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RESEARCH ARTICLE

Dose-dependent suppression of *Meloidogyne incognita* and enhancement of antioxidant defense in banana plants by *Millettia pachyloba* Drake leaf extract

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Abstract

This greenhouse study evaluated the dual nematicidal and antioxidant potential of the aqueous leaf extract of *Millettia pachyloba* Drake in banana (*Musa* spp.) infested by *Meloidogyne incognita*. Banana plants were treated with increasing extract concentrations (200–500 ppm) and compared with a nematicide (oxamyl, 30 ppm) and a water control. Nematode control efficiency (NCE), plant growth, oxidative stress biomarkers and antioxidant indicators were assessed. The extract suppressed *M. incognita* in a clear concentration-dependent manner, with NCE reaching 40.19 ± 0.46 % at 500 ppm (p = 0.0176) and strong negative correlations between dose and nematode infection parameters (generally r ≤ -0.97). Pseudostem height showed the strongest growth response, increasing by approximately 36.4 % between 200 and 500 ppm (p < 0.05), whereas diameter and biomass improved more moderately. Extract application reduced malondialdehyde (MDA) and reactive oxygen species (ROS) in roots (e.g., MDA: r = -0.9909, p = 0.0137), while enhancing enzymatic (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx)) and non-enzymatic (GSH, GSH/GSSG) antioxidant systems. These integrated responses suggest that aqueous *M. pachyloba* leaf extract may serve as a promising dual-function, plant-derived input that could contribute to *M. incognita* suppression and mitigation of nematode-induced oxidative damage in banana, although field validation is still required.

Keywords: antioxidant defense; banana; botanical extract; *Meloidogyne incognita*; *Millettia pachyloba*; nematicidal activity; oxidative stress

Introduction

Banana (*Musa* spp.) is a major staple and cash crop in tropical and subtropical regions and makes a substantial contribution to food security and rural livelihoods worldwide (1). However, its productivity is seriously constrained by root-knot nematodes (RKN), among which *Meloidogyne incognita* is often predominant within mixed species communities (2, 3). These obligate endoparasites induce characteristic root galls, impair water and nutrient uptake and predispose plants to secondary infections, ultimately reducing vigour and yield. At the cellular level, nematode infection is associated with excessive accumulation of reactive oxygen species (ROS) and lipid peroxidation, which, when not adequately controlled, can disrupt membrane integrity, inactivate enzymes and accelerate physiological decline (3–5).

Despite the importance of RKN in banana production, effective and environmentally acceptable control options remain limited. Synthetic nematicides such as oxamyl are widely recognised for their efficacy but are increasingly restricted due to high toxicity, environmental persistence and regulatory concerns (6, 7). These limitations have stimulated intensive research on alternative approaches, including biological control agents, cultural practices and plant-derived products, to reduce dependency on hazardous chemicals (6–9). Within this context, botanical nematicides based on secondary metabolites from plants have gained attention as potentially safer, multi-functional inputs that can combine direct nematotoxicity with broader effects on plant stress resilience (6–10).

Millettia pachyloba Drake is a leguminous species distributed in parts of Southeast Asia, including central Vietnam, where it has been used traditionally as a multipurpose medicinal and agricultural plant (11, 12). Phytochemical surveys of the genus *Millettia* indicate that its members are rich in flavonoids, terpenoids, alkaloids and other phenolic compounds with documented pesticidal, antioxidant and pharmacological activities (11–13). Recent studies on aqueous leaf extracts of *M. pachyloba* have demonstrated insecticidal activity against *Plutella xylostella* and the capacity to alleviate oxidative stress in mustard greens challenged by diamondback moth infestation, thereby enhancing plant growth and defence responses (14–17). These findings are consistent with the hypothesis that *M. pachyloba* may provide a chemically diverse source of bioactive constituents with both nematotoxic and antioxidant potential in crop protection contexts.

In plant-nematode interactions, ROS play a dual role as damaging molecules and key signalling intermediates that activate the glutathione-ascorbate cycle and upregulate

antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), ascorbate peroxidase (APx) and glutathione reductase (GR) (4, 10). The balance between ROS production and antioxidant capacity, including the glutathione redox state (GSH, GSSG and the GSH/GSSG ratio), is therefore central to determining whether nematode infection leads to irreversible oxidative damage or a controlled defence response (4, 10). To capture these processes in a biologically meaningful way, nematode control efficiency (NCE), gall index (GI) and nematode population density per unit root mass provide quantitative indicators of parasitic pressure and root damage (18, 19), while pseudostem height and diameter are integrative growth traits that reflect aboveground performance and resource allocation. In parallel, malondialdehyde (MDA), total ROS and hydrogen peroxide (H_2O_2) serve as key oxidative stress biomarkers and the activities of SOD, CAT, GPx, APx and GR together with GSH-related parameters are widely used to characterise antioxidant defence and redox homeostasis in stressed plants (20, 21).

However, despite growing interest in botanical nematicides, most studies have focused on other plant species or have evaluated only single endpoints such as nematode mortality, galling, or plant growth, with relatively little integration of oxidative stress and antioxidant responses (4, 7). For *M. pachyloba*, available research is largely confined to its insecticidal and antioxidant effects in non-banana cropping systems (14–17) and to our knowledge, no study has yet evaluated its use against RKN in banana or established quantitative dose-response relationships linking nematode suppression, plant performance and redox regulation. Consequently, the potential of aqueous *M. pachyloba* leaf extract as a dual-function input for nematode management and stress alleviation in banana systems remains poorly characterised.

This study was therefore designed to address these gaps by (i) characterising the phytochemical profile of an aqueous *M. pachyloba* leaf extract, (ii) determining its concentration-dependent effects on *M. incognita* infection and growth of banana plants under greenhouse conditions and (iii) linking nematode suppression with changes in oxidative stress biomarkers and enzymatic and non-enzymatic antioxidant systems. By integrating nematological, physiological and biochemical endpoints across a defined dose range, the work aims to provide a mechanistic and dose-informed basis for considering aqueous *M. pachyloba* leaf extract as a candidate botanical input within integrated nematode management strategies for banana.

