Journal of Helminthology

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Infectivity of entomopathogenic nematodes against the legume pod-borer, *Maruca vitrata* Fabricius, infesting pigeon pea

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Research Paper

Cite this article: Pervez R, Rao U (2021). Infectivity of entomopathogenic nematodes against the legume pod-borer, *Maruca vitrata* Fabricius, infesting pigeon pea. *Journal of Helminthology* **95**, e15, 1–5. https://doi.org/10.1017/S0022149X21000043

Received: 21 October 2020
Revised: 11 January 2021
Accepted: 18 January 2021

Key words:
Biological control; insects; *Heterorhabditis*; *Steinernema*; *Oscheius*

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Abstract

The legume pod-borer, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) (LPB), is an important insect pest of pigeon pea. Chemical pesticides are generally employed to manage this pest, but because of the soil residue issues and other environmental hazards associated with their use, biopesticides are also in demand. Another benign alternative is to use entomopathogenic nematodes (EPNs) to manage this vital pest. In the present study, the infectivity of ten native EPNs was evaluated against LPB by assessing their penetration and production in the LPB. The effectiveness of the promising EPNs against second-, third- and fourth-instar LPB larvae was also studied. *Heterorhabditis* sp. (Indian Agricultural Research Institute-Entomopathogenic Nematodes Rashid Pervez (IARI-EPN RP) 06) and *Oscheius* sp. (IARI-EPN RP 08) were found to be most pathogenic to LPB, resulting in about 100% mortality within 72 h, followed by *Steinernema* sp. (IARI-EPN RP 03 and 09). *Oscheius* sp. (IARI-EPN RP 04) was found to be the least pathogenic to LPB larva with 67% mortality. Maximum penetration was exhibited by *Heterorhabditis* sp. (IARI-EPN RP 06) followed by *Oscheius* sp. (IARI-EPN RP 08), whereas the lowest rate of penetration was exhibited by *Oscheius* sp. (IARI-EPN RP 01). The highest rate of production was observed with *Oscheius* sp. (IARI-EPN RP 08), followed by *Oscheius* sp. (IARI-EPN RP 04 and 10). Among the tested instars of the LPB larvae, second-instar larvae were more susceptible to EPNs, followed by third- and fourth-instar larvae. The results indicate that *Heterorhabditis* sp. (IARI-EPN RP 06) and *Oscheius* sp. (IARI-EPN RP 08) have a good potential to the manage LPB.

Introduction

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is one of the most important grain legumes in India and is cultivated on 3.9 million hectares with a production of 2.4 million tons (FAOSTAT, 2018). This constitutes 63% of the overall world production. Pigeon pea provides nutritious food, feed and fodder, and is an integral component of the subsistence farming system of the country. It is vital for human health as it contains high levels of protein (important amino acids – methionine, lysine and tryptophan), carbohydrate, fat, vitamins (vitamins B1, B2, B3, B5, B6, B9, C, E, K) and minerals (calcium, iron, potassium, magnesium, phosphorus, sodium, manganese, zinc).

Pigeon pea cultivation is affected by several biotic constraints and, of these, infestation and damage caused by the legume pod-borer, *Maruca vitrata*, is of utmost significance. It feeds on flower buds, flowers and young pods, and has several alternate hosts, which serve as the primary source of the infestation. *Maruca vitrata* has been estimated to cause a loss of 26–28% to the pigeon pea crop (Randhawa & Verma, 2011). Chemical insecticides have been employed to manage this pest, but they often prove to be ineffective, and, as a result, their indiscriminate use by farmers has led to serious health and environmental problems. Therefore, there is an urgent need to identify suitable eco-friendly alternatives for the management of the legume pod-borer *M. vitrata* (LPB). As entomopathogenic nematodes (EPNs) have been reported as potential candidates for use as biopesticides against lepidopteran insect pests (Ali *et al.*, 2008; Pervez & Rao, 2018). Hence, investigations were conducted to test the efficacy of ten native EPNs for managing LPB. Studies were conducted to record infectivity, penetration and production of these EPNs on LPB. In addition, infectivity of promising EPNs against second-, third- and fourth-instar LPB larvae was also evaluated.

