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Control of Banana-Parasitic Nematode Populations Using *Azadirachta indica* and *Ocimum gratissimum* Extract-based Bionematicides in Côte d'Ivoire

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ABSTRACT

Plant-parasitic nematodes are a major constraint for banana cultivation worldwide. The control methods against this pest are mainly based on the use of chemical nematicides whose effects are harmful to the environment and human health. This study aims at assessing the nematicidal effect of plant extracts in order to control the populations of the main plant-parasitic nematodes found in banana tree plantations in Côte d'Ivoire. The effect of aqueous extracts of *Azadirachta indica* seed powder and the aqueous emulsion of the essential oil of *Ocimum gratissimum* were tested in on-farm by root application in a completely randomized Fischer block design from 2015 to 2016 over two cycles of industrial banana cultivation in the Abidjan area. The populations of *Radopholus similis* were reduced by 87.75% using the aqueous emulsion of the essential oil of *Ocimum gratissimum*. The aqueous extract and the *Azadirachta indica* seed powder caused 84.3% and 81.24% mortality respectively for the same nematode species. As for *Pratylenchus coffeae*, it had a reduction of 86.5% with the aqueous emulsion of *Ocimum gratissimum* and 86% with the aqueous extract of the *Azadirachta indica* powder. As for the *Azadirachta indica* seed powder and the chemical nematicide (Cadusafos 10%), the reduction rates were 80.06% and 79.8%, respectively. The results obtained suggest that the aqueous extract of the powder and the *A. indica* seed powder as well as the essential oil of *O. gratissimum* have the capacity to control plant-parasitic nematode populations.

Introduction

Banana is a source of food and provides substantial income to people. In Côte d'Ivoire, the dessert banana sector contributes about 3% to national GDP and 8 to 10% to agricultural GDP (FIRCA, 2016). However, this enterprise practiced for many years on the same site, without rotation, is subject to constraints related to agro-industrial intensive monoculture and requires large quantities of inputs (Lescot, 2006). This type of exploitation, less environmentally friendly, contributes to soil degradation and biological imbalances making the crop sensitive to diseases and pests. Also, telluric pathogens such as plant parasitic nematodes, which are specific to the crop, tend to increase in the absence of a break in their life cycle, leading to increased risks of resistance to pesticides (Gowen et al., 2005)

Plant-parasitic nematodes are microscopic worms measuring less than 1 mm in length (Quénéhervé, 2008). Those who attack banana trees are strict parasites that mostly feed on plant roots, causing symptoms such as necrosis, lesions and scab on the roots (Quénéhervé et al., 2009; Chabrier et al., 2005). The latter become unable to ensure water, mineral nutrition and plant anchorage. These effects result in the fall of banana trees, which leads to a decline in yield (Sarah et al., 1996). The most devastating plant nematodes or plant-parasitic nematodes are migratory endoparasites (*Radopholus similis* and *Pratylenchus coffeae*), semi-endoparasites (*Helicotylenchus multicinctus*) as well as sedentary endoparasites, *Meloidogyne* (Gowen and Quénéhervé, 1990; Araya, 1995).

Until now, chemical control has been the most effective and the most popularized among the various methods (Sarah et al., 1996, Chabrier et al., 2005 and 2008). However, despite its effectiveness, the misuse of synthetic chemicals has severe consequences for humans and their environment (Aubertot et al., 2005, Kosma et al., 2013).

Also, many research works have helped explore alternatives for controlling plant pests using plants

that have antiparasitic properties such as neem (*Azadirachta indica* A. Juss.) and African basil (*Ocimum gratissimum* Lam.) (Ioannina et al., 2004; Jothi et al., 2004; Orafidiya, 2006; Oussou et al., 2004; Kassi et al., 2014). Several plants having nematicidal or nematofuge properties have been identified. However, very little works using plant extracts in the protection of banana dessert against plant-parasitic nematodes have been carried out.

The main objective of this study is to assess the effect of *A. indica* seed and *O. gratissimum* essential oil-based formulations on plant-parasitic nematode populations in dessert banana fields in Côte d'Ivoire.

