ISSN(O): 2320-5407 | ISSN(P): 3107-4928

ISSN NO. 2320-5407

International Journal of Advanced Research

Publisher's Name: Jana Publication and Research LLP

www.journalijar.com

REVIEWER'S REPORT

Manuscript No.: IJAR-55829

Title: Exploring the nematicidal potential of cashew nut shell liquid against root-knot nematodes: in vitro and greenhouse evaluations

Recommendation:
Accept as it is

Rating	Excel.	Good	Fair	Poor
Originality		√		
Techn. Quality		√		
Clarity		√		
Significance			√	

Reviewer Name: Dr. Manju M

Detailed Reviewer's Report

1. Background and importance of root-knot nematodes

Root-knot nematodes (Meloidogyne spp.) are among the most damaging soil-borne pests affecting vegetable crops globally. They invade plant roots and induce gall formation, which severely disrupts water and nutrient uptake. This leads to stunted growth, reduced vigor, and substantial yield losses. Their impact is particularly severe in tropical and subtropical regions where climatic conditions favor rapid reproduction. Smallholder farmers are disproportionately affected due to limited access to effective control measures. The economic losses caused by these nematodes are often underestimated. Effective management of RKNs is therefore essential for sustainable vegetable production.

2. Hidden nature and diagnostic challenge of nematode damage

Unlike foliar pests, root-knot nematodes cause damage below ground, making early detection difficult. Above-ground symptoms such as wilting or yellowing are often mistaken for nutrient deficiencies. By the time visible symptoms appear, nematode populations are already well established. This hidden nature allows infestations to persist unnoticed across cropping cycles. Farmers often respond with inappropriate inputs, further delaying proper control. As a result, nematode pressure accumulates in the soil. Improved awareness and effective management tools are therefore critical.

3. Relevance to peri-urban and urban vegetable production

Urban and peri-urban vegetable systems are highly intensive and characterized by continuous cropping. These systems play a vital role in supplying fresh vegetables to growing urban populations. However, repeated cultivation of susceptible crops creates ideal conditions for nematode buildup. Limited land availability restricts crop rotation and fallow practices. Soil health often declines due to intensive input use. Consequently, nematode infestations become chronic and difficult to manage. Addressing RKNs in these systems is a major agricultural priority.

4. Socio-economic implications for smallholder farmers

Vegetable farming is a key source of income for smallholder producers in developing regions. Yield losses caused by nematodes directly reduce household income and food availability. Farmers often lack access to diagnostic services and effective management options. The cost of commercial nematicides further limits adoption. Crop failure due to nematodes increases financial risk and vulnerability. Women

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and youth engaged in urban farming are particularly affected. Sustainable nematode control can therefore improve livelihoods and resilience.

5. Limitations of synthetic nematicides

Chemical nematicides are widely used but present serious challenges. Their high cost restricts accessibility for small-scale farmers. Improper handling and lack of protective equipment pose health risks. Many nematicides are toxic to beneficial soil organisms and contaminate the environment. Regulatory restrictions have led to the withdrawal of several active ingredients. Overreliance on chemicals undermines long-term soil health. These limitations emphasize the need for safer and affordable alternatives.

6. Emergence of Meloidogyne enterolobii as a major threat

Meloidogyne enterolobii is recognized as one of the most aggressive RKN species. It can infect a wide range of host plants and reproduces rapidly. Importantly, it overcomes resistance genes used in many tomato cultivars. This renders host resistance strategies largely ineffective. Its spread in Sub-Saharan Africa poses a serious risk to vegetable production. Conventional control measures often fail against this species. Novel management approaches are urgently required.

7. Need for environmentally friendly nematode control

Growing concern over pesticide residues in vegetables has increased demand for eco-friendly solutions. Consumers are increasingly aware of food safety and environmental sustainability. Environmentally benign control options reduce risks to farmers and ecosystems. Such approaches align with integrated pest management principles. They also support compliance with regulatory and export standards. Botanical-based nematicides offer a promising pathway. Their development is consistent with sustainable agriculture goals.

8. Potential role of botanical extracts

Botanical extracts have long been used in traditional pest management. They are biodegradable and generally less harmful to non-target organisms. Many plants contain secondary metabolites with nematicidal activity. Local availability makes botanicals attractive to smallholder farmers. Their use can reduce dependence on synthetic chemicals. However, scientific validation is necessary to ensure efficacy. Standardization and dose optimization remain key challenges.

9. Cashew nut shell liquid as an underutilized resource

Cashew nut shell liquid is a by-product of cashew processing industries. Large quantities are generated annually in cashew-producing regions. Traditionally, CNSL is treated as industrial waste. However, it contains valuable bioactive compounds. Utilizing CNSL for pest control adds economic value to waste materials. This supports circular economy concepts. Its local abundance makes it particularly attractive for West Africa.

10. Chemical composition and biological activity of CNSL

CNSL contains anacardic acids, cardanol, and cardol as major components. These phenolic compounds are known for antimicrobial and pesticidal properties. Their chemical structure allows interaction with biological membranes and enzymes. Previous studies have demonstrated insecticidal and antifungal effects. However, nematicidal activity has received limited attention. Understanding this potential fills an important research gap. The present study addresses this knowledge deficiency.