Materials and methods

EPNs and insect sources

Ten native EPNs, *viz.* *Oscheius* sp. (Indian Agricultural Research Institute-Entomopathogenic Nematodes Rashid Pervez (IARI-EPN RP) 01), *Oscheius* sp. (IARI-EPN RP 02), *Steinernema*

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sp. (IARI-EPN RP 03), *Oscheius* sp. (IARI-EPN RP 04), *Steinernema* sp. (IARI-EPN RP 05), *Heterorhabditis* sp. (IARI-EPN RP 06), *Oscheius* sp. (IARI-EPN RP 07), *Oscheius* sp. (IARI-EPN RP 08), *Steinernema* sp. (IARI-EPN RP 09) and *Oscheius* sp. (IARI-EPN RP 10), were obtained from the EPN repository of the Division of Nematology, Indian Council of Agricultural Research (ICAR) – Indian Agricultural Research Institute (IARI), New Delhi. All these EPNs were cultured as per the procedure described by Kaya & Stock (1997). Fresh harvested Infective juveniles (IJ) were surface sterilized with 0.1% Hyamine solution and stored in sterilized distilled water in tissue culture flasks.

LPB was collected from the pigeon pea fields of the ICAR-IARI experimental farm (28°07′N, 77°13′E) during October 2019. No experimental site was treated with either pesticide or biopesticides during the cultivation. The fifth-instar larvae were used for the infectivity, penetration and multiplication assays. The greater wax moth, *Galleria mellonella* L. (GWML), was reared on an artificial diet as per the procedure described by David & Kurup (1988).

Bioassay

Infectivity of EPNs

The infectivity of EPNs, *viz*. *Oscheius* sp. (IARI-EPN RP 01), *Oscheius* sp. (IARI-EPN RP 02), *Steinernema* sp. (IARI-EPN RP 03), *Oscheius* sp. (IARI-EPN RP 04), *Steinernema* sp. (IARI-EPN RP 05), *Heterorhabditis* sp. (IARI-EPN RP 06), *Oscheius* sp. (IARI-EPN RP 07), *Oscheius* sp. (IARI-EPN RP 08), *Steinernema* sp. (IARI-EPN RP 09) and *Oscheius* sp. (IARI-EPN RP 10), were tested against LPB larvae in a Petri dish (9 cm diameter) lined with a moistened filter paper at the base of the dish. Sterilized distilled water was used to moisten the filter paper used in Petri dish in all the studies mentioned in this research paper. One hundred IJs of each EPN were suspended in 0.5 ml of water and evenly distributed on the Petri dish. A single fifth-instar LPB larva was kept in each dish, along with a pigeon pea flower as larva feed. The dishes were then sealed with parafilm to avoid moisture loss and incubated at 28 ± 2°C in a biochemical oxygen demand (BOD) incubator. Twelve replicates for each treatment along with the control were evaluated in the study. LPB mortality was recorded 72 h after IJ inoculation. The mortality data were converted into percentages and the mean values were calculated.

Penetration of EPNs

The penetration of EPNs into LPB larva was tested in a Petri dish (9 cm diameter) lined with filter paper at the base of the dish previously moistened with sterilized distilled water. Ten fifth-stage instar LPB larvae were released in the Petri dish along with the three pigeon pea flowers as larvae feed. One hundred IJs of each EPN were suspended in 0.5 ml of water and spread evenly on the Petri dish. To assess the IJ penetration, upon larval death, LPB cadavers were transferred to a separate Petri dish (9 cm diameter) containing dry filter paper, and maintained in darkness for 24 h. After 24 h the cadavers were rinsed with distilled water to remove the nematodes from the surface of their bodies and then dissected in Ringer's solution under a stereomicroscope to count the number of IJs penetrated inside each cadaver. Each larva was considered as a replicate. The penetration rate was then determined as per Pervez & Ali (2011).

