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Research Article

Penetration and pathogenicity of entomopathogenic nematodes to sugarcane early shoot borer, *Chilo infuscatellus* Snellen (Lepidoptera: Crambidae)

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ABSTRACT: Penetration and pathogenicity of seven species of entomopathogenic nematodes (EPN), *viz*., *Heterorhabditis indica* (LN2), *H. bacteriophora* (LN8), *Heterorhabditis* sp. (HII), *Steinernema carpocapsae, S. glaseri*, *S. riobrave* and *S. feltiae* to sugarcane early shoot borer, *Chilo infuscatellus* Snellen, was studied under laboratory conditions with bioassay techniques. Significant differences were observed in mean penetration of the EPNs in *C. infuscatellus* larvae. The penetration rate recorded was highest in *S. glaseri* followed by *S. carpocapsae* (29 and 18% respectively), while it was lowest in the three species of heterorhabditids, *Heterorhabditis* sp. (HII), *H. indica* (LN2) and *H. bacteriophora* (LN8). In pathogenicity studies, mortality of the early shoot borer larvae increased with increase in dosage. The larval mortality was 83.3 per cent at the dose of 50 infective juveniles (IJs) of *H. indica* and *S. glaseri*. It was least at 10 IJs/larva of all EPNs tested. The LD_{50} values showed that *S. glaseri* was superior to all other EPN species (24.2 IJs/larva) followed by *S. feltiae* (26.6 IJs/larva).

KEY WORDS: *Chilo infuscatellus,* early shoot borer, entomopathogenic nematodes, *Heterorhabditis*, *Steinernema*, sugarcane

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INTRODUCTION

Early shoot borer, *Chilo infuscatellus* Snellen (Lepidoptera: Crambidae), is an important pest in the early stages of sugarcane growth in India. Among the organisms involved in the biological control of this pest, entomopathogenic nematodes (EPN) are gaining much attention in recent times (Sankaranarayanan *et al*., 2003). EPNs from the families Heterorhabditidae and Steinernematidae have already been successfully used throughout the world for the control of agricultural pests (Gaugler, 1981). Bedding *et al*. (1983) indicated the importance of screening several nematode species against the target insect in the laboratory before commencing field evaluations.

Lethal dosage (LD_{50}) estimation is a relative measure of susceptibility of a host population and is a convenient and commonly used index of relative efficacy of EPN. However, the actual number of nematodes that invade a host and cause mortality in a susceptible host generally is not known. Epsky and Capinera (1994) recommended determination of percentage host mortality and nematode invasion efficacy to be done simultaneously for assessment of nematode efficacy. The susceptibility of economically important insect pests has been tested in a wide range of laboratory assays. The most commonly used bioassays are Petri dish dose response assay (Kaya and Hara, 1980) and penetration rate assay (Caroli *et al.,* 1996). The objective of our studies was to test the penetration and pathogenicity of seven EPN species to sugarcane early shoot borer in laboratory conditions with standard bioassay techniques.

MATERIALS AND METHODS

Insect and nematode culture

For generating the culture, the larvae of sugarcane early shoot borer (ESB), *C. infuscatellus*, were collected from sugarcane fields in and around Coimbatore and reared in sugarcane shoots at laboratory conditions. Fully grown larvae were selected for the bioassay tests. Three indigenous isolates of heterorhabditids, *viz*., *Heterorhabditis indica* (LN2), *Heterorhabditis* sp. (HII), *H. bacteriophora* (LN8) and four steinernematids, *viz*., *Steinernema glaseri, S. carpocapsae*, *S. riobrave* and *S. feltiae* obtained from Dr. Ralf-Ehlers, Germany were used in this study.

Fifth instar larvae of greater wax moth, *Galleria mellonella* L. reared on artificial diet (David and Kurup, 1988) were used for *in vivo* production of EPN based on the method outlined by Woodring and Kaya (1988). The infective juveniles (IJs) of EPN were collected in 0.01 per cent formalin and the cultures were maintained at 11°C.

