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ORIGINAL ARTICLE

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Efficacy of indigenous entomopathogenic nematodes from Meghalaya, India against the larvae of taro leaf beetle, *Aplosonyx chalybaeus* (Hope)

Arun K. Yadav · Lalramliana

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Abstract The efficacy of three entomopathogenic nematode (EPN) species, *Heterorhabditis indica*, *Steinernema thermophilum*, and *S. glaseri*, from Meghalaya, India was studied against the larvae of taro leaf beetle, *Aplosonyx chalybaeus* (Hope) (Coleoptera: Chrysomelidae), under the laboratory conditions. The beetle larvae (grubs) were exposed to 25, 50, 75, 100 and 200 infective juveniles (IJs) of each nematode species for different time periods and they were found to be susceptible to all the EPNs tested. However, the susceptibility of grubs to nematode infection varied according to the dosages of IJs and their exposure periods. Appreciably good performance was achieved by *S. glaseri*, which showed 100 % mortality of insect larvae in 48 h exposure time. At 48 h of incubation, its LC_{50} value was 90.3 IJs/larva, which was lower than that of *S. thermophilum* (115.0 IJs/larva) and *H. indica* (186.0 IJs/larva), at the same exposure time. All the tested nematode species were also found to reproduce within the host and produced infective juveniles. *H. indica*, however, showed comparatively more production of IJs per cadaver of infected host (168.9×10^3 IJs/larva), as compared to the other two tested nematode species. The production of IJs per cadaver of infected host by *S. thermophilum* was recorded to be 82.0×10^3 IJs/larva. In case of *S. glaseri*, while production of IJs increased initially to 18.9×10^3 IJs/larva at concentration of 100 IJs/larva, it declined thereafter to 14.7×10^3 IJs/larva at the dose of 200 IJs/larva. In conclusion, the evidence obtained in this study suggests that all the three indigenous EPN species are virulent enough to produce 100 % mortality in the last instar larvae of *A. chalybaeus*. These EPN species thus have potential scope for the management of *A. chalybaeus* in taro crops.

Keywords *Aplosonyx chalybaeus* · Biological control · Entomopathogenic nematodes · *Heterorhabditis indica* · *Steinernema glaseri* · *Steinernema thermophilum* · Taro

A. K. Yadav (✉) · Lalramliana
Department of Zoology, North-Eastern Hill University, Shillong 793022, India
e-mail: akynehu@hotmail.com

Introduction

Taro (*Colocasia esculenta* (L.) Schott) is an important staple crop in developing countries, especially in African and Southeast Asian countries. It is widely cultivated in South Africa, Asia, Oceania, Central Africa, West Indies and the islands of the Caribbean and Central America (Chandra 1984). Both, the wild and cultivated forms of taro are found in India (Kuruvilla and Singh 1981). All the plant parts, i.e. the leaves, petioles, corms and cormels are eaten in some or other forms in different regions of the world (Chandra 1984; Onwueme 1999). It is also considered as an important source of food during lean period, and feed for livestocks. Taro is susceptible to attack by about two dozens of pathogens and pests, but only a few causes serious reduction in its growth and production (Ooka 1990). In the Northeastern region of India, a chryomelid beetle, *Aplosonyx chalybaeus* (Hope) (Coleoptera: Chrysomelidae) is a serious pest of taro which damages its roots and corms (Swamy et al. 2002; Sanwal 2008). Elsewhere in the East Asia, these beetles have also been reported as deleterious pest of this crop in Vietnam and the Philippines (Lazell et al. 1991; Szinicz et al. 2005). The adult beetle feeds on the foliage of the host plant and produces several small circular holes. Whereas, the larvae, commonly called chrysomelid corm

borer, are found in the soil around the damaged plant, where they feed mainly on corms, making numerous holes and tunnels. The diseased corms possess numerous holes and tunnels that not only make them unmarketable in the local markets, but also unfit for human or livestock consumption. Although chemical insecticides, such as carbofuran, can be used in the management of pest, there are many concerns because of contamination of the corns and adverse effects of chemical pesticides on the human health and environment. Therefore, some suitable and environment friendly methods for the management of pest is urgently required.

Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are the potential alternatives to chemical control for many economically important insect pests (Georgis 1990; Kaya and Gaugler 1993). The infective third stage juveniles (IJs) of these nematodes, found in the soil, carries mutualistic bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp.) in their intestine, for steinernematids and heterorhabditids, respectively (Poinar 1979). Upon finding a susceptible host, the IJs enter the host's body through spiracles, mouth and anus, and release the specific symbiont bacteria that kill the host by septicemia, usually within 24–48 h (Kaya and Gaugler 1993). Many species of EPNs belonging to both genera have been used with variable success as biocontrol agents against a number of insect pests occupying different habitats. However, considerable success has been achieved against soil-dwelling pests or pests in cryptic habitats (Begley 1990; Klein 1990; Williams and Walters 1999; Tomalak et al. 2005; Valle et al. 2008). Therefore, many studies have highlighted the usefulness of EPNs as suitable biological control agents against soil-dwelling pests (Hominick and Reid 1990). In this connection, researchers have reported that while developing a long-term strategy for the inundative release of a nematode species against pest species, it is always advisable to employ the indigenous strains of EPNs as they are comparatively better adapted to the local climate and also host population, as compared to the exotic EPN strains (Gaugler 1988; Bedding 1990). Further, it has also been argued that an ideal EPN strain must also have a good pathogenicity and should be able to propagate well on a large scale (Bedding 1990). Our recent studies on prevalence of EPNs in Meghalaya (India) have yielded three indigenous strains of nematodes (Lalramliana 2007), which we believe may be potentially effective against local pest species. Therefore, the aim of the present study was to evaluate the efficacy of these three locally isolated EPN strains, namely *Heterorhabditis indica* Poinar, Karunakar and David, *Steinernema thermophilum* Ganguly and Singh, and *S. glaseri* (Steiner) against the last instar larva of taro beetle, *Aplosonyx chalybaeus*. The virulence of EPN species to the grubs and their reproduction in host species was studied under the laboratory conditions.

Materials and methods

Nematode sources

The three strains of EPNs, *H. indica,* Karunakar and David, *S. thermophilum* Ganguly and Singh, and *S. glaseri* (Steiner), used in this study were isolated from forest soils in Meghalaya. These EPN strains have been characterized in previous studies, using the late instar larvae of the greater wax moth, *Galleria mellonella* Linnaeus as host (Lalramliana, 2007). For experimental study, the nematodes were reared in the laboratory on late instar larvae of *G. mellonella* at 25 °C, as described by Woodring and Kaya (1988). The IJs that emerged from wax moth larval cadavers were collected using modified White traps (Kaya and Stock 1997), and stored in darkness at 15 °C in deionized water. Before being used for assay, the IJs were allowed to acclimatize for 1 h at room temperature and their viability was checked by observation of movements under a stereomicroscope.

Insect sources

Last instar larvae of *A. chalybaeus* 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. During this period, larvae were observed for feeding behavior and movements, and only the healthy looking larvae were included in the study.

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, following the methods of Kaya and Stock (1997). Nematodes in 0.5 ml of deionized water were added to the filter paper in concentrations of 25, 50, 75, 100 and 200 IJs/larva. After 30 min, a single larva of *A. chalybaeus* 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 checked at every 24 h for up to 120 h. The cause of larval death was confirmed by body colour change of the cadaver which being evident due to the presence of symbiotic bacteria.

Reproduction of EPNs

Last instar larvae of *A. chalybaeus* were exposed to 25, 50, 75, 100 and 200 IJs concentrations of each EPN in separate

Petri dishes and total number of IJs produced per larva for up to a period of 20 days was counted. In brief, the nematode-infected dead insect larvae were removed from dishes, rinsed in deionized water and transferred individually on to White traps for their emergence from the body (White 1927). 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 bioassay 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. LC_{50} values were calculated using Probit analysis with SPSS software.

Results and discussion

The aim of this study was to evaluate the efficacy of three species of EPNs, namely *H. indica*, *S. thermophilum* and *S. glaseri*, against the last instar larvae of *A. chalybaeus*, a serious pest of taro in the Northeastern region of India. Each nematode species was evaluated for its virulence on the basis of dose and time required by nematode to kill the insect larvae and also on the basis of nematode's ability to propagate within the body of infected host and produce infective juveniles.

