

NEMATICIDAL EFFECT OF *EUPATORIUM ODORATUM* LINN. AQUEOUS EXTRACT ON BURROWING NEMATODES (*RADOPHOLUS SIMILIS*) AND ITS APPLICATION TO CONTROL TOPPLING DISEASE ON CAVENDISH BANANA (*MUSA ACUMINATA*)

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ABSTRACT

Radopholus similis (Cobb 1893) (Rhabditida, Pratylenchidae) is a common and destructive pest that causes significant yield losses in Cavendish bananas. The repeated use of synthetic pesticides to control this nematode has raised concerns about toxicity to users and the environment. An alternative approach to managing this harmful species involves the use of nontoxic plant-derived extracts that enhance protection against various biological constraints. In this study, aqueous extract from the leaves and stems of *Eupatorium odoratum* Linn. (EAEQ) was used to control *R. similis* in banana roots under in vitro and on-plant conditions. Through direct contact and penetration experiments on roots, as well as on-plant trials, concentrations of 12500, 6250, 3120, 1560, and 780 mg DW·L⁻¹ EAEQ were used to evaluate nematode mortality rates, infection levels in root segments (in vitro experiment), root infection, root necrosis index, shoot and root fresh weight, and growth parameters such as stem length, diameter, leaf number, and leaf surface area (on-plant experiment). The results showed that the nematode mortality rate due to EAEQ ranged from 35.3% to 90.1%, which is statistically significant compared to the mortality rate in the control treatment (16%). Significant nematode inhibition was observed in banana roots infected with *R. similis* and exposed to EAEQ for 48 h. There were no statistically significant differences in root infection, root necrosis, and plant fresh weight between plants treated with fenamiphos and EAEQ. Maximum effectiveness was observed at the 12500 mg DW·L⁻¹ EAEQ concentration. These findings provide valuable insights into the nematicidal metabolites of EAEQ against *R. similis*. Thus, the potential of EAEQ as a biocontrol agent against *R. similis* has been demonstrated. For future research, investigations will be extended to elucidate the mechanisms of action of the essential compounds in EAEQ against nematodes, determine the optimal EAEQ dosage for nematode control without harm to banana plants and the environment, assess the long-term effects of EAEQ on banana plants and the environment, explore the ecological impact of EAEQ use in agricultural ecosystems, study the effectiveness of EAEQ on various plant species, and consider the development of commercial products or formulations based on EAEQ.

Key words: Cavendish bananas, banana root burrowing nematodes, *Radopholus similis*, pesticides, fenamiphos, plant-based nematicides

INTRODUCTION

Banana plants are often considered the world's first globally traded commodity and continue to be the most exported fruit on the planet. Banana cultivation and exportation have provided livelihoods for thousands of small and large farms.

Despite the advantages of a warm tropical climate, this industry is threatened by fungal populations, insects, and plant-parasitic nematodes. These factors have led to significant declines in banana production and exports (Bartholomew et al. 2014). Plant-parasitic nematodes significantly threaten banana production in tropical and subtropical regions.

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Nematode infestation in bananas leads to lower bunch weight and longer crop cycles, which can significantly affect yield (Kisaakye et al. 2023). *Radopholus similis* is a migratory endoparasitic nematode primarily found in plant roots and is widely known as a destructive pest of bananas, citrus, and black pepper. It has been found in over 250 plant species in tropical and subtropical regions, with bananas being its primary host plant. Globally, *R. similis* considered bananas' most dangerous nematode pest (Haegeman et al. 2010). *R. similis* feeds on the cell walls and creates tunnels in the roots and stems, leading to lesions and necrotic root damage that affects the plant's ability to absorb water and nutrients, resulting in reduced banana bunch development and decreased fruit yield. Damaged and necrotic root tissues also affect the anchorage of the plant, leading to plant toppling or uprooting. Therefore, effective control of *R. similis* is crucial for the sustainability of the banana industry (Jesus et al. 2015).

Nematicides (chemical agents) such as carbofuran and methyl bromide have been used to manage the damaging nematode *R. similis* in banana plants. However, these chemicals are associated with numerous health and environmental hazards, including soil pollution, plant, and groundwater contamination, and risks to the health of animals, farmers, and consumers. Therefore, their use is discouraged or banned (WHO 2010). There is a growing demand for plant-based extracts with biological activity as an alternative to synthetic chemicals to control of nematodes in agricultural systems. Some plant-derived compounds have shown nematicidal activity, cost effectiveness, and environmental safety, making them suitable for organic farming systems (Mwamula et al. 2022). Cultivating clean and healthy plant materials is vital to managing the parasitic nematode *R. similis* in bananas (Jesus et al. 2015).

