health and hormone balance. Together, they work together to increase yield and stress resistance in improving fruit biochemical composition, marketable yield and postharvest shelf life in strawberry (10, 11). However, systematic research under north Indian agro- climatic conditions for evaluating the synergistic potential of these components on strawberry quality is still limited.

Hence, the present investigation was undertaken to evaluate the "Influence on yield and quality traits of strawberry cv. Flamenco by nano chitosan, encapsulated PGPR Bio- capsules and *T. harzianum* Bio- capsules". This study aims to elucidate the role of nano- bio formulations in enhancing strawberry's physiological, morphological and biochemical traits under subtropical field conditions in North India.

Materials and Methods

The experiments were conducted over two consecutive growing seasons (2023- 24 and 2024- 25) in the Garden, Department of Fruit Science, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India. The site is situated in a subtropical zone at an elevation of 135 meters above mean sea level, positioned between 25.26° and 26.58° North latitude and 79.31° and 80.34° East longitude. The area falls under a humid subtropical climate zone, experiencing temperature ranging from 2 to 45 °C. Rainfall is primarily received during the monsoon months (June - September), averaging between 850 and 1000 mm annually. The soil at the experimental site is sandy loam in texture, with a neutral to slightly alkaline reaction (pH 6.5- 7.4), moderate levels of organic carbon (0.85- 1.45 %) and electrical conductivity ranging from 0.45 to 0.78 dS/m. It also maintains a balanced nutrient profile, with available nitrogen (208- 298 kg/ha), phosphorus (12- 46 kg/ha) and potassium (104- 250 kg/ha). The present investigation comprised ten different treatment combinations involving soil- drenching with nano chitosan, Plant Growth Promoting Rhizobacteria (PGPR) bio- capsules and *Trichoderma harzianum* (MTCC- 5179) bio- capsules, either individually or in various combinations and concentrations including one control are given below:

Treatment	Treatment Description
T_1	Control (Water spray)
T_2	Nano chitosan @ 500 ppm
T_3	PGPR bio- capsules @ 500 ppm
T_4	*Trichoderma harzianum* (MTCC- 5179) bio- capsules @ 500 ppm
T_5	Nano chitosan @ 250 ppm + *T. harzianum* bio- capsules @ 250 ppm
T_6	*T. harzianum* bio- capsules @ 250 ppm + PGPR bio- capsules @ 250 ppm
T_7	Nano chitosan @ 250 ppm + PGPR bio- capsules @ 250 ppm
T_8	Nano chitosan @ 100 ppm + *T. harzianum* bio- capsules @ 200 ppm + PGPR bio- capsules @ 200 ppm
T_9	*T. harzianum* bio- capsules @ 100 ppm + Nano chitosan @ 200 ppm + PGPR bio- capsules @ 200 ppm
T_{10}	PGPR bio- capsules @ 100 ppm + *T. harzianum* bio- capsules @ 200 ppm + Nano chitosan @ 200 ppm

Planting material source

One- year- old runners of strawberry (*Fragaria × ananassa* Duch.) cv. Flamenco was brought from ICAR- National Bureau of Plant Genetic Resources (NBPGR), substation Bhowali, Uttrakhand. Growers prefer Flamenco, a late season ever bearing strawberry, specially for pick your own (PYO) options. Dr David Simpson bred this variety at East smelling research to deliver high yields and excellent fruit quality. Flamenco plants, flower in spring and produce fruit from March until the first frost, they yield medium to large, uniformly conical berries with a glossy orange, red appearance, the fruit offer a firm, texture, strong skin and rich flavour. Growers consistently achieve over 1 kg of fruit per plant with about 55% of berries exceeding 35 mm in diameter. Additionally, flamenco resist *Verticilium wilt* effectively.

Field preparation

The experimental field was deep ploughed using a Disc harrow one week before transplanting and plants were planted at a spacing of 45x30 cm. The recommended dose of NPK at a ratio of 100:60:140 kg/ha was applied as a basal dose along with FYM. Subsequent doses were applied as per the treatment requirements.

Nano chitosan and bioformulation preparation

Nano chitosan was procured from the Nano Research Facility, Haryana and prepared using 1 % acetic acid and ethanol as a solvent carrier. Bio- capsules of *T. harzianum* and PGPR (N Fixers, P solubilisers, mobilisers) were sourced from CADAGU Agritech Pvt. Ltd., Bengaluru and patented by ICAR- Indian Institute of Spices Research (IISR), Kozhikode, Kerala. Each bio-capsule was formulated to inoculate 100 kg of farmyard manure and contained an effective concentration of viable microbial spores or cells. For use, one capsule was first dissolved in 1 L of luke worm water and incubated for 24 hrs, then thoroughly mixed with 100 L of water for field application applied in three split doses corresponding to the seedling, vegetative and reproductive stages of crop growth.

Application schedule

The first application was done immediately after transplanting, followed by three more applications at 20- day intervals during early vegetative growth to ensure optimum colonization and physiological response.

