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### RESEARCH ARTICLE

## EXPLORING THE NEMATICIDAL POTENTIAL OF CASHEW NUT SHELL LIQUID AGAINST ROOT-KNOT NEMATODES: IN VITRO AND GREENHOUSE EVALUATIONS

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### Abstract

Root-knot nematodes (*Meloidogyne* spp., RKNs) remain a major threat to vegetable production and a reason for the overuse of pesticides, particularly in Sub-Saharan Africa. The objective of this study was therefore to investigate the nematocidal properties of cashew nut shell liquid (CNSL) against RKNs on vegetables. Laboratory bioassays and greenhouse pot experiment were conducted to assess the suppressive effects of CNSL on RKN reproduction and damage. Four concentrations (0.01%, 0.1%, 1%, and 10%) of cold- and hot-extracted CNSL were used in the laboratory bioassays to evaluate their nematocidal activity against *M. enterolobii* juveniles and eggs. The pot experiment consisted of eight treatments (two CNSL types, one concentration, three applications on doses, and two controls) assessed in a completely randomized block design with five replicates, using tomato (cv. Padma 108 F1) and soil naturally infested with RKNs. Results from the in vitro bioassays showed that CNSL induced juvenile mortality by up to 90% after 48 h of exposure against 2% in the SDW used as control. Egg hatching was inhibited by up to 88% after 7 days of exposure to CNSL against 6% for SDW. In pots, application of 100 ml of CNSL induced reductions in reproduction factor by up to 78.77% and 91.47% compared with *Crateva adansonii* leaf aqueous extract and positive control treatments, respectively. Cold-extracted CNSL was more effective in suppressing nematode reproduction and root galling than hot-extracted CNSL. Tomato plant growth was negatively affected by CNSL application, indicating the need for lower concentrations. These initial results are positive and show promise.

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## Introduction:-

Root-knot nematodes (*Meloidogyne* spp.; RKNs) are a malignant soilborne curse to vegetables, particularly in tropical and subtropical areas where vegetable production is highly dependent on proper nematode control (Coyne et al., 2018; Hallmann and Meressa, 2018). In Sub-Saharan Africa, RKNs were identified as the single greatest biotic threat in the highly intensive urban and peri-urban vegetable production systems, where vegetable production contributes significantly to food security, family income and employment. These nematodes reduce the absorption and assimilation capacity of the root system, resulting in a decrease in the plant's mineral nutrition, flowering, and fructification (Nilusmas, 2020). Furthermore, several authors have reported that initial infestation of tomato plants by RKNs inhibits the expression of resistance genes to some major diseases, thereby predisposing infested crops to secondary infections by fungi, bacteria, and viruses, leading to increased yield losses (Kidane et al., 2020; Walia and Khan, 2023).

Various strategies are available to protect vegetables in these cropping systems against RKNs, but the use of synthetic nematicides remains the most common coping strategy relied upon by growers. According to Trudgill and Blok (2001), RKNs are very difficult to eradicate due to the wide diversity of their hosts, their very short life cycle, very high reproduction rate, and endoparasitic nature. This could also be due to the presence of a mixture of RKN species in the same fields, including the highly pathogenic and invasive species *M. enterolobii* (Pagan et al., 2015; Affokpon et al., 2017). For example, *M. enterolobii* has been found to exhibit resistance to standard control measures against RKNs (Coffi et al., 2025; Poudel et al., 2025). However, pesticide use in Sub-Saharan Africa presents a number of challenges, among which the limited or inaccurate understanding by farmers has led to their misuse and overuse (Coyne et al., 2010). Vegetables produced under such situations are delivered directly to markets with little or no care for quality or consumer health and safety. Because of their detrimental effects on the environment and human health, environmentally sound alternatives are being sought and investigated. The use of botanical extracts appears to be an important component of integrated methods for managing these nematodes.

Recent research has addressed the pesticidal effects of cashew nut shell liquid (CNSL), a by-product of cashew (*Anacardium occidentale*, L.) nuts abundantly available in any cashew-producing countries. CNSL, either cold- or hot-extracted, has been shown to have pesticidal effects on different developmental stages of various insect pests and pathogenic fungal growth (Garcia et al., 2018; Lekha et al., 2020; Sotondji et al., 2020). Its insecticidal efficacy against *Aphis craccivora* and *Riptortus pedestris* infesting cowpea, was reported earlier in in vitro assay where topical application of concentration 0.1% caused mortality comparable to that of the chemical insecticide Thiamethoxam 0.03% (Lekha et al., 2020). These authors observed that the survived *R. pedestris* exhibited developmental abnormalities and the formation of nymphal adult intermediary, and suggested a possible insect growth regulatory effect of CNSL.

Recently, the CNSL has been proven to have insecticidal effects on *Spodoptera frugiperda* larvae and eggs, and antifeedant properties with average lethal concentrations ( $LC_{50}$ ) of 1.92% and 2.50% after 2 h for larvae and eggs, respectively, and effective concentrations ( $EC_{50}$ ) of 0.34% for antifeedant effects (Nyirenda et al., 2024). At a concentration of 320  $\mu\text{g ml}^{-1}$ , CNSL was found to inhibit sporulation and mycelial growth of *Collectotrichum gloeosporioides* and *Lasioidiplodia theobromae* on papaya fruit (Garcia et al., 2018). They reported no reduction in fungal growth in the absence of direct contact between the fungus and CNSL, indicating that the inhibitory effect of CNSL was not due to volatile substances. Anacardic acids are the main constituents of the CNSL, which forms cardol, cardanol, and polymeric material during the decarboxylation process; some phenolic compounds being known for their biological activity against various pests and diseases (Maia et al., 2015; Garcia et al., 2018). However, reports on the bioactivity of CNSL against plant-parasitic nematodes are scarce. This study was conducted to investigate the nematocidal properties of CNSL against RKNs on vegetable crops. The specific objectives were to assess in vitro the inhibitory effect of both cold- and hot-extracted CNSL on juveniles and eggs of *M. enterolobii* and to determine, under greenhouse conditions, their effectiveness in protecting tomato against RKNs.

