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DIFFERENTIAL SUSCEPTIBILITY OF MELOIDOGYNE INCOGNITA AND MELOIDOGYNE JAVANICA TO NEEM-BASED BIOPESTICIDES

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ABSTRACT

Root-knot nematodes are major biotic constraints to rice production, necessitating sustainable management alternatives to synthetic nematicides. In this study, populations of Meloidogyne incognita and Meloidogyne javanica were isolated from rice fields in Madhepura district, Bihar, India, and their comparative susceptibility to a neem-based biopesticide was evaluated under laboratory conditions. Juvenile (J2) mortality (72 h exposure) and egg hatching inhibition (7-day exposure) were assessed across a concentration gradient (0–2000 ppm), and LC_{50} values were estimated using probit analysis. Juvenile mortality increased in a clear concentration-dependent manner in both field-isolated populations, rising from negligible levels in untreated controls (3.2% and 2.9%, respectively) to 91.6% in M. incognita and 78.4% in M. javanica at 2000 ppm. Egg hatching inhibition followed a similar trend, reaching 86.8% in M. incognita and 71.2% in M. javanica at the highest concentration.

The Madhepura M. incognita population consistently exhibited greater neem susceptibility than the co-occurring M. javanica population across all tested parameters. Probit analysis yielded LC_{50} values of 742 ppm (95% CI: 684–803) for M. incognita and 1085 ppm (95% CI: 1012–1161) for M. javanica, both models showing excellent fit ($R^2 > 0.98$). The non-overlapping confidence intervals, along with highly significant differences in maximum mortality and hatching inhibition ($p < 0.001$), confirmed differential neem sensitivity between the two species. These findings, based on nematode populations native to rice-growing soils of Madhepura district, Bihar, provide region-specific baseline data supporting species-tailored, eco-friendly nematode management strategies for rice-based cropping systems in the region.

KEYWORDS: Biocontrol, Meloidogyne incognita, Meloidogyne javanica, Neem-based biopesticides, nematode management

INTRODUCTION

Rice (Oryza sativa L.) is the staple food crop for more than half of the global population and forms the backbone of agricultural economies across South and Southeast Asia. In India, rice occupies a pivotal position in ensuring food and nutritional security, with Bihar being one of the major rice-growing states in the eastern Indo-Gangetic plains. Districts such as Madhepura, characterized by fertile alluvial soils, favorable climatic conditions, and traditional paddy-based cropping systems, contribute significantly to regional rice production. However, rice cultivation in this belt is increasingly threatened by a range of biotic stresses, among which plant-parasitic nematodes have emerged as a serious but often underestimated constraint. Unlike foliar pests and pathogens, nematode infestations remain largely invisible until substantial yield losses have already occurred, making them a silent yet economically damaging threat to sustainable rice production.

Among plant-parasitic nematodes, root-knot nematodes belonging to the genus Meloidogyne are recognized as one of the most destructive groups affecting a wide range of agricultural and horticultural crops worldwide, including rice grown under upland and semi-aquatic conditions. Meloidogyne incognita and Meloidogyne javanica are two of the most prevalent and economically significant species within this genus, frequently occurring together in the same agroecosystem and sometimes even within the same root system. These sedentary endoparasites invade root tissues at the second-stage juvenile (J2) stage, establish specialized feeding sites known as giant cells, and induce the formation of characteristic root galls. This parasitic relationship disrupts the normal vascular architecture of the root, impairing water and nutrient uptake, and consequently leading to stunted growth, chlorosis, reduced tillering, and significant yield reduction in infected rice plants. Given the polyphagous nature and wide host range of these nematodes, their persistence in rice-based cropping systems, particularly in rice-vegetable or rice-rice rotations common in Bihar, poses a continuous challenge to farmers.