As shown in Fig. 1, the larvae of *A. chalybaeus* were found to be susceptible to all the three tested EPNs. However, the degree of susceptibility of insect larvae to nematode infection varied according to the doses of their infective juveniles as well as the exposure period. Also, a positive correlation was found between the doses of infective juveniles applied and time of larval mortality for all the three tested EPN species. A moderate larval mortality was observed due to infection with *S. thermophilum* in 24 h exposure time at 200 IJs/insect (Fig. 1b), whereas, *H. indica* and *S. glaseri* caused larval mortality only after 48 h of incubation time (Fig. 1a, c). After 48 h of incubation, both *S. thermophilum* and *S. glaseri*, at 200 IJs/insect, caused cent percent larval mortality, while *H. indica* gave comparatively low larval mortality after first 48 h incubation time. A further increase in incubation time to 72 h led to a complete mortality of insect larvae by *S. glaseri* only at a dose of 100 IJs/insect. However, at the same dose and incubation time, *H. indica* gave about 65 % of larval mortality. Larval mortality due to *H. indica*, however, continued to increase further with an increase in exposure time and 100 % larval mortality was recorded only after 120 h at 100 IJs/insect. The calculated values of LC_{50} are presented in Table 1. The increase in exposure time resulted in reduction of the values of LC_{50} for all nematodes. Among the EPNs tested, *S. glaseri* was the most virulent with minimum values of LC_{50} at all exposure time. After 48 h of incubation, *S. glaseri* showed a LC_{50} value of 90.3 IJs/larva (95 % fiducial limit (FL): 72.5–141.3) which was lower than that of *S. thermophilum* (115.0, 95 % FL: 92.3–162.7). At the same exposure time, *H. indica* was comparatively less virulent with the LC_{50} value of 186.0 IJs/larva (95 % FL: 122.9–212.7). A number of workers have used these assays to adjudge the efficacy of EPNs against various insect pests (Ricci et al. 1996; Sims et al. 1992; Bhatnagar et al. 2004). In the present study, the beetle larvae were found to be susceptible to all the three tested EPNs. Also, it emerged that there exists a positive correlation between the dose of IJs and host mortality for all the three nematodes. Among all nematodes, *S. glaseri* was found to be the most virulent species. There has been no study to date on the efficacy of entomopathogenic nematodes against the larvae of taro beetle, *A. chalybaeus*. In general, the findings of the present study on efficacy of EPNs against *A. chalybaeus* larvae are in agreement with other insect hosts. For example, Hominick and Reid (1990) reported that, besides the intrinsic qualities of the species, EPN efficiency is greatly influenced by its dose. In a similar manner, researchers have also recorded a positive relationship between nematode concentration and host mortality in many previous studies (Forschler and Nordin 1988; Glazer and Navon 1990; Peters and Ehlers 1994). The present findings on virulence of *S. glaseri* are in tune with Toepfer et al. (2005), who also reported this species to be the most virulent one among all the studied EPNs against western corn rootworm, *Diabrotica virgifera virgifera*. Further, Karunakar et al. (1999) also recorded almost similar trends in the parasitism of larvae of white grub at different doses of *S. glaseri* and *H. indica*. Differences in infectivity between nematode species or strains have been also been documented for many other insect species (Forschler and Nordin 1988; Griffin et al. 1989). This study also confirms the results of previous studies made by Converse and Grewal (1998) and Seth and Barik (2009) who demonstrated that *S. glaseri* is much more virulent nematode than *H. indica*. The susceptibility of soil-inhabiting white grubs to *S. glaseri* was first demonstrated by Glaser (1932). In that study, too, *S. glaseri* was reported to be more effective than other