## Material and Methods:-

### Experimental site:-

The experiments were carried out in the laboratory facilities and greenhouse at the Nematology Unit (UNema) research station in Sekou, Benin (06°37'30.8" N; 002°13'56.9" E; 74 m asl). The research station is located in the Guinean biogeographical zone and is characterized by a sub-equatorial climate with two wet seasons (April to mid-July and mid-September to end October) alternated by two dry seasons (Affokpon et al., 2021).

**Preparation of nematode inoculum:-**

Second stage juveniles and eggs of *M. enterolobii* were used for the in vitro bioassays. They were obtained from a pure population maintained on celosia (*Celosia argentea* L.) in the greenhouse of the Nematology Unit following the method described by Coffi et al. (2025).

For the pot experiment, infested soil was collected from in vivo RKN multiplication plots of the UNema research station. These plots were initially inoculated with a mixture of *M. enterolobii*, *M. incognita* and *M. javanica* and grown with celosia for RKN inoculum production purpose.

**Preparation of CNSL and leaf aqueous extract of *Crateva adansonii*:-**

Two types of CNSL, cold and hot extracts, were produced from cashew (*Anacardium occidentale*, L.) shells. The products were provided by a local supplier located at Savalou, Benin. For the experiments, a stock solution of the CNSL was diluted in sterile distilled water (SDW) using the serial dilution method to obtain  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  suspensions, corresponding to 10%, 1%, 0.1% and 0.01%, respectively (hereafter referred to as concentrations). For the pot experiment, aqueous suspension from leaves of *C. adansonii* (Capparaceae; DC, 1824) was additionally used as a control treatment. Compost from *C. adansonii* has recently been found to have a nematocidal effect on *M. enterolobii* (Sero et al., 2025a). To prepare the suspension, freshly harvested leaves collected from the plantation of Nematology Unit were carefully washed and cut into small pieces. Then, 200 g was macerated in 500 ml SDW using a hand blender (BOSCH, 400 W). The resulting solution was filtered through a 200  $\mu$ m mesh sieve 24 hours later and used for the experiment.

**In vitro bioassays:-**

Two in vitro experiments, repeated once, were conducted to assess the suppressive effect of the CNSL on *M. enterolobii* J2 mortality and its inhibitory effect on egg hatching.

**Juvenile mortality bioassay:-**

The effect of the CNSL on *M. enterolobii* J2s was assessed in vitro after 12, 24, and 48 hours of exposure (Ahmad and Siddiqui, 2018) to four concentrations of each CNSL type. Nine treatments were assessed: cold-extracted CNSL at 0.01%, 0.1%, 1%, and 10%; hot-extracted CNSL at 0.01%, 0.1%, 1%, and 10%; and SDW used as control. Using an Eppendorf micropipette, a 100  $\mu$ l of aliquot 50 J2s was inoculated into individual 55-mm diameter Petri dishes containing 1 ml of a single treatment. The Petri dishes were arranged on the laboratory bench at ambient temperature ( $28 \pm 1^\circ\text{C}$ ) following a completely randomized design with four replicates. The number of dead J2s was counted using the technique described by Gebrehana et al. (2025) after 12, 24, and 48 hours of exposure to CNSL. For this, nematode suspensions from the CNSL treatments were washed through nested 20  $\mu$ m sieves using SDW, and J2s were transferred to a counting dish for observation and estimation under a light microscope (20 x magnification, Olympus CX31). For each exposure time, J2 mortality was determined as follows: J2 mortality (%) = (Dead J2 number / Total J2 number) x 100

**Egg hatching inhibition bioassay:-**

The egg hatching inhibition potential of the CNSL was assessed using nine treatments as for the J2 mortality bioassay: two CNSL types (cold and hot extracts), four concentrations per CNSL type (0.01%, 0.1%, 1%, and 10%), and a control (SDW). Using a tong, one egg mass was placed in individual Petri dishes (55-mm diameter) containing 1 ml of a single treatment. The Petri dishes were arranged in a completely randomized design with four replicates on the laboratory bench at ambient temperature ( $28 \pm 1^\circ\text{C}$ ). The number of hatched J2s was counted 7 days after exposure to each treatment (Machal et al., 2025) using a light microscope following the procedure described for the J2 mortality experiment. The hatching inhibition rate was determined as follows: Egg hatching inhibition (%) = (number of unhatched eggs / Total number of eggs) x 100.

**Greenhouse pot experiment setup:-**

The pot experiment, repeated once, included eight treatments: two CNSL types (cold and hot extracts), three application doses (25 ml, 50 ml, and 100 ml), one concentration (10%, based on the in vitro bioassay), a positive control (no CNSL application), and a reference control (application of leaf aqueous extract of *C. adansonii* at 50 ml). Plastic pots (1.5 l capacity) were individually filled with 1000  $\text{cm}^3$  of unsterilized soil infested with RKNs collected from UNema experimental field, as indicated above. For both experiments, the initial nematode population densities were adjusted to 1140 individuals per pot by adding sterilized soil to the field soil. Depending on the treatments, pots were then inoculated with CNSL and aqueous extracts of *C. adansonii* and thoroughly mixed. Twenty-four

hours later, four week-old tomato seedlings (cv. Padma 108 F1) were singly transplanted into each pot. Pots were arranged in a completely randomized design with five replications. Plants were watered manually (twice a day) with tap water and maintained for eight weeks before harvest.

