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RESEARCH ARTICLE

Improving strawberry 'Flamenco' performance with nano-chitosan, PGPR and *Trichoderma harzianum* bio-capsules

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Abstract

A two-year study (2023-24 and 2024-25) assessed the effect of nano-chitosan, plant growth-promoting rhizobacteria (PGPR) and *Trichoderma harzianum* bio-capsule, individually and in combination on the growth, morphology and yield of strawberry (*Fragaria* × *ananassa* Duch.) cv. Flamenco in a subtropical environment. Among the treatments, T_8 (nano-chitosan 100 ppm + *Trichoderma harzianum* (MTCC-5179) bio-capsule 200 ppm + PGPR Bio-capsule 200 ppm) was found to be significantly superior, achieving plant height (18.59 cm), leaves (20.68 plant^{-1}), leaf area (87.74 cm^2), runners (7.20 plant^{-1}), crowns (2.78 plant^{-1}), flowers (18.22 plant^{-1}), fruit volume (19.16 cm^3), diameter (43.81 mm), weight (18.55 g), length (56.95 mm), specific gravity (0.98 g cm^{-3}), shelf-life (2.61 days) and yield (22.22 t ha^{-1}) ($p \leq 0.05$). The plants in this treatment exhibited a shorter time to bloom, with the first flowering observed on day 57.25 and fruit set (6.14 days after bloom) occurred soon after flowering. The PCA showed that the first principal component (PC1) explained 96.21 % of the variance integrating vegetative, reproductive and yield traits while the results of the Pearson correlation showed a strong positive association ($r > 0.97$) of the yield with morphological and fruit traits and a strong negative association ($r < -0.96$) with flowering delays. Concurrent application of nano-chitosan, PGPR and *T. harzianum* bio capsule has been established as an effective technology for strawberry vegetation, productivity, quality and postharvest performance under subtropical conditions.

Keywords: *Fragaria* × *ananassa* Duch.; nano-chitosan; PGPR bio-capsule; sustainable strawberry production; *Trichoderma harzianum* bio-capsule

Introduction

The strawberry (*Fragaria* × *ananassa* Duch.) is an herbaceous plant and is a hybrid of *F. virginiana* which is native to North America and *F. chiloensis* from Chile, which was formed through a natural hybridization and belongs to the family Rosaceae. After going through the process of double octoploid (2n = 8x = 56), the plant was formed. Other earlier cultivated types were *F. vesca*, *F. moschata* and *F. viridis* which had practical or decorative value for their users. The United States, China and Spain were frontrunners in global strawberry production in the year 2021, with a total of 9.17 million metric tons produced. Strawberries are cultivated in India on over 3031 ha, with a production of around 19840 MT, mainly in Mahabaleshwar, Nainital, Dehradun, Kashmir and Kalimpong (1). They are grown in the regions with the temperate, subtropical and high-altitude tropical climates due to their adaptability. For several reasons (high water content, soft fruit tissue, high respiration rate etc.), the fruit has short shelf life.

Nutritionally, strawberries are made up of about 91 % water and provide only 32 kcal per 100 g, making them a very light and hydrating fruit. They are a good source of vitamin C (58.8 mg) and essential minerals such as potassium (153 mg) and calcium (16 mg), which contribute both flavor and nutritional value, as well as phenolic antioxidants like pelargonidin 3-O-glucoside, which is the main anthocyanin that gives the red color and is also associated with other health benefits (2-4). A total of 100 g of the fresh product contains only 24 calories and is eaten fresh or transformed into juices, jams, yogurts and confectionery (5). Sandy or sandy-loam soils with pH 5.5-6.5 are the best. These soils should have good drainage and balanced nutrients levels (6). Light, temperature (10 °C-26 °C) and photoperiod (> 14 hr) are the factors that affect the growth (7). The main way of propagation is by stolons; runner plants are kept in a storage at -2 °C before they are planted. Strawberry farming is affected by pests (aphids, thrips, whiteflies), diseases (anthracnose, powdery mildew) and abiotic stresses.

To overcome these limitations, sustainable strategies such as integrated pest management, organic farming and high-tech tools such as nano-chitosan, PGPR and *T. harzianum* are being employed. Nano-chitosan is a polymer derived from chitin and it is a biodegradable, biocompatible and bioactive polymer which improves soil health by enhancing the microbial diversity, nutrient retention and stability of the organic matter. It also contains necessary macro- and micronutrients and indirectly, through the stimulation of photosynthesis, hormone balance, antioxidant enzymes and stress-tolerance pathways, promotes plant growth. As an antimicrobial agent, it disrupts pathogen membranes, complexes with metal ions, binds nucleic acids and induces systemic plant defense via PR proteins, lignin synthesis, ROS signaling and secondary metabolites. Nano-chitosan is a flexible delivery system that not only allows the

controlled application of agrochemicals but also increases their bioavailability and decreases pesticide toxicity and environmental residues. It is used all along the crop cycle—in the seed coating, soil amendment, foliar spraying and post-harvest coating and thus increases yields, disease resistance, food quality and sustainability (8). PGPR are plant-associated microbes, including *Bacillus* and *Pseudomonas* spp., which fix the nitrogen and make it available to plants (9). *T. harzianum* is a fungus that can increase nutrient absorption, root growth and systemic resistance (10). The use of nanotechnology not only helps with the transport of nutrients but also manages diseases and pests (11).

The main objective of this research was to determine the most effective approaches that promote the growth, physical traits and fruit quality of strawberry with sustainable production through assessing shelf life under ambient conditions.

