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Isolation, Identification and Pathogenicity of Native Entomopathogenic Nematode Strains Collected from Hyderabad, Telangana State in India

S. Manickam[1*], S. Suneel Kumar[1], P. Neha Reddy[1] and P. Sudha Reddy[1]

[1]KN Bioscience (India) Pvt. Ltd., Bachupally, Hyderabad, Telangana State, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author SM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SSK and PNR managed the analyses of the study. Author PSR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Entomopathogenic nematodes (EPNs) are a promising alternative to integrated biocontrol control agent in many agricultural crop pests. Isolation and identification of indigenous species of entomopathogenic nematodes are necessary for successful implementation in the IPM (integrated pests management) program. The study was aimed to isolate native entomopathogenic nematode strain under genus *Steinernema* and *Heterorhabditis* in Hyderabad region to determine their potential control of sugarcane root grub (*Holotrichia serrata*), cotton and vegetable crops pod borer (*Helicoverpa armigera*), leaf-eating caterpillar (*Spodoptera litura*), potato cutworm (*Agrotis segitum*) etc. Hundred 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*) found to be highly virulent and more pathogenic effect to the major borer pests of sugarcane, cotton, vegetable, potato and

**Corresponding author: Email: shankar_ms10@rediffmail.com;*

groundnut resulting lowest lethal dosages and lethal times. These entomopathogenic nematodes carrying symbiotic bacteria represent one of the best non-chemical strategies for insect control. Infective juveniles (IJs) 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 for the development of predictive models for the control programmes.

Keywords: Entomopathogenic nematode; Steinernema; Heterorhabditis; pathogenicity; bioassay.

1. INTRODUCTION

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* 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.* Ruiz-Vega et al. [1]. 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 mouthparts of the body within 24 -48 h. The associated symbiotic 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 (Eleftherianos et al. [2]). Thus providing nutrients for the nematodes development and then reproduction occurs within the host insect cadaver. Rosa et al. [3] reported that in the last two decades, the survey has been carried out and several species of nematode species isolated. 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 in India.

Considering the major economic source of Hyderabad farmers cultivating various crops like, sugarcane, cotton, vegetable, potato and groundnut, the borer pests are become major threat causing more damage. Hence, the use of entomopathogenic nematodes cannot be generalized, but experimentation is required to determine which kind of nematode species is most suitable for controlling a certain species of larva to implement under the crop management program in that particular region. Therefore, when a new entomopathogenic species is isolated from field samples, laboratory assessments of ineffectiveness and control efficacy have to be done before recommendation in the field. It also an important to know the host range and lethal dosages of the EPN, as well as their interactions with pest populations. This knowledge is necessary to use them effectively to alternate synthetic pesticides. These nematodes possess many desirable attributes, including wide host range, safety for non-target organisms and the environment, exemption from registration in many countries, easy mass production and application, ability to find the pest, and potential to persist in the environment, among others.

Due to serious concerns associated with the use of pesticides, alternatives strategies are being emphasized throughout the world for the management of insect pests. Therefore, the use of entomopathogenic nematodes can be one of the feasible alternatives to the detrimental chemicals. In this manuscript the authors have isolated, identified and tested the pathogenicity of native entomopathogenic nematode strains collected from Hyderabad, Telangana state in India.

2. MATERIALS AND METHODS

2.1 Surveys for Detection of Entomopathogenic 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 such as, cotton, chilies, sugarcane, tomato, potato, brinjal, maize, ridge guard, zinger, bhendi

and ground nut, (11 crops) and the 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).

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, India). 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.

Table 1. Isolation of entomopathogenic nematodes stains from the soil samples collected in and around Hyderabad (India) 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

2.2 Isolation and Propagation of Nematodes

Isolated nematodes strains from the 33 positive samples were propagated with the laboratory host insect, *G. mellonella* Linnaeus larvae in 4.5 cm dia Petri dish lined with Whatman No. 5. The Petri dishes were incubated at room temperature 25ºC ± 2 temperature for 8 to 10 days until the IJs emerged from the cadavers. Collected nematodes were stored at 20 to 25℃ at the rate 10000 IJs/ ml in distilled water with 0.1% formalin in tissue culture flask (250 ml).

Collected soil samples were processed EPN strain identification with the laboratory host, the Greater wax moth, *Galleria mellonella* L (Bedding et al. [4]). 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 25ºC ± 2 temperature. The dead cadaver (if any) was collected, transferred gently into a white trap reported by White [5] 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* L, 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 [6].

