

Occurrence and distribution of plant-parasitic nematodes associated with rice in the south-eastern Agro-climatic Zone (IIIA) of Bihar, India

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Summary

Rice (*Oryza sativa* L.) is a major staple cereal crop that sustains nearly two-thirds of the global population, particularly in Asia. India is one of the leading producers and consumers of rice, with the state of Bihar playing a significant role in national rice production. However, rice productivity is constrained by several biotic and abiotic factors, among which plant-parasitic nematodes (PPNs) represent an important yet often overlooked limitation. Rice grown under waterlogged conditions is vulnerable to infestation by various PPNs, resulting in substantial yield losses. The present study was conducted to assess the occurrence, distribution, and community structure of PPNs associated with rice cropping systems across six districts of agro-climatic zone IIIA of Bihar. Nematodes were extracted from both root and soil samples and analyzed for population density and community parameters. The results revealed that *Meloidogyne* spp. were the predominant nematodes in both root and soil samples. Community analysis further demonstrated that the frequency, density, and dominance of the root-knot nematode (*Meloidogyne* spp.) were significantly higher than those of other PPNs in the rice ecosystem. These findings highlight the widespread prevalence of *Meloidogyne* spp. in rice fields of Bihar and provide a scientific basis for developing region-specific nematode management strategies to enhance rice productivity.

Keywords: Rice; root knot nematode; *Meloidogyne* sp.; distribution; community analysis

Introduction

The family Poaceae includes rice (*Oryza sativa* L.), one of the most important cereal crops worldwide in terms of both production and human nutrition. Rice serves as the primary staple food for more than two-thirds of the global population, particularly in Asia, where nearly 90 % of global rice production and consumption occur (Bridge *et al.*, 1990; Abodolereza & Racionzer, 2009). India ranks first among the world's leading producers, consumers, and exporters of rice (Anonymous, 2022). Within India, Bihar is a major rice-growing state, producing approximately 7.06 million tonnes annually from more than 3 million hectares; however, rice

productivity in Bihar remains below the national average.

Rice productivity is constrained by multiple biotic and abiotic stresses, among which plant-parasitic nematodes (PPNs) constitute a major yield-limiting factor. Globally, PPNs are responsible for approximately 21.3 % yield losses, accounting for economic losses of about 102,039.79 million (USD 1.58 billion) (Kumar *et al.*, 2020). Nematode-induced crop losses are reported to be considerably higher in developing countries (14.6 %) than in industrialized nations (8.8 %) (Sasser & Freckman, 1987). The impact of phytonematodes on rice production is therefore a growing concern for global food security (Jones *et al.*, 2013).

PPNs are microscopic, worm-like, multicellular organisms charac-

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terized by bilateral symmetry and a pseudocoelomic body cavity. These nematodes primarily parasitize plant roots, although certain species may also attack stems, leaves, and other vegetative parts. They complete their parasitic life cycle by extracting cell sap from host tissues. The semi-aquatic nature of rice makes it particularly vulnerable to infestation by diverse phytonematode species, resulting in significant yield losses across different agro-climatic regions. Several PPNs have been reported to infest rice, with *Meloidogyne* spp. being the most prevalent, followed by *Ditylenchus* spp. and others (Prasad *et al.*, 1992).

Among these, the rice root-knot nematode (*Meloidogyne graminicola*) is one of the most destructive species, capable of causing yield losses exceeding 50 % under various rice-growing ecosystems (Lorenzana *et al.*, 1999; Kumar *et al.*, 2020). This nematode is a serious parasite of upland, lowland, and deep-water rice. It is well adapted to flooded conditions, surviving for extended periods in waterlogged soils either as eggs within egg masses or as juveniles. The infective second-stage juveniles penetrate rice roots near the root cap region and migrate into the vascular tissues, where they induce the formation of specialized feeding structures known as giant cells. This activity results in the development of characteristic hook or horseshoe-shaped galls at the root tips, leading to impaired nutrient uptake and stunted plant growth. Above-ground symptoms include chlorosis, patchy growth, stunting, and ultimately significant yield reduction (Kyndt *et al.*, 2014). The nematode feeding cells function as a continuous nutrient source throughout the parasite's life cycle, facilitating its growth and reproduction.

