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Productivity of the entomopathogenic nematode, *Heterorhabditis indica* isolates in *Galleria mellonella*

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ABSTRACT

Heterorhabditis indica, CICR-HI-MN and CICR-HI-CL isolates were tested over the fifth larval instar of the Greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), at nine different dosages, i.e., at 10, 20, 40, 60, 80, 100, 120, 140, and 160 infective juveniles (IJs)/larva in 100 µl of sterile water under laboratory condition at the district Nagpur of Vidharbha Region, India, during 2022. When the LC50 and LC95 values of 5th instar larvae were analyzed, the CICR-HI-CL isolate was quite more efficient than CICR-HI-MN isolate. Moreover, when compared to various treatments, given isolates showed greater reproduction rate in the fifth instar, although the CICR-HI-MN isolate produced a higher emergence of infective juveniles with a statistically significant difference (P = 0.001). Native isolates of *H. indica* tested against fifth instar of *Galleria mellonella* larvae exhibited excellent results and can be employed as the principal model for entomopathogenic nematodes maintenance and multiplication.

Key words*: Galleria mellonella, Heterorhabditis indica, Entomopathogenic nematode, Larval mortality, Biological control.*

Introduction

Entomopathogenic nematodes (EPNs) are the obligatory pathogens of insects from the genera *Steinernema* spp. and *Heterorhabditis* spp. conferred by the symbiotic complex formed with *Xenorhabdus* spp. and *Photorhabdus* spp., of bacteria (El-Sadawy *et al*., 2020) has been utilized as biocontrol agents throughout the worldwide because they have a special set of qualities that make them a promising alternative for pest control (Abd-Elgawad, 2019).

These nematodes, which parasitize insects, offer great potential for use in pest management seeing as they act as carriers of bacteria that quickly kill the insects and pests of various crops (Grewal *et al*., 2001; El- Sadawy *et al*., 2020).

The mass generation process of entomopathogenic nematodes from the tissues (cadaver) of a range of vulnerable insects might further support the uniqueness of these parasites. Productivity is a crucial factor in the long-term survival of EPNs. The most widely preferred host for growing entomopathogenic nematodes is the *Galleria mellonella* (Lepidoptera: Pyralidae) (Kumar, 2015). So, the purpose of the current investigation was to determine the reproductive ability of two isolates of EPN in *Galleria mellonella* larvae.

Materials and Method

Rearing of *Galleria mellonella*

The Central Institute for Cotton Research (CICR), Nagpur, Maharashtra, India, provided the initial culture (egg masses) of *G. mellonella*. The eggs were

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collected, and the larvae were raised on an artificial diet made of wheat and corn (Singh, 1994) and maintained under controlled conditions (65 ± 5% relative humidity (RH); 14L:10D photoperiod, 27 ± 1 °C temperature) until they pupated in a plastic container measuring 50 cm×30 cm and stuffed with 20–35 g of freshly prepared diet and covered with muslin cloth. Adult moths were served honey solution by cotton swabs, while folded paper strips were provided for egg laying by the female moths.

Nematode culture

Pure culture of *Heterorhabditis indica* isolates used in the study were provided by Crop Protection Division of ICAR-CICR, Nagpur. The infective juveniles were cultivated in the last instar larvae of *Galleria mellonella*, and were stored at 15 ± 1 °C temperature.

Laboratory experiment

Virulence of EPN isolates against *G. mellonella*

Infectivity of *Heterorhabditis indica* isolates was evaluated against 5th instar larvae of *G. mellonella*. Ten larvae were taken in multi-cell plastic trays lined with filter paper moistened with sterile water. Each larva was inoculated with 100 µl of sterile water contained different nine concentrations viz., 10, 20, 40, 60, 80, 100, 120, 140, 160 IJs/larva with water as control of tested nematode isolates. All the trays were incubated at 25±2 °C and larval mortality was observed regularly upto 96 hours. Each treatment was replicated five times that is total 50 larvae were used per treatment concentration.

