The Pharma Innovation Journal 2023; SP-12(12): 384-387

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(12): 384-387

www.thepharmajournal.com
Received: 11-10-2023
Accepted: 27-11-2023

Sonia Soni
Department of Entomology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Jagadeesh Patil
ICAR–National Bureau of Agricultural Insect Resources, Bengaluru, Karnataka, India

Jayalaxmi Ganguli
Department of Entomology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

RN Ganguli
Department of Entomology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Corresponding Author:
Sonia Soni
Department of Entomology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

First report of *Steinernema surkhetense* (Nematoda: Rhabditida: Steinernematidae) from Chhattisgarh state of India

Sonia Soni, Jagadeesh Patil, Jayalaxmi Ganguli and RN Ganguli

Abstract
Entomopathogenic nematodes belonging to the family Steinernematidae and Heterorhabditidae are potent biocontrol agents of insect pests particularly those inhabiting in soil or in concealed environment. Native isolates are more efficacious against insect pests than exotic ones because of their adaptation to local environment. Aiming to isolate local EPNs of Chhattisgarh, 200 soil samples were collected of which three samples were found to be positive for EPNs. An EPN isolated from Chitapur village of Bastar district belonging to family Steinernematidae was identified as *Steinernema surkhetense* after sequence analysis of internal transcribed spacer (ITS) region of ribosomal DNA. The physiochemical properties of the soil sample from which *S. surkhetense* was isolated, when tested revealed clay loam soil texture, 6.97 pH, 0.16 dSm^{-1} EC, 0.84% organic carbon and 1.45% organic matter content. This is the first record of *S. surkhetense* from Chhattisgarh state of India. Further exploration into diverse habitat of the state will likely to result in more previously unrecorded EPN species.

Keywords: Entomopathogenic nematodes, *Steinernema surkhetense*, Biological control, Chhattisgarh, internal transcribed spacer (ITS) regions, GenBank

Introduction
Entomopathogenic nematodes (EPNs) are obligate parasites of insects, which are pathogenic to insects and have significant potential in their biological control. They are capable of killing insect pests without having any adverse effect on humans and other vertebrate animals (Nguyen and Hunt, 2007) [10]. They have been largely excluded from pesticide registration requirements in many countries as they are considered safe to humans, non-target organisms, and the environment (Ehlers, 2005; Piedra-Buena *et al*., 2015) [5, 12]. Entomopathogenic nematode accommodate two families:- Steinernematidae (represented by two genera- *Steinernema* and *Neosterinernema*) and Heterorhabditidae (represented by genus *Heterorhabditis*). These nematodes are symbiotically associated with gram negative bacteria of family Morganellaceae (Adeolu *et al*., 2016) [2], where *Steinernema* is associated with bacteria of genus *Xenorhabdus* while *Heterorhabditis* is associated with bacteria of genus *Photorhabdus*. The symbiotic bacteria are carried by only the soil dwelling, free-living, non-feeding stage i.e. J3 stage called infective juvenile (IJs) or Dauer stage. After finding a suitable insect host, IJs enter the host hemocoel via natural openings or by penetrating the cuticle, release the bacteria into insect hemocoel and resume development. The bacteria multiply rapidly and kill the insect host often within 24-48 hrs. After insect's death the IJs feed on the symbiont biomass and the insect tissues metabolized by the bacteria in order to develop and reproduce. The IJs of *Steinernema* develop into amphimictic males and females, so at least two IJs should infect the host to mate and reproduce inside the insect. On the contrary, the IJs of *Heterorhabditis* develop into hermaphroditic females and subsequently into amphimictic males and females, exhibiting a heterocyclic life cycle. Therefore, even single IJ is capable of multiplication inside the infected host cadaver. Nematodes reproduction continues within the host until resources in the cadaver are depleted. As the resources within host cadaver become scarce then again IJs are produced, which exit from the depleted host and search for new host. These IJs are adapted for survival, dispersal, host finding and infection of fresh new hosts. Native EPNs species are well adapted to local environment and climatic conditions, which make them promising candidates for use as biological control agents in comparison to exotic once, therefore to isolate native species of EPNs naturally occurring in soils of Chhattisgarh, an extensive study was carried out in 2021-22.

