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Research Article

In Vitro Nematocidal Effect of Leaf and Seed Extracts of Jimsonweed (*Datura stramonium*) against *Meloidogyne incognita*

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ABSTRACT
This study assessed the *in vitro* nematocidal effectiveness of Jimsonweed (*Datura stramonium*) leaf and seed extracts against *Meloidogyne incognita*, a major root-knot nematode infesting tomato crops in Katsina State, Nigeria. *Meloidogyne incognita* was isolated from infected tomato roots and identified through morphological and perineal pattern analysis. Phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, saponins, sterols, tannins, and terpenoids. *In vitro* bioassays showed that extracts, especially the combined leaf and seed extract (JWLSE), achieved significantly higher nematode mortality compared to controls, with a mean mortality of 10.40 (± 8.45) at 24 hours. ANOVA results indicated that plant part, concentration, exposure time, and their interactions significantly affected nematode mortality ($p < 0.001$), explaining 99.6% of the variability ($R^2 = 0.996$). Mortality increased progressively with time, underscoring the time-dependent efficacy of the extracts. The findings support the potential of *D. stramonium* as a natural, environmentally friendly nematicide. It is recommended that further research could focus on field trials to optimize application rates and formulations, promoting sustainable nematode management in tomato production.

Keywords: *Datura stramonium; In vitro; M. incognita; Phytochemical;* Tomato

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INTRODUCTION

Root-knot nematode infestation is widely seen as one of the biggest challenges to agricultural productivity, especially when it comes to vegetable crops. This is largely due to its high occurrence, broad range of host plants, and its tendency to interact with other plant pathogens in ways that can amplify the damage (Walia and Khan, 2023). Root-knot nematodes are a type of plant-parasitic nematode belonging to the genus Meloidogyne. They are typically found in the soil, especially in regions with warm climates or mild winters (Khan, 2023). These pesky little creatures are responsible for about 5% of the global crop loss (Khan, 2023). The larvae of Root-knot nematodes invade plant roots, causing the development of distinct root knots or galls. These growths can disrupt the flow of photosynthates and vital nutrients from the host plant (Adriana *et al.,* 2023). If young plants suffer a severe infestation, it can lead to their death. In contrast, mature plants typically experience stunted growth and significant reductions in yield (Darling *et al.,* 2021).

Root-knot nematodes are a real problem for plants, as they latch onto root cells with their needle-like mouthparts, called stylets, and start feeding. This feeding disrupts the root system's normal functions, leading to significant damage that hinders the plant's ability to take in water and nutrients effectively

(Adriana *et al.,* 2023). These pesky nematodes were first spotted back in 1855 in a greenhouse in England, and since then, they have turned into a major agricultural issue around the globe. They can be found in various agroecological regions, making them quite the cosmopolitan pests (Khan, 2023). Among the different species, *Meloidogyne incognita* stands out as the most destructive, wreaking havoc and causing substantial economic losses across a wide array of important crops (Kavitha *et al.,* 2025).

Meloidogyne incognita is a type of endoparasitic nematode that needs to invade plant roots to form a successful host-parasite relationship. The infective juveniles easily make their way into host roots, particularly near the growing tips, drawn in by the root exudates (Shivakumara *et al.,* 2018).

Over the last twenty years, plant and soil samples sent to the University of Maryland Nematology Laboratory have consistently shown significant populations of root-knot nematodes. Among the species found, *M. incognita* stands out as the most common, with vegetable crops being the most frequently affected. In Maryland, this nematode has been linked to damage in several key crops, including Corn, Muskmelon, Soybean, Sweet potato, Tobacco, Tomato, Vetch, and Wheat (Tiwari *et al.,* 2024). There have also been notable yield losses reported in Potato and other vegetable crops grown in the area due to root-knot nematode infestations.

In agriculturally developing countries like Nigeria, Ghana, and Kenya, it has been discovered that among root-knot nematodes, *Meloidogyne incognita* causes most damage to vegetable crops. *Meloidogyne spp* (root-knot nematodes), are important pests of *Lycopersicon esculentum* (Tomato) worldwide (Baale *et. al*., 2021).

