# Nematicidal Efficacy of *Carissa carandas* Extracts against *Meloidogyne incognita*

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***Abstract***

*Plant-parasitic nematodes (PPNs), especially root-knot nematodes (RKNs) of the genus Meloidogyne, significantly diminish the productivity of horticultural crops, leading to global yield losses valued at approximately $157 billion. Traditional chemical treatments for PPNs pose environmental hazards, prompting the need for eco-friendly alternatives. This study explores the nematicidal potential of Carissa carandas L. (Karonda) plant extracts against Meloidogyne incognita. Various parts of C. carandas were collected, processed and extracted using solvents such as methanol, ethanol, ethyl acetate, chloroform and water. The efficacy of these extracts was tested on egg-hatching inhibition and juvenile mortality of M. incognita. Methanol extracts, particularly from seeds, demonstrated the highest nematicidal activity, with significant juvenile mortality and egg-hatching inhibition of M. incognita.*

*Results indicated that the nematicidal properties are likely due to phytochemicals such as alkaloids, flavonoids and organic acids present in the extracts. This study suggests that C. carandas extracts, especially methanolic seed extracts, are promising eco-friendly alternatives for managing RKNs and promoting sustainable agricultural practices. Further research is recommended to isolate and to identify the active compounds responsible for the nematicidal activity.*

**Keywords:** *Meloidogyne incognita*, *Carissa carandas*, Juvenile mortality, Egg-hatching inhibition. Methanol extract.

## Introduction

Plant-parasitic nematodes (PPNs) are among the most damaging parasites that significantly decrease the productivity of horticultural crops[4]. PPNs are pseudocoelomate, unsegmented, worm-like animals that constitute about 15% of all nematode species[6]. The symptoms they cause in plants resemble those of fungal infections, water stress, or other physiological disorders, earning them the moniker "hidden enemy" of farmers. Root-knot nematodes (RKNs), a prevalent type of plant-parasitic nematode, belong to the genus Meloidogyne and affect plants globally. They infect over 3,000 plant species including many cultivated plants[1], leading to an estimated worldwide yield loss of 12.3%, equivalent to 157 billion dollars, with 40.3 million dollars reported from India alone[17]. Of the 106 described species of RKN, only four - *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla* account for 95% of infestations[12,16].

Various methods can manage root-knot nematodes including chemical, physical, biological and cultural land management practices. The most effective method has been chemical treatment. However, these chemicals are hazardous, accelerate biodegradation and cause environmental contamination, leading to long-term negative environmental impacts[7]. Therefore, an eco-friendly strategy to combat plant-parasitic nematodes is the best solution, providing an alternative to chemical pesticides. Plant-derived products are an effective, eco-friendly method for mitigating nematode infestations in various crops.

*Carissa carandas* L. (family Apocynaceae), commonly known as Karonda, is an important minor fruit that grows wild in bushes throughout India. Various parts of the plant including the fruit, leaves and roots, are used to treat different diseases and have nutraceutical value[5]. *C. carandas* is known to contain a wide range of phytochemicals in its fruits, leaves and roots, giving it significant medicinal value. The pharmacological importance of crude extracts from these parts and isolated chemical compounds has been evaluated by several researchers using *in vitro* and *in vivo* methods[2]. The plant exhibits a range of biological activities, such as antidiabetic, antimicrobial, hepatoprotective, analgesic, anti-inflammatory, antipyretic, antiviral and anthelmintic properties[2,14].

This study aims to develop sustainable and eco-friendly plant protection strategies to reduce crop losses caused by root-knot nematodes and to improve plant health. To address these issues, the research focuses on utilizing various parts of the *Carissa carandas* plant to suppress *Meloidogyne incognita* infection under laboratory conditions.

## Material and Methods

The present study was conducted at the Department of Studies in Botany, Manasagangotri, Mysuru, Karnataka, India. Various parts of the *C. carandas* plant (leaves, unripened fruit, ripened fruit and seeds) were collected from the Gopinatham forest region, Chamarajanagara district, Karnataka, India. The collected plant parts were thoroughly washed with sterile distilled water, shade-dried in the laboratory for two weeks, powdered using a blender and sieved to obtain a fine powder. The powdered plant material

was then stored in an air-tight container for future use. Fifty grams of the powdered plant parts were extracted using a Soxhlet apparatus with different solvents such as water (aqueous), methanol, ethanol, ethyl acetate and chloroform. The solvent was regularly changed until no coloration was observed and the extract was concentrated using a rotary evaporator.

