Isolation, identification and biocontrol potential of entomopathogenic nematodes occurring in Purvanchal and Bundelkhand regions of Uttar Pradesh, India

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Abstract

Entomopathogenic nematodes (EPNs) are being used for the management of insect pests occurring in both agricultural and horticultural crops. But native EPNs are reasonably more efficacious in controlling insect pests than introduced EPN species because they are adapted to local environmental conditions and insect pests. Therefore, in the present study, a survey was conducted to isolate EPNs at 4 districts of Purvanchal Region and 1 district of Bundelkhand Region of Uttar Pradesh, India, between 2016 and 2017 years. Out of 130 soil samples, EPNs were recovered from 3 soil samples (2.3%). Morphological characters examination and ITS-rDNA region revealed that the 3 EPNs such as Steinernema sp. (IIVR JNC01 strain) Steinernema sp. (IIVR JNC02 strain), and Steinernema sp. (IIVR EPN03 strain) are belonging to Steinernema siamkayai. Further pathogenicity of all these strains was tested on Galleria mellonella Linnaeus and the most effective strain was used to determine the biocontrol potential against lepidopteran and coleopteran pests of major vegetable crops in comparison to commercially available Heterorhabditis indica (NBAIIH38 strain). Results revealed that S. siamkayai (IIVR JNC01 strain) caused 100, 100, and 85%, mortality of 3rd instar larvae of Spodoptera litura Fabricius, Spilosoma obliqua Walker, and Spoladea recurvalis Fabricius, respectively. Similarly, S. siamkayai (IIVR JNC01 strain) caused 92.5% mortality of 2nd instar grubs of Myllocerus subfaciatus Gurein under laboratory conditions. The present study revealed that EPNs commonly present at Purvanchal and Bundelkhand regions of Uttar Pradesh and S. siamkayai (IIVR JNC01 strain) isolated from the Bundelkhand Region showed a good biocontrol potential against major insect pests of vegetable crops.

Keywords: Entomopathogenic nematodes, Isolation, Molecular characterization, Biological control, India

Background

With the rapid development and advancement of synthetic chemistry in the early decades of twentieth century, a range of new chemical insecticides had been developed. These chemical insecticides are being used to control insect pests occurring in both agricultural and horticultural crops. However, indiscriminate use of chemical insecticides leads to resistance in insect pest population and non-target effects. Therefore, search of alternative methods to scale down the use of chemical insecticides is gaining importance (Bohinc et al. 2019). Biological control program is one of the alternative methods to chemical insecticides. It exploits insects, bacteria, viruses, fungi, and entomopathogenic nematodes (EPNs). Among these biocontrol agents, EPNs are lethal obligate parasites belonging to Steinernematidae and Heterorhabditidae families (Ishibashi and Choi 1991). They are mutually associated with gram-negative bacteria Xenorhabdus spp. and Photorhabdus spp., respectively, and kill infected insect host within 24–48 h by causing
septicemia (Ciche et al. 2006). These nematodes naturally occur in soil and epigal habitats, and have been isolated from most regions of the world (Kaya and Gaugler 1993). Many laboratory and field studies have indicated that EPNs have potential to control insect pests of vegetable crops (Park et al. 2001; Somvanshi et al. 2006; Trdan et al. 2007; Laznik et al. 2011; Ebssa and Koppenhofer 2011 and Gowda et al. 2016). However, for the effective management of insect pests, native EPN species/strains play a major role because they are easily adapted to local climatic conditions and insect pests (Gaugler 1988 and Noosidum et al. 2010).

Occurrence and distribution of EPNs have been studied in some isolated parts of Uttar Pradesh (Istkhar and Chaubey 2017 and Devindrappa et al. 2019). However, occurrence and distribution of EPNs in Purvanchal and Bundelkhand regions of Uttar Pradesh, India, are previously unexplored.

In this connection, the present study is designed to isolate, identify, and characterize the native EPNs from Purvanchal and Bundelkhand regions of Uttar Pradesh, India, and also to evaluate their biocontrol potential against major lepidopteran and coleopteran pests of major vegetable crops grown in these regions.

Material and methods

Insect culture

Greater wax moth, Galleria mellonella Linnaeus, was reared on artificial diet according to the method (Patil et al. 2020). The last larval instars of G. mellonella were used for the experiment.

