



Available Online at EScience Press

International Journal of Phytopathology

ISSN: 2312-9344 (Online), 2313-1241 (Print)

<https://esciencepress.net/journals/phytopath>

IN VITRO ASSESSMENT OF AQUEOUS EXTRACTS OF CASTOR PLANT (*RICINUS COMMUNIS*) AGAINST ROOT-KNOT NEMATODES (GENUS *MELOIDOGYNE*) INFECTING TOMATO

Coulibaly Kafondja Estelle, Assiri Kouamé Patrice, Karamoko Ben, Hortence Atta Diallo*Phytopathology Laboratory, Plant Health Unit, University Nangui Abrogoua, Côte d'Ivoire.*

ARTICLE INFO

Article History

Received: April 12, 2023

Revised: June 16, 2023

Accepted: August 23, 2023

Keywords

Castor plant

Nematicidal activity

Root-knot nematode

Tomato

ABSTRACT

Root-knot nematodes represent a real problem for tomato cultivation throughout the world. The aim of this study was to develop a method of controlling root-knot nematodes with aqueous extracts of castor bean, and to evaluate the effect of these extracts on these agents. Firstly, a sampling which consisted of taking the roots presenting galls and the soil was carried out in a tomato plantation in the region of Tchologo (Côte d'Ivoire) more precisely at Camp Agropastoral Sika (CapSIKA). The analysis carried out showed that there is a significant difference between the number of nematodes in the soil and in the roots of the samples taken, (p -value <0.05). A large number of nematodes were observed in the roots compared to the soil. Then, the extracted nematodes were used for a pathogenicity test to verify their involvement in the appearance of galls in tomato roots. Galls were observed on inoculated tomato plants following this test. Subsequently, the individuals were exposed to the aqueous extract of castor bean (*Ricinus communis*) at concentrations of 100, 75, 50 and 25%, the control group was not brought into contact with the aqueous extract of castor bean. Mortality and immobility rates were determined. A significant difference between the number of immobile and dead individuals in the extracts ($P<0.05$) was obtained following the statistical analyses. The results made it possible to show the nematicidal nature of the aqueous extract of castor bean under controlled conditions (*in vitro*). This extract could be used for the control of *Meloidogyne* after testing its effectiveness in open fields.

*Corresponding Author: Coulibaly K. Estelle**Email: coulibaly7789@gmail.com**© The Author(s) 2023.*

INTRODUCTION

The tomato (*Solanum lycopersium* Mill.) is an annual plant of the solanaceae family. It is cultivated for its fruits rich in minerals, lycopene, carotenoid, vitamins A, C and E (Choudourou *et al.*, 2012; Sawadogo *et al.*, 2015). World tomato production in 2021, according to FAOSTAT amounted to more than 189 million tons. Globally, the top three producing countries are China with 67.63 million tonnes, followed by India with 21.18 million tonnes and Turkey with 13.09 million tonnes. In Côte d'Ivoire, the annual tomato production was 48,804

tonnes in 2021 (FAO, 2021). Tomato cultivation represents a viable economic activity for many rural, urban and peri-urban producers (Djidji *et al.*, 2010). Its consumption is associated with the reduction of several types of cancers and certain cardiovascular diseases (Outis *et al.*, 2016). Despite its nutritional and therapeutic properties, tomato cultivation is faced with many biotic constraints including plant-parasitic nematodes (PPN) and especially root-knot nematodes (RKN).

Although less integrated into research programs, NPPs

are among the most important causes of crop yield reduction (Koffi *et al.*, 2017). Indeed, root-knot nematodes of the genus *Meloidogyne* are highly harmful agents and the most worrying on vegetable crops (Mokrini, 2017) especially tomatoes. Tariq-Khan *et al.* (2020) in their work showed that among several vegetable crops, the severity of the disease caused by root-knot nematodes was very pronounced on tomato roots. Studies have also shown that the plant-parasitic nematodes contribute annually to significant economic losses of approximately \$173 billion in damage to the yield and quality of agricultural production worldwide (Heflish *et al.*, 2021). These nematodes are responsible for producing knots that disrupt nutrient and water circulation, resulting in reduced growth, root system damage and reduced yield (Barbary, 2014).