Materials and Methods

Collection and isolation of Entomopathogenic nematodes: A total of 200 soil samples were collected from few different district belonging to three different agroclimatic zones of Chhattisgarh *viz*.- Bilaspur, Raipur, Rajnandgaon, Mohla-Manpur-Ambagarh Chowki (from Chhattisgarh Plain zone); Surajpur and Surguja (from Northern Hill zone) and Kanker, Kondagaon and Bastar (from Bastar Plateau Zone) in 2021-2022. Each soil sample (approximately 1kg) from a site consisted of 5 subsamples, taken atleast 8-10 m apart, from the surface to a depth of 15-20 cm. All sub-samples were mixed together and were placed in a polythene bag and information like sampling site, type of vegetation, date of sampling were recorded. Then the samples were brought to the laboratory. EPNs were recovered from the soil samples using the insect baiting method of Bedding and Akhurst (1975) [3]. To examine the presence of EPNs, about 250 gm of soil was transferred to 500 ml plastic container and was baited with five full grown larvae of *Galleria mellonella*. Small pin sized holes were made in the lid to facilitate aeration. The containers were then incubated in dark at 27 ± 2 °C and checked daily for larval mortality up to one week. Cadaver showing signs of EPN infection, recognized by change in colour (red/ purple for Heterorhabditis and orche/ brown/ black for Steinernematids) were removed and were rinsed with sterile distilled water and individually placed in modified White traps (Kaya and Stock, 1997) [7] for emergence of IJs. Emerging nematodes were collected for each sample and were used to infect fresh *G. mellonella* larvae to confirm Koch's postulates of pathogenicity and to obtain nematodes for identification and establishment of cultures. A small portion of soil sample was used for soil analysis in order to determine few physiochemical properties of soil (*viz*. - pH, EC, soil texture, OC and OM).