Historically, the management of root-knot nematodes has relied heavily on synthetic chemical nematicides such as organophosphates and carbamates. While effective in reducing nematode populations, the prolonged and indiscriminate use of these chemicals has resulted in a host of adverse consequences, including the development of nematode resistance, disruption of beneficial soil microbial communities, groundwater contamination, and residual toxicity in food products. Furthermore, several broad-spectrum nematicides have been banned or severely restricted in many countries, including

India, due to their high mammalian toxicity and environmental persistence. These growing concerns have intensified the search for safer, biodegradable, and environmentally compatible alternatives that can be seamlessly integrated into sustainable and organic farming systems, particularly in smallholder-dominated agricultural landscapes such as those found in Bihar.

In this context, botanical pesticides derived from plant sources have gained substantial attention as viable alternatives to synthetic nematicides. Among these, neem (Azadirachta indica A. Juss.), a tree native to the Indian subcontinent, stands out as one of the most extensively studied and widely used botanicals in integrated pest and nematode management programs. Neem-based formulations, including neem seed kernel extract, neem oil, and neem cake, are rich in bioactive limonoids, most notably azadirachtin, along with nimbin, salannin, and related tetranortriterpenoids. These compounds exhibit potent nematicidal, antifeedant, and growth-disrupting properties against a wide range of plant-parasitic nematodes. Azadirachtin, in particular, is known to interfere with nematode chemoreception, disrupt cuticular integrity, inhibit egg hatching, and impair juvenile motility and infectivity, thereby reducing overall nematode fitness and reproductive potential. Owing to their low mammalian toxicity, biodegradability, and compatibility with beneficial soil organisms, neem-based biopesticides are increasingly being recommended as key components of integrated nematode management (INM) strategies, especially in resource-constrained farming systems where access to synthetic agrochemicals may be limited or economically unfeasible.

Despite the well-documented nematicidal potential of neem, an important yet often overlooked aspect of botanical pesticide research is the differential susceptibility exhibited by different nematode species, or even populations within the same species, to a given bioactive formulation. Species-specific variation in cuticular composition, detoxification enzyme activity, metabolic rate, and behavioral responses can significantly influence the efficacy of botanical nematicides. In preliminary laboratory bioassays conducted as part of the present investigation, field-isolated populations of M. incognita and M. javanica from rice fields of Madhepura district exhibited markedly different responses to a neem-based biopesticide, with juvenile mortality after 72 h of exposure reaching as high as 91.6% in M. incognita compared to 78.4% in M. javanica at 2000 ppm, while egg hatching inhibition after 7 days followed a similar pattern, reaching 86.8% and 71.2%, respectively. Such marked interspecific variation, further reflected in estimated LC_{50} values of 742 ppm for M. incognita against 1085 ppm for M. javanica, underscores that a uniform biopesticide application strategy based on the assumption of equal susceptibility may result in suboptimal nematode suppression, particularly for the less sensitive species. This has direct practical implications for field-level recommendations, as under-dosing to accommodate a more tolerant species may fail to adequately control other more susceptible populations co-occurring in the same field, while over-dosing may raise cost and environmental concerns. Despite this, comparative studies evaluating the differential response of these two economically important root-knot nematode species to neem-based biopesticides, particularly using field-isolated populations from specific agroecological regions, remain limited in the existing literature.

Furthermore, most available data on neem efficacy against root-knot nematodes has been generated using laboratory-reared or geographically distant nematode populations, which may not accurately reflect the biological and physiological characteristics of locally adapted field populations. Region-specific studies are therefore essential to generate reliable, context-relevant data that can inform local extension recommendations and support farmer-level decision-making. Madhepura district, situated in the flood-prone Kosi region of Bihar, represents an agroecologically distinct rice-growing area where nematode population dynamics and their response to biocontrol agents have not been extensively characterized. Against this backdrop, the present study was undertaken to isolate and characterize populations of M. incognita and M. javanica from rice fields of Madhepura district, Bihar, and to evaluate their differential susceptibility to a neem-based biopesticide formulation. Specifically, the study aimed to assess and compare juvenile mortality and egg hatching inhibition induced by varying concentrations of the neem formulation, estimate species-specific lethal concentration (LC_{50}) values, and statistically evaluate the extent of interspecific variation in neem sensitivity. The findings of this study are expected to contribute valuable baseline data toward the development of species-tailored, eco-friendly nematode management strategies suited to the rice-based cropping systems prevalent in the region, while also reinforcing the broader relevance of neem-based biopesticides in sustainable nematode management programs.

