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ORIGINAL ARTICLE

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Evaluation of the efficacy of three indigenous strains of entomopathogenic nematodes from Meghalaya, India against mustard sawfly, *Athalia lugens proxima* Klug (Hymenoptera: Tenthredinidae)

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Abstract The objective of this study was to evaluate the efficacy of three indigenous strains of entomopathogenic nematodes (EPN) from Meghalaya, India, namely *Heterorhabditis indica* Poinar, Karunakar and David, *Steinernema thermophilum* Ganguly and Singh, and *Steinernema glaseri* (Steiner) against the last instar larva of mustard sawfly, *Athalia lugens proxima* Klug, a serious pest of mustard and radish in India. The larvae of *A. lugens proxima* were exposed to 10, 25, 50, 75 and 100 infective juveniles (IJs) concentration of each nematode species in Petri dishes. Percentage larval mortality and nematode reproduction in insect larvae was studied. The sawfly larvae were found to be susceptible to all the three EPNs tested, but the degree of susceptibility to infection varied from among nematode species. Based on LC50 value, *H. indica* was the most pathogenic species. Nevertheless, *S. thermophilum and S. glaseri* also showed a high insect mortality. This study also revealed that all the three test nematodes are also able to propagate in the host cadaver and produce first generation infective juveniles. However, *H. indica* produced significantly more number of IJs per insect larva than the other two nematode species. The progeny production was recorded to be the least in case of *S. glaseri*. In conclusion, our findings suggest that of the three indigenous EPNs studied, *H. indica* and *S. thermophilum* have good potential as biological control agents against mustard sawfly, *A. lugens proxima*.

Keywords Entomopathogenic nematodes · Biological control · Mustard sawfly · *Athalia lugens proxima* Klug · *Heterorhabditis indica* · *Steinernema thermophilum* · *Steinernema glaseri* · Meghalaya · India

Introduction

Mustard sawfly, *Athalia lugens proxima* Klug (Hymenoptera: Tenthredinidae) has become a serious pest of mustard and radish in several regions of India, including the north-east India (Narayanan and Gopalakrishnan 2003; Chowdhury 2009). It is a pest of cold weather and is generally active during October to March. The female fly lays the eggs singly on the young leaves, close to the margin. Under favourable conditions, hatching takes place in 5–7 days, and the larval stage lasts about 13–15 days (Patil and Pokharkar 1973). There are six larval instars, and the pupation takes place in the soil. The whole life-cycle is completed in about 30–39 days (Dhillon 1966). The larvae alone are destructive and feed from the margin of the leaf towards centre. The grown up larvae make holes, preferably on young leaves, and skeletonise them. Sometimes, they also feed on the epidermis of the tender shoots, flowers and fruits (Chowdhury 2009). The severity of infestation varies according to season, and in severe cases of major attack at the seedling stage, the crop may even need resowing. There are several chemical control means, such as spray application of carbaryl, endosulfan, phosalone, etc. which are currently employed in the management of pest on mustard crops (Ramoliya et al. 2011). However, there are concerns about the use of pesticides, because of their negative effects on the environment and human health. Furthermore, dependence on pesticides is discouraged, as in the event of their indiscriminate use, they may also cause other problems,

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such as the elimination of natural enemies, resurgence of pests, development of insecticide resistance and outbreak of secondary pests (Hardin et al. 1995). Further, in many cases, these pesticides are also expensive and out of reach to the poor farmers. Therefore, there is a need to develop alternative methods of pest management.

Insect pest management by biopesticides is an alternative strategy that results in pesticide-free production with no hazards to the environment. Patel and Jhala (1999) explored the possibility of developing some neem-based formulations (*Azadirachta indica* and *A. Juss*) on sawfly. In this study, Repelin and Margocide CK showed good potentials against the pest, under laboratory conditions. Patnaik et al. (1987) observed that 3.0% neem oil spray can result into a 100% larval mortality of *A. proxima*. On the other hand, Singh et al. (1993) tested some plant products to control the sawfly and reported that spray applications of *A. indica*, *Saussurea lappa* and *Lantana camera* were more effective in reducing the pest incidence and increasing the crop yield. Recently, Srivastava and Singh (2003) reported that soil application of neem leaf powder, at the time of sowing in furrows, also helps in reducing the population of mustard sawfly.

