

## SYNERGISTIC NEMATOCIDAL AND PHYSIOLOGICAL EFFECTS OF COMBINED EXTRACTS OF *MILLETTIA PACHYLOBA* AND *MELIA AZEDARACH* IN BANANA CHALLENGED BY *MELOIDOGYNE INCOGNITA*

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### ABSTRACT

The root-knot nematode *Meloidogyne incognita* severely threatens banana production by impairing root function and overall plant vigor. In this study, a combined aqueous leaf extract of *Millettia pachyloba* and *Melia azedarach* (MPMA) at a 1 : 1 ratio (v/v) was evaluated for nematode suppression and oxidative stress alleviation under in vitro and greenhouse conditions. Using single extract comparators at equivalent total doses, the mixture consistently exceeded the highest single agent (HSA) benchmark for key endpoints, including in vitro J2 mortality, root galling, and plant nematode density. Interaction model analyses showed endpoint-dependent responses: in vitro Bliss independence and Loewe/Chou–Talalay analyses indicated mainly additive or near-additive effects, with  $\Delta\text{Bliss}$  values  $\leq 0$  and combination indices close to 1, whereas in-planta Bliss analysis showed a mild positive interaction signal at intermediate to high doses. Greenhouse experiments revealed that MPMA significantly reduced root galling, rhizosphere and root nematode populations, and improved host physiology by lowering ROS,  $\text{O}_2^-$ , and  $\bullet\text{OH}$ , while enhancing ascorbate peroxidase, glutathione reductase, and the reduced-to-oxidized glutathione ratio. These changes resulted in greater biomass accumulation and higher composite growth indices in infected plants. Correlation analyses demonstrated strong negative relationships between nematode burden and antioxidant defenses, and positive correlations between antioxidant status and growth performance. Overall, MPMA provided reproducible benefits beyond the best single extract under the HSA benchmark, with quantitative interaction metrics supporting an additive to near-additive interpretation in vitro and mild, endpoint-dependent synergy under greenhouse conditions. These findings underscore its potential as a practical botanical strategy for integrated nematode management in banana cultivation.

**Key words:** HSA benchmark, additive interaction, extract synergy, nematode control, oxidative stress mitigation, phytochemical composition

### INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.), particularly *Meloidogyne incognita*, are among the most destructive soil-borne pathogens affecting horticultural crops. This study specifically focused on the second-stage juveniles (J2) of *M. incognita* infecting Cavendish banana, with no other nematode taxa investigated. Their ability to

induce root galling, disrupt water and nutrient uptake, and impair physiological performance severely limits crop growth and yield. Cavendish banana (*Musa acuminata*, AAA group), a staple fruit in Vietnam and other tropical regions, is especially vulnerable to *M. incognita*, making nematode management a critical challenge for sustainable banana production (De Waele & Davide 1999; Ha et al. 2020).

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Conventional nematode management still relies heavily on synthetic nematicides such as oxamyl and fosfiazate. Although effective in the short term, these chemicals pose serious environmental and health concerns, including disruption of soil microbiota, phytotoxicity, and the emergence of resistant nematode populations (Qiao et al. 2020). Consequently, research efforts have increasingly shifted toward eco-friendly alternatives, particularly botanical nematicides, which offer effective biocontrol while ensuring safety and sustainability. For instance, garlic-derived polysulfides and neem-based formulations have been shown to reduce *Meloidogyne* infestation and enhance host performance in horticultural systems (Eder et al. 2021; Bui & Tran 2026).

Plant-derived extracts are valued not only for their direct nematicidal activity but also for their ability to prime plant defenses and alleviate oxidative stress. *Millettia pachyloba* Drake (Fabaceae), a leguminous shrub native to Southeast Asia, is rich in rotenoids, flavonoids, and coumarins, phytochemicals with documented insecticidal, nematicidal, and antioxidant activities (Heinz et al. 2017; Yan et al. 2019). Previous studies have demonstrated that *M. pachyloba* extracts mitigate oxidative injury in *Brassica juncea* under insect stress and exhibit potent insecticidal effects against *Plutella xylostella* (Nhung & Quoc 2024a; Bui & Tran 2026). However, the role of *M. pachyloba* in integrated nematode management, particularly against *M. incognita* in banana, remains poorly understood under realistic agronomic conditions.

*Melia azedarach* L. (Chinaberry), a member of the *Meliaceae* family, contains limonoids and azadirachtin analogs with reported nematicidal and insecticidal potential. Previous work has shown that *M. azedarach*-based amendments can reduce *Meloidogyne* pressure, influence rhizosphere nematode communities, and improve plant vigor in tomato and ornamental systems (Theofilidou et al. 2023; Sarri et al. 2024). These bioactive components operate via mechanisms distinct from those of *M. pachyloba*, providing a complementary spectrum of biological activity.

The combined application of *M. pachyloba* and *M. azedarach* extracts (MPMA) thus offers a promising strategy that integrates the rotenoids

and polyphenols from *M. pachyloba* with the limonoids from *M. azedarach*, potentially enhancing nematicidal efficacy and host recovery through synergistic interactions. This dual botanical approach aligns with sustainable horticultural practices by reducing dependence on synthetic nematicides and promoting integrated pest and stress management.

To address these research gaps, the present study aimed to: (i) evaluate the nematicidal efficacy of *M. pachyloba* and *M. azedarach* aqueous leaf extracts, individually and in combination, against *M. incognita*; (ii) assess their roles in modulating oxidative stress markers. (iii) determine their capacity to promote physiological recovery in infected banana plants; and (iv) explore correlations between nematode suppression, antioxidant responses, and plant growth performance.

The findings are expected to support the development of synergistic botanical formulations for sustainable nematode control in the banana-based system.

## MATERIALS AND METHODS

### Plant materials and extract preparation

Mature leaves of *Millettia pachyloba* and *Melia azedarach* were collected in August 2024 from Huong Hoa and Cam Lo Districts, respectively (Quang Tri Province, Vietnam). The leaves were thoroughly washed with distilled water, shade-dried at ambient temperature, and finely ground into powder.

For aqueous extraction, 500 g of each powdered sample was mixed separately with 5 L of distilled water (1 : 10 w/v) and incubated at 60 °C for 2 h with intermittent stirring. The mixtures were filtered, and the filtrates were designated as *Millettia pachyloba* extract and *Melia azedarach* extract, respectively. Equal volumes of both extracts were subsequently combined to produce a mixed formulation, referred to as MPMA (combined *M. pachyloba* and *M. azedarach* extract).

The mixed extract (MPMA) was diluted to 50, 100, 150, and 200 ppm for in vitro assays and to 100, 200, 300, and 400 ppm for greenhouse trials. Two single extracts – *M. pachyloba* (MP) and *M. azedarach* (MA) – were prepared and tested at the same concentration ranges to allow direct comparison with the mixture.

### Phytochemical screening and quantification

Qualitative phytochemical screening was performed to detect the presence of alkaloids, flavonoids, polyphenols, tannins, terpenoids, saponins, steroids, and cardiac glycosides using standard chemical assays (Tran & Tran 2023). Total flavonoid content was determined using the aluminum chloride colorimetric method, while total phenolic and tannin contents were quantified by the Folin–Ciocalteu method (Nhung 2025). All results were expressed as milligram equivalents per gram of extract, using quercetin, gallic acid, and tannic acid as calibration standards.

### LC-MS/MS profiling

Liquid chromatography-mass spectrometry (LC-MS/MS) analysis was performed to identify the major phytochemical constituents of MPMA. An aliquot of the extract was filtered and injected into an LC-MS system equipped with a C18 reversed-phase column. The mobile phase consisted of water containing 0.1% formic acid and acetonitrile, using a gradient elution. Spectra were acquired in positive ionization mode, and compounds were tentatively identified based on retention time and  $m/z$  ratio, comparing them with reference databases and published literature (Yan et al. 2019; Meesakul et al. 2022).

### Plant and nematode preparation

Tissue-cultured Cavendish plantlets (6–8 weeks old, 5–6 true leaves, 10–12 cm height) were obtained from SIHOVI Technology, Ho Chi Minh City, Vietnam (Certificate No. 2908/2024SINO). *Meloidogyne incognita* populations were maintained on infected banana roots in sterilized soil. Second-stage juveniles (J2) were extracted using the Baermann funnel technique and stored at 4 °C for use (Lu et al. 2023).

