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Efficacy of native strains of entomopathogenic nematode, *Heterorhabditis indica* against the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) from India

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Abstract

Background: The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a notorious polyphagous pest that has recently invaded India in 2018. Within a short period, this pest has spread throughout the country, causing a significant damage to maize. In order to manage this menace, farmers rely on chemical insecticides, but due to concealed feeding nature of this pest insecticides remain ineffective in reaching the target site. In this context, the present study aimed to isolate, characterize and evaluate the reproductive potential of the native strains of entomopathogenic nematode, *Heterorhabditis indica* against larval instars of *S. frugiperda* under laboratory conditions.

Results: Two strains of *H. indica* isolated and characterized from the rhizosphere of mango and curry leaf trees were designated as CICR-HI-MN and CICR-HI-CL, respectively. Both strains, CICR-HI-CL and CICR-HI-MN showed differential mortality against 3rd (LC_{50} value of 21.65, 48.91 IJs/larva), 4th (LC_{50} value of 25.46, 52.36 IJs/larva) and 5th (LC_{50} value of 59.20, 71.04 IJs/larva) instar larvae of *S. frugiperda,* respectively. The instar-wise mortality of *S. frugiperda* showed that 3rd instar was relatively more susceptible compared to 4th and 5th instar larvae. In terms of efficacy, CICR-HI-CL was almost twice effective than CICR-HI-MN when LC_{50} and LC_{95} values of 3rd instar larvae were compared. However, the reproductive potential of both the strains was significantly higher against 5th instar than the 3rd and 4th instar larvae. Strain CICR-HI-CL was more effective in larval mortality, while nematode reproduction was higher in CICR-HI-MN.

Conclusions: Native strains of *H. indica* evaluated in the present study were found effective against larval instars of fall armyworm, *S. frugiperda*. Higher reproduction on 5th instar larvae demonstrates the ability of these strains to multiply, sustain and perpetuate on late instars of this polyphagous pest and may serve as viable option in integrated pest management program.

Keywords: Entomopathogenic nematodes, *Heterorhabditis indica*, *Spodoptera frugiperda*, Pest management, Reproductive potential

Background

In India, maize is mainly grown for food and fodder by small and marginal farmers. Maize is third most important food grain crop in India next to rice and wheat contributing 10% to total food production (Shukla

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et al. 2018). Due to its shorter duration and high yields under Indian climatic conditions, the crop can be grown throughout the year (Joshi et al. 2005). This marks the importance of the maize crop in the Indian scenario.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), is a notorious pest that has invaded India and was first reported in 2018 (Sharanabasappa et al. 2018). It is a polyphagous pest that feeds on more than 353 hosts belonging to 76 plant families across the globe (Montezano et al. 2018). The highly adaptive nature of this pest coupled with its migratory nature and high reproduction rate could make it possible for the pest to invade more than 70 countries across the globe (CABI 2022). The pest has posed economic loss to the extent of 22–67% annually across the world (Baudron et al. 2019).

The sudden invasion of this pest in India has led to several recommendations of chemical insecticides as a foliar application. But concealed feeding nature of this pest in the whorl portion of maize makes insecticides non-reachable to the target site. Coupled with this fall armyworm has developed resistance to many insecticides (Gutiérrez-Moreno et al. 2019). Due to the indiscriminate use of chemical insecticides in the recent invasion, the pest might have developed resistance in Indian landscapes as well along with raising concerns of insecticide residues for human and animal health. In view of the adverse effect of insecticides to the natural ecosystem, utilization of biocontrol agents, especially entomopathogens proved to be a viable option for environment responsive pest management. Fall armyworm found to be the most susceptible to entomopathogens like fungi, viruses, bacteria and nematodes as well (Molina-Ochoa et al. 2003) and use of entomopathogens has been proven as effective management strategy under severe outbreaks (De Faria and Wraight 2007).

Under these circumstances, there is an urgent need to identify an eco-friendly, effective and sustainable management strategy to curtail the spread of fall armyworm in India. The full grown larvae fall off to the ground and pupate in soil. As the larvae remains concealed in the whorl portion and pupate in soil, the use of entomopathogenic nematodes (EPNs) serves as one of the unexplored and best options for its management. The EPN of the genera, *Heterorhabditis* and *Steinernema* are among the most known and important biological control agents (associated with mutualistic symbiotic bacteria *Photorhabdus* and *Xenorhabdus*, respectively) against insect pests worldwide (Chavan et al. 2019). The EPN species harbor bacteria, bacterial retention occurs in a vesicle (*Steinernema*), or the anterior and middle part of the intestine (*Heterorhabditis*) in the third-stage infective juvenile (IJ) which enter the insects' hemolymph through natural openings or also with assistance of dorsal tooth in the mouth region. Symbiotic bacteria associated with nematodes are released in haemocoel and resulting in septicemia, thereby killing the insect host within 48–72 h. (Laznik et al. 2011). The EPNs are widely used in insect pest management, especially for management of pests that has soil dwelling stage in its life cycle (Kaya and Gaugler 1993). Several researchers across the globe have studied genera *Heterorhabditis* for management of lepidopteran pests like *S. frugiperda* (Patil et al. 2022); *S. litura* (Gokte-Narkhedkar et al. 2019); *S. littoralis* (Sobhy et al. 2020). In this context, present study was conducted to isolate, characterize and evaluate the efficacy of two strains of *H. indica* (ICAR-CICR-HI-MN and ICAR-CICR-HI-CL) against larval stages of fall armyworm under laboratory conditions.

