



## Nematicidal effectiveness of products stemming from dried leaves of castor-oil plant (*Ricinus communis* L.) on *Meloidogyne* and *Pratylenchus*, yam pathogenic nematodes in Côte d'Ivoire

Yadom Yao François Régis Kouakou\*, Kouamé Daniel Kra, Kouamé Patrice Assiri, Hortense Atta Diallo

*Unité de Phytopathologie, Pôle de Recherche Production Végétale, Unité de Formation et de Recherche des Sciences de la Nature, Université Nangui Abrogoua, Abidjan, Côte d'Ivoire*

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**Key words:** Castor-oil plant, Côte d'Ivoire, Phytopathogenic nematodes, Yam

### Abstract

The damage caused by phytopathogenic nematodes on yam and the harmful effects of chemical nematicides on the consumer encourage the search for an alternative nematode-control method. The objective of this study is to assess the nematicidal effectiveness of products stemming from leaves of castor-oil plant (*Ricinus communis* L.) on yam pathogenic nematodes. Populations of *Meloidogyne* and *Pratylenchus* from yam cultivation plot soils were exposed to five concentrations of the aqueous extract of castor-oil plant dried leaves. The rates of immobility, mortality and recovery of the nematodes exposed to the extract were determined. The effectiveness of formulations (powder and liquid) of castor-oil plant leaves were compared to that of Carbofuran in reducing the total number of nematodes. The 50,000 ppm concentration extract was the most effective on *Meloidogyne* and *Pratylenchus* with over 92% mortality. The powder formulation reduced the total number of *Meloidogyne* (79%) and *Pratylenchus* (84%) more than the liquid formulation. The powder formulation reduced the total number of *Meloidogyne* and *Pratylenchus* in soils as well as carbofuran (79% reduction).

\*Corresponding Author: Yadom Yao François Régis Kouakou ✉ [yadomregis@yahoo.fr](mailto:yadomregis@yahoo.fr)

## Introduction

Phytopathogenic nematodes are a major constraint on agricultural production worldwide (Rubino *et al.*, 2008). They attack several crops including yam, the first food crop in Côte d'Ivoire in terms of yield (Ettien and Tschannen, 2003). Indeed, with a yield of 5.8 million tons, Côte d'Ivoire is the world's third largest producer after Nigeria and Ghana (Faostat, 2013). The phytopathogenic nematodes *Scutellonema bradys*, *Pratylenchus coffeae*, *Meloidogyne incognita* and *Rotylenchulus reniformis* are economically more damaging than other species to yam (Osei *et al.*, 2013). They cause over 80% loss of yam tubers during storage (Claudius-Cole, 2015). Their actions damage the nutritional and commercial quality of yam tubers (Ogara and Bina, 2010). The application of chemical nematicides is the most effective strategy for controlling phytopathogenic nematodes (Naserinasab *et al.*, 2012). However, these chemicals are very harmful to human health as well as the environment (Bissadou *et al.*, 2012). In the face of increasingly demanding environmental standards imposed by the international community in agriculture, the search for an alternative to chemical control is strongly encouraged. Thus, the use of antagonistic microorganisms (Mervat *et al.*, 2012) or plant species having nematocidal properties (Tsay *et al.*, 2004) are control methods developed against phytopathogenic nematodes. In order to contribute to this non-chemical fight against phytopathogenic nematodes that guarantee a sustainable environment, the nematotoxic properties of plant extracts were studied. The objective of this study is to assess the nematocidal effectiveness of the aqueous extract and the powder of dried leaves of castor-oil plant (*Ricinus communis* L.) on *Meloidogyne* and *Pratylenchus*, yam pathogenic nematodes in Côte d'Ivoire.

## Materials and methods

*Assessment of the in vitro effect of the aqueous extract of dried leaves of castor-oil plant on nematodes*

*Collection and drying of castor-oil plant leaves*

The healthy fresh leaves of castor-oil plants (*Ricinus communis* L., Euphorbiaceae) were collected in the

suburbs of the District Autonome d'Abidjan in southern Côte d'Ivoire. They were washed with tap water and then dried at room temperature ( $27 \pm 2^\circ\text{C}$ ) for 14 days. The dried leaves were transformed into powder using the mixer (Fadelux Y44). The resulting powder was used to prepare the aqueous extract of dried leaves of castor-oil plant.