Four major species, namely *M. arenaria, M. hapla, M. javanica* and *M. incognita* have been reported to infect tomatoes in the tropics (Coyne *et al.,* 2018). These species cause gall or root-knot on infected plants. Other symptoms include stunted growth, wilting, and poor fruit yield. Infection by *Meloidogyne incognita* can increase root weight and decrease shoot weight. In the tropics estimated production losses of tomatoes due to *Meloidogyne spp* reach as high as 50%. However, the overall impact on tomato is highly variable, as disease intensity is influenced by many biotic and abiotic factors (Luka, 2017). While root-knot nematodes alone are capable of causing severe plant injury and reduction in crop production, they are also often involved with pathogenic fungi and bacteria. These combine actions often result in more than additive effects such as the breaking down of resistance or production of symptoms differing from those usually produced by other organisms alone. Such associations are sometimes referred to as disease complexes (Luka 2015, Coyne *et al.,* 2018).

Several control strategies such as host plant resistance, rotation with non-hosts, sanitation and avoidance, destruction of residual crop root, ands judicious use of nematicides have been reported to effectively control root-knot nematodes. However, the use of resistant varieties remains the most viable option, particularly for small-scale farmers with limited resources (Luka, 2017). Even with these control strategies root-knot nematodes are often cited as major limiting factor of crop production. Despite their relative importance in the biology and growth and yield of crops, root-knot nematodes have not been fully addressed in Nigeria.

Taking into account the world-wide distribution of root-knot nematodes, it is necessary to find out the most effective and feasible control measure.

The use of chemicals (nematicides) which are the most effective method of controlling nematodes is, however, not economical, because these chemicals are very expensive particularly on large scale farming and most farmers cannot afford them. Also they are currently being reappraised with respect to the environmental hazards and human health (Tiwari, 2024).

Indiscriminate use of synthetic pesticides for controlling nematodes is likely to give rise to phytoxicity, environmental pollution and nematode resistance. Unsafe use of pesticides may result in poisoning of humans and is a problem especially in developing countries (Ndala, *et al*., 2019).

There is a need to develop naturally occurring nematicides, which may be less toxic to man and animals but as effective against nematodes of various crops as synthetic ones.

Toxicity of plants extracts of different plants against nematodes has been reported by many researchers (Mwamula *et al.,* 2022; Spiegler *et al.,* 2022). Management is an important concept for dealing with nematodes problems. Nematode control refers to specific tactics applied to reduce or eliminate nematode population, while management describes efforts to reduce nematode numbers to non-damaging level through the application of severe control procedures in combination or in sequence (İlker *et al*., 2016).

Identification of plants with nematocidal properties facilitates safer, cheaper, practical and profitable control of nematodes through botanical management with such plant extracts or biological control by

cultivation of such plants with nematocidal properties on highly infected agricultural soils to reduce or bring the population of root-knot nematodes down to safe levels. Generally, in pest control, the method used must be of economic value, that is the increase in monetary value of the crop must be more than enough to offset the cost of control measures. Hence, this study investigates the efficacy of jimsonweed (*Datura stramonium*) leaves and seed extracts in the *In Vitro* management of root-knot nematodes (*Meloidogyne incognita*).

MATERIALS AND METHODS

Collection and Preparation of Jimsonweed Leaves and Seeds

This study was carried out at the Microbiology Laboratory of Umaru Musa Yar'adua University Katsina, Katsina State Nigeria. The leaves and seeds of Jimsonweeds were cut from the branches of the plant located at Bioresources Development Centre, Katsina, and placed into a clean polythene bag. The plant samples were taken to the Department of Plant Science and Biotechnology, Federal University, Dutsinma, Katsina State and a voucher number FUDMA/PSB/00099 was obtained and deposited. The plant samples were then kept at room temperature in the laboratory until further use (Elisha *et al.,* 2017).

Extraction of Jimsonweed Leaves and Seeds

Datura stramonium leaves and seeds were dried under room temperature (25^0C -27^0C) for 2-4 weeks in the laboratory and ground into fine powder using mortar and pestle (Kepenekçi *et al.*, 2016) and stored in a clean polythene bag. Crude extracts from Jimsonweed leaves and seed samples were extracted using the modified method of Ndala *et al.* (2019). Five hundred grams of the powdered plant samples were dissolved in 2000 mL of ethanol in the ratio of (1:4 wt/vol, dry powder/solvent) followed by soaking for 48 h. The extracts were filtered using clean muslin cloth, then, the filtrates were transferred from flat bottom flasks to beakers and allowed to evaporate to dryness at room temperature.

