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Screening of newly isolated indigenous entomopathogenic nematodes against *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae)

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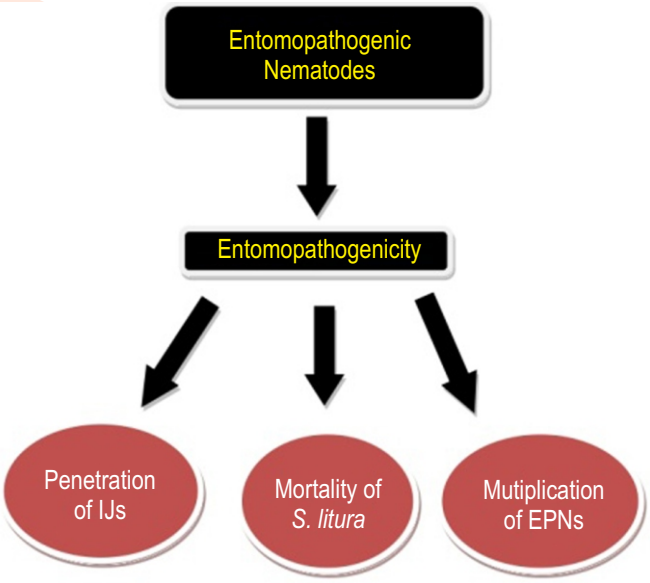
Abstract

Aim: Insecticides have traditionally been used to control *S. litura,* although bio-pesticides as alternative control measures are also in demand due to soil residual issues and other environmental dangers. Another safe technique for controlling this important pest is entomopathogenic nematodes (EPNs).

Methodology: This study evaluated the infectivity of ten native recently isolated entomopathogenic nematodes against *S. litura* by assessing their penetration and multiplication in *S. litura*. The efficiency of the promising EPNs against second, third and fourth instars *S. litura* larvae was further examined.

Results: Among the tested EPNs, *Steinernema* sp. (IARI-EPN RP 03), *Heterorhabditis* sp. (IARI-EPN RP 06), and *Oscheius* sp. (IARI-EPN RP 07) were intensely harmful to *S. litura,* producing 100 percent mortality in 72 hrs, followed by *Steinernema* sp. (IARI-EPN RP 09), which caused 92 percent mortality, and *Oscheius* sp. (IARI-EPN RP 10) was the least pathogenic recording 58 percent mortality. *Heterorhabditis* sp. (IARI-EPN RP 06) showed the maximum penetration, followed by *Oscheius* sp. (IARI-EPN RP 07) and *Oscheius* sp. (IARI-EPN RP 08), with *Oscheius* sp. (IARI-EPN RP 04) showed the least penetration. The second instar larvae of *S. litura* were highly vulnerable to EPNs, followed by third and fourth instar larvae. According to the findings, *Steinernema* sp. (IARI-EPN RP 03) and *Heterorhabditis* sp. (IARI-EPN RP 06) were most virulent against *S. litura.*

Interpretation: As a result, both EPNs have the potential to act as biological control agents for *S. litura.* Furthermore, the fifth instar *S. litura* larva were more suited for multiplication, indicating that this insect is suitable for EPN production. Field testing of these promising EPNs will reveal their efficacy in managing *S. litura* in a sustainable manner.



Key words: Bio-pesticides, Entomopathogenic nematodes, *Heterorhabditis*, Insects, *Oscheius, Steinernema*

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Introduction

Tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is one of the most destructive polyphagous insect pest due to wide host range, damaging 112 species of host plants (60 species from India) like tobacco, castor, cotton, soybean and groundnut throughout tropical and temperate world (Fand *et al.,* 2015; Sahu *et al.,* 2020). However, it is primarily a tobacco pest, it also targets corn, chili, cotton, sunflower, peanut, and legumes (Yadav *et al.,* 2013). *S. litura* damages tobacco nurseries to the extent of 80 to 100 percent and field crop up to 10 to 25 percent, reducing tobacco production by 23 to 50 percent (Patil *et al.,* 2014). As an adult, this insect has a tremendous reproductive potential and may travel considerable distances (Armes *et al.,* 1997). This species is a migratory species that may be found on all continents and is a major problem because of its wide host range, nocturnal nature, high mobility of adult moths and reproductive capability (Feng *et al.,* 2005). Larvae cause significant losses in agricultural production during outbreaks by feeding on the leaves, buds, flowers and fruits of field crops (Sun *et al.,* 2016).