#### **Estimation of nematode reproduction and root damage:-**

At harvest, the effect of the treatments on nematode reproduction and root galling were assessed through the following parameters: soil and root final nematode population densities, galling index and number of egg masses per g root. The galling index was assessed per plant on the entire root system after plants were uprooted using a 0–10 rating scale, where 0 = no galling damage and 10 = 91–100% galled roots (Affokpon et al., 2012). To estimate the production of egg masses, part of the root systems was stained with 20% McCormick red food colour for 15 min (Coffi et al., 2025). Then, 1 g root was removed and the egg masses were counted under a stereomicroscope. Final nematode population densities were determined in roots and soil at harvest. Briefly, J2s and eggs were extracted from the total root system and from 250 cm<sup>3</sup> soil using the centrifugation technique described by Affokpon et al. (2011). Nematodes were counted under a compound microscope (Olympus CX31) from 3 ml aliquots removed from 50 ml and 100 ml of the soil and root suspensions, respectively. Nematode reproductive factor (RF) was then calculated per pot as the ratio between the final number of soil and root nematodes (J2s and eggs) and the initial nematode inoculum.

#### **Measurement of crop growth characters:-**

Eight weeks after tomato transplanting, shoot length, stem girth, fresh shoot weight, and fresh root weight were measured to determine the effect of the CNSL application on tomato growth.

#### **Statistical analysis:-**

R software version 4.4.2 (R Core Team, 2024) was used to perform the statistical analyses. Data on nematode counts were transformed to log (x+1) to conform to normal distribution prior to statistical analysis (Gomez and Gomez, 1984). All data were subjected to ANOVA and means were separated using the Fisher LSD procedure at 5%. The lethal concentrations (LC<sub>50</sub>) were calculated from J2 mortality and egg inhibition percentages, which were corrected using Abbott's formula; the "drm" function from the drc package was used (Ritz et al., 2016), as it allows the fitting of different non-linear dose-response models to binary data. The arguments fct = LL.2 and type = 'binomial' were applied, indicating that the two-parameter logistic function and binomial data type were selected. The LC<sub>50</sub> was obtained using the ED function from the 'drc' package. For both in vitro and pot bioassays, data from the repeated experiments were pooled for the ANOVA after determining that the interaction treatment × experiment was not significant (Affokpon et al., 2011).

### **Results:-**

#### **In vitro bioassays:-**

##### **Effect of cashew balm on *M. enterolobii* juveniles and eggs:-**

Regardless of the type of CNSL, in most cases, their suppressive effect on *M. enterolobii* J2s increased consistently with exposure time and CNSL concentration (Figure 1). Comparing the two CNSL types, for the same concentration, cold-extracted CNSL induced higher J2 mortality rates than hot-extracted CNSL, except for the concentration 10%. Cold-extracted CNSL at concentrations 1% and 10% caused more than 50% dead of J2s at 12h exposure and reached approx. 85% dead at 48h exposure time for the concentration 10%. In hot-extracted CNSL treatments, only the concentration 10% caused J2 mortality rate higher than 50%, regardless of the exposure time, but reached 90% at 48 hours. In contrast, the J2 mortality rate in the SDW average 2%. The linear regression analysis showed that the lethal concentrations (LC<sub>50</sub>) against *M. enterolobii* J2s after 48 h of exposure were 3.368 µl/ml and 18.156 µl/ml for cold- and hot-extracted CNSL, respectively (Figure 2).

##### **Effect of CNSL on *M. enterolobii* eggs:-**

Regarding the effects of CNSL on *M. enterolobii* eggs, the hatching inhibition rate in CNSL suspensions after 7 days of exposure was significantly higher ( $P < 0.05$ ) than that recorded in SDW (6.13%) for the same period, except for the cold-extracted CNSL at concentration 0.01% (Figure 3). The highest egg hatching inhibition rates were observed at 10% CNSL concentration, with 88.25% and 84.88% for cold and hot extracted CNSL, respectively. The lethal concentrations (LC<sub>50</sub>) for hatching inhibition of *M. enterolobii* eggs after 7 days of exposure were 1.133 µl/ml and 4.156 µl/ml for cold- and hot-extracted CNSL, respectively (Figure 3).

**Greenhouse pot experiment:-****Effect of cashew balm on nematode reproduction and root infestations:-**

Application of CNSL in unsterilized soil planted with tomato resulted, in most cases, in significant reductions of nematode final population densities, reproductive factor, gall index, and number of egg masses compared with the untreated positive control (Table 1). The highest reductions of the final nematode (J2s and eggs) population densities were recorded from soil treated with 100 ml of cold-extracted CNSL with 78.77% and 91.47% reductions, respectively, compared with *C. adansonii* leaf aqueous extract and untreated positive controls. For the same CNSL application doses, the final nematode population densities in the cold-extracted CNSL treatments were at least 20% lower than those in the hot-extracted CNSL treatments. Nematode reproductive factor (RF) was also significantly inhibited following CNSL application, with reduction rates similar to those of nematode population densities (Table 1).

The same trends were also observed when the average RF values were compared between CNSL types for the same application dose. Regarding the gall index, significant inhibition of root galling was observed in tomato plants from CNSL-treated pots compared with the untreated positive pots, except for the low dose (25 ml of CNSL). Cold-extracted CNSL applied at 100 ml/pot induced the highest gall index reduction by up to 54%, 78%, and 85% compared with the same dose for hot-extracted CNSL, *C. adansonii* treatment, and the positive control, respectively (Table 1). Moreover, egg mass production was significantly inhibited following CNSL application at doses 50 and 100 ml per pot for the cold extract and 100 ml/pot for the hot extract. However, there was no significant difference in number of egg masses per g roots between CNSL types at a dose of 100 ml/pot (Table 1).