Penetration and pathogenicity studies

The penetration rate and dose response assays of EPN against ESB were conducted in 1.5 cm diameter 24 well plates (Corning, USA). Each well was padded with two filter paper discs (Whatman No. 1). One ESB larva was placed in the well and 200 IJs of respective EPN were transferred to each well in 75µl suspension. Control wells were maintained with 0.01% formalin in distilled water. The penetration rate assay was conducted as described by Caroli *et al*. (1996). About 200 IJs of respective EPNs were inoculated in the well plate containing one ESB larva/well. Each treatment consisted of fifty replicates. Larval mortality was recorded 48h after nematode inoculation and the number of penetrated nematodes was determined by dissecting the dead cadaver in Ringer's solution.

In pathogenicity study, fifth instar ESB larvae were exposed to five dosages (0, 10, 20, 30, 40 and 50 IJs /larva) of EPN and mortality of the larvae was recorded 96h after nematode inoculation. Each treatment consisted of three replications and each replication accommodated 20 ESB larvae. The mortality data (expressed in percentage) were transformed to arc sine and subjected to analysis of variance. The means were separated by Duncan's Multiple Range Test (SPSS, 2007). The LD_{50} was calculated using probit analysis and all comparisons were made at $P = 0.05$ level of significance.

RESULTS AND DISCUSSION

All entomopathogenic nematodes tested penetrated into ESB larvae and caused mortality ranging between 50 and 96.6 per cent. Steinernematids recorded 50–96.6 per cent mortality of ESB larvae, with *S. glaseri* recording the maximum (96.6 per cent). It was followed by *S. carpocapsae* (76.6%) and *S. feltiae* (70%) being on par with each other. The heterorhabditid nematodes recorded 50–66.6 per cent mortality of the ESB larvae, of which *H. indica* recorded 66.6 per cent mortality of the larvae (Fig. 1a). Significant differences were observed in the mean penetration of the IJs of steinernematids at 48h. *S. glaseri* showed highest penetration (29.0%) and was superior to other EPN species, followed by *S. carpocapsae* (18.0%) (Fig. 1b). The penetration was least in the case of heterorhabditids.

In pathogenicity study, all the EPN tested in the laboratory bioassay caused mortality of *C. infuscatellus* with great variation (Fig. 2). In general, mortality of the larvae increased with increase in the dosage of EPN. *S. glaseri* and *H. indica* recorded 83.3 per cent mortality of ESB @ 50 IJs/larva. The lowest mortality (10 to 13.3%) of ESB was observed for *H. bacteriophora, Heterorhabditis* sp. (HII) and *S. riobrave* at a dosage of 10 IJs/larva. The LD_{50} values determined from different dosage levels for all EPN indicated that *S. glaseri* was superior to other EPN species with the lowest LD_{50} (24.2 IJs/larva), followed by *S. feltiae* (26.6 IJs/larva). *Heterorhabditis* sp.(HII) was found least effective in this respect (Table 1).

In the present study, *S. glaseri* was found to be superior in terms of penetration, mortality of ESB and lowest LD_{50} value. The number of heterorhabditid IJs found in ESB larva was two to five folds lower than the number of steinernematids. The differences in