Materials and Methods

The field trials were conducted at the Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh, India, for two consecutive cropping seasons, 2023-24 and 2024-25. The experimental site was in the humid subtropical agro-climatic zone, 135 m above sea level, between 25.26° N and 26.58° N latitude and 79.31° E and 80.34° E longitude. This region has a clear seasonal shift, with temperatures ranging from 2 °C in the winter season to 45 °C in the summer season. The major amount of rainfall occurs during the southwest monsoon (June-September) and the total annual precipitation varies from 850 to 1000 mm. The experimental field soil is classified as sandy loam and has a slightly alkaline to neutral pH reaction (6.5-7.4), moderate organic carbon content (0.85 %-1.45 %) and electrical conductivity that dissolves nutrients, takes nitrogen from the air and produces phytohormones within the range of 0.45-0.78 dS/m. The soil fertility is moderate for nitrogen, phosphorus and potassium, at 208-298 kg/ha, 12-46 kg/ha and 104-250 kg/ha, respectively.

The current research consisted of ten different treatment combinations using soil drenching with nano-chitosan, PGPR and *T. harzianum* (MTCC- 5179) bio-capsule, either independently or in different combinations and concentrations. One control was also included. The details are provided in Table 1.

Planting material

One-year-old strawberry runners (*Fragaria* × *ananassa* Duch.) cultivar Flamenco was procured from the ICAR- National Bureau of Plant Genetic Resources (NBPGR), Substation Bhowali, Uttarakhand. Cultivators choose Flamenco, a late-season ever-bearing strawberry, particularly for pick-your-own (PYO) opportunities. Dr. David Simpson developed this variety at East Malling Research to achieve large yields and superior fruit quality. Flamenco bushes bloom in spring and bear fruit from March until the initial frost, yielding medium to large, consistently conical berries with a lustrous orange-red hue. The fruit possess a firm texture, robust skin and rich flavor. Producers routinely attain over 1 kg of fruit per plant, with over 55 % of berries measuring above 35 mm in diameter. Furthermore, Flamenco has effective resistance to *Verticillium* wilt.

Field preparation

The experimental field was tilled with a disc harrow a week before transplanting and the plants were spaced at 45 × 30 cm. The basal NPK dose was 100:60:140 kg/ha, supplemented with FYM. Subsequent doses were applied as per the treatment requirements.

Nano-chitosan and bioformulation preparation

Nano-chitosan was obtained from the Nano Research Facility, Haryana and prepared using 1 % acetic acid and ethanol as the solvent carrier. PGPR bio-capsule (nitrogen fixers, phosphorus solubilizes and mobilizers) and *T. harzianum* bio-capsule was procured from CADAGU Agritech. Pvt. Ltd., Bangalore and are patented by ICAR-Indian Institute of Spices Research (IISR), Kozhikode, Kerala. Each bio-capsule was formulated with a viable concentration of microbial spores or cells, sufficient for inoculating 100 kg of farmyard manure (FYM). The first one was dissolved in 1 L of warm water and incubated for 24 hr. They were thereafter mixed with 100 L of water for field application, applied in three split doses at sowing, vegetative and reproductive phases of the crop.

Application schedule

The application schedule consisted of an initial application immediately following transplant, followed by three more applications at 20 days intervals during the early stages of vegetative growth for colonization and physiological response optimization.

Observations recorded

The noted characteristics were classified into four sections. The growth traits which include plant height, number of leaves, leaf area, number of runners, crowns and number of flowers. The indicators that related to the phenological traits was days it took to first flowering and how many days there were from flowering to fruit set. The fruit physical traits included fruit volume, diameter, weight, specific gravity, length and shelf life, while those yield traits were total yield per hectare.

Table 1. Treatment combinations

Treatments	Description
T_1	Control (water spray)
T_2	Nano-chitosan 500 ppm (soil drenching)
T_3	PGPR bio-capsule 500 ppm (soil drenching)
T_4	*T. harzianum* (MTCC-5179) bio-capsule 500 ppm
T_5	Nano-chitosan 250 ppm + *T. harzianum* (MTCC-5179) bio-capsule 250 ppm
T_6	*T. harzianum* (MTCC-5179) bio-capsule 250 ppm + PGPR bio-capsule 250 ppm
T_7	Nano-chitosan 250 ppm + PGPR bio-capsule 250 ppm
T_8	Nano-chitosan 100 ppm + *T. harzianum* (MTCC-5179) bio-capsule 200 ppm + PGPR bio-capsule 200 ppm
T_9	*T. harzianum* (MTCC-5179) Bbio-capsule 100 ppm + nano-chitosan 200 ppm + PGPR bio-capsule 200 ppm
T_{10}	PGPR bio-capsule 100 ppm + *T. harzianum* (MTCC-5179) bio-capsule 200 ppm + nano-chitosan 200 ppm

Statistical analysis

Experimental design

The experiment was designed with three replications based on a randomized block design (RBD) to reduce error and ensure statistical significance among the treatments.

Significance Testing

Data were analysed by an AOAC analysis of variance (ANOVA) test (12). The significance between treatment means was assessed at the 5 % probability level by calculating critical differences (CD) using F-test.

Multivariate analysis

Plots of the traits with and without treatment, as well as data arcs, are given. Principal component analysis using XLSTAT software was performed to analyse interrelationships among traits with treatment. Thus, it also identified the main factors of variation and groups of treatments along the principal axes.

Results

Vegetative growth

In both seasons, the simultaneous use of nano-chitosan (100 ppm) + *T. harzianum* (200 ppm) + PGPR bio-capsule (200 ppm) (T_8) was the treatment showed the highest vegetative growth, consistently, followed by T_9 (*T. harzianum* (MTCC-5179) bio-capsule 100 ppm + nano-chitosan 200 ppm + PGPR bio-capsule 200 ppm), whereas T_1 (control) attained the lowest vegetative growth. In 2023-24, the height of the plants was 18.17 cm in T_8 and 17.98 cm in T_9, compared with 12.89 cm in T_1; in 2024-25, the respective values were 19.01, 18.08 and 12.02 cm, with pooled means of 18.59, 18.03 and 12.45 cm ($p \leq 0.05$) (Table 2; Fig. 1 & 2).

