OPEN ACCESS Plant Protection, 10 (04) 2026. 707-720 DOI:10.33804/pp.010.04.5927

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Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

Research Article

Integrated Management of *Meloidogyne* spp. in Cucumber Using Plant Extracts

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ARTICLE INFO

Article history
Received: 8th February, 2026
Revised: 10th July, 2026
Accepted: 14th July, 2026

Keywords
Meloidogyne spp.
Root-knot nematode
Plant extracts
Biological nematicides
Sustainable nematode management

ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) are among the most destructive plant-parasitic nematodes, and the search for environmentally friendly alternatives to synthetic nematicides is essential. This study evaluated the *in vitro* and greenhouse nematicidal potential of aqueous extracts of selected plant species against *Meloidogyne* sp. juveniles and their effects on cucumber growth. *In vitro* assays revealed significant differences among treatments ($P < 0.0001$), with *Tithonia diversifolia* and *Ricinus communis* extracts at 100% concentration causing the highest corrected juvenile mortalities of 64.07% and 57.24%, respectively, after 72 h. Oxamyl showed comparatively lower mortality (51.27%). Greenhouse experiments demonstrated significant reductions in *Meloidogyne* sp. populations in cucumber roots and soil following extract applications. The nematode population densities in roots were significantly reduced, with the highest reduction rate reaching 96.93% in treated plants compared with untreated controls. Root gall index and severity were also significantly affected by treatments, with the lowest galling observed in the most effective treatment (gall index 0.67 and severity 6.67%). However, plant growth parameters, including stem height, collar diameter, and number of leaves, were not significantly influenced by treatments. Overall, aqueous extracts of *T. diversifolia* and *R. communis* demonstrated promising nematicidal activity against *Meloidogyne* sp. and may serve as potential eco-friendly alternatives for integrated management of root-knot nematodes. Further studies are required to identify active compounds and validate their field-scale efficacy.

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Introduction

The cucumber (*Cucumis sativus* L.), a plant belonging to the Cucurbitaceae family, is one of the most widely grown and consumed vegetables globally (Samba et al., 2024). Due to its significant nutritional value, this vegetable is utilized in food, cosmetics, and medicines (Aminu-Taiwo, 2017; Romo-Tovar et al., 2024). Grown across many tropical and subtropical countries (Blasco, 2016; Yao et al., 2021), it ranks fourth among vegetables worldwide after tomatoes, cabbage, and onions (Ismael and Mahmood, 2020). Annual global production is estimated at over 87 million tons, while production in Côte d'Ivoire is estimated at 20,550 tons (FAOSTAT, 2024).

However, cucumber production is threatened by numerous pests, including plant-parasitic nematodes, among which root-knot nematodes of the genus *Meloidogyne* are the most damaging. Root-knot nematodes are sedentary parasites of plant roots. By targeting the vascular system, they cause nutrient deprivation and impair water transport, leading to above-ground symptoms such as stunted growth, wilting, chlorosis, and reduced crop yields (Tapia-Vázquez et al., 2021). They are particularly difficult to manage due to their subterranean lifestyle. Nematodes of this genus cause significant economic losses in cultivated plants, especially vegetable crops (Anwar and McKenry, 2012). Damage caused by *Meloidogyne* sp. in cucumber crops has been estimated at 80% in Afghanistan (Nimgarri et al., 2023). In Côte d'Ivoire, this nematode was identified at a frequency of 91% in roots and 67% in soils across cucumber plots spread over six agroecological zones (Yao, 2025).

To limit losses caused by plant-parasitic nematodes, chemical control using synthetic nematicides remains the most common method. However, despite their effectiveness, these synthetic products present major disadvantages for health and the environment due to their high toxicity (Cayrol et al., 2010). This situation has led to the regulatory withdrawal of many active substances from the market (Barroso, 2012).

To improve environmental and food safety, alternative or complementary methods to chemical control are being implemented for the sustainable management of plant-parasitic nematodes. These include the use of plant extracts with nematicidal effects (Njenga et al., 2019). In France, asparagus (*Asparagus officinalis*) has been evaluated as a trap plant against *Meloidogyne* sp. (Védié and Madani, 2009). In Nigeria, extracts from neem (*Azadirachta indica*) and vernonia (*Vernonia amygdalina*) leaves were shown to kill 95% and 86.8% of *Meloidogyne* populations, respectively (Afouda et al., 2012). In addition to being effective, nematicidal plant extracts improve crop quality, decompose quickly, cause minimal environmental pollution, and are readily available locally (Yarou et al., 2017).

In Côte d'Ivoire, the high pressure of phytoparasitic nematodes on vegetable crops, the limited amount of specialized nematological research, and the urgent search for environmentally friendly control methods motivated this study. The objective of this study was to evaluate the nematicidal effect of extracts from *Ricinus communis*, *Tithonia diversifolia*, *Crotalaria retusa*, and *Chromolaena odorata* both *in vitro* and *in vivo* alone and in combination with a half-dose of oxamyl against *Meloidogyne* sp. in greenhouse cucumber crops.

Materials and methods

Plant material

The botanical material comprised four local plants chosen for their proven availability, accessibility to local farmers, and documented fungicidal or insecticidal effects: *Ricinus communis*, *Tithonia diversifolia*, *Crotalaria retusa*, and *Chromolaena odorata*. Leaves were the primary organ used. Greenhouse trials were conducted under semi-controlled conditions in a shade house using the F1 TOKYO cucumber variety. This variety was selected because it is widely cultivated by vegetable growers and is highly susceptible to root-knot nematodes (Yao, 2025).

Animal material

The biological material consisted of egg masses and second-stage juveniles (J2) of root-knot nematodes (*Meloidogyne* sp.). These nematodes were isolated from root galls collected from commercial lettuce plots on the shores of the lakes of Yamoussoukro, located in central Côte d'Ivoire. This site was selected due to its history of intensive agriculture and continuous rotation of susceptible crops, which strongly favours the development of *Meloidogyne* sp. populations (Djian-Caporalino et al., 2009).

Chemical product

A commercial synthetic nematicide formulated with Oxamyl (30 g/kg) was used as the positive reference control. This is the most widely used approved active ingredient in Côte d'Ivoire against nematodes in vegetable crops.