2.4 Nematode Identification and Morphological Studies

Morphological characterization of the isolated, nematode strain was individually examined by *'heat kill'* method in 60℃ 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 Seinhorstj [7].

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 to7 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 Lab Bioassay Study

2.5.1 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* (Lepidoptera : Pyraustidae), Dimand back moth, *Plutella xylostella* (Lepidoptera : Plutellidae), The white grub Holotrichia serrata (Coleoptera: Scarabaeidae), American boll worm, *Helicoverpa armigera (*Lepidoptera : Noctuidae), Tobacco leaf eating caterpillar worm, *Spodoptera litura (*Lepidoptera : Noctuidae) and Potato cut worm, *Agrotis segitum (*Lepidoptera : Noctuidae) 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. Data were analysed and subjected to ANOVA and statistical significance was judged at the level ($P < 0.05$) to test for comparing mortality.

3. RESULTS

3.1 Surveys for Detection of Entomopathogenic Nematodes

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 (Kaya et al. [8]; Nyamboli [9]).

3.2 Isolation and Propagation of Nematodes

Nematodes were routinely cultured in *G. mellonella* larvae (Woodring and Kaya [10]). The collected IJs were checked regularly for their pathogenicity with the *G. mellonella* larvae once in ten days (Table 3).

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

S. no.	Locality	Crop	EPN strain isolated	% mortality	Code
1	Jeerlapally	Sugarcane	*Heterorhabditis sp*	100	KN EPNH1
2	Jeerlapally	Chillies	*Steinernema sp*	95	KN EPNS1
3	Jeerlapally	Tomato	*Steinernema sp*	100	KN EPNS2
4	Jharasangam	Cotton	*Steinernema sp*	80	KN EPNS3
5	Sadasivpet	Sugarcane	*Steinernema sp*	75	KN EPNS4
6	Sadasivpet	Potato	*Heterorhabditis sp*	100	KN EPNH2
7	Sadasivpet	Maize	*Heterorhabditis sp*	98	KN EPNH3
8	Sadasivpet	Bhendi	*Steinernema sp*	90	KN EPNS5
9	Sadasivpet	Cotton	*Steinernema sp*	96	KN EPNS6
10	Kamareddy	Sugarcane	*Heterorhabditis sp*	100	KN EPNH4
11	Kamareddy	Maize	*Steinernema sp*	78	KN EPNS7
12	Medchal	Brinjal	*Heterorhabditis sp*	80	KN EPNH5
13	Medchal	Tomato	*Heterorhabditis sp*	100	KN EPNH6
14	Medchal	Chillies	*Steinernema sp*	95	KN EPNS8
15	Koyyur	Sugarcane	*Heterorhabditis sp*	100	KN EPNH7
16	Koyyur	Zinger	*Steinernema sp*	100	KN EPNS9
17	Koyyur	Potato	*Steinernema sp*	100	KN EPNS10
18	Koyyur	Cotton	*Steinernema sp*	85	KN EPNS11
19	Koyyur	Maize	*Steinernema sp*	90	KN EPNS12
20	Zaheerabad	Sugarcane	*Heterorhabditis sp*	100	KN EPNH8
21	Zaheerabad	Potato	*Heterorhabditis sp*	100	KN EPNH9
22	Zaheerabad	Zinger	*Steinernema sp*	100	KN EPNS13
23	Toopran	Brinjal	*Heterorhabditis sp*	89	KN EPNH10
24	Toopran	Tomato	*Heterorhabditis sp*	100	KN EPNH11
25	Toopran	Chillies	*Heterorhabditis sp*	80	KN EPNH12
26	Kotpally	Sugarcane	*Heterorhabditis sp*	100	KN EPNH13
27	Kotpally	Cotton	*Heterorhabditis sp*	95	KN EPNH14
28	Kotpally	Ground nut	*Steinernema sp*	100	KN EPNS14
29	Kalakkal	Ridge guard	*Heterorhabditis sp*	98	KN EPNH15
30	Kalakkal	Bitter guard	*Steinernema sp*	80	KN EPNS15
31	Kalakkal	Sugarcane	*Steinernema sp*	100	KN EPNS16
32	Vikarabad	Cotton	*Steinernema sp*	85	KN EPNS17
33	Vikarabad	Maize	*Heterorhabditis sp*	75	KN EPNH16

3.3 Nematode Identification and Morphological Studies

3.3.1 Description of entomopathogenic nematodes

Steinernema carpocapsae: *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 (1^{st} 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.

