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ORIGINAL ARTICLE

# Evaluation of the nematicidal efficacy of an aqueous extract of *Eupatorium odoratum* on *Radopholus similis* nematode infestation in banana (*Musa acuminata* "Cavendish") in a micro plot experiment and under field trial conditions

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**Abstract**

*Eupatorium odoratum* is known for its ability to resist nematode infestations that attack the root systems of banana plants. An aqueous extract of the leaves and stems of *E. odoratum* (named EEOL) represents a natural solution that we investigated for its potential to control the harmful nematode, *Radopholus similis*, in Cavendish banana plants. Our research into EEOL's efficacy spanned two distinct environments: a micro plot experiment model and a field model. Various concentrations of EEOL were examined to assess its efficacy in alleviating *R. similis* infestations and in mitigating their adverse effects on Cavendish banana plants. In the micro plot experiment model, the concentration of the original solution, diluted at ratios of 1:30×, 1 : 16×, 1 : 8×, 1 : 4×, and 1 : 2×, ranged from 1.76 to 28.16 mg · ml$^{-1}$. In the field model, the corresponding rates varied from 6.03 to 96.54 l · ha$^{-1}$. Key parameters, including infection rates, root necrosis indices, plant growth metrics, percentage of fallen trees, and harvest yields, were meticulously monitored and assessed. The results demonstrated that EEOL significantly reduced infection rates ($p < 0.05$), decreased root necrosis indices ($p < 0.05$), and promoted increased plant height, pseudostem circumference, and leaf area ($p < 0.05$) in both models. Furthermore, it lowered the percentage of fallen trees ($p < 0.05$) and enhanced harvest yields ($p < 0.05$) in the field model. Notably, observations in the field model revealed that EEOL, particularly at a dosage of 96.54 l · ha$^{-1}$, exhibited effectiveness equivalent to the conventional chemical nematode control method, fenamiphos ($p > 0.05$). The study's findings underscore the promising potential of EEOL in effectively managing *R. similis* infestations and improving the yield and quality of Cavendish banana plants. The aqueous extract of the stem and leaves of *E. odoratum* emerged as an effective nematode management solution for banana cultivation, in both the micro plot experiment and field conditions.

**Keywords:** biological control, field trial conditions, micro plot experiment trials, nematicidal efficacy, nematode management

## Introduction

The nematode *Radopholus similis*, commonly referred to as the burrowing nematode, is responsible for causing considerable harm to banana plants (Brooks 2004). This nematode invades the banana plant's root system, leading to root damage and rot, which subsequently reduces the plant's ability to absorb water and nutrients. Ultimately, this results in the wilting, weakening, and even death of the plant. *R. similis* is particularly detrimental to young banana seedlings, impeding their growth and development. Additionally, it serves as a vector for a variety of bacteria and fungi, elevating the risk of disease transmission and compromising the plant's adaptability to its surroundings (Jesus *et al.* 2015). Utilizing chemical pesticides for nematode control may give rise to a multitude of side effects and latent risks. These chemical substances

can accumulate within the soil, thereby affecting the soil's ecosystem and causing harm to beneficial microorganisms, consequently diminishing soil quality. Furthermore, the application of chemical pesticides can result in environmental pollution, which has implications for groundwater contamination, ecosystem disturbances, and human health. In scenarios where safety protocols are not strictly adhered to, farmers may find themselves in direct contact with hazardous substances. Additionally, consumers can encounter risks through the consumption of contaminated food products. Moreover, excessive pesticide usage can foster nematode resistance, rendering nematode control more intricate (Wezel *et al.* 2014). An effective alternative solution to synthetic nematicides is the utilization of plant-derived compounds. These compounds typically possess the ability to combat nematodes without inflicting detrimental effects on the environment and come with cost-efficiency while ensuring human safety (Motha *et al.* 2010).

*Eupatorium odoratum*, is an herbaceous plant belonging to the Asteraceae family. This species is native to tropical and subtropical regions of the Americas and Africa. *E. odoratum* typically ranges from 30 to 150 cm in height, featuring elongated ovate leaves with a distinct fragrance. Its flowers are often white or pink and attract various pollinators (Zachariades *et al.* 2009). *Eupatorium odoratum* has a history of traditional medicinal use and offers a wide range of applications. Various parts of the plant, particularly the leaves and stems, are employed for treating conditions such as the common cold, or abdominal pain. It can be used for anti-inflammatory purposes and pain relief. Furthermore, numerous chemical compounds isolated from *E. odoratum* exhibit therapeutic properties, including anti-diarrheal, diuretic, wound-healing, antimicrobial, and insecticidal activities (Jitendra *et al.* 2011). *E. odoratum* emerges as a promising natural resource for combating harmful agricultural pests, encompassing both insects and nematodes. The plant houses chemical compounds renowned for their insecticidal attributes, which act upon the nervous and digestive systems of insects, thereby, effectively serving as a natural insect repellent. Additionally, studies indicate its potential in controlling nematodes such as *R. similis*, the burrowing nematode. *E. odoratum*'s arsenal of compounds, including eupolin, saponin, flavonoids, and sesquiterpenes, possesses the capacity to induce damage in nematodes and disrupt their intracellular environment (Udebuani *et al.* 2015). Given the current context, this study sought to evaluate the efficacy of water extracts from *Eupatorium odoratum* L. in combating *R. similis*, which affects Cavendish banana plants. The research was conducted in both micro plots and field environments. The findings have the potential to lead to the development of bioactive products aimed at promoting sustainable agricultural practices.

## Materials and Methods

### Plant material

#### Collection of plant material

In February 2023, *E. odoratum* leaves and stems were gathered from Phu My Hung commune, Cu Chi district, in Ho Chi Minh City, Vietnam. After meticulous washing, the fresh leaves and stems were finely chopped, air-dried, and subsequently subjected to a 3-day drying process in a Memmert drying cabinet (Germany) at 60°C, until a consistent weight was attained. The obtained dried material was pulverized into a fine powder, securely stored in a moisture-resistant container, shielded from light, and maintained at room temperature for use in subsequent experiments.

#### Preparation of aqueous extract (EEOL)

We soaked 3 kg of dried *E. odoratum* stems and leaves in 40 liters of tap water for 48 h, then boiled it for 5 min, cooled it, and filtered it through a thin cotton cloth, followed by vacuum filtration. The resulting aqueous plant extract, with a concentration of 0.046 g · ml$^{-1}$ (w/v, the plant extract solution contained 0.046 grams of extract per milliliter) and is referred to as EEOL, was stored in a sealed glass container at 4°C, shielded from light, for future use (Cerda *et al.* 2019). Our choice of the water extraction method was based on the native growth of *E. odoratum* in natural habitats near agricultural regions. This approach provides a practical, cost-effective solution for farmers, reducing the need for labor-intensive or financially burdensome extraction processes.

### Qualitative phytochemical screening of EEOL extract

We employed extracts from *E. odoratum* leaves and stems to qualitatively evaluate essential phytochemical categories, encompassing alkaloids, saponins, flavonoids, phenols, terpenoids, and tannins. The experiments adhered to established standard procedures described by Ramya and Dhamotharan (2015). In addition, the total alkaloid, flavonoid, polyphenol, and tannin contents were also determined according to the procedures of Rahman *et al.* (2023).