The same trend was observed in leaf production, as T_8 produced 19.99 and 21.37 leaves per plant over the two years, T_9 produced 18.76 and 19.96 and T_1 only 10.04 and 10.81 leaves per plant (Table 2, Fig. 1 & 2). The pooled means were 20.68 for T_8, 19.36 for T_9 and 10.43 for T_1 leaves ($p \leq 0.05$). The maximum leaf area was reported for T_8, 95.56 cm^2 and 79.92 cm^2, followed by T_9, while T_1 recorded 50.10 cm^2 and 49.56 cm^2. The pooled means were 87.74, 79.15 and 49.83 cm^2, respectively ($p \leq 0.05$).

Runner and crown production

Maximum runner production was obtained in plants receiving T_8, which produced 7.23 and 7.16 runners per plant in 2023-24 and 2024-25 respectively, followed by T_9, with 5.89 and 6.91 and T_1 with 2.86 and 2.91. The pooled means were 7.20, 6.88 and 2.89, respectively ($p \leq 0.05$).

As for crowns per plant, T_8 was found to be the most appropriate and effective treatment, producing 2.78 consistently, while T_9 produced 2.69 and 2.68 and T_1 produced 1.78 and 1.81. The pooled means of 2.78, 2.69 and 1.80, respectively ($p \leq 0.05$).

Phenology

In the 2023-24 experiment, the first flower initiation was observed in treatment T_8 at 57.21 days, followed by T_9 at 57.34 days, whereas T_1 required 65.65 days (Table 3; Fig. 1 & 2). For the experiment of 2024-2025, flowering commenced at 55.71 days in T_8, 57.15 days in T_9 and 65.49 days in T_1, resulting in pooled means of 56.46, 57.25 and 65.57 days ($p < 0.05$).

In the 2023–24 experiment documented, the number of flowers per plant was the highest in T_8 (18.97 flowers/plant), followed by T_9 (17.63 flowers/plant), while the minimum was observed in T_1 (7.00 flowers/plant). In the 2024–25 experiment, T_8 (17.46 flowers/plant) again recorded the highest flower count, closely followed by T_9 (16.22 flowers/plant), whereas T_1

Table 2. Effect of different treatments on various vegetative growth attributes of strawberry cv. Flamenco

Treatments	Plant height (cm)			Number of leaves per plant			Leaf area (cm^2)			Runners per plant		
	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled
T_1	12.89	12.02	12.45	10.04	10.81	10.43	50.1	49.56	49.83	2.86	2.91	2.89
T_2	13.59	12.6	13.09	11.08	12.19	11.64	69.1	53.42	61.26	3.72	3.69	3.7
T_3	14.11	13.46	13.78	12.33	13.21	12.77	71.74	56.48	64.11	3.76	3.83	3.8
T_4	14.92	14.24	14.58	13.08	14.57	13.83	72.37	59.59	65.98	3.81	3.75	3.78
T_5	16.48	16.72	16.6	16.46	17.42	16.94	77.33	70.14	73.74	5.55	5.63	5.59
T_6	16.17	15.94	16.06	15.33	16.39	15.86	76.1	66.73	71.42	5.65	5.61	5.63
T_7	15.34	15.01	15.18	14.26	15.69	14.98	73.53	63.43	68.48	4.56	4.65	4.6
T_8	18.17	19.01	18.59	19.99	21.37	20.68	95.56	79.92	87.74	7.23	7.16	7.2
T_9	17.98	18.08	18.03	18.76	19.96	19.36	81.17	77.12	79.15	6.85	6.91	6.88
T_{10}	17.41	17.62	17.52	17.53	19.1	18.32	80	73.3	76.65	6.7	6.65	6.68
F test	S	S	S	S	S	S	S	S	S	S	S	S
S.Ed. (±)	0.13	0.14	0.08	0.28	0.27	0.16	0.21	0.35	0.19	0.74	0.02	0.37
C.D. at 5 % level	0.28	0.3	0.17	0.6	0.57	0.33	0.44	0.74	0.4	1.56	0.05	0.78

Table 3. Effect of different treatments on flowering and reproductive traits of strawberry cv. Flamenco

Treatments	Crowns per plant			Days to first flowering			Number of flowers per plant			Flowering to fruit set (days)		
	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled
T_1	1.78	1.81	1.80	65.65	65.49	65.57	7.00	6.46	6.73	7.03	7.21	7.12
T_2	1.93	1.91	1.92	67.08	63.29	65.19	8.36	7.73	8.05	6.92	7.08	7.00
T_3	2.04	2.00	2.02	62.97	61.54	62.26	9.67	8.92	9.30	6.84	6.92	6.88
T_4	2.12	2.19	2.16	62.81	63.52	63.16	11.00	10.19	10.59	6.75	6.83	6.79
T_5	2.44	2.43	2.43	58.19	56.81	57.50	14.99	13.82	14.41	6.51	6.43	6.47
T_6	2.34	2.40	2.37	59.44	57.92	58.68	13.67	12.58	13.13	6.57	6.59	6.58
T_7	2.22	2.26	2.24	60.13	60.20	60.17	12.31	11.37	11.84	6.64	6.70	6.67
T_8	2.78	2.78	2.78	57.34	57.15	57.25	18.97	17.46	18.22	6.20	6.07	6.14
T_9	2.69	2.68	2.69	57.21	55.71	56.46	17.63	16.22	16.92	6.29	6.21	6.25
T_{10}	2.59	2.55	2.57	57.60	57.05	57.33	16.31	15.01	15.66	6.36	6.35	6.36
F test	S	S	S	S	S	S	S	S	S	S	S	S
S.Ed. (±)	0.02	0.03	0.02	1.73	1.6	1.04	0.03	0.03	0.02	0.02	0.03	0.02
C.D. at 5 % level	0.05	0.07	0.04	3.63	3.36	2.18	0.06	0.05	0.05	0.05	0.05	0.04

