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Evaluation of the Nematicidal Activity of *Millettia pachyloba* Drake Aqueous Extract Against *Meloidogyne incognita* in Cavendish Banana

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ABSTRACT

Root-knot nematodes (*Meloidogyne incognita*) pose a significant threat to banana production, while conventional chemical controls raise concerns due to their environmental impact. *Millettia pachyloba* Drake, known for its bioactive compounds, offers a sustainable alternative for pest management. In this study, various concentrations (50, 100, 150, and 200 ppm) of aqueous extract from *M. pachyloba* (EAMP) were evaluated for their effects on *M. incognita* in Cavendish banana plants. Parameters assessed included egg hatching, nematode mortality, enzyme activity, and overall plant health. Egg hatching and nematode mortality were measured using in vitro assays, whereas enzyme activities (AChE, SOD, CAT) and plant health indicators were analyzed three days post-treatment under greenhouse conditions. Statistical analysis was conducted to determine the efficacy of each treatment. The water control group showed no inhibition of egg hatching (0.00 ± 0.00%; $P < 0.05$), whereas the chemical nematicide Oxamyl significantly reduced hatching to 13.11 ± 0.27% ($P < 0.05$). EAMP exhibited a dose-dependent reduction in egg hatching, with the 200 ppm concentration (EAMP200) showing the highest efficacy (23.13 ± 0.23%; $P < 0.05$). Similarly, nematode mortality increased with higher EAMP concentrations, reaching 71.74 ± 7.34% at 72 hours for EAMP200 ($P < 0.05$). In vivo results revealed that EAMP significantly reduced the root-knot index (RKI) and egg mass in a concentration-dependent manner. Notably, EAMP500 lowered the RKI to 1.33 ± 0.58 and egg mass to 82.67 ± 2.08 ($P < 0.05$). In addition, EAMP treatments improved chlorophyll and carotenoid contents in banana leaves, with EAMP500 yielding the highest recovery (chlorophyll: 2.23 ± 0.04 mg g^{-1}; carotenoid: 0.18 ± 0.01 mg g^{-1}; $P < 0.05$). These findings highlight the potential of EAMP as a promising, eco-friendly alternative to chemical nematicides for managing root-knot nematodes in banana cultivation. The extract effectively suppresses nematode activity, enhances plant physiological health, and minimizes environmental impact. However, yet practical aspects such as extraction scalability need further evaluation.

Abbreviations: Acetylcholinesterase (AchE), Acetylthiocholine iodide (ATChI), Catalase (CAT), The aqueous extract of M. pachyloba (EAMP), Egg Hatch Inhibition Rate (EIR), Final volume of the extract (FE), Fresh weight of leaf sample (FS), Length of the path of light (LPL), Nitro Blue Tetrazolium (NBT), Number of Dead Nematodes (NDN), Mortality Rate (NMR), Number of nematodes in soil (NNS), Number of nematodes counted (NS), Number of hatched juveniles in the control group (NHC), Number of hatched juveniles in the treatment group (NHT), Quercetin (QE), Root-knot index (RKI), Reactive Oxygen Species (ROS), Sodium

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Eluate (SE), Superoxide dismutase (SOD), Tannic Acid (TE), Total Number of Nematodes (TNN), Volume used for counting (VCN), Total extracted solution (VES), Weight of the soil (WSE)

Introduction

Bananas are among the most important fruit crops worldwide, primarily cultivated in tropical and subtropical regions (Gert et al., 2021). The Cavendish cultivar (*Musa acuminata* Cavendish) dominates global banana production due to its high yield, adaptability, and consistent fruit quality (Nhung & Quoc, 2024a). However, this variety is highly susceptible to the root-knot nematode *Meloidogyne incognita*, a major pest that significantly impairs plant growth and productivity (Guzman et al., 2023). *M. incognita* infects banana roots by forming galls, which interfere with water and nutrient uptake, resulting in stunted growth, reduced vigor, and increased vulnerability to environmental stressors. In cases of prolonged infestation, banana plants may fail to flower or bear fruit, with yield losses reaching 30–50% under severe conditions. Additionally, nematode damage reduces fruit size and weight, thereby decreasing market value (Sudeep et al., 2020).

Current management practices largely rely on chemical nematicides. However, these compounds present significant environmental and health risks, including soil degradation, contamination of water sources, disruption of beneficial soil organisms, and potential harm to human health (David et al., 2022). Moreover, the widespread use of chemical nematicides has led to the emergence of nematode resistance, further diminishing their efficacy (Abd-Elgawad, 2024). These limitations underscore the urgent need for alternative nematode management strategies that are both effective and environmentally sustainable. Plant-based extracts, particularly those derived from medicinal or pesticidal plants, are gaining attention as promising biocontrol agents due to their low toxicity to non-target organisms, biodegradability, and potential to reduce resistance development (Nhung & Quoc, 2024b).

Millettia pachyloba Drake, a large woody species belonging to the Fabaceae family and native to the tropical forests of Africa, has been traditionally valued for its medicinal properties. This species is especially notable for its chemically diverse profile, including flavonoids, alkaloids, saponins, and triterpenoids, which are associated with antioxidant, antibacterial, antifungal, and pest-inhibitory activities (Wei et al., 2019; Rasmita et al., 2020). Several studies have demonstrated the pest-suppressive potential of *M. pachyloba* extracts. For instance, they have been shown to inhibit the development of the diamondback moth (*Plutella xylostella*) across its life stages, reducing growth and reproductive capacity (Nhung & Quoc, 2024c). These extracts have also been reported to decrease pest populations in green mustard crops, while alleviating oxidative stress and immune suppression caused by pest infestation, thereby improving plant health and productivity (Nhung & Quoc, 2024d).