### In vitro evaluation

#### Experimental design

To dissect the individual and combined nematicidal effects, twelve treatments were evaluated alongside controls. Control groups included sterile water (negative control) and oxamyl at 7.5 ppm (positive control). Test treatments comprised two single extract series and one combined series as follows: (i) *Milletia pachyloba* (MP) aqueous extract at 50, 100,

150, and 200 ppm; (ii) *Melia azedarach* (MA) aqueous extract at 50, 100, 150, and 200 ppm; and (iii) the combined extract MPMA (MP : MA = 1 : 1, v/v) at 50, 100, 150, and 200 ppm.

For each well, the exact initial number of second-stage juveniles (J2) was recorded before treatment (approximately 100 J2 per well; accepted range 95–105). Mortality percentages were calculated using the recorded initial count for that well. J2 were  $\approx 3$  days post-hatch and synchronized by Baermann extraction. All assays were conducted at  $25 \pm 1$  °C. Mortality was assessed at 24, 48, 72, and 96 h. After 72 h of exposure, J2 from the recovery series were transferred to fresh distilled water for an additional 24 h to assess recovery and compute the survival index (SI).

J2 were classified as “dead” if immobile and unresponsive to gentle probing with a fine needle after 30 s; otherwise, they were scored as live. All individuals within each well were examined. Observation at 96 h captured late-mortality kinetics before the 24-h recovery step used for SI computation.

Assays were performed in two independent experimental series, each with triplicate wells per condition ( $n = 6$  per condition). Mortality scoring followed the live/dead criteria described below and the standardized J2 bioassay approach of Pontes et al. (2024).

To verify and quantify pharmacological interactions between MP and MA, a fixed-ratio combination design was implemented using five volumetric ratios (MP : MA = 1 : 0, 3 : 1, 1 : 1, 1 : 3, 0 : 1) across three to four total concentration levels (100, 150, 200, 250 ppm) (Abd-Elgawad 2025). This design enabled subsequent Loewe additivity and Chou–Talalay combination index (CI) analyses ( $CI < 1$ ,  $= 1$ ,  $> 1$  indicate synergism, additivity, and antagonism, respectively).

All assays were carried out under identical laboratory conditions ( $25 \pm 1$  °C,  $70 \pm 5\%$  RH) to minimize variability. Data from single and combination treatments were jointly analyzed to establish concentration-response relationships and determine the synergistic effects of MPMA.

### Antioxidant assays

Antioxidant activity was evaluated using DPPH, ABTS, and FRAP assays following previously established protocols (Chaves et al. 2020; Baliyan et al. 2022; Hussen & Endalew 2023). Absorbance was measured at characteristic wavelengths for each assay, and radical scavenging activity (%) and IC<sub>50</sub> values were determined for the DPPH and ABTS methods. FRAP assay results were expressed as  $\mu\text{mol Fe}^{2+}$  equivalents per gram of extract.

### Nematicidal assays

For mortality assays, two parallel readings were used to separate post-exposure mortality from recovery. Continuous exposure series were maintained in the original treatment solutions and scored at 24, 48, 72, and 96 h. Recovery series were exposed for 72 h, transferred to distilled water for 24 h, and then scored for SI only. J2 mortality was calculated as:

$$\text{MR (\%)} = \frac{\text{Number of dead J2 at time } t}{\text{Exact initial number of J2 in that well}} \times 100; (1)$$

where  $t = 24, 48, 72$ , or  $96$  h.

A J2 was considered dead if it was immobile and unresponsive to gentle needle probing within 30 s; otherwise, it was classified as live. All individuals in each well were examined.

LC<sub>50</sub> and LT<sub>50</sub> values were estimated from pre-recovery mortality data using the dose-response and time-mortality models.

LC<sub>50</sub> denotes the concentration causing 50% mortality at a fixed exposure time, while LT<sub>50</sub> denotes the exposure time required to reach 50% mortality at a fixed concentration. IC<sub>50</sub> was used only for antioxidant assays.

The survival index (SI) was calculated from the recovery series as:

$$\text{SI (\%)} = \frac{\text{Number of live J2 after 24 h recovery}}{\text{Number of live J2 before transfer at 72 h}} \times 100. (2)$$

This separation prevented the 24 h recovery period from affecting LC<sub>50</sub> and LT<sub>50</sub> estimates.

### Synergy analysis

Combination performance was evaluated to determine whether the MPMA mixture suppressed *M. incognita* more effectively than either extract used alone at the same total dose.

The highest single agent (HSA) comparison was used as the primary biological benchmark: MPMA was considered beneficial when its mortality or disease-suppression effect exceeded that of the better-performing single extract (MP and MA) at the matched concentration.

Bliss independence and Chou–Talalay indices were retained as supportive checks for additivity or synergy. These analyses were applied mainly at 48 and 72 h before the recovery step, so that interaction estimates reflected direct nematicidal activity rather than post-transfer recovery.

### **In vivo evaluation**

#### Greenhouse experimental design

Banana seedlings were transplanted into 2.5 L pots filled with sterilized soil and acclimatized for 10 days under greenhouse conditions. Treatments included sterile water (negative control), oxamyl at 30 ppm (positive control), and MPMA at 100, 200, 300, and 400 ppm. Each plant was inoculated with 200 freshly collected J2, applied as an aqueous suspension to the root zone. Extracts and controls were applied as soil drenches after inoculation, and root galling, root nematode density, and rhizosphere nematode density were assessed after 9 weeks.

In addition to MPMA, two series of single extracts were included to allow direct comparison at identical concentration ranges: MP at 100, 200, 300, and 400 ppm, and MA at 100, 200, 300, and 400 ppm.

Pots were arranged in a completely randomized design with three biological replicates per treatment ( $n = 3$ ), and pot positions were re-randomized weekly to minimize positional bias. Environmental conditions were maintained at  $25 \pm 2^\circ\text{C}$ , 55–60% relative humidity, and a natural photoperiod.

For in-planta benchmarking against single extracts, key doses of MP, MA, and MPMA (1 : 1, v/v) were compared at matched total concentrations and performance was summarized as excess over the best single extract for gall index and root nematode density.

The greenhouse trials were conducted in two independent experimental runs, each with  $n = 3$  plants per treatment.

### Nematicidal efficacy

Nematode populations were quantified using the modified Baermann method. Rhizosphere nematode density was determined from a 100-g composite soil sample per pot, extracted using the modified Baermann technique and expressed as the number of individuals per 100 g of soil. Root galling was assessed under a stereomicroscope. Root gall severity was evaluated using a validated 0–10 ordinal scale for *Meloidogyne* on horticultural hosts, following Terefe et al. (2009), ensuring consistency across treatments.

Root nematode density was measured for the entire root system of each plant. After harvest, roots were gently washed free of soil, chopped, and extracted. The total eluate per plant was adjusted to a fixed volume, and J2 individuals were counted from duplicate 1-mL aliquots (averaged), yielding results expressed as individuals per plant.

Rhizosphere nematode populations were determined from standardized soil samples of 100 g per pot, extracted and expressed as the number of individuals per 100 g of soil (Pontes et al. 2024).

The reduction rate of nematode population (RRNP) was calculated with respect to the negative control as follows:

$$\text{RRNP (\%)} = \frac{N_c - N_t}{N_c} \times 100;$$

where  $N_t$  and  $N_c$  are the mean number of nematodes in the treated and control groups, respectively, expressed as the number of individuals per plant (roots) and the number of individuals per 100 g of soil (rhizosphere).

Combination effects in-plant were interpreted by comparing MPMA with the best single extract at the same total dose (HSA benchmark). Bliss additivity was used as a secondary validation, with positive values indicating performance better than expected based on the independent action of each extract.

### Oxidative stress and antioxidant defense

In planta, oxidative stress markers, including reactive oxygen species (ROS), superoxide ( $\text{O}_2^-$ ), and hydroxyl radical ( $\bullet\text{OH}$ ), as well as antioxidant defense parameters such as ascorbate peroxidase (APx), glutathione reductase (GR), and the ratio of

reduced to oxidized glutathione (GSH/GSSG), were quantified in root and pseudostem tissues following standardized plant biochemistry protocols (Nhung & Quoc 2025).

For each assay, 0.5 g of fresh tissue (root or pseudostem) was homogenized in ice-cold extraction buffer. Protocols followed previously established plant methods (e.g., for ROS,  $\text{O}_2^-$ ,  $\bullet\text{OH}$  quantification, and APx, GR enzyme assays), with minor modifications to buffer composition, pH, and incubation time. ROS,  $\text{O}_2^-$ , and  $\bullet\text{OH}$  levels were measured spectrophotometrically and fluorometrically in root and pseudostem extracts (Nhung & Quoc 2024b). Antioxidant enzyme activity (APx, GR) and the GSH/GSSG ratio were determined using standardized protocols, with minor adjustments where required.