The resulting crude extracts were stored in a refrigerator in an air-tight container until needed. A pure population of *M. incognita* was maintained on tomato plants in the glasshouse of the Department of Studies in Botany, Manasagangotri, University of Mysore. This population was initially established from root-knot nematode-infected fields of tomato plants. The identification of *M. incognita* was based on perineal patterns[8]. Egg masses were picked and hatched in a beaker filled with sterile distilled water at 27±2°C in an incubator. The suspension containing hatched juveniles was collected daily and fresh distilled water was added. The concentration of freshly hatched second-stage juveniles was standardized as required.

**The egg-hatching inhibition experiment:** A single healthy egg mass of *M. incognita* was picked from the roots of infected tomato plants and transferred to Petri dishes containing 10 ml of each extract of the *C. carandas* plant. Egg mass in distilled water served as the control. All Petri dishes were kept at room temperature on the laboratory bench for egg hatching. Each treatment was replicated three times. The Petri dishes were incubated at 28°C for up to 72 hours and the total number of hatched juveniles was recorded using a binocular microscope[13].

**The juvenile mortality test:** 2 ml of double-distilled water containing 200 freshly hatched second-stage juveniles of *M. incognita* were transferred into Petri dishes containing 8 ml extracts of different solvents of *C. carandas* plant parts. Double-distilled water served as the control. Each treatment was replicated three times. The Petri dishes were incubated at 28°C and after incubation, all Petri dishes were observed using a stereoscopic microscope. The numbers of living and dead juveniles were recorded after 24, 48 and 72 hours. Nematodes showing any mobility or appearing in winding shapes were considered alive[10]. Nematodes showing no movement and having a straight body shape were considered dead. The number of dead and alive nematodes was counted and recorded. The values presented are the average (± SE) of three independent measurements per experiment.

Analysis of variance (ANOVA) was conducted on all experimental data to evaluate the significance of differences. Data processing and result presentation were performed using OPSTAT, an online tool available on the CCSHAU, Hisar website (www.hau.ac.in). The percentage inhibition of egg hatching and mortality was calculated according to the method described by Khan et al[13]:

For egg hatching: $(Co - T\alpha)/Co \times 100$

For juvenile mortality: $(T\alpha - Co)/T\alpha \times 100$

where $Co$ is the number of juveniles hatched in the control or the number of dead nematodes in the control Petri dish and $T\alpha$ is the number of juveniles hatched or the number of dead nematodes after 24, 48 and 72-hour exposure in each treatment.

## Results and Discussion

This study investigated the effectiveness of extracts from different parts of the *C. carandas* plant (leaves, unripened fruit, ripened fruit and seeds) using various solvents on juvenile mortality and egg-hatching inhibition of *M. incognita*. The data on juvenile mortality over 24, 48 and 72 hours, compared to control groups (distilled water) which showed no mortality, revealed significant differences based on the type of extract and solvent used. In general, juvenile mortality rates increased with longer exposure times. Methanol extracts were particularly effective, with seed extracts in methanol resulting in 100% mortality at both 48 and 72 hours. Leaf extracts in methanol also showed high mortality rates, reaching 92.5% at 72 hours.

On the other hand, aqueous extracts had much lower mortality rates with unripened fruit aqueous extract showing only 4.5%, 13.83% and 30.83% mortality at 24, 48 and 72 hours respectively. Chloroform extracts were also effective, especially seed extracts, which reached 80.33% mortality at 72 hours (Table 1). Overall, methanol extracts from seeds, leaves and unripened fruits were highly toxic to *M. incognita* juveniles after 72 hours of exposure, indicating methanol's superior ability to extract and maintain the toxic components responsible for juvenile mortality. The increase in exposure time from 24 to 72 hours led to a corresponding increase in juvenile mortality. Similar findings were reported by Elbadri et al[9] and Khan et al[13].

The egg-hatching inhibition results (Table 2) showed that the control group in distilled water had a steady increase in egg hatching, reaching 243.33 after 72 hours. In contrast, leaf extracts in methanol showed the highest inhibition rates with 75.32% at 24 and 48 hours and 70.5% at 72 hours. The unripened fruit extract in methanol also exhibited strong inhibitory effects, with 82.56% at 24 hours and 80.82% at 72 hours. Ripened fruit extracts in ethanol had lower inhibition rates, particularly after 72 hours (52.73%). The seed extract in methanol displayed the highest inhibition rates, achieving 94.47% at 24 hours, 96.01% at 48 hours and 95.61% after 72 hours (Table 2).

These results are consistent with the mortality studies where methanol extracts of all tested plant parts were significantly effective against egg-hatching of *M. incognita*, indicating that methanol not only enhances the extract's inhibitory effects but also preserves these effects over extended periods. The results clearly indicate that the nematicidal potential of *C. carandas* extracts is due to the presence of certain nematotoxins or phytochemicals that are soluble in

the solvents used for extraction. The *in vitro* studies showed that methanolic extracts from all plant parts had significant nematicidal efficacy against egg-hatching and juvenile mortality of *M. incognita*.

