

Enhancement of physiological and biochemical attributes of Blackgram (*Vigna mungo* L.) through foliar nutrient supplementation

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Background: Blackgram (*Vigna mungo* L.) is an important pulse crop valued for its nutritional quality, contribution to biological nitrogen fixation and role in sustainable cropping systems. However, its productivity is frequently constrained by inadequate nutrient availability during critical stages of crop growth, particularly flowering and pod development. Foliar nutrient supplementation offers a rapid approach for improving nutrient availability and physiological efficiency by supplying nutrients directly to the foliage.

Methods: The present study evaluated the influence of foliar nutrient supplementation on the physiological and biochemical attributes of blackgram cv. VBN 11 under field conditions. The experiment was conducted in a randomized block design with seven treatments and three replications. Foliar nutrient treatments were applied at 55 days after sowing, and physiological and biochemical observations were recorded at 60 days after sowing.

Results: The treatments significantly influenced total chlorophyll content, chlorophyll stability index, relative water content, soluble protein, proline, catalase and peroxidase activities. Among the treatments, T5 (T2 + NAA 40 ppm + salicylic acid 100 ppm) recorded the highest total chlorophyll content (1.49 mg g⁻¹ FW), chlorophyll stability index (88.3%), relative water content (96.5%), soluble protein content (15.7 mg g⁻¹ FW), proline content (360.1 µg g⁻¹ FW) and peroxidase activity (8.1 nmol min⁻¹ mg protein⁻¹). Principal component analysis further showed that PC1 explained 91.40% of the standardized variance and was positively associated with chlorophyll, CSI, RWC, soluble protein, proline and peroxidase.

Conclusion: The findings demonstrate that foliar nutrient supplementation, particularly the combination of nutrients with NAA and salicylic acid, influenced the physiological and biochemical responses of blackgram cv. VBN 11 under the conditions of the present experiment.

Keywords: Blackgram, VBN 11, foliar nutrition, physiological attributes, chlorophyll, soluble protein, proline

Introduction

Blackgram (*Vigna mungo* L.) is one of the important grain legumes cultivated in India and forms an integral component of pulse-based cropping systems. The crop is valued for its high-quality protein and nutritional importance and contributes to soil fertility through biological nitrogen fixation. Its relatively short duration and adaptability to different production systems make blackgram an important crop for sustainable agricultural production (Perumal et al., 2025). It is rich in nutrients and provides several health benefits (Gunasekaran et al., 2024). Black gram is an excellent source of protein, dietary fiber, and various vitamins and minerals. It is particularly rich in iron, which is important for maintaining healthy blood hemoglobin levels and preventing anemia. The high fiber content supports digestive health and helps regulate blood sugar levels. The legume also provides a good amount of potassium, which is essential for heart and muscle function, as well as magnesium for bone health. Including black gram in diet can contribute to your overall nutrient intake, especially for plant-based sources of protein and essential nutrients. Despite its importance, the productivity of blackgram frequently remains below its attainable potential. Crop performance is influenced by several environmental and management factors, among which nutrient availability during critical growth and reproductive stages is particularly important. Nutrient deficiency can adversely affect leaf development, chlorophyll formation, photosynthetic activity, assimilate production and partitioning towards reproductive organs (Philips et al., 2024). The physiological performance of a crop is closely associated with its ability to capture and utilize solar radiation, maintain adequate leaf water status and sustain photosynthetic activity. Chlorophyll content is an important indicator of the photosynthetic apparatus and nutritional status of plants (Subiramaniyan et al., 2025). Similarly, relative water content provides an indication of plant water status, whereas biochemical attributes such as soluble protein and proline reflect important aspects of plant metabolic activity and physiological adjustment (Senthil et al., 2018). Foliar nutrient application has emerged as an efficient approach for supplementing nutrients during periods of high crop demand. Unlike soil application, foliar application provides nutrients directly to the leaf surface, from where they can be absorbed and utilized relatively rapidly. This approach can therefore complement conventional soil fertilization, particularly when nutrient availability through the root zone is restricted or when the crop has a high nutrient requirement during a specific growth stage (Chandrasekaran et al., 2024). The response to foliar nutrient supplementation may vary with crop species, cultivar, nutrient source, concentration and time of application (Rajeshkumar et al., 2024). Therefore, cultivar-specific evaluation is essential for identifying suitable nutrient-management strategies. Blackgram cv. VBN 11 is an important high-yielding cultivar, and optimization of foliar nutrition may help improve its physiological and biochemical performance. The present study was therefore undertaken to evaluate the effect of different foliar nutrient supplementation treatments on the physiological attributes of blackgram cv. VBN 11 and to identify the most effective treatment for improving physiological and biochemical efficiency.