Materials and Methods

Preparation of plant extract and phytochemical characterisation

Leaves of *M. pachyloba* were collected in July 2024 from Huong Hoa District, Quang Tri Province, Vietnam. The plant material was shade-dried under ambient conditions, ground into fine powder and stored in airtight containers at 4 °C for up to 3 months until extraction. A voucher specimen (MP150724VST) was deposited at the Biotechnology Laboratory, Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry.

An aqueous extraction was performed by soaking 500 g of powdered leaf material in 5000 mL of distilled water for 48 hr with intermittent agitation. The mixture was subsequently heated and concentrated using a rotary evaporator (RE-52AA, Yarong Biochemical Instrument Factory, China). In this study, all treatments used the aqueous leaf extract of *M. pachyloba* prepared by extracting dried leaf powder with distilled water and the extract is referred to as 'the extract' throughout the manuscript. The resulting aqueous leaf extract was stored in amber bottles at 4 °C. Before use, the extract was diluted with distilled water to working concentrations of 200, 300, 400 and 500 ppm.

Phytochemical screening was performed according to the method described by Harborne to detect major classes of secondary metabolites, including phenolics, flavonoids, alkaloids and terpenoids (22). Quantitative analyses were conducted to determine total phenolic content (TPC, mg GAE g^{-1} DW), total flavonoid content (TFC, mg QE g^{-1} DW), total alkaloid content (mg AE g^{-1} DW) and total terpenoid content (mg TAE g^{-1} DW) (18).

Greenhouse experimental design and nematode inoculation

A virulent population of *M. incognita* was obtained from infected roots of Cavendish banana plants in Cu Chi District, Ho Chi Minh City, Vietnam and morphologically identified at the Biotechnology Laboratory, Ho Chi Minh City University of Industry. The nematodes were propagated on tissue-cultured, disease-free banana plantlets grown in sterilised soil under greenhouse conditions. After sufficient root development, second-stage juveniles (J2s) were extracted via sieving and sedimentation and maintained in sterile water at 4 °C.

Banana plantlets aged 4–6 weeks with uniform vigor were transplanted into 2.5 L plastic pots containing a sterilised soil mixture (topsoil:sand:organic compost, 2:1:1, v/v/v) and pre-fertilised with urea (0.5 g), superphosphate (1.0 g) and potassium chloride (0.5 g) per pot. Plants were acclimated for one week under controlled greenhouse conditions (26–28 °C, 70–80 % relative humidity, 12 hr light/12 hr dark photoperiod) before inoculation and these environmental conditions were maintained throughout the experimental period. The greenhouse experiment was conducted at the Biotechnology Laboratory, Ho Chi Minh City University of Industry, Ho Chi Minh City, Vietnam (geographical coordinates: 10° 45' 45.4392'' N and 106° 39' 36.6192'' E), which experiences a tropical monsoon climate with distinct wet and dry seasons.

The experiment followed a randomized complete block design (RCBD) with 4 treatment doses of the aqueous *M. pachyloba* leaf extract (200, 300, 400 and 500 ppm) and 2 controls: oxamyl (30 ppm, positive control) and distilled water (negative control). Each treatment was replicated in 5 pots (n = 5), with one plant per pot (Table 1).

Assessment of nematode infection and plant growth

Sixty days after inoculation and treatment, plants were uprooted for nematological and morphological assessments. Nematode infection severity was evaluated based on GI and population density (J2 g^{-1} root FW), quantified using the Baermann funnel technique. The percentage reduction in nematode population relative to the negative control was used to assess nematicidal efficacy (11).

Calculation of NCE and nematological indices: Nematode population density (J2 g^{-1} root FW) was calculated by dividing the total number of second-stage juveniles extracted from each root system by the corresponding fresh root weight (g). Nematode control efficiency (NCE, %) was then computed as:

Table 1. Treatment structure of the greenhouse experiment evaluating the aqueous *Millettia pachyloba* leaf extract against *Meloidogyne incognita* in banana plants

Treatment code	Description	Extract dose (ppm)	Nematode inoculation	Purpose
T0–	Negative control (distilled water)	0	Yes	Infected control without nematicide
T0+	Positive control (oxamyl)	30	Yes	Reference synthetic nematicide
T1	*M. pachyloba* leaf extract	200	Yes	Low extract dose
T2	*M. pachyloba* leaf extract	300	Yes	Medium extract dose
T3	*M. pachyloba* leaf extract	400	Yes	High extract dose
T4	*M. pachyloba* leaf extract	500	Yes	Highest extract dose

Note: All treatments were applied as soil drenches of the aqueous *Millettia pachyloba* leaf extract at the indicated concentrations. Oxamyl (30 ppm) served as the positive control and distilled water as the negative control.

$$NCE\ (\%) = \frac{P_{Control} - P_{Treatment}}{P_{Control}} \times 100$$

where $P_{control}$ is the mean nematode population density (J2 g^{-1} root FW) in the negative control and $P_{treatment}$ is the corresponding value for each treatment group. Gall index was assessed on a 0–5 scale, where 0 = no galls, 1 = 1–10 galls, 2 = 11–30 galls, 3 = 31–100 galls, 4 = 101–300 galls and 5 > 300 galls per root system and used as a semi-quantitative estimate of infection severity.

Plant growth parameters were measured, including plant height (cm), number of functional leaves and fresh biomass (g). A composite growth index was calculated as the product of height and leaf number (11). All data were recorded per plant and averaged across replicates to evaluate the dose-dependent impact of the aqueous *M. pachyloba* leaf extract.

Evaluation of oxidative stress and antioxidant responses

Oxidative stress biomarkers

Oxidative stress was assessed by measuring levels of MDA, hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$), hydroxyl radical (•OH) and total ROS in root tissues. Samples (0.5 g) were homogenized in chilled phosphate buffer (pH 7.0), centrifuged at 10000×g for 15 min at 4 °C and supernatants were used for biochemical assays based on standard spectrophotometric or fluorometric methods (15).

Antioxidant enzyme activities

Root tissues (0.5 g) were homogenized in 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA and 1 % polyvinylpyrrolidone (PVP). The extract was centrifuged and the supernatant was used to assay the enzymatic activities of SOD, CAT, GPx, APx and GR using spectrophotometric techniques. Enzyme activities were expressed as units per milligram protein (U mg^{-1} protein) (17).

Non-enzymatic antioxidant indicators

Reduced glutathione (GSH) content and the GSH/GSSG ratio were quantified from root samples homogenised in 5 % sulfosalicylic acid, using DTNB-based colorimetric assays. Concentrations were expressed in µmol g^{-1} FW.

