Research Article

Effect of Aqueous Extracts of Some Aromatic Plants on the Main Parasitic Nematodes of Banana (*Musa* spp.) *In Vitro*

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Abstract | Banana cultivation is subject to several constraints, including nematodes. A study was conducted in the laboratory to identify plants that have nematicidal effects. Aqueous extracts of four aromatic plants (*Cymbopogon citratus*, *Ocimum gratissimum*, *Azadirachta indica*, and *Cacia siamea*) were evaluated for their effect on the mortality of two species of nematodes. The methodology consisted of preparing aqueous extracts of different parts of aromatic plants (leaves, stems, roots), tested at four concentrations (5, 10, 25 and 50%) on 20 nematodes per Petri dish. After 48 hours incubation at an ambient laboratory temperature of 25 ± 2°C, nematode mortality was assessed and corrected using the Schneider-Orelli formula. A statistical analysis (ANOVA) was used to compare the effects of the different extracts. The results obtained showed a significant efficacy of aqueous extracts on both nematode species, particularly those of *C. citratus* leaves (up to 94.44% mortality for *P. coffeae* and 76.47% for *R. similis* at 50% concentration). The aqueous extracts of *O. gratissimum* and *A. indica* were also found to be effective, although with some variability. The aqueous extracts of *C. citratus* had the lowest mortality rates. The concentrationt of the aromatic plants used depended on the plant species, the organ used and the concentration. Aqueous extracts of these aromatic plants could constitute a basis for the formulation of nematicides and an alternative to the use of synthetic chemicals in the control of plant-parasitic nematodes.

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Introduction

Plant-parasitic nematodes are a major, but often underestimated, component of the pathogens affecting the health of cultivated plants. These small, microscopic worms, belonging to the Ecdysozoa group, are ubiquitous and colonize virtually all humid terrestrial ecosystems. Their taxonomic diversity and numerical abundance make them formidable pests in many agricultural systems, with significant economic repercussions worldwide (Lambert and Bekal, 2002). In banana cropping systems, plant-parasitic nematodes, particularly migratory endoparasites, are a major factor limiting productivity. By attacking the root system, they induce necrotic lesions that disrupt water and mineral uptake, compromising plant stability and promoting premature fall (Vawa, 2017; Yeo, 2019). The most virulent species identified in banana agrosystems are *Radopholus similis* and *Pratylenchus coffeae*. The yield losses they cause in tropical and subtropical countries are estimated at 14.6% (Nicol *et al*., 2011). These recurrent losses often force growers to replant banana plantations at high frequency, with significant economic and environmental impacts (Gnonhouri *et al*., 2009).

In Côte d'Ivoire, the presence of *R. similis* and *P. coffeae* has been known for several decades, with the first detections dating back to the 1960s and 1980, respectively (Luc and Vilardebo, 1961; Fargette and Quénéhervé, 1988). Their polyphagous nature, which enables them to survive on various weeds outside the banana-growing periods, makes them particularly difficult to eradicate (Duyck *et al*., 2009). For decades, the management of these plant-parasitic nematodes has essentially relied on the use of chemical nematicides and synthetic fumigants because of their effectiveness and rapid action (Odeyemi *et al*., 2013). However, the toxicity of these products for humans and the environment and their gradual withdrawal from the international market, as in the case of methyl bromide, have highlighted the need to develop more sustainable control strategies (Giannakou *et al*., 2004; Rich *et al*., 2004). Alternative approaches have been explored, including cropping practices (rotations, organic amendments, resistant varieties) and biological control, which uses natural agents such as antagonistic microorganisms, biofumigants and plant extracts (Meadows *et al*., 2018; Yeo, 2019). Plant extracts with nematicidal properties are a promising solution. Rich in bioactive compounds, these extracts have low toxicity for non-target organisms, are biodegradable, and can be easily integrated into agroecological production systems (Wiratno *et al*., 2009; Singh, 2015). This is the background to the present study, which assesses the nematicidal activity of aqueous extracts of four aromatic plants on two species of plant-parasitic nematodes, *Radopholus similis* and *Pratylenchus coffeae*, under control conditions. The aim of this study was to contribute to the development of sustainable management strategies for nematodes in banana plantations, compatible with the principles of ecological and resilient agriculture.