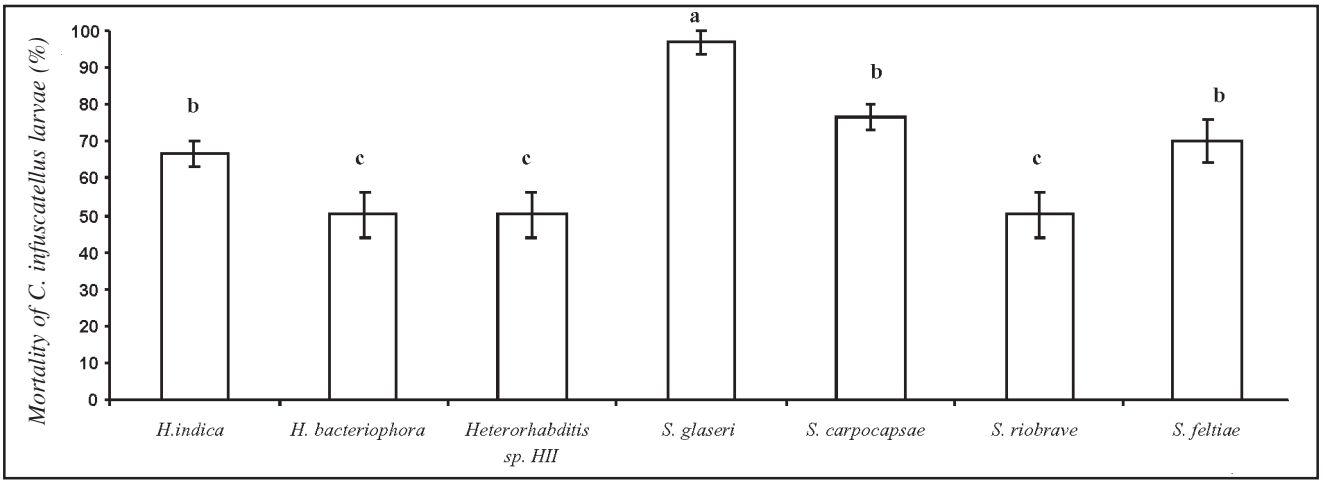

Fig. 1a. Per cent mortality of *C. infuscatellus* due to EPN at 48h exposure in penetration assay. Bars with same letter are not significantly different (DMRT, $P = 0.05$)

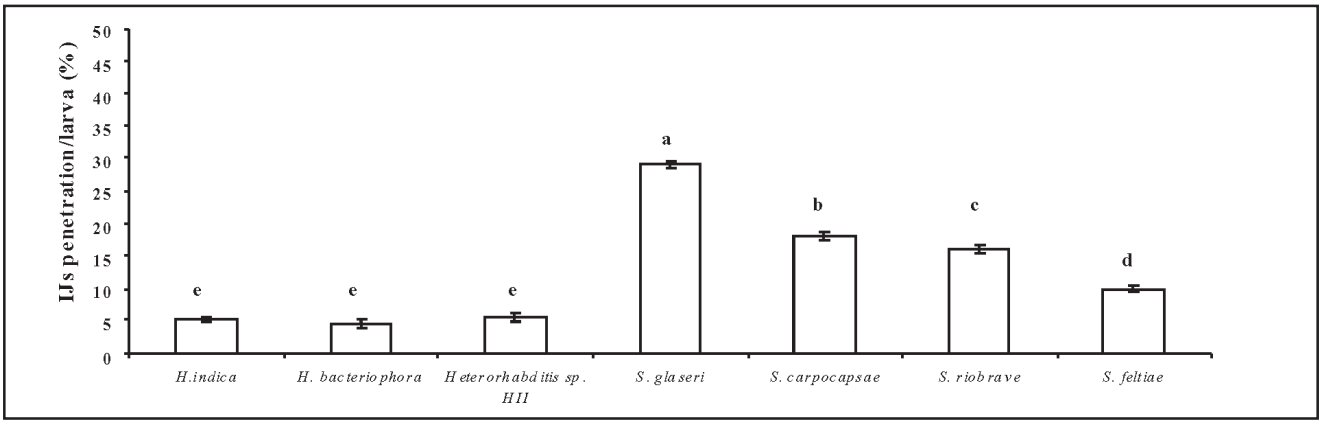

Fig. 1b. Average number of IJs of EPN in infected larvae of *C. infuscatellus* at 48h exposure. Bars with same letter are not significantly different (DMRT, *P* = 0.05)

