

ISOLATION, IDENTIFICATION AND PATHOGENICITY OF NATIVE ENTOMOPATHOGENIC NEMATODE STRAINS COLLECTED FROM HYDERABAD, TELANGANA STATE

ABSTRACT

Entomopathogenic nematodes (EPNs) are a promising alternative to integrated control in many agricultural crop pests. Isolation and identification of indigenous species of Entomopathogenic nematodes are necessary for successful control of various agriculturally important crop pests. The study was aimed to isolate native entomopathogenic nematodes (EPNs) strain under genus mostly known as EPNs: *Steinernema* and *Heterorhabditis* in Hyderabad region to determine their potential for control of sugarcane root grub (*Holotrichia serrata*), cotton and vegetable crops pod borer (*Helicoverpa armigera*), leaf eating caterpillar (*Spodoptera litura*), potato cut worm (*Agrotis segitum*) etc. Hundreden sixty soil samples were collected in 11 communities during the period Sep., 2016 to March, 2017, in that 33 samples were found to be positive for EPNs. All the isolates were selected for their apparent of pathogenicity against the greater wax moth, *Galleria mellonella*. Seventeen samples of the genus *Steinernema* and sixteen samples of *Heterorhabditis* were isolated in the same geographical region of various crop ecosystems. The native isolates of KN EPNH1 (*Heterorhabditis indica*) and KN EPNS17 (*Steinernema carpocapsae*) was found to be highly virulent and more pathogenic effect to the major borer pests of sugarcane, cotton, vegetable, potato and groundnut resulting lowest lethal dosages and lethal times. Using polymerase chain reaction (PCR) procedures, the species identification was done for *Heterorhabditis indica* and *Steinernema carpocapsae*. These entomopathogenic nematodes carrying symbiotic bacteria represent one of the best non-chemical strategies for insect control. Infective juveniles of Heterorhabditidae and Steinernematidae nematodes actively seek the host in the soil which is employed as biocontrol agents. The study of population dynamics of entomopathogenic nematodes is fundamental to understanding their persistence, distribution and effect on insect populations and for the development of predictive models for the control programmes.

Key words: entomopathogenic nematode, *Steinernema*, *Heterorhabditis*, pathogenicity, bioassay,

1. INTRODUCTION

Entomopathogenic nematodes are parasites and ubiquitous in distribution throughout the world. It searches the suitable insect host with the help of damaged stem and root portions, carbon dioxide and host insect pheromones *etc.* The non-feeding infective juvenile (IJs) 3rd stage of entomopathogenic nematodes act as a biological control agents by killing the insects at different stages *viz.*, egg, larva, pupa, and adult depend upon the species of nematodes and the host insects. The nematode penetrates into the insect spiracle, cuticle, anus and mouth parts of body within 24 -48 h. The symbiotic associated bacteria in the genera *Xenorhabdus* Poinar and *Photorhabdus* vomit inside the host and multiply, release a number of virulence factors, including complexes of toxins, hydrolytic enzymes, hemolysins, and antimicrobial compounds [3]. Thus providing nutrients for the nematodes development and then reproduction occurs within the host insect cadaver. In the last two decades, survey has been carried out and several species of nematode species isolated [10]. Environmental factors influence the nematode occurrences and distribution and their survival. Biotic and abiotic factors cause the distribution of EPN to differ across different regions. Major factors namely temperature and host availability are thought to be important in determining their distribution. Therefore, EPNs are considered as one of the most relevant non-chemical alternatives to insect pest control due to their high reproductive potential, ease of mass production and their harmlessness to microbes, animals, humans and plants. In this study, a survey was conducted to isolate and identify EPNs from disturbed habitats in the Hyderabad region of Telangana State, Morphology, morphometrics and ITS region of rRNA of nematodes was studied.

2. MATERIALS AND METHOD

2.1 Surveys for detection of entomopathogenic nematodes

The basic principle in the isolation of a potential strain of entomopathogenic nematodes (EPNs) for agriculturally important crop pests is an extensive collection of soil samples drawn

from various geographical locations. Indigenous entomopathogenic nematodes are highly specific against pests, more virulent and highly adaptable to particular region or locality. Soil samples were collected from different habitats, altitudes and rainfall by random sampling technique. The occurrence and distribution of EPNs was surveyed in crop fields of Hyderabad district (Telangana State). Study area was segregated into four geographical locations and the soil samples were collected at different crop ecosystems. EPNs distribution in relation to the environmental changeable, soil physical and chemical characteristic of soil samples were analyzed which were collected from different crops.

