PLANT SCIENCE TODAY
Vol 12(sp4): 01–07
https://doi.org/10.14719/pst.10793

eISSN 2348-1900

RESEARCH ARTICLE

GC-MS based phytochemical profiling of bhendi hybrid CO4 leaves under Nano DAP treatment

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Received: 21 July 2025; Accepted: 09 September 2025; Available online: Version 1.0: 17 October 2025

Cite this article: Suriya R, Indu Rani C, Savitha BK, Janaki P, Djanaguiraman M. GC-MS based phytochemical profiling of bhendi hybrid CO4 leaves under Nano DAP treatment. Plant Science Today. 2025;12(sp4):01–07. https:/doi.org/10.14719/pst.10793

Abstract

Abelmoschus esculentus L. (bhendi or okra) is widely cultivated for its nutritional, economic and therapeutic value. This study investigates the phytochemical composition of bhendi hybrid CO4 leaves treated with nano diammonium phosphate (Nano DAP) using Gas Chromatography-Mass Spectrometry (GC-MS). Comparative profiling identified 44 metabolites in Nano DAP-treated samples compared with 37 metabolites in untreated control. The identified compounds include hydrocarbons, esters, alcohols, diterpenes and fatty acid methyl esters, reflecting diverse metabolic activities. Notably, compounds such as neophytadiene (13.93 %), isopropyl myristate (13.63 %), squalene (7.23 %) and 9, 12, 15-octadecatrienoic acid methyl ester (5.16 %) were detected in high abundance, known for their antioxidant, anti-inflammatory and antimicrobial properties. The nano DAP application triggered the biosynthesis of several unique metabolites do not present in control samples, indicating enhanced secondary metabolism. The enriched terpenoid and lipid profiles suggest a shift in biochemical pathways associated with nutrient assimilation, oxidative stress mitigation and defence mechanisms. These findings highlight the potential of nano-based phosphorus delivery to modulate metabolic dynamics and promote crop resilience in bhendi. This study establishes a foundation for further research into nano-fertilizer-mediated metabolomic shifts and their role in improving vegetable crop physiology and phytochemical richness.

Keywords: Bhendi hybrid CO4; GC-MS; metabolomic profiling; nano DAP; neophytadiene; phytochemicals; secondary metabolites

Introduction

Bhendi (*Abelmoschus esculentus* L.) is a major vegetable crop in India, renowned for its high dietary fiber, mucilage and antioxidant content. India is the world's largest producer of okra, with an annual production of 7288.51 thousand metric tons cultivated over 554.49 thousand hectares, resulting in an average productivity of 13.14 metric tons per hectare (Indiastat, 2024). The bhendi hybrid CO4, released by Tamil Nadu Agricultural University (TNAU), is a high-yielding variety known for its adaptability under diverse agro-climatic conditions. It produces long, slender, dark green fruits with good tenderness, making it highly preferred in fresh markets. The hybrid is characterized by early flowering, uniform fruit set and a prolonged harvesting window, contributing to its superior productivity compared with traditional varieties.

Optimizing nutrient use, particularly phosphorus, is critical for enhancing crop productivity and quality. Phosphorus deficiency can limit crop growth by impairing energy transfer, photosynthesis and metabolic functions. Foliar fertilization with nano formulations, such as nano DAP, has gained popularity as a method to improve nutrient uptake, reduce fertilizer losses and stimulate plant metabolism (1, 2). These inefficiencies not only reduce nutrient availability but also increase production costs and environmental risks.

Nano DAP offers a promising alternative by addressing these limitations. Engineered at the nanometer scale (20-50 nm), Nano DAP provides a greater surface area-to-volume ratio, enhancing reactivity and bioavailability (3). Its nanoscale properties enable rapid dissolution, controlled nutrient release and improved penetration through the leaf cuticle and stomatal openings, thereby enhancing foliar uptake compared with conventional formulations (4). Recent studies have demonstrated that Nano DAP can improve phosphorus uptake by 15 %-30 %, reduce the required fertilizer dose and enhance photosynthetic performance without yield penalties (5, 6). Beyond nutrient supplementation, nanoformulations also act as elicitors of secondary metabolism, triggering stress-responsive pathways that contribute to crop resilience. These unique physicochemical and physiological advantages justify the exploration of Nano DAP in metabolomic investigations.

Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful analytical tool for detecting low-molecular weight metabolites, enabling both qualitative and semi-quantitative identification of diverse plant secondary metabolites such as terpenoids, fatty acids, alcohols and hydrocarbons. Previous studies using GC-MS have reported significant metabolic shifts in crops treated with nanofertilizers including enhanced antioxidant and therapeutic properties (7). However, there is limited information on how Nano DAP influences the metabolome of vegetable crops, particularly in high-yielding bhendi hybrids.

The present study was therefore undertaken to investigate the effect of Nano DAP foliar application on the metabolic profile of bhendi hybrid CO4 leaves using GC-MS and to compare the phytochemical constituents with those of untreated control plants. This work provides new insights into the secondary metabolic reprogramming induced by Nano DAP and its implications for improving crop quality, resilience and sustainability.

Materials and Methods

Plant material and treatments

The study was conducted at the Department of Vegetable Science, Tamil Nadu Agricultural University, Coimbatore. Seeds of the Bhendi hybrid CO4 were sown in field conditions and managed as per the guidelines of TNAU Crop Production Guide (CPG). The experiment included two treatments:

T_1 (Control): 100 % Recommended Dose of Phosphorus (RDP) with no foliar spray.

T_2 (Nano DAP): 75 % RDP combined with nano DAP foliar sprays applied at 25 and 45 days after sowing (DAS)

T_3 (Nano DAP): 50 % RDP combined with nano DAP foliar sprays applied at 25 and 45 DAS.

The field experiment was laid out in a Randomized Block Design (RBD) with four replications to ensure adequate precision and to account for field variability. Each treatment was assigned randomly within each replication. Individual plots measured 5 × 4 m and were separated by suitable inter-plot alleys to minimize cross-contamination. Standard agronomic practices were followed uniformly across plots. Leaves for GC-MS analysis were sampled from each plot 3 hr after the second foliar spray (as described in the treatment schedule) and processed as described in the sample extraction section.

Sample extraction

The collected leaves were washed, shade-dried and ground into a fine powder. Ten grams of powdered sample were extracted with methanol using Soxhlet extraction for 6 hr. The extract was filtered through Whatman No. 40 filter paper and concentrated using a rotary evaporator. A 2 µL aliquot of each sample was utilized for GC-MS analysis.

GC-MS analysis

The GC-MS analysis of leaf extracts from the bhendi hybrid CO4 was performed using an Agilent GC 8890 system which was coupled with an MS 5977C detector and an Autosampler 7693A (Agilent Technologies, USA). A DB-5ms capillary column (30 m in length, 0.25 mm internal diameter and 0.25 µm film thickness) was employed for the separation of phytochemicals. Helium (99.999 % purity) served as the carrier gas, maintaining a constant flow rate of 1.0 mL/min. The oven temperature program was set as follows: an initial temperature of 50 °C for 1 min, then ramped at 10 °C/min to 300 °C, where it was held for 1 min. The injection port temperature was kept at 250 °C and 1 µL of the methanol-concentrated crude plant extract was injected using the splitless mode.

Mass spectra were recorded over the m/z range of 0.6–1091 and compound identification was performed by comparing the mass spectra with entries in the NIST20 mass spectral library using MassHunter software (Agilent Technologies).

