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PHYTOCHEMICAL SCREENING AND NEMATICIDAL ACTIVITY OF LIXIVIATE FROM PLANTAIN AND BANANA RACHIS

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ABSTRACT

Nematodes are among the most economically damaging parasites of bananas (*Musa AAA*) and plantains (*Musa AAB*). The restriction on the use of nematicides has encouraged the development of alternative strategies. This work aimed to study the effects of plantain and banana lixiviate on *Pratylenchus coffeae* and *Radopholus similis in vitro* essay. *P. coffeae* and *R. similis* were exposed to four concentrations (5%, 15%, 25% and 35%) of each lixiviate for 48 hrs. These effects were compared with a water control and Fluopyram at 10%. Then they were transferred to water for 24 hours to assess the viability of the immobile nematodes. The percentages of immobility and mortality of the nematodes were determined. A phytochemical screening of plantain and banana lixiviate to identify their composition in secondary metabolites was also performed. Lixiviate presented nematostatic activity at 15%. Also, lixiviate showed nematicide activity at 25% and 35%. The percentage of mortality was higher in *Pratylenchus coffeae* than in *Radopholus similis* (40% against 30% for plantain lixiviate and 39% against 29% for banana lixiviate). The phytochemical screening revealed the presence of secondary metabolites in proportions which vary few in the two compounds. In this study, we showed that plantain and banana lixiviate can be encouraging for developing methods for the biological control of banana parasitic nematodes.

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INTRODUCTION

The migratory endoparasitic nematodes *Pratylenchus coffeae* and *Radopholus similis* are the major parasites of bananas and plantains in Côte d'Ivoire (Gnonhouri and Adiko, 2005). Root necrosis caused by these two nematodes reduce water and mineral uptake of the root functions by killing root tissues resulting in growth reduction and ultimately affecting bananas and plantains production. To date, the use of chemical nematicides is the main method, however it has limitations: from an ecological point of view, synthetic nematicides have a negative impact on the biological activity of soils and represent a long-term source pollution for the environment (Aktar *et al.*, 2009; Tilman *et al.*, 2002). For the small producer with low incomes, nematicide treatments are often out of reach. These various constraints have made the search for more economical and environmentally friendly alternatives a priority. Thus, the use of organic matter constitutes a potential alternative to the use of nematicides in banana plantations (Oka and Yermiyahu, 2002). Several studies suggested that certain organic materials have nematotoxic and nematostatic compound which successfully reduce nematode infection and reduce plant parasitic nematode populations. Lixiviate, extracted from banana rachis, has reported with anti-microbial substances (Osorio Gutiérrez *et al.*, 2012). The effectiveness of lixiviate against several pathogenic fungi and bacteria has been demonstrated by several studies (Sikirou *et al.*, 2010; Maritza and Jimenez, 2016). Lixiviate against nematodes has been very little explored. Study objective was to evaluate *in vitro* effect of lixiviate extracted from plantain and banana rachis against potential endo-parasitic migratory plant parasitic species *R. similis* and *P. coffeae*.

MATERIALS AND METHODS

Plant material

The plant material consisted of rachis of plantain (*Musa AAB* "Corne 1") and banana (*Musa AAA* "Grande naine") were to extract lixiviate.

Nematodes

Isolates of the two nematodes species were collected from different sites. *P. coffeae* isolated from roots of plantain (*Musa AAB* "Corne 1") in Azaguié and *R. similis* isolated from roots of banana (*Musa AAA* "Grande naine") in Aboisso. These different areas are known to harbor the most virulent pathotypes of each of the two nematode species in Côte d'Ivoire (Konan, 2016).

Lixiviate preparation

The preparation of the lixiviate from the plantain and banana rachis was done anaerobic composting. Lixiviate was prepared according to the method described by Seri *et al.* (2018). The rachis were chopped as (3 to 4 cm thick and 10 to 15 cm in length). Eight kilograms of these cut rachis were mixed with 15 L of distilled water in a 20 L barrel, then the barrel was sealed and stored at 27 °C at the CNRA Nematology Laboratory. The mixture was stirred manually and vigorously every day for 2 min. Three months of storage, the resulting solution was filtered through a sieve (250 µ mesh diameter) to obtain the lixiviate.

