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Evaluation of nematicidal potential of botanicals for sustainable management of *Meloidogyne incognita* population

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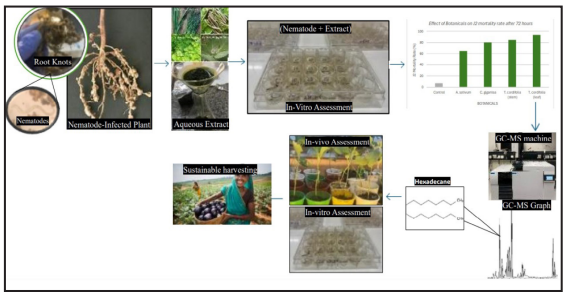
Abstract

Meloidogyne incognita, a root-knot nematode (RKNs), poses a significant economic threat to various agriculturally important crops as an endoparasite. The use of botanicals as a component of integrated pest management (IPM) offers an eco-friendly alternative to chemical pesticides. In this study we evaluated the nematicidal potential of *Calotropis gigantea, Tinospora cordifolia*, and *Allium sativum* L. against *M. incognita* juveniles (J2s) at concentration of 0.2 g/ 10 mL, 0.4 g/ 10 mL, 0.6 g/ 10 mL, 0.8 g/ 10 mL and 1 g/ 10 mL over exposure periods of 24 hours, 48 hours and 72 hours. Among the tested extracts, *T. cordifolia* exhibited the highest J2s mortality (100.00% and 99.27%), while *A sativum* L. extract showed the lowest efficacy (41.19%). Furthermore, *T. cordifolia* showed remarkable phytonematicidal compounds. To enhance the efficacy of phytonematicides, we investigated the bioactive compounds present in the aqueous extract of *T. cordifolia* , which showed the highest mortality rate. In vitro and in vivo assays were conducted to assess the impact of hexadecane, a crude hydrocarbon present prominently in the aqueous extract of *T. cordifolia* leaf. Its 0.002 mL/ L and 0.004 mL/ L concentrations were tested for different time durations against *M. incognita* . Hexadecane at 0.004 mL/ L exhibited the highest J2s mortality (85.09% after 72 h of treatment, as compared to the control 3.46%). These findings highlight the potential of *T. cordifolia* based bio-nematicide formulations. GC-MS profile of *T. cordifolia* revealed the prominant presence of hexadecane, thus it was asses to test its bioeffacy as a bionematicide which would show a path of sustainable strategy for plant parasitic nematode management. This novel approach offers an environmentally friendly and cost-effective alternative to synthetic pesticides, potentially leading to improved crop yield and sustainable agricultural practice.

Keywords: Plant parasitic nematode (PPNs), Botanicals, *Meloidogyne*, Root-knot nematode (RKNs), *Tinospora cordifolia*, Hexadecane



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Introduction

Economies important plants such as *Calotropis gigantea* (estimated for industrial, medicinal, and agricultural potential), *Tinospora cordifolia* , and *Allium sativum* L. (known

for their nutraceutical and pharmaceutical) offer promising solutions for the sustainable management of plant-parasitic nematodes (PPNs) (1,2). Since 2600 BC, humans have harnessed plant metabolites with secondary metabolites predominantly utilized over the next 4,000 years for food, medicine, and biopesticides (3,4,5). In the present scenario, agriculture is the cornerstone of our food supply, and maintaining food security necessitates efficient and sustainable farming techniques. In agricultural settings, pest infestation has a significant warning impact on yield and stands as one major obstacle to global food security. Among pests, PPNs pose a substantial threat to global food security (6). *Meloidogyne incognita* stands out as one of the most economically devastating species due to its wide host range and causative agent of huge loss in crop productivity (21.3%) globally (7). This issue highlights the urgent need for the development of effective control measures (8). Several ways have been used to minimize the impact of PPNs, including cultural practices, biological control strategies, and chemical nematicides. However, the widespread use of harmful chemical pesticides has aroused major concerns about their negative environmental effects and probable health risks (9). Chemical pesticides frequently accumulate in the ecosystem, contaminating soil, water, and harming non-targeted creatures. As a result, there is a growing need for environmentally friendly, sustainable products, notably plant-derived bioactive substances with effective nematicidal effects. Plants including *C. gigantea , T. cordifolia ,* and *A. sativum* L. have shown promise for long-term PPNs management (10). These plants are known to produce a range of secondary metabolites, including alkanes, alkaloids, and flavonoids, which exhibit potent nematicidal activity (11). Thus, the study aimed to explore quantitatively and qualitatively the nematicidal properties of *C. gigantea* (leaf), *T. cordifolia* (leaves and stem), and *A. sativum* L. (leaf). It also focuses on chemical profiling, screening, and testing of prominent bioactive phytochemical compounds present in the most efficient botanical (12,13).