2.2 Isolation and Propagation of nematodes

Collected soil samples were processed EPN strain identification with the laboratory host, *Galleria mellonella* (Greater wax moth) followed by [1]. Five hundred gram of each soil sample placed in a plastic box and released ten final instar larvae of *G. mellonella*. The boxes were stored at $25^{\circ}\text{C} \pm 2$ temperature. The dead cadaver (if any) was collected, transferred gently into a white trap [17] to observe the presence of EPN. All the soil samples were baited three times with larvae to get the maximum number of positive nematodes available in the sample.

2.3 Host insect culture maintenance

Culture of the laboratory host insect, Greater wax moth, *G. mellonella*, collected from the abandoned beehives under a banyan tree in Professor Jayashankar Telangana State Agricultural University, Hyderabad. The larvae were cultured using artificial diet for further multiplication. The adults were kept in the oviposition cage for egg laying. The eggs were transferred to the artificial diet and mass reared [12].

2.4 Nematode Identification and Morphological studies

Morphological characterization of the isolated, nematode strain was individually examined by 'heat kill' method in 60°C by using Ringer's solution. The heat-killed nematodes place in triethanolamine formalin (TAF) fixative and processed to anhydrous glycerine for

mounting. The morphological features of males and IJs and hermaphroditic female randomly selected from different *G. mellonella* under light microscopy [14].

The identity was verified by comparing its morphometric with the data original descriptions in our KN Bioscience laboratory at Hyderabad. Manual observation of the species in two families, *Heterorhabditidae* and *Steinernematidae* was found most of the soils. The life cycle of nematodes includes an egg, four juvenile, adult for *Steinernematidae* and egg, four juvenile, adult and entotokiametricida were observed for *Heterorhabditidae* which were compared both the genera. The 3rd juvenile stage of entomopathogenic nematodes is referred to as the infective juvenile or dauer stage and is the only free-living stage used as biocontrol agent due to its capability of survives in the soil, which locates the host insect and attack. We have also segregate the genus level of EPN strains under optimal conditions like, the developmental stage which takes 5 to 7 days for *Steinernematid* and 8 to 10 days for *Heterorhabditid* species to emergence of IJs. Isolated EPN strains were sent to National Bureau of Agricultural Insect Resources (NBAIR), Bangalore. Identified strains were maintained in incubator for further mass production.

2.5 Molecular Characterization

The isolates were molecularly characterized by analysis of the internal transcribed spacer region (ITS) ribosomal DNA sequences. DNA extraction was performed [15]. The IJs were crushed using extraction buffer (0.05M EDTA, 1%SDS, 400µl Proteinase K) and centrifuged for 10-15 minutes for 12,000 rpm. The supernatant was collected and add equal volume of phenol: chloroform: isoamylalchol at the ratio 25:24:1. Then the tubes mixed well and centrifuge 12,000 rpm and 3M ammonium precipitate was added for better DNA precipitation. The suspensions freeze for 2 hours and centrifuge for 30 minutes at 12,000 rpm. DNA was precipitated from aqueous phase and after drying re-suspended in TE buffer. The DNA sample was run by agarose gel electrophoresis using 1kb markers of lambda DNA as standard. For polymerase chain reaction (PCR) amplification and sequencing of the ITS, primers were designed [5].

2.6 Lab bioassay study

Comparative bio-efficacy study of native EPN strains (KN EPNS17 and KN EPNH1)

Experiment were carried out to verify the pathogenic effect of native virulent two stains of KN EPNS17 (*Steinernema carpocapsae*) and KN EPNH1 (*Heterorhabditis indica*) with 25 larvae of tests insects such, Bhendi fruit and shoot borer (*Leucinodes orbonalis*), Dimand back moth (*Plutella xylostella*), Sugarcane root grub (*Holotrichia serrata*), American boll worm (*Helicoverpa armigera*), Tobacco leaf eating caterpillar worm (*Spodoptera litura*) and Potato cut worm (*Agrotis segitum*) which were collected from the respective crop plots. After 48 hrs of exposure the percentage of larval mortality was determined and each larva was placed in a White trap to verify their ability to produce IJs in adequate numbers. This experiment was laid out in a completely randomized design (RBD) and replicated five times to confirm the bioassay result.