Similarly, other Fabaceae members exhibit promising biopesticidal properties. Extracts from *Acacia nilotica* seeds, which contain functional groups such as alkane, alkyl, aromatic, and amide, have reduced insect population growth by up to 50% in greenhouse trials, highlighting their agricultural potential (Vivekanandhan et al., 2023). Flavonoids from Fabaceae species have also been shown to affect the nervous systems and physiological processes of plant-parasitic nematodes, including *Meloidogyne spp.*, *Globodera pallida*, and *G. rostochiensis* (Sabrina, 2018). In addition, saponins derived from five *Millettia* species (*M. heyniana*, *M. hybrida*, *M. lupulina*, *M. murex*, and *M. truncatula*) have demonstrated nematicidal activity against *M. incognita*, *Xiphinema index*, and *G. rostochiensis*, primarily through disruption of nematode cell structure (Trifone et al., 2020). Collectively, these findings underscore the biocontrol potential of Fabaceae species, including *M. pachyloba*, as viable alternatives to chemical nematicides.

Given the promising bioactive properties of *M. pachyloba* and its established efficacy against insect pests, its application against plant-parasitic nematodes warrants further investigation. This is particularly relevant in the context of *M. incognita*, which poses a significant threat to Cavendish banana production by reducing yield and compromising plant health. Therefore, the present study aims to evaluate the nematicidal activity of *M. pachyloba* aqueous extracts against *M. incognita* under both in vitro and greenhouse conditions. In addition, the study seeks to elucidate the potential mechanisms of action of the plant's bioactive compounds. The findings are expected to contribute to the development of safe and sustainable nematode management strategies, reducing dependence on chemical inputs and promoting long-term productivity in banana cultivation.

Materials and Methods

Collection of material and preparation of the extract

Millettia pachyloba was collected in October 2023 from the Dakrông district, Quảng Trị, Vietnam. A voucher specimen of dried plant material

(MPD221023VST) was stored at the Biotechnology Laboratory, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City. The plant was cleaned, air-dried in the shade for 3-5 d, and ground into powder. The powder was stored in sealed containers under dry, cool, and light-protected conditions. For extraction, 1000 g of the powder was mixed with 10 L of distilled water (1:10 ratio, w/v) and macerated at room temperature for 24 h. Following filtration, the EAMP was obtained with an extraction efficiency of 68%, yielding 680 mL. The extraction efficiency was calculated using the following formula:

$$Extraction\ efficiency\ (\%) = \frac{The\ amount\ of\ extract\ obtained}{The\ initial\ amount\ of\ material} \times 100$$

(Niloofar et al., 2023).
This extract was used for subsequent experimental analyses.

Phytochemical analysis
Phytochemical analysis of EAMP followed a procedure outlined by Nhung et al. (2023), identifying alkaloids, flavonoids, tannins, steroids, saponins, cardiac glycosides, and terpenoids. Alkaloids were quantified using picric acid and UV-Vis spectrophotometry at 520 nm (Nhung and Quoc, 2023a). Tannins were measured with $FeCl_3$ at 510 nm (Trang et al., 2021). Saponins were determined by foam height and a standard curve (Nhung and Quoc, 2023b). Flavonoids were analyzed with $AlCl_3$ at 415 nm as quercetin equivalents (Nhung and Quoc, 2024e).

Meloidogyne incognita culture and preparation
Meloidogyne incognita nematodes were inoculated on young banana plants in a greenhouse at the Faculty of Biotechnology, Industrial University of Ho Chi Minh City. A nematode population was established from infected roots collected from a banana field in Cu Chi District, Ho Chi Minh City. Eggs or second-stage juveniles (J2) were extracted and incubated in distilled water at 27 ± 2 °C to facilitate hatching. J2 larvae were collected daily, and their suspension was used for inoculating banana plant roots. The inoculated plants were maintained under controlled laboratory conditions (25-28 °C) for 4-6 weeks to allow nematode development, with root galls indicating sufficient nematode population for further experiments (Leidy et al., 2023).

In vitro experiments
Experimental design
The EAMP extract was prepared as a stock solution and diluted to concentrations of 50, 100, 150, and 200 ppm (EAMP50, EAMP100, EAMP150, and EAMP200) based on preliminary screening tests (data not shown) and literature references (Faryad et al., 2019). Control groups included a negative control (Water group) and a positive control (Oxamyl 7.5 ppm) (Faryad et al., 2019). All treatments were conducted in triplicate to ensure reliable results.

Egg hatching rate of nematodes
Five egg masses of *Meloidogyne incognita* were collected from infected banana roots and placed in Petri dishes with 10 mL of each extract concentration. After 7 d at room temperature, the dishes were incubated at 27.5 °C. Hatched juveniles were counted under an Olympus microscope, and egg hatch inhibition was calculated using the formula:

$$\text{EIR}\ (\%) = \frac{\text{NHC} - \text{NHT}}{\text{NHC}} \times 100$$

where NHC is the number of hatched juveniles in the control, and NHT is in the treatment group (Faryad et al., 2019).