### Growth and physiological parameters

A composite growth index (CGI) was calculated from plant height, pseudostem diameter, and leaf area to integrate multiple growth traits into a single metric. Each parameter was standardized across all plants and then averaged to generate the CGI.

Plant height (cm), pseudostem diameter (mm, measured 2 cm above the collar), and leaf area ( $\text{cm}^2$ ) were recorded at harvest. Leaf area was measured by digital planimetry for all plants to ensure that one consistent method was applied across the full experimental series.

This composite index followed the approach described by Nhung & Quoc (2024b) and ensured comparability across traits with different measurement units.

The trial did not measure relative nutritional index (RNI), yield, or percentage of fallen plants, but these are recognized as limitations of the study (Nhung & Quoc 2024b).

### **Correlation analysis**

To investigate mechanistic associations, both Spearman's rank and Pearson's correlation analyses were performed to evaluate relationships between nematode burden (root galling and population density), oxidative stress markers (ROS,  $\text{O}_2^-$ ,  $\bullet\text{OH}$ ), antioxidant defenses (APx, GR, GSH/GSSG), and plant growth parameters (biomass and composite growth index). Correlation strength and direction were interpreted according to standard thresholds ( $|r| \geq 0.7$  – strong; 0.4–0.69 – moderate;  $< 0.4$  – weak).

### Biostimulant efficacy analysis

The biostimulatory potential of MPMA under nematode-induced stress was assessed by comparing growth-related parameters (root and pseudostem biomass and composite growth index) of MPMA-treated, oxamyl-treated, and untreated control plants.

The enhancement of growth performance relative to the control was interpreted as an indicator of biostimulant activity, complementing the nematocidal effect of MPMA.

### Statistical analysis

Data are presented as mean  $\pm$  SD. Each plant was considered one experimental unit in greenhouse assays ( $n = 3$  biological replicates per treatment), while in vitro wells were averaged within each run before aggregation into independent runs.

Normality and homogeneity of variance were checked using the Shapiro–Wilk and Brown–Forsythe tests. The data were analyzed using ANOVA, and where significant differences were found for a given effect, the Duncan Multiple Range Test was applied. Because several percentage and count endpoints were nonnormal or heteroscedastic, overall treatment effects were assessed using Kruskal–Wallis tests followed by Dunn’s post-hoc comparisons with Benjamini–Hochberg adjustment (adjusted  $p < 0.05$ ).

LC<sub>50</sub> and LT<sub>50</sub> values were estimated from pre-recovery mortality data, whereas SI was calculated from the 24 h recovery data. Combination effects were summarized primarily using HSA, with Bliss and Chou–Talalay outputs used as supportive interaction metrics.

## RESULTS

### Phytochemical composition

Table 1 summarizes the phytochemical profile of the MPMA. The extract exhibited a rich spectrum of secondary metabolites, including flavonoids ( $39.57 \pm 1.42$  mg QE·g<sup>-1</sup>; including genistein, kaempferol, quercetin, formononetin, biochanin A glucoside, and apigenin),

terpenoids ( $41.55 \pm 1.62$  mg QE·g<sup>-1</sup>), polyphenols ( $70.47 \pm 2.12$  mg GAE·g<sup>-1</sup>), alkaloids ( $5.38 \pm 0.33$  mg AE·g<sup>-1</sup>), tannins ( $7.79 \pm 0.43$  mg TE·g<sup>-1</sup>), and rotenoids detected by LC-MS/MS (pachylobin, rotenone, and millettone). Saponins and steroids were qualitatively detected, whereas cardiac glycosides were absent.

These bioactive constituents are widely recognized for their nematicidal, antioxidant, and growth-promoting properties, thereby highlighting the potential of MPMA as a multifunctional botanical formulation for sustainable banana cultivation.

Targeted LC-MS/MS analysis confirmed the presence of representative phytoconstituents listed in Table 1, thereby defining the chemical fingerprint of the MPMA mixture used in all subsequent bioassays.

### LC-MS/MS profiling

LC-MS/MS analysis of MPMA revealed nine major peaks in the total ion chromatogram (Figure 1). Identified metabolites included rotenoids (pachylobin, rotenone, millettone), isoflavones (genistein, formononetin), flavonols (kaempferol, quercetin), and other flavonoids such as biochanin A glucoside and apigenin, all of which are integrated into the representative-compound descriptions in Table 1.

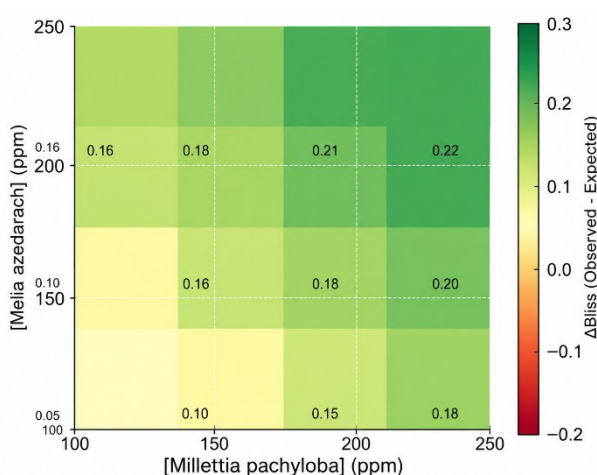


Figure 1. Heat map of the  $\Delta$ Bliss effect (48 h)

Retention times (RT) ranged from 2.7 to 9.1 min, with mass-to-charge ratios ( $m/z$ ) from 270.08 to 448.15, and VIP scores from 1.55 to 1.84.

Relative abundances varied from 9.4% to 13.0%, and calibration curves showed high linearity ( $R^2 = 0.995\text{--}0.998$ ). These phytoconstituents are functionally associated with nematotoxicity and redox modulation, supporting a synergistic mechanism of action in the MPMA combination.

### In vitro evaluation

#### Synergy metrics for single extract vs. mixture

Both single aqueous extracts exhibited dose- and time-dependent increases in J2 mortality over

24–96 h (Table 2). At 96 h, *Millettia pachyloba* (MP) induced 55.33–78.33% mortality, while *Melia azedarach* (MA) caused 52.33–76.33% mortality across 50–200 ppm.  $LT_{50}$  values decreased with increasing concentrations (MP: 92.85 → 68.91 h; MA: 95.77 → 71.32 h), confirming faster nematode mortality at higher doses. Four-parameter logistic (4PL) dose-response and time-mortality models corroborated these trends (Figure 2A–B).

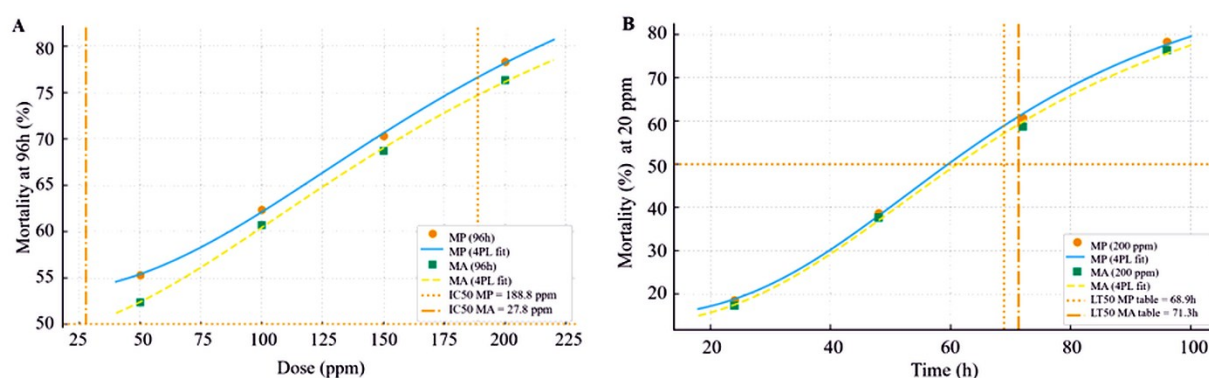


Figure 2. Single extract reference curves for *M. pachyloba* (MP) and *M. azedarach* (MA): dose-response at 96 h for MP and MA, with fitted 4PL curves and estimated median effect area (A); mortality kinetics over time at 200 ppm for MP and MA, with estimated  $LT_{50}$  (B)