Methods

Rearing of *Spodoptera frugiperda*

Various larval instars of *S. frugiperda* were collected from infested maize fields located at ICAR-Central Institute for Cotton Research (ICAR-CICR), Nagpur (21° 04′ 48.39″ N 78° 06′ 58.02″ E), Maharashtra, India during 2020–21. The collected larvae were initially reared on natural food, i.e., maize stem and subsequently transferred to an artificial diet (Prasanna et al. 2018) with slight modifications (Additional file 1: Table S1) and maintained under controlled conditions (65 ± 5% relative humidity (RH); 14L:10D photoperiod, 27 ± 1 °C temperature). The larvae were identified after reaching 5th instar based on morphological characteristics including presence of distinct inverted "Y" shape on the head capsule and the patterns of four black spots on the abdominal segments in the form of square and trapezoidal, the species was confirmed as *S. frugiperda* (Sharanabasappa et al. 2018). Adult moths after emergence were paired and transferred to a plastic container (20 lit capacity with 27 cm height and 24 cm diameter), covered with the muslin cloth. Moths were supplied with 10% honey solution dipped cotton plug on every alternate day as a sugar source (Sharanabasappa et al. 2018). The eggs were collected and larvae were transferred to 25 well multi-cell plates (Innovative Biosciences Private Limited, Nagpur, India) containing diet and maintained till pupation. The laboratory culture of greater wax moth, *Galleria mellonella* (L.) was available at ICAR-CICR, Nagpur and maintained on wheat and corn based artificial diet (Metwally et al. 2012) with slight modifications (Additional file 1: Table S2) under controlled conditions as mentioned above. The EPN populations of both the strains were maintained on larvae of *G. mellonella* under laboratory conditions (Patil et al. 2020).

Isolation and maintenance of EPN strains

The baiting technique was used for isolating the EPN using *G. mellonella* as bait modified after Bedding and Akhurst (1975). In 2020–21 cropping season, during months of August–September, sampling and baiting was done at the farms of ICAR-CICR, Nagpur. About ten 5th instar larvae of *G. mellonella* were placed in the bottle, which had small pin holes on the lid and subsequently filled with moist soil positioned in the maize field (rotated with cotton in previous year) including the root zone of mango (*Mangifera indica*) and curry leaf (*Murraya koenigii*) trees grown on field bunds. Our previous experience on pupation of *S. frugiperda* on bunds of main field and well established fact about higher populations of EPN in undisturbed cropping system, the sampling was performed in bunds inhabiting mango and curry leaf trees. Though the sampling was also carried out in the main field of maize, EPN's were recovered only from the baits placed on bunds (exactly the root zone of mango and curry leaf trees). These baits were placed 15 cm below soil depth near the root zone in a slanting position at 45° angle and were labeled properly and the baiting sites were marked for easy retrieval. Traps were observed on 4th day after initial installation and after 8 days these baits were taken out and inspected for the larval mortality (Khashaba et al 2020). Dead insects were placed on top of filter paper draped inverted watch glass containing sterilized water. This setup was maintained at 25 °C and inspected on alternate days (White 1927). Infective juveniles of nematode were harvested from the White traps by rinsing them in 0.1% formaldehyde solution and stored at 15 °C (White 1927). Preliminary confirmation about infection of *Heterorhabditis* was made based on the larvae of *Galleria* infected with the isolated strain turning dark red color (Ashwini et al 2022). EPN isolates were identified based on morphological/morphometric observations and molecular characterization.

Identification and characterization of EPN strains

Morphological characterization

Larval cadavers were collected from the baits installed into rhizosphere soils of mango and curry leaf trees growing in maize fields. Larval cadavers turned into dark reddish color indicated the probable association of EPN, *Heterorhabditis* with baited *Galleria* larvae. These baited cadavers were washed thoroughly with sterile water and subsequently the larval cadavers were placed on White trap (White, 1927) and incubated in growth chamber at 25 °C and 75–80% RH. The IJs emerging from the White trap had characteristic slow moving nature; such IJs were collected and re-inoculated to fresh 5th instar *Galleria* larvae to confirm pathogenic nature of these strains. The larval mortality was recorded within 48 h. post-inoculation and the *Galleria* larvae turned brick red in color indicting possible *Heterorhabditis* infection (Additional file 2: Fig. S1). The fresh IJs emerging from this second round of infection showing virulence against *Galleria* larvae were re-infected to fresh batch of *Galleria,* which was used for morphological identification (Hoy et al. 2008). The dissection of larval cadaver was planned in batches every day post-inoculation for recovery of males, first generation was hermaphroditic female and was obtained 3–4 days post-inoculation. Second generation was amphimictic where males and females were recovered 7–8 days post-inoculation. Larval cadavers were dissected (Nguyen and Smart, 1995); nematodes were killed and fixed in 4% formalin (at 50–60 °C). Nematode mounts were prepared in glycerin according to Seinhorst's (1959) rapid method. Adult nematodes were mounted on slides using cover glass supports to avoid flattening. All measurements for male and IJs were made using a drawing tube attached to Leica DMLB light microscope (Leica Microsystems, Wetzlar, Germany) (Gokte-Narkhedkar et al. 2019). Infective juveniles started emerging from the insect cadaver on 10th day after inoculation and juveniles emerging in the first week of emergence were used for morphological characterization. Taxonomic keys from Nguyen and Hunt were used in identification of nematode species (Nguyen and Hunt 2007).

Molecular characterizations

For molecular characterizations of EPN strains, rDNA sequences from the large subunit, small subunit and the internal transcribed spacer (ITS) regions were considered. DNA was extracted from IJs and adults as described (Smits et al. 1991). The PCR reaction mixture contained 5X reaction buffer—5 µl, $MgCl_2$ (25 mM)—3 µl, dNTP's mix (10 mM)—1.5 µl, Taq polymerase—0.3 µl (5U/µl), BSA (10 mg/ml)—0.5 µl, forward primer (10 µM)—1 µl, reverse primer (10 µM)—1 µl, nematode DNA(10 ng)—1 µl and double-distilled sterile H_2O 11.7 µl in a final 25 µl of reaction mixture. PCR conditions included an initial denaturation step of 95 °C for 10 min, followed by 35 cycles of 94 °C for 45 s, 62 °C for 45 s and 72 °C for 1 min, and finished with one cycle at 72 °C for 10 min. Primer pairs used were: Forward 5′-TTGATTACGTCCCTGCCCTTT-3′ and reverse 5′-TTCACTCGCCGTTACTAAGG-3′ (Vrain et al. 1992). This primer set amplifies complete ITS regions of rDNA, including complete ITS1, ITS2, and 5.8S rDNA subunit and partial 18S and 28S. Gels were stained with ethidium bromide and gel images were captured with a Syngene Gel Documentation System (Cambridge, United Kingdom). Sanger dideoxy sequencing was done from Barcode Life Sciences, Hyderabad. The partial ITS