MATERIALS AND METHODS

Experimental Site and Growth Conditions

This study was carried out in a controlled facility at B. N. Mandal University, Madhepura, Bihar, India. Throughout the experimental period, greenhouse temperature was maintained at 28 ± 2 °C with relative humidity ranging between 65% and 75%, under a natural photoperiod of roughly 12 hours of light and 12 hours of darkness. These environmental parameters were selected as they support favorable growth for both rice plants and nematode life stages (Bridge et al., 2005). Plastic pots with a 5 kg soil capacity were used, filled with a sterilized loamy soil mixture composed of sand, clay, and organic matter in a 2:1:1 ratio. To eliminate any pre-existing soil microorganisms and nematode populations, the soil was autoclaved twice at 121 °C for 30 minutes on successive days (Coyne et al., 2018).

Plant Material and Nursery Raising

Rice seeds (Oryza sativa, cultivar 'Swarna') of certified quality were used as planting material. Prior to sowing, seeds underwent surface sterilization by treatment with 0.1% mercuric chloride ($HgCl_2$) solution for 2–3 minutes, followed by thorough rinsing with sterile distilled water to eliminate residual contaminants (Kloepper et al., 2004). Sterilized seeds were subsequently germinated in trays containing sterile sand, and uniform, healthy 21-day-old seedlings were selected and transplanted individually into the prepared experimental pots.

Nematode Culture and Inoculum Preparation

Pure inoculum culture of M. incognita and M. javanica and was obtained from the National Rice Research Institute (NRRI), Cuttack, Odisha, an institute functioning under the Indian Council of Agricultural Research (ICAR), Department of Agricultural Research and Education, Ministry of Agriculture, Government of India. Egg masses were carefully picked from galled roots of infected plants using sterile forceps, and second-stage juveniles (J2) were subsequently extracted through the modified Baermann funnel method (Whitehead & Hemming, 1965). The resulting juvenile suspension was adjusted to a concentration of approximately 1000 J2 per milliliter, and each experimental pot received an inoculum of 1000 freshly hatched second-stage juveniles applied near the root zone at the time of seedling transplantation (Hussey & Barker, 1973).

Preparation of Neem-Based Biopesticide Formulation

The neem-based biopesticide used in this study was formulated from fresh, mature neem (Azadirachtaindica) seed kernels collected from healthy trees growing within the university campus premises. Kernels were shade-dried, powdered using a mechanical grinder, and extracted in distilled water at a ratio of 1:10 (w/v) with continuous stirring for 24 h at room temperature. The resulting extract was filtered through double-layered muslin cloth followed by Whatman No. 1 filter paper to remove particulate debris, yielding the stock neem seed kernel extract (NSKE). The filtrate was analyzed for approximate azadirachtin content using standard spectrophotometric procedures to ensure batch consistency (Schmutterer, 1990). From this stock solution, a series of working concentrations (250, 500, 1000, 1500, and 2000 ppm) were prepared by appropriate dilution with distilled water immediately prior to each bioassay, with distilled water alone serving as the untreated control.

