RESEARCH

Open Access

Susceptibility of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), to four species of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from Mizoram, North-Eastern India

Hrang Chal Lalramnghaki[1,2], Lalramliana[1*] , Hmar Tlawmte Lalremsanga[2], Vanlalhlimpuia[3], Mary Lalramchuani[1,2] and Vanramliana[1]

Abstract

Background: Outbreak of the fall armyworm *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) occurred in Mizoram, North-Eastern India. The infestation spread in the entire state covering a total area of around 2840 hectares of maize cultivated land. Entomopathogenic nematodes (EPNs) represent potential candidates for the biological control of *S. frugiperda*. In the study, the susceptibility of the pest against 4 locally isolated EPN species *Heterorhabditis indica*, *H. baujardi*, *Steinernema sangi* and *S. surkhetense* was evaluated.

Results: The results indicated that all the isolated EPN species showed a high rate of larvicidal and pupicidal activities against the pest. Mortality between 43.75–100.00 and 25.00–100.00% of 3rd and 5th larval instars, respectively (at concentrations 10–800 IJs/larva), and 37.50–68.75% mortality of pupae (at concentrations 200–1600 IJs /pupa) were found after exposure to the EPN species. The mortality rate of the pest showed significant variations with life stages of the host insect, nematode concentrations and incubation time. Based on the median lethal concentration (LC_{50}), *H. indica* was the most pathogenic species, followed by *S. sangi*, *H. baujardi* and *S. surkhetense*. The LC_{50} values of *H. indica* at 72 h post-incubation were 20.26 and 62.07 IJs/larva for the 3rd and 5th larval instars, respectively, and 913.34 IJs/ pupa. The penetration assay showed that *H. indica* had the highest penetration rate into the hosts, 27.24, 21.30 and 20.00% in the 3rd, 5th larval instars and pupae, respectively. Furthermore, all the EPN isolates were capable of successful multiplication inside the cadaver of *S. frugiperda* that showed significant differences with the EPN isolates and life stages of the pest. Among the isolates, *H. indica* showed the highest multiplication rates, 17,692.25 ± 2103.59, 8345.63 ± 785.34 and 79,146.38 ± 5943.73 IJs per 3rd instar larva, 5th instar larva and pupa, respectively.

Conclusions: The study revealed that the 4 species of EPNs showed a high potency against *S. frugiperda*, thereby having the potential to be developed as a biological control agent against the pest. Moreover, the isolated EPN

*Correspondence: lrl_zoo@yahoo.co.in
[1] Department of Zoology, Pachhunga University College, Mizoram
University, Aizawl 796001, India
Full list of author information is available at the end of the article

Lalramnghaki *et al. Egypt J Biol Pest Control* (2021) 31:110

Page 2 of 11

species could potentially serve as alternatives for chemical insecticides and could further be incorporated into the Integrated Pest Management (IPM).

Keywords: *Spodoptera frugiperda*, Entomopathogenic nematodes, Potential, Biological control, LC_{50}

Background

Maize (*Zea mays* L.) is one of the most important cultivated cereal crops in the world and the third most important agricultural crop in India after rice and wheat (Joshi et al. 2005). In India, a total loss of 13.20% of the crop has been estimated due to insect pests and diseases, thereby adversely affecting the production of maize in the country (Kumar et al. 2014).

Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith, 1797) (Family: Noctuidae) is an invasive lepidopteran pest native to America (Todd and Poole 1980). It is a polyphagous pest of more than 350 species of plants, causing serious damage to economically important cultivated crops such as maize, rice, sorghum, sugarcane, cotton and other vegetable crops (CABI 2020). Subsequently, FAW was reported to invade Central and Western Africa in the year 2016 (Goergen et al. 2016), the Indian subcontinent in Asia in the year 2018 (Sharanabasappa et al. 2018) and currently with worldwide distribution (CABI 2020). In India, FAW was firstly reported in maize fields in Shivamogga, Karnataka, in May 2018 (Sharanabasappa et al. 2018) with subsequent scientific reports from other regions of the country (Repalle et al. 2020).

The use of chemical insecticides is the most effective approach for the control of FAW (Belay et al. 2012). However, the pest was reported to develop resistance against major classes of commonly used insecticides (Zhu et al. 2015) and *Cry* proteins of *Bacillus thuringiensis* Berliner (Bacillaceae) as well (Murúa et al. 2019); therefore, safer, eco-friendly control strategies need to be developed and further implemented. Biological control offers a promising strategy against a wide range of insect pests, and biopesticides being environmentally safer are potential alternatives to chemical pesticides. Like other biological control agents, entomopathogenic nematodes (EPNs) are potential and promising agents for controlling insect pests (Lacey and Georgis 2012). EPNs of the family Steinernematidae and Heterorhabditidae are parasitic nematodes of insects with natural occurrence in the soil. Many researchers have reported the application of indigenous EPN strains with better adaptation to the prevailing local climatic conditions in comparison with exotic strains (Bedding 1990). The study on the susceptibility of *S. frugiperda* to EPNs has been conducted by some workers (Viteri et al. 2018). However, to date, no studies have been conducted on the pathogenicity of the EPN isolates, *Heterorhabditis baujardi*, *Steinernema sangi* and *S. surkhetense* against *S. frugiperda*.

The present study aimed to evaluate the susceptibility of *S. frugiperda* to locally isolated EPN species.

Methods

Collection of *S. frugiperda*

Larvae of FAW were collected from infested maize plants and reared in the laboratory using their natural diet at 26 ± 2 °C. Different life stages were obtained for morphological and molecular identification of the pest. In addition, 3rd and 5th larval instars and pupal stage were selected for the evaluation of their susceptibility against the locally isolated EPN species.

Morphological and molecular identification

Morphological characteristics of different life stages of *S. frugiperda*, including female genitalia were examined and compared to previous studies of the pest (Ganiger et al. 2018). Adult females were dissected under an Olympus CX41 microscope and the female genitalia was studied for further identification.

Molecular characterization of the pest involved extraction of DNA from adult insects by using QIAamp DNA mini kit (Qiagen). The mitochondrial gene, cytochrome C oxidase subunit I (COI) was amplified by using a ProFlex™ 3 × 32-Well PCR System (Applied Biosystems). The COI (M1–M6 partition) primers, LCO 1490 (5′-GGT CAACAAATCATAAAGATATTGG-3′) forward and HCO 2198 (5′-TAAACTTCAGGGTGACCAAAAAAT CA-3′) reverse (Folmer et al. 1994) were used for amplification of the gene. PCR conditions were: 1 cycle of 94 °C for 1 min followed by 35 cycles at 94 °C for 60 s; 55 °C for 60 s; and 72 °C for 2 min. The last step was 1 cycle at 72 °C for 10 min. The PCR product was directly sequenced in both directions at AgriGenome Labs Private Limited, Kakkanad, Kerala, India. The sequence was edited using FinchTV 1.4.0 software packages (Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com), aligned using MEGA version X (Kumar et al. 2018) and submitted to NCBI GenBank. Nucleotide sequences of *Spodoptera* along with one out-group sequence (*Helicoverpa armigera*) were retrieved from GenBank. The phylogenetic tree was constructed using maximum likelihood tree (ML), in MEGA X with a total of 1000 bootstrap data sets.