### Experimental design

#### Micro plot experiment design

Preparation of *Radopholus similis* nematodes: *Radopholus similis* nematodes were identified through

a dual approach, combining microscopic examination of key features for morphological identification and extracting genetic information from nematode samples for DNA analysis. *R. similis* nematodes were harvested from Cavendish banana roots and extracted from carrot disk culture medium (Bartholomew *et al.* 2014). Specifically, carrot agar disks were prepared by overlaying a layer of agar (a gel medium for nematode cultivation) on the surface of the disk. The agar contained essential nutrients to support the growth of the nematodes. The nematode sample was placed on the agar surface of the carrot disk. Conditions were meticulously controlled, maintaining a temperature of 22–25°C, relative humidity of 70–80%, and complete absence of light to create an optimal environment for nematode development. Carrot disks were incubated for 3 days. After the incubation period, nematodes were separated from the carrot tissues through sieving (mesh size of 420 mm and 25 mm) and filtration through cloth. Subsequently, glass dishes with a depth of 2.5 cm ("Beltsville dish") were prepared, each containing 100 nematodes and 50 ml of sterile, deionized water. Proceeding, an additional 250 ml of the test solution was introduced into each dish, elevating the total volume to 300 ml per dish. The nematodes within the dishes were further incubated for 48 hours in a humidity chamber, maintaining a relative humidity of 100% and a temperature of 25°C. The resultant solution obtained after incubation was utilized for subsequent experiments.

Design of micro plot experiment: Each treatment method was designed with ten 4-week-old Cavendish banana plants, grown in a compact area measuring 40 $m^2$, spaced 2 × 2 m apart. The growth medium consisted of well-draining loamy soil, stable moisture levels, and adequate nutrients for the banana plants. A complete nutrient solution (Chem-Gro, Hydro-Gardens, Inc., Colorado Springs, CO, USA) was supplemented once a week to ensure plant growth. Immediately upon planting, the banana plants were shielded with insect-proof mesh. The experimental design included a positive control treatment (standard insecticide, fenamiphos, at 5.25 mg · $ml^{-1}$, concentration 40% EC), a negative control treatment (tap water), and five EEOL concentrations (1.76, 3.52, 7.04, 14.08, and 28.16 mg · $ml^{-1}$). The experiment was carried out in a completely randomized block design with five repetitions. Five milliliters of nematode inoculum (approximately 200 nematodes) were introduced into the soil at the base of each plant. Control measures were implemented 1 week after introducing nematodes into the base of each plant. The application of control measures occurred daily, and the plants were maintained for 9 weeks at a temperature of approximately 27°C and a relative humidity of 80% within the experimental garden. Observational parameters were monitored and recorded every 3 weeks.

**Field experiment design**

Location of field experiment: The Cavendish banana cultivation research site is located in Phu My Hung commune, Cu Chi district, Ho Chi Minh City, Vietnam. This region features a tropical monsoon climate with well-defined wet (May to November) and dry (December to April of the following year) seasons. The average temperature remains relatively stable at approximately 26.6°C year-round. The site's elevation varies from 8 to 10 m above sea level, sloping gently in two directions, from northwest to southeast and from northeast to southwest. Climate data, including temperature and rainfall, were continuously monitored at a local weather station situated within a 2 km radius of the research site. Standard agricultural practices for cultivating Cavendish bananas, such as plowing, weed management, irrigation, and organic soil enhancement, were carried out following the technical guidelines of the Department of Biotechnology, Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry. Recognizing the fluctuating nature of the soil in Phu My Hung, Cu Chi, between the dry and wet seasons, experiments were conducted in both seasons, spanning from April 2022 to May 2023, to thoroughly evaluate the treatment's impact on *R. similis* population dynamics.

Preparation of plant extract: The aqueous extract from *E. odoratum* stems and leaves was prepared in accordance with the aforementioned protocol (Cerda *et al.* 2019). The exploration of *E. odoratum* aqueous extract (EEOL) in combatting *R. similis* nematodes had the overarching goal of mitigating the nematode population, impeding their growth, or disrupting their reproductive processes. Furthermore, the investigation aimed to evaluate the impact of the extract on crop growth and soil systems. Concurrently, it delved into the potential formulation of nematode-resistant products derived from *E. odoratum* extract, laying the groundwork for plant protection solutions or biofertilizers. Throughout the course of the study, any identified deviations in outcomes suggested potential modifications in nematode-suppressing activities, environmental interactions, or the influence of specific chemical constituents within the extract. These distinctions may unveil new avenues for optimizing the efficacy of *E. odoratum* extract in the control of *R. similis* nematodes. For the application of the extract, a 5-liter sprayer (Petrul FUT-5LP; Truper SA, Jilotepec, México) was utilized to ensure comprehensive coverage, encompassing both the upper and lower leaf surfaces, as well as the base of the plant, until the point of saturation.

Experimental design: The primary mode of infestation of *R. similis* nematodes in banana plants occurs through the soil and the root system. Nematodes infiltrate the plant through wounds on the roots or small

openings in the banana root system. The banana plantation followed a systematic row-row system, with a spacing of 1.8 meters between rows (measured from the midpoint of one row to the midpoint of the next row). Within each row, the spacing between adjacent banana plants was 3.6 meters (measured from the midpoint of one banana plant to the midpoint of the next plant in the same row). Additionally, between adjacent banana plants in the same row, there was a gap of 2.05 meters (the space between neighboring plants within the same row, measured from the end point of one plant to the starting point of the next). This gap serves to create a sufficiently large space for the convenience of care, harvesting, and other cultivation activities without compromising the plant's development. This initial layout established a planting density of 1806 banana plants · $ha^{-1}$. The experimental plots were systematically arranged in a completely randomized design, with five repetitions. Each research area, corresponding to each experimental treatment, covered an area of approximately 500 $m^2$, cultivating 90 banana plants. These plants were meticulously monitored for nematode infestation and yield status. To prevent cross-contamination due to drift between neighboring plots, 2-meter-wide alleys were maintained between the experimental plots. The experiment was structured as a completely randomized block design, comprising five treatment methods and two control methods. Administering extracts to plants was executed through such methods as foliar spray, enhancing absorption capabilities through the leaves, and direct contact with nematodes on the leaf surface. This aids in controlling nematodes in the upper part of the plant and diminishes the risk of infection from the surrounding environment. Spraying on the trunk and base of the plant creates conditions for the extract to be absorbed through the bark and to be in contact with the root system, inhibiting the nematode's growth in the soil and on the plant's body. Additionally, directly applying the extract to the soil around the base of the plant helps establish an environment in the soil unsuitable for nematode development and reproduction. To apply the extract to the plants, we employed a combination of methods, including foliar spraying, stem and root spraying, and direct soil application around the base of the plant. The concentrations of the extract in the field model were 6.03, 12.07, 24.13, 48.27, and 96.54 l · $ha^{-1}$. These concentrations were achieved by diluting the original EEOL extract with water at ratios of 1:30×, 1:16×, 1:8×, 1:4×, and 1:2×. Negative control plots received tap water, while positive controls employed the standard nematode-killing agent, fenamiphos (concentration 40% EC, formula $C_{15}H_{22}NO_3PS$) (at a rate of 1.8 l · $ha^{-1}$). Both the EEOL and fenamiphos treatments commenced 30 days after the initial banana planting and were consistently applied every 7 days over 12 months. Each main plot comprised 54 out of 90 plants, and their nematode infestation status and productivity were closely monitored. Observations, recordings, and evaluations were conducted every 3 months