Phytochemical screening

The characterization tests of the lixiviate from the plantain and banana rachis were carried out at the Laboratory of Organic Chemistry and Natural Substances of the UFHB. The detection of secondary metabolites called upon a set of revealers:

- Dragendorff and Nessler reagents for alkaloids,
- Reaction to cyanidin for visualization of flavonoids,
- Demonstration of the presence of foam for the saponins,
- 2% ferric chloride reagent for the detection of polyphenols,
- Lieberman and Burchard reaction for steroid research,
- Stiasny reagent for the detection of gallic tannins.

The results were classified according to the techniques described by Loe *et al.* (2018) -: none; +: low; ++: moderately rich; +++: rich.

Nematodes rearing and extraction

Nematode inoculum was obtained according to the method described by Quénéhervé *et al.* (2012) and Lescot and Chabrier (2016). *P. coffeae* populations were maintained on the susceptible plantain (*Musa AAB* "Corne 1") while *R. similis* was maintained on the susceptible banana (*Musa AAA* "Grande naine"). The culture of nematodes took place *in vivo* conditions for 8 weeks. The nematodes were extracted by the Baerman's technique modified by Chabrier (2008).

In vitro study

A suspension 5 ml containing 50 live individuals of *R. similis* and 4 ml suspension containing 50 live individuals of *P. coffeae* were placed in petri dishes. By dilution of the stock suspensions, solutions of 5%, 15%, 25% and 35% concentrations of each type of lixiviate were obtained. The maximum concentration tested was 35% because previous studies have demonstrated the phytotoxicity of lixiviate at high concentrations (Seri *et al.*, 2018). The effect of the lixiviate was compared to controls represented by distilled water and a solution of the nematicide Fluopyram at 10%. In total, the trial consisted of 20 treatments repeated five times. After 48 h of incubation at room temperature (27 °C) the mobile and immobile nematodes were counted under a binocular magnifying glass. They were transferred to 5 ml of distilled water for 24 h to assess the viability of immobile nematodes. For the viability test, the nematodes were considered dead when, at the prick of a needle, they remain immobile. The percentage of immobility (Pim) and mortality (Pm) of the nematodes was noted and corrected compared control.

Immobility rate (IRc)was calculated by using formula:

$$\text{IRc} = \frac{IR - IRW}{100 - IRWR} \times 100$$

IR : Immobility rate of treatment
IRw : Immobility rate of water

Mortality rate (MRc) computed by formula;

$$\text{MRc} = \frac{MR - MRW}{100 - MRwR} \times 100$$

MR : Mortality rate of treatment
MRw : Mortality rate of water

Statistical analysis

The results were processed using software Statistica 7.1. The percentages of mobility and mortality were transformed by the arcsin √x/100 function before statistical analysis. In the event of a significant difference between the averages at 5% level, Fisher's LSD test was used so as to get homogeneous groups.

RESULTS

Phytochemical Estimation

The phytochemical screening carried out on lixiviate from plantain and banana rachis enabled us to highlight the secondary metabolites in these two products. The composition of secondary metabolites does not vary qualitatively from one product to another (Table 1). Thus, the main common compounds present in large quantities (+++) are polyphenols and flavonoids. On the other hand, the alkaloids are present in moderate quantity (++) at the level of the two products while the terpenoids are in low quantity (+). Saponosides are low (+) in plantain lixiviate and medium (++) in banana lixiviate. Gallic tannins are abundant (+++) in plantain lixiviate and in moderate amounts (++) in banana lixiviate.

Table 1. Qualitative and quantitative comparison of secondary metabolites in the two lixiviate.

Metabolites	Lixiviate	
	Plantain	Dessert
Alkaloids	++	++
Polyphenols	+++	+++
Flavonoids	+++	+++
Saponins	+	++
Gallic tannins	+++	++
Terpenoids	+	+
Catechic tannins	-	-

-: none; +: low; ++: moderately rich; +++: rich.

Effect of lixiviate on nematodes

Except for the percentages of mortality recorded at the 15% concentration of the plantain and banana lixiviate, the percentages of immobility and mortality of the two species increase significantly for concentrations ranging from 5% to 25% of the two types of products. For a concentration of 5%, the percentages of immobility and mortality were statistically similar to the water control (Table 2).

Immersed in 15% lixiviate, the two species which showed significant immobility in plantain lixiviate (43.8% immobility for *P. coffeae* and 27.4% for *R. similis*) and in the banana lixiviate (43% immobility for *P. coffeae* and 26.8% for *R. similis*), became mobile again after viability test in water (Figure 1). The highest percentages of immobility and mortality are observed when the two species were in contact with the concentrations of 25% and 35%. For these two concentrations, the percentages of mortality obtained was not statistically different for each type of lixiviate.