Despite the economic importance of rice and the damaging effects of PPNs, information on the occurrence and distribution of plant nematodes associated with rice in Bihar remains limited. However, a study has reported the distribution of PPNs in rice fields of the southeastern region (Agro-climatic Zone IIIA) of Bihar, highlighting the need for further systematic investigations across other rice-growing areas of the state.

Materials and Methods

Location of survey and sample collection

The survey was conducted during the rice-growing season in the major rice-producing areas of Agro-climatic Zone IIIA of Bihar, India. Six districts, Sheikhpura, Lakhisarai, Jamui, Munger, Banka, and Bhagalpur, were selected for the study, all of which have a long history of rice cultivation. From each district, two blocks were selected for survey and sampling. From each block, a total of six samples were collected, comprising three soil samples and three corresponding plant (root) samples, following a random sampling approach. Soil samples (200 g each) were collected from the rhizosphere at a depth of approximately 20 cm from three locations within each block. Root samples were collected from the same plants from which soil samples were obtained. In total, 78 samples were collected, consisting of 39 soil samples and 39 root samples. All soil and root samples were placed in properly labeled polythene bags and stored in a refrigerator at cool temperatures to prevent desiccation and loss of nematodes prior to analysis. The samples were subsequently processed in the Nematology Laboratory of the Department of Entomology, Bihar Agricultural College, Sabour.

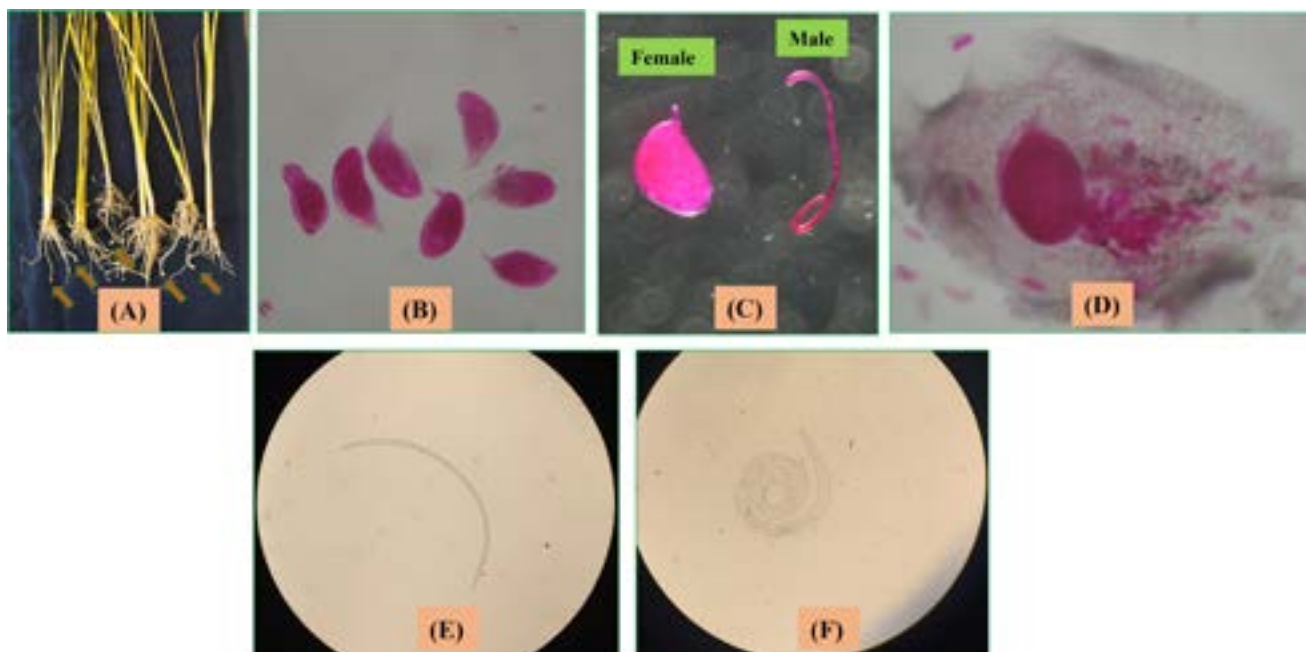


Fig. 1. Gall formation on rice root by root knot nematode, *Meloidogyne* sp. (arrow indicated) (A), Immature stages of female of *Meloidogyne* sp. (B), Mature stage of female and male (C), Female of *Meloidogyne* sp. with eggmasses (D), Adult of *Hirshmanniella* sp. (E), and Adult of *Helicotylenchus* sp. (F).

