

## Original Research Article

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## Report of Novel Entomopathogenic Nematode (*Heterorhabditidoides* sp.) in Telangana state, India

Amit. U. Paschapur<sup>1\*</sup>, K. Vijaya Lakshmi<sup>2</sup>, Khushbu Chauhan<sup>3</sup>, B. S. Sunanda<sup>2</sup>, Chetna Mathur<sup>3</sup>, Chaitra Bhat<sup>3</sup>, Vishal Somavanshi<sup>3</sup>, Uma Rao<sup>3</sup> and Vinod Pawar<sup>2</sup>

<sup>1</sup>Division of Entomology, <sup>3</sup>Division of Nematology, ICAR-IARI, New Delhi- 110012, India

<sup>2</sup>National Institute of Plant Health Management, Rajendranagar, Hyderabad-500030, India

\*Corresponding author

### ABSTRACT

#### Keywords

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#### Article Info

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A soil survey was carried out during 2016-2017 in four predominantly Sugarcane growing districts of Telangana state, viz., Sangareddy, Kamareddy, Nizamabad and Khammam, for the presence of entomopathogenic nematodes (EPNs) from Sugarcane ecosystem. The soil samples collected from Kamareddy district showed the highest occurrence of EPNs in 33.33% of the soil samples, followed by Sangareddy (23.33%), Khammam (16.67%) and Nizamabad (5.55%) districts. The isolates of EPNs were utilized for molecular characterization and identification. The genomic DNA of EPN isolates was extracted and 18S and 28S ITS rDNA region of genomic DNA was amplified by PCR technique using a universal primer. The molecular studies shown the occurrence of *Heterorhabditis indica* in 41.18% of the soil samples, followed by *Heterorhabditidoides* sp. in 23.53% and *Heterorhabditis bacteriophora* in 17.65% of the soil samples. The results concluded that *H. indica* was dominant EPN species occurring in Sugarcane ecosystem. The new EPN species *Heterorhabditidoides* spp. was reported for the first time in India.

### Introduction

Entomopathogenic nematodes are soft bodied, non-segmented roundworms that are obligate or sometimes facultative parasites of insects. Entomopathogenic nematodes are widely distributed throughout the world and have wide range of insect hosts (Hominick, 2002). Introduction of EPNs as biological control agents in a particular site requires prior knowledge on their occurrence and proper identification of native species. Introduction of exotic EPNs may induce exclusion of the local populations and/or species, thus eroding

natural diversity and be inefficient towards local insect pests as they may not be adapted to local environmental conditions (Miller and Barbercheck, 2001). Therefore, Isolation and identification of native entomopathogenic nematodes existing in the field is necessary before embarking upon their exploitation as biological control agents. Until now, more than 84 species in the Steinernematidae and about 20 species in the Heterorhabditidae (Lacey, 2012) family have been described throughout the world. But, keeping in view of the diverse agro-climatic conditions in the country, isolation and molecular identification

of native entomopathogenic nematodes in the field level are necessary for successful control of endemic pests in a particular location without causing any imbalance in biodiversity of the locality. With this basic knowledge, a soil survey was conducted during 2016-2017 in four predominantly Sugarcane growing districts of Telangana state, viz., Sangareddy, Kamareddy, Nizamabad and Khammam, for the presence of entomopathogenic nematodes (EPNs) from Sugarcane ecosystem. Once the EPNs are isolated from soil, accurate identification is fundamental to understand the geographical distribution and habitat specificity of entomopathogenic nematodes. The distinctions based on molecular characterization will elucidate species and groupings, which can then be studied for morphological characters that distinguish them from each other.