Observations recorded

The following traits were recorded during the study:

Quality attributes

Ascorbic acid content (mg/100 g), Total Soluble Solids (°Brix), titratable acidity (%), total sugar (%), TSS: acid ratio, sugar: acid ratio, total anthocyanin (mg/100 g), fruit pH, juice content (%), pulp content (%), total flavonoids (mg/100 g), total phenol content (mg GAE/100 g).

Yield attributes

Number of fruits per plant, fruit yield per plant (g).

Statistical analysis

Experimental design

The experiment was laid out in a Randomized Block Design (RBD) with three replications to minimize variability and ensure reliable comparisons among treatments.

Significance testing

Data were subjected to statistical analysis following Fisher's analysis of variance (ANOVA) method (12). Treatment effects were tested using the F- test and Critical Differences (C.D.) at a 5 % probability level were computed to assess the significance between treatment means.

Multivariate analysis

To examine interrelationships among traits and treatments, PCA was performed using XLSTAT software. This helped identify major contributing variables and treatment groupings across principal axes.

Results and Discussion

Quality attribute

Ascorbic acid (mg/100 g)

As data presented in Table 1, treatment T_8 demonstrated a statistically significant ($p < 0.05$) elevation in ascorbic acid content, recording 58.89 mg/100 g (2023- 24), 59.74 mg/100 g (2024- 25) and 59.32 mg/100 g (pooled), followed by T_9 and T_{10} with pooled values of 56.45 mg/100 g and 54.31 mg/100 g, respectively. Conversely, the lowest ascorbic acid content was observed in plants under T_1- Control (46.41 mg/100 g in pooled). The superior performance of plants in T_8 may be attributed to enhanced physiological activity and induced systemic resistance. PGPR improves vitamin C content by modulating rhizosphere conditions and enhancing antioxidant enzymes in strawberry (10), while biocapsule treatments upregulate ascorbate- related genes (11). Increased ascorbic acid content due to bio- fertilizers was also reported (13, 14). Nano chitosan enhances micronutrient uptake and GLDH pathway activity in apple (15), improves ROS detoxification and stimulates ascorbate biosynthesis under microbial consortia (16, 17).

Total soluble solids (°Brix)

The data given in Table 1 indicated that T_8treatment led to a significant ($p < 0.05$) enhancement in TSS, achieving 11.84 °Brix (2023- 24), 11.86 °Brix (2024- 25) and 11.85 °Brix (pooled) followed by $T_9$and T_{10}, whichrecorded pooled TSS values of 11.41 °Brix and 10.77 °Brix, respectively. Control (T_1) registered the minimum TSS (7.50°Brix pooled). The significant increase under T_8 reflects improved sugar accumulation due to enhanced photosynthesis and assimilate partitioning. Nano chitosan delays senescence aiding prolonged sugar translocation in strawberry (18). *Azotobacter* and PSB application increased TSS contents by accelerating metabolic transformation in tissue cultured banana (19). Bio- capsules and nano- treatments improve nutrient uptake, chlorophyll content and reduce transpiration loss in strawberry (17, 20). Hormonal regulation and enhanced nitrate reductase activity contributed to °Brix improvement in strawberry (11, 21).

Titratable acidity (%)

As shown in Table 1, the lowest titratable acidity was attained under T_8, maintaining a significant ($p < 0.05$) value of 0.89 % across 2023-24, 2024-25 and in pooled analysis. $T_9$and T_{10} recorded pooled acidity levels of 0.91 % and 0.93 %, respectively, whereas the maximum acidity was observed in T_1 (1.02 % pooled). The marked reduction under T_8 indicates a more favorable sugar: acid balance and improved palatability. *T. harzianum* mitigates acid accumulation by modulating TCA cycle enzymes in banana and strawberry respectively (19, 22) and PGPR lowers acidity by improving nutrient uptake and altering organic acid biosynthesis in isabgol (23), banana (24, 25) and in strawberry (26). Trichoderma and organic bio capsule treatments further enhanced flavour in strawberry (27, 28).

Total sugar (%)

Treatment T_8, as reflected in Table 1, recorded a highly appreciable gain in total sugar content, measuring 9.18 % (2023- 24), 10.10 % (2024- 25) and 9.64 % pooled, followed by T_9 and T_{10}, with 9.32 % and 8.80 % pooled sugar contents, respectively. The lowest total sugar was found under T_1 (5.72 % pooled). The significant at $p < 0.05$ enhancement in T_8suggests the synergistic role of nano chitosan and microbial bio- formulations in promoting sugar biosynthesis and accumulation. PGPR improves nitrogen

Table 1. Influence of nano bioformulation treatments on biochemical attributes of strawberry (*Fragaria* × *ananassa*) cv. "Flamenco"