Several methods are used by producers to control these nematodes, but the most used method is chemical control with synthetic nematicides. However, it has adverse effects for both humans and the environment. To overcome these shortcomings, we are moving more and more towards control methods that are more respectful of the environment and have no effect on producers. Indeed, plant extracts with a nematicidal effect have shown their effectiveness on *Meloidogyne* spp. Studies by Koffi *et al.* (2017), showed that treatments carried out with aqueous extracts of castor bean (*Ricinus communis*), significantly reduced the impact of *Meloidogyne* on gall formation on tomato. In these works, castor beans were dried and ground for the

preparation of the aqueous extract and this extract was brought into contact with root-knot nematodes.

Moreover, El-Nagdi and Youssef (2013) in their work in Egypt, recorded a reduction of 15 to 21% in the number of *M. incognita* in soils and tomato roots from soils treated with aqueous castor extract. The use of castor oil could be a method appropriate alternative control to control *Meloidogyne* spp. Indeed, ricin and ricinusaglutinin contained in castor bean are both capable of strongly adhering to the vital organs of plant-parasitic nematodes and modifying their chemotactic behavior or even causing their death (Jaramillo Orellana, 2019). The general objective of our study was to develop a method of controlling root-knot nematodes with aqueous extracts of castor bean, and to evaluate the effect of these extracts on these agents.

MATERIAL AND METHODS

Sampling site

The sampling was carried out in the Camp Agropastoral Sika (CapSika) (Latitudes 9.6411 and Longitudes -5.3202) located in the locality of Comonkaha. This locality is located in the Sudanian savannah zone, in the Tchologo region (Ferkéssédougou), in the north of Côte d'Ivoire. The Tchologo region is limited to the south by the Hambol region, to the east by the Boukani region, to the west by the Poro region, to the north by Burkina Faso and Mali. Its area is estimated at 40,323 km² (Koffi *et al.*, 2022). The plot visited has an area of approximately 3 Ha. It was subdivided into four subplots to facilitate sampling (Figure 1).

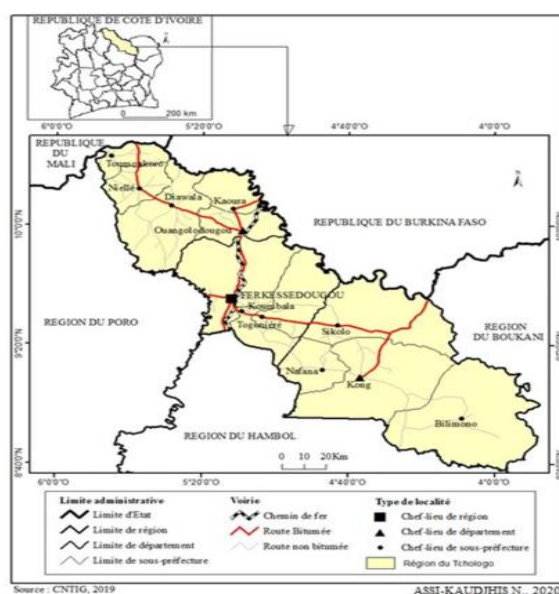


Figure 1. Location of the sampling region (source: CNTIG 2019).

Root-knot nematode inoculum

All species of root-knot nematodes extracted from soil and tomato roots were used in order to have a broad idea of the effectiveness of aqueous extracts on these nematodes (Mukhtar *et al.*, 2021).