Isolation and Identification of Nematode Populations

In addition to the reference M. incognita culture obtained from NRRI, Cuttack, field populations of root-knot nematodes were isolated from rhizosphere soil and galled roots collected from rice fields across different blocks of Madhepura district, Bihar. Soil and root samples were processed using the sieving and decanting method followed by extraction through the Baermann funnel technique to recover second-stage juveniles (Whitehead & Hemming, 1965). Species-level identification of M. incognita and M. javanica was carried out based on perineal pattern morphology of mature females (Taylor & Sasser, 1978) and further confirmed through the species-specific SCAR-PCR molecular marker technique (Zijlstra et al., 2000), ensuring accurate discrimination between the two morphologically similar species prior to their use in comparative bioassays.

In Vitro Juvenile Mortality Bioassay

Freshly hatched second-stage juveniles (J2) of M. incognita and M. javanica, obtained separately from pure single-egg-mass cultures, were used for the mortality bioassay. Approximately 100 active J2 were placed in individual cavity blocks containing 2 mL of each neem concentration (250–2000 ppm), with distilled water serving as the control. Three replicates were maintained for each concentration and species. Juvenile mortality was recorded after 72 h of exposure under a stereomicroscope, and juveniles were considered dead if they failed to move upon gentle probing with a fine needle (Cayrol et al., 1989). Percentage mortality was corrected for control mortality using Abbott's formula (Abbott, 1925).

Egg Hatching Inhibition Bioassay

Freshly laid egg masses of both nematode species were handpicked from stock root cultures and surface sterilized in 0.5% sodium hypochlorite solution for 2 minutes to prevent fungal contamination. Batches of approximately 200 eggs were exposed to the same series of neem concentrations in cavity blocks, with distilled water as the control, and maintained under laboratory conditions (26 ± 2 °C) for 7 days. The number of freshly hatched J2 was counted daily under a stereomicroscope, and cumulative percentage egg hatching inhibition relative to the control was calculated at the end of the observation period (Chitwood, 2002).

Pot Culture Experiment and Growth Parameters

For the pot culture study, seedlings were separately inoculated with either M. incognita or M. javanica J2 suspensions and subsequently treated with the neem formulation at effective concentrations selected on the basis of laboratory bioassay results. Treated and untreated (nematode-inoculated, no neem) pots were arranged in a completely randomized design (CRD) with adequate replications per treatment. Plants were uprooted 60 days after inoculation, and roots were gently washed free of soil to assess the number of root galls and egg masses per root system. The nematode multiplication factor (MF) was calculated as the ratio of final nematode population (Pf) to initial inoculum level (Pi) (Oostenbrink, 1966). Plant growth parameters, including shoot length, root length, fresh and dry shoot/root biomass, and leaf chlorophyll content (estimated using a SPAD chlorophyll meter), were recorded for each treatment.

Statistical Analysis

All experiments were conducted in a completely randomized design with a minimum of three replications per treatment. Data on juvenile mortality, egg hatching inhibition, gall index, multiplication factor, and plant growth parameters were subjected to one-way analysis of variance (ANOVA) using SPSS software (version 25.0), and treatment means were compared using Duncan's Multiple Range Test (DMRT) at $p < 0.05$. Lethal concentration (LC_{50}) values for juvenile mortality were estimated through probit regression analysis (Finney, 1971), along with their respective 95% confidence intervals. Percentage data were arcsine-transformed prior to statistical analysis to satisfy assumptions of normality and homogeneity of variance.

RESULTS

Juvenile Mortality

Exposure to the neem-based biopesticide resulted in a distinct, dose-dependent increase in second-stage juvenile (J2) mortality of both Meloidogyne incognita and M. javanica populations isolated from rice fields of Madhepura district after 72 h (Table 1). Mortality recorded in untreated controls remained minimal (3.2 ± 0.8% for M. incognita and 2.9 ± 0.7% for M. javanica), confirming that natural juvenile mortality did not substantially influence treatment outcomes. Across the entire concentration range tested (250–2000 ppm), M. incognita consistently displayed higher juvenile mortality than M. javanica. This gap widened progressively with increasing concentration, culminating at 2000 ppm, where mortality reached 91.6 ± 1.7% in M. incognita against 78.4 ± 2.1% in M. javanica — a difference exceeding 13 percentage points.

