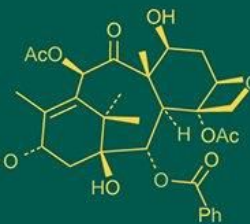
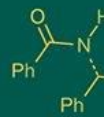


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 NAAS Rating (2025): 5.29
 IJABR 2025; 9(9): 668-670
www.biochemjournal.com
 Received: 14-07-2025
 Accepted: 20-08-2025

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Ecological survey and isolation of native entomopathogenic nematodes in Chhattisgarh agro-ecosystems

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DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i9i.5806>

Abstract

Entomopathogenic nematodes (EPNs) from *Steinernematidae* and *Heterorhabditidae* families are potent biocontrol agents against insect pests, yet their natural diversity in Chhattisgarh remains largely unexplored. A systematic survey of 150 soil samples across five agro-ecosystems viz., orchard, field crops, forestry, vegetable, and plantation, yielded EPNs in 8 samples (5.33% recovery). Orchard soils showed the highest prevalence (4 samples, 0.4%), followed by agroforestry (3 samples, 0.3%), while crop and plantation soils had minimal recovery (1 sample each, 0.1%). No EPNs were detected in vegetable ecosystems, likely due to intensive cultivation and chemical inputs. These results suggest that EPN distribution is influenced by habitat type, land use intensity, and ecological conditions. The presence of native strains in less disturbed ecosystems highlights their potential for sustainable pest management and warrants further molecular and ecological characterization to assess their virulence, adaptability, and compatibility with integrated pest control strategies.

Keywords: Entomopathogenic nematodes, *Steinernematidae*, *Heterorhabditidae*, biocontrol agents, agro-ecosystems

Introduction

Entomopathogenic nematodes (EPNs) of the families *Steinernematidae* and *Heterorhabditidae* are lethal pathogens of insects and have been widely used as biological control agents. Nematodes in genera *Steinernema* and *Heterorhabditis* are unique among insect parasitic nematodes due to several reasons. These pathogens contribute to the regulation of natural populations of insects, but the main interest in them is as an inundatively applied biocontrol agent. These beneficial organisms maintain a mutualistic relationship with bacteria—*Xenorhabdus* in *Steinernema* and *Photorhabdus* in *Heterorhabditis*. The bacteria reside within the intestine of the infective stage nematode (a third-stage, free-living juvenile) (Hazir *et al.*, 2003) [9]. Together, this nematode-bacterium partnership collaborates to kill the insect host using the infective juvenile

The age and habitat of EPNs, such as soil type, pesticide use, agricultural methods, and location, have an impact on their number (Mietkiewskiet *et al.*, 1997; Chidawanyika *et al.*, 2012; Usta, 2013) [20, 4, 19]. Multiple studies have been carried out over the world in search of EPNs to get new resources for biological management of insect pests (Bhat *et al.*, 2023) [2]. EPNs have been detected in a wide range of ecologically diverse soil habitats, including cultivated fields, forests, grasslands, deserts, and even ocean beaches on every continent except Antarctica, as a result to the decades of applied research and hundreds of surveys (Griffin *et al.*, 1990; Tarasco *et al.*, 2015) [8, 19]. the isolation of the native species and populations is considered a valuable resource, not only from a biodiversity perspective but also from an environmental perspective because of their adaptability and utility in biological control. In view of this a survey was conducted throughout the state of Chhattisgarh for potential strains of EPN. Chhattisgarh- a land locked state in central India, possess treasure of diverse flora and fauna. Approximately 44.21% of the state's total area is under forest cover. The state rank third for its area under forest cover in India the climate of Chhattisgarh is tropical. It is hot and humid because of its proximity to Tropic of Cancer. The state has vast diversity and natural heritage. But very little work has been carried out to explore the natural diversity of EPNs in this state and there is tremendous scope to explore in this

direction. Considering all the above parameters the present investigation was undertaken aiming to isolate native EPNs of Chhattisgarh and to study their ecological characterisation.

Materials and Methods

Rearing of bait insect, greater wax moth

Greater wax moth larvae, *Galleria mellonella* was used as the bait insect for trapping entomopathogenic nematodes present in the collected soil samples and for the mass multiplication of isolated EPNs in the laboratory. *Galleria* larvae were reared under laboratory conditions (27 ± 2 °C temperature; $65 \pm 5\%$ RH; L12:D12 photoperiod), as per the method suggested by Ellis *et al.* (2013) [5] on standard artificial diet. It was reared on artificial diet as per the method described by David and Kurup.

Collection of soil samples

Field surveys were conducted to identify native species and strains of EPNs. Soil samples collected from horticultural, agroforestry, vegetables, plantation and crop field. Sub samples were taken from a depth of 20 cm, by using a hand spade, from underneath the canopy of ten trees, randomly at least 10 metres apart and 3 sub samples from a diameter of 1 metre around each tree were combined to form one composite sample of approximately 500 grams. In the similar manner soil samples were taken for rice and maize fields. Each field was divided in 10 plots by mere visual observation and 3 sub samples from each plot were collected which was made to one composite sample of 500 grams.

The samples were collected in plastic zip lock pouches to prevent moisture loss and were brought to the laboratory for further processing. Information on sampling location, vegetation, longitude, latitude and altitude were recorded.