3. RESULTS

3.1 Surveys for detection of nematodes

Total 160 soil samples were collected in the polythene bag of 1.0 kg size of 500 g soils from the depth of 10 to 15 cm from the various crop ecosystem such as cotton, chilies, sugarcane, tomato, potato, brinjal, maize, ridge guard, zinger, bhendi and ground nut, sampling interval was about 15 meters per sample with 4 replications of each plot of in and around Hyderabad region (Table-1). The area of collection had different altitude and longitude and various soil types and the soil type varied from gravelly back, red, sand to clay. The crop wise distribution of soil was presented (Table-2).

In the study, the results of the soil samples conducted 160 samples from 7 locations, 33 samples were showed positive for nematode infection to *Galleria mellonella* larvae in soil bait method which is around 20% of the total sample. According to the color of the 'dead cadaver' we recognized 17 samples were positive for the genus *Steinernema* and 16 samples showed as the genera of *Heterorhabditis*. The host insect *G. mellonella* larvae infected with EPN of the

genus *Heterorhabditidae* have showed orange color in the beginning first 2 days then turn reddish and brick red after 4th day and those infected with *Steinernema* sp., show gray-brown coloring [6-8].

3.2 Isolation and Propagation of nematodes

Isolated nematodes strains from the 33 positive samples were propagated with the laboratory host insect, *G. mellonella* larvae in 4.5 cm dia Petri dish lined with Whatman No.5. The Petri dishes were incubated at room temperature $25^{\circ}\text{C} \pm 2$ temperature for 8 to 10 days until the IJs emerged from the cadavers. Collected nematodes were stored at 20 to 25 ⁰C at the rate 10000 IJs/ ml in distilled water with 0.1% formalin in tissue culture flask (250 ml). Nematodes were routinely cultured in *G. mellonella* larvae [18]. The collected IJs were checked regularly for their pathogenicity with the *G. mellonella* larvae once in ten days (Table-3).

3.3 Nematode Identification and Morphological studies

Diagnosis characters of *Steinernema* sp: i) *Infective juvenile*: Cephalic region truncate, body slender, straight/lightly curved in heated specimen with or without a sheath. Oesophagus and intestine appear reduced. Excretory pore anterior to nerve ring, rectum and anus distinct, Tail filiform. ii) *Female*: Larger than male, cuticle may be smooth, excretory pore anterior to nerve ring, head rounded. Six lips present and reproductive system didelphic, amphidelphic, reflexed. Vulva at mid- body sometimes on a protuberance, with or without epiptygma. iii) *Male*: Body always curved posterior J/C shaped, smaller than female, tail conoid with tail tip rounded, digitate or mucronate, number of genital papillae is diagnostic, one single and 10-14 pairs of genital papillae present with 7-10 pairs precloacal. Morphometric analysis and other characteristic features of infective juveniles (1st generation) extracted from positive samples (Table-4). Slides of fresh nematodes were mounted in Ringer's solution and sealed with paraffin. At least 15 males, 5 females, 5 hermaphrodites and 30 IJs of each isolate were observed, drawn and measured. Measurements were made using a drawing camera (*camera lucida*) (Axioplan Axiophoto) attached to a Zeiss (MC100 SPOT) light microscope [16-4].

3.4 Lab bioassay study

Bioefficacy of native EPN strains (KN EPNS17 and KN EPNH1): The Bioefficacy of selective two stains of KN EPNS17 (*Steinernema carpocapsae*) and KN EPNH1 (*Heterorhabditis indica*) KN were found to be promising result against bhendi fruit and shoot borer (*Leucinodes orbonalis*) and the diamondback moth (*Plutella xylostella*) and sugarcane root grub (*Holotrichia serrata*) with 100% mortality with in 48 hrs of post exposure. The other test insects like American boll worm (*Helicoverpa armigera*), Tobacco cut worm (*Spodoptera litura*) and Potato cut worm (*Agrotis segitum*) showed 85 to 100% mortality at 48 hrs of post exposure however, all these insects were showed 100% mortality after 72 hrs of exposure (Table-5). Native species of entomopathogenic nematodes that are adapted to local environmental and climatic conditions are especially good candidates for use as biological control agents. Our report of the study revealed that the ventricular portion of the intestine of the Steinernematidae infective juvenile is specifically modified for storage of symbiotic bacteria and is called an intestinal vesicle. In the infective stage of heterorhabditid nematodes, symbiotic bacteria are located in the esophagus and in the ventricular portion of the intestine [9].

