



RESEARCH ARTICLE

Biofertilisers and biostimulants mediated modulation of fruit quality traits in guava (*Psidium guajava* L.) cv. G-27

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Received: 22 December 2025; Accepted: 11 May 2026; Available online: Version 1.0: 08 September 2026

Cite this article: Astha, Gurjar PKS, Ramawatar C, Raj N, Nindiya B, Mohammad A, Farooq AK. Biofertilisers and biostimulants mediated modulation of fruit quality traits in guava (*Psidium guajava* L.) cv. G-27. Plant Science Today. 2026;13(sp5):01–06. <https://doi.org/10.14719/pst.13304>

Abstract

The present investigation evaluated the interactive effects of biofertilisers and Jeevamrit on the physicochemical and biochemical fruit quality traits of guava (*Psidium guajava* L.) cv. G-27. A field experiment was conducted over 2 years (2023–24 and 2024–25) at Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya (RVSKV), Gwalior, Madhya Pradesh, India, using a factorial randomised block design (FRBD) comprising 16 treatment combinations (4 biofertiliser levels × 4 Jeevamrit levels) with 3 replications on 10-year-old guava trees. Biofertiliser treatments consisted of graded doses of *Azotobacter*, phosphate-solubilising bacteria (PSB) and potassium solubilising bacteria (KSB), applied at 0, 200, 250 and 300 mL tree⁻¹, in combination with Jeevamrit levels of 0, 1.90, 2.50 and 3.125 L tree⁻¹. The integrated treatment T₁₆ (*Azotobacter* 300 mL tree⁻¹ + PSB 300 mL tree⁻¹ + KSB 300 mL tree⁻¹ + Jeevamrit 3.125 L tree⁻¹), applied at the pea stage, resulted in the highest total soluble solids (11.48 °Brix), ascorbic acid (160.10 mg 100 g⁻¹ pulp) and total sugars (11.39 %), along with enhanced pulp weight (249.91 g) and bioactive compound content, while recording the lowest titratable acidity (0.17 %). These improvements are attributed to microbially mediated nutrient mobilisation through nitrogen, phosphorus and potassium solubilisation, enhanced assimilate partitioning to fruits and the activation of antioxidant metabolism driven by microbial metabolites and phytohormones. The pooled results confirm the effectiveness of integrated biofertiliser-biostimulant application in improving guava fruit quality under sustainable nutrient management.

Keywords: biofertilisers; biostimulants; fruit quality; guava; nutrient management; physicochemical characters

Introduction

Guava (*Psidium guajava* L.), a member of the family Myrtaceae, ranks fourth among India's major fruit crops after citrus, banana and mango (1). Known as the “apple of the tropics”, it offers exceptional nutrition, including high ascorbic acid (210 mg 100 g⁻¹) and pectin (0.60 %), along with essential minerals and B-complex vitamins (2). In 2024, guava occupied approximately 357 thousand hectares, with a production of 5.26 million tonnes and a productivity of 15.6 t ha⁻¹ (3). Its wide adaptability to diverse soils (pH 4.5–8.2) and climatic conditions makes it a key crop for sustainable horticulture (4).

However, intensive cultivation practices relying on chemical fertilisers often result in soil degradation, nutrient imbalance and environmental concerns, necessitating eco-friendly nutrient management strategies (2). Biofertilisers such as nitrogen-fixing bacteria (e.g., *Azotobacter*), phosphate-solubilising bacteria (PSB) and potassium-solubilising bacteria (KSB) enhance nutrient availability and uptake, leading to

improved photosynthetic efficiency and fruit quality, while also stimulating soil enzymatic activity and rhizosphere microbial dynamics (5, 6). Biostimulants, on the other hand, regulate metabolism via bioactive compounds, improving nutrient use efficiency, antioxidant activity and source-sink relationships (6, 7). Among these, Jeevamrit, a microbial-organic formulation, is gaining attention for its role in stimulating soil microbial activity and plant metabolic functions.

Although the individual benefits of biofertilisers and biostimulants are well documented, their interactive effects under field conditions remain poorly understood, particularly in perennial fruit crops. Moreover, the systematic evaluation of graded combinations of microbial consortia with Jeevamrit is lacking for guava cv. G-27 under semi-arid conditions. Therefore, the present study aimed to assess the combined effects of biofertilisers and Jeevamrit on physicochemical and biochemical fruit quality traits to develop an efficient and sustainable nutrient management strategy.