Phytochemical Screening of Jimsonweed Leaves and Seed

The phytochemical analyses of *Datura stramonium* (Leaves and seeds) were carried out to determine the presence of alkaloids, flavonoids, cardiacglycosides, saponins, sterols, tannins and terpenes, based on standard methods (Luka, 2017).

Collection of Infected Tomato Roots for Isolation and Identification of Root-Knot Nematode

Galled tomato roots symptomatic of root-knot nematode infection were randomly collected from farm fields in Ajiwa, Katsina State for isolation and identification of the root-knot nematodes. Root samples were taken by lifting whole tomato plants from the soil using a spade (Elisha *et al.,* 2017) so that the galls and root lesions could be observed from the roots in situ. Roots were collected and placed in a sample bag. The samples were then transported to the laboratory and stored at 10^0C for nematode laboratory assay.

Whole root systems collected from the plants were freed of soil by washing under a gentle stream of tap water and mopped dry with a clean towel. The roots were separated into live (functional) and dead (non-functional) roots. Thereafter, live roots were cut transversely with scissors into about 1-2 cm pieces, mixed carefully and 10 g sub-sample was assayed for nematodes using the Whitehead and Hemming (1965) tray modification of Baermann technique. A 50 g sub-sample of the roots was put in a blender with 200 mL of water and macerated for 30 s. The macerated suspension was poured into a Whitehead and Hemming (1965) set up comprising rubber tubes, tissue paper, sieve support, fine mesh sieve, water, beaker, funnel, microscope for observation and counting and measuring cylinder. Thereafter, the plastic sieves containing the macerated roots were removed briskly, and the nematode suspension in the bowl was poured into a 500 mL nalgene wash bottle and allowed to settle (Ogunsola, 2018). The supernatant was siphoned out with a rubber tube, and the suspension containing nematodes was then poured into a nematode-counting dish and examined under a stereo and compound microscope. Identification of root-knot nematodes was done with the aid of a compound microscope using the simplified pictorial nematode key of Mai and Lyon (1975). Nematode population was determined by counting, and population data was expressed in percentages**.**

Identification of Root-knot Nematodes

A slide mount was prepared by placing three drops of clear nail polish on a clean microscope slide. Using a dropper, a suspension filled with nematodes was placed right in the center of a clean glass slide. The slide was passed over a warmed flame of an alcohol lamp six times to relax and immobilize the nematodes. After that, the cover slip was carefully mounted and secured it with nail polish to avoid putting too much pressure on the specimens. The nematodes were examined under a compound microscope and a standard manual was used for identification, especially focusing on the stylet-

bearing species of plant-parasitic nematodes (Bogale *et al.*, 2020).

Preparation of Extract Concentrations for *In Vitro* Assay of Nematocidal Effect

The resulting root-knot nematode *Meloidogyne incognita* obtained from the infected root sources were introduced at the rate of 20 juveniles per plate into petri dishes containing the various concentrations of the test plants extracts as follows; 1g each of the extract was dissolved in 5 mls of distilled water. Subsequent concentrations of each extract were prepared by dissolving 1g in 10 mL, 15 mL, and 20 mL of distilled water. These were replicated in triplicates and kept at room temperature (25^0 C – 27^0 C) with mouths closed to reduce evaporation of the water. The dead and surviving nematodes were counted after an interval of 6, 12 and 24 h. The mortality was assessed by touching the nematode with a fine needle to trigger movement and observed for signs of life (Liang *et al.*, 2022). Root-knot nematodes (20 in number) were also introduced into distilled water without the test plant extract and kept in separate Petri dishes to serve as control. The average number of dead and surviving nematodes were computed.

RESULTS

Phytochemical Screening of Jimsonweed Leaves and Seeds (Table 1) showed that both leaves and seeds of Jimsonweed contained phyto-constituents, including alkaloids, flavonoids, glycosides, saponins, sterols, tannins, and terpenoids. Carbohydrates and phenols were not detected in either plant parts.

