Original Research

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Laboratory evaluation of the pathogenicity of three entomopathogenic nematodes against larvae of cabbage butterfly, *Pieris brassicae* Linnaeus (Lepidoptera: Pieridae)

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ABSTRACT

The development of indigenously isolated entomopathogenic nematodes as biological control agents was investigated. The study involved three nematode species (*Heterorhabditis indica*, *Steinernema thermophilum* and *S. glaseri*) and their pathogenicity against larvae of cabbage butterfly, *Pieris brassicae*, under laboratory conditions. Nematodes of different concentrations (0, 10, 25, 50, 75, and 100 IJs/larva in 0.5 ml of distilled water) were applied against the insect pest using Petri dish assay. Progeny production of IJs was determined by the number of IJs produced per larva/pupa (within 20 days), following their exposure to IJs of EPN species at different concentrations. The data were analyzed statistically and the significance of the difference was determined by one way analysis of variance (ANOVA) and student's *t*-test. LC_{50} and LT_{50} values were determined and estimated. Among the three species *S. thermophilum* caused larval mortality at 24 HAI (hours after inoculation) at 50 IJs/larva. However, at 48 HAI in addition to *S. thermophilum, H. indica* also revealed 100% mortality at 100 IJs/larva. In case of *S. glaseri* no mortality was observed at 24 HAI. The study thus concluded that both on the basis of mortality and LC_{50} value (30.2 IJs/larva at 48 HAI) *S. thermophilum* emerged as the most potent species. The progeny production by larvae of *P. brassicae* was noted to be highest only in case of *H. indica*. The production increased along the concentrations till the highest concentration for both *H. indica* and *S. thermophilum* but declined from 50 IJs/larva onwards in case of *S. glaseri*.

Key words: Entomopathogenic nematodes (EPNs); *Heterorhabditis indica*; IJs (infective juveniles); *Pieris brassicae*; *Steinernema glaseri*; *S. thermophilum.*

INTRODUCTION

One of the major constraints in agriculture production in India is sustained losses due to attack by pests and diseases. The agricultural pests include mites, disease causing pathogens, weeds and other organisms causing damage to crops, but in many instances it has been used to denote insects alone. One or the other insect pests are always associated with every crop grown, but not all these pests are of economic importance. When the pest abundance crosses the action threshold or the economic injury lev-

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el, their control is one of the main agricultural requirements for increase in crop productivity.

Rapidly increase in knowledge regarding biology, host range and epidemiology has laid groundwork for the eventual use of entomopathogenic nematodes (EPNs) as effective biological control agents world-wide. In developed countries like USA, Australia and Europe, commercial nematode based products are available and are being utilized for biological control of insects.[1] EPNs have emerged as excellent candidates for biological control of insect pests. Attributes making the nematodes ideal biological insecticides includes their broad host range, high virulence, safety for non-target organisms and high efficacy in favorable habitats.

The cabbage butterfly, *Pieris brassicae* Linnaeus (Lepidoptera: Pieridae), is a common insect pest of cruciferous including broccoli, brussel, sprouts, cabbage, cauliflower and other important crops. Young larvae graze away the lower epidermis of the leaves whereas the older larvae cause extensive defoliation and often reduce plants to a skeleton of stems and major veins; it may also kill the plant. Besides, the plants are also contaminated with large quantity of its faeces.[2,3] The larvae pass through five instars and feed gregariously; and are fully grown in about 24 days. They leave the plant to pupate on a solid substrate nearby such as wall, fence, tree trunks, etc. Pupation takes 10-15 days and second generation emerges. Eggs are laid in batch of 20-100 mostly on the underside of the leaves. They hatch about 1-2 weeks later depending on the temperature.[4]

The objective of the present study was to provide basic information necessary for the utilization of indigenously isolated EPNs as biological control agents. The study dealt with the three nematode species such as *Heterorhabditis indica* Poinar, Karunakar & David (Rhabditida: Heterorhabditidae), *Steinernema thermophilum* Ganguly & Singh (Rhabditida: Steinernematidae) and *S. glaseri* Steiner (Rhabditida: Steinernematidae), and their pathogenicity against *P. brassicae* under laboratory conditions.