Eupatorium odoratum Linn., also known as *Chromolaena odorata* (Asteraceae family), was first identified in Vietnam. It is a common herbaceous plant found in various parts of the world. Previous studies have reported that various components of *E. odoratum* extracts exhibit antibacterial, anti-inflammatory, antioxidant, analgesic, cell-protective, antimicrobial, and other significant medicinal properties (Vijayaraghavan et al. 2018). Furthermore, the

plant's soft and easily degradable characteristics make it an ideal botanical for effective weed and pest control. Extracts from *E. odoratum* increase the mortality rate of various insects and parasitic pests while reducing their population. These attributes of *E. odoratum* in protecting plants against natural enemies contribute to the potential discovery of a new plant source with biological activities for pest management (Kato-Noguchi & Kato 2023). Although *E. odoratum* is known to increase the mortality rate of several nematode species, to our knowledge, there have been no reports on its nematicidal effect against *R. similis*. Therefore, this study aims to investigate the efficacy of aqueous extract of *E. odoratum* (EAEO) in controlling burrowing nematodes (*R. similis*) and its application in controlling root rot disease in Cavendish bananas.

MATERIALS AND METHODS

The leaves and stems of *E. odoratum* were collected in Phu My Hung commune, Cu Chi district, Ho Chi Minh City, Vietnam, in December 2022. The plant specimens were sent to the Plant Biotechnology Laboratory with the voucher EO101222VST for future reference. The fresh leaves and stems were washed, chopped, air-dried in the shade, and subsequently dried at 40 °C for three days until reaching a moisture content of approximately 10–12%. For further research, the dried product was ground into powder and stored in moisture-proof bags at 25 °C.

Preparation of EAEO

The powdered plant material (1.5 g) was added to 20 mL of distilled water and boiled for 5 min, then allowed to cool and filtered through a thin cotton cloth and filter paper (Whatman No. 1). The volume of water was adjusted to 25 mL to obtain 6% (w/v) extract. The extract was then collected, sterilized using a 0.22 µm Millipore filter (Merck-Millipore, Burlington, MA, USA), and frozen until use. The stock solution was diluted with distilled water at 1:2, 1:4, 1:8, 1:16, and 1:30. The five dilution ratios corresponded to five concentrations: 12500, 6250, 3120, 1560, 780 mg DW·L⁻¹ (mg dry weight per liter).

Phytochemical EAEO compounds

A standard phytochemical screening method was used to evaluate the phytochemical profile of EAEO.

Chemical reactions and color changes were used as indicators to determine the presence of major chemical groups, such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, coumarins, and steroids in EAEO (Table 1) (Nascimento et al. 2013; Hodges & Minich 2015; Kumar et al. 2016; Loman & Ju 2017; Wang et al. 2018).

***R. similis* culture and preparation**

The population of burrowing nematodes (*R. similis*) was collected from the roots of Cavendish bananas grown at a banana farm in Phu My Hung commune, Cu Chi district, Ho Chi Minh City, Vietnam. The process of culturing *R. similis* was carried out in a growth chamber on fresh organic carrots that had been washed and soaked in a 2% sodium hypochlorite solution, following the method described by Koshy and Sosamma (1980). The carrot cores were prepared according to Moody et al. (1973). Centrifuge tubes of 1.5 mL were used to collect and multiply the nematodes. Twenty-five female nematodes were selected under a dissecting microscope and transferred to tubes with 600 µL of 1% ampicillin using a fine needle. After ten minutes, most of the ampicillin solution was removed and replaced with 200 µL of sterile water. Five minutes later, most of the tube water was removed and replaced with 200 µL of antibiotic solution. After a ten-minute rest, 150 µL of the antibiotic solution was removed, and the remaining 50 µL of nematode cyst suspension was pipetted onto the surface of carrot cores. The carrot cores were inoculated with 25 females and maintained in an adjusted growth chamber at 28°C. After five days, each flask was checked for any contaminating organisms, and contaminated flasks were discarded. After 20 days, nematodes were extracted from carrot tissue as Coolen and D'Herde (1972) described. The nematodes were collected in a 400-mesh sieve. The sieve was washed with sterile water, and the nematodes were recovered. The number of nematodes (including eggs) was estimated by counting them in a counting chamber under a dissecting microscope.

