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RESEARCH PAPER

Concentration-dependent nematicidal activity of *Millettia pachyloba* extract against the root-knot nematode *Meloidogyne incognita* in banana (*Musa* spp.)

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ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) are among the most destructive plant-parasitic nematodes affecting banana (*Musa* spp.), causing substantial yield losses worldwide. This study evaluated the concentration-dependent nematicidal activity of *Millettia pachyloba* ethanolic extract (MP-EA) against *Meloidogyne incognita*. LC-MS/MS profiling indicated that MP-EA was rich in flavonoids, terpenoids, and polyphenols. The extract was evaluated at concentrations ranging from 50 to 500 ppm using in vitro bioassays and greenhouse experiments to assess egg-hatching inhibition, juvenile mortality, enzymatic biomarkers, nematode infestation, and photosynthetic pigment content. Under in vitro conditions, MP-EA inhibited egg hatching by up to 52% relative to the negative control and induced more than 70% juvenile mortality after 72 h. In greenhouse experiments, the highest concentration reduced the root-knot index and soil nematode populations by more than 60% compared with the nematode-inoculated untreated control, while increasing catalase activity and carotenoid content by up to 28% and 24%, respectively. These findings indicate that MP-EA can suppress *M. incognita* and is associated with recovery of host physiological traits under greenhouse conditions. Collectively, the results highlight the potential of MP-EA as a botanical nematicide for sustainable management of root-knot nematodes in banana. However, further studies on formulation optimization, non-target safety, and field validation are required before practical application can be considered.

Keywords: Acetylcholinesterase, antioxidant enzymes, botanical nematicide, LC-MS/MS profiling, soil drench

INTRODUCTION

Banana (*Musa* spp.), particularly Cavendish cultivars, is one of the world's most important fruit crops, providing an essential source of food security and income for both smallholder and commercial farming systems in tropical and subtropical regions (Chukwu et al., 2025). In Southeast Asia, including Vietnam, banana production is increasingly threatened by root-knot nematodes (RKNs; *Meloidogyne* spp.), which rank among the most economically important plant-parasitic nematodes affecting perennial cropping systems (Sousa et al., 2024). Among these species, *Meloidogyne incognita* is particularly destructive, causing extensive root galling, root tissue deformation, and disruption of water and nutrient uptake. These effects result in stunted growth, premature leaf senescence, and yield losses of up to 20-60%, depending on cultivar, environment conditions, and nematode population density (Sousa et al., 2024).

Beyond direct yield reduction, chronic infestation accelerates root turnover, predisposes plants to secondary pathogen infections, and impairs soil ecosystem services, thereby reducing the long-term productivity and resilience of banana-based agroecosystems (Abd-Elgawad, 2024; Ghareeb et al., 2024). The problem is particularly severe in monoculture plantations and continuously cultivated orchards, where intensive management practices, elevated temperatures, and repeated planting favor the persistence and population build-up of *Meloidogyne* spp. (Mukesh et al., 2024; Zhang et al., 2025).

Management of plant-parasitic nematodes has long depended on synthetic nematicides. However, many conventional active ingredients have been restricted or withdrawn because of concerns regarding environmental contamination, human health, and adverse impacts on non-target soil organisms (Pires et al., 2022). Current Integrated Pest Management (IPM) frameworks, therefore, emphasize reduced pesticide dependence and the use of safer alternatives, including host resistance, cultural practices, biological control agents, and plant-derived biopesticides (Abd-Elgawad, 2024; Ibrahim et al., 2024; Nur et al., 2016; Swibawa et al., 2024). Among these approaches, phytopesticides and nanobiopesticides have attracted increasing attention owing to their biodegradability,

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lower environmental impact, and compatibility with sustainable crop protection programs.

Plant-based nematicides, especially those derived from medicinal and aromatic species, often show strong in vitro activity but more variable performance under greenhouse or field conditions because of limitations related to stability, bioavailability, and interactions with the soil environment (Khosa et al., 2020). Phytochemicals such as phenolics, flavonoids, and terpenoids have been reported to disrupt nematode neuromodulation, induce oxidative stress, and impair reproduction (Williams et al., 2021). However, relatively few studies have established clear concentration-response relationships linking phytochemical composition with nematicidal efficacy across laboratory and greenhouse conditions while simultaneously evaluating plant physiological responses. Such information is essential for determining whether botanical extracts can serve as reliable IPM-compatible nematicides for tropical crop production.

The genus *Millettia* (Fabaceae) comprises numerous species traditionally used in ethnomedicine and plant protection because of their abundance of flavonoids, terpenoids, saponins, and other biologically active secondary metabolites (Jena et al., 2020). Among them, *Millettia pachyloba*, na species native to Southeast Asia, has recently demonstrated promising insecticidal activity against *Plutella xylostella*, highlighting its potential as a botanical pesticide (Nhung & Quoc, 2024). Metabolite profiling using LC-MS has further revealed that *Millettia* extracts contain abundant flavonoids, terpenoids, and polyphenols, compounds that have been associated with nematicidal activity and induction of plant defense responses in several crop systems (Jena et al., 2020). Despite this promising phytochemical profile, no study has comprehensively evaluated the nematicidal potential of *M. pachyloba* against *M. incognita* or established concentration-dependent relationships between its phytochemical composition, nematode suppression, and host physiological responses. Furthermore, evidence remains scarce regarding whether the efficacy observed under laboratory conditions can be translated into greenhouse performance in the banana–*M. incognita* pathosystem.

Therefore, this study investigated the nematicidal potential of *Millettia pachyloba* ethanolic extract (MP-EA) using an integrated approach combining LC-MS phytochemical profiling with complementary laboratory and greenhouse experiments. Specifically, the study aimed to: (i) characterize the phytochemical composition of MP-EA using LC-MS; (ii) evaluate its concentration-dependent effects on egg hatching, second-stage juvenile (J2) mortality, and key nematode biochemical biomarkers, including acetylcholinesterase, superoxide dismutase, and catalase, under *in vitro* conditions; and (iii) assess its effects on root-knot severity, soil nematode populations, and photosynthetic pigment contents in greenhouse-grown Cavendish banana plants inoculated with *M. incognita*. We hypothesized that MP-EA would exhibit concentration-dependent inhibition of egg hatching and increased juvenile mortality, disrupt essential biochemical pathways involved in nematode survival, suppress root-knot development and soil nematode populations, and mitigate nematode-induced physiological impairment in banana, as reflected by improved photosynthetic pigment content. By integrating phytochemical, biochemical, nematological, and physiological assessments, this study provides a comprehensive evaluation of the concentration-dependent nematicidal efficacy of MP-EA and its potential as an environmentally compatible component of integrated management strategies for root-knot nematodes in banana production.

