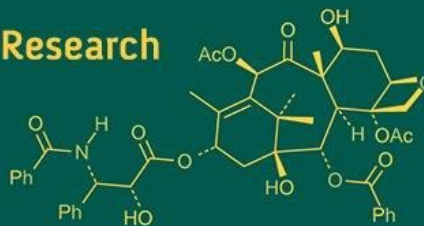


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Isolation of entomopathogenic nematodes across diverse ecosystems of Chhattisgarh

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Abstract

Entomopathogenic nematodes (EPNs) are increasingly recognized as promising agents for sustainable pest management, yet their natural occurrence in Indian agro-ecosystems remains insufficiently documented. In the present survey conducted across 24 districts of Chhattisgarh, 780 soil samples were examined to determine the diversity and prevalence of EPNs. The results revealed a low overall frequency, with only five samples (0.64%) testing positive, all belonging to *Heterorhabditis* spp. The highest recovery was recorded in sericulture farms (1.05%) and agroforestry systems (0.9%), particularly associated with tree species such as *Terminalia arjuna*, *Terminalia tomentosa*, *Tectona grandis*, *Gmelina arborea*, and *Shorea robusta*. In contrast, no EPNs were detected in sugarcane, vegetable, or other crop-based habitats, underscoring the influence of ecosystem type and soil conditions on nematode distribution. Although the prevalence was comparatively low, the detection of indigenous *Heterorhabditis* strains adapted to local agro-climatic conditions highlights Chhattisgarh's potential as a reservoir of native EPNs. These findings provide important baseline data for future research aimed at molecular characterization, mass production, and field evaluation of these isolates, ultimately supporting their integration into eco-friendly pest management strategies.

Keywords: Entomopathogenic nematodes, *Heterorhabditis* spp., agroforestry ecosystems, biological control, soil diversity

Introduction

Entomopathogenic nematodes (EPNs) are soil-dwelling roundworms of the genera *Steinernema* and *Heterorhabditis* that serve as effective biological control agents against diverse insect pests. Their success lies in the infective juvenile (IJ), a non-feeding third-stage juveniles that carries symbiotic bacteria (*Xenorhabdus* in *Steinernema* and *Photorhabdus* in *Heterorhabditis*). Upon entering the host hemocoel through natural openings, the IJ releases bacteria that kill the host within 48 hours by septicemia, produce antibiotics to suppress competitors, and digest host tissues to support nematode growth (Sankaranarayanan and Singaravelu, 2013; Sajnaga and Kazimierczak, 2020) [36, 35].

Research in India began in 1966 with Rao and Manjunath, who studied exotic species such as *Steinernema carpocapsae*. Subsequent trials with imported strains (*S. carpocapsae*, *S. glaseri*, *S. feltiae*, *Heterorhabditis bacteriophora*) yielded inconsistent results due to poor adaptability to Indian agro-climatic conditions (Kaya *et al.*, 2006) [18]. This highlighted the need for indigenous strains, as factors like soil type, pesticide use, and agricultural practices strongly influence EPN distribution (Mietkiewski *et al.*, 1997) [25]. Native isolates such as *Heterorhabditis indica* and *Steinernema abbasi* have since shown promise against local pests (Patil *et al.*, 2016; Sharma *et al.*, 2016) [28, 37].

Despite global advances in EPN-based pest management, knowledge of their diversity remains limited in developing regions. In Chhattisgarh, India, surveys are urgently needed to identify locally adapted strains that can provide sustainable, eco-friendly solutions for soil-dwelling insect pests (Afroz, 2023) [1].

Materials and Methods

Collection of Soil Samples

Soil samples were collected from three distinct agro-climatic zones of Chhattisgarh, namely the Northern Hills, the Chhattisgarh Plains, and the Bastar Plateau.

In addition to these zonal collections, samples were also obtained from diverse agroecosystems including sugarcane fields, agroforestry systems, vegetable cultivation areas, and sericulture farms. Sampling was conducted randomly by following the zig-zag method to ensure representative coverage of each site. Soil was collected from a depth of 15-20 cm around the root zone of crops using a spade, employing the standard 'V-notch method' for precision. At each sampling site, soil was collected from ten different spots, and approximately 250 g of soil was finally pooled to constitute a composite sample for baiting.

Isolation of Entomopathogenic Nematodes (EPNs) via Insect Baiting

Entomopathogenic nematodes (EPNs) were isolated from collected soil samples using the *in situ* insect-baiting technique. Each experimental unit was baited with five last-instar larvae of the greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), which serve as highly susceptible terrestrial hosts. Systematic observations were conducted at 24-hour intervals over a seven-day period to monitor larval mortality. Diagnostic criteria for EPN infection included the absence of putrefaction, lack of malodorous decomposition, and maintenance of cuticle integrity under physical pressure, without visible mycelial proliferation. Confirmed cadavers were subsequently transferred into sterile Petri dishes and incubated for an additional 4-7 days to facilitate intra-host nematode development.

