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Effect of Neem (*Azadirachta indica*) and Castor (*Ricinus communis*) Leaf Extracts on Root-knot Nematodes of the Genus *Meloidogyne* in Tomato (*Solanum lycopersicum*) Culture

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Authors' contributions

This work was carried out in collaboration among all authors. Author CKE conceived the study, and wrote it in its entirety. All authors read and approved the final manuscript.

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ABSTRACT

Aims: During production, root-knot nematodes of the *Meloidogyne* genus, probably the most harmful, attack tomatoes. Given the harmful effect of certain nematicides used to control this nematode, the use of plant extracts could be an alternative for sustainable tomato production. The

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aim of this study was to evaluate the efficacy of neem and castor leaf extracts on root-knot nematodes in the field.
Place and Study Duration: Côte d'Ivoire, from April to August 2023.
Methodology: A plot previously infested with root-knot nematodes set up using a randomized complete block design. Neem and castor extracts are applied at a dose 100 g per tomato plant and furasol at 5 g, planted once a month until harvest. The density of root-knot nematodes was estimated in the soil each month before extract application and in tomato roots at harvest. Parameters such as severity and prevalence of galls, nematode density in soil and roots, plant height and fruit yield were measured during the study.
Results: The prevalence and severity of root galls on tomato plants were statistically identical on plants treated with furasol and castor (P>0,05). The results showed that neem and castor extracts had similar effects to furasol on tomato plants regarding plant height (from 72,40 ± 18,38 to 78,40 ± 11,27) and fruit yield (from 4,20 ± 0,63 to 4,80 ± 0,42). These plant extracts induced a low rate of multiplication of these nematodes in the same way as furasol, and reduced the number of nematodes in the soil and roots of treated tomato plants.
Conclusion: The results obtained in this study are encouraging, and the long-term use of these plant extracts could be a sustainable control method against root-knot nematodes.

Keywords: Meloidogyne; plant extracts; prevalence; severity; tomato.

1. INTRODUCTION

Market garden produce plays a vital role in the general diet of human beings. Among these products, tomatoes are an important source of minerals, essential amino acids, sugars, dietary fiber and vitamins, as they contain vitamin A in the form of carotene, vitamin B1 (thiamine), B2 (riboflavin), niacin and vitamin C (Sesso et al., 2002). World production in 2022 amounted to over 186 million tonnes, compared with 44578.83 tonnes for Côte d'Ivoire (Faostat, 2022). Although tomatoes are important both nutritionally and economically, biotic and abiotic factors limit their growth (Oluwatayo et al., 2019). During cultivation and after harvest, tomatoes can be affected by over 200 pathogens, including fungi, bacteria, viruses, and viroids, and parasitic nematodes (Panno et al., 2021). In sub-Saharan Africa, particularly Côte d'Ivoire, tomatoes are attacked by several pests, including nematodes (Doga et al., 2022). These are microscopic worms, mostly in the soil, parasitising plants. They severely infect almost all crops worldwide, causing an estimated annual global crop loss of around 80 billion USD (Jones et al., 2013). Historically, the main method of controlling nematode infestations was chemical control, such as the fumigant methyl bromide (Desaeger et al., 2020). These chemicals are known to have a negative impact on the crop environment, such as a decrease in the distribution of beneficial microorganisms in the soil, an increase in chemical-resistant pests, environmental pollution due to residual toxicity and toxicity to users (Kiewnick and Sikora, 2006) and persistence of chemicals in the environment (Swaruparani et al*.,* 2024) . Recently, alternative products for nematode control have come into use (Jagdale and Grewal, 2002). These include botanical extracts with a nematicidal effect, which are one of the potential alternatives for sustainable control of root-knot nematodes (Gahukar, 2012). This natural pest control strategy can improve both soil quality and human health by reducing harmful residues or contamination (Hulot and Hiller, 2021). Authors such as Alam (1989) and Mojumder (1995) have confirmed the efficacy of soil-incorporated neem (*Azadirachta indica*) and castor (*Ricinus communis*) cakes against *Meloidogyne* spp, as well as other plant-parasitic nematode species. The aim of the present study was to evaluate the efficacy of neem and castor leaf extracts on root-knot nematodes in the field.

