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Chaitra G Bhat
Assistant Scientist, Department
of Nematology, CCS Haryana
Agricultural University, Hisar,
Haryana, India

Anil Kumar
Department of Nematology, CCS
Haryana Agricultural
University, Hisar, Haryana,
India

Prakash Banakar
Department of Nematology, CCS
Haryana Agricultural
University, Hisar, Haryana,
India

Sardul S Mann
Department of Nematology, CCS
Haryana Agricultural
University, Hisar, Haryana,
India

Vinod Kumar
Department of Nematology, CCS
Haryana Agricultural
University, Hisar, Haryana,
India

Lochan Sharma
Department of Nematology, CCS
Haryana Agricultural
University, Hisar, Haryana,
India

Priyanka Duggal
Department of Nematology, CCS
Haryana Agricultural
University, Hisar, Haryana,
India

Akshay J
Department of Nematology, CCS
Haryana Agricultural
University, Hisar, Haryana,
India

Rohit Kumar
Department of Nematology, CCS
Haryana Agricultural
University, Hisar, Haryana,
India

Corresponding Author:
Chaitra G Bhat
Assistant Scientist, Department
of Nematology, CCS Haryana
Agricultural University, Hisar,
Haryana, India

Morpho-molecular evidence reveals Indian jujube (*Ziziphus mauritiana*) rhizosphere as a rich reservoir of entomopathogenic and necromenic nematode fauna in semi-arid ecosystems

Chaitra G Bhat, Anil Kumar, Prakash Banakar, Sardul S Mann, Vinod Kumar, Lochan Sharma, Priyanka Duggal, Akshay J and Rohit Kumar

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Abstract

Entomopathogenic and necromenic nematodes, which exhibit pathogenic or parasitic associations with insects, play significant roles in maintaining soil ecological balance and biological pest management. The present study explored insect-associated nematode communities in the semi-arid agroecosystems of Haryana, India. Although the overall recovery frequency of nematode fauna was 5.14%, a remarkably higher occurrence (30%) was recorded in the rhizosphere of Indian jujube or ber (*Ziziphus mauritiana*), indicating its potential as a reservoir of beneficial nematode diversity. Integrated morphological observations and ITS-rDNA-based molecular characterization provided reliable identification and phylogenetic placement of the recovered isolates. Molecular analysis confirmed distinct nematode lineages corresponding to *Heterorhabditis indica*, *Metarhabditis amsactae*, *Distolabrellus veechi*, and *Acrobeloides* spp., validating preliminary morphological identification. The coexistence of multiple taxa within Indian jujube rhizosphere samples highlights the complexity of insect-associated nematode communities and demonstrates that single ecological niches can support diverse obligate and facultative insect-parasitic strategies. Despite the harsh climatic conditions of semi-arid regions, the recovery of multiple nematode genera suggests that perennial fruit tree systems provide favourable ecological niches for their survival and persistence. These native isolates represent valuable genetic resources for developing region-specific and ecologically adapted biological control strategies.

Keywords: Entomopathogenic nematodes, necromenic nematodes, biocontrol agents, Internal transcribed spacer marker, rhizosphere

Introduction

Nematodes represent one of the most abundant and ecologically diverse groups of multicellular metazoans inhabiting terrestrial ecosystems. Soil nematode communities occupy almost every trophic level and comprise bacterivores, fungivores, predators, omnivores, plant parasites and animal-associated forms. Among their diverse ecological adaptations, association with insects represents one of the most fascinating evolutionary strategies. These interactions range from random temporary associations to phoretic dispersal relationships, necromenic lifestyles, facultative parasitism and highly specialized obligate parasitic interactions^[1].

Entomopathogenic nematodes (EPNs) are a unique group of insect-parasitic nematodes that have evolved highly specialized obligate mutualistic associations with pathogenic bacteria, enabling them to rapidly infect and kill insect hosts. Among EPNs, the genera *Steinernema* and *Heterorhabditis* have received maximum scientific attention because of their strong insecticidal activity and association with the bacterial genera *Xenorhabdus* and *Photorhabdus*, respectively. Their ability to kill insect pests within a short period, host-seeking behaviour, compatibility with integrated pest management (IPM) strategies and relative safety towards plants, vertebrates and many non-target organisms have resulted in their development as sustainable biological control agents. Nearly 14 species belonging to these genera have been commercialized worldwide and are being utilized for the management of various insect pests^[2].