Results and Discussion

Phytochemical profile of control and Nano DAP samples

A diverse array of metabolites was detected in both Nano DAP-treated and control bhendi (okra) leaves, with each sample

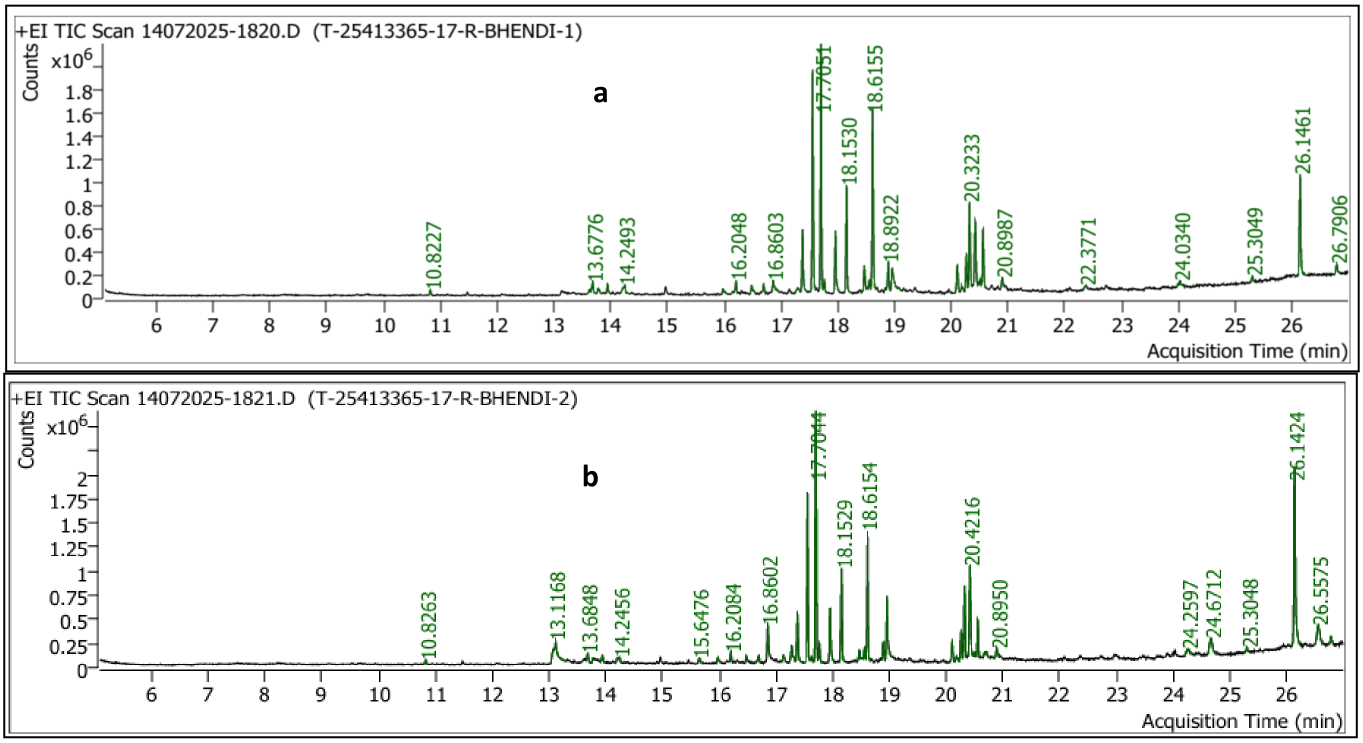


Fig. 1 a & b. Gas Chromatogram of Nano DAP-treated (a) control (b) bhendi samples using GC-MS analysis. The plot represents ion counts (y-axis) against acquisition time in min (x-axis), illustrating a rich spectrum of retention peaks.

exhibiting distinct retention times and peak intensities across the GC-MS chromatograms (Fig. 1 a & b). A total of 37 compounds were identified in the control sample, while 44 were detected in the nano DAP treatment.

Identified compounds

The GC-MS analysis of methanolic extracts from Nano DAP-treated and untreated (control) bhendi hybrid CO4 leaves revealed significant variations in both the qualitative and quantitative presence of metabolites. The major compounds identified in Nano DAP-treated and control bhendi leaves are summarized in Table 1 and 2. Notably, there was a 5.3 % higher abundance of neophytadiene in the Nano DAP-treated okra (13.93 % vs. 13.23 %), which upregulates the MEP pathway, enhancing diterpenoid synthesis under abiotic stress. Isopropyl myristate was significantly more abundant in treated samples, reaching 13.63 % compared to just 8.93 % in the control group, indicating an upregulation of lipid biosynthesis pathways. Squalene, a terpenoid compound known for its antioxidant and membrane-stabilizing properties, surged to 13.00 %