Biocontrol potential of EPNs was tested against Spodoptera littura Fabricius infesting cabbage Brassica oleracea var. capitata, Spilosoma obliqua Walker on brinjal, Solanum melongena Linnaeus, and Spoladea recurvalis Fabricius on amaranthus, Amananthus tricolor Linnaeus in this study. A continuous culture of these insect hosts was maintained in an insectary at 26 ± 2 °C and 70 ± 5% RH, a photo phase of 14 h and scoto phase of 10 h. For initial establishment of the culture, different larval stages of these insects were collected from their respective host plants grown in research farm of ICAR- Indian Institute of Vegetable Research, Varanasi (25°10′55.6″ N, 82°52′37.2″ E; 80.71 m above sea level), Uttar Pradesh, India. The larvae were individually reared on their natural diet viz., S. littura on cabbage, S. obliqua on brinjal, and S. recurvalis on amaranthus in Petri dish (diam. 9 cm, depth 1.8 cm). Third instar larvae of these insect species were used for the bioassays.

Second instar grubs of Myllocerus subfaciatus Gurein were collected from naturally infested eggplants grown in ICAR-NBAIR research farm, Bengaluru (13° 05′ 48.7″ N, 77° 34′ 02.8″ E; 920 m above sea level), Karnataka, India. Grubs were kept in 100 ml plastic containers (diam. 5.8 cm, height 8 cm, soil capacity 115 g, total surface area 198.61 cm², shape; round) containing 100 g of autoclaved soil with 12% moisture for 2 days at 25 ± 1 °C. Potato (Solanum tuberosum L.) pieces were provided to feed the grub. Three days after apparently healthy grubs were used for bioassay.

Isolation of native EPNs

In the present study, a survey was conducted during 2016 and 2017 in four districts of Purvanchal Region, i.e., Varanasi, Mirzapur, Sonbhadra, Deoria, and one district at Bundelkhand Region, i.e., Jhansi of Uttar Pradesh; these areas were previously unexplored. Soil samples were collected randomly using hand shovel from agricultural, horticultural, and forest ecosystems from early August to late November. In total, 130 soil samples were collected. Each representative sample (approximately 1 kg each) consists of 5 soil samples were taken at a depth of 15–20 cm. Then, soil samples were placed in polythene bags and transferred to the laboratory for investigation (Yuksel and Canhilal 2019). To isolate EPNs from the soil samples, soils were placed in 1000 ml plastic container (diam. 10.5 cm, height 14.5 cm, soil capacity 1100 g, total surface area 731.59 cm², shape is round) with 4 individuals of last instar G. mellonella larvae. The plastic containers were covered with a lid having a small pin holes for allowing air flow, then containers were incubated at 25 ± 1 °C for 1 week. Soil samples were checked on a daily basis for 7 days to observe the cadavers of the larvae of G. mellonella. All recovered dead larvae of Galleria were placed individually on White’s traps (White 1927). Infective juveniles (IJs) emerged from these dead larvae were collected and stored at 15 °C. Two- to 3-day-old IJs of each nematode strain was tested against 20 individuals of the last instar G. mellonella larvae to confirm Koch’s postulates for pathogenicity (Pelczar and Reid 1972 and Kaya and Stock 1997).

Morphology and morphometry of native EPN strains

To study the morphology and morphometrics of EPNs strains, last instar larvae of Galleria were inoculated at rate of 200 IJs larva⁻¹ and incubated 25 ± 1 °C in the dark. Three days after larval death, first generation adults were collected by dissecting the cadavers in Ringer’s solution. Similarly, second generation adults were collected 5th day after larval death. Infective juveniles were harvested from cadavers of the larvae of G. mellonella with the help of White’s traps. The adults of both generations and IJs were killed by pouring hot fixative and then fixed in TAF and processed, using Seinhorst I and Seinhorst II reagents (Seinhorst, 1959). The adults of both generations and IJs were mounted in dehydrated glycerin, using appropriate size glass support. The mounted specimens were used for detailed microscopic studies (Poinar 1990). The measurements
and examination of morphology were completed, using Trinocular Research Microscope (model Axio Imager Z2 by Carl Zeiss Microscopy GmbH provided with DIC optics) with suitable photomicrographs. For each *Steinernema* IIIVR strain, 20 specimens of each developmental stage were examined. The results of morphometric and morphological characters of EPN strains isolated in this study were compared to the original description of *S. siamkayai* Stock, Somsook, and Reid.