Heterorhabditis indica*:* i) *Infective juveniles:* Third stage juveniles inside second stage cuticles, body shorter than those of previously described species. Labial region with a large dorsal tooth. Stoma walls opened and not collapsed at base. Outer second stage cuticle strongly ribbed longitudinally and closely appressed to the third stage cuticle. Hemizonid quite distinct and located just anterior to excretory pore. The smaller less distinct hernizonion is located in the anterior intestinal region. Ventricular portion of intestine devoiding of intestinal cells and forming an intestinal pouch which is filled with symbiotic bacteria. These bacteria also occur in the lumen of the intestine, mainly the anterior portion. i) *Females*: Head and structures in anterior end similar to those of the species *H. bacteriophora.* Gonads amphidelphic reflexed with sperm occurring in the proximal portion of the ovotestis of hermaphroditic females. Amphimictic females with sperm in the proximal portion of the oviduct. Vulva of amphimictic females narrow, surrounded with copulation plug after mating. Anal region of amphimictic females and especially hermaphrodites conspicuously swollen. The anus occurs approximately at the anterior third of the anal swelling. Rectum heavily cuticularized and a conspicuous valve separates it from the intestine. ii) *Males* : Anterior region similar to that of females, but smaller. Nerve ring near the basal bulb. Testis single, reflexed. Spicules paired and separate, with pointed tips; capitulum usually set off from shaft; blade with single medial rib. Gubernaculum flat, narrow, approximately half the spicule length, not reflexed at tip. Bursa open, peloderan, with a double membrane, one running external and the other internal to the bursal papillae; bursa with only seven normal papillae, the last two (Nyamboli [9] and Poinar et al. [11]) are generally atrophied, highly modified (round or very slender) or absent. Measurements were made using a drawing camera (*camera lucida*) (Axioplan Axiophoto) attached to a Zeiss (MC100 SPOT) light microscope (Hominick et al. [12]; SAS [13]; Stock et al. [14]).

3.4 Lab Bioassay Study

3.4.1 Bioefficacy of native EPN strains, KN EPNS17 and KN EPNH1

The Bioefficacy of selective two stains of *Steinernema carpocapsae (*KN EPNS17) and *Heterorhabditis indica* (KN EPNH1) 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 [11].

4. DISCUSSION

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. Stock et al. [14] that conducted a sampling of different habitats in the state of California and found 26.3% of positive samples. In Oaxaca Ruiz-Vega et al. [1] 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 Ruiz-Vega et al. [1] 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 [14] who reported 80% in *Steinernema* and 20% in *Heterorhabditis* on the other hand, Ruiz-Vega et al. [1] 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 by Rosa et al. [3] 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 (Joyce et al. [15]; Nguyen, [16]). 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 (Claudia [17]; Stock [18]).

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

Parameter (mm)	KN EPNS17 *(Steinernema carpocapsae)*	KN EPNH1 *(Heterorhabditis indica)*
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 *Steinernema carpocapsae* (KN EPNS17) and *Heterorhabditis indica* (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	*Helicoverpa armigera*	Cotton, Tomato and Bhendi	85	100
2	Tobacco caterpillar	*Spodoptera litura*	Cotton and Tomato	90	100
3	Bhendi fruit and shoot borer	*Leucinodes orbonalis*	Bhendi	100	100
4	Dimand back moth	*Plutella xylostella*	Cauliflower	100	100
5	Potato cut worm	*Agrotis segitum*	Potato	100	85
6	Sugarcane root grub	*Holotricha serrata*	Sugarcane	100	100

Data were analysed and subjected to ANOVA and statistical significance was judged at the level (P < 0.05) to test for comparing mortality

5. CONCLUSION

The use of insect parasitic nematodes and other biological control agents to manage insect pests has grown in popularity. Many pests have developed resistance to certain pesticides, new pests have arisen to replace those successfully controlled, the effectiveness of biocontrol agents such as 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 hope that establishing entomopathogenic nematodes in Hyderabad agricultural region and other states of India would bring several benefits. Firmly establishing insect parasitic nematodes will promote the sustained use of agriculture and develop a better understanding of biodiversity. In our investigations, the Heterorhabditid nematode species *H. indica* (KN EPNS17) and Steinernematid species *S. carpocapsae* (KN EPNH1) are so far the most promising biocontrol agent which are isolated from the local soils in various cropping ecosystem, further studies to establish EPN utilities to the farmers recommendation, mass production in soil media technology and identifying suitable formulations technologies are in active process.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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