Treatment Notation	Ascorbic acid (mg/100 g)			TSS (°Bx)			Titratable acidity (%)			Total sugar (%)		
	2023- 24	2024- 25	Pooled	2023- 24	2024- 25	Pooled	2023- 24	2024- 25	Pooled	2023- 24	2024- 25	Pooled
T_1	46.28	46.54	46.41	7.53	7.47	7.50	1.01	1.03	1.02	5.78	5.65	5.72
T_2	49.35	49.57	49.46	7.99	7.97	7.98	1.02	1.01	1.01	6.29	6.15	6.22
T_3	51.45	53.12	52.29	8.59	8.42	8.50	0.98	0.98	0.98	6.66	6.75	6.71
T_4	51.01	50.01	50.51	9.02	8.94	8.98	0.97	0.98	0.98	7.02	7.19	7.11
T_5	52.57	55.48	54.03	10.34	10.44	10.39	0.95	0.94	0.95	8.09	8.68	8.39
T_6	51.52	52.35	51.94	9.98	9.87	9.93	0.97	0.95	0.96	7.79	8.16	7.98
T_7	51.41	53.44	52.43	9.31	9.42	9.36	0.98	0.97	0.98	7.46	7.61	7.53
T_8	58.89	59.74	59.32	11.84	11.86	11.85	0.89	0.89	0.89	9.18	10.10	9.64
T_9	56.14	56.75	56.45	11.44	11.38	11.41	0.91	0.91	0.91	8.92	9.72	9.32
T_{10}	54.39	54.23	54.31	10.80	10.75	10.77	0.93	0.93	0.93	8.57	9.03	8.80
F test	S	S	S	S	S	S	S	S	S	S	S	S
S.Ed. (±)	0.67	2.26	1.20	0.11	0.07	0.05	0.02	0.01	0.01	0.07	0.08	0.05
C.D. at 5% level	1.40	4.75	2.53	0.22	0.16	0.11	0.04	0.03	0.03	0.15	0.16	0.10

assimilation and activates sugar- metabolizing enzymes in strawberry (29) and banana (24, 25). Nano chitosan prolongs photosynthetic duration, facilitating greater carbohydrate accumulation in strawberry (18). PGPR and *T. harzianum* bio capsules enhance assimilate translocation in strawberry (11), while nano- carriers boost potassium uptake critical for sugar transport in apple (15). Microbial consortia improve enzymatic activity and hormonal regulation in *Fragaria* (17, 18, 30).

TSS: acid ratio

The maximum TSS: acid ratio was observed under T_8, recording 13.35 (2023- 24), 13.27 (2024- 25) and 13.31 pooled, indicating a pronounced superiority in flavor quality (Table 2). $T_9$and T_{10} also achieved pooled ratios of 12.50 and 11.61, respectively. The control (T_1) had the lowest TSS: acid ratio (7.36 pooled) (Table 2). The increase in the TSS: acid ratio under T_8 indicates an improved sugar- acid balance, enhancing fruit flavour. PGPR formulations influence organic acid metabolism and sugar accumulation in strawberry (13, 29 31). *T. harzianum* modulates TCA cycle enzymes to enhance sweetness in strawberry (32). Organic bio-capsules improve °Brix- to- acid ratio in strawberry (28). Microbial treatments increase the expression of sugar transporter proteins, facilitating sugar dominance in kinnow (33).

Sugar: acid ratio

The maximum sugar: acid ratio was recorded under T_8, with values of 10.31 (2023- 24), 11.35 (2024- 25) and 10.83 pooled, highlighting its superior impact on flavor quality (Table 2). Treatments T_9 and T_{10} also showed favorable results, with pooled ratios of 10.02 and 9.45, respectively, while the control (T_1) recorded the lowest (5.61) (Table 2). Such improvements may be attributed to the role of PGPR in boosting sugar levels while modulating organic acid content through rhizosphere-mediated biochemical changes. *Trichoderma harzianum* is also known to influence acidity by altering the activity of TCA cycle enzymes and improving rhizospheric dynamics, which has been observed in crops such as banana (24) and strawberry (34). Moreover, combined applications of microbial and organic inputs have been linked to enhanced sugar: acid balance in strawberry (29). Nano-chitosan has similarly been found to promote sugar accumulation by stimulating sugar transporter genes and downregulating organic acid biosynthesis pathways, as demonstrated in peach (35) and strawberry (36).

Anthocyanin content (mg/100 g)

As presented in Table 2, the treatment T_8 exhibited a statistically significant ($p < 0.05$) superiority in total anthocyanin content, recording 29.82 mg/100 g during 2023- 24, 32.39 mg/100 g during 2024- 25 and a pooled mean of 31.10 mg/100 g. It was closely followed by T_9 (28.82 mg/100 g, 29.55 mg/100 g and 29.19 mg/100 g pooled) and T_{10} (27.08 mg/100 g, 27.26 mg/100 g, 27.17 mg/100 g pooled). The increase in anthocyanin content under T_8 indicates enhanced pigmentation and antioxidant properties. *T. harzianum* stimulates phenylpropanoid metabolism boosting anthocyanin accumulation (37). PGPR and *T. harzianum* interactions improved colour and antioxidant profiles in strawberry (38). Nano chitosan induces PAL and CHS gene expression critical for flavonoid biosynthesis (39). Secondary metabolites were significantly upregulated in nano- treated in basil (40).