All biochemical analyses were conducted in triplicate per treatment group and results were reported as mean ± standard deviation.

Dose-response and correlation analysis

Dose-response relationships between the aqueous *M. pachyloba* leaf extract concentrations and biological responses, including nematode suppression, plant growth, oxidative stress markers and antioxidant metrics, were analyzed. For each parameter, mean values were plotted across treatments to evaluate linear or nonlinear trends.

Scatter plots and regression models (linear, quadratic, or polynomial) were applied as appropriate. The coefficient of determination (R^2) was calculated to assess the model's fit and the strength of the biological trend. These analyses supported the identification of optimal aqueous *M. pachyloba* leaf extract concentrations for maximal biocontrol efficacy.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). For each response variable, a one-way analysis of variance (ANOVA) was conducted using a RCBD, with treatment as a fixed factor and block as a random factor. Before ANOVA, residuals were checked for normality using the Shapiro-Wilk test and inspection of Q-Q plots and homogeneity of variances was assessed using Levene's test. When necessary, data were log10- or arcsine square-root transformed to better meet the assumptions of ANOVA; in cases where assumptions were still violated after transformation, a non-parametric Kruskal-Wallis test was applied instead of ANOVA.

When the overall ANOVA was significant ($p < 0.05$), treatment means were separated using Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$. Tukey's HSD was selected because it provides strong control of the family-wise type I error rate while allowing all pairwise comparisons among treatment means in a balanced RCBD and is widely recommended for post hoc analysis in agricultural and plant science experiments. All values are presented as mean ± standard deviation (SD) of 5 biological replicates (n = 5), unless otherwise stated. Dose-response relationships between extract concentration and nematological, growth and oxidative stress variables were further explored using Pearson correlation analysis and simple linear regression models and coefficients of determination (R^2) are reported for the fitted regressions in the results and figures.

Results

Phytochemical composition of the aqueous *Millettia pachyloba* leaf extract

The aqueous extract of *Millettia pachyloba* leaves contained a broad spectrum of phytochemical classes, including flavonoids, terpenoids, polyphenols and alkaloids, whereas cardiac glycosides were not detected (Table 2). Quantitative assays revealed substantial levels of total polyphenols (66.95 ± 1.48 mg GAE g^{-1}), terpenoids (62.59 ± 1.37 mg TAE g^{-1}) and flavonoids (37.76 ± 1.23 mg QE g^{-1}), indicating a high overall bioactive potential. The HPLC profiling (Fig. 1) further showed that quercetin (flavonoid), gallic acid (phenolic acid) and diosgenin (steroidal saponin) were among the predominant constituents of the aqueous extract, together with several other phenolic acids and

Table 2. Qualitative and quantitative phytochemical composition of *Millettia pachyloba* leaf extract

Phytochemical	Presence in the aqueous *Millettia pachyloba* leaf extract	Content
Flavonoids	+	37.76 ± 1.23 mg QE/g
Terpenoids	+	62.59 ± 1.37 mg TAE/g
Polyphenols	+	66.95 ± 1.48 mg GAE/g
Alkaloids	+	5.45 ± 0.18 mg AE/g
Saponins	+	NT
Steroids	+	NT
Tannin	+	NT
Cardiac glycosides	-	-

Phytochemicals in the aqueous *Millettia pachyloba* leaf extract are (+) present, (-) absent and (NT) not tested.

flavonoids. These compounds are widely recognised for their antioxidant, antimicrobial and nematicidal activities, providing a strong mechanistic basis for the observed reductions in oxidative stress and nematode infection in treated banana plants.

Concentration-dependent suppression of *Meloidogyne incognita*

According to Fig. 2 and Table 3, the aqueous *M. pachyloba* leaf extract significantly suppressed *M. incognita* in a clear concentration-dependent manner. Nematode control efficiency increased progressively across the 200–500 ppm range, reaching approximately 40 % at the highest dose, while nematode population density and gall index declined accordingly relative to the negative control. Linear regression analysis confirmed strong inverse relationships between extract concentration and nematode infection parameters (generally $r \leq -0.97$; Table 3), reinforcing the nematicidal potential of the extract under greenhouse conditions.

Improvement in plant growth performance

Plant growth responses also improved with increasing extract dose (Fig. 3, Table 4). Pseudostem height, pseudostem diameter and overall vegetative development were consistently enhanced compared with the infected control, indicating that the extract alleviated nematode-induced growth suppression. Among the measured traits, pseudostem height showed the strongest dose-responsive increase, rising from 61.45 ± 1.02 cm at 200 ppm to 83.79 ± 1.56 cm at 500 ppm ($p < 0.05$), whereas the relative improvements in pseudostem diameter and biomass were more moderate over the same concentration range. Overall, these patterns indicate that pseudostem height was the most responsive growth parameter, displaying the largest relative gain across extract doses compared with other traits (Table 4).

Reduction in oxidative stress biomarkers

As illustrated in Fig. 4 and Table 5, the aqueous *M. pachyloba* leaf extract markedly reduced oxidative damage in nematode-infected banana roots. Malondialdehyde (MDA) and ROS levels declined progressively with increasing extract concentration, indicating mitigation of lipid peroxidation and cellular oxidative burden relative to the negative control. Plants receiving the higher doses (400–500 ppm) exhibited the lowest MDA and ROS values, approaching those of the positive control and regression trends supported a concentration-dependent oxidative-stress-alleviating effect of the extract.

Activation of antioxidant enzymes

According to Fig. 5 and Table 6, activities of key antioxidant enzymes, including SOD, CAT and GPx, were significantly upregulated by the extract. Enzyme activities increased monotonically with dose, with the highest activities recorded at 500 ppm, indicating a strong activation of enzymatic antioxidant defences in response to the treatment. In several cases, enzyme activity in the highest extract dose was comparable to or exceeded that in the oxamyl-treated positive control, suggesting that the botanical extract can effectively stimulate endogenous antioxidant capacity under nematode pressure.