In vitro experiments

Approximately 800 nematodes of *R. similis*, second-stage juveniles (J2) were tested for their survival ability after exposure to EAEO for 48 h. Five dilutions of EAEO were prepared corresponding to concentrations of 12500, 6250, 3120, 1560, 780 mg DW·L⁻¹

(EAEO₁₂₅₀₀, EAEO₆₂₅₀, EAEO₃₁₂₀, EAEO₁₅₆₀, and EAEO₇₈₀ treatment, respectively), and two controls (fenamiphos at a concentration of 4.65 mg·mL⁻¹ – positive control, water – negative control). Three replicates were prepared in test tubes for each dilution and maintained at room temperature. Both mobile and immobile nematodes were counted under a microscope. Dead nematodes (immobile and stained with tryptophan) exhibited various peculiar body shapes, such as S-shaped and coiled, with a fluorescent greenish hue indicating tryptophan. The mortality rate was calculated using the following formula (Kesba et al. 2021):

$$\text{Mortality (\%)} = \frac{\text{Number of death nematode in a treatment}}{\text{Number of total tested nematode in the same treatment}} \times 100.$$

Effects of EAEO on *R. similis* infection of root segments

The penetration assays of *R. similis* (J2) into the host plant roots were conducted using the method described by Marin et al. (2006). 75 wells of the 96-well flat bottom microtiter plate (7 mm diameter and 10 mm depth) was filled with 0.6 g of sterile fine sand (pre-autoclaved). Root segments 4 mm long were excised from the elongation zone of banana plants in the greenhouse. A volume of 25 mL of test EAEO, fenamiphos, and water was distributed to each well, before placing a single root segment horizontally in the growth medium, and adding an extra 0.28 g of dry sand. Nematode cysts were extracted from carrot disk cultures, quantified, and resuspended in sterile deionized water at 8,000 nematodes per mL concentration. A 25 mL of the nematode cyst suspension (~200 nematodes) was added to each well. The microtiter plates were covered with plastic lids and placed in a humidity chamber (100% RH at 25°C) for 24 h. The root pieces were then collected, and the active nematodes were extracted by soaking in 300 mL of sterile deionized water in separate microtiter plate wells. The plates were then incubated in the humidity chamber (100% RH at 25°C) for 48 h. The nematode extracts were quantified under a compound microscope using phase-contrast illumination. To detect any remaining nematodes, the root segments were soaked in 1% NaOCl for 10 min, rinsed under running tap water for 15 min, and stained for 30 seconds at boiling temperature with acid fuchsin solution in water. The root segments were placed between two microscope slides (25 × 75 mm), and all nematodes were counted. The experiment was designed and analyzed

in a completely randomized design with three replicates. The data were transformed with $\log_{10}(x + 1)$ before analysis.

Greenhouse experiment

Cavendish banana plants from tissue culture were grown in 414 mL foam cups (top diameter 8.9 cm, height 13.6 cm). A 1:1 mixture of sterile coarse river sand and 254 μm sand (autoclaved for one hour at 121 °C) was used as the growth medium. A complete nutrient solution (Hydro Umat F, Vietnam) was supplemented twice a week. Before nematode inoculation, the plants were grown for four weeks at 27 °C and 80% relative humidity (RH) in the greenhouse. Nematode cysts were extracted from carrot disk culture medium, quantified, and resuspended in sterile deionized water (40 cysts per mL). Five milliliters of nematode cyst suspension (~200 nematodes) were added to the base of each plant. The experiment was designed with seven treatments as above. The EAEO and fenamiphos solution were distributed in 40 mL aliquots and applied to the plants at zero and five days after inoculation. The plants were maintained for eight weeks at approximately 27 °C and 80% RH in the greenhouse (Marin et al. 2000).

At the end of the experiment, the plants were excavated, their roots and stems were cleaned to remove any soil particles, and the root necrosis index (RNI) and the fresh weight of roots and corm (RCFW) were determined. To assess the extent of root decay, the RNI was scored according to Bartholomew et al. (2014) on a scale from 0 to 4 [0: no damage; 1: 1–25% of the total root cortex necrotic; 2: 26–50% of the total root cortex necrotic; 3: 51–75% of the total root cortex necrotic; 4: more than 75% of the total root cortex necrotic].

Plant growth

Throughout the research process, the following plant measurements were recorded on a weekly basis: (a) pseudostem length (measured from the lowest leaf point to the base of the pseudostem, cm); (b) pseudostem circumference (girth measured at a point halfway along the pseudostem length, cm); (c) total number of fully opened functional leaves; (d) leaf area (LA, cm^2) predicted using regression models: $\text{LA} = 0.0266 + (\text{L} \times \text{W} \times 0.7629)$ ($r = 0.98$), where L represents leaf length and W represents leaf width. The third leaf from the top was selected as the reference leaf for measurement (Potdar & Pawar 1991).