Materials and Methods

The study was conducted over two consecutive winter seasons (2023–24 and 2024–25) on the same 48 uniformly grown guava (*P. guajava*) cv. G-27 trees (10 years old, spaced 5 × 5 m) at the Fruit Orchard, Department of Horticulture, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya (RVSKW), Gwalior, Madhya Pradesh, India (26°13' N, 78°14' E, 211.5 m above mean sea level (amsl)). The semi-arid subtropical climate of Gwalior featured mean annual rainfall of 719.6 mm (2023–24) and 1120.1 mm (2024–25), with temperature extremes of 5.2–44.1 °C; however, the experiment was conducted during the winter season under comparatively moderate temperature conditions. The soil was sandy loam (66.0 % sand, 22.5 % silt and 15.1 % clay), slightly alkaline (pH 7.3), with EC of 0.44 dS m⁻¹, low organic carbon (0.48 g kg⁻¹) and medium available nitrogen (235.63 kg ha⁻¹) and phosphorus (14.55 kg ha⁻¹), but high potassium (251.23 kg ha⁻¹), indicating moderately fertile and well-drained conditions.

A factorial randomised block design (FRBD) was used to test two factors, biofertiliser levels (B: B₀ = control; B₁ = 200 mL tree⁻¹; B₂ = 250 mL tree⁻¹; B₃ = 300 mL tree⁻¹ of *Azotobacter* + PSB + KSB) and Jeevamrit levels (J: J₀ = control; J₁ = 1.90 L tree⁻¹; J₂ = 2.50 L tree⁻¹; J₃ = 3.125 L tree⁻¹), across 16 combinations (T₁–T₁₆) with 3 replications (*n* = 48 trees).

All bioinputs were prepared in the laboratory under aseptic conditions. Liquid cultures of *Azotobacter chroococcum*, PSB and KSB were maintained at a population density of approximately 10⁸ CFU mL⁻¹, verified using standard serial dilution and plate count techniques on selective media (8). Jeevamrit, a microbial-organic biostimulant, was prepared following standard organic farming protocols using fresh cow dung (10 kg), cow urine (10 L), jaggery (2 kg), pulse flour (2 kg) and water (200 L) and incubated for 5–7 days under shade with regular stirring (9, 10). The microbial load of Jeevamrit (approximately 10⁶–10⁸ CFU mL⁻¹) was confirmed using serial dilution and viable count methods (11).

Biofertiliser and Jeevamrit solutions were mixed in 10 L water tree⁻¹ and applied both as soil drench in the rhizosphere and as foliar spray at the pea stage during each season using a pneumatic foot-operated sprayer (16:00–18:00 hr). Polythene sheets were placed beneath tree canopies during foliar application to prevent contamination of soil-applied treatments.

Four uniform, ripe fruits per tree were harvested, washed and analysed for total soluble solids (°Brix), titratable acidity (12), ascorbic acid (12), total sugars (13), pulp and seed weight (g), pectin content (14), antioxidant activity (15) and total phenolics (16) using standard analytical procedures. Data from both years were pooled for analysis after confirming non-significant year effects through preliminary analysis of variance (ANOVA). Treatment effects and interactions were evaluated using two-factor ANOVA (FRBD) with

OPSTAT software. When the F-test indicated significant differences, treatment means were compared using the critical difference (CD) test [least significant difference (LSD)] at *p* = 0.05.

Results and Discussion

Individual effect of biofertilisers

The individual application of biofertilisers exerted a significant influence on all physicochemical quality attributes of guava fruits (Table 1). A consistent and progressive improvement was observed from B₀ (control) to B₃, with most parameters showing statistically significant differences (*p* ≤ 0.05). Total soluble solids (TSS) increased significantly from 8.74 °Brix in B₀ to 10.68 °Brix in B₃, with the difference exceeding the CD value (1.29), indicating a significant treatment effect. Similarly, total sugars increased from 8.70 to 10.62 % and the increase was statistically significant (CD = 1.09).

