



Effects of Two-Dried Castor Leaf Formulations on the Population Dynamics and Pathological Activities of Root-Lesion and Root-Knot Nematodes on Water Yam

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Authors' contributions

This work was carried out in collaboration among all authors. Author YYFRK designed the study, performed the statistical analysis and wrote the manuscript. Authors KDK and HAD managed the analysis of the study. All authors read and approved the final manuscript.

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ABSTRACT

The pathological activities of plant-parasitic nematodes affect the marketable value of yam tubers. This study intends to evaluate the nematicidal effects of dried castor leaf powder and dried castor leaf extract on root-lesion and root-knot nematodes under field conditions. Trials were conducted in the Autonomous District of Yamoussoukro in Côte d'Ivoire during the 2015 and 2016 growing seasons. Before yam seed planting, dried castor leaf powder, dried castor leaf extract (suburbs of Abidjan), and carbofuran (Callivoire S.A., Abidjan) were applied at the top of mounds in a randomized complete block design with three replicates. Symptom prevalence, symptom severity index, nematode numbers, and yam yield were determined depending on the treatments. The gall

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(28.1%) and dry rot (48.9%) prevalences on yam tubers from untreated soils were higher than those from product-incorporated soils (1.11–12.1%) ($P < 0.05$). The dry rot prevalence (2.78%) was only lower on yam tubers from soil incorporated with dried castor leaf powder compared to that (10.1%) obtained with dried castor leaf extract ($P = 0.000$). The nematode numbers were lower in the product-incorporated soils and treated yam plants (3–105 individuals) than in the untreated soils and untreated yam plants (37–423 individuals) ($P = 0.000$). The nematode numbers in the soil, root, and tuber samples were statistically similar regardless of the dried castor leaf formulations. The highest yields (10.1 to 10.48 t/ha) were obtained from yam plants on soils that were incorporated with dried castor leaf powder and carbofuran ($P = 0.000$). Dried castor leaf powder is an excellent tool to manage water yam nematodes.

Keywords: Castor plant; Leaf formulations; Nematodes; Population dynamics; Water yam.

1. INTRODUCTION

There are more than 600 species of yam, ten of which are grown for their tubers [1]. Yam takes pride of place in the socio-economic lives of people through the celebration of the new yam [2]. In Côte d'Ivoire, several yam species, including *Dioscorea cayenensis-rotundata* and *Dioscorea alata*, are cultivated [3]. However, *Dioscorea alata* occupies 55–60% of the yam yielded [4]. The majority of domestic yam yield comes from the central and northern areas above the 8th parallel of northern latitude [5]. However, the yield in the forest zone is not negligible [4]. The domestic yam yield was estimated at 7.65 million tons of tubers [6].

Despite the foregoing, yam yield faces enormous constraints, including low availability of fertile land, expensive planting material, production costs, pests and diseases [7]. Among these constraints, plant-parasitic nematodes hold an important place. Plant-parasitic nematodes are microscopic, worm-like organisms with a stylet. The stylet is the unique organ used by nematodes to puncture the cell walls of host plants and suck the contents [8]. Plant-parasitic nematodes are biotrophic organisms that attack the root system and tubers of yam plants, causing damage and degrading the nutritional and commercial quality of the tubers [9].

Plant-parasitic nematodes are responsible for economic losses on yam worldwide, estimated at 17.7% [10]. They are responsible, among other things, for dry rot, cracks, and galls on yam, depending on the nematode species and the agroecological zones [11]. In Côte d'Ivoire, *Pratylenchus coffeae* and *Scutellonema bradys* have been associated with dry rot on tubers of *Dioscorea alata* and the *Dioscorea cayenensis-rotundata* complex, respectively [12,13].

Meloidogyne arenaria and *Meloidogyne javanica* have been associated with galls on *Dioscorea alata* tubers [12].