## Evaluation of infection rate and nematode quantity in banana plant roots

Each banana plant was subjected to regular assessments to determine the rate of nematode infection. The presence of *R. similis* was evaluated before inoculating the soil with nematode suspension. This procedure was consistently carried out every week. Five grams of fresh roots were utilized for each plant to establish the presence of nematodes. The rate of banana plants infected with nematodes (IPI) was calculated according to the following formula:

$$\text{IPI (\%)} = \frac{\text{The number of plants infected with nematodes/treatment}}{\text{The total number of plants/treatment}} \times 100.$$

The enumeration of nematode populations in banana roots employed the hydrogen peroxide nematode extraction technique. This method entailed extracting nematodes from root samples using hydrogen peroxide, effectively eliminating them from the specimens and facilitating subsequent quantification. A standardized amount of 5 grams of root per plant was utilized to ensure consistent and representative measurements of nematodes. Nematode density was expressed as the count per 100 grams of fresh roots (applicable to bananas), establishing a standardized metric for comparing infection levels across samples, irrespective of specific root masses.

Subsequent to the enumeration process, root samples underwent a 24-hour air-drying period in a cabinet set at 60°C. This drying procedure aimed to eliminate moisture from the samples, preserving solely the essential components and retaining the inherent properties of the samples for storage and future analysis (Chabrier and Queneherve 2003).

## Root necrosis index (RNI)

After the field and micro plot experiments, the banana roots and corms were collected and meticulously cleansed to eliminate soil particulates. They were then employed for the assessment of the root necrosis index (RNI). The root necrosis index, initially outlined by Bartholomew *et al.* (2014), was subject to minor adjustments to align with the specific experimental conditions. This index was used to evaluate the extent of root rot, with a scale ranging from 0 to 4

["0" = no damage; "1" ≤ 25% of the total root cortex damaged; "2" = 26–50% of the total root cortex damaged; "3" = 51–75% of the total root cortex damaged; "4" ≥ 75% of the total root cortex damaged]. The RNI was computed using the following formula:

$$\text{RNI} = \frac{\text{No. of roots with nematode induced necrosis}}{\text{Total number of roots sampled}} \times 100.$$

## Monitoring plant growth

Throughout our research, we regularly performed the following plant measurements every week: (i) Pseudostem length (cm): This measurement was taken from the lowest point of a leaf to the base of the pseudostem. (ii) Circumference/Pseudostem circumference (cm): Measured from a point located midway along the pseudostem's length. (iii) Total functional leaves: This count was recorded once all the leaves had fully unfurled (Bartholomew *et al.* 2014). (iv) Leaf area (LA): Calculated using the formula LA ($m^2$) = (L × W × 0.8), where L corresponds to leaf length, and W denotes leaf width. The third leaf from the top of the plant was consistently chosen as the reference leaf for measurement (Jadhav *et al.* 2019).

## Observing the percentage of fallen trees

Detailed information for each individual plant in the sample was collected, including such characteristics as health, growth stage, and any noticeable symptoms. A meticulous examination to identify plants displaying indications of burrowing nematode infestation was conducted. This process entailed scrutinizing the roots, evaluating the overall health of the plant, or documenting specific symptoms linked to nematode intrusion. Subsequently, relevant details concerning plants impacted by burrowing nematodes, including the extent of infection, visible damage, or any distinctive features signifying nematode presence were documented. The number of banana plants that had fallen due to burrowing nematodes which compromised root systems was quantified and recorded. The proportion of fallen plants was calculated using the formula (Engwali *et al.* 2013):

$$\text{Percentage of fallen trees (\%)} = \frac{\text{Number of fallen trees}}{\text{Total number of trees}}.$$

## Determining harvest yield

The productivity parameters were measured during the harvest season (number of hands/bunch, number of fingers/bunch, finger weight, bunch weight, yield factor, and total yield) (Lamessa 2021).

i/Number of hands/bunch:

$$\text{No. of hands/bunch} = \frac{\text{No. of bananas in the bunch}}{\text{No. of bananas per hand}}.$$

ii/ Number of fingers/bunch:

$$\text{No. of fingers/bunch} = \frac{\text{No. of bananas in the bunch}}{\text{No. of bananas per finger}}.$$

iii/ Finger weight (FW):

$$\text{FW (g)} = \frac{\text{Bunch weight}}{\text{No. of fingers}}.$$

iv/ Bunch weight (BW):

$$\text{BW (kg)} = \frac{\text{Total weight of the entire bunch}}{\text{No. of bananas in the bunch}}.$$

v/ Yield factor (YF):

$$\text{YF (\%)} = \frac{\text{Harvested banana weight}}{\text{Ideal banana weight}} \times 100.$$

vi/ Total yields (TY):

$$\text{TY (t/}ha\text{/yr)} = \frac{\text{Total harvested weight}}{\text{Cultivated area}} \times \text{Time period}.$$

## Statistical analysis

The data is presented in the form of mean values (Mean) along with standard deviations (SD). Count data for nematode infestation were processed using the $\log_{10}$ (x + 1) to reduce variance. The RNI (Root Necrosis Index) ratios from both the micro plot experiment and field surveys were adjusted by applying a logarithmic transformation [log(x)] to ensure data adherence to a normal distribution. All datasets were subjected to analysis of variance (ANOVA) using the Statgraphics Centurion XIX software and were separated by Tukey's multiple comparison test of significance ($p < 0.05$). The correlation between the density of *R. similis* nematodes in banana roots and root damage was evaluated using the Spearman rank correlation coefficient.

# Results

## Qualitative and quantitative analysis of phytochemicals in *Eupatorium odoratum* leaves and stems

The qualitative analysis of the phytochemical components in the extract of *E. odoratum* leaves and stems revealed the presence of tannins, flavonoids, alkaloids, saponins, terpenoids, and phenolics. The qualitative results for the plant-derived chemical compounds in

EEOL are represented with a symbol (+) to indicate their presence, as shown in Table 1. The presence of tannin, flavonoid, alkaloid, saponin, terpenoid, and phenolic in EEOL holds significant importance in utilizing this extract for nematode control. These compounds often exhibit anti-parasitic and toxic properties, potentially influencing the survival and reproduction of nematodes. The combination of these compounds may result in an extract that proves effective in regulating and inhibiting the growth of nematodes within the crop environment.