Materials and methods

Dessert banana tree

In this study, dessert banana trees of the Cavendish AAA (Grande Naine) subgroup were used for assessing the effect of neem seed and *O. gratissimum* essential oil-based formulations on plant-parasitic nematodes on-farm. The Grande naine cultivar, variety JOBO 902, was used for planting in 2015. The study was carried out under conditions of natural infestation by plant nematodes in three agro-industrial farms located in the regions of Grands-ponts and Sud-Comoé. The experiment was repeated over two crop cycles.

Production of the different *Azadirachta indica* seed-based formulations

The ripe fruits of *Azadirachta indica* (neem) were harvested in the locality of Korhogo in northern Côte d'Ivoire. The collected fruits were placed in the open air under a shade for two weeks (14 days) and then manually decorticated so as to extract the seeds. The seeds thus obtained were ground to a fine powder using an electric grinder. The neem seed powder (PGN) obtained was stored in opaque glass flasks.

The principle for producing aqueous extracts of powder was that of Kosma et al. (2013) modified.

100 g of *A. indica* seed powder was dissolved in one liter of water. After 48 hours of maceration, the solution obtained was the aqueous extract of neem (EAN) which was manually applied around the banana tree (100 g/l) using a dosette at a rate of 500 ml/ banana tree plant. The neem seed powder (PGN) was applied around the banana trees at a rate of 100 g/plant in a 20 to 30 cm radius.

Production of the *Ocimum gratissimum* essential oil-based formulation

The essential oil of *O. gratissimum* was obtained from fresh leaves, by saturated steam distillation carried out with a Clevenger-type apparatus for three hours (Kassi et al., 2014). The aqueous emulsion was obtained by dissolving the essential oil at a dose of 3 ppm to which was added 1 % Tween 20. This emulsion was applied at a dose of 400 ml per banana tree plant.

Method of application and assessment of the nematoregulatory effect of formulated products

Four hundred and fifty (450) plants of dessert banana tree of the JOBO 902 variety were used to assess the nematicidal effect of the neem seed and *O. gratissimum* essential oil-based formulations on-farm. Before the trials were set up, a preliminary nematological analysis made it possible to determine the level of initial natural infestation at the experimental site by *R. similis*, *P. coffeae*, *H. multicinctus* and *Meloidogyne* spp. nematodes. A Fischer block experimental design with five modalities repeated three times was adopted. The following treatments were applied to each group of plants: aqueous extract of neem (EAN); neem seed powder (PGN); aqueous emulsion of *O. gratissimum* (EAO) essential oil, a reference product represented by the chemical treatment with Cadusafos 10% (TC) and a control without any product application were also assessed.

The PGN and TC treatments were spread like rings around each plant on the succeeding sprout side within a radius of about 30 cm and at doses of 100 and 30 g/plant respectively. The EAN and EAO

formulations were applied at doses of 0.5 and 0.4 l/plant respectively, using a knapsack sprayer equipped with an extract-blast nozzle. The solutions were spread all around each plant towards the succeeding sprout forming a circle of about 30 cm diameter. Nematode populations were counted monthly in the roots after application of the different treatments. The monthly reduction rate (TRM) of nematode populations which help assess the effect of each formulation was calculated according to the following formula:

$$TRM = \frac{T_0 - T_n}{T_0} \times 100$$

T_0 = nematode population during the initial natural infestation (100g of Roots).

T_n = nematode population of recorded during the month of experiment (100g of Roots).

Data analysis

Statistical analyses were performed using Statistica version 7.1 software. The numbers of individuals of each nematode species observed in banana roots were subjected to an analysis of variance (ANOVA). When the effects of the factors tested (bionematicides) were significant, the average numbers of individuals of each nematode species in the roots were compared according to the Newman and Keuls test at 5% threshold.