Table 1. Average number of plant parasitic nematodes (root samples) in surveyed blocks of rice fields of zone III A region, Bihar. The values of the number of plant parasitic nematodes are the mean of three replicates.

Blocks	Average no. of plant parasitic nematodes per 10 gm of rice roots		
	Plant parasitic nematode genera		
	<i>Meloidogyne</i> sp.	<i>Hirshmanniella</i> sp.	<i>Helicotylenchus</i> sp.
Sheikhpura	123.4	33.2	23.1
Chewara	103.3	16.9	26.5
Halsi	146.6	23.1	16.6
Ramgarh Chowk	106.8	36.5	23.1
Jamui	46.5	33.4	36.8
Barhat	56.6	33.4	23.6
Munger	53.2	36.9	23.4
Tarapur	116.7	16.4	36.9
Rajoun	123.5	13.1	23.2
Sambhuganj	103.1	16.6	16.4
Jagdishpur	146.9	23.4	26.8
Shakhund	76.6	13.5	23.5
Sabour	116.8	13.6	16.4
C. D.	11.2	6.2	5.1
SE(m)	3.8	2.1	1.7
SE(d)	5.4	3.0	2.4
C. V.	6.5	15.7	12.4

Extraction of plant parasitic nematodes from plant and soil materials
For the extraction of nematodes from plant tissues, 10 g of rice roots were washed thoroughly under running water to remove adhering soil particles. The roots were cut into 1 – 2 cm segments and macerated briefly in a blender. Nematodes were extracted from the macerated root material using the Modified Baermann Funnel Technique (Baermann, 1917). The nematode suspension was collected in 100 ml of water, and population counts were determined from three 10 ml subsamples. PPN's were extracted from soil samples using Cobb's Decanting and Sieving Method (Cobb, 1918). A composite sample of 100 cm³ soil was prepared from three randomly collected subsamples from each block. The soil was placed in a plastic container, mixed thoroughly with water, and allowed to stand for 15 – 20 seconds to permit the heavier soil particles to settle. The soil water suspension was then passed

sequentially through a series of sieves with mesh sizes of 20, 60, 350, and 400 to recover nematodes of different sizes. The nematodes retained on the sieves were collected and brought to a final volume of 100 ml. Nematode counts were performed by withdrawing 10-ml aliquots from the thoroughly mixed suspension, with counts repeated 3 times to obtain a mean population density. The mean number of nematodes recovered from root and soil samples was used to estimate population density and for subsequent community analysis.

Nematodes were identified up to the genus level under an Olympus stereozoom microscope using standard morphological and taxonomic characters, following the identification keys described by Siddiqi (2000).

The formulas used for community analysis are summarised in the list of formulas.

List of formulas used for community analysis:

$$\text{Absolute Frequency (AF, \%)} = \frac{\text{Number of samples containing a species}}{\text{Number of samples collected}} \times 100$$

$$\text{Relative Frequency (RF, \%)} = \frac{\text{Frequency of a species}}{\text{Sum of frequency of all species}} \times 100$$

$$\text{Absolute Density (AD)} = \frac{\text{Number of specimen of the species in the sample}}{\text{Volume or mass of the sample}}$$

$$\text{Relative Density (RD, \%)} = \frac{\text{Number of individuals of a species in a sample}}{\text{Total of all individuals in a sample}} \times 100$$

$$\text{Prominence Value (PV)} = \text{Absolute density} \times \sqrt{\text{Absolute frequency}}$$

$$\text{Relative Prominence Value (RPV, \%)} = \frac{\text{Prominence value of genus}}{\text{Sum of prominence value of all genera}} \times 100$$

Table 2. Average number of plant parasitic nematodes (soil samples) in surveyed blocks of rice fields of zone III A region, Bihar. The values of the number of plant-parasitic nematodes are the mean of three replicates.