Production of EPNs

For the production of EPNs, dead larvae infected by EPNs were removed from the Petri dish and rinsed with sterilized distilled water to remove any EPNs adhering to the body surface. Then the LPB larvae were transferred individually onto the modified White trap (White, 1927) and incubated at 28 ± 2°C in a BOD incubator. Each larva was considered as a replicate. The total number of IJs that emerged from each larva was counted three times under a stereo microscope, with the help of a Syracuse counting dish, and the mean values were determined.

Infectivity of EPNs against different instar larva of LPB

Based on the results obtained in the infectivity assays, two EPNs were identified as promising. The infectivity of these promising EPNs, *Heterorhabditis* sp. (IARI-EPN RP 06) and *Oscheius* sp. (IARI-EPN RP 08), against second-, third- and fourth-instar LPB larva was tested in a Petri dish (9 cm diameter) lined with moistened filter paper at the base of the dish. A single instar LPB larva, along with a pigeon pea flower, was placed in each dish as larval feed and 100 IJs of the promising EPN in 0.5 ml water were added to it. The experiment was conducted at 28 ± 2°C in a BOD incubator and replicated ten times along with the control. The observations on their mortality were recorded at 24 h intervals up to 72 h. Each test instar LPB larva, as well as EPN, was tested singly and on an individual basis. The mortality data were transformed into percentages and the mean values were calculated.

Statistical analysis

Percentage data were normalized using arcsine transformation, and numerical data were square-root transformed before analysis. The analysis was undertaken on the transformed data, and only the back-transformed data are presented. Infectivity, penetration and production data were assessed using analysis of variance (ANOVA). Standard deviation and standard error were also calculated.

Results

Infectivity of EPNs

The result showed that all test EPNs were pathogenic against LPB, though the levels of mortality varied markedly among the tested EPNs (ANOVA; $F = 8.31$; df = 10, 119; $P = 0.001$) (fig. 1). Among the EPNs, *Heterorhabditis* sp. (IARI-EPN RP 06) and *Oscheius* sp. (IARI-EPN RP 08) were found to be the most pathogenic to LPB, causing 100% mortality within 72 h, followed by *Steinernema* sp. (IARI-EPN RP 03 and 09), which brought about 92% mortality. *Oscheius* sp. (IARI-EPN RP 04) was the least pathogenic, recording 81% mortality.

Penetration of EPNs

Results (table 1) indicated that the rate of EPN penetration in the LPB larva body was significant (ANOVA; $F = 10.19$; df = 8, 99; $P = 0.0001$). Among the tested EPNs, the highest number of penetration was registered by *Heterorhabditis* sp. (IARI-EPN RP 06; 11.16 IJs/larva), followed by *Oscheius* sp. (IARI-EPN RP 08; 8.23 IJs/larva). The lowest rate of penetration was registered by *Oscheius* sp. (IARI-EPN RP 01; 3.64 IJs/larva).

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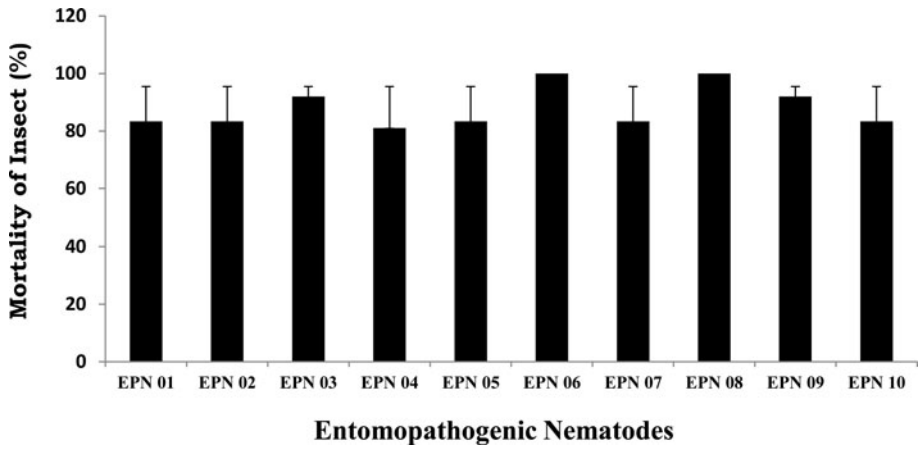