Knowing the importance of insect pests importance as a limiting factor, numerous solutions have been developed and implemented to combat it (Zhou *et al.,* 2010, Acharaya, 2020). Pesticides may initially appeal to growers because of their easy availability, ability to quickly suppress insect pest populations, and increased productivity, but they often do not prove to be effective, resulting in the development of pest resistance (Ahmad *et al.,* 2009; Abbas *et al.,* 2012; Ahmad and Mehmood, 2015). Furthermore, indiscriminate pesticide use has grossly poisoned almost every component of the biosphere, including the resurgence of pests and a reduction in natural eutrophication. As a result, it is critical to develop viable environmentally friendly options for controlling this insect pest. Entomopathogenic nematodes (EPNs) have been identified as possible bio-pesticide candidates against *S. litura* (Ali *et al.,* 2008; Radhakrishnan and Shanmugam, 2017; Gokte-Narkhedkar *et al.,* 2019, Khan *et al.,* 2020; Yan *et al.,* 2020). Hence, investigations were conducted to test the efficacy of ten native EPNs for managing *S. litura*. Studies were conducted to record larva susceptibility, penetration and production of these EPNs on *S. litura*. In addition, infectivity of promising EPNs against 2nd, 3rd, and 4th, instar larvae of *S. litura* was also evaluated.

Materials and Methods

Entomopathogenic nematodes and Insect sources: There are ten native entomopathogenic nematodes, among them six belong to *viz., Oscheius* sp. (IARI-EPN RP 01, 02, 05,07,08 and 10), three belong to *Steinernema* sp. (IARI-EPN RP 03,05 and 09) and one belong to *Heterorhabditis* sp. (IARI-EPN RP 06). They were obtained from the EPN repository of the Division of Nematology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi. All of the EPNs were cultured as per procedure of by Kaya and Stock (1997). Infective juveniles (IJs) were surface sterilized with 0.1% hyamine solution and kept in tissue culture flasks in sterilized distilled water. *S. litura* was collected from Insect Reared Laboratory, Division of Entomology, ICAR-Indian Agricultural Research Institute (IARI), New Delhi.

Larval susceptibility: Screening of ten native EPNs against *S. litura* larvae were analyses in a Petri dish (9 cm diameter) lined with filter paper at the bottom and wet with sterilized distilled water. Each EPN had 100 IJs suspended in 0.5 ml water and equally dispersed over the Petri dish. In each dish, a single fifth instar *S. litura* larva was released with small piece of artificial diet as larva nutrition. To prevent moisture loss, the plates were then sealed with parafilm and kept at 28±2°C in a BOD incubator. The study looked at ten replicates of each treatment with a control. *S. litura* mortality was measured 72 hrs following inoculation with Infective juveniles. The data on mortality was converted into percentages, and the mean values were calculated.

Penetration of EPNs: EPNs were tested for penetration into *S. litura* larvae in a Petri dish (9 cm diameter) lined with filter paper at the bottom and wet with sterilized distilled water. In the Petri dish, single *S. litura* fifth instar larvae were released with small piece of artificial diet as larvae food. In 0.5 ml of water, 100 Infective juveniles of each EPN were suspended and equally distributed across the Petri dish. *S. litura* cadaver were placed in a separate Petri dish (9 cm diameter) containing dry filter paper and kept in the dark for 24 hrs to test Infective juveniles penetration. After 24 hrs, the cadaver were rinsed with distilled water to remove the nematodes from their body surfaces, and then dissected in Ringer's solution under a stereomicroscope to count the number of Infective juveniles that had penetrated each cadaver. Each larva was treated as a separate replicate. The penetration rate was then calculated as per described by Pervez and Ali (2011).

Multiplication of EPNs: Dead larvae infected with EPNs were removed from the Petri dish and rinsed with sterilized distilled water to remove EPNs sticking to the body surface in order to produce EPNs. The *S. litura* larvae were then individually put onto a modified White trap (White, 1927) and incubated in a BOD incubator at 28±2°C. Each larva was treated as a separate replicate. Under a stereo microscope, the total number of IJs that emerged from each larva was counted three times using a Syracuse counting plate and the mean values were determined.