Among the other biopesticides, entomopathogenic nematodes (EPNs) are promising agents for the control of various insect pests (Grewal et al. 2005). EPNs of the genera *Steinernema* and *Heterorhabditis* are obligate pathogens of insects (Poinar 1979) that have been found in many diverse climates throughout the world (Hominick et al. 1996). They possess many of the attributes of an ideal biological control agent, including broad host range, high virulence, host seeking capability, ease of mass production, recycling ability, non- hazardous to environment, etc. (Gaugler and Kaya 1990; Kaya and Gaugler 1993). Many species of EPNs belonging to both genera have been used with variable success as biological control agents against insects occupying different habitats (Gaugler 1988). However, most success has been achieved against soil-dwelling pests or pests in cryptic habitats (Williams and Walters 1999; Tomalak et al. 2005; Valle et al. 2008). Numerous studies have found that the susceptibility of different developmental stages of insect hosts shows great variations to different species or strains of EPNs (Bedding and Molineux 1982; Geden et al. 1985; Fuxa et al. 1988; Glazer et al. 1991; Smits et al. 1994; Jansson 1996; Simões and Rosa 1996; Khatri-Chhetri et al. 2011). While developing a sustainable management strategy for any local insect pest, it is therefore always advisable to use the right indigenous EPN isolate, as it is more likely to be adapted to the local climatic conditions and host population. In addition, an ideal nematode species or isolate should also have good potential to recycle and propagate in insect host. A recent survey on indigenous EPNs in Meghalaya, India recovered three new EPN isolates (Lalramliana 2007). The aim of this study was to evaluate these indigenous strains of EPNs, namely, *Heterorhabditis indica* Poinar, Karunakar and David, *Steinernema thermophilum* Ganguly and Singh, and *Steinernema glaseri* (Steiner) against the last instar larva of mustard sawfly, *Athalia lugens proxima* Klug, a serious pest of mustard and radish in India. The virulence of nematodes to sawfly larvae and their reproduction in insect host was studied, under the laboratory conditions.

Materials and methods

Insects

The larvae of *A. lugens proxima* were obtained from the experimental farms of Indian Council of Agriculture Research, Shillong, India and maintained in the laboratory on natural diets. The collected larvae were kept for at least 5 days in the laboratory to check, whether or not, there are any other infections before using them for experiments.

Nematodes

The three nematodes, *H. indica*, *S. thermophilum* and *S. glaseri*, used in this study, were originally isolated from forest soils in Meghalaya, India. These EPNs have been characterized in previous studies (Lalramliana 2007). The nematodes were reared at 20°C in last instar larvae of wax moth, *Galleria mellonella* L. (Pyralidae), according to Woodring and Kaya (1988). The infective juveniles (IJs) emerging from the wax moth larval cadavers were collected in deionized water using a modified White's trap, and stored in darkness at 15°C (Kaya and Stock 1997). Before being used for assay, IJs were allowed to acclimatize at room temperature for 1 h and their viability was checked by observing movements under a stereomicroscope.

Larval mortality bioassay

Larval mortality bioassays were carried out in Petri dishes (35 × 10 mm) lined with double layer of Whatman No. 1 filter paper, using the methods of Kaya and Stock (1997). Nematodes in 0.5 ml of deionized water were added to the filter paper in concentrations of 10, 25, 50, 75 and 100 per larva. After 30 min, a single sawfly larva was placed in each of the Petri dish. The dishes were sealed with Parafilm and maintained in a climatic chamber at 27 ± 2°C in the dark. For each nematode species and concentration, there were eight replicates and the experiment was repeated thrice. Untreated controls were identical to the treatment except that no IJs were added. Larval mortality was