Materials and Methods

Establishment of Nematode Culture

The culture of *M. incognita* was maintained in the Biological Control Laboratory, Amity University, Noida. India (28°29′46″ N latitude and 77°32′09″ E longitude, 212 m above mean sea level, mean rainfall: 700 mm/ year). The population of the nematodes was collected from the infected brinjal plants from the Organic Agriculture experimental field of Amity University. Filtrate was obtained by blending these roots and inoculating them into healthy brinjal plants grown under laboratory conditions. After 45 days of inoculation, the perineal pattern of the female was examined to identify the tested species. For all the assessments, second-stage juveniles were obtained from the egg masses by the sieving method (14).

Aqueous extract preparation

C. gigantea (leaves), *T. cordifolia* (leaf and stem), and *A. sativum* L. (leaves) were washed and air dried after being taken from the field of Amity University, Uttar Pradesh. 10 g leaves/stem were grinded in a pestle and mortar. 10 mL of distilled water was added. The resulting slurry was proceeded to prepare a homogenizer mixer. Homogenized mixers were centrifuged at 5000 rpm for 15 minutes at 4ºC. The supernatant was collected and filtered through a Whatman filter paper (Grade 1). Finally, the obtained extracts (1 g leaf/ stem sample/ 10 mL water) were treated as 100%. Five aliquots of each sample were prepared: 0.2 g/ 10 mL, 0.4 g/ 10 mL, 0.6 g/ 10 mL, 0.8 g/ 10 mL, and 1 g/10 mL. Prepared leachates were consumed within 24 h for bioassay studies.

Assessment of nematicidal activity of aqueous plant extract against M. incognita under in vitro conditions:

A culture plate containing 24 wells was used for assessing the effect of various concentrations of plant extracts on nematodes. For this, in each well, 990 µl distilled water

(containing approximately 70 J2s) was poured. 10 µl of each concentration of the selected extract was used for further studies. Untreated wells were maintained as a control containing distilled water and approximately 70 J2s. Five such replicas were maintained to explore the behaviour of *M. incognita* in all selected treatments and controls. To estimate the nematicidal property of each extract at different time intervals, *i.e*., 24 h, 48 h, and 72 h, mortality was calculated from the outcome of in vitro assessment. This entire volume of each treatment and control was examined drop by drop under a microscope (Stereo Zoom Microscope Leica), and the number of live as well as dead nematodes was counted carefully, and the data obtained was tabulated. Percent mortality was calculated using the following formula (15,6).

% Mortality = Total no. of dead nematodes in each treatment / Total no. of nematodes (live and dead) in each treatment *100

Identification of compounds in the aqueous extract of T. cordifolia leaf

The chemicals present in the *T. cordifolia* leaf extract were identified by GC-MS analysis using SHIMADZU-GCMSQP2010ULTRA. It had a Rtx-5 MS column with dimensions of 30 m × 0.25 mm I.d. × 0.25 µm film thickness. A device for ionizing electrons was used, and worked in the form of an electron hit, producing 70 eV of ionization energy. Helium (99.9% pure) as a carrier gas was used with a flow rate of 1 milliliter per minute for analysis purposes. With a split ratio of 10:1, the extract injection volume was 1 µl. Each sample was given a total run time of 60 minutes, and the mass-to-charge ratio (m/z) was 40. The National Institute of Standards and Technology (NIST) database was used to illuminate the mass spectrum (16).