Infectivity of EPNs against different instar larva of *S. litura*: Three EPNs were identified as promising based on the results of the infectivity assays. In a Petri dish (9 cm diameter) lined with wet filter paper at the bottom, the infectivity of these promising EPNs, *Steinernema* sp. (IARI-EPN RP 03), *Heterorhabditis* sp. (IARI-EPN RP 06), and *Oscheius* sp. (IARI-EPN RP 07), against 2nd, 3rd, and 4th instar *S. litura* larvae was investigated. Each dish was put up a single instar *S. litura* larva and chickpea leaves as larval nutrition, as well as 100 IJs of the promising EPN in 0.5 ml water. The experiment was carried out in a BOD incubator at 28±2°C and replicated 10 times with the control. Observations on their mortality were made every 24 hrs for up to 72 hrs. *S. litura* larvae

of each test instar, as well as EPN, were examined separately and on an individual basis. The data on mortality was converted into percentages, and the mean values were determined.

Statistical analyses: Before analysis, percentage data was standardized using the arcsine transformation and the numerical data was square-root converted. Only the back converted data is shown because the analysis was done on the transformed data. The data on larval susceptibility, penetration, and multiplication were analyzed by ANOVA. In addition, the standard deviation and standard error were calculated.

Results and Discussion

The results demonstrated that all of the test EPNs were harmful to *S. litura*, while the levels of mortality differed significantly among them (ANOVA; F= 34.31; df = 9, 90; p = 0.0001). Out of tested EPNs, *Steinernema* sp. (IARI-EPN RP 03), *Heterorhabditis* sp. (IARI-EPN RP 06), and *Oscheius* sp. (IARI-EPN RP 07) found to be most harmful to *S. litura,* causing 100 percent mortality in 72 hrs, followed by *Steinernema* sp. (IARI-EPN RP 09), which caused 92 percent mortality in 72 hrs. *Oscheius* sp. (IARI-EPN RP 10) was least pathogenic, with a mortality rate of 58 percent (Fig. 1).

The rate of EPN penetration in the body of *S. litura* larvae was found to be significant (ANOVA; F= 11.02; df = 9, 99; p = 0.0001). *Heterorhabditis* sp. (IARI-EPN RP 06; 17.4 Ijs per larva) had the highest number of penetration among the studied EPNs, followed by *Oscheius* sp. (IARI-EPN RP 07; 11.7 IJs/larva). *Oscheius* sp. (IARI-EPN RP 04; 2.9 IJs/larva) had the lowest rate of penetration (Table 1). EPNs were found to be able to grow within the haemocoel of *S. litura* larvae, and their production was differed significantly (ANOVA; F= 65.13; df = 9, 90; p = 0.0001). With 1.7 x 105 Ijs per larva, *Oscheius* sp. (IARI-EPN RP 07) had the highest output among the studied EPNs, followed by *Steinernema* sp. (IARI-EPN RP 05 and 10; 1.3 x 105 Ijs per larva). *Oscheius* sp. (IARI-EPN RP 09; 0. 3 x 105 Ijs per larva) had the lowest productivity (Fig. 2).

Table1: Rate of EPN penetration into *S. litura.*

EPN	No. of Ijs per larva
Oscheius sp. (IARI-EPN RP 01)	5.7±2.79 (0.88)
Oscheius sp. (IARI-EPN RP 02)	3.8±2.57(0.81)
Steinernema sp. (IARI-EPN RP 03)	10.2±4.96 (1.59)
Oscheius sp. (IARI-EPN RP 04)	2.9±2.18 (0.69)
Steinernema sp. (IARI-EPN RP 05)	8.4±4.94 (1.56)
Heterorhabditis sp. (IARI-EPN RP 06)	17.4±7.15 (2.26)
Oscheius sp. (IARI-EPN RP 07)	11.7±4.57 (1.44)
Oscheius sp. (IARI-EPN RP 08)	6.4±3.37 (1.06)
Steinernema sp. (IARI-EPN RP 09)	4.2±3.49 (1.10)
Oscheius sp. (IARI-EPN RP 10)	7.6±3.27 (1.03)

Values are mean ±S.D. (S.E.)