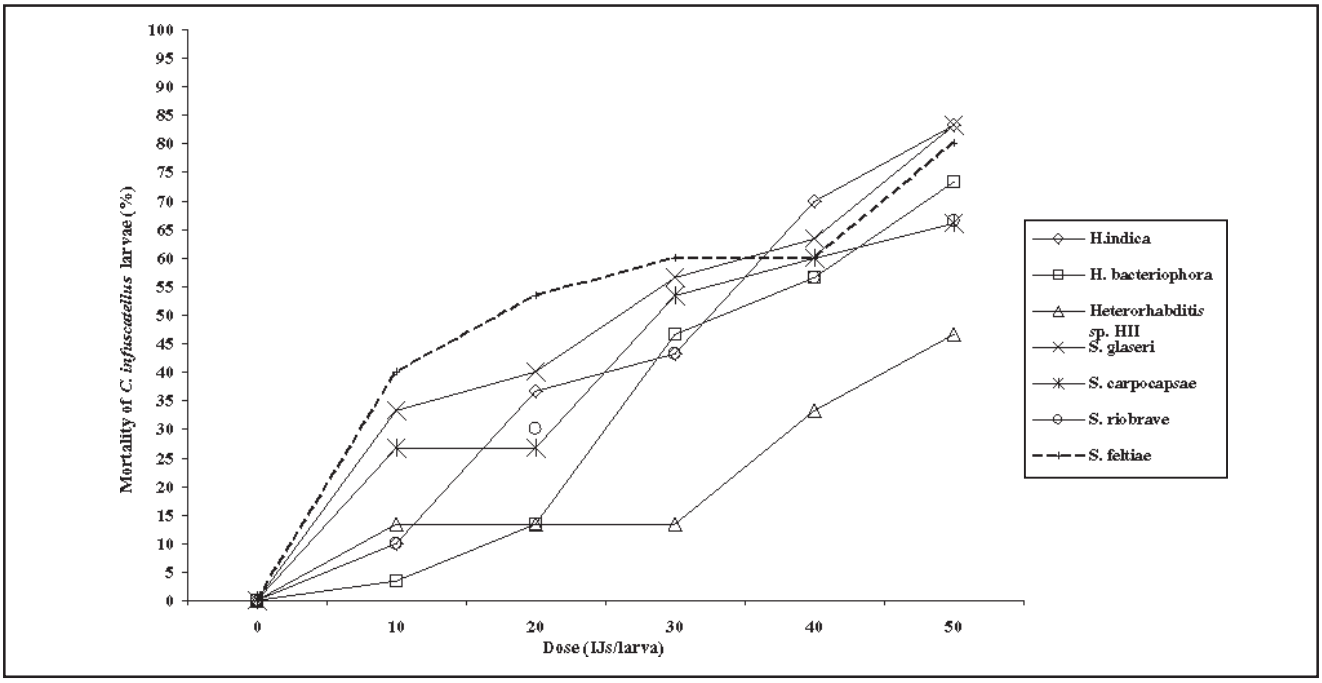

Fig. 2. Per cent mortality of *C. infuscatellus* larvae following exposure to different concentrations of EPN at 96h

Table 1. LD_{50} value of EPN against larvae of *C. infuscatellus*

EPN species	LD50 (No. IJs/ESB larva)	95% confidence limit	
		Lower limit	Upper limit
Heterorhabditis indica (LN2)	30.2	24.9	35.9
H. bacteriophora (LN8)	34.6	32.0	37.6
Heterorhabditis sp. (HII)	45.8	38.6	51.3
Steinernema glaseri	24.2	17.7	31.1
S. carpocapsae	29.8	20.2	34.7
S. riobrave	33.8	30.2	38.5
S. feltiae	26.6	18.4	32.6

penetration by EPN in the present study might be due to the infection strategies of nematodes. The adults developing from invading IJs of heterorhabditids reproduce hermaphroditically and a single invading IJ could potentially reproduce. Therefore, few invaders will be sufficient to establish the next generation. Steinernematids are amphimictic and mating is essential for further reproduction (Poinar, 1990), thus an invasion of high numbers of IJs increased the probability of mating and further reproduction. Even at lower penetration levels, the heterorhabditid nematodes could cause 50–66.6 per cent mortality of ESB larvae. The observed levels of penetration were similar to those reported by earlier investigators (Caroli *et al*., 1996; Ricci *et al*., 1996; Epsky and Capinera, 1993).

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