Preparation of powdered and aqueous extracts

Fresh leaves of *R. communis*, *T. diversifolia*, *C. retusa*, and *C. odorata* were shade-dried for two weeks and ground into a fine powder. For extraction, 50 g of powder from each plant was mixed with 500 mL of distilled water in 1.5-liter jars. The mixtures were agitated and left to stand for three days, with manual shaking every 6 h to prevent sedimentation. After 72 h, the solutions were filtered three times through a funnel lined with 3 cm thick cotton wool to yield purified aqueous extracts at a stock concentration of 100 g/L. These solutions were sequentially diluted using a two-fold serial dilution method to obtain concentrations of 50%, 25%, 12.5%, and 6.25% (corresponding to 50, 25, 12.5, and 6.25 g/L, respectively).

Preparation of inoculum

Infested lettuce roots displaying severe galling were transferred to the Agronomy, Forestry, and Crop Protection Laboratory at the INP-HB northern site. Egg masses were carefully detached from the roots and placed in Petri dishes containing distilled water (Figure 1). After 48 h, emerged juveniles were collected in graduated beakers. A 1 mL aliquot of the suspension was sampled to determine the population density using a gridded counting plate mounted on a MOTICAM X3 (TECLAST, China) optical microscope. The suspension was subsequently calibrated with distilled water to achieve an inoculum density of 100 ± 10 juveniles per 4 mL aliquot.

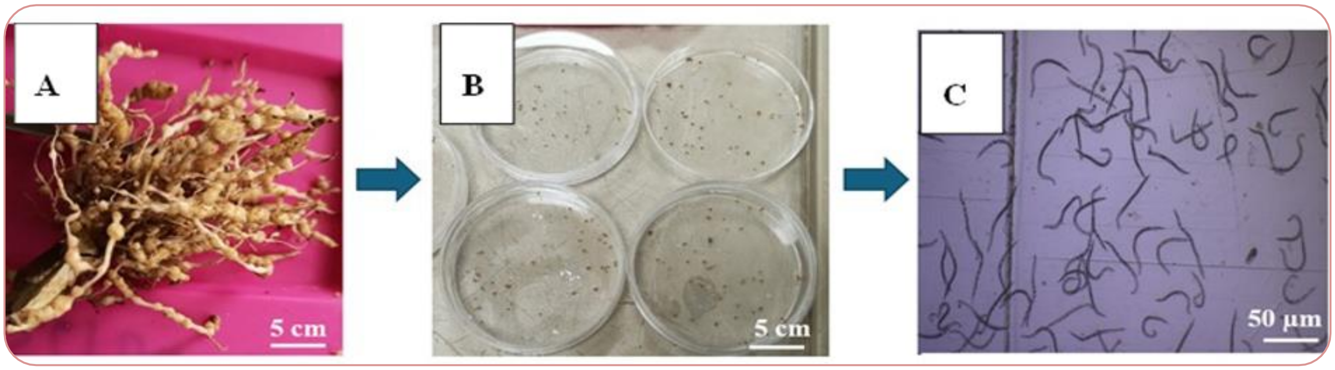

Figure 1. Inoculum preparation steps. (A) Roots galls. (B) Eggs masses in Petri dishes. (C) *Meloidogyne* sp. juveniles.

***In vitro* evaluation**

Experimental setup

A completely randomized design (CRD) with four replicates was used for the laboratory efficacy testing of the aqueous extracts, where the different extract concentrations served as the treatment factors. The effects of the stock solution (100%) and its four dilutions (50%, 25%, 12.5%, and 6.25%) were evaluated against 48-h-old J2s of *Meloidogyne* sp. These botanical treatments were compared against a 0.75 g dose of the synthetic nematicide (oxamyl), corresponding to half of the recommended field application rate.

A 4 mL volume of each plant extract treatment was combined with 4 mL of the juvenile suspension (containing approximately 100 juveniles) in Petri dishes. The dishes were incubated in the dark at 24°C (Figure 2). Immobilized and dead nematodes were counted under an optical microscope at 24, 48, and 72 h post-application. Completely straight, immobile juveniles were considered dead if they failed to respond to physical prodding with a fine needle. Natural mortality was monitored using a control group containing 4 mL of the juvenile suspension and 4 mL of sterile distilled water.

Calculation of adjusted mortality

The effectiveness of a treatment on nematodes was assessed by estimating the mortality caused by this treatment. For each concentration tested, the average mortality rate of the larvae was calculated. The results were expressed as corrected mortality, considering natural mortality in the population (control: distilled water), in addition to mortality due to the extract. Mortality and corrected mortality rates were expressed according to the formulas of El-Ashry et al. (2021) and Ribeiro et al. (2015), respectively.

$$\%\ \text{Mortality} = \frac{\text{Number of dead nematodes}}{\text{Total number of individuals}} \times 100$$

$$\text{Mc} = \frac{\text{M2} - \text{M1}}{100 - \text{M1}} \times 100$$

Where Mc = corrected mortality, M1= mortality recorded in the control group, M2 = mortality recorded in the treated groups.

Greenhouse evaluation

Experimental setup

The trial was conducted in the greenhouse located at the INP-HB farm, specifically in the northern site. The setup used was a completely randomised design with four replicates for each treatment. The best concentrations of *R. communis* and *T. diversifolia* extracts, which had shown the greatest effectiveness against *Meloidogyne* sp. larvae in the laboratory, were used in the greenhouse alone and in combination with the reference oxamyl-based nematicide (30 g/kg). Powders from these plants were also used. Quantities of 50 g of powder were tested. However, *R. communis* powder, which completely inhibited the germination of cucumber seeds, was not selected. The test, therefore, involved a dose of 100 g/L for the aqueous extracts of the two plants and 50 g for the *T. diversifolia* powder. Water was used as a control in the treatments of soil infested and soil not artificially infested with nematodes. The nematicide was used at a dose of 0.75 g/plant, which is half the approved dose. The experiment involved nine treatments and four replications (Table 1).