Table 1. Phytochemical composition of combined *M. pachyloba*-*M. azedarach* aqueous extract (MPMA), including representative LC-MS/MS-detected compounds grouped within the phytochemical profile

| Phytochemical      | Presence in MPMA | Content                                                                                                                                                 |
|--------------------|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|
| Flavonoids         | +                | $39.57 \pm 1.42$ mg QE·g <sup>-1</sup> ; examples detected by LC-MS/MS: genistein, kaempferol, quercetin, formononetin, biochanin A glucoside, apigenin |
| Terpenoids         | +                | $41.55 \pm 1.62$ mg QE·g <sup>-1</sup> ; includes terpenoid/limonoid-related constituents associated with <i>M. azedarach</i>                           |
| Polyphenols        | +                | $70.47 \pm 2.12$ mg GAE·g <sup>-1</sup> ; includes flavonoid and phenolic fractions                                                                     |
| Alkaloids          | +                | $5.38 \pm 0.33$ mg AE·g <sup>-1</sup>                                                                                                                   |
| Saponins           | +                | NT                                                                                                                                                      |
| Steroids           | +                | NT                                                                                                                                                      |
| Tannins            | +                | $7.79 \pm 0.43$ mg TE·g <sup>-1</sup>                                                                                                                   |
| Rotenoids          | +                | Detected by LC-MS/MS: pachylobin, rotenone, millettone                                                                                                  |
| Cardiac glycosides | -                | -                                                                                                                                                       |

Note: (+) present, (-) absent, and (NT) not tested; Representative compounds were assigned from LC-MS/MS profiling and grouped under the corresponding phytochemical class

Table 2. Mortality (%) of *Meloidogyne incognita* J2 exposed to single aqueous extracts of *Millettia pachyloba* (M) and *Melia azedarach* (A) at 24–96 h

| Treatment | 24 h Mortality (%)         | 48 h Mortality (%)        | 72 h Mortality (%)        | 96 h Mortality (%)        | LT <sub>50</sub> (h) |
|-----------|----------------------------|---------------------------|---------------------------|---------------------------|----------------------|
| M50       | 12.33 ± 0.58 <sup>a</sup>  | 25.33 ± 0.58 <sup>a</sup> | 40.67 ± 0.58 <sup>a</sup> | 55.33 ± 0.58 <sup>a</sup> | 92.85                |
| M100      | 14.67 ± 0.58 <sup>b</sup>  | 30.33 ± 0.58 <sup>b</sup> | 47.67 ± 0.58 <sup>b</sup> | 62.33 ± 0.58 <sup>b</sup> | 82.41                |
| M150      | 16.33 ± 0.58 <sup>bc</sup> | 34.33 ± 0.58 <sup>c</sup> | 53.67 ± 0.58 <sup>c</sup> | 70.33 ± 0.58 <sup>c</sup> | 75.26                |
| M200      | 18.67 ± 0.58 <sup>c</sup>  | 38.67 ± 0.58 <sup>d</sup> | 60.67 ± 0.58 <sup>d</sup> | 78.33 ± 0.58 <sup>d</sup> | 68.91                |
| A50       | 11.67 ± 0.58 <sup>a</sup>  | 24.33 ± 0.58 <sup>a</sup> | 38.33 ± 0.58 <sup>a</sup> | 52.33 ± 0.58 <sup>a</sup> | 95.77                |
| A100      | 13.67 ± 0.58 <sup>b</sup>  | 29.33 ± 0.58 <sup>b</sup> | 45.33 ± 0.58 <sup>b</sup> | 60.67 ± 0.58 <sup>b</sup> | 84.55                |
| A150      | 15.33 ± 0.58 <sup>bc</sup> | 33.67 ± 0.58 <sup>c</sup> | 51.33 ± 0.58 <sup>c</sup> | 68.67 ± 0.58 <sup>c</sup> | 77.84                |
| A200      | 17.33 ± 0.58 <sup>c</sup>  | 37.67 ± 0.58 <sup>d</sup> | 58.67 ± 0.58 <sup>d</sup> | 76.33 ± 0.58 <sup>d</sup> | 71.32                |

Values are mean ± SD (n = 3). Different superscript letters within a column indicate significant differences between treatments at a given time point ( $p < 0.05$ , ANOVA with Tukey's test); LT<sub>50</sub> (h) was estimated from a time–mortality model (probit/logit/Weibull)

At matched total doses, the MPMA mixture consistently produced 3–7% higher mortality than either of the single extracts, yielding a positive excess over HSA at all concentrations tested. Data on single extract mortality are presented in Table 2, while the MPMA mixture mortality is shown in Table 3 and Figure 2A.

Based on the HSA model, the mixture outperformed the best single extract in the range of 50–200 ppm. However, under Bliss model independence,  $\Delta E_{(\text{Bliss})}$  values were  $\leq 0$ , indicating that the mixture enhancement did not surpass the expected additive effect. Chou–Talalay analysis yielded CI values close to 1 at medium to high effect levels, consistent with near additivity. Overall, MPMA showed measurable improvement over single extracts in the HSA benchmark, whereas Bliss and Chou–Talalay analyses supported an additive or near-additive interpretation in vitro (Figures 3 & 4).

#### Antioxidant activity

Kruskal–Wallis tests followed by Dunn's post-hoc comparisons showed that MPMA exhibited significant, concentration-dependent antioxidant activity across the DPPH, ABTS, and FRAP assays (adjusted  $p < 0.05$ ; Table 4). DPPH scavenging increased from  $40.66 \pm 0.45\%$  at 50 ppm

to  $84.74 \pm 1.29\%$  at 200 ppm, while ABTS scavenging increased from  $49.16 \pm 1.33\%$  to  $83.66 \pm 1.82\%$ . FRAP values also rose from  $606.12 \pm 27.55$  to  $1106.01 \pm 34.51 \mu\text{mol Fe}^{2+} \cdot \text{L}^{-1}$ . These data clearly demonstrate a dose-dependent redox modulating effect of the MPMA mixture.

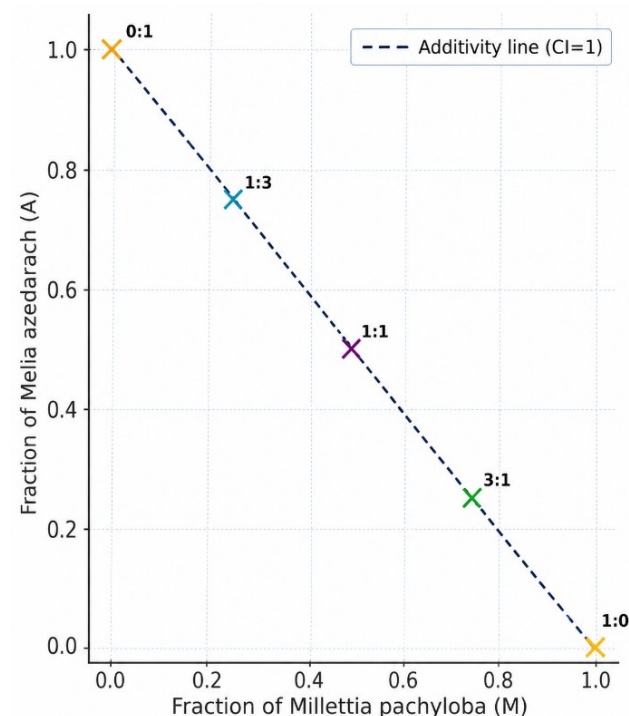


Figure 3. Isobologram and combination index (CI<sub>0.75</sub>)

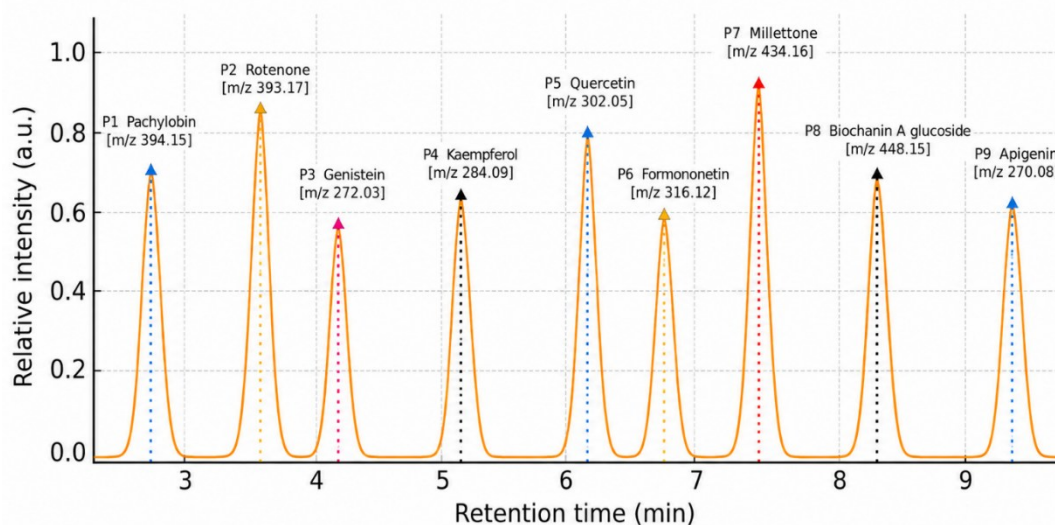


Figure 4. Chromatographic profile (LC-MS/MS) of *Millettia pachyloba-Melia azedarach* extract (MPMA)