Materials and Methods

Plant material

The different organs of the following aromatic plants were used: *Ocimum gratissimum* L. (Lamiaceae), *Cacia siamea* L. (Fabaceae), *Cymbopogon citratus* L. (Poaceae) and *Azadirachta indica* L. (Meliaceae). The choice of the four plant species studied was based mainly on their biochemical compositions and their biocidal effects, as demonstrated by several previous studies. These plants, belonging to 4 different botanical families, were harvested before flowering (March-April) in different regions of Ferkessédougou during the dry season in northern Côte d'Ivoire.

Animal material

The animal biological material used in this experiment consisted of migratory endoparasitic nematodes (*Radopholus similis* and *Pratylenchus coffeae*). These were obtained from infested banana roots collected from industrial dessert banana plantation in the Abidjan region in southern Côte d'Ivoire.

Plant extracts preparation

The aqueous solutions were extracted from 50 g of the organ (leaves, stems and roots) used after grinding it in 100 mL sterile distilled water to a concentration of 50% (S). The solution obtained was filtered and then centrifuged at 3,000 rpm for 5 min to remove any debris (Kumar, 2003).

Evaluation of the nematicidal potential of aqueous extracts on plant-parasitic nematodes

The nematicidal activity of aqueous extracts of aromatic plants was evaluated *in vitro* on the plant-parasitic nematodes *Radopholus similis* and *Pratylenchus coffeae*. For each test, twenty (20) individuals of nematodes were introduced into 90 mm diameter Petri dishes containing 5 mL of extract solutions at concentrations of 5, 10, 25 and 50% (Kosma *et al*., 2011). The control consisted solely of sterile distilled water. All plates were incubated at 25 ± 2°C for 48 h in a temperature-controlled oven. Each treatment was replicated five times. After incubation, the nematodes were observed under a light microscope (magnifications × 100 and × 400) to determine the mortality rate. Individuals were considered dead in the absence of spontaneous movement or movement induced by mechanical stimulation and in the presence of a characteristic rigid posture. The mortality rate was expressed as a percentage of the total number of individuals treated.

Estimation of the corrected mortality of nematodes under the effect of aqueous extracts

The assessment of the nematicidal efficacy of the aqueous extract was based on the quantification of induced larval mortality. For each concentration of aqueous extract tested, the average mortality rate was determined to estimate the lethal effect of the extract on the nematode larval stages. However, natural mortality, observed in the negative controls (distilled water), could have influenced the interpretation of the results. A correction for mortality was therefore essential to isolate the real effect of the extract tested. Observed mortality was calculated using the following formula:

$$\text{mortality observed } (\%) = \frac{\text{Number of dead nematodes}}{\text{Total number of individuals}} \text{ x } 100$$

To correct this mortality for that observed in the control, the following formula from Schneider-Orelli (1947), adapted by Püntener (1981), was used:

$$\text{Corrected mortality } (\%) = \frac{\text{mortality observed in the treatment} - \text{mortality in the control}}{100 - \text{mortality in the control}} \text{x}100$$

This method provides a more accurate estimate of the intrinsic efficacy of the extract, by neutralizing the confounding effects of the basic experimental conditions.

Statistical analysis

The data collected during experiments conducted under controlled conditions (*in vitro*) were analyzed using a two-factor analysis of variance (ANOVA) with aromatic plants and formulation as independent variables. STATISTICA software version 7.1. was used to evaluate the effect of treatments on nematode mortality. The Student Newmane-Keuls (SNK) test was used to determine if means were significantly different ($P < 0.05$).

Results

The effect of two types of aqueous extracts of the aromatic plants *O. gratissimum* (Lamiaceae), *C. siamea* (Fabaceae), *C. citratus* (Poaceae) and *A. indica* (Meliaceae) tested at different concentrations and exposure times showed an efficacy on the mortality rate of *R. similis* and *P. coffeae*.