4. DISCUSSION

Entomopathogenic nematodes carrying symbiotic bacteria represent one of the best non-chemical strategies supporting insect control [19]. In the soil, the infective juveniles of these nematodes (*Heterorhabditis*/*Steinernema* genera) actively seek the host in the soil, penetrate through insect's natural openings, travel to the hemocoel and release symbiotic bacterial cells (mostly species of *Photorhabdus*/*Xenorhabdus* respectively). The bacteria multiply and release a number of virulence factors, including complexes of toxins, hydrolytic enzymes, hemolysins, and antimicrobial compounds that kill the insect host usually within 48 h [3].

Entomopathogenic nematodes have been detected with various frequencies in most terrestrial habitats. In this study, native isolates of 33 EPN virulent strains of *S. carpocapsae* and *H. indica* in Hyderabad are potentially effective in controlling various crop pests plantation. The percentage of positive samples for nematodes obtained in this study was 20.0%, which might

seem high when compared with other studies such as [16] who conducted a sampling of different habitats in the state of California and found 26.3% of positive samples. In Oaxaca [11] found a maximum of 8.9% of positive samples in the region. The difference with the last study mentioned above may be because the sampling was random, including natural vegetation areas, while in the current study sampling was directed to agricultural fields where pest's infestation had occurred and thus the presence of EPN was more likely. This idea is supported by the study [11] who found that the highest percentages of nematode soil samples were found. In terms of percentage of positive samples by genera, this study found 51.52% of EPN in the genus *Steinernema* and 48.48 % in the *Heterorhabditis*, which concurs with [16] who reported 80% in *Steinernema* and 20% in *Heterorhabditis* on the other hand, [11] found 67% in *Steinernema* and 33% in *Heterorhabditis*. The isolated nematodes were subject to an evaluation of relative pathogenicity to various tests insects. Those who were attacked by fungus or produced IJ's in small numbers were considered not entomopathogenic. It is known that when an insect is infected with the nematode's symbiotic bacteria, it is protected against the attack of other organisms. Revealed the study of our report of [10] consider that when larvae are infected by EPN, these will not rot, nor have foul odors. Also, each host will produce IJ's in large numbers. However, temperature and inoculums level have been found to affect the amount of IJ's produced.

The host insect, *Galleria mellonella* recovered *H. indica* and *S. carpocapsae* from 33 positive soil samples, two strains were showed highly virulent and pathogenic effect with *G. mellonella* as bait. These results again justify the popularity of *G. mellonella* as the bait insect of choice for recovering EPNs from soil. For morphological studies, third-stage IJs were obtained within 7th day of emergence of IJs of *H. indica* and 5th day of *S. carpocapsae* from insect cadavers [7]. Other nematode stages were obtained by infecting ten *G. mellonella* larvae with 100 IJs/ml in Petri dishes. First generation hermaphrodites and second generation females and males were dissected from the cadavers 6-7 and 8-9 days after infection, respectively. All observations and measurements were performed within one week of harvest similar [2].

5. CONCLUSION

The use of insect parasitic nematodes and other biological control agents to manage insect pests has grown in popularity. This is primarily due to the changing problems associated with pest control. Many pests have developed resistance to certain pesticides, new pests have arisen to replace those successfully controlled, the effectiveness of natural control agents (predators, parasites and pathogens) has been reduced by pesticide use, pesticides are no longer inexpensive to use, and there is increased concern about pesticide safety and environmental quality. These beneficial organisms can be an important component of an integrated pest management (IPM) program for agriculturally important crop pests. We conclude that establishing entomopathogenic nematodes in Hyderabad and other states in India would bring several benefits to the region. Firmly establishing insect nematodes will promote the sustained use of agriculture and develop a better understanding of biodiversity. In our investigations, the Heterorhabditid nematode, *H. indica* (KN EPN1) and Steinernematid, *S. carpocapsae* (KN EPN17) are so far the most promising biocontrol agent to establish our studies on mass production and formulation.