Sampling methodology

Sampling of root-knot nematodes in tomato fields was carried out in February 2022. Withered tomato plants were observed on the four subplots, and 30 withered plants with galls on the roots were collected in order to extract the nematodes that were there. About 2 kg of soil was taken from the infested root zones. Then, the soil and tomato roots were packed in plastic bags labeled (date, area and plot) and then transported to the Plant Health Unit laboratory at Nangui University, Abrogoua. The average prevalence of these plants was calculated using formula 1. And finally, the severity of the galls was evaluated using the Zeck (1971) (ranging from 1 to 10), and their average was calculated using formula.

$$PM(\%) = \frac{Pt}{N} \times 100$$

PM: Average prevalence of galls, Pt = number of plants with galls, N = total number of plants selected

$$S(\%) = \frac{\sum(ni \times n)}{Nt \times nie} \times 100$$

S (%): severity of galls, ni: severity score assigned to galls on the plant, n: number of plants to which the score ni was assigned, Nt: total number of plants used, nie: highest severity score recorded in this study.

Extraction and identification of nematodes from soil and roots

Soil samples were sieved and homogenized. Then, the extraction was carried out with 100 g of soil according to the method of Whitehead and Hemming (1965). As for the root samples, they were rinsed with tap water, cut into explants of approximately 2 mm and 100 g were ground in a household blender according to the Baerman method. The experiments were repeated four times.

After 48 hours, the nematodes were collected in 50 ml capacity pillboxes using a 25 μ m mesh sieve, and were counted and described by observation under an optical microscope using the Perry *et al.* (2009) key. This description has only been carried out on a genus scale only in order to have a wide range of nematodes. The following formula was used to calculate the average number of individuals extracted in 50 ml of nematode suspension:

$$EMi = \frac{1}{n} \sum(e_i) \times 10$$

EMi : Average number of individuals of a genus i in 50 ml

of nematode suspension

ei: Number of individuals of genus i in 5 ml aliquot

n: Number of repetitions performed.

Pathogenicity test

Nematodes extracted from tomato roots were used for this test. To this end, juveniles of the second larval stage (J2) of root-knot nematodes were selected because this is the most infectious stage. 20 pots containing autoclaved soil were inoculated with 100 J2 and 30-day-old tomato plants were transplanted into this soil. The pots were placed under an insect shelter within the university. The experiment was repeated three times and, 45 days after transplanting the plants, parameters such as the number of nematodes in the roots and in the soil, the number of galls on the roots, the severity of the galls, the size and finally the number of leaves on the seedlings were collected.

Preparation of the aqueous extract of castor

The seeds were dried for 14 days in the sun and then ground in a mortar. 100 g of the ground product obtained were macerated in 1 L of tap water in a transparent plastic bottle for 72 hours at ambient laboratory temperature ($28 \pm 2^\circ$ C.) and in the dark. After 72 hours, the solution obtained was filtered by a series of three filtrations with a densified layer (approximately 3 cm thick) of absorbent cotton placed in a 60 ml syringe. The cotton layer was renewed each time the crude extract was filtered, and the filtrate was collected in an Erlenmeyer flask. The stock solution was then diluted in order to obtain concentrations of 25% (25 ml of the stock solution + 75 ml of water), 50% (50 ml of the stock solution + 50 ml of water) and 75% (75 ml of the stock solution + 25 ml of water).

In vitro evaluation of the effect of aqueous castor extracts on second stage root-knot nematodes

In order to have a generalized effect of the aqueous extracts, all the root-knot nematodes extracted were used for this test. Volumes of 2 ml of nematode suspension containing an average of 30 J2 individuals (second stage root-knot nematodes) were taken from the nematode suspension after extraction and placed in pillboxes (a total of five pillboxes for each concentration). To these 2 ml were added 2 ml of each concentration of the aqueous extract of castor bean in the pillboxes and separately. Control individuals were exposed to 2 ml of distilled water. The pill boxes were gently shaken and placed in the laboratory at room temperature. The experiment was repeated four times.

