Short Research Article

THE EFFICIENCY OF SOME NATURAL ALTERNATIVES AGAINST PRATYLENCHUS COFFEAE, PEST OF PLANTAIN IN CÔTE D'IVOIRE

ABSTRACT

Aims: Analyze in vitro test the efficiency of aqueous extracts nematicidal activities of four selected medicinal plants (*Tithonia diversifolia, Vernonia colorata, Piper sarmentosum,* and *Lantana camara*) against *Pratylenchus coffeae.* Analyse in greenhouse assay the efficiency of aqueous leaf extract of *T. diversifolia* and *V. colorata* against *P. coffeae.*
Study design: The study took place in vitro and greenhouse assay.
Place and Duration of Study: Department of Nematology, plantain banana, and pineapple program, National Research Agronomic Center of Côte d'Ivoire, between January to July 2022.
Methodology: In vitro assay, the efficiency of aqueous leaf extract was tested against *P. coffeae* after 30 min, 1h, 1h30, 2h, 2h30, 3h, 3h30, and 4h of exposure. Two parameters were measured nematostatic paralysis and mortality rates. Each treatment has been replicated four times. Bananas variety Corne 1 has been used in greenhouse assay. After the inoculation of 100 nematodes of *P. coffeae*, reproduction factor, and agronomics parameters have been studied.
Results: All plants caused significant nematostatic and mortality effects ($P<0.05$). *V. colorata* extract was the most effective with a 90% of nematostatic rate and 87% mortality. This was followed by: *P. sarmentosum* (93%; 83%), *T. diversifolia* (95%; 80%), and *L. camara* (86%; 73%) in nematostatic and mortality effects. Aqueous leaf extract of *T. diversifolia* and *V. colorata* has affected some agronomic parameters compared to the blank. No significant difference in nematode populations has been detected for the two extracts.
Conclusion: Leaves of *V. colorata* and *T. diversifolia* plants could be used for the management of *P. coffeae*.

Keywords: Pratylenchus coffeae*, leaves extracts, Lantana camara, Tithonia diversifolia, Piper sarmentosum, Vernonia colorata.*

1. INTRODUCTION

Bananas (*Musa* spp.) are one of the most consumed and cheapest fruits worldwide: they are the most traded fruit and the fifth most traded agricultural product. The global export value of the banana trade was estimated to be US $8 billion in 2016, with a retail value between $20 and 25 billion (https://www.bananalink.org.uk/all-about-bananas/). Côte d'Ivoire is one of Africa's biggest banana-producing and exporting countries. This herbaceous flowering plant is susceptible to certain plant diseases, pests, and nematodes which significantly reduced production. *Pratylenchus coffeae* is one of the major root nematodes of banana yield. It is plant-parasitic nematode species affecting the quantity and quality of crop production in many annual and perennial crops, bananas included (Vaganan *et al.,* 2014; Zhu *et al.,* 2014; Lau *et al.,* 2018; Wang *et al.,* 2020). Infected plants show typical symptoms including brown lesions on the root, stunting, and nutrient deficiency, particularly nitrogen deficiency (Faheem *et al.,* 2010). Therefore, the control of nematodes is very important to enhance plant productivity. Indiscriminate use of chemicals nematicides to control nematodes causes great injuries to human beings, animals, vegetation, and to the environment as a whole due to their non-target effect, and hazardous nature besides they are expensive. The production of healthy bananas and plantains is one of the main concerns for many plantain and banana holders. So with the increasing awareness of the possible deleterious effects of chemicals, biological controls of plant pathogens have received considerable attention (Kerry, 1990 ; Duong et *al.,* 2021). Extract from plants is used to control nematodes because of environmental considerations and costs of nematicides that other methods of control may be investigated, an alternative method is the use of antagonistic plants in rotation with or interplanted with crop plants. Some plant extracts and their constituent were experimentally used for such an aim (Jeyaprakash *et al.,* 2011; Azhagumurugan *et al*., 2014). The current study was designed to evaluate the potential beneficial effects of some plant leaf extracts such as *Lantana camara, Tithonia diversifolia*, *Vernonia colorata* and *Piper sarmentosum*, through their toxic effects on nematodes in vivo and in greenhouse assay.