Nematode mortality rate
A 0.2 mL aliquot of water containing 100 second-stage juveniles was added to 9.8 mL of plant extract (50-200 ppm). Water and Oxamyl (7.5 ppm) served as controls. Mortality was observed at 24, 48, and 72 h, with movement indicating live nematodes. Mortality was calculated using the formula:

$$\text{NMR}\ (\%) = \frac{\text{NDN}}{\text{TNN}} \times 100$$

where NDN is the number of dead nematodes and TNN is the total number of nematodes. Probit analysis was used to calculate the LC50 values for all treatments (Faryad et al., 2019).

Enzyme activity of nematodes
Acetylcholinesterase (AChE)
Nematodes (100 g) were homogenized in phosphate buffer to extract AChE. Enzyme activity was measured using 1 mM ATChI at 412 nm and calculated from a standard calibration curve (Abdullah et al., 2023).

Superoxide dismutase (SOD)
Nematodes (100 g) were homogenized and centrifuged to extract SOD. The activity was assessed by illuminating a reaction mixture with 0.3 mM NBT, riboflavin, and phosphate buffer, measuring absorbance at 560 nm, and calculating from a standard calibration curve (Yuh et al., 2024).

Catalase (CAT)

Nematodes (100 g) were homogenized and centrifuged to extract CAT. Activity was measured by the decrease in absorbance of 20 mM hydrogen peroxide at 240 nm, using a standard calibration curve (Yuh et al., 2024). Nematodes were collected from all treatment groups (EAMP50, EAMP100, EAMP150, and EAMP200) and the control group after 3 d of treatment to evaluate the dose-dependent effects of EAMP on AChE, SOD, and CAT activity.

Greenhouse experiment

Experimental design

Clay pots (25 cm in diameter and 30 cm in height) were used for the greenhouse experiment, each filled with 2 kg of sterilized soil prepared by mixing sandy loam and well-decomposed manure in a 3:1 ratio. To enhance soil quality and microbial activity, 50 g of finely chopped fresh *Millettia pachyloba* leaves were incorporated into each pot and regularly watered to facilitate decomposition. The experimental setup included seven treatments, each replicated three times: four concentrations of *M. pachyloba* aqueous extract (EAMP) at 200, 300, 400, and 500 ppm; a negative control (water); a positive control (Oxamyl 30) (Faryad et al., 2019); and a normal control (no treatment and no nematode inoculation).

Each pot was planted with one micropropagated Cavendish banana plant aged 4 to 6 weeks. Subsequently, 3000 freshly hatched second-stage juveniles (J2) of *Meloidogyne incognita* were introduced into each pot through three evenly spaced holes around the root zone. The experiment was repeated three times to ensure reliability and reproducibility of the findings. This design was intended to rigorously evaluate the effects of EAMP under controlled greenhouse conditions.

Root-knot index

Sixty days after nematode inoculation, banana plants were uprooted, and the roots were gently rinsed with water to remove adhering soil. The roots were then examined for the presence of galls caused by *Meloidogyne incognita*. The root-knot index (RKI) was assessed on a scale of 0 to 5, where 0 indicated no galls and 5 represented more than 100 galls, to quantify the severity of nematode infection and evaluate the effectiveness of the treatments (Sithole et al., 2021).

Egg mass on the root system

Banana plants were carefully uprooted, and the root systems were gently rinsed to preserve attached egg masses. The roots were then stained with acid fuchsin and examined under a magnifying lens to count the number of egg masses present on the root surface (Faryad et al., 2019).

Nematode population in soil

A 250 g soil sample was collected from around the root zone of the banana plant, sieved, and placed in a Baermann funnel. After 24 h, nematodes were counted in the collected water using a microscope. The total number of nematodes per 250 g of soil was calculated using the following formula:

$$\mathrm{NNS} = \frac{\mathrm{NS}}{\mathrm{VCN}} \times \mathrm{VES} \times \frac{1}{\mathrm{WSE}} \times 100$$

where NS is the number of nematodes counted, VCN is the volume used for counting, VES is the total extracted solution, and WSE is the weight of the soil (Faryad et al., 2019).

Chlorophyll and carotenoid content

Fresh banana leaves (1 g) were ground and extracted with 20 mL of 80% acetone, centrifuged, and absorbance was measured at 645 and 663 nm for chlorophyll, and 480 and 510 nm for carotenoids. Chlorophyll and carotenoid concentrations were calculated using the following formulas:

$$\text{Total chlorophyll content} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \left(\frac{\mathrm{FE}}{1000 \times \mathrm{FS}}\right)$$

$$\text{Carotenoid content} = 7.6\,(A_{480}) - 1.49\,(A_{510}) \times \left(\frac{\mathrm{FE}}{1000 \times \mathrm{LPL} \times \mathrm{FS}}\right)$$

A_{480}, A_{510}, A_{645}, A_{663} = Absorbance of extract at given wavelengths (480, 510, 645, and 663 nm, respectively), FE = Final volume of the extract, FS = Fresh weight of leaf sample, LPL = Length of the path of light (Faryad et al., 2019).

Statistical analysis

Data were analyzed using one-way ANOVA with Statgraphics Centurion XIX software. Differences among treatments were assessed using Duncan's multiple range test, with significant differences set at $P < 0.05$. Data were presented as mean ± standard deviation (SD).