**Effect of the CNSL application on plant growth:-**

The potential effect of the CNSL on plant growth was assessed using tomato (cv. Padma 108 F1). There were significant reductions in shoot length (SL), stem girth (SG), fresh shoot weight (FSW), and fresh root weight (FRW) in CNSL treated pots compared with the control pots, except for the hot-extracted CNSL applied at 25 ml/pot for SL and SG (Table 2). Notable reductions were particularly observed for cold-extracted CNSL, regardless of the application doses, and for hot-extracted CNSL applied at 50 ml and 100 ml per pot. SL, SG, FSW, and FRW were 74%, 82%, 86%, and 69.13%, respectively, lower in pots treated with cold-extracted CNSL at 100 ml than in untreated control pots (Table 2).

**Discussion:-**

*Meloidogyne enterolobii* has been reported to overcome standard control measures developed against RKNs, including chemical control and use of resistant varieties (Coffi et al., 2025; Poudel et al., 2025). This aggressive RKN species is spreading in Sub-Saharan Africa (Pagan et al., 2015; Kolombia et al., 2016; Affokpon et al., 2017), and, is therefore considered a serious threat for food security in this region. The results of the current study indicated the suppressive effect of CNSL against *M. enterolobii* juveniles and an inhibitory effect on egg hatching. To the best of our knowledge, no or very few studies have been conducted on the nematicidal properties of CNSL. In contrast, this product has been tested for its pesticidal effect against nematode parasites of animal (Ademola and Eloff, 2011), insect pests (Sotondji et al., 2022; Nyirenda et al., 2024; Sivaranjani et al., 2026), mites (De Carvalho Castro et al., 2019), and pathogens (Garcia et al., 2018). Phytochemical analysis of CNSL indicated the presence of various bioactive compounds, including anacardic acid, phenols, tannins, terpenoids, amino acids, flavonoids, and saponins (Kumar et al., 2022; Ashong et al., 2025).

According to Garcia et al. (2018), after the entrance of CNSL into the intracellular environment facilitated by the formation of chains, its phenol groups work to inactivate enzymes of energy synthesis, therefore possessing toxic potential for pathogens. This is in agreement with Dix (1995), who explained that the phenol groups inactivate enzymes related to energy production and the synthesis of basic metabolites. Assessing the insecticidal potential of CNSL on *Aedes aegypti* larvae, Passari et al. (2015) observed severe change in the midgut, leading to irreversible disruption of the complete larval development. In our study, the in vitro exposure of *M. enterolobii* to CNSL resulted in up to 90% mortality rate of J2s and inhibition of up to 88% of egg hatching. These results reflected the low LC<sub>50</sub> values for both J2 mortality and egg hatching inhibition, providing evidence of the high nematicidal potential of CNSL. Interestingly, the suppressive effects recorded in this study outperformed the nematode suppression rates observed in the *C. adansonii* control treatment and those reported on some plant extracts. Sero et al. (2025a) observed a maximum of 61.40% *M. enterolobii* J2 mortality after 72 h of exposure to compost tea produced from *C. adansonii* and 29.80% inhibition of egg hatching after 8 days of exposure. In the best cases, Mishra et al. (2017)

observed over 50% inhibition of *M. incognita* egg hatching after 15 days of exposure to vermicompost tea from agricultural residues, which is lower than that caused by CNSL.

At some concentrations (below or equal to 1%), the larvicidal effects on *M. enterolobii* were higher in cold extracted CNSL than in hot extracted, indicating that at medium concentrations, the effectiveness of CNSL is enhanced by the extraction methods. These findings aligned with previous observations which indicated that the composition of CNSL is determined by the mode of extraction (Maia et al., 2015; Ashong et al., 2025). Several authors revealed differences in chemical compositions of CNSL following the extraction methods, with cold extracted CNSL having the highest content of anacardic acid (Patel et al., 2006; Rodrigues et al., 2010, Sharma et al., 2020).

In the greenhouse pot experiment, CNSL application at some doses was found to inhibit nematode reproduction better than *C. adansonii*. Recently, Sero et al. (2025a) reported the nematocidal effect of compost tea produced from *C. adansonii* against *M. enterolobii* in an in vitro bioassay. The present study confirms the superiority of CNSL over *C. adansonii* in a naturally nematode-infested environment. In a pot experiment assessing the suppressive effect of compost produced from *Chromolaena odorata* on *M. enterolobii*, Sero et al. (2025b) reported a reproduction factor of 2.91 over 10, while it was 1.15 in our study. Interestingly, this outperformance of CNSL was observed using unsterilized soil infested with mixture species of *Meloidogyne* spp., reflecting the nematode situation in the West African vegetable production systems. These impressive results position CNSL as a promising alternative for RKN management in vegetable crops. Between the two types, cold-extracted CNSL, particularly at doses 50 ml and 100 ml per pot proved more effective than hot-extracted CNSL in suppressing nematode reproduction and inhibiting root gall formation. These results reflected those recorded in the in vitro bioassays, confirming that the mode of extraction affects the CNSL composition (Maia et al., 2015) and, in this case, the content of anacardic acid (Patel et al., 2006; Rodrigues et al., 2010, Sharma et al., 2020).