Materials and Methods

In the present study the EPNs used for pathogenicity tests include, *H. indica*, *S. thermophilum* and *S. glaseri*, which were isolated from the Ri-Bhoi District of Meghalaya, India, and reared *in vivo* on larvae of the wax moth, *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae), according to Woodring and Kaya.[5]

Collection and laboratory maintenance of the nematodes

The insect larvae were collected along with their natural diets from experimental farms of the Indian Council of Agriculture Research, Umiam, Meghalaya, India, and kept for at least 5 days in the laboratory to check, whether or not, there are any other infections before using them for experiments.

Mortality test for larvae

Eight numbers of Petri dishes (35 x 10 mm) were lined with double layer of Whatman No. 1 filter paper for each nematode concentration. Nematodes of different concentrations (0, 10, 25, 50, 75, and 100 IJs/larva in 0.5 ml of distilled water, DW) were evenly distributed on the filter paper and kept at least for 30 minutes. One larva was placed in each of the Petri plates and sealed with parafilm. Number of insect pest larvae showing mortality were recorded every 24 hrs till 120 hrs. Larvae placed on wetted filter papers without IJs served as control. Three (3) replicates (containing 8 insects each) for each nematode species and concentration were set.

Progeny production

Progeny production of IJs was determined by the number of IJs produced per larva/pupa (within 20 days), following their exposure to IJs of EPN species at different concentrations. A Petri dish was lined with double layer of

Whatman No.1 filter paper for each nematode concentration. Nematodes of different concentrations (10, 25, 50, 75 and 100 IJs/larva in 0.5 ml of DW) were evenly distributed on filter papers and kept for at least 30 minutes. One insect larva was placed in each of the Petri plates and sealed with parafilm.

Larval mortality was checked at 24 hrs interval. The dead larvae/pupae were picked up from each nematode species and concentration. They were rinsed in DW and kept in White traps separately to determine the total number of IJs produced in each case. There were 8 replicates for each nematode species and concentrations. To each concentration, one Petri dish/container, prepared as described above but without IJs was kept as control.

Statistical analysis

The data were analyzed statistically and were represented as mean ± standard error of mean (SEM). The significance of the difference was determined by the one way analysis of variance (ANOVA) and student's *t*-test. Probability less than 5% (p value < 0.05) was accepted as statistically significant. Correlation between the parameters was determined by regression analysis. LC_{50} and LT_{50} values were determined and estimated by probit analysis using SPSS software.

RESULTS

Larval mortality

The concentrations of IJs were found to be positively correlated with the time of larval mortality (Table 1). At 10 IJs/larva, *H. indica* caused a mortality of 12.5 ± 4.13% from 48 hours after inoculation (HAI) and the same reached to 95.8 ± 4.13%

Table 1. Correlations between the concentrations of EPNs and larval mortality time of *P. brassicae* larva.

A.

Concentrations (IJs/larva)	Time		
	H. indica	*S. thermophilum*	*S. glaseri*
10	0.97**	0.96**	0.99**
25	0.95**	0.97**	0.99**
50	0.95**	0.95**	0.95**
75	0.70	0.73	0.95**
100	0.70	0.70	0.88*

B.

Time (hrs)	Concentrations		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	0.90*	-
48	0.93**	0.96**	0.97**
72	0.88*	0.88*	0.95**
96	86*	0.91*	0.95**
120	0.64	0.64	0.84

** significant at 0.01% and * significant at 0.05%.

Table 2. LC_{50} and LT_{50} values of EPNs against *P. brassicae* larva.

A.

Hours after inoculation	Time		
	H. indica	*S. thermophilum*	*S. glaseri*
24	-	87.5	-
48	41.1	30.2	104.9
72	10.0	17.3	17.6
96	-	-	-
120	-	-	-

B.