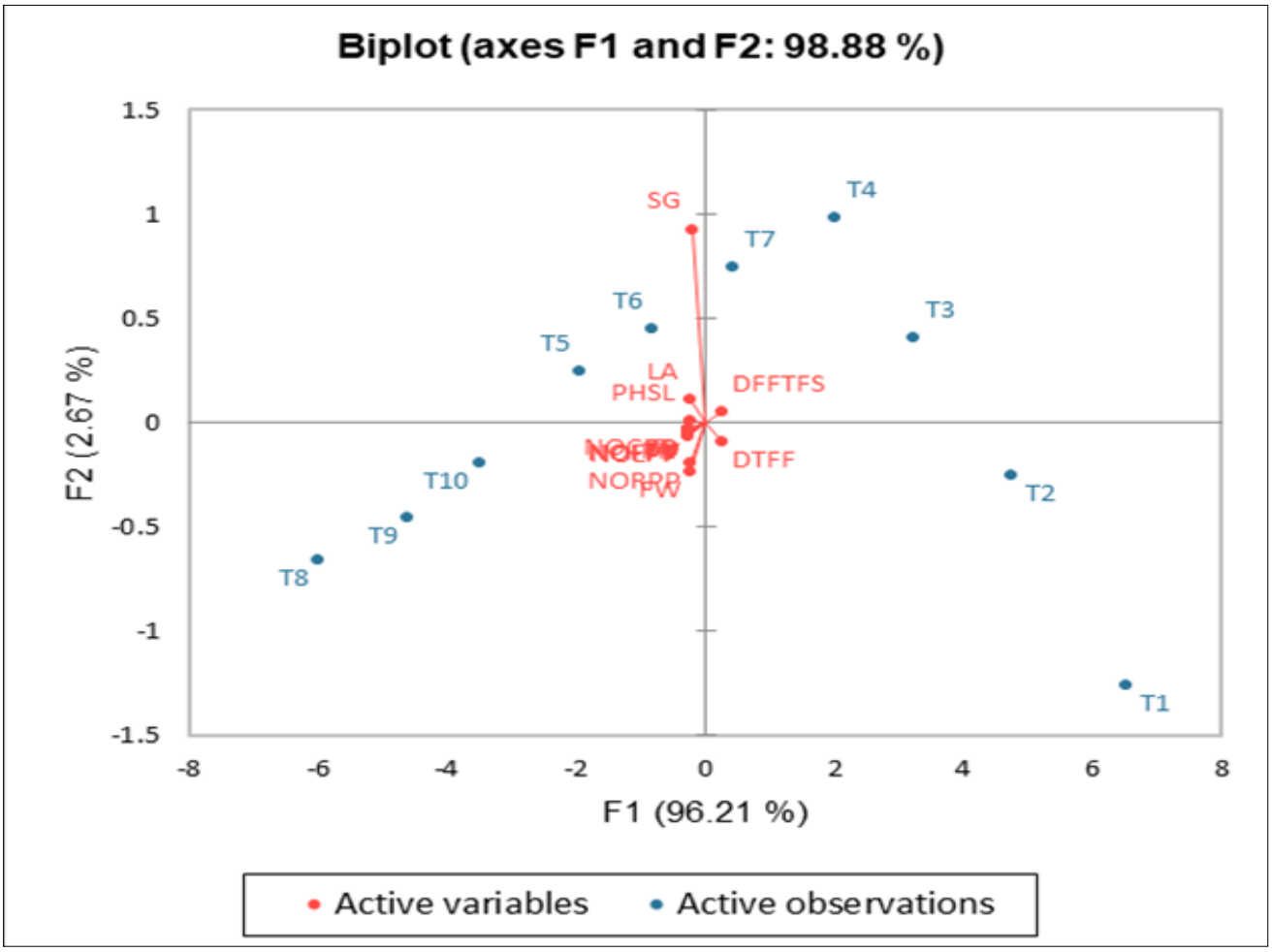

Fig. 1. Loading biplot of PCA for growth and yield characters of strawberry.