Assessment of nematicidal activity of Hexadecane against M. incognita under in vivo and in vitro conditions:

To estimate the nematicidal impact of a prominent saturated hydrocarbon hexadecane, against *M. incognita* in vivo and in vitro Bioassays were conducted. Two concentrations of hexadecane *i.e*. 0.002 mL/ L and 0.004 mL/ L were selected for assessment across multiple exposure durations.

For in vivo evaluation, brinjal (Hybrid BS6-793) plants were grown in a glasshouse under controlled conditions (14:10 h L:D, 25-27°C, 65-70% RH). Plants were grown in 18 cm diameter pots containing sterile, nematode-free soil. Brinjal plants aged 45-60 days with a well-developed root system were selected for further testing. To assess the influence of hydrocarbon on *M. incognita* under controlled glasshouse conditions, a standardized inoculation methodology was used (6). Each experimental pot was included with a 10 mL suspension of 1,000 second-stage juveniles (J2s) of *M. incognita* . For inoculation, five equidistant, 1-inch-deep trenches were prepared along the pot's periphery, each about 1 inch away from the main stem. After three days of incubation period, selected hydrocarbon treatments were administered at certain concentrations 0.002 mL/ L and 0.004 mL/L). To ensure specific exposure, 50 µL of each concentration was injected into the same pits. To control variability, five replicates of each treatment were performed. The treated plants were grown in a controlled glasshouse environment using typical agronomic procedures. Growth conditions were set at a 14:10 h light-dark (L:D) photoperiod, 25-27°C temperature, and 65-70% relative humidity (RH). A subset of plants was carefully uprooted 10 and 20 days after treatment to allow nematode extraction. Nematodes were extracted from the Soil intact roots by the baermann funnel method and examined using a Stereo Zoom Microscope (Leica, 40× magnification) (6, 15). In vitro bioassays were performed in parallel using 24-well polystyrene culture plates, following the protocol previously mentioned (6) for evaluating plant extract efficacy. Hexadecane was tested at the same concentration (0.002 mL/ L and 0.004 mL/L) against *M. incognita* J2s to determine its direct nematicidal effect under controlled laboratory conditions.

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Statistical Analysis

The experimental data were analyzed using one-way analysis of variance (ANOVA) using the OPSTAT. Multiple comparison tests to compare the significance of each treatment group with the control were also conducted, and to observe the actual impact of the extract on the mortality of PPNs, Abbott's values were calculated (17). The tests were analysed using GraphPad Prism Software version 8.0.2 (18).

Results and Discussion

In this study, we evaluated the nematicidal activity of *C. gigantea* , *T. cordifolia* , and *A. sativum* L. under different concentrations (0.2 g/ 10 mL, 0.4 g/ 10 mL, 0.6 g/ 10 mL, 0.8 g/ 10 mL and 1 g/ 10 mL) and time durations (24 h, 48 h and 72 h) through in vitro analysis as an ecofriendly sustainable alternative. The efficacy of plant-derived nematicides is well established, with existing literature emphasizing their dual function in suppressing plant-parasitic nematode populations and promoting plant growth parameters by mitigating nematode-induced stress (19,20). Several studies also demonstrated the nematicidal properties of plant extracts, including those derived from *A.* sativum L.*, T. cordifolia ,* and *C. gigantea* against *M. incognita* . (21) evaluated the effect of eight plants: Neem (*Azadirachta indica* (A.Juss.)) leaves, Aloe vera (*Aloe barbadense*) leaves, Aak (*Calotropis gigantea*) leaves, Sadabahar (*Vinca rosea* L.) leaves, Jatropha (*Jatropha curcas* L.) leaves, Garlic (*Allium sativum* L.) clove, Onion (*Allium cepa* L.) bulb, and Jatropha (*Jatropha curcas* L.) seeds. on the mortality of *M. incognita* and observed significant nematicidal activity across all tested plant extracts. Similarly, the nematocidal potential of *C. gigantea* and *T. cordifolia* extracts, as demonstrated in our study, supports previous findings of (22) who reported the significant suppressive effects on PPNs population, thereby highlighting the disruptive effects of these botanicals on key life table parameters of PPNs.