In addition to classical EPNs, several nematode species belonging to genera such as *Metarhabditis*, *Osccheius*, and *Acrobelloides* are increasingly being recovered during EPN surveys and have been reported to exhibit lifestyles ranging from necromeny to highly virulent entomopathogenic associations [3, 4, 5]. Members of these groups can exploit insect cadavers as nutrient resources and may contribute to insect mortality either independently or through interactions with their associated microbial communities. Therefore, the exploration of insect-associated nematode diversity provides broader insights into soil biodiversity, host-microbe interactions, and potential biological control resources.

In the current investigation, survey and sampling were undertaken to explore the diversity of native entomopathogenic nematodes in semi-arid regions of Haryana and adjoining areas. Soil samples were baited using susceptible insect larvae, and recovered nematodes were characterized using morphological observations as well as molecular identification based on the internal transcribed spacer (ITS) region to assess and document the diversity of insect-associated nematode fauna.

Materials and Methods

Soil sampling and isolation of entomopathogenic nematodes

A systematic survey was conducted across different locations of Haryana during 2024-2025, covering spring, summer, and humid monsoon seasons, to explore naturally occurring populations of entomopathogenic nematodes. Soil samples were collected from diverse habitats, including the rhizosphere of annual crops (sugarcane, rice, tomato, and chilli), perennial fruit trees (Indian jujube/ber, sapota, jamun and citrus), and fallow lands. Samples were collected from a depth of 10-20 cm, and 4-5 subsamples from each site were pooled to obtain representative composite samples. The samples were placed in labelled polyethylene bags containing information regarding location, host vegetation and sampling details, transported to the laboratory, and processed for nematode isolation using the insect baiting technique.

Soil samples were baited with late-instar larvae of *Galleria mellonella* (waxworm) using 10 larvae per 200 cc of soil from the composite sample and incubated under laboratory conditions at 28-30 °C. The samples were monitored regularly for one week, and dead larvae showing symptoms of nematode infection were collected, gently cleaned, and transferred individually onto modified White traps for recovery of emerging infective juveniles [6]. Individual cadavers were maintained separately to avoid mixing of different nematode populations originating from the same soil sample. Special attention was given to variation in insect cadaver colouration, as different nematode-microbe associations can produce distinct cadaver appearances. Recovered nematode populations were maintained separately as individual isolates. Each isolate was multiplied *in vivo* using fresh live *Galleria* larvae, and newly emerged infective juveniles were harvested and stored at 15 °C in aerated tissue culture flasks at the Department of Nematology laboratory for further morphological and molecular characterization.

Morpho-molecular characterization and phylogenetic analysis of recovered insect-associated nematode isolates

Preliminary identification of recovered nematode isolates was performed based on insect cadaver colouration and

morphological observations of infective juveniles using light microscopy. Molecular characterization was carried out for representative isolates to confirm their species level taxonomic identity.

Genomic DNA was extracted from representative nematode individuals of each isolate using worm lysis buffer (200 mM NaCl, 200 mM Tris-HCl pH 8.0, 1% β-mercaptoethanol and 800 µg/ml proteinase K) following the protocol of Holterman *et al.* [2006] with minor modifications. Briefly, individual nematodes were incubated in 25 µl lysis buffer at 65 °C for 1.5 h, followed by proteinase K inactivation at 99 °C for 5 min [7]. The crude DNA extract was centrifuged to remove debris, and the supernatant was stored at -20 °C until PCR amplification. The internal transcribed spacer (ITS) region of ribosomal DNA was amplified using primers TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3'). PCR reactions were performed using GoTaq® Green Master Mix (Promega, USA) in a final volume of 50 µl containing 2 µl template DNA, 25 µl master mix, 2.5 µl each primer (10 µM), and nuclease-free water. Amplification conditions consisted of initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 45 s, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 10 min.