Results

The results showed that the extracts of *Ocimum gratissimum* and *Azadirachta indica* had an impact on the population dynamics of *Radopholus similis*, *Pratylenchus coffeae*, *Helicotylenchus multicinctus* and *Meloidogyne* spp. In fact, their activity resulted in a decline in the nematode population whose reduction rate depended on the product applied and the nematode assessed. During this first month of assessment (M1), the application of treatments of the aqueous emulsion of *Ocimum gratissimum* essential oil (EAO) at the dose of 3 ppm caused

significant differences ($p < 0.002$). Indeed, a 38.13 % reduction in the population of *Radopholus similis* was recorded (Fig. 1). In the same period, the aqueous extract of the neem powder (EAN) at 100g/l and the neem seed powder (PGN) at 100 g/plant caused a reduction by 87.84 % and 86.25 % of the *Radopholus similis* population respectively while the reduction rate recorded with Cadusafos 10 % (chemical nematicide) was only 9.09 % (Table 1). In the second month of assessment (M2),

the nematoregulatory effect of both plant extracts faded and a significant repopulation of *Radopholus similis* was observed on the plots treated with the aqueous emulsion of *O. gratissimum* essential oil (EAO) and *A. indica* seed powder, which remained, however, significantly lower than the population observed on the control plots. A significant reduction effect ($p < 0.002$) was observed with the populations on the plots treated with the chemical nematicide (TC) which was 43.76%.

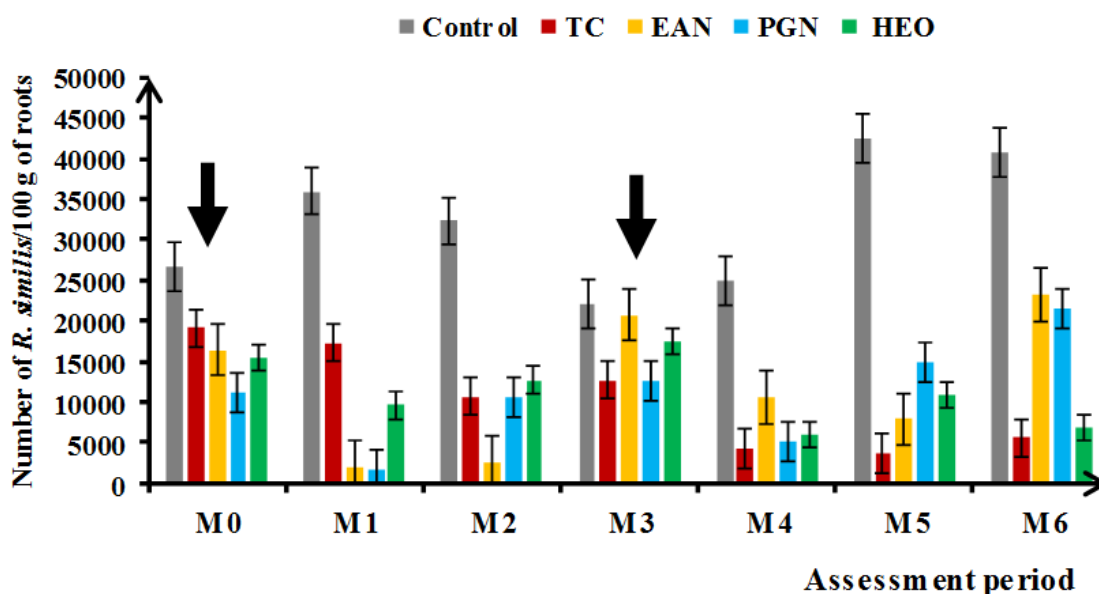


Fig. 1: Effect of treatments with *O. gratissimum*, *A. indica* (neem) plant extracts and Cadusafos (TC) on the evolution of *Radopholus similis* population in dessert banana industrial plantation.

Table 1. Monthly reduction rate of *Radopholus similis* population.