MATERIALS AND METHODS

Research Site. Leaves of *Millettia pachyloba* were collected in January 2024 from Dakrong Commune, Dakrong District, Quang Tri Province, Vietnam, (16.5801° N, 106.903° E). Plant identity was authenticated by a qualified taxonomist, and a voucher specimen (No. MP220124VST) was deposited in the Biotechnology Laboratory, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City.

Plant Material and Ethanol Extract Preparation. Fresh leaves of *M. pachyloba* were washed thoroughly, shade-dried to constant weight (approximately 3–5 days), and ground into a fine powder). The powder was stored in sealed, light-protected containers at 25 ± 2 ºC until extraction. A total of 1000 g of leaf powder was macerated in 10 L of 95% ethanol (1:10, w/v) at 40 ± 2 ºC for 24 hours under continuous magnetic stirring. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at 45 ºC to obtain the crude ethanolic extract, designated MP-EA. The extract was stored at 4 ºC until further analyses.

LC-MS/MS Analysis and Metabolite Annotation. The phytochemical profile of MP-EA was analyzed using an Agilent 6545 Q-TOF LC-MS/MS system (Agilent Technologies, USA). For each detected metabolite, the observed m/z, proposed molecular formula, retention time (RT), mass error (ppm), ion adduct type, and characteristic MS/MS fragment ions were recorded.

Metabolites were putatively annotated using accurate-mass measurements, isotope pattern matching, and MS/MS spectral comparisons with available mass spectral libraries and published literature. Unless confirmed using authentic reference standards, all compounds were considered putatively identified. Metabolite annotation focused primarily on flavonoids, terpenoids, and polyphenols because these metabolite classes are frequently associated with botanical nematicidal activity and plant defense induction (Kesba et al., 2021).

Phytochemical Screening and Quantification. Qualitative phytochemical screening was performed to detect alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, polyphenols, and cardiac glycosides according to Tran et al. (2023). Total phenolic content was determined using the Folin–Ciocalteu assay and expressed as mg gallic acid equivalents (GAE)/g extract. Total flavonoid content was determined using the aluminum chloride colorimetric assay and expressed as mg quercetin equivalents (QE)/g extract. Total alkaloid and terpenoid contents were determined following Tran & Tran (2021) and expressed as mg alkaloid equivalents (TAE)/g extract, respectively. (Tran & Tran, 2021).

Nematode Source and Inoculum Preparation. Second-stage juveniles (J2s) of *Meloidogyne incognita* were obtained from naturally infested banana roots collected in Long An Province, Vietnam. Species identity was established using morphological characteristics, including perineal pattern and stylet morphology, and confirmed by PCR amplification of the ITS region (GenBank accession: OR123456). Egg masses were excised under a stereomicroscope and incubated using a modified Baermann funnel technique to obtain freshly hatched J2s. Juvenile suspensions were filtered, and inoculum density was adjusted to 200 J2/mL before use.

In Vitro Bioassays. MP-EA was evaluated at concentrations of 50, 100, 150, and 200 ppm. This concentration range was selected to establish a measurable concentration-response relationship for botanical extracts against *Meloidogyne* spp. in aqueous bioassays while minimizing potential confounding effects associated with solubility limitations and medium turbidity at higher concentrations (Mezerket et al., 2025). In contrast, higher concentrations (200–500 ppm) were used in the greenhouse experiment because phytochemicals generally exhibit lower bioavailability in soil owing to dilution in the soil solution, adsorption to organic matter and clay minerals, and degradation processes. Consequently, higher concentrations are often required under greenhouse conditions to achieve nematode suppression comparable to that observed in in vitro assays (Kesba et al., 2021).

Oxamyl (7.5 ppm; commercial formulation, Vydate® L) and distilled water were used as positive and negative controls, respectively. The oxamyl concentration was selected based on preliminary range-finding experiments to provide measurable, but incomplete, nematicidal activity within 72 hours, thereby enabling discrimination among MP-EA treatments. This concentration is also consistent with oxamyl levels commonly used in short-term Meloidogyne bioassays.

Egg-hatching inhibition was evaluated by incubating approximately 100 eggs per treatment at 25 ± 2 ºC, 65–70% relative humidity, and a 12:12 hours light/dark photoperiod for 72 hours. Juvenile mortality was assessed after 24, 48, and 72 hours. Juveniles were considered dead when they remained immobile and failed to respond to gentle probing (Furmanczyk et al., 2025). Enzymatic biomarkers, including acetylcholinesterase (AChE), superoxide dismutase (SOD), and catalase (CAT), were quantified spectrophotometrically and expressed as U/mg protein.

Enzymatic Biomarker Assays. Following 72 hours of exposure, J2s from each biological replicate were washed three times with ice-cold phosphate buffer to remove residual extract and homogenized individually in phosphate buffer (pH 7.0) on ice. Homogenates were centrifuged at 4 ºC, and the resulting supernatants were used for enzyme assays.

Activities of acetylcholinesterase (AChE), superoxide dismutase (SOD), and catalase (CAT) were determined spectrophotometrically and normalized to total soluble protein. Protein concentration was determined using the Bradford assay. Enzyme activities were expressed as U/mg protein. Each biological replicate was analyzed in triplicate, and the average value of the technical replicates was used for statistical analysis (Mezerket et al., 2025).

Greenhouse Experiment. The greenhouse experiment was conducted using a completely randomized design. Four- to six-week-old Cavendish banana plantlets were transplanted into 5-L pots containing sterilized loamy soil:sand (2:1:1, v/v/v). Each treatment consisted of three biological replicates, with six plants per replicate. The treatments comprised: (i) non-inoculated plants receiving distilled water (healthy control); (ii) plants inoculated with *M. incognita* and receiving distilled water (negative control); (iii) inoculated plants treated with oxamyl at 30 ppm (positive control); and (iv–vii) inoculated plants treated with MP-EA at 200, 300, 400, or 500 ppm.