The root-knot nematodes taken from diseased tomato plants, which showed the typical gall symptoms linked to root-knot nematode infestation (Plate 1), were identified as Meloidogyne incognita (Plate 2). A single juvenile nematode, specifically the second-stage juvenile (J2) of M. incognita, viewed under a compound microscope at a magnification of ×40 is the crucial stage in the nematode's life cycle as it is the infective phase, responsible for penetrating host roots and establishing infection. The juvenile had a slender, elongated, and slightly curved (C-shaped) body, which is typical of second-stage juveniles, with noticeable tapering at both the front and back ends, especially at the tail region.

The image (Plate 3) showed a microscopic view (magnification x40) of egg masses of *M. incognita*, a root-knot nematode extracted from an infected tomato plant. The central region of the image (Plate 3) showed a dense aggregation of eggs surrounded by a gelatinous matrix. The egg masses were likely secreted by adult female nematodes embedded in plant root tissue. Numerous oval to elliptical structures with clearly defined boundaries were visible. The eggs appeared transparent to slightly opaque and were arranged loosely or in small groupings, some of which showed partial hatching or degradation. There were amorphous, granular materials surrounding the egg masses, which likely consisted of organic root debris, mucilage, and other residues from the host plant.

Table 1: Phytochemical Screening of Jimsonweed (*Datura stramonium*) Leaves and Seeds

Parameters	Test plant parts	
	Leaves	Seed
Alkaloids	+	+
Carbohydrates	-	-
Flavonoids	+	+
Glycosides	+	+
Phenols	-	-
Saponins	+	+
Sterols	+	+
Tannins	+	+
Terpenoids	+	+