The Internal Transcribed Spacer region (ITS), located between the repeating array of nuclear 18S and 28S ribosomal DNA genes, is a versatile genetic marker, among eukaryotes, including organisms as diverse as protozoa, plants, vertebrates, nematodes and fungi. ITS data have been used in constructing phylogenetic trees, estimating genetic population structures, evaluating population level evolutionary processes, and determining taxonomic identity. The application of the ITS region for identification of nematodes has received the most attention by nematologists (Campbell *et al.*, 1995; Cherry *et al.*, 1997; Joyce *et al.*, 1994; Vrain and McNamara, 1994). The majority of these studies have focussed upon agriculturally important plant-parasitic species, animal parasites, or beneficial insect parasites. Yet, there is not a single nematode species that has failed to provide an amplification product of the ITS region when amplified with "Universal" PCR primer sets. Universal amplification coupled with the ability to amplify ITS from individual nematodes suggests that any species,

population, or ecological community of nematodes can be analyzed using a molecular approach based on the rDNA ITS region (Vrain and McNamara, 1994).

## Materials and Methods

For identification of isolated EPNs, molecular characterization studies were carried out. DNA was extracted using the DNA extraction method developed by Holterman *et al.*, (2006) and DNA was purified using a protocol of Purelink® genomic DNA mini Kit (Fig. 1).

Genomic DNA from nematodes was extracted and PCR amplification of nematode ITS rDNA region using universal primers was carried out. Universal primers used for amplification of ITS rDNA region was SC356 (primer code) designed by Vrain *et al.*, (1992).

Forward primer: TTGATTACGTCCCTGCC  
CTTT

Reverse primer: TTTCACCTCGCCGTTAC  
TAAGG

Product size: 1000-1100 bp

Once the ITS rDNA region was amplified by PCR, the PCR (polymerase chain reactions), products were sent for sequencing to AgriGenome laboratories private limited, Kochi, Kerala. Nucleotide BLAST (Basic Local Alignment Search Tool) of the sequenced samples was performed using NCBI database programme BLASTN 2.6.1+ (Fig. 2, 3 and 4).

## Results and Discussion

In the soil survey, out of 90 soil samples collected from four districts of Telangana state for studying the prevalence of EPNs using bating technique, 17 isolates tested positive for EPNs, which constituted 18.89% recovery. These isolates were subjected to molecular characterization studies to identify the

different species of EPNs and the results obtained from the studies are presented in the table 1 and figure 4.

The 17 isolates which showed positive reaction for EPNs were subjected to molecular characterization and identification of the EPNs was done by amplifying the ITS region found between the 18S and 28S rDNA using universal primer SC356 (primer code) designed by Vrain *et al.*, (1992). Based on universal primer, the amplification of 18S and 28S rDNA genomic region confirmed that, out of 17 isolates, 14 isolates were EPNs belonging to order Rhabditida and the genomic DNA of three isolates did not amplify, indicating them as nematodes belonging to the orders other than Rhabditida. These results were recorded based on the presence of PCR amplified ITS region of DNA found between the 18S and 28S rDNA by visualizing the DNA bands obtained on the Agarose gel cube under UV light with an Alpha Imager EP Gel documentation system and banding pattern (Plate 1).

Based on molecular characterization and nucleotide BLAST results, three species of EPNs were identified in the soil samples. They were *Heterorhabditis indica*, *Heterorhabditis bacteriophora* and *Heterorhabditoides* sp. Among the three species, *H. indica* was the dominant species comprising 41.18% of the total population, followed by *Heterorhabditoides* sp. (23.53%) and *H. bacteriophora* comprising 17.65%. Among the three species, the occurrence of *Heterorhabditoides* sp. was reported for the first time in India.

The occurrence of different species of EPNs from four districts of Telangana state indicated that, in Sangareddy, out of the seven samples tested positive for EPNs, five samples had *H. indica* and two samples recorded the presence of *H. bacteriophora*. In Kamareddy out of

four samples tested positive for EPNs, the molecular analysis results indicated the existence of *H. indica* in two samples, and *Heterorhabditoides* sp. in one sample. While in one sample the nematode identification was not confirmed. In Nizamabad district one sample which tested positive for EPNs was found to be *Heterorhabditoides* sp. In Khammam among the five samples which gave the positive results for EPNs, two samples belong to *Heterorhabditoides* sp., one sample was *H. bacteriophora*, while two samples did not reveal the presence of EPN species.