*Preparation of the aqueous extract*

A crude extract of 100 g/L concentration was prepared by maceration of 100 g powder of dried leaves of castor-oil plant in 1L of distilled water for 72 hours at room temperature ( $27 \pm 2^\circ\text{C}$ ). The crude extract was filtered three times with a densified layer of about 3 cm thick hydrophilic cotton placed in a 60 ml syringe. The aqueous extract was used for testing the sensitivity of yam pathogenic nematodes.

*Preparation of Meloidogyne and Pratylenchus inoculum*

The nematodes were obtained from soil samples collected from yam cultivation plots (*Dioscorea alata* L.) from four localities (Babadougou, Soubré, Tanda and Toumodi) located in yam production areas in Côte d'Ivoire. The soils of these plots are infested with nematodes belonging to the *Meloidogyne* and *Pratylenchus* genera. These nematodes are yam pathogens in Côte d'Ivoire (Kouakou *et al.*, 2016). The tray method of Whitehead (Coyné *et al.*, 2010) was used to extract nematodes from soils.

*Amendment of aliquots of nematodes*

Four (4) ml aliquots containing 58 to 67 individuals of *Meloidogyne* and 49 to 55 individuals of *Pratylenchus* were removed and placed in tubes containing 4; 3.2; 2.4; 1.6 and 0.8 ml of the aqueous extract of castor-oil plant so as to obtain the respective concentrations of 50,000; 40,000, 30,000; 20,000 and 10,000 ppm. Aliquots of nematodes placed in the tubes containing 4; 3.2; 2.4; 1.6 and 0.8 ml of sterile distilled water served as respective controls for the aforementioned treatments. The aliquots were incubated in the dark at room temperature ( $27 \pm 2^\circ\text{C}$ ) for six days.

*Determination of nematode immobility, mortality and recovery rates*

A count of the immobile individuals exposed to the extracts was carried out daily under an optical microscope (AmScope). The number of immobile individuals NI (E) of a genus *i* in the extract and the number of immobile individuals of the same genus in the corresponding control NI (T) were determined. A subtraction of NI (T) from NI (E) was made to obtain the number of immobile individuals P (E) of the genus *i* due to the extract.

This number was divided by the total number of individuals of the genus *i* in the starting extract TN (E); the whole was multiplied by 100 so as to obtain the immobility rate IR (E) of the individuals of the genus *i* in the extract as indicated below:

$$IR (E) = \frac{P(E)}{TN (E)} \times 100$$

IR (E): Immobility rate of individuals of a genus *i* in the extract,

P (E): Number of immobile individuals of the genus *i* due to the extract,

TN (E): Total number of individuals of the genus *i* in the extract.

To determine the dead or recovered status of each immobile individual, the immobile individuals P (E) were transferred into sterile distilled water and incubated for 24 hours in the dark at laboratory temperature ( $27 \pm 2^\circ\text{C}$ ).

Dead or restored individuals were identified and counted by stimulation with a needle. The individual was considered dead in the absence of movement following the injection (Cayrol *et al.*, 1989). Finally, the mortality and recovery rates were calculated as follows:

$$MR (E) = \frac{ND (E)}{P (E)} \times 100$$

MR (E): Mortality rate of individuals of a genus *i* in the extract,

ND (E): Number of dead individuals of a genus *i* after exposure to the extract,

P (E): Number of immobile individuals of a genus *i* due to the extract.

$$RR (E) = 100 - MR (E)$$

RR (E): Recovery rate of individuals of a genus *i* in the extract,

MR (E): Mortality rate of individuals of a genus *i* in the extract.

*Comparative effects of products stemming from dried leaves of castor-oil plant on Meloidogyne and Pratylenchus under field conditions*