(+) = Present**; (–)** = Absent

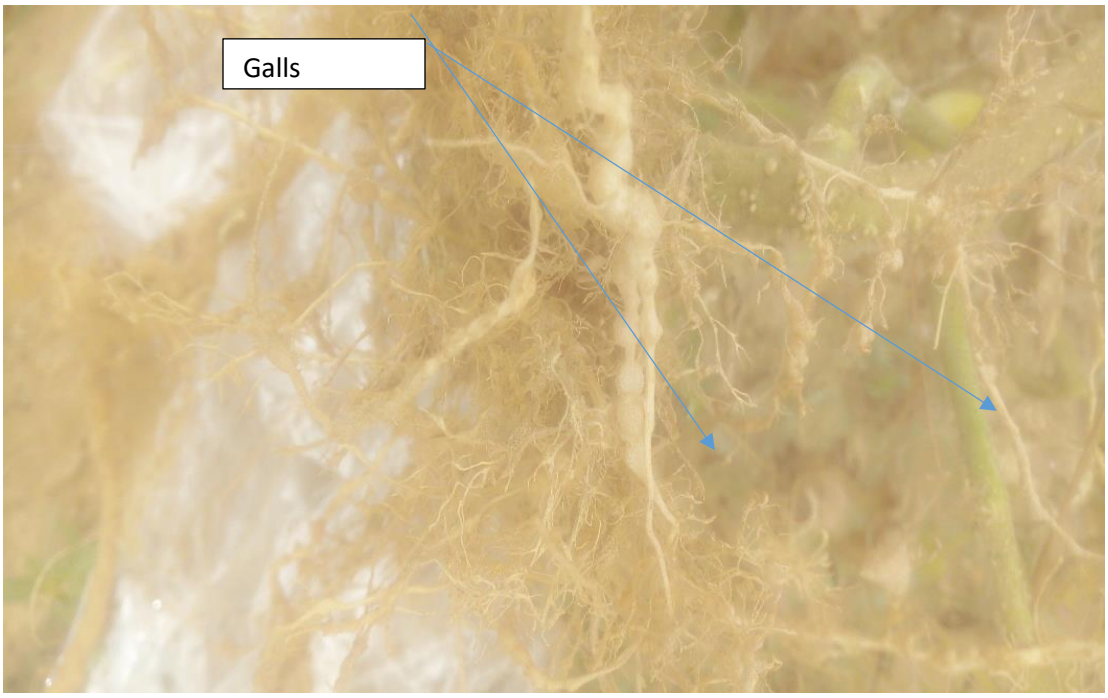

Plate 1: Diseased tomato plant with galls

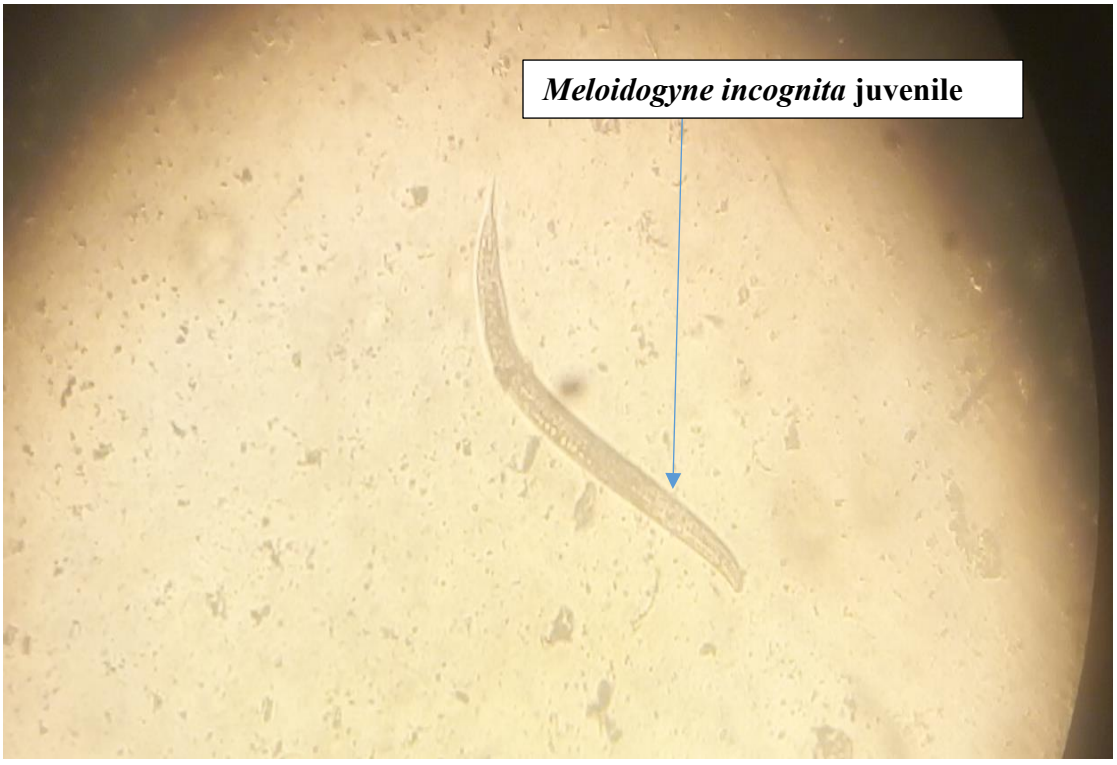

Plate 2: A live *Meloidogyne incognita* juvenile extracted from infected tomato plant (x 40).