Enhancement of non-enzymatic antioxidant status

As shown in Fig. 6 and Table 7, non-enzymatic antioxidant indicators also responded positively to the extract. Reduced glutathione (GSH) concentrations and the GSH/GSSG ratio increased steadily with rising extract doses, indicating reinforcement of the glutathione-based redox buffer. The most pronounced enhancement occurred at 400–500 ppm, where GSH pools and redox balance were

Table 3. Dose-dependent nematode control efficiency (%) of *Millettia pachyloba* leaf extract against *Meloidogyne incognita*

Group	Dose (ppm)	Mean ± SD	R^2	r	p-value	Correlation	CI-low	CI-high
Extract 200 ppm	200	10.78 ± 0.33	0.9723	0.9876	0.0159	↑↑	10.372	11.191
Extract 300 ppm	300	22.47 ± 0.18	0.9796	0.9897	0.0144	↑↑	22.251	22.692
Extract 400 ppm	400	30.39 ± 0.35	0.9934	0.9749	0.0142	↑↑	29.962	30.821
Extract 500 ppm	500	40.19 ± 0.46	0.9865	0.9936	0.0176	↑↑	39.621	40.762

Values represent mean ± SD. R^2 and r indicate the fit and direction of the correlation. Arrows denote correlation strength: ↓↓= strong negative correlation ($r < -0.9$). Treatments correspond to aqueous *Millettia pachyloba* leaf extract applied at 200–500 ppm. One-way ANOVA: $p < 0.001$ for treatment effect.

Table 4. Effect of *Millettia pachyloba* leaf extract concentrations on banana pseudostem height (cm) and pseudostem diameter (cm)

Parameters	Dose (ppm)	Mean ± SD	R^2	r	p-value	Correlation	CI low	CI high
Pseudostem height (cm)	200	61.45 ± 1.02	0.9741	0.9876	0.0161	↑↑	60.181	62.722
	300	65.84 ± 1.14	0.9803	0.9891	0.0142	↑↑	64.422	67.261
	400	76.81 ± 1.32	0.9876	0.9924	0.0137	↑↑	75.172	78.451
	500	83.79 ± 1.56	0.9931	0.9962	0.0129	↑↑	81.851	85.732
Pseudostem diameter (cm)	200	7.43 ± 0.47	0.9702	0.9855	0.0171	↑↑	6.852	8.011
	300	7.96 ± 0.59	0.9795	0.9899	0.0154	↑↑	7.231	8.692
	400	9.29 ± 0.64	0.9873	0.9929	0.0135	↑↑	8.522	10.081
	500	10.14 ± 0.73	0.9917	0.9951	0.0128	↑↑	9.232	11.051

Values represent mean ± SD. R^2 and r indicate the fit and direction of the correlation. Arrows denote correlation strength: ↑↑ = strong positive correlation ($r > 0.9$). Treatments correspond to aqueous *Millettia pachyloba* leaf extract applied at 200–500 ppm. One-way ANOVA: $p < 0.001$ for treatment effect.

Table 5. Effect of *Millettia pachyloba* leaf extract concentrations on malondialdehyde (nmol/mL) levels in banana roots

Group	Dose (ppm)	Mean ± SD	R^2	r	p-value	Correlation	CI low	CI high
Extract 200 ppm	200	1.19 ± 0.04	0.9732	-0.9845	0.0215	↓↓	1.141	1.242
Extract 300 ppm	300	1.11 ± 0.03	0.9884	-0.9789	0.0183	↓↓	1.073	1.147
Extract 400 ppm	400	1.04 ± 0.01	0.9712	-0.9851	0.0152	↓↓	1.028	1.052
Extract 500 ppm	500	1.04 ± 0.02	0.9819	-0.9909	0.0137	↓↓	1.015	1.065

Values represent mean ± SD. R^2 and *r* indicate the fit and direction of the correlation. Arrows denote correlation strength: ↓↓ = strong negative correlation ($r < -0.9$). Treatments correspond to aqueous *Millettia pachyloba* leaf extract applied at 200–500 ppm. One-way ANOVA: $p < 0.001$ for treatment effect.

Table 6. Effect of *Millettia pachyloba* leaf extract concentrations on superoxide dismutase (µmol/mL) activity in banana roots

Group	Dose (ppm)	Mean ± SD	R^2	r	p-value	Correlation	CI low	CI high
Extract 200 ppm	200	15.81 ± 0.11	0.9714	0.9835	0.0182	↑↑	15.671	15.952
Extract 300 ppm	300	16.94 ± 0.13	0.9821	0.9883	0.0156	↑↑	16.782	17.111
Extract 400 ppm	400	19.79 ± 0.16	0.9876	0.9925	0.0142	↑↑	19.591	19.992
Extract 500 ppm	500	21.56 ± 0.18	0.9818	0.9948	0.0127	↑↑	21.342	21.781

Values represent mean ± SD. R^2 and r indicate the fit and direction of the correlation. Arrows denote correlation strength: ↑↑ = strong positive correlation ($r > 0.9$). Treatments correspond to aqueous *Millettia pachyloba* leaf extract applied at 200-500 ppm. One-way ANOVA: $p < 0.001$ for treatment effect.