Materials and Methods

Experimental material

The field experiment was conducted at the experimental farm of the School of Agricultural Sciences, Dhanalakshmi Srinivasan University, Samayapuram, Tiruchirappalli, Tamil Nadu, India. The experimental site was located at approximately 10°55' N latitude and 78°42' E longitude, at an elevation of approximately 78 m above mean sea level. Blackgram (*Vigna mungo* L.) cv. VBN 11 was used as the experimental material.

Crop establishment and agronomic management

Blackgram cv. VBN 11 were sown at a seed rate of 20 kg ha⁻¹ with a spacing of 25 cm × 10 cm. Basal fertilizer was applied at 25 kg N, 50 kg P₂O₅ and 25 kg K₂O ha⁻¹. Weeding and other agronomic practices were carried out uniformly in all treatments. No differential management was imposed among treatments except for the foliar nutrient treatments.

Experimental design and treatments

The experiment consisted of different foliar nutrient supplementation (Table 1) treatments imposed on blackgram cv. VBN 11. The experiment was laid out in a Randomized Block Design (RBD) with three replications. Each experimental plot measured 4 m × 3 m, providing a gross plot area of 12 m². The experiment comprised seven foliar nutrient supplementation treatments, including an untreated control, imposed on blackgram (*Vigna mungo* L.) cv. VBN 11. The treatments were applied as foliar sprays at the specified crop growth stage, while uniform agronomic practices were followed throughout the experimental period. Foliar spray treatments were applied once at 55 days after sowing (DAS). Physiological and biochemical observations were recorded at 60 days after sowing (DAS), i.e. five days after foliar application.

Table 1. Treatment details

S. No	Treatments
1.	Treatment 1 - Control
2.	Treatment 2 - Urea + MOP + Borax + FeSO ₄ + MnSO ₄ + ZnSO ₄ , each at 1% (w/v)
3.	Treatment 3 - Urea + MOP + FeSO ₄ + CuSO ₄ + Borax, each at 1% (w/v)
4.	Treatment 4 - Urea + MOP + MgSO ₄ + Borax + FeSO ₄ + ZnSO ₄ , each at 1% (w/v)
5.	Treatment 5 - (T2 + NAA 40 ppm +SA 100 ppm)
6.	Treatment 6 - (T3 + NAA 40 ppm +SA 100 ppm)
7.	Treatment 7 - (T4 + NAA 40 ppm +SA 100 ppm)

Observations

Total chlorophyll content

Chlorophyll was extracted using 80% acetone, and absorbance was measured at 652 nm using a spectrophotometer (Arnon, 1949).

Chlorophyll stability index

Murphy (1962) was used to calculate the chlorophyll stability index. Heat-treated and control sets of leaf samples were separated. Chlorophyll was extracted using 80% acetone, and absorbance was measured at 652 nm using a spectrophotometer. CSI was expressed as percentage.

Relative water content

$$RWC (\%) = \frac{FW - DW}{TW - DW} \times 100$$

The relative water content was calculated using the Barrs & Weatherley (1962) approach. Following sampling, the fresh weight (FW) of the leaf discs was noted right away. Turgid weight (TW) was obtained by floating the discs on distilled water for four hours. Dry weight (DW) was then determined by oven drying at 80°C.

Soluble protein content

Soluble protein content was estimated by the method of Lowry et al. (1951). Leaf samples were homogenized in phosphate buffer and centrifuged. The supernatant was reacted with alkaline copper reagent followed by Folin–Ciocalteu reagent, and absorbance was measured at 660 nm. Bovine serum albumin (BSA) was used as the standard to express the protein content as mg g⁻¹ fresh weight.

Proline content

The Bates et al. (1973) method was used to determine the proline content. After homogenizing leaf tissue in 3% sulfosalicylic acid, the extract was treated with glacial acetic acid and acid ninhydrin. Following a one-hour incubation period at 100°C, the chromophore was extracted using toluene, and absorbance at 520 nm was recorded. The unit of measurement for proline content was µg g⁻¹ fresh weight.