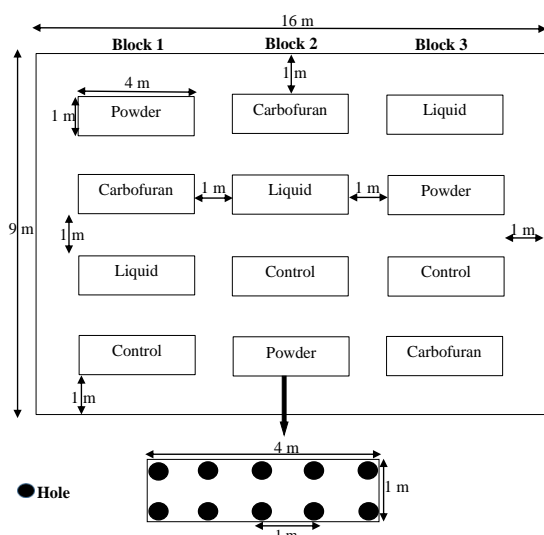
*Study site*

The study site is located in N'gattakro, a village of the District Autonome de Yamoussoukro (central Côte d'Ivoire). The climate is equatorial transitional attenuated between the Guinean and Sudanian climates (N'guessan, 1990). It is characterized by two rainy seasons (March to June and September to October) and two dry seasons, from July to August and from November to February (Bla *et al.*, 2015). The rainfall depth varies from 1 200 to 1 600 mm/year (N'guessan, 1990).

The average annual temperature is  $26^\circ\text{C}$  (Alui *et al.*, 2011). The vegetation consists of mesophilic forests, gallery forests, shrub savannahs (Soro *et al.*, 2014). The geographical coordinates of the study site are: N  $06^\circ52.950'$ ; W  $005^\circ19.992'$ . The cropping history of the study site was a three-year fallow consisting mainly of *Chromolaena odorata* (Asteraceae), *Manihot esculenta* (Euphorbiaceae) and *Panicum maximum* (Poaceae) plants.

*Establishment of the experimental plot*

The plot was cleared, freed from stumps and subdivided into three blocks of four elementary plots of  $1 \text{ m} \times 4 \text{ m}$  area (Fig. 1.). Openings of about 20 cm in diameter and 30 cm in depth were made, at the rate of ten per elementary plot arranged on two rows spaced 1 m apart and also 1 m apart on the row. The experimental design was a completely randomized block with three repetitions. The plot was set up in May 2015, marking the beginning of the rainy season.



**Fig. 1.** Experimental design of the trial set up under field conditions.

#### Soil treatment with products

Four treatments were evaluated: powder formulation of dried leaves of castor-oil plant, liquid formulation (aqueous extract) of dried leaves of castor-oil plant, Diafuran 5G (chemical nematicide, active ingredient: Carbofuran) and non-treated control.

The applications rates of the products were:

**Control:** Untreated soil,

**Carbofuran:** Soil treated with Carbofuran (10 g per hole),

**Powder formulation:** Soil treated with the powder of dried leaves of castor-oil plant (100 g per hole),

**Liquid formulation:** Soil treated with 500 ml of crude extract of 100 g/L concentration of dried leaves of castor-oil plant per hole.

The products were applied to soils removed from the holes, and then a mixture was made before putting them back into the holes. The control soils were removed and put back into the holes without treatment. No planting of plant material was carried out on soils. Three manual weeding of the plot were done after treatment of soils at one-month interval.

#### Soil samples collection

The collection of soil samples was done before soil treatment, then every month after treatment until the 4<sup>th</sup> month. Soil samples were collected with a soil corer. It was driven in the treated or non-treated soil down to 30 cm deep.

The soil was removed and put in a sachet, then labeled. A total of 120 soil samples were collected each period, that is, 30 per treatment.

#### Extraction, identification and counting of phytopathogenic nematodes

The method used to extract nematodes from soil samples was the tray method of Whitehead (Coyne *et al.* 2010). The genera of phytopathogenic nematodes extracted from the different soil samples were identified using the keys of Hunt *et al.* (2005) and Castillo and Vovlas (2007). The total number of each nematode genus was determined from 100 ml of soil.

#### Determination of the reduction rate in the total number of nematodes

The reduction rate (Rr) in the total number of individuals of each nematode genus was calculated according to the formula of Mahdy *et al.* (2014):

$$Rr = \frac{TNC - TNT}{TNC} \times 100$$

Tr (%): Rate of reduction in the total number of individuals of a genus *i*,

TNC: Total number of individuals of a genus *i* obtained in 100 ml of untreated soil samples,

TNT: Total number of individuals of a genus *i* obtained in 100 ml of treated soil samples

#### Statistical analyses

Statistica 7.1 software was used for statistical analyses. Immobility, mortality and recovery rates and reduction rates in the total number of nematodes were transformed by  $\arcsin \sqrt{p/100}$  function prior to statistical analysis.