For *P. coffeae* previously immersed in plantain lixiviate, the mortality percentages are 40% nematodes at 25% concentration and 41% nematodes at 35%. For the same species, the results obtained were 39% of dead nematodes at 25% and 41% of dead nematodes at 35% on contact with the banana lixiviate.

For *R. similis* previously immersed in plantain lixiviate, the mortality percentages were 30% nematodes at 25% and 31% nematodes at 35%. For the same species, the results obtained were 29% dead nematodes and 30%

dead nematodes respectively at concentrations of 25% and 35% in contact with the banana lixiviate.

Table 2. Percentage of immobility and mortality obtained with each product

Treatments	*Pratylenchus coffeae*		*Radopholus similis*	
	% Immobility	% Mortality	% Immobility	% Mortality
Water	7,8 c	7,7 c	7 d	7,6 c
Fluopyram	60,2 a	52,8 a	5,4 a	40,6 a
5% lixiviate plantain	7,6 c	8,2 c	7,2 d	8,6 c
5% lixiviate dessert	7 c	8 c	8,8 d	7,8 c
15% lixiviate plantain	43,8 b	7,4 c	27,4 c	9 c
15% lixiviate dessert	43 b	8 c	26,8 c	9,2 c
25% lixiviate plantain	51,6 ab	40 b	42,4 b	30,4 b
25% lixiviate dessert	51 ab	39,4 b	41,4 b	29,2 b
35% lixiviate plantain	52 ab	41 b	40,4 b	31 b
35% lixiviate dessert	51,4 ab	41,4 b	41 b	30 b

Means in columns followed by different letters are significantly different at $p<0.05$ according to Fisher's LSD Test.

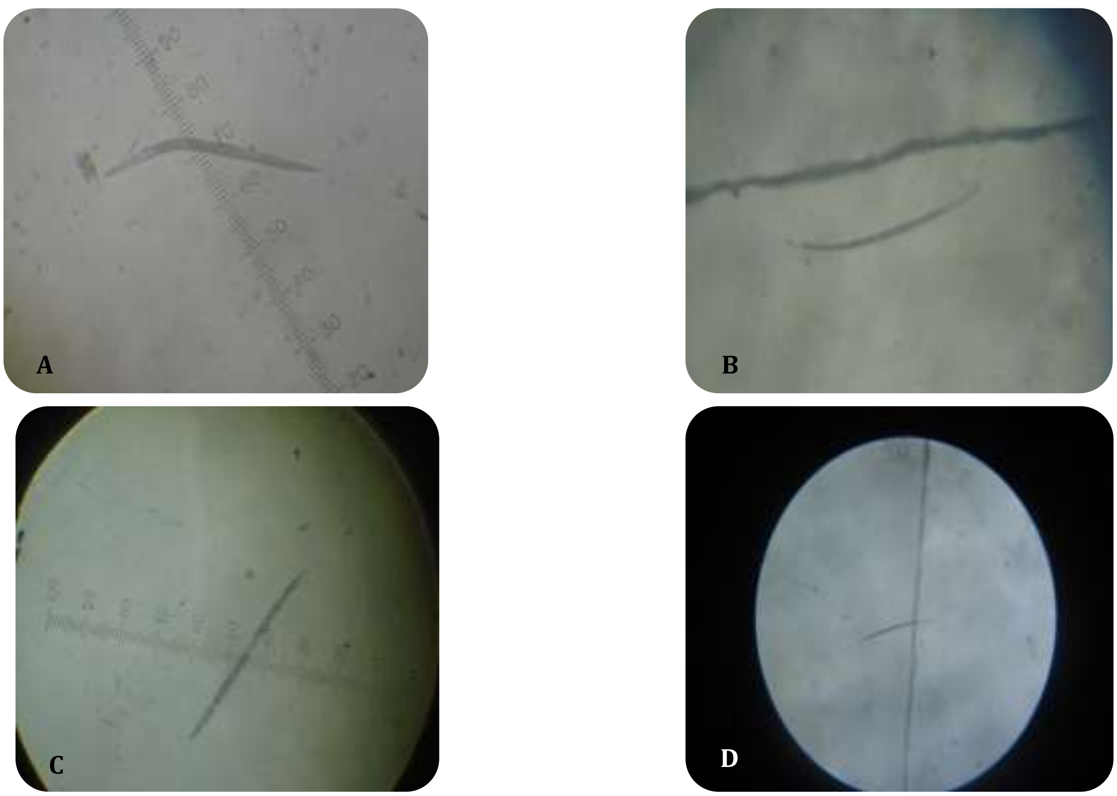

Figure 1. Microscopic image of nematodes after viability test; A) *Radopholus similis* B) *Pratylencus coffeae* which become mobile again after contact with 15% lixiviate C) *Radopholus similis* D) *Pratylencus coffeae* dead after contact with 25% lixiviate