**Molecular characterization of native EPN strains**

The genomic DNA was extracted from a single female of each *Steinernema* IIIVR strain, using a modified method of Joyce et al. (1994). The ITS region of the EPN strains DNA was amplified by polymerase chain reaction (PCR) mixture containing 10 μl of the DNA suspension, 2.5 μl 10× PCR buffer with MgCl₂, 0.5 μl dNTP mixture (10 mM each), 0.5 μl (100 pM/μl) of each primer, 0.3U Taq polymerase, and 10.7 μl double distilled water to make volume of 25 μl. The forward primer TW81 (5'-GTGGTGAACCTGC-3') and the reverse primer AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') were used in the PCR reaction for amplification of the complete ITS. The amplified PCR products were purified using a Qiagen Gel Purification Kit. The fragments of DNA were sequenced by Sanger’s method (Eurofins Genomics India Pvt., Ltd., Bengaluru, India). Sequences of the ITS-rDNA region of the EPN were used to study the phylogenetic relationships between the *Steinernema* IIIVR strains and other related 21 *Steinernema* species, and *Caenorhabditis elegans* was used as an out group. The DNA sequences were edited using BioEdit with sequences of related species/strains. The accession numbers are cited in the phylogenetic tree out group. The DNA sequences were edited using BioEdit with sequences of related species/strains. The DNA sequences were edited using BioEdit with sequences of related species/strains.

**Source of nematodes for laboratory bioassays**

Entomopathogenic nematode species/strains such as *Steinernema* sp. (IIVR JNC01 strain), *Steinernema* sp. (IIVR JNC02 strain), *Steinernema* sp. (IIVR EPN03 strain), and *Heterorhabditis indica* (NBAIIH38 strain) were cultured in last instar larvae of *G. mellonella* (Kaya and Stock 1997). Two to 3-day-old IJs were used for laboratory bioassays. Virulence of all three *Steinernema* spp. IIIVR strains was tested on last instar larvae of *G. mellonella*. Based on the better virulence, *Steinernema* sp. (IIVR JNC01 strain) was selected to test the biocontrol potential against lepidopteran and coleopteran pests in comparison to commercially available *H. indica* (NBAIIH38 strain).

**Evaluation of biocontrol potential against lepidopteran pests of vegetable crops**

In this study, *Steinernema* sp. (IIVR JNC01 strain) was tested against *S. litura*, *S. obliqua* and *S. recurvalis* in comparison to *H. indica* (NBAIIH38 strain). The study was conducted in Petri dish (diam. 9 cm, depth 1.8 cm), was filled with 20 g of sterilized soil and moisture was adjusted to 15% by adding water. Fresh leaf discs of individual host plant were placed in a Petri dish to feed insects. Each Petri dish was inoculated by each nematode species at 0, 25, 50, 100, 200 IJs larva⁻¹. After an hour, 4 individuals of 3rd larval instar of each insect host species were released to each dish. Larval mortality was recorded 2 days after nematode inoculation, and nematode infection was confirmed by dissecting cadavers under a stereomicroscope. The whole experiment was repeated with 5 replicates.

**Evaluation of biocontrol potential against eggplant ash weevil**

In this experiment, biocontrol potential of *Steinernema* sp. (IIVR JNC01 strain) was tested against 2nd instar grubs of *M. subfasciatus* in comparison to *H. indica* (NBAIIH38 strain). For this study, 30 ml plastic cups (diam. 3.5 cm, height 3.5 cm, soil capacity 35 g, total surface area 153.93 cm², shape is round) were filled by 25 g of sterilized soil, and moisture was adjusted to 12%. Potato pieces were added to each plastic cup to feed the grub. A single 2nd instar grub of *M. subfasciatus* was placed in each cup. After 24 h, grub was inoculated with each nematode species at 0, 100, 300, and 500 IJs grub⁻¹. Then cups were placed in incubator at 25 ± 1 °C. Each treatment had 20 replicates and grub mortality was assessed on daily basis up to 7 days. Cadavers were placed on White’s traps and confirmed the death is due to EPNs by observing the nematode emergence from cadavers. The whole experiment was repeated with 20 replicates.