Table 3. Effect of *Millettia pachyloba-Melia azedarach* extract (MPMA) on mortality rate (MR) and median lethal time (LT<sub>50</sub>) of *Meloidogyne incognita* J2 juveniles

| Experimental group         | 24 h                      | 48 h                      | 72 h                      | 96 h                         | LT <sub>50</sub> (h) |
|----------------------------|---------------------------|---------------------------|---------------------------|------------------------------|----------------------|
| Water                      | 11.33 ± 0.58 <sup>a</sup> | 23.67 ± 0.58 <sup>a</sup> | 35.33 ± 0.58 <sup>a</sup> | 46.67 ± 0.58 <sup>a</sup>    | 111.09               |
| Oxamyl 7.5                 | 21.33 ± 0.58 <sup>c</sup> | 44.67 ± 0.58 <sup>f</sup> | 67.33 ± 0.58 <sup>f</sup> | 88.67 ± 0.58 <sup>f</sup>    | -                    |
| MPMA50                     | 14.67 ± 0.58 <sup>b</sup> | 30.67 ± 0.58 <sup>b</sup> | 46.67 ± 0.58 <sup>b</sup> | 61.67 ± 0.58 <sup>b</sup>    | 83.42                |
| MPMA100                    | 16.67 ± 0.58 <sup>c</sup> | 34.67 ± 0.58 <sup>c</sup> | 52.33 ± 0.58 <sup>c</sup> | 69.33 ± 0.58 <sup>c</sup>    | 75.23                |
| MPMA150                    | 17.67 ± 0.58 <sup>d</sup> | 37.33 ± 0.58 <sup>d</sup> | 55.67 ± 0.58 <sup>d</sup> | 73.67 ± 0.58 <sup>d</sup>    | 70.88                |
| MPMA200                    | 19.67 ± 0.58 <sup>d</sup> | 41.33 ± 0.58 <sup>c</sup> | 62.33 ± 0.58 <sup>c</sup> | 82.33 ± 0.58 <sup>c</sup>    | 64.99                |
| LC50 (mg L <sup>-1</sup> ) | >300                      | >300                      | >300                      | 126.68 (95% CI: 110.2–144.3) | -                    |

Values are expressed as mean ± SD (n = 3); Different letters within a column indicate groups that differ significantly according to the Kruskal–Wallis test followed by Dunn’s multiple comparisons test with Benjamini–Hochberg false discovery rate adjustment (adjusted p < 0.05); Mortality in the water group represents natural/background mortality during the 96-h handling and observation period and was retained as a negative baseline control; Overall omnibus effects (two-sided) were MR 24 h: p = 0.0037, LT<sub>50</sub> between groups: p = 0.0021; LC<sub>50</sub>/LT<sub>50</sub> values are reported with 95% confidence intervals; LC<sub>50</sub> was estimated from pre-recovery mortality data, while LT<sub>50</sub> was derived from time-mortality regression fits

Table 4. Antioxidant activity of the combined *Millettia pachyloba-Melia azedarach* aqueous extract (MPMA) evaluated by DPPH, ABTS, and FRAP assays

| Experimental group | DPPH assay<br>% DPPH<br>scavenging | DPPH assay<br>IC <sub>50</sub> (mg per L) | ABTS assay<br>% ABTS<br>scavenging | ABTS assay<br>IC <sub>50</sub> (mg per L) | FRAP assay<br>(μmol Fe <sup>2+</sup> per L) |
|--------------------|------------------------------------|-------------------------------------------|------------------------------------|-------------------------------------------|---------------------------------------------|
| Water              | 0.00 ± 0.00 <sup>a</sup>           | —                                         | 0.00 ± 0.00 <sup>a</sup>           | —                                         | 284.23 ± 18.66 <sup>a</sup>                 |
| Oxamyl 7.5         | 14.32 ± 2.44 <sup>b</sup>          | —                                         | 29.75 ± 3.70 <sup>b</sup>          | —                                         | 410.62 ± 24.31 <sup>b</sup>                 |
| MPMA50             | 40.66 ± 0.45 <sup>c</sup>          | 142.31 (95% CI: 130.02–156.21)            | 49.16 ± 1.33 <sup>c</sup>          | 171.44 (95% CI: 159.33–185.18)            | 606.12 ± 27.55 <sup>c</sup>                 |
| MPMA100            | 59.71 ± 1.16 <sup>d</sup>          | 74.51 (95% CI: 67.42–82.12)               | 64.66 ± 2.39 <sup>d</sup>          | 116.22 (95% CI: 103.87–128.45)            | 793.51 ± 28.73 <sup>d</sup>                 |
| MPMA150            | 76.85 ± 1.73 <sup>e</sup>          | 51.62 (95% CI: 46.91–56.78)               | 76.52 ± 0.60 <sup>e</sup>          | 83.44 (95% CI: 78.22–88.93)               | 1006.28 ± 32.37 <sup>e</sup>                |
| MPMA200            | 84.74 ± 1.29 <sup>f</sup>          | 38.11 (95% CI: 34.28–42.65)               | 83.66 ± 1.82 <sup>f</sup>          | 64.71 (95% CI: 60.19–69.84)               | 1106.01 ± 34.51 <sup>f</sup>                |

Values are expressed as mean ± SD (n = 3); Different superscript letters within a column indicate groups that differ significantly according to the Kruskal–Wallis test followed by Dunn’s multiple comparisons test with Benjamini–Hochberg false discovery rate adjustment (adjusted p < 0.05)

### Nematicidal activity

Exposure of *Meloidogyne incognita* J2 juveniles to MPMA caused a significant, dose- and time-dependent increase in mortality (Table 3; Figure 5). At 200 ppm, mortality reached 82.33% at 96 h, with an  $LT_{50}$  of 64.99 h and an  $LC_{50}$  of  $126.68 \text{ mg} \cdot \text{L}^{-1}$  (95% CI:  $110.2\text{--}144.3 \text{ mg} \cdot \text{L}^{-1}$ ). The survival index declined to 48.00%, closely approximating the oxamyl-treated positive control (45.33%) (Figure 6).

### Physiological and biochemical responses

Greenhouse trials confirmed that the use of MPMA substantially reduced nematode infection and enhanced the physiological resilience of banana plants.

#### In planta: single-extract vs mixture and HSA/Bliss

At matched total doses, the 1 : 1 mixture (MPMA) consistently produced lower root gall indices than either single extract (*M. pachyloba* or *M. azedarach*), with the HSA line showing increased benefits at higher concentrations (Figure 7). When expressed as excess over HSA on the fractional reduction scale (relative to the negative control), the mixture outperformed the best single extract between 100 and 300 ppm, with modest but positive effects at 100–200 ppm and the highest benefit at 300 ppm (Figure 7).

When in-planta endpoints were normalized to fractional effects (gall and nematode population

reductions in roots and rhizosphere),  $\Delta E_{(\text{Bliss})}$  values were positive at  $\geq 200 \text{ ppm}$ , indicating performance above Bliss expectations, and remained near zero around 100 ppm. Representative  $\Delta E_{(\text{Bliss})}$  values ranged from +0.10 to +0.18 at intermediate-high doses (two-sided bootstrap 95% CIs excluding zero at the upper end), confirming a mild but consistent synergy signal under the pre-specified in-planta criteria (Figure 7).

In the physiological comparison (Table 5), MPMA at 300 ppm produced lower ROS,  $\text{O}_2^-$ , and  $\bullet\text{OH}$  levels and higher APx, GR, and GSH/GSSG values than the single extract treatments at 200 ppm. Accordingly, the composite growth index of the mixture was higher than that of *M. pachyloba* or *M. azedarach* alone, reflecting a physiological advantage consistent with the trend of disease suppression.

#### Nematicidal efficacy in planta

MPMA treatment markedly reduced rhizosphere nematode populations, achieving up to 82.27% suppression at 400 ppm, comparable to the oxamyl benchmark (86.30%) (Figure 8). Root galling declined significantly, and root nematode density decreased from 980 (control) to 250 individuals per plant (Figure 9), confirming the strong in-planta nematicidal potential of the formulation.