Statistical analysis

Differences in nematode mortality, infection nematode of root segments, root necrosis index, roots and corm fresh weight, and growth parameters were analyzed using ANOVA. Transformed means were reported in figures and tables, and only significant differences ($p < 0.05$) were considered. A post hoc analysis, such as Tukey's HSD test, was conducted to examine specific group differences further. All statistical analyses were performed using the statistical software Statgraphics Centurion XIX.

RESULTS AND DISCUSSION

EAEO phytochemicals

The presence of plant chemicals in EAEO was demonstrated through positive chemical reactions (Table 1), indicated by notable color changes. Phytochemical screening of EAEO revealed the presence of plant chemicals belonging to the following groups: phenols, flavonoids, terpenoids, alkaloids, tannins, and saponins. Glycoside, steroids, and coumarins were absent in the extract.

Table 1. Components of aqueous extracts of leaves and stems of *Eupatorium odoratum*

Chemical compound	Test	Observations	Presence in EAEO
Tannin (Braymer test)	2 mL extract + 2 mL H_2O + 2–3 drops FeCl_3 (5%)	green precipitate	+
Flavonoid	1 mL extract + 1 mL $\text{Pb}(\text{OAc})_4$ (10%)	yellow	+
Terpenoid	2 mL extract + 2 mL $(\text{CH}_3\text{CO})_2\text{O}$ + 2–3 drops conc. H_2SO_4	dark red	+
Saponins (foam test)	5mL extract + 5 mL H_2O + heat	foam	+
Steroids (Salkowski test)	2 mL extract + 2 mL CHCl_3 + 2 mL H_2SO_4 (conc.)	sepia circle at the junction	-
Glycosides (Liebermann test)	2 mL extract + 2 mL CHCl_3 + 2 mL CH_3COOH	purple to blue to green	-
Alkaloids (Hager test)	2 mL extract + few drops of Hager's reagent	yellow precipitate	+
Phenols	2 mL extract + 2 mL FeCl_3	bluish color	+
Coumarin	1 mL extract + 1 mL NaOH 10%	yellow formation	-

Presence of phytochemicals in EAEO: (+) present and (-) absent

The nature of the structure and mode of action of most plant chemicals with pesticidal properties has been extensively discussed. The EAEO has been reported to have the ability to increase mortality rates and reduce populations of various insects and parasitic nematodes (Kato-Noguchi & Kato 2023). It is known that extracts from the *E. odoratum* plant are rich in alkaloids, phenolics, glycosides, and flavonoids. Extracts containing alkaloids have been found to exhibit ovicidal properties against nematode eggs. Alkaloids can affect and paralyze the central nervous system of nematodes, exhibiting strong antibacterial effects. Phenolic compounds interfere with energy production mechanisms by disrupting the process of oxidative phosphorylation and inhibiting glycoprotein on the cell surface of parasites, leading to their death. The nematicidal effect of glycosides is attributed to their function as inhibitors of cholinesterase enzymes, preventing the typical accumulation of nematodes. Flavonoids, as low-molecular-weight secondary metabolites, have diverse functions, including auxin transport inhibition and protection, and are associated with the ability to resist nematodes' limited movement and migration (Kato-Noguchi & Kato 2023).

Effect of EAEO on *R. similis* mortality *in vitro*

The effects of EAEO on the mortality rate of 800 J2 juveniles were tested in a laboratory experiment using five dilutions. Overall, after 48 h of exposure, all doses of EAEO used showed nematicidal activity against *R. similis* (Table 2). The percentage of mortality rate reduction varied with the degree of extract concentration. However, the first two dilutions, 1:2 and 1:4 (corresponding to 12500 and 6250 mg DW·L⁻¹, respectively), exhibited high toxicity with

90.1% and 78% mortality rates, respectively. Nevertheless, the mortality rate decreased to approximately 35.3% at the 1:30 dilution (780 mg DW·L⁻¹), the lowest mortality rate among the tested doses. However, the number of dead nematodes and the mortality rate in the experimental treatments were significantly higher compared to the water control.