Amplified PCR products were confirmed through gel electrophoresis and purified PCR products were subjected to bidirectional Sanger sequencing. Raw sequences were quality checked, trimmed, and assembled into consensus sequences using BioEdit Sequence Alignment Editor [8]. Species identity was determined through similarity searches against reference sequences available in the NCBI database using BLAST analysis. Representative sequences were submitted to GenBank. Phylogenetic relationships of recovered isolates with reference nematode species were inferred using MEGA v12.0 [9]. Sequences were aligned, and Maximum Likelihood (ML) phylogenetic analysis was performed using the best-fit nucleotide substitution model with 1000 bootstrap replicates to evaluate branch support. The ITS sequence of a plant-parasitic nematode *Meloidogyne arenaria* was included as an outgroup to root the phylogenetic tree.

Results and Discussion

Occurrence and diversity of entomopathogenic and necromenic nematodes from surveyed habitats

Considering the significance of entomopathogenic nematodes in integrated pest management programmes and maintaining soil ecological balance, the exploration and characterization of indigenous EPN resources have become essential for identifying locally adapted strains with improved survival and biological control potential. In the current study, survey was carried out across Hisar, Fatehabad, Kaithal, Karnal, and Rohtak districts of Haryana to isolate the native entomopathogenic nematode fauna for further use in biological plant protection programs. The surveyed locations represent hot semi-arid to humid subtropical climatic conditions and belong predominantly to the alluvial plains agro-ecological sub-region, with sandy loam to loamy soil types. A total of 175 soil samples collected from diverse habitats, including the rhizosphere of fruit trees (Indian jujube, sapota, citrus, and jamun), vegetable crops (tomato and chilli), field crops (rice and sugarcane), and fallow lands, were screened using the insect

baiting technique. Insect-associated nematodes were recovered from infected waxworm cadavers using modified White traps. Entomopathogenic and necromenic nematodes were recovered from nine soil samples, resulting in an overall frequency of occurrence of 5.14% (Table 1). Among different crop habitats, rhizosphere soils from Indian jujube or also called the ber tree (*Ziziphus mauritiana*) yielded the highest recovery of insect-associated nematodes, with a frequency of occurrence of 30%, indicating its potential as a rich habitat of nematode diversity. Recovered juveniles were further reinoculated into fresh live larvae and it confirmed their ability to establish insect pathogenic and/or necromenic associations and complete their life cycle *in vivo* on waxworm larval hosts.

Morphological characterization of recovered nematode isolates

Initial characterization of recovered nematode isolates was carried out based on infected insect cadavers and morphological observations of infective juveniles or the dauer juvenile stages. Cadavers obtained from sugarcane, jamun, and two ber rhizosphere samples developed characteristic brick-red colouration. Juveniles recovered from these cadavers possessed typical morphological characters of EPN *Heterorhabditis*, including the presence of a dorsal mural tooth, retained second-stage cuticle (sheathed IJs), and rosette formation during storage of IJs, which is a collective behavior where IJs aggregated, creating a rosette-like appearance (Figure 1: a-b and i). Reinfection of freshly emerged IJs into *Galleria* larvae reproduced similar brick-red cadaver symptoms (Figure 1j), confirming their entomopathogenic nature. The isolate recovered from the sapota rhizosphere produced black cadavers, and juvenile morphology showed resemblance to *Steinernema* spp.

Distinct variations in cadaver colouration were observed when Indian jujube rhizosphere samples were baited with *Galleria* larvae. Individual processing of cadavers with different colouration from the same habitat (creamish white, tan brownish, and dark necrotic cadavers) resulted in the recovery of multiple nematode taxa. Brownish-black cadavers yielded nematodes morphologically resembling *Metarhabditis*, characterized by the presence of a distinct stoma with metastegostomal structures and an elongated conical tail with an acute terminus (Figure 1: c-d and k). Light yellowish cadavers that later developed a tan-brown necrotic appearance yielded nematodes identified morphologically as belonging to the genus *Distolabrellus* (Figure 1l). These nematodes were characterized by a well-developed muscular corpus, a distinct swollen median bulb (metacarpus), and a conoid tail tapering towards a finely pointed terminus (Figure 1: e-f). Creamish-white cadavers that gradually became flaccid and collapsed during nematode multiplication, eventually turning off-white, yielded rhabditid nematodes exhibiting morphological characteristics similar to *Acrobelloides* spp. (Figure 1m). The recovered nematodes possessed a typical cephaloboid pharynx divided into three regions, a strongly cuticularized spheroid basal bulb with valvular apparatus, and a conoid tail with a slightly rounded terminus (Figure 1: g-h).