sequences of CICR-HI-MN (Accession No. ON965330) and CICR-HI-CL (Accession No. ON965310) strains were used to retrieve the homologous ITS sequences from different species of genus *Heterorhabditis* using National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLASTn) (Altschul et al. 1990). Multiple sequence alignment was generated, using the clustalW program and subsequently Maximum Likelihood (ML) phylogenetic tree was constructed using Tamura–Nei model, with 1000 bootstrap replications in MEGA11 (Tamura et al. 2021).

Virulence of EPN strains CICR-HI-MN and CICR-HI-CL against *Spodoptera frugiperda*

Virulence of *H. indica* strains (CICR-HI-MN and CICR-HI-CL) was evaluated against 3rd, 4th and 5th instar larvae of *S. frugiperda* (Additional file 2: Fig. S2). Ten larvae of each instar were placed in 25 well multi-cell plates (Innovative Biosciences Private Limited, Nagpur, India) filled with 1 ml of semi-synthetic diet per cell as a source of food. Each larva was exposed to 100 µl of nematode suspension spread directly on larval surface. A preliminary virulence test was conducted using a wider dose range (50, 100 and 200 IJs/larva) to ascertain the mortality. Subsequently the doses were fine-tuned with total of nine concentrations viz., 10, 20, 40, 60, 80, 100, 120, 140, 160 IJs/larva with water control. All the plates were incubated at 25 ± 2 °C and larval mortality was recorded on daily basis up to 4 days. Each treatment consisted of 50 larvae ($n = 10$/replication; five replications per treatment). No mortality was observed for all control setups.

Reproduction potential of EPN strains CICR-HI-MN and CICR-HI-CL on *Spodoptera frugiperda*

The nematode infected cadavers ($n = 3$; 5 replications) of 3rd, 4th and 5th instar larvae were removed from rearing trays and transferred to the White trap for the emergence of IJs from the cadavers (White, 1927) (Additional file 2: Fig. S1). Water suspension containing IJs were transferred to a tissue culture flask of 500 ml capacity (Tarson, Kolkata, India). The suspension taken out was subjected to nematode population count by observing 100 µl suspension under stereo zoom binocular microscope (Leica Microsystems, Wetzlar, Germany). The total quantity of nematode suspension taken out from the Petri plate was noted and instar-wise total population count was calculated and converted to unit of IJs/larva. These observations for population count were taken for all treatments and replications of two different strains separately (Aryal et al. 2022). The pictures of IJs multiplying inside the body of 4th and 5th larval instars were captured using stereo zoom binocular microscope (Leica Microsystems, Wetzlar, Germany) (Additional file 2: Fig. S3). IJs counting were done 10 days after inoculation until 30 days when no IJs emerged from the cadavers.

Statistical analysis

Experimental data recorded on larval mortality was subjected to probit analysis (Finney 1971) using Polo Plus (Version 2.0; LeOra software) for calculation of LC_{50}, LC_{90} and LC_{95} values (Thube et al. 2022). The fiducial limits were taken at 95% confidence interval. The data on mean reproduction potential against various larval instars and across concentrations were analyzed using one way ANOVA in WASP (Web Agri Stat Package) software. Web-based Agricultural Statistics Package (WASP, version 2.0) developed by ICAR-Central Coastal Agricultural Research Institute, Goa, India which is an open source software available online at http://www.ccari.res.in/waspnew.html.

Results

Morphological and molecular characterization

Both the strains of *H. indica* (CICR-HI-CL and CICR-HI-MN) were characterized based on morphological and molecular parameters. Morphological parameters of males and IJs were within the reported range (Poinar et al. 1992) and their identity as *H. indica* was confirmed (Tables 1 and 2; Fig. 1). For molecular characterization the ITS regions of rDNA, including complete ITS1, ITS2, 5.8S rDNA subunit, partial 18S and 28S were PCR amplified and sequenced. Generated sequences were BLASTn-searched to compare with all sequences deposited in the NCBI (Altschul et al. 1990) for closely related species. All obtained sequences were trimmed, annotated and then deposited at the NCBI GenBank. The amplicons of 1032 bp for CICR-HI-CL strain and 902 bp for CICR-HI-MN strain were deposited in NCBI GenBank with accession No. ON965310 and ON965330, respectively. The NCBI-BLAST analysis of the sequences revealed that CICR-HI-CL was 99% similar to *H. indica* strain CICR-COTNG2 (Accession No. JQ359018) isolated from cotton soils, while CICR-HI-MN showed 99% similarity with *H. indica* isolate DH13 (Accession No. MK271285) isolated from Uttar Pradesh, India. In the phylogenetic tree, both the sequences also showed a tight clustering with the Indian *H. indica* isolates, separate from other species of *Heterorhabditis* (Fig. 2), thus suggesting that the identified and evaluated species of *Heterorhabditis* in the present study belong to the *H. indica*.