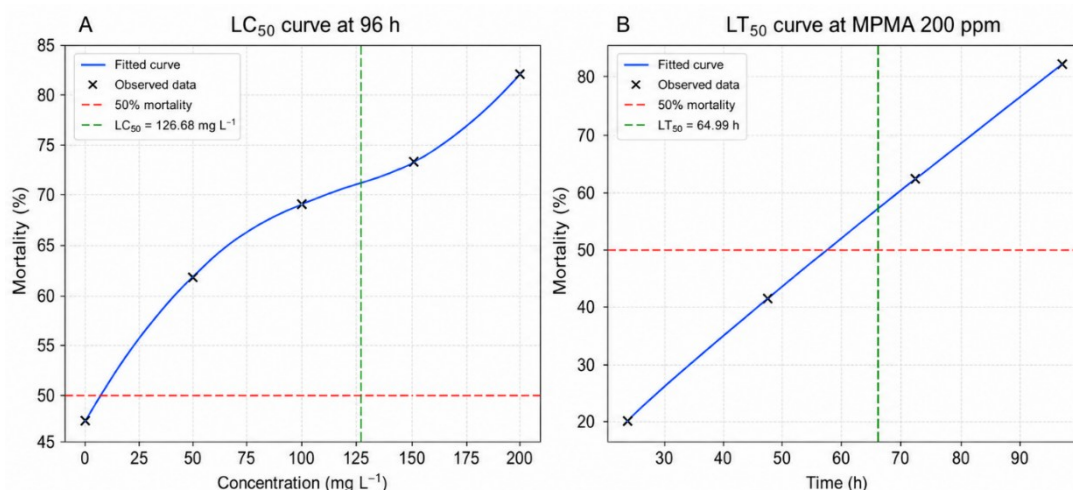


Figure 5. Mortality responses as a function of concentration and time of the combined *Millettia pachyloba*-*Melia azedarach* aqueous extract (MPMA 1 : 1, v/v) against *Meloidogyne incognita* J2:  $LC_{50}$  curve at 96 h (A);  $LT_{50}$  curve at MPMA 200 ppm (B). Points are mean  $\pm$  SD ( $n = 3$ ), and dashed guide lines indicate 50% mortality and estimated  $LC_{50}$  or  $LT_{50}$ ;  $LC_{50} = 126.68 \text{ mg} \cdot \text{L}^{-1}$  (95% CI:  $110.2\text{--}144.3$ );  $LT_{50} = 64.99 \text{ h}$  (95% CI:  $58.2\text{--}72.1$ )

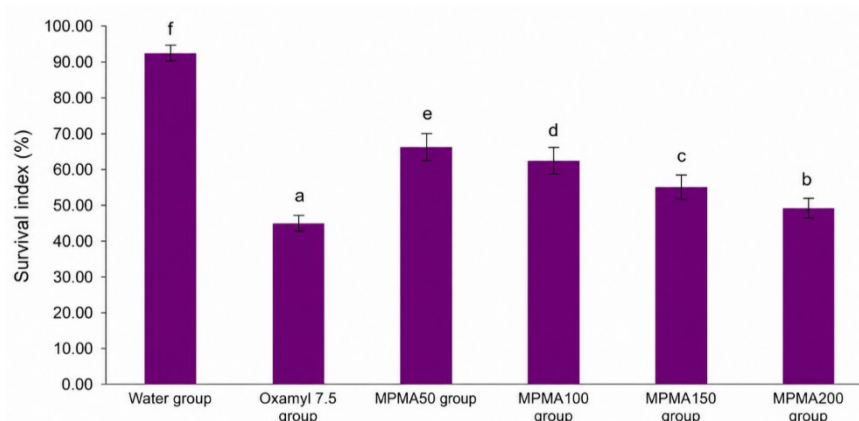


Figure 6. Survival index (SI) of *Meloidogyne incognita* juveniles exposed to a series of concentrations of *Milletia pachyloba*-*Melia azedarach* extract (MPMA) and controls

Bars represent mean  $\pm$  SD ( $n = 3$ ); Different letters above the bars indicate groups that differ significantly according to the Kruskal–Wallis test followed by Dunn’s multiple comparison test with Benjamini–Hochberg adjustment (adjusted  $p < 0.05$ )

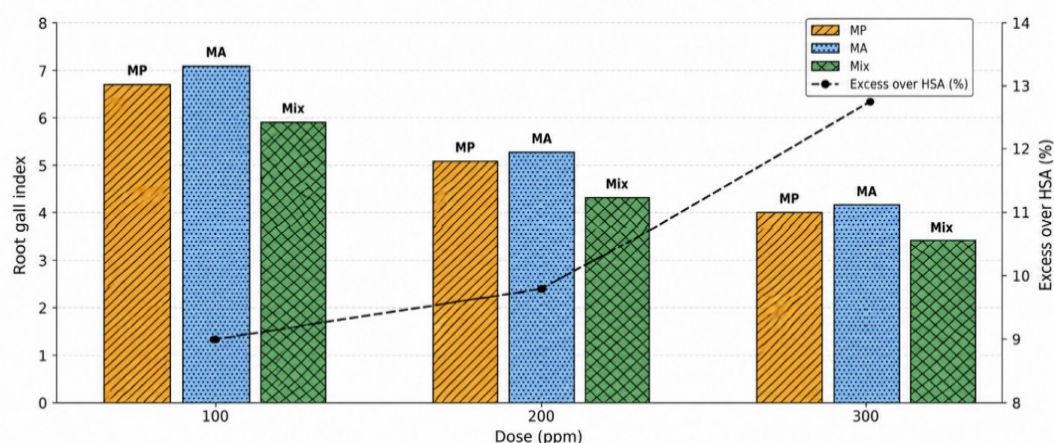


Figure 7. Descriptive comparison of the highest single agent (HSA) in-plant

Bars show root gall index for *M. pachyloba* (MP), *M. azedarach* (MA), and *Milletia pachyloba*-*Melia azedarach* extract – MPMA 1 : 1 (Mix) at 100, 200, and 300 ppm (left axis – lower is better); The dashed line (right axis) shows the excess to HSA (%), calculated from fractional reductions relative to the negative control; Treatment effects for root galling were significant in the related Kruskal–Wallis analysis ( $p < 0.01$ ); this panel serves as a descriptive HSA benchmark and not a standalone inferential plot

Table 5. Physiological responses of *Musa acuminata* to *M. pachyloba* (MP), *M. azedarach* (MA), and *Milletia pachyloba*-*Melia azedarach* extract (MPMA) under *Meloidogyne incognita* stress

| Treatment | ROS<br>(nmol per g FW)          | O <sub>2</sub> <sup>-</sup><br>(nmol per g FW) | •OH<br>(nmol per g FW)        | APx<br>(U per mg protein)    | GR<br>(U per mg protein)     | GSH/GSSG                     | Growth index                    |
|-----------|---------------------------------|------------------------------------------------|-------------------------------|------------------------------|------------------------------|------------------------------|---------------------------------|
| Control   | 205.46 $\pm$ 17.63 <sup>e</sup> | 88.56 $\pm$ 5.15 <sup>e</sup>                  | 37.55 $\pm$ 2.61 <sup>e</sup> | 0.48 $\pm$ 0.03 <sup>a</sup> | 0.28 $\pm$ 0.03 <sup>a</sup> | 1.38 $\pm$ 0.08 <sup>a</sup> | 245.11 $\pm$ 9.41 <sup>a</sup>  |
| Oxamyl 30 | 114.14 $\pm$ 15.78 <sup>a</sup> | 49.21 $\pm$ 4.27 <sup>a</sup>                  | 20.86 $\pm$ 2.25 <sup>a</sup> | 0.86 $\pm$ 0.06 <sup>d</sup> | 0.51 $\pm$ 0.04 <sup>d</sup> | 2.48 $\pm$ 0.15 <sup>d</sup> | 465.69 $\pm$ 19.35 <sup>e</sup> |
| MP200     | 152.63 $\pm$ 14.82 <sup>d</sup> | 66.74 $\pm$ 3.95 <sup>d</sup>                  | 27.41 $\pm$ 1.86 <sup>d</sup> | 0.64 $\pm$ 0.04 <sup>b</sup> | 0.37 $\pm$ 0.03 <sup>b</sup> | 1.83 $\pm$ 0.10 <sup>b</sup> | 326.11 $\pm$ 10.08 <sup>b</sup> |
| MA200     | 148.25 $\pm$ 14.26 <sup>c</sup> | 63.22 $\pm$ 3.88 <sup>c</sup>                  | 26.38 $\pm$ 1.74 <sup>c</sup> | 0.66 $\pm$ 0.04 <sup>b</sup> | 0.38 $\pm$ 0.03 <sup>b</sup> | 1.91 $\pm$ 0.11 <sup>b</sup> | 335.55 $\pm$ 10.62 <sup>c</sup> |
| MPMA300   | 136.97 $\pm$ 14.55 <sup>b</sup> | 59.04 $\pm$ 3.58 <sup>b</sup>                  | 25.03 $\pm$ 1.59 <sup>b</sup> | 0.72 $\pm$ 0.04 <sup>c</sup> | 0.42 $\pm$ 0.02 <sup>c</sup> | 2.07 $\pm$ 0.12 <sup>c</sup> | 367.65 $\pm$ 11.03 <sup>d</sup> |