Treatments	Assessment period						
	M1	M2	M3	M4	M5	M6	
Control	-34.99	-21.04	17.00	6.33	-59.49	-52.74	
TC	9.09	43.76	33.57	77.85	80.77	70.63	
EAN	87.84	84.59	-32.03	35.81	51.57	-41.49	
PGN	86.25	5.67	-12.75	54.78	-34.46	-93.03	
HEO	38.13	18.04	-12.86	61.09	30.05	56.44	

M= month; HEO= Essential oil of *Ocimum gratissimum*; TC= Chemical treatment; EAN = Aqueous extract of Neem seeds; PGN= Neem Seed Powder.

A loss of effectiveness of both plant extracts called for a second treatment at month three (M3). This led to a significant decrease ($p < 0.000$) of *R. similis* stands in the treated plots in month 4. This second treatment resulted in a fall by 30.05% and 51.57% in the *R. similis* population at month five (M5)

respectively on the plots treated with the aqueous emulsion of *O. gratissimum* (EAO) and the aqueous extract of neem powder (EAN) compared to the fourth month of assessment. The *Radopholus similis* population recorded by chemical treatment (TC) with Cadusafos was 80.77%. This

nematoregulatory property of the *O. gratissimum* aqueous emulsion was confirmed at the sixth month of assessment (Table 1).

The analysis of variance performed on *Pratylenchus coffeae* populations, showed a significant effect ($p < 0.026$) during month one (M1) to month two (M2) of the assessment, compared to the values obtained during the assessment of the initial level of infestation (T0) of the experimental sites (Fig. 2). On the plots treated with plant extracts, a reduction by 59.10 % (EAO) and 27.17 % (EAN) was observed respectively with the aqueous emulsion of *O. gratissimum* essential oil

and the aqueous extract of *A. indica* powder. As for *A. indica* seed powder and Cadusafos 10% the reduction rates were 28.45% (PGN) and 32.24% (TC) respectively.

A reduction in the stand of plant-parasitic nematodes in the plots was observed during months one and two (Fig. 2). The second treatment applied at month three of the assessment at doses of 3 ppm of *O. gratissimum* essential oil and 100 g/l of the aqueous extract of *A. indica* and 100 g/l plant for *A. indica* seed powder caused a significant decline ($p < 0.002$) in the *P. coffeae* population on the treated plots.