For treatments ii-vii, plants were inoculated with 100 J2s per pot and treated weekly for four weeks with 100 mL of MP-EA at the designated concentration. The inoculum level of 100 J2s per pot was selected to produce measurable root galling within the 4-week pot assay while avoiding severe plant stunting that could confound physiological assessments. This inoculum level falls within the range commonly used in short-duration pot experiments evaluating botanical extracts against *Meloidogyne* spp. (Khosa et al., 2020).

Oxamyl (30 ppm; 30 mg/L) was applied as a soil-drench positive control to provide a benchmark for nematicidal efficacy under greenhouse conditions. This concentration was selected based on previous studies demonstrating the sensitivity of *M. incognita* to oxamyl at similar concentrations in controlled pot experiments and was not intended to represent the conditions were maintained at 25-30 °C, 65-75% relative humidity, under natural daylight.

Statistical Analysis. Dose-response relationships were examined using Pearson correlation and ordinary least-squares linear regression. For each endpoint, analyses used the individual biological-replicate values across all four tested MP-EA concentrations (n = 12), not the four treatment means. For greenhouse endpoints, each observation was the replicate mean of six plants. For enzyme assays, the mean of technical readings within each biological replicate was used. Statistical analyses were performed using Statgraphics Centurion version 19.1.01 (StatPoint Technologies, USA).

RESULTS AND DISCUSSION

Phytochemical Composition of *Millettia pachyloba* Extract. LC-MS/MS profiling of the ethanolic extract of *M. pachyloba* (MP-EA) revealed a phytochemical profile dominated by flavonoids, terpenoids, and polyphenols, together with detectable alkaloids and saponins, whereas cardiac glycosides were not detected. Quantitative analysis demonstrated relatively high contents of total phenolics (70.64 ± 1.56 mg GAE/g extract), terpenoids (62.78 ± 1.66 mg TAE/g extract), and flavonoids (41.57 ± 1.44 mg QE/g extract) (Table 1). LC-MS/MS analysis further putatively annotated several bioactive metabolites, including azadirachtin, lupeol, genistein, rutin, gallic acid, and ferulic acid (Table 2), compounds that have previously been associated with nematicidal activity and induction of plant defense responses (Table 2).

The diverse phytochemical composition of MP-EA provides a plausible explanation for its biological activity. Azadirachtin has been reported to interfere with molting and reproductive signaling in *Meloidogyne* spp. by disrupting ecdysteroid-related pathways (Ibrahim et al., 2024). Lupeol has been associated with mitochondrial dysfunction and membrane disruption, leading to oxidative stress and structural damage in nematodes (Barbosa et al., 2024). Likewise, flavonoids such as rutin and genistein possess antioxidant properties and may regulate phenylpropanoid metabolism, thereby reducing reactive oxygen species (ROS) accumulation and suppressing gall formation (Bui & Tran, 2026; Chen et al., 2024). Phenolic acids, including gallic and ferulic acids, have also been implicated in strengthening cell-wall integrity and stimulating defense-related enzymes, such as catalase (CAT), peroxidase (POD), and polyphenol oxidase (PPO), thereby contributing to reduced nematode establishment (Kesba et al., 2021).

Although the present study did not directly evaluate the biological activity of individual compounds or their interactions, the combined occurrence of these metabolite classes provides a reasonable chemical basis for the nematicidal activity observed in subsequent laboratory and greenhouse experiments. Therefore, the biological responses reported here should be interpreted as the collective effects of multiple phytochemical constituents rather than evidence of confirmed synergistic interactions.

In Vitro Nematicidal Activity

Egg Hatching Inhibition and Juvenile Mortality. MP-EA exhibited pronounced concentration-dependent nematicidal activity against *M. incognita* (Table 3; Figures 1A-B). Egg-hatching inhibition increased progressively with extract concentration, exceeding 50% at 200 ppm compared with the untreated control. Similarly, juvenile (J2) mortality increased with both extract concentration and exposure duration, reaching more than 70% after 72 h at the highest concentration

Table 1. Phytochemical constituents detected and quantified in the ethanolic extract of *Millettia pachyloba* (MP-EA)

Phytochemical	Presence	Content
Flavonoids	+	41.57 ± 1.44 mg QE/g
Terpenoids	+	62.78 ± 1.66 mg TAE/g
Polyphenols	+	70.64 ± 1.56 mg GAE/g
Alkaloids	+	4.85 ± 0.24 mg AE/g
Saponins	+	NT
Steroids	+	NT
Tannins	+	NT
Cardiac glycosides	-	ND

+ = Presence; − = Absence; NT = not quantitatively determined; ND = not detected. Quantitative values are presented as mean ± SD (n = 3).

Table 2. Selected bioactive compounds putatively annotated in the ethanolic extract of *Millettia pachyloba* (MP-EA) by LC-MS/MS

No.	Putatively annotated compound	Chemical class	Major phytochemical group	Estimated content	Reported biological activity
1	Azadirachtin	Limonoid	Terpenoids (mg TAE/g)	15.82 ± 0.40	Nematicidal activity
2	Lupeol	Triterpenoid	Triterpenes	17.95 ± 0.28	Anthelmintic and anti-inflammatory activities
3	Genistein	Isoflavone	Flavonoids (mg QE/g)	8.95 ± 0.35	Inhibition of nematode development
4	Rutin	Flavonoid	Flavonoids	9.88 ± 0.44	Antioxidant and nematicidal activities
5	Gallic acid	Phenolic acid	Phenolics (mg GAE/g)	24.85 ± 0.53	Plant defense induction and antioxidant activity
6	Ferulic acid	Phenolic acid	Phenolics	26.74 ± 0.47	Antioxidant activity and cell-wall reinforcement

tested.

The progressive increase in egg hatching inhibition and juvenile mortality across the tested concentration range (50–200 ppm) demonstrated a clear concentration-response relationship. Regression for egg-hatching inhibition, analysis of the replicate-level observations confirmed a strong linear concentration response relationship (R^2 = 0.9906; Table 3).The complete 0–100% effect window, median lethal (LC_{50}) or effective (EC_{50}) concentrations were not estimated. Instead, regression coefficients were used to characterize the strength and consistency of the concentration-response relationship.