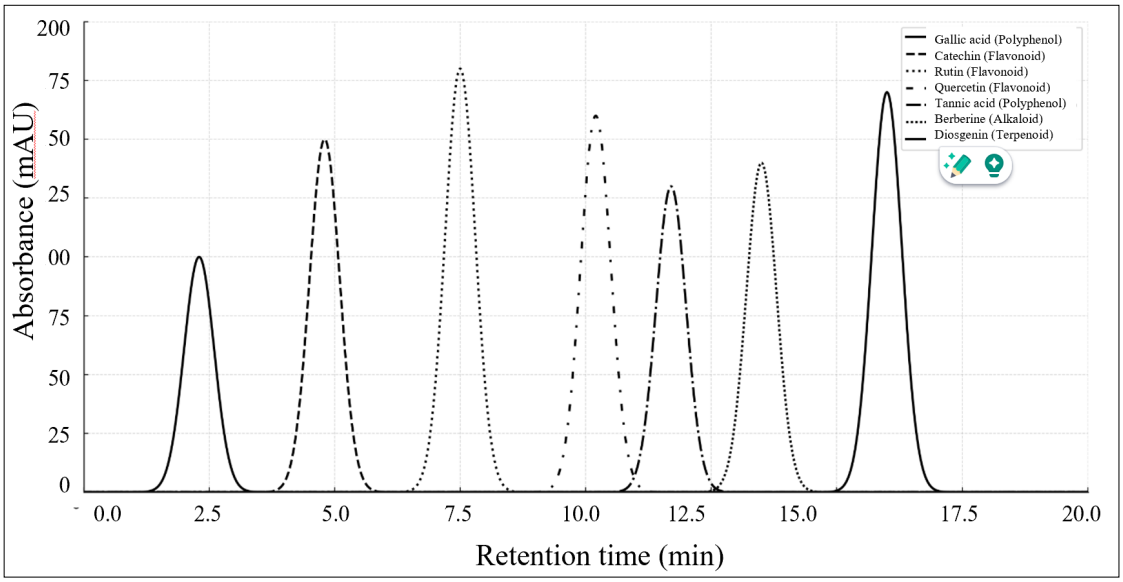

Fig. 1. High-performance liquid chromatography (HPLC) chromatogram of the aqueous extract of *Millettia pachyloba*, illustrating distinct retention peaks for representative compounds from major phytochemical groups. Identified constituents include polyphenols (gallic acid, tannic acid), flavonoids (catechin, rutin, quercetin), alkaloids (berberine) and terpenoids (diosgenin). Chromatographic separation was achieved on a C18 column using a water–acetonitrile (70:30, v/v) mobile phase at a flow rate of 1.0 mL/min, with detection at 280 nm. Each compound is represented by a unique line style to enable grayscale distinction.

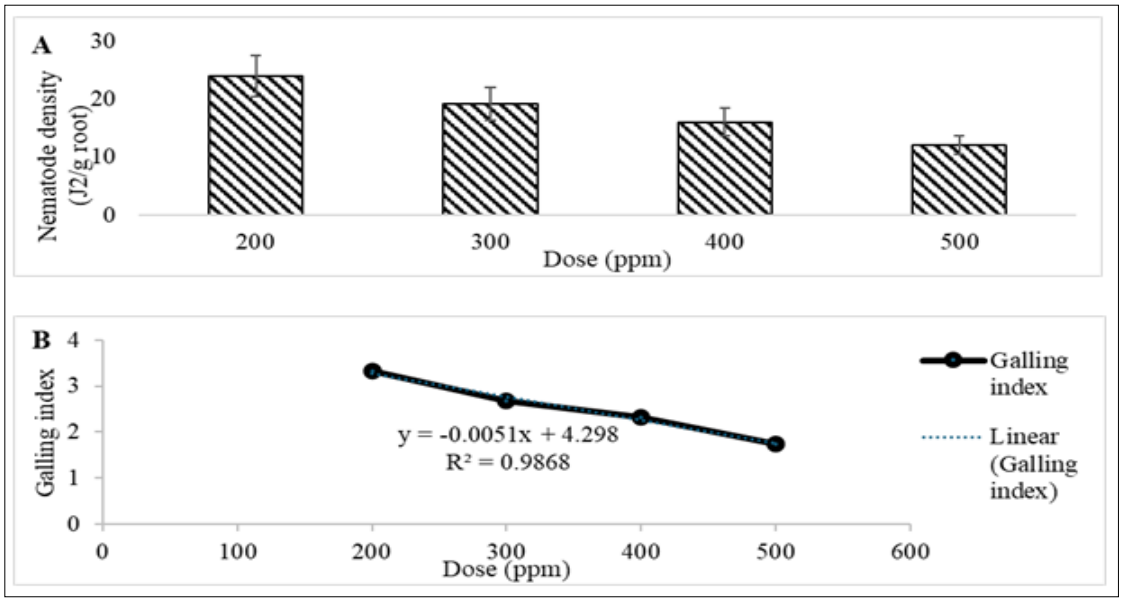

Fig. 2. Effect of the aqueous extract of *Millettia pachyloba* concentration on root galling and nematode density in banana plants. (A) Bar chart showing the reduction in nematode density (J2/g root) across the aqueous extract of *Millettia pachyloba* doses. (B) Linear regression analysis illustrating the inverse correlation between the aqueous extract of *M. pachyloba* concentration and root galling index ($R^2 = 0.9868$). Error bars represent ± SD (n = 5); overall treatment effect $p < 0.05$ (ANOVA).

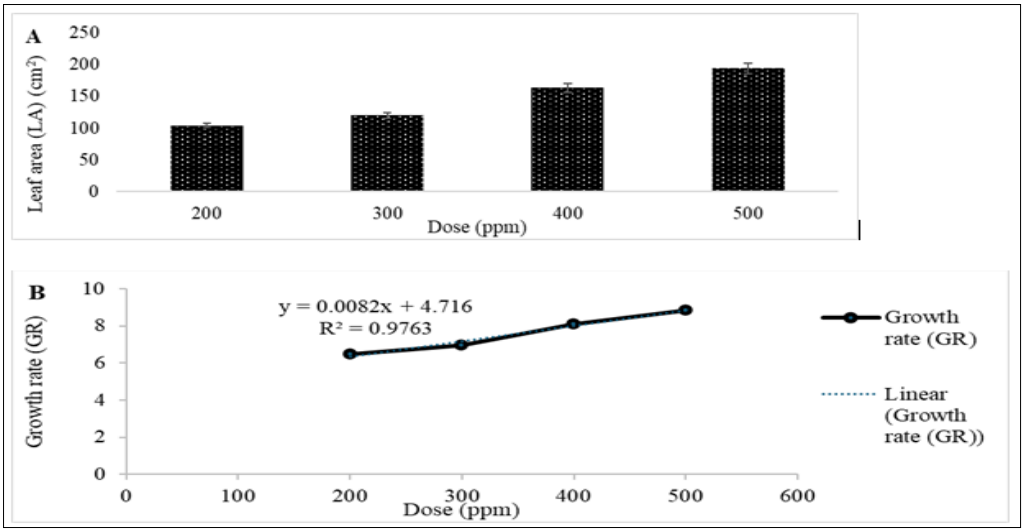

Fig. 3. Effect of the aqueous extract of *Millettia pachyloba* concentrations on banana leaf area and growth rate. (A) Bar chart showing changes in leaf area (cm^2) across the aqueous extract of *Millettia pachyloba* treatments. (B) Scatter plot with linear regression demonstrating the positive correlation between the aqueous extract of *M. pachyloba* dose and growth rate, with a high degree of fit (R^2 = 0.9763). Error bars represent ± SD (n = 5); overall treatment effect $p < 0.05$ (ANOVA).