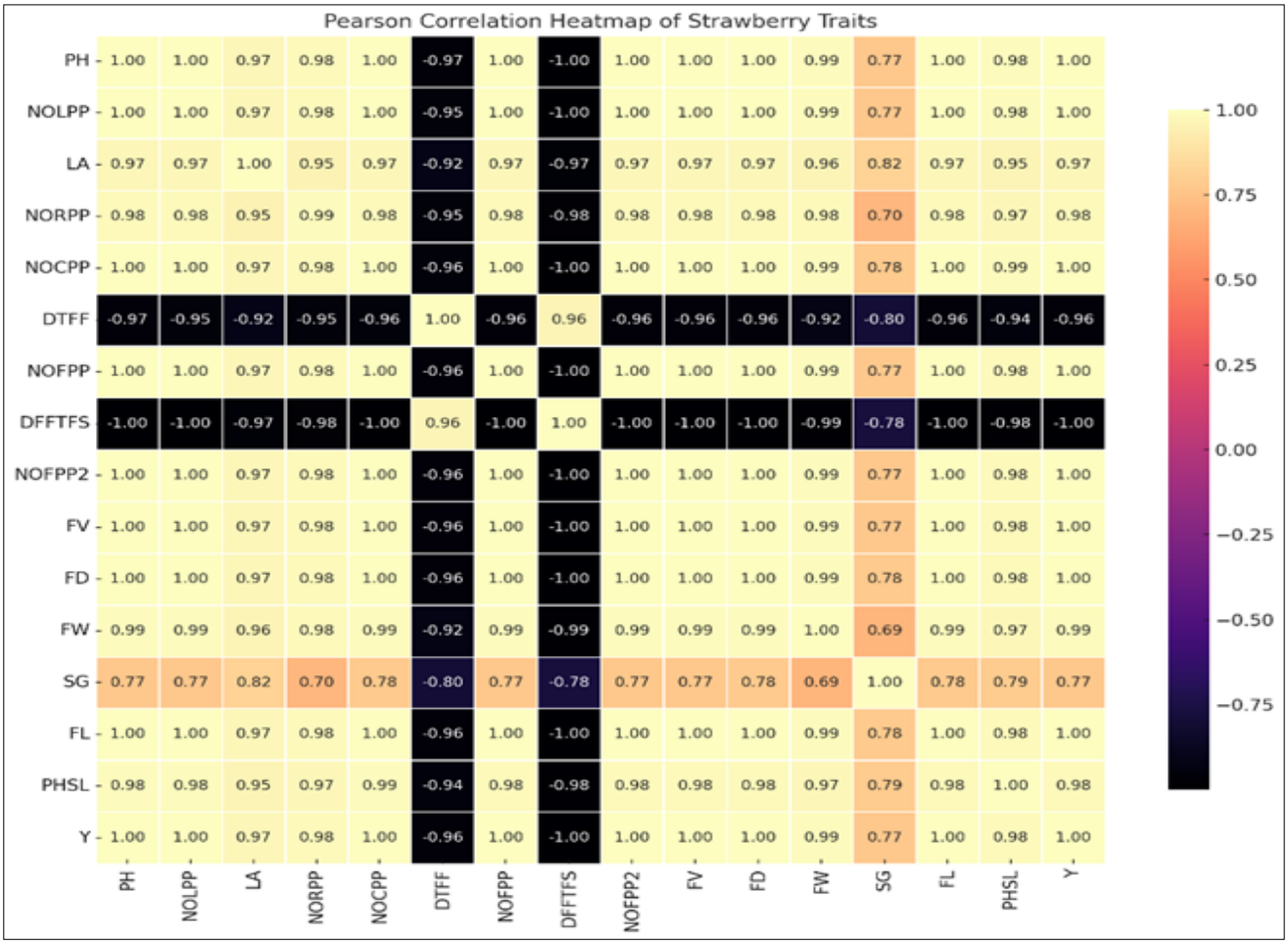

Fig. 2. Correlation matrix of growth, physical and yield characters of strawberry.

(6.46 flowers/plant) produced the least. The pooled means showed that T_8 (18.22 flowers/plant) and T_9 (16.92 flowers/plant) were superior, whereas T_1 (6.73 flowers/plant) remained the lowest ($p < 0.05$).

Additionally, fruit set was also earlier in T_8 (6.20 and 6.07 days after flowering in the two years) than T_9 (6.29 and 6.21 days) and T_1 (7.03 and 7.21 days) with pooled means of 6.14, 6.25 and 7.12 days ($p \leq 0.05$) as shown in Table 3 and Fig. 1 & 2.

Fruit physical traits

The treatment T_8 (19.83 cm^3) had the highest fruit volume in the 2023–2024 season, followed closely by T_9 (19.36 cm^3), while lowest volume was observed in T_1 (15.52 cm^3) as shown in Table 4 and Fig. 1 & 2. In 2024–2025, the corresponding values were 18.48, 18.02 and 13.97 cm^3, giving pooled means of 19.16, 18.69 and 14.74 cm^3, respectively ($p \leq 0.05$).

The fruit weight (g) showed the same trend. In 2023–2024, T_8 produced 17.01 g, T_9 produced 14.98 g and T_1 produced 10.92 g, while in 2024–2025 the values were 20.09, 19.80 and 9.95 g, with pooled means of 18.55, 17.39 and 10.44 g, respectively ($p < 0.05$). In terms of fruit length, T_8 reached 55.84 mm and 58.06 mm, with a pooled mean of 56.95 mm across the two seasons, followed by T_9 (51.93, 56.16 and 54.55 mm), while smallest fruits were observed in T_1 (31.32, 31.64 and 31.48 mm) ($p < 0.05$).

The fruit diameter followed a similar pattern: T_8 produced 42.95 and 44.66 mm in the two seasons (pooled mean 43.81 mm), T_9 produced 40.71 and 43.20 mm (41.96 mm) and lowest in T_1 produced 24.09 and 24.34 mm (24.22 mm) ($p < 0.05$). Specific gravity (g/cm^3) also showed this trend (Table 5, Fig. 1 & 2). In 2023–2024, T_8, T_9 and T_1 had 0.86, 0.77 and 0.68, respectively. In 2024–25, the values were 1.10, 1.09 and 0.71, with pooled means of 0.98, 0.93 and 0.70, respectively ($p \leq 0.05$).

Postharvest shelf life and yield

Shelf life has undergone a clear and continuous enhancement through the application of integrated strategies in both seasons. In 2023-24, the fruits produced in T_8 showed the better average value of 2.52 days for the marketable time of the fruits, while T_9 had the average of 2.36 days for the marketable time (Table 5; Fig. 1 & 2). Nevertheless, the produce from the untreated control (T_1) showed quality for just 1.11 days before revealing maturity and microbial spoilage. A similar scenario was observed in 2024-25, T_8 fruits expressed marketable phase for 2.70 days, T_9 fruits for 2.51 days and lowest in T_1 fruits for merely 1.19 days. The mean pooled data, T_8 achieved the maximum shelf life of 2.61 days, while T_9—2.44 days and T_1 was the lowest, 1.15 days.

The statistical analysis depicted at $p \leq 0.05$, showing these differences significant, emphasized the potentiality of the use of nano-chitosan and the partnership with the beneficial microbial inoculants in the extension of strawberry postharvest life through prolonged senescence and decreased microbial decay and with similar trend in subsequent year yield (t/ha). Table 6 and Fig. 1 & 2 shown that T_8 recorded with 22.22 t/ha and 20.74 t/ha in T_9 whereas, T_1 yielded only 8.75 t/ha.