Microscopic observations revealed pronounced morphological alterations in treated juveniles, including cytoplasmic vacuolization, body shrinkage, and cuticular deformation, indicating substantial cellular and structural damage following MP-EA exposure (Jomova et al., 2024). Similar morphological abnormalities have previously been reported following exposure of plant-parasitic nematodes to botanical extracts rich in phenolics and terpenoids, suggesting disruption of cellular integrity and physiological homeostasis (Barbosa et al., 2024). However, because no ultrastructural or molecular analyses were conducted in the present study, these observations should be regarded as phenotypic evidence of toxicity rather than confirmation of specific mechanisms of action.

Overall, MP-EA produced consistent concentration- and time-dependent suppression of egg hatching together with increased juvenile mortality, demonstrating substantial nematicidal activity under controlled laboratory conditions. These findings provide a strong foundation for subsequent greenhouse

Table 3. Concentration-response relationship between MP-EA concentration and egg-hatching inhibition of *M. incognita*

MP-EA concentration (ppm)	Egg-hatching inhibition (%)
50	22.47 ± 0.18
100	30.39 ± 0.35
150	40.19 ± 0.46
200	52.21 ± 0.52
R^2 = 0.9906; Pearson r = 0.9953; P < 0.0001.	

Values are mean ± SD (n = 3). Overall statistics used all 12 individual biological-replicate observations across four concentrations.

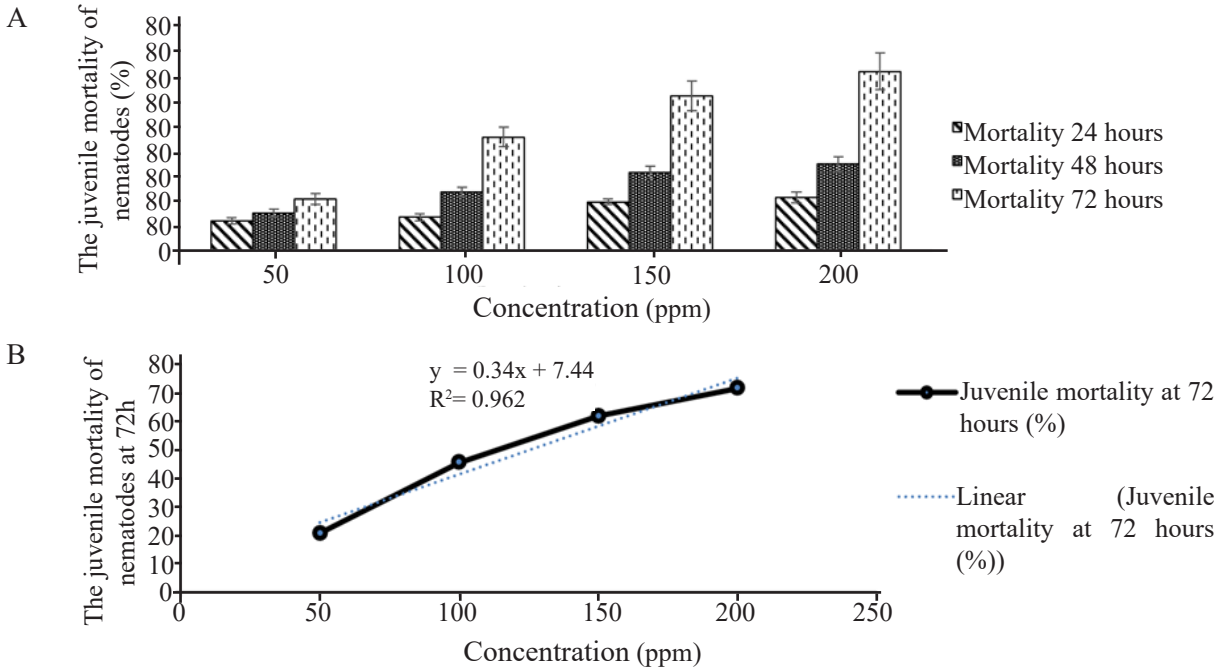

Figure 1. Effect of MP-EA on juvenile mortality of *M. incognita*. A. Juvenile mortality at 24, 48, and 72 hours after treatment across MP-EA concentrations; B. Concentrations-response regression for juvenile mortality at 72 hours (R^2 = 0.962). Error bars represent standard deviation (SD; n = 3). Different letters above the bars indicate significant differences among treatments (P < 0.05; Tukey's HSD test). Graphs were generated using GraphPad Prism version 9.

evaluation and support the potential of MP-EA as a promising botanical nematicide for the management of *M. incognita* (Kesba et al., 2021).

Enzymatic Biomarkers in Nematodes. Exposure of *M. incognita* second-stage juveniles (J2) to MP-EA induced significant concentration-dependent (Table 4; Figures 2A–B). Acetylcholinesterase (AChE) activity decreased progressively with increasing extract concentration, whereas the activities of superoxide dismutase (SOD) and catalase (CAT) increased significantly relative to the untreated control. This coordinated biomarker response, characterized by inhibition of AChE accompanied by activation of antioxidant enzymes, provides biochemical evidence that MP-EA affects both neuromuscular function and cellular redox homeostasis in *M. incognita*, complementing the mortality responses observed in the in vitro bioassays.

The marked reduction in AChE activity is consistent with previous reports indicating that botanical metabolites, particularly terpenoids and polyphenols, may interfere with cholinergic neurotransmission, thereby impairing nematode movement and normal physiological function (Mezerket et al., 2025). Conversely, the increased activities of SOD and CAT suggest the induction of oxidative stress and activation of antioxidant defense mechanisms in response to elevated intracellular reactive oxygen species (ROS) generated during MP-EA exposure (Jomova et al.,

Table 4. Concentration-response relationship between MP-EA concentration and acetylcholinesterase (AChE) activity in *M. incognita* juveniles

MP-EA concentration (ppm)	AChE activity (U/mg protein)
50	141.89 ± 10.48
100	131.76 ± 9.76
150	115.29 ± 8.92
200	108.51 ± 7.98
R^2 = 0.7327; Pearson r = -0.8560; P = 0.0004.	

Values are mean ± SD (n = 3). Overall statistics used all 12 individual biological-replicate observations across four concentrations.