Table 1. Major compounds identified in Nano DAP-treated bhendi leaves (GC-MS)

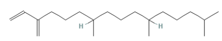
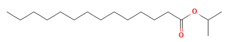
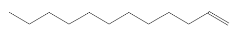
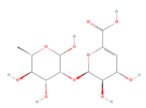
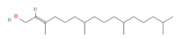
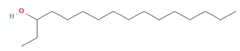
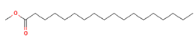
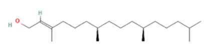
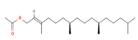
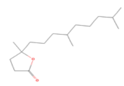
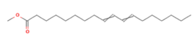
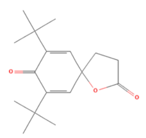
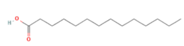
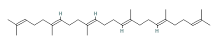
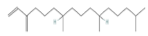
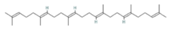
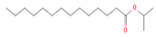
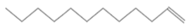
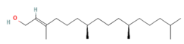
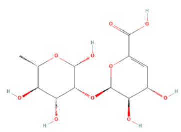
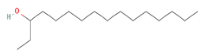
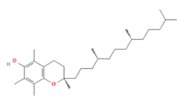
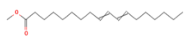
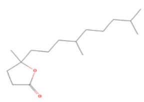
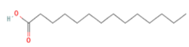
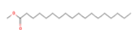
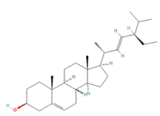
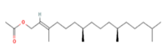
S.No	Compound	Retention time (min)	Formula	Peak area (%)	Class	Structure	Biological significance
1.	Neophytadiene	17.71	$C_{20}H_{38}$	13.93	Diterpenoid hydrocarbon		Antimicrobial, anti-inflammatory, plant growth promoter
2.	Isopropyl myristate	17.56	$C_{17}H_{34}O_2$	13.63	Fatty acid ester		Used in pharmaceuticals and cosmetics, facilitates transdermal drug delivery
3.	Hexadecanoic acid, methyl ester	18.61	$C_{17}H_{34}O_2$	11.34	Fatty acid methyl ester		Antioxidant, hypocholesterolemic, nematicide, pesticide
4.	9,12,15-Octadecatrienoic acid, methyl ester	20.32	$C_{19}H_{32}O_2$	5.16	Fatty acid ester (PUFA)		Anti-inflammatory, antioxidant, membrane fluidity enhancer
5.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	17.96	C20H40O	4.13	Diterpene alcohol		Vitamin E precursor, antioxidant properties
6.	3-Hexadecanol	17.38	$C_{16}H_{34}O$	4.04	Fatty alcohol		Cuticular wax component; water barrier
7.	Methyl stearate	20.56	$C_{19}H_{38}O_2$	3.73	Fatty acid methyl ester		Lubricant and emollient; industrial fatty acid derivative
8.	Phytol	20.42	$C_{20}H_{40}O$	5.45	Diterpene alcohol		Chlorophyll degradation product; precursor of vitamins E and K
9.	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	18.15	$C_{22}H_{42}O_2$	6.26	Fatty alcohol ester		Volatile, potential pheromone component in some species
10.	4,8,12-Trimethyltridecan-4-olide	20.10	$C_{16}H_{30}O_2$	1.93	Macrocyclic lactone		Fragrance compound, antimicrobial activity
11.	1,3-Propanediol, ethyl tetradecyl ether	18.89	$C_{19}H_{40}O_2$	1.85	Ether alcohol		Cosmetic and surfactant use, moisturizing agent
12.	9,11-Octadecadienoic acid, methyl ester	20.26	$C_{19}H_{34}O_2$	2.12	Polyunsaturated fatty acid ester		Essential fatty acid with anti-inflammatory benefits
13.	7,9-Di-tert-butyl-1-oxaspiro...	18.47	C17H24O3	1.66	Spiro compound		Synthetic antioxidant, scavenges reactive oxygen species
14.	Tetradecanoic acid	16.86	$C_{14}H_{28}O_2$	1.18	Saturated fatty acid		Antimicrobial, membrane structure integrity
15.	Squalene	26.14	$C_{30}H_{50}$	7.23	Triterpene hydrocarbon		Antioxidant, precursor for sterol and hormone biosynthesis