Titrate acidity showed a significant decreasing trend with increasing biofertiliser levels, declining from 0.36 % in B₀ to 0.19 % in B₃ (*p* ≤ 0.05), indicating an improvement in sugar-acid balance. Ascorbic acid content increased significantly from 122.10 mg 100 g⁻¹ pulp in the control to 149.10 mg 100 g⁻¹ pulp in B₃. Fruit pulp weight showed a pronounced and significant increase from 157.8 g in B₀ to 224.3 g in B₃, while seed weight also increased significantly from 3.48 to 4.24 g (*p* ≤ 0.05). Biochemical quality traits such as pectin content, antioxidant activity and total phenolic content were significantly enhanced under higher biofertiliser levels. Pectin content increased from 0.68 to 0.83 %, antioxidant activity from 83.05 to 101.48 mg 100 g⁻¹ and total phenols from 131.9 to 161.2 mg 100 g⁻¹ (*p* ≤ 0.05). Overall, B₃ recorded significantly superior values for most quality parameters compared to B₀, B₁ and B₂.

Individual effect of bioinputs (Jeevamrit)

The individual application of Jeevamrit significantly influenced fruit quality parameters of guava (Table 2). Increasing levels of Jeevamrit (J₀–J₃) resulted in statistically significant improvements in most traits when compared with the CD values. The TSS increased significantly from 8.52 °Brix in J₀ to 10.81 °Brix in J₃ (*p* ≤ 0.05), while total sugars increased from 8.48 to 10.75 % (*p* ≤ 0.05).

Titrate acidity declined significantly from 0.36 to 0.24 % (*p* ≤ 0.05), reflecting improved fruit palatability. Ascorbic acid content increased significantly with Jeevamrit application, rising from 119.0 mg 100 g⁻¹ pulp in J₀ to 150.9 mg 100 g⁻¹ pulp in J₃ (*p* ≤ 0.05). Fruit pulp weight showed a significant increase from 155.7 to 213.6 g (*p* ≤ 0.05), while seed weight increased from 3.39 to 4.29 g (*p* ≤ 0.05). Pectin content increased significantly from 0.67 % in the control to 0.84 % in J₃ (*p* ≤ 0.05). Antioxidant activity and total phenols also showed significant enhancement, increasing from 80.92 to 102.7 mg 100 g⁻¹ (*p* ≤ 0.05) and from 128.6 to 163.1 mg 100 g⁻¹ (*p* ≤ 0.05), respectively. Overall, J₃ recorded the most favourable fruit quality attributes.

Table 1. Individual effects of biofertilisers application on fruit quality of guava (*Psidium guajava*) cv. G-27

Trait	B ₀	B ₁	B ₂	B ₃	Mean	CD (<i>p</i> ≤ 0.05)
TSS (°Brix)	8.74	9.62	9.85	10.68	9.72	1.29
Acidity (%)	0.36	0.3	0.23	0.19	0.27	0.019
Ascorbic acid (mg 100 g ⁻¹ pulp)	122.1	134.5	137.4	149.1	135.8	17.15
Total sugars (%)	8.7	9.58	9.78	10.62	9.67	1.09
Pulp weight (g)	157.8	185.2	214.3	224.3	195.4	17.18
Seed weight (g)	3.48	3.84	3.92	4.24	3.87	0.54
Pectin (%)	0.68	0.75	0.76	0.83	0.76	0.10
Antioxidant (mg 100 g ⁻¹)	83.05	91.41	93.48	101.48	92.36	9.12
Total phenols (mg 100 g ⁻¹)	131.9	145.2	148.5	161.2	146.7	17.63

TSS: total soluble solids; CD: critical difference. B₀ = control, B₁ = 200 mL tree⁻¹, B₂ = 250 mL tree⁻¹, B₃ = 300 biofertilisers levels mL tree⁻¹.

Table 2. Individual effects of bioinputs application on fruit quality of guava (*Psidium guajava*) cv. G-27

Trait	J ₀	J ₁	J ₂	J ₃	Mean	CD ($p \leq 0.05$)
TSS (°Brix)	8.52	9.62	9.95	10.81	9.73	1.35
Acidity (%)	0.36	0.27	0.21	0.24	0.27	0.021
Ascorbic acid (mg 100 g ⁻¹ pulp)	119.0	134.3	139.0	150.9	135.8	20.22
Total sugars (%)	8.48	9.56	9.9	10.75	9.67	1.16
Pulp weight (g)	155.7	199.5	212.9	213.6	195.4	17.18
Seed weight (g)	3.39	3.83	3.96	4.29	3.87	0.48
Pectin (%)	0.67	0.75	0.77	0.84	0.76	0.11
Antioxidant (mg 100 g ⁻¹)	80.92	91.32	94.47	102.7	92.35	15.01
Total phenols (mg 100 g ⁻¹)	128.6	145.1	150.1	163.1	146.72	17.33

TSS: total soluble solids; CD: critical difference. J₀ = control, J₁ = 1.90 L tree⁻¹, J₂ = 2.50 L tree⁻¹, J₃ = 3.125 are Jeevamrit levels L tree⁻¹.