Synthetic nematicides remain one of the most effective means for controlling plant-parasitic nematodes [14]. However, these nematicides are very harmful to both the environment and human health [15]. The search for an alternative to conventional chemical control is currently encouraged. Thus, the use of plant extracts with antimicrobial properties is on the rise [16]. Osei et al. [17] used, in Ghana, dried neem (*Azadirachta indica*), dried mucuna (*Mucuna pruriens*) seed powder, and dried cocoa pod (*Theobroma cacao*) powder against yam nematodes.

Thus, as part of the project "Development of disease, pest, and weed control strategies in cassava and yam fields", Kouakou et al. [18] revealed the *in vitro* nematicidal potential of the aqueous extract of castor leaves on pathogenic yam nematodes. They showed that dried castor leaves in the form of powder and aqueous extract had, in the absence of a host (yam), reduced the numbers of these nematodes in the soil. This study aims at assessing the nematicidal effectiveness of the powder and extract of dried castor leaves on water yam nematodes.

2. MATERIALS AND METHODS

2.1 Study Site and Climatic Conditions

The field trials were conducted in the Autonomous District of Yamoussoukro in Côte d'Ivoire. This district is located in the forest-savannah transitional zone. It is one of the main water yams (*Dioscorea alata*) production zones in the country. The climate is an equatorial transitional type [19]. It is characterized by two

rainy seasons (March to June and September to October) and two dry seasons (July to August and November to February) [20]. The total rainfall varied from 1200 to 1600 mm per year [19]. The average annual temperature was 26°C [21].

2.2 Trial Set-up

2.2.1 Experimental design

A 23 m × 23 m plot was set up to evaluate the effectiveness of dried castor leaf powder and dried castor leaf extract. The plot was a 3-year fallow. The vegetation of the plot was mainly composed of *Chromolaena odorata*, *Manihot esculenta*, and *Panicum maximum* plants. The plot soil was infested with yam nematodes. Before planting, a 100-ml soil sample contained 72–85 *Pratylenchus coffeae* individuals and 67–79 *Meloidogyne javanica* individuals.

The trial comprised one factor, viz. nematicidal products, with three levels (ca. dried castor leaf powder, dried castor leaf extract, and carbofuran), and an untreated control. The plot was divided into three blocks of four elementary plots, each measuring 5 m by 4 m. Mounds about 50 cm high were made at the rate of 20 mounds per elementary plot. A randomized complete block design was set up with three replicates. The trial was set up in April, marking the start of the rainy season in this area. The trial was conducted twice (in 2015 and 2016).

2.2.2 Preparation of dried castor leaf products

Castor leaves were collected in the suburbs of the Autonomous District of Abidjan. Leaf samples were air-dried in a room for two weeks. The dried leaves were powdered in a household mixer. Two formulations of dried castor leaves were prepared. These included the liquid formulation in the form of an aqueous extract and the powder formulation.

One kilogram of dried castor leaf powder was macerated in 10 liters of water for 72 hours to produce the 100 g/l dried castor leaf extract. The dried castor leaf extract was filtered with a clean cloth. Ten containers of 10-liter extract were obtained to satisfy the needs of the trial.

Batches of 100-g dried castor leaf powder were made after processing the dried leaves. These batches of powder were intended to be applied on top of mounds. A total of 150 batches of 100-

gram dried castor leaf powder were used for this trial.

2.2.3 Supplying yam seeds

The water yam (*Dioscorea alata*, cv. *Bètè bètè*) tubers were used in this trial. This cultivar was chosen because of its great extension in Côte d'Ivoire and the long shelf life of its tubers. Ten yam tubers with no apparent symptoms were collected from yam farmers in the Autonomous District of Yamoussoukro. Several pieces of peel were taken from several places on all the yam tubers in order to detect nematodes. Yam tubers without nematodes were used for seed supply. The yam tubers were cut into seeds weighing about 200 g.

2.2.4 Application of products and planting of yam seeds

Carbofuran (10 g/mound) from the Callivoire S.A. company in Abidjan (Côte d'Ivoire), dried castor leaf powder (100 g/mound), and dried castor leaf extract (500 ml/mound) were applied and incorporated into the soil at the top of the mounds, depending on the treatments. Yam seeds were inserted into the mounds in the area where products were incorporated. Yam seeds were planted at the rate of one seed per mound. The unincorporated mounds were the control treatment.