EPN-infected insect cadavers generally remain intact due to the rapid killing of the host and suppression of secondary microbial decomposition by their associated symbiotic bacteria. In the present study, cadavers infected with

Heterorhabditis and *Metarhabditis* isolates remained intact, whereas cadavers infected with other nematode isolates showed rapid collapse and flaccidity. Furthermore, cadaver colour serves as an important diagnostic feature in classical EPNs such as *Heterorhabditis*, where infected insects typically develop a characteristic brick-red colouration due to pigments produced by their bacterial symbiont *Photobacterium* [10]. However, in necromenic nematodes, cadaver colour is not considered a reliable diagnostic trait, as these nematodes generally lack an obligate association with a specific pigment-producing bacterial symbiont. Instead, colour changes in necromenic nematode-infected cadavers largely reflect the activity of associated microbial communities, bacterial proliferation, and the progression of host decomposition.

Molecular identification and phylogenetic characterization of recovered nematodes

Considering the higher frequency of recovery and diverse cadaver phenotypes observed from the ber rhizosphere samples, representative isolates of nematodes recovered from this habitat were further subjected to molecular characterization using the ribosomal ITS DNA marker to determine their taxonomic identity to the species level and explore the diversity of associated nematode taxa. Amplification and sequencing of the ITS region generated high-quality sequences containing internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2, and partial large subunit ribosomal RNA for species-level identification, with sequence lengths ranging from 679 bp to 900 bp among different isolates. The sequences were submitted to GenBank and accession numbers were obtained for a total of nine nematode isolates (Table 2). BLAST analysis and phylogenetic reconstruction supported the identity and placement of recovered isolates with corresponding reference nematode species. Two of the isolates from Indian jujube soil samples, initially identified as *Heterorhabditis* based on morphology, were confirmed as species *H. indica* based on the ITS marker. Four isolates were identified as *Metarhabditis amsactae*, two isolates as *Acrobelloides* spp., and one isolate as *Distolabrellus veechi*. Molecular analysis reconfirmed the coexistence of multiple insect-associated nematode taxa within individual ber rhizosphere samples. Co-occurrence of *Metarhabditis* and *Acrobelloides* was detected in one of the Ber samples from Kaul, Kaithal, whereas another sample from Hisar, Haryana, harboured *Metarhabditis*, *Acrobelloides*, and *Distolabrellus*, highlighting the entomopathogenic and necromenic nematode diversity associated with ber rhizosphere ecosystems.

Further, phylogenetic analysis based on ITS rDNA sequences resolved the nematode isolates recovered from ber rhizosphere into distinct taxonomic groups corresponding to *Heterorhabditis*, *Metarhabditis*, *Distolabrellus*, and *Acrobelloides*. The isolates HI-3 and HI-4 clustered within the *H. indica* clade along with previously reported reference isolates, confirming their species identity. Four isolates (RBR1, HBR2, HBR3, and KBR4) grouped with reference sequences of *M. amsactae*. The recovered *Distolabrellus* isolate HDSV-1 formed a distinct cluster with available *D. veechi* sequences, whereas *Acrobelloides* isolates (HACR1 and KACR2) grouped within the *Acrobelloides* clade and KACR2 showed close association with reported *A. saeedi* species. The bootstrap analysis

strongly supported the inferred taxonomic relationships within the phylogenetic tree.

EPN *H. indica* is well adapted to tropical and subtropical conditions and one of the commercially exploited EPN species in India ^[11]. In addition to classical EPNs, several other insect-associated nematodes, including *M. amsactae*, *D. veechi*, and *A. saeedi*, have been reported from different regions of India, particularly Uttar Pradesh and Punjab. These nematodes are regarded as facultative entomopathogens or necromenic associates of insects and have demonstrated biocidal potential against several insect pests such as *Spodoptera* and poplar defoliator ^[3, 12, 13, 14]. In the present study, isolates belonging to these nematode groups were successfully recovered from soil samples of Haryana using the insect baiting approach, thereby expanding the knowledge on the ecological distribution of these emerging insect-associated nematode taxa. Furthermore, their successful multiplication within insect cadavers and ability to induce mortality upon reinfection of healthy insect larvae under laboratory conditions indicate their potential insect-associated pathogenic behaviour.