**Table 1.** Examination of the chemical components in the leaves and stems extract of *Eupatorium odoratum* (EEOL) through both qualitative and quantitative analysis

| Phytochemicals in EEOL | Qualitative of phytochemical | Quantitative of phytochemical |
|---|---|---|
| Tannins | + | 27.68 ± 2.51 (mg TAE · $g^{-1}$) |
| Flavonoids | + | 18.89 ± 3.75 (mg QE · $g^{-1}$) |
| Alkaloids | + | 9.04 ± 0.85 (mg · $g^{-1}$) |
| Phenolic compounds | + | 34.24 ± 4.62 (mg GAE · $g^{-1}$) |
| Saponins | + | not tested |
| Terpenoids | + | not tested |

(+) – present; TAE – tannic acid equivalent; QE – quercetin equivalent; GAE – gallic acid equivalent

The results of the phytochemical analysis of the leaf and stem extract of *E. odoratum* were quantitatively estimated and are presented in Table 1. The findings indicate that the content of phenolic compounds, tannins, flavonoids, and alkaloids in EEOL was 34.24 ± 4.62 mg gallic acid equivalent (GAE) · $g^{-1}$, 27.68 ± 2.51 mg tannic acid equivalent (TAE) · $g^{-1}$, 18.89 ± 3.75 mg quercetin equivalent (QE) · $g^{-1}$, and 9.04 ± 0.85 mg · $g^{-1}$, respectively. These concentrations are significant in the context of using the extract for nematode control. Phenolic compounds, tannins, flavonoids, and alkaloids are known for their bioactive properties, including anti-parasitic and toxic effects. The measured concentrations suggest that EEOL contains substantial amounts of these bioactive compounds, which could contribute to its effectiveness in controlling and mitigating nematode infestation in the crop environment.

## Micro plot experiment

### Infection rate and nematode abundance in banana plant roots

The incidence of nematode infestation in banana plants exhibited a noteworthy escalation, reaching statistical significance just 3 weeks after nematode inoculation at the base of each banana plant, as demonstrated in Table 2. The water treatment conspicuously displayed the most elevated nematode density in the 9th week, with a corresponding plant infestation rate soaring to a staggering 98% ($p < 0.05$). In contrast, the utilization of EEOL, administered at concentrations of 7.04, 14.08, and 28.16 mg · $ml^{-1}$, applied through both foliar spray and root injection modalities, yielded a substantial mitigation in nematode infestation. This reduction encompassed the percentage of plants besieged by *R. similis*, which diminished to 52%, 36%, and 18%, respectively ($p < 0.05$), along with a marked decrease in root infestation ($p < 0.05$). The pinnacle of nematode control efficacy was attained at an EEOL dose of 28.16 mg · $ml^{-1}$, rendering its nematocidal potency equivalent to fenamiphos ($p > 0.05$).

### The root necrosis index (RNI)

Figure 1 depicts the results of the Root Necrosis Index (RNI) in the micro plot experiment model. The highest RNI was observed in the water treatment, with a rate of 3.0% ± 0.71% ($p < 0.05$), indicating the initial level of root damage in this treatment before any treatment. The gradual decrease in RNI with increasing EEOL doses (1.76, 3.52, 7.04, 14.08, and 28.16 mg · $ml^{-1}$) was

**Table 2.** The impact of *Eupatorium odoratum* (EEOL) leaves and stems extract on plant infection incidence and *Radopholus similis* population in micro plots experiment

| Experimental treatments | Rate of banana plants infected with nematodes [%] | | | Nematode quantity in banana plant roots [nematodes] | | |
|---|---|---|---|---|---|---|
| | 3 weeks | 6 weeks | 9 weeks | 3 weeks | 6 weeks | 9 weeks |
| Water treatment | 58 ± 4.47 e | 78 ± 8.37 f | 98 ± 4.47 f | 422 ± 32.84 f | 563 ± 31.68 f | 707 ± 42.59 f |
| Fenamiphos treatment | 8 ± 8.37 a | 12 ± 4.47 a | 16 ± 5.48 a | 61 ± 7.04 a | 82 ± 7.04 a | 103 ± 16.84 a |
| $EEOL_{1.76}$ | 52 ± 8.94 de | 70 ± 7.07 e | 84 ± 8.94 e | 301 ± 26.64 e | 402 ± 33.67 e | 504 ± 32.61 e |
| $EEOL_{3.52}$ | 42 ± 8.37 cd | 56 ± 5.48 d | 68 ± 8.37 d | 250 ± 17.26 d | 334 ± 27.63 d | 419 ± 27.63 d |
| $EEOL_{7.04}$ | 32 ± 8.37 bc | 42 ± 4.47 c | 52 ± 4.47 c | 192 ± 11.4 c | 251 ± 20.77 c | 322 ± 22.62 c |
| $EEOL_{14.08}$ | 22 ± 8.37 b | 28 ± 8.37 b | 36 ± 5.48 b | 132 ± 12.79 b | 177 ± 15.73 b | 222 ± 15.73 b |
| $EEOL_{28.16}$ | 10 ± 7.07 a | 14 ± 5.48 a | 18 ± 4.47 a | 70 ± 10.3 a | 94 ± 12.79 a | 118 ± 15.86 a |

Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between treatment ($p < 0.05$)

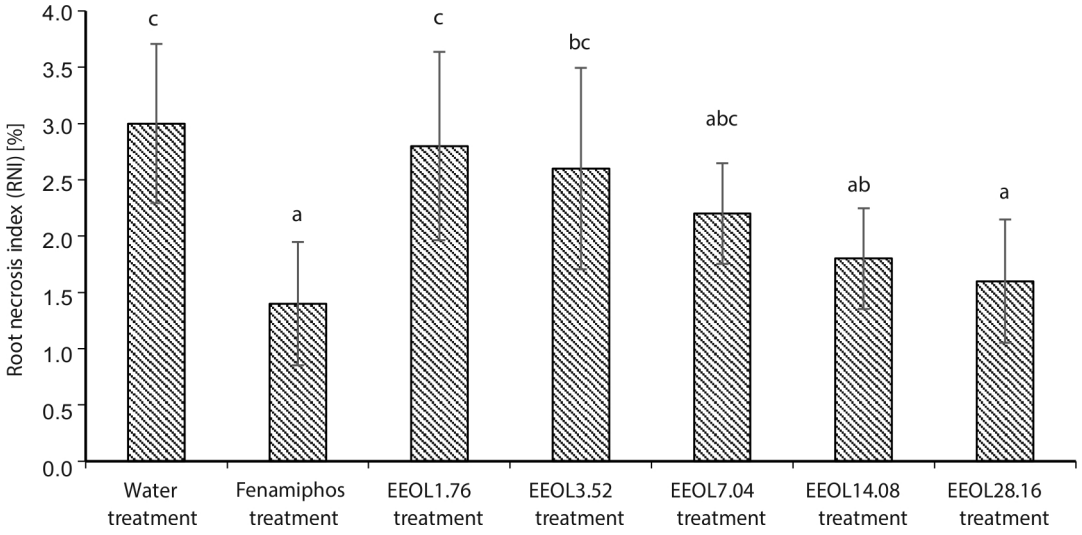


**Fig. 1.** The influence of *Eupatorium odoratum* leaf and stem extract on the root necrosis index (RNI) of banana plants in a micro plot experiment model. Values are expressed as Mean ± SD, letters (a, b, and c) represent the difference between treatments ($p < 0.05$)

2.8 ± 0.84%, 2.6 ± 0.89%, 2.2 ± 0.45%, 1.8 ± 0.45%, and 1.6 ± 0.55%, respectively ($p < 0.05$). This demonstrates the effectiveness of EEOL in reducing root necrosis as the dosage increases. Notably, the performance of EEOL at a concentration of 28.16 mg · ml$^{-1}$ showed no significant difference compared from fenamiphos (1.4% ± 0.55%) ($p > 0.05$), suggesting competition between EEOL and chemical pesticides in root nematode control.