Results

Qualitative and quantitative phytochemical analysis of bioactive compounds in extracts

Preliminary screening of the phytochemical composition of the aqueous extract of *M. pachyloba* (EAMP) identified the presence of several secondary metabolites, including alkaloids, flavonoids, phenolics, steroids, tannins, terpenoids, and saponins. Notably, cardiac glycosides were absent (Table 1). Quantitative analysis indicated significant concentrations of tannins (7.95 ± 0.12 mg TE g^{-1}), flavonoids (40.73 ± 1.24 mg QE g^{-1}), alkaloids (182.37 ± 4.45 µg mL^{-1}), and saponins (14.26 ± 0.28

mg SE g^{-1}) (Table 1). These findings highlight the potential applications of EAMP in agriculture and medicine, particularly due to its diverse bioactive compounds.

Table 1. Phytochemical screening and quantification of secondary metabolites in the aqueous extract of *Millettia pachyloba* (EAMP).

Phytoconstituents	Test	Observation	Present in EAMP	Quantification of phytochemicals
Tannins	2 mL EAMP + 2 mL H_2O + 2-3 drops $FeCl_3$ (5%)	Green precipitate	+	7.95 ± 0.12 (mg TE g^{-1})
Flavonoids	1 mL EAMP + 1 mL $Pb(OAc)_4$ (10%)	Yellow coloration	+	40.73 ± 1.24 (mg QE g^{-1})
Terpenoids	2 mL EAMP + 2 mL $(CH_3CO)_2O$ + 2-3 drops conc. H_2SO_4	Deep red coloration	+	-
Polyphenol	2 mL EAMP + 2 mL $FeCl_3$	Bluish-green appearance	+	-
Saponins	5 mL EAMP + 5 mL H_2O + heat	Froth appears	+	14.26 ± 0.28 (mg SE g^{-1})
Steroids	2 mL EAMP + 2 mL $CHCl_3$ + 2 mL H_2SO_4 (conc.)	The reddish-brown ring at the junction	+	-
Cardiac glycosides	2 mL EAMP + 2 mL $CHCl_3$ + 2 mL CH_3COOH	Violet to Blue to Green coloration	-	-
Alkaloids	2 mL EAMP + a few drops of Hager's reagent	Yellow precipitate	+	182.37 ± 4.45 (µg mL^{-1})

Phytochemicals in EAMP are (+) present and (-) absent.

In vitro experiments

Egg-hatching inhibition

Figure 1 illustrates the inhibitory effects of various concentrations of *Millettia pachyloba* aqueous extract (EAMP) on the egg hatching of *Meloidogyne incognita*, in comparison with a water control and the chemical nematicide Oxamyl (7.5 mg L^{-1}). The water control group exhibited no inhibition of egg hatching (0.00 ± 0.00%), serving as a baseline for untreated conditions ($P < 0.05$). In contrast, oxamyl significantly suppressed egg hatching, resulting in a hatching rate of 13.11 ± 0.27% ($P < 0.05$), thus confirming its potent nematicidal activity.

EAMP exhibited a clear concentration-dependent inhibitory effect. At the lowest concentration (EAMP50), egg hatching remained relatively high (45.97 ± 0.44%; $P < 0.05$). However, increasing the concentration led to progressively greater inhibition: hatching rates decreased to 39.40 ± 0.20% for EAMP100, 31.10 ± 0.17% for EAMP150, and 23.13 ± 0.23% for EAMP200 ($P < 0.05$). These results indicate that higher concentrations of EAMP effectively reduce *M. incognita* egg hatching, supporting its potential as a botanical nematicide.

Nematode mortality

Table 2 describes the nematode mortality rate (NMR) of *Meloidogyne incognita* across different treatments over 24, 48, and 72 h, alongside the 72 h LC50 value of EAMP. The water control group exhibited no nematode mortality (0.00 ± 0.00%) ($P < 0.05$), whereas oxamyl (7.5 mg L^{-1}) showed the highest efficiency, with mortality rates increasing from 22.20 ± 1.64% (24 h) to 84.67 ± 9.47% (72 h) ($P < 0.05$). EAMP treatments displayed concentration- and time-dependent effects. EAMP50 exhibited the lowest mortality rates (11.80 ± 1.30% at 24 h to 20.58 ± 2.14% at 72 h) ($P < 0.05$), while EAMP200 recorded the highest nematicidal activity among EAMP treatments (21.20 ± 2.17% at 24 h to 71.74 ± 7.34% at 72 h) ($P < 0.05$). The LC50 of EAMP at 72 h was calculated as 120 ppm, indicating significant efficiency at higher concentrations.