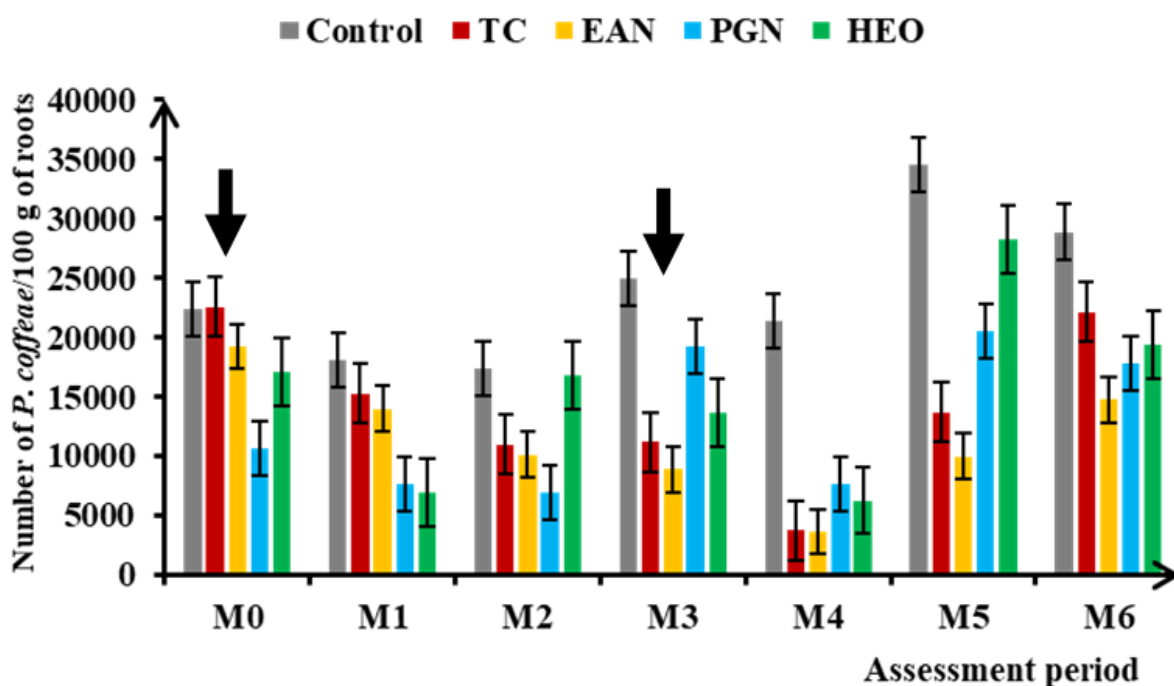


Fig. 2: Effect of treatments with *O. gratissimum*, *A. indica* (neem) plant extracts and Cadusafos (TC) on the evolution of *Pratylenchus coffeae* population in dessert banana industrial plantation.

Table 2. Monthly reduction rate of *Pratylenchus coffeae* population.

Treatments	Assessment period					
	M1	M2	M3	M4	M5	M6
Control	19.05	31.29	-11.61	44.94	-54.16	-28.87
TC	32.24	51.25	50.36	83.45	39.43	1.91
EAN	27.17	47.28	53.87	81.04	47.98	23.35
PGN	28.45	34.73	-81.59	27.62	-92.68	-68.20
HEO	59.10	1.43	2.13	63.25	-65.19	-12.99

M= month; HEO= Essential oil of *Ocimum gratissimum*; TC= Chemical treatment; EAN = Aqueous extract of Neem seeds; PGN= Neem Seed Powder.

A decrease by 59.10% and 28.45% in the *P. coffeae* population was caused respectively by treatment with the aqueous emulsion of *O. gratissimum* essential oil and with the powder of *A. indica* against a fall by 27.17% and 32.24% for the aqueous extract of *A. indica* and the treatment with chemical nematicide as from the first month after application of the products (Table 2). This effect of controls by plant extracts began to fade during the fifth month (M5) after the second treatment. A loss of efficiency was observed by an increase in plant-parasitic nematode populations. As for *Helicotylenchus multicinctus*, the root application of the different treatments (aqueous

emulsion with the essential oil of *O. gratissimum* at 3 ppm, aqueous extract of *A. indica* powder at 100 g/l and *A. indica* seed powder) had a significant control effect ($p < 0.005$) in the first five months of assessment (Fig. 3). However, the root application of the aqueous emulsion of the essential oil of *O. gratissimum* at the dose of 3 ml/l and 100 g/l of aqueous extract of *A. indica* powder showed an effect of significant control by reducing *H. multicinctus* populations by the fifth month of assessment compared to the control (no treatment). At month six of the assessment (M6), a very strong repopulation of the control plots was observed (Table 3).