**Statistical analysis**

Before statistical analysis, percentage mortality data were normalized, using arcsine transformation. Analysis was undertaken on the transformed data. An ANOVA was conducted using PROC ANOVA (SAS version 9.3; SAS institute 2011, Cary, NC, USA). When ANOVA was significant, relevant means were compared to Tukey’s significance test values at the 5% level of significance.
Results and discussion

Isolation, identification, and characterization of native EPN strains

Detailed information of EPN strains and the locations from where they were isolated is given in Table 1. Out of 130 soil samples collected, EPNs were recovered only from 3 samples (2.3%). Among these 3 samples, 2 (Steinernema sp. IIVR JNC01 strain, Steinernema sp. IIVR JNC02 strain) belongs to Jhansi District of Bundelkhand Region and 1 (Steinernema sp. IIVR EPN03 strain) from Varanasi District of Purvanchal Region. EPN survey revealed that there is relatively low occurrence of EPNs in these regions of Uttar Pradesh. Similar to this study, previous studies also revealed a low occurrence of EPNs in western part of Uttar Pradesh (Istkhar and Chaubey 2017 and Devindrappa et al. 2019). The present study was conducted as a regional survey, which frequently has rational sampling, which could maximize nematode isolation (Abd-Elgawad 2020). The other possible reasons for low occurrence of EPNs from collected soil samples might be influenced by climatic factors of particular region. Prevalence of long dry spell, low relative humidity, and high atmospheric temperature may suppress the abundance of EPNs in these regions. Previous studies revealed that temperature is one factor that regulates the temporal and spatial distribution of EPNs because these nematodes are highly sensitive to environmental extremes such as temperature and relative humidity (Gaugler 1981; Ehlers and Peters 1996 and Laznik and Trdan 2012).

Morphology and morphometric studies of different life stages (IJs, adults of 1st and 2nd generations) of 3 Steinernema IIVR strains isolated in this study revealed closer resemblance with S. siamkayai Stock, Somsook and Reid (Fig. 1). The morphometric analysis of S. siamkayai (IIVR JNC01 strain) was given in Table 2. Morphometric characteristics of S. siamkayai (IIVR JNC01 strain) were compared with originally described S. siamkayai (Stock et al. 1998). Morphometrically, body length of IJs of S. siamkayai (IIVR JNC01 strain) was comparatively longer than the original description 464 (452–472) vs 446 (398–495) μm. Apart from this, distance from anterior end to nerve ring 78 (70–86) vs 72 (68–80) μm. The first generation females also showed difference in body length 4921 (3897–4921) vs 3937 (3161–5172) μm, and body width 231.4 (181–273) vs 198 (170–280) μm. Females of second generation also showed variation in body length 1867 (1812–1919) vs 1836 (1410–2560) μm. Similarly, the 1st and 2nd generation males’ body length also varied with original description. Nevertheless, most of the characters such as distance from anterior end to excretory pore, distance from anterior end to nerve ring, tail length, macro length, a, b, c, SL, GL SW, GS, and D% were not showed much differences with original description. A comparison of important morphometric parameters between S. siamkayai (IIVR JNC01 strain) and original description of S. siamkayai Stock, Somsook, and Reid is shown in Table 3. In addition, molecular characterization revealed that the
Fig. 1 LM photos of first generation female. a Anterior part with excretory pore. b Vulva with epitygma. c Tail with mucro and first generation male. d Anterior part with excretory pore. e Spicule lateral view with mucro. f Gubernaculum lateral view

Table 2 Morphometrics of *Steinernema siamkayai* (IIVR JNC01 strain) (measurements are in μm and in the form of mean ± SD (range))