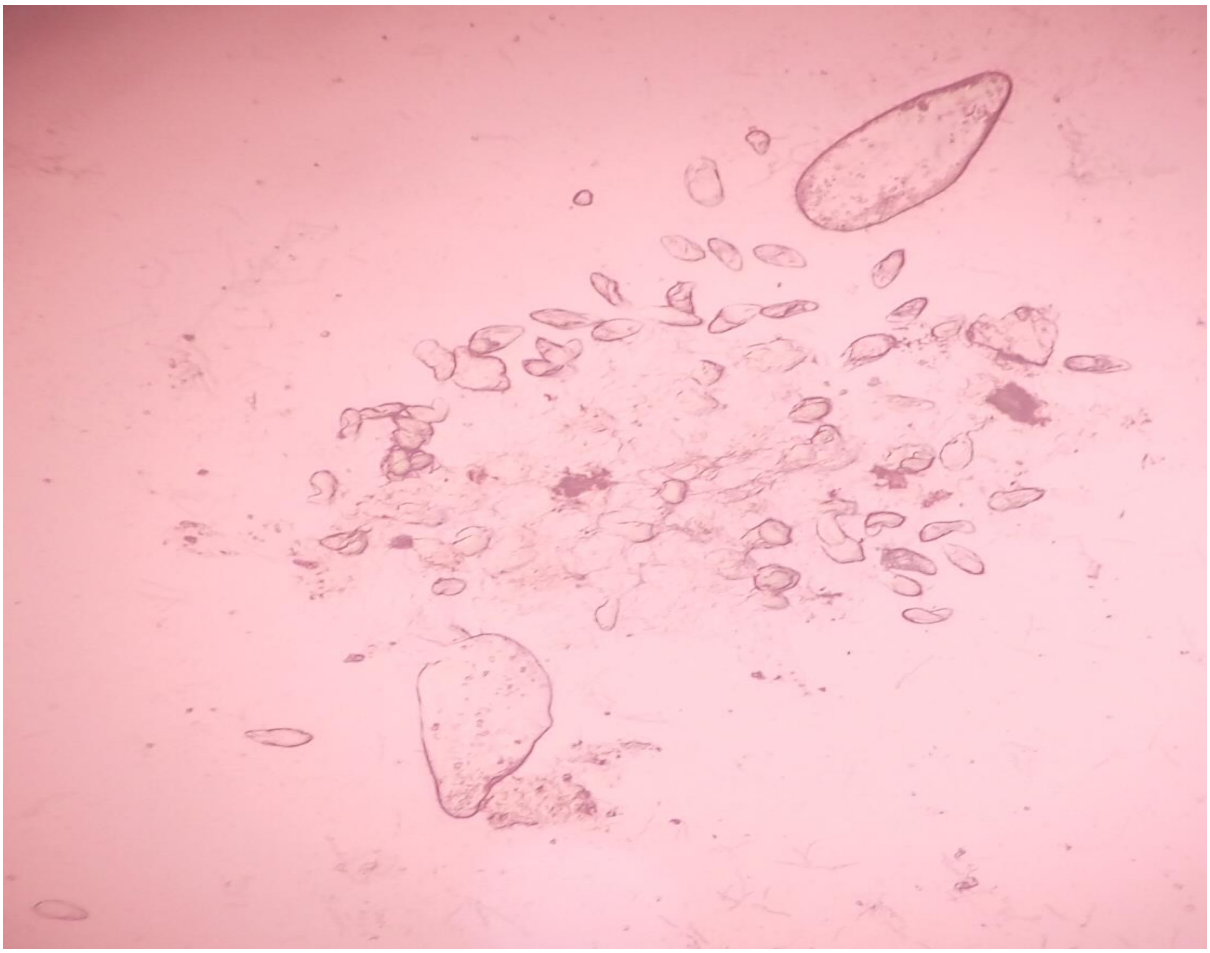

Plate 3: Egg masses of *Meloidogyne incognita* extracted from infected tomato plant (x 40).

The descriptive statistics in Table 2 reveal that nematode mortality varied markedly across treatments, concentrations, and exposure times. Among plant parts, the Jimson Weed Leaf and Seed Extract (JWLSE) produced the highest mean number of mortalities (10.40), followed by JWSE (9.54) and JWLE (8.94). The control group recorded zero mortality, confirming the extracts' nematocidal effects. Across concentrations, mean mortality ranged between 6.19 (1g/20 mL) and 8.21 (1g/10 mL), indicating that concentration influenced nematode mortality but not in a strictly linear fashion. Notably, exposure time had a strong effect, with mortality progressively increasing from 0 at 0 hours to 14.75 at 24 hours**,** indicating a time-dependent response to treatment.

The ANOVA results in Table 3 revealed highly significant main effects of plant part, concentration, and exposure time on nematode mortality (all $p < 0.001$). Moreover, all two-way and three-way interactions were significant ($p < 0.001$), suggesting that the effect of extract type depended on both the dosage and the length of nematode exposure. The exceptionally high R^2 value (0.996) indicates that nearly all the variability in mortality was explained by the model, confirming the strong impact of these factors.

The post hoc analysis (Table 4) confirmed that all Jimson Weed extracts produced significantly higher nematode mortality compared to the control ($p < 0.001$). Among them**,** JWLSE was the most effective, causing an average of 10.40 more nematode deaths than the control.