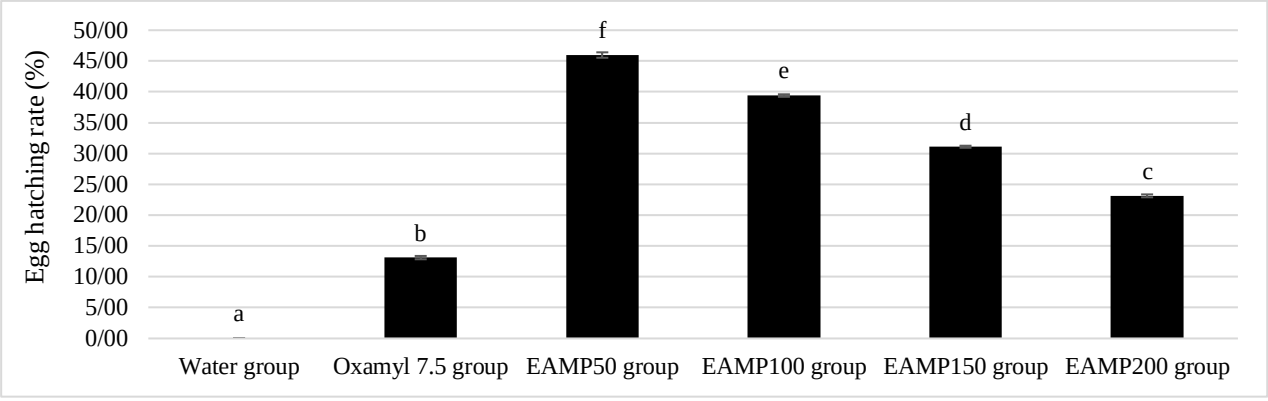

Fig. 1. Comparative egg-hatching inhibition rates (%) of *Meloidogyne incognita* after treatment with different concentrations of *Millettia pachyloba* Drake aqueous extract (EAMP) and controls. Values are presented as mean ± SD. Different letters (a–f) indicate statistically significant differences between treatments ($P < 0.05$).

Table 2. Time-dependent nematicidal activity of EAMP against *Meloidogyne incognita* and LC_{50} determination.

Groups	The nematode mortality rate (NMR) (%)			72 h LC50 of the EAMP
	24 h	48 h	72 h	
Water group	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	120 ppm
Oxamyl 7.5 group	22.20 ± 1.64^{d}	39.35 ± 1.92^{f}	84.67 ± 9.47^{f}	
EAMP50 group	11.80 ± 1.30^{b}	14.98 ± 1.60^{b}	20.58 ± 2.14^{e}	
EAMP100 group	13.20 ± 1.30^{b}	23.29 ± 1.86^{c}	45.46 ± 3.95^{c}	
EAMP150 group	19.40 ± 1.14^{c}	31.29 ± 2.43^{d}	61.96 ± 5.92^{d}	
EAMP200 group	21.20 ± 2.17^{cd}	34.58 ± 2.98^{e}	71.74 ± 7.34^{e}	

Values are expressed as Mean ± SD, and letters (^{a}, ^{b}, ^{c}, ^{d}, ^{e}, and ^{f}) represent the difference between treatments ($P < 0.05$).

Enzymatic activity

EAMP's mode of action was further investigated by analyzing enzyme activity in *Meloidogyne incognita* (Table 3). Acetylcholinesterase (AChE), which is crucial for nematode neural function, was significantly reduced in a concentration-dependent manner. EAMP200 caused the most substantial reduction (108.51 ± 7.98 U mL^{-1}) ($P < 0.05$), closely approaching the inhibitory effect of oxamyl (102.48 ± 6.25 U mL^{-1}) ($P < 0.05$). Additionally, the activity of antioxidant enzymes, i.e., superoxide dismutase (SOD) and catalase (CAT), known as markers of oxidative stress, increased in EAMP-treated groups. SOD activity rose from 2.78 ± 0.05 U mL^{-1} (water control) to 4.73 ± 0.01 U mL^{-1} (EAMP200) ($P < 0.05$), while CAT activity increased from 1.64 ± 0.01 U mL^{-1} to 2.79 ± 0.03 U mL^{-1}, respectively ($P < 0.05$).

Table 3. Changes in enzymatic activity (AChE, SOD, CAT) of *Meloidogyne incognita* following EAMP treatment.

Group	Acetylcholinesterase (AChE) (U mL^{-1})	Superoxide dismutase (SOD) (U mL^{-1})	Catalase (CAT) (U mL^{-1})
Water group	184.46 ± 11.56^{f}	2.78 ± 0.05^{a}	1.64 ± 0.01^{a}
Oxamyl 7.5 group	102.48 ± 6.25^{a}	5.01 ± 0.05^{f}	2.95 ± 0.04^{f}
EAMP50 group	141.89 ± 10.48^{e}	3.61 ± 0.02^{b}	2.13 ± 0.04^{b}
EAMP100 group	131.76 ± 9.76^{d}	3.89 ± 0.01^{c}	2.31 ± 0.01^{c}
EAMP150 group	115.29 ± 8.92^{b}	4.45 ± 0.01^{d}	2.62 ± 0.02^{d}
EAMP200 group	108.51 ± 7.98^{c}	4.73 ± 0.01^{e}	2.79 ± 0.03^{e}

Values are expressed as Mean ± SD, and letters (^{a}, ^{b}, ^{c}, ^{d}, ^{e}, and ^{f}) represent the difference between treatments ($P < 0.05$).

Greenhouse experiment

Root-knot index and egg mass

Figures 2 and 3 illustrate the effects of EAMP on the Root-Knot Index (RKI) and egg mass in Cavendish banana plants. The water control group had the highest RKI (4.33 ± 0.58) and egg mass count (132.67 ± 4.51) (P < 0.05), reflecting severe nematode infestation. Oxamyl significantly reduced these values to 0.67 ± 0.58 and 73.67 ± 1.53, respectively (P < 0.05). EAMP treatments caused dose-dependent reductions in both parameters. At the highest concentration (EAMP500), RKI and egg mass were reduced to 1.33 ± 0.58 and 82.67 ± 2.08, respectively (P < 0.05), approaching the efficiency of oxamyl.