The occurrence, distribution, and persistence of EPNs in soil ecosystems are governed by complex interactions among abiotic and biotic factors, including soil texture, temperature, moisture availability, vegetation type, host insect abundance, microbial communities, and agricultural disturbances ^[15]. Semi-arid regions represent challenging habitats for entomopathogenic nematodes because elevated temperatures, low soil moisture, and intense solar radiation can adversely affect the free-living infective juvenile stages.

However, the rhizosphere microenvironment of perennial trees, especially of fruit trees are generally well buffered due to canopy shade, accumulated organic residues, leaf litter, and relatively stable soil moisture conditions, thereby creating natural refugia for soil-dwelling organisms. The higher recovery and diversity of insect-associated nematodes from ber rhizosphere soils may be attributed to the unique ecological characteristics of these habitats. Ber trees are highly adapted to hot arid and semi-arid regions and generally represent comparatively undisturbed ecosystems compared with intensively managed annual cropping systems. Continuous deposition of leaf litter, decomposing fruits, and organic matter enriches the rhizosphere environment, enhances microbial activity, and supports diverse soil invertebrate communities. The presence of soil-dwelling insects attracted by decaying organic substrates may provide continuous host availability, facilitating long-term survival, recycling, and establishment of insect-associated nematode populations. Furthermore, the coexistence of multiple entomopathogenic and necromenic taxa within the same ecological niche indicates that natural soil ecosystems harbour a diverse nematode community involved in insect-nematode interactions, thereby contributing to ecological functions in soil food web dynamics and biological regulation of insect populations. The native isolates recovered from the ber rhizosphere represent valuable genetic resources that can be further explored for the development of region-specific and ecologically adapted biological control strategies.

Table 1: Occurrence of entomopathogenic and necromenic nematodes across different crop rhizosphere habitats in surveyed semi-arid regions of Haryana

Cropping system/Habitat	No. of soil samples collected	No. of samples positive for entomopathogenic and necromenic nematodes	Frequency of occurrence (%)	Recovered nematode taxa
Rice	25	-	-	
Sugarcane	25	1	4	<i>Heterorhabditis</i> sp.
Tomato	15	-	-	
Chilli	15	-	-	
Indian jujube/Ber	20	6	30	<i>H. indica</i> , <i>M. amsactae</i> , <i>Acrobelloides saeedi</i> , <i>Distolabrellus veechi</i>
Sapota	20	1	5	<i>Steinernema</i> sp.
Jamun	20	1	5	<i>Heterorhabditis</i> sp.
Citrus	20	-	-	
Fallow lands	15	-	-	
Total	175	9	5.14	

Table 2: Molecular characterization of entomopathogenic and necromenic nematode isolates recovered from Indian jujube rhizosphere in Haryana

Nematode isolate identified from ber rhizosphere	Sampling location	ITS rDNA GenBank accession number	ITS sequence length (bp)
<i>Heterorhabditis indica</i> isolate HI-3	Hisar, Haryana	PZ534495	766 bp
<i>Heterorhabditis indica</i> isolate HI-4	Karnal, Haryana	PZ534496	784 bp
<i>Metarhabditis amsactae</i> isolate RBR-1	Rohtak, Haryana	PZ543643	890 bp
<i>Metarhabditis amsactae</i> isolate HBR-2	Barwala, Hisar, Haryana	PZ543644	899 bp
<i>Metarhabditis amsactae</i> isolate HBR-3	Hisar, Haryana	PZ543645	900 bp
<i>Metarhabditis amsactae</i> isolate KBR-4	Kaul, Kaithal, Haryana	PZ543646	737 bp
<i>Acrobelloides saeedi</i> isolate HACR-1	Hisar, Haryana	PZ543647	780 bp
<i>Acrobelloides saeedi</i> isolate KACR-2	Kaul, Kaithal, Haryana	PZ543648	840 bp
<i>Distolabrellus veechi</i> isolate HDSV-1	Hisar, Haryana	PZ543649	679 bp



Fig 1: Morphological characteristics of entomopathogenic and necromenic nematodes recovered from Indian jujube rhizosphere and infected insect cadavers with these nematode isolates.