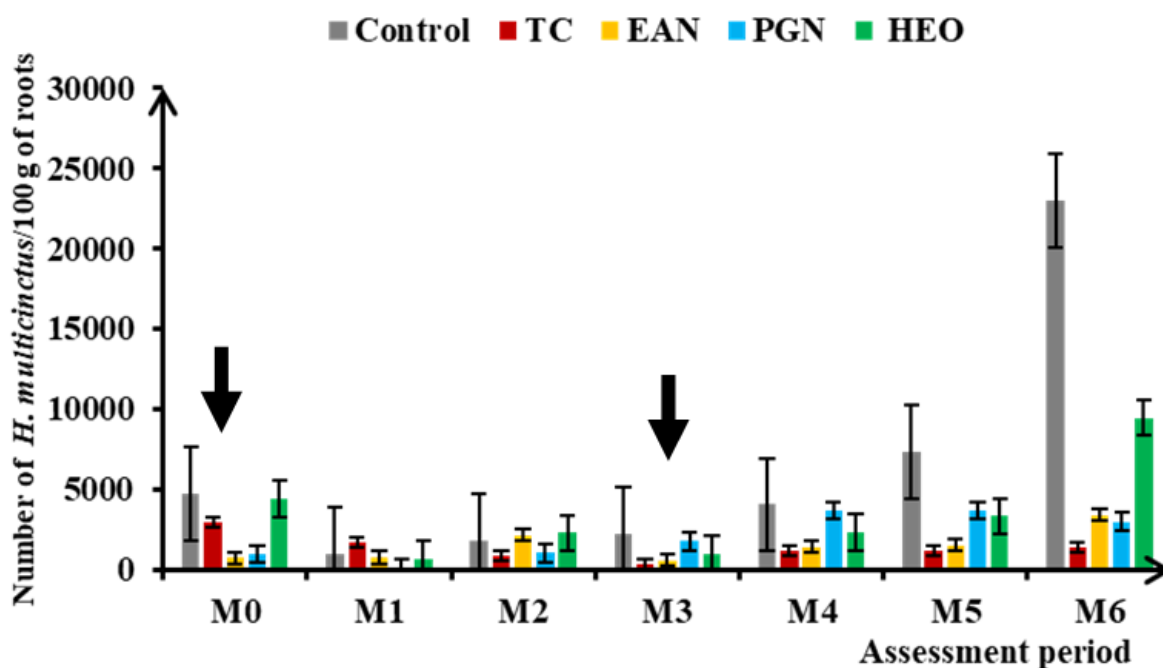


Fig. 3: Effect of treatments with *O. gratissimum*, *A. indica* (neem) plant extracts and Cadusafos (TC) on the evolution of *H. multicinctus* population in dessert banana industrial plantation.

Table 3. Monthly reduction rate of *Helicotylenchus multicinctus* population.

Treatments	Assessment period					
	M1	M2	M3	M4	M5	M6
Control	63.94	51.88	53.52	14.08	-54.93	-385.92
TC	65.93	72.23	87.41	62.23	60.00	53.33
EAN	66.15	65.13	72.31	34.36	29.23	-56.92
PGN	97.58	71.82	52.12	72.73	0.00	18.18
HEO	85.05	58.33	77.77	46.46	24.24	-115.15

M= month; HEO= Essential oil of *Ocimum gratissimum*; TC= Chemical treatment; EAN = Aqueous extract of Neem seeds; PGN= Neem Seed Powder.

Regarding the population of *Meloidogyne* spp., there was a significant effect of the extracts ($p < 0.003$) on the number of *Helicotylenchus multicinctus* individuals in the plant roots. A decrease by 26.67 % and 40.74 % of the population was recorded respectively after the application of the aqueous emulsion of *O. gratissimum* and the aqueous extract of *A. indica* in the first month of assessment compared to the values obtained during the assessment of the

initial level of infestation of the experimental sites (Fig. 4). This reducing effect of populations by plant extracts was confirmed until the sixth month. *Ocimum gratissimum* even caused a 73.33 % reduction in the sixth month. However, the chemical nematicide (TC) had no effect on the *Meloidogyne* population in the first month of the trial (M1). But from the second month onwards, this population fell to 86.67 % in month six (Table 4).