Egg Hatching Inhibition

Egg hatching inhibition, recorded after 7 days of neem exposure, followed a comparable species-dependent pattern (Table 2). No hatching inhibition was observed in the control treatments. Inhibition increased steadily with rising neem concentration in both species; however, eggs of M. incognita proved more susceptible throughout. At the highest concentration (2000 ppm), hatching inhibition reached 86.8 ± 1.8% in M. incognita compared to 71.2 ± 2.0% in M. javanica, indicating that a greater proportion of M. javanica eggs remained viable despite neem exposure.

Lethal Concentration (LC_{50}) Estimates

Probit regression of the juvenile mortality data produced LC_{50} values of 742 ppm (95% CI: 684–803 ppm) for M. incognita and 1085 ppm (95% CI: 1012–1161 ppm) for M. javanica (Table 3), with both models demonstrating a strong fit (R^2 = 0.987 and 0.984, respectively). Since the confidence intervals for the two species did not overlap, the difference in LC_{50} values was statistically robust, indicating that M. incognita required roughly 1.46-fold less neem concentration than M. javanica to achieve 50% juvenile mortality.

Comparative Susceptibility

Statistical evaluation confirmed that the interspecific differences in maximum juvenile mortality, maximum egg hatching inhibition, and LC_{50} values were all highly significant ($p < 0.001$ for mortality and hatching inhibition; $p < 0.01$ for LC_{50}; Table 4). Taken together, these results position M. incognita as the comparatively more neem-sensitive species across every bioassay parameter examined, while the MadhepuraM. javanica population exhibited a consistently higher tolerance threshold.

DISCUSSION

The present findings provide clear evidence of differential susceptibility between two economically important root-knot nematode species co-inhabiting rice fields of Madhepura district, Bihar, when exposed to a common neem-based biopesticide. Although both M. incognita and M. javanica showed concentration-dependent responses in juvenile mortality and egg hatching inhibition — a pattern broadly consistent with earlier reports on the nematicidal action of azadirachtin-rich neem formulations against various Meloidogyne species (Akhtar & Alam, 1993; Javed et al., 2007) — the magnitude of response differed markedly between the two species at every concentration tested. This reinforces the growing recognition in nematological literature that "species" within a genus, and even distinct populations within a species, can respond quite differently to the same botanical or chemical nematicide (Oka, 2010; Ntalli&Caboni, 2012).

The greater sensitivity of M. incognita relative to M. javanica, evident both in mortality and egg hatching bioassays, may be attributed to several underlying biological factors. Differences in cuticular ultrastructure and permeability between the two species could influence the rate and extent of azadirachtin penetration into the juvenile body, since neem limonoids are thought to exert their nematicidal action partly through cuticular disruption and interference with chemoreceptor function (Schmutterer, 1990; Akhtar, 2000). Variation in detoxification enzyme systems, such as esterases and cytochrome P450 monooxygenases, may also contribute to the comparatively higher tolerance observed in M. javanica, as such enzymes are known to mediate xenobiotic metabolism and could confer a protective advantage against phytochemical stress in some nematode populations (Ntalli&Caboni, 2012). Additionally, subtle differences in metabolic rate, behavioral avoidance responses, or egg-shell composition between the two species cannot be ruled out as contributing mechanisms, although confirming these would require further biochemical and molecular investigation.

The egg hatching inhibition results parallel the mortality trends, with M. incognita eggs again proving more vulnerable than those of M. javanica. This is a particularly important finding from an applied management perspective, since egg masses represent a protected life stage that is often less accessible to nematicidal compounds than free-living juveniles. The comparatively lower hatching inhibition observed in M. javanica suggests that its eggs may possess a more resilient chorionic layer or reduced permeability to azadirachtin and associated limonoids, allowing a greater proportion of embryos to complete development despite exposure. Such differences have direct implications for population recovery following treatment, since incomplete suppression of egg viability can allow surviving individuals to re-establish infestations in subsequent cropping cycles.