2.2.5 Conduct of the trial

The staking of the yam plants was carried out in the second month after seed planting. The trial was manually weeded five times, two months apart, starting with the first one. No fertilizer was applied during the trial. Yam tubers were harvested in the 9th month after seed planting.

2.3 Sampling

Soil sampling was carried out before and at 3, 6, and 9 months after the seed planting. Before yam seed planting and product application, soil was sampled from 10 mounds per elementary plot. In the 3rd, 6th, and 9th months after seed planting, soil, root, and tuber samples were collected according to the destructive model. Five plants were selected from each elementary plot and carefully uprooted at each sampling date. The root system of each plant and the adhering soil were removed and bagged. All the samples were sent to the laboratory for nematode extraction.

2.4 Evaluation of the Nematicidal Effects of Dried Castor Leaf Formulations

2.4.1 Evaluation of the effects of treatments on symptom development

Yam tubers were harvested in the 9th month after seed planting. Observations were made to identify the characteristic symptoms of plant-parasitic nematode infections (dry rot, cracks, and galls). The prevalence and severity index of symptoms were determined according to the formulas of Ogara & Bina [9] and Zewain [22], respectively.

2.4.2 Evaluation of the effects of treatments on nematode numbers

All samples were classified according to their treatment. Nematodes from soil samples were extracted using Whitehead's tray method. Those from yam root and tuber samples were extracted using the maceration method [23]. Only *Pratylenchus coffeae* and *Meloidogyne javanica* individuals were counted from a 100-ml soil sample or a 5-g root or yam peeling sample. The numbers of individuals of each species of nematode in the samples were determined depending on the dried castor leaf formulations (formula 1).

$$ANI = \frac{1}{n} \sum_{i=1}^n ni \quad (1)$$

ANI: average number of individuals in 100 ml of soil samples or 5 g of yam root or tuber peel samples; **ni:** number of individuals in 100 ml of nematode suspension; **n:** number of repetitions

2.4.3 Evaluation of the effects of treatments on nematode population dynamics

The numbers of *P. coffeae* and *M. javanica* individuals in 100 ml of soil, in 5-g roots, and in 5-g yam peelings were determined before and at 3, 6, and 9 months after the seed planting.

2.4.4 Evaluation of the effects of treatments on yam yield

At harvest, the yield of the yam plants was determined according to Rodriguez's formula [24]. The yield was determined with 10 yam plants selected per elementary plot.

2.5 Data Analysis

The data were analyzed with Statistica 7.1 software. Nematode numbers, then symptom

prevalence and symptom severity index, were normalized by the functions $\text{Log}(x+1)$ and $\text{arcsin}\sqrt{p/100}$, respectively. Data were analyzed depending on the treatments. To identify the most effective treatment, means were separated using Dunnett's test at the 5% level.

3. RESULTS

3.1 Effects of Dried Castor Leaf Formulations on Symptom Development

Galls, cracks, and dry rot were observed on freshly harvested yam tubers. The prevalence of symptoms varied from 1.11 to 48.9% depending on the treatment (Table 1). Treatments influenced the prevalence of galls and dry rot ($P < 0.05$). Galls and dry rot were less common on tubers from soils incorporated with dried castor leaf powder, dried castor leaf extract, and carbofuran compared to those from untreated soils. The prevalence of galls was less than 5% on tubers from treated soils. However, it was 28.1% on tubers from untreated soils. The prevalence of dry rot on tubers varied from 2.78 to 12.1% depending on the products, compared to 48.9% in untreated soils. However, the prevalence of crack varied from 2.16 to 2.78% depending on the products.