11. Influence of extraction method on CNSL efficacy

CNSL can be obtained through cold or hot extraction methods. Cold extraction preserves higher levels of anacardic acids. Hot extraction increases cardanol content due to decarboxylation. These compositional differences influence biological activity. Comparing both extracts allows understanding of structure–activity relationships. Extraction method selection is therefore critical. This comparison strengthens the scientific rigor of the study.

12. Objectives and scope of the study

The study was designed with clear and comprehensive objectives. It evaluated juvenile mortality and egg hatching inhibition in vitro. Greenhouse experiments assessed efficacy under more realistic conditions.

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Cold- and hot-extracted CNSL were compared systematically. Potential phytotoxic effects on tomato plants were also examined. This holistic approach enhances relevance and applicability. The scope addresses both efficacy and safety.

13. Experimental location and environmental relevance

Experiments were conducted at the UNema research station in Benin. The site represents typical vegetable-growing conditions in West Africa. The sub-equatorial climate favors nematode development. Conducting experiments under such conditions increases ecological validity. Both laboratory and greenhouse facilities were utilized. Controlled conditions ensured reliable data generation. The location enhances regional relevance of findings.

14. Preparation and standardization of nematode inoculum

A pure population of M. enterolobii was maintained on a host plant. Standard extraction protocols ensured consistent juvenile and egg collection. Using a pure culture improved experimental accuracy. For greenhouse trials, naturally infested soil was included. This reflected real farming conditions more closely. Inoculum density was carefully standardized. Such rigor ensures reproducibility and reliability.

15. Preparation and application of CNSL treatments

CNSL extracts were prepared using precise dilution methods. Multiple concentrations allowed dose-response evaluation. Sterile water minimized contamination risks. Proper handling ensured consistency across treatments. Both extracts were applied uniformly in assays. This systematic preparation strengthens data reliability. Concentration gradients facilitated meaningful comparisons.

16. Inclusion of botanical reference control

Crateva adansonii leaf extract was used as a reference botanical. This plant is known for nematicidal properties. Including it allowed benchmarking CNSL performance. Preparation followed established protocols. The comparison enhanced interpretability of results. It provided a realistic alternative control option. Such inclusion improves study credibility.

17. Juvenile mortality bioassay and observations

Second-stage juveniles were exposed to CNSL treatments. Mortality was assessed at multiple time intervals. Microscopic examination ensured accurate scoring. Results showed concentration- and time-dependent effects. Cold-extracted CNSL caused higher mortality. Controls showed minimal natural mortality. The assay demonstrated direct toxic effects clearly.

18. LC_{50} determination and comparative toxicity

Dose–response analysis was conducted to calculate LC_{50} values. Cold-extracted CNSL showed significantly lower LC_{50} values. This indicates higher potency at lower concentrations. Such efficiency is advantageous for field application. Statistical analysis confirmed significant differences. These results highlight extraction method importance. Lower doses reduce cost and risk.

19. Egg hatching inhibition methodology

Egg masses were exposed to CNSL for extended periods. Hatching juveniles were counted carefully. Inhibition rates were calculated relative to controls. This method assessed impact on reproduction. Replication ensured robustness. The assay complemented juvenile mortality results. Together, they provided a full life-stage assessment.

20. Egg hatching inhibition results and implications

CNSL significantly inhibited egg hatching across concentrations. Cold-extracted CNSL consistently showed stronger effects. High inhibition rates indicate strong ovicidal activity. Even moderate doses were effective. Control treatments showed normal hatching. This demonstrates disruption of nematode life cycles. Such effects are crucial for long-term control.

21. Greenhouse experimental design

Pot experiments were conducted under controlled greenhouse conditions. Treatments were arranged in a randomized design. Naturally infested soil increased realism. Different CNSL doses were tested. Controls were included for comparison. Plant and nematode parameters were measured. The design allowed efficacy and safety evaluation.

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22. Effect on nematode populations and reproduction

CNSL significantly reduced nematode populations in soil and roots. Reproductive factor values declined sharply. Cold-extracted CNSL performed best. Reduced reproduction limits future infestations. Root galling and egg mass production decreased. These outcomes indicate strong suppressive effects. CNSL outperformed the reference botanical.

23. Phytotoxic effects and possible explanations

CNSL negatively affected tomato growth at higher doses. Shoot length and biomass were reduced. Effects were more pronounced with cold-extracted CNSL. Confined pot conditions may have intensified exposure. Phenolic compounds can interfere with root metabolism. Limited leaching increased toxicity. These observations highlight the need for caution.

24. Overall conclusion and future perspectives

The study demonstrates strong nematicidal potential of CNSL. Cold-extracted CNSL was consistently more effective. Dual action on juveniles and eggs is a major advantage. Greenhouse results confirm practical relevance. However, phytotoxicity at high doses is a limitation. Dose optimization and formulation improvement are required. Field trials are essential before recommendation.