Reproduction of EPN isolates on *Galleria mellonella*

The dead larvae that had been infected by nematodes were taken out of the rearing trays and placed in batches of three on the white traps for IJs emergence from the body (White, 1927). (Fig. 2). Then, a stereozoom binocular microscope was used to inspect these petri plates and white traps for nematode emergence counts. The nematodes after emerged from cadavers moved into surrounding water in the petri dish and this water containing infective juveniles were taken out in a tissue flask daily for upto 25 days. The suspension taken out was subjected to check for nematode population count, which was taken by observing 100 µl suspension under stereozoom binocular microscope for number of IJs in the droplet. Total population count was computed after noting the total count of nematode suspensions collected from the petri plate. These observations for population count were taken for all treatments and replications separately.

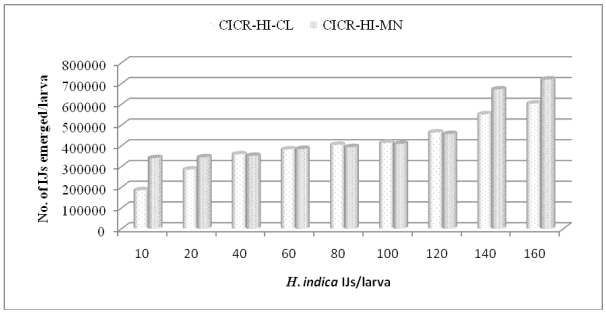

Fig 1. Number of infective juveniles emerged under different treatments imposed on 5th instar of *G. mellonella*

Statistical analysis

To perform Probit analysis on experimental data pertaining to larval mortality in order to determine the LC50 and LC95 values Polo Plus (Version 2.0; LeOra software) was used. The fiducial limits were taken at 95% confidence intervals. In WASP (webagristat package) software, one-way ANOVA was used to examine the results on the average reproduction rate against fifth larval instar and between dosages.

Results

Pathogenicity of EPN isolates CICR-HI-MN and CICR-HI-CL against *Galleria mellonella*

Galleria larva inoculated with different EPN concentrations resulted in considerably high mortality at 72 and 96 hours after inoculation. The *in-vitro* performance of the CICR-HI-MN isolate against *G. mellonella* exhibits LC50 values (8.65, 5.91 IJs/larva) and LC95 values (39.13, 29.98 IJs/larva) at 72 and 96 hours after inoculation. There was no noticeable difference between the two isolates, even though the CICR-HI-CL isolate was quite more pathogenic, with LC50 values (7.23 and 6.58 IJs/larva) and LC95 values (38.52 and 22.13 IJs/larva), respectively, at 3rd and 4th day of post inoculation.

Reproductive potential of EPN isolates CICR-HI-MN and CICR-HI-CL on *Galleria mellonella*

The present findings showed that *H. indica* reproduced efficiently in the fifth larval instar of *G. mellonella*, and their offspring were emerged from

the larval cadavers. The reproduction of *H. indica* CICR-HI-MN and CICR-HI-CL isolates (F=18.64; df=8; P 0.001, F=18.69; df=8; P 0.001) was not significantly affected by the varied quantities of IJs injected to 5th instar larvae. Despite the fact that the ability of *H. indica* to reproduce in *G. mellonella* larvae dramatically increased as the rate of IJs raised. The CICR-HI-MN isolate recorded (341281 IJs/larva, 720448 IJs/larva) at the lower and higher concentrations, which was substantially greater than CICR-HI-CL, which recorded (186131 IJs/larva, 604145 IJs/larva) (Table 1).

Discussion

In this research, we investigated IJs productivity of two EPN isolates in *G. mellonella* larvae. Within 96 hours of inoculation, the current investigation indicated 100% mortality of *G. mellonella* at 60 IJs/larva, which is comparable to the results of Ottum *et al*. (2021), who discovered 60% mortality of *G. mellonella* at 50 IJs/larva of *Heterorhabditis* spp. Similar to this, Ibrahim *et al*. (2019) reported the pathogenicity of *H. zealandica* on the 5th instar of *G. mellonella* and found a positive relationship between the concentration and percentage of host mortality.