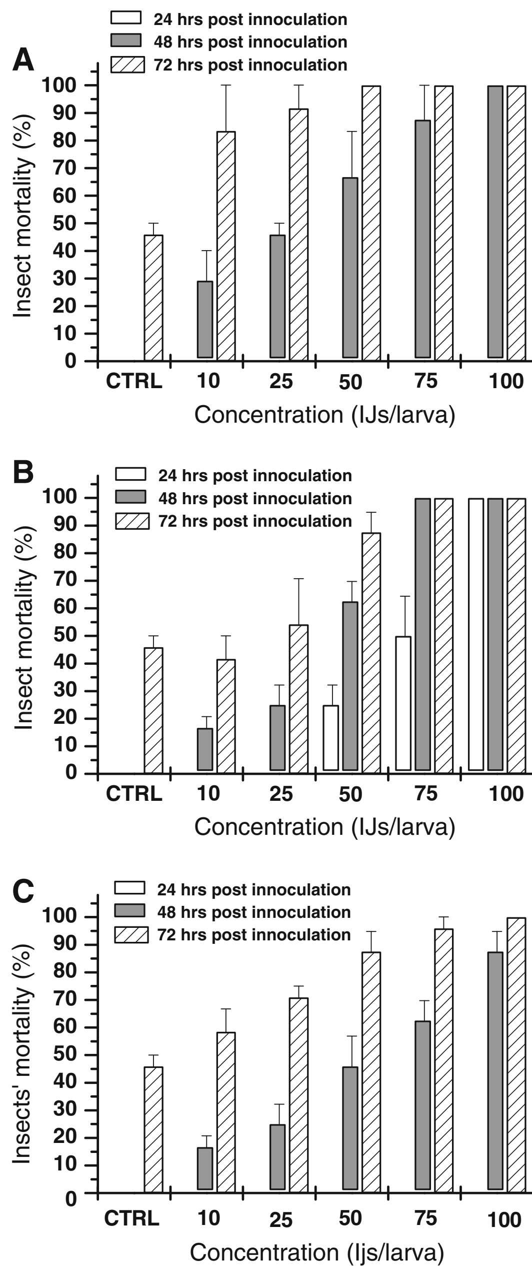

Fig. 1 The percentage mortality of *A. lugens proxima* larvae following exposure to different concentrations of infective juveniles (IJs) of nematodes. **a** *H. indica* **b** *S. thermophilum* **c** *S. glaseri*

recorded at every 24 h for up to 72 h. The cause of larval death was confirmed by body colour change of the cadaver due to symbiotic bacteria observed under stereomicroscope.

Reproduction of nematodes

Last instar larvae of *A. lugens proxima* were exposed to 10, 25, 50, 75 and 100 IJs concentrations of each nematode species in separate Petri dishes and total number of IJs produced per larva for up to a period of 20 days were counted. In this experiment, the nematode-infected dead larvae of test insect were removed from dishes, rinsed in deionized water and transferred individually on to White traps for their emergence from the body. The larvae were collected daily for up to a period of 20 days, till the emergence of IJs was stopped from insect cadavers and total number of IJs produced per larva was then determined. There were eight replicates for each nematode species and concentration and the experiment was repeated thrice. To each concentration, one Petri dish, prepared as described above but without IJs served as control.

Statistical analysis

All experimental data were analyzed statistically and are presented as mean ± standard error of mean (SEM). The significance of the difference in all experiments was determined by one way analysis of variance (ANOVA) and student's *t*-test. P values < 0.05 were accepted as statistically significant. Correlation between the parameters was determined by regression analysis. LC50 values were calculated using Probit analysis with SPSS software.

Results

In this study, *A. lugens proxima* larvae were found to be susceptible to all the three EPNs tested (Fig. 1 a–c). The degree of susceptibility to nematode infection varied from species to species and also on the exposure time. Also, a positive correlation was found between the doses of infective juveniles and larval mortality time for all the EPNs studied.

At 24 h post-exposure to nematode IJs, only *S. thermophilum* could cause insect mortality. On the other hand, neither *H. indica* nor *S. glaseri* showed larval mortality at this exposure time. The lowest dose of IJs that could cause larval mortality at 24 h post-exposure in *S. thermophilum* was registered to be 50 IJ/larva. When the dose of infective juveniles was increased to 100 IJs/larva, it caused a 100% larval morality in 24 h exposure time. At 48 h post-exposure to nematode infective juveniles, all isolates killed the insect larvae, although mortality rates of insect larvae varied. While *S. thermophilum* caused 100% mortality at 75 IJs/larva, *H. indica* could do so at 100 IJs/Lara dose. In case of *S. glaseri*, about 90% of insect mortality was recorded 100 IJ/larva dose. At 72 h post-exposure to nematode infective juveniles, mortality of insect larva (about 50%) was also recorded in control group. Nevertheless, both *H. indica* and *S. thermophilum* also showed 100% larval mortality at this exposure time, while *S. glaseri* revealed about 90% insect mortality. Based on LC50 at 48 h post-exposure to infective juveniles, *H. indica* was

Table 1 LC50 values calculated from dosage response assays conducted with different nematode species and last instar larvae of *A. lugens proxima*

Hours after inoculation	LC50 (IJs/larva)		
	H. indica	*S. thermophilum*	*S. glaseri*
24	–	68.3	–
48	30.6	37.3	50.7
72	–	17.4	5.6
96	–	–	–

judged to be the most virulent species, with the minimum value of LC50 (30.6 IJs/larva), followed by *S. thermophilum* and *S. glaseri*, with LC50 values of 37.3 and 50.7 IJs/larva, respectively (Table 1) .