Most *R. similis* populations thrive best at an average temperature of 25°C. Their ability to survive in adverse conditions is enhanced by three factors: (i) a wide host range, (ii) a short life cycle that allows for rapid reproduction during favorable periods, and (iii) female reproductive capabilities without the need for males. However, the nematodes become rigid and immobile in plant extracts when exposed to secondary metabolites such as terpenoids, flavonoids, glucosinolates, etc. In vitro experiments have shown that certain common flavonoids exhibit nematicidal activity, with kaempferol inhibiting the hatching of *R. similis* eggs. In contrast, kaempferol, quercetin, and myricetin all have nematicidal effects and can attract or repel juveniles (J2) (Haegeman et al. 2010). Tannins are a diverse group of polyphenolic compounds, showing strong sensitivity to *R. similis* at high extract concentrations. Some plant extracts rich in saponins and alkaloids have demonstrated strong nematicidal activity (Kirwa et al. 2018). In the current study, EAEO secondary metabolites significantly increased the mortality rate of *R. similis* compared to water control. The production of biologically active secondary metabolites by *E. odoratum* may play an important role in the control of *R. similis*. It is consistent with the findings of Kato-Noguchi and Kato (2023) regarding the interaction and nematicidal activity of *E. odoratum*.

Table 2. Mortality percentages of *Radopholus similis* as influenced by aqueous extract of leaves and stems of *Eupatorium odoratum* at different dilutions

Trait	Treatments (mg DW·L ⁻¹)						
	water	fenamiphos (4.65 mg·mL ⁻¹)	EAEO ₁₂₅₀₀	EAEO ₆₂₅₀	EAEO ₃₁₂₀	EAEO ₁₅₆₀	EAEO ₇₈₀
Number of dead nematodes	129 ± 2.5 ^a	761 ± 4.5 ^k	723 ± 6.8 ^f	624 ± 6.8 ^e	512 ± 5 ^d	409 ± 6.7 ^c	282 ± 7.5 ^b
Mortality (%)	16.1 ± 0.3 ^a	95.1 ± 0.6 ^k	90.1 ± 0.7 ^f	78 ± 0.9 ^e	64 ± 0.6 ^d	51.1 ± 0.8 ^c	35.3 ± 0.9 ^b

Values are expressed as Mean ± SD, using Tukey's HSD test to compare Means and letters (a, b, c, d, e, f, k) represent the difference between treatments (p < 0.05)

Effects of EAEO on *R. similis* infection of root segments

Separate analyses were conducted on nematodes recovered from disease-infected roots and nematodes remaining in the roots after a 48-hour incubation in EAEO, fenamiphos, and water. In both analyses, all treatments significantly reduced nematode infection in the roots compared to the water control (Fig. 1). The number of live nematodes inside the roots for the EAEO treatments at 780 and 1560 mg DW·L⁻¹ (31.3 ± 4 and 27.7 ± 4.2 , respectively) was higher than the EAEO at 12500 mg DW·L⁻¹ and the fenamiphos treatments (14.7 ± 3.5 and 11.7 ± 2.5 , respectively). However, the number of live nematodes in these treatments was still significantly lower than in the water control group (Fig. 1A). The highest number of nematodes escaping from the roots was observed in the treatment with EAEO at 780 mg DW·L⁻¹ (36.7 ± 2.5), which was significantly lower than in the water control group (78.7 ± 4.7). EAEO at 12500 and 6250 mg DW·L⁻¹ (18.7 ± 4.5 and 22.7 ± 3.2 , respectively) provided nematode control similar to fenamiphos (21.7 ± 3.5) (Fig. 1B).

All developmental stages of *R. similis* can be found inside banana roots. The nematodes puncture the root epidermal cells with a sharp stylet, digesting and extracting cell contents as they penetrate the roots. Invasion typically occurs at the tips of soft, nonwoody, lateral roots or in the region of the feeding site. Once inside the root, the nematode feeds and reproduces. With its rudimentary stylet, the male cannot penetrate the root or cause any damage to the root at any level, while the females and second-stage juveniles (J2) feed on the root and cause significant damage. The nematodes only leave the root and move away when the root decays or their density becomes too high (Haegeman et al. 2010). However, plants possess a complex defense system to resist invading agents, including constitutive mechanisms, preformed structures, and inducible immune responses that occur upon recognition of the invading factors. The immune and constitutive defense systems against nematodes rely on the presence of plant secondary metabolites with antinematode activity (Sato et al. 2019). Particularly in roots, leaves, and stems, a variety of plant secondary metabolites, such as phenolic compounds, terpenoids, saponins, benzoxazinoids, organosulfur compounds, alkaloids, and glucosinolates are present and released, acting

as attractants, repellents, stimulants, or nematode inhibitors (Sikder & Vestergård 2020). After a 48-hour incubation in EAEO, fenamiphos, and water, the number of surviving nematodes inside the roots and some nematodes emerging from the roots showed variations among the treatments. The number of nematodes remaining in and emerging from EAEO-treated roots was significantly lower compared to the negative control, and roots treated with high concentrations of the extract exhibited similar efficacy to those treated with fenamiphos. These results once again affirm the role of EAEO in controlling *R. similis*.