Virulence of EPN strains CICR-HI-MN and CICR-HI-CL against *Spodoptera frugiperda*

Inoculation of various concentrations of EPN suspensions showed significantly high mortality at 72 and 96 h. Instar-wise mortality data of various EPN concentrations

Table 1 Morphometric data for *H. indica* (CICR-HI-CL and CICR-HI-MN strains) males compared with original description

Isolate	Body length	Body width	Tail length	Anal body width	Distance to Ex. Pore	Distance to nerve ring	Length of esophagus	Reflexion of testis	Gubernaculum length	Spicule length
CICR-HI-CL	715 ± 15	42 ± 0.7	30 ± 0.6	22 ± 1.0	130 ± 4.0	76 ± 3.9	105 ± 6.6	95 ± 4.5	21 ± 0.8	43 ± 2.9
	610–735	41–44	29–31	21–25	122–136	72–80	93–110	85–96	19–22	41–46
CICR-HI-MN	675 ± 19	38 ± 0.8	28 ± 0.7	21 ± 0.8	119 ± 3.2	75 ± 3.7	92 ± 5.8	82 ± 3.9	22 ± 0.9	40 ± 2.4
	590–720	37–42	27–31	20–24	112–127	72–82	90–99	78–85	20–23	39–44
Poinar et al. (1992)	721 ± 64	42 ± 7	28 ± 2	23 ± 8	123 ± 7	75 ± 4	101 ± 4	91 ± 26	21 ± 3	43 ± 3
	573–788	35–46	24–32	19–24	109–138	72–85	93–109	35–144	18–23	35–48

All measurements are in µm and expressed as means ± standard deviation (range) ($N = 20$)

Table 2 Morphometrics data for *H. indica* (CICR-HI-CL and CICR-HI-MN strains) infective juveniles compared with original description

Isolate	Body length	Body width	Tail length	Distance from anterior end to excretory pore	Distance to nerve ring	Length of esophagus	Anal body diameter
CICR-HI-CL	555 ± 19	19 ± 0.7	102 ± 4.1	103 ± 3.5	82 ± 3.6	120 ± 3.4	12 ± 0.9
	520–600	18–21	99–108	92–108	79–90	113–132	11.0–13.0
CICR-HI-MN	510 ± 20	21 ± 0.6	96 ± 5.0	95 ± 4.0	80 ± 3.0	115 ± 3.3	11.5 ± 0.8
	490–585	19–22	93–100	91–100	75–88	100–129	11–13.0
Poinar et al. (1992)	528 ± 26	20 ± 6	101 ± 6	98 ± 7	82 ± 4	117 ± 3	–
	479–573	19–22	93–109	88–107	72–85	109–123	

All measurements are in µm and expressed as means ± standard deviation (range) ($N = 20$)

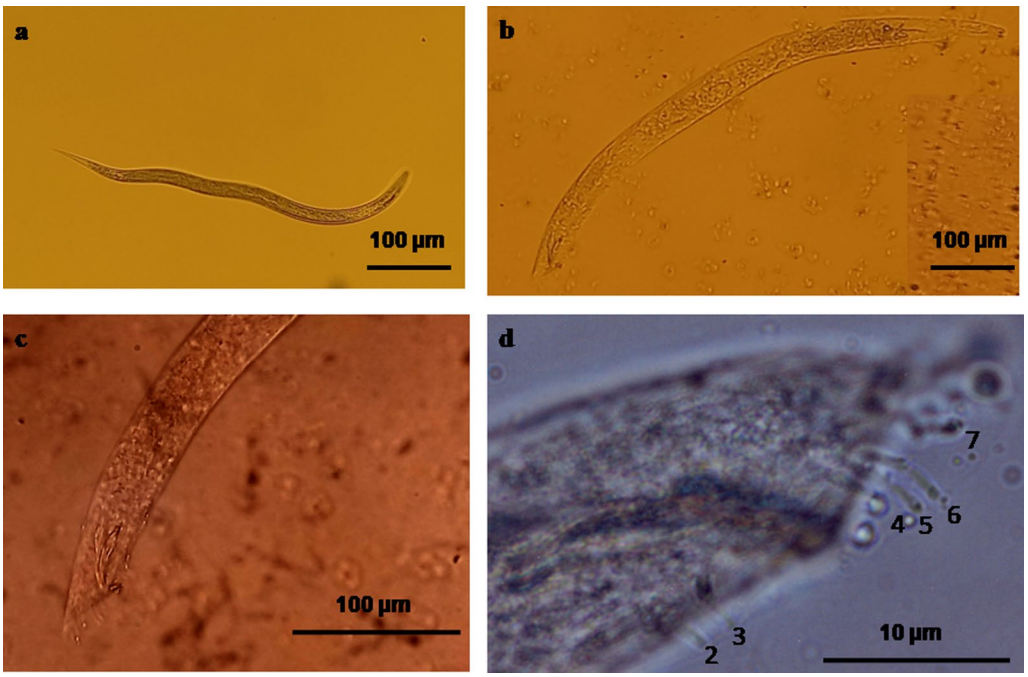

Fig. 1 *H. indica* **a**. Infective juvenile; **b**. Male; **c**. Tail end of male; **d**. Pattern of bursal papillae

for CICR-HI-MN strain is presented in Table 3. The *in vitro* efficacy assay of CICR-HI-MN strain against different instars of *S. frugiperda* clearly showed that 3rd instar was relatively more susceptible with LC_{50} value (48.91, 35.16 IJs/larva) and LC_{95} values (215.96, 162.45 IJs/larva) at 72 and 96 h. post-inoculation, respectively. The CICR-HI-CL strain was more virulent, with LC_{50} value (21.65, 13.79 IJs/larva) and LC_{95} values (119.80, 86.03 IJs/larva) at 72 and 96 h. post infection, respectively (Table 3). Comparatively 5th instar larvae recorded a higher LC_{50} (71.04, 55.64 IJs/larva) and LC_{95} values (618.58, 518.43 IJs/larva) at 72 and 96 h. post infection, respectively, for CICR-HI-MN strain (Table 3). The 5th instar larvae of *S. frugiperda* found to be more susceptible to CICR-HI-CL strain than to CICR-HI-MN strain with LC_{50} (59.20, 41.82 IJs/larva) and LC_{95} values (540.32, 398.31 IJs/larva) at 72 and 96 h. post infection, respectively (Table 3). A strain CICR-HI-CL proved more virulent with almost two times reduced LC_{50} and LC_{95} values than CICR-HI-MN against 3rd instar larvae (Table 3).