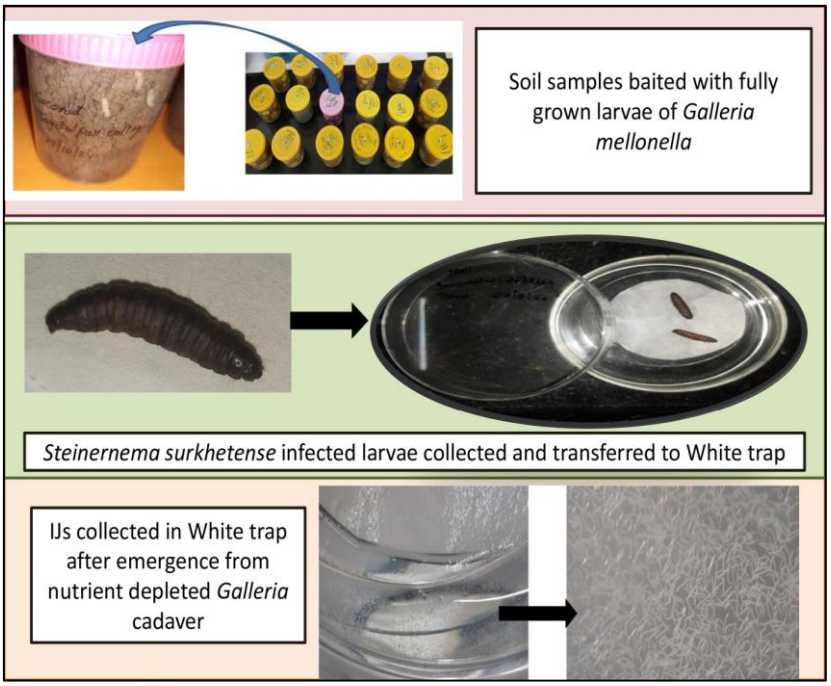

Fig 1: Isolation of EPN from collected soil samples

Molecular Characterization of isolated nematode: DNA was extracted from single female. Each female was transferred into a sterile eppendorf tube (1.5 ml) with 20 µl of extraction buffer (17.7 µl of dd H_2O, 2 µl of 10 × PCR buffer, 0.2 µl of 1% Tween, and 0.1 µl of proteinase K (20 mg/ml) Buffer and nematode were frozen at -20 °C for 20 min and then immediately incubated at 65 °C for 1h, followed by 5 min at 95 °C. The lysates were cooled on ice, centrifuged (2min, 9000 g) and 1 µl of supernatant was used for PCR. Primers were synthesized by Bioserve Biotechnologies Pvt. Ltd. (Telangana, India). A fragment of rDNA containing the internal transcribed spacer regions was amplified using primers 18S: 5′TTGATT ACGTCC CTGCCC TTT- 3′ (forward) and 28S: 5′TTTCACT CGCCGTT ACTAA GG-3′ (reverse) (Vrain *et al*., 1992) [15]. PCR reaction 1 cycle of 95 °C for 5 min followed by 35 cycles of 94 °C for 60 s, 55.4 °C for 30 s, 72 °C for 60 s and a final extension at 72 °C for 10 min; PCR was followed by electrophoresis (120 min 70 V) of 2 µl of PCR product in a 1% TAE-buffered agarose gel stained with ethidium bromide (10 µl ETB per 100 ml of gel). The PCR products were sequenced by Eurofins Genomics (Karnataka, India). The PCR products were sequenced and deposited in GenBank with accession number OR447669. The sequence was blasted in GenBank for comparison. A phylogenetic tree was constructed using the ITS rDNA sequence.

Result and Discussion

In the survey conducted in 2021 in Chhattisgarh state of India, aiming to determine the occurrence and distribution of EPNs, 3 soil samples out of 200 samples collected were found to be positive for EPNs. Out of the three EPNs isolates obtained, the one obtained from Chitapur village of Bastar District resulted into dark-brown to black coloration of the infected *Galleria* larvae, which indicated that the isolate belonged to the family Steinernematidae. The IJs of the isolate-*Steinernema* strain NBAIRS81 passed Koch's Postulate when tested for its pathogenicity on larvae of *G. mellonella*. The isolate was recovered from the soil having clay loamy textured with 50%, 22% and 28% of sand, silt and clay respectively. Soil pH was 6.97, EC was 0.16 dsm^{-1}, organic carbon and organic matter content was 0.84% and 1.45% respectively. The frequency of recovery of EPN is higher in sandy soil (Abd-Elbary *et al*., 2012; Hatting *et al*., 2014;

Valadas *et al*., 2014) [1, 6, 14]. However other soil types *viz*. - clay, silty clay, loam and silty loam are also found to harbor EPNs (Nyasami *et al*., 2008; Ma *et al*., 2010; Abd-Elbary *et al*., 2012) [11, 9, 1]. Stock *et al*., 1999 [13] reported a pH range of 4.2-7.2 for soil samples found positive for EPNs and observed that Steinernematids were mostly recovered from acidic (pH-0.5) to neutral soil, while heterorhabditis were mostly recovered from slightly acidic to slightly alkaline soil (pH 6.3-7.1), which is in accordance with the present findings.

Table 1: Site of isolation of *Steinernema Surkhetense* NBAIRS81 along with some basic physio-chemical properties of the soil sample

EPNs isolated	***Steinernema surkhetense* NBAIRS81**							
Isolation site	Chitapur village, Bastar							
GPS coordinates	18º57'36"N, 81º49'50"E							
Type of vegetation	Maize							
Soil physico-chemical parameters	pH	EC dSm^{-1}	% OC	% OM	Soil texture	% Sand	% Silt	% Clay
	6.97	0.16	0.84	1.45	Clay loam	50	22	28

In various studies it has been seen that land subjected to intensive cultivation, frequent tillage and high agrochemical inputs and frequent fluctuations in environmental conditions, impose detrimental effects on EPNs (Nyasani *et al*., 2008) [11]. *Steinernema* strain NBAIRS81 was recovered from maize field which was subjected to minimum soil disturbance, as Bastar being a tribal area where majority of the farmers practice natural cultivation with minimum agricultural inputs and tillage operations, resulting into a favorable environment for occurrence of these beneficial nematodes.