Light microscopic observations showing diagnostic morphological features of recovered nematode isolates: (a-b) *Heterorhabditis indica* infective juveniles (IJs) showing anterior region with characteristic dorsal tooth and posterior region with an elongated tail and presence of double cuticle; (c-d) *Metarhabditis amsactae* juveniles showing rhabditid stoma with metastegostome and posterior region with conoid-elongate tail ending in a pointed terminus; (e-f) *Distolabrellus veechi* showing anterior region with prominent rhabditid pharyngeal structures, prominent metacarpus and posterior region with tapering tail morphology; (g-h) *Acrobeloides* sp. showing cephaloboid anterior morphology and conoid with slight rounded tail region; (i) characteristic rosette aggregation behaviour by *H. indica* infective juveniles; (j) *H. indica*-infected insect cadavers exhibiting typical reddish-brown coloration; (k) *M. amsactae*-infected insect cadavers; (l) *D. veechi*-infected insect cadavers; and (m) *Acrobeloides* sp.-infected insect cadavers showing characteristic infection-associated changes. Scale bars = 50 µm (for microscopic images).

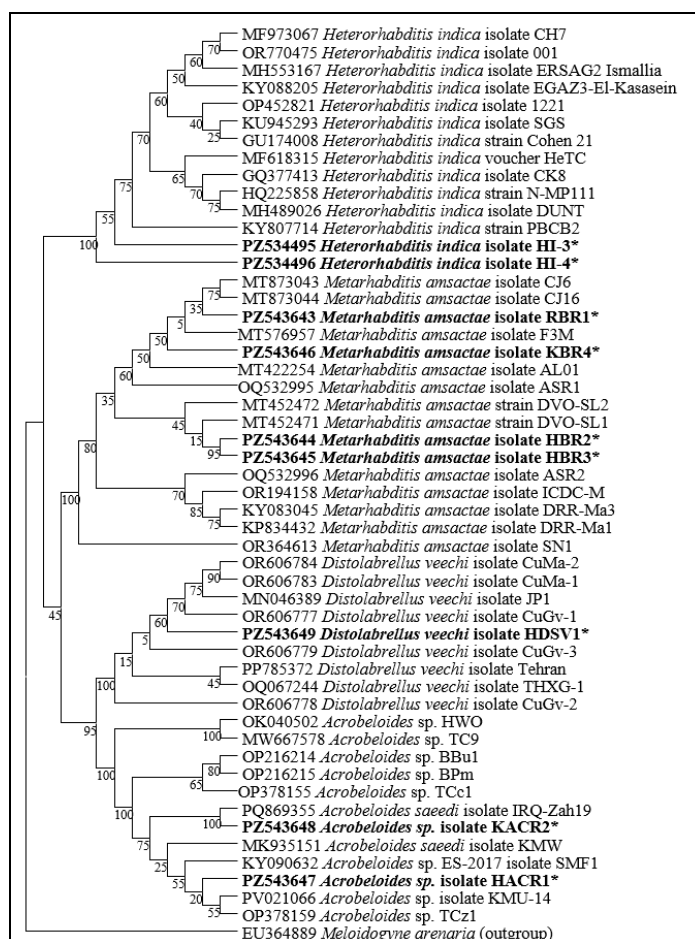


Fig 2: Molecular phylogenetic relationships of entomopathogenic and necromenic nematodes recovered from the rhizosphere of Indian jujube based on ITS-rDNA sequences.

The phylogeny was inferred using the Maximum Likelihood method with the Kimura 2-parameter nucleotide substitution model. The phylogenetic analysis included 52 nucleotide sequences representing *Heterorhabditis indica*, *Metarhabditis amsactae*, *Distolabrellus veechi*, and *Acrobeloides* spp., with a total of 1,459 positions in the final dataset. The percentage of replicate trees in which associated taxa clustered together is indicated at branch nodes. Newly characterized isolates obtained in the present study are highlighted in bold and marked with an asterisk (*). The tree confirms the phylogenetic placement of recovered isolates within their respective species-level clades, supporting morphological identification. *Meloidogyne arenaria* was included as an outgroup to root the phylogenetic tree.