Nematicidal impact assessment observations revealed that all botanicals exhibited significantly higher mortality percentage compared to the control across all tested concentrations and exposure durations. Notably, the highest concentration tested (1 g/ 10 mL) consistently resulted in maximum mortality. Among the tested botanicals *T. cordifolia* leaf extract recorded the highest mortality (93.18±2.34 p=0.0001, 99.27±0.73 p=0.0001 and 100±0.00 p=0.0001) followed by *T. cordifolia* stem (83.44±2.35 p=0.0001, 90.18±1.40 p=0.0001,95.39±2.18 p=0.0001) and *A. sativum* L. leaf extract (41.19±2.58 p=0.0001, 76.30±3.66 p=0.0001,92.54±2.28 p=0.0001) in varied time duration *i.e*., 24 h, 48 h, and 72 h. In contrast, *C. gigantea* showed the lowest mortality (84.98±0.52 p=0.0001, 86.42±0.55 p=0.0001, 90.17±1.88 p=0.0001) even at the highest concentration tested at similar duration. The highest mortality was recorded in *T. Cordifolia* leaf extract at 72 h exposure (100±0.00 p=0.0001).

It is evident that the efficacy of botanical nematicides is primarily influenced by two factors: the concentration of extracts and the duration of nematode exposure (23). In the present study, *M. incognita* mortality rates increased with increasing plant extract concentration and longer exposure duration, demonstrating a clear dose-dependent nematicidal effect. (Table 1) As the concentration of aqueous leaf extract increased, *M. incognita* mortality rate also showed a corresponding rise, aligning with the previous findings (24).

The present study revealed that the aqueous leaf extract of *T. cordifolia* possesses a significantly higher nematicidal effect compared to its stem extract and the other two tested extracts.

This supports its potential as a most effective botanical alternative for managing plant-parasitic nematodes. The results are aligned with the findings of (22), who reported 82.74% mortality of second-stage juveniles (J2s) of *M. incognita* (22). Additionally, he reported the nematicidal potential of *T. cordifolia*

Table 1. Effect of Aqueous Extract of various Botanicals on the mortality of *Meloidogyne* species

Botanicals	Dilution / Duration	Percent Mortality		
		24 h	48 h	72 h
Giloy	0.2g/10mL	5.26±0.77	6.49±1.02	15.23±2.09
Stem	0.4g/10mL	25.87±4.67	26.40±0.93	35.27±2.55
	0.6g/10mL	40.00±3.23	45.91±0.83	60.40±2.57
	0.8g/10mL	73.09±2.86	76.40±2.38	88.21±4.61
	1g/10mL	83.44±2.35	90.18±1.40	95.39±2.18
Giloy	0.2g/10mL	76.27±1.16	78.68±0.91	90.35±1.49
Leaves	0.4g/10mL	84.09±1.43	86.00±1.25	97.06±1.36
	0.6g/10mL	90.13±1.37	91.24±1.40	98.03±1.27
	0.8g/10mL	93.49±2.29	94.65±1.64	99.31±0.68
	1g/10mL	93.18±2.34	99.27±0.73	100±0.00
Calotropis	0.2g/10mL	66.82±0.76	71.61±0.54	81.39±0.95
Leaves	0.4g/10mL	71.21±2.72	76.55±1.57	83.39±1.22
	0.6g/10mL	73.16±2.25	76.80±2.10	86.42±0.37
	0.8g/10mL	77.69±1.96	83.22±0.39	89.57±1.67
	1g/10mL	84.98±0.52	86.42±0.55	90.17±1.88
Garlic	0.2g/10mL	16.06±2.24	52.85±0.70	68.61±1.72
Leaves	0.4g/10mL	25.54±4.18	60.44±4.76	75.51± .91
	0.6g/10mL	27.60±1.19	75.07±2.86	79.54±3.81
	0.8g/10mL	30.68±4.56	79.71±3.36	87.60±2.99
	1g/10mL	41.19±2.58	76.30±3.66	92.54±2.28

*Values of each interaction is mean Abbotts value of five replicates.