2. MATERIALS AND METHODS

2.1 Material Composition

The material used in this study consisted of neem (*Azadirachta indica*) and castor (*Ricinus communis*) leaves, as well as the chemical nematicide (furasol) for the treatments and tomato seeds of the Cobra 26 variety.

2.2 Choice of Plot

The choice of experimental plot is based on its culture history. Previous use of this plot for tomato cultivation revealed heavy infestation by root-knot nematodes. Before setting up the trial, soil samples were taken and analyzed in the laboratory, to assess the proportion of root-knot nematodes in 100 g of soil.

2.3 Preparation of Neem and Castor Extracts

The extracts used in this study were castor and neem leaf powders. The preparation of these extracts involves harvesting fresh leaves from each plant, followed by drying in the shade for 2 weeks, then ground to powder using a blender. The dose of 100 g/plant for the tests is chosen for each powder.

2.4 Setting Up the Experimental Plot

The experiment was carried out on a 282 m^2 (23.5 m × 12 m) plot from April to August 2023, following a complete randomized block design with four blocks separated by 1.5 m each. Each block consisted of four elementary plots measuring 2 m × 2 m. Each elementary plot comprised thirty plants planted in the. The individual pots were spaced 0.5 m apart on the row and between rows. Each elementary plot thus comprised 30 tomato plants.

A nursery was set up by sowing seeds in honeycomb plates containing soil amended with 50 g of each plant extract and 2 g for the synthetic nematicide. In the case of control plants, the soil was left untreated.

At the same time, the plant extracts were applied to the planting plots at a depth of 10 cm, depending on the treatment. The plots watered daily to promote the decomposition of the plant extracts. Twenty-one days later, the vigorous seedlings transplanted into the pits. The treatments carried out were as follows:

- T^-: tomato plants not treated with plant extracts or synthetic nematicides;
- T^+: tomato plants treated with synthetic nematicide (furasol);
- T_{Fn}: tomato plants treated with neem leaf powder;
- T_{Fr}: tomato plants treated with castor leaf powder.

The experiment was carried out under natural conditions, without the applying of chemical fertilizers, and repeated twice.

After transplanting the tomato seedlings, 100 g of plant extracts were applied monthly to the plants until harvest to assess their efficacy against root-knot nematodes. For furasol, 5 g were applied.

2.5 Determination of Nematode Density as a Function of Treatments Carried Out

The density of root-knot nematodes estimated in soil and tomato roots. In the case of soil, this density was determined each month before the application of plant extracts, to assess the evolution of nematode density in 100 g of sample. For this purpose, 10 plants per elementary plot were randomly selected and soil samples were taken from their roots. The density of nematodes in the roots are determined at harvest on the same tomato plants in 100 g of root samples.

2.6 Determination of Tomato Agronomic Parameters According to Treatments

The following parameters are determined at harvest on 10 randomly selected plants per elementary plot:

- Plant height, measured using a tape measure;
- Fruit yield, determined according to treatments using the following formula:

$$\text{Yield (t/ha)} = \frac{\text{Total weight of harvested fruit (t)}}{\text{Surface area (ha)}}$$

- Degree of fruit firmness, determined on a scale from 1 to 5 (1 = completely soft fruit, 2 = soft fruit, 3 = moderately firm fruit, 4 = firm fruit, 5 = very firm fruit) from Noupé et al*.* (2019).