		Average no. of plant parasitic nematodes per 100 CC of soil									
		Plant parasitic nematode genera									
Blocks		<i>Meloidogyne</i> sp.	<i>Hirschmanniella</i> sp.	<i>Helicotylenchus</i> sp.	<i>Hoplolaimus</i> sp.	<i>Tylenchorhynchus</i> sp.	<i>Criconeimoides</i> sp.	<i>Xiphinema</i> sp.	<i>Longidorus</i> sp.	<i>Trichodorus</i> sp.	
Sheikhpura	66.6	13.2	13.2	13.2	13.2	13.1	23.5	13.1	13.1	13.1	13.1
Chewara	83.2	16.4	13.2	13.2	23.4	23.2	33.4	23.2	23.5	23.5	23.4
Halsi	73.5	16.7	16.4	16.4	16.6	13.4	36.2	13.4	13.4	13.4	26.6
Ramgarh	46.4	36.5	13.1	13.1	33.1	16.5	23.4	16.6	16.4	16.4	36.4
Chowk											
Jamui	76.8	16.4	16.4	16.4	13.4	26.6	33.5	33.4	13.2	13.2	16.5
Barhat	66.7	23.4	13.6	13.6	16.8	23.4	16.8	16.7	23.2	23.2	13.2
Munger	43.1	23.6	13.4	13.4	13.5	13.2	26.4	13.9	13.4	13.4	13.4
Tarapur	63.6	33.4	23.8	23.8	16.4	23.2	26.6	16.4	33.6	33.6	13.1
Rajoun	56.9	13.8	13.5	13.5	13.2	13.4	16.4	23.8	26.4	26.4	13.6
Sambhuganj	53.7	16.6	16.8	16.8	23.3	23.4	33.2	23.4	16.5	16.5	23.4
Jagdishpur	36.8	13.4	13.4	13.4	13.5	13.5	23.4	13.1	23.6	23.6	13.5
Shakhund	46.9	36.7	23.4	23.4	16.4	26.4	13.5	16.4	13.4	13.4	16.7
Sabour	43.2	16.3	16.5	16.5	16.7	23.1	16.9	13.1	13.8	13.8	16.8
C. D.	7.6	5.0	4.3	4.3	5.7	3.9	3.5	3.5	3.7	3.7	3.7
SE(m)	2.6	1.7	1.4	1.4	1.9	1.3	1.2	1.2	1.2	1.2	1.2
SE(d)	3.6	2.4	2.1	2.1	2.7	1.9	1.7	1.7	1.8	1.8	1.7
C. V.	7.7	14.1	16.3	16.3	19.1	12.0	8.5	11.4	12.0	12.0	11.8

Statistical analysis

The recorded observations were analyzed using one-way analysis of variance (ANOVA) under a completely randomized design (CRD). Statistical significance was evaluated at the 5 % ($P < 0.05$) and 1 % ($P < 0.01$) probability levels.

Ethical Approval and/or Informed Consent

No studies involving human participants or animals were conducted in this research; therefore, ethical approval was not applicable.

Results

Three genera of PPN's, *Meloidogyne*, *Hirshmanniella*, and *Helicotylenchus*, were recovered from rice root samples collected across the surveyed fields (Fig. 1). Among these, *Meloidogyne* was the most prevalent genus, followed by *Hirshmanniella* and *Helicotylenchus*. Root samples were collected from 13 blocks to assess the distribution of PPN's. The mean population density of *Meloidogyne* per 10 gm of rice roots was highest in Hali and Jagdishpur blocks (146.6 and 146.9) and lowest in Jamui block (46.5) (Table 1). In contrast, the population densities of *Hirshmanniella* and *Helicotylenchus* did not differ significantly among the surveyed blocks. The mean population of *Hirshmanniella* ranged from 13.1 to 36.9, while that of *Helicotylenchus* ranged from 16.4 to 36.9 across blocks.