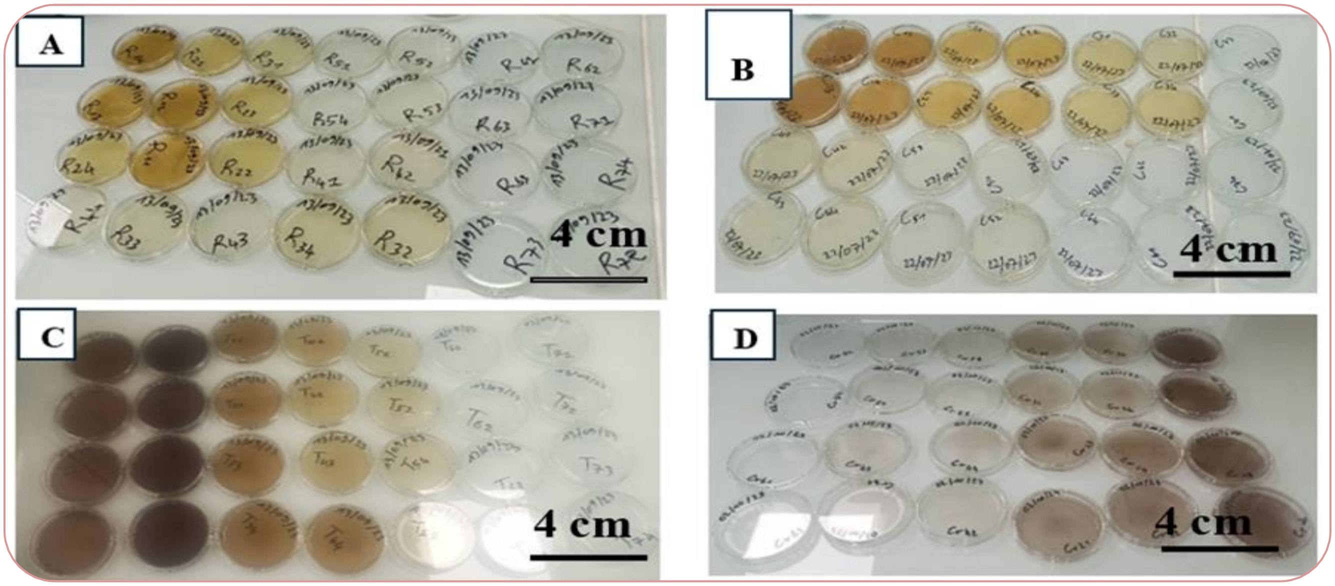

Figure 2. Petri dishes containing aliquots and various aqueous extracts. (A) Aliquot + aqueous extract of *R. communis.* (B) Aliquot + aqueous extract of *C. odorata*. (C) Aliquot + aqueous extract of *T. diversifolia*. (D) Aliquot + aqueous extract of *C. retusa.*

Table 1. Details and description of treatments.

Treatments	Description of Treatments
T0	infested soil + water only
T1	Blank control: soil untreated
T2	Infested soil treated with oxamyl (30 g/kg) at 0.75 g/plant
T3	Infested soil treated with 15 ml/plant of aqueous extract of *R. communis* 100 g/l
T4	Infested soil treated with 15 ml/plant of aqueous extract of *T. diversifolia* 100 g/l
T5	Infested soil treated with 50 g/plant of powdered extract of *T. diversifolia*
T6	Infested soil treated with 15 ml/plant of aqueous extract of *R. communis* 100 g/l + 0.75 g/plant of oxamyl at 30 g/kg
T7	Infested soil treated with 15 ml/plant of aqueous extract of *T. diversifolia* 100 g/l + 0.75 g/plant of oxamyl at 30 g/kg
T8	Infested soil treated with 50 g/plant of powdered extract of *T. diversifolia* + 0.75 g/plant of oxamyl at 30 g/kg

Soil preparation and sterilisation

The soil used for this test was taken from the experimental plot in Djahakro (a village in Yamoussoukro) and sterilised in an autoclave at 121°C for 60 min, then cooled for 24 h (Mazurkiewicz-Zapałowicz et al., 2002). One-litre plastic pots capable of holding at least 1 kg of soil were used. The sterilised soil was distributed among these pots. A total of 36 pots were filled.

Cucumber cultivation

Tokyo F1 cucumber seeds were used for this test and were sown directly into pots at a rate of one seed per pot. After sowing, the pots were watered to moisten them and encourage seed germination.

Application of treatments and inoculation of cucumber plants

Two weeks after sowing the cucumbers, the plants' roots were inoculated by making five holes in a triangular arrangement, each 2 cm from the stem. A 5 ml aliquot containing approximately 100 ± 10 juveniles of *Meloidogyne* sp. was inoculated at a rate of 1 mL per hole.

The holes were then covered with soil. One day (24 h) after inoculation, 15 mL of the aqueous extract was applied around the plants. For the powdered extracts, 50 g was applied two weeks before sowing in the pots, followed by daily watering to allow the organic matter to decompose. The synthetic oxamyl-based nematicide was applied two days after sowing at a rate of 0.75 g per plant. In addition, 15 mL of distilled water was applied around the plants 24 h after inoculation and served as a positive control. The blank control was untreated soil (Ghnimi, 2018).

Collection of agronomic and nematological parameters

The diameter at the collar and the height of the stems of the plants were measured (Baghestani et al., 2007). The stem height of cucumber plants in each pot was measured using a measuring tape, from the base (collar) to the terminal bud, six weeks after sowing the seeds. The collar diameter was measured using callipers at the same time.

The plants were dug up 4 weeks after inoculation with juvenile root-knot nematodes. The soil was then removed from the roots by gently shaking the plants. The galls were scored using the Bridge and Page (1980) assessment table. These scores were used to calculate the severity of root galls using the Zeck (1971) formula.

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

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

J2s of *Meloidogyne* sp. in the soil and roots were extracted using the modified Baermann method of Whitehead and Hemming (1965) and the double centrifugation method of Coolen and d'Herde (1972), respectively. The densities of *Meloidogyne* sp. per unit of soil and roots were determined using the method employed by Yéo et al. (2018):

$$\mathrm{N} = \frac{\mathrm{n} \times \mathrm{v}}{\mathrm{m}} \times 100$$

Where N = number of nematodes per 100 g of roots or soil; n = number of nematodes per 2 ml of suspension; v = volume of nematode suspension obtained (ml); m = mass of roots or soil used for extraction.