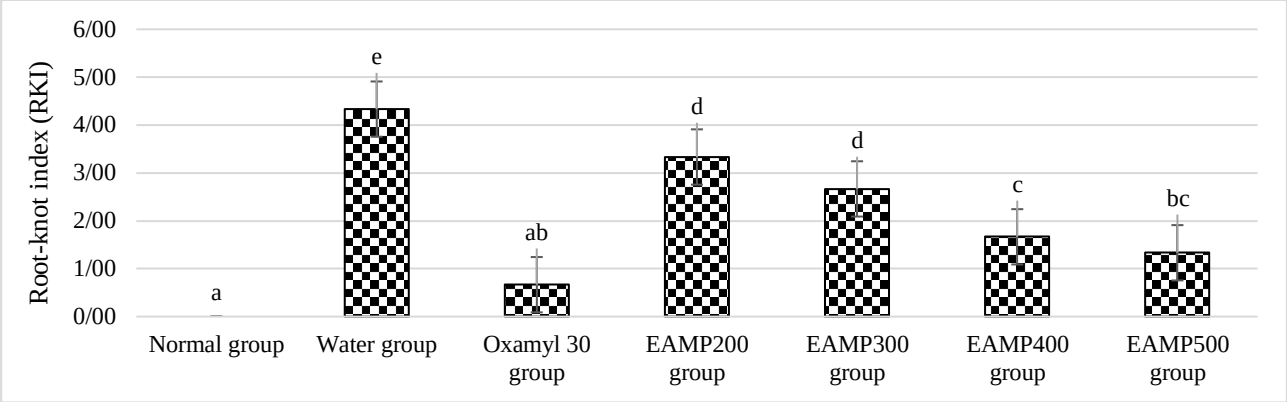

Fig. 2. Dose-dependent reduction in root-knot index of *Meloidogyne incognita* in Cavendish banana plants following treatment with different concentrations of *Millettia pachyloba* aqueous extract (EAMP) compared to controls. Values are presented as mean ± SD. Different letters (a–e) indicate statistically significant differences between treatments (P < 0.05).

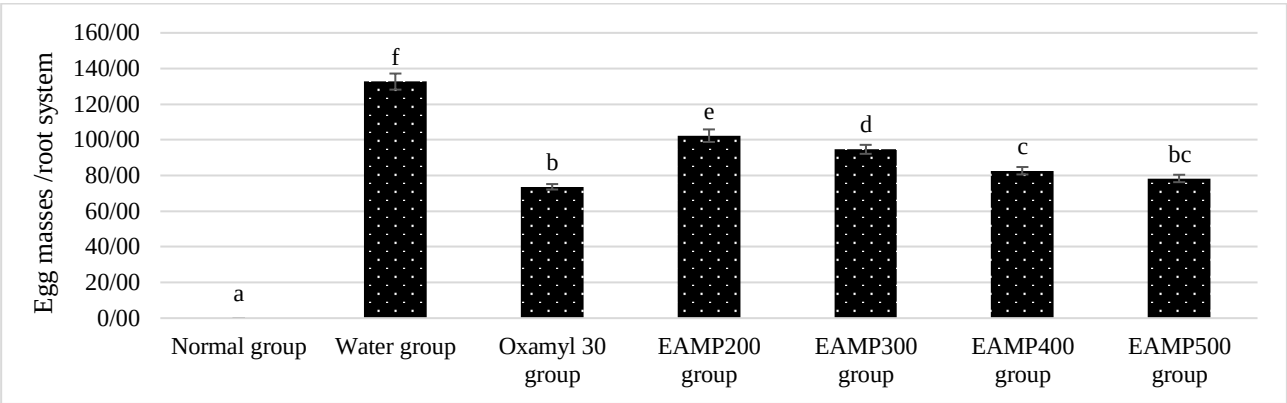

Fig. 3. Quantitative assessment of egg masses on Cavendish banana root systems following treatment with different concentrations of *Millettia pachyloba* aqueous extract (EAMP), compared to the water control and Oxamyl. Values are presented as mean ± SD. Different letters (a–f) indicate statistically significant differences between treatments (P < 0.05).

Soil nematode population

Figure 4 shows the soil nematode density in different treatment groups. The water control group recorded the highest nematode density (2107 ± 42.43 mg g^{-1}) (P < 0.05), whereas oxamyl reduced the population to 1171 ± 4.24 mg g^{-1} (P < 0.05). EAMP treatments reduced nematode density in a dose-dependent manner, with EAMP500 achieving the lowest density (1239 ± 4.24 mg g^{-1}) (P < 0.05), comparable to oxamyl.

Photosynthetic pigment recovery

Table 4 presents the chlorophyll and carotenoid contents in banana leaves across different treatment groups. The water-treated (negative control) group exhibited the lowest pigment levels, with chlorophyll at 1.57 ± 0.06 mg g^{-1} and carotenoid at 0.13 ± 0.01 mg g^{-1} (P < 0.05), indicating the detrimental impact of *Meloidogyne incognita* infection on plant health. In contrast, the normal control group (no nematode inoculation) showed the highest pigment levels (chlorophyll: 2.67 ± 0.07 mg g^{-1}; carotenoid: 0.24 ± 0.01 mg g^{-1}; P < 0.05), reflecting optimal physiological conditions.

Oxamyl-treated plants demonstrated significant recovery in pigment content, with chlorophyll and carotenoid levels reaching 2.43 ± 0.05 mg g^{-1} and 0.21 ± 0.01 mg g^{-1}, respectively ($P < 0.05$). Similarly, EAMP treatments improved pigment concentrations in a dose-dependent manner. The highest recovery was observed in the EAMP500 group, which recorded chlorophyll and carotenoid levels of 2.23 ± 0.04 mg g^{-1} and 0.18 ± 0.01 mg g^{-1}, respectively ($P < 0.05$). These results suggest that EAMP not only mitigates nematode-induced damage but also supports the restoration of photosynthetic capacity in banana plants.