### Effect of *Eupatorium odoratum* leaf and stem extract on banana plant growth

In the micro plot experiment model, banana plant growth was evaluated using a range of parameters (Table 3). The control treatment, which received only water, exhibited the least favorable growth trends ($p < 0.05$). Conversely, the treatments treated with *E. odoratum* extract (EEOL) demonstrated a noteworthy enhancement in these parameters. Pseudostem length and leaf area exhibited a gradual increase in these treatment treatments ($p < 0.05$), while pseudostem circumference and the total number of active leaves showed a decrease ($p < 0.05$). The most significant improvement was observed in those treated with fenamiphos ($p < 0.05$), and this result was also similar to the treatment with EEOL treatment at a concentration of 28.16 mg · ml$^{-1}$. These findings underscore the positive impact of EEOL on banana plant development in the micro plot experiment model, underscoring its potential to boost banana plant growth under the study's conditions.

## Field experiment

### Infection rate and nematode abundance in banana plant roots

The outcomes from the investigation of nematode infestation rates in banana plants grown under field conditions are meticulously outlined in Table 4. With the water treatment, a substantial augmentation

**Table 3.** The impact of *Eupatorium odoratum* leaves and stems extract on banana plants growth in a micro plots experiment

| Experimental treatments | Banana plants growth | | | |
|---|---|---|---|---|
| | pseudostem length [cm] | pseudostem circumference [cm] | total functional leaves [leave] | leaf area [m$^2$] |
| Water treatment | 298.85 ± 26.54 c | 51.96 ± 2.19 a | 9.2 ± 0.45 e | 0.35 ± 0.01 a |
| Fenamiphos treatment | 212.18 ± 15.8 a | 66.63 ± 3.77 e | 6.6 ± 0.55 a | 0.58 ± 0.04 g |
| $EEOL_{1.76}$ | 265.98 ± 22.92 bc | 56.58 ± 2.75 b | 8.4 ± 0.55 d | 0.39 ± 0.01 b |
| $EEOL_{3.52}$ | 257.03 ± 26.38 b | 58.58 ± 3.21 bc | 8.2 ± 0.45 cd | 0.43 ± 0.01 c |
| $EEOL_{7.04}$ | 245.07 ± 32.86 ab | 60.47 ± 2.29 bcd | 7.6 ± 0.55 bc | 0.46 ± 0.02 d |
| $EEOL_{14.08}$ | 233.12 ± 29.75 ab | 62.38 ± 3.51 cd | 7.4 ± 0.55 b | 0.51 ± 0.02 e |
| $EEOL_{28.16}$ | 218.17 ± 24.15 a | 64.21 ± 4.12 de | 7 ± 0.71 ab | 0.55 ± 0.01 f |

Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between treatment ($p < 0.05$)

in the incidence of nematode-infected plants and the quantification of nematodes within the root system was manifested in banana plant development ($p < 0.05$). By the 12th month, nematode infestation in banana plants of the water treatment had reached an alarming 93.33%, with the number of nematodes inhabiting the root zone amounting to 3178 ± 84.18. Conversely, upon implementing the EEOL treatment approach, a statistically significant decline was witnessed in the prevalence of *R. similis*-infected plants ($p < 0.05$) and the extent of nematode infestation in the roots ($p < 0.05$). The most substantial reduction was ascertained at an EEOL concentration of 96.54 l · ha$^{-1}$, resulting in the nematode infection rate plummeting to a mere 33.33% and the nematode population within the roots decreasing to 537 ± 25.64 ($p < 0.05$).

### The root necrosis index (RNI)

In the field experiment model, the level of root necrosis (RNI) progressively decreased in treatments receiving EEOL treatments (Fig. 2). We observed a significant difference in RNI rates among treatments that received EEOL treatments (6.03, 12.07, 24.13, 48.27, and 96.54 l · ha$^{-1}$), with RNI values of 3.0 ± 0.71%, 2.8 ± 0.84%, 2.4 ± 0.55%, 2 ± 0.71%, and 1.8 ± 0.45%, respectively, compared to the negative control treatment (water treatment) (3.2 ± 0.45%) ($p < 0.05$). This illustrates the effectiveness of EEOL in reducing root necrosis in a dose-dependent manner. Notably, the effectiveness of EEOL at a dose of 96.54 l · ha$^{-1}$ showed no significant difference compared to the chemical pesticide fenamiphos (1.6 ± 0.55%) ($p > 0.05$). This suggests the potential of EEOL as a viable alternative in root necrosis control.

### Effect of *Eupatorium odoratum* leaf and stem extract on plant growth

In the field model, the efficacy of *E. odoratum* extract (EEOL) was evident from the data presented in Table 5. The application of EEOL for nematode control significantly reduced nematode-induced damage to banana plants, leading to a marked improvement in growth parameters ($p < 0.05$). Banana plants treated with EEOL at various concentrations exhibited a substantial increase in pseudostem length, pseudostem circumference, and leaf area compared to the control treatment receiving water treatment ($p < 0.05$). The most effective treatment was observed in the fenamiphos treatment ($p < 0.05$), which closely resembled the treatment treated with EEOL at a concentration of 96.54 l · ha$^{-1}$ ($p > 0.05$). These results indicate the potential of EEOL as a promising alternative for managing root nematode damage and promoting the growth of banana plants in the field model.

### Percentage of fallen plants

The observation of the percentage of banana plants falling due to the impact of nematodes revealed a significant improvement following the utilization of *E. odoratum* extract (Fig. 3). The percentage of banana plants falling dramatically decreased in treatments with EEOL 6.03–96.54 l · ha$^{-1}$ (7.41%, 6.3%, 4.81%, 3.33%, and 1.85%, respectively) compared to the control treatment with water (10.13%) ($p < 0.05$), with this effect being particularly notable in the micro plot experiment model. The effectiveness of EEOL 96.54 l · ha$^{-1}$ (1.85%) was almost equivalent to that of fenamiphos (1.48%), a commonly used chemical pesticide for nematode control. This highlights the potential of EEOL in reducing the percentage of falling banana plants