The severity index of symptoms ranged from 1.11 to 33.7% (Table 1). Treatments significantly affected the severity index of symptoms ($P = 0.01$). Symptoms were more severe on tubers from untreated soils compared to those on treated soils. Indeed, the symptom severity index varied from 24.2 to 33.7% on control tubers. In contrast, it oscillated between 0.28 and 6.94% depending on the products incorporated into soils. Dried castor leaf extract and dried castor leaf powder limited gall and crack development similarly. However, dried castor leaf powder limited dry rot development more than dried castor leaf extract.

3.2 Effects of Dried Castor Leaf Formulations on Nematode Numbers

The number of *M. javanica* and *P. coffeae* individuals varied from 3 to 423 individuals depending on the applied products (Table 2). Carbofuran, dried castor leaf powder, and dried castor leaf extract reduced nematode numbers in the soil, roots, and tubers compared to the control. Treatments significantly influenced the

nematode numbers in the soil, roots, and tubers (P = 0.000). Nematode numbers in the soil (14–54 individuals), roots (3–105 individuals), and tubers (14–27 individuals) of treated soils were lower than those of control soils (37–423 individuals). Dried castor leaf powder and dried castor leaf extract reduced the number of nematodes in the same way as carbofuran.

Table 1. Prevalence and severity index of symptoms depending on the dried castor leaf formulations

Castor leaf formulations	Prevalence of Symptoms (%)			Severity Index of Symptoms (%)		
	Galls	Cracks	Dry rot	Galls	Cracks	Dry rot
Control	28.1 ± 11.6a	2.78 ± 1.39a	48.9 ± 11.6a	33.7 ± 10.1a	24.2 ± 7.23a	30.8 ± 11.2a
Carbofuran	1.11 ± 1.11b	2.16 ± 1.45a	12.1 ± 5.22b	0.28 ± 0.28b	0.59 ± 0.39b	5.37 ± 2.92b
Leaf powder	1.11 ± 1.11b	2.44 ± 1.63a	2.78 ± 2.78c	1.11 ± 1.11b	0.28 ± 0.28b	5.56 ± 5.56b
Leaf extract	4.92 ± 2.16b	2.78 ± 1.47a	10.1 ± 6.01b	3.89 ± 1.77b	1.11 ± 0.61b	6.94 ± 3.56b
P	.02	.98	.000	.01	.01	.01

Average ± standard deviation; Values with the same letter in each column are statistically identical at the 5% level; P: probability value; Leaf powder: dried castor leaf powder formulation (100 g/mound); Leaf extract: dried castor leaf extract formulation (500 ml/mound); Carbofuran: carbofuran (10 g/mound); Control: untreated mounds

Table 2. Nematode numbers depending on the dried castor leaf formulations

Castor leaf formulations	Nematode Numbers					
	<i>Meloidogyne javanica</i>			<i>Pratylenchus coffeae</i>		
	Soils	Roots	Tubers	Soils	Roots	Tubers
Control	84 ± 14a	52 ± 22a	37 ± 6a	194 ± 42a	423 ± 183a	75 ± 30a
Carbofuran	14 ± 2c	15 ± 4b	15 ± 3b	23 ± 3c	92 ± 42b	21 ± 05b
Leaf powder	18 ± 3b	03 ± 1c	18 ± 4b	31 ± 15c	105 ± 23b	27 ± 06b
Leaf extract	21 ± 5b	28 ± 4b	14 ± 4b	54 ± 6b	95 ± 40b	20 ± 04b
P	.000	.000	.000	.000	.000	.000

Average ± Standard deviation; Values with the same letter in each column are statistically identical at the 5% level; P: probability value; Leaf powder: dried castor leaf powder formulation (100 g/mound); Leaf extract: dried castor leaf extract formulation (500 ml/mound); Carbofuran: carbofuran (10 g/mound); Control: untreated mounds

Table 3. Yam yield depending on the dried castor leaf formulations

Castor leaf formulations	Yield (t/ha)
Control	7.67 ± 0.44b
Carbofuran	10.01 ± 0.42a
Leaf powder	10.48 ± 0.43a
Leaf extract	8.89 ± 0.40b
P	.000