Different instar larval mortality of *S. litura* differed significantly among *Steinernema* sp. (IARI-EPN RP 03; F = 13.4; df = 2, 27; P = 0.0001), *Heterorhabditis* sp. (IARI-EPN RP 06; F = 7.5; df = 2, 27; P = 0.001) and *Oscheius* sp. (IARI-EPN RP 07; F =

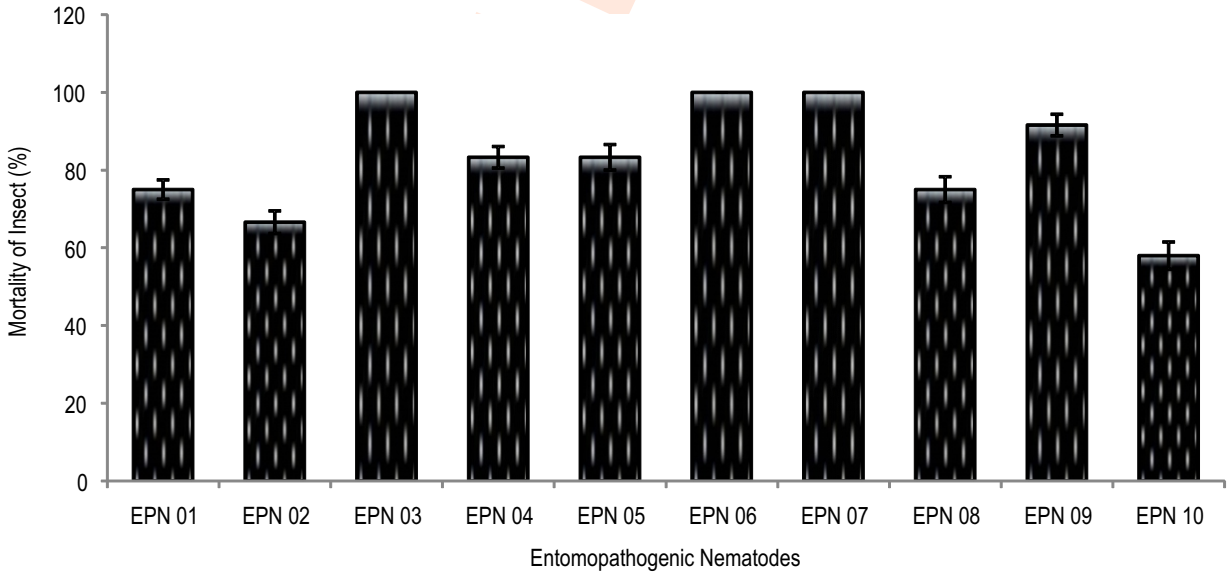

Fig. 1: Mortality of *S. litura* larvae (mean±standard eror) through EPNs(n=10); EPN 01-*Oscheius* sp. (IARI-EPN RP 01), EPN 02-*Oscheius* sp. (IARI-EPN RP 02), EPN 03-*Steinernema* sp. (IARI-EPN RP 03), EPN 04-*Oscheius* sp. (IARI-EPN RP 04), EPN 05-*Steinernema* sp. (IARI-EPN RP 05), EPN 06-*Heterorhabditis* sp. (IARI-EPN RP 06), EPN 07-*Oscheius* sp. (IARI-EPN RP 07), EPN 08-*Oscheius* sp. (IARI-EPN RP 08), EPN 09-*Steinernema* sp. (IARI-EPN RP 09), and EPN 10-*Oscheius* sp. (IARI-EPN RP 10).