Fruit Ph

As per the findings summarized in Table 2, the maximum fruit pH was recorded under T_8, measuring 3.72 (2023- 24), 3.68 (2024 - 25) and 3.70 pooled followed by in T_9 and T_{10} (pooled pH values of 3.64 and 3.59, respectively). The minimum pH was observed under T_1 (3.22 pooled). The increase in fruit pH under T_8 was statistically significant ($p < 0.05$), indicating reduced organic acid accumulation. PGPR reduces acid- synthesizing enzyme activity in strawberry (29). Bio- formulations buffer internal acidity by improving ionic homeostasis (41). *T. harzianum* improves vacuolar pH through potassium uptake in strawberry (28). Microbial inoculants modify acid homeostasis favouring neutral metabolites in isabgol (23). Bioinoculant treatments suppressed citric acid, elevating pH in strawberry (34).

Juice content (%)

As reflected in Table 3, T_8 recorded the highest juice content, achieving 72.53% (2023- 24), 70.53% (2024- 25) and 71.53% pooled. This was followed by T_9 (70.22% pooled) and T_{10} (68.50% pooled). The minimum juice content was recorded under T_1 (60.72% pooled). The increase in juice content under T_8 was statistically significant ($p < 0.05$), likely due to enhanced water uptake, improved membrane fluidity and osmotic balance. PGPR induces aquaporin activation improving cellular hydration in strawberry (16). Nano chitosan maintains turgor and delays cell wall collapse in strawberry (18). Environmental and agronomic inputs stimulate flavonoid biosynthesis (42). Nano chitosan boosts flavonoids by enhancing antioxidant enzyme expression (43). Chitosan stimulates defence responses enhancing fruit's phenolic and flavonoid content (44). PGPR and *T. harzianum* activate plant secondary metabolism pathways in strawberry (45) and citrus (46).

Table 2. Influence of nano bioformulation treatments on biochemical attributes of strawberry (*Fragaria* × *ananassa*) cv. "Flamenco

Treatment Notation	TSS: acid ratio			Sugar: acid ratio			Total Anthocyanin (mg/100g)			Fruit pH		
	2023- 24	2024- 25	Pooled	2023- 24	2024- 25	Pooled	2023- 24	2024- 25	Pooled	2023- 24	2024- 25	Pooled
T_1	7.44	7.27	7.36	5.72	5.49	5.61	18.61	18.77	18.69	3.20	3.25	3.22
T_2	7.84	7.92	7.88	6.17	6.09	6.16	21.01	21.41	21.21	3.25	3.29	3.27
T_3	8.77	8.62	8.70	6.8	6.89	6.85	19.52	21.80	20.66	3.30	3.32	3.31
T_4	9.27	9.16	9.21	7.24	7.34	7.26	23.24	24.47	23.86	3.37	3.36	3.36
T_5	10.85	11.15	11.00	8.52	9.23	8.83	29.56	24.39	26.98	3.52	3.48	3.50
T_6	10.33	10.40	10.36	8.03	8.59	8.31	26.37	27.43	26.90	3.48	3.44	3.46
T_7	9.50	9.68	9.59	7.61	7.85	7.68	24.36	26.47	25.42	3.42	3.41	3.42
T_8	13.35	13.27	13.31	10.31	11.35	10.83	29.82	32.39	31.10	3.72	3.68	3.70
T_9	12.54	12.46	12.50	9.81	10.68	10.24	28.82	29.55	29.19	3.69	3.60	3.64
T_{10}	11.61	11.61	11.61	9.22	9.71	9.46	27.08	27.26	27.17	3.61	3.57	3.59
F test	S	S	S	S	S	S	S	S	S	S	S	S
S.Ed. (±)	0.27	0.20	0.19	0.18	0.12	0.10	1.78	1.48	1.10	0.01	0.01	0.01
C.D. at 5% level	0.56	0.42	0.40	0.37	0.31	0.28	3.74	3.11	2.31	0.03	0.03	0.02

Table 3. Assessment of nano chitosan, PGPR bio- capsules and *Trichoderma harzianum* on enhancing fruit quality traits in strawberry (*Fragaria × ananassa*) cv. "Flamenco"

Treatment Notation	Juice content (%)			Pulp content (%)			Total Flavonoids (mg/100g)		
	2023- 24	2024- 25	Pooled	2023- 24	2024- 25	Pooled	2023- 24	2024- 25	Pooled
T_1	60.58	60.87	60.72	29.63	24.76	27.19	25.37	23.38	24.38
T_2	60.42	60.22	60.32	26.02	25.77	25.90	22.19	27.55	24.87
T_3	60.89	63.69	62.29	31.94	27.17	29.56	23.84	28.66	26.25
T_4	63.12	61.95	62.54	26.81	28.53	27.67	28.03	24.90	26.46
T_5	67.25	67.85	67.55	28.87	33.62	31.25	27.86	29.28	28.57
T_6	66.02	63.42	64.72	33.14	32.79	32.96	30.89	30.27	30.58
T_7	65.63	65.03	65.33	28.40	29.98	29.19	29.64	27.29	28.47
T_8	72.53	70.53	71.53	36.86	37.00	36.93	33.21	34.63	33.92
T_9	70.19	70.24	70.22	33.79	32.92	33.36	32.62	31.43	32.03
T_{10}	68.87	68.13	68.50	34.48	33.67	34.07	27.03	32.58	29.81
F test	S	S	S	S	S	S	S	S	S
S.Ed. (±)	1.56	2.52	1.76	2.10	2.16	1.57	1.97	1.54	1.25
C.D. at 5% level	3.28	5.30	3.70	4.42	4.53	3.30	4.14	3.24	2.64

Nano biological consortia stimulate root hydraulic conductivity (11). Microbial treatments sustain cell water potential and improve strawberries' internal water- solute balance in strawberry (10).