Thus the results obtained from the molecular analysis studies clearly revealed the presence of two genera of EPNs, *viz.*, *Heterorhabditis* and *Heterorhabditoides*. Among the *Heterorhabditis* genera, two species were recorded, *viz.*, *H. indica* and *H. bacteriophora*. In the present study new EPN belonging to the new genus *Heterorhabditoides* was identified for the first time in India. This nematode has provided evidence for its being entomopathogenic, as it killed *G. mellonella* quickly and efficiently. Existence of this nematode genus *Heterorhabditoides* was reported from China by Zhang *et al.*, (2008 and 2012) based on morphological characters combined with molecular data. In the present study the occurrence of *H. indica* in Sugarcane field supports the findings made by Poinar (1983) who documented the prevalence of *H. indica* from sugarcane fields at Coimbatore.

The *H. indica* species has been commonly isolated from sub-tropical and tropical regions of the world such as Srilanka (Amarasinghe *et al.*, 1994), Cuba (Joyce *et al.*, 1994) and Egypt (Shamseldean and Abd-Elgaw, 1994). In India the species has been reported to occur in Coimbatore and Kanyakumari districts (Ambika, 1995), Kerala (Gulsar *et al.*, 2004), Bangalore (Hussaini *et al.*, 2000) and

Telangana state (Sunanda *et al.*, 2016). The prevalence of *H. bacteriophora* was recorded from Czech republic (Mracek *et al.*, 2008). Singh *et al.*, (2015) reported the occurrence of *H. bacteriophora* and *H. indica* from cultivated fields, forests and garden soils of Gorakhpur divisions of India.

In the present study, the documentation of the prevalence of *H. indica* in greater proportions and identification of a novel EPN species

*Heterorhabditoides* sp., in Sugarcane fields of Telangana state has paved the path for further exploitation of these species in biological control of Sugarcane root grubs. The future studies on identification of symbiotic bacteria associated with *Heterorhabditoides* sp. can also provide valuable information regarding the use of this novel genus for the management of Sugarcane root grubs (Fig. 5).

**Table.1** Occurrence of different species of entomopathogenic nematodes from Sugarcane growing districts of Telangana state

Sl. no.	District surveyed	Samples tested positive for EPNs	Major EPN species identified	Samples tested positive for different EPN species	Samples tested positive for different species (%)
1.	Sangareddy	7	<i>H. indica</i>	5	71.43
			<i>H. bacteriophora</i>	2	28.57
2.	Kamareddy	4	<i>H. indica</i>	2	50.00
			<b><i>Heterorhabditoides</i> sp.</b>	1	25.00
			Not confirmed	1	25.00
3.	Nizamabad	1	<b><i>Heterorhabditoides</i> sp.</b>	1	100.00
4.	Khammam	5	<i>H. bacteriophora</i>	1	20.00
			<b><i>Heterorhabditoides</i> sp.</b>	2	40.00
			Not confirmed	2	40.00
Total samples		17	<i>H. indica</i>	7	41.17
			<i>H. bacteriophora</i>	3	17.64
			<b><i>Heterorhabditoides</i> sp.</b>	4	23.52
			Not confirmed	3	17.64