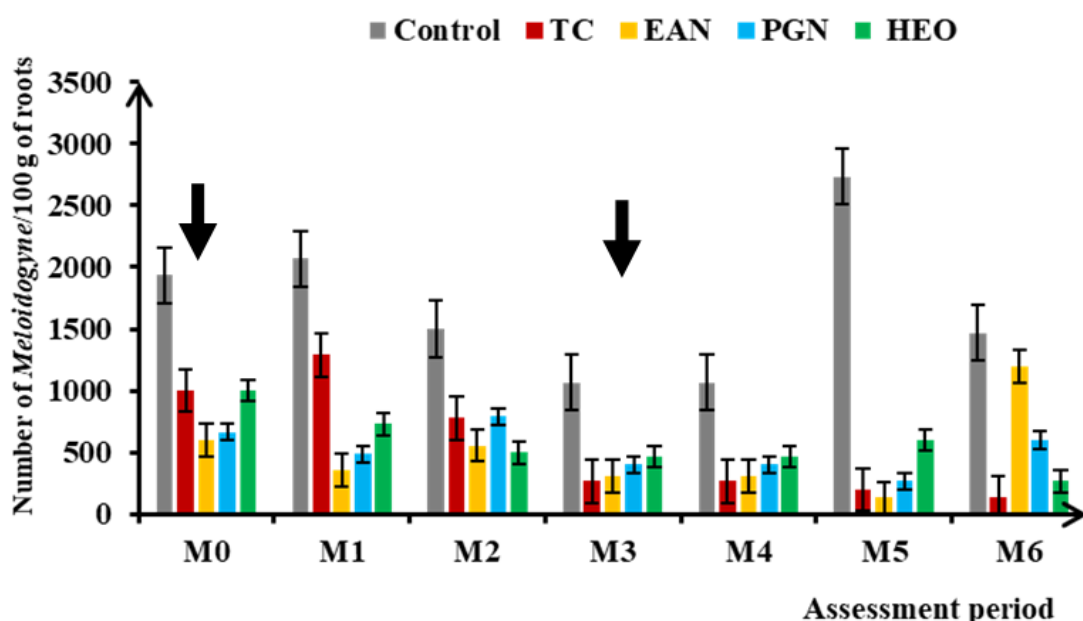


Fig. 4: Effect of treatments with *O. gratissimum*, *A. indica* (neem) plant extracts and Cadusafos (TC) on the evolution of *Meloidogyne* spp. population in dessert banana industrial plantation.

Table 4. Monthly reduction rate of *Meloidogyne* spp. population.

Treatments	Assessment period					
	M1	M2	M3	M4	M5	M6
Control	63.94	51.88	53.52	14.08	-54.93	-385.92
TC	65.93	72.23	87.41	62.23	60.00	53.33
EAN	66.15	65.13	72.31	34.36	29.23	-56.92
PGN	97.58	71.82	52.12	72.73	0.00	18.18
HEO	85.05	58.33	77.77	46.46	24.24	-115.15

M= month; HEO= Essential oil of *Ocimum gratissimum*; TC= Chemical treatment; EAN = Aqueous extract of Neem seeds; PGN= Neem Seed Powder.

Discussion

On-farm trials have shown that the aqueous extract of *Ocimum gratissimum* essential oil had a significant regulatory effect on the reduction of

nematode populations. For the genera *Radopholus similis*, *Pratylenchus coffeae*, *Helicotylenchus multicinctus* and *Meloidogyne* spp. the root application of the *Ocimum gratissimum* essential oil-based formulation caused a sharp reduction in

the populations of those nematodes. This could be explained by the fact that the active substances contained in the essential oil of *O. gratissimum* had a repulsive or toxic effect on those types of nematodes. The extracts applied were therefore nematocidal and/or nematofuge. They killed the worms by poisoning them and/or forcing them to leave the treated area. Our results corroborate those of Kassi et al. (2014), Hamilton-Kemp et al. (2000) and Uribe et al. (1985). The results of these authors showed a similar effect of the aqueous extract of the essential oil of *O. gratissimum* on a pathogenic fungus of banana tree (*Mycosphearella fijiensis*) responsible for black leaf streak disease (BLSD). They highlighted the major chemical constituents of *O. gratissimum* essential oil and showed that they are dominated by thymol, a phenolic terpene which seems to exert a direct action on the reduction in fungus growth. Phenolic compounds such as thymol, mainly found in the essential oil of *O. gratissimum* are known to be toxic and would target the envelopes of microorganisms such as the cytoplasmic membrane and the wall of the organism. The essential oil of *O. gratissimum* would also have an effect on the membrane structure and the development of juvenile nematode species. Indeed, the works of Hamilton-Kemp et al. (2000) and Archbold (2000) have shown that the constituents of this oil such as phenolic compounds, terpene alcohol and aldehydes alter the permeability of membranes, denature and precipitate the proteins.

