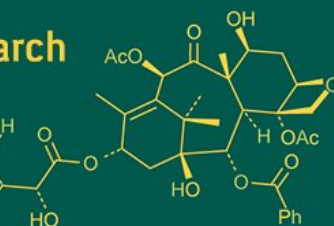
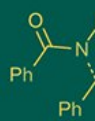


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(5): 986-993
www.biochemjournal.com
Received: 10-03-2026
Accepted: 12-04-2026

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Multivariate correlation and clustering analysis reveal nutrient-physiological interactions in guava under nano zinc oxide applications

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5SI.8501>

Abstract

Correlation and multivariate statistical analyses were employed to elucidate nutrient-physiological interactions in Guava under foliar application of conventional zinc sulphate and biogenic zinc oxide nanoparticles (ZnO-NPs). The experiment was conducted during 2023-24 and 2024-25 using two guava cultivars, Lucknow-49 and Lalit, with treatments comprising ZnSO₄ at 5,000 and 7,500 ppm and nano ZnO at 100, 150, 200 and 250 ppm applied at flowering and one month later. Correlation heatmap and hierarchical clustering analyses revealed strong positive associations among zinc accumulation, photosynthetic rate, chlorophyll fluorescence, stomatal conductance, transpiration and major nutrient concentrations, whereas phosphorus exhibited an antagonistic association with zinc uptake. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) effectively differentiated treatments and cultivars based on nutrient and physiological traits. Variable importance projection (VIP) scores identified Zn, Ca, B, photosynthetic rate and chlorophyll fluorescence as the major contributors governing treatment discrimination and physiological efficiency. Nano ZnO at 200-250 ppm formed distinct clusters associated with enhanced growth and physiological performance, recording maximum annual shoot growth (52.08 cm), plant height increase (16.56%), leaf area (52.55 cm²), chlorophyll content (SPAD 20.72), and net photosynthetic rate (12.30 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Network analysis further demonstrated strong synergistic relationships among Zn, Ca and B under nano zinc application. Overall, multivariate analyses successfully characterized complex nutrient-physiological relationships and demonstrated that foliar application of biogenic ZnO-NPs at 200-250 ppm improved nutrient use efficiency and physiological performance in guava under semi-arid conditions.

Keywords: Correlation analysis, PCA, PLS-DA, hierarchical clustering, nano zinc oxide, nutrient interaction, physiological traits, guava

1. Introduction

Fruits play a vital role in human nutrition by supplying essential vitamins, minerals, dietary fibre and bioactive compounds that contribute to food security and human health. Among tropical fruit crops, Guava occupies a prominent position due to its high nutritional value, adaptability and economic importance. Commonly referred to as the “Apple of the Tropics” or “Poor Man’s Apple”, guava is rich in vitamin C, vitamin A, minerals, antioxidants and dietary fibre (Yadav *et al.*, 2022) [19]. Native to Central and South America, guava is now extensively cultivated across tropical and subtropical regions worldwide. India is the largest producer of guava, with major cultivation concentrated in Uttar Pradesh, Bihar, Madhya Pradesh, Andhra Pradesh and West Bengal (NHB, 2021-22). Owing to its hardy nature and wide adaptability, guava is considered an important fruit crop for semi-arid and resource-poor regions. However, sustained productivity and fruit quality largely depend on balanced nutrient management, particularly micronutrient availability. Among micronutrients, zinc (Zn) is one of the most critical elements regulating plant growth and metabolism. Zinc is involved in chlorophyll biosynthesis, enzyme activation, protein synthesis, auxin metabolism and photosynthetic regulation (Babu *et al.*, 2022) [3]. Deficiency of zinc results in reduced shoot growth, chlorosis, impaired photosynthetic activity, poor fruit development and inferior fruit quality. Zinc deficiency has emerged as a widespread nutritional disorder in Indian soils, especially under alkaline and calcareous conditions where zinc fixation reduces

nutrient availability (Takkar *et al.*, 2018) [17]. Conventional zinc fertilization through zinc sulphate (ZnSO_4) often exhibits low nutrient use efficiency because of leaching, fixation and limited foliar absorption, necessitating the development of more efficient nutrient delivery systems (Patel *et al.*, 2022) [10].

Nanotechnology has emerged as a promising approach for improving nutrient management in agriculture. Nano-fertilizers possess unique physicochemical properties such as higher surface area, increased reactivity, enhanced solubility and controlled nutrient release, which improve nutrient uptake and utilization efficiency (Solanki *et al.*, 2015) [16]. Among nano-fertilizers, zinc oxide nanoparticles (ZnO -NPs) have gained considerable attention due to their ability to enhance photosynthetic efficiency, nutrient accumulation, enzymatic activity and crop productivity at comparatively lower application rates (Dimkpa *et al.*, 2017; Raliya *et al.*, 2015) [4, 12]. Foliar application of ZnO -NPs has also been reported to improve physiological performance and nutrient use efficiency in several horticultural crops under stress-prone environments.