In addition to these agronomic parameters, the number of nematode-induced galls on tomato plant roots are determined by counting, and the nematode multiplication rate was determined using the following formula:

$$\text{Tm (\%)} = \frac{\text{Nnf}}{\text{Nni}} \times 100$$

Tm: multiplication rate;
Nnf: final number of nematodes;
Nni: initial number of nematodes.

2.7 Evaluation of the Severity and Prevalence of Galls on the Root System of Tomato Plants According to Treatments

In order to assess the effect of the different treatments on root-knot nematode damage on treated tomato plants, severity and prevalence were assessed on their root systems at harvest.

2.7.1 Assessment of gall prevalence

Gall prevalence is assessed using an X pattern, i.e. by selecting 60 plants along the two diagonals of each plot. On each diagonal, 30 plants of roughly equal distance are selected. The number of plants showing galls was then recorded, and the prevalence of this symptom was calculated using the following formula:

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

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

2.7.2 Assessment of gall severity

The Zeck scale (1971) was used. This scale corresponds to a rating of root systems from 0 to 10, based on observation of the state of the root system, and reflects the reality of symptomatological situations observed on tomatoes. It is summarized as follows:

0 = root system uttery free of galls; 1 = a few rare and small galls detected during close observation; 2 = a few rare and small galls easily detected; 3 = numerous small galls; 4 = numerous small galls, a few large galls ; 5 = 25% of root system gall-affected and non-functional (no rootlets); 6 = 50% of root system gall-affected; 7 = 75% of root system gall-affected; 8 = no root unaffected, plant still green; 9 = root system decaying; 10 = plant dead. The severity of the galls is calculated using the formula of Mckinney (1923):

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

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

2.8 Statistical Analysis

The data collected in this study were analyzed using SPSS version 26 software. Tests of homogeneity of variance and distribution of data were used to determine the choice between a parametric or non-parametric test. The severity and prevalence of galls, the number of nematodes in soil and roots, nematode multiplication rates, plant height, fruit yield, and fruit firmness are analyzed using the non-parametric Kruskal-Wallis test (comparison of several means). In the event of a significant difference, at the 5% threshold, the Mann-Whitney U test was used to compare modalities in pairs to form homogeneous groups.

3. RESULTS

3.1 Description of Plants Obtained According to Treatments

The tomato plants obtained showed different aspects. Plants treated with neem, castor, and furasol generally showed green leaves with pretty vigorous stems (Fig. 1 A) and firm fruit (Fig. 1 B). The roots of these plants showed small galls that were barely detectable at first glance. Untreated plants, on the other hand, showed a rather different appearance for the most part. They showed yellow leaf discoloration, dwarfing on some plants (Fig. 1 C), and galls on the roots (Fig. 1 D).

3.2 Agronomic Parameters Obtained According to Treatments

These parameters changed with each treatment.

Plant height varied from 48.60 ± 17.50 cm to 78.40 ± 11.27 cm. Plant height was highest in plants treated with castor extract, at 78.40 ± 11.276. However, no difference was observed in plants treated with neem, castor, and furasol. On the other hand, statistical tests revealed a significant difference between these three treatments and the control (Table 1).

Regarding fruit firmness, the plants treated with furasol, neem, and castor were firmer. With these three products, firmness was greater than 4. Statistical tests showed a significant difference between the firmness of fruit obtained with these three extracts and fruit from untreated plants (Table 1).

Tomato plant yields ranged from 10.32 ± 2.89 t/ha to 17.80 ± 2.54 t/ha (Fig. 2). untreated plants showed the lowest yield. On the other hand, higher yields were observed in plants treated with castor extract, followed by those treated with neem and finally furasol. However, the yields obtained from plants treated with these three products were statistically identical. Nevertheless, the yields obtained with these three products were statistically different from those obtained with untreated plants (Fig. 2).

3.3 Prevalence and Severity of Symptoms Observed

The prevalence of nematode-induced galls ranged from 30.33 ± 2.94% to 71.23 ± 3.62% according to the different treatments.