Rearing of *Galleria mellonella* (L.)

Larvae of greater wax moth, *Galleria mellonella* L. were collected from local beekeepers and reared in the laboratory by using their natural diet.

Nematode sources

Four species of locally isolated EPNs, *H. indica*, *H. baujardi*, *S. sangi* and *S. surkhetense*, were used for the study. The 2 species, *S. sangi* and *H. baujardi*, were reported by previous studies of Vanlalhlimpuia et al. (2018). Meanwhile, the study represents the first report of *H. indica* and *S. surkhetense* from Mizoram, India. The nematodes were isolated from soil samples by baiting methods, following Bedding and Akhurst (1975). A survey was done in different areas of Mizoram, NE India, covering undisturbed and disturbed forest areas. From each collection site, a total of 400–500 g of soil sample was collected from a depth of 10–15 cm. The collected soil samples were transferred to the laboratory and immediately baited with the last instar larvae of *G. mellonella* in a container and incubated at room temperature. For 10 consecutive days, larval mortality was checked every 24 h of time and dead larvae were transferred to a modified white trapped as per Kaya and Stock (1997). The nematodes that emerged from the dead cadaver were used for re-infection of fresh host for confirmation. The emerged IJs from the re-infection plate were collected and stored with aerated water in an incubator at 12–15 °C. For the experiment, only freshly emerged IJs from dead cadaver were used.

Susceptibility test

Four species of EPN, *Heterorhabditis indica*, *H. baujardi*, *Steinernema sangi* and *S. surkhetense*, were used against *S. frugiperda* to assess the difference in virulency among the nematode isolates. Meanwhile, 3rd, 5th larval instars and pupae of the pest were selected to assess whether susceptibility varies with advancement in life stages of the host.

Larval mortality assay

The experiment was performed using Petri dish assay, following Kaya and Stock (1997). Different concentrations of nematodes (10, 25, 50, 100, 200, 400 and 800 IJs/larva) were selected, and healthy larvae (3rd and 5th instars) of *S. frugiperda* were used for this assay. A Petri dish (35 × 10 mm) was lined by double-layer Whatman filter paper No. 1., and 0.5 ml of distilled water containing each concentration of nematode was introduced into an individual Petri dish and incubated for 30 min. A single larva was introduced into individual Petri plate pre-inoculated with nematodes. For both larval stages, 8 replicates were set for each concentration of nematodes and EPN isolates, and the experiment was repeated thrice. A Petri dish lined with filter paper wetted only with distilled water was set for each concentration of nematode to serve as a control plate.

Pupal mortality assay

Different concentrations of nematodes (200, 400, 800 and 1600 IJs/pupa) were used to examine the susceptibility of pupa against the EPN isolates. The assay was performed by heating sandy loam soil to dry for 24 h and the moisture content was then adjusted at 15–20% (v/w) by adding distilled water (including pipetted water in nematode suspension). For all the EPN isolates, each of the nematode concentrations was added to an individual Petri dish (35 × 10 mm) filled with pre-wetted soil sample containing burrowed one-day-old pupa of *S. frugiperda*. Control plates and replicates were set as in the larval assay and the experiment was repeated thrice.

For both the assays, the experiments were carried out in an incubator at 28 ± 2 °C and mortality rate was check at 24 h intervals time for 120 h (5 days). Colour change, no movement and the smell emanating from the dead body of larvae and pupae were used for primary confirmation of death due to EPNs. After 48 h of mortality, the dead insects were rinsed with distilled water and individually dissected under an Olympus CX41 microscope for further confirmation.

Host penetration

After 48 h of mortality, dead larvae and pupae from mortality assays were rinsed by distilled water, individually dissected in Ringer's solution and the total number of adult nematodes in the dead cadaver was counted and recorded.

Nematode multiplication in host

A concentration of 200 IJs/insect was used for examining the reproductive potential of the EPN isolates in *S. frugiperda*. The experiment was set up as in mortality assay. Dead larvae (3rd and 5th instar larvae) and pupae were placed individually on a modified white trap for multiplication. The experiment was maintained at 28 ± 2 °C in an incubator. The date of emergence of IJs from a dead cadaver was recorded, and a total emerged IJs were counted at 15–30 days post-emergence from individual plates to record the multiplication rate of the EPN isolates.

Statistical analysis

For all the experimental data, statistical data analysis was performed. One-way analysis of variance (ANOVA) was conducted to determine significant differences (at the

Lalramnghaki *et al. Egypt J Biol Pest Control*   (2021) 31:110

Page 4 of 11

level of $p \leq 0.05$) in the parameters of the experiment. To determine a correlation among different experimental parameters, regression analysis was performed. Values of both median lethal concentration (LC_{50}) and median lethal time (LT_{50}) were calculated by using Probit regression analysis (SPSS 20.0 software).

Results

Morphological and molecular identification of *S. frugiperda*

The analysis of morphological characters along with that of female genitalia strongly suggests that the pest belongs to the species *S. frugiperda* (Ganiger et al. 2018). The generated partial sequence of the mitochondrial COI gene consisting of 677 bp long was deposited in GenBank under accession number MT677868. The BLAST search result of the sequence showed 100% similarity with sequences (MN640599, MK790611, MK318297, MT180097, MT881755 and MN630563) available for *S. frugiperda* in NCBI GenBank. The developed and database sequences of *S. frugiperda* showed an intraspecific (K2P) distance of 0.00%. However, based on the K2P distance, the developed sequence and sequence of closely related species studied: *S. litura* (KX863232), *S. littoralis* (KJ634300.1) and *S. picta* (HQ950412) exhibit interspecific distance of 4–6%. The phylogenetic relationship of the developed sequence and sequences acquired from GenBank given in Fig. 1 revealed that upon comparison, the insect was identified as *S. frugiperda*.

Susceptibility of *S. frugiperda*

The study revealed that larvae and pupae of *S. frugiperda* were highly susceptible to the EPN isolates, *H. indica*, *H. baujardi*, *S. sangi* and *S. surkhetense* (Figs. 2, 3, 4). The overall mortality rate showed significant differences with life stages of the host insect ($F = 14.85$, df $= 2$, 69, $p < 0.001$), nematode concentrations ($F = 4.79$, df $= 6$, 61, $p < 0.001$) and incubation time ($F = 4.002$, df $= 4$, 135, $p < 0.001$). However, non-significant differences in *S. frugiperda* mortality rates were observed among the studied EPN isolates ($p > 0.05$). Overall, the mortality rates of *S. frugiperda* ranged from 43.75 to 100.00% in 3rd instar larvae, 25.00–100.00% in 5th instar larvae and 37.50–68.75% in the pupal stage. Also, the application of nematode concentrations showed a positive correlation with host mortality for all the 4 EPN isolates (values are 0.91, 0.96 and 1.0 in *H. indica*; 0.94, 0.92 and 0.98 in *H. baujardi*; 0.95, 0.91 and 0.99 in *S. sangi*; 0.93, 0.89 and 0.98 in *S. surkhetense*, for 3rd and 5th larval instars and pupa, respectively).