**Table 4.** The influence of *Eupatorium odoratum* (EEOL) leaves and stems extract on plants infection incidence and *Radopholus similis* population in field-based experimental model

| Experimental treatments | Rate of banana plants infected with nematodes [%] | | | | Nematode quantity in banana plant roots [nematodes] | | | |
|---|---|---|---|---|---|---|---|---|
| | 3 months | 6 months | 9 months | 12 months | 3 months | 6 months | 9 months | 12 months |
| Water treatment | 56.3 ± 3.84 f | 65.56 ± 3.36 f | 84.07 ± 1.01 f | 93.33 ± 2.11 f | 1909 ± 68.83 g | 2226 ± 72.55 g | 2864 ± 73.58 g | 3178 ± 84.18 g |
| Fenamiphos treatment | 6.67 ± 2.11 a | 7.78 ± 1.55 a | 10 ± 1.01 a | 10.74 ± 2.41 a | 278 ± 14.75 a | 325 ± 13.58 a | 418 ± 14.88 a | 462 ± 21.9 a |
| $EEOL_{6.03}$ | 45.93 ± 3.56 e | 53.33 ± 2.41 e | 69.26 ± 1.01 e | 76.67 ± 9.85 e | 1361 ± 53.61 f | 1586 ± 44.69 f | 2042 ± 53.57 f | 2266 ± 63.17 f |
| $EEOL_{12.07}$ | 35.93 ± 5.34 d | 41.48 ± 2.11 d | 54.07 ± 0.83 d | 60 ± 3.84 d | 1131 ± 43.63 e | 1318 ± 35.6 e | 1695 ± 47.58 e | 1883 ± 54.66 e |
| $EEOL_{24.13}$ | 27.04 ± 3.84 c | 31.85 ± 1.55 c | 40.37 ± 0.83 c | 44.81 ± 4.79 c | 896 ± 37.6 d | 1014 ± 26.71 d | 1304 ± 34.66 d | 1447 ± 43.58 d |
| $EEOL_{48.27}$ | 17.41 ± 3.61 b | 20 ± 2.41 b | 26.3 ± 0.83 b | 29.26 ± 4.22 b | 598 ± 28.7 c | 698 ± 23.65 c | 898 ± 34.66 c | 999 ± 36.8 c |
| $EEOL_{96.54}$ | 7.41 ± 2.93 b | 8.89 ± 1.55 b | 12.59 ± 2.41 b | 33.33 ± 8.28 b | 323 ± 16.72 b | 377 ± 17.99 b | 485 ± 24.65 b | 537 ± 25.64 b |

Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between treatment ($p < 0.05$)

**Table 5.** The influence of *Eupatorium odoratum* leaves and stems extract on banana plants growth in a field-based experiment

| Experimental treatments | Banana plants growth | | | |
|---|---|---|---|---|
| | pseudostem length [cm] | pseudostem circumference [cm] | total functional leaves [leave] | leaf area [$m^2$] |
| Water treatment | 664.14 ± 43.71 a | 231.55 ± 17.22 a | 14.2 ± 0.45 e | 0.71 ± 0.02 a |
| Fenamiphos treatment | 936.39 ± 52.28 f | 293.68 ± 23.97 d | 10.2 ± 0.45 a | 0.92 ± 0.03 f |
| $EEOL_{6.03}$ | 743.82 ± 46.69 b | 251.65 ± 20.63 ab | 13 ± 0.71 d | 0.74 ± 0.01 b |
| $EEOL_{12.07}$ | 770.41 ± 35.95 bc | 260.11 ± 24.08 bc | 12.6 ± 0.55 d | 0.77 ± 0.02 b |
| $EEOL_{24.13}$ | 810.24 ± 32.2 cd | 263.05 ± 16.23 bc | 11.8 ± 0.45 c | 0.81 ± 0.01 c |
| $EEOL_{48.27}$ | 850.08 ± 41.05 d | 272.35 ± 22.44 bcd | 11.4 ± 0.55 bc | 0.85 ± 0.03 d |
| $EEOL_{96.54}$ | 909.82 ± 45.05 e | 283.51 ± 18.57 cd | 10.8 ± 0.45 ab | 0.88 ± 0.01 e |

Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between treatment ($p < 0.05$)

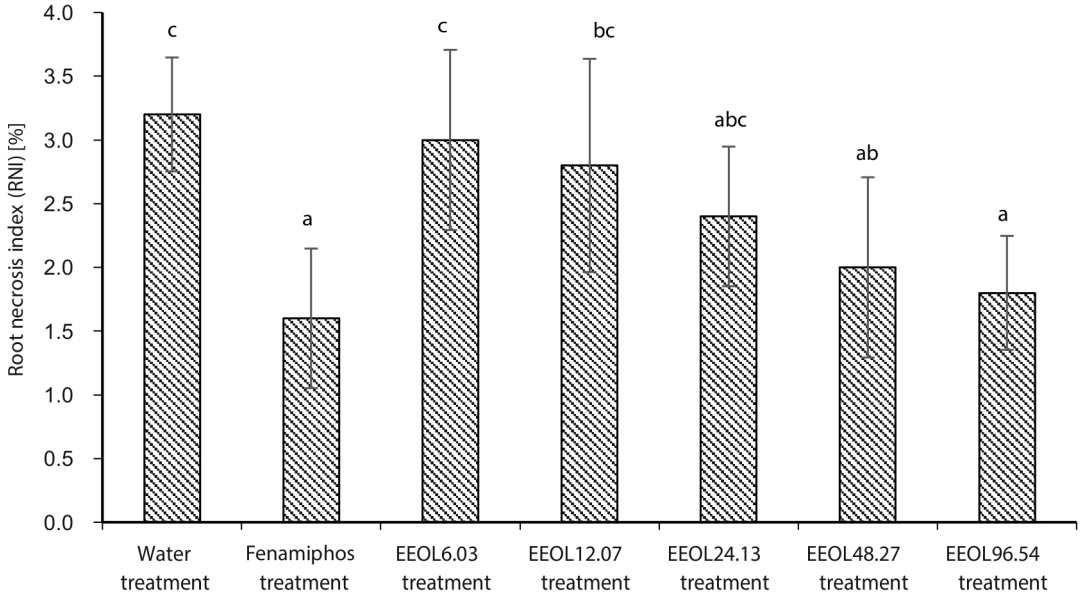


**Fig 2.** The effects of *Eupatorium odoratum* leaf and stem extract on the root necrosis index (RNI) in banana plants in a field-based experimental model. Values are expressed as Mean ± SD, letters (a, b, and c) represent the difference between treatments ($p < 0.05$)

due to nematode impact. These results demonstrate that EEOL has the capability to mitigate the adverse effects of nematodes on banana plant stability, thereby enhancing crop productivity and quality.

### Effect of *Eupatorium odoratum* leaf and stem extract on the harvest yield

Regarding the harvest yield parameters (Table 6), the presence of burrowing nematodes after 12 months of planting showed a significant decrease in harvest yield in the water control treatment compared to the other treatments ($p < 0.05$). The numbers hands/bunch, fingers/bunch, finger weight, bunch weight, yield factor, and total yield all exhibited improvements in the EEOL treatments ($p < 0.05$). The highest improvements were observed in the 96.54 l · $ha^{-1}$ dose treatment after 12 months of cultivation, which were equivalent to the fenamiphos treatment ($p > 0.05$). These parameters demonstrate the effectiveness of EEOL in enhancing harvest yields and the differences compared to the water control treatments.