A total of nine genera of PPN's were identified from soil samples collected from rice fields, namely *Meloidogyne*, *Hirshmanniella*, *Helicotylenchus*, *Hoplolaimus*, *Tylenchorhynchus*, *Criconeimoides*, *Xiphinema*, *Longidorus*, and *Trichodorus*. Among these, *Meloidogyne* was the dominant genus in soil samples. The mean population density of root-knot nematodes per 100 CC of soil was highest in Chewara blocks (83.2) (Table 2), while the lowest density was recorded in Jagdishpur (36.8). The remaining nematode genera

were detected at comparatively lower densities, ranging from 13.1 to 36.7.

Community analysis of PPN's was conducted using a total of 78 root and soil samples. In root samples, *Meloidogyne* exhibited the highest absolute frequency (89.74 %), followed by *Hirshmanniella* (79.49 %) and *Helicotylenchus* (66.67 %) (Fig. 2A). Similarly, *Meloidogyne* showed the highest relative frequency (38.04 %), compared with *Hirshmanniella* (33.70 %) and *Helicotylenchus* (28.26 %) (Fig. 2B). The absolute density of *Meloidogyne* was markedly higher (1.02) than that of *Hirshmanniella* and *Helicotylenchus* (0.24 each) (Fig. 2C). A comparable trend was observed for relative density, with *Meloidogyne* accounting for 67.80 % of the total nematode population, while *Hirshmanniella* contributed 15.90 % (Fig. 2D). The prominence value was highest for *Meloidogyne* (96.15 %), whereas lower values were recorded for *Hirshmanniella* (21.22 %) and *Helicotylenchus* (19.92 %) (Fig. 2E). Likewise, *Meloidogyne* exhibited the highest relative prominence value (70.03 %), while *Helicotylenchus* showed the lowest (14.51 %) (Fig. 2F).

Community analysis of soil samples revealed a similar pattern. *Meloidogyne* showed the highest absolute frequency (89.74 %), followed by *Hirshmanniella* (71.79 %) and *Hoplolaimus* (64.10 %) (Fig. 3A). The lowest absolute frequency was observed for *Trichodorus* (23.08 %). In terms of relative frequency, *Meloidogyne* again dominated (21.47 %), whereas *Trichodorus* contributed the least (5.52 %) (Fig. 3B). The highest absolute density was recorded for *Meloidogyne*, while the lowest densities were observed for *Longidorus* and *Trichodorus* (0.19 each) (Fig. 3C). Relative density analysis showed that *Meloidogyne* accounted for 27.35 % of the total nematode population, whereas *Helicotylenchus* had a lower relative density (7.47 %) (Fig. 3D). Prominence value analysis further confirmed the dominance of *Meloidogyne* (26.97 %), while *Trichodorus* exhibited the lowest value (8.87 %) (Fig. 3E). Similarly, the highest relative prominence value was observed in *Meloidogyne* (36.11 %), and the lowest in *Trichodorus* (5.82 %) (Fig. 3F).