At a concentration of 10 IJs/larva, all the studied EPN species could cause larval mortality after 24 h of incubation (Figs. 2, 3). A further increase in nematode concentration and incubation period resulted in high mortality

of the pest. At a concentration of 100 IJs/larva and incubation periods (24–120 h), mortality rate of the 3rd instar ranged between 62.50–100.00%, 68.75–93.75%, 56–100.00% and 56.25–87.50%, respectively, for *H. indica*, *H. baujardi*, *S. sangi* and *S. surkhetense*. For the same conditions, mortality rate of the 5th larval instar ranged between 50.00–100.00% and 43.75–75.00%, respectively, for *H. indica* and *H. baujardi*; 43.75–81.25% for both *S. sangi* and *S. surkhetense*. Meanwhile, for the same nematode concentration, the isolates *H. indica* and *S. sangi* caused 100 percent mortality for 3rd instar larvae at 96 and 120 h post-incubation, respectively, while *H. baujardi* and *S. surkhetense* caused comparatively low mortality of 87.50 and 93.75%, respectively, at 120 h post-incubation. A 100 percent mortality of the 5th larval instar was initially observed at 100 IJs/larva after 120 h of incubation for *H. indica*. However, for the isolates of *H. baujardi*, *S. sangi* and *S. surkhetense*, the same mortality rate was observed at 200 IJs/larva after 96 h of incubation.

Against the pupal stage, similar mortality patterns were observed with a comparatively low mortality rate as given in the Fig. 4. The highest mortality rate (68.75%) was observed with *S. sangi* at a concentration of 1600 IJs/pupa after 96 h of incubation. With the exposure of 400 IJs/pupa and 72 h post-incubation, the three EPN isolates of *H. indica*, *H. baujardi* and *S. surkhetense* caused 37.50% mortality while *S. sangi* caused low mortality of 31.25%.

LC_{50} and LT_{50}

Based on the calculated LC_{50} values, *H. indica* was the most pathogenic among the EPN isolates with minimum values (Table 1). At 72 h post-incubation, *H. indica* showed LC_{50} values of 20.26 and 62.07 IJs/larva for the 3rd and 5th larval instars, respectively, and 913.34 IJs/pupa. For the same incubation period, *S. surkhetense* was the least virulence that caused the highest LC_{50} values of 35.08 and 80.50 IJs/larva for the 3rd and 5th larval instars, respectively, and 1111.75 IJs/pupa. Meanwhile, at 72 h post-incubation, the LC_{50} values of *S. sangi* were lower (22.64 and 70.07 IJs/larva, respectively) compared to that of *H. baujardi* (30.62 and 96.44 IJs/larva) for the 3rd and 5th larval instars, respectively. However, for the same incubation period, the LC_{50} values of *H. baujardi* (963.44 IJs/pupa) in the pupae was lower than that of *S. sangi* (1106.72.75 IJs/pupa).

The calculated LT_{50} values are given in Table 2. At a concentration of 100 IJs, the LT_{50} value of *H. indica*, *H. baujardi*, *S. sangi* and *S. surkhetense* in the 3rd larval instar was 15.99, 16.12, 14.33 and 17.64 h, respectively. For the same nematode concentration, in the 5th larval instar, a comparatively high LT_{50} value of 21.74, 28.98, 28.90 and 32.39 h was recorded for *H. indica*, *H. baujardi*, *S. sangi* and *S. surkhetense*, respectively. In the case
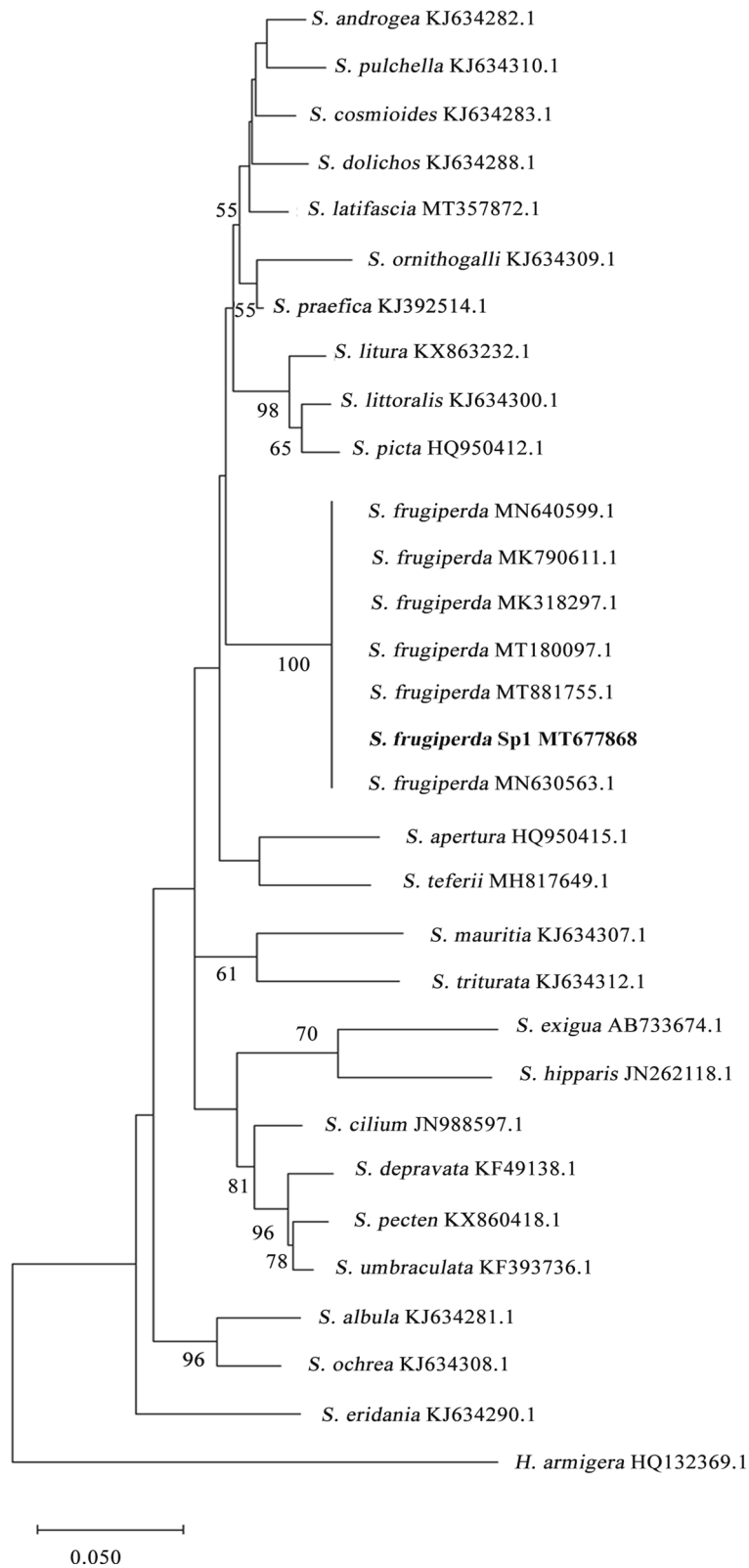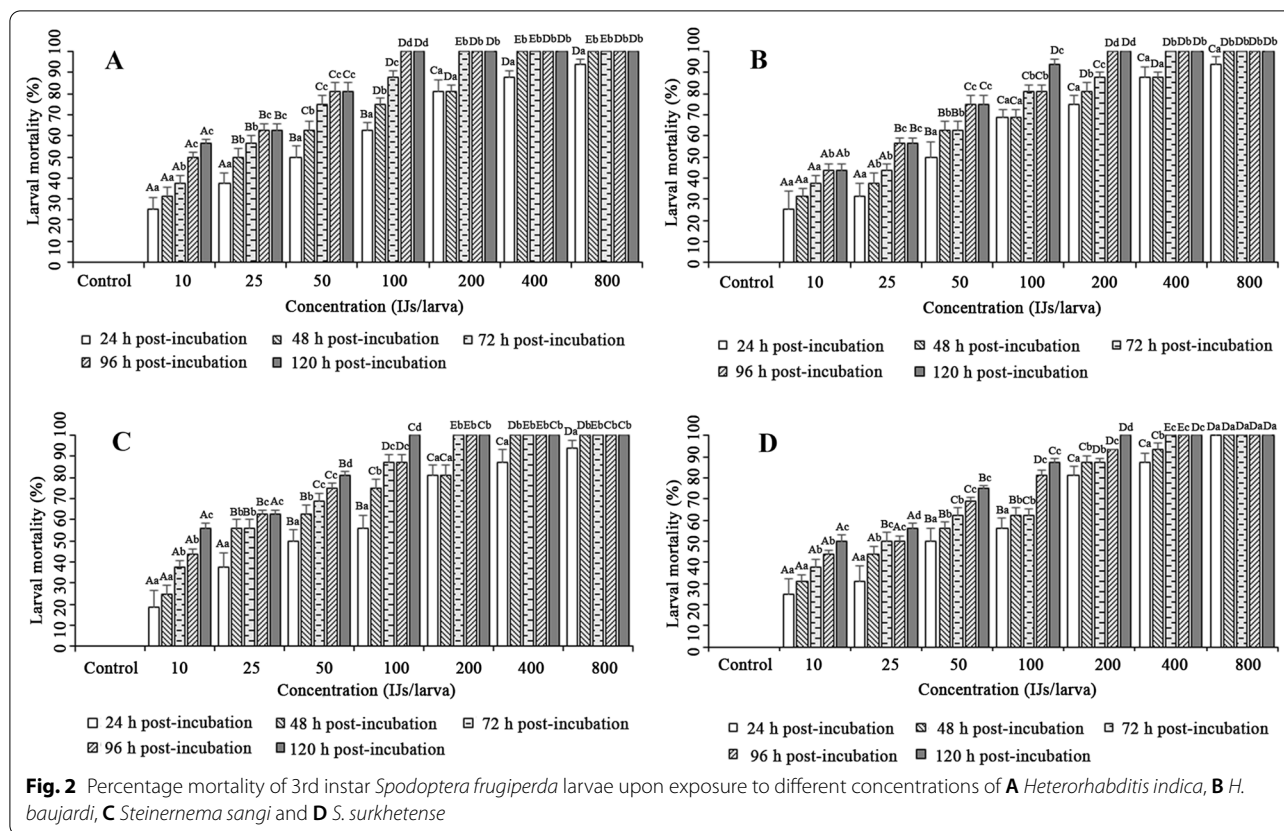
Fig. 1 Phylogenetic relationship of *Spodoptera frugiperda* with other *Spodoptera* spp. based on distance analysis of COI DNA regions. Maximum likelihood tree was constructed by using General Time Reversal model. Numbers at the nodes indicate bootstrap values (> 50%, 1000 replicates). GenBank Accession numbers are given after each species