Pulp content (%)

The data shown in Table 3 revealed that T_8 significantly ($p < 0.05$) enhanced pulp content, recording 36.86 % (2023- 24), 37.00 % (2024- 25) and 36.93 % pooled closely followed by T_9 with a pooled pulp content of 33.36 % and T_{10} recorded 34.07 % pooled. In contrast, T_1 had the lowest pulp content (27.19 % pooled). T_8 significantly improved pulp content, indicating increased dry matter accumulation. Microbial consortia promote calcium and boron uptake, strengthening cellular structure (29). *T. harzianum* improves cation absorption (6). Nano chitosan supports cellulose biosynthesis and membrane integrity in HRMN apple (15). Chitosan reduces water loss, increasing the pulp- to- juice ratio (26). PGPR and nano elicitors enhance carbohydrate partitioning into structural tissues (25).

Total flavonoids (mg/100 g)

As evident from Table 3, T_8 recorded the highest total flavonoid content, with values of 33.21 mg/100 g (2023- 24), 34.63 mg/100 g (2024- 25) and 33.92 mg/100 g pooled followed by T_9 and T_{10}, with pooled flavonoid contents of 32.03 mg/100 g and 29.81 mg/100 g, respectively. Control (T_1) registered the minimum flavonoid content (23.88 mg/100 g pooled). Fruits of treatment T_8 have high flavonoid concentrations, likely resulting from stress priming and microbial elicitor effects.

Total Phenol Content (mg GAE/100 g)

The data from Table 4 showed that the maximum total phenol content was recorded under T_8, measuring 78.85 mg GAE/100 g (2023- 24), 75.23 mg GAE/100 g (2024- 25) and 77.04 mg GAE/100 g pooled closely followed by T_9 (73.86 mg GAE/100 g pooled) and T_{10} (75.16 mg GAE/100 g pooled). The minimum phenol content was observed under T_1 (65.81 mg GAE/100 g pooled). The increased phenol content observed under T_8 may be attributed to the activation of the phenylpropanoid pathway, a key biosynthetic route for phenolic compounds. PGPR inoculation is known to trigger PAL (phenylalanine ammonia-lyase) gene expression, thereby enhancing systemic resistance and antioxidant capacity (29). Nano chitosan enhances systemic resistance and antioxidant compounds in peach (43). *T. harzianum* treatments increased flavonoid and phenol synthesis in strawberry (36, 37). Nano chitosan upregulates ROS- scavenging genes (38). Nano- and bio - formulations synergistically enhance phenolic richness in strawberry (10).

Yield attributes

Number of fruits per plant

As presented in Table 4, the maximum number of fruits per plant was recorded under T_8, attaining 18.89 fruits in 2023- 24, 17.48 fruits in 2024- 25 and a pooled mean of 18.19 fruits. It was closely followed by T_9 (17.40, 16.07, 16.74 pooled) and T_{10} (15.91, 14.61, 15.26 pooled). Conversely, the control T_1 recorded the minimum number of fruits (5.50, 4.51, 5.00). The increase in the number of fruits per plant under T_8 was likely due to enhanced flowering, pollination efficiency and fruit set mediated by microbial modulation of phytohormones. PGPR improved fruit sets via nutrient bioavailability and hormonal stimulation in strawberry (7, 41 and 47). PGPR consortia activate auxin signalling genes enhancing floral architecture (21). Biofertilizer- treated strawberries showed higher flowering density (48). Encapsulated PGPR and *T. harzianum* improved reproductive sink strength in strawberry (49). *Trichoderma*- based bio capsules significantly increased fruit numbers under polyhouse conditions (50).

Table 4. Assessment of nano chitosan, PGPR bio- capsules and *Trichoderma harzianum* on enhancing fruit yield traits in strawberry (*Fragaria × ananassa*) cv. "Flamenco

Treatment Notation	Total Phenol Content (mg GAE/100g)			Number of fruits per plant			Fruit yield per plant (g)		
	2023- 24	2024- 25	Pooled	2023- 24	2024- 25	Pooled	2023- 24	2024- 25	Pooled
T_1	66.80	64.82	65.81	5.50	4.51	5.00	79.97	77.47	78.72
T_2	65.91	66.76	66.33	7.00	5.93	6.46	94.44	89.99	92.22
T_3	68.84	71.80	70.32	8.48	7.43	7.95	108.91	102.51	105.71
T_4	69.18	66.65	67.92	9.97	8.80	9.39	123.34	115.02	119.18
T_5	69.56	71.25	70.41	14.44	13.18	13.81	166.65	152.51	159.58
T_6	70.37	70.55	70.46	12.93	11.74	12.34	152.24	140.02	146.13
T_7	73.23	68.49	70.86	11.47	10.26	10.87	137.77	127.49	132.63
T_8	78.85	75.23	77.04	18.89	17.48	18.19	209.99	189.99	199.99
T_9	74.24	73.48	73.86	17.40	16.07	16.74	195.59	177.52	186.55
T_{10}	74.19	76.13	75.16	15.91	14.61	15.26	181.09	164.99	173.04
F test	S	S	S	S	S	S	S	S	S
S.Ed. (±)	2.03	1.78	1.30	0.03	0.02	0.02	0.02	0.03	0.01
C.D. at 5% level	4.27	3.73	2.72	0.06	0.04	0.03	0.05	0.07	0.03