Concentrations (IJs/larva)	Concentrations		
	H. indica	*S. thermophilum*	*S. glaseri*
10	74.3	76.8	75.4
25	59.7	60.4	72.4
50	57.1	28.5	58.1
75	39.0	29.7	57.1
100	39.0	-	47.9

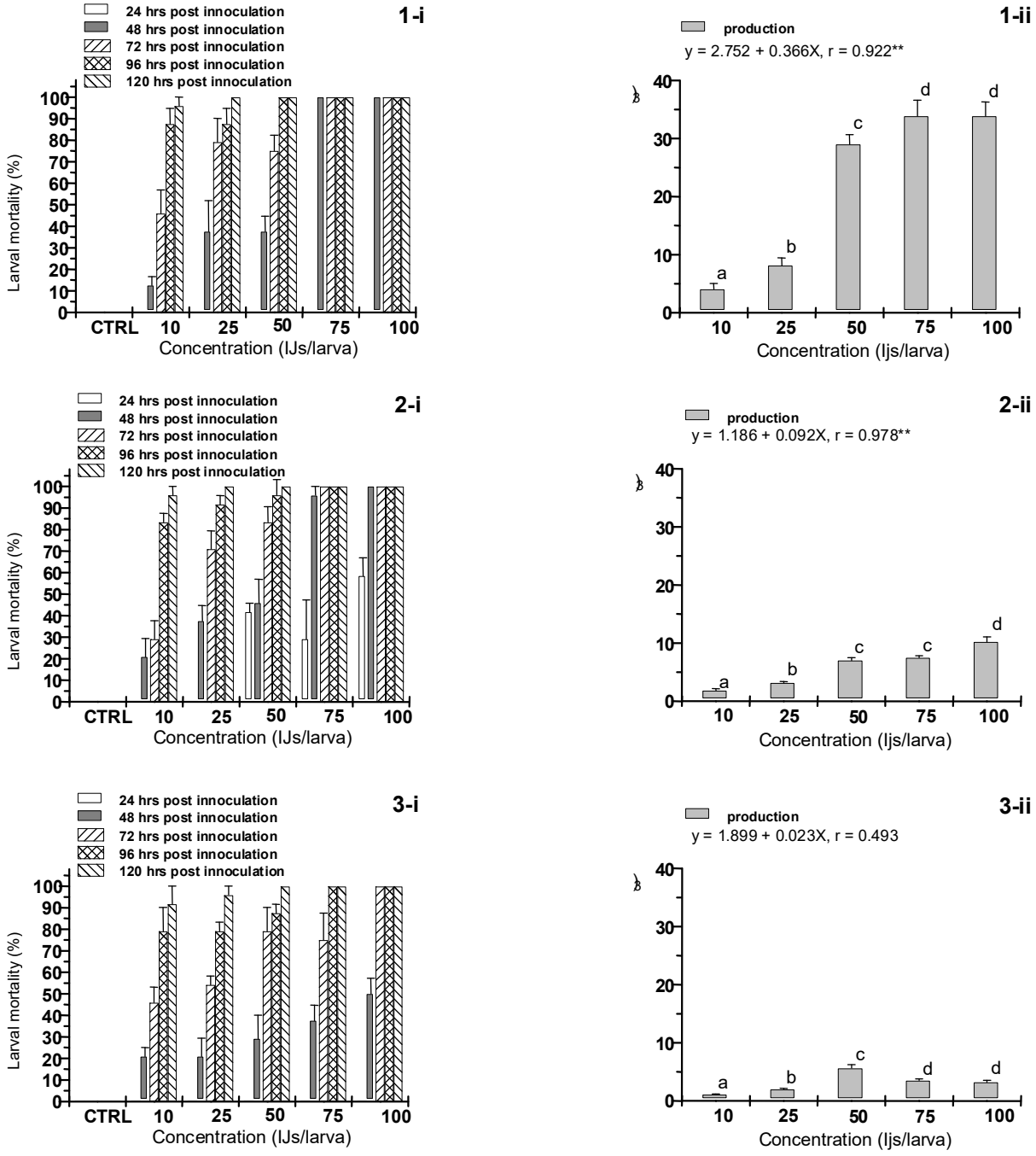
within 120 HAI (Fig. 1-i). The mortality induced by *S. thermophilum* and *S. glaseri* was first observed at 48 HAI and it reached up to 95.8 ± 4.13% in case of *S. thermophilum* and 91.6 ± 8.5% for S. *glaseri* within 120 HAI (Fig. B-i & C-i). At 25 IJs/larva, mortality was first observed at 48 HAI for all the three species; *H. indica* (37.5 ± 14.38%), *S. thermophilum* (37.5 ± 7.25%) and *S. glaseri* (20.8 ± 8.5%). Within 120 HAI, mortality reached upto 100% in case of both *H. indica* and *S. thermophilum,* whereas up to 95.8 ± 4.13% in case of *S. glaseri.* At 50 IJs/larva, mortality was first observed at 48 HAI in case of *H. indica* and *S. glaseri,* whereas it was observed at 24 HAI for *S. thermophilum.* At this concentration, 100% mortality was observed within 96 HAI for *H. indica,* whereas *S. glaseri* and *S. thermophilum* showed 100% mortality at 120 HAI. At 75 IJs/larva, *H. indica* did not cause any mortality within 24 HAI but 100% mortality was observed at 48 HAI. Mortality started at 24 HAI in case of *S. thermophilum,* and it reached to 100% within 72 HAI. *S. glaseri* also caused a 100% larval mortality within 72 HAI.