Nematode populations were used to determine the nematode reduction rate in different treatments using the formula described by (Elkelany et al., 2020):

$$R\ (\%) = \frac{C1 - C2}{C1} \times 100$$

Where R (%) = reduction rate, C1 = number of juvenile nematodes in the soil or roots of the control (untreated soil), C2 = number of juvenile nematodes in the soil or roots of the treated groups (treated soil).

Data analysis

Before carrying out the analysis of variance, the Shapiro-Wilk test was performed to check the normality of the variables. Data from laboratory and greenhouse experiments were analysed using one-way analysis of variance (ANOVA). These data were corrected for nematode mortality for the *in vitro* test. *In vivo*, the data analysed were nematode densities in the soil and roots, nematode reduction rates, root gall index, root gall severity, stem height and diameter, and growth parameter increase rates. The means of these data were compared using the Tukey HSD test at a significance level of 5% using XLSTAT software version 2014 in cases of significant difference.

Results

In vitro nematicidal effect of plant extracts

Effect of plant extracts on juvenile mortality

The results of the application of plant extracts to juveniles of *Meloidogyne* sp. are presented in Table 2. Analysis of variance (ANOVA) showed a significant difference between treatments 72 h after inoculation ($P < 0.0001$). The 100% doses of *T. diversifolia* and *R. communis* recorded the highest corrected mortality rates, of 64.07% and 57.24%, respectively. These were followed by the 0.75 g dose of synthetic nematicide (oxamyl), with a corrected mortality rate of 51.27%. The 12.5% dose of *R. communis* recorded a mortality rate of 43.20%. Furthermore, the 50% doses of *R. communis* and *T. diversifolia* recorded mortality rates of 38.19% and 33.83%, respectively. All doses of *C. odorata* and *C. retusa,* and the two lowest doses of *T. diversifolia* (12.5 and 6.25%) recorded the lowest mortality rates, below 20%.

Changes in mortality rates over the observation period

The mortality dynamics of *Meloidogyne* sp. juveniles are shown in Figure 3. Mortality rates varied according to time and dose. For the 6.25% dose, mortality rates for *R. communis* increased from 15.02% to 24.41% after 72 h. The other extracts showed irregular and less marked changes. The 12.5%, 25%, 50%, and 100% doses showed irregular mortality rate dynamics over the three observation periods and for all doses. Castor bean had the highest mortality rate. However, for the 25% and 100% doses, *T. diversifolia* had the highest rates, with values of 53.70% and 64.07%, respectively.

Table 2. Corrected mortalities of J2s of *Meloidogyne* sp. 72 h after application of treatments.

Treatments	Corrected % mortality J2s of *Meloidogyne* sp.
T100	64.07 ± 12.88 a
R100	57.24 ± 11.50 ab
OX0.75	51.27 ± 16.13 b
R12.5	43.20 ± 9.30 bc
R50	38.19 ± 15.38 cd
T50	33.83 ± 11.41 cd
R25	31.53 ± 18.47 cde
R6.25	24.42 ± 13.46 def
T25	16.99 ± 3.96 efg
C12.5	13.52 ± 7.54 fg
C100	9.82 ± 5.34 fgh
C50	9.05 ± 9.65 fg
C25	8.54 ± 6.69 gh
CR25	7.57 ± 5.72 gh
CR12.5	7.33 ± 4.60 gh
T12.5	6.98 ± 2.91 gh
C6.25	6.40 ± 3.71 gh
CR6.25	4.79 ± 3.81 gh
T6.25	4.13 ± 0.87 gh
CR50	2.95 ± 9.65 gh
CR100	2.95 ± 4.10 gh
T0	0.00 ± 0.00 h
Mean	20.21
P-value	< 0.0001

The values in columns followed by the same letters are statistically identical at the 5% significance level according to Tukey's HSD test.

T100, T50, T25, T12.5, and T6.25: *T. diversifolia* applied at doses of 100, 50, 25, 12.5, and 6.25%. R100, R50, R25, R12.5, and R6.25: *R. communis* applied at doses of 100, 50, 25, 12.5, and 6.25%. C100, C50, C25, C12.5, and C6.25: *C. odorata* applied at doses of 100, 50, 25, 12.5, and 6.25%. CR100, CR50, CR25, CR12.5, and CR6.25: *C. retusa* applied at doses of 100, 50, 25, 12.5, and 6.25%. T0: negative control by application of distilled water only. OX0.75: synthetic nematicide with active ingredient oxamyl (30 g/Kg) applied at 0.75 g/L.

Effect of nematicidal plant extracts on agromorphological parameters

No significant differences were observed among different treatments in terms of agromorphological parameters of cucumber (F1 Tokyo) such as stem height (P = 0.6564), collar diameter (P = 0.6100), and number of leaves (P = 0.2720) assessed four weeks after treatment application (Table 3). Average heights ranged from 16.33 cm (T5) to 25.33 cm (T8). Collar diameters ranged from 2.87 cm (T1) to 3.97 cm (T8). The extreme values for the number of leaves were recorded for treatments T6 (3.33 leaves/plant) and T8 (6 leaves/plant).

Effect of nematicidal plant extracts on *Meloidogyne* sp. in greenhouse

The ANOVA test could be carried out because the variables followed a normal distribution c Root galls were more pronounced in treatment T0, untreated soil, unlike pots containing treated soil, particularly for T4 and T8, as shown in Figure 4. The study of the nematicidal effects of local plant extracts against *Meloidogyne* juveniles in greenhouse revealed significant differences among different treatments in terms of nematode numbers in the soil (P < 0.0001), as shown in Table 3. In fact, nematode population densities were higher for treatment T0 (positive control: infested soil + water only), followed by T1 (negative control: uninfested soil without treatment), with respective densities of 19.67 and 9.00 individuals/100 g of soil. The other treatments recorded population densities lower than or equal to 3 individuals/100 g of soil. These were the lowest values.