Catalase activity

In accordance with Rubio et al. (2002), catalase activity was calculated. A spectrophotometer was used to track the breakdown of H₂O₂ for one minute at 240 nm. Catalase activity was measured in µg H₂O₂ g⁻¹ min⁻¹ of the sample (Aebi, 1984).

Peroxidase activity

The estimation of peroxidase activity was based on Hammerschmidt et al. (1982). The components of the reaction mixture were hydrogen peroxide, phosphate buffer, guaiacol, and enzyme extract. At 420 nm, an increase in absorbance was noted, and the activity was measured in nmol min⁻¹ mg protein⁻¹.

Statistical analysis

The experiment was conducted in a Randomized Block Design (RBD) with seven treatments and three replications. The data recorded for total chlorophyll content, chlorophyll stability index (CSI), relative water content (RWC), soluble protein, proline, catalase and peroxidase activity were subjected to analysis of variance (ANOVA) appropriate for RBD. Treatment means were compared using the critical difference (CD) at the 5% probability level when the treatment effect was significant. The standard error of difference (SE(d)) was calculated to assess the precision of comparison between treatment means. The significance of treatment effects was determined using the F-test at $P = 0.05$. Where significant differences occurred, means were separated using Fisher's least significant difference (LSD/CD) test. For multivariate interpretation, Principal Component Analysis (PCA) was performed on the standardized treatment means of the physiological and biochemical traits, and principal components were interpreted based on their eigenvalues, variance explained and trait loadings.

Results and Discussion

Treatment effects were highly significant for all seven measured traits ($P < 0.001$), whereas the block effects were non-significant for all traits ($P > 0.05$) (Table 2).

Table 2. ANOVA Summary

Trait	Treatment df.	Error df.	Treatment MS	Error MS	F-value	P-value	SE(d.)	CD (5%)
Total chlorophyll	6	12	0.319543	0.000702164	455.083	1.95e-13	0.0216	0.0471
CSI	6	12	33.4971	2.00887	16.675	3.49e-05	1.1573	2.5214
RWC	6	12	105.674	2.30517	45.842	1.39e-07	1.2397	2.7010
Soluble protein	6	12	9.05429	0.180484	50.167	8.32e-08	0.3469	0.7558
Proline	6	12	9598.37	130.947	73.299	9.39e-09	9.3434	20.3574
Catalase	6	12	867.25	17.579	49.335	9.15e-08	3.4233	7.4588
Peroxidase	6	12	2.64714	0.0787323	33.622	7.95e-07	0.2291	0.4992

RBD analysis of variance revealed significant treatment effects ($P < 0.001$) for total chlorophyll, chlorophyll stability index (CSI), relative water content (RWC), soluble protein, proline, catalase and peroxidase activities. The corresponding F-values were 455.083, 16.675, 45.842, 50.167, 73.299, 49.335 and 33.622, respectively. Error mean squares were 0.000702, 2.008866, 2.305170, 0.180484, 130.947327, 17.578951 and 0.078732, respectively. Total chlorophyll, CSI and RWC varied markedly among the foliar nutrient treatments (Table 3). T5 (T2 + NAA 40 ppm + SA 100 ppm) recorded the highest chlorophyll content (1.49 mg g⁻¹ FW), significantly superior to all other treatments. T7 (0.94 mg g⁻¹ FW) and T3 (0.75 mg g⁻¹ FW) followed T5. CSI was highest in T5 (88.3%), followed by T7 (86.7%) and T3 (85.6%). T5 and T7 were statistically at par for CSI based on the calculated CD (2.52%). RWC was maximum in T5 (96.5%), followed by T7 (94.5%) and T3 (92.6%). T5 and T7 were also statistically at par for RWC, with a CD of 2.70%. The enhanced chlorophyll and RWC may reflect improved nutrient availability and physiological regulation by NAA and salicylic acid. Similar improvements in chlorophyll and RWC following foliar nutrient and salicylic-acid application have been reported in blackgram (Banerjee et al., 2023; Rawat et al., 2023). Overall, T5 showed the most favourable physiological response among the treatments.