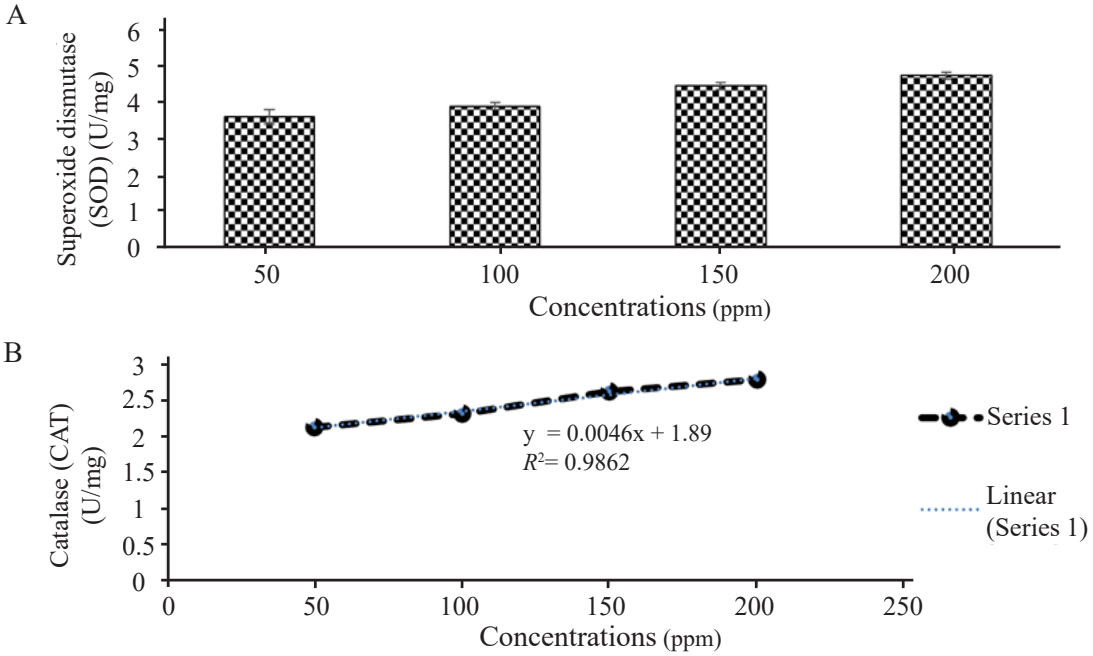

Figure 2. Effect of MP-EA on antioxidant enzyme activities in *M. incognita* juveniles. A. Superoxide dismutase (SOD) activity (U/mg protein); B. Catalase (CAT) activity (U/mg protein) and concentration-response regression (R^2 = 0.9862). Error bars represent standard deviation (SD; n = 3). Different letters indicate significant differences among treatments ($P < 0.05$; Tukey's HSD test). Graphs were generated using GraphPad Prism version 9.

2024). Similar enzymatic responses have been reported following exposure of plant-parasitic nematodes to phytochemical-rich extracts, in which flavonoids and phenolic compounds disrupt cellular redox balance through mechanisms involving mitochondrial dysfunction, lipid peroxidation, and oxidative damage, ultimately contributing to nematode mortality (Zhou et al., 2024; Barbosa et al., 2024).

Although the present study did not directly investigate the molecular mechanisms underlying these enzymatic responses, the observed concentration-dependent changes in AChE, SOD, and CAT were closely associated with the progressive increases in juvenile mortality and egg-hatching inhibition. These findings indicate that enzymatic biomarkers provide valuable complementary evidence of MP-EA-induced physiological stress beyond conventional toxicity endpoints. Nevertheless, the proposed mechanisms should be regarded as interpretations supported by previous studies rather than experimentally confirmed pathways in the present investigation. Further studies incorporating ROS quantification, mitochondrial function assays, and transcriptomic or proteomic analyses would be valuable for elucidating the molecular targets underlying the nematicidal activity of MP-EA.

Greenhouse Evaluation in Banana Plants

Root-knot Index and Egg Masses. Under greenhouse conditions, MP-EA significantly reduced both root-knot index (RKI) and egg-mass production of *M. incognita* in a concentration-dependent manner (Table 5; Figures 3A–B). The highest concentration (500 ppm) reduced the RKI by more than 60% relative

to the nematode-inoculated untreated control and produced the lowest egg-mass density among all treatments. These findings indicate that the nematicidal activity observed under in vitro conditions was effectively translated into suppression of root infection severity and nematode reproduction under greenhouse conditions. Comparable suppression of root-knot nematodes under controlled and pot conditions has been reported for Purpureocillium lilacinum, which reduced J2 populations in soil and roots and mitigated root damage in tomato and guava plants (Swibawa et al., 2024).

The reduction in gall formation and egg-mass production is consistent with previous studies demonstrating that botanical extracts rich in flavonoids, terpenoids, and phenolic compounds can suppress nematode infection by impairing feeding, development, and reproduction while simultaneously enhancing host defense responses (Mezerket et al., 2025). Similar reductions in galling and nematode reproduction have been reported for plant-derived nematicidal materials, including extracts of *Azadirachta indica* and *Tagetes* spp. under greenhouse conditions, as well as Brassicaceae plant residues used as biofumigants under field microplot conditions (Khosa et al., 2020; Williams et al., 2021; Nur et al., 2016). These findings support the potential of MP-EA as a botanical nematicide for managing root-knot nematodes in banana. Nevertheless, the present study evaluated these responses at the phenotypic level, and the molecular mechanisms underlying reduced gall formation and reproduction remain to be elucidated.

Overall, increasing MP-EA concentration from 200 to 500 ppm resulted in progressive reductions in both root-knot severity and egg-mass production, with the greatest suppression consistently observed at 500

Table 5. Concentration-dependent effects of MP-EA on the root-knot index of banana plants infested with *M. incognita*

MP-EA concentration (ppm)	Root-knot index
200	3.52 ± 0.43
300	3.29 ± 0.35
400	1.87 ± 0.28
500	1.25 ± 0.19
R^2 = 0.8675; Pearson r = -0.9314; P < 0.0001.	

Values are mean ± SD (n = 3). Overall statistics used all 12 greenhouse replicate means (three per concentration)..