| Character                  | Infective juveniles (IJ’s) (n = 20) | First generation | Second generation |
|----------------------------|------------------------------------|------------------|-------------------|
|                            | Females (n = 20)                   | Males (n = 20)   | Females (n = 20)  | Males (n = 20)  |
| Body length (L)            | 464 ± 6.6 (452–472)                | 4492 ± 264.8 (3897–4921) | 1193 ± 84.7 (1098–1324) | 1867 ± 37.6 (1812–1919) | 1855 ± 44.4 (1784–906) |
| Greatest body width        | 20.8 ± 1.3 (18–23)                 | 231.4 ± 22.1 (181–273) | 142.1 ± 10.5 (102–152) | 109 ± 10.3 (85–124) | 65.4 ± 9.9 (48–78) |
| EP                         | 34.2 ± 1.2 (32–36)                | 64.4 ± 2.6 (61–68)                      | 60.3 ± 2.2 (59–66)                      | 66.9 ± 1.5 (65.5–69) | 60.8 ± 2.1 (59–66) |
| ES                         | 101.1 ± 8.9 (83–112)              | 177.2 ± 14 (149–196)                  | 138.3 ± 4.2 (132–145)                  | 153.6 ± 6.4 (142–164) | 123.4 ± 6.5 (110–131) |
| NR                         | 78.0 ± 4.2 (70–86)                | 186 ± 2.4 (159–205)                  | 86.6 ± 7.1 (79–102)                   | 124 ± 5.5 (115–134) | 84.0 ± 3.3 (78–89) |
| Tail length (TL)           | 35.3 ± 2.6 (32–40)                | 31 ± 4.9 (23–37)                      | 26.1 ± 3.8 (20–32)                   | 389 ± 4.7 (31–49) | 22.2 ± 2.2 (20–27) |
| Body width at cloaca       | –                                | 47.5 ± 6.6 (35–58)                  | –                                 | 28.6 ± 1.2 (27–30) |                                    |
| Spicule length             | –                                | 77.7 ± 1.2 (76–79)                  | –                                 | 59.7 ± 2.5 (55–63) |                                    |
| Spicule width              | –                                | 8.5 ± 0.5 (7.7–9.3)                 | –                                 | 5.9 ± 0.4 (5.0–6.8) |                                    |
| Gubernaculum length        | –                                | 55.8 ± 7.3 (45–67)                  | –                                 | 42.2 ± 2.3 (39–46) |                                    |
| Gubernaculum width         | –                                | 5.4 ± 0.8 (3.8–6.7)                 | –                                 | 3.7 ± 0.4 (3.0–4.7) |                                    |
| Mucro length               | –                                | 7.9 ± 1.4 (5.2–10.4)                | 3.1 ± 0.3 (2.6–3.7)                 | 4.4 ± 1 (3.2–6.2) | 2.8 ± 0.5 (2.2–3.8) |
| a                          | 22.4 ± 1.5 (19.9–26.1)            | –                                | –                                 | –                                 |                                    |
| b                          | 46.0 ± 0.4 (42.4–5.5)             | –                                | –                                 | –                                 |                                    |
| c                          | 13.2 ± 0.9 (11.4–14.3)            | –                                | –                                 | –                                 |                                    |
| SW                         | –                                | 1.67 ± 0.2 (1.36–2.17)              | –                                 | 2.09 ± 0.1 (1.87–2.37) |                                    |
| GS                         | –                                | 0.72 ± 0.1 (0.57–0.88)              | –                                 | 0.7 ± 0.1 (0.66–0.75) |                                    |
| V%                         | –                                | 48.7 ± 1.0 (47–50)                 | –                                 | 53.4 ± 2.0 (50–56.5) |                                    |
| D% ([EP/ES]*100])          | 34.0 ± 2.9 (30–40)               | –                                | 43.2 ± 1.7 (40–45)                 | –                                 | 49.4 ± 3.0 (47–54) |
| E% ([EP/TL]*100])          | 97.4 ± 7.0 (82–106)              | –                                | 237.2 ± 38.5 (184.3–309)             | –                                 | 276.6 ± 21.5 (226–300) |

*EP* distance from anterior end to excretory pore, *NR* distance from anterior end to nerve ring, *ES* distance from anterior end to end of pharynx/esophagus, *a* body length/body width, *b* body length/ES, *c* body length/tail length, *SW* spicule length divided by anal body diameter, *GS* gubernaculum length divided by spicule length, *V%* distance from anterior end to end vulva as percentage of length
Table 3  Comparative morphometrics *Steinernema siamkayai* (IVR JNC01 strain) with original description. All measurements are in micrometers (μm) (except percentage) and in the form of mean (range) for *Steinernema siamkayai*.