## Discussion

Plant extracts are acknowledged as a valuable natural resource for the research and development of bio-based products aimed at controlling burrowing nematodes that afflict banana plants. In this study, we present an inaugural investigation into the bioactivity of aqueous extracts from the leaves and stems of *E. odoratum* in

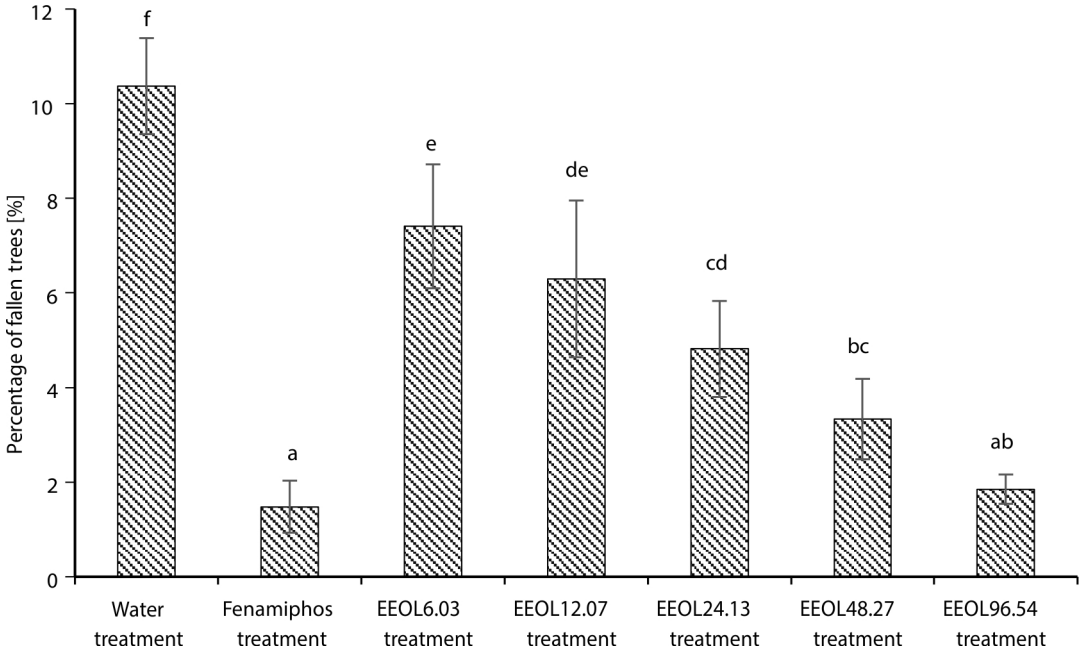


**Fig. 3.** The influence of *Eupatorium odoratum* leaf and stem extract on the incidence of fallen banana trees in a field-based experimental framework. Values are expressed as Mean ± SD, letters (a, b, c, d, and e) represent the difference between treatments ($p < 0.05$)

**Table 6.** The impact of *Eupatorium odoratum* leaves and stems extract on banana harvest yield in a field experiment

| Experimental treatments | Harvest yield | | | | | |
|---|---|---|---|---|---|---|
| | No. of hands/bunch [hand/bunch] | No. of fingers/bunch [finger/bunch] | finger weight [gm] | bunch weight [kg] | yield factor [%] | total yield [t · ha$^{-1}$ · yr$^{-1}$] |
| Water treatment | 7.6 ± 1.01 a | 107.45 ± 9.75 a | 120.48 ± 8.82 a | 21.61 ± 1.07 a | 61.64 ± 3.18 a | 61.69 ± 4.93 a |
| Fenamiphos treatment | 10.75 ± 1.04 e | 159.44 ± 12.7 f | 179.76 ± 10.6 f | 28.54 ± 1.05 f | 95.14 ± 5.26 e | 77.26 ± 7.58 b |
| $EEOL_{6.03}$ | 8.32 ± 1.04 ab | 119.71 ± 8.45 ab | 130.68 ± 7.82 ab | 23.25 ± 1.04 b | 67.75 ± 4.96 b | 68.18 ± 5.89 ab |
| $EEOL_{12.07}$ | 8.88 ± 1.14 abc | 128.24 ± 8.17 bc | 141.27 ± 9.27 bc | 24.36 ± 1.03 bc | 72.48 ± 3.8 b | 72.84 ± 13.79 ab |
| $EEOL_{24.13}$ | 9.38 ± 1.07 bcd | 137.28 ± 10.56 cd | 152.18 ± 9.63 cd | 25.35 ± 1.09 cd | 78.65 ± 4.44 c | 65.38 ± 6.74 ab |
| $EEOL_{48.27}$ | 9.83 ± 0.98 cde | 145.35 ± 9.02 de | 163.45 ± 10.11 de | 26.25 ± 1.08 de | 84.57 ± 4.23 d | 75.37 ± 13.04 b |
| $EEOL_{96.54}$ | 10.32 ± 1.01 de | 151.28 ± 10.73 ef | 174.26 ± 0.17 ef | 27.35 ± 1.04 ef | 93.46 ± 4.02 e | 76.4 ± 13.48 b |

Values are expressed as Mean ± SD, letters (a, b, c, d, e, and f) represent the difference between treatment ($p < 0.05$)

combatting *R. similis*, a prominent nematode species that poses a significant menace to banana cultivation in the field.

Secondary metabolites in plants, encompassing tannins, flavonoids, alkaloids, saponins, terpenoids, and phenolics, play pivotal roles in diverse biological processes. These processes include conferring resistance to insects, attracting pollinators, and responding to abiotic stressors (Desmedt *et al.* 2020). Among these compounds, phenolic compounds hold particular importance in defense mechanisms against various plant-damaging insects, including nematodes. For example, phenylphenalenone phytoalexin, a subclass of phenolic compounds, has been demonstrated to have a pivotal role in fending off root-knot nematodes, such as *R. similis*, which pose threats to banana plants. Phenylphenalenone forms complexes with lipids within the nematode, culminating in the development of substantial lipid-anigorufone droplets, ultimately leading to the nematode's demise (Hölscher *et al.* 2014). Flavonoids also manifest nematocidal properties, including the inhibition of *R. similis* egg development and the repellence and lethality of J2 nematodes (Kirwa *et al.* 2018). In the context of banana plants, nematode-resistant cultivars frequently exhibit elevated levels of condensed tannins. These

tannins can induce alterations in pH levels, nutrient concentrations, or the production of compounds capable of combating nematodes (Collingborn *et al.* 2000). Aldehyde terpenoids (TA), a subtype of terpenoids, are being extensively studied in the context of plant-nematode interactions. TA accumulates within root tissues and can migrate to feeding sites of developing juvenile nematodes, restraining their growth (Veech 1979). Alkaloids, recognized for their high toxicity, play a pivotal role in plant protection against insects and pathogens. Pyrrolizidine alkaloids, in particular, exert essential nematode resistance by affecting their respiratory and muscular systems (Thoden and Boppré 2010). Alkaloids, saponins, triterpenes, steroids, etc., have also been identified in neem seed extract (*Azadirachta indica* A. Juss) (Kosma *et al.* 2011), as well as in extracts from *Azadirachta indica* and *Allium sativum* (Bartholomew *et al.* 2014). These extracts have been reported to possess the capability to control and eradicate detrimental nematode species affecting banana plants. So, the presence of these plant compounds, such as tannins, flavonoids, alkaloids, saponins, terpenoids, and phenolics in the *E. odoratum* leaf and stem extract (EEOL), underscores their potential in countering *R. similis* nematodes