REFERENCES

1. Bedding RA, Akhurst RJ. A simple technique for the detection of insect parasitic nematodes in soil. *Nematologica*. 1975; 21: 109-110.
2. Claudia D, Fernando LK, Inês RM, Carlos EW. Molecular and morphological characterization of Heterorhabditid entomopathogenic nematodes from the tropical rainforest in Brazil. *Mem Inst Oswaldo Cruz, Rio de Janeiro*. 2008 ; 103(2): 150-159.
3. Eleftherianos I, Joyce S, Ffrench-Constant RH, Clarke DJ, Reynolds SE. Probing the tritrophic interaction between insects, nematodes and *Photorhabdus*. *Parasitol*. 2010; 137: 1695–1706.
4. Hominick WM, Briscoe BR, Garcia Del-Pino F, Heng J, Hunt DJ, Kozodoy E, Mracek Z, Nguyen KB, Reid AP, Spiridonon S, Stock P, Sturhan D, Waturu C, Yoshida M. Biosystematics of entomopathogenic nematodes: current status, protocols and definitions. *J Helminthol*. 1997; 71: 271-298.
5. Joyce S.A, Reid A, Driver F, Curran J. Application of polymerase chain reaction (PCR) methods to the identification of entomopathogenic nematodes. In: Burnell, A.M., Ehlers,

- R.-U. & Masson, J.-P. (Eds). *COST 812 Biotechnology: Genetics of entomopathogenic nematodesbacterium complexes*. Proceedings of symposium and workshop, St Patrick's College, Maynooth, County Kildare, Ireland. Luxembourg, European Commission, DGXII, 1994; 178- 187.
6. Kaya HK, Stock SP. Techniques in insect nematology. *In* L. A. Lacey (ed.) Manual of techniques in insect pathology. *Academic Press*, New York, 1997; 281-324.
7. Nguyen KB, Smart Jr GC. Morphometrics of infective juveniles of *Steinernema* spp. and *Heterorhabditis bacteriophora* (Nemata: Rhabditida. *J Nematol* 1995; 27: 206-212.
8. Nyamboli MA. Mass production of entomopathogenic nematodes for plant protection. 2008. Master of Science dissertation, University of the Witwatersrand, Johannesburg, 2008; 193.
9. Poinar Jr, GO, GR Thomas, Hess R. Characteristics of the specific bacterium associated with *Heterorhabditis bacteriophora*. *Nematologica*. 1977; 23: 97-102.
10. Rosa J, Bonfassi E, Amaral J, Lacey LA, Simoes N, Laumond C. Natural occurrence of entomopathogenic nematodes (Rhabditida: *Steinernema*, *Heterorhabditis*) in the Azores. *J. Nematol*. 2000; 32: 215-222.
11. Ruiz-Vega J, Aquino-Bolaños T, Kaya HK, Stock P. Colecta y evaluación de nematodos entomopatógenos para el control de gallinas ciegas *Phyllophaga vetula* (Horn) en Oaxaca, México. *Folia Entomol Mex*. 2003; 42 (2): 169-175.
12. Sankar M. Investigation of an indigenous entomopathogenic nematode, *Heterorhabditis indica* Poinar, Karunakar and David (1992) as a potential biocontrol agent of insect pest in rice. *PhD thesis, Osmania Univ., Hyderabad*. 2009; 168-79.
13. SAS. SAS Institute Inc., SAS/STAT. User's Guide, Version 9.0, Cary NC. Statistical Analysis System Institute Inc., North Caroline 2002.
14. Seinhorstj W. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, 1959; 4: 67-69.
15. Stock SP, Kaya HK. A multivariate analysis of morphometric characters of *Heterorhabditis* species (Nemata: Heterorhabditidae) and the role of morphometrics in the taxonomy of species of the genus. *J Parasitol*. 1996; 82: 806-813.
16. Stock SP, Pryor BM, Kaya HK. Distribution of entomopathogenic nematodes (*Steinernematidae* and *Heterorhabditidae*) in natural habitats in California, USA. *Biodivers. Conserv*. 1999; 8: 535-549.