Table 3. Effect of foliar nutrient supplementation on total chlorophyll content, relative water content and chlorophyll stability index of blackgram cv. VBN 11

Treatment	Total Chl. Content (mg g ⁻¹ FW)	CSI (%)	RWC (%)
T ₁	0.46 ^a	78.5 ^a	78.2 ^a
T ₂	0.70 ^d	83.8 ^b	90.5 ^{bc}
T ₃	0.75 ^b	85.6 ^{bd}	92.6 ^{bd}
T ₄	0.72 ^{bd}	84.8 ^{bd}	91.1 ^{bc}
T ₅	1.49 ^c	88.3 ^c	96.5 ^c
T ₆	0.69 ^d	81.2 ^c	88.6 ^c
T ₇	0.94 ^c	86.7 ^{de}	94.5 ^{de}
SE(d)	0.0216	1.1573	1.2397
CD (P=0.05)	0.0471	2.5214	2.7010

Soluble protein and proline contents varied markedly among the foliar nutrient treatments. T5 (T2 + NAA 40 ppm + SA 100 ppm) recorded the highest soluble protein content (15.7 mg g⁻¹ FW), significantly superior to all other treatments based on the CD of 0.756. T7 (14.0 mg g⁻¹ FW) and T3 (13.9 mg g⁻¹ FW) followed T5, while T1 recorded the lowest

value (10.3 mg g⁻¹ FW). Proline content was also maximum in T5 (360.1 µg g⁻¹ FW), followed by T7 (320.3) and T3 (284.1 µg g⁻¹ FW). The difference between T5 and T7 (39.8 µg g⁻¹ FW) exceeded the CD of 20.36, indicating a significant difference (Table 4). The higher soluble protein may reflect improved nutrient availability and enhanced metabolic activity under foliar supplementation. Increased proline accumulation may indicate enhanced osmotic adjustment and physiological adaptation to environmental stress. The combined application of nutrients with NAA and salicylic acid in T5 may have promoted protein synthesis and stress-responsive metabolism. Similar improvements in biochemical traits following nutrient and salicylic-acid application have been reported in blackgram (Banerjee et al., 2023 and Rawat et al., 2023). Overall, T5 was the most effective treatment for improving soluble protein and proline contents in blackgram cv. VBN 11.

Table 4. Effect of foliar nutrient supplementation on soluble protein content and proline content of blackgram cv. VBN 11

Treatment	Soluble Protein Content (mg g ⁻¹ FW)	Proline Content (µg g ⁻¹ FW)
T ₁	10.3 ^a	180.2 ^a
T ₂	12.8 ^b	259.5 ^d
T ₃	13.9 ^{cf}	284.1 ^b
T ₄	13.2 ^{bc}	267.0 ^{bd}
T ₅	15.7 ^d	360.1 ^c
T ₆	11.7 ^e	252.0 ^d
T ₇	14.0 ^f	320.3 ^e
SE(d)	0.3469	9.3434
CD (P=0.05)	0.7558	20.3574

Catalase and peroxidase activities showed considerable variation among the foliar nutrient treatments (Table 5). Catalase activity was highest in the control (T₁; 130.1 µg H₂O₂ g⁻¹ min⁻¹), whereas the lowest activity was recorded in T₅ (81.5 µg H₂O₂ g⁻¹ min⁻¹). T₇ and T₃ also recorded comparatively lower catalase activities. In contrast, peroxidase activity was highest in T₅ (8.1 nmol min⁻¹ mg protein⁻¹), compared with 5.5 nmol min⁻¹ mg protein⁻¹ in the control. The contrasting responses of catalase and peroxidase indicate a differential regulation of antioxidant enzyme activities in response to the combined nutrient, NAA and salicylic acid treatment. However, the lower catalase activity alone does not necessarily indicate enhanced antioxidant capacity. The increased peroxidase activity may suggest greater involvement of peroxidase-mediated H₂O₂ metabolism under T₅, although further assessment of additional antioxidant enzymes and oxidative stress markers would be required to establish an overall enhancement of antioxidant defence.