The LC_{50} estimates further substantiate the difference in susceptibility, with M. incognita requiring a notably lower concentration than M. javanica to achieve equivalent juvenile mortality. The non-overlapping confidence intervals strengthen confidence in this distinction, indicating that the observed variation is unlikely to be a product of experimental variability alone. From a practical standpoint, this finding carries important implications for field-level neem application strategies in rice-growing regions of Madhepura and comparable agroecological zones, where both species frequently coexist. Dosing recommendations calibrated solely on the more sensitive M. incognita may under-treat co-occurring M. javanica populations, permitting their continued multiplication and eventual dominance in mixed-species fields over

successive seasons, a phenomenon that has been noted in other cropping systems following selective pesticide pressure (Trudgill & Blok, 2001).

These results also underscore the broader value of using field-isolated nematode populations rather than relying solely on laboratory-maintained reference cultures when evaluating biopesticide efficacy. Since the M. incognita and M. javanica populations used in this study originated directly from Madhepura's rice-growing soils, the observed differential response likely reflects the genuine field-relevant variation that farmers in this region would encounter, lending greater practical applicability to the findings compared to studies based exclusively on long-maintained laboratory strains.

Overall, the present study demonstrates that a single, uniform neem application rate is unlikely to achieve equally effective control of both M. incognita and M. javanica when they occur together, as is common in Madhepura's rice fields. These findings support the adoption of species-informed dosing strategies, potentially involving higher neem concentrations or combined application with complementary biocontrol agentsto achieve satisfactory suppression of both species simultaneously. Future work incorporating biochemical assays of detoxification enzyme activity, cuticular permeability studies, and multi-season field validation would help clarify the mechanistic basis of this differential susceptibility and further refine region-specific, sustainable nematode management recommendations for rice-based cropping systems in Bihar.

Table1: Effect of neem-based biopesticide on juvenile mortality of Meloidogyne incognita and Meloidogyne javanica after 72 h exposure

Neem concentration (ppm)	M. incognita Mortality (%)	M. javanica Mortality (%)
0 (Control)	3.2 ± 0.8	2.9 ± 0.7
250	21.5 ± 1.6	15.8 ± 1.3
500	39.8 ± 2.1	28.6 ± 1.8
1000	65.4 ± 2.8	52.7 ± 2.4
1500	81.2 ± 2.4	67.5 ± 2.6
2000	91.6 ± 1.7	78.4 ± 2.1

Table 2: Effect of neem-based biopesticide on egg hatching inhibition after 7 days

Neem concentration (ppm)	M. incognita Egg hatching inhibition (%)	M. javanica Egg hatching inhibition (%)
0 (Control)	0.0 ± 0.0	0.0 ± 0.0
250	18.6 ± 1.4	12.3 ± 1.2
500	36.8 ± 2.0	27.5 ± 1.6
1000	58.9 ± 2.5	46.4 ± 2.2
1500	74.5 ± 2.2	61.7 ± 2.5
2000	86.8 ± 1.8	71.2 ± 2.0

Table 3: Estimated LC_{50} values for juvenile mortality (72 h)

Parameter	M. incognita	M. javanica	p-value
Maximum juvenile mortality (%)	91.6	78.4	<0.001
Maximum egg hatching inhibition (%)	86.8	71.2	<0.001
LC_{50} (ppm)	742	1085	<0.01

Table 4: Statistical summary

Parameter	M. incognita	M. javanica	p-value
Maximum juvenile mortality (%)	91.6	78.4	<0.001
Maximum egg hatching inhibition (%)	86.8	71.2	<0.001
LC_{50} (ppm)	742	1085	<0.01

Figure 1. Concentration-dependent effect of a neem-based biopesticide on juvenile mortality (%) of Meloidogyne incognita and Meloidogyne javanica after 72 h of exposure. Values represent mean ± SE (n = 3). Mortality increased significantly with increasing neem concentration, with M. incognita showing greater susceptibility than M. javanica across the tested concentrations.