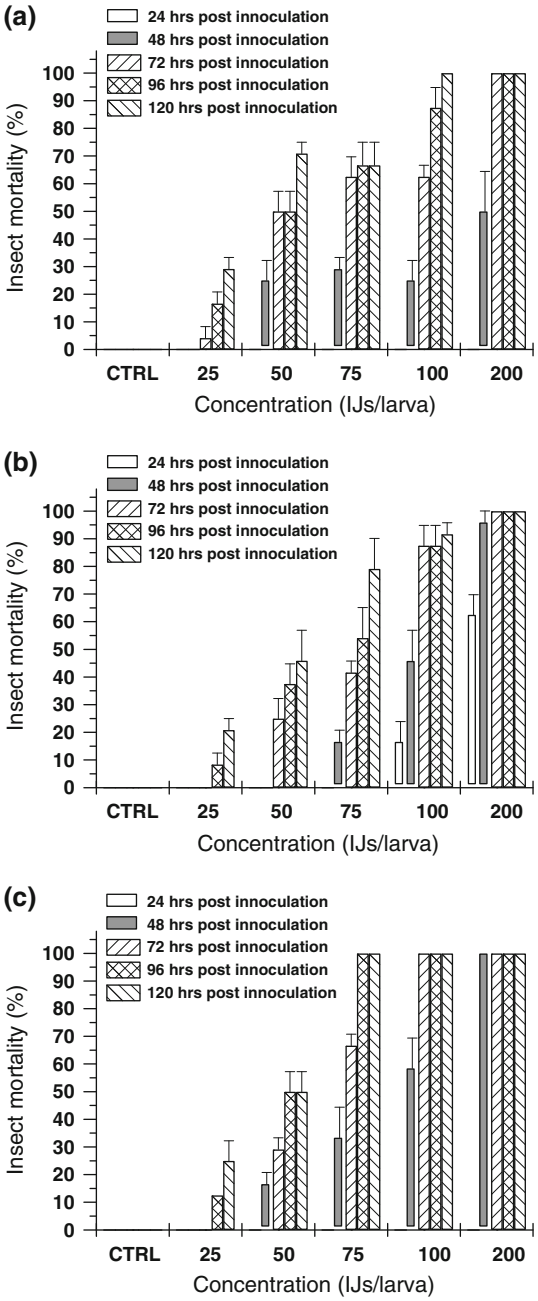

Fig. 1 Percentage mortality of *A. chalybaeus* larvae following exposure to different concentrations of infective juveniles (IJs) of nematodes. **a** *H. indica*, **b** *S. thermophilum* and **c** *S. glaseri*

steinernematids and heterorhabditids against scarab larvae. The pathogenicity of EPNs 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 (Forschler and Nordin 1988; Griffin et al. 1989). In many investigations, the virulence of EPNs has also been found to be dependent on other factors such as host invasion and penetration ability of the nematode infective juveniles (Gaugler 1988; Lewis et al. 1992; Glazer et al. 2001). For example, while studying the pathogenicity and penetration of five EPNs against sugarcane internode borer, Sankaranarayanan et al. (2008) reported a higher penetration ability and pathogenicity of *S. glaseri* than *H. indica*. A good virulence of *S. glaseri*, therefore, may also be explained in part due to its efficient penetration ability into the insect host.

Table 1 LC_{50} values calculated from dosage response assays conducted with different nematode species and last instar larvae of *A. chalybaeus*

Nematode species	Incubation period (h)	LC_{50} (IJs)	Fiducial limit (95 %)
H. indica	24	–	–[a]
	48	186	122.9–212.7
	72	70.1	42.4–105.1
	96	56.4	27.4–77.8
	120	48.7	17.4–67.3
S. thermophilum	24	177.9	142.4–252.8
	48	115.0	92.3–162.7
	72	74.4	59.4–93.6
	96	65.9	46.5–87.9
	120	52.6	22.5–72.4
S. glaseri	24	–	–[a]
	48	90.3	72.5–141.3
	72	63.4	50.3–76.9
	96	46.2	33.3–59.7
	120	42.7	25.5–56.9

[a] Values of LC_{50} could not be calculated

In order to study the reproductive potential of EPNs, the insect larvae were exposed to different concentrations of IJs of the three test nematodes. Following host mortality, the emerging IJs were collected from host cadavers and counted. The data revealed that all the three species were able to invade and propagate within the host and produce infective juveniles (Fig. 2a–c). It was also evident that, except *S. glaseri*, the other two nematode species exhibited a linear relationship between the concentrations of IJs applied and total number of infective juveniles produced per infected larva. In this study, *H. indica* produced significantly more number of infective juveniles per insect larva as compared to other tested nematodes (Fig. 2a). At the dose of 200 IJ/larva, the progeny production in *H. indica* was recorded to be 168.9×10^3 IJs/larva. This was followed by *S. thermophilum*, which produced 82.0×10^3 IJs/larva (Fig. 2b). In case of *S. glaseri* though progeny production initially increased with the increase in IJs dose, it declined thereafter to 14.7×10^3 IJs/larva at 200 IJs/larva (Fig. 2c).