After 24 h, mobile and immobile individuals were counted and transferred to distilled water. 24 hours later, another observation was made in order to verify the nematocidal or nematostatic activity of the aqueous extracts of castor bean on the larvae. At the end of the test, the immobility and mortality rates were determined using the following formulas:

$$Ti(E) = \frac{NI(E) - NI(T)}{Nt(E)} \times 100$$

Ti(E) = rate of immobility of the individuals in the extract;
 NI(E) = number of immobile individuals in the extract;
 NI(T) = number of immobile individuals in the control;
 Nt(E) = total number of individuals in the extract.

$$Tm(E) = \frac{NM(E)}{Nt(E)} \times 100$$

Tm = mortality rate of individuals in the extract; NM(E) = number of dead individuals in the extract; Nt(E) = total number of individuals in the extract.

Statistical analysis

The data obtained were analyzed using Statistica 7.1 software. After their transformation into logs, the mean mortality and immobility rates and the number of J2, disease development parameters and agronomic parameters were subjected to one-way analysis of variances (ANOVA). The classes were determined with Fisher's LSD test at the 5% level of significance.

RESULTS

Health status of tomato plantations

Symptoms observed in the field were as follows: color change appearing as chlorosis on the leaves; physiological dysfunction materialized by the withering of certain tomato plants and the reduction of the root system; deformation of the organs materialized by the presence of rounded nodules 1 to 2 cm in diameter (root galls) on the roots and the dwarfism of certain plants (Figure 2). The average prevalence of galls was 91.67%, while their average severity was 75.55%.



Figure 2. A: wilting of the plant B: galls on the roots C: root rot D: reduction of the root system E: discoloration of the leaves F: dwarfism of a foot tomato.

Nematodes extracted from soil and tomato roots

The nematodes obtained after extraction from the soil and the roots were root-knot nematodes (*Meloidogynes*) belonging to different stages. These were eggs, individuals of the 1st, 2nd and 4th larval stage (Figure 3). The data obtained were estimated in 50 ml of nematode suspension using formula 3. In the roots,

the number of individuals of the second stage (J2) was higher than that in the soil. Indeed, in the soil the average number of J2 nematodes was 395.33 ± 46.31 while in the roots it was 83013.33 ± 3194.45 . Statistical analysis showed a significant difference between the workforce average of the nematodes J2 at above and below ground levels.