The total number of nematodes were transformed by the  $\log_{10} (x + 1)$  function (Jayaraman, 1999). The transformed data were subjected to one-way analysis of variance. A two-way analysis was carried out to compare the average total numbers of nematodes according to time and treatments. In case of a significant difference at 5% level of probability, Fisher's Least Significant Difference (LSD) test was used to obtain homogeneous groups.

**Results**

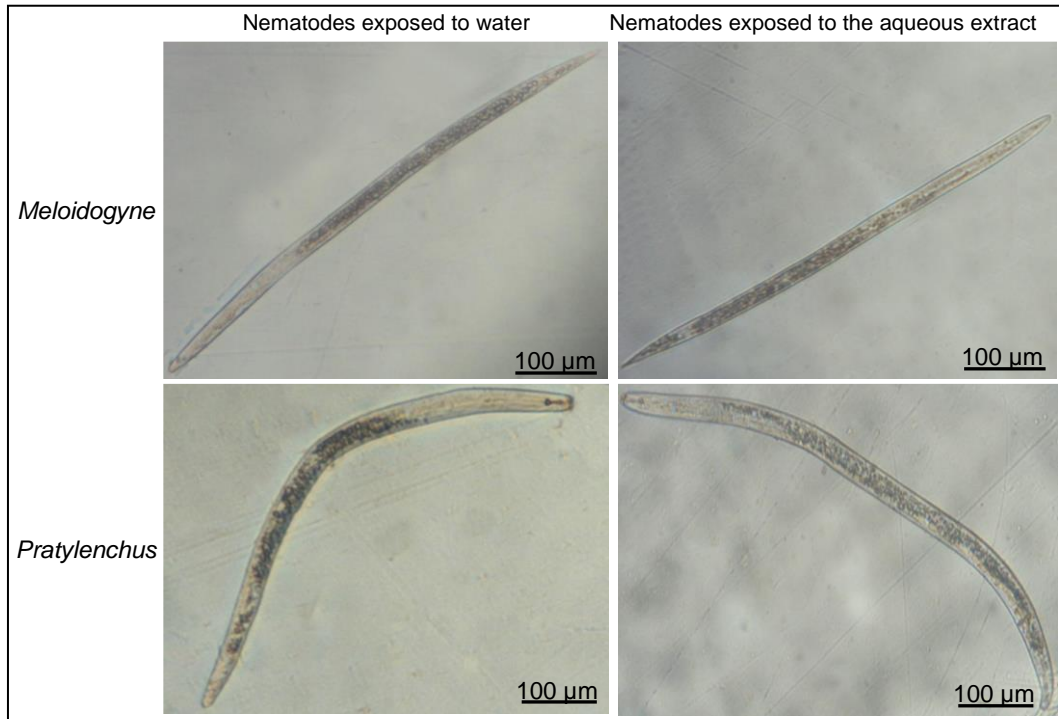
*In vitro* antiparasitic activities of the aqueous extract of dried leaves of castor-oil plant on *Meloidogyne* and *Pratylenchus*

*Change in the pigmentation of nematode cuticle*

Exposure of *Meloidogyne* and *Pratylenchus* individuals for six days to the aqueous extract of dried leaves of castor-oil plant caused a change in the pigmentation of their cuticle (Fig. 2.). The anterior and caudal end of individuals exposed to water were

hyaline, while their posterior region was dark brown. In individuals exposed to the aqueous extract, the anterior region and the caudal end were light brown.

A brown staining of the stylet of *Meloidogyne* individuals exposed to the aqueous extract was noted, while it was hyaline and almost invisible for those exposed to water. The posterior region of individuals of each genus exposed to the aqueous extract was dark brown as for those of control individuals.



**Fig. 2.** Pigmentation of the cuticle of nematodes belonging to the *Meloidogyne* and *Pratylenchus* genera exposed for six days to the aqueous extract of dried leaves of castor-oil plant.