The population sizes of *Meloidogyne* sp. in cucumber roots (F1 Tokyo variety) were statistically different depending on the treatment (P < 0.0001). Treatment T1 recorded the highest population size of 49,354.84 individuals/100 g of roots. It was followed by treatment T0 (37,366.67 individuals/100 g of roots). However, the lowest nematode population densities were recorded in treatments T2 (6,363.64 individuals/100 g of roots) to T8 (1,333 individuals/100 g of roots).

In terms of gall indices and severity on the F1 Tokyo variety of cucumber crop (Table 3), a significant difference was observed among treatments (P < 0.0001). Indeed, treatment T3, which corresponded to a 100% dose of the aqueous extract of *R. communis*, recorded a higher gall index and severity of 9.33 and 93.33%, respectively. The lowest gall index and severity was recorded for T8 (0.67 and 6.67%, respectively). Treatments T0, T4 and T5 recorded average gall indices and severities.

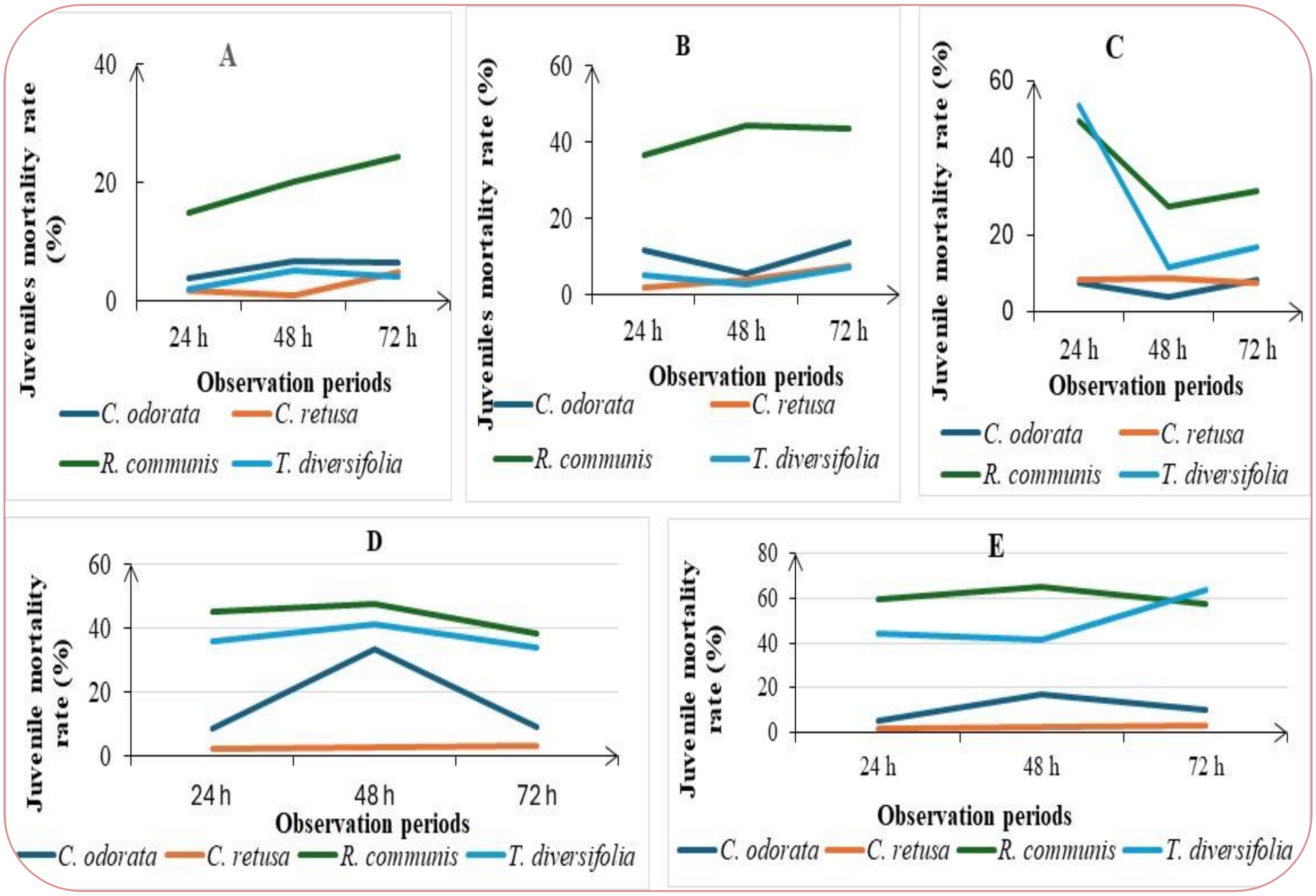

Figure 3. Mortality dynamics of juveniles treated with aqueous extracts of plants with nematicidal effects at different concentrations. (A) 6.25 %. (B) 12.5 %. (C) 25 % (D) 50 %. (E) 100 %.