Table 2. Major compounds identified in control bhendi leaves (GC-MS)

S.No	Compound Name	Retention time (min)	Formula	Peak area (%)	Class	Structure	Biological significance
1.	Neophytadiene	17.70	$C_{20}H_{38}$	13.23	Diterpenoid hydrocarbon		Anti-inflammatory, antimicrobial
2.	Squalene	26.14	$C_{30}H_{50}$	13.00	Triterpene hydrocarbon		Antioxidant, sterol precursor
3.	Isopropyl myristate	17.56	$C_{17}H_{34}O_2$	8.93	Fatty acid ester		Drug delivery enhancer
4.	Hexadecanoic acid, methyl ester	18.62	$C_{17}H_{34}O_2$	7.10	Fatty acid methyl ester		Antioxidant, lipid component
5.	Phytol	20.42	$C_{20}H_{40}O$	5.95	Diterpene alcohol		Vitamin E/K precursor
6.	9,12,15-Octadecatrienoic acid, methyl ester	20.32	$C_{19}H_{32}O_2$	4.18	PUFA ester		Anti-inflammatory
7.	3-Hexadecanol	17.38	$C_{16}H_{34}O$	2.88	Fatty alcohol		Barrier wax component
8.	Vitamin E	24.67	$C_{29}H_{50}O_2$	2.28	Tocopherol		Membrane antioxidant
9.	9,11-Octadecadienoic acid, methyl ester	20.27	$C_{19}H_{34}O_2$	1.69	Fatty acid ester		Anti-inflammatory
10.	1,3-Propanediol, ethyl tetradecyl ether	18.89	$C_{19}H_{40}O_2$	1.11	Ether alcohol		Moisturizer, emollient
11.	4,8,12-Trimethyltridecan-4-olide	20.11	$C_{16}H_{30}O_2$	1.35	Lactone		Flavorant, bioactive
12.	Tetradecanoic acid	16.86	$C_{14}H_{28}O_2$	1.74	Saturated fatty acid		Antimicrobial, lipid
13.	Methyl stearate	20.56	$C_{19}H_{38}O_2$	2.00	Fatty acid ester		Emollient, biodiesel component
14.	Stigmasterol	26.56	$C_{29}H_{48}O$	2.65	Phytosterol		Anti-inflammatory, cholesterol-lowering
15.	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate	18.15	$C_{22}H_{42}O_2$	5.05	Long-chain ester		Semiochemical, wax component

under Nano DAP influence, in contrast to 7.23 % in the untreated sample. Additionally, phytol, a precursor in the synthesis of tocopherols, showed a substantial decrease from 5.95 % in the control to 5.45 % post-treatment.

Nano DAP treatment significantly increased the abundance of key bioactive metabolites. Several new compounds, such as 9,12,15-octadecatrienoic acid methyl ester and tetramethyl hexadecenol were exclusively detected in the treated samples, highlighting enhanced fatty acid and chlorophyll metabolism. These metabolite shifts collectively underscore the treatment's role in enhancing stress resilience and metabolic efficiency in bhendi.

Compound-level comparison

The application of Nano DAP significantly elevated the levels of esters and fatty acids, including isopropyl myristate, hexadecanoic acid methyl ester and methyl stearate. This indicates the activation of Acetyl-CoA–dependent lipid biosynthesis pathways which are essential for membrane development and serve as energy reserves that support fruit enlargement and Vigor (6). Additionally, treated bhendi showed pronounced accumulation of terpenoid compounds such as squalene and neophytadiene. A total of twelve key phytoconstituents were selected for comparison based on their peak area abundance and known physiological roles (Table 3). Notably, neophytadiene was the most dominant compound detected in both treated and control samples, exhibiting 100 % relative peak area in both. However, the Nano DAP-treated sample displayed slightly higher total chromatographic peak intensity, suggesting a possible enhancement in terpenoid metabolism. Neophytadiene, a diterpenoid, is recognized for its antimicrobial and anti-inflammatory properties and plays a role in plant defense mechanisms (7).