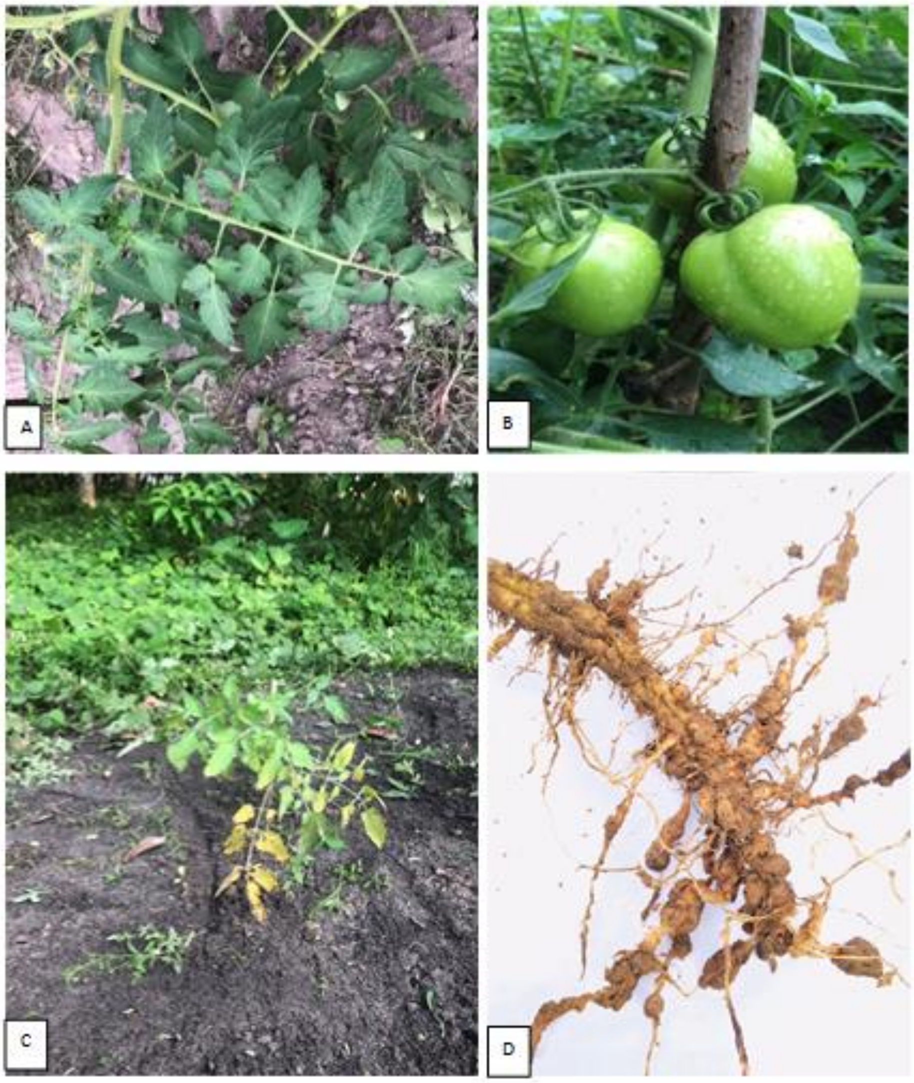

Fig. 1. Aspect of tomato plants observed according to extract treatments

A: leaves of a tomato plant treated with neem; B: tomato fruit of a plant treated with castor; C: untreated plant showing symptoms of leaf yellowing; D: galls on the roots of an untreated plant

Table 1. Tomato plant height and fruit firmness as a function of treatments

Traitements	Plant height (cm)	Fruit firmness
T^- (Indicator)	48,60 ± 17,506[a]	3,02 ± 0,48[a]
T^+ (Furasol)	72,40 ± 18,38[b]	4,20 ± 0,632[b]
Neem	73,00 ± 17,506[b]	4,80 ± 0,422[b]
Ricin	78,40 ± 11,276[b]	4,70 ± 0,483[b]