Table 5. Effect of foliar nutrient supplementation on catalase and peroxidase activities of blackgram cv. VBN 11

Treatment	Catalase (µg H ₂ O ₂ g ⁻¹ min ⁻¹)	Peroxidase activity (nmol min ⁻¹ mg protein ⁻¹)
T ₁	130.1 ^d	5.5 ^c
T ₂	113.9 ^b	6.1 ^d
T ₃	101.7 ^a	7.2 ^{ac}
T ₄	110.3 ^b	6.8 ^a
T ₅	81.5 ^c	8.1 ^b
T ₆	123.1 ^d	5.9 ^{cd}
T ₇	92.6 ^c	7.5 ^c
SE(d)	3.4233	0.2291
CD (P=0.05)	7.4588	0.4992

Principal component analysis

Table 6. Eigenvalues and percentage variance explained by principal components

Principal component	Eigenvalue	Variance explained (%)	Cumulative variance (%)
PC1	6.7179	91.40	91.40
PC2	0.3378	4.60	96.00
PC3	0.1439	1.96	97.95
PC4	0.1133	1.54	99.50
PC5	0.0282	0.38	99.88
PC6	0.0079	0.11	99.99
PC7	0.0009	0.01	100.00

Principal component analysis (PCA) was performed for total chlorophyll, chlorophyll stability index (CSI), relative water content (RWC), soluble protein, proline, catalase and peroxidase. PC1 explained 91.40% of the standardized variance and PC2 explained 4.60%; together they accounted for 96.00% of the total variance (Table 6).

Table 7. PCA loading matrix (correlations between standardized traits and principal components)

Trait	PC1	PC2	PC3	PC4
Total chlorophyll	0.908	0.355	0.217	0.052
CSI	0.965	-0.229	-0.025	0.095
RWC	0.941	-0.274	0.090	-0.174
Soluble protein	0.991	-0.064	-0.011	0.044
Proline	0.990	-0.001	0.094	-0.062
Catalase	-0.925	-0.253	0.238	0.155
Peroxidase	0.970	-0.013	-0.124	0.191

Table 8. PCA scores of treatments for the first four principal components

Treatment	PC1	PC2	PC3	PC4
T1	-4.285	0.589	-0.152	0.422
T2	-0.761	-0.368	0.200	-0.305
T3	0.840	-0.432	-0.451	0.041
T4	-0.049	-0.447	-0.133	0.092
T5	4.124	0.839	0.283	0.168
T6	-1.815	-0.079	0.579	-0.284
T7	1.945	-0.102	-0.327	-0.134

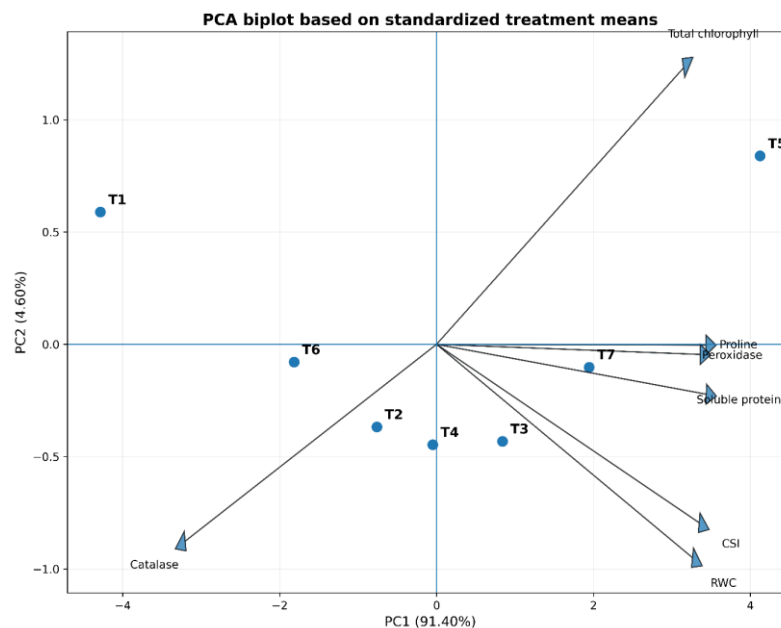


Figure 1. PCA biplot

The PC1 axis was strongly and positively associated with total chlorophyll (loading = 0.908), CSI (0.965), RWC (0.941), soluble protein (0.991), proline (0.990) and peroxidase (0.970), whereas catalase showed a strong negative loading (−0.925) (Table 7). Thus, PC1 represents a broad physiological and biochemical response gradient in which the treatments with higher chlorophyll, water status, soluble protein, proline and peroxidase tended to occur at the positive end, while catalase contributed to the opposite direction. Similar multivariate associations among physiological and biochemical traits have been reported under drought and water-deficit conditions, where chlorophyll, RWC, proline, soluble proteins and antioxidant enzymes collectively contributed to the discrimination of stress responses and tolerant plant materials (Santos et al., 2023; Alzahrani, 2024; Iqbal & Yaning, 2024). PC2 contributed 4.60% of the variance and was characterized principally by total chlorophyll (0.355), RWC (−0.274), catalase (−0.253) and CSI (−0.229). Because PC2 explained a relatively small proportion of the variance, it should be interpreted as a secondary source of differentiation rather than a dominant treatment-response axis. The use of PCA to integrate multiple physiological and biochemical