2. MATERIAL AND METHODS

2.1 Material

2.1.1 Plant material

As shown in (Table 1), plant materials of *Lantana camara, Tithonia diversifolia, Piper sarmentosum*, and *Vernonia colorata* were collected from mature plants grown in the Bimbresso area.

Table 1: Information about the five plant species used in the present study.

Vernacular name	*Scientific name*	*Family*	*Plant part used*	*Reference of previous use*
kro-déni (Dioula)	*Lantana camara*	Verbenaceae	Leaves	Hassanain A. E. H. *et al.,* 2019
Abowi (Baoulé) Brahia (Dioula)	*Vernonia colorata*	Asteraceae	Leaves	Haidara M. *et al.*, 2016
Poivrier sarmenteux,	*Piper sarmentosum*	Piperaceae	Leaves	Salehi B. *et al.,* 2019

Bétel marron (Français)				
Tournesol américain (Français)	*Tithonia diversifolia*	Asteraceae	Leaves	Gitahi S. M. *et al.*, 2018

2.1.1 Inoculum preparation

Isolates of *P. coffeae* used were extracted from roots taken from plantain (variety Big Ebanga). Give reference here. The root samples were taken from a banana plantation located at Anguededou.

2.2 Methods

2.2.1 in vitro assay

2.2.1.1 Preparation of plant extracts

Fresh green leaves were plucked from their branches. 100 g of leaves were mixed with 1 L of purified water using a blender. The solution was filtered through muslin cloths and then through Whatman No. 1 filter paper

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2.2.1.2 Nematode extraction, identification, counting, determination of immobility and mortality

Nematodes from roots were extracted by the modified Baermann technique and 50g of roots was described by Hooper *et al*. Macerated roots were incubated for 72h. Microscopy was used for the morphological identification of structures on P. coffeae. Nematode extracts were counted using a 1 ml aliquot on a counting slide under a Leica 2500 (Leica Microsystems CMSGmbH, Wetzler, Germany) compound microscopeat×20 magnification. *Pratylenchus coffeae* were identified and isolated. For each sample, one hundred nematodes were isolated and exposed to 10 ml of leaf extract for 30 minutes, 1 hour, 1 hour and 30 minutes, 2 hours, 2 hours and 30 minutes, 3 hours, 3 hours and 30 minutes, and 4 hours. Each treatment has been replicated write repeated four times. Control samples with 100 nematodes were exposed to distilled water at the same time of exposure.

Corrected mortaltity and immobility rates of plant extract were calculated using:

Pim= $number\ of\ immobiles\ nematodes * 100 / number\ of\ nematodes\ inoculated$

Pm= $number\ of\ dead\ nematodes * 100 / number\ of\ nematodes\ inoculated$

Pimc= $\frac{(Pim - Pimt) * 100}{(100 - Pimt)}$ Pmc= $\frac{(Pm - Pmt) * 100}{(100 - Pmt)}$

Pimc: Corrected immobility rate Pmc: Corrected mortality rate

Pim: immobility rate of treatment. Pm: mortality rate

Pimt: immobility rate of control sample Pmt mortality rate of the control sample

2.2.2 Greenhouse assay

2.2.2.1 Preparation of plant extracts and inoculation

Fresh green leaves were plucked from their branches. 1 kg of leaves was cut up and soaked into 25 L at atmospheric temperature for 10 days. For vivo assay, *Tithonia diversifolia* and *Vernonia colorata* have been used.
Bananas variety Corne 1 is it susceptible to nematodes? Mention here. has been used for vivo assay. The plants were put individually in a polyethylene bag of 20 cm-diam and of 25 cm in depth, containing sterilized soil by steam. Soil has been collected in Bimbresso, belonging to the Ferralsols class is deep and tertiary sandy (Essehi *et al*., 2021). Three treatments have been studied in this experience:experiment

- T0: experimental Control with mineral fertilizer, no-inoculated;inoculation.
- T1: add of aqueous leaf extract of *T. diversifolia* concentrated to 50%;
- T2: add of aqueous leaf extract of *V. colorata* concentrated to 50%.