Despite increasing interest in nano-enabled fertilization, systematic information regarding nutrient-physiological interactions induced by ZnO nanoparticles in guava remains limited, particularly under semi-arid agro-climatic conditions of the Bundelkhand region. Moreover, conventional statistical approaches often fail to comprehensively explain the complex relationships among nutrient dynamics, physiological responses and treatment discrimination. Advanced multivariate statistical tools such as correlation analysis, hierarchical clustering, principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and variable importance projection (VIP) analysis provide robust approaches for understanding multidimensional interactions among plant traits and identifying key contributing variables. Therefore, the present investigation was undertaken to evaluate the influence of foliar-applied conventional zinc sulphate and biogenic zinc oxide nanoparticles on nutrient dynamics and physiological responses of guava cultivars using correlation and multivariate analytical approaches. The study aimed to identify key nutrient contributors regulating physiological efficiency and to elucidate the interrelationships among growth, gas exchange and nutrient accumulation traits under nano zinc fertilization.

2. Materials and Methods

2.1 Experimental site and treatment details

The field experiment was conducted during 2023-24 and 2024-25 at the Experimental Farm of the Department of Fruit Science, Rani Lakshmi Bai Central Agricultural University, Jhansi (25.31°N, 78.33°E; 285 m amsl), located in the semi-arid Bundelkhand region of Uttar Pradesh, India. The experimental site possessed sandy loam soil and experiences a semi-arid subtropical climate characterized by hot summers, cold winters and average annual rainfall of approximately 714.2 mm, with most precipitation occurring during the southwest monsoon season. Two guava cultivars, Guava Lucknow-49 (V_1) and Guava Lalit (V_2), were subjected to seven zinc treatments comprising control (N_0), ZnSO_4 at 5,000 ppm (N_1), ZnSO_4 at 7,500 ppm (N_2), and nano ZnO at 100 ppm (N_3), 150 ppm (N_4), 200 ppm (N_5) and 250 ppm (N_6). Foliar sprays were applied twice, at flowering and one month later during mrig bahar stage, resulting in 14

cultivar $\times$ nutrient combinations arranged in a Factorial Randomized Block Design (FRBD) with three replications.

2.2 Synthesis and characterization of ZnO nanoparticles

Biogenic zinc oxide nanoparticles (ZnO -NPs) were synthesized using 0.01 M zinc sulphate as precursor and fresh Sahiwal cow urine as reducing and stabilizing agent. The reaction mixture was centrifuged, washed with distilled water and lyophilized to obtain nanoparticle powder. Dynamic light scattering (DLS) analysis conducted at IGFRJ-Jhansi revealed an average hydrodynamic diameter of 99.1 nm with low polydispersity, while scanning electron microscopy (SEM) analysis performed at CSIR-National Physical Laboratory, New Delhi confirmed the formation of flower-like nanostructures with radially arranged petal-like morphology, indicating successful nanoscale synthesis suitable for foliar application.

2.3 Morpho-physiological and nutrient observations

Morphological observations included annual shoot growth, plant height increment and leaf area. Annual shoot growth was measured from the point of emergence to the apex at fruit set stage, while plant height increment was calculated on percentage basis. Leaf area was determined using a digital leaf area meter from mature leaves collected from tagged plants. Chlorophyll content was estimated using a SPAD-502 Plus chlorophyll meter, whereas chlorophyll fluorescence (F_v/F_m) was recorded using a pulse amplitude-modulated fluorometer (FMS1, Hansatech, UK). Gas exchange parameters including net photosynthetic rate, stomatal conductance, transpiration rate and internal CO_2/O_2 concentration were measured using a portable infrared gas analyser system (Li-Cor 6200, Li-Cor Biosciences, USA) between 9:00 and 11:00 AM on fully expanded mature leaves. For nutrient analysis, recently matured leaves and commercially mature fruits were collected, washed thoroughly, oven dried at $65 \pm 2^\circ\text{C}$ and ground into fine powder. Samples were digested using triacid and diacid digestion methods for determination of nitrogen, phosphorus, potassium, calcium, sulphur, zinc and boron using standard analytical procedures involving spectrophotometry, flame photometry and atomic absorption spectrophotometry following Jackson (1973) [17].

2.4 Statistical and multivariate analysis

Successive year data were subjected to analysis of variance (ANOVA) for Factorial Randomized Block Design using R software (v4.3.2) with the agricolae package. Treatment means were compared using Least Significant Difference (LSD) test at $p \leq 0.05$. To elucidate interrelationships among morpho-physiological and nutrient traits, Pearson's correlation analysis and hierarchical clustering were performed using OriginPro 2021. Principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and variable importance projection (VIP) analyses were conducted using MetaboAnalyst 5.0 to identify key variables contributing to treatment discrimination and nutrient-physiological interactions under nano zinc application.