Table 4. Effect of different treatments on physical characteristics of strawberry cv. Flamenco

Treatments	Fruit volume (cm^3)			Fruit weight (g)			Fruit length (mm)			Fruit diameter (mm)		
	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled
T_1	15.52	13.97	14.74	10.92	9.95	10.44	31.32	31.64	31.48	24.09	24.34	24.22
T_2	15.96	14.48	15.22	10.91	11.14	11.02	34.32	34.74	34.53	26.4	26.72	26.56
T_3	16.44	14.99	15.72	11.25	12.29	11.77	37.59	37.9	37.75	28.92	29.16	29.04
T_4	16.91	15.52	16.22	11.57	13.26	12.42	39.24	41.49	40.36	30.18	31.91	31.05
T_5	18.40	17.01	17.71	12.94	16.71	14.83	48.2	49.89	49.04	37.07	38.38	37.73
T_6	17.92	16.54	17.23	12.26	15.44	13.85	43.72	47.02	45.37	33.63	36.17	34.9
T_7	17.44	16.00	16.72	11.83	14.53	13.18	42.5	44.38	43.44	32.69	34.14	33.42
T_8	19.83	18.48	19.16	17.01	20.09	18.55	55.84	58.06	56.95	42.95	44.66	43.81
T_9	19.36	18.02	18.69	14.98	19.80	17.39	52.93	56.16	54.55	40.71	43.2	41.96
T_{10}	18.89	17.48	18.18	13.80	18.94	16.37	51.56	52.23	51.89	39.66	40.17	39.92
F test	S	S	S	S	S	S	S	S	S	S	S	S
S.Ed. (±)	0.02	0.02	0.02	0.1	0.15	0.09	0.71	0.65	0.61	0.55	0.5	0.44
C.D. at 5 % level	0.05	0.05	0.03	0.22	0.32	0.19	1.5	1.37	1.29	1.15	1.05	0.93

Table 5. Effect of different treatments on specific gravity and shelf life of strawberry

Treatments	specific gravity (g/cm^3)			Post harvest shelf life (days)		
	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled
T_1	15.52	13.97	14.74	10.92	9.95	10.44
T_2	15.96	14.48	15.22	10.91	11.14	11.02
T_3	16.44	14.99	15.72	11.25	12.29	11.77
T_4	16.91	15.52	16.22	11.57	13.26	12.42
T_5	18.40	17.01	17.71	12.94	16.71	14.83
T_6	17.92	16.54	17.23	12.26	15.44	13.85
T_7	17.44	16.00	16.72	11.83	14.53	13.18
T_8	19.83	18.48	19.16	17.01	20.09	18.55
T_9	19.36	18.02	18.69	14.98	19.80	17.39
T_{10}	18.89	17.48	18.18	13.80	18.94	16.37
F test	S	S	S	S	S	S
S.Ed. (±)	0.02	0.02	0.02	0.1	0.15	0.09
C.D. at 5 % level	0.05	0.05	0.03	0.22	0.32	0.19

Table 6. Effect of different treatments on yield of strawberry cv. Flamenco

Treatments	Yield (t/ha)
T_1	8.75
T_2	10.24
T_3	11.74
T_4	13.24
T_5	17.74
T_6	16.24
T_7	14.74
T_8	22.22
T_9	20.74
T_{10}	19.22

Discussion

Vegetative growth

The vegetative and reproductive performance of strawberry is significantly improved by nano-chitosan, PGPR and *Trichoderma*, each having distinct but complementary actions. For plant height, nano-chitosan enhances nitrate reductase activity and stimulates auxin production, which induces elongation of internodes, PGPR produce auxins and gibberellins that stimulate shoot elongation, while *Trichoderma* regulates gibberellins and cytokinins in the rhizosphere to promote early apical dominance (13-15). The increase in leaf number is linked to nano-chitosan's promotion of cell cycle progression and chlorophyll synthesis, PGPR through the secretion of auxin and cytokinin to hasten leaf initiation and expansion and *Trichoderma* through the enhancement of phosphorus and iron uptake in addition to the release of cytokinin for rapid foliar initiation (16-18). There are various ways that leaf area expansion is promoted, among which nano-chitosan acts as the activator of aquaporin and osmotic regulation, lamina widening is induced by PGPR through IAA and gibberellins and *Trichoderma* enhances photosynthetic surface area by coupling root down-conductivity, potassium uptake and auxin-like metabolite production (19-21).

Runner and crown production

Runner formation is promoted by nano-chitosan-induced auxins and sugar mobilization in strawberry, PGPR-mediated auxin-cytokinin balance for stolon bud differentiation and *Trichoderma*-driven root growth and carbon allocation in strawberry (19-22). Similarly, crown multiplication is managed by nano-chitosan via meristem proliferation and carbohydrate partitioning, both of which auxin-mediated, PGPR via the cytokinin-auxin synergy and nitrogen fixation in strawberry and *Trichoderma* with hormone-like metabolites and phosphorus solubilization that support the meristematic activity of the crown-root intersection (16, 18, 23). Reproductive development is also enhanced.