*Values after ± indicate Standard Error.

extracts against *M. incognita* and *Rotylenchulus reniformis* (Linford and Oliveira). The nematicidal activity of plant extracts in this study was found to be dose-dependent, with mortality rates increasing with higher extract concentration and longer exposure durations. Notably, the significantly highest mortality rate of PPNs was observed at the highest concentrations at 72 h of exposure. The nematicidal activity of six plant extracts was evaluated by (25) against *M. incognita* through in vitro analysis with exposure to various concentrations over 12 h, 24 h, and 48 h durations. Plant extracts from the leaves of six plant species, namely *Jatropha pandurifolia* L., *Polyalthia longifolia* (Sonn.), *Wedelia chinensis* , *Nerium indicum* L., *Duranta repens* L., and *Cassia fistula* L., showed highly promising mortality of 99.00-72.00% after 48 h of exposure. Varied concentrations of aqueous extracts of leaves of *J. pandurifolia* L., *P. longifolia* (Sonn.), and *W. chinensis* applied for different time durations were found to be highly

efficient in inhibiting egg hatching and increasing juvenile mortality of *M. incognita* . Similarly, (26) identified n-hexane *A. Sativum* L. extract as the most potent nematicide, and (22) tested the nematicidal activity of *Aegle marmelos* and *T. cordifolia* extracts against *M. incognita* in vitro, resulting in 86.48% and 82.47% mortality, respectively. These findings further highlight the diverse mechanisms through which different plant extracts exert their nematicidal effects.

The potent nematicidal activity of *T. cordifolia* may be attributed to the presence of several compounds in its aqueous phase. The GC-MS analysis of *T. cordifolia* aqueous extract revealed a total of 41 chemical fractions. The major compounds were Octadecadienoic acid, Phytol, Palmitic Acid, Pentanol, Hexadecane, Methyl stearate, Stearic acid, Phytol acetate, Butanamine

Hexadecanoic acid, Cyclopentyl propionic acid, Fumaric acid, Diisooctyl phthalate and Hexadecane reflect greater than 90% similarity index (Table 2). These findings are consistent with previous studies (27), which reported similar groups of compounds in *T. cordifolia* during their study. GC-MS profile indicates that benzopyrone has a maximum retention area, reinforcing the observations of (28) who emphasized the nematicidal role of secondary metabolites such as alkaloids, terpenoids, coumarins, glycosides, and flavonoids, in which benzopyrone exhibits particularly prominent nematicidal properties.

Table 2. Quantification of volatile compounds by GC-MS of *T. cordifolia* leaf extracts

Name of Compound	Retention Time in minutes	Area covered by the peak
Octadecadienoic acid	19.78	1152348
Phytol	19.946	777842
Palmitic Acid	19.278	5092831
Pentanol	6.551	452135
Hexadecane	17.677	576930
Methyl stearate	20.072	483217
Stearic acid	21.062	673221
Phytol, acetate	17.225	1559371
Butanamine	6.765	278135
Hexadecanoic acid	18.137	663242
Cyclopentylpropionic acid	21.477	543660
Fumaric acid	22.943	437372
Diisooctyl phthalate	23.542	118781
Hexadecane	10.695	70900

Additionally, the nematocidal potential of *A. sativum* L. leaf extract was evident, likely due to the presence of bioactive compounds that suppress egg hatching and delay juvenile emergence (29). Phytochemical investigations have confirmed the existence of alkaloids, flavonoids, and terpenoids that contribute to nematode toxicity (30). He also highlighted the significance of *T. cordifolia* as a potential therapeutic agent and biopesticide owing to its chemical constituents respectively.

The findings of this study confirm that the bioactive compounds of *T. cordifolia* are responsible for nematotoxic properties. The

strong nematicidal effect of the tested extracts may be attributed to their high contents of certain oxygenated compounds, which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (31). The mechanism of plant extract action may involve protein denaturation and degradation, enzyme inhibition, and interfering with the electron flow in the respiratory chain or with ADP phosphorylation.

Previous studies have demonstrated the antibiotic activity of Eucalyptus spp. Leaves (32) further support the hypothesis that plant-derived components can effectively inhibit nematode development. The inhibitory effect of the extract may be due to the chemicals present in the extract. These chemicals either affected the embryonic development, killed the eggs, or dissolved the egg masses. Several reports (33,34,20) indicated that plant extracts contained alkaloids, flavonoids, saponins, amides, including benzamide and ketones, that singly and in combination inhibited hatching.