| Species                        | L  | GW | EP  | NR  | TL  | α   | b   | c   | D%  |
|--------------------------------|----|----|-----|-----|-----|-----|-----|-----|-----|
| *Steinernema siamkayai*       | 446| 21 | 35  | 72  | 35.5| 21  | 4.7 | 11.3| 37  |
|                               | (398–495) | (18–24) | (29–38) | (68–80) | (31–41) | (19–23) | (4.0–6.1) | (10.3–14.8) | (31–43) | (85–112) |
| *Steinernema siamkayai* (IVR JNC01 strain) | 464| 20.8 | 34.2 | (32–36) | 78 | 35.3 | 22.4 | 4.6 | 13.2 | 34 |
|                               | (452–472) | (18–23) | (70–86) | (32–40) | (199–261) | (199–261) | (4.2–5.5) | (11.4–14.8) | (30–40) | (82–106) |

| First generation female (n = 20) | L  | GW | EP  | NR  | TL  | M  |
|-----------------------------------|----|----|-----|-----|-----|---|
| *Steinernema siamkayai*           | 3937| 198 | 66  | 133 | 31  | 70 |
| (3161–5172) | (170–280) | (50–84) | (116–147) | (22–37.5) | (45-11) | (48–53) |
| *Steinernema siamkayai* (IVR JNC01 strain) | 4492| 231.4 | 644 | 137 | 31  | 79 |
| (3897–4921) | (181–273) | (61–68) | (119–151) | (23–37) | (5.2–10.4) | (47–50) |

| Second generation female (n = 20) | L  | GW | EP  | NR  | TL  | M  |
|-----------------------------------|----|----|-----|-----|-----|---|
| *Steinernema siamkayai*           | 1836| 105 | 68  | 123 | 31  | 79 |
| (1410–2560) | (83–128) | (65–70.5) | (113–130) | (22–37) | (5.2–10.4) | (47–50) |
| *Steinernema siamkayai* (IVR JNC01 strain) | 1867| 109 | 669 | 124 | 38.9| 4.4 |
| (1812–1919) | (85–124) | (65.5–69) | (115–134) | (31–49) | (32–6.2) | (50–56.5) |

| First generation male (n = 20) | L  | GW | EP  | NR  | TL  | ML  | SL  | GL  | SW  | GS  | D%  |
|--------------------------------|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| *Steinernema siamkayai*        | 1135| 190 | 57  | 81 | 22.5 | 75.5 | 53.5 | 1.70 | 0.7 | 43 |
| (1035–1278) | (107–159) | (47.5–67) | (81–101) | (22–32) | (25.3) | (197–80) | (47–65) | (14–2.2) | (0.6–0.8) | (35–49) |
| *Steinernema siamkayai* (IVR JNC01 strain) | 1193| 142 | 60.3 | 79 | 20.5 | 77.7 | 55.8 | 1.67 | 0.72 | 43.2 |
| (1098–1324) | (102–152) | (59–66) | (79–102) | (20–32) | (26–3.7) | (76–79) | (45–67) | (13.3–17.1) | (0.57–0.88) | (40–45) |

| Second generation male (n = 20) | L  | GW | EP  | NR  | TL  | ML  | SL  | GL  | SW  | GS  | D%  |
|--------------------------------|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| *Steinernema siamkayai*        | 830| 57  | 58  | 85  | 22  | 3   | 62  | 41.5 | 2.13 | 0.69 | 51 |
| (716–952) | (47–75) | (54–68) | (80–91) | (19–33) | (2–4) | (55–73) | (37–55) | (22–2.35) | (0.66–0.75) | (49–56) |
| *Steinernema siamkayai* (IVR JNC01 strain) | 855| 65.4 | 60.8 | 84  | 22.2 | 2.8 | 59.7 | 42.2 | 2.09 | 0.70 | 49.4 |
| (785–906) | (48–78) | (59–66) | (78–89) | (22–38) | (156–53) | (39–46) | (18.7–2.37) | (0.67–0.75) | (47–54) |
native EPN strains yielded ~ 800 bp fragment upon PCR amplification with the ITS primers. Ribosomal DNA (ITS-rDNA region of EPNs) sequences generated for the strains were aligned and matched with available sequences in NCBI GenBank. The BLAST search analysis of DNA sequence of native EPN strains showed > 99% matches to authenticated reference sequence of *S. siamkayai*. Based on the morphological examinations and information supported by molecular tool, *Steinernema* strains isolated in this study were identified as *S. siamkayai* (IIVR JNC01 strain) (GenBank accession no. MH208855), *S. siamkayai* (IIVR JNC02 strain) (GenBank accession no. MH208856), and *S. siamkayai* (IIVR EPN03 strain) (GenBank accession no. MG976754). Phylogenetic relationship between all the three IIVR strains of *S. siamkayai* and previously identified *S. siamkayai* and other *Steinernema* species are presented in Fig. 2.