*Immobility, motility and recovery rate of nematodes according to concentrations of the aqueous extract*

The immobility and mortality rates of *Meloidogyne* and *Pratylenchus* increased significantly with the concentration of the aqueous extract of dried leaves of castor-oil plant, in contrast to the recovery rate (Table 1). The extract at 50,000 ppm concentration was more effective than the others on both nematode genera, with 100% immobility, mortality and 0% recovery on *Meloidogyne*, then 98.67% immobility, 98.11% mortality and 1.89% recovery on *Pratylenchus*. From 40,000 ppm, the aqueous extract of dried leaves of castor-oil plant had a total nematocidal activity on the *Meloidogyne* genus, unlike

that of *Pratylenchus* where 94 to 98% of immobile individuals died of the aqueous extract.

*Comparative sensitivity of Meloidogyne and Pratylenchus populations from different localities*

*Meloidogyne* populations from the different study sites reacted similarly to the aqueous extract of dried leaves castor-oil plant at 50,000 ppm concentrations. Indeed, the immobility and mortality rates of *Meloidogyne* individuals exposed to the extract at 50,000 ppm concentrations were 100% irrespective of their origins. In contrast, *Pratylenchus* populations from the different sites reacted differently to the 50,000 ppm extract (Table 2).

**Table 1.** Immobility, mortality and recovery rates of *Meloidogyne* and *Pratylenchus* according to aqueous extract concentrations of dried leaves of castor-oil plant.

Concentrations (ppm)	<i>Meloidogyne</i>			<i>Pratylenchus</i>		
	IR (%)	MR (%)	RR (%)	IR (%)	MR (%)	RR (%)
10 000	89.10 ± 1.54c	75.07 ± 0.48d	24.93 ± 0.48a	89.11 ± 1.53c	58.70 ± 1.17e	41.30 ± 1.17a
20 000	92.00 ± 0.70c	83.40 ± 1.63c	16.60 ± 1.63b	92.90 ± 1.09bc	77.00 ± 0.97d	23.00 ± 0.97b
30 000	93.90 ± 1.74b	89.80 ± 1.82b	10.20 ± 1.82c	93.90 ± 1.49b	86.00 ± 1.68c	14.00 ± 1.68c
40 000	97.00 ± 0.74b	100.00 ± 0.00a	0.00 ± 0.00d	95.00 ± 0.74b	94.50 ± 0.72b	5.50 ± 0.72d
50 000	100.00 ± 0.00a	100.00 ± 0.00a	0.00 ± 0.00d	98.67 ± 0.71a	98.11 ± 0.77a	1.89 ± 0.77e
F	21.77	173.79	173.79	13.59	131.23	131.23
P	0.00	0.00	0.00	0.00	0.00	0.00

ppm: Parts per million, IR: Immobility rate, MR: Mortality rate, RR: Recovery rate, F: Value of Fischer, P: Value of probability. In each column, values having the same letter are statistically identical at 5% level of probability according to Fisher's LSD test.

All populations were sensitive, but the one from the soils of Soubré was more sensitive than the other populations with 98% immobile individuals, 99.50% dead individuals and 0.50% recovered individuals.

**Table 2.** Sensitivity of *Pratylenchus* populations exposed for six days to the aqueous extract of dried leaves of castor-oil plant at 50.000 ppm concentrations.

Population origins	IR (%)	MR (%)	RR (%)
Babadougou	99.00 ± 0.17a	98.00 ± 0.29b	2.00 ± 0.29a
Soubré	98.00 ± 0.29b	99.50 ± 0.08a	0.50 ± 0.08b
Toumodi	98.00 ± 0.29b	97.94 ± 0.32b	2.06 ± 0.32a
Tanda	99.68 ± 0.10a	97.00 ± 0.38b	3.00 ± 0.38a
F	18.15	12.00	12.00
P	0.00	0.00	0.00

ppm: Parts per million, IR: Immobility rate, MR: Mortality rate, RR: Recovery rate, F: Value of Fischer, P: Value of probability in each column, values having the same letter are statistically identical at 5% level of probability according to Fisher's LSD test.

*Comparative sensitivity of Meloidogyne and Pratylenchus genera*

The *Meloidogyne* and *Pratylenchus* genera reacted differently to the aqueous extract of dried leaves of castor-oil plant at 50,000 ppm concentrations (Table 3). The *Meloidogyne* genus was more sensitive than the *Pratylenchus* one of with 100% immobility and mortality, and no recovered individual.

**Table 3.** Sensitivity of yam pathogenic nematodes exposed for six days to the aqueous extract of dried leaves of castor-oil plant at 50,000 ppm concentrations.