Values are mean  $\pm$  SD ( $n = 3$ ); Different superscript letters within a column indicate significant differences between treatments ( $p < 0.05$ , ANOVA followed by Duncan’s multiple range test); 300 ppm of the MPMA reduced ROS levels by ~9% compared with the mean of the individual extracts and increased APx and GR activity by ~9–14%, indicating a physiological synergy in mitigating oxidative stress and growth recovery

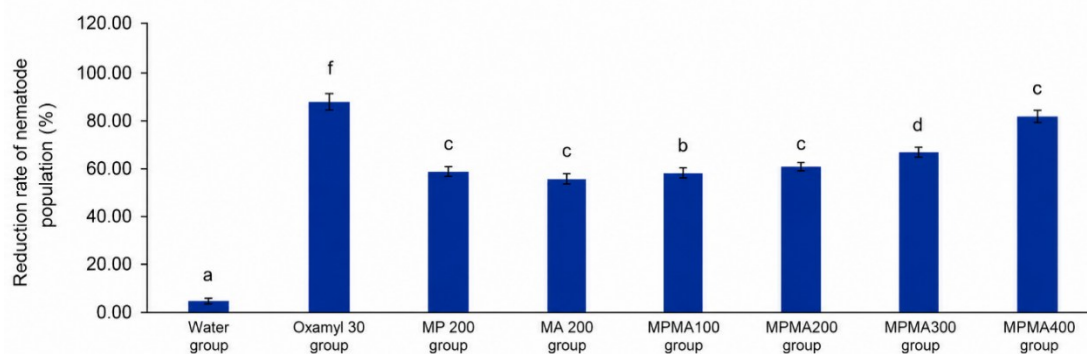


Figure 8. Reduction rate of rhizosphere nematode population (%) in banana plants infected with *Meloidogyne incognita* following treatment with water, 30 ppm of oxamyl, 200 ppm of *M. pachyloba* (MP), 200 ppm of *M. azedarach* (MA), and 100–400 ppm of *Milletia pachyloba*-*Melia azedarach* extract (MPMA). Values are mean  $\pm$  SD ( $n = 3$ ); Different letters indicate significant differences between treatments [Kruskal–Wallis test followed by Dunn’s test with Benjamini–Hochberg adjustment (adjusted  $p < 0.05$ )]

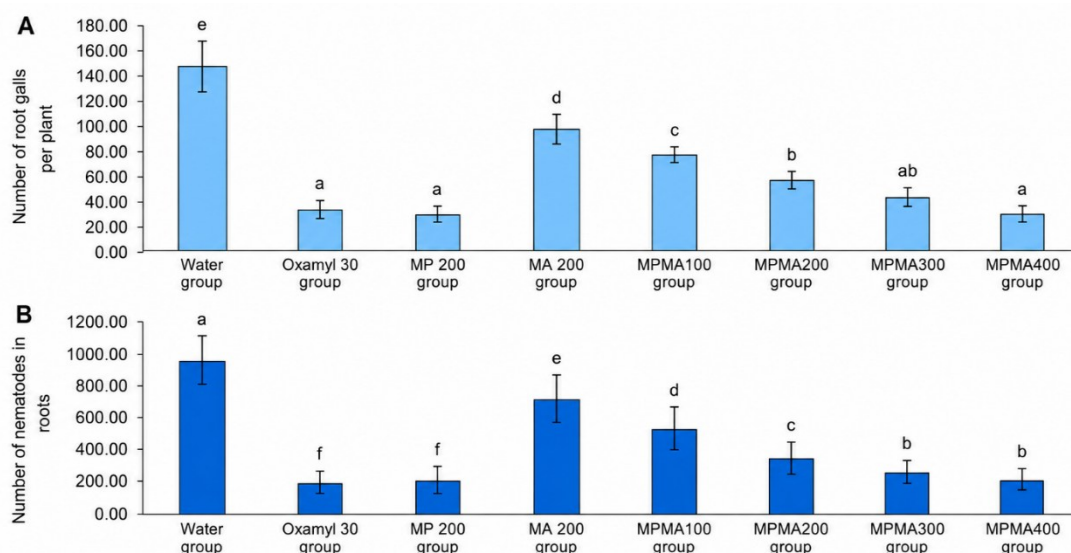


Figure 9. Effects of water, 30 ppm of oxamyl, 200 ppm of *M. pachyloba* (MP), 200 ppm of *M. azedarach* (MA), and 100–400 ppm of *Milletia pachyloba*-*Melia azedarach* extract (MPMA) on nematode infection in banana plants challenged with *Meloidogyne incognita*: number of root galls per plant (A) and number of nematodes in roots (B), measured 9 weeks after inoculation. Values are mean  $\pm$  SD ( $n = 3$ ); Different letters within each panel indicate significant differences between treatments [Kruskal–Wallis test followed by Dunn’s test with Benjamini–Hochberg adjustment (adjusted  $p < 0.05$ )]

### Oxidative stress and antioxidant responses

Across treatments, Kruskal–Wallis tests followed by Dunn’s post hoc comparisons showed that MPMA significantly decreased oxidative stress biomarkers (ROS,  $O_2^-$ ,  $\bullet OH$ ) in both root and pseudostem tissues (adjusted  $p < 0.05$ ). Simultaneously, antioxidant enzymes activities (APx, GR) and the GSH/GSSG ratio increased concentration-dependently (Table 6), indicating an enhanced redox buffering capacity and activation of antioxidant defense mechanisms in host tissues.

### Plant growth and physiological parameters

Across treatments, Kruskal–Wallis tests followed by Dunn’s post hoc comparisons showed that MPMA treatment resulted in significant increases in biomass accumulation and composite growth index values (adjusted  $p < 0.05$ ). At 400 ppm, root and pseudostem fresh weights, as well as the overall growth index, were substantially higher than those of the untreated control and comparable to the oxamyl group (Table 6). These outcomes suggest that MPMA not only suppresses nematode infection but also promotes host growth and physiological recovery.

### Correlation between nematode suppression, oxidative stress, and growth parameters

Two-sided Pearson correlations revealed that root nematode density was negatively associated with antioxidant status: APx activity ( $r = -0.86$ ,  $p = 0.0026$ ), GR activity ( $r = -0.84$ ,  $p = 0.0045$ ), and the GSH/GSSG ratio ( $r = -0.81$ ,  $p = 0.0031$ ). In contrast, antioxidant indices were positively correlated with plant performance, as reflected by the growth index: APx ( $r = 0.79$ ,  $p = 0.0019$ ), GR ( $r = 0.82$ ,  $p = 0.0027$ ), and GSH/GSSG ( $r = 0.85$ ,  $p = 0.0052$ ). These relationships indicate that greater antioxidant capacity is linked to lower nematode burden and improved growth (Table 7).

## DISCUSSION

### Quantitative evidence beyond single extracts

Head-to-head comparisons at matched total doses demonstrated that MPMA consistently outperformed

the better-performing single extract in the HSA benchmark, indicating a reproducible advantage over each constituent alone. Reference data for the single extract are presented in Table 2, mixture mortality is shown in Table 3, and HSA/Bliss comparisons in planta are summarized in Figure 7. In vitro analyses, Bliss and Chou–Talalay supported an additive to near-additive interpretation, with  $\Delta$ Bliss values  $\leq 0$  and CI values close to 1. In planta, however,  $\Delta$ Bliss values were positive at  $\geq 200$  ppm (+0.10 to +0.18; bootstrap 95% CIs excluding 0 in the upper end), which is consistent with the HSA-based improvement results observed for root galling and nematode density. Collectively, MPMA provided repeatable gains beyond those of either extract, with mainly additive to near-additive interactions in vitro and mild, endpoint-dependent synergy under greenhouse conditions, in line with current quantitative synergy analysis frameworks (Abd-Elgawad 2025).