Fig.1 Procedure for extraction of genomic DNA from EPNs

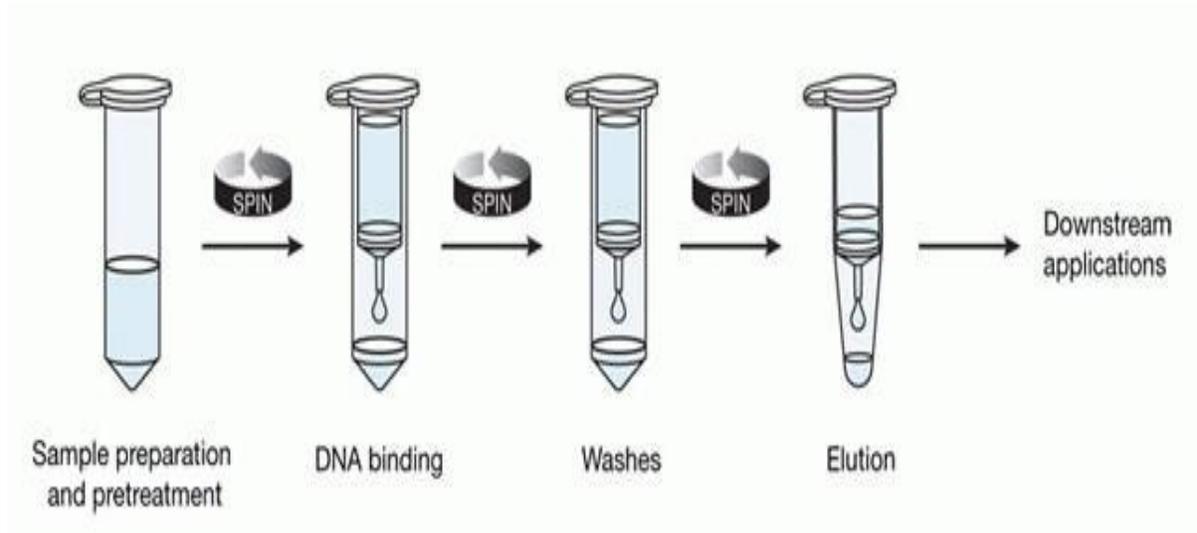


Fig.2 Trimmed sequence (879 bp) of *Heterorhabditoides* sp.

TGCTTAAGTTCGGCGGGTAATCACGACTGAGCTCAGGTTGCTAAGACAAGGCGC  
AAGCTCAGAACGGCATCATAAGCGACCGAAGGCTACTCAACCAGAAGCAGCGGT  
AGAGAGCCGACGGCAATGCACGTTTCGACGAGCACACCTACAGCGAGACTAGAA  
GTCTCATGTTGCTTTCATTCTTTACTAGAACGAGTACGCTTGCGCATAGTCGCTCG  
ACAAGCCAACGCCGAAGCGATGGCAGTTAAGAAAACAACCCTGAACCAGACGT  
GCCGACGGGAAAACCCGACGGCGCTGTGCGTTCCAAATTTCACTCAAAAGC  
GTCTGCAATTCGTGGTAAATAACGGAGCTAGCTCCGTTGTTTCATCGATATGCGAA  
TCGACCGATCCATCCCAATGGGTAGTAAGAGTATGTTCTTTCGAACAAGCCAAAA  
CATAAGTTAAGTAAAAGGAAAGGGGGTTTGCCGCCACGTTCTCTTCCACGAAA  
GAAGAGCAACGCAAGTCATTAAGGCTTCCACCGCCAATGGATGGTAATATAGCC  
AGCAAGGGAAACATTGCGAGGCGGTAGCCATAGCTGCTACACTTTACGTTCCGCC  
GAACCACTGCACACTGAAGCGCGCTGTGGAATCAGACTGTTGCGCCCGAGTCCG  
AAAACCCAGATGCTGCGGTCGCATAAAGCCGCCAGACTGATGAACGAACACCTC  
ACGTTATGCTGCACGTGGCAAGTATCGGAAGGAGACAGACCAAAGCCCGTTCC  
CATTTGCGACCGACACTCAACCGTATCAAAGCCAGAGTTCCGTTAGGAATCAGC  
GATGATCCACCTGCAGGTTACCTACAGGTACCTTGTTACGACTTTTGCCCGGTT  
CAAGCCATTGCG