Furthermore, the aqueous extract of *Azadirachta indica* seeds also showed a control effect on nematode populations. For the different phytonematodes, the effect of *A. indica* is significant as the level of nematode infestation was higher for the untreated control plants than for the *A. indica* treated plants. This effect of *A. indica* powder-based formulations was not significantly different from that of cadusafos 10%. Untreated control plants showed the highest level of infestation, reflecting the positive impact of *A. indica* extracts in reducing nematode populations. Actually, the application of *A. indica* has prevented those species of nematodes from

feeding on banana tree roots because they became toxic. Neem is having an antifeedant effect. These results are similar to those of de Hafeez et al. (2000), Kumar and Khanna (2006). They tested *A. indica* against *Meloidogyne incognita* on tomato roots. Akhtar and Alam (1990) also observed the reduction of the free parasitic nematode population on sweet potato. This reduction would be due to treatments with *A. indica* seed oilcake and leaves. The nematodes therefore move to other horizons for feeding, where they stay on the treated plot, they stop feeding and they die. The works of Johnson et al. (2016) and Berger (2011) demonstrated antifeedant effects of azadirachtin contained in *A. indica*. These molecules induce a decrease in food intake or an inhibition of food intake reflex and the organisms die subsequently. Insects and nematodes turn away from treated crops and foods. When the pest ingests plants treated with *A. indica*, this is followed by a digestive imbalance paralyzing its digestive tract, causing it to stop feeding and die. Berger (2011) was thus able to highlight the four active components of *A. indica* that are azadirachtin, salanine, nimbidin and melandriol. Nimbidin is an effective repellent that can be used for soil crops. The latter seems to be the most toxic for nematodes.

According to Srivastava and Raizada (2007), the compounds identified as biologically active in *A. indica* extracts are azadirachtin, nimbidin, salanine, and meliantriol. Azadirachtin is the main active ingredient in *A. indica* and has the property of disrupting the morphogenesis and embryonic development of nematodes. The active ingredients of *A. indica* would then cause disorders in the diet of the pest preventing its normal development and growth. The high nematocidal activity observed in treatments with *A. indica* seed powder and *A. indica* seed aqueous extracts is potentially due to the presence of these secondary metabolites which have nematocidal and/or nematostatic properties. The results obtained are similar to those of (Kapil et al., 1994). The *A. indica* seed powder-based formulation would therefore limit the reproductive capacity of nematodes. These results are in accordance with those of Kosma et al. (2013) who

determined the effectiveness of *A. indica* seed formulations against *R. similis* nematodes affecting plantain tree plants. *A. indica* seed powder showed a better destructive capacity than the aqueous extract. The higher level of biological activity of *A. indica* seed powder than that of the aqueous extract might result from the presence of some secondary metabolites that could be released into the soil upon decomposition by microorganisms.

However, authors including Hoitink and Grebus (1994) as well as Zhang et al. (1998) have discussed another mode of action in plant extracts: The induction of Systemic Acquired Resistance (SAR) in the plant. Indeed, it has been shown that organic composts and their plant extracts can induce the expression of certain defense genes in the plant when the latter is exposed to a pathogen (Vallad et al., 2003). Thus, the reduction of nematode multiplication on the roots of plants treated with plant extracts could be due to a kind of resistance induced by such extracts.

The results obtained show that plant extracts could be an environmentally friendly way and a sustainable alternative method for controlling nematodes in banana cultivation.

Conclusion

The results clearly show the effectiveness of *Azadirachta indica* seed and *Ocimum gratissimum* essential oil-based formulations against the main pest nematodes of dessert banana trees in Côte d'Ivoire. The high level of biological control of *A. indica* seeds and *O. gratissimum* essential oil suggests that they could be used as a basic substrate for the formulation of nematicides. Given the environmental concerns that global agriculture is faced with, these extracts may be a sustainable, interesting and promising alternative for Ivorian banana agriculture.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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