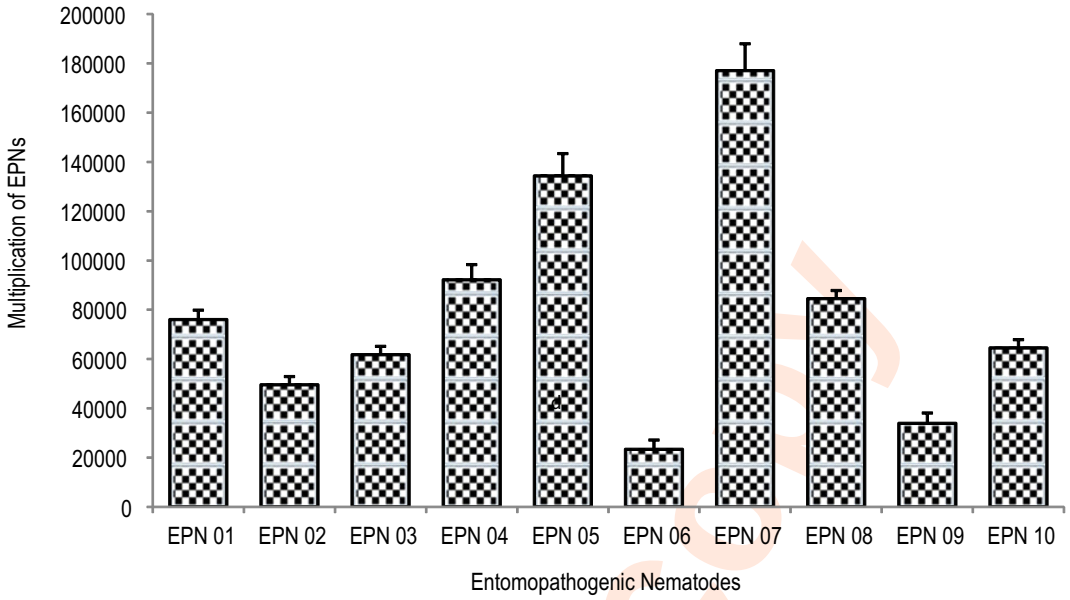

Fig. 2: Multiplication of entomopathogenic nematodes (EPNs) on *S. litura* larvae. Values are mean ±S.D.

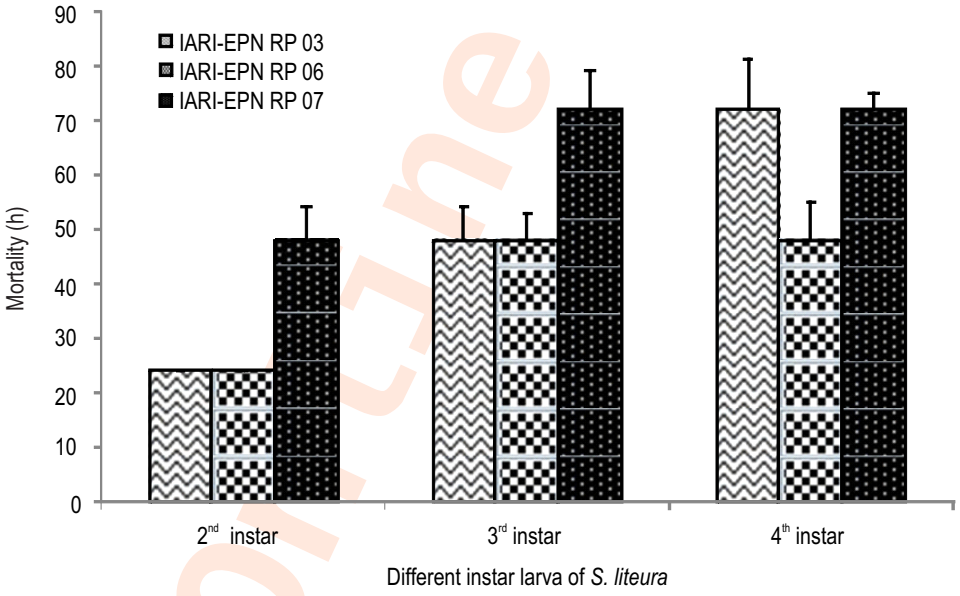

Fig. 3: Mortality (mean±standard eror) of different instar larvae of *S. litura.*

3.2; df = 2, 27; P=0.05). Among the three tested promising EPNs, *Heterorhabditis* sp. (IARI-EPN RP 06) was the most pathogenic exhibiting 100 percent killing of 2nd, 3rd, and 4th instar *S. litura* larva within 24 to 48hr, but *Oscheius* sp. (IARI-EPN RP 07) took 48 to 72hr to kill *S. litura* larvae. The second instar larva of *S. litura* was the most vulnerable to EPNs, followed by the third and fourth instar larvae (Fig. 3). EPN pathogenicity is determined by inherent traits as well as host circumstances, penetration, production ability and differences in bacterial symbionts (Yan *et al.,* 2020; Kapranas 2020; Pervez *et al.,* 2012). The results of this study, revealed that EPN virulence differed significantly among EPN species. Among the ten tested EPNs, *Steinernema* sp. (IARI-EPN RP 03), *Heterorhabditis* sp. (IARI-EPN RP 06), and *Oscheius* sp. (IARI-EPN RP 07) were virulent to varying extents in

S. litura larvae. Because one of the main reasons attributed to the failure of EPNs for biological control of insect pests is the incorrect choice of EPNs (Georgis and Gaugler, 1991), screening EPNs for infectivity in the laboratory is an important step in developing a biological control programme for a particular pest before beginning field studies (Shapiro *et al.,* 2002; Pervez *et al.,* 2007).