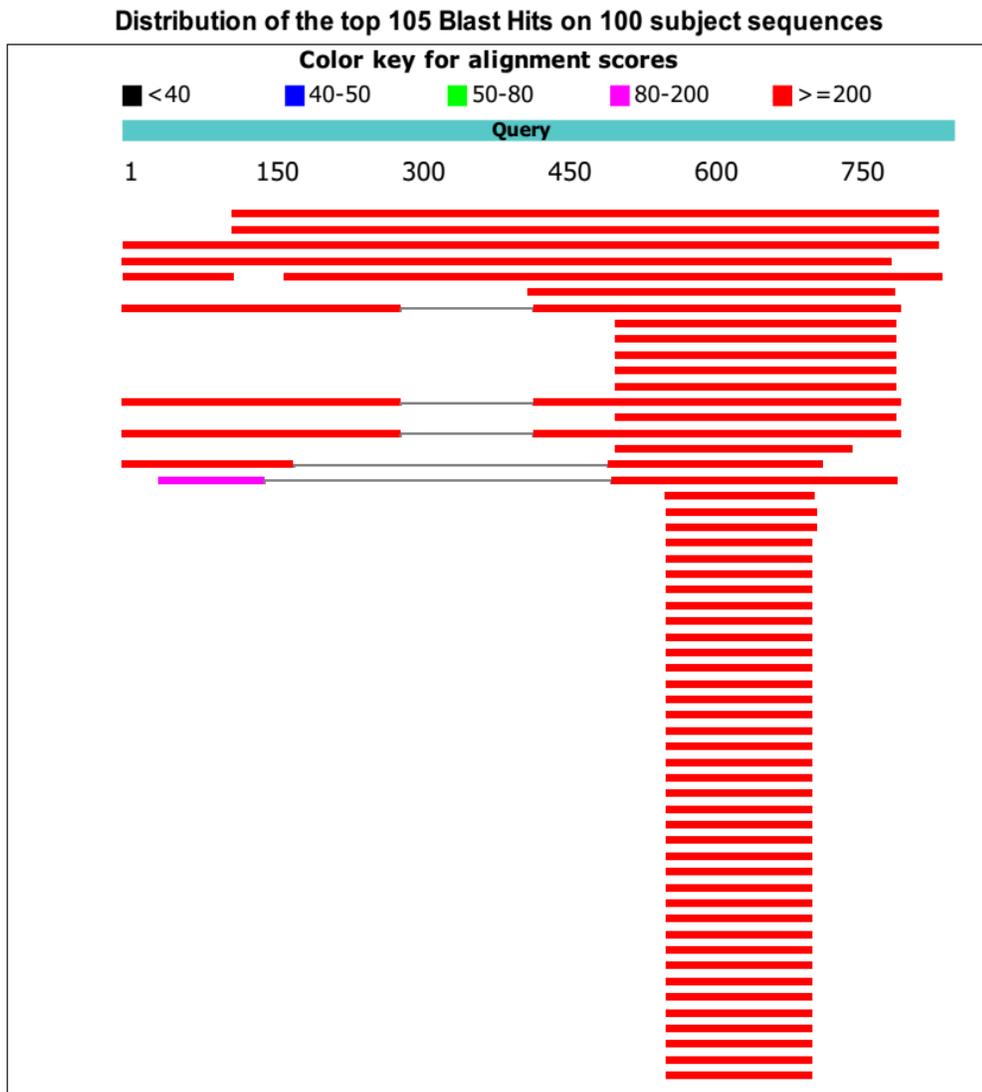
**Fig.3** Nucleotide BLAST (Basic Local Alignment Search Tool) of the sequenced samples

**BLAST Results**

Job title: AH11\_VS3.F\_1668-21\_P0199,Trimmed Sequence(832...