Reproductive potential of EPN strains CICR-HI-MN and CICR-HI-CL on *Spodoptera frugiperda*

Results of the study indicate a successful reproduction of *H. indica* strains on all the larval instars of *S. frugiperda*. The reproduction potential of both the strains varied significantly among different instars of *S. frugiperda*. The number of IJs emerged from larval cadavers of 3rd and 4th instars did not differ significantly in both strains. However, the reproduction

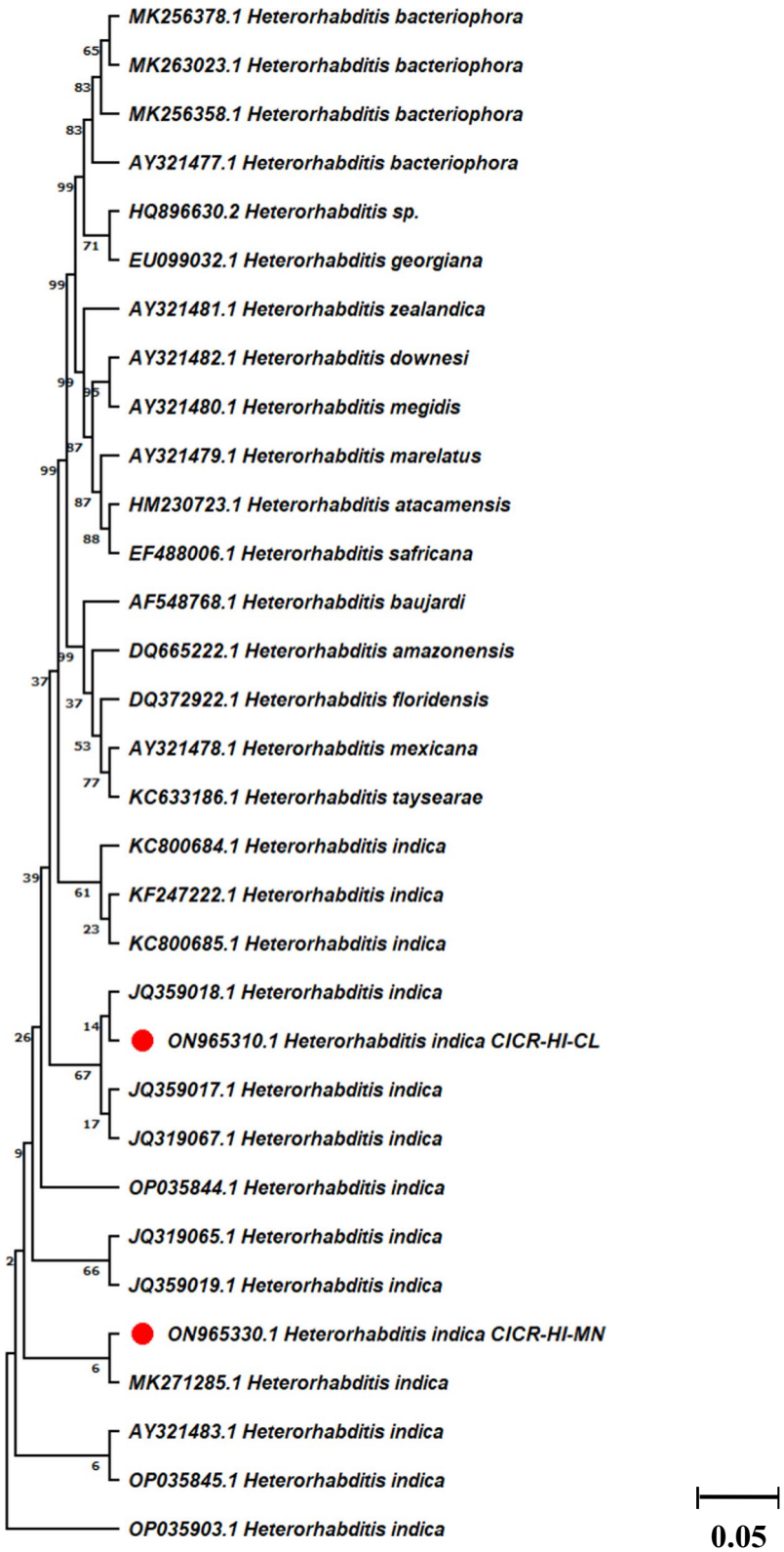

Fig. 2 Maximum likelihood based phylogenetic tree of *Heterorhabditis* spp. based on the analysis of ITS sequences with bootstrap values of 1000 replicates. GenBank accession numbers are given prefix to the *Heterorhabditis* species

Table 3 Dose–mortality response of third, fourth and fifth-instar larvae of *S. frugiperda* to *H. indica* strains at 72 and 96 h after treatment ($n=50$) under laboratory conditions expressed as lethal concentration (LC)