Effect of the different plants tested on the adjusted mortality rates of Radopholus similis and Pratylenchus coffeae

The analysis of variance showed a significant difference ($p \leq 0.000$) in the influence of the tested plants on the corrected mortality rates of *Radopholus similis* and *Pratylenchus coffeae*. *C. citratus* recorded the highest corrected mortality rates for *R. similis* and *P. coffeae*, with values of 54.40% and 62.38%, respectively (Figure 1). For *O. gratissimum* and *A. indica*, moderate mortality rates were observed, with values around 40% for both nematode species. The lowest corrected mortality rates were induced by *C. siamea*, with 22.22% and 15.24% for *R. similis* and *P. coffeae*, respectively (Figure 1).

Influence of different plant organs on the corrected mortality rate of Radopholus similis and Pratylenchus coffeae

The two-factor analysis of variance (concentrations and organ used for extraction) showed a significant effect ($p = 0.023$) on the corrected mortality of *Radopholus similis* induced by the organs extracts from the four aromatic plants used. Leaf extract at a concentration of 50% was the most effective, inducing a corrected mortality of 54.17%. Roots at this same concentration showed a low corrected mortality rate of 27.78% (Table 1). Notably, even at 5%, leaf extract induced a mortality of 38.89%. In general, aqueous extracts of roots showed reduced larvicidal efficacy, not exceeding 35% corrected mortality (Table 1), regardless of the plant organ used (leaves, stems or roots).

Table 1: *Effect of different plant organs on the corrected mortality rate of Radopholus similis and Pratylenchus coffeae.*

Organs	Time of exposure (Hours)	Concentrations (%)	Mortalité *R. similis* (%)	Mortalité *P. coffeae* (%)
Leaves	48	5	38.89 ± 9.79 abc	40.71 ± 6.91 b
		10	53.47 ± 8.00 a	61.43 ± 8.50 a
		25	49.31 ± 5.92 ab	68.57 ± 6.52 a
		50	54.17 ± 5.35 a	63.57 ± 8.29 a
Stems	48	5	39.58 ± 4.75 abc	40.00 ± 11.36 b
		10	41.67 ± 7.55 abc	39,29 ± 8.08 b
		25	22.92 ± 8.00 c	41,43 ± 8.67 b
		50	43.06 ± 4.78 abc	23.57±10.51bc
Roots	48	5	23.61 ± 10.01 c	19,29 ± 7.24 c
		10	31.94 ± 5.63 c	29.29 ± 9.41 bc
		25	31.11 ± 7.53 abc	29.29±10.30bc
		50	27.78 ± 6.76 c	25.00 ± 7.24 bc
F			2.73	3.47
P			0.023	0.006

Means with the different letters are significantly different according to the Newman-Keuls test (P ≤ 0.05). Values with ± represent standard errors.

The two-factor analysis of variance (concentrations and organ types) showed statistically significant effects (p = 0.006) of aqueous extracts from the different organs of the four aromatic plants on the corrected mortality of *Pratylenchus coffeae* (Table 1). The results showed variable efficacy depending on the aromatic plant organs and the aqueous extract concentration. Of all the aqueous extracts tested, that derived from the leaves induced the strongest nematicidal activity, with increasing mortality rates ranging from 61.43, 68.57 to 63.57 % at concentrations of 10, 25 and 50%, respectively. In contrast, aqueous extracts from the stems and roots were the least effective, inducing only low mortality rates ranging from 25.00% and 19,29%, even at 50 and 5% concentration after 48 hours of exposure (Table 1). These results highlight the bioinsecticidal variability depending on the organ used and the concentration of the extract.