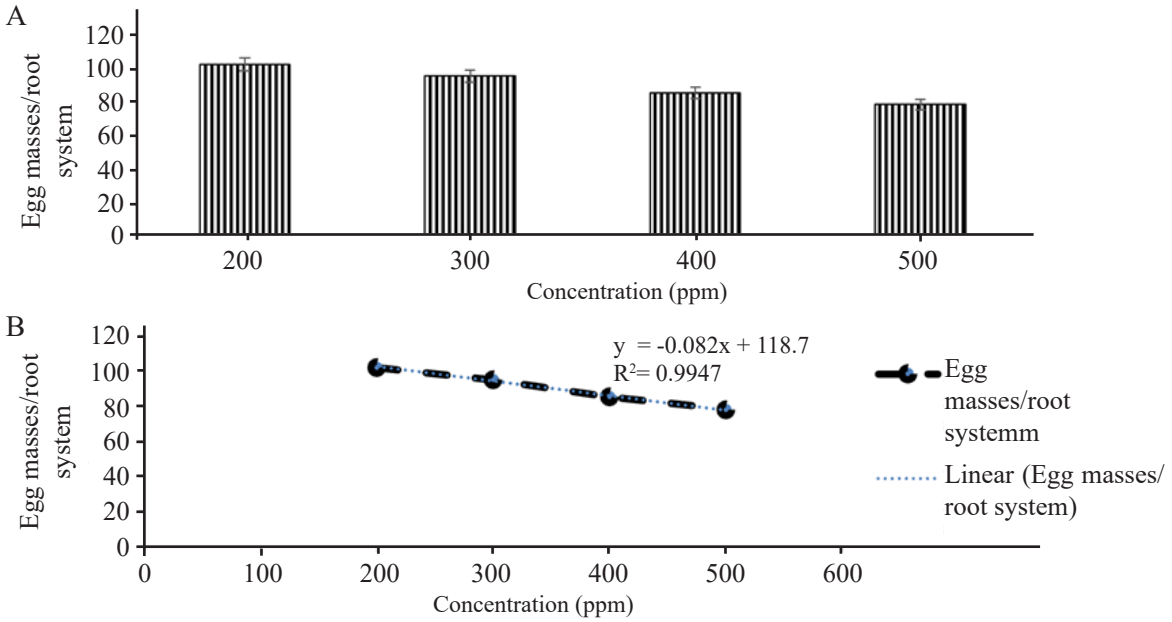

Figure 3. Effect of MP-EA on egg mass formation in banana roots. A. Egg mass counts per root system; B. Regression analysis showing the inverse relationship between MP-EA concentration and egg mass counts (R^2 = 0.9947). Error bars represent standard deviation (SD; n = 3). Different letters indicate significant differences among treatments (P < 0.05; Tukey's HSD test). Graphs were generated using GraphPad Prism version 9.

ppm. These results demonstrate that MP-EA effectively limited nematode establishment and reproductive success under greenhouse conditions.

Soil Nematode Population. Soil nematode density declined consistently with increasing MP-EA concentration (Table 6; Figures 4A–B). The greatest suppression was observed at 500 ppm, where the combined population of eggs and second-stage juveniles (J2) declined by more than 60% relative to the nematode-inoculated untreated control. This reduction in soil inoculum complemented the decreases in root-knot severity and egg-mass production, indicating that MP-EA suppressed both root infection and the rhizosphere nematode reservoir. The observed reduction in soil nematode density is consistent with previous greenhouse studies of bionematicides, in which *P. lilacinum* application reduced J2 populations in both soil and roots and consequently reduced root damage (Swibawa et al., 2024).

The observed decline in soil nematode density agrees with previous greenhouse studies reporting that phytochemical-rich botanical extracts, including those derived from *Origanum* and *Tagetes*, can reduce nematode persistence through direct nematicidal activity and indirect effects on the rhizosphere environment (Zhou et al., 2024). However, because soil microbial communities, enzyme activities, and rhizosphere processes were not evaluated in the present study, these mechanisms remain speculative and should not be regarded as experimentally confirmed.

Collectively, the greenhouse results demonstrate

Table 6. Concentration-dependent effects of MP-EA on soil nematode population density (eggs + J2 per 100 g soil) in banana pots

MP-EA concentration (ppm)	Soil nematode population density (eggs + J2 per 100 g soil)
200	1,750 ± 50
300	1,600 ± 45
400	1,450 ± 40
500	1,250 ± 35
R^2 = 0.9601; Pearson r = -0.9799; P < 0.0001.	

Values are mean ± SD (n = 3). Overall statistics used all 12 greenhouse replicate means (three per concentration).