indicators is increasingly reported in drought-tolerance studies, where the first few components are used to identify major trait associations while secondary components capture additional sources of treatment or genotype differentiation (Santos et al., 2023). Treatment centroids placed T5 at the positive extreme of PC1 (4.124), whereas T1 occurred at the negative extreme (−4.285) (Table 8). T7 (1.945) and T3 (0.840) were also positioned on the positive side of PC1, while T2, T4 and T6 remained closer to the negative side. This multivariate pattern is broadly consistent with the univariate treatment means, particularly the strong response of T5 across chlorophyll, CSI, RWC, soluble protein, proline and peroxidase. Recent studies have similarly demonstrated that combinations of photosynthetic pigments, plant water status, osmolytes and antioxidant enzymes provide complementary information for characterizing plant responses to water deficit. For example, Iqbal & Yaning (2024) reported that PCA grouped proline, soluble protein, total chlorophyll and RWC among the physiological and biochemical traits closely associated with improved drought performance in quinoa. Likewise, rapeseed genotypes exhibiting better photosynthetic performance, water status, pigment concentration and biochemical attributes were effectively differentiated using PCA and related multivariate approaches.

The positive association of proline with the major PCA axis is also physiologically meaningful because proline accumulation is widely recognized as an important component of osmotic adjustment and stress acclimation. Recent studies have reported enhanced proline accumulation together with improved antioxidant activity and maintenance of chlorophyll and water status in plants exhibiting greater tolerance to drought or combined abiotic stress (Khan et al., 2024; Zaman et al., 2024). The strong loading of peroxidase on PC1 further indicates the contribution of antioxidant defence to the multivariate treatment response, consistent with recent evidence showing coordinated changes in peroxidase, catalase, proline and photosynthetic traits under water deficit. Overall, the PCA indicates that the major variation among the treatments was associated with an integrated physiological and biochemical response involving photosynthetic pigment retention, cellular water status, osmotic adjustment, soluble protein accumulation and antioxidant defence. The close positioning of T5 toward the positive end of PC1 therefore reflects its comparatively strong multivariate response across these measured attributes, while the separation of T1 toward the negative end indicates a contrasting overall physiological profile. Similar integration of multiple physiological and biochemical traits through PCA has been demonstrated in recent drought-response studies in cereals, legumes and horticultural crops, supporting the usefulness of multivariate approaches for interpreting complex plant stress responses.

Conclusion

The study demonstrated that foliar nutrient supplementation influenced the physiological and biochemical attributes of blackgram cv. VBN 11 under the experimental field conditions. Among the evaluated treatments, T5 (T2 + NAA 40 ppm + SA 100 ppm) recorded higher numerical values for total chlorophyll, chlorophyll stability index, relative water content, soluble protein, proline and peroxidase activity, together with lower catalase activity. The ANOVA indicated significant treatment effects for all measured physiological and biochemical traits. The contrasting responses of catalase and peroxidase suggest differential regulation of antioxidant enzyme activities following combined nutrient and growth-regulator application. These findings indicate that foliar nutrient supplementation, particularly in combination with NAA and salicylic acid, influenced the physiological and biochemical responses of blackgram cv. VBN 11 under the conditions of the present experiment. Further studies involving multiple seasons, concentrations and additional oxidative-stress indicators are required to validate these responses.

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Author contributions

Vinothini Seemaichamy, YogeshKhan Mukesh and Yuvaraj Srinivasan contributed to field experimentation, data collection and laboratory observations. Ashok Subiramaniyan, Chozhan Kannan, Chandrasekaran Perumal and Selvakumar Gurunathan contributed to conceptualization, supervision, interpretation of the results and critical revision of the manuscript. All authors read and approved the final manuscript.

Competing interest

The authors declare no competing interest.

Ethics approval

Not applicable.

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AI tool usage declaration

The authors used Grammarly AI tool for improving grammar, language clarity, and spelling.

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