Nematodes	IR (%)	MR (%)	RR (%)
<i>Meloidogyne</i>	100.00 ± 0.00a	100.00 ± 0.00a	0.00 ± 0.00b
<i>Pratylenchus</i>	98.67 ± 0.71a	98.11 ± 0.77b	1.89 ± 0.77a
t	-1.87	-2.82	2.82
P	0.09	0.02	0.02

ppm: Parts per million, IR: Immobility rate, MR: Mortality rate, RR: Recovery rate, t: Value of Student, P: Value of probability in each column, values having the same letter are statistically identical at 5% level of probability according to t test.

*Comparative effectiveness of products stemming from dried leaves of castor-oil plant in reducing the total number of Meloidogyne and Pratylenchus in soils*

*Main nematodes extracted from soils*

In total, six genera of phytopathogenic nematodes were extracted from the soil. They included *Gracilacus*, *Helicotylenchus*, *Meloidogyne*,

*Pratylenchus*, *Tylenchorhynchus* and *Xiphinema*.

The total numbers of these nematodes were significantly different (Table 4). *Pratylenchus*, *Meloidogyne*, *Helicotylenchus* and *Xiphinema* were the four main genera of phytopathogenic nematodes extracted with 160; 53; 40 and 33 individuals respectively in 100 ml of soil, with isolation rates ranging from 51.78 to 10.68%.

**Table 4.** Total numbers and average isolation rates of phytopathogenic nematodes found in the soil of the locality of N’gattakro.

Nematodes genera	Total number/100 ml soil	Isolation rate (%)
<i>Pratylenchus</i>	160 ± 12.91a	51.78 ± 4.17a
<i>Meloidogyne</i>	53 ± 4.64b	17.15 ± 1.50b
<i>Helicotylenchus</i>	40 ± 4.71b	12.95 ± 1.53b
<i>Xiphinema</i>	33 ± 4.33b	10.68 ± 1.40b
<i>Tylenchorhynchus</i>	14 ± 4.39c	4.53 ± 1.42c
<i>Gracilacus</i>	09 ± 3.09c	2.91 ± 1.00c
F	18.43	50.25
P	0.00	0.00

In each column, values having the same letter are statistically identical at 5% level of probability according to Fisher’s LSD test, F: Value of Fisher, P: Value of probability.

**Table 5.** Reduction rates of *Meloidogyne* and *Pratylenchus* in the treated soils.

Treatments	<i>Meloidogyne</i>	<i>Pratylenchus</i>
Carbofuran	85.14 ± 3.17a	83.54 ± 3.28a
Powder formulation	79.73 ± 5.04a	84.81 ± 4.05a
Liquid formulation	43.24 ± 6.89b	46.84 ± 4.51b
F	19.48	37.79
P	0.00	0.00

F: Value of Fischer, P: Probability, in each column, values having the same letter are statistically identical at 5% level of probability according to Fisher’s LSD test.

Control: Untreated soil

Carbofuran: Soil treated with Carbofuran (10 g per hole).

Powder formulation: Soil treated with the powder of dried leaves of castor-oil plant (100 g per hole).

Liquid formulation: Soil treated with 500 ml of crude extract of 100 g/L concentration of dried leaves of castor-oil plant per hole.

*Reduction of the total number of Meloidogyne and Pratylenchus according to products*

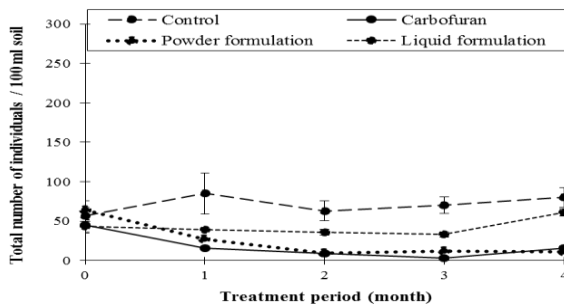
The total numbers of *Meloidogyne* and *Pratylenchus* numbers were significantly reduced in soils treated with products compared to control soils.

This reduction was influenced by the products applied to soils ( $P < 0.05$ ) (Table 5).

It appeared that the powder formulation of dried leaves of castor-oil plant (100 g per hole) was more effective than the liquid formulation (500 ml of crude extract of 100 g/L concentration) on the one hand, and of similar effectiveness to that of Carbofuran (10 g per hole) on the other hand, *Meloidogyne* and *Pratylenchus* populations in soils with 79 and 84% reduction rate respectively.