Adversely, all the growth parameters assessed in this study were negatively affected by CNSL, regardless the types. These results suggest a negative effect of CNSL soil application on tomato plants. Investigation of the phytotoxicity effect of CNSL on the germination and growth of lettuce, tomato and coffee revealed that CNSL negatively affected the germination and vigor of lettuce and tomato, particularly the root development (Matias et al., 2017), reflecting the situation in the present study. In contrast, Sotondji et al. (2022) has reported an increase of marketable cabbage yields (average 14 t/ha vs. 1.70 t/ha for untreated plots) following application of cold-extracted CNSL at 15%. However, the CNSL has been applied as foliar treatment, while it has been applied in soil in the current study. Likewise, the assessment of the effects of CNSL on rice in pot cultivation, showed no significant effect on the plant growth and dry matter production following soil application of CNSL at 0.1% (Minamikawa et al., 2021). Therefore, the harmful effect of CNSL observed in our study might be due to its application in close environment (pot experiment) at a high concentration (10%) with no possibility of leaching. These observations call for further studies to assess various concentrations (dilution rates) and application doses.

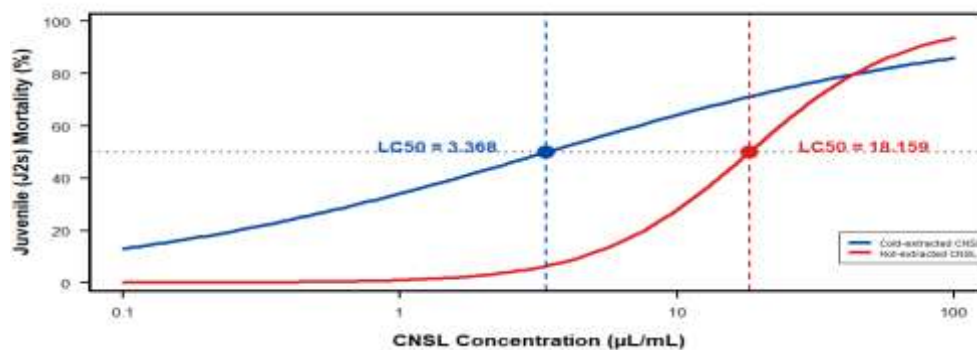
### **Conclusion:-**

The present study provides evidence that CNSL has nematocidal properties against *M. enterolobii* juveniles and eggs in vitro. Furthermore, this cashew nut by-product clearly demonstrates its inhibitory effects on RKN reproduction and tomato root galling in soil naturally infested with mixed species of RKNs. It is important to note that cold-extracted CNSL outperforms hot-extract CNSL in suppressing RKNs and the damage they cause to tomato roots. However, at a concentration of 10%, CNSL, regardless of the extraction mode, has a negative effect on the development of tomato plants, particularly fresh shoot and root weights. Hence, further investigations are required to identify concentrations suitable to suppress nematode reproduction while ensuring plant growth.

### **Acknowledgements:-**

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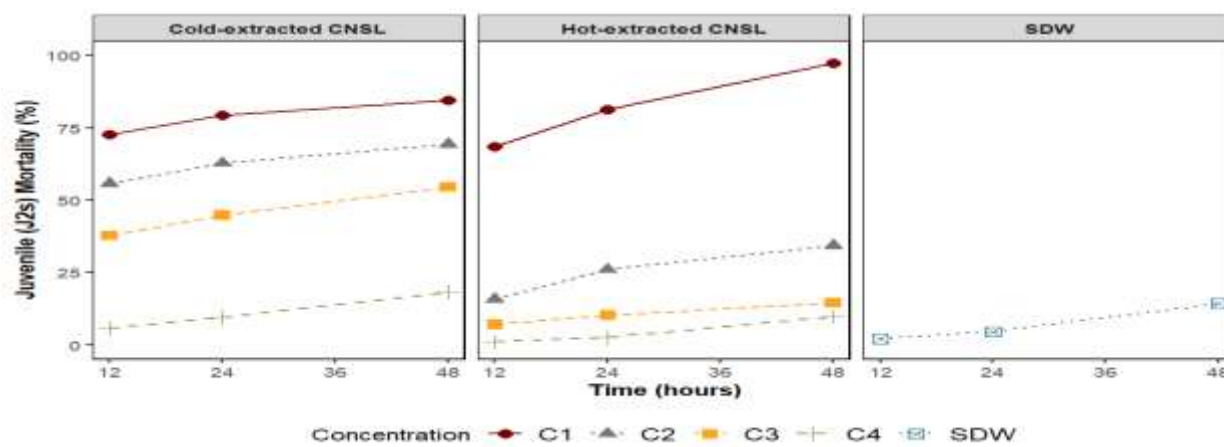
## Figures



**Figure 1: Percentage of dead second-stage juveniles (J2s) of *Meloidogyne enterolobii* at different exposure times following treatment with different concentrations of cold- and hot- extracted cashew nut shell liquid (CNSL)**

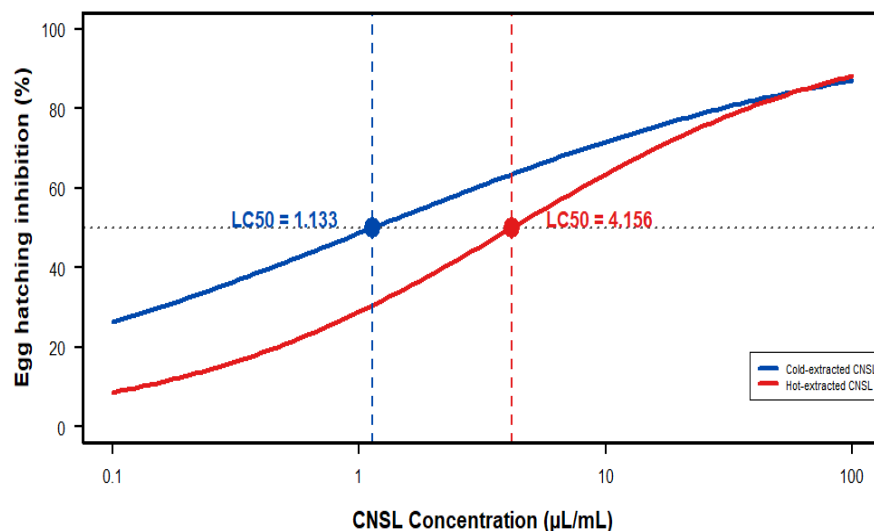
C1, C2, C3, and C4 represent CNSL concentrations of 10%, 1%, 0.1%, and 0.01%, respectively.