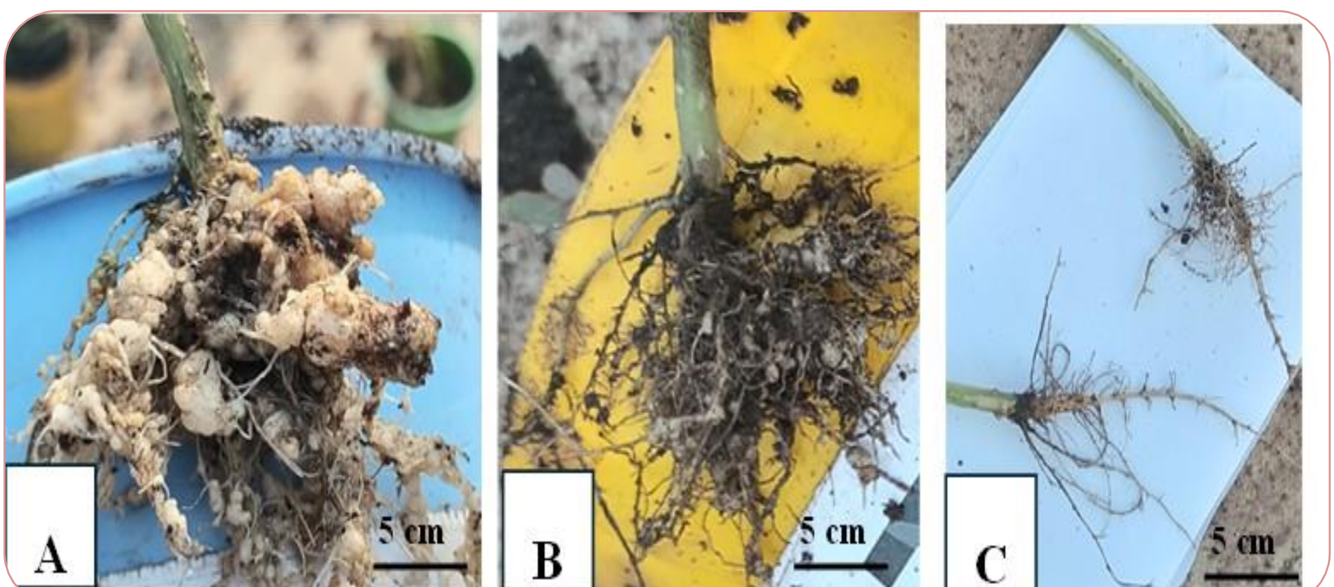

Figure 4. Root conditions of cucumber plants 4 weeks after treatment application. (A) Roots of T0 = positive control infested soil + water only. (B) Roots of T4 = infested soil treated with 15 ml/plant of aqueous extract of *T. diversifolia* 100 g/L. (C) Roots T8 = infested soil treated with 50 g/plant of powdered extract of *T. diversifolia* + 0.75 g/plant of oxamyl at 30 g/Kg.

Table 3. Effects of treatments on nematological and agromorphological parameters of cucumber cv. F1 Tokyo.

Treatment	Number of juveniles in 100 g of soil	Number of juveniles in 100 g of roots	Gall index	Severity (%)	Stem height (cm)	Stem diameter (mm)	Number of leaves per plant
T0	19.67 ± 2.08 a	37 366.67 ± 2 283 b	6.67±4.04 ab	66.66 ±5 .77 ab	19.00±1.00 a	3.63±0.65 a	3.67±0.58 a
T1	9.00 ± 2.64 b	49 354.84 ± 8 936 a	1.33±0.58 ab	13.33 ± 5.77 ab	20.33±3.51 a	2.87±0.23 a	4.00±1.00 a
T2	1.33 ± 1.15 c	6 363.64 ± 4 209 c	8.33±0.58 ab	83.33 ± 5.77 ab	19.00±1.00 a	3.03±0.60 a	4.33±1.15 a
T3	2.33 ± 1.00 c	2 396.40 ± 1 185 c	9.33±0.58 a	93.33 ± 5.77 a	25.00±2.64 a	3.27±0.15 a	4.00±1.00 a
T4	3.00 ±1.53 c	8 769.23 ± 1 687 c	6.67±0.58 ab	66.67 ± 15.27 ab	22.33±2.89 a	2.93±0.39 a	4.33±0.67 a
T5	1.67 ± 0.57 c	3 968.25 ± 2 999 c	5.33±1.52 abc	53.33 ± 20.00 abc	16.33±2.52 a	3.53±0.70 a	4.67±0.58 a
T6	0.67 ± 1.03 c	1 184.74 ± 594.66 c	4.00±2.00 bc	40.00±15.27 bc	22.00±2.64 a	3.17±0.40 a	3.33±0.58 a
T7	2.00 ± 1.19 c	3 043.48 ± 2 324 c	3.67±1.53 bc	36.67±15.27 bc	19.33±5.68 a	3.4±0.26 a	3.67±0.58 a
T8	1 ± 1.03 c	1 333.33 ± 1 309 c	0.67± 0.57 c	6.67 ± 5.77 c	25.33±15.69 a	3.97±1.61 a	6.00±2.65 a
Means	4.52	12 642.29	5.11	51.11	20.96	3.31	4.22
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.6564	0.6100	0.2720

In the same column, values followed by the same letters are statistically identical at the 5% significance level according to Tukey's HSD test.

T0: infested soil + water only; T1: blank control: untreated soil; T2: infested soil treated with oxamyl (30 g/kg) at 0.75 g/plant; T3: infested soil treated with 15 ml/plant of aqueous extract of *R. communis* 100 g/L; T4: infested soil treated with 15 ml/plant of aqueous extract of *T. diversifolia* 100 g/L; T5: infested soil treated with 50 g/plant of powdered extract of *T. diversifolia;* T6: infested soil treated with 15 ml/plant of aqueous extract of *R. communis* 100 g/l + 0.75 g/plant of oxamyl at 30 g/Kg; T7: infested soil treated with 15 ml/plant of aqueous extract of *T. diversifolia* 100 g/l + 0.75 g/plant of oxamyl at 30 g/Kg; T8: infested soil treated with 50 g/plant of powdered extract of *T. diversifolia* + 0.75 g/plant of oxamyl at 30 g/Kg.

Effect of treatments on the reduction rate of *Meloidogyne* sp.

Figure 5 shows the reduction rates of *Meloidogyne* sp. populations in roots and soil 4 weeks after application of the treatments. Analysis of variance performed on the reduction rates showed a significant difference among treatments for roots (P < 0.0001). The reduction rates for treated plants ranged from 76.51% to 96.93% for treatments T4 and T2, respectively, which were higher than the untreated control, where the rate was zero. In the soil, no significant difference was observed (P = 0.2555) among treatments for reduction rates. These ranged from 0 to 73.33% for treatments T0 and T1, respectively.