The burrowing nematode (*R. similis*) is a common nematode species in large-scale banana cultivation worldwide. It inflicts severe damage on commercial banana crops, particularly the Cavendish banana variety. *Radopholus similis* is an endoparasitic nematode species, completing its life cycle within 20–25 days in banana plant roots and pseudostem tissues. Adult nematodes are mobile and can exit the roots under unfavorable conditions, easily colonizing new root tissue. This endoparasitic nematode creates cavities in the roots and pseudostems, causing damage that impairs the plant's water and nutrient uptake, leading to banana plant debilitation, reduced fruit yield, plant toppling, and favorable conditions for the development of pathogenic microorganisms (Sarah *et al.* 1996). The plant's ability to resist nematode infestation is often contingent upon the activity of plant compounds found in extracts from the leaves and stems of *E. odoratum*. These compounds can impede nematode movement and feeding, as well as disrupt their reproductive processes. This limits the nematodes' ability to infest banana roots and pseudostems. Phenolic compounds in the EEOL exhibit antioxidant properties and can be toxic to juvenile nematodes or adversely affect their development. These compounds act as mechanical barriers to nematodes, reducing infestation and preventing nematode penetration into the plant (Sankar *et al.* 2017).

*Radopholus similis* can readily propagate through the dissemination of infected planting materials or in regions where disease symptoms are manifested, potentially resulting in extensive harm to the root structure. Upon introduction to a new cultivation area, the infection process is initiated as *R. similis* infiltrates the root system, causing harm. Evidence of damage becomes apparent when roots are longitudinally split or when tree trunks are stripped, revealing brown-red wounds that subsequently evolve into blackened tissue due to bacterial proliferation. Root system impairment diminishes the plant's ability to uptake water and essential nutrients, ultimately leading to plant desiccation and collapse (Mwaka *et al.* 2023). Advancements in plant biotechnology have unlocked substantial prospects for mitigating nematode-induced yield losses. Secondary metabolites present in plant extracts exert specific influences on nematode development, penetration, and the damage they inflict (Desmedt *et al.* 2020). Alkaloids can establish an adverse environment, disrupting the nematode's nutrient absorption process or impeding its mobility. Flavonoids possess antioxidant properties and anti-inflammatory characteristics, reducing inflammation and root damage caused by nematodes. Tannins form complexes with nematode proteins, compromising their structural integrity. Furthermore, tannins can impede nematode infiltration into plant roots and curtail their activity (Desmedt *et al.* 2020). EEOL holds the potential for controlling nematodes detrimental to plants through compounds such as alkaloids, flavonoids, and tannins, thus safeguarding and fortifying the plant's root system against nematode infestations.

*Radopholus similis* induces a slow decline in many plant species but exhibits particularly severe symptoms in bananas. *R. similis* causes fatalities in intermediary roots and instigates brown-red discoloration on larger root surfaces, potentially leading to severe root wrapping and destruction. Ultimately, they migrate from the roots into the root stem, causing circular black lesions, a condition known as "blackhead disease", resulting in water deficiency, nutrient scarcity, diminished support, and growth in the affected plants (Brooks 2008). Sustainable control of banana nematodes has been explored through the application of plant extracts, as previously demonstrated by Jesus *et al.* (2015) and Marin *et al.* (2000), both of which yielded highly promising results, showing improved banana plant growth following treatment with plant extracts. In this study, *E. odoratum* extract significantly enhanced pseudostem length, pseudostem circumference, and leaf area. Remarkably, in the field model, the efficacy of *E. odoratum* extract closely paralleled that of the chemical nematicide fenamiphos. This underscores the potential of *E. odoratum* extract as a safe and effective alternative for nematode control.

The burrowing nematode, as it infiltrates the roots of banana plants, inflicts severe harm by dismantling root tissues along their length. This results in the root

system's atrophy, rendering it less robust, marked by necrosis, and substantially weakened. Bananas afflicted with this nematode infection encounter a shortage of water and nutrients, hampering their growth. These banana plants exhibit symptoms commonly referred to as "banana toppling". When these plants are affected by nematode infestation, it provides a window for other pests to proliferate, causing significant harm, particularly during periods of heavy rainfall and strong winds. This phenomenon arises due to the root system's considerable debilitation and damage, rendering it incapable of firmly anchoring the plant in the soil (Wang *et al.* 2001). The extract from *E. odoratum* has demonstrated its effectiveness in eradicating these nematodes, thereby curbing the destruction they impose upon banana plants.

The parameter "Number of hands per bunch" serves as an essential metric for gauging the developmental progress of banana plants and quantifying the number of banana clusters on each pseudostem. Meanwhile, "Number of fingers per bunch" is closely associated with the dimensions of these banana clusters and serves as a measure of the individual banana quality within each cluster. "Finger weight" enables the assessment of the dimensions and mass of individual bananas, whereas "Bunch weight" provides a comprehensive measure of the aggregate harvest weight from each cluster. The "Yield factor" denotes the banana plant's productivity, taking into account both the number of clusters and the banana size. Finally, the "Total yield" stands as a pivotal parameter for appraising the overall output of a banana field. These parameters greatly facilitate the evaluation of banana plant performance and allow for the quantification of changes following various interventions (Sora and Guji 2023). The research findings convincingly establish the adverse influence of burrowing nematodes on banana crop yields. In the water control treatment, banana yields were notably lower than in treatments involving the application of EEOL and fenamiphos. The most substantial improvement in yield was observed in the EEOL treatments at a concentration of 96.54 l · ha$^{-1}$. These results underscore the efficacy of EEOL in enhancing banana harvest yields and mitigating the nematode-induced impact. Notably, the effectiveness of EEOL closely parallels that of the conventional chemical pesticide fenamiphos, implying that EEOL holds potential to serve as a secure and efficacious alternative approach to bolster banana crop yields while curbing nematode-inflicted damage.

This study highlights the effectiveness of *E. odoratum* aqueous extract (EEOL) in controlling the destructive nematode *R. similis* in Cavendish banana plants, both in micro plot experiments and under field conditions. Nematode presence negatively impacted crop yield and plant growth, but significant improvements were observed with EEOL application. Key parameters, including infection rates, root damage, plant growth, and harvest yield, all showed substantial enhancements, especially in the field model. At a dosage of 96.54 l · ha$^{-1}$, EEOL proved as effective as the traditional chemical nematode control method, fenamiphos. This emphasizes EEOL's potential as a safe and efficient alternative for nematode management. In summary, the use of *E. odoratum* aqueous extract effectively combats *R. similis* nematodes, leading to improved Cavendish banana plant yield and quality in both micro plot experiments and field settings. EEOL offers a secure and effective alternative for nematode control.

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