Fig. 2 Percentage mortality of 3rd instar *Spodoptera frugiperda* larvae upon exposure to different concentrations of **A** *Heterorhabditis indica*, **B** *H. baujardi*, **C** *Steinernema sangi* and **D** *S. surkhetense*

of pupal stage, at a concentration of 400 IJs, LT_{50} value for *H. indica*, *H. baujardi*, *S. sangi* and *S. surkhetense* was 112.00, 114.52, 114.69 and 105.00 h, respectively.

Host penetration

Host penetration (Fig. 5) showed significant differences among the studied EPN isolates ($F = 19.16$, df = 3, 68, $p = 0.00$) and non-significant among life stages of the host insect ($p > 0.05$). Furthermore, the study recorded positive correlation between mean penetration rates and nematode concentrations for all EPN isolates tested ($r = 0.987$ in *H. indica*, $r = 0.97$ in *H. baujardi*, $r = 0.951$ in *S. sangi* and $r = 0.97$ in *S. surkhetense*). Among the 4 studied EPN isolates, the total mean penetration of the 2 heterorhabditids was 27.24, 21.30 and 20.00% for *H. indica* and 25.25, 20.60 and 18.00% for *H. baujardi* in 3rd, 5th instars and pupae, respectively. It showed significantly higher values than the steinernematids, *S. sangi* (17.74, 15.93 and 13.00%) and *S. surkhetense* (19.17, 17.45 and 14.00%) in 3rd, 5th larval instars and pupae, respectively.
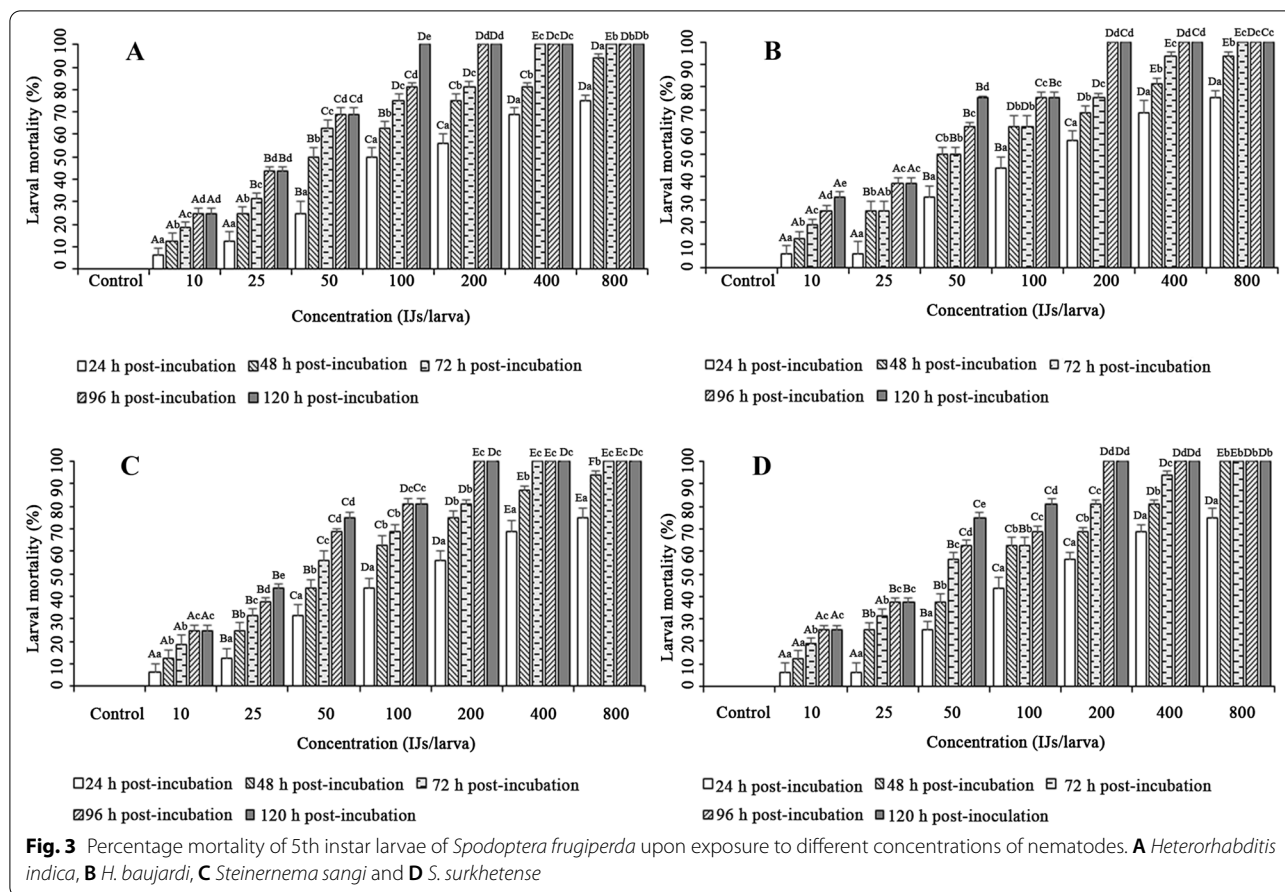
Nematode multiplication in host