The present study also focuses on the in vitro and in vivo assessment of prominent hydrocarbons present in *T. cordifolia* aqueous extract impact assessment on *M. incognita* mortality. The result indicated > 76% to 86% mortality (Table 3). This approach signifies the role of active compounds in nematicidal activity (30). Our findings also reinforce the potential of readily available plant extracts as cost-effective and sustainable alternatives for managing plant-parasitic nematodes (35). The tested botanicals, which are easily available to farmers at almost negligible cost, demonstrated the ability to reduce the nematode population below the economic threshold level, highlighting their practical applicability in integrated pest management strategies.

Table 3. Effect of Hexadecane on the Mortality of *Meloidogyne* species

	In vivo Analysis		In Vitro Analysis		
	10 days	20 days	24 h	48 h	72 h
Hexadecane 20 ppm	77.36±2.43	79.19±0.40	82.18±0.98	83.32±2.12	84.88±0.72
Hexadecane 40ppm	86.00±1.58	86.45±0.93	83.09±1.24	84.19±1.69	85.09±0.85
Hexane	4.58±0.70	4.52±0.32	5.49±1.34	5.30±0.85	5.24±0.44
Water	2.93±0.54	3.29±0.48	3.33±0.58	3.57±0.69	3.46±0.40
C.D.	4.580	1.760	3.255	4.416	1.907
SE(m)	1.515	0.582	1.076	1.460	0.631
SE(d)	2.142	0.823	1.522	2.065	0.892
C.V.	7.929	3.001	5.530	7.405	3.157

*Values of each interaction is mean of five replicates,
*Values after ± indicates Standard error
*C.D.- Critical Difference; SE(m)- Standard Error of mean; SE(d)- Standard Error of Deviation; C.V.- Coefficient of Variation

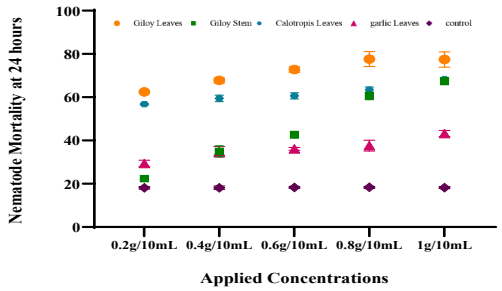

Figure 1. Effect of various concentrations of botanicals on nematode mortality at 24 h

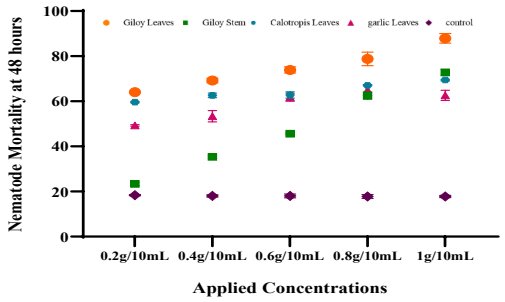

Figure 2. Effect of various concentrations of botanicals on nematode mortality at 48 h

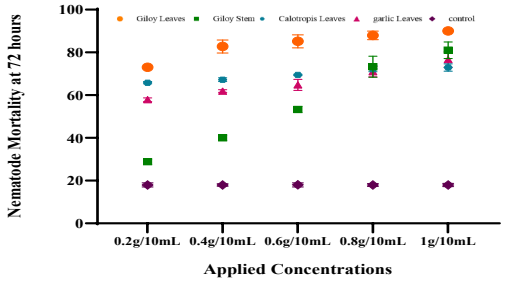

Figure 3. Effect of various concentrations of botanicals on nematode mortality at 72 h

Conclusion

The research findings suggest that among the 4 tested plant extracts, *T. cordifolia* aqueous extract showed the highest nematicidal activity, and hexadecane, a prominent bioactive compound present in *T. cordifolia* extract, may be applied as a soil amendment to reduce nematode infestations effectively. It also promotes plant health, which aligns with the principles of sustainable agriculture by minimizing chemical inputs. The farmers can achieve efficient nematode control, making it a promising strategy for sustainable agriculture by integrating *T. cordifolia* extracts into integrated pest management (IPM) programs.

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