A quantity of 10 ml of extracts was applied to plant once by week.
The nematodes were inoculated into 5 different holes 2-3cm deep, uniformly distributed in the soil near the stipe stem using a pipette. Each plant received 100 nematodes.

2.2.2.2 Measured parameters
Forty-five days after inoculation, the evolution of nematodes population has been done by modified methods of Baerman. Reproduction Factor (RF = initial nematode density/final nematode density) values.

Agronomic parameters such as have been evaluated. The parameters evaluated for this experiment were: the mass of the root system, collar circumference and plant height were evaluated Thirty-six bananas have been tested by each treatment, and four plants by treatment repeated nine times in a completely randomized block.

2.2.3 Statistical analysis

Experimental data were statistically analysed using Analysis of variance (ANOVA) was used to determine differences between treatments with respect. A comparison of means was performed by the Duncan multiple range test with a significance level of P< 0.05 using Jamovi (2022).

3. RESULTS AND DISCUSSION

3.1 Results

3.3.1 Interactive effect of plant extract and exposure time in vitro assay

3.3.1.1 Corrected immobility rate

P. coffeae exhibited the nematostatic reaction in vitro for leaf extracts (Fig 1). Time of exposure and species used for extract has been an effect on the rate. Significant differences were found between the different leaf extract (table 2). The nematostatic effect of extracts was observed with all species. In Figure 1, the highest value of immobility rate (nematostatic effect) was recorded with the extract of *Tithonia diversifoli*a followed by *Piper sarmentosum* and *Vernonia colorata*. The lowest value was recorded with the extract of Lantana camara. Immobility rates were progressively increased with exposure time from 30 minutes to 4 hours. Illustrate the interactive effect of plant extract and exposure time on nematode (P< .01).

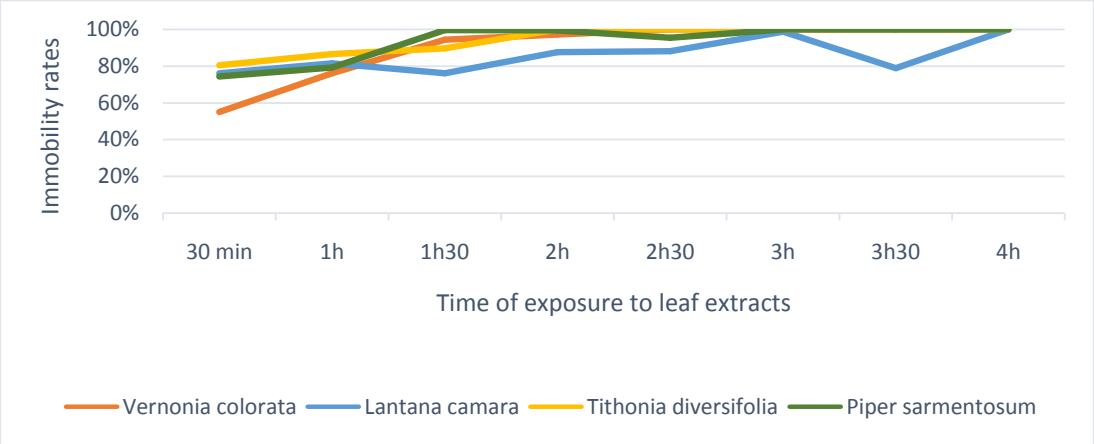

Fig. 1: Mean performance (± SE) of plant extract, immobility rates on nematode

Table 2. Test of Significant differences between the different leaf extracts.

	sum of squares	Ddl I think it is degrees of freedam	Mean squares	F	P
Aqueous treatment	2718	3	906.1	20.23	< .001
exposure time	10622	7	1517.4	33.87	< .001
Aqueous treatment ⁕ exposure time	3735	21	177.8	3.97	< .001
Résidus What is this?	5600	125	44.8		

3.3.1.1 Corrected mortality rate

In Fig. 2 ,the nematode was recorded with the highest value with the extract *Vernonia colorata*, followed by *Piper sarmentosum*, and *Tithonia diversifolia*. The lowest value was recorded with the extract of *Lantana camara*. The nematode mortality and immobility rates (%) were progressively increased with exposure time from 30 minutes to 4h. Illustrate by the interactive effect of plant extract and exposure time on nematode (P< .01).