The multiplication ability of any biological control agent is an important feature for their extended persistence and pathogenecity to the targeted insect pests (Blanco–Pérez, 2017; Patil and Rangasamy, 2018). It not only results in insect pest mortality but also determines the recycling ability of the EPNs to tackle the succeeding generations of the targeted insect pests (Patil *et al.,* 2019). EPNs can be produced *in vivo,* wherein the insect serves as a small biological reactor. Among GWML (*Galleria mellonella* L.) insects have been frequently used for *in vivo* EPN generation. Several insect larvae were used in earlier studies to examine the potential of different EPNs to produce EPNs. Among them, *Helicoverpa virescens* Fabricius, *H. armigera* F., *Chilo sacchariphagus indicus K*., *Spodoptera exigua* Hübner, *S. litura* Fabricius (Karunakar *et al*., 1999; Elawad *et al*., 2001), *Corcyra cephalonica S*. (Pervez and Ali, 2009), *Trichopulsia ni Hübner* (Khan *et al*., 2020; Acharya *et al.,* 2020), *Athalia proxima K.* (Pervez *et al*., 2007), Conogethes *punctiferalis G.* and *Maruca vitrata F.* (Pervez and Rao, 2021) were reported to produce a large number of EPNs and were thus considered as good hosts for producing EPNs.

Various species of *Steinernema, Heterorhabditis* and *Oscheius* have been multiplied using these insects, with varied yields of IJs depending on the insects' larvae. The greater EPN production rate observed in this study could aid in dealing with future generations in the field. The penetration rate of EPNs into *S. litura* larvae varied depending on EPN species. Among the ten EPNs tested, *Heterorhabditis* sp. (IARI-EPN RP 06) had a higher penetration rate than the other EPNs. This could be due to the nematodes' foraging behavior (Cardoso *et al.,* 2015; Safdar *et al.,* 2018; Sahu *et al.,* 2020). Differences in the toxicity of bacterial symbionts are related to differences in their cell wall substances, which results in the relative destruction of host hemocytes and, eventually, host's death (Javed *et al.,* 2022; Pervez and Rao, 2021; Tabassum and Salma, 2020; Dunphy and Webster, 1991).

The penetration rate can be used to determine the vulnerability of the host. Heterorhabditis sp. (IARI-EPN RP 06) and *Oscheius* sp. (IARI-EPN RP 07) were able to penetrate the insect body in greater quantities in this investigation, and so can be considered promising biocontrol agents for *S. litura* management. Three EPNs were tested for virulence against 2nd, 3rd and 4th instars *S. litura* larvae in this study. The virulence of EPNs differed between *S. litura* instars larvae. Among the three tested EPNs, virulence of *Steinernema* sp. (IARI-EPN RP 03) and *Heterorhabditis* sp. (IARI-EPN RP 06) against *S. litura* instars larvae was higher than that of *Oscheius* sp. (IARI-EPN RP 07). Various developmental phases have different susceptibilities to EPN. The 2nd instar *S. litura* larvae were the most sensitive, followed by the 3rd and 4th instar *S. litura* larvae, according to our findings. These findings are consistent with those of Banu *et al.* (2007) and Pervez (2010) who found that 1st and 2nd instar larvae of *H. armigera* were extremely vulnerable to *H. indica. H. bacteriophora* HY was also found to be more efficient against the second and third instars of *S. litura* (Javed *et al.,* 2022; Park *et al.,* 2001). Similarly, the second instar larvae of the pod borer, *H. armigera,* and the legume pod borer, *M. vitrata,* were vulnerable to EPNs, followed by the third, fourth, and fully grown larvae (Pervez, 2010; Patil *et al.,* 2020). For instance, early larval stages were reported to be the most susceptible stages in *Tipula paludosa* (Meigen), and *T. oleracea* (Meigen) was recorded for the first instars (Peters *et al.,* 1994).

Based on the findings of this study, it can be concluded that out of ten native EPN strains tested, *Heterorhabditis* sp. (IARI-EPN RP 06) and Steinernema sp. (IARI-EPN RP 03) were the most virulent against *S. litura.* As a result, both EPNs have the potential to be effective biological control agents for *S. litura.* Furthermore, the fifth instar *S. litura* larva was more suited for multiplication, indicating that this insect is suitable for EPN production. Further field testing of these promising EPNs will reveal their efficacy in managing *S. litura* in a sustainable manner.

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