In each column, means marked with the same letter are statistically similar according to the Mann-Whitney U test at the 5% threshold
p = probability value

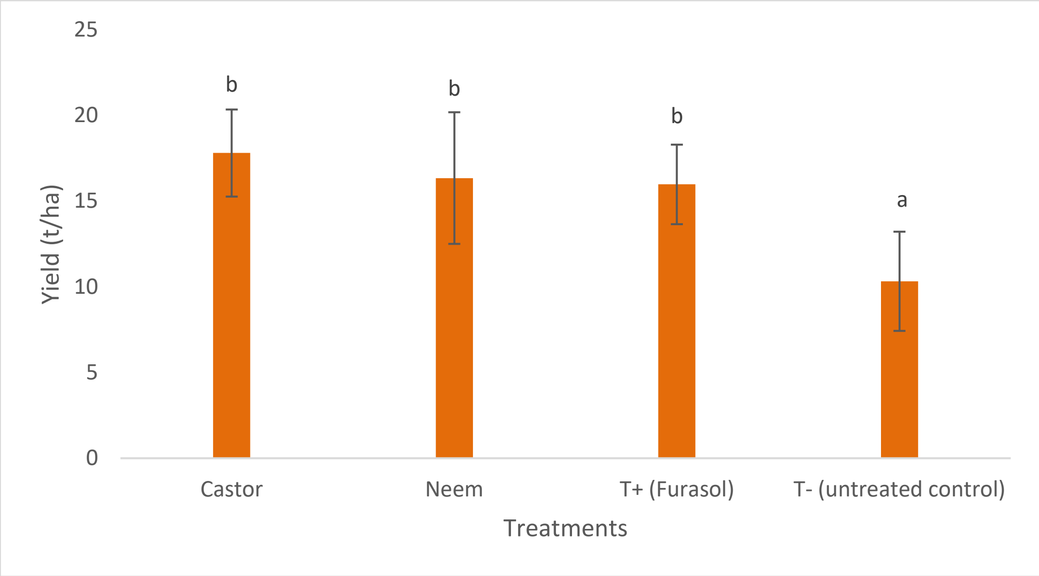

Fig. 2. Average yield of tomato fruit according to treatments carried out

Means marked with the same letter are statistically similar according to the Mann-Whitney U test at the 5% threshold

The prevalence of nematode-induced galls was higher in untreated tomato plants (controls) than those treated with aqueous extracts of neem, castor, and furasol. In the case of treated plants, the prevalence of galls was statistically lower in those treated with castor and furasol than in those treated with neem (Table 2).

The severity of galls varied from 25.89 ± 1.33% to 60.51 ± 2.58%, depending on the different treatments. Untreated tomato plants, showed the highest severity. Statistical analysis showed a significant difference between the severity of these plants and those treated with neem, castor and furasol. On the other hand, tomato plants treated with castor and furasol had statistically lower severities than those treated with neem (Table 2).

3.4 Evolution of Nematode Density in the Soil and their Numbers in the Roots

At harvest, the number of nematodes in the roots of tomato plants with galls ranged from 4380.67 ± 47.39 to 16280.35 ± 1327.734. Untreated tomato plants, showed the highest value. Statistical analysis showed a significant difference between the number of nematodes extracted from tomato roots of untreated plants and those treated with neem, castor, and furasol ($P<0.05$). In contrast, the number of nematodes extracted from tomato plants treated with neem, castor, and furasol was statistically identical (Table 3).

The rate of nematode multiplication in roots was higher in untreated plants than in those treated with neem, castor, and furasol. Statistical analysis confirmed a significant difference in nematode multiplication rates between untreated plants and those treated with neem, castor, and furasol. For all three treatments, the multiplication rate was statistically identical (Table 3).