17. White GF. A method for obtaining infective nematode larvae from cultures. Science. 1927; 66: 302-303.
18. Woodring JL, Kaya HK.. *Steinernematid* and *Heterorhabditid* nematodes: hand book of biology and techniques. South cooperative Ser. Bulletin Arkans Agricultural Exp Stn Fayetteville. 1998; 331: 1-30.
19. Zhu H, Grewal PS, Reding ME. Development of a dessicated cadaver delivery system to apply entomopathogenic nematodes for control of soil pests. Appl Eng Agric. 2011; 27: 317–324.

Table 1. Isolation of entomopathogenic nematodes stains from the soil samples collected in and around Hyderabad agricultural cropping system from Sep., 2016 to March, 2017.

S.No	Location	Number of samples collected (nos.)	No. of samples positive to EPN (nos.)	Percent of samples showed EPN positive (%)
1	Jeerlapally,	10	2	20.0
2	Sadasivpet	18	5	27.0
3	Kamareddy	15	3	33.3
4	Medchal	20	2	10.0
5	Koyyur	16	4	25.0
6	Zaheerabad	20	2	10.0
7	Tupran	06	1	16.6
8	Kotpally	15	6	40.0
9	Kalakkal	16	4	25.0
10	Vikarabad	20	2	10.0
11	Jharasangam	10	2	20.0
Total=		160	33	20.0

Table 2. Soil sample collected from various geographical area and types of soils.

S.No	Locality	Latitude	Longitude	Soil type
1	Jeerlapally	17°42'33.66"N/ 17°43'49.96"N	77°46'19.14"E/ 77°46'49.95"E	Black cotton soils
2	Jharasangam	17°42'33.66"N/ 17°44'14.53"N	77°45'38.38"E/ 77°46'49.95"E	Black cotton soils
3	Sadasivpet	17°37'20.57"N/ 17°37'24.96"N	77°56'52.50"E/ 77°56'51.50"E	Red sandy soils
4	Kamareddy	18°18'52.00"N/ 18°18'54.79"N	78°20'02.78"E/ 78°20'02.95"E	Black cotton soils
5	Medchal	17°37'44.06"N/ 17°37'41.40"N	78°29'30.28"E/ 78°29'23.35"E	Deep red loamy soils
6	Koyyur	12°56'31.66"N/ 12°56'59.97"N	75°17'56.75"E/ 75°18'00.50"E	Black cotton soils
7	Zaheerabad	17°40'08.77"N/ 17°40'21.57"N	77°37'11.35"E/ 77°37'12.05"E	Clay loams
8	Tupran	17°51'00.71"N/ 17°51'12.12"N	78°28'16.44"E/ 78°28'23.72"E	Black cotton soils
9	Kotpalli	17°22'52.07"N/ 17°23'04.05"N	77°44'29.38"E/ 77°45'18.51"E	Deep red loamy soils
10	Kalakkal	17°42'35.20"N/ 17°42'48.05"N	78°29'04.84"E/ 78°29'08.26"E	Black cotton soils
11	Vikarabad	17°20'29.93"N/ 17°19'47.64"N	77°54'41.78"E/ 77°54'50.29"E	Red sandy soils

Table 3. Native EPN strain isolates from soil samples collected from in and around Hyderabad agricultural regions showed the insect mortality with *Galleria mellonella* larvae.