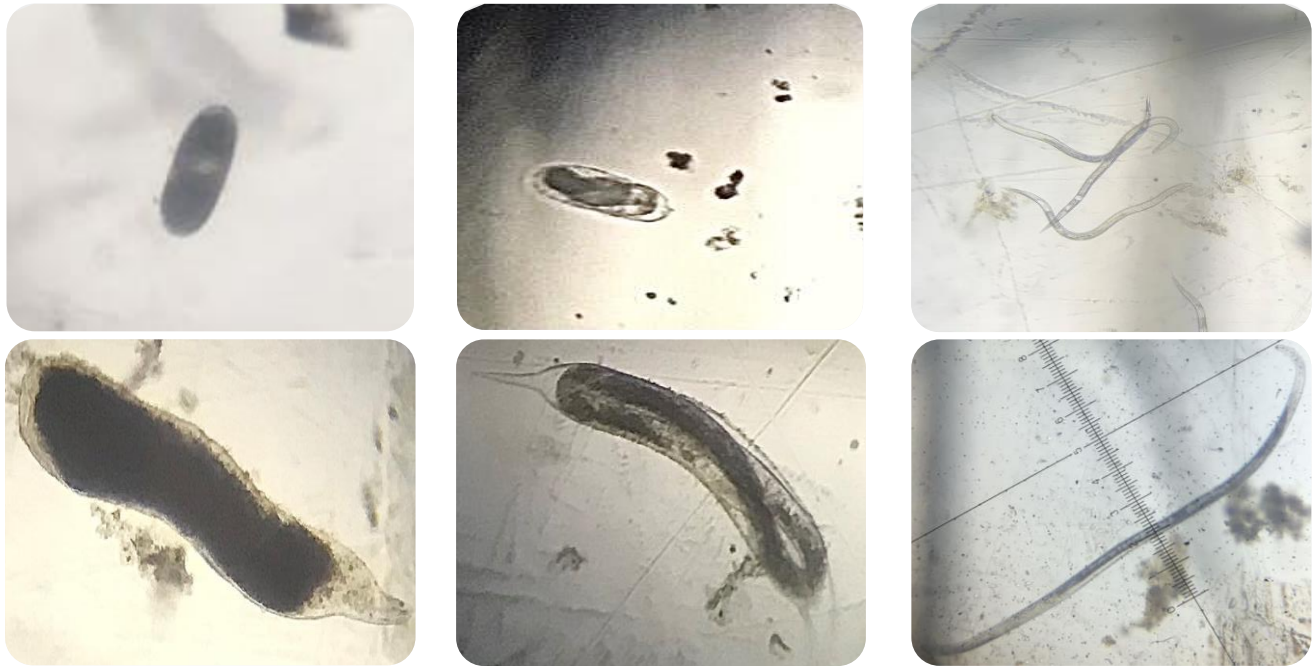


Figure 3. Nematodes extracted from soil and tomato roots (*Solanum lycopersium* Mill.) A: egg; B: stage 1 juvenile (J1); C: stage 2 juvenile (J2); D: stage 4 (J4) female juvenile; E: stage 4 (J4) male juvenile; F: adult male

Pathogenicity of extracted nematodes

The symptoms observed in the field on the underground part of the tomato plants (namely the galls) were identical to those observed following the pathogenicity test. Discolorations were also observed on the leaves of these plants. In addition, the nematodes extracted from these roots were the J2 nematodes of *Meloidogyne* which constituted our inoculum.

After the pathogenicity test, no presence of galls was observed on the plants coming from uncontaminated soil. On the other hand, galls were observed on the plants coming from the contaminated soils. Statistical analysis showed a significant difference between the average number of galls, the severity and the number of nematodes between plants from inoculated soils and those from non-inoculated soils (Table 1).

Table 1. Parameters observed on tomato plants grown or not on soil contaminated with *Meloidogyne* spp. at the end of the test.

Treatments	Average number of J2 (ground)	final number of J2s (root)	Average final number of J2s (root)	Average severity	gall	Average gall count	Average height (cm)	Average number of leaves
Control (T0)	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	95.72± 2.05 ^a	78.79± 5.18 ^b
Treated (T1)	43.94 ± 3.22 ^b	26746.91 ± 1122.36 ^b	3,08±0,16 ^b	61,09±4,1 ^b	78,79 ± 5,18 ^b	50,33±5,96 ^b		
P	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05	< 0,05

In each column, the averages assigned the same letter are statistically similar according to the Fisher LSD test at 5% threshold; T0: control plants; T1: plants whose soil has received the inoculum.

Regarding the average height and the average leaf number of the plants, they were significantly higher for the plants grown on the uncontaminated soil and lower in the plants grown on the contaminated soil. Statistical analysis showed a significant difference between the mean number of leaves of inoculated and non-inoculated plants (Table 1).

In vitro* effect of aqueous extract of castor bean on J2 of *Meloidogyne

Aqueous extracts of castor had a nematicidal effect on root-knot nematodes J2. The immobility rates of

Meloidogyne J2 nematodes varied according to the concentrations of the aqueous extracts of castor. The highest immobility rates were obtained with the 100% crude castor extract. The same result was observed for the mortality rates which were different depending on the concentrations of the aqueous extract of the plant. Mortality rates were higher in either crude extract than in other aqueous extracts. Statistical analysis showed a significant difference between the different rates depending on the concentrations (Table 2).