3. Results and Discussion

3.1 Correlation analysis of morpho-physiological traits

Pearson's correlation analysis revealed strong interrelationships among growth, photosynthetic and gas

exchange parameters of Guava under foliar zinc treatments. Annual shoot growth, plant height increment and leaf area exhibited significant positive correlations with chlorophyll content, chlorophyll fluorescence (Fv/Fm), photosynthetic rate and CO₂/O₂ ratio, indicating that enhanced photosynthetic efficiency directly contributed to improved vegetative growth. Similarly, photosynthetic rate showed a strong positive association with stomatal conductance and transpiration rate, reflecting coordinated regulation of gas exchange processes. The correlation heatmap further demonstrated close clustering among chlorophyll content, photosynthetic rate, stomatal conductance and transpiration (Fig. 1D), suggesting synchronized physiological functioning under nano ZnO application. In contrast, phosphorus exhibited a negative association with zinc accumulation, indicating Zn-P antagonism under higher zinc concentrations. These relationships collectively suggest that nano ZnO-mediated enhancement of nutrient availability improved carbon assimilation, chloroplast activity and overall physiological efficiency.

The observed enhancement in growth and physiological traits under nano ZnO treatments may be attributed to improved foliar absorption, greater surface reactivity and higher bioavailability of nanoparticles compared with conventional ZnSO₄. Zinc acts as an essential cofactor for enzymes involved in chlorophyll biosynthesis, auxin metabolism, protein synthesis and photosynthetic regulation, thereby improving plant vigour and physiological performance (Alloway, 2008; Laware and Raskar, 2014)^[1, 8]. Similar positive associations among zinc nutrition, photosynthesis and vegetative growth have also been reported in fruit crops and vegetables under nano-fertilizer applications (Raliya *et al.*, 2015; Dimkpa *et al.*, 2017)^[12, 4].

3.2 Hierarchical clustering and nutrient interaction analysis

Hierarchical clustering analysis of leaf and fruit nutrient profiles revealed distinct grouping patterns among macro- and micronutrients under different zinc treatments (Fig. 2A). Strong positive associations were observed among potassium, nitrogen, calcium, sulphur and boron in both leaf and fruit tissues, indicating coordinated nutrient assimilation and synergistic interactions under nano ZnO application. Potassium in leaves and fruits showed close clustering with nitrogen, calcium and boron, highlighting its central role in nutrient mobilization and physiological regulation.

Conversely, zinc accumulation in leaves and fruits exhibited negative correlations with phosphorus, confirming antagonistic Zn-P interactions under elevated zinc concentrations. The correlation network plot further supported these findings, where Zn formed separate negatively associated nodes, while K, Ca, N and B formed strongly interconnected positive clusters (Fig. 2B). These interactions suggest that nano ZnO not only enhanced zinc accumulation but also altered overall nutrient dynamics and metabolic coordination within guava plants.

The enhanced accumulation of nitrogen, potassium, calcium and sulphur under nano ZnO treatments may be associated with improved membrane permeability, activation of nutrient transporters and enhanced enzymatic activity induced by zinc. Nano-sized particles possess greater penetration ability and controlled nutrient release characteristics, facilitating efficient nutrient uptake and translocation (Rizwan *et al.*, 2019; Semida *et al.*, 2021)^[14].

^[15]. However, excessive zinc accumulation may interfere with phosphorus uptake due to competition at transport sites and formation of insoluble zinc phosphate complexes, as also reported by Ahmed *et al.* (2023)^[2] and Gitika *et al.* (2019)^[6].

3.3 Principal component analysis (PCA) of morpho-physiological traits

Principal component analysis (PCA) effectively discriminated nutrient treatments and guava cultivars based on morpho-physiological attributes (Fig. 1B and 1E). The first two principal components explained a major proportion of total variability, clearly separating nano ZnO treatments from conventional ZnSO₄ and untreated control. Treatments receiving nano ZnO at 200 and 250 ppm formed distinct clusters associated with enhanced photosynthetic rate, chlorophyll content, plant height and stomatal conductance, whereas control treatments clustered separately with comparatively poor physiological performance (Fig. 1B and 1E).

The PCA biplot demonstrated strong positive loading of chlorophyll fluorescence, photosynthetic rate, transpiration and plant growth traits towards higher nano ZnO concentrations, indicating that these variables contributed substantially to treatment differentiation (Fig. 1C). The separation of treatments in multivariate space confirms the strong influence of nano ZnO on nutrient-mediated physiological regulation and demonstrates the effectiveness of PCA in summarizing multidimensional trait variation. Similar multivariate discrimination patterns under nano nutrient applications have been reported in tomato, wheat and brinjal crops (Patel *et al.*, 2019; Rasouli *et al.*, 2022)^[11, 13].

3.4 PLS-DA and VIP analysis of nutrient profiles

Partial Least Squares Discriminant Analysis (PLS-DA) further validated treatment-wise discrimination of nutrient profiles in guava cultivars (Fig. 3A-F). In cv. Lucknow-49, Component 1 explained 82.0% of total variance, whereas in cv. Lalit, the first component alone contributed 88.5% variance, indicating strong treatment separation based on nutrient accumulation patterns. High-dose nano ZnO treatments (200 and 250 ppm) formed distinct clusters separated from control and lower concentration treatments, confirming substantial modification of nutrient profiles under nano zinc fertilization (Fig. 3A, 3B, 3D and 3E).