Phenology

The number of days to first flowering is decreased as nano-chitosan induces auxin and gibberellin biosynthesis and flowering gene expression in coffee, PGPR supply gibberellins and cytokinin for early bud differentiation in strawberry and *Trichoderma* improves P and K uptake to speed up the bud initiation in strawberry (20, 21, 24). For that reason, flower number is increased via nano-chitosan-induced auxin-gibberellin activity in strawberry, PGPR-enhanced cytokinin production and nitrogen fixation in strawberry and *Trichoderma*-mediated cytokinin mimicry and improved carbohydrate utilization (17, 18, 23). The period of flowering and fruiting is reduced due to the nano-chitosan's reinforcement of reproductive tissues and the auxin -gibberellin connection for ovary growth, PGPR improving pollen viability and fertilization efficiency through hormones and nutrient cycling and *Trichoderma* facilitating embryo development through K and Ca uptake along with auxin-like secondary metabolites in strawberry (14, 25, 26). Thus, all the characteristics have been positively affected-height, leaf development, runners, crowns, flowering and fruiting-together with the application of nano-chitosan, PGPR and *Trichoderma*, which has resulted in improved nutrient uptake, hormonal regulation and assimilate partitioning, thus sustaining vegetative vigor and accelerating reproductive success in strawberry.

Fruit physical traits

Strawberries are capable of increasing fruit volume in this regard as a result of high sink strength, assimilate partitioning and cell expansion. This is supported using nano-chitosan, which, by loosening the cell walls, enhances osmotic regulation and improves water uptake, results in the production of denser and larger fruits in strawberry (13, 19). PGPR not only enhances nutrient assimilation, but also, produces auxins and gibberellins, which regulate sink-source biomass translocation, making it is one of the ways for strawberry fruit volume to be improved, as also observed in watermelon, apricot and sweet oranges (27-29). *T. harzianum* also enhances nutrient mobilization in the rhizosphere, with its hormonal activity being modulated by the plant, thereby increasing fruit size; its application has been proven in citrus and strawberry (30, 31).

Fruit diameter increases as a result of the expansion of pericarp cells, vascular supply and their division. Nano-chitosan takes part in this through osmotic balance and mitotic activity in strawberry and okra (13, 19, 32). Moreover, the PGPR treatment promotes diameter growth by stimulating their hormones and using photosynthates effectively, as proven in strawberry (33-36). In addition, *Trichoderma* contributes to diameter improvement through nutrient translocation and bioformulations, which consistently shown increased fruit diameter in citrus (30).

Since the weight of fruit is a direct determinant of the potential yield, it is linked to food supply as means of transfer and improved sink strength. The application of nano-chitosan adds weight due to chlorophyll content, photosynthetic efficiency and nutrient assimilation, particularly in the case of nano-chitosan in strawberry (13, 19, 36, 37). PGPR-bioagents such as IAA and gibberellin generate excess photosynthate transfer, as a result, fruits get heavier in strawberry) and in sweet orange (29, 33, 34, 38). *Trichoderma* is known to improve root environments and nutrient uptake, beside the increase of fruit weight, through microbial consortia in strawberry (30, 31).

As for the internal quality, nano-chitosan increases specific gravity and fruit density because of higher carbohydrate accumulation and osmotic regulation in strawberry (13, 19). PGPR contributes not only to the enhancement of sucrose but also hexose deposition, thereby reinforcing the fruit tissues in strawberry and in papaya (33, 34, 39, 40). *Trichoderma* increases fruit density and sugar accumulation, producing thicker and firmer fruits, for instance, improvements have been recorded in strawberry and banana, as well as in mushroom (41).

Fruit length depends on the basal cell division and how auxin and gibberellin interact. Nano-chitosan increases elongation by the activity of nitrate reductase and the synthesis of GA in strawberry and in okra (13, 19, 32). PGPR also increases length

through the secretion of auxin, as observed in strawberry (42), while *Trichoderma,* through improved nutrient availability, contributes to the production of longer berries (21). To conclude with shelf life and storability, fruits benefit synergistically from these agents. Nano-chitosan act as a coating against microbial infections while also being an antimicrobial agent. Due to this aspect, it reduces the spoilage and weight loss of fruits in addition to improving in firmness (13, 19, 37, 43).

Postharvest shelf life and yield

The storability is improved by PGPR, which is a stimulator of antioxidants that keeps decay in check (28, 38, 44). *Trichoderma* enzymes are induced and pest resistance rises; thus, the strawberries and other crops have a longer fruit life with reduced postharvest spoiling (21).

The increase in the integrated outcome of strawberry yield per hectare is due to the enhanced plant biomass, higher flowering ratio, successful fruit set and good berry maturation, which are promoted by nano-chitosan, PGPR and *Trichoderma*. The basic principle of nano-chitosan is based on its potential to promote photosynthesis, nutrient uptake and stress tolerance. Chitosan foliar sprays applied to the plants remarkably increased berry size and number, thus making a big contribution to the total yield in strawberry (13). Conclusive discoveries revealed that the yields of chitosan-treated products increased significantly in strawberry (37). Nano-chitosan, further strengthened the yield per hectare by improving nutrient mobilization and berry quality (19). PGPR is a primary method of enhancing production through phytohormone secretion (IAA, GA, cytokinin), nitrogen fixation and phosphorus solubilization, all of which influence flowering and fruit development.

The better flower and fruit set due to flower retention makes it easy to obtain higher yields, as was seen in strawberry and apricot (28, 33). In the same way, microbial consortia were demonstrated to not only stabilize but also raise the yield of different crops-PGPR-based bioformulations were consistent in strawberry achieve high performance (45, 46). *T. harzianum* contributes greatly to this process through root growth promotion, nutrient uptake improvement and hormone balance. The applications of *Trichoderma* bioformulations in strawberry were succeeded in achieving better production and were also proven effective in banana under mixed bio-formulation (41).

Moreover, the combination of PGPR and *Trichoderma* has demonstrated yield benefits such as increased yield stability and improvement in strawberry (47). In brief, nutrient mobilization, hormonal regulation and assimilate partitioning are the main physiological roles of the complementary actions of nano-chitosan, PGPR and *Trichoderma,* which lead to significant improvements in strawberry yield per hectare.