Fig. 1. Mortality of LPB larvae (mean ± standard error) through EPNs ($n = 12$). Abbreviations: EPN 01, *Oscheius* sp. (IARI-EPN RP 01); EPN 02, *Oscheius* sp. (IARI-EPN RP 02); EPN 03, *Steinernema* sp. (IARI-EPN RP 03); EPN 04, *Oscheius* sp. (IARI-EPN RP 04); EPN 05, *Steinernema* sp. (IARI-EPN RP 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); EPN 10, *Oscheius* sp. (IARI-EPN RP 10).

Table 1. Rate of EPN penetration into LPB.

EPN	No. of IJs/larva Mean ± SD (SE)
Oscheius sp. (IARI-EPN RP 01)	3.6 ± 1.51 (0.47)
Oscheius sp. (IARI-EPN RP 02)	4.3 ± 2.11 (0.66)
Steinernema sp. (IARI-EPN RP 03)	5.0 ± 1.63 (0.52)
Oscheius sp. (IARI-EPN RP 04)	6.7 ± 2.75 (0.87)
Steinernema sp. (IARI-EPN RP 05)	4.3 ± 2.26 (0.71)
Heterorhabditis sp. (IARI-EPN RP 06)	11.2 ± 2.61 (0.83)
Oscheius sp. (IARI-EPN RP 07)	7.9 ± 3.03 (0.96)
Oscheius sp. (IARI-EPN RP 08)	8.2 ± 2.70 (0.85)
Steinernema sp. (IARI-EPN RP 09)	6.6 ± 2.27 (0.71)
Oscheius sp. (IARI-EPN RP 10)	4.9 ± 1.79 (0.56)

SD, standard deviation; SE, standard error.

Production of EPNs

The results revealed that EPNs were able to grow within the haemocoel of LPB larva (fig. 2) and production was significant (ANOVA; $F = 67.41$; df = 8, 99; $P = 0.0001$). Among the tested EPNs, the highest production was observed with *Oscheius* sp. (IARI-EPN RP 08) with 1.3×10^5 IJs/larva, followed by *Oscheius* sp. (IARI-EPN RP 05 and 10; 1.1×10^5 IJs/larva). The lowest production was registered by *Steinernema* sp. (IARI-EPN RP 03; 0. 4×10^5 IJs/larva).

Infectivity of EPNs against different instar larva of LPB

Different instar larval mortality of LPB differed significantly among *Heterorhabditis* sp. (IARI-EPN RP 06) ($F = 3.21$; df = 2, 27; $P = 0.05$) and *Oscheius* sp. (IARI-EPN RP 08) ($F = 7.51$; df = 2, 27; $P = 0.001$). Among the two promising EPNs, *Oscheius* sp. (IARI-EPN RP 08) was most pathogenic, exhibiting 100% mortality of second-, third- and fourth-instar LPB larva within 24–48 h, whereas *Heterorhabditis* sp. (IARI-EPN RP 06) took more time to kill LPB (i.e. 48–72 h). Among the different instars of the LPB larva, the second-instar larva was more susceptible to EPNs, followed by third- and fourth-instar larva (fig. 3).

Discussion

One of the main reasons attributed to the failure of EPNs for the biological control of insect pests is the inaccurate choice of EPNs (Georgis & Gaugler, 1991) since the virulence can vary greatly, even among strains of the same species (Shapiro *et al.*, 2002; Pervez & Rao, 2018). Hence, *in vitro* screening of EPNs for infectivity is an important step in developing a biological control program for a particular pest (Ricci *et al.*, 1996) before commencing field studies. This study revealed that EPNs *Heterorhabditis* sp. (IARI-EPN RP 06) and *Oscheius* sp. (IARI-EPN RP 08) were highly virulent to the LPB larva. Variations in infectivity among the tested EPNs could be related to several factors, including the host insect, penetration, production ability (Kaya & Gaugler, 1993) and difference in the bacterial symbionts (Boemare, 2002; Pervez *et al.*, 2012).