Reproduction of nematodes

In order to study the reproduction of EPNs, the sawfly larvae were exposed to 10, 25, 50, 75 and 100 IJs/larva of teach nematode species. Following host mortality, the emerging IJs from host cadavers were collected and counted. The data revealed that all the three test nematodes are able to successfully invade and propagate in the insect larvae and produce first generation infective juveniles (Figs. 2a–c). It was also evident that except *S. thermophilum*, the other two nematode species exhibit a linear relationship between the concentrations of IJs applied and total number of IJs produced per infected larva. In this study, *H. indica* produced significantly more number of infective juveniles per insect larva than the other two nematode species (Fig. 2a). For *H. indica*, the maximum production of infective juveniles per larva ($29.3 \pm 1.39 \times 10^3$ IJs/larva) was obtained at 75 IJ/larva dose. In case of *S. thermophilum*, though the progeny production initially increased with the increase in IJs dose reaching to its maximum of $10.8 \pm 0.79 \times 10^3$ IJs/larva at 50 IJs/larva, it declined thereafter to $7.6 \pm 0.6 \times 10^3$ IJs/larva at 100 IJs/larva dose. Among the three EPNs studied, the least progeny production was recorded for *S. glaseri.* It increased linearly with an increase in IJ's dose, reaching to its maximum of $1.3 \pm 0.12 \times 10^3$ IJs/larva at concentration of 100 IJs/larva.

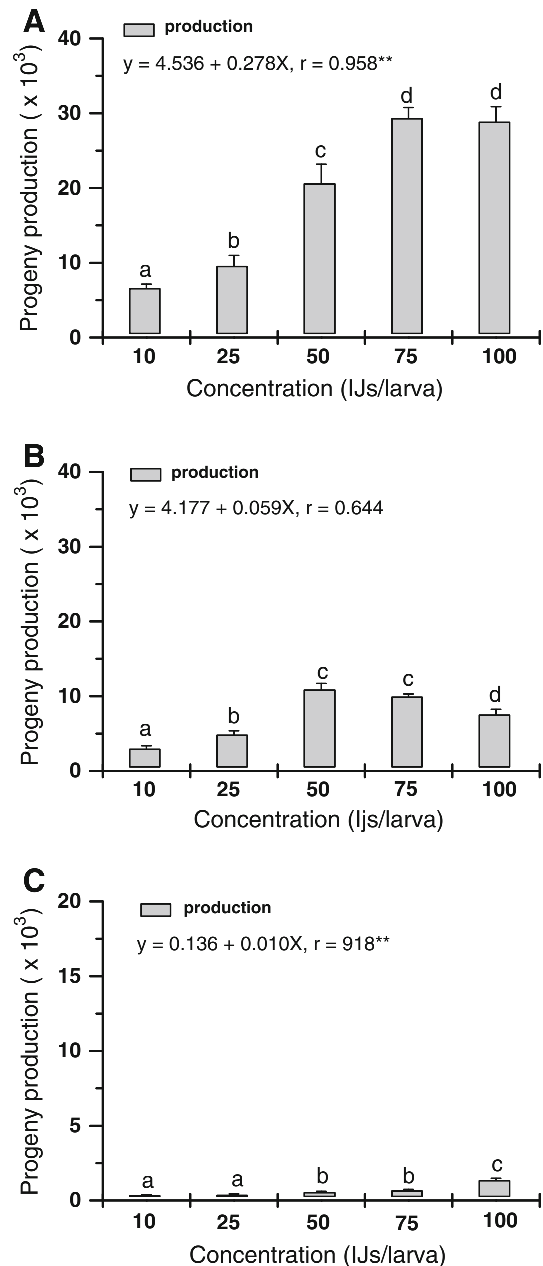

Fig. 2 Production of first generation infective juveniles in *A. lugens proxima* larvae at different dosages of infective juveniles. **a** *H. indica* **b** *S. thermophilum* (C) *S. glaseri*. **$p < 0.01\%$; Means shown by the same letter are not significantly different ($p > 0.05$)