Jimsonweed leaves and seeds extract (Table 5) was the most effective with 10.40 ± 8.45 mortality rate, followed by JWSE (9.54 ± 7.80) and JWLE (8.94 ± 7.34), while the control had no mortality effect. This indicates that Jimson Weed extracts possess strong nematocidal properties, with combined leaf and seed extracts (JWLSE) showing superior efficacy.

Table 2: *In Vitro* Nematocidal Effect of Leaf and Seed Extracts of Jimsonweed by Plant Part, Concentration, and Exposure Time

Factor	Levels	Mean Mortality (NM)	Std. Dev.
Plant Part (Group)	JWLE (Jimson Weed Leaf Extract)	8.94	7.34
	JWSE (Jimson Weed Seed Extract)	9.54	7.80
	JWLSE (Jimson Weed Leaf and Seed Extract)	10.40	8.45
	Control	0.00	0.00
Concentration	1g/5 mL	7.48	8.81
	1g/10 Ml	8.21	8.11
	1g/15 mL	7.00	7.77
	1g/20 mL	6.19	7.24
Time of Exposure	0 Hours	0.00	0.00
	6 Hours	9.58	6.37
	12 Hours	4.54	4.35
	24 Hours	14.75	8.64

Table 3: Analysis of Variance (ANOVA) for Nematode Mortality

Source of Variation	df	SS	MS	F	Sig. (p)
Plant Part (Group)	3	3386.60	1128.87	3234.97	<0.001 **
Concentration	3	103.60	34.54	98.97	<0.001 **
Time of Exposure	3	5836.23	1945.41	5574.91	<0.001 **
Group × Concentration	9	79.77	8.86	25.40	<0.001 **
Group × Time	9	2062.98	229.22	656.87	<0.001 **
Concentration × Time	9	344.65	38.29	109.74	<0.001 **
Group × Concentration × Time	27	282.31	10.46	29.96	<0.001 **
Error	128	44.67	0.35		
Total (Corrected)	191	12140.81			

**= Significant at p<0.001.

Table 4: Post Hoc Comparisons (Dunnett's Test) of Plant Parts against Control

Treatment (vs Control)	Mean Difference	Std. Error	Sig. (p)	95% CI (Lower, Upper)
JWLE	+8.94	0.12	<0.001 **	8.65, 9.22
JWSE	+9.54	0.12	<0.001 **	9.26, 9.83
JWLSE	+10.40	0.12	<0.001 **	10.11, 10.68

KEY: **= Significant at p<0.001, JWLE= Jimsonweed Leaves Extract, JWSE= Jimsonweed Seed Extract, JWLSE= Jimsonweed Leave and Seed Extracts

Table 5: Effect of Jimson Weed Extracts on Nematode Mortality

Treatment (Group)	Mortality (Mean ± SD)
JWLE	8.94 ± 7.34^{b}
JWSE	9.54 ± 7.80^{ab}
JWLSE	10.40 ± 8.45^{a}
Control	0.00 ± 0.00^{c}

Means followed by the same superscripts within the same column are not significantly different.

DISCUSSION

The phytochemical screening showed that *Datura stramonium* leaves and seeds possess a number of bioactive secondary metabolites including alkaloids, flavonoids, glycosides, saponins, sterols, tannins, and terpenoids; carbohydrates and phenols are absent. This profile is broadly consistent with what other researchers have reported, though there are some divergences in the presence/absence and relative abundances of certain classes. For example, Sharma *et al.* (2024) in a recent review note that *D. stramonium* typically contains alkaloids, flavonoids, cardiac glycosides, tannins, phenolic compounds, terpenoids and carbohydrates among its principal phytochemicals. The absence of carbohydrates and phenols in both leaves and seeds contrasts with these findings, suggesting that either extraction or detection methods may lead to non-detection of

these compounds under the experimental conditions, or that local environmental or genetic variation has led to their suppression.