Among the tested plant parts, the seed extract in all solvents was the most effective in reducing egg-hatching and juvenile mortality. The nematicidal potential was found to be directly proportional to the exposure period. These results align with the findings of Mukhtar et al[15] and Asif et al[3]. The plant's nematicidal properties are attributed to the presence of chemical compounds such as alkaloids, flavonoids, saponins, glycosides, triterpenoids, phenolic compounds, tannins and organic acids. These bioactive compounds were previously reported in methanolic extracts by Hegde et al[11].

**Table 1**
**Effect of *C. carandas* extracts on second-stage juveniles of *M. incognita* with respect to different exposure times.**

| Type of Extract | Solvent | (Mean± SE) Juvenile mortality in different time intervals | | |
|---|---|---|---|---|
| | | 24 hrs | 48 hrs | 72 hrs |
| Control (Distilled Water) | | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Leaf Extract | Aqueous | 17.33 ± 1.85 (8.66) | 50.00 ± 3.78 (25) | 109.66 ± 2.18 (54.83) |
| | Methanol | 95.00 ± 2.08 (47.5) | 147.33 ± 9.26 (73.66) | 185.00 ± 2.64 (92.5) |
| | Ethanol | 46.00 ± 2.30 (23) | 104.00 ± 4.35 (52) | 146.00 ± 2.88 (73) |
| | Ethyl acetate | 37.00 ± 3.78 (18.5) | 78.33 ± 1.85 (39.16) | 128.33 ± 1.45 (64.16) |
| | Chloroform | 46.66 ± 2.96 (23.33) | 113.00 ± 1.52 (56.5) | 160.66 ± 5.04 (80.33) |
| Unripen Fruit Extract | Aqueous | 9.00 ± 2.64 (4.5) | 27.66 ± 1.76 (13.83) | 61.66 ± 1.20 (30.83) |
| | Methanol | 33.66 ± 1.45 (16.83) | 67.33 ± 1.76 (33.66) | 114.00 ± 3.60 (57) |
| | Ethanol | 23.66 ± 1.45 (11.83) | 44.00 ± 2.51 (22) | 89.00 ± 3.60 (44.83) |
| | Ethyl acetate | 29.33 ± 0.88 (14.66) | 46.66 ± 1.20 (23.33) | 84.66 ± 2.33 (42.33) |
| | Chloroform | 28.33 ± 0.88 (14.16) | 62.33 ± 0.88 (31.16) | 99.66 ± 2.18 (49.83) |
| Ripen Fruit Extract | Aqueous | 24.66 ± 2.18 (12.33) | 41.66 ± 2.33 (20.83) | 82.00 ± 2.08 (41) |
| | Methanol | 34.00 ± 2.08 (17) | 78.66 ± 5.04 (39.33) | 134.00 ± 2.08 (67) |
| | Ethanol | 78.00 ± 4.58 (39) | 95.33 ± 2.33 (47.66) | 111.66 ± 1.45 (55.83) |
| | Ethyl acetate | 23.33 ± 1.20 (11.66) | 48.33 ± 1.45 (24.16) | 93.33 ± 2.33 (46.66) |
| | Chloroform | 26.33 ± 0.88 (13.16) | 55.33 ± 2.33 (27.66) | 94.00 ± 1.73 (47) |
| Seed Extract | Aqueous | 34.00 ± 3.05 (17) | 82.66 ± 2.90 (41.33) | 128.66 ± 4.05 (64.33) |
| | Methanol | 94.66 ± 3.71 (47.33) | 200.00 ± 0.00 (100) | 200.00 ± 0.00 (100) |
| | Ethanol | 44.66 ± 4.05 (22.33) | 104.00 ± 3.05 (52) | 142.66 ± 1.76 (71.33) |
| | Ethyl acetate | 37.33 ± 2.40 (18.66) | 74.66 ± 2.40 (37.66) | 110.66 ± 4.05 (55.33) |
| | Chloroform | 66.66 ± 2.40 (33.33) | 111.33 ± 4.05 (55.66) | 160.66 ± 2.40 (80.33) |

(Each value is an average of three replications, SE – Standard error, Values given in parentheses represent the percent mortality of $J_2$ over control. Values given without parentheses represent the dead $J_2$s of *M. incognita*)