Average ± Standard deviation; Values with the same letter are statistically identical at the 5% level; P: probability value; Leaf powder: dried castor leaf powder formulation (100 g/mound); Leaf extract: dried castor leaf extract formulation (500 ml/mound); Carbofuran: carbofuran (10 g/mound); Control: untreated mounds

3.3 Effect of Dried Castor Leaf Formulations on Yam Yield

Yam yield varied from 7.67 to 10.48 t/ha depending on the dried castor leaf formulations (Table 3). Treatments significantly influenced the yam yield. The highest yields (10.01–10.48 t/ha) were obtained from plants on soils incorporated with carbofuran and dried castor leaf powder.

3.4 Effects of Dried Castor Leaf Formulations on Nematode Dynamics

3.4.1 Nematode population dynamics in soil

The number of nematodes varied depending on the yam development stages and dried castor

leaf formulations. The number of nematodes increased in untreated soils, while it decreased in soils incorporated with the product. Thus, the number of *M. javanica* individuals in the untreated soils was 67 before the yam seed planting (Fig. 1A). It increased to reach 98 and 80 individuals in the 6th and 9th months, respectively. However, the number of *M. javanica* individuals was significantly lower in the treated soils compared to the untreated soils. The number of individuals was not more than 21 in the 3rd month and less than 20 and 10 individuals in the 6th and 9th months, respectively, regardless of the applied product.

In untreated soils, the number of *P. coffeae* individuals was 85 before seed planting. It

increased to reach a peak of 352 individuals in the 6th month before decreasing to 216 individuals in the 9th month (Fig. 1B). However, it was very low in the treated soils. The number of *P. coffeae* individuals was maintained at less than 50 individuals in the 3rd month, regardless of the applied products. It was stabilized at 50 individuals from the 3rd to the 9th month in soils treated with carbofuran.

The number of *P. coffeae* individuals increased significantly in soils incorporated with dried castor leaf powder and dried castor leaf extract. It increased from 30 to 48 individuals between the 3rd and 9th months in the soil incorporated with dried castor leaf powder. However, it increased from 43 to 86 individuals during the yam growing season in soils treated with dried castor leaf extract.

3.4.2 Nematode population dynamics in yam roots

The application of products did not prevent the increase in nematode numbers in the roots of yam plants. The nematode number increased slightly in the roots of plants on the treated soils compared to the control plants.

Thus, the number of *M. javanica* individuals increased from 3 to 89 individuals in the roots of the plants on untreated soils in the 3rd and 6th months, respectively (Fig. 2A). In the roots of the yam plants, the number of *M. javanica* individuals, although low compared to the control plants, also increased. It rose from not more than

13 individuals in the 3rd month to less than 40 individuals in the 6th month for all the applied products.

Similar results were obtained for *P. coffeae*. Its number increased considerably, reaching a peak of 812 individuals in the 6th month in the roots of untreated soils (Fig. 2B). However, there were fewer than 10 and 200 individuals in the 3rd and 6th months, respectively, regardless of the applied product.

3.4.3 Nematode population dynamics in yam tubers

In yam tubers, nematode numbers increased despite the products applied to the soil. The number of *M. javanica* individuals increased considerably in the tubers from the control treatment, with 0, 12, and 97 individuals in the 3rd, 6th, and 9th months, respectively (Fig. 3A). In yam tubers from treated soils, nematode numbers increased slightly compared to the control treatment, with less than 10 and 50 individuals in the 6th and 9th months, respectively.

In the tubers from the control treatment, the number of *P. coffeae* individuals increased significantly during the yam growing season, with 5, 25, and 194 individuals in the 3rd, 6th, and 9th months, respectively (Fig. 3B). In contrast, it was relatively low in the tubers from treated soils. The number of *P. coffeae* individuals in yam tubers from treated soils was less than 5 and 70 individuals in the 6th and 9th months, respectively.