Comparison of the effect of 25% lixiviate on nematode mortality

The percentages of mortality were not statistically different regardless of the type of lixiviate in which the nematode species have been immersed. For *P. coffeae,* 40% of nematodes dead in contact with plantain lixiviate and 39% of nematodes dead in contact with banana lixiviate were found. For the species *R. similis* 30% mortality after exposure to plantain lixiviate and 29% mortality after exposure to banana lixiviate were presented. The two lixiviate showed the same effectiveness.

Sensitivity of nematodes to lixiviate at 25%

For the same lixiviate, the mortality percentages are significantly higher for *P. coffeae* compared to those for *R. similis*. The results indicate 40% of dead nematodes for *P. coffeae* against 30% for *R. similis* when both species are exposed to plantain lixiviate. In contact with the banana lixiviate, 39% mortality was observed for *P. coffeae* against 29% for *R. similis. Pratylenchus coffeae* is more sensitive to both compounds than *Radopholus similis*.

DISCUSSION

The results of our study *in vitro* indicate that lixiviate from plantain and banana rachis may provide protection against *Radopholus similis* and *Pratylenchus coffeae*. Furthermore, secondary metabolites were found in the two compounds. Application of both types of lixiviate at the 15% concentration revealed nematostatic activity. The compounds such as alkaloid and saponins are responsible for nematostatic activity observed, as reported by other authors (Mayad *et al.*, 2019; Ahmed, 2015). These authors suggested that alkaloids and saponins are the most important groups of natural substances playing an important role in nematostatic activity. In our work, alkaloids and saponosides caused paralysis of nematodes at low concentrations. Comparison of the nematicidal effect of 25% and 35% plantain and banana lixiviate revealed similar effects due to the secondary metabolite composition of the two compounds.

The potential toxicity of phenols on nematodes has been recognized and reported in the literature (Denilson *et al.*, 2019; Aissani *et al.*, 2018). Others authors reported that tannins are also natural secondary metabolites found in several botanical families with the highest nematicidal activity (Rubabura *et al.*, 2020; d'Errico *et al.*, 2018). In our study, phenolic compounds, and tannins caused the death of nematodes at relatively high concentrations.

The highest percentage of mortality was obtained with chemical treatment and the effect of 25% lixiviate on nematode mortality is close to this result. These observations support the assertion that the plantain and banana lixiviate provided effective control of *P. coffeae* and *R. similis* under 25% *in vitro* conditions. The two nematode species exhibited a diversity of sensitivity to the same lixiviate. The difference in cuticular structure, an organ that acts as a physical barrier to external attacks, could be responsible for the variable nematicidal effect. Indeed, in *R. similis*, the body is entirely covered with a 0.55 µm thick cuticle (Chabrier, 2008), while this organ is practically non-existent in *P. coffeae* (Tuyet, 2010).

CONCLUSION

Plantain and banana lixiviate, two compounds rich in secondary metabolites, had similar effects on the nematodes studied. However, these effects varied depending on the concentration used: at 15%, a nematostatic effect was observed. While from 25% nematicidal activity has been detected. In a nematode control program, 25% is the minimum efficient rate. *Pratylenchus coffeae* is more sensitive than *Radopholus similis* when in contact with the same product.

REFERENCES

Ahmed, S. M. 2015. Nematicidal activity of extracts from *Phytolacca americana* on five plant-pathogenic nematode species of economic importance, Clemson University.

Aissani, N., R. Balti and H. Sebai. 2018. Potent nematicidal activity of phenolic derivatives on *Meloidogyne incognita*. Journal of helminthology, 92: 668-73.

Aktar, W., D. Sengupta and A. Chowdhury. 2009. Impact of pesticides use in agriculture: Their benefits and hazards. Interdisciplinary toxicology, 2: 1-12.

Chabrier, C. 2008. Survival and dissemination of the nematode *Radopholus similis* (Cobb) Thorne in rusty-brown soils with halloysites (nitisols): Effects of water status and water fluxes, Université des Antilles.

d'Errico, G., S. L. Woo, N. Lombardi, G. Manganiello and P. F. Roversi. 2018. Activity of chestnut tannins against the southern root-knot nematode *Meloidogyne incognita*. Journal of Zoology, 101: 53-59.