Conclusion

Overall, this study expands the current understanding of insect-associated nematode diversity in semi-arid agroecosystems and identifies the Indian jujube (ber) rhizosphere as a previously underexplored hotspot of insect-associated nematode biodiversity. The findings suggest that relatively undisturbed perennial fruit tree ecosystems can serve as important conservation reservoirs for beneficial nematode communities. Targeted exploration of such habitats could enhance the efficiency of future EPN biodiversity surveys and increase the likelihood of recovering ecologically adapted native strains with promising potential for biological pest management. Further studies focusing on host range, virulence potential, bacterial associations, stress tolerance, and formulation development of these native isolates will facilitate their future utilization in sustainable pest management programmes.

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Conflict of interest statement

The authors declare no conflict of interest.

References

1. Trejo-Meléndez VJ, Ibarra-Rendón J, Contreras-Garduño J. The evolution of entomopathogeny in nematodes. *Ecol Evol.* 2024;14(2):e10966.
2. Ramatsitsi N, Manyevere A, Khosa MC. A systematic review on research trends and commercialised entomopathogenic nematodes: A global perspective. *Agric For Entomol.* 2026;28(2):298-313.
3. Kour S, Sharma N, Singh R, Gandhi SG, Ohri P. *Metarhabditis amsactae*: A potential biopesticide isolated from Punjab (India) with potent insecticidal activity and immunomodulatory effects against *Galleria mellonella* (Lepidoptera: Pyralidae). *J Invertebr Pathol.* 2023;196:108046.
4. Kumar P, Jamal W, Somvanshi VS, Chauhan K, Mumtaz S. Description of *Oscieus indicus* n. sp. (Rhabditidae: Nematoda) from India. *J Nematol.* 2019;51:e2019-04.
5. Singh P, Kumar A, Singh Somvanshi V, Kumar P, Prakash V. Biocidal potential of nematodes *Acrobeloides saeedi* (Rhabditida, Cephalobidae), *Metarhabditis amsactae*, *M. rainai* and *Oscieus myriophilus* (Rhabditida, Rhabditidae) against poplar defoliator, *Clostera cupreata*. *Biocontrol Sci Technol.* 2026;36(5):528-537.
6. Kaya HK, Stock SP. Techniques in insect nematology. In: Lacey LA, editor. *Manual of Techniques in Insect Pathology*. London: Academic Press; 1997. p. 281-324.
7. Holterman M, van der Wurff A, van den Elsen S, van Megen H, Bongers T, Holovachov O, *et al.* Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Mol Biol Evol.* 2006;23(9):1792-1800.
8. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser.* 1999;41:95-98.
9. Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S, Tamura K. MEGA12: Molecular Evolutionary Genetic Analysis Version 12 for Adaptive and Green Computing. *Mol Biol Evol.* 2024;41(12):msae263.
10. An R, Grewal PS. Molecular mechanisms of persistence of mutualistic bacteria *Photorhabdus* in the entomopathogenic nematode host. *PLoS One.* 2010;5(10):e13154.
11. Bhat CG, Somvanshi VS, Budhwar R, Godwin J, Rao U. Nematode genome announcement: the draft genome sequence of entomopathogenic nematode *Heterorhabditis indica*. *J Nematol.* 2022;53:e2022-21.
12. Bhat AH, Srivastava S, Rana A, Chaubey AK, Machado RAR, Abolafia J. Morphological, morphometrical, and molecular characterization of *Metarhabditis amsactae* (Ali, Pervez, Andrabi, Sharma and Verma, 2011) Sudhaus, 2011 (Rhabditida, Rhabditidae) from India and proposal of *Metarhabditis longicauda* as a junior synonym of *M. amsactae*. *J Nematol.* 2020;52:e2020-116.
13. Parihar RD, Dhiman U, Bhushan A, Gupta PK, Gupta P. *Heterorhabditis* and *Photorhabdus* symbiosis: a natural mine of bioactive compounds. *Front Microbiol.* 2022;13:824462.
14. Rana A, Bhat AH, Bhargava S, Chaubey AK, Abolafia J. Morphological and molecular characterization of *Acrobeloides saeedi* Siddiqi, De Ley and Khan, 1992 (Rhabditida, Cephalobidae) from India and comments on its status. *J Nematol.* 2020;52:e2020-27.
15. Matuska-Łyżwa J, Duda S, Nowak D, Kaca W. Impact of abiotic and biotic environmental conditions on the development and infectivity of entomopathogenic nematodes in agricultural soils. *Insects.* 2024;15(6):421.