CICR-HI-MN strain		Lethal concentrations 72 h after treatment ($n=50$)					Lethal concentrations 96 h after treatment ($n=50$)				
Larval instar	DF[a]	Slope ± SE[b]	χ^{2c}	LC_{50}[d] (FL)[e]	LC_{90}[d] (FL)[e]	LC_{95}[d] (FL)[e]	Slope ± SE[b]	χ^{2c}	LC_{50}[d] (FL)[e]	LC_{90}[d] (FL)[e]	LC_{95}[d] (FL)[e]
3rd	7	2.55 ± 0.22	12.95	48.91 (38.34–59.91)	155.57 (119.02–235.71)	215.96 (156.50–364.41)	2.48 ± 0.21	5.82	35.16 (29.89–40.43)	115.86 (98.05–143.01)	162.45 (132.83–211.49)
4th	7	2.40 ± 0.21	16.38	52.36 (39.44–66.31)	178.83 (129.39–310.93)	253.32 (171.46–509.25)	2.18 ± 0.20	9.48	33.21 (25.22–41.13)	128.64 (99.57–186.39)	188.83 (138.24–304.10)
5th	7	1.75 ± 0.19	5.77	71.04 (60.14–84.21)	383.54 (273.63–633.21)	618.58 (408.72-1153.98)	1.70 ± 0.18	3.92	55.64 (46.38–65.90)	316.67 (230.55–503.79)	518.43 (349.61–931.73)
CICR-HI-CL strain		**Lethal concentrations 72 h after treatment ($n=50$)**					**Lethal concentrations 96 h after treatment ($n=50$)**				
Larval instar	**DF[a]**	**Slope ± SE[b]**	**χ^{2c}**	**LC_{50}[d] (FL)[e]**	**LC_{90}[d] (FL)[e]**	**LC_{95}[d] (FL)[e]**	**Slope ± SE[b]**	**χ^{2c}**	**LC_{50}[d] (FL)[e]**	**LC_{90}[d] (FL)[e]**	**LC_{95}[d] (FL)[e]**
3rd	7	2.21 ± 0.20	4.24	21.65 (17.30–25.91)	82.10 (68.55–102.84)	119.80 (96.42–159.66)	2.07 ± 0.22	4.59	13.788 (10.06–17.41)	57.41 (47.24–72.96)	86.03 (68.27–117.50)
4th	7	2.13 ± 0.20	4.24	25.46 (20.54–30.30)	101.89 (84.47–129.38)	150.96 (120.08–205.07)	1.62 ± 0.19	4.64	14.15 (9.48–18.73)	88.00 (69.54–120.39)	147.73 (109.66–227.64)
5th	7	1.71 ± 0.18	8.36	59.20 (46.63–74.05)	331.55 (221.25–650.29)	540.32 (328.02–1262.60)	1.68 ± 0.18	7.51	41.82 (32.02–52.02)	242.11 (170.79–419.46)	398.31 (257.36–808.50)

Data analyzed using PoloPlus software; [a]Degrees of freedom; [b]Slope ± SE (standard error); [c]$\chi 2$ indicates a good fit of the line to the data; [d]lethal concentrations are expressed in IJs/larva; [e]FL (fiducial limits)

potential on 5th instar larvae differed significantly than the other two instars at all the inoculation concentrations [For concentration 10 IJs/larva: $F=175.51$; df = 2; $P<0.0001$; 20 IJs/larva: $F=202.77$; df = 2; $P<0.0001$; 40 IJs/larva: $F=177.71$; df = 2; $P<0.0001$; 60 IJs/larva: $F=178.47$; df = 2; $P<0.0001$; 80 IJs/larva: $F=173.23$; df = 2; $P<0.0001$; 100 IJs/larva: $F=212.53$; df = 2; $P<0.0001$; 120 IJs/larva: $F=205.67$; df = 2; $P<0.0001$; 140 IJs/larva: $F=205.00$; df = 2; $P<0.0001$; 160 IJs/larva: $F=206.73$; df = 2; $P<0.0001$] (Fig. 3). The reproductive potential was proportionate to the number of IJs inoculated. A higher reproduction potential of CICR-HI-MN was reported compared to CICR-HI-CL strain (Table 4). The larval mortality was influenced by initial inoculation of IJs that intern influence the reproduction. Reproduction potential of CICR-HI-MN on 3rd instar larvae differed significantly ($F=57.35$; df = 8; $P<0.001$) in the case of higher concentrations (120–160 IJs/larva)