Discussion

The present study tested the nematicidal effects of four plants viz., *R. communis*. *T. diversifolia*, *C. odorata* and *C. retusa* against root-knot nematodes of the genus *Meloidogyne*. *R. communis* and *T. diversifolia* induced mortality in *Meloidogyne* juveniles *in vitro*. This was confirmed by the experiment conducted by Curto et al. (2015), who demonstrated the ability of *C. retusa* to reduce the reproduction factor of *Meloidogyne* when used as green manure. Furthermore, aqueous extracts of *T. diversifolia* inhibited the hatching of *M. incognita* eggs by 98% to 100%, 2 and 9 days after inoculation in *in vitro* tests (Odeyemi and Adewale, 2011). Furthermore, dried leaves of *T. diversifolia* have been shown to have a nematicidal effect on root-knot nematodes (*M. incognita*) in tomato crops by reducing infestation by these nematodes. In fact, 0.2 g/mL of *T. diversifolia* was very effective in killing more than 50% of juveniles within 48 h (Premachandra and Amarasinghe, 2016). Furthermore, Cayrol et al. (2010) reported that castor oil blocks the development and reproduction of nematodes leading to a drastic reduction in nematode populations. This is thought to be due to the presence of a group of compounds with nematicidal properties in the aqueous extract of *R. communis.* Juvenile mortality increased with increasing concentration and duration of exposure, a result consistent with the findings of Kepenekçi et al. (2016). This can be explained by differences in the biochemical content of the plants.

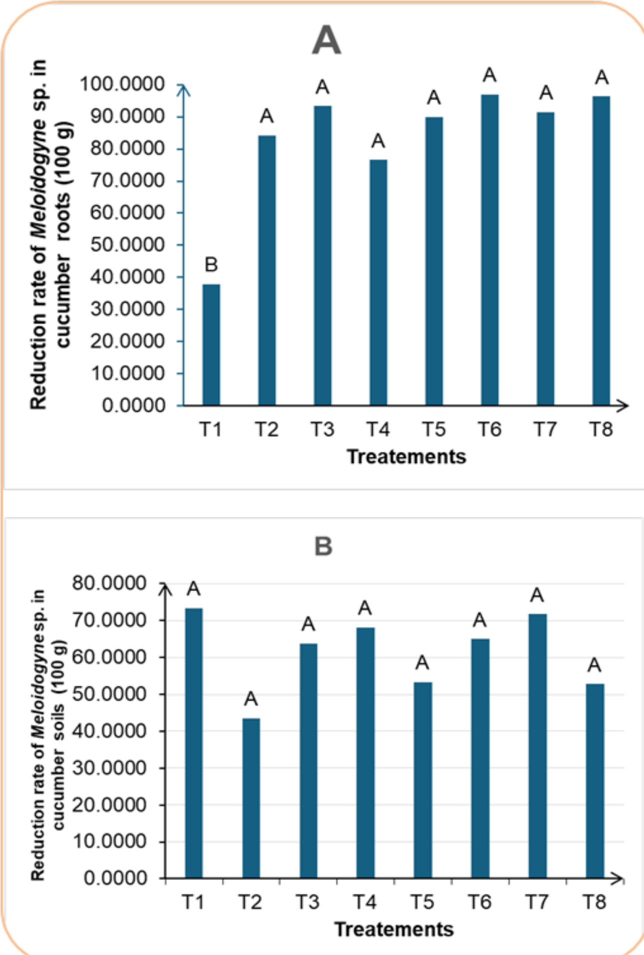

Figure 5. Effect of treatments on the reduction rate of *Meloidogyne* sp. Roots (A) and soil (B). Histograms marked with the same letters are statistically identical according to Tukey's HSD test at the 5% threshold. T0 = infested soil + water only, T1 = blank control, soil untreated, T2 = Infested soil treated with oxamyl (30 g/Kg) at 0.75 g/plant, T3 = Infested soil treated with 15 mL/plant of aqueous extract of *R. communis* 100 g/L, T4 = Infested soil treated with 15 mL/plant of aqueous extract of *T. diversifolia* 100 g/L, T5 = Infested soil treated with 50 g/plant of powdered extract of *T. diversifolia*, T6 = Infested soil treated with 15 mL/plant of aqueous extract of *R. communis* 100 g/L + 0.75 g/plant of oxamyl at 30 g/Kg, T7 = Infested soil treated with 15 mL/plant of aqueous extract of *T. diversifolia* 100 g/L + 0.75 g/plant of oxamyl at 30 g/Kg and T8 = infested soil treated with 50 g/plant of powdered extract of *T. diversifolia* + 0.75 g/plant of oxamyl at 30 g/Kg.

Furthermore, in this study, extracts of *C. odorata* and *C. retusa* showed lesser effects on the juvenile mortality of *Meloidogyne* sp. The contradictory effect was observed in a study where *C. odorata* (2 tons/hectare of compost) significantly reduced root-knot nematode populations in the soil (Abolusoro et al., 2020). However, our results are consistent with those of Agaba and Fawole (2015) who found that the synthetic carbofuran-based nematicide was more effective than powdered and aqueous extracts of *C. odorata*. Studies conducted with the aqueous extract of *C. retusa* have shown its effectiveness in killing *Meloidogyne* juveniles at a minimum dose of 12.5 mg/L (Doga et al., 2022).

The effectiveness of synthetic nematicides has already been proven in numerous previous studies (Doga et al., 2022; Kakou, 2022). The results obtained in this study confirm the work of these authors. Oxamyl resulted in a 90% inhibition rate of egg hatching and a 95% juvenile mortality in the experiment conducted by El-Ashry et al. (2021). Oxamyl is a carbamate that acts on the nervous system of nematodes by contact and ingestion, inhibiting acetylcholinesterase and thus disrupting the transmission of nerve impulses at the synapses. It is absorbed by the roots and migrates through the vascular system to the growing organs of the stems or roots (Dubé et al., 2013).

Due to their *in vitro* efficacy, aqueous extracts of *T. diversifolia* (powdered and aqueous extract) and *R. communis* (aqueous extract) were tested *in vivo* alone and in combination with the synthetic nematicide (oxamyl) in cucumber cultures. The results obtained in this experiment confirm those obtained *in vitro*. Indeed, the number of juveniles in the soil and roots 4 weeks after application of the treatments showed a significant difference between the treated plants and the control. In addition, the reduction rates of the treated plants were higher than those of the untreated control. Our results corroborate those of Coulibaly et al. (2023) who demonstrated the nematicidal activity of castor bean.