Interaction effect of biofertilisers and Jeevamrit

The interaction effects of biofertilisers and Jeevamrit were significant for all studied parameters, indicating strong synergism between the two inputs (Table 3). The TSS ranged from 6.14 °Brix in T₁ (control) to 11.48 °Brix in T₁₆, with the difference exceeding the CD value ($p \leq 0.05$). Total sugars showed a similar trend, increasing from 6.13 to 11.39 %. Titratable acidity decreased significantly from 0.49 % in T₁ to as low as 0.14 % in T₁₅ ($p \leq 0.05$). Ascorbic acid content increased significantly from 85.83 mg 100 g⁻¹ pulp in the control to 160.10 mg 100 g⁻¹ pulp in T₁₆. Pulp weight exhibited a marked increase from 103.00 to 249.91 g, while seed weight increased from 2.45 to 4.55 g ($p \leq 0.05$). Pectin content increased significantly from 0.48 to 0.89 %. Antioxidant activity and total phenols increased from 58.33 to 109.05 mg 100 g⁻¹ and from 92.67 to 173.26 mg 100 g⁻¹ ($p \leq 0.05$), respectively. Among all treatments, T₁₆ consistently recorded significantly superior values for all quality parameters.

Discussion

The improvement in fruit quality under the integrated application of biofertilisers and Jeevamrit can be attributed to enhanced nutrient dynamics and microbially mediated metabolic regulation. Biofertiliser consortia (*Azotobacter*, PSB and KSB) enhance nutrient availability through biological nitrogen fixation and the solubilisation of phosphorus and potassium, thereby optimising plant nutrition and strengthening source-sink relationships. This promotes efficient assimilate partitioning to fruits. In addition, potassium plays a key role in regulating acid metabolism by activating enzymes involved in organic acid conversion (e.g., malate dehydrogenase), facilitating the transformation of organic acids into sugars during ripening and maintaining cellular pH balance. Similar improvements in sugar accumulation have been reported in guava (17, 18); however, the stronger response observed in the present study indicates a synergistic effect of combining biofertilisers with biostimulants, which has not been consistently reported.

The reduction in titratable acidity may be associated with accelerated conversion of organic acids into sugars during ripening, mediated by potassium-activated enzymes and microbial activity. Comparable trends have been observed in guava and citrus (19, 20), although inconsistent responses under sole biofertiliser application suggest that integrated inputs are more effective. Enhancement of ascorbic acid and antioxidant compounds appears to result from improved nitrogen assimilation and activation of biosynthetic pathways. Microbial metabolites and phytohormone-like substances in Jeevamrit

likely act as signalling molecules, stimulating enzymes involved in L-ascorbic acid biosynthesis and limiting oxidative degradation. These findings align with reports in guava and aonla (21, 22), although variability across studies indicates environmental and management influences.

Improved pulp development reflects enhanced cell division and enlargement driven by microbially produced growth regulators and improved nutrient uptake (23, 24). Moreover, the increased pectin content suggests enhanced cell wall biosynthesis and stability, likely mediated by improved calcium and potassium availability (25, 26). The enhancement of antioxidant activity and total phenolics content indicates the stimulation of secondary metabolism, particularly the phenylpropanoid pathway. Biofertilisers and Jeevamrit may act as elicitors, enhancing phenylalanine ammonia-lyase activity and phenolic synthesis (27, 28), although responses vary with crop and environment.

Overall, the integrated application of biofertilisers and biostimulants exerts a coordinated influence on primary and secondary metabolism, thereby improving fruit quality under sustainable nutrient management.