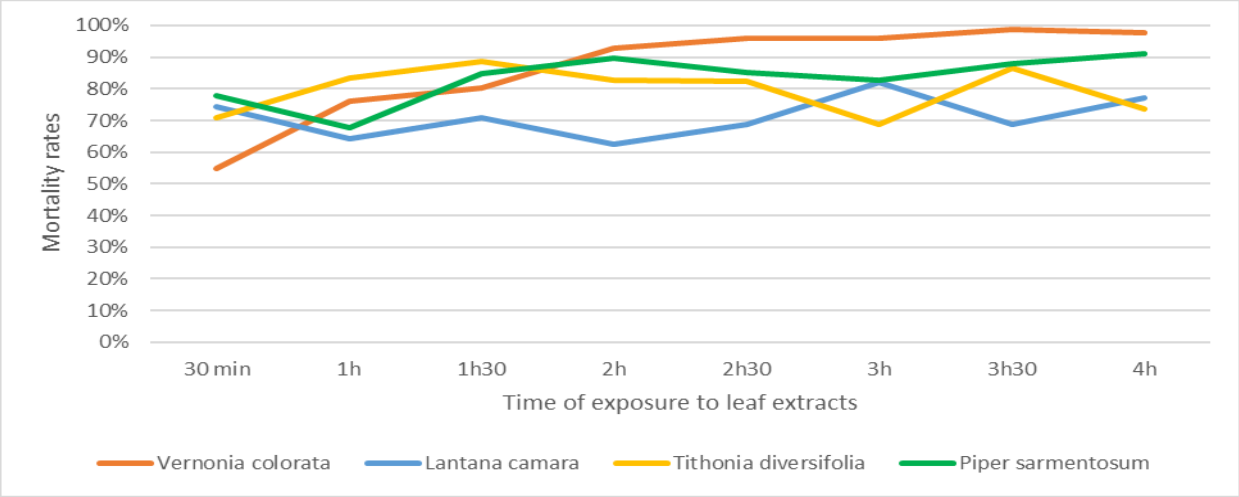

Fig. 2: Mean performance (± SE) of plant extract mortality rates.

Table 3: Results of ANOVA test for mortality rate

	sum of squares	Ddl I think it is df, degrees of freedam	Mean squares	F	P
Modèle What is this? general	16336	31	527	4.62	< .001
Aqueous treatment	5105	3	1702	15.01	< .001
exposure time	4080	7	583	5.14	< .001
Aqueous treatment ✻ exposure time	7150	21	340	3.00	< .001
Résidus	14176	125	113		

3.3.2 Interactive effect of plant extract in vivo assay

Significant variation in the growth was observed according to treatments where plants that received extracts have the best result than the others. *T. diversifolia* and *V. colorata* extracts resulted in significantly higher weights of vegetative parts and roots, height, and circumference. (table 3). Based on the data, it appears that the null hypothesis cannot be dismissed as the p-value surpasses .05. No-significant effect on nematode populations has been detected between the two aqueous extracts (Table 3).

Table 4: Results of ANOVA test in a greenhouse assay

Treatment	weight of the vegetative part,gm.	Weight of roots,gm.	Number of roots	Reproduction factor	Height,cm.	Circumference,cm.
T0	51.3a	12.8a	11.3a	-	4.76a	1.66a
T1	87.3b	27.3b	12.8a	9.07a	8.48b	2.11b
T2	76.5b	24.0b	12.7a	8.59a	8.67b	2.30b

Data are means ±S.E. different lower or upper letters in a column indicate significant differences between the treatments at P ≤ 0.05.