**Biocontrol potential of EPNs against lepidopteran pests of vegetable crops**

Laboratory bioassays were designed to evaluate the biocontrol potential of *S. siamkayai* (IIVR JNC01 strain) in comparison to commercially available *H. indica* (NBAIHH38 strain) against 3rd instar larvae of *S. litura*, *S. obliqua*, and *S. recurvalis*. Bioassays data revealed that when *H. indica* (NBAIHH38 strain) inoculated at rate of 200 IJs larva⁻¹ caused 100% mortality in 3rd instar of all the 3 insect host species tested in this study. Similarly, *S. siamkayai* (IIVR JNC01 strain) also caused 100% mortality in 3rd instar of *S. litura* and *S. obliqua* whereas 85% mortality in 3rd instar of *S. recurvalis* (Fig. 3a–c). Analysis of variance revealed that, among the nematode species, irrespective of IJ concentrations, *H. indica* (NBAIHH38 strain) caused greater mortality only in *S. recurvalis* compared to *S. siamkayai* (IIVR JNC01 strain) (*F* = 13.86, df = 1, 90, *P* = 0.0003). However, there was non-significant difference in mortality caused by both EPN species on 3rd instar larvae of *S. litura* (*F* = 2.82, df = 1, 90, *P* = 0.09) and *S. obliqua* (*F* = 1.69, df = 1, 90, *P* = 0.20). The calculated LC₅₀ and LC₉₀ values for *S. siamkayai* (IIVR JNC01 strain) and *H. indica* (NBAIHH38 strain) on *S. recurvalis*, *S. litura*, and *S. obliqua* are shown in Table 4. Adioubane et al. (2010) found that *S. siamkayai* isolated from Karaikal Region of...
Puducherry, India, had greater biocontrol potential against larval stages and pre-pupae of *S. litura*. *Steinernema longicaudum* X-7, *Steinernema* sp. 64-2, four isolates of *S. carpocapsae*, and 2 isolates of *H. indica* caused > 90% mortality in 2nd, 3rd, and 4th larval instars of *S. litura* (Yan et al. 2019). Similarly, in the present study, it is also evidenced that *S. siamkayai* (IIVR JNC01 strain) had greater biocontrol potential against 3rd larval instar of *S. litura*, *S. obliqua*, and *S. recurvalis*, and the efficacy was quite comparable to commercially available *H. indica* (NBAIIH38 strain). In addition, present findings provide the first insight into the biocontrol potential of *S. siamkayai* (IIVR JNC01 strain) and *H. indica* (NBAIIH38 strain) against 3rd instar larvae of *S. recurvalis*, the major pest of leafy vegetables, particularly on amaranthus grown in Purvanchal and Bundelkhand regions of Uttar Pradesh.

**Biocontrol potential of EPNs against eggplant ash weevil**

This assay revealed that both EPN species were able to kill 2nd larval instar of grubs of *M. subfasciatus*. When a concentration of 500 IJs grubs⁻¹ was applied, the greatest mortality of 2nd larval instar of grubs was observed for *H. indica* (NBAIIH38 strain) (100%) and *S. siamkayai* (IIVR JNC01 strain) (92.5%) (Fig. 4). Analysis of variance showed that biocontrol potential of *S. siamkayai* (IIVR JNC01 strain) was comparable with *H. indica* (NBAIIH38 strain) against 2nd larval instar of grubs of *M. subfasciatus*. The calculated LC₅₀ and LC₉₀ values for *S. siamkayai* (IIVR JNC01 strain) and *H. indica* (NBAIIH38 strain) on 2nd larval instar of grubs were shown in Table 4. Similarly, Gowda et al. (2016) reported that *S. carpocapsae* and *H. indica* had greater biocontrol potential against 3rd instar larvae and pre-pupae of *M. subfasciatus*. In another study, Nagesh et al. (2016) demonstrated greater biocontrol potential of *H. bacteriophora* NBAIIHb105, *H. indica* NBAIIHi101, *H. indica* NBAIIHiMah, *S. abbasii* NBAIIIs01, *S. abbasii* NBAIIIS04, *S. carpocapsae* NBAIISc04, and *S. glaseri* NBAIIISg01 against *M. subfasciatus*. Inclusively, earlier and present studies indicated that EPNs are the best candidates for the management of *M. subfasciatus*.
Conclusion
Search and identification of native EPN species/strains, adapted to local agro-climatic conditions, as a biocontrol potential agent against major insect pests, serve as a critical component in developing integrated pest management programs of a particular pest. In this study, *S. siamkayai* (IIVR JNC01 strain) and *H. indica* (NBAIIH38 strain) showed greater biocontrol potential against *S. recurvalis, S. litura, S. obliqua*, and *M. subfaciatus*. Moreover, high virulence of EPNs attained under laboratory conditions cannot be generalized for the field efficacy. Further evaluations of their biocontrol potentials should be tested under poly greenhouse and field conditions.