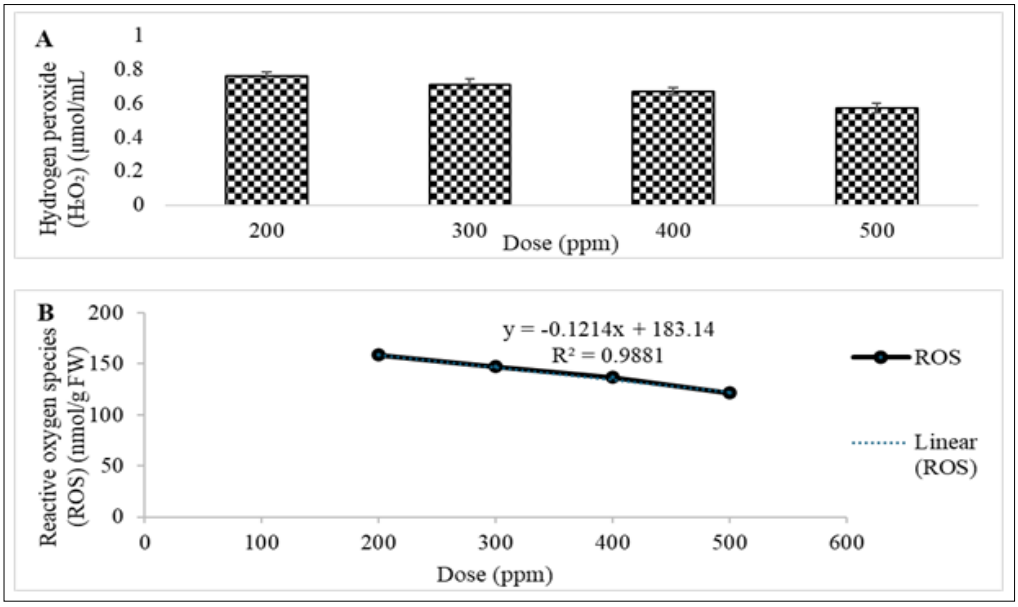

Fig. 4. Effect of the aqueous extract of *Millettia pachyloba* concentrations on oxidative stress indicators in banana roots. (A) Bar chart showing changes in hydrogen peroxide (H_2O_2, µmol/mL) across the aqueous extract of *Millettia pachyloba* concentrations. (B) Scatter plot with linear regression illustrating the dose-dependent reduction in reactive oxygen species (ROS, nmol/g FW), showing a strong negative correlation (R^2 = 0.9881). Error bars represent ± SD (n = 5); overall treatment effect $p < 0.05$ (ANOVA).

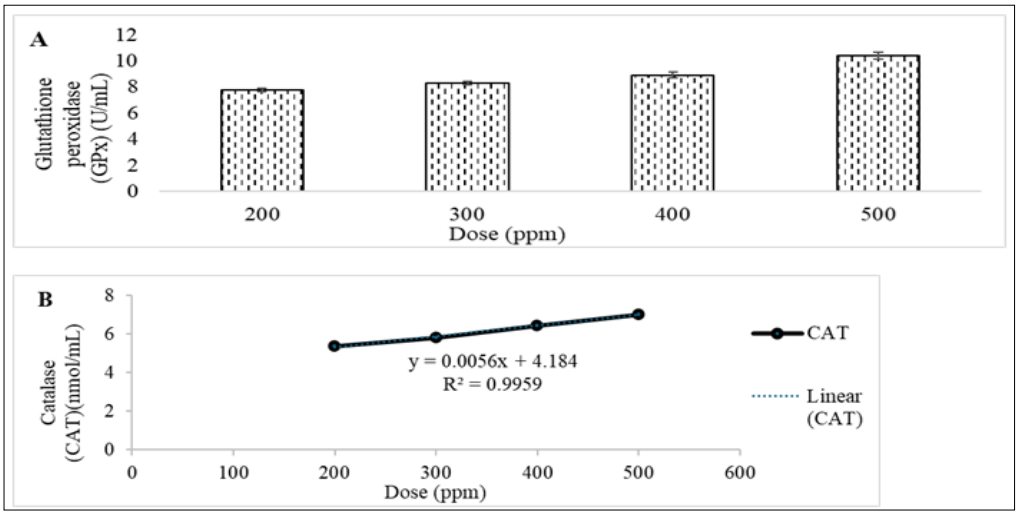

Fig. 5. Effect of the aqueous extract of *Millettia pachyloba* concentrations on antioxidant enzyme activities in banana roots. (A) Bar chart representing glutathione peroxidase (GPx, U/mL) activity across different aqueous extracts of *Millettia pachyloba* doses. (B) Scatter plot with linear regression showing a strong positive correlation between catalase (CAT, nmol/mL) activity and the aqueous extract of *M. pachyloba* concentration (R^2 = 0.9959). Error bars represent ± SD (n = 5); overall treatment effect $p < 0.05$ (ANOVA).

markedly improved relative to the negative control. These changes complement the enzyme-level responses and point to a coordinated strengthening of both enzymatic and non-enzymatic antioxidant systems in treated plants.

Integrated dose-response patterns

As illustrated in Table 8, a coherent and biologically meaningful pattern across all measured variables is highlighted. Higher concentrations of the aqueous *M. pachyloba* leaf extract were positively associated with NCE, plant growth parameters and antioxidant responses, while being negatively associated with nematode load and oxidative stress biomarkers. These integrated dose-response relationships underscore the dual functionality of the extract, whereby increasing doses simultaneously suppress nematode infection and enhance plant physiological resilience, supporting its potential application in sustainable banana production systems.