Isopropyl myristate, a fatty acid ester, demonstrated a significant increase from 67.49 % in the control to 97.79 % in the treated sample. This indicate enhanced lipid biosynthesis or esterification, potentially triggered by the nanoformulated phosphorus source. Isopropyl esters are involved in cuticle development and the surface lipid composition of plants (8). Moreover, squalene, a triterpenoid intermediate in sterol biosynthesis, also showed an elevation, rising to 98.24 % in the Nano DAP treatment compared to 51.88 % in the control group. This suggests that the mevalonate (MVA) pathway is upregulated under the influence of Nano DAP. Squalene not only serves as a precursor for sterols but also possesses antioxidant properties, which contribute to plant stress resilience (9).

Interestingly, phytol exhibited identical peak areas in both treatments (44.99 %), indicating stable expression of this compound regardless of nutrient treatment. Phytol, a chlorophyll-derived diterpene alcohol, plays a role in tocopherol biosynthesis (10). Fatty acid methyl esters (FAMEs), such as methyl stearate, hexadecanoic acid methyl ester and methyl tetradecanoate, showed increased abundance in the treated sample. Notably, hexadecanoic acid methyl ester (methyl palmitate) rose from 53.68 % to 81.37 % under Nano DAP treatment, suggesting intensified membrane lipid turnover or biosynthesis. FAMEs are vital for maintaining cellular membrane integrity, especially under abiotic stress (11). The unsaturated fatty acid methyl ester 9,12,15-octadecatrienoic acid methyl ester (linolenic acid methyl ester) displayed a moderate increase in treated leaves, rising from 31.59 % to 37.05 %. This compound is associated with oxylipin biosynthesis and stress signal transduction in plants (12).

Additionally, a slight elevation in 2,4-di-tert-butylphenol was observed, increasing from 4.05 % in the control to 4.39 % in the treated sample. This phenolic antioxidant is known to enhance plant defence and suppress pathogen activity (13). Similarly, 3-hexadecanol, a long-chain fatty alcohol, showed increased levels in the treated leaves, reaching 29.01 %. This may contribute to cuticular wax deposition and environmental stress tolerance. Higher levels of 3-hexadecanol indicate the synthesis of long-chain alcohols, which likely aid in forming cuticular wax, a protective adaptation to minimize water loss and resistant against pathogens.

Biological relevance of upregulated compounds

The foliar application of Nano DAP markedly enhanced the accumulation of key bioactive compounds, particularly neophytadiene and squalene which have well-documented roles in plant stress physiology and growth. Neophytadiene, a diterpenoid hydrocarbon, is recognized for its antimicrobial and anti-inflammatory activities and is associated with the activation of the MEP pathway which enhances diterpenoid synthesis under stress conditions. Elevated levels of neophytadiene in treated leaves may

Table 3. Compound-level comparison between Nano DAP-treated and control bhendi samples

S. No.	Compound	Nano DAP (% area-M)	Control (% area-M)	Interpretation
1.	Neophytadiene	100.00	100.00	Present in both samples as a dominant peak, but the total peak area is higher in the treated
2.	Isopropyl myristate	97.79	67.49	Lipid biosynthesis is significantly amplified by Nano DAP
3.	Squalene	98.24	51.88	Possible basal sterol pathway activity
4.	Phytol	44.99	44.99	Identical abundance; consistent tocopherol precursor levels in both
5.	Vitamin E	17.21	17.21	Equal percentage peak abundance; Nano DAP may impact rate or timing more than magnitude
6.	Methyl stearate	26.75	15.14	Treated fruits show enhanced fatty acid methylation
7.	Hexadecanoic acid, methyl ester	81.37	53.68	Markedly elevated in the treated sample, reflecting upregulated membrane lipid synthesis
8.	9,12,15-Octadecatrienoic acid methyl ester	37.05	31.59	Slight increase under treatment, linked to stress response
9.	Razoxane	3.80	Absent	Unique to the treated sample; potential signaling molecule or induced secondary compound
10.	2,4-Di-tert-butylphenol	4.39	4.05	Slightly elevated antioxidant phenolic in the treated sample
11.	3-Hexadecanol	29.01	21.80	Higher in the treated sample; part of the long-chain alcohols for cuticular wax
12.	Methyl tetradecanoate	4.95	1.56	Upregulated lipid ester via Nano DAP