The isolated EPNs successfully reproduced inside cadaver of *S. frugiperda* (Fig. 6). Progeny production showed significant variations with life stages of

the host ($F = 228.628$, df = 2, 189, $p = 0.00$) and among the EPN isolates ($F = 3.432$, df = 2, 189, $p = 0.018$). However, progeny production in host insect did not show significant difference within the same EPNs family (Heterorhabditidae and Steinernematidae) ($p > 0.05$). Among the studied EPNs, the highest progeny production was recorded for *H. indica*, followed by *H. baujardi*, *S. surkhetense* and *S. sangi*. Against 3rd instar larvae, a total of $17,692.25 \pm 2103.59$, $16,375.31 \pm 1688.37$, 9862.68 ± 1031.45 and $12,218.63 \pm 1501.93$ IJs/larva was recorded for *H. indica*, *H. baujardi*, *S. sangi* and *S. surkhetense*, respectively. Furthermore, a correspondent total progeny production of 8345.63 ± 785.34, 7357.44 ± 832.56, 5506 ± 608.56 and 4827.38 ± 707.91 IJs/5th instar larva was recorded. In case of pupal stage, comparatively high multiplication rate was recorded as $79,146.38 \pm 5943.73$, $77,999 \pm 7432.20$, $42,747.44 \pm 3934.22$ and $52,383.63 \pm 4331.35$ IJs/pupa *H. indica*, *H. baujardi*, *S. sangi* and *S. surkhetense*, respectively.

Discussions

The study involved evaluation on the susceptibility of *S. frugiperda* to 4 species of EPNs isolated from Mizoram, North-Eastern India. A susceptibility test was performed

Lalramnghaki *et al. Egypt J Biol Pest Control* (2021) 31:110

Page 7 of 11

Fig. 3 Percentage mortality of 5th instar larvae of *Spodoptera frugiperda* upon exposure to different concentrations of nematodes. **A** *Heterorhabditis indica*, **B** *H. baujardi*, **C** *Steinernema sangi* and **D** *S. surkhetense*

by using different concentrations of each EPN isolates against the pest. Mortality of host insect was evaluated based on nematode concentration and incubation period; nematode penetration rate of host insect was evaluated based on nematode concentration. Several workers have conducted scientific studies to evaluate the susceptibility of *S. frugiperda* to different EPN species under laboratory and field conditions. It is not surprising that the susceptibility varied among the different species of the nematodes. Andaló et al. (2010) reported that at a dosage of 200 IJs/5th instar larva of *S. frugiperda*, 96.07 and 100.00% mortality rates were recorded when treated with *Heterorhabditis* sp. and *Steinernema arenarium*, respectively. At 48 h post-incubation, *H. indica* and *S. sangi* caused 75.00% mortality, while *H. baujardi* and *S. surkhetense* caused 68.75% host mortality at a concentration of 200 IJs/5th instar larva. Caccia et al. (2014) reported that concentrations of 50 and 100 IJs of *S. diaprepesi* caused 93.00 and 100.00% mortality rates of last instar larvae of *S. frugiperda* at 144 h post-incubation. However, at the concentration of 50 IJs/5th instar larva, the present study recorded 68.78% host mortality due to *H. indica*, while *S. sangi*, *H. baujardi* and *S. surkhetense*

caused 75.00% mortality at 120 h post-incubation. At 100 IJs/5th instar larva, both *S. sangi* and *S. surkhetense* caused 81.25% mortality, while *H. indica* and *H. baujardi* caused 100.00 and 75.00% mortality, respectively. In another study, Garcia et al. (2008) reported that against 3rd instar larvae of *S. frugiperda*, *Steinernema* sp. (280 IJs/larva) and *H. indica* (400 IJs/larva) caused 100.00 and 75.00% mortality rates at 48 h post-incubation. In the present study, at the concentration of 400 IJs/3rd instar larva, *S. sangi* and *H. indica* caused 100.00% mortality; *S. surkhetense* and *H. baujardi* caused 93.75 and 87.50%, respectively. The slight variation in the mortality rate with other studies may be attributed to the difference in the nematode species and life stage of the pest. The present study showed that *H. indica* was found to be the most pathogenic in terms of LC_{50} and LT_{50} values, rate of host penetration and reproduction in host cadaver. This may be attributed, in part, to the fact that *H. indica* showed generally the highest prevalence among the locally isolated EPN isolates, thereby predicting its high adaptability in the prevailing climatic conditions.