Variable Importance Projection (VIP) scores identified zinc, calcium and boron as the most influential variables governing treatment discrimination, with VIP values exceeding 1.0. Phosphorus exhibited comparatively lower discriminatory contribution due to its antagonistic association with zinc accumulation (Fig. 3C and 3F). The PLS biplot revealed strong positive association of Zn, Ca and B vectors with high-dose nano ZnO treatments, emphasizing their critical role in physiological regulation and nutrient interaction.

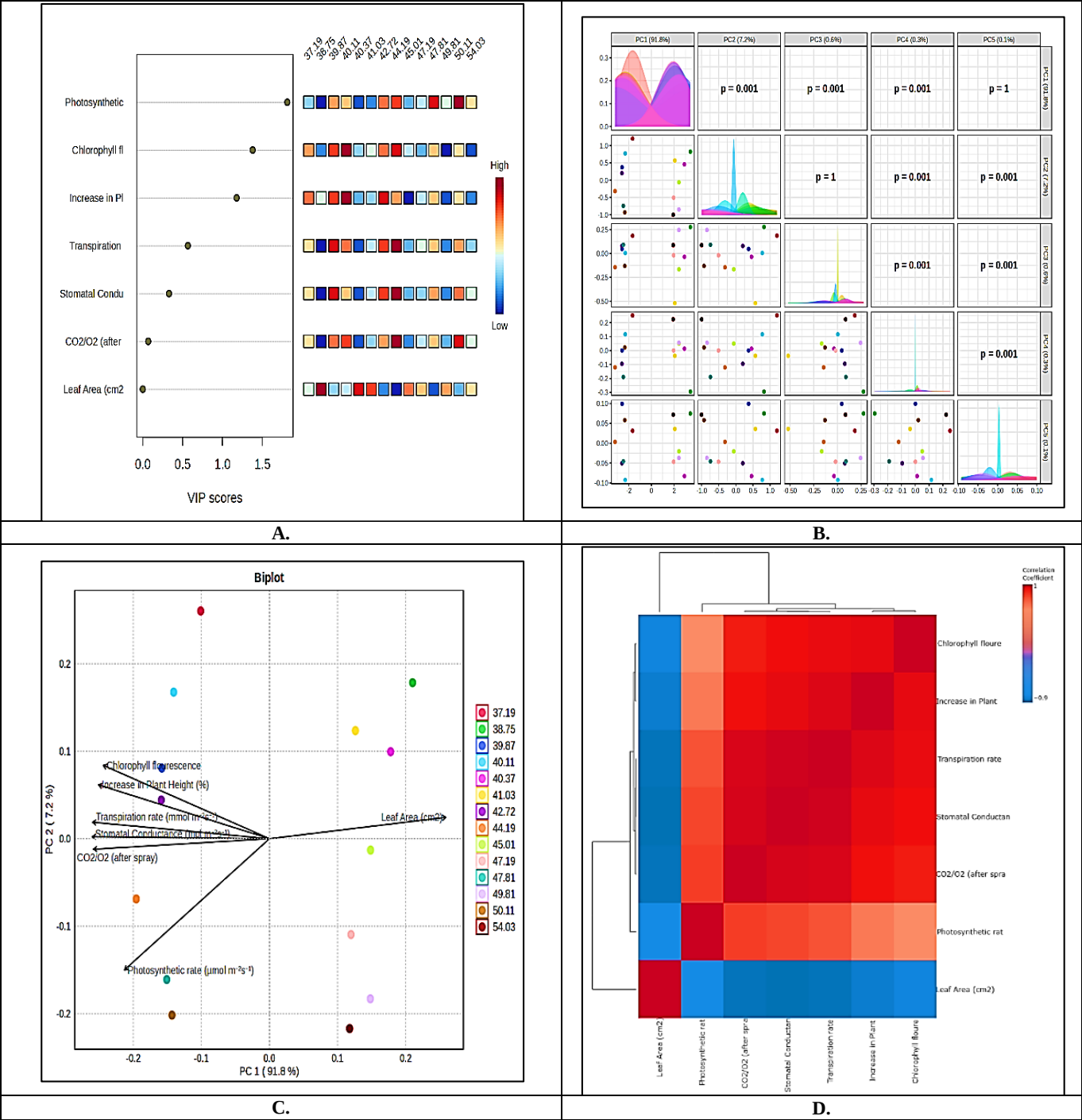
These findings indicate that nano ZnO significantly modulated nutrient dynamics and physiological efficiency in guava through coordinated enhancement of micronutrient accumulation and gas exchange processes. Cultivar Lalit exhibited superior nutrient assimilation and physiological responsiveness, whereas Lucknow-49 showed stronger vegetative growth responses, reflecting genotype-dependent nutrient utilization efficiency under nano zinc fertilization.

Similar genotype-dependent nutrient discrimination patterns using PLS-DA and PCA approaches have been reported in horticultural crops under micronutrient management studies (Tryfon *et al.*, 2023; Guo *et al.*)^[185].