At 100 IJs/larva, *H. indica* and *S. glaseri* did not cause any larval mortality at 24 HAI; however, *S. thermophilum* showed 58.3 ± 8.5% mortality at 24 HAI. 100% larval mortality was observed at 48 HAI for both *H. indica* and *S. thermophilum* whereas, *S. glaseri* caused 100% mortality at 72 HAI.

No larval mortality was observed in the control groups. The calculated values of LC_{50} and LT_{50} are presented in Table 2.

Progeny production

The production of IJs was positively correlated



Figures 1-3. Bioefficacy of EPNs against *P. brassicae* larvae. 1. *H. indica*; 2. *S. thermophilum*; 3. *S. glaseri*. i. Larval mortality; ii. Progeny production.
***Significant at 0.01%* and **Significant at 0.05%.*
Means shown by the same letter are not significantly different ($p < 0.05$).

with the concentrations for *H. indica* ($y = 2.752 + 0.366\ x$, $r = 0.922$) (Fig. 1-ii), *S. thermophilum* ($y = y = 1.186 + 0.092\ x$, $r = 0.978$) (Fig. 2-ii) and *S. glaseri* ($y = 1.899 + 0.023\ x$, $r = 0.493$) (Fig. 3-ii). The production of IJs at different concentrations increased from $4.1 \pm 0.946 \times 10^3$ IJs/larva at 10 IJs/larva concentration to $33.8 \pm 2.46 \times 10^3$ IJs/larva at 100 IJs/larva for *H. indica;* for *S. thermophilum* IJs production varied from $1.8 \pm 0.31 \times 10^3$ IJs/larva at 10 IJs/larva concentration to $10.2 \pm 0.85 \times 10^3$ IJs/larva at 100 IJs/larva concentration. In case of *S. glaseri,* it was $1.1 \pm 0.11 \times 10^3$ IJs/larva at 10 IJs/larva concentration and reached to a highest production of $5.6 \pm 0.58 \times 10^3$ IJs/larva at 50 IJs/larva concentration.