therefore strengthen innate defense mechanisms, enabling plants to withstand pathogen pressure and oxidative damage (7). Similarly, squalene, a triterpene hydrocarbon and intermediate in sterol biosynthesis, exhibited a substantial increase under Nano DAP application. Squalene is known to function as a membrane stabilizer and antioxidant, scavenging reactive oxygen species and protecting cellular components from oxidative stress (9). Its upregulation indicates an activation of the mevalonate (MVA) pathway, which contributes not only to sterol and hormone biosynthesis but also to enhanced abiotic stress tolerance. Together, the enrichment of neophytadiene and squalene reflects a metabolic adjustment towards stress resilience and improved physiological efficiency, suggesting that Nano DAP application does more than provide phosphorus. It also stimulates protective secondary metabolism that supports growth and productivity.

Novel Compounds in Nano DAP-treated plants

A unique detection in Nano DAP-treated leaves was Razoxane (3.80 %), a derivative of bis-dioxopiperazines, not observed in the control. The presence of Razoxane which was absent in the control, suggests that Nano DAP plays a role in inducing secondary metabolism, potentially as part of a defense response or elicitation effect (1). Razoxane and its related bis-dioxopiperazine derivatives are known to possess cytoprotective and stress-modulating properties, particularly in regulating oxidative stress and stabilizing cellular functions under adverse conditions (e.g. DNA-protective and anti-oxidative roles) (9, 11). Their occurrence in plants exposed to Nano DAP could therefore signify an adaptive metabolic reprogramming, wherein phosphorus at the nanoscale may trigger stress-responsive pathways leading to the synthesis of protective secondary metabolites. Such induction not only reflects the elicitor-like effect of Nano DAP but also suggests that nano-phosphorus fertilization can enhance the resilience of plants against abiotic and biotic stresses by activating secondary metabolism.

Overall, these findings indicate that the foliar application of Nano DAP enhances lipid, terpenoid and antioxidant metabolism in bhendi leaves. The upregulation of bioactive compounds such as squalene, isopropyl myristate and methyl esters demonstrates a physiological adjustment that may contribute to improved growth, stress tolerance and yield quality.

Conclusion

GC-MS profiling revealed that nano DAP foliar application significantly enhances the biosynthesis of key secondary metabolites in bhendi hybrid CO4 leaves. Treated plants exhibited an enrichment of key compounds such as neophytadiene which enhances diterpenoid metabolism and supports defense signaling; squalene, a triterpenoid intermediate with strong antioxidant and membrane-stabilizing roles; and isopropyl myristate and other fatty acid methyl esters which reflect the activation of lipid biosynthesis and membrane turnover. The detection of Razoxane, a bis-dioxopiperazine derivative absent in the control, further highlights the elicitor-like effect of Nano DAP in inducing stress-responsive secondary metabolism. Together, these metabolite shifts indicate that Nano DAP not only improves phosphorus utilization but also upregulates terpenoid and lipid pathways that contribute to oxidative stress mitigation, enhanced defense capacity and overall physiological efficiency. Thus, Nano DAP acts both as a nutrient source and a metabolic modulator, offering a sustainable strategy to improve crop quality, resilience and productivity in bhendi.

Acknowledgements

The authors would like to acknowledge the Department of Vegetable Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu for the support rendered in preparation of this research article.

Authors' contributions

Research work and preparation of the original draft were contributed by SR and IRC. The visualization process was carried out by SBK, JP and DM. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

Declaration of generative AI and AI assited technologies in the writing process

During the preparation of this manuscript the author(s) used grammar correcting tools to improve the language. After using this tool, the author(s) reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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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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