Influence of the plant organs on the efficacy of aqueous extracts of aromatic plants on nematode mortality

The results relating to the nematicidal efficacy of aqueous extracts of the different parts (leaves, stems and roots) of the aromatic plants *Cymbopogon citratus*, *Ocimum gratissimum*, *Azadirachta indica* and *Cassia siamea* are reported in Table 2. The corrected mortality rates of the nematodes *Radopholus similis* and *Pratylenchus coffeae* varied significantly depending on the plant species, the organ used and the type of nematode targeted. For the aromatic plant *Cymbopogon citratus*, aqueous extracts from leaves induced the highest mortalities with 66.67% for *R. similis* and 80.71% for *P. coffeae* followed respectively by extracts from stems (53.47% and 59.29%) and roots (43.06% and 47.14%). A similar trend was observed in *A. indica*, where leaf extracts caused 50.69% mortality in R. *similis* and 59.29% in *P. coffeae*, compared with stem extracts (23.61% and 40.00%) and root extracts (25.69% and 29.29%). On the other hand, in the aromatic plant *O. gratissimum*, the nematicidal effect of the leaf and stem extracts on the nematode *R. similis* was identical (50%), while the leaf extracts were the most effective against the nematode *P. coffeae* (67.14%), unlike the stem (37.14%) and root (15.71%) extracts (Table 2). Finally, *C. siamea* extracts showed low overall efficacy, although leaf extracts showed the highest mortality rates (25.69% for the *R. similis* nematode and 27.14% for the *P. coffeae* nematode), while stem extracts were the least effective (17.36%; 7.86% respectively). These results confirmed that the plant organ used has a strong influence on the nematicidal activity of aqueous extracts, with leaves generally being the most active, regardless of the plant species considered.

Table 2: *Effectiveness of different parts of plant organs on the corrected mortality of Radopholus similis and Pratylenchus coffeae.*

Plants	Time of exposure (Hours)	Organs	Mortalité *R. similis* (%)	Mortalité *P. coffeae* (%)
Cymbopogon citratus	48	Leaves	66.67 ± 6.86 a	80.71 ± 3.57 a
		Stems	53.47 ± 6.07 ab	59.29 ± 4.29 bc
		Roots	43.06 ± 8.09 bc	47.14 ± 9.54 cd
Ocimum gratissimum	48	Leaves	52.78 ± 4.32 ab	67.14 ± 1.69 ab
		Stems	52.78 ± 6.03 ab	37.14 ± 4.55 cd
		Roots	27.08 ± 3.23 ab	15.71 ± 8.2 d
Azadirachta indica	48	Leaves	50.69 ± 2.52 ab	59.29 ± 6.82 ab
		Stems	23.61 ± 7.12 c	40.00 ± 9.56 bc
		Roots	25.69 ± 5.94 c	29.29 ± 8.71 cd
Cacia siamea	48	Leaves	25.69 ± 4.58 c	27.14 ± 2.81cd
		Stems	17.36 ± 4.32 c	7.86 ± 5.39 d
		Roots	23.61 ± 5.92 c	10.71 ± 3.96 d
F			3.65	2.76
P			0.0043	0.022

Means with the different letters are significantly different according to the Newman-Keuls test (P≤ 0.05). Values with ± represent standard errors.

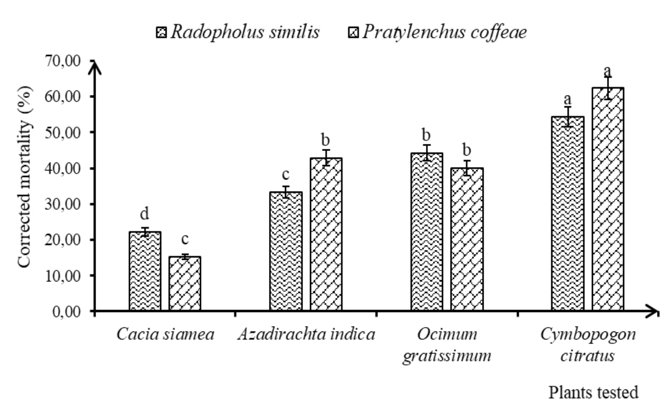

Figure 1: *Effect of plants on corrected mortality of Radopholus similis and Pratylenchus coffeae.*

Discussion

This investigation aimed at determining the nematicidal potential of four plant species (*Cymbopogon citratus*, *Ocimum gratissimum*, *Azadirachta indica* and *Cacia siamea*) using aqueous extracts from different plant organs, tested at four concentrations (5, 10, 25 and 50%) and at a standardized exposure time of 48 hours. The efficacy of the aqueous extracts was analyzed

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in vitro on the mortality of two plant-parasitic nematodes of agronomic importance, *Radopholus similis* and *Pratylenchus coffeae*.