EPNs can be produced *in vivo*, wherein the insect serves as a small biological reactor. Among the insects, GWML has been widely used for *in vivo* production of EPNs. In previous studies, the production ability of different EPNs was assessed in several insect larvae. Among them, *Helicoverpa virescens* Fabricius, *Chilo sacchariphagus indicus* K, *Spodoptera exigua* Hübner, *Spodoptera litura* Fabricius, *Corcyra cephalonica* S., *Trichopulsia ni* Hübner and *Athalia proxima* K. were reported to produce a large number of EPNs and were thus considered as good hosts for producing EPNs (Karunakar *et al.*, 1999; Elawad *et al.*, 2001; Pervez *et al.*, 2007; Pervez & Ali, 2009; Khan *et al.*, 2020). These insects have been utilized for the multiplication of various species of *Steinernema*, *Heterorhabditis* and *Oscheius* with varying yields of IJs depending upon the size of the larva of the test insects. The multiplication capability of any biological control agent is an important feature for their extended persistence and pathogenicity to the targeted insect pests (Blanco-Pérez *et al.*, 2017; Patil & Rangasamy, 2018). It not only results in the mortality of insect pests but also determines the recycling ability of the EPNs to tackle the succeeding generations of the targeted insect pests (Patil *et al.*, 2019). The higher production rate of EPNs noticed in this study may help in tackling the succeeding generations in the field. While there are reports on the susceptibility of *M. vitrata* larva to EPNs, but the data on their production in LPB larva is not available, as a result, this is the first record of the production of any EPN on LPB larva.

The rate of penetration could be utilized as a real measure of host susceptibility. Dunphy & Webster (1991) reported that the

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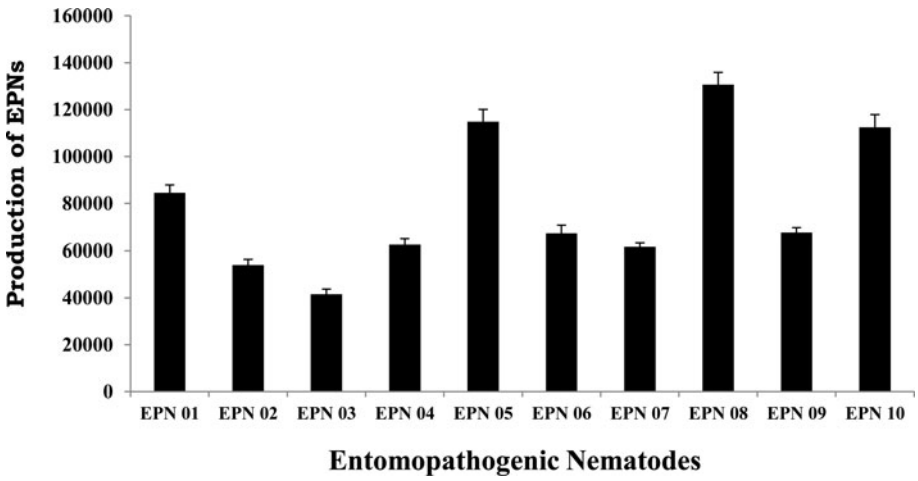

Fig. 2. Production of EPNs (mean ± standard error) on the LPB larvae ($n = 10$).