3.5 Integrated multivariate interpretation

Integrated correlation, clustering and multivariate analyses collectively demonstrated that foliar application of nano ZnO at 200-250 ppm substantially improved nutrient accumulation, photosynthetic efficiency and physiological performance in guava. Strong positive interactions among zinc, calcium, boron, chlorophyll content, photosynthetic rate and stomatal conductance suggest coordinated metabolic regulation under nano zinc nutrition. The clear

separation of nano ZnO treatments in PCA and PLS-DA score plots further confirms their distinct physiological and nutritional influence compared with conventional zinc sulphate. Overall, the multivariate analytical approaches successfully characterized complex nutrient-physiological relationships and identified Zn, Ca and B as key contributors governing treatment differentiation and physiological efficiency in guava under semi-arid conditions. The findings further support the utility of advanced multivariate tools in deciphering nutrient interaction networks and physiological responses under nano-enabled fertilization strategies (Dimkpa *et al.*, 2019; Semida *et al.*, 2021)^[4, 15].



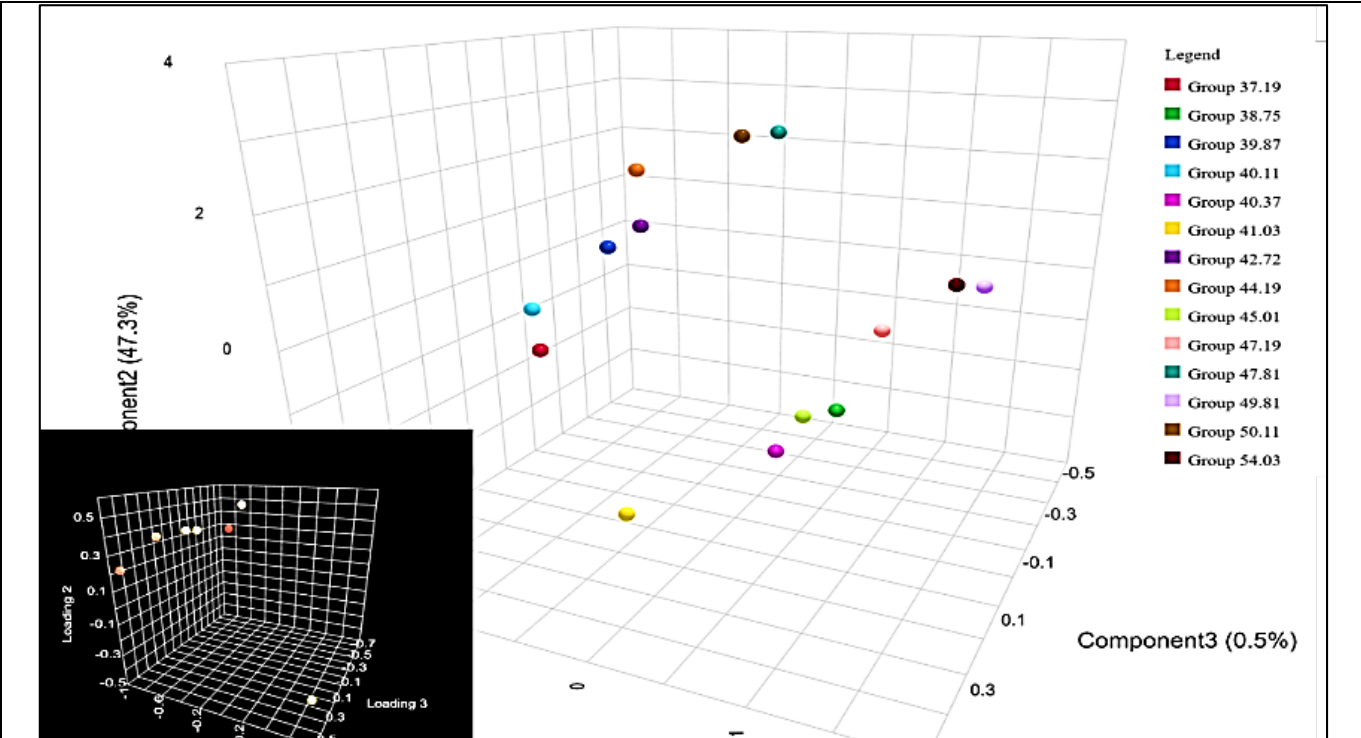
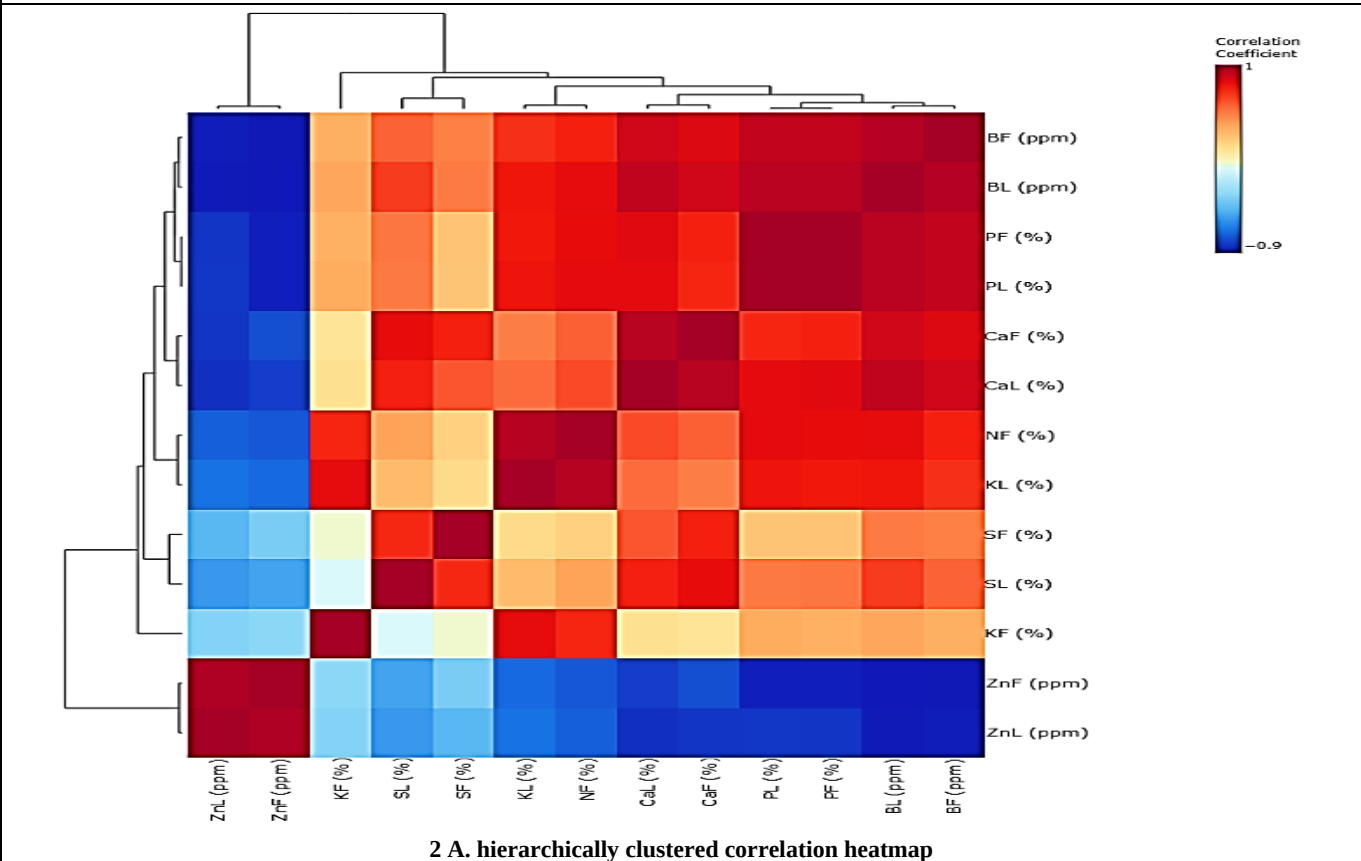
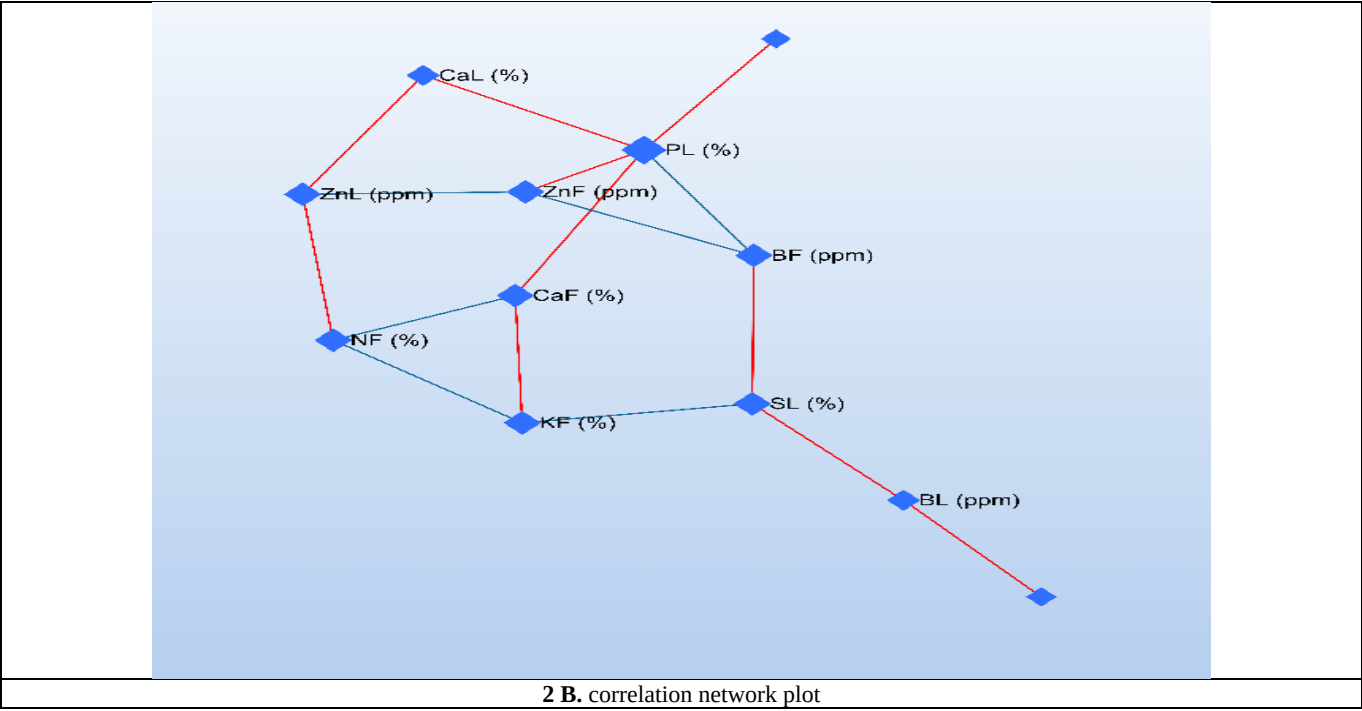
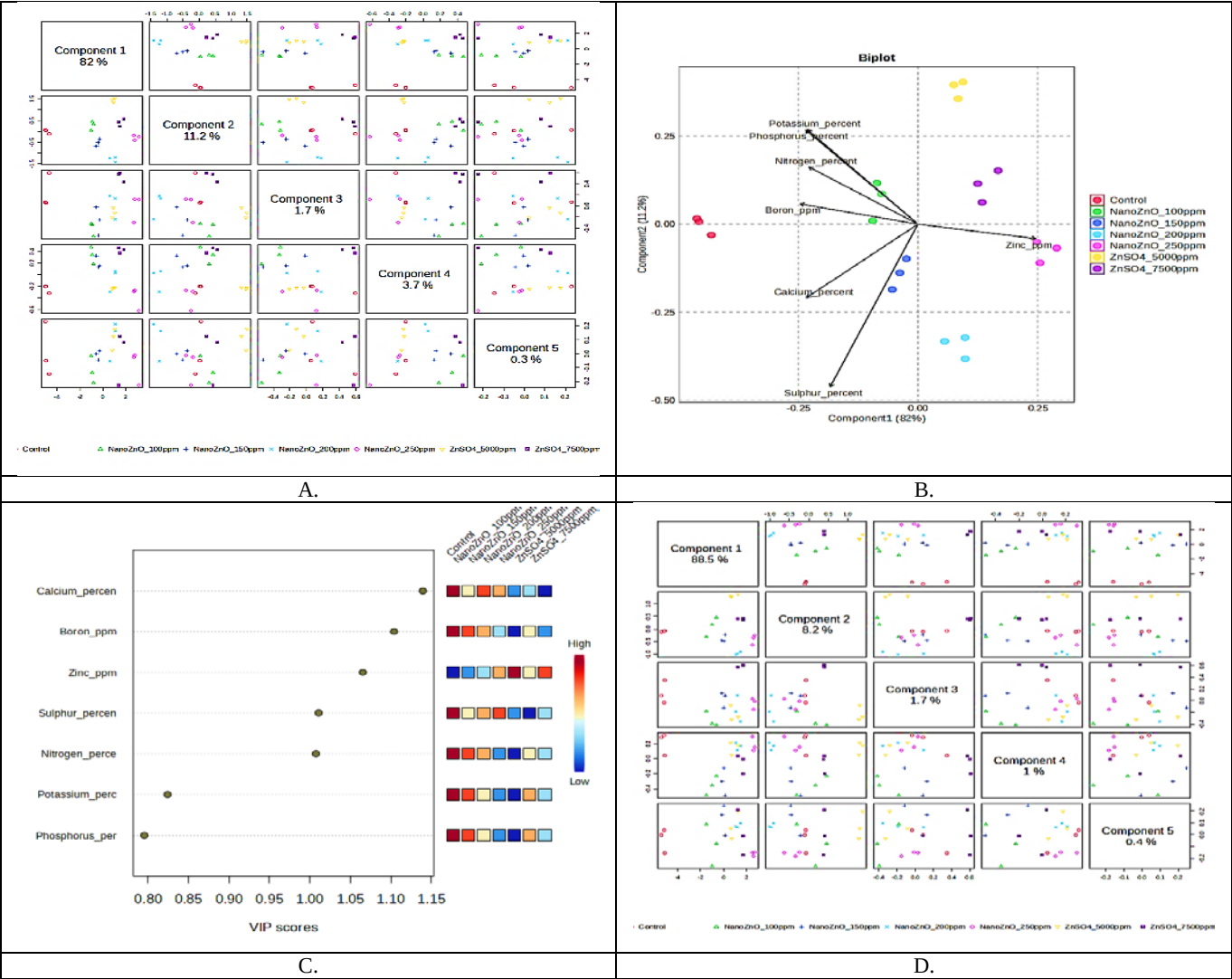