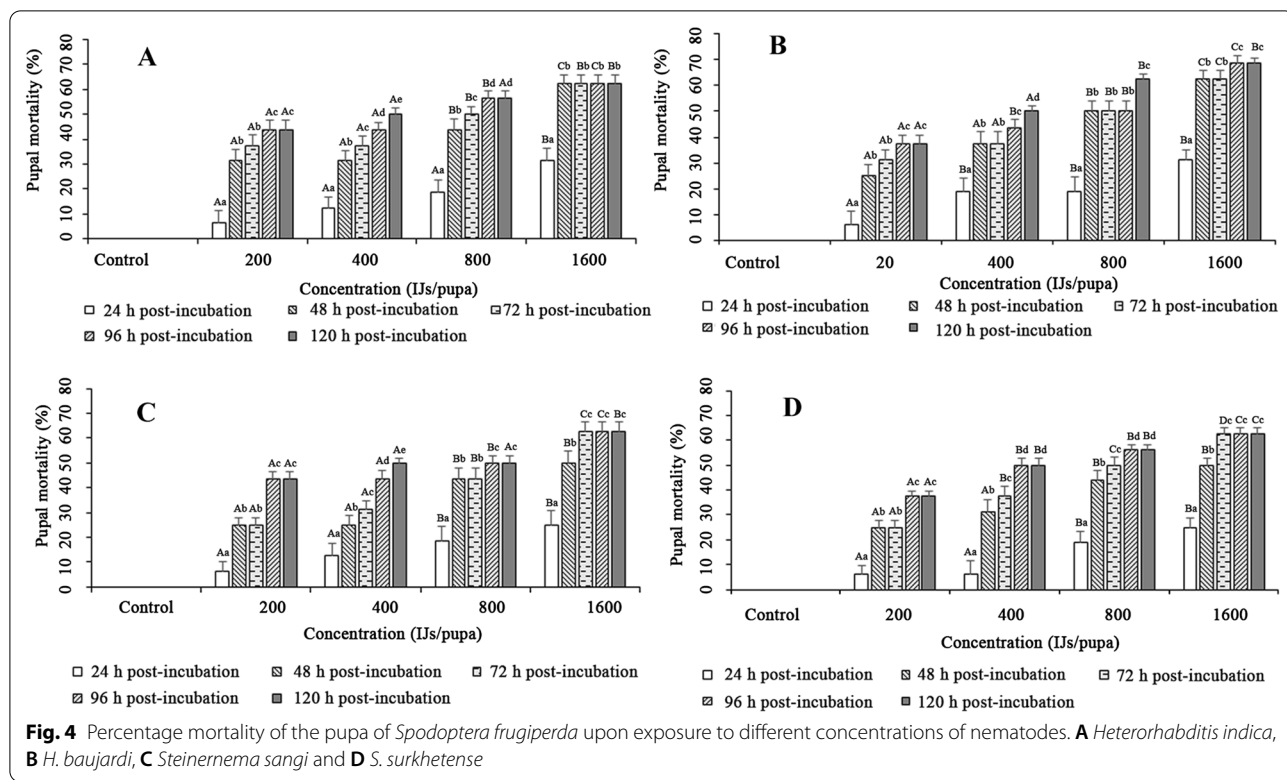Host penetration is one of the important factors related to the pathogenicity of EPNs (Kaya and Gaugler 1993).

Lalramnghaki *et al. Egypt J Biol Pest Control*   (2021) 31:110

Page 8 of 11

Fig. 4 Percentage mortality of the pupa of *Spodoptera frugiperda* upon exposure to different concentrations of nematodes. **A** *Heterorhabditis indica*, **B** *H. baujardi*, **C** *Steinernema sangi* and **D** *S. surkhetense*

Table 1 Lethal concentration (LC$_{50}$) of the four isolated EPN species on *Spodoptera frugiperda*

Time (h)	Host life stages	LC$_{50}$ (IJs/larva)			
		Heterorhabditis indica	*H. baujardi*	*Steinernema sangi*	*S. surkhetense*
72	Instar larvae				
	3rd	20.26	30.62	22.64	35.08
	5th	62.07	96.44	70.07	80.50
	Pupa	913.34	963.44	1106.72	1111.75

Table 2 Lethal time (LT$_{50}$) for the four isolated EPN species against *S. frugiperda*

Nematode concentrations	Host life stages	LT$_{50}$ (h)			
		H. indica	*H. baujardi*	*S. sangi*	*S. surkhetense*
100 IJs/larva	Instar larvae				
	3rd	15.99	16.12	14.33	17.64
	5th	21.74	28.98	28.90	32.39
400 IJs/pupa	Pupa	112.00	114.52	114.69	105.00

The potency of host invasion and penetration ability of EPN species may be attributed to variations in their virulence against insect pests (Glazer et al. 2001). The observed host invasion rates of the studied EPN isolates are within the range as per reports of other scientific investigations (Phan et al. 2005). A high penetration rate may be correlated with high production of toxins (Akhurst and Boemare 1990) that will ultimately result

Fig. 5 Percentage penetration of the EPN isolates on **A** 3rd instar larvae, **B** 5th instar larvae and, **C** pupa of *Spodoptera frugiperda* upon exposure to different concentrations of nematodes