Reproduction and recycling of entomopathogenic nematodes in host play an important role in their persistence in

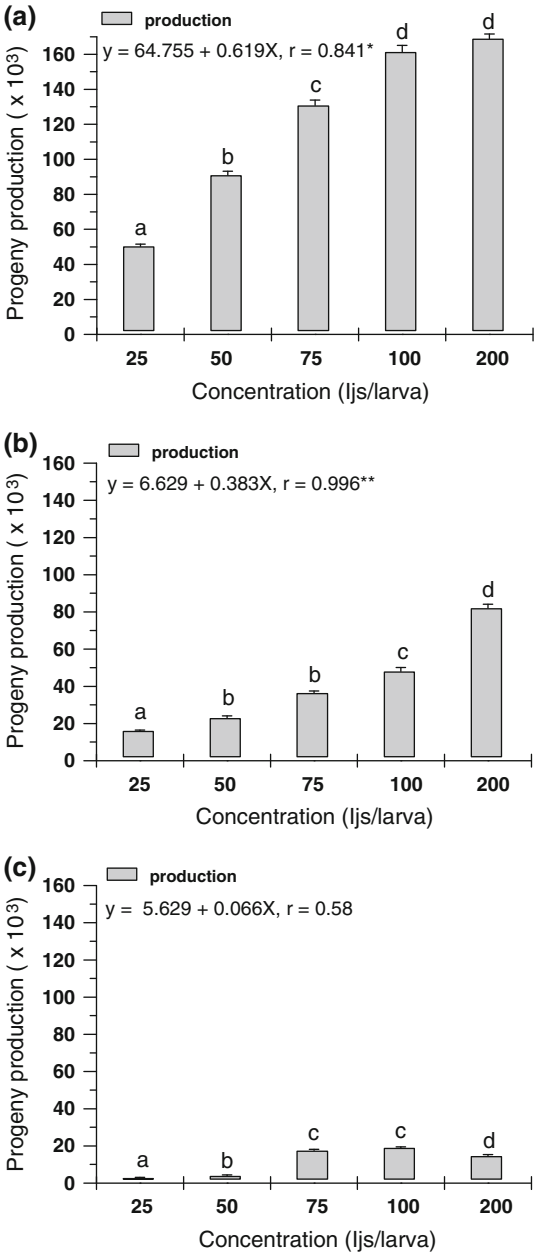

Fig. 2 Progeny production by insect larvae at different dosages of infective juveniles (IJs) of entomopathogenic nematode species. **a** *H. indica,* **b** *S. thermophilum* and **c** *S. glaseri*. Significant at ** 0.01 and * 0.05 %, *(a–d)* Mean values shown by different letters are significantly different at 0.05 %

the soil, and also in their overall effectiveness in pest control (Harlan et al. 1971; Georgis and Hague, 1981). A prior knowledge about reproduction and recycling of nematode is considered important in determining the time and dose of subsequent EPN application, which may be useful in reducing the cost of application. The data in this study suggest that following application, all the tested species of nematodes were able to infect and propagate within the insect host and produce infective juveniles. However, the highest production of IJs was obtained with *H. indica*, which yielded 168.9 $\times$ 10^3 IJs/larva at 200 IJs post exposure time, followed by *S. thermophilum* which yielded 82.0 $\times$ 10^3 IJs/larva at the same dose and exposure period. In case of *S. glaseri*, though the production of IJs increased initially with increase in IJs dose, it declined thereafter to 14.7 $\times$ 10^3 IJs/larva at the dose of 200 IJ/larva. Differences in reproduction (i.e., production of F_1 generation infective juveniles) between nematode species have been documented for many insect hosts (Ali et al. 2006; Karunakar et al. 1999). The size and behaviour of nematode species may account for differences in nematode ability to reproduce in the host (Loya and Hower 2003). Bhatnagar et al. (2004) also reported that *H. bacteriophora* being the smaller in size, produces more IJs per cadaver of infected host 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 also 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* in the present study may also be attributed, in part, to the fact that heterorhabditids being hermaphroditic are likely to contribute to more progeny than steinernematids which are amphimictic (Poinar 1990; Mannion and Jansson 1992).

In conclusion, the evidence obtained in this study suggests that all the three tested indigenous species of EPNs are virulent enough to produce 100 % mortality to the larvae of *A. chalybaeus*. Furthermore, all EPNs can also propagate in the infected larva and produce F_1 generation infective juveniles. Considering these attributes, it could be suggested that these EPNs have potential use as biocontrol agents for the management of *A. chalybaeus* in taro crops.

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