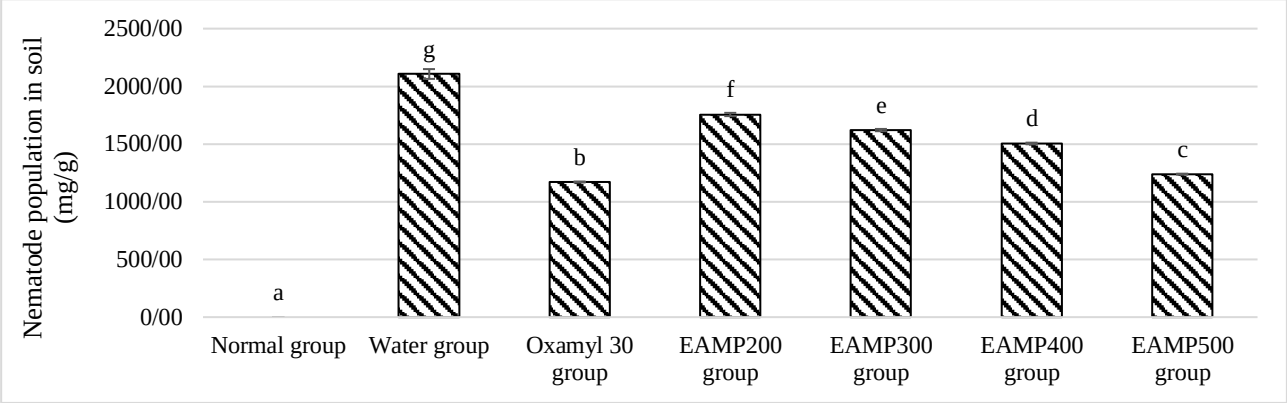

Fig. 4. Soil nematode population density (mg g^{-1}) in Cavendish banana cultivation following application of *Millettia pachyloba* aqueous extract (EAMP) at different concentrations (200–500 ppm) compared to control treatments. Values are presented as mean ± SD. Different letters (a–g) indicate statistically significant differences between treatments ($P < 0.05$).

Table 4. Effect of EAMP treatment on chlorophyll and carotenoid contents in banana leaves under nematode stress.

Group	Chlorophyll content (mg g^{-1})	Carotenoid content (mg g^{-1})
Normal group	2.67 ± 0.07^{g}	0.24 ± 0.01^{f}
Water group	1.57 ± 0.06^{a}	0.13 ± 0.01^{a}
Oxamyl 30 group	2.43 ± 0.05^{f}	0.21 ± 0.01^{e}
EAMP200 group	1.78 ± 0.06^{b}	0.14 ± 0.01^{ab}
EAMP300 group	1.91 ± 0.03^{c}	0.15 ± 0.01^{bc}
EAMP400 group	2.05 ± 0.04^{d}	0.16 ± 0.01^{c}
EAMP500 group	2.23 ± 0.04^{e}	0.18 ± 0.01^{d}

Values are expressed as Mean ± SD, and letters (^{a}, ^{b}, ^{c}, ^{d}, ^{e}, ^{f}, and ^{g}) represent the difference between treatments ($P < 0.05$).

Discussion

Secondary metabolites play a critical role in plant defense against pathogens and pests (Sithole et al., 2021). This study highlights both the nematicidal efficacy and the phytochemical composition of *Millettia pachyloba* aqueous extract (EAMP) in managing *Meloidogyne incognita* infestations in Cavendish banana. EAMP was found to contain several key bioactive metabolites, including alkaloids (182.37 ± 4.45 µg mL^{-1}), flavonoids (40.73 ± 1.24 mg QE g^{-1}), tannins (7.95 ± 0.12 mg TE g^{-1}), saponins (14.26 ± 0.28 mg SE g^{-1}), as well as phenolics, steroids, and terpenoids. Each of these compounds contributes to the overall nematicidal activity of the extract.

Alkaloids are known to interfere with the nematode nervous system, leading to paralysis and eventual death (Kelly et al., 2022). Flavonoids disrupt nematode metabolism and enhance plant defenses through their antioxidant properties (Elisa et al., 2022). Tannins exert anti-nematode effects by binding to proteins, thereby impairing digestion and reproduction (Luise et al., 2022). Saponins compromise the integrity of the nematode cuticle and disrupt osmotic regulation, resulting in mortality (Maria et al., 2021). Phenolic compounds strengthen plant cell walls, limiting nematode penetration (Sivakumar et al., 2022), while steroids and terpenoids reduce nematode activity through anti-inflammatory and deterrent mechanisms (Julio, 2024). Collectively, these metabolites act synergistically to disrupt nematode physiology and enhance host plant defense, establishing EAMP as a

promising eco-friendly alternative to synthetic nematicides.

The nematicidal potential of EAMP is evident through its strong inhibition of egg hatching, increased juvenile mortality, and an LC_{50} value of 120 ppm after 72 h. EAMP significantly reduced egg hatching in a concentration-dependent manner, with higher concentrations (e.g., EAMP200) demonstrating greater inhibition than lower concentrations (e.g., EAMP50). This suggests that EAMP's bioactive compounds interfere with embryonic development and create unfavorable hatching conditions, thereby limiting early-stage population growth (Amir & Tariq, 2024).