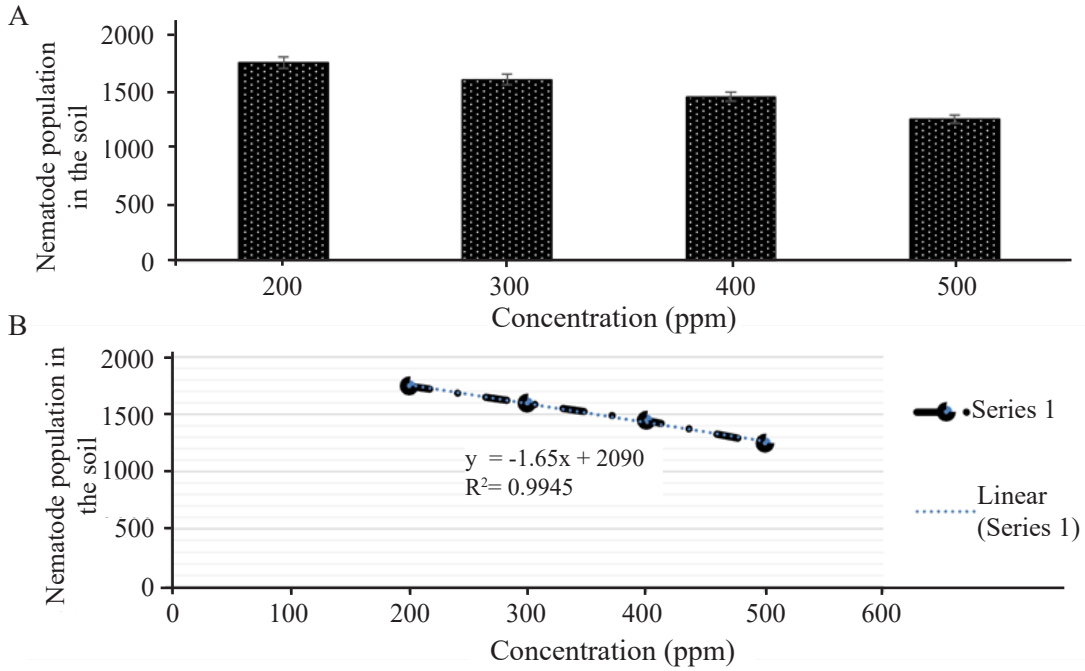

Figure 4. Effect of MP-EA on soil nematode population density in banana pots. A. Nematode population density (eggs + J2 per 100 g soil); B. Regression analysis showing the negative relationship between MP-EA concentration and soil nematode population density (R^2 = 0.9945). Error bars represent standard deviation (SD; n = 3). Different letters indicate significant differences among treatments (P < 0.05; Tukey's HSD test). Graphs were generated using GraphPad Prism version 9.

that increasing MP-EA concentration produced consistent reductions in soil nematode populations, root galling, and egg-mass production. The close agreement between greenhouse and *in vitro* responses further supports the potential of MP-EA as an effective botanical nematicide for suppressing *M. incognita*. Nevertheless, validation under field conditions is required to determine whether similar levels of efficacy can be achieved under more complex environmental conditions.

Photosynthetic Pigment Content. Application of MP-EA significantly increased the contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in *Meloidogyne incognita*-infected banana plants (Table 7; Figures 5A–B). The highest concentration (500 ppm) produced the greatest improvement in photosynthetic pigment content, with total chlorophyll and carotenoid levels increasing by more than 20% compared with the nematode-inoculated untreated control. These results indicate that, in addition to suppressing nematode infection, MP-EA contributed to the recovery of the photosynthetic apparatus under greenhouse conditions.

The restoration of photosynthetic pigments is consistent with reduced biotic stress resulting from the suppression of nematode infection. Root-knot nematodes impair root function, thereby restricting water and nutrient uptake, which subsequently reduces chlorophyll biosynthesis and photosynthetic efficiency (Sikandar et al., 2024). Accordingly, the reduction in root-knot severity and soil nematode populations observed following MP-EA application likely alleviated these constraints, enabling improved physiological performance and pigment accumulation. Similar improvements in chlorophyll and carotenoid contents have been reported in nematode-infected plants treated with botanical extracts rich in flavonoids, terpenoids, and phenolic compounds, reflecting enhanced plant vigor following successful nematode suppression (Sikandar et al., 2024).

Table 7. Concentration-response effects of MP-EA on chlorophyll content in banana leaves

MP-EA concentration (ppm)	Chlorophyll content (mg/g fresh weight)
200	0.14 ± 0.01
300	0.15 ± 0.01
400	0.16 ± 0.01
500	0.18 ± 0.01
R^2 = 0.7401; Pearson r = 0.8603; P = 0.0003.	

Values are mean ± SD (n = 3). Overall statistics used all 12 greenhouse replicate means (three per concentration)..

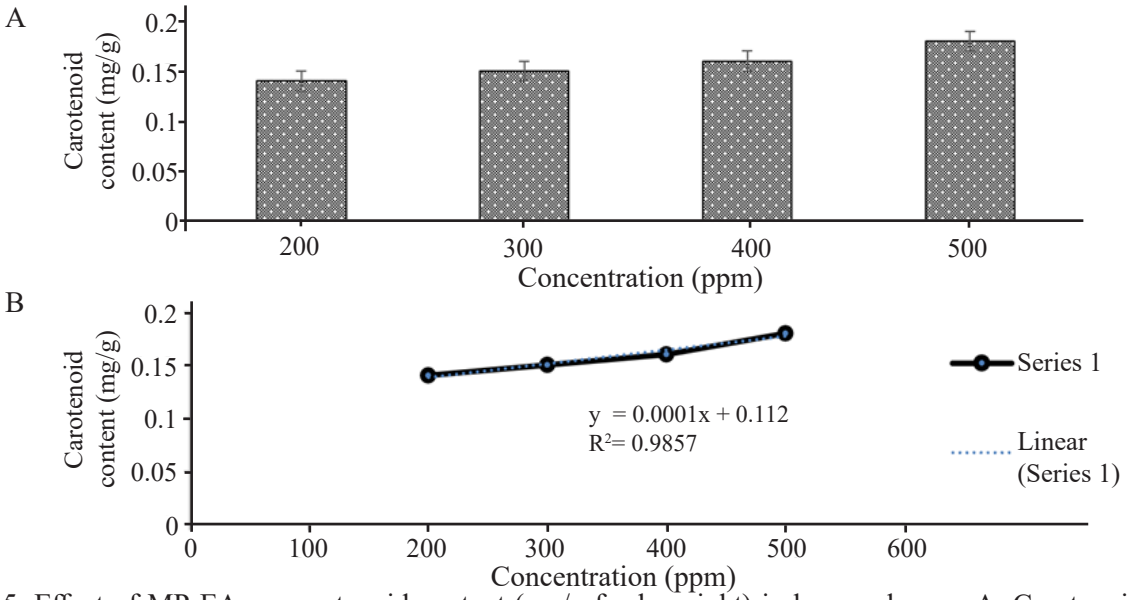

Figure 5. Effect of MP-EA on carotenoid content (mg/g fresh weight) in banana leaves. A. Carotenoid content across MP-EA concentrations; B. Descriptive regression through the four treatment means (R^2 = 0.9857). Inferential Pearson correlation and regression used all 12 replicate-level observations and are reported in Table 7. Error bars represent SD (n = 3). Different letters indicate significant differences among treatments (P < 0.05; Tukey?s HSD test).

Although the present study did not directly assess chloroplast ultrastructure, photosynthetic efficiency, or oxidative damage within leaf tissues, the concurrent improvement in pigment content and reduction in nematode infection support an association between effective nematode management and recovery of host physiological status. Therefore, the proposed mechanisms should be regarded as interpretations supported by previous studies rather than direct evidence generated in the present investigation.

Overall, the concentration-dependent improvement in photosynthetic pigments closely paralleled reductions in root-knot severity, egg-mass production, and soil nematode populations. These findings demonstrate that the beneficial effects of MP-EA extended beyond nematode suppression to include recovery of plant physiological performance, further supporting its potential as a botanical nematicide for sustainable management of *M. incognita* in banana.