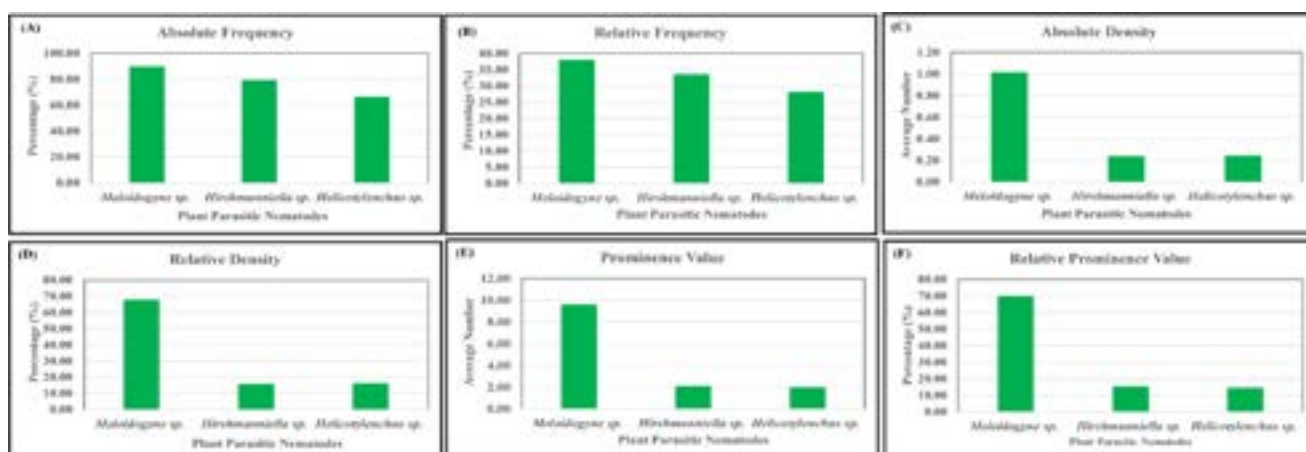


Fig. 2. Community analysis of plant-parasitic nematodes (root samples). Absolute frequency (A), Relative frequency (B), Absolute density (C), Relative density (D), Prominence value (E) and Relative prominence value (F).

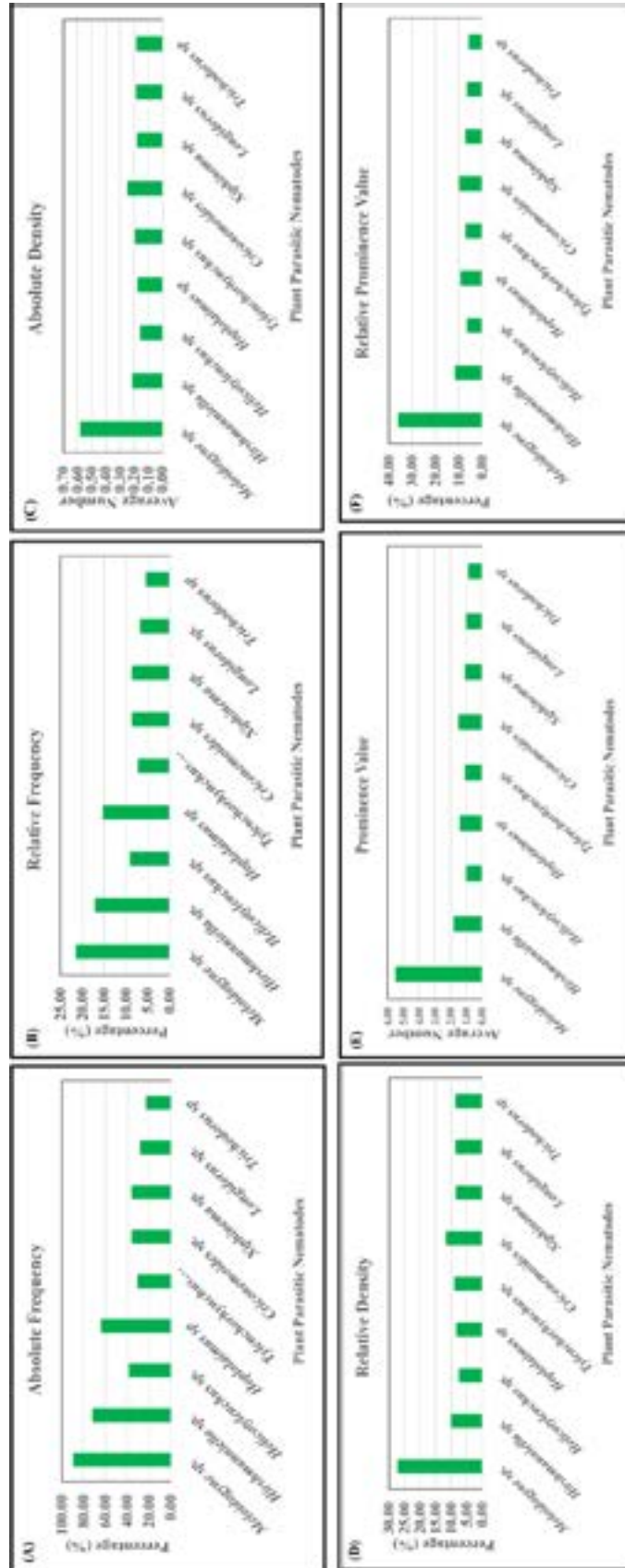


Fig. 3. Community analysis of plant-parasitic nematodes (soil samples). Absolute frequency (A), Relative frequency (B), Absolute density (C), Relative density (D), Prominence value (E) and Relative prominence value (F).