*Reduction of the total number of Meloidogyne and Pratylenchus according to products and time*

Before application of products, the total numbers of *Meloidogyne* were 57 individuals in 100 ml of untreated soil (Fig. 3.). They increased to 85 individuals before remaining substantially constant during the trial. But, in treated soils, the total numbers of *Meloidogyne* were reduced to less than 40 in 100 ml of soil in the first month. In soils treated with Carbofuran and the powder formulation of dried leaves of castor-oil plant, the total numbers of *Meloidogyne* were reduced to less than 20 individuals in the 4<sup>th</sup> month. Concerning the soils treated with the liquid formulation of dried leaves of castor-oil plant, the total numbers started increasing from the 3<sup>rd</sup> to reach 61 individuals in 100 ml of soil in the 4<sup>th</sup> month.



**Fig. 3.** Number of *Meloidogyne* individuals in soil according to applied products and time.

Control: Untreated soil,

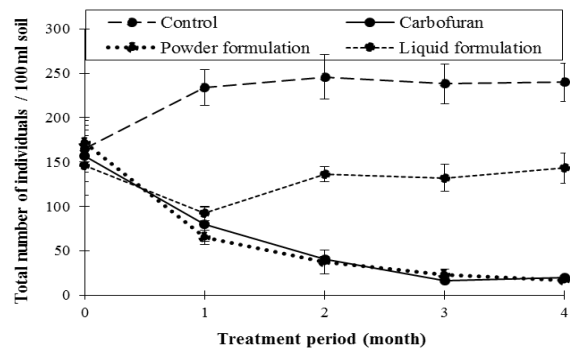
Carbofuran: Soil treated with Carbofuran (10 g per hole),

Powder formulation: Soil treated with the powder of dried leaves of castor-oil plant (100 g per hole),

Liquid formulation: Soil treated with 500 ml of crude extract of 100 g/L concentration of dried leaves of castor-oil plant per hole.

As for *Pratylenchus*, its total numbers were 165 individuals in 100 ml of untreated soil before application of products (Fig. 4). These total numbers increased to 246 individuals in the 2<sup>nd</sup> month, then remained relatively constant until the 4<sup>th</sup> month with 240 individuals in 100 ml of soil. However, the total numbers of *Pratylenchus* were greatly reduced in soils treated with less than 100 individuals in 100 ml of soil in the first month.

This reduction rose to 20 and 17 individuals in 100ml of soil in the 4<sup>th</sup> month respectively with Carbofuran and the powder formulation of dried leaves of castor-oil plant. In contrast, in soils treated with the liquid formulation of dried leaves of castor-oil plant, the total numbers of *Pratylenchus* started increasing from the second month to reach 132 and 143 in 100ml of soil in the 3<sup>rd</sup> and 4<sup>th</sup> months respectively.



**Fig. 4.** Number of *Pratylenchus* individuals in soil according to applied products and time.

Control: Untreated soil,

Carbofuran: Soil treated with Carbofuran (10 g per hole),

Powder formulation: Soil treated with the powder of dried leaves of castor-oil plant (100 g per hole),

Liquid formulation: Soil treated with 500 ml of crude extract of 100 g/L concentration of dried leaves of castor-oil plant per hole.

**Discussion**

The exposure of nematodes to the aqueous extract of dried leaves of castor-oil plant showed a change in the pigmentation of their cuticle and organs. This would be explained by the diffusion of the extract through the cuticle to accumulate in the nematode organs. Indeed, the extract of castor-oil plant leaves contains certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knobloch *et al.*, 1989). Phospholipids are an important component of cellular membranes (Jena and Gupta, 2012). In addition, the cuticle of nematodes is composed of lipids, glycoproteins (Proudfoot *et al.*, 1990), collagens and cuticulins (Gravato and Evans, 1998).



These situations might have favored the diffusion and accumulation of the extract in nematode organs, and might disrupt their functioning.