Fig. 6 Progeny production of the studied EPN isolates on 3rd, 5th larval instars and pupae of *Spodoptera frugiperda*
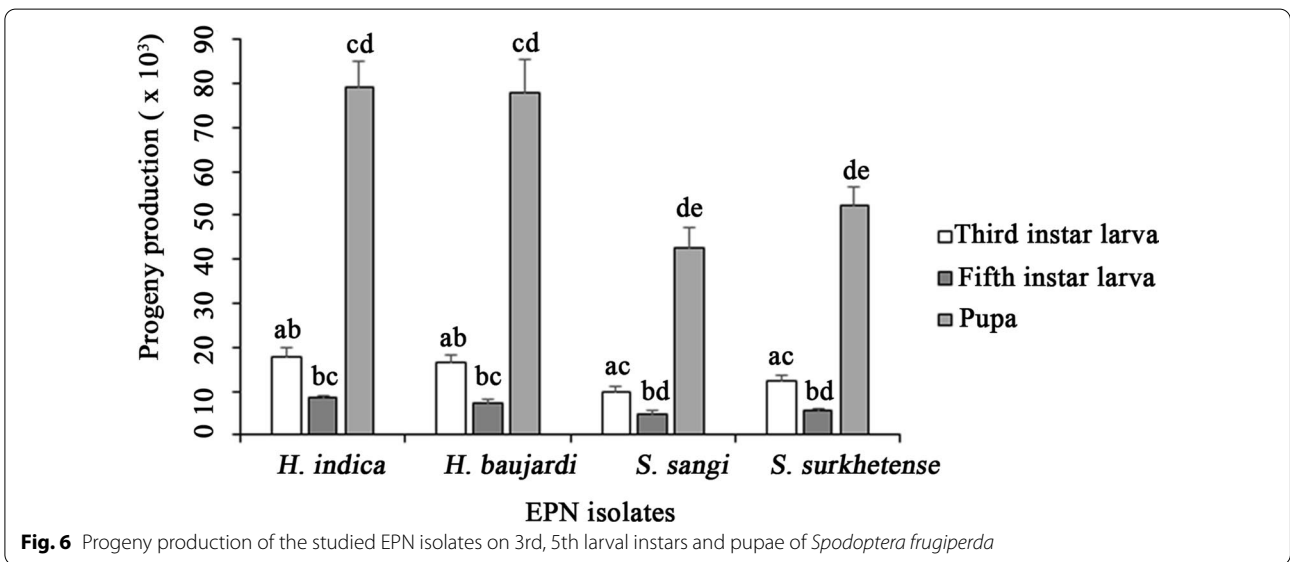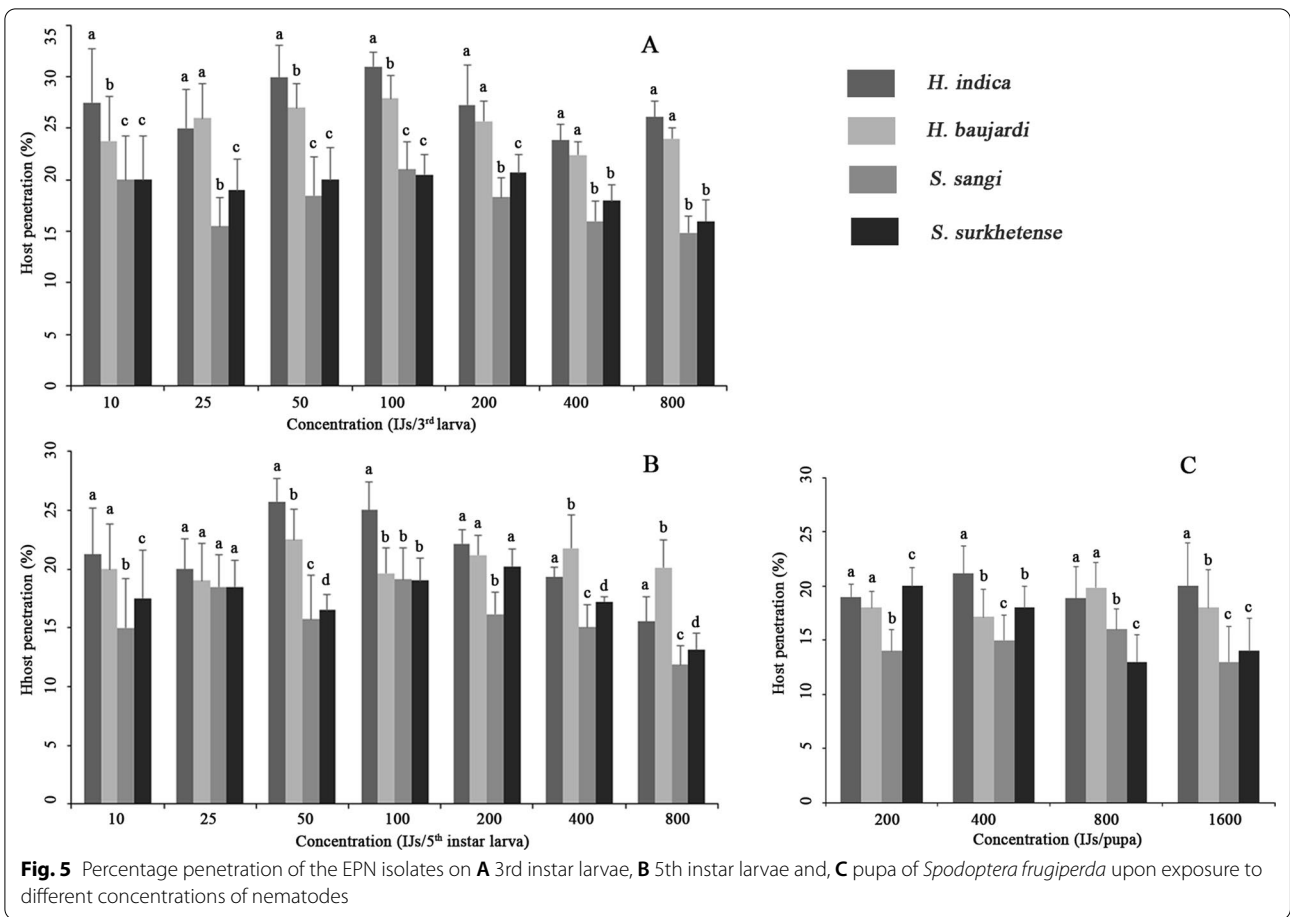
in rapid killing of the insect. Therefore, high mortality caused by *H. indica* and *H. baujardi* in the present study may be correlated with a high rate of penetration in heterorhabditids. However, the study recorded that *S. sangi* with a comparatively low penetration rate showed a similar level of pathogenicity with heterorhabditids. It

Lalramnghaki *et al. Egypt J Biol Pest Control*   (2021) 31:110

Page 10 of 11

is thus clear that, even though many studies reported the dependent relationship of nematode virulence and penetration rate (Glazer et al. 2001), the determination of virulence varied with the species since some workers still reported the lack of relationship between penetration and mortality rate (Ricci et al. 1996).

Reproductive efficacy inside the host insect plays an important role in the effectiveness of EPNs as a biological control agent. The nematode upon infecting the host undergoes growth and reproduction inside the cadaver for multiple generations after which the IJs emerged and seek new hosts. The progeny production data of the present study showed a successful host infection and reproduction of the EPN isolates, thereby revealing their potency as effective biopesticides. Caccia et al. (2014) evaluated the capacity of *S. diaprepesi* on the larvae of *S. frugiperda* and reported that the nematode produced 11,329 and 27,155 IJs at a concentration of 50 and 100 IJs/larva, respectively. Obtained findings on progeny production by EPN isolates showed comparatively significant differences among the 4 species, which agree with the results of Rahoo et al. (2018), where reproductive rate significantly varied among EPN isolates. Besides, a high rate of multiplication in the pupal stage as per obtained observation may be correlated with the mass and nutrient content of the host (Loya and Hower 2003). Moreover, the 2 heterorhabditids, *H. indica* and *H. baujardi*, showed comparatively higher production of progeny than the 2 steinernematids, *S. sangi* and *S. surkhetense*. Hermaphroditic reproduction of the first generation in heterorhabditids (Glazer et al. 1994) may be attributed to the higher reproductive rate in comparison with steinernematids that reproduce sexually in the first generation (Kondo and Ishibashi 1987) as this may strongly correlate with an initial establishment in the host.

Conclusions

The EPN isolates evaluated in the present study showed a high pathogenicity against *S. frugiperda* with successful reproductions, thereby indicating potential candidates to control the pest. This is the first scientific study on the evaluation of the susceptibility of *S. frugiperda* against the locally isolated EPNs.

Author details
[1]Department of Zoology, Pachhunga University College, Mizoram University, Aizawl 796001, India. [2]Department of Zoology, Mizoram University, Aizawl 796004, India. [3]Department of Environmental Studies, Mizoram University, Aizawl 796004, India.