The results showed that nematicidal activity varied significantly depending on the plant species, the organ used for extraction and the concentration applied. All the aqueous extracts induced mortality rates of more than 50%, indicating marked bioactivity. This observed mortality is probably attributable to toxic secondary metabolites present in plant extracts, such as terpene aldehydes, monoterpenes, alkaloids, tannins, phenols, triterpenes and saponins, whose modes of action may include the inhibition of larval penetration into the roots, direct intoxication of nematodes or alteration of their reproduction (Coyne *et al*., 2018). Of the compounds identified, neral and geranial are characteristic of lemongrass (*Cymbopogon citratus*) regardless of the geographical origin. These two aldehydes, present at 39.7% and 60.3% respectively in the essential oils of the Brazilian variety (Barreira *et al*., 2004), have demonstrated significant antiparasitic activity against Plasmodium bergheir (Buena *et al*., 2007). The essential oil of *Ocimum gratissimum* is dominated by thymol (43.14%), a monoterpene with well-established antiseptic properties (Kassi *et al*., 2014). In addition, phytochemical analyses of *Azadirachta indica* have revealed a diversity of bioactive compounds such as alkaloids, saponins, triterpenes and steroids, known for their nematicidal or nematostatic effects (Faouzi, 2002), while tannins and phenolic compounds, although more widespread in the plant kingdom, have antimicrobial effects and inhibit the development of nematodes (Faouzi, 2002). The heterogeneity of the responses depending on the plant organ used for extraction is probably explained by the variability of the intracellular biochemical composition, influenced by the differential solubility of the metabolites in water, the main solvent in the study. Despite the undeniable advantages of plant extracts (biodegradability, low environmental toxicity and specificity of action) their adoption by farmers remains limited. This low uptake is linked to several constraints: preparation time considered excessive, the need for repeated treatments, delayed effect, low persistence, and a narrow spectrum of action compared with synthetic plant protection products (Adékambi *et al*., 2010; Tounou, 2011).

Conclusion

The results obtained show that the four aromatic plants evaluated (*Cymbopogon citratus*, *Ocimum gratissimum*, *Azadirachta indica* and *Cacia siamea*) have significant nematicidal activity, particularly through the marked biocidal efficacy of their aqueous extracts derived from the leaves. Evaluation of their effects on the mortality of the plant-parasitic nematodes *Radopholus similis* and *Pratylenchus coffeae* revealed a high potential for these extracts to have a lasting effect on nematode populations. These results suggest that these aqueous extracts could be promising biocontrol agents that can be incorporated into agroecological nematode management strategies, thereby helping to reduce the use of synthetic chemical nematicides in sustainable production systems.

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Novelty Statement

This study provides the first comparative evaluation of the nematicidal potential of aqueous extracts from selected aromatic plants against the main parasitic nematodes of banana (*Musa* spp.) under in vitro conditions, highlighting eco-friendly alternatives to synthetic nematicides.

Author's Contribution

Gnénakan Yeo: Wrote the protocol, conducted the study, collected data, performed the statistical analysis and wrote the first draft of the manuscript.
Seydou Tuo: Analyzed and interpreted data.
Lassina Fondio: Supervised the research work, read and approved the final manuscript.

Generative AI and AI-assisted technology statement
The authors declare that no artificial intelligence tools were used in the writing, linguistic editing, or

enhancement of the manuscript's clarity. Likewise, no algorithmic or AI-based systems were employed to generate scientific ideas, interpret data, or formulate conclusions. The authors take full and sole responsibility for the integrity, accuracy, and originality of the scientific content presented herein.

Conflict of interest

The authors have declared no conflict of interest.

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