SDW: Sterile Distilled Water (control)



**Figure 2: Lethal concentrations (LC<sub>50</sub>) of cold- and hot- extracted cashew nut shell liquid (CNSL) causing 50% mortality of *M. enterolobii* J2s after 48 hours of exposure**





**Figure 3: Lethal concentrations ( $LC_{50}$ ) of cold- and hot- extracted cashew nut shell liquid (CNSL) inhibiting 50% hatching of *M. enterolobii* eggs after 7 days of exposure**

## Tables

**Table 1: Effect of different doses of cold- and hot-extracted cashew nut shell liquid (CNSL) on root-knot nematode reproduction and gall index eight weeks on tomato plants (cv. Padma 108 F1) 8 weeks after transplanting in *Meloidogyne* spp. infested soil**

Doses of CNSL (ml/pot)	Final nematode densities per pot <sup>a</sup>	RF = Pf/Pi <sup>b</sup>	Gall index <sup>c</sup> (0-10)	no egg masses/ g of roots <sup>a</sup>
Cold-extracted CNSL 100	1301 f	1.15 e	1.2 d	4 c
Cold-extracted CNSL 50	3586 d	3.17 d	2.6 c	6 c
Cold-extracted CNSL 25	12094 b	10.69 b	7.6 a	18 ab
Hot-extracted CNSL 100	1642 e	1.45 e	2.6 c	4 c
Hot-extracted CNSL 50	5944 c	5.25 c	5.0 b	17 ab
Hot-extracted CNSL 25	15034 a	13.28 a	7.8 a	22 a
<i>C. adansonii</i> leaf extract (20g/50 ml)	6128 c	5.41 c	5.6 b	10 b
Positive control (nematode alone)	15251 a	13.47 a	8.0 a	26 a
Fisher	<b>249.4</b>	<b>280.5</b>	<b>156.5</b>	<b>16.12</b>
P value	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>

Data are means of ten replicates. Values in columns followed by different letters are significantly different ( $P \leq 0.05$ ) based on Fisher's Least Significant Difference (LSD) test using one-way ANOVA. <sup>a</sup>Statistical analysis based on  $\log_{10}(x+1)$ , backtransformed data presented. <sup>b</sup>RF (nematode reproductive factor) = final nematode density (eggs and juveniles) per pot / initial nematode inoculum per pot. <sup>c</sup>Gall index was assessed on a scale of 0-10, where 0 = no galling and 10 = 91-100 % galled roots (Affokpon et al., 2012).



**Table 2: Shoot length (SL), stem girth (SG), fresh shoot weight (FSW) and fresh root weight (FRW) of tomato plants (cv. Padma 108 F1) 8 weeks after transplanting in Meloidogyne spp. infested soil**

Doses of CNSL (ml/pot)	SL (cm)	SG (mm)	FSW (g)	FRW (g)
Cold-extracted CNSL 100	15.3 d	1.11 d	2.69 f	2.76 e
Cold-extracted CNSL 50	39.5 c	2.97 c	7.50 e	3.30 de
Cold-extracted CNSL 25	51.6 b	4.04 b	13.24 c	7.09 c
Hot-extracted CNSL 100	15.0 d	1.59 d	3.12 f	2.66 e
Hot-extracted CNSL 50	39.9 c	3.03 c	10.62 d	4.67 d
Hot-extracted CNSL 25	52.5 b	5.81 a	14.12 c	6.99 c
C. adansonii leaf extract (20 g/50 ml)	64.4 a	6.26 a	26.77 a	11.80 a
Positive control (nematode alone)	59.1 ab	6.34 a	19.44 b	8.94 b
Fisher	<b>30.99</b>	<b>55.5</b>	<b>78.55</b>	<b>44.89</b>
P value	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>

Data are means of ten replicates. Values in columns followed by different letters are significantly different ( $P \leq 0.05$ ) based on Fisher's Least Significant Difference (LSD) test using one-way ANOVA