**Table 2**
**Effect of *C. carandas* extracts on egg-hatching of *M. incognita* with respect to different exposure times.**

| Type of Extract | Solvent | (Mean± SE) Egg-hatching inhibition in different time intervals | | |
|---|---|---|---|---|
| | | **24 hrs** | **48 hrs** | **72 hrs** |
| Control (Distilled Water) | | 78.33 ± 2.33 | 167.00 ± 3.21 | 243.33 ± 1.20 |
| Leaf Extract | Aqueous | 35.33 ± 1.85 (54.89) | 63.33 ± 2.78 (61.88) | 98.00 ± 1.52 (41.31) |
| | Methanol | 19.33 ± 1.20 (75.32) | 35.33 ± 2.33 (75.32) | 71.66 ± 1.20 (70.55) |
| | Ethanol | 27.66 ± 0.88 (64.68) | 53.00 ± 1.52 (68.26) | 88.00 ± 2.08 (63.83) |
| | Ethyl acetate | 34.00 ± 1.52 (56.59) | 65.00 ± 2.08 (61.07) | 105.00 ± 2.64 (56.84) |
| | Chloroform | 27.00 ± 1.15 (65.53) | 46.00 ± 2.51 (72.45) | 82.66 ± 1.85 (66.02) |
| Unripen Fruit Extract | Aqueous | 35.00 ± 2.30 (55.31) | 77.66 ± 2.40 (52.49) | 121.00 ± 4.04 (50.27) |
| | Methanol | 13.66 ± 1.20 (82.56) | 28.66 ± 1.45 (82.83) | 46.66 ± 2.84 (80.82) |
| | Ethanol | 21.66 ± 1.20 (72.34) | 32.00 ± 2.08 (80.83) | 45.66 ± 1.85 (81.23) |
| | Ethyl acetate | 28.66 ± 1.20 (63.41) | 49.00 ± 1.52 (70.65) | 81.33 ± 1.45 (66.57) |
| | Chloroform | 23.66 ± 2.02 (69.79) | 41.66 ± 1.20 (75.05) | 67.33 ± 2.02 (72.32) |
| Ripen Fruit Extract | Aqueous | 25.66 ± 2.40 (67.24) | 55.33 ± 2.33 (66.86) | 95.33 ± 2.96 (60.82) |
| | Methanol | 18.33 ± 1.45 (76.59) | 37.00 ± 2.30 (77.84) | 64.00 ± 1.73 (73.69) |
| | Ethanol | 38.33 ± 1.76 (51.06) | 73.66 ± 1.45 (55.89) | 115.00 ± 2.08 (52.73) |
| | Ethyl acetate | 34.66 ± 2.33 (55.75) | 65.66 ± 2.02 (60.68) | 112.00 ± 2.51 (53.95) |
| | Chloroform | 27.00 ± 1.73 (65.53) | 54.33 ± 1.20 (67.46) | 84.33 ± 2.40 (65.34) |
| Seed Extract | Aqueous | 24.66 ± 2.33 (68.51) | 54.00 ± 1.52 (67.66) | 85.00 ± 2.08 (65.06) |
| | Methanol | 4.33 ± 0.33 (94.47) | 6.66 ± 0.88 (96.01) | 11.33 ± 0.88 (95.61) |
| | Ethanol | 16.00 ± 1.52 (79.57) | 20.33 ± 1.85 (87.82) | 30.00 ± 2.08 (82.03) |
| | Ethyl acetate | 23..33 ± 1.76 (70.21) | 44.00 ± 1.52 (73.65) | 62.66 ± 0.88 (74.38) |
| | Chloroform | 24.00 ± 2.02 (69.36) | 42.33 ± 2.02 (74.65) | 60.33 ± 1.85 (75.20) |

(Each value is an average of three replications, SE – Standard error, Values given in parentheses represent the percent inhibition of $J_2$ hatching over control. Values given without parentheses represent the hatched $J_2$s of *M. incognita*)

Similarly, Elbadri et al[9] reported that neem releases phenols, amino acids, aldehydes and fatty acids, which are antagonistic to root-knot nematodes. The nematicidal efficacy of *C. carandas* seed extracts against *M. incognita* may be attributed to the presence of toxic organic acids and fatty acids.

## Conclusion

The study has revealed that *C. carandas* extracts inhibit egg hatching and cause mortality of second-stage juveniles of *M. incognita*. The inhibitory effect of these extracts is likely due to the presence of chemicals with nematicidal properties. Additionally, these plant parts can improve plant health and enhance yield attributes. The metabolites or chemicals produced by the plant parts could serve as a potential source of new organic nematicidal compounds. However, further research is needed to identify the active ingredients in the plant.

In the study, seed extracts were particularly effective against *M. incognita* under *in vitro* conditions. Therefore, this plant can be recommended for promoting organic agriculture and for use in environmentally friendly and sustainable nematode management programs.

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