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

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**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 (LC₅₀), *H. indica* was the most pathogenic species, followed by *S. sangi*, *H. baujardi* and *S. surkhetense*. The LC₅₀ 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...
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 sahari* 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 CAAACAAATCATAAAGATATTGG-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 container and incubated at room temperature. For 10 larvae, mortality was checked every 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.

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 virulence among the nematode isolates. Meanwhile, 3rd, 5th larval instars and pupae of the pest were selected to assess the difference in virulency among S. surkhetense and S. indiaca, were used against S. frugiperda and H. indica.

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 loamy 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
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.
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 ± 2103.59, 16,375.31 ± 1688.37, 9862.68 ± 1031.45 and 12,218.63 ± 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 ± 5943.73, 77,999 ± 7432.20, 42,747.44 ± 3934.22 and 52,383.63 ± 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.
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).
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. 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) |
|----------|------------------|-----------------------|
|          |                  | H. indicia | H. baujardi | S. 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) |
|-------------------------|------------------|---------------|
| 100 IJs/larva           | Instar larvae    | H. indica     | H. baujardi | S. sangi | S. surkhetense |
| 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        |
in rapid killing of the insect. Therefore, high mortality caused by \textit{H. indica} and \textit{H. baujardi} in the present study may be correlated with a high rate of penetration in heterorhabditids. However, the study recorded that \textit{S. sangi} with a comparatively low penetration rate showed a similar level of pathogenicity with heterorhabditids. It
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. diapreps* 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.

**Abbreviations**

EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; LC: Lethal concentration; LT: Lethal time; NE: North-Eastern; hr: Hour.

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**Authors’ contributions**

HCL, VLL and MLR did the field survey, collection and identifications of the pest. HCL, LRL and HTL involved in the isolation and identification of the EPN isolates. HCL, LRL and VRL designed the research experiment, data analysis and manuscript writings. The final manuscript was read and accepted by all the authors.

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**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare no competing interests.

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