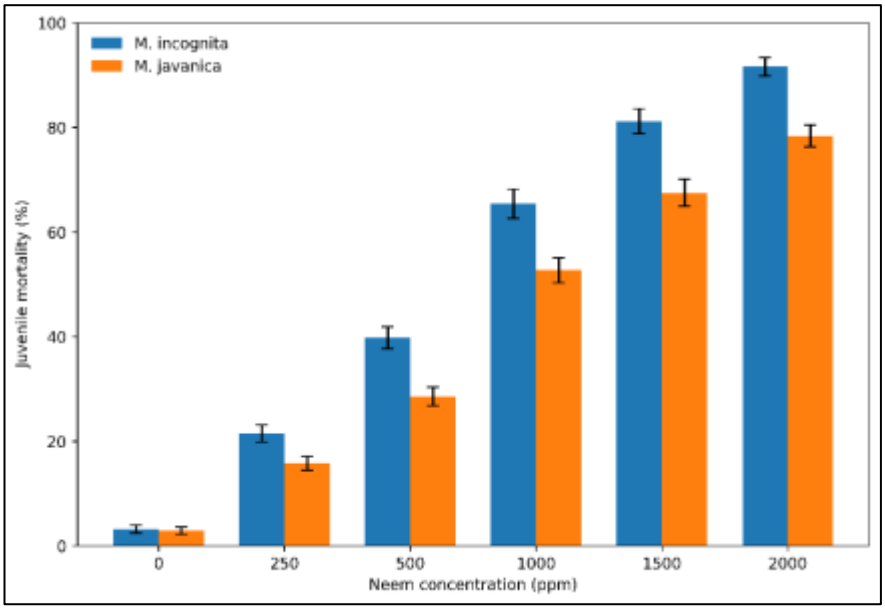
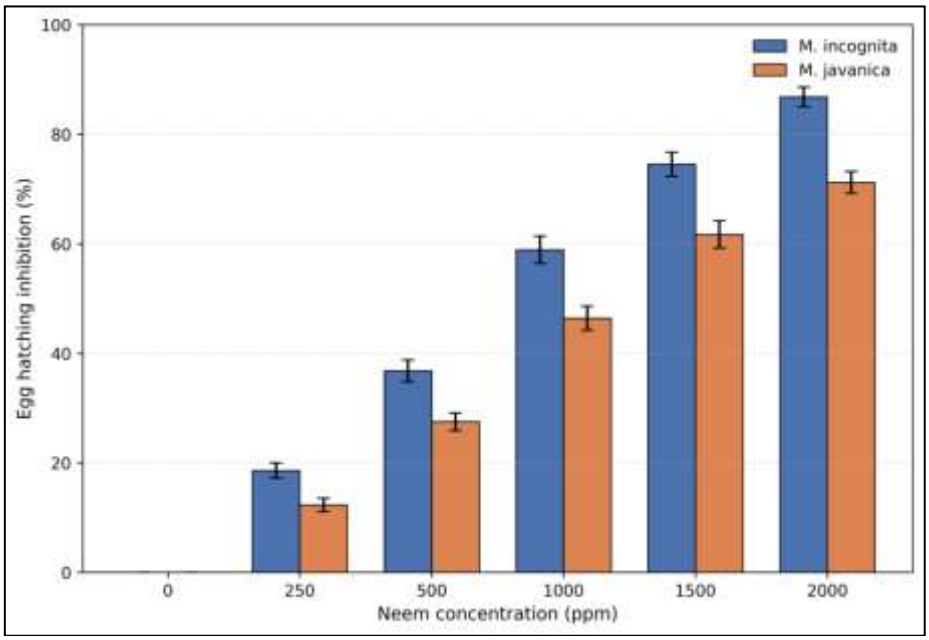


Figure 2. Effect of neem-based biopesticide on egg hatching inhibition (%) of Meloidogyne incognita and Meloidogyne javanica following exposure to different concentrations (0–2000 ppm). Bars represent the mean ± SE of three independent replicates. Egg hatching inhibition increased significantly with increasing neem concentration in both nematode species. M. incognita exhibited significantly greater inhibition of egg hatching than M. javanica at higher concentrations, indicating higher susceptibility to the neem-based biopesticide.