Fruit yield per plant (g)

According to Table 4, T_8 demonstrated a highly substantial increase in fruit yield per plant, registering 209.99 g (2023- 24), 189.99 g (2024- 25) and a pooled yield of 199.99 g followed by T_9 with 195.59 g, 177.52 g and 186.55 g pooled and T_{10} with 181.09 g, 164.99 g and 173.04 g pooled. In contrast, the lowest yield per plant was observed under T_1 (79.97 g, 77.47 g and 78.72 g pooled). Yield boost in T_8 is attributed to improvements in vegetative growth, fruit number, weight and quality traits like TSS and ascorbic acid. PGPR and *T. harzianum* enhance nutrient mobilization, antioxidant metabolism and stress regulation leading to sustained yield. Similar yield increases with bio-capsules have been observed in strawberry (10, 11), while the synergy between Nano chitosan and microbes in improving productivity was noted in capegooseberry (51), peach (43). *T. harzianum* enhances vascular efficiency (52). These results are consistent with those reported in Isabgol (Plantago ovata) (23) and strawberry (29), papaya (53) following the application of *Azotobacter* and PSB.

Principal Component Analysis (PCA)

PCA was conducted to examine the multivariate relationships among fruit quality and yield attributes under different treatments. The first two principal components (F1 and F2) together accounted for 98.88 % of the total variation, with PC1 alone contributing 98.01 % and PC2 contributing 0.87 % (Table 5). This indicates that most of the trait variation was effectively captured by the first component, allowing reliable two-dimensional visualization. The biplot (Fig. 1) revealed clear differentiation among treatments based on their association with key traits. PC1 was positively associated with total soluble solids (TSS), total sugars (TS), sugar: acid ratio (SAR), TSS: acid ratio (TAR), fruit pH (FP), juice content (JC), pulp content (PC), total flavonoids (TF), total phenol content (TPC), number of fruits per plant (NFPP) and fruit yield per plant (FYPP). These traits showed strong positive loadings (>0.97) on PC1, indicating that treatments on the positive side of this axis were associated with greater sweetness, nutritional content and productivity. Conversely, titratable acidity (TA) and ascorbic acid (AA) showed negative loadings, suggesting that treatments with negative PC1 scores were associated with higher acidity and vitamin C content but lower overall sweetness and yield.

PC2 contributed marginally to trait separation but distinguished treatments with relatively higher total anthocyanin content (TAC) and TA, which showed slight positive loadings, whereas AA showed a notable negative association with this component. Thus, PC2 helped to explain limited variability related to antioxidant and acidity parameters. Among the treatments, T8 was positioned farthest on the positive side of PC1, indicating its superiority in terms of sugar accumulation, phenolic content and yield attributes. In contrast, T_3 and T_2, located on the negative side of PC1, were associated with higher acidity and ascorbic acid content but lower sweetness and yield. Treatments such as T_5, T_6 and T_{10} clustered near the center-right of the biplot, indicating moderate to good performance across multiple traits. Meanwhile, T_1, T_4 and T_7 showed intermediate profiles, suggesting a balance between quality and yield traits. Overall, the PCA highlighted strong correlations among sweetness-related attributes and yield, while traits like acidity and antioxidants showed independent patterns of variation. These findings underscore the effectiveness of PCA in identifying superior treatment combinations, with T_8 emerging as the most promising in terms of both quality and yield performance.

Correlation analysis

The Pearson correlation matrix among the measured quality and yield attributes of cape gooseberry revealed several highly significant associations (Fig. 2). Total soluble solids (TSS) showed strong positive correlations with total sugars ($r > 0.98$), sugar: acid ratio (SAR), TSS: acid ratio (TAR), juice content, phenolic traits (total flavonoids and total phenols) and yield components (number of fruits per plant and fruit yield per plant). These relationships indicate that enhanced sweetness and biochemical richness are closely linked with improved fruit productivity. In contrast, titratable acidity (TA) exhibited strong negative correlations with nearly all other traits, particularly TSS ($r < -0.95$), SAR, TAR and yield attributes. This suggests that higher acidity is associated with reduced sugar accumulation and lower yield, confirming its antagonistic role in determining fruit palatability and market quality. Juice content and fruit pH were also positively associated with sweetness and phenolic composition, further supporting their physiological coordination during fruit ripening. The uniformly strong positive correlations observed among most traits reflect a coordinated enhancement in quality and yield under effective treatments, while the negative associations with acidity highlight the importance of its modulation for overall improvement in fruit traits.