Molecular Identification: The ITS region sequence (OR447669) of this EPN- *Steinernema* strain NBAIRS81 when BLASTN searched showed 99% resemblance with sequences of *S. surkhetense* isolates present in the GenBank (accession no.- MW365746, MH822627, MH822626, MG976890, HQ317503, HQ190042, KP219886, KR029844, MF618312 and MF618308). The nematode *S. surkhetense* (Khatri-Chhetri *et al*., 2011) [8] was originally described from Nepal and belongs to "*carpocapsae*" group. The type sequence HQ190042 is the only one from Nepal. The GenBank records showed the presence of this species in four Asian countries- Nepal, India, China and Vietnam with its maximum records from Indian subcontinent. From India the species has been reported from Uttar Pradesh, Uttrakhand, Mizoram, Assam, West Bengal and Punjab (Bhat *et al*., 2020) [4]. This study presents the first report of presence of *S. surkhetense* from Chhattisgarh.

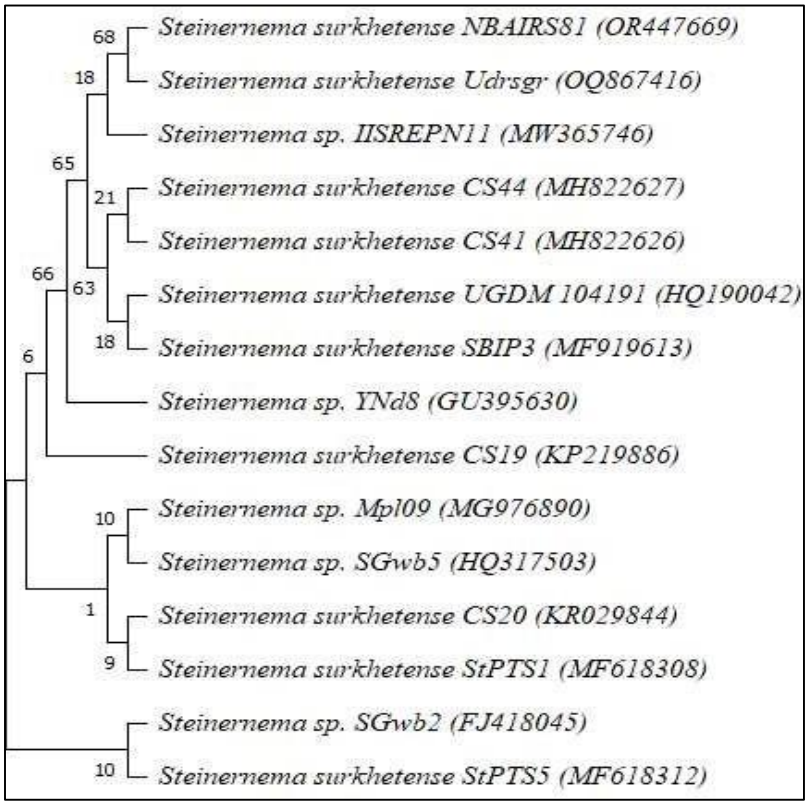

Fig 2: Phylogenetic relationship of *Steinernema surkhetense* NBAIRS81 with other isolates of *Steinernema surkhetense* (information retrieved from GenBank) based on ITS regions of rDNA. Numbers at the nodes indicate bootstrap values.