### References:-

- Ademola, I. O., & Eloff, J. N. (2011). Anthelmintic activity of acetone extract and fractions of Vernonia amygdalina against Haemonchus contortus eggs and larvae. Onderstepoort Journal of Veterinary Research, 43, 521–527.
- Affokpon, A., Coyne, D. L., Htay, C. C., Agbèdè, R. D., Lawouin, L., & Coosemans, J. (2011). Biocontrol potential of native Trichoderma isolates against root-knot nematodes in West African vegetable production systems. Soil Biology and Biochemistry, 43(3), 600–608. <https://doi.org/10.1016/j.soilbio.2010.11.029>
- Affokpon, A., Dan, C. B. S., Houedjissi, M. E., Hekpazo, B. A., & Tossou, C. (2012). L'efficacité des dérivés de graines de neem contre les nématodes à galles en cultures maraîchères diffère en fonction du type de dérivé. Bulletin de la Recherche Agronomique du Bénin, 72, 48–58.
- Affokpon, A., Waeyenberge, L., Afoha, S. A. P., Coffi, D. N. E., Dossou-Yovo, D., Dansi, A., Viaene, N., & Coyne, D. L. (2017). Nematode parasites of yam (Dioscorea spp.) in Benin: prevalence and species diversity. 69<sup>th</sup> International Symposium on Crop Protection. Communications in Agricultural and Applied Biological Sciences, Ghent, Belgium, p. 255.
- Affokpon, A., Coffi, D. N. E., Atchadé, J. M. C. O., Tossou, C., & Coyne, D. L. (2021). Population build-up and damage of Scutellonema bradys and Meloidogyne spp. on local yam cultivars under field and storage conditions in Benin. RUFORUM Working Document Series, 19(1), 33–42. <http://repository.ruforum.org>
- Ahmad, L., & Siddiqui, M. A. (2018). Efficacy of botanicals and carbofuran for the control of Meloidogyne incognita affecting Solanum lycopersicum L. International Journal of Phytopathology, 7(2), 69–75. <https://doi.org/10.33687/phytopath.007.02.2522>
- Ashong, G. W., Darko, C. E., Pappoe, E., Ababio, B. A., & Kwaansa-Ansah, E. E. (2025). Exploration of the phytochemical evaluation, chemical profile, and antimicrobial activities of cashew nut shell oil, a potential medicinal plant for various applications. Pharmacological Research – Natural Products, 8, 100291.
- Coffi, E. N. D., Affokpon, A., Hounkponou, B. E., Azonkpin, S., Djihinto, C. A., & Quenum, F. J. B. (2025). Do Ralstonia solanacearum-resistant tomato varieties withstand root-knot nematodes? A greenhouse evaluation of Meloidogyne enterolobii impact. International Journal of Plant & Soil Science, 37(9), 1–18. <https://doi.org/10.9734/ijpss/2025/v37i95680>
- Coyne, D. L., Nicol, J. M., & Claudius-Cole, B. (2010). Les nématodes des plantes : Un guide pratique des techniques de terrain et de laboratoire. SP-IPM, International Institut of Tropical Agriculture (IITA), Cotonou, Benin. 93 p.
- Coyne, D. L., Cortada, L., Dalzell, J. J., Claudius-Cole, A. O., Haukeland, S., Luambano, N., & Talwana, H. (2018). Plant-parasitic nematodes and food security in Sub-Saharan Africa. Annual Review of Phytopathology, 56(1), 381–403. <https://doi.org/10.1146/annurev-phyto-080417-045833>
- De Carvalho, F. G., dos Santos, L. M., Monnerat, R., Andrade, M. D., de Andrade, M., dos Santos, M. A., Bastos, M. D. I., & de Santana, J. M. (2019). Ovicidal and deleterious effects of cashew (Anacardium

- occidentale) nut shell oil and its fractions on *Musca domestica*, *Chrysomya megacephala*, *Anticarsia gemmatalis* and *Spodoptera frugiperda*. *Chemistry & Biodiversity*, 16(5), e1800468. <https://doi.org/10.1002/cbdv.201800468>
12. Dix, N. J. & Webster, J. 1995. *Fungal Ecology*. Chapman & Hall, London, UK. 549 p. <https://doi.org/10.1007/978-94-011-0693-1>
  13. Garcia, N. Z. T., Barbosa, G. F., Matias, R., Pedrinho, D. R., Bono, J. A. M., & Martini, D. (2018). Antifungal potential of cashew nut shell liquid in the control of plant pathogens. *Bioscience Journal*, 34(1), 95–103.
  14. Gebrehana, Z. G., Gebremikael, M. T., Beyene, S., Wesemael, W. M., & De Neve, S. (2025). Suppression potential of selected vermicomposts against root-knot nematode (*Meloidogyne incognita*) under in vitro, pot, and field conditions. *Frontiers in Plant Science*, 16, 1532800. <https://doi.org/10.3389/fpls.2025.1532800>
  15. Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research*. Wiley J. & Sons Inc., New York, USA.
  16. Hallman, J., & Meressa, B. H. (2018). Nematode parasites of vegetables. In Sikora, R. A., Coyne, D., Hallman, J., & Timper, P. (Eds.), *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture* (pp. 346–410). CABI International, Wallingford. <https://doi.org/10.1079/9781786391247.0346>
  17. Kidane, E., Seid, A., & Kebede, M. (2020). Reaction of tomato (*Solanum lycopersicum*) cultivars towards root-knot nematode (*Meloidogyne incognita*) and bacterial wilt (*Ralstonia solanacearum*). *Nematropica*, 49, 246–253.
  18. Kolombia, Y. A., Lava Kumar, P., Claudius-Cole, A. O., Karssen, G., Viaene, N., Coyne, D., & Bert, W. (2016). First report of *Meloidogyne enterolobii* causing tuber galling damage on white yam (*Dioscorea rotundata*) in Nigeria. *Plant Disease*, 100(10). <https://doi.org/10.1094/PDIS-03-16-0348-PDN>
  19. Kumar, V. N., Prabhakumari, C., & John, S. (2022). Phytochemical screening and antibacterial activity of leaf extracts of *Anacardium occidentale* L. *The Journal of the Indian Botanical Society*, 102(4), pp. 326-333. <https://jibs.mripub.com/index.php/JIBS/article/view/49>
  20. Lekha, M., Faizal, M. H., & Anitha, N. (2020). Insecticidal activity of cashew nut shell liquid against sucking pests of cowpea (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdc.). *Entomology*, 45(2), 107–114. <https://doi.org/10.33307/entomon.v45i2.518>
  21. Maia, F. J. N., Ribeiro, P. F. W., Rangel, G. J. H., Lomonaco, D., Luna, F. W. P., de Lima-Neto, P., & Mazzetto, S. E. (2015). Evaluation of antioxidant action by electrochemical and accelerated oxidation experiments of phenolic compounds derived from cashew nut shell liquid. *Industrial Crops and Products*, 67, 281–286. <https://doi.org/10.1016/j.indcrop.2015.01.034>
  22. Matias, R., Rosa, A. C., Oliveira, A. K. M., Pereira, K. C. L., Rizzi, E. S., & Machado, A. A. (2017). Cashew nut shell liquid and formulation: toxicity during the germination of lettuce, tomato seeds and coffee senna and seedling formation. *Acta Scientiarum. Agronomy*, 39(4), 487–495. <https://doi.org/10.4025/actasciagron.v39i4.32603>
  23. Kazunori, M., Masahiro K., & Hisayoshi H. (2021). Effect of cashew nut shell liquid on rice growth and methane emission from paddy soil. *Soil Science and Plant Nutrition*, 67 (3), 323-331, DOI: 10.1080/00380768.2021.1887710
  24. Mishra, V., Desai, J., & Patel, K. I. (2018). (UV/Oxidative) dual curing polyurethane dispersion from cardanol based polyol: synthesis and characterization. *Industrial Crops and Products*, 111, 165–178. <https://doi.org/10.1016/j.indcrop.2017.10.015>
  25. Nilusmas, S. (2020). Gestion durable des nématodes à galles en culture maraîchère par la modélisation et l'optimisation du déploiement de variétés résistantes. Thèse de doctorat, Université Côte d'Azur. NNT: 2020COAZ4086.
  26. Nyirenda, J., Kadango, Z., Funjika, E., & Chipabika, G. (2024). Larvicidal, ovicidal and antifeedant activity of crude cashew nutshell liquid against fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). *Crop Protection*, 179, 106619. <https://doi.org/10.1016/j.cropro.2024.106619>
  27. Pagan, C., Coyne, D., Carneiro, R., Kariuki, G., Luambano, N., Affokpon, A., & Williamson, V. M. (2015). Mitochondrial haplotype-based identification of ethanol-preserved root-knot nematodes from Africa. *Phytopathology*, 105(3), 350–357. <https://doi.org/10.1094/PHYTO-08-14-0225-R>
  28. Passari, A. K., Mishra, V. K., Gupta, V. K., & Singh, B. P. (2015). Effects of cashew nut shell liquid (CNSL) component upon *Aedes aegypti* Lin. (Diptera: Culicidae) larvae's midgut. *African Journal of Biotechnology*, 14(9), 829–834. <https://doi.org/10.5897/AJB2014.14347>
  29. Patel, R. N., Bandyopadhyay, S., & Ganesh, A. (2006). Extraction of cashew (*Anacardium occidentale*) nut shell liquid using supercritical carbon dioxide. *Bioresource Technology*, 97(6), 847–853.