Table 5. PCA factor loadings for quality traits

	F1	F2	F3	F4	F5
Ascorbic acid (mg/100 g)	0.955	- 0.268	- 0.120	0.034	0.005
TSS (°Bx)	0.998	0.043	- 0.006	- 0.018	- 0.019
Titratable acidity (%)	- 0.988	0.108	- 0.073	0.009	0.068
TSS: acid ratio	0.999	- 0.001	0.022	- 0.018	- 0.024
Total sugar (%)	0.997	0.047	- 0.010	- 0.016	- 0.011
Sugar: acid ratio	0.999	0.002	0.022	- 0.017	- 0.016
Total Anthocyanin (mg/100g)	0.973	0.159	- 0.130	0.103	0.002
Fruit pH	0.997	0.029	0.042	- 0.006	0.025
Juice content (%)	0.993	0.005	0.021	- 0.098	0.062
Pulp content (%)	0.990	0.034	- 0.034	- 0.032	- 0.059
Total Flavonoids (mg/100g)	0.994	- 0.033	- 0.013	- 0.019	0.087
Total Phenol Content (mg GAE/100g)	0.979	- 0.023	0.135	0.145	0.025
Number of fruits per plant	0.998	0.052	- 0.004	- 0.022	- 0.004
Fruit yield per plant (g)	0.998	0.052	- 0.003	- 0.021	- 0.004
Eigenvalue	13.722	0.122	0.060	0.046	0.022
Variability (%)	98.012	0.869	0.425	0.328	0.157
Cumulative (%)	98.012	98.882	99.307	99.635	99.792

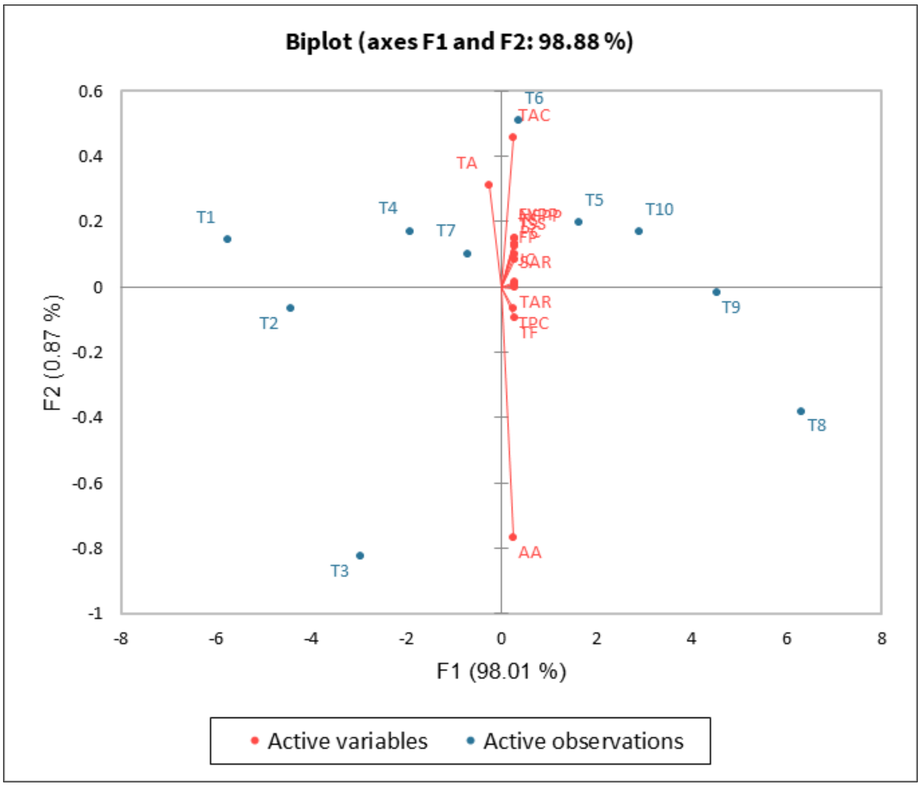

Fig. 1. PCA biplot displaying the relationships between treatments and measured traits of strawberry. The first two principal components (PC1 and PC2) accounted for 98.88 % of the total variance (98.01 % and 0.87 %, respectively). Vectors represent the direction and contribution of each fruit quality and yield trait, while the spatial distribution of treatments indicates their multivariate association with these traits.