According to Shapiro-Ilan *et al*. (2002b), choosing the proper EPN species against a specific pest is crucial that would be found successful in a particular ecosystem. The results of the present study showed that *H. indica* exhibited the highest productivity of

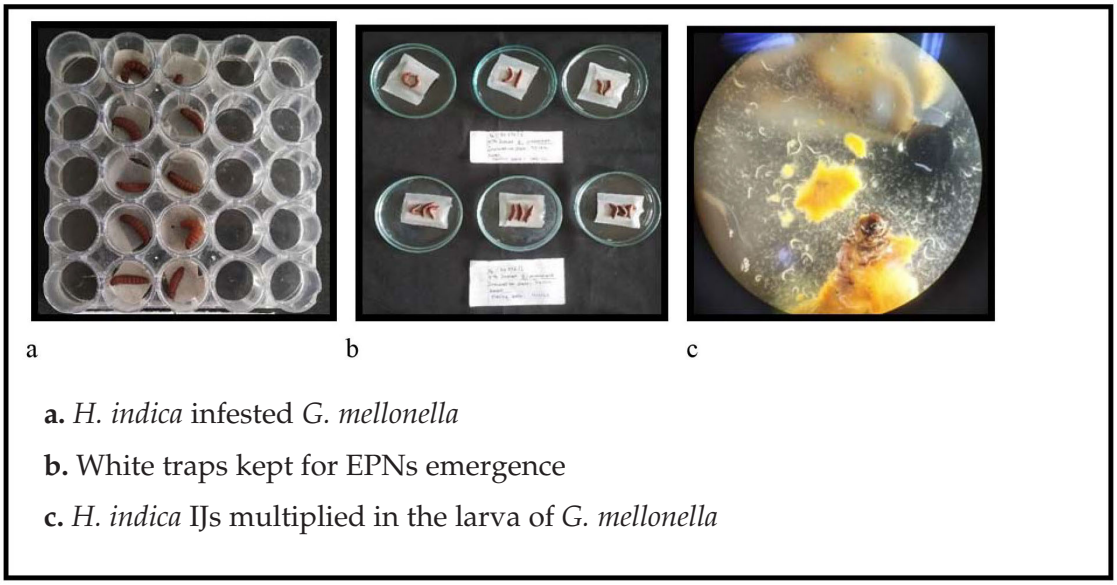

Fig. 2. Virulence of *H. indica* isolates against fifth instar of *G. mellonella*

Table 2. Reproductive potential of EPN infective juveniles emerged under different treatments imposed on 5thinstar of *Galleria mellonella*

NO. of IJs/larva	CICR-HI-MN strain	CICR-HI-CL strain
10	341281±25007.22 c	186131±11626.08 f
20	345208±27686.1 c	286167±17666.4 e
40	352280±25597.38 c	359908±24573.06 de
60	384953±27971.18 bc	383233±25791.11 cd
80	394313±27587.76 bc	405452±24319.73 cd
100	409593±28531.68 bc	414700±29776.31 cd
120	457650±32095.05 b	465350±33171.49 bc
140	672883±44929.78 a	552344±35067.57 ab
160	720448±49507.6 a	604145±39832.5 a

Different lowercase letters denote statistical significance of means compared between the concentrations at P 0.001 level of significance.

IJs which is important factor to consider in insect biological control agents supported by Nouh *et al*. (2021) results indicated that reproduction of *Heterorhabditis* sp. strain TAN5 was the highest (1,40,402 IJs/larva) from the fifth instar larvae of *G. mellonella* as well as Mhatre *et al*. (2020) reported reproduction capacity of *S. cholashanense* on fourth-instar larvae *G. mellonella* (5075.08). Whereas findings on progeny production in *G. mellonella* larvae by Rahoo *et al*. (2018) indicated greater emergence of IJ from the *H. bacteriophora* (1,99,894/larva at 55 IJs/larva on the 23rd day) than those from the *S. carpocapsae* (21,407/larva at 50 IJs/larva on the 20th day). As per the Noitubtim *et al*. 2022 in *G. mellonella* larvae *Heterorhabditis* spp. produced higher IJ than *Steinernema* spp. All these studies have close relevance with the present findings.

Conclusion

With lower LC50 and LC95 values, *G. mellonella* provided significantly higher susceptibility and productivity of EPN isolates against fifth instar larvae. This has the added benefit that with smaller doses, we can mass culture the infective juveniles and maintain them to be used in laboratory bioassays or areas for further research including novel approach of field applications of EPNs in the form of desiccated cadaver of *G. mellonella* which may serve as inundative or inoculative release of EPNs in Integrated Pest Management.

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Competing interests

The authors declare that they have no competing interest.

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