<b>RID</b>	<a href="#">HRP94ZMC013</a> (Expires on 05-18 13:01 pm)	<b>Database Name</b>	nr
<b>Query ID</b>	Id Query_216187	<b>Description</b>	Nucleotide collection (nt)
<b>Description</b>	AH11_VS3.F_1668-21_P0199,Trimmed Sequence(832 bp)	<b>Program</b>	BLASTN 2.6.1+
<b>Molecule type</b>	nucleic acid		
<b>Query Length</b>	832		

**Graphic Summary**



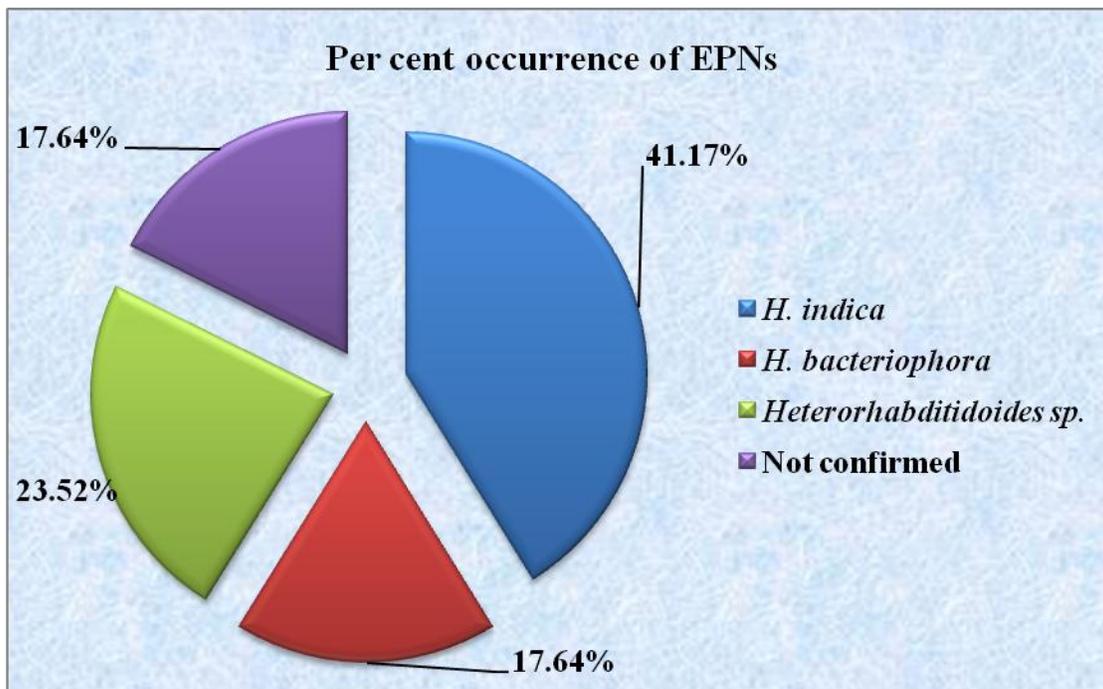
**Fig.4** The new sequences showing 99% resemblance to *Heterorhabditoides* sp.