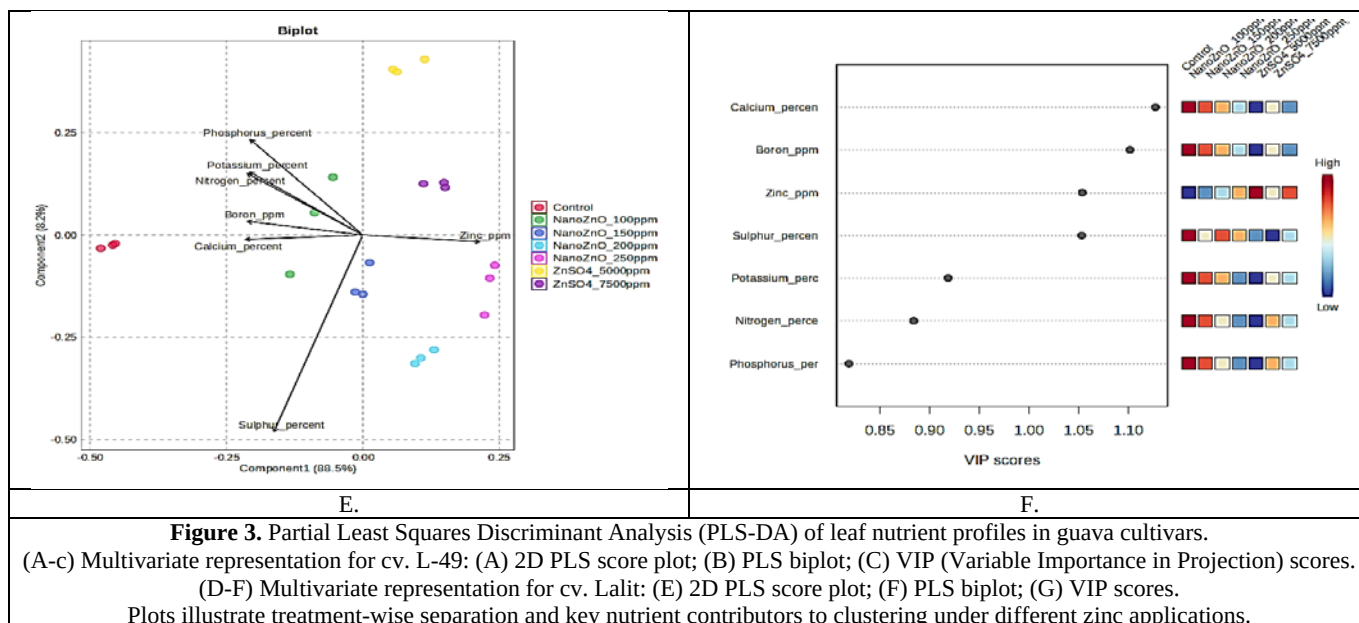
Fig 1: Multivariate analysis of morpho-physiological traits of guava A. VIP Score plot B. Principle Component Analysis C. Biplot Analysis D. Correlation heatmap E. 3-dimensional score plot.