Table 6. Physiological responses and plant performance of *M. incognita*-infected banana plants following *Millettia pachyloba*-*Melia azedarach* extract (MPMA) treatment

| Experimental group | ROS (nmol per g FW)          | O <sub>2</sub> <sup>-</sup> (nmol per g FW) | •OH (nmol per g FW)        | APx (U per mg protein)    | GR (U per mg protein)     | GSH/GSSG                  | Root FW (g)               | Pseudostem FW (g)          | Growth index                |
|--------------------|------------------------------|---------------------------------------------|----------------------------|---------------------------|---------------------------|---------------------------|---------------------------|----------------------------|-----------------------------|
| Water (control)    | 205.46 ± 17.63 <sup>d</sup>  | 88.56 ± 5.15 <sup>c</sup>                   | 37.55 ± 2.61 <sup>c</sup>  | 0.48 ± 0.03 <sup>a</sup>  | 0.28 ± 0.03 <sup>a</sup>  | 1.38 ± 0.08 <sup>a</sup>  | 28.45 ± 1.18 <sup>a</sup> | 67.68 ± 1.45 <sup>a</sup>  | 245.11 ± 9.41 <sup>a</sup>  |
| Oxamyl 30          | 114.14 ± 15.78 <sup>a</sup>  | 49.21 ± 4.27 <sup>a</sup>                   | 20.86 ± 2.25 <sup>a</sup>  | 0.86 ± 0.06 <sup>d</sup>  | 0.51 ± 0.04 <sup>d</sup>  | 2.48 ± 0.15 <sup>d</sup>  | 54.06 ± 1.37 <sup>f</sup> | 128.59 ± 3.38 <sup>f</sup> | 465.69 ± 19.35 <sup>f</sup> |
| MPMA100            | 158.05 ± 15.37 <sup>c</sup>  | 68.12 ± 4.14 <sup>d</sup>                   | 28.88 ± 2.13 <sup>d</sup>  | 0.62 ± 0.04 <sup>b</sup>  | 0.36 ± 0.04 <sup>b</sup>  | 1.79 ± 0.09 <sup>b</sup>  | 36.99 ± 1.22 <sup>b</sup> | 87.98 ± 2.13 <sup>b</sup>  | 318.63 ± 10.14 <sup>b</sup> |
| MPMA200            | 146.76 ± 14.88 <sup>bc</sup> | 63.26 ± 3.85 <sup>cd</sup>                  | 26.82 ± 1.98 <sup>cd</sup> | 0.67 ± 0.03 <sup>bc</sup> | 0.39 ± 0.06 <sup>b</sup>  | 1.93 ± 0.11 <sup>bc</sup> | 39.83 ± 1.35 <sup>c</sup> | 94.75 ± 2.24 <sup>c</sup>  | 343.14 ± 9.41 <sup>c</sup>  |
| MPMA300            | 136.97 ± 14.55 <sup>b</sup>  | 59.04 ± 3.58 <sup>c</sup>                   | 25.03 ± 1.59 <sup>c</sup>  | 0.72 ± 0.04 <sup>c</sup>  | 0.42 ± 0.02 <sup>bc</sup> | 2.07 ± 0.12 <sup>c</sup>  | 42.68 ± 1.44 <sup>d</sup> | 101.52 ± 2.58 <sup>d</sup> | 367.65 ± 11.03 <sup>d</sup> |
| MPMA400            | 120.86 ± 13.34 <sup>ab</sup> | 52.09 ± 3.22 <sup>b</sup>                   | 22.09 ± 1.35 <sup>b</sup>  | 0.81 ± 0.05 <sup>d</sup>  | 0.47 ± 0.03 <sup>cd</sup> | 2.34 ± 0.12 <sup>d</sup>  | 48.37 ± 1.53 <sup>e</sup> | 115.06 ± 2.75 <sup>e</sup> | 416.68 ± 11.30 <sup>e</sup> |

Values are expressed as mean ± SD (n = 3); Different letters within a column indicate groups that differ significantly according to the Kruskal–Wallis test followed by Dunn's multiple comparisons test with Benjamini–Hochberg false discovery rate adjustment (adjusted  $p < 0.05$ )

Table 7. Correlation coefficients between nematode density, antioxidant markers, and growth index in banana plants

| Parameter 1           | Parameter 2    | Correlation coefficient (r) | p-value |
|-----------------------|----------------|-----------------------------|---------|
| Root nematode density | APx activity   | -0.86                       | < 0.01  |
| Root nematode density | GR activity    | -0.84                       | < 0.01  |
| Root nematode density | GSH/GSSG ratio | -0.81                       | < 0.05  |
| APx activity          | Growth index   | 0.79                        | < 0.01  |
| GR activity           | Growth index   | 0.82                        | < 0.01  |
| GSH/GSSG ratio        | Growth index   | 0.85                        | < 0.01  |

### Mechanistic plausibility

The combined efficacy of MPMA aligns with the complementary phytochemistry of its components. Rotenoids from *M. pachyloba* inhibit mitochondrial complex I, depleting ATP and inducing J<sub>2</sub> mortality (Heinz et al. 2017), whereas limonoids from *M. azedarach* disrupt hormonal signaling and membrane integrity (Theofilidou et al. 2023). Additionally, alkaloids, saponins, and terpenoids modulate neuromuscular and reproductive processes, perturb cuticular stability, and reinforce host defense responses (Wang et al. 2023; Ibrahim et al. 2025). This partially nonoverlapping target profile provides a biochemical rationale for the observed HSA-positive and ΔBliss-positive outcomes (Tables 6–7) and coheres with the antioxidant and defense-priming responses recorded (↓ROS, O<sub>2</sub><sup>•−</sup>, •OH; ↑APx, GR, GSH/GSSG), which correlated strongly with growth recovery (Hassanpour & Doroudi 2023; Jomova et al. 2024). These findings indicate that redox homeostasis plays a pivotal role in mediating recovery from nematode-induced stress and promoting physiological resilience. Together, they highlight the coordinated regulation of nematode antioxidant defense and suppression as the mechanistic core of MPMA bioactivity.

### Limitations and scope

A benchmarked analysis of in-planta synergy was performed primarily using HSA, with the ΔBliss and Loewe models as supporting frameworks in which mapping of fractions and effects was feasible. Future work should employ denser fixed-ratio designs (above 1 : 1) and broader effect level coverage to improve CI precision, especially at low response levels, and validate performance via multi-site field trials, formulation stability testing, and integration with microbial/organic amendments.

### Applied and practical relevance

From a practical perspective, the water-based and nonphytotoxic MPMA is suitable for soil drenching or fertigation in organic and integrated pest-management (IPM) systems. Its compatibility with microbial inoculants and organic amendments requires

further validation at field scale under diverse edaphic and climatic conditions. Longer-term studies should also evaluate formulation stability, storage feasibility, and cost-effectiveness to support practical implementation.

### Concluding remarks

In summary, the dual application of *Milletia pachyloba* and *Melia azedarach* extracts confers synergistic nematicidal and antioxidant benefits, establishing MPMA as a dual-function botanical agent for sustainable banana cultivation. Its combined action mitigates nematode-induced oxidative stress while enhancing host growth and resilience, offering a viable, eco-compatible alternative for integrated root-knot nematode management.

## CONCLUSIONS

This study provides evidence that combined *Milletia pachyloba* and *Melia azedarach* aqueous leaf extracts (MPMA) produced reproducible benefits that exceeded the best single extract in the highest single agent benchmark, with mainly additive to near-additive interactions in vitro and mild, endpoint-dependent synergy under greenhouse conditions. The formulation significantly enhanced nematode suppression, restored redox homeostasis, and promoted growth in banana plants infected with *Meloidogyne incognita*. These dual effects support a complementary mode of action, both direct nematicidal activity and physiological recovery. Our findings were obtained under controlled greenhouse conditions, so field validation of performance in multiple sites will be required. Due to its water-based formulation, ecological safety, and compatibility with organic practices, MPMA represents a practical and sustainable botanical option for integrated root-knot nematode management in banana cultivation. Future work should focus on formulation refinement, field-scale validation, and integration with biofertilizers to maximize efficacy and facilitate farmer adoption.

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## Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## Ethical statement

This study did not involve experiments on animals or humans. All experiments were conducted on cultivated banana plants under greenhouse conditions and complied with institutional and national guidelines for research on plants. No endangered or protected species were collected.

## Data availability

All data supporting the findings of this study are included in the article. Additional raw data (e.g., primary spreadsheets, analysis scripts, and uncropped images) are available from the corresponding author upon reasonable request.

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