The dead cadavers were transferred to White Trap and stored in a Biological Oxygen Demand (BOD) incubator maintained at 25 ± 2 °C and emergence of IJs were observed.

Harvesting, Quantification, and storage of IJs

IJs were harvested in distilled water, filtered, and stored as suspensions (≈ 2000 IJs/ml) in vented tissue culture flasks at 15 °C. Flasks were acclimated to room temperature before use.

Identification and Taxonomic Authentication

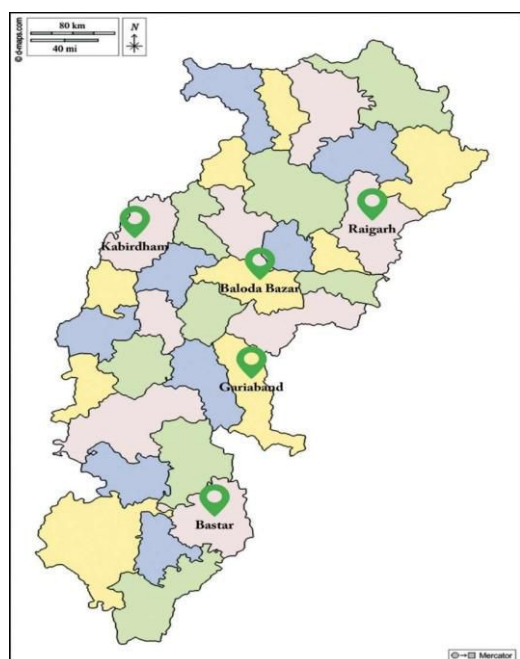
Preliminary identification was based on cadaver color. EPN-infected larvae exhibit distinct chromogenic shifts—transitioning to brick red (indicative of *Heterorhabditis* spp.) or grey/ochre (indicative of *Steinernema* spp.). The detailed identification was conducted at the National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, using inoculated soil-sand mixtures with *G. mellonella* larvae.

Result

The survey was conducted in 24 districts for diversity and occurrence of EPNs, out of which only 5 districts have the presence of EPNs. A total of 780 soil samples were collected from different locations with various microclimate, soil types, moisture regime and plant groups *i.e.*, Sugarcane, brinjal, jackfruit, ridge guard, broccoli, potato, tomato, sweet potato, cabbage, cowpea, *Terminalia arjuna*, *Terminalia tomentosa*, mulberry, sagon, eucalyptus, sarai, mahua, jamun, amla, sheesham, bamboo, gamhar, semialata, *Peltophorum pterocarpum*. Out of 780 soil samples collected from different habitats, 5 samples (0.64%) were found to be positive for EPN (Table 1). The highest frequency of occurrence of EPNs (1.05 %) recorded in sericulture farms with 2 samples out of 190 samples infected for EPNs followed by agroforestry ecosystem (0.9%) from 3 samples out of 340 samples.

Table 1: Entomopathogenic Nematode Recovery in Different Districts

S. No.	Districts	Sampling Sites	GPS location	Crops
1.	Raigarh	Sericulture farm, Boirdadar, Raigarh	21.9105°N, 83.4278°E	<i>Terminalia tomentosa</i>
2.	Baloda Bazar	Sericulture farm, Bhatapara, Baloda Bazar	21.7371°N, 81.9473°E	<i>Terminalia arjuna</i>
3.	Gariyaband	Forest Area, Rajim, Gariyaband	20.9636°N, 81.8823°E	<i>Tectona grandis</i>
4.	Kabirdham	Krishi Vigyan Kendra, Kabirdham	22.0268°N, 81.2538°E	<i>Gmelina arborea</i>
5.	Bastar	Forest Area, Jagdalpur, Bastar	19.0927°N, 81.9610°E	<i>Sorea robusta</i>



Districts with positive samples for Entomopathogenic nematodes

Amongst them *Heterorhabditid* nematodes, were recovered from all 5 soil samples, the frequency of occurrence was 0.64 percent *viz.* *Terminalia arjuna* (Baloda Bazar), *Terminalia tomentosa* (Raigarh), *Tectona grandis* (Gariyaband), *Gmelina arborea* (Kabirdham) and *Sorea robusta* (Bastar). Several samples collected from the fields *viz.*, Sugarcane, Brinjal, jackfruit, Ridge Guard, broccoli, potato, tomato, sweet potato, cabbage, cowpea, mulberry, eucalyptus, mahua, jamun, amla, sheesham, bamboo, semialata, *Peltophorum pterocarpum* showed no presence of the EPNs. This highlights the importance of conducting a more discursive survey.