The density of nematodes in the soil varied according to the sampling periods and treatments. In fact, density increased over time for tomato plants that had not undergone any treatment. It rose from 176 ± 15.05 in the first month to 673 ± 84.72 in the fourth month. On the other hand, in plants treated with neem, castor, and furasol, a decrease in density is observed from the first to the fourth month after sowing. For tomato plants treated with castor, density fell from 86 ± 10.75 to 70 ± 8.16. For plants treated with neem, density was 91 ± 16.63 in the first month after sowing and 69 ± 7.37 in the fourth month after sowing. For plants treated with furasol, density fell from 99 ± 13.70 to 76 ± 9.66 between the first and fourth months (Fig. 3).

Statistical analysis showed a significant difference in nematode density between untreated tomato plants and those treated with neem, castor, and furasol. However, no significant difference is observed between tomato plants treated with neem, castor and furasol at the different sample collection periods.

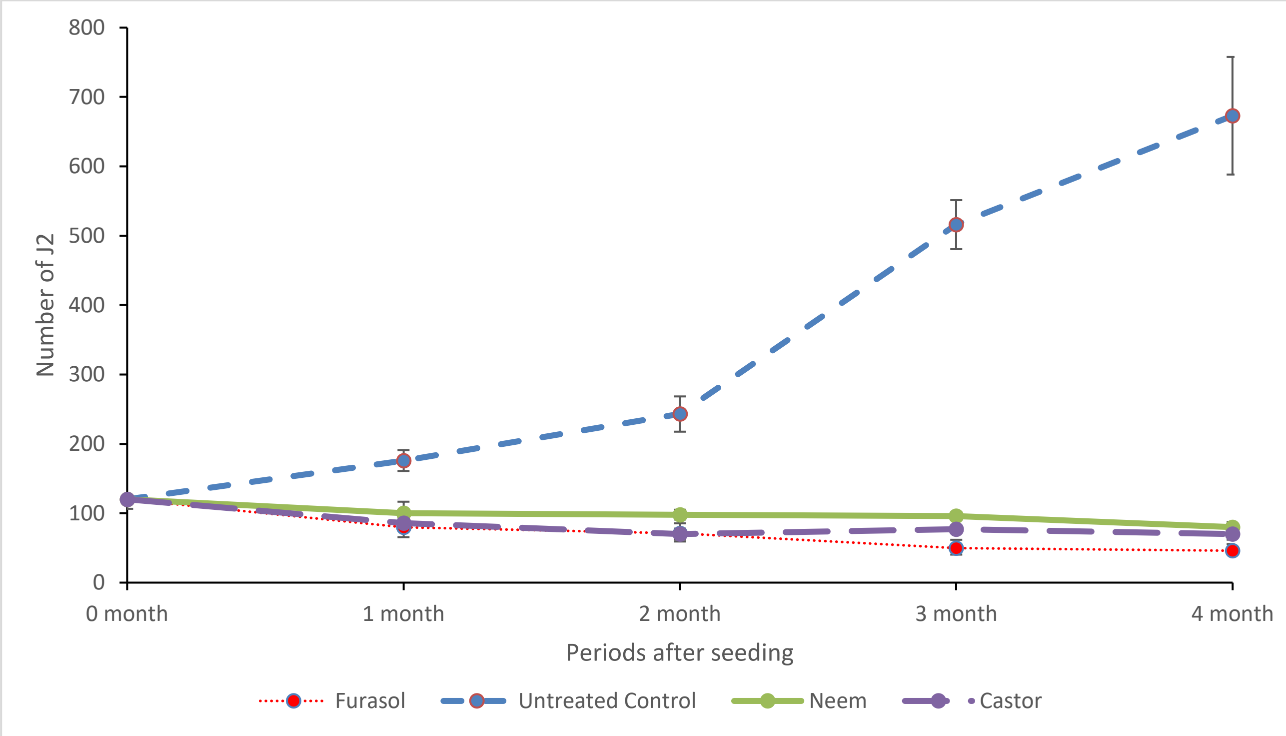

Fig. 3. Number of *Meloidogyne* J2 in the soil according to treatment and period after seeding