Several limitations have been identified in the experimental study that should be considered and addressed in future research endeavors. The relatively narrow range of dilutions used in the conducted experiments can be viewed as a limitation, and the short exposure time of only 48 hours following EAEO contact may have impacted our understanding of EAEO's effects. Additionally, the exclusive focus on the *R. similis*, without extending the investigation to other nematode species, presents a limitation that should be overcome. Notably, the ecological implications of using EAEO for nematode control should have been addressed in depth, warranting further examination in future studies. Future research directions should encompass a more extensive series of experiments to establish a detailed dose-response relationship. Such investigations will aid in determining the optimal dosage for effective and economically viable nematode control. Concurrently, research into the persistence and potential development of drug resistance by nematodes under EAEO conditions is essential. Expanding the scope beyond *R. similis*, experiments on various nematode species are warranted to evaluate the broad-spectrum efficacy of EAEO. Furthermore, delving into the molecular mechanisms through which EAEO impacts nematodes represents a crucial research avenue. Gaining insights into these mechanisms can facilitate the optimization of EAEO's application. Lastly, the environmental impact of EAEO usage, encompassing soil health, effects on nontarget organisms, and the overall dynamics of ecosystems, should be rigorously evaluated. This approach will ensure that using EAEO as a nematode control method is positive and sustainable in the long term.

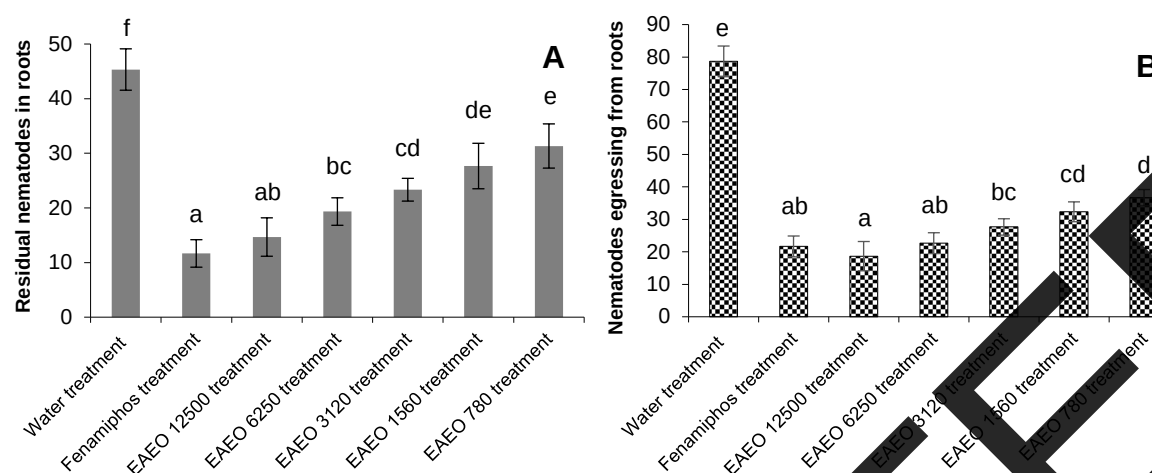


Figure 1. Effects of EAO compared to fenamiphos and water control on rate of nematode infection of banana-root segments: residual nematodes in roots (A), nematodes egressing from roots (B)

Values are the average of three replicates (mean $\pm$ standard deviation) and using Tukey's HSD test to compare means; Bars with various letters (a, b, c, d, e, and f) are significantly different ($p < 0.05$)

Greenhouse experiment

Root necrosis index

Differences in RNI between treatments (fenamiphos and EAO₁₂₅₀₀) were statistically significant compared to the water control in all experiments (Fig. 2). All other EAO treatments resulted in an RNI that was not significantly greater than the control. An increase in the number of *R. similis* in the roots leads to increased root damage, which aligns well with the observed differences in the number of nematodes remaining inside the roots and emerging from the roots.