![Graph](image)

**Fig. 4** Mortality (mean% ± SE) of 2nd instar grub of *Myllocerus subfaciatus* Guerin at different concentrations of entomopathogenic nematodes, *Steinernema siamkayai* (IIVR JNC01 strain), and *Heterorhabditis indica* (NBAIIH38 strain) at 7 days after treatment. Different letters on the top of error bars indicate statistically different values for different nematode concentrations at (*P* < 0.05) using Tukey’s test. Bars = standard error (*n* = 40).

**Table 4** The lethal concentration (LC50 and LC90) of *Steinernema siamkayai* (IIVR JNC01 strain) and *Heterorhabditis indica* (NBAIIH38 strain) against lepidopteran pests at 2 days after treatment and eggplant ash weevil (*Myllocerus subfaciatus* Guerin) at 7 days after treatment

| Lepidopteran insect pests | Entomopathogenic nematode species | LC50 95% Fiducial limits | LC90 95% Fiducial limits | Slope ± SE | χ² | P (≤ 0.05) |
|---------------------------|----------------------------------|--------------------------|--------------------------|------------|----|-------------|
| *Spodoptera recurvalis*   | *Steinernema siamkayai* (IIVR JNC01 strain) | 60 42–81 | 385 221–1300 | 1.58 ± 0.32 | 24.15 | < 0.0001 |
|                           | *Heterorhabditis indica* (NBAIIH38 strain) | 39 24–53 | 205 116–1244 | 1.78 ± 0.49 | 12.92 | 0.0003 |
| *Spodoptera litura*       | *Steinernema siamkayai* (IIVR JNC01 strain) | 20 11–28 | 68 52–109 | 2.46 ± 0.53 | 21.59 | < 0.0001 |
|                           | *Heterorhabditis indica* (NBAIIH38 strain) | 27 16–35 | 103 77–178 | 2.19 ± 0.42 | 26.97 | < 0.0001 |
| *Spilosoma obliqua*       | *Steinernema siamkayai* (IIVR JNC01 strain) | 19 10–26 | 55 43–84 | 2.83 ± 0.65 | 18.79 | < 0.0001 |
|                           | *Heterorhabditis indica* (NBAIIH38 strain) | 20 11–25 | 43 35–65 | 3.83 ± 1.00 | 14.73 | < 0.0001 |
| *Myllocerus subfaciatus*  | *Steinernema siamkayai* (IIVR JNC01 strain) | 105 49.9–150.3 | 519.74 369.8–1482 | 1.85 ± 0.44 | 12.91 | 0.0003 |
|                           | *Heterorhabditis indica* (NBAIIH38 strain) | 95.04 – | 335.9 – | 2.33 ± 1.05 | 03.67 | 0.055 |

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Abbreviations
EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; ICAR: Indian Council of Agricultural Research; IIVR: Indian Institute of Vegetable Research; NBARI: National Bureau of Agricultural Insect Resources; ANOVA: Analysis of variance; TAF: Triethanolamine formalin; LC: Lethal concentration; ITS: Internal transcribed spacer; BLAST: Basic Local Alignment Search Tool; NCBI: National Center for Biotechnology Information; PCR: Polymerase chain reaction

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Authors’ contributions
MTG, JP, and ABR designed the research. MTG, PAD, and VK collected soil samples. VR molecularly characterized the EPNs. JP studied the morphological characters of EPNs. MTG, JP, JH, VK, and PAD conducted the experiments. MTG and JP analyzed the data. MTG, JP, JH, ABR, and JS wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
All experimental works were approved by ICAR-Indian Institute of Vegetable Research, Varanasi and ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India.

Consent for publication
The agreement of publication was taken, and as a corresponding author, I confirm that.

Competing interests
The authors declare that they have no competing interests.

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