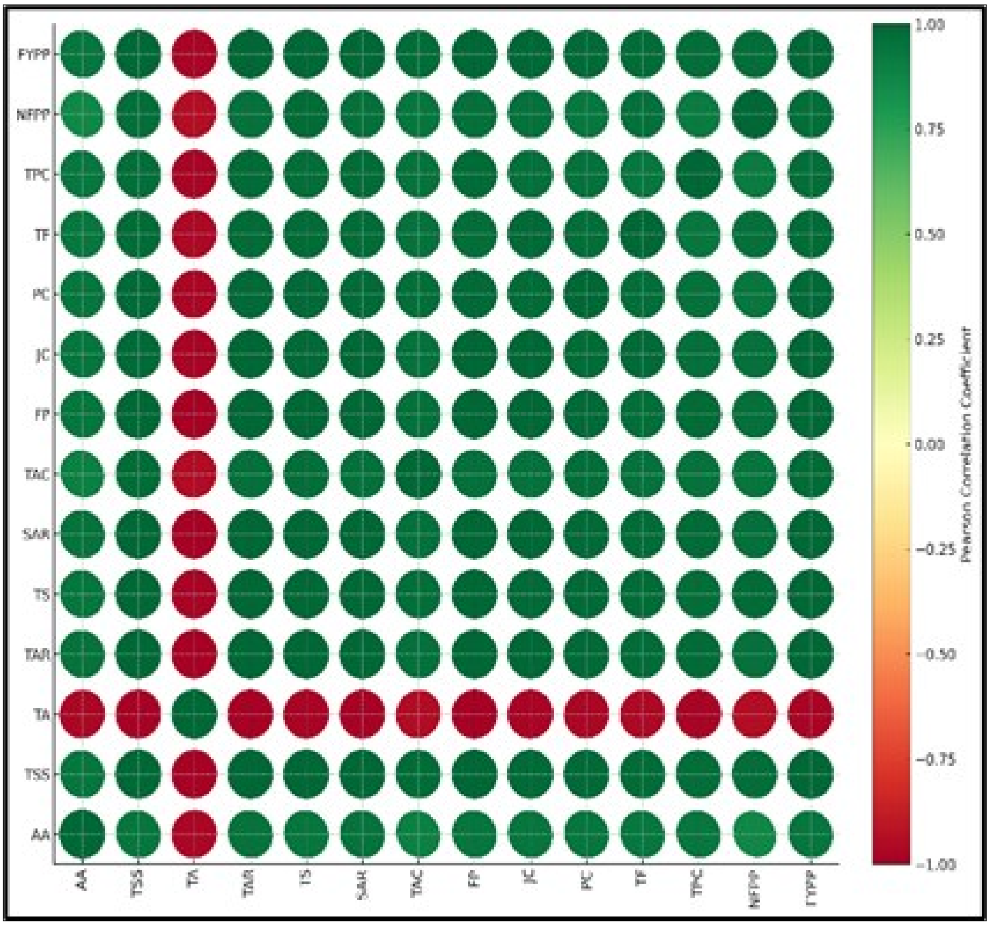

Fig. 2. Pearson's correlation coefficients between the measured fruit quality and yield attributes. Ellipse color and shape represent the strength and direction of correlation. Green ellipses indicate positive correlations, red ellipses indicate negative correlations and circle size reflects the magnitude. Trait abbreviations: AA - Ascorbic acid content (mg/100 g), TSS - Total soluble solids (°Brix), TA - Titratable acidity (%), TAR - TSS: acid ratio, TS - Total sugars (%), SAR - Sugar: acid ratio, TAC - Total anthocyanin (mg/100 g), FP - Fruit pH, JC - Juice content (%), PC - Pulp content (%), TF - Total flavonoids (mg/100 g), TPC - Total phenol content (mg GAE/100 g), NFPP - Number of fruits per plant, FYPP - Fruit yield per plant (g).

Conclusion

The present study demonstrated that the integrated application of nano chitosan (100 ppm), encapsulated Plant Growth-Promoting Rhizobacteria (PGPR, 200 ppm) and *Trichoderma harzianum* bio-capsules (200 ppm) significantly improved fruit quality and yield traits of strawberry (*Fragaria* × *ananassa* Duch.) cv. Flamenco under subtropical field conditions in the North Indian plains. Among the ten treatments evaluated, the three-way combination (T_8) consistently outperformed all others, exhibiting maximum enhancement in ascorbic acid, total soluble solids, total sugars, anthocyanin, flavonoid and phenolic content, along with increased fruit number (18.19) and fruit yield per plant (199.99 g). Multivariate analysis confirmed that PC1, accounting for 98.01 % of the total variation, was positively associated with sweetness, phenolic richness and yield-related traits, while titratable acidity and ascorbic acid showed negative loadings. Correlation analysis further supported strong positive linkages between sugar-related attributes and yield and inverse associations with acidity, suggesting improved organoleptic quality and market potential under T_8. The synergistic effects observed under T_8 can be attributed to the physiological roles of each component: Nano chitosan enhanced membrane permeability, antioxidant response and nutrient uptake; PGPR improved root architecture, nitrogen assimilation and hormonal regulation; while T. harzianum contributed to systemic resistance and organic acid modulation. Together, these inputs triggered coordinated metabolic and biochemical responses favouring both fruit nutritional quality and productivity.

These findings highlight the potential of nano-biotechnological formulations as a sustainable alternative to conventional agrochemicals. The adoption of such integrated approaches can improve yield, enhance fruit quality and support eco-friendly practices in commercial strawberry cultivation.

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Authors' contributions

JC conducted the experiment, carried out the investigation, performed the formal analysis, curated the data and drafted the original manuscript. VKT provided substantial guidance, supervised the study, facilitated the research by offering necessary laboratory facilities, assisted with data analysis, contributed to the methodology and participated in reviewing and editing the manuscript. Both JC and VKT contributed to the visualization of the data. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no competing interests.

Ethical issues: None

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