Discussion

Interest in the promotion of EPN as biological agents for agricultural pests has provoked a number of studies on optimization of EPN for their bio-efficacy.[6] The nematodes in the genera *Heterorhabditis* and *Steinernema* are being used for the biological control of soil-dwelling insects.[1] In order to formulate tests for the evaluation of nematodes against a particular host species, a better understanding of their pathogenesis is essential.[7] The need to evaluate insecticidal activity in the laboratory has resulted in the development of a variety of assays that measure nematode infectivity by host mortality.[8] One such assay which has been commonly applied in many studies includes dose-response test, i.e., to determine the percent mortality of insect pest following its exposure to different doses of IJs for different time periods.[9]

In the present study, the larvae of cabbage butterfly, *P. brassicae*, were found to be very susceptible to EPNs. Although all the nematode species tested caused 100% mortality, their infectivity levels varied. This finding agreed with other report that the efficacy of various EPN species or strains for controlling a particular insect pest may differ significantly.[10,11] It is concluded here that both on the basis of mortality and LC_{50} value (30.2 IJs/larva at 48 HAI), *S. thermophilum* emerged as the most potent species. In other studies, Hussaini *et al*. demonstrated the susceptibility of *Leucinodes orbonalis* larvae to entomopathogenic nematodes and reported that *H. indica* causes 100% mortality within 72 hrs at 25 IJs/larva.[12] Narayanan and Gopalakrishnan in their study on susceptibility of *Athalia proxima* by *S. feltiae* have reported a high mortality rate of the larvae. The difference in the pathogenicity level may be due to different insect pest species.[13] Differences in the susceptibility among insect life-cycle stages have also been observed in the family Pyralidae, with the pupae being less susceptible than the larvae.[14,15]

Once the rate of pest mortality is established, one can also go for reproduction capability tests by accessing the total number of IJs of the test EPNs emerging from the dead pest in a limited time period. The assessment of reproduction capability of EPN becomes important as its gives an idea about total production of IJs that can emerge from pest and in turn can recycle in the environment to infect other surrounding pests. Of all the EPNs, the progeny production by larvae of *P. brassicae* was noted to be highest in case of *H. indica*. The same was noticed to be considerably low in case of *S. thermophilum* and and *S. glaseri*. The production increased along the concentrations till the highest concentration for both *H. indica* and *S. thermophilum* but declined from 50 IJs/larva onwards in case of *S. glaseri.*

These observations are in agreement with the findings of Mahar *et al*., who studied the production and infectivity of *S. carpocapsae, S. feltiae, H. indica* and *H. bacteriophora* against *P. brassicae* and reported that the progeny production was highest in case of *H. indica* as compared to the other species.[16] The decline in the production of *S. glaseri* may be because of their IJs size which is bigger as compare to others. This is due to the fact that the development of parasites in a host may be adversely affected by high infection density.

Changes in sex ratio, reduction in body size, progeny production and survival of entomogenous nematodes have been attributed to crowding of the

parasites in their host.[17,18]

This study observed that concentrations of IJs had a significant effect on the numbers of IJs established per host, which in turn affect their reproduction potential for all the species. This observation agreed with Selvan *et al*. who reported that the proportion of *S. carpocapsae* and *H. bacteriophora* infecting *G. mellonella* larvae declines with increasing dose.[19] Danilov, using longer exposure periods, also found that *S. carpocapsae* percentage infection declines with increasing dose.[20] In contrast, Fan and Hominick found that *Heterorhabditis* sp. percentage infection was relatively constant over a range of doses.[8] However, they used a relatively low dose range of IJs (10-300) and a series of host exposures.

From a pest management standpoint, the main goal is to kill a large number of the target pests to bring it below the economic threshold level; however, if nematode reproduction can occur successfully in the target insect, then longer term management might be achievable. The present study observed that the EPNs reproduction performance is satisfactory. In general, a high progeny production was recorded in case of *H. indica* which was followed by *S. thermophilum*. In contrast, *S. glaseri* did not reveal a better progeny production.

In summarizing the findings, it can be stated that in terms of biocontrol agents, *H. indica* appears to be the most promising EPN, followed by *S. thermophilum* and *S. glaseri* against *P. brassicae*. Further, these EPNs showed good compatibility with most of the tested chemical pesticides.[21] Hence, they could be incorporated into the integrated pest management strategies to control insect pests. It is suggested that in order to estimate the practical value of applying these nematodes in the control of insect pests field studies should be continued.

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