Denilson, O. F., V. A. Costa, W. C. Terra, V. P. Campos, P. M. Paula and S. J. Martins. 2019. Impact of phenolic compounds on *Meloidogyne incognita in vitro* and in tomato plants. Experimental parasitology, 199: 17-23.

Gnonhouri, G. P. and A. Adiko. 2005. Results of nematological surveys carried out in banana cultivation in Côte d'Ivoire Corus-Nematologie. Report of the first stage. pp. 4-7.

Konan, E. 2016. Diversité pathotype de *Pratylenchus coffeae* en Côte d'Ivoire, Université Lorougnon Guédé

Lescot, T. and C. Chabrier. 2016. Amélioration de la culture de la banane en Haiti Projet de sécurité alimentaire Secal-Ouest Rapport de mission. Haiti. pp. 39.

Loe, G. E., C. O. Ebongue, G. P. Ngaba, M. Mpai, C. K. Pouka, N. J. P., C. C. Ngoule, J. Yinyang, S. Elisée, E. S. Tankeu and S. D. Dibong. 2018. Evaluation of the antioxidant and anti-inflammatory activities of the aqueous extract of the haustoria of *Phragmanthera capitata* (Sprengel) *S. Balle* (Loranthaceae) collected on *Psidium guajava* in adult female rats of the wistar strain. Journal of Animal and Plant Sciences, 36: 5933-41.

Maritza, F. B. O. and M. I. Jimenez. 2016. Evaluation of the lixiviate activity of banana rachis (Musa AAA), banana (Musa AAB), and banana orito (Musa AA) on the agent of black sigatoka (*Mycosphaerella fijiensis* morelet) in vitro conditions. Revista Tecnologia ESPOL, 20: 200-08.

Mayad, E. H., B. Khadija, J. N. Furze, N. Heimeur, B. Senhaji, B. Chebli, E. H. Miloud, T. Mateille, L. I. Hassani and Z. Ferji. 2019. Reversible nematostatic effect of *Peganum harmala* L.(nitrariaceae) on *Meloidogyne javanica*: Effect of Syrian rue on root-knot nematodes. Journal of AgriSearch, 6: 29-33.

Oka, Y. and U. Yermiyahu. 2002. Suppressive effects of composts against the root-knot nematode *Meloidogyne javanica* on tomato. Nematology, 4: 891-98.

Osorio Gutiérrez, L., J. Castaño Zapata and L. Gutiérrez Ríos. 2012. In-vitro efficacy of plantain lixiviates on *Fusarium oxysporum* Schlecht, causal agent of pea (*Pisum sativum* Linneo) roots rot. Agronomía, 20: 17-25.

Quénéhervé, P., M. Godefroid, P. Topart, S. Marie-Luce, F. Salmon, P. Marie and C. Chabrier. 2012. Differential responses to plant-feeding nematodes among sibling cultivars of dessert bananas (*Cavendish* subgroup) and a synthetic hybrid. Crop protection, 42: 30-35.

Rubabura, J. A. K., J. J. M. Bagalwa, A. C. Lorena, C. L. Masunga and J. B. L. Mugisho. 2020. Biocidal Activities of *Chenopodium ambrosioides* and *Tagetes minuta* against *Antestiopsis orbitalis* ghesquierei carayon (Heteroptera: Pentatomoidae) *in vitro*. International Journal of Pharmacognosy and Phytochemical Research, 12: 60-65.

Seri, S. P., P. Gnonhouri, A. P. Kouame, L. Turquin, S. T. Vawa, S. Koutoua and H. A. Diallo. 2018. Bio-control of the lesion nematode *Pratylenchus coffeae* using lixiviate from banana rachis (*Musa* sp.). International Journal of Phytopathology, 7: 111-21.

Sikirou, R., Z. Afio, G. H. Gualbert, T. Felicien and O. A. K. Franccedil. 2010. Fungicide effect of banana column juice on tomato southern blight caused by *Sclerotium rolfsii*: Technical and economic efficiency. African Journal of Agricultural Research, 5: 3230-38.

Tilman, D., K. G. Cassman, P. A. Matson, R. Naylor and S. Polasky. 2002. Agricultural sustainability and intensive production practices. Nature, 418: 671-77.

Tuyet, N. T. 2010. A comparative polyphasic study of 10 *Pratylenchus coffeae* populations from Vietnam, Katholieke Universiteit.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

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