The exposure of nematodes to the aqueous extract of dried leaves of castor-oil plant resulted in immobility and mortality of nematodes. The reaction of the nematodes to the product in this study might be due to the activity of one or more secondary compounds with nematostatic and/or nematicidal properties present in the aqueous extract of castor-oil leaves and which might have disrupted the functioning of the vital organs of nematodes. This nematotoxic potential might be attributed to phenolic compounds (Koul, 2008), alkaloids and terpenoids whose nematicidal activity has been indicated by Goswami and Vijayalakshmi (1986). These compounds were indeed detected by Jena and Gupta (2012) in the leaf extract of *R. communis*, as well as ricin, a compound with a significant lipolytic activity.

Furthermore, these immobility and mortality rates increased with the concentration of the extract, contrary to the nematode recovery rates. This would be explained by the increase in the amount of nematicidal compounds in the extract as the concentration increases. Thus, the nematicidal activity of the aqueous extract of dried leaves of castor-oil plant increases with its concentration on nematodes belonging to the *Meloidogyne* and *Pratylenchus* genera.

Except for *Meloidogyne* populations, those of *Pratylenchus* reacted differently to the aqueous extract of dried leaves of castor-oil plant at 50 000ppm concentration. This difference would be explained by the existence of at least one species of *Pratylenchus* in the soils of yam production areas. Indeed, this crop is attacked by several species of *Pratylenchus*, notably *P. coffeae*, *P. sudanensis* (Bridge *et al.*, 2005) and *P. brachyurus* (Muniz *et al.*, 2012).

The high concentrations (from 40 000ppm) of the aqueous extract of the dried leaves of castor-oil plant have a significant *in vitro* nematicidal activity on yam pathogenic nematodes in Côte d'Ivoire, but a

nematostatic activity with low concentrations (less than 40,000 ppm). Thus, the application of this product under field conditions on these nematodes, in the absence of the host plant (yam) has also given satisfactory results.

Under field conditions, soil treatments with Carbofuran followed by products stemming from dried leaves of castor-oil plant (powder and aqueous extract) have reduced the total numbers of *Meloidogyne* and *Pratylenchus*, two pathogenic nematodes of yam found in the soil of the study site. The reduction in the total numbers of *Meloidogyne* and *Pratylenchus* in soils treated with chemical nematicide might be due to the anticholinesterase property of carbofuran. In fact, carbofuran in contact with nematodes might inhibit the activity of acetylcholinesterase, the enzyme responsible for the hydrolysis of acetylcholine (Bertrand *et al.*, 1998). This inhibition causes a disruption of the transmission of nerve impulse, thus creating perceptual disturbances, muscle paralysis or even death of nematodes (Daramola *et al.*, 2013).

The reduction in the total numbers of these nematodes in soils treated with the aqueous extract of dried leaves of castor-oil plant might be due to the presence of a set of secondary metabolites having nematicidal properties. They might directly or not interfere with the vital organs functions of nematodes causing death, hence the reduction in total numbers.

The reduction in the total numbers of nematodes in soils amended with powder of dried leaves of castor-oil plant could be explained in two ways. Once incorporated into the soil, the organic matter decomposes resulting in the release of ammonium ions (NH<sub>4</sub><sup>+</sup>), formaldehyde, phenols and volatile fatty acids (Walker 2004, Wang *et al.*, 2004). Such compounds are toxic to phytopathogenic nematodes, thus reducing their total numbers in soils. These compounds might act individually or in combination to stimulate the proliferation of microorganisms antagonizing phytopathogenic nematodes (Akhtar and Malik, 2000).

The powder formulation of dried leaves of castor reduced the total numbers of *Meloidogyne* and *Pratylenchus* in the same way as Carbofuran in soils. The result obtained in our study shows that dried leaves of castor-oil plant, in powder formulation could be used as a replacement for chemical nematicides in the control of yam pathogenic nematodes. In fact, the organic amendment of soils has the advantage of improving the physicochemical and biological properties of soils, and then improving the growth and development of plants (Renčo, 2013).

### Conclusion

The aqueous extract of dried leaves of castor-oil plant has a strong *in vitro* nematicidal potential on yam pathogenic nematodes in Côte d'Ivoire. Under field conditions, in the soil and in the absence of the host plant, this potential is very high in the reduction of the total numbers of *Meloidogyne* and *Pratylenchus* with the powder formulation of dried leaves of castor-oil plant as well as Carbofuran. This nematicidal potential of the powder formulation of dried leaves of castor-oil plant is to be checked on these nematodes in the presence of the host, in particular yam.

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