A significant difference was also observed between the gall indices and severity levels induced by different treatments. Low severity levels and gall indices were observed for plants treated with a combination of oxamyl and aqueous extracts of *R. communis* and *T. diversifolia*, and powdered extract of *T. diversifolia*. This can be explained by the low numbers of juveniles in the soil and roots for these treatments. Juveniles in the second stage represent the infectious stage of nematodes of the genus *Meloidogyne*. Once the roots are attacked by these nematodes, above-ground and below-ground symptoms appear on the plant. Typical above-ground symptoms are root galls caused by cell dedifferentiation and the formation of giant cells induced by J2s (Djian-Caporalino et al., 2018). The nematicidal effect of several plants has already been proven by previous studies. To this end, the nematicidal effect of *R. communis* seed extracts was demonstrated by Oluwatayo et al. (2019) by the significant reduction in the final nematode population and root gall index.

Furthermore, extracts from the leaves and dry stems of *T. diversifolia* significantly reduced the reproduction factor number of galls and hatching of *Meloidogyne* eggs in an *in vitro* experiment on tomato plants (Tefu et al., 2023). The nematicidal effect of plants is thought to be due to their chemical composition which contains substances with nematicidal properties. Indeed, chemical studies of plants in the Asteraceae family indicate the presence of chemical compounds such as phenolic compounds (flavonoids and tannins) and terpenoids (Nunes et al., 2023). *T. diversifolia* contains tannins and alkaloids, triterpenes, steroids, and saponins (Lezcano et al., 2012). Furthermore, Negrel et al. (2018) observed that tannins strongly inhibit the development of parasitic nematodes, particularly *Xiphinema index*. The nematicidal activity of plants containing flavonoids has already been proven in a study by Allagui et al. (2007). According to the work reported by Diby et al. (2015), *T. diversifolia* has insecticidal, larvicidal, and repellent properties, which could explain the results obtained in this study. The study by Ghnimi (2018) concluded that castor oil contains ricinoleic acid, which has the property of inhibiting acetylcholinesterase (AChE) and can therefore be used in biological control. Castor bean also contains other substances, including skatole, γ-decalactone, 4-methylphenol, and phenol, which have nematicidal properties against *M. incognita*, as proven by Pedroso et al. (2019). In addition, the biological activity (fungicidal, insecticidal, nematicidal, and medicinal) of castor oil has been proven by numerous authors (Zaidat, 2021; Iglesias et al., 2024; Rodríguez-Cervantes et al., 2024).

The treatment containing powdered extract of *T. diversifolia* showed the best nematological and agromorphological parameters. Indeed, the use of appropriate organic matter improves the chemical, physical, and biological parameters of the soil. This results in nutrient availability and improved soil texture and structure (Camara et al., 2023). This improves soil fertility and reduces pathogen population densities (Faye et al., 2023). The degradation of organic matter can release toxic and nematicidal substances that contribute to nematode control (Ashraf and Khan, 2010). According to Dauphinais et al. (2024), adding *Crotalaria* to the soil reduced populations of the root-knot nematode, *M. hapla*, tenfold and significantly increased carrot yields the following year.

In this study, no significant differences were observed among treatments in terms of height, diameter and number of leaves. Contrary results were found in the study by Kakou (2022), in which tomato plants treated with *R. communis* and *T. diversifolia* developed better than those in the control group. In a greenhouse experiment with bean crops, the application of raw *T. diversifolia* extract significantly influenced agronomic parameters (N'jenga et al., 2019). The lack of any significant difference between the agronomic parameters could be explained by the fact that the products' response time depends on environmental conditions, the host plant, and time.

The nematicidal effect of combining bioproducts and synthetic nematicides observed in this study has already been demonstrated in previous studies. Radwan et al. (2011) showed that combining oxamyl with the bioproduct Biozeid reduced root galls caused by *M. incognita* by 95.5% in the soil. Similarly, Ismael and Mahmood (2020) showed that combining the bioproduct Nemakey with humic acid reduced juvenile population densities in 100 g of soil in greenhouse cucumber crops. Based on the results of this study, *T. diversifolia* and *R. communis* plants could be used in the integrated management of plant-parasitic nematodes.

Conclusion

In vitro testing of aqueous extracts from *R. communis*, *T. diversifolia*, *C. odorata*, and *C. retusa* against *Meloidogyne* sp. confirmed the strong nematicidal potential of these plants, with *R. communis* and *T. diversifolia* showing the highest efficacy. This primary efficacy depended on extract concentration and exposure duration, a trend subsequently validated under semi-controlled greenhouse conditions in cucumber crops.

The greenhouse trials proved that applying aqueous extracts of castor bean or powdered *T. diversifolia* in combination with a half-dose of oxamyl significantly reduces *Meloidogyne* sp. juvenile populations in cucumbers. For sustainable nematode management, we recommend combining synthetic products like oxamyl with *T. diversifolia* powder or *R. communis* aqueous extract. This integrated approach offers a practical alternative or complementary measure to exclusive chemical control, encouraging the adoption of local botanical resources for sustainable vegetable production.

Future research should focus on field-scale trials to confirm these greenhouse findings across diverse agroecological environments. Moreover, screening other local plants with nematicidal potential will expand the options available to growers. Finally, comprehensive phytotoxicity assessments are recommended to ensure these botanical extracts do not cause adverse effects across different crop development stages.

Authors' Contributions

KA conceptualized and supervised the research, and contributed to the correction and preparation of the final manuscript draft. ASY developed the protocol, collected the data, performed data analysis, and prepared the first draft of the manuscript. AMJK, EOT, YK, MD, and KKT contributed to data analysis, interpretation of results, and manuscript preparation. All authors read and approved the final version of the manuscript.

Research Funding

This research did not receive any grant from funding agencies.

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

Policy Addressed

This study supports the integration of plant-based nematicides into sustainable crop protection programs. Policies should promote research, registration, and commercialization of botanical extracts, along with farmer training on integrated nematode management. Standardization, safety evaluation, and field validation of plant-derived products are essential to reduce reliance on synthetic nematicides and improve environmental sustainability.

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