The percentage of positive samples for nematodes obtained in this study was 0.64 percent and were comparable with other studies, *i.e.*, the findings of EPN recovery were comparatively average in India, where Bharath (2019) [4] who revealed that out of 200 samples, 2 samples were positive for EPNs (1%) with 1 sample containing *Oscheius* sp. (0.5%), and another sample containing *Heterorhabditis* sp. (0.5%). Kalitha *et al.*, (2019) [17] surveyed that out of 200 soil samples, eight soil samples were positive for entomopathogenic nematodes (4%), two isolates were

assigned to the genus *Heterorhabditis* (1%). Kary *et al.*, (2009) ^[19] reported that from 833 soil samples studied, 27 were positive for entomopathogenic nematodes (3.2%), with 17 (2%) containing *Heterorhabditis* and ten (1.2%) *Steinernema* isolates. Parihar *et al.*, (2002) ^[27] undertook another survey in Rajasthan and reported the presence of EPN in 8 samples out of 477 samples (1.68%). Bruck (2004) ^[6] reported that recovery frequency of EPNs may vary from 0.7 % to 70.1%. For instance, similar results were obtained in the research of Laznik *et al.*, (2009a) ^[23], where they confirmed the occurrence of EPNs in Slovenian soils in only 2.5% of soil samples. Gowda *et al.*, (2020) ^[11] reported that out of 130 soil samples collected, EPNs were recovered only from 3 samples (2.3%).

The current research findings are in partial conformity with Raj Kumar *et al.*, (2001) who reported that out of 105 soil samples collected from Rajasthan, only 5 (4.76%) were found to be positive for Steinernematids and Heterorhabditids. Vashisth (2014) ^[38] revealed that out of 131 locations, entomopathogenic nematodes were recovered from 5 locations (3.82%), mainly from hilly areas. Globally, EPN prevalence in soil samples has been reported as ranging from 0.7% to 50% (Hominick, 2002; Pillay *et al.*, 2009) ^[25, 29]. Kumar *et al.*, (2003) ^[20] also found entomopathogenic nematodes in only 5 samples out of 105 samples *i.e.*, 4.76%. Devi *et al.*, (2017) ^[9] documented that out of the 100 soil samples, Entomopathogenic nematodes were recovered from 5 samples (5%) and were found in all the three agroclimatic regions of Assam. Whereas, surveys conducted elsewhere in the world have reported the recovery frequency of as low as < 1% (Bruck, 2004) ^[6], 2% (Hazir *et al.*, 2003) ^[14], 2.6 % (Yakovlev *et al.*, 2014) ^[39]. Lalramliana and Yadav (2010) ^[21] reported that the entomopathogenic nematodes were recorded from 89 samples (5.37%) out of 1656 samples in Meghalaya, India. Benseddik *et al.*, (2020) ^[3] observed the prevalence was low EPN were isolated from 14 of 169 soil samples (8%).

The findings are in consonance with the results of Hominick (2002b) ^[25] who had stated that steinernematids are generally recovered more often than heterorhabditids during non-targeted surveys but in the non-targeted survey of Viti Levu, no steinernematids were encountered. IJs of different species occupy different strata of soil (delPino and Palomo, 1997; Lawrence *et al.*, 2006) ^[8, 22] during sampling. *H. indica* species was first isolated from India (Poinar *et al.*, 1992a) ^[30]. Since then various studies have demonstrated that *H. indica* is cosmopolitan and is widely distributed in tropical and subtropical regions *e.g.* Brazil (Dolinski *et al.*, 2008) ^[10], Vietnam (Nguyen and Sturhan, 2006) ^[26], Benin (Zadji *et al.*, 2013) ^[40], Cuba, Guadeloupe (Mauleon *et al.*, 2006) ^[24], Jamaica, Puerto Rico (Roman and Figueroa, 1995) ^[32], Trinidad, Venezuela (Rosales and Suarez, 1998) ^[34], India (Razia and Sivaramakrishnan, 2014) ^[31], and Indonesia (Griffin *et al.*, 2000) ^[13].

The dominance of heterorhabditids over steinernematids has been found in rather few surveys Rosa *et al.*, (2000) ^[33]. Similarly, Griffin *et al.*, (1994) ^[12] also reported a dominance of heterorhabditids over steinernematids in Britain and Ireland. Predominance of heterorhabditids over steinernematids has also been reported by Hara *et al.*, (1991) ^[17] in Hawaiian Islands.

Conclusion

The survey across 24 districts of Chhattisgarh revealed the

presence of entomopathogenic nematodes (EPNs) in five districts, with isolates recovered exclusively from *Heterorhabditis* spp. Although the overall frequency was low (0.64%), their occurrence in diverse agroforestry ecosystems—*Terminalia arjuna*, *Terminalia tomentosa*, *Tectona grandis*, *Gmelina arborea*, and *Shorea robusta*—demonstrates that Chhattisgarh possesses promising native reservoirs of EPNs. The relatively higher recovery from sericulture and agroforestry systems highlights the influence of microclimate, soil type, and vegetation on nematode distribution. While no EPNs were detected in sugarcane, vegetable, or other crop-based habitats, the detection of indigenous strains adapted to local conditions is significant. These findings suggest that Chhattisgarh holds strong potential for developing EPN-based biocontrol strategies tailored to its agro-climatic diversity. Expanding surveys, coupled with molecular characterization and field evaluation, will be essential to harness this potential and integrate native EPNs into sustainable integrated pest management (IPM) programs, thereby reducing reliance on chemical pesticides and promoting eco-friendly agriculture.

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