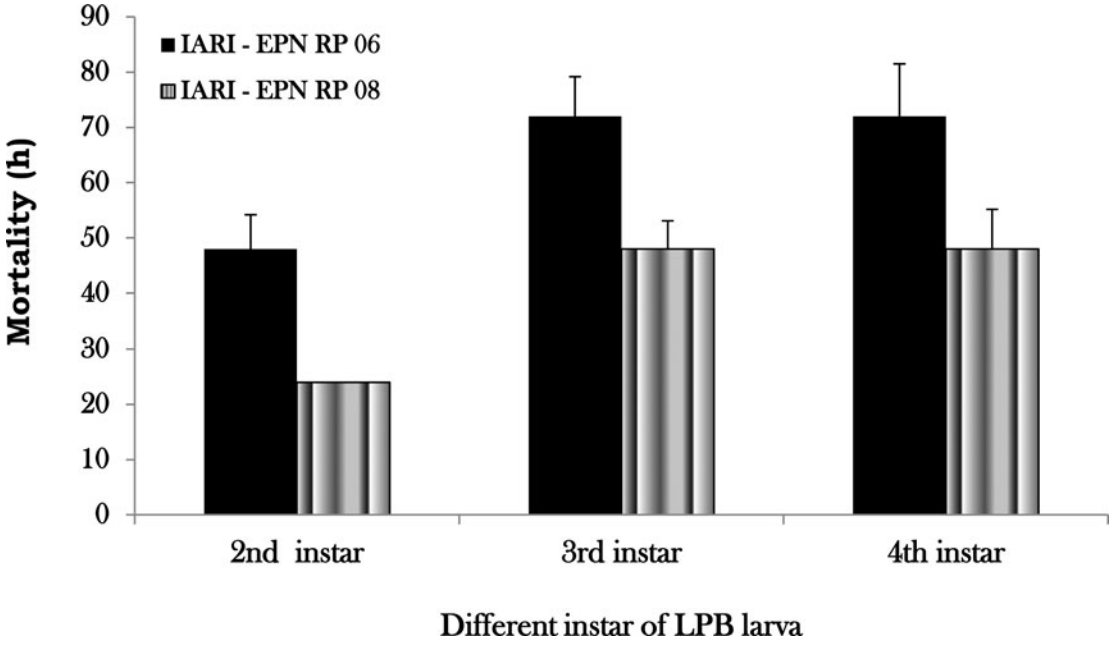

Fig. 3. Mortality (mean ± standard error) of different instar larvae of the LPB ($n = 10$).

difference in the toxicity of bacterial symbionts is related to the difference in their cell wall substances, which may have led to the relative destruction of host haemocytes and, finally, to the death of the host. In the present study, *Heterorhabditis* sp. (IARI-EPN RP 06) and *Oscheius* sp. (IARI-EPN RP 08) were able to penetrate the insect body in more numbers and, hence, can be considered as promising biocontrol agents for the management of LPB.

Our study revealed that second-instar larvae were highly susceptible, followed by third- and fourth-instar LPB larvae. These results are consistent with the findings of Banu *et al.* (2007), who reported that the first- and second-instar larvae of *Helicoverpa armigera* were highly susceptible to *Heterorhabditis indica*. Similarly, the second-instar larva was highly susceptible, followed by the third-instar, fourth-instar and fully grown larva of pod-borer to EPNs (Pervez, 2010).

Conclusions

From the present study, it can be concluded that *Heterorhabditis* sp. (IARI-EPN RP 06) and *Oscheius* sp. (IARI-EPN RP 08) were most virulent against LPB among the ten native EPN strains tested. Furthermore, the fifth-instar LPB larva was more suitable for the multiplication of *Oscheius* sp. (IARI-EPN RP 08), suggesting the suitability of this insect for EPN production. Further evaluation of these promising EPNs under field conditions will indicate their utility in the integrated management of LPB.

Acknowledgements. The authors express their gratitude to the Director and Joint Director (Research) at ICAR – Indian Agricultural Research Institute, New Delhi, for providing all the facilities.

Financial support. Resources of the ICAR – Indian Agricultural Research Institute, New Delhi, India, were utilized for this research study.

https://doi.org/10.1017/S0022149X21000043 Published online by Cambridge University Press

Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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