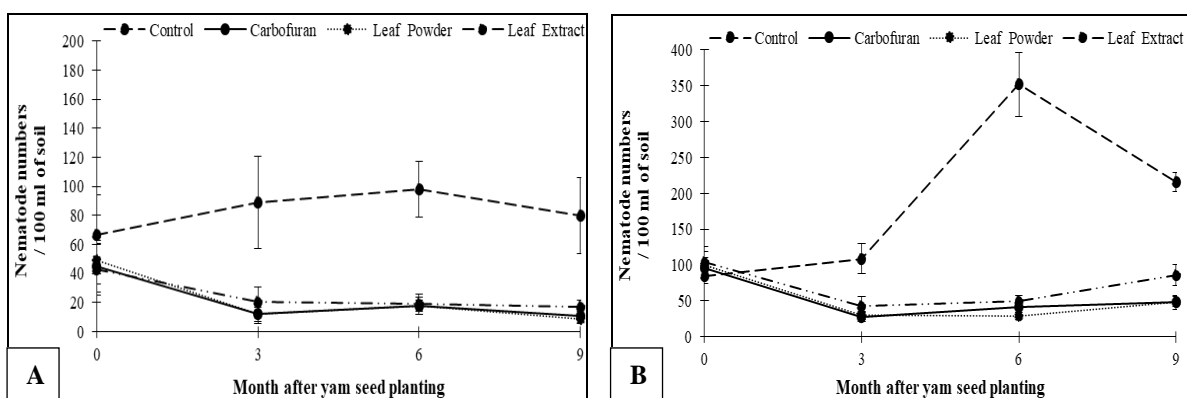


Fig. 1. *Meloidogyne javanica* (A) and *Pratylenchus coffeae* (B) population dynamics in soil depending on the dried castor leaf formulations

Leaf powder: dried castor leaf powder formulation (100 g/mound); Leaf extract: dried castor leaf extract formulation (500 ml/mound); Carbofuran: carbofuran (10 g/mound); Control: untreated mounds

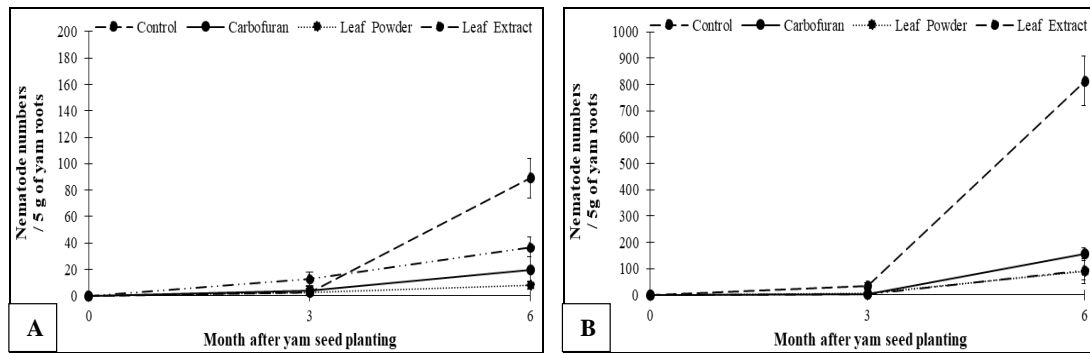


Fig. 2. *Meloidogyne javanica* (A) and *Pratylenchus coffeae* (B) population dynamics in yam roots depending on the dried castor leaf formulations

Leaf powder: dried castor leaf powder formulation (100 g/mound); Leaf extract: dried castor leaf extract formulation (500 ml/mound); Carbofuran: carbofuran (10 g/mound); Control: untreated mounds