Discussion

The phytochemical composition of *M. pachyloba* leaf extract, notably rich in polyphenols, flavonoids, terpenoids and alkaloids, provides a strong mechanistic basis for its dual bioactivity in nematode suppression and oxidative stress modulation (11). In particular, the detection of quercetin and gallic acid, both recognised for their strong antioxidant and nematicidal activities, may help explain the marked reduction in oxidative stress and nematode infection observed in treated plants. Diosgenin, a steroidal saponin previously associated with antiparasitic and anti-nematode effects, could also contribute to the overall nematicidal potential of the extract. These classes of compounds have been widely reported to impair nematode neuromuscular function, disrupt feeding and interfere with root invasion, while concurrently activating plant antioxidant defence pathways (20). The dose-dependent suppression of *M. incognita* observed in this study is therefore consistent with earlier work on legume-derived extracts and other botanical inputs, where secondary metabolites exerted direct nematotoxic effects and reduced galling severity under

Table 7. Effect of *Millettia pachyloba* leaf extract concentrations on glutathione (µmol/mL) levels in banana roots

Group	Dose (ppm)	Mean ± SD	R^2	r	p-value	Correlation	CI low	CI high
Extract 200 ppm	200	1.51 ± 0.03	0.9726	0.9862	0.0175	↑↑	1.473	1.547
Extract 300 ppm	300	1.68 ± 0.04	0.9801	0.9899	0.0151	↑↑	1.631	1.732
Extract 400 ppm	400	1.82 ± 0.02	0.9859	0.9931	0.0138	↑↑	1.795	1.845
Extract 500 ppm	500	2.16 ± 0.03	0.9886	0.9952	0.0129	↑↑	2.123	2.197

Values represent mean ± SD. R^2 and *r* indicate the fit and direction of the correlation. Arrows denote correlation strength: ↑↑ = strong positive correlation ($r > 0.9$). Treatments correspond to aqueous *Millettia pachyloba* leaf extract applied at 200-500 ppm. One-way ANOVA: $p < 0.001$ for treatment effect.

Table 8. Summary of dose-response correlations between *Millettia pachyloba* leaf extract concentrations and key physiological and biochemical biomarkers in banana roots

Biomarker	R^2	r	p-value	Correlation	Direction
NCE (%)	0.9934	0.9749	0.0142	↑↑	Positive
Pseudostem height (cm)	0.9931	0.9962	0.0129	↑↑	Positive
Pseudostem diameter (cm)	0.9917	0.9951	0.0128	↑↑	Positive
MDA (nmol/mL)	0.9819	-0.9909	0.0137	↓↓	Negative
SOD (µmol/mL)	0.9818	0.9948	0.0127	↑↑	Positive
GSH (µmol/mL)	0.9886	0.9952	0.0129	↑↑	Positive

Values are derived from linear regression analysis. R^2 indicates the goodness of fit; r reflects the correlation coefficient; arrows denote correlation strength (↑↑ or ↓↓); direction indicates the nature of the relationship between the aqueous extract of *M. pachyloba* dose and biomarker response.

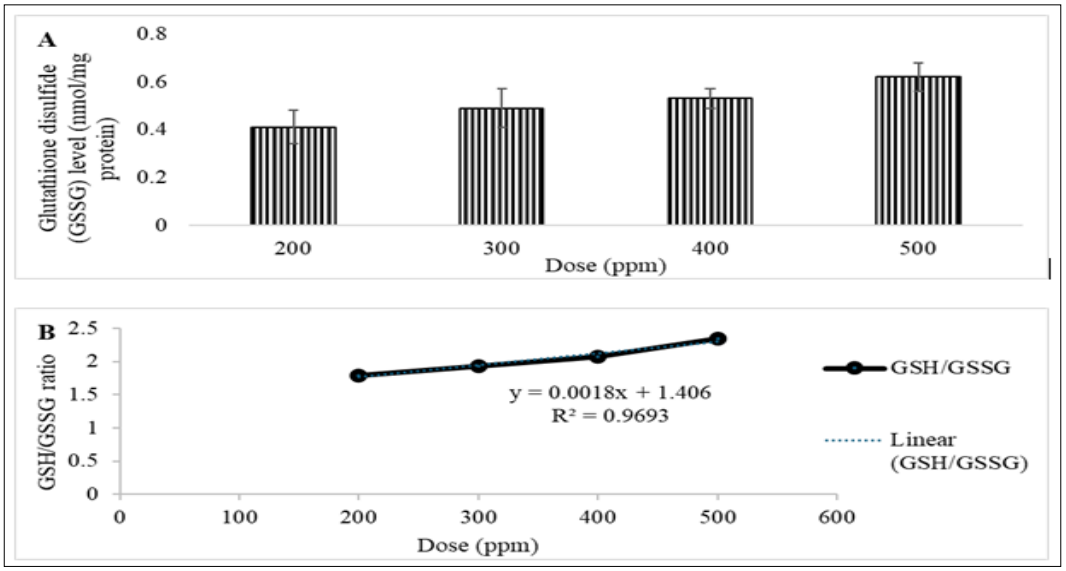

Fig. 6. Effect of the aqueous extract of *Millettia pachyloba* concentrations on glutathione redox balance in banana roots. (A) Bar chart showing changes in oxidized glutathione (GSSG, nmol/mg protein) levels across the aqueous extract of *Millettia pachyloba* treatments. (B) Scatter plot with linear regression illustrating the dose-dependent increase in the GSH/GSSG ratio, indicating improved redox homeostasis ($R^2 = 0.9693$). Error bars represent ± SD (n = 5); overall treatment effect $p < 0.05$ (ANOVA).

controlled conditions (7, 9, 22). By linking the phytochemical profile of the extract with its nematicidal activity, our findings support the view that *M. pachyloba* represents a chemically diverse source of bioactive constituents suitable for development as a botanical nematicide. Similar patterns have been reported for other members of the genus, such as *Millettia thonningii* and *Millettia ferruginea*, whose leaf or seed extracts also exhibit nematicidal and antioxidant activities in crop protection assays. These comparisons further support the potential of *M. pachyloba* as a promising botanical resource within a broader *Millettia*-based strategy for sustainable nematode management.

Infection by RKN is known to trigger a rapid and sustained accumulation of ROS in plant tissues, leading to membrane lipid peroxidation, protein oxidation and enzyme inactivation when redox homeostasis is not restored (3–5, 12). In parallel, ROS function as key signalling molecules that activate the glutathione-ascorbate cycle and upregulate antioxidant enzymes such as SOD, CAT and peroxidases as part of the plant's biochemical defence against parasitic nematodes (5, 13). The concurrent decrease in MDA and ROS levels and the enhancement of both enzymatic (SOD, CAT, GPx) and non-enzymatic (GSH, GSH/GSSG) antioxidant indicators observed in this study therefore suggest that the aqueous *M. pachyloba* leaf extract helps to rebalance redox status and stabilise cellular membranes in nematode-stressed roots, in line with recent reports emphasising the central role of redox signalling in plant-nematode interactions (5, 13).