2 B. correlation network plot





4. Conclusion

The present investigation demonstrated that correlation and multivariate statistical approaches effectively elucidated the complex nutrient-physiological interactions in Guava under foliar application of conventional zinc sulphate and biogenic zinc oxide nanoparticles (ZnO-NPs). Correlation heatmaps and hierarchical clustering revealed strong positive associations among zinc accumulation, chlorophyll content, photosynthetic rate, stomatal conductance, transpiration and major nutrient concentrations, indicating coordinated regulation of physiological processes under nano zinc fertilization. In contrast, phosphorus exhibited a negative association with zinc, confirming Zn-P antagonistic interaction under elevated zinc levels. Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) clearly discriminated nano ZnO treatments from conventional zinc sulphate and untreated control, highlighting the pronounced influence of nano zinc on nutrient dynamics and physiological performance. Variable Importance Projection (VIP) analysis identified zinc, calcium and boron as the most significant contributors governing treatment differentiation and physiological efficiency. Nano ZnO at 200-250 ppm consistently formed distinct clusters associated with improved nutrient accumulation, enhanced photosynthetic activity and superior morpho-physiological performance. Overall, the study confirmed that foliar application of biogenic ZnO-NPs substantially improved nutrient use efficiency and physiological functioning in guava under semi-arid conditions. The integration of correlation analysis, PCA, hierarchical clustering and PLS-DA provided comprehensive insights into nutrient interaction networks and identified key nutrient drivers regulating plant performance. These findings highlight the potential of nano zinc fertilization as an efficient and sustainable nutrient management strategy for guava cultivation and demonstrate the usefulness of multivariate analytical tools in understanding complex plant nutrient relationships.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments: The authors are sincerely thankful to Rani Lakshmi Bai Central Agricultural University, Jhansi (U.P.), for providing the necessary facilities, academic support, and research environment required to carry out this study.

Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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