Discussion

Rice is the primary source of dietary calories for a large proportion of Bihar's population; however, its productivity remains suboptimal. Among the biotic constraints, PPNs constitute a major yet often overlooked threat to rice production due to their microscopic size and cryptic nature. Symptoms caused by PPN infestation frequently resemble those associated with water stress or nutrient deficiencies, leading to underdiagnosis in farmers' fields. Therefore, understanding the distribution and population dynamics of PPNs is essential for farmers, extension personnel, and policymakers to design effective nematode management strategies. Despite their economic importance, information on the occurrence and spatial distribution of nematodes associated with rice cultivation in Bihar is limited. Consequently, the present study was undertaken in the rice-growing areas of agro-climatic zone IIIA of Bihar to elucidate nematode diversity, distribution patterns, and quantitative parameters associated with the rice ecosystem.

The present investigation documented the occurrence of nine genera of PPNs in rice fields of agro-climatic zone IIIA, namely *Meloidogyne*, *Hirshmanniella*, *Helicotylenchus*, *Hoplolaimus*, *Tylenchorhynchus*, *Criconeoides*, *Xiphinema*, *Longidorus*, and *Trichodorus*. Among these, *Meloidogyne* emerged as the predominant genus, indicating a potential risk to rice productivity in the region. The nematode community structure varied between root and soil samples, reflecting differences in habitat preference and feeding behavior. Similar patterns of dominance have been reported by Pervez *et al.* (2022), who observed *Meloidogyne graminicola* as the predominant species in integrated pest management fields under a rice–wheat cropping system in Gautam Buddh Nagar, Uttar Pradesh. In contrast, *Aphelenchoides besseyi* was more prevalent in Rohtak, Haryana. The predominance of *Meloidogyne* spp. in both root and soil samples in the present study is consistent with these earlier observations.

Khan (2005) reported the presence of fifteen PPN species in a jute-rice cropping system, including *M. graminicola*, *Hirshmanniella oryzae*, *H. mucronata*, *Hoplolaimus indicus*, *Pratylenchus coffeae*, and *P. brachyurus* in rice, many of which were associated with substantial yield losses. In the present study, three genera, *Meloidogyne*, *Hirshmanniella*, and *Helicotylenchus*, were consistently detected in root samples, while soil samples harbored a broader diversity comprising nine nematode genera. These findings underscore the importance of soil as a reservoir for nematode diversity and highlight the need for soil-based diagnostic approaches in rice ecosystems. Musarrat *et al.* (2016) further emphasized the complexity of rice nematode communities by reporting 39 nematode genera across herbivorous, fungivorous, bacterivorous, omnivorous, and predatory groups, indicating a highly dynamic soil food web.

PPNs are characterized by slow movement and aggregated distribution patterns, which prevent rapid epidemic development. Instead, they cause chronic yield losses that intensify gradually

over successive cropping seasons. Continuous rice monoculture, as commonly practiced in the region, favours the build-up of nematode populations, leading to a progressive increase in disease severity over time. These findings highlight the need for long-term nematode management strategies, including crop rotation, resistant varieties, and integrated nematode management practices, to sustain rice productivity in Bihar.

Conclusion

The present investigation on the occurrence and distribution of PPNs elucidated their association with rice cultivation in agro-climatic zone IIIA of Bihar. Community analysis clearly revealed the predominance of *Meloidogyne* spp. in the surveyed rice fields, highlighting the urgent need for region-specific, sustainable management strategies to effectively suppress this economically important nematode. Although other plant-parasitic nematode genera, including *Hirshmanniella*, *Helicotylenchus*, *Hoplolaimus*, *Tylenchorhynchus*, *Criconeoides*, *Xiphinema*, *Longidorus*, and *Trichodorus*, were detected at comparatively lower population densities, their gradual build-up over time cannot be overlooked. Therefore, integrated nematode management approaches should consider the entire nematode complex rather than focusing solely on the dominant species. Overall, the widespread occurrence and higher frequency of root-knot nematode (*Meloidogyne* spp.) in both root and soil samples underscore its major role in limiting rice productivity in the region.

Conflict of Interest

The authors declare that there are no competing interests associated with this study.

Acknowledgment

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