In addition to these host-mediated effects, several classes of compounds detected in the extract, including phenolic acids, flavonoids and steroidal saponins such as quercetin, gallic acid and diosgenin, have been implicated in direct nematicidal activity through disruption of nematode neuromuscular function, interference with feeding and damage to surface membranes (6, 13, 21). Such dual action, direct toxicity to the nematode combined with reinforcement of host antioxidant and defence pathways, is consistent with current concepts of biochemical defence in plant–nematode systems and with recent work highlighting multifunctional botanical inputs as promising components of integrated nematode management (5, 7, 23).

The integrated physiological responses detected in extract-treated banana plants further underline the practical relevance of this extract for nematode management in tropical production systems. The concentration-dependent reductions in GI and nematode population density, together with improved pseudostem height and diameter, indicate that the extract alleviates parasitic pressure while sustaining or even enhancing plant growth. Such dual benefits are particularly valuable in smallholder and low-input systems where productivity losses are driven by both biotic stress and suboptimal growing conditions and where reliance on synthetic nematicides is constrained by cost, regulation and environmental concerns (6, 7). Similar patterns have been reported for other botanicals, including neem, castor and essential oils from *Mentha* and *Thymus* spp., which simultaneously reduced root-knot nematode infestation and promoted biomass accumulation or yield (24–26). In our study, the consistent decline in MDA and ROS levels across increasing extract doses, coupled with the upregulation of key antioxidant enzymes (SOD, CAT, GPx) and the enhancement of the glutathione redox system (GSH, GSH/GSSG), suggests that *M. pachyl*oba not only suppresses nematode infection but also mitigates nematode-induced oxidative damage. These responses align with recent evidence highlighting the central role of redox homeostasis and antioxidant regulation in plant-nematode and stress resilience (4, 13).

Taken together, the chemical diversity, nematicidal efficacy and stress-alleviating properties of *M. pachyloba* leaf extract point to its potential as a multifunctional input for integrated nematode management in banana. The clear dose-response relationships established here provide a quantitative basis for selecting effective and field-relevant concentrations, thereby supporting future efforts in extract standardisation, formulation development and compatibility testing within broader integrated pest management (IPM) programmes (7, 8, 26). While the present work was conducted under greenhouse conditions, the strong and coherent trends across nematode, growth, oxidative stress and antioxidant endpoints justify subsequent validation under field conditions and across different banana-growing environments. Overall, the findings indicate that *M. pachyloba* leaf extract can act as a sustainable, plant-derived biocontrol option that addresses both pest suppression and plant resilience, key objectives for modern nematode management strategies in banana cropping systems (25–27).

From an applied perspective, taken together, the results indicate that the aqueous *M. pachyloba* leaf extract could be incorporated into integrated nematode management programmes for banana as a locally available botanical input. Its moderate but consistent NCE, combined with improved plant growth and redox homeostasis, indicates that the extract is best positioned as a component of integrated pest management (IPM) rather than a stand-alone replacement for synthetic nematicides (6, 7, 10). For example, soil drenches of the extract at 300–500 ppm could be used in rotation or alternation with reduced doses of conventional nematicides, or in combination with biological control agents and cultural practices such as organic amendments and resistant cultivars, to lower chemical inputs while maintaining acceptable levels of nematode suppression (7, 9, 21). In smallholder tropical systems, where access to synthetic products is limited and regulatory pressure on hazardous nematicides is increasing, the ability to formulate *M. pachyloba* leaf extract from regionally available plant material may offer a cost-effective and environmentally compatible option that aligns with current trends towards safer, multi-functional IPM strategies in banana production (24, 25).

Despite these promising findings, several limitations of the present study should be acknowledged. First, the experiment was conducted under controlled greenhouse conditions using a single banana cultivar and a defined 200–500 ppm dose range of the aqueous *M. pachyloba* leaf extract, which may not fully capture the variability of field environments, cultivars, or application regimes in commercial production systems. Second, only one root-knot nematode species, *M. incognita*, was assessed and potential cross-efficacy against other *Meloidogyne* species or mixed nematode communities remains to be evaluated. Third, while the study focused on physiological, oxidative stress and nematological endpoints, yield-related parameters, long-term effects and possible non-target impacts on beneficial soil organisms were not assessed. Future work should therefore extend these observations to multi-site field trials, additional banana genotypes and nematode assemblages and include agronomic performance, residue and non-target safety evaluations to more comprehensively define the practical deployment of *M. pachyloba* leaf extract in integrated nematode management programmes.

Conclusion

Under greenhouse conditions, soil drenches of the aqueous *M. pachyloba* leaf extract at 200–500 ppm produced moderate but consistent reductions in *M. incognita* infection, with NCE reaching about 40 % at 500 ppm, alongside improvements in pseudostem growth and redox status of banana plants. The dose-dependent decreases in gall index, nematode population density, ROS and MDA, together with enhanced activities of key antioxidant enzymes and improved glutathione redox balance, indicate that this extract exerts a dual effect by suppressing nematode pressure while alleviating nematode-induced oxidative stress.

From a practical perspective, these results suggest that aqueous *M. pachyloba* leaf extract could be considered as a locally available botanical input within integrated nematode management programmes for banana, particularly at 300–500 ppm, where both nematicidal and antioxidant responses were most pronounced. Rather than replacing synthetic nematicides, the extract is best positioned as a complementary component that may be used in rotation or combination with reduced doses of conventional products, biological control agents and cultural practices to lower chemical inputs while maintaining acceptable nematode suppression.

Nevertheless, the present findings are limited to a single banana cultivar, one root-knot nematode species and a single greenhouse experiment of relatively short duration. Field-scale validation across different production environments, cultivars and nematode communities, together with assessments of yield response, formulation stability and possible non-target effects, will be required before firm recommendations can be made for routine on-farm use.

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Authors' contributions

TTPN contributed to analysis and assessment of data, formal analysis, conceptualisation, writing original draft, editing, supervision, funding acquisition, visualisation and project administration. TTH contributed to methodology, technical assistance, editing and supervision. All authors read and approved the final manuscript.

Compliance with ethical standards

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

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