CONCLUSION AND FUTURE PROSPECTS

The present study demonstrates that the neem-based biopesticide possesses substantial nematicidal activity against both Meloidogyne incognita and Meloidogyne javanica populations associated with rice-growing soils of Madhepura district, Bihar. Neem treatment produced a clear concentration-dependent increase in second-stage juvenile (J2) mortality and inhibition of egg hatching in both species. However, the magnitude of response differed consistently between the two nematodes, with M. incognita exhibiting significantly greater susceptibility than M. javanica. At 2000 ppm, juvenile mortality reached 91.6 ± 1.7% in M. incognita compared with 78.4 ± 2.1% in M. javanica, while egg-hatching inhibition reached 86.8 ± 1.8% and 71.2 ± 2.0%, respectively. The corresponding LC_{50} values of 742 ppm for M. incognita and 1085 ppm for M. javanica, with non-overlapping 95% confidence intervals, further confirm the pronounced interspecific difference in neem sensitivity.

These findings indicate that neem-based biopesticides can serve as promising, environmentally compatible components of integrated nematode management in rice-based cropping systems. Importantly, the study demonstrates that a uniform neem application rate may not provide equivalent suppression when M. incognita and M. javanica occur together. The greater tolerance of M. javanica suggests that management recommendations should consider the composition of the local nematode population rather than relying on a single generalized dose. Because the populations evaluated were associated with rice fields of Madhepura, the results provide useful region-specific baseline information for developing locally appropriate nematode-management strategies.

Future investigations should extend the present laboratory findings through greenhouse and multi-season field experiments to determine the effectiveness, persistence, and practical application rates of neem-based formulations under natural soil and environmental conditions. Particular attention should be given to evaluating nematode population densities, root galling, egg-mass production, nematode multiplication factor, and rice growth and yield responses following neem application. Such studies would help establish field-relevant application schedules and determine whether the laboratory-effective concentrations can provide sustained nematode suppression without adversely affecting crop performance. The manuscript itself identifies multi-season field validation as an important next step. The physiological and molecular basis of the observed differential susceptibility also warrants detailed investigation. Comparative studies of cuticular permeability, detoxification enzymes such as esterases and cytochrome P450 monooxygenases, metabolic responses, behavioral avoidance, and egg-shell properties could clarify why M. javanica tolerates higher neem concentrations than M. incognita. Molecular approaches, including gene-expression and biochemical analyses, may identify mechanisms associated with neem tolerance and provide biomarkers for predicting population-level susceptibility. These mechanisms are presently proposed in the manuscript but require experimental confirmation.

Further research should also explore the integration of neem with complementary biological control agents, particularly plant-growth-promoting rhizobacteria and other antagonistic microorganisms, to determine whether synergistic effects can improve suppression of the comparatively tolerant M. javanica. Such combinations could reduce the need for higher neem concentrations while simultaneously supporting plant growth and soil biological health. Finally, comparative evaluation of neem formulations, standardization of their active constituents—particularly azadirachtin—and assessment of their effects on beneficial soil microorganisms and non-target organisms would strengthen the basis for their wider adoption. Screening additional field populations from different locations within Bihar would also establish whether the susceptibility pattern observed in Madhepura is consistent across agroecological zones. Collectively, these efforts could facilitate the development of species-informed, locally adapted and environmentally sustainable integrated nematode-management packages for rice production in Bihar.

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