Table 2. Mean prevalence and severity of galls caused by nematodes on tomato roots according to treatments carried out

Treatments	Prevalence (%)	Severity (%)
T^- (Conrol)	$71{,}23\pm 3{,}62^c$	$60{,}51 \pm 2{,}58^c$
T^+ (Furasol)	$30{,}33\pm 2{,}94^a$	$26{,}62 \pm 1{,}03^a$
Neem	$35{,}43\pm 2{,}55^b$	$30{,}5 \pm 1{,}67^b$
Ricin	$30{,}67 \pm 3{,}91^a$	$25{,}89 \pm 1{,}33^a$

In each column, means marked with the same letter are statistically similar according to the Mann-Whitney U test at the 5% threshold
p = probability value

Table 3. Number of nematodes extracted from the roots of tomato plants and their reproduction rate as a function of the treatments applied

Treatments	Number of nematodes in roots	Nematode multiplication rate
T^- (Control)	$26280{,}35 \pm 1327{,}73^c$	$2{,}24 \pm 0{,}52^b$
T^+ (Furasol)	$4380{,}67 \pm 47{,}39^a$	$0{,}37 \pm 0{,}00^a$
Neem	$5860{,}53 \pm 50{,}35^b$	$0{,}49 \pm 0{,}00^a$
Ricin	$5381{,}33 \pm 11{,}66^b$	$0{,}45 \pm 0{,}00^a$

In each column, means marked with the same letter are statistically similar according to the Mann-Whitney U test at the 5% threshold
p = probability value

4. DISCUSSION

In the field, neem and castor leaf extracts, together with the chemical nematicide furasol, reduced the number of nematodes in the soil and roots of tomato plants, as well as their rate of multiplication. Neem contains bioactive substances that may be responsible for nematicidal activity (Danahap et al., 2024). Indeed, neem contains azadirachtin, which may induce nematostasis, a process that prevents nematodes from invading plants without killing them directly, according to Rehma et al*.* (2009). Such an effect was observed by Gharras et al. (2011) in zucchini. These authors showed that treatments with castor oil cake, argan oil cake, argan leaves and neem oil cake reduced nematodes in the soil by more than 25% and the gall index by more than 50%. Affokpon et al*.* (2012) also obtained similar results in their study. They showed that using neem seed derivatives significantly reduced the rate of root-knot nematode multiplication in tomato and more excellent nightshade crops. Similarly, this rate reduced by 81% in plots of greater nightshade treated with neem seed derivatives. Castor powder also reduced the root-knot nematodes of the *Meloidogyne* genus in tomato plots, with a rate of around 51% compared with that of untreated plots.

These powders also improved certain agronomic traits of the plants as well as tomato fruit yield. For Khan et al. (2012), the improvement in crop yields on root-knot nematode-infested soil following neem derivatives was due to nematode control and improved soil fertility levels. For Akhtar and Alam (1993), the triterpene compounds in neem seeds inhibit the nitrification process and increase the quantity of available nitrogen, in addition to their nematicidal effects, which explains the plants growth and good yield. Gharras et al. (2011) showed that argan, neem and castor oil cakes resulted in significant vegetative growth. Indeed, adding these extracts significantly improved plant height and tomato fruit yield. Moreover, an increase in plant height compared with the control is observed with castor oil and neem.

5. CONCLUSION

The study showed that neem and castor leaf extracts significantly reduced the evolution of nematodes in the soil and in tomato roots, and their effect was comparable to that of the synthetic nematicide used. These extracts also improved specific agronomic parameters, such as fruit yield and firmness, as well as the size of treated tomato plants. These plants offer a promising option for sustainable control of root-knot nematodes.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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