Principal Component Analysis (PCA)

Analysis of principal components on the 15 quantitative traits in *Fragaria × ananassa* Duch. cv. Flamenco revealed that the first five principal components encompassed 99.84 % of the total phenotypic variance. The first principal component (PC1) accounted for 96.21 % of the total deviation, while the remainder was distributed among PC2 (2.67 %), PC3 (0.55 %), PC4 (0.24 %) and PC5 (0.17 %). PC1 represented mostly high negative loadings for the majority of growth and yield-related attributes, such as plant height (-0.998), number of leaves per plant (-0.998), leaf area (-0.975), number of runners per plant (-0.980), number of crowns per plant (-0.998), number of flowers per plant (-0.999), fruit volume (-0.999), fruit diameter (-0.999), fruit weight (-0.999), fruit length (-0.795), fruit shelf life (-0.999) and yield (-0.987) (Fig. 1).

This strong clustering of vegetative, reproductive and yield components under PC1 clearly shows the existence of an integrated morphological-yield axis, which implies that vegetative vigor can drive superior reproductive output, better fruit quality and longer shelf life in tandem. Conversely, days from flowering to fruit set (0.966) and days to first flowering (0.999) were found to be negatively loaded with respect to PC1. They were more directly with yield and earliness; delayed initiation of flowers and fruit set were instead relatively associated with higher productivity losses. Specific gravity (-0.984) also carried significant weight on PC1, rendering it significant in reference to the total fruit density and internal quality metrics.

PC2, on the other hand, was mainly characterized by a large positive contribution from fruit length (0.605), while leaf area (0.071) and yield (0.003) made minor contributions to it. This suggests that the concept was primarily related to fruit shape and elongation, rather than performance measures of total yield. PC3 had a major focus on days from flowering to fruit set (0.237) and leaf area (0.153), which were the drivers of a secondary phenological-morphological axis that had a small impact on total variability. PC4 and PC5 were too weak (< 0.5 % combined variance) to assert their own descriptors, but they were, slightly influenced by residual traits that remain positive-leaf area (0.137 in PC4) and yield (0.125 in PC5)-though to a lesser extent than in PC 1.

Overall, the ordination representation denotes that PC1 plays the chief role in determining treatment differentiation by shaping the pattern of vegetative growth, phenology, fruit morphology, yield potential and postharvest. The clustering of these traits epitomizes the collaborative result of nano-chitosan, PGPR and *T. harzianum* bio-capsules in maximizing the crop productivity in a subtropical field setting.

Correlation matrix

The Pearson correlation analysis conducted on the 15 quantitative traits of strawberry (*Fragaria × ananassa* Duch. cv. Flamenco) and the first five principal components of PCA showed that the association patterns were clear and consistent. F1 was the only component that showed consistently strong negative correlations ($r \approx -0.97$ to -1.00) with most traits associated with morphology and yield, such as plant height (trait 1: -0.998), number of leaves per plant (trait 2: -0.998), number of runners per plant (trait 4: -0.980), number of crowns per plant (trait 5: -0.998), fruit volume (trait 9: -0.999), fruit diameter (trait 10: -0.999), fruit weight (trait 12: -0.984), fruit length (trait 13: -0.795), fruit shelf life (trait 14: -0.987) and yield (trait 15: -0.999) (Fig. 2). The fact that these correlations are both of high magnitude and uniform shows that F1 is an integrated factor of vegetative-reproductive-yield relationships, where high trait values equal to lower F1 scores.

Phenological traits, such as days of head flowering first (trait 8: 0.999) and days of fruit set from flowering (trait 6: 0.966), were very highly and positively correlated with F1, with their values suggesting the inverse relationship between early phenology and late yield performance. Specific gravity (trait 11: -0.984) also revealed the negative association with F1, linking fruit density and morphological vigor closely to yield.

F2 was mainly defined by a strikingly strong positive correlation with fruit length (trait 13: 0.605) and a few weaker positive associations with leaf area (trait 3: 0.071) and yield (trait 15: 0.003). This implies that F2 is a fruit elongation-canopy dimension axis; practically it is not well connected to vegetative -yield factors and therefore it is not the primary axis. F3 was primarily associated with days from flowering to fruit set (trait 6: 0.237) and leaf area (trait 3: 0.153) giving high positive loading, but the overall variances remained unaffected, as it only represented a small phenology-canopy interaction.

F4 and F5 explained less than 0.5 % of the whole variability. F4 had a moderate positive association with leaf area (trait 3: 0.137) and the number of runners per plant (trait 4: 0.081) was moderate. Moreover, F5 was strongly positively associated with fruit shelf life (trait 14: 0.125). These minor axes may represent residual variation not captured by the primary phenotypic patterns.

Overall, the correlation structure supports F1 as the major phenotypic variability factor in strawberry. It governs vegetative growth, reproductive capacity and yield quality, while F2-F5 contribute weaker in a trait-specific manner.

Conclusion

The two-year field study confirmed that the application of nano -chitosan, PGPR and *T. harzianum* bio-capsules significantly improved ($p < 0.05$) the growth and yield of strawberry cv. Flamenco in subtropical conditions. The most efficient treatment was T_8, which resulted in an impressive plant height of 18.59 cm, a leaf area of 87.74 cm^2 flowers per plant of 18.22, a fruit weight of 18.55 g and a yield of 22.22 t/ha, all surpassing the control. PCA results showed that total variation was best explained by PC1 (98.28 %) and for plant height, crowns, yield and shelf life, it indicated strong negative loadings of -0.999 for height, -0.998 for crowns, -0.999 for yield and -0.986 for shelf life. The correlation analysis indicated that fruit yield was strongly and positively correlated ($r > 0.97$) with plant height, flower number, fruit weight and fruit volume, while days until first flowering were negatively correlated ($r = -0.96$). These findings indicate that earlier flowering and the stronger growth are key determinants yield and the mixtures behave synergistically to increase the quality and productivity of strawberries and thus serve as an ecological alternative to the conventional inputs.

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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.

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