Table 2. Mean rate of immobility and mortality of J2 larvae of *Meloidogyne* spp. depending on the different concentrations of aqueous extracts of castor.

Concentration extracts aqueous	Tim (%)	Tm (%)
T-	0	0
25%	65.12± 1.49 ^a	56.42± 1.87 ^a
50%	68.39± 1.35 ^a	63,59 ± 1,12 ^b
75%	81,16 ± 2,38 ^b	72,38 ± 2,32 ^c
100%	100	92,31 ± 1,54 ^d
<i>p</i>	< 0,05	< 0,05

In each column, the averages assigned the same letter are statistically similar according to the Fisher LSD test at 5% threshold; Tim = Average immobility rate of *Meloidogyne* spp. larvae; Tm = Average mortality rate of *Meloidogyne* spp. larvae; T- = control.

DISCUSSION

During this study, different symptoms were observed in the tomato plantations visited. These include root galls, root rot, wilting of plants, discoloration of leaves, reduction of the root system and dwarfing of some plants. Then, the nematodes extracted from the soil and root samples were the nematodes of the genus *Meloidogyne* at different stages of development. Indeed, the nematodes penetrate the roots thus causing the formation of galls which prevent the diffusion of water and nutrients to the rest of the plant. This consequently causes dwarfing of the plants and reduction in yield. The plants also become vulnerable to other stress factors such as drought or disease (Asghar *et al.*, 2020; Aslam and Mukhtar, 2023).

The pathogenicity test made it possible to extract these same agents following the presence of these same symptoms. According to Eder *et al.* (2010) gall formation is a typical symptom of attack by nematodes belonging to the genus *Meloidogyne*. These are caused by the juveniles of the second stage (J2) which represents the only mobile and exophyte stage of *Meloidogyne* spp.

Indeed, second-stage juveniles (J2) attack the vascular bundles of plants and produce multinucleated giant cells for an uninterrupted supply of nutrients (Jones *et al.*, 2013). They weaken plant cells by secreting cell wall degradation enzymes to facilitate intercellular migration. Once the vascular system of the plant has been reached, these nematodes will then become sedentary and induce the dedifferentiation of plant cells into giant cells (Subedi *et al.*, 2020). In short, the infected plants then show symptoms on the aerial part (wilting of the plants, yellowing of the foliage, stunting of the plant) and on the underground part (lesions or root necrosis, reduction of the root system, proliferation of secondary roots and the presence of galls) (Haougui *et al.*, 2013). The results of the tests *in vitro* revealed the nematicidal effect of aqueous extracts of castor bean on nematodes. Studies have shown that this extract had nematicidal activity (*in vitro*) at many different concentrations, which reduces egg hatch and increases J2 mortality (Frederick *et al.*, 2015; Siddiqui and Alam, 1987). This could be due to the toxic and nematicidal properties contained in castor oil. The inhibitory effect of medicinal

plants according to Adegbite and Adesiyan (2006) could be due to the chemicals present in the extracts which possess ovicidal and larvicidal properties. Indeed, castor bean tissues release toxic compounds capable of strongly adhering to the amphidia of plant-parasitic nematodes and modifying their chemotactic behaviors (Jaramillo Orellana, 2019). In addition, ricin, which is the main toxin in castor seeds, is a phytotoxin protein capable of inhibiting protein synthesis (Audi *et al.*, 2005).

The 100% concentration of castor extract recorded the greatest rate of immobility and mortality. This increase in the rate of immobility and mortality could be explained by the high concentration of compounds with nematicidal potential in the extract. Studies carried out by Kepenekci *et al.* (2016), also showed that juvenile mortality and egg hatch inhibition increased with increasing concentration and exposure time.

CONCLUSION

The present study shows the effectiveness of aqueous extracts of castor bean on root-knot nematodes. The highest concentration of this extract resulted in the highest mortality rate for these agents. These extracts could therefore be used against nematodes next to the tomato.

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