S.No	Locality	Crop	EPN strain isolated	% mortality	Code
1	Jeerlapally	Sugarcane	<i>Heterorhabditis sp</i>	100	KN EPNH1
2	Jeerlapally	Chillies	<i>Steinernema sp</i>	95	KN EPNS1
3	Jeerlapally	Tomato	<i>Steinernema sp</i>	100	KN EPNS2
4	Jharasangam	Cotton	<i>Steinernema sp</i>	80	KN EPNS3
5	Sadasivpet	Sugarcane	<i>Steinernema sp</i>	75	KN EPNS4
6	Sadasivpet	Potato	<i>Heterorhabditis sp</i>	100	KN EPNH2
7	Sadasivpet	Maize	<i>Heterorhabditis sp</i>	98	KN EPNH3
8	Sadasivpet	Bhendi	<i>Steinernema sp</i>	90	KN EPNS5
9	Sadasivpet	Cotton	<i>Steinernema sp</i>	96	KN EPNS6
10	Kamareddy	Sugarcane	<i>Heterorhabditis sp</i>	100	KN EPNH4
11	Kamareddy	Maize	<i>Steinernema sp</i>	78	KN EPNS7
12	Medchal	Brinjal	<i>Heterorhabditis sp</i>	80	KN EPNH5
13	Medchal	Tomato	<i>Heterorhabditis sp</i>	100	KN EPNH6
14	Medchal	Chillies	<i>Steinernema sp</i>	95	KN EPNS8
15	Koyyur	Sugarcane	<i>Heterorhabditis sp</i>	100	KN EPNH7
16	Koyyur	Zinger	<i>Steinernema sp</i>	100	KN EPNS9
17	Koyyur	Potato	<i>Steinernema sp</i>	100	KN EPNS10
18	Koyyur	Cotton	<i>Steinernema sp</i>	85	KN EPNS11

19	Koyyur	Maize	<i>Steinernema sp</i>	90	KN EPNS12
20	Zaheerabad	Sugarcane	<i>Heterorhabditis sp</i>	100	KN EPNH8
21	Zaheerabad	Potato	<i>Heterorhabditis sp</i>	100	KN EPNH9
22	Zaheerabad	Zinger	<i>Steinernema sp</i>	100	KN EPNS13
23	Toopran	Brinjal	<i>Heterorhabditis sp</i>	89	KN EPNH10
24	Toopran	Tomato	<i>Heterorhabditis sp</i>	100	KN EPNH11
25	Toopran	Chillies	<i>Heterorhabditis sp</i>	80	KN EPNH12
26	Kotpally	Sugarcane	<i>Heterorhabditis sp</i>	100	KN EPNH13
27	Kotpally	Cotton	<i>Heterorhabditis sp</i>	95	KN EPNH14
28	Kotpally	Ground nut	<i>Steinernema sp</i>	100	KN EPNS14
29	Kalakkal	Ridge guard	<i>Heterorhabditis sp</i>	98	KN EPNH15
30	Kalakkal	Bitter guard	<i>Steinernema sp</i>	80	KN EPNS15
31	Kalakkal	Sugarcane	<i>Steinernema sp</i>	100	KN EPNS16
32	Vikarabad	Cotton	<i>Steinernema sp</i>	85	KN EPNS17
33	Vikarabad	Maize	<i>Heterorhabditis sp</i>	75	KN EPNH16

Table 4. Morphometric characteristic features native virulent isolates of infective juveniles (IJs) of KN EPNS17 and KN EPNH1 isolated from the sample.

Parameter (mm)	KN EPNS17	KN EPNH1
Length	558.3	573.41
Width	29.03	21.24
Stoma length	2.30	2.03
Stoma width	2.21	1.48
EP	38.69	64.38
EPW	0.8	1.70
NR	45.62	62.02
ES	60.52	72.77
Anal body width	15.02	13.11
Tail length	62.69	75.72
Ratio A	19.23	26.99
Ratio B	9.23	7.88
Ratio C	8.91	7.57
Ratio D	0.64	0.88
Ratio E	0.62	0.85

All measurements in microns: **A:** Length/width; **B:** Length / distance from head to pharynx base; **C:** Length/tail length; **D:** Distance from head to excretory pore/Distance from head to pharynx base; **E:** Distance from head to excretory pore/tail length

Table 5. Bioefficacy of virulent EPN strains of KN EPNS17 and KN EPNH1 against various crop pests in the lab study.

S.No	Insect common name	Scientific name	Associated crop	KN EPNH1 mortality (%)	KN EPNS17 mortality (%)
1	American Boll worm	<i>Helicoverpa armigera</i>	cotton, tomato and Bhendi	85	100
2	Tobacco caterpillar	<i>Spodoptera litura</i>	cotton and tomato	90	100
3	Bhendi fruit and shoot borer	<i>Leucinodes orbonalis</i>	Bhendi	100	100
4	Dimand back moth	<i>Plutella xylostella</i>	Cauliflower	100	100
5	Potato cut worm	<i>Agrotis segitum</i>	Potato	100	85
6	Sugarcane root grub	<i>Holotricha serrata</i>	Sugarcane	100	100