30. Poudel, N., Torres, L., Davis, R. F., Jagdale, G. B., McAvoy, T., & Chowdhury, I. A. (2025). Effect of non-fumigant nematicides on reproduction of recently detected Meloidogyne species in Georgia under greenhouse conditions in tomato. *Horticulturae*, 11(1), 36. <https://doi.org/10.3390/horticulturae11010036>
31. Ritz, C., Strebig, J. C., & Ritz, M. C. (2016). Package 'drc'. Creative commons: mountain view, CA, USA.
32. Rodrigues, F. H. A., França, F. C. F., Souza, J. R. R., Ricardo, N. M. P. S. & Feitosa, J. P. A. (2010). Comparison between physico-chemical properties of the technical Cashew Nut Shell Liquid (CNSL) and those natural extracted from solvent and pressing. *Polímeros*, 21(2), 156-160.
33. Sero, S., Affokpon, A., Mensah, S. E. P., Garadima, A., Lahamy, O., Assogba, M. F., & Zandjanakou-Tachin, M. (2025a). In vitro nematicidal activity of composts from *Crotalaria retusa* and *Crateva adansonii* on *Meloidogyne enterolobii* and assessment of their phytotoxicity on tomato under greenhouse conditions. *Journal of Advances in Biology & Biotechnology*, 28(10), 1117–1132. <https://doi.org/10.9734/jabb/2025/v28i103131>
34. Sero, S., Azize, G., & Affokpon, A. (2025b). Nematicidal effects of compost produced from *Chromolaena odorata* L. against *Meloidogyne enterolobii* under laboratory and greenhouse conditions. *International Journal of Applied Science and Research*, 8 (6): 244–254. <https://doi.org/10.56293/IJASR.2025.6827>
35. Sharma, A. K., & Sharma, P. (2020). *Host Pathogen Interactions and Applications*. Springer, Singapore. <https://doi.org/10.1007/978-981-15-3321-1>
36. Sivaranjani, K., Raja, K., Umarani, R., Preetha, G., Chandrakumar, K., & Janaki, P. (2025). Potential bioactivity of cashew nut shell liquid (CNSL) against storage pests in paddy (*Oryza sativa* L.). *Annals of Phytomedicine*, 14(2), 651–661. <http://dx.doi.org/10.54085/ap.2025.14.2.65>
37. Sotondji, F. A., Douro Kpindou, O. K., Djihinto, A. C., Dannon, E. A., Zodome, G., Sagbo, R., Dossou, J., Adjou, E., Hougourou, D. C., & Soumanou, M. M. (2020). Efficacité du baume de cajou et des huiles végétales pour la lutte contre les populations de *Plutella xylostella* L. 1758 (Lepidoptera : Plutellidae). *African Crop Science Journal*, 28(2), 151–168. <https://doi.org/10.4314/acsj.v28i2.2>
38. Trudgill, D. L., & Blok, V. C. (2001). Apomictic, polyphagous root-knot nematodes: Exceptionally successful and damaging biotrophic root pathogens. *Annual Review of Phytopathology*, 39, 53–77. <https://doi.org/10.1146/annurev.phyto.39.1.53>
39. Walia, R. K., & Khan, M. R. (2023). Root-knot nematodes (*Meloidogyne* spp.). In *Root-Galling Disease of Vegetable Plants* (pp. 1–60).