References
Akhurst RJ, Boemare NE (1990) Biology and taxonomy of *Xenorhabdus*. In: Gaugler R, Kaya HK (eds) Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, pp 75–90

Andaló V, Santos VA, Moreira GF, Moreira CC, Junior AM (2010) Evaluation of entomopathogenic nematodes under laboratory and greenhouses conditions for the control of *Spodoptera frugiperda*. Ciênc Rural 40(9):1860–1866. https://doi.org/10.1590/S0103-84782010005000151

Bedding RA (1990) Logistics and strategies for introducing entomopathogenic nematode technology in developing countries. In: Gaugler R, Kaya HK (eds) Entomopathogenic nematodes in biological control. CRC Press, Boca Raton, pp 233–248

Bedding RA, Akhurst RJ (1975) A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21:109–110

Belay DK, Huckaba RM, Foster JE (2012) Susceptibility of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), at Santa Isabel, Puerto Rico, to different insecticides. Fla Entomol 95:476–478. https://doi.org/10.1653/024.095.0232

CABI (2020) Invasive Species Compendium, Wallingford. https://www.cabi.org/isc. Accessed 26 Oct 2020.

Caccia MG, Del Valle E, Doucet ME, Lax P (2014) Susceptibility of *Spodoptera frugiperda* and *Helicoverpa gelotopoeon* (Lepidoptera: Noctuidae) to the entomopathogenic nematode *Steinernema diaprepesi* (Rhabditida: Steinernematidae) under laboratory conditions. Chil J Agric Res 74(1):123–126. https://doi.org/10.4067/S0718-58392014000100019

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial Cytochrome C oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3:294–299

Lalramnghaki *et al. Egypt J Biol Pest Control*   (2021) 31:110

Page 11 of 11

Ganiger PC, Yeshwanth HM, Muralimohan K, Vinay N, Kumar ARV, Chandrasekhar K (2018) Occurrence of new invasive pest, fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera Noctuidae), in the maize field of Karnataka, India. Curr Sci 115:621–623

Garcia LC, Raetano CG, Leite LG (2008) Application technology for the entomopathogenic nematodes *Heterorhabditis indica* and *Steinernema* sp. (Rhabditida: Heterorhabditidae and Steinernematidae) to control *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) in corn. Neotrop Entomol 37(3):305–311. https://doi.org/10.1590/s1519-566x2008000300010

Glazer I, Alekseev E, Samish M (2001) Factors affecting the virulence of entomopathogenic nematodes to engorged female *Boophilus annulatus* ticks. J Parasitol 87:808–812. https://doi.org/10.1645/0022-3395(2001)087[0808:fatvoe]2.0.co;2

Glazer I, Koltai H, Zioni, CNS, Segal D (1994) Life cycle and reproduction in *Heterorhabditis*. In: Burnell AM, Ehlers RU, Masson JP (eds) Genetics of entomopathogenic nematodes-bacterium complex. European Commission, Luxembourg

Goergen G, Kumar PL, Sankung SB, Togola A, Tamò M (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS ONE 11(10):e0165632. https://doi.org/10.1371/journal.pone.0165632

Joshi PK, Singh NP, Singh NN, Gerpacio RV, Pingali PL (2005) Maize in India: production systems, constraints, and research priorities. CIMMYT, Mexico

Kaya HK, Gaugler R (1993) Entomopathogenic nematodes. Annu Rev Entomol 38:181–206. https://doi.org/10.1146/annurev.en.38.010193.001145

Kaya HK, Stock SP (1997) Techniques in insect nematology. In: Lacey LA (ed) Manual of techniques in insect pathology. Academic Press, San Diego, pp 281–324. https://doi.org/10.1016/B978-012432555-5/50016-6

Kondo E, Ishibashi N (1987) Comparative infectivity and development of the entomopathogenic nematodes *Steinernema* spp. on the lepidopterous insect larvae, *Spodoptera litura* (Noctuidae) and *Galleria* mellonella (Galleridae). Jpn J Nematol 17:35–41. https://doi.org/10.14855/jjn1972.17.35

Kumar S, Stecher G, Li M (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549

Kumar S, Kumar P, Bana JK, Shekhar M, Sushil SN, Sinha AK, Asre R, Kapoor KS, Sharma OP, Bhagat S, Sehgal M, Boopathi T, Amaresan N, Chattopadhyay C, Satyagopal K, Jeyakumar P (2014) Director, Integrated Pest Management Package, New Delhi

Lacey LA, Georgis R (2012) Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. J Nematol 44:218–225

Loya LJ, Hower AA Jr (2003) Infectivity and reproductive potential of the Oswego strain of *Heterorhabditis bacteriophora* associated with life stages of the clover root curculio, *Sitona hispidulus*. J Invertebr Pathol 83:63–72. https://doi.org/10.1016/s0022-2011(03)00044-2

Murúa MG, Vera MA, Michel A, Casmuz AS, Fatoretto J, Gastaminza G (2019) Performance of field-collected *Spodoptera frugiperda* (Lepidoptera: Noctuidae) strains exposed to different transgenic and refuge maize hybrids in Argentina. J Insect Sci 19:21. https://doi.org/10.1093/jisesa/iez110

Phan KL, Tirry L, Mones M (2005) Pathogenic potential of six isolates of entomopathogenic nematodes (Rhabditidia: Steinernematidae) from Vietnam. Biocontrol 50:477–491

Rahoo M, Mukhtar T, Abro SI, Bughio BA, Rahoo RK (2018) Comparing the productivity of five entomopathogenic nematodes in *Galleria melonella*. Pak J Zool 50(2):679–684. https://doi.org/10.17582/journal.pjz/2018.50.2.679.684

Repalle N, Jethva DM, Bhut JB, Wadaskar PS, Kachot A (2020) Present status of new invasive pest fall armyworm, *Spodoptera frugiperda* in India: a review. J Entomol Zool Stud 8(2):150–156. https://doi.org/10.22271/j.ento

Ricci M, Glazer I, Campbell JF, Gaugler R (1996) Comparison of bioassays to measure virulence of different entomopathogenic nematodes. Biocontrol Sci Technol 6(2):235–246. https://doi.org/10.1080/09583159650039421

Sharanabasappa KCM, Asokan R, Mahadeva Swamv HM, Marutid MS, Pavithra HB, Kavita H, Shivaray N, Prabhu ST, Georg G (2018) First report of the fall armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. Pest Manag Hort Ecosyst 24:23–29

Todd EL, Poole RW (1980) Keys and illustrations for the armyworm moths of the noctuid genus *Spodoptera Guenée* from the Western Hemisphere. Ann Entomol Soc Am 73(6):722–738.

Vanlalhlimpuia L, Lalramnghaki HC, Vanramliana, (2018) Morphological and molecular characterization of entomopathogenic nematode, *Heterorhabditis baujardi* (Rhabditida, Heterorhabditidae) from Mizoram, northeastern India. J Parasit Dis 42(3):341–349

Viteri D, Linares A, Flores L (2018) Use of the entomopathogenic nematode *Steinernema carpocapsae* in combination with low-toxicity insecticides to control fall armyworm (Lepidoptera: Noctuidae) larvae. Fla Entomol ss101(2):327–329. https://doi.org/10.1653/024.101.0228

Zhu YC, Blanco CA, Portilla M, Adamczyk J, Luttrell R, Huang F (2015) Evidence of multiple/cross resistance to Bt and organophosphate insecticides in Puerto Rico population of the fall armyworm, *Spodoptera frugiperda*. Pestic Biochem Phys 122:15–21. https://doi.org/10.1016/j.pestbp.2015.01.007

Publisher's Note