The nematodes isolated from galled tomato roots exhibiting characteristic symptoms of root-knot nematode infection were successfully identified as *Meloidogyne incognita*, based on both morphological and anatomical features. The use of the Baermann funnel technique facilitated effective extraction of motile juvenile stages from infected root tissues, which is consistent with standard nematological protocols (Huang, 2025). The second-stage juvenile (J2), displayed typical diagnostic features such as an elongated, slightly curved (C-shaped) body with tapering at both anterior and posterior ends, features well documented for *M. incognita* J2s, the infective and root-penetrating stage of the life cycle (Jaiman *et al.,* 2023; Sengar *et al.*, 2024). These juveniles are critical as they initiate root invasion and gall formation after hatching from the eggs. Furthermore, the perineal pattern analysis of adult females provided reliable confirmation of species identity. The distinctive perineal configuration characterized by wavy striae and low dorsal arch is a standard taxonomic feature for differentiating *M. incognita* from other root-knot nematode species (Mahmoud *et al.,* 2026). An elongated, slightly curved (C-shaped) body which tapered at both anterior and posterior ends, are features well documented for *M. incognita* J2s, the infective and root-penetrating stage of the life cycle (Jaiman *et al.,* 2023; Sengar *et al.*, 2024). The gelatinous matrix serves to protect eggs from desiccation and predators, aiding in the persistence and spread of the nematode in soil environments (Jhamta *et al.,* 2024). These observations corroborate earlier reports by Mondal and Khan (2026), who emphasized the significance of combining J2 morphology and perineal patterns for accurate root-knot nematode identification. Together, the morphological features along with extraction and identification methods, validate the nematode species as *Meloidogyne incognita*, a widely distributed and economically important pathogen of tomato. The in vitro evaluation of Jimsonweed (*Datura stramonium*) extracts revealed a clear nematocidal effect. All treatments, namely leaf extract, seed extract and combined leaf and seed extract induced varying degrees of mortality in second-stage juveniles (J2) of *Meloidogyne incognita*, while the control group recorded zero mortality. The highest mean mortality was observed in the combined extract, suggests a possible synergistic effect between leaf and seed bioactive compounds. This aligns with findings by Oplos *et al*. (2018), who reported enhanced nematocidal activity in composite plant extracts compared to individual parts, likely due to the complementary action of alkaloids, saponins, and terpenoids.

Regarding concentration, the 1g/10 mL extract produced the highest average mortality, followed by 1g/5mL, indicating a non-linear dose-response relationship. Surprisingly, mortality declined at higher dilution levels (1g/15mL and 1g/20mL), possibly due to reduced bioavailability of active compounds. Similar trends were observed by Natarajan *et al.* (2021), who noted that optimal concentration thresholds exist for plant-based nematicides, beyond which efficacy may decline due to dilution or compound instability.

Exposure time significantly influenced mortality outcomes. At 0 hours, there was no nematode death, but mortality sharply increased at 6 hours and peaked at after 24 hours. This time-dependent increase is consistent with the typical mode of action of phytochemicals, which often require sustained contact to disrupt nematode physiology (Degroote *et al*. 2024). The progressive mortality also underscores the systemic action of phytochemicals such as alkaloids and terpenoids found in *D. stramonium*, which are known to impair nematode nervous and digestive systems over time (Jhamta *et al.,* 2024). Compared to other botanicals studied in recent years, *D. stramonium* showed promising nematocidal efficacy. For instance, in a similar study by Jawhari *et al*. (2025), *Croton bonplandianus* extract achieved a mortality rate of 12.6 at 24 hours against *M. incognita*, slightly lower than the 14.75 recorded in this study. Thus, *D. stramonium*, particularly the combined leaf and seed extract, presents strong potential as a natural alternative to synthetic nematicides, with notable activity even at moderate concentrations and short exposure durations.

CONCLUSION

The root-knot nematodes isolated from galled tomato roots were identified as *Meloidogyne incognita* based on morphological characteristics and perineal pattern analysis, affirming the pathogen's identity and its role in disease expression. *In vitro* assays demonstrated that all plant parts extracts particularly the combined leaf and seed extract induced significant mortality in second-stage juveniles (J2) of *M. incognita*, with mortality rates increasing over exposure time. This time dependent toxicity suggests that phytochemicals from *D. stramonium* may have a cumulative or systemic mode

of action against nematodes. The study demonstrated that *D. stramonium* especially when both leaves and seeds are combined, offers a natural, effective, and potentially environmentally friendly alternative to synthetic nematicides.

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