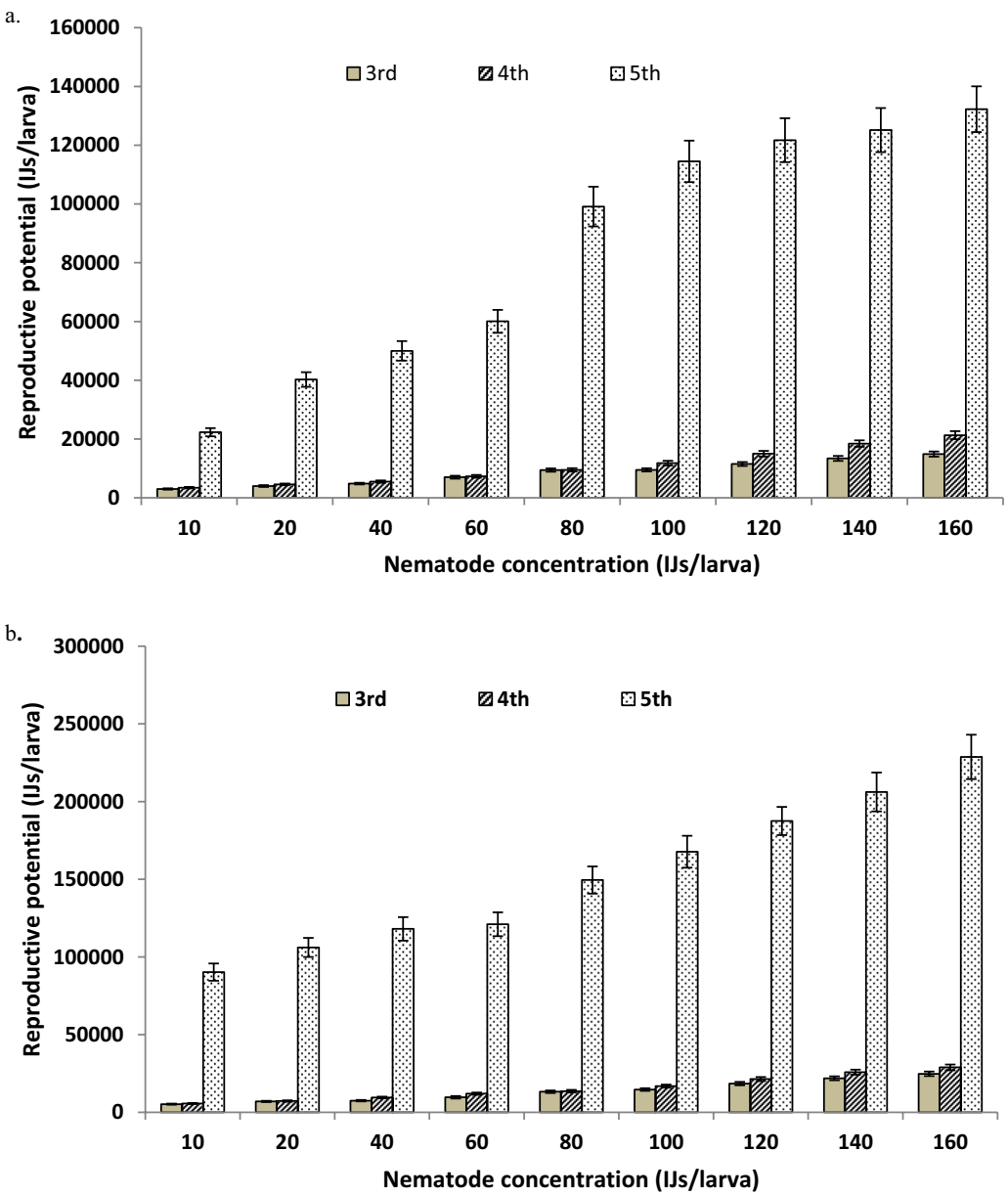

Fig. 3 Reproductive potential of *H. indica* strains on different larval instars *of S. frugiperda* in laboratory assay. Error bars indicate standard error of mean (SEM). **a**. CICR-HI-CL strain, **b**. CICR-HI-MN strain. No significant difference was observed across concentrations in reproductive potential of 3rd and 4th instar, however 5th instar differed significantly at $P \leq 0.01$ level of significance

Table 4 Reproductive potential of *H. indica* infective juveniles emerged under different treatments imposed on 3rd, 4th and 5th instar larvae of *Spodoptera frugiperda*

No. of IJs/larva	CICR-HI-MN strain number of infective juveniles emerged/larva			CICR-HI-CL strain number of infective juveniles emerged/larva		
	3rd Instar	4th Instar	5th Instar	3rd Instar	4th Instar	5th Instar
10	5250 ± 370.05f	5665 ± 344.47g	90,240 ± 5545.33f	3000 ± 231.59f	3435 ± 252.80g	22,310 ± 1400.07f
20	7000 ± 406.01ef	7250 ± 476.07fg	106,120 ± 6141.06ef	4000 ± 313.22ef	4560 ± 299.05g	40,250 ± 2488.88e
40	7500 ± 469.76ef	9550 ± 554.91ef	118,055 ± 7567.10ef	4830 ± 289.03e	5500 ± 404.42fg	50,000 ± 3326.65de
60	9690 ± 815.16e	12,000 ± 735.19de	121,050 ± 7675.08e	7000 ± 496.79d	7300 ± 481.17ef	60,055 ± 3899.36d
80	13,250 ± 831.95d	13,600 ± 852.82d	149,500 ± 8746.48d	9430 ± 565.66c	9500 ± 571.67de	99,115 ± 6763.84c
100	14,665 ± 855.97d	16,875 ± 982.94c	167,700 ± 10,278.51cd	9500 ± 552.29c	11,800 ± 791.07d	114,500 ± 7059.21bc
120	18,500 ± 1092.58c	21,435 ± 1276.96b	187,500 ± 9077.77bc	11,500 ± 666.13b	15,000 ± 959.61c	121,700 ± 7474.40ab
140	21,900 ± 1267.89b	25,840 ± 1627.70a	206,165 ± 12,504.72ab	13,400 ± 850.18a	18,450 ± 1096.97b	125,140 ± 7506.87ab
160	24,800 ± 1465.07a	28,925 ± 1847.17a	228,740 ± 14,281.06a	14,875 ± 879.91a	21,300 ± 1371.55a	132,250 ± 7780.73a

Values in the table indicate mean reproductive potential (± Standard error of mean), different lower case letters indicate statistical significance of means compared across concentrations within same larval instar using WASP software in completely randomized design at $P \leq 0.01$ level of significance

at 96 h. post-inoculation. However the difference in the reproduction potential of CICR-HI-MN was non-significant at higher concentrations (140–160 IJs/larva) in 4th ($F = 57.43$; df = 8; $P < 0.001$) and 5th ($F = 25.52$; df = 8; $P < 0.001$) instars larvae. The reproduction potential of CICR-HI-CL strain on 4th instar larvae at higher concentrations (120–160 IJs/larva) differed significantly from each other ($F = 65.91$; df = 8; $P < 0.001$). However, the reproduction potential of the strain on 3rd and 5th instar was non-significant at higher two (140–160 IJs/larva; $F = 52.27$; df = 8; $P < 0.001$) and three (120–160 IJs/larva; F = 52.17; df = 8; $P < 0.001$) concentrations, respectively.

Discussion

The use of EPN in insect pest management has been successfully demonstrated across the globe (Kumar et al. 2022). Isolating and identifying native EPN strains is a key step in attaining effective pest control due to their better adaptability to the local environment (Nikdel and Niknam 2015). *In vitro* evaluation remains an important step in identifying the most virulent strain of EPN that can be further utilized for field application. In the present study, efforts were made to isolate, characterize and evaluate the native virulent strain of EPN against *S. frugiperda*. This investigation revealed that both the strains significantly vary with respect to virulence against larval stage of *S. frugiperda*. The strain CICR-HI-CL was found more efficient than CICR-HI-MN.