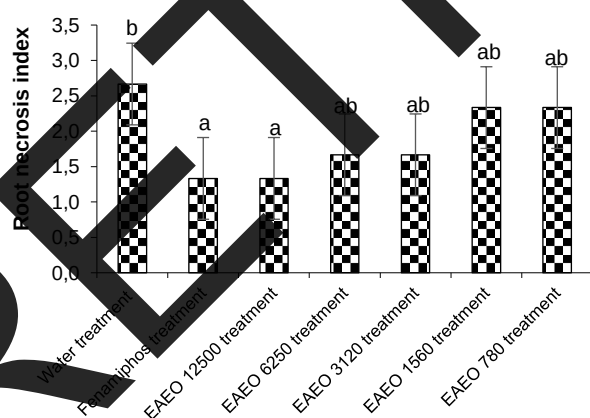


Figure 2. Effects of EAO compared to fenamiphos and water control on roots necrosis index (RNI) values

Values are the average of three replicates (mean $\pm$ standard deviation) and using Tukey's HSD test to compare means; Bars with various letters (a and b) are significantly different ($p < 0.05$)

The burrowing nematode is an endoparasitic migratory nematode that invades banana roots, feeds, migrates, and reproduces within the root tissues. *R. similis* causes characteristic symptoms of root damage, including reddish-brown to blackish-brown lesions due to disruption of cell walls as they move between and within cells, deep cavities in the roots, and destruction of phloem and cambium, leaving behind pockets filled with nematodes. External cracks may appear on the lesions, and tissue decay occurs after secondary fungal and bacterial infections. The root system becomes stunted, poorly developed, and necrotic (EPPO 2008). These symptoms correspond well with the condition of the infected and damaged roots observed in the surveyed banana groups affected by nematode infestation. Particularly in the water-treated group, the plant roots were severely damaged with a high RNI value (2.7). However, this outcome was reversed in the groups treated with EAO and fenamiphos. The RNI also significantly decreased in the groups treated with *Azadirachta indica* and *Allium sativum* extracts, which is consistent with the findings of Bartholomew et al. (2014) and supports the results of the current study.

Banana root and corm fresh weight

All EAO treatments were effective in maintaining healthy banana roots and stems. EAO-treated plants consistently showed higher root and corm fresh weight (RCFW) than plants treated with water.

The plants treated with the highest concentration of EAEO and fenamiphos had the greatest weight (Fig. 3). The quantity of *R. similis* in the roots and stems corresponds to their RCFW. According to Moens et al. (2001), root weight decreases linearly with increasing *R. similis* inoculum density. Marin et al. (1999) also observed a reduction in root weight ranging from 8% to 80% after *R. similis* inoculation. The data obtained in this study align with the reported literature, as the water treatment exhibited the highest quantity of *R. similis* in the roots – 45.3 ± 3.8 , and RNI – 2.7 ± 0.6 (Fig. 1A & 2), resulting in the lowest total RCFW (1.5 ± 0.1 kg) (Fig. 3). However, the data wholly reversed for the groups treated with EAEO and fenamiphos. In these groups, the quantity of *R. similis* in roots decreased significantly in the EAEO₁₂₅₀₀ 14.7 ± 3.5 and RNI 1.33 ± 0.58 , and fenamiphos treatments 11.7 ± 2.5 and RNI 1.3 ± 0.6 (Fig. 1A & 2), leading to the highest total RCFW EAEO₁₂₅₀₀ 2.6 ± 0.1 and fenamiphos treatments 2.4 ± 0.1 kg.

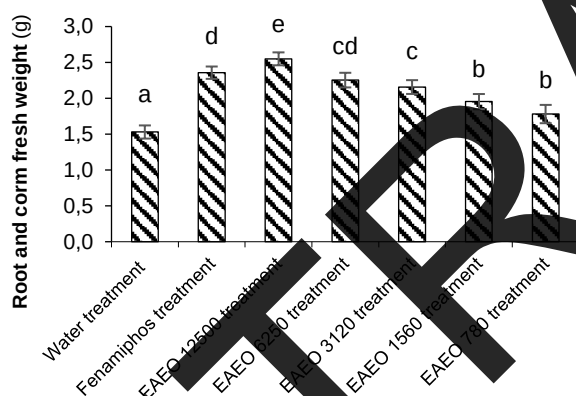


Figure 3. Effects of EAEO compared to fenamiphos and water control on root and corm fresh weight (RCFW)

Values are the average of three replicates (mean $\pm$ standard deviation) and using Tukey's HSD test to compare means; Bars with various letters (a, b, c, d, and e) are significantly different ($p < 0.05$)

R. similis is considered one of the top 10 most dangerous plant-parasitic nematodes worldwide, causing devastating diseases in banana plants. According to the European and Mediterranean Plant Protection Organization (EPPO 2008), *R. similis* is a quarantine organism with A2 status due to the disease it causes in bananas. The burrowing nematodes destroy the root system and make the plants susceptible to toppling over. *R. similis* typically invades the nearby root tips, killing off lateral roots and causing reddish-brown lesions on the root surface, extending along the entire root length. They move between the cells of the root cortex, damaging the roots until necrotic lesions form. Eventually, the burrowing nematodes can move from the roots into the stems, causing blackhead disease (formation of circular dark lesions in the stem). Secondary plant metabolites such as alkaloids, phenols, terpenoids, flavonoids, saponins, etc., have the potential to control and eliminate nematodes (Kosma et al. 2011), and these compounds have also been found in EAEO. It can explain the increased RCFW observed in the groups treated with EAEO.