Discussion

The aim of this study was to evaluate the efficacy of three indigenous strains of EPNs (*H. indica*, *S. thermophilum* and *S. glaseri*) isolated from the forest soils in Meghalaya, India against the last instar larvae of mustard sawfly, *A. lugens proxima*, a serious pest of mustard and radish in north-east India. Each EPN isolate was evaluated for its virulence on the basis of dose and time required to cause insect mortality and also on the basis of nematodes' ability to propagate within the body of infected host and produce first generation infective juveniles. Several previous studies have made use of these assays to evaluate the efficacy of EPNs against various insect pests (Sims et al. 1992; Ricci et al. 1996; Bhatnagar et al. 2004; Phan et al. 2005). In the present study, *A. lugens proxima* larvae showed a high

susceptibility to all the three tested nematodes. Further, a positive correlation was found between the dose of IJs applied and insect mortality for all the EPNs studied. On the basis of LC50, *H. indica* emerged out to be the most effective species. At almost all exposure doses, *H. indica* showed the maximum larval mortality.

This is the first study to demonstrate that EPNs can act as potential biocontrol agents against mustard sawfly, *A. lugens proxima*. The positive relationship between the dose of infective juveniles and host mortality found in the present study has also been recorded in several other studies (Forschler and Nordin 1988; Glazer and Navon 1990; Peters and Ehlers 1994). The differences in the infectivity between nematode species or strains as registered in the present study have also been found for many insect hosts (Forschler and Nordin 1988; Griffin et al. 1989). The pathogenicity of EPNs is considered to be a complex process, which depends upon many biotic and abiotic factors, like host invasion, penetration, reproduction, etc. (Kaya and Gaugler 1993). Thus different nematode species have been found to differ in their pathogenicity against a specific insect host owing to one or other biotic or abiotic factors (Forschler and Nordin 1988; Griffin et al. 1989). In many studies, the virulence of EPNs has also been found to be dependent on host invasion and penetration ability of the nematode species (Gaugler 1988; Lewis et al. 1992; Glazer et al. 2001).

Reproduction and recycling of EPNs in the host play a vital role in their persistence in the environment after application, and thus in overall effectiveness of pest control (Harlan et al. 1971; Georgis and Hague 1981). The information is considered important in determining the time and dose of subsequent EPN application, which may be useful in reducing the cost of EPN application in the field. The data obtained in this study suggest that all the three species of EPNs are able to infect and propagate within the insect host and produce first generation infective juveniles. However, the highest production of IJs was obtained with *H. indica* than the rest two nematode species. In case of *S. thermophilum*, though the production of IJs increased initially with increase in IJs dose, it declined thereafter to minimum at the highest dose of IJs tested. In contrast, *S. glaseri* showed an extremely low production of first generation infective juveniles. Many earlier studies have documented the differences in the ability of different nematode species to produce the first generation infective juveniles (Ali et al. 2006; Karunakar et al. 1999). It has been found that besides factors on the part of host, the size and behaviour of nematode species also affect the nematode's reproduction inside the host body (Loya and Hower 2003). For example, Bhatnagar et al. (2004) reported that *H. bacteriophora,* being the smaller, produces more IJs per cadaver of infected larva than *S. glaseri*, which is larger in size, against the final instar grubs of *Maladera insanabilis*. Our findings on production of IJs by EPNs are in agreement with the results of Jothi and Mehta (2006), where *H. bacteriophora* was recorded to produce comparatively more IJs per infected insect larva than *S. glaseri* (Stuart et al. 1996). A higher production of IJs by *H. indica* as recorded in the present study may also be attributed, in part, to the fact that heterorhabditids being hermaphroditic are likely to contribute more progeny production than steinernematids which are amphimictic (Poinar 1990; Mannion and Jansson 1992).

In conclusion, our findings demonstrate that all the three indigenous strains of EPNs are virulent to *A. lugens proxima* larvae, however, *H. indica* and *S. thermophilum* show better efficacy than *S. glaseri*. Similarly, except *S. glaseri*, the other two EPN isolates also show good reproductive potentials in sawfly larvae. It may, therefore, be concluded from this study that these EPN isolates have good potential as biocontrol agents against mustard sawfly *A. lugens proxima*.

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