In addition to affecting egg development, EAMP induced time- and concentration-dependent mortality among second-stage juveniles (J2), with EAMP200 achieving mortality rates comparable to those of the synthetic nematicide Oxamyl after 72 h. The synergistic action of alkaloids, flavonoids, and saponins likely underpins these effects by targeting neural pathways, metabolic processes, and cellular structures (Hashim et al., 2024). Although the LC_{50} of EAMP is higher than that of Oxamyl, it is within the range reported for other plant-based nematicides, supporting its potential as a sustainable alternative (Ping et al., 2024).

Furthermore, EAMP was found to modulate key enzymatic activities essential for nematode survival, including acetylcholinesterase (AChE), superoxide dismutase (SOD), and catalase (CAT). EAMP inhibited AChE activity in a concentration-dependent manner, disrupting neurotransmission and leading to nematode paralysis and death (Sean et al., 2022). Concurrently, increased SOD and CAT activities indicated elevated oxidative stress levels, reflecting the nematodes' attempt to counteract reactive oxygen species (ROS). Despite these responses, the ROS accumulation induced by EAMP overwhelmed the nematodes' antioxidant defenses, resulting in extensive cellular damage and mortality. This dual mechanism—combining neurotoxicity with oxidative stress—underscores the potent nematicidal action of EAMP (Andrey et al., 2024).

In addition to its direct nematicidal effects, EAMP effectively mitigates root-knot nematode damage in Cavendish banana plants by significantly reducing both the Root-Knot Index (RKI) and egg mass on the roots. A lower RKI reflects reduced gall formation and improved root function, while decreased egg mass indicates suppression of nematode reproduction. These reductions correlate with decreased infestation severity and reproductive success of *Meloidogyne incognita*, ultimately supporting plant vigor and yield potential (Nhung & Quoc, 2024a; 2024b).

Furthermore, EAMP enhances chlorophyll and carotenoid content in banana leaves under nematode-induced stress by suppressing nematode populations and minimizing root damage. This recovery of pigment biosynthesis is attributed to the antioxidant properties of flavonoids and phenolic compounds in the extract, which help neutralize ROS generated during nematode infection (Mirza et al., 2020). Beyond its biochemical effects, EAMP also promotes root health by fostering beneficial soil microbiota, enhancing nutrient uptake, and restoring hormonal balance disrupted by nematode activity (Ntombikhona et al., 2022).

These physiological improvements are consistent with previous reports showing that effective nematode control enhances plant growth, stress tolerance, and photosynthetic efficiency (Fatemy & Faryad, 2019; Nhung & Quoc, 2024a; Feiyan et al., 2022). Collectively, the findings highlight the multifaceted efficacy of EAMP in managing *M. incognita*, through mechanisms including disruption of nematode physiology, inhibition of reproduction, and reinforcement of plant defense responses. Overall, EAMP demonstrates strong potential as a sustainable and environmentally friendly alternative to conventional chemical nematicides. Its ability to reduce nematode burden while promoting plant health makes it a valuable tool for improving crop productivity and supporting environmentally conscious agricultural practices.

Conclusion

The findings of this study demonstrated that *Millettia pachyloba* aqueous extract (EAMP) possesses potent nematicidal properties and can serve as an effective biocontrol agent against *Meloidogyne incognita*, a major pest threatening banana cultivation. EAMP significantly reduced egg hatching, increased juvenile mortality, and suppressed nematode population density in a concentration-dependent manner. These direct effects were complemented by notable improvements in plant health, including enhanced root development and restoration of chlorophyll and carotenoid levels, which are typically compromised under nematode-induced stress. The observed physiological recovery is attributed to the antioxidant properties of EAMP's bioactive constituents—particularly flavonoids, phenolics, alkaloids, and saponins—which not only inhibit nematode activity but also strengthen the plant's own defense systems. These multifaceted effects position EAMP as a viable and eco-friendly alternative to synthetic nematicides such as Oxamyl. Unlike chemical controls, which are associated with environmental degradation, toxicity to non-target organisms, and increasing resistance among nematode populations, EAMP offers a safer and more sustainable option for integrated pest management in banana production systems. However, despite its efficacy, several limitations

must be addressed before EAMP can be recommended for widespread agricultural application. The preparation of the extract is time-intensive and may require substantial labor and raw plant material, potentially limiting its practicality for large-scale use. Furthermore, standardization of active compound concentrations and formulation stability must be established to ensure consistent performance across different environmental and agronomic conditions. Additional field-based studies are also necessary to validate the greenhouse findings under real-world farming scenarios. In conclusion, EAMP represents a promising botanical nematicide with broad-spectrum benefits for plant protection and health restoration. Its use aligns with current goals in sustainable agriculture, promoting reduced reliance on synthetic chemicals while enhancing crop productivity and environmental safety. Future research should focus on optimizing extraction methods, assessing long-term field efficacy, and exploring the potential of EAMP in integrated pest management programs.

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Author contributions
Conceptualized and designed the study, BHQ; conducted experiments, BHQ; interpreted the data, BHQ; drafted the manuscript, BHQ; conducted revisions. BHQ; provided methodological guidance, TTPN; performed statistical analyses, TTPN; assisted in data interpretation, TTPN; reviewed the manuscript for intellectual content, TTPN; served as the corresponding author, TTPN. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest
The authors indicate no conflict of interest in this work.

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