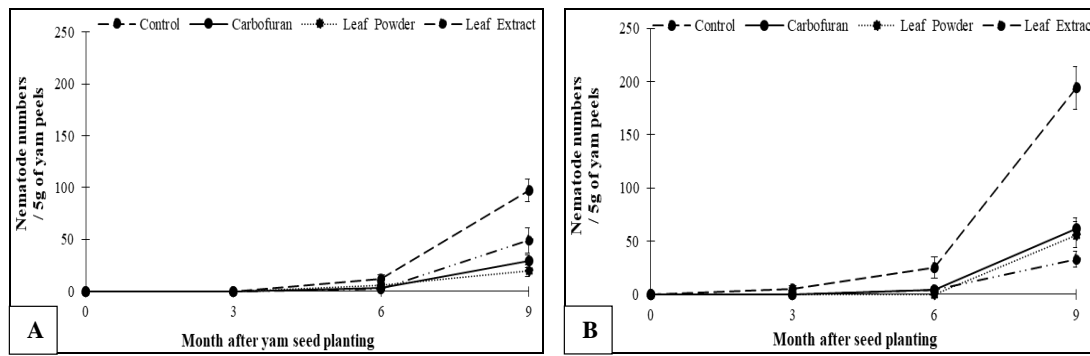


Fig. 3. *Meloidogyne javanica* (A) and *Pratylenchus coffeae* (B) population dynamics in yam tubers depending on the dried castor leaf formulations

Leaf powder: dried castor leaf powder formulation (100 g/mound); Leaf extract: dried castor leaf extract formulation (500 ml/mound); Carbofuran: carbofuran (10 g/mound); Control: untreated mounds

4. DISCUSSION

This study revealed that the application of dried castor leaves in the form of powder and aqueous extract reduces nematode numbers in yam soils, roots, and tubers. A reduction in nematode numbers after application of dried castor leaf extract might be due to the nematicide secondary metabolites found in the extract. Indeed, plant extracts contain, among others, phenolic compounds, terpenes, and terpenoids [25]. Several of these compounds have nematicidal properties, although their modes of action are unknown. Thus, they might directly or indirectly alter the respiratory functions of the nematodes, sometimes leading to death, hence the reduction in their number. Meanwhile, the reduction in nematode numbers after application of the dried castor leaf powder might be due to the decomposition of organic matter, which results in the release of ammonium ions, formaldehyde, phenols, and volatile fatty acids [26,27]. These compounds, toxic to nematodes, might cause their deaths and reduce their number in soils.

The number of *P. coffeae* and *M. javanica* individuals in yam roots and tubers from treated soils were lower than that from untreated soils. This would be attributable to a significant decrease in nematode numbers in soils after product application. Thus, the yam roots would have absorbed more water and nutrients compared to the roots of control plants. This might account for the high yield noted in plants from soils treated with carbofuran and dried castor leaf powder.

Furthermore, dried castor leaf powder and dried castor leaf extract reduced nematode numbers in yam soils, roots, and tubers like carbofuran. This means that dried castor leaf powder and dried castor leaf extract could be used as a replacement for carbofuran. Indeed, chemical nematicides are increasingly withdrawn from the market because of their persistence as residues in the food web, their toxicity to humans [28], and their high cost for low-income households [29]. Organic amendments have several advantages, including the improvement of the physical,

chemical, and biological properties of soils. They have a beneficial effect on plant growth and are low-cost [30]. In addition, the farmer could have several choices regarding yam nematode control strategies.

However, this study revealed an increase in nematode numbers in tubers after the third month after seed planting and after a single application of the products. This increase in nematode numbers is due to the depletion of nematicidal residues from the dried castor leaf powder and extract applied to the soil. Indeed, three months after application, the compounds of these products would have been totally degraded and would no longer be effective. The soil conditions would have become favourable for the development of nematodes and, consequently, an increase in their numbers. This study reveals the time of effectiveness of dried castor leaf powder and dried castor leaf extract. Thus, the effect of the frequency of product application on nematode population dynamics is significant.

5. CONCLUSION

The dried castor leaf powder and dried castor leaf extract reduce nematode numbers in yam soils, roots, and tubers. They keep the development of symptoms at a significantly low level and allow a gain in yam yield. Dried castor leaf powder and dried castor leaf extract are promising means for controlling yam nematodes. There is therefore an interest in using these technologies in an integrated management program for yam diseases.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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