Conclusion

Out of 200 soil samples collected from few districts of Chhattisgarh, three samples were found to be positive for EPNs. Among the three obtained isolates, one isolate obtained from Chitapur village of Bastar district belonged to the family Stainernematidae resulting into dark-brown to blackish color change of the infected *Galleria mellonella* larvae. Sequencing of ITS region of rDNA revealed that the Stainernematid belonged to *S. surkhetense* (with more that 99% sequence similarity to *S. surkhetense* isolates deposited in Genbank). The soil sample from which *S. surkhetense* was recovered had clay loam soil texture, pH 6.97, EC 0.16 dSm^{-1}, organic

carbon 0.84% and organic matter 1.45%. The isolate was obtained from maize ecosystem, subjected to minimum agricultural inputs and minimum soil disturbance, which provided congenial environment for survival of EPNs. Use of EPNs in pest management has several advantages like movement ability, high virulence, ability to kill hosts quickly, easy mass rearing high reproductive potential, broad host range and safety to non-target organisms. As *S. surkhetense* is indigenous to the Indian subcontinent, thus this species has a high scope and better prospect as an important component of biocontrol in future eco-friendly management of insect pest in India.

Acknowledgement
We are grateful to ICAR- National Bureau of Agricultural Insect Resources Bengaluru and Indira Gandhi Krishi Vishwavidyalaya for providing the research facilities and financial support throughout the work.

References

1. Abd-Elbary NA, Shamseldean MS, Stock SP, Abu-Shady NM. Diversity of entomopathogenic nematode species (Heterorhabditidae and Steinernematidae) in Egypt, Egyptian Journal of Agronematology, (Egypt), 2012;11(2):333-353.
2. Adeolu M, Alnajar S, Naushad S, Gupta SR. Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. International Journal of Systematic and Evolutionary Microbiology. 2016;66(12):5575-5599.
3. Bedding RA, Akhurst RJ. A simple technique for the detection of insect paristic rhabditid nematodes in soil. Nematologica. 1975;21(1):109-110.
4. Bhat AH, Sharma L, Chaubey AK. Characterisation of *Steinernema surkhetense* and its symbiont *Xenrorhabdus stockiae* and a note on its geographical distribution. Egyptian Academic Journal of Biological Sciences. A, Entomology. 2020;13(1):105-122.
5. Ehlers RU. Forum on safety and regulation. In Nematodes as biocontrol agents; c2005, p. 107-114. Wallingford UK: CABI publishing.
6. Hatting J, Stock SP, Hazir S. Diversity and distribution of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in South Africa. Journal of Invertebrate Pathology. 2009;102(2):120-128.
7. Kaya HK, Stock SP. Techniques in insect nematology. In Manual of techniques in insect pathology; c1997. p. 281-324. Academic Press.
8. Khatri-Chhetri HB, Waeyenberge L, Spiridonov S, Manandhar HK, Moens M. Two new species of Steinernema Travassos, 1927 with short infective juveniles from Nepal. Russian Journal of Nematology. 2011;19(1):53-74.
9. Ma J, Chen S, Zou Y, Li X, Han R, Clercq DP, *et al.* Natural occurrence of entomopathogenic nematodes in North China. Russian Journal of Nematology. 2010;18(2):117-126.
10. Nguyen K, Hunt D Entomopathogenic nematodes: systematics, phylogeny and bacterial symbionts. Brill. 2007, 5.
11. Nyasani JO, Kimenju JW, Olubayo FM. Occurrence of entomopathogenic nematodes and their potential in the management of diamondback moth in Kale; c2008.
12. Piedra-Buena A, López-Cepero J, Campos-Herrera R. Entomopathogenic nematode production and application: regulation, ecological impact and non–target effects. In Nematode Pathogenesis of Insects and Other Pests: Ecology and Applied Technologies for Sustainable Plant and Crop Protection. 2015, p. 255-282. Cham: Springer International Publishing.
13. Stock SP, Pryor BM, Kaya HK. Distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in natural habitats in California, USA. Biodiversity & Conservation. 1999;8:535-549.
14. Valadas V, Laranjo M, Mota M, Oliveira S. A survey of entomopathogenic nematode species in continental Portugal. Journal of Helminthology. 2014;88(3):327-341.
15. Vrain TC, Wakarchuk DA, Levesque AC, Hamilton RI. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology. 1992;15:563-573.