Integrated Concentration-Response Relationships. Correlation analysis demonstrated that increasing MP-EA concentrations were positively associated with egg-hatching inhibition and host physiological indicators, including CAT activity, chlorophyll, and carotenoid contents, whereas negative correlations were observed with AChE activity, root-knot index (RKI), egg-mass production, and soil nematode populations (Table 8). Regression analysis further revealed strong linear relationships among the evaluated endpoints, including a particularly close association between RKI and selected enzymatic biomarkers (e.g., R^2= 0.92), indicating coordinated concentration-dependent responses at nematological, biochemical, and physiological levels.

The integration of laboratory and greenhouse data suggests that MP-EA exerts multiple biological effects throughout the banana–*M. incognita* pathosystem. Concentration-dependent inhibition of AChE activity and induction of antioxidant enzymes in juveniles were accompanied by reduced egg hatching, increased juvenile mortality, lower root-knot severity, diminished soil nematode populations, and improved photosynthetic pigment contents. Collectively, these responses indicate that suppression of nematode infection was closely associated with recovery of host physiological performance under greenhouse conditions.

The phytochemical composition of MP-EA, characterized by abundant flavonoids, phenolics, and terpenoids, provides a plausible explanation for these coordinated responses. Previous studies have shown that these metabolite classes can interfere with nematode neuromuscular function, disrupt cellular redox homeostasis, and stimulate plant defense responses (Zhou et al., 2024; Chen et al., 2024). However, the present study evaluated the biological activity of the crude extract rather than individual constituents. Therefore, the observed responses should be interpreted as the combined effects of multiple phytochemical classes rather than evidence of specific compounds or confirmed synergistic interactions.

Although the concentration-dependent trends observed across multiple endpoints provide strong support for the biological activity of MP-EA, the correlation analyses remain descriptive and do not establish causal relationships among enzymatic responses, nematode suppression, and host physiological recovery. Future studies integrating direct ROS quantification, mitochondrial function assays, transcriptomic or proteomic analyses, and bioassay-guided fractionation will be valuable for identifying the principal bioactive constituents and elucidating the molecular mechanisms underlying

Table 8. Summary of concentration-response correlations between MP-EA concentration and biological parameters of *M. incognita* and banana plants

Parameter	Pearson r	*P*-value
Egg-hatching inhibition	0.9953	< 0.0001
AChE activity	-0.8560	0.0004
CAT activity	0.9897	< 0.0001
Root-knot index	-0.9314	< 0.0001
Egg masses per root system	-0.9587	< 0.0001
Soil nematode population	-0.9799	< 0.0001
Chlorophyll content	0.9735	< 0.0001
Carotenoid content	0.8603	0.0003

r is the overall Pearson correlation coefficient calculated from all 12 replicate-level observations per endpoint. The sign of r indicates the direction of association; therefore, the former 'Relationship' column was removed.

MP-EA-induced nematicidal activity (Nhung, 2025; Barbosa et al., 2024).

From an applied perspective, greenhouse evaluation identified MP-EA concentrations between 300 and 500 ppm as the most promising range for suppressing *M. incognita* while improving plant physiological performance under the conditions tested. Nevertheless, these findings should not be directly extrapolated to field application because environmental variability, soil characteristics, and formulation stability may substantially influence efficacy. Accordingly, further optimization of extract formulation, application strategies, and multi-location field validation will be essential before MP-EA can be incorporated into integrated pest management (IPM) programs for banana production (Ayilara et al., 2023; Jeevan et al., 2025).

Limitations and Future Perspectives. The present study provides comprehensive evidence that MP-EA exhibits concentration-dependent nematicidal activity against *M. incognita* under both laboratory and greenhouse conditions. Nevertheless, several limitations should be acknowledged. Metabolite identification was based primarily on putative LC-MS/MS annotation and was not confirmed using authentic reference standards. Furthermore, the molecular mechanisms underlying the observed biochemical responses were inferred from enzymatic biomarkers rather than directly investigated. In addition, the efficacy of MP-EA was evaluated only under controlled greenhouse conditions, and its persistence, environmental stability, and performance under field conditions remain unknown.

Future studies should focus on confirming metabolite identities using authentic standards, conducting bioassay-guided fractionation to identify the principal nematicidal constituents, elucidating molecular mechanisms through biochemical and omics-based approaches, optimizing formulation to improve stability and soil bioavailability, evaluating non-target safety, and validating efficacy under diverse field conditions. These efforts will facilitate the development of MP-EA as an environmentally compatible botanical nematicide for sustainable management of root-knot nematodes in banana production.

CONCLUSION

This study demonstrates that *Millettia pachyloba* ethanolic extract (MP-EA) exhibits concentration-dependent nematicidal activity against *Meloidogyne incognita* under both in vitro and greenhouse conditions. MP-EA significantly inhibited egg hatching, increased juvenile mortality, and induced concentration-dependent changes in enzymatic biomarkers, characterized by reduced acetylcholinesterase activity together with enhanced superoxide dismutase and catalase activities. Under greenhouse conditions, MP-EA suppressed root-knot severity, egg-mass production, and soil nematode populations while improving photosynthetic pigment contents in banana plants. Collectively, these findings demonstrate that MP-EA possesses substantial potential as an environmentally compatible botanical nematicide for the sustainable management of root-knot nematodes within an integrated pest management (IPM) framework. Nevertheless, because the present study was conducted under controlled laboratory and greenhouse conditions, further research is required to validate field efficacy, optimize formulation and application strategies, evaluate environmental persistence and non-target safety, and elucidate the molecular mechanisms underlying its nematicidal activity before practical agricultural application.

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AUTHORS' CONTRIBUTIONS

TPNT conceived and designed the study, developed the methodology, conducted the investigation, performed formal data analysis and data curation, administered and supervised the project, prepared the original manuscript draft, reviewed and edited the manuscript, and served as the corresponding author. HQB conducted the investigation, performed data curation, formal and statistical analyses, and reviewed and edited the manuscript. VTV contributed to the study methodology, provided resources, validated the results, prepared data visualization, and reviewed and edited the manuscript.

COMPETING INTEREST

The authors declare no conflict of interest.

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