**Descriptions**

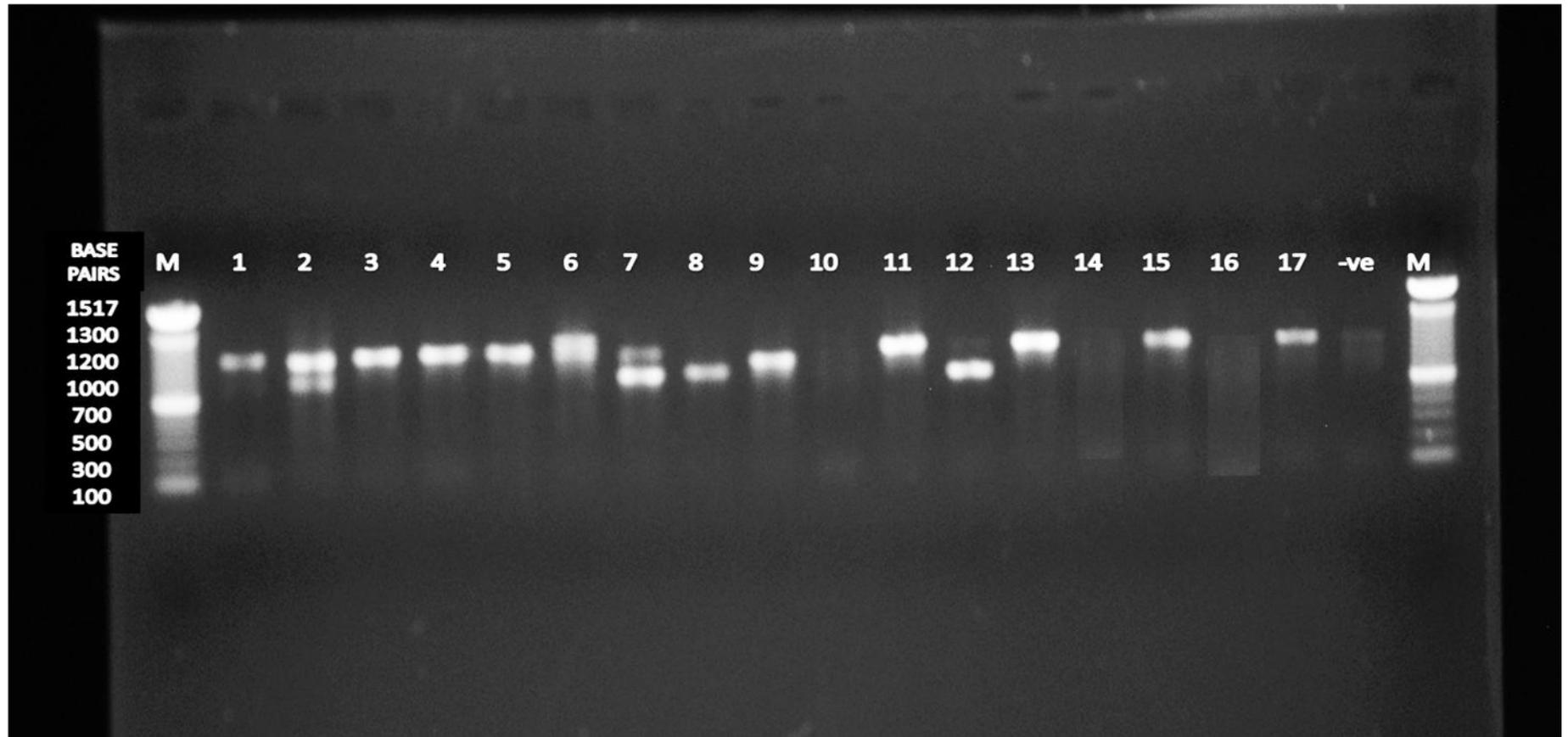
Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Heterorhabditoides sp. BRA6 genomic DNA sequence contains 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene, strain BRA6, clone BRA6_11	1293	1293	86%	0.0	99%	<a href="#">LN611142.1</a>
Oscheius sp. MCB 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1277	1277	86%	0.0	99%	<a href="#">KF684370.1</a>

**Fig.5** Occurrence of different species of entomopathogenic nematodes in the soil surveyed from Sugarcane growing districts of Telangana state



**Plate.1** Bands of PCR amplified ITS region of DNA obtained on the agarose gel after electrophoresis



Note: Isolates 10, 14 and 16 did not form DNA bands when amplified using universal primer indicating that these isolates do not belong to order Rhabditida

In conclusion, the present study constitutes the first report of *Heterorhabditoides* sp. as one of the promising bioagents against Sugarcane root grubs in the Telangana state of India. The research findings made from the present study may open up the prospects of using *Heterorhabditis* and *Heterorhabditoides* species in the biological control programme against Sugarcane root grubs. Future studies need to be carried out on the efficacy of the different species of entomopathogenic nematodes isolated from Telangana region for developing effective management strategies by choosing the right isolate suitable for their use in biological pest management of root grubs.

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