Plant growth

Banana growth data confirmed that EAEO treatment can be comparable to fenamiphos regarding growth parameter values (Table 3). The pseudostem length of EAEO-treated banana plants was significantly greater compared to the negative control. The highest values were recorded in plants treated with fenamiphos and EAEO₁₂₅₀₀ and decreased with lowering EAEO concentration. It indicates the effectiveness of controlling *R. similis* nematodes, protecting and ensuring the growth of bananas. Similarly, parameters such as stem diameter, leaf number, and leaf surface area also yield favorable results in the groups treated with EAEO and fenamiphos.

Table 3. Effects of the treatments on the growth parameters of banana plant

Trait	Treatments (mg DW·L ⁻¹)						
	water	fenamiphos (4.65 mg·mL ⁻¹)	EAEO ₁₂₅₀₀	EAEO ₆₂₅₀	EAEO ₃₁₂₀	EAEO ₁₅₆₀	EAEO ₇₈₀
Pseudostem length (cm)	67.8 $\pm$ 2 ^a	95.9 $\pm$ 3.1 ^d	92.7 $\pm$ 2 ^d	86.6 $\pm$ 2 ^c	82.6 $\pm$ 2.1 ^c	78.6 $\pm$ 2 ^b	75.9 $\pm$ 2.4 ^b
Girth (cm)	23.4 $\pm$ 1 ^a	28.6 $\pm$ 1 ^c	27.4 $\pm$ 1 ^{cde}	28.2 $\pm$ 1.6 ^{de}	26.6 $\pm$ 1.1 ^{bcd}	25.4 $\pm$ 1.1 ^{bc}	25.9 $\pm$ 1 ^b
Leaf numbers	8.3 $\pm$ 0.6 ^c	6.3 $\pm$ 0.6 ^a	6.7 $\pm$ 0.6 ^{ab}	7 $\pm$ 1 ^{ab}	7.3 $\pm$ 0.6 ^{abc}	7.7 $\pm$ 0.6 ^{bc}	7.7 $\pm$ 0.6 ^{bc}
Leaf area (cm ²)	2071.7 $\pm$ 23.7 ^a	2589.3 $\pm$ 36.6 ^c	2510.3 $\pm$ 27.5 ^d	2490.3 $\pm$ 34.8 ^d	2397.7 $\pm$ 43.9 ^c	2341 $\pm$ 29.5 ^{bc}	2286.7 $\pm$ 36.3 ^b

Note: see Table 2

The experimental study presented here still bears certain limitations. A more comprehensive elucidation of the mechanisms by which EAEO influences nematode reproduction and root damage is needed. Furthermore, the sustainability of the control method needs to be adequately assessed, and there has been no discussion regarding potential hidden environmental consequences, including soil health and nontarget organisms, associated with using EAEO. Assessment of plant development, fruit, and overall plant health must also be improved and there is no specific quantitative data or statistical comparisons to substantiate the claim of equivalence between EAEO and fenamiphos. Future research endeavors should consider the potential for synergy between EAEO and other nematode control methods (biological control and cultivation practices) to formulate integrated nematode management strategies. Field trials should be conducted in authentic banana cultivation environments to evaluate the practicality and effectiveness of treatment methodologies. A thorough cost-effectiveness analysis of EAEO usage compared to alternative nematode control methods should be undertaken. Lastly, a priority should be disseminating research findings to the agricultural community to encourage responsible and informed use of EAEO as a nematode control tool in banana cultivation.

CONCLUSIONS

EAEO was effective in controlling *R. similis* on banana plants both in laboratory and greenhouse tests, especially at concentrations of 12500 and 6250 mg DW L⁻¹. Treatment with EAEO has increased the mortality rate of nematodes, significantly reduced the infection of *R. similis* in root segments, and consequently stimulated growth parameters, such as stem length, diameter, leaf number, and leaf surface area. The application of EAEO has proven to be an effective alternative to synthetic nematicides, as it is comparable with fenamiphos in preventing the development of *R. similis*.

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