3.2 Discussion

The current study is part bioprospecting study on native plant extracts against *P. coffeae*. Several natural plants growing all over the world produce chemicals that immobilize and kill worms. These chemicals are most likely secondary metabolic products that, while not involved in primary metabolism, contribute to plant defense. The majority of case studies have focused on *Meloidogyne* sp. All of the plant extracts evaluated in this study have an antagonistic action in vitro against *P. coffeae* and greater nematicidal activity. According to the findings of the current investigation, leaf extract can paralyze and kill worms. The efficiency increases increasing exposure time. According to Nidhi and Trivedi (2002), exposure time plays an important role in nematode mortality. As a result, they probably contain natural nematotoxic chemicals capable of killing the worm. Secondary metabolites such as alkaloids, phenolic chemicals, glycosidic saponins, flavonoids, and tannins are found in them (Bokesch *et al*., 2011; Omokhua *et al.,* 2018). These chemicals have been linked to plant defense systems as well as biocidal activity, including nematicidal activity. As a result, the current study sought to investigate the fatal effect of an aqueous leaf extract. The discrepancies in the efficiency of the various plant extracts examined could be attributed to variances in the chemical compositions and quantities of poisonous components present in the plant material. *L. camara* nematostatic and nematicidal activity against root-knot nematodes, *Meloidogyne* spp., have also been studied in vitro and soil (in vivo) tests (Faheem *et al.,* 2010; Qamar *et al.,* 2005). Camaric acid, lantanilic acid, and oleanolic acids

are pentacyclic triterpenoids found in *L. camara*. According to Faheem *et al.* (2010), *L. camara* aqueous leaf extract did not operate as a potent nematicide on juveniles, who were only paralyzed rather than killed by the plant leaf extract.

Essential oil, alkaloids, flavonoids, lignans, and steroids have all been identified as phytochemical elements of *P. sarmentosum* (Sun *et al.,* 2020). In vitro, nematicidal effects of alkaloids piperine, this molecule, have been observed at concentrations up to 500 g/ml, but no indication of the concentration of these metabolites in planta was given (Kang *et al*., 2018). According to Osman and Ewees (2008), nematifuge plants are especially beneficial in worm management tactics, and fresh leaves of *Vernonia* spp. induce mortality of more than 86% after 6 days of nematode exposure. The phythonematoxic characteristics and nematicidal potential of *T. diversifolia* extract and residue on *Meloidogyne* sp. Chitwood infecting yam was investigated in laboratory research and a screen house experiment. *Tithonia* ethanol extract components were discovered to contain alkaloids and saponins. In addition, *Tithonia* aqueous extract significantly (P 0.05) reduces *M. incognita* egg hatch by 98% from 2 days after incubation (DAI), with 100% inhibition at 9 DAI (Odeyemi et Adewale, 2011).

In the greenhouse assay, the contribution of aqueous leaf extract to growth is likely to be related to the addition of nitrogen where *T. diversifolia* (Endris, 2019) is known to produce nitrogen-rich green biomass. The nutrients in *Tithonia* biomass are rapidly released in plant-available forms during decomposition (Gachengo *et al.,* 1999). *Tithonia* residues have also been shown to reduce P sorption sites, P metal complexes, and Al toxicity, and ameliorate soil aggregation (Cong, 2000). The study did Aboyeji (2019) showed that the incorporation of *Vernonia* sp. of green manures increased soil availability of OM, N, P, K, and Mg.

3. CONCLUSION

The recent approach to nematode control is a direct method toward the possibility of reducing populations of plant-parasitic nematodes in soil by using natural substances extracted from some plants. Such methods don't lead to the disturbance of the biological balance of nature. Thus, this finding is important in the identification and development of alternative strategies for controlling bananas and plantains' nematodes. Other studies have to be done to improve the application of aqueous leaf extract in greenhouse assay in bananas and plantain.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

"All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

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