Conclusion

The study demonstrates that integrated application of biofertilisers and Jeevamrit markedly improves fruit quality of guava cv. G-27 by enhancing sugars, ascorbic acid and antioxidant attributes while reducing acidity, indicating a coordinated regulation of primary and secondary metabolism. The superior response observed under the combined application, particularly with *Azotobacter* + phosphate-solubilising bacteria (PSB) + potassium solubilising bacteria (KSB) (300 mL each per tree) and Jeevamrit at 3.125 L per tree, confirms a synergistic interaction between microbial nutrient mobilisation and biostimulant-driven metabolic activation, representing a key advancement over sole bioinput application. From an applied perspective, this approach offers a viable and sustainable nutrient management strategy that can improve fruit quality and economic returns while reducing dependence on chemical fertilisers and associated environmental risks.

However, the findings are based on short-term evaluation under specific agro-climatic conditions and on a single cultivar (G-27), which may limit broader generalisation. Further long-term, multi-location studies are needed to validate the consistency of these results across environments, refine application protocols and elucidate the underlying physiological and molecular mechanisms, particularly in relation to soil microbial dynamics and plant metabolic pathways.

Table 3. Interaction effect of various treatments on physicochemical characteristics of guava (*Psidium guajava*) cv. G-27

Treatments	TSS (°Brix)	Titratable acidity (%)	Ascorbic acid (mg/100 g pulp)	Total sugar in fruits (%)	Pulp weight (g)	Seed weight (g)	Pectin (%)	Antioxidant (mg/100 g)	Total phenol (mg/100 g)
T ₁	6.14	0.49	85.83	6.13	103.00	2.45	0.48	58.33	92.67
T ₂	9.69	0.35	135.27	9.62	176.66	3.86	0.75	91.97	146.16
T ₃	9.05	0.28	126.24	8.98	178.43	3.60	0.70	85.88	136.46
T ₄	10.10	0.30	141.03	10.06	173.25	4.01	0.79	96.02	152.49
T ₅	8.20	0.39	114.48	8.15	138.60	3.26	0.64	77.85	123.70
T ₆	10.14	0.31	141.57	10.07	201.61	4.04	0.79	96.24	152.94
T ₇	9.79	0.24	137.15	9.79	204.33	3.93	0.76	93.05	147.76
T ₈	10.37	0.27	144.86	10.33	196.32	4.13	0.81	98.52	156.46
T ₉	8.62	0.30	120.31	8.55	178.36	3.43	0.67	81.79	129.98
T ₁₀	9.17	0.24	128.03	9.11	213.01	3.65	0.71	87.06	138.32
T ₁₁	10.30	0.17	143.79	10.24	230.72	4.10	0.80	97.84	155.43
T ₁₂	11.30	0.20	157.62	11.22	235.03	4.49	0.88	107.21	170.34
T ₁₃	11.13	0.26	155.46	11.07	202.93	4.43	0.87	105.72	167.95
T ₁₄	9.46	0.20	132.21	9.44	206.51	3.76	0.74	90.01	142.89
T ₁₅	10.65	0.14	148.63	10.59	238.00	4.23	0.83	101.12	160.64
T ₁₆	11.48	0.17	160.10	11.39	249.91	4.55	0.89	109.05	173.26
CD (0.05)	1.41	0.018	19.61	1.39	16.82	0.553	0.111	13.41	21.36

TSS; total soluble solids; CD: critical difference. T₁ = (B₀J₀), T₂ = (B₀J₁), T₃ = (B₀J₂), T₄ = (B₀J₃), T₅ = (B₁J₀), T₆ = (B₁J₁), T₇ = (B₁J₂), T₈ = (B₁J₃), T₉ = (B₂J₀), T₁₀ = (B₂J₁), T₁₁ = (B₂J₂), T₁₂ = (B₂J₃), T₁₃ = (B₃J₀), T₁₄ = (B₃J₁), T₁₅ = (B₃J₂); T₁₆ = (B₃J₃).

Acknowledgements

The authors express their sincere gratitude to the Department of Horticulture (Fruit Science), College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya (RVSKVV), Gwalior, for providing the essential facilities and institutional support throughout the study. The authors also acknowledge the Department of Biotechnology, RVSKVV, Gwalior, for its technical assistance. Special thanks are extended to the research supervisors for their valuable guidance, support and encouragement, which significantly contributed to the successful completion of this work.

Authors' contributions

A conducted the experimental trial. PKSG conceived the research idea and designed the study. RC assisted with the laboratory analysis, while RN contributed to the statistical analysis of the data. NB assisted in conducting the experiment and recording observations. MA and FAK contributed to the discussion of the results and the drafting of the final manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used perplexity AI Tool in order to discuss the results of the study with scientific reasoning. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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