Isolation of EPN through baiting technique

Approximately 250 grams of soil were filled in a 500 ml plastic container along with five mature *Galleria* larvae as bait so as to assess the presence of entomopathogenic nematodes (EPNs). The container lids were perforated with pin-sized holes to allow for proper air circulation. The receptacles were subsequently maintained at 27 ± 2 °C in the dark for a week. Regular observations were carried out to monitor the *Galleria* larvae's mortality. Any dead larvae discovered were transferred one at a time to a modified White trap. (Woodring and Kaya, 1988) [21] to determine the cause of death. The emergence of EPNs was monitored in cadavers that were undamaged and did not exhibit any unpleasant odour, while those that displayed signs of bacterial or fungal infection were disposed. The emerging nematodes *i.e.* infective juveniles (IJ's) were re-inoculated into *G. mellonella* larvae, to get pure IJs.

Preparation of white trap

Watch glass (52 mm diameter) was placed on large petri dish (110×25 mm) in inverted position and then a circular filter paper (Whatman no.1) was placed on watch glass in such a way that, the lower edge of the filter paper touched the bottom of the petri dish. After that, distilled water was poured over the filter paper, always keeping the water level in touch with the edges of the paper. Then petridishes were incubated in BOD incubator at ambient temperature 25 ± 2 °C.

Harvesting and Storage of Entomopathogenic nematodes

The first emergence was re-inoculated and pure IJs were harvested with distilled water in to a beaker and filtered using vacuum filtration unit. The harvesting of IJs could be done up to one week. The White trap was rinsed 4-5 times to ensure that all infective juveniles were harvested before they were pooled sample by sample and concentrated by vacuum filtration. All nematodes emerging from dead larvae in the same sample were grouped together and treated as a single isolate. The IJs emerged were collected and were re-inoculated to the *Galleria* larvae to confirm Koch's postulates for pathogenicity (Pelczar and Reid, 1972; Kaya and Stock, 1997) [16, 12].

Results and Discussion

Isolation of entomopathogenic nematodes from soil

A comprehensive survey across diverse agro-ecosystems of Chhattisgarh was undertaken to evaluate the diversity and prevalence of entomopathogenic nematodes (EPNs). Investigating various landscapes to isolate and identify local EPN populations is essential for developing biological pest control strategies tailored to specific regions. Native nematode species are highly effective in reducing insect populations in their natural habitats. When these nematodes work in synergy with other parasites and pathogens, they play a significant role in managing widespread pest infestations (Mracek *et al.*, 2009). Their adaptability eliminates the need for insect populations to develop immunity against them, thereby making them highly efficient pest control agents (Gaugler, 1981) [6].

Among 150 soil samples collected, ten from each of the Agroecosystem *i.e.* orchard, field crops, forestry, vegetable and plantation, eight samples (recovery percentage 5.33%) tested positive for the presence of EPNs. The highest frequency of EPN occurrence was recorded in orchard soils, with 4 positive samples with recovery percentage of (0.4%), followed by agroforestry ecosystems with 3 positive samples with recovery percentage of (0.3%). The lowest frequency was observed in crop and plantation ecosystems, with 1 positive sample each with recovery percentage of (0.1%) for both of them. Interestingly, no EPNs were recovered from vegetable agro-ecosystems, which may reflect the effects of intensive agricultural practices.

Table 1: Recovery per centage of different agroclimatic zones of Chhattisgarh during survey of 2022-23

Agroclimatic zone	Number of samples	Occurrence	Recovery %
Orchard	10	4	0.4
Field crops	10	1	0.1
Vegetable	10	0	0.0
Forestry	10	2	0.2
Plantation	10	1	0.1
Total	150	8	5.33

The percentage of positive samples for nematodes obtained in this study was 5.33 per cent, were comparable with other studies, *i.e.*, the findings of EPN recovery were comparatively average in India, where Bharath (2019) [1] 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) 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) ^[11] 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) ^[15] undertook another survey in Rajasthan and reported the presence of EPN in 8 samples out of 477 samples (1.68%). Bruck (2004) ^[3] reported that recovery frequency of EPNs may vary from 0.7 per cent to 70.1 per cent. For instance, similar results were obtained in the research of Laznik *et al.* (2009) ^[13], where they confirmed the occurrence of EPNs in Slovenian soils in only 2.5 per cent of soil samples. Gowda *et al.*, (2020) ^[7] reported that out of 130 soil samples collected, EPNs were recovered only from 3 samples (2.3%).

Conclusion

The study documented the occurrence and distribution of entomopathogenic nematodes (EPNs) across different agro-ecosystem in Chhattisgarh, marking the first comprehensive survey of its kind. Out of 150 collected soil samples, only 8 (5.33%) tested positive for EPNs, representing ecologically diverse agro-ecosystem. EPNs were recovered from eight locations, including highest recovery in orchard soil (0.4%), followed by forestry (0.2%), then for both plantation and field crop recovery was 0.1%.

The recovery of EPNs confirmed their occurrence in Chhattisgarh soils, with the highest prevalence in orchard soils. This suggests that orchard management practices may create favorable conditions for EPN survival and proliferation. In contrast, EPNs were absent in vegetable soils, likely due to the adverse impacts of intensive agricultural practices such as frequent tillage, pesticide use, and heavy fertilization.

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