The present investigation revealed that the 3rd and 4th instar larvae of *S. frugiperda* were more susceptible than 5th instar. Studies on similar line showed that 3rd instar larvae of *S. frugiperda* were highly susceptible to *H. indica* and *Steinernema* sp. (Garcia et al. 2008). In complementary to the present investigations, identified both strains of *H. indica* were more virulent against 3rd instar larvae of *S. frugiperda*. In agreement to this, strain CICR-HI-CL proved most virulent with lowest LC_{95} value (86.03 IJs/larva) at 96 h. post treatment. Obtained findings about higher virulence of EPN against 3rd instar larvae are in conformity with other researchers (Patil et al. 2022).

Virulence is one of the important traits of a good biological control agent. Patil et al. 2022, reported *H. indica* 1 NBAIIH38 as the most virulent strain against *S. frugiperda* with 3rd instar LC_{50} value of 44 IJs/larva 5 days after treatment. However, the studied strains (CICR-HI-CL and CICR-HI-MN) proved more virulent with the reduction in LC_{50} value by 3.19 times (13.79 IJs/larva) and 1.25 times (35.16 IJs/larva), respectively, at 4 days after treatment. Evaluation of IJs of EPN caused a complete mortality of 3rd instar larvae of *S. frugiperda* at concentration of 280 IJs/larva (*Steinernema* sp.) and 75% mortality with concentration of 400 IJs/larva (*H. indica*) (Garcia et al. 2008). As low as 200 IJs/larva of *S. arenarium* and *Heterorhabditis* sp. RSC02 caused 100 and 97.6% mortality rates under laboratory conditions (Andalo et al. 2010). In confirmation to these findings in present study, LC_{95} values were 215.96; 162.45 IJs/larva for CICR-HI-MN strain and 119.80; 86.03 IJs/larva for CICR-HI-CL strain at 72 and 96 h post-inoculation. Larval and pupal stages of *S. frugiperda* were found highly susceptible to three EPN species viz., *S. carpocapsae*, *H. indica* and *S. longicaudum* (Acharya et al. 2020). Pre-pupae of *S. frugiperda* were very effectively managed by *Steinernema carpocapsae* and *S. riobravis* (Molina-Ochoa et al. 2003).

Ibrahim et al. (2019) reported that virulence of *H. zealandica* against *G. mellonella* is positively correlated with initial concentration of inoculation. Radhakrishnan and Shanmugam (2017) reported that the mortality rate of

S. litura is directly proportional to the initial concentration of *H. indica* and *Steinernema glaseri*. However, *H. indica* proved to be more effective than *S. glaseri*. Similar results were reported that showed direct relationship of nematode concentration with insect mortality (Javed et al. 2022). Obtained results about increasing concentration of IJs/larva with mortality of *S. frugiperda* are in agreement with these reports. The reproductive potential is a measure of suitability of target host insect for multiplication and also an indicator for its persistence under field conditions (Blanco-Pérez et al. 2017). Higher reproductive potential of EPN is attributed to virulence against the target pest with success of perpetuation under field conditions (Patil et al. 2022). But in contrast our present study reported CICR-HI-CL to be more virulent with less reproductive potential than CICR-HI-MN. Among the various factors, preferable developmental stage of the host insect is a key factor governing reproductive potential of EPN (Park et al. 2001). The present study reported that 5th instar larvae were most preferred and suitable stage for reproduction of both the strains with production of 228,740 ± 14,281.06 IJs/larva (CICR-HI-MN) and 132,250 ± 7780.73 IJs/larva (CICR-HI-CL) at inoculation concentration of 160 IJs/larva. The results about higher reproduction potential in later instars are in line with findings of Acharya et al. 2020. Our findings about higher initial concentration resulted in higher reproduction potential are in concurrence with Kumar et al. (2022). Successful evaluation of these native strains under field conditions would be important to manage the menace of this polyphagous pest in Indian landscapes.

Conclusions

In the present study, two native strains of *H. indica* (CICR-HI-CL and CICR-HI-MN) were isolated, characterized and evaluated against larval instars of the fall armyworm, *S. frugiperda* provided with promising results. Both strains of *H. indica* showed a great potential in managing the late larval stages of *S. frugiperda* under laboratory conditions. The concealed feeding nature of fall armyworm coupled with soil dwelling pupal stage makes EPN as excellent option for its management as it protects nematode from direct exposure to the sunlight. In future whorl application of water/gel/alginate bead based EPN formulation in maize may prove as an excellent management strategy.

Abbreviations

CICR-HI-CL: Central Institute for Cotton Research-*Heterorhabditis indica*-curry leaf strain; CICR-HI-MN: Central Institute for Cotton Research-*Heterorhabditis indica*-mango strain; EPN: Entomopathogenic nematodes; *H. indica*: *Heterorhabditis indica*; IJs: Infective juveniles; NCBI: National Center for Biotechnology Information; *S. frugiperda*: *Spodoptera frugiperda*; WASP: Web Agri Stat Package software.

Supplementary Information

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Additional file 1: **Table S1**. Composition of artificial diet for fall armyworm, *S. frugiperda*. **Table S2**. Composition of artificial diet for grater wax moth, *Galleria mellonella*

Additional file 2: **Fig. S1**. *Heterorhabditis indica* a. Infected *Galleria* larvae turned brick red in color; b. Infected *Galleria* larvae on White trap; c. *Spodoptera frugiperda* 5th instar infected larvae; d. Infected *Spodoptera frugiperda* larvae on White trap. **Fig. S2**. Larval instars of *Spodoptera frugiperda* used for evaluation a. Third instar b. Forth instar c. Fifth instar. **Fig. S3**. *Heterorhabditis indica* infective juveniles (IJs) multiplication inside a. Fourth and b. Fifth instar larvae of *Spodoptera frugiperda*

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Author contributions

SPS, NGN, VKB, NVL, SV, YGP and DBI involved in conceptualization and conducted the experiments; SPS, NGN, DBI, VKB and SV in methodology; SPS, NGN, SHT, YGP and SV involved in analyzing the data, writing original draft preparation, and editing. All authors have read and agreed to the published version of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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