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Pathogenicity of entomopathogenic nematodes to sugarcane internode borer, *Chilo sacchariphagus indicus* Kapur (Lepidoptera: Crambidae)

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ABSTRACT: The penetration and pathogenicity of five species of entomopathogenic nematodes (EPN), *viz.*, *Heterorhabditis indica* (isolate LN2), *H. bacteriophora*, *Steinernema glaseri*, *S. riobrave* and *S. feltiae* to the sugarcane internode borer (INB), *Chilo sacchariphagus indicus* (Kapur), were studied under laboratory conditions. Significant differences were observed among mean penetration of the EPNs. The highest penetration (42.2%) was observed for the steinernematids, *S. glaseri* and *S. riobrave* (28.3%) and the lowest penetration for the two heterorhabditids. *H. indica* (5.5%) and *H. bacteriophora* (5.2%). In pathogenicity studies, mortality of the INB increased with increase in dosage. The borer suffered 100 per cent mortality with 40 and 50IJs of *H. indica* and with 50IJs larva^{-1} of *S. glaseri*. The lowest mortality (10 to 26.6%) of INB was observed at a dosage of 10IJs larva^{-1} of all EPNs. *H. indica* was found to be superior among all EPN species with an LD_{50} of 14.7IJs larva^{-1}. The LD_{50} values for other EPNs were 17.9IJs larva^{-1} for *S. glaseri*, 24.4IJs larva^{-1} for *H. bacteriophora*, 27.5IJs larva^{-1} for *S. riobrave* and 33.1IJs larva^{-1} for *S. feltiae*.

KEY WORDS: *Chilo sacchariphagus indicus*, entomopathogenic nematodes, *Heterorhabditis*, *Steinernema*, sugarcane internode borer

INTRODUCTION

The sugarcane internode borer (INB), *Chilo sacchariphagus indicus* Kapur, can inflict serious losses in cane yield in tropical India. There are several organisms involved in the biological control of this pest, among which the entomopathogenic nematodes (EPN) belonging to the families Heterorhabditidae and Steinernematidae are gaining much attention in recent years (Karunakar *et al.*, 1992). Entomopathogenic nematodes (*Steinernema* spp. and *Heterorhabditis* spp.) and their symbiotic bacteria (*Xenorhabdus* and *Photorhabdus* spp.) are obligate pathogens of insects in nature. Many qualities make them excellent biocontrol agents; they have a broad host range, possess the ability to search for the hosts actively, present no hazard to mammals and are exempt from registration and regulation requirements by the US Environmental Protection Agency (EPA) (Gaugler, 1988).

Estimation of LD_{50} is a relative measure of susceptibility of a host population and 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 that determination of percentage host mortality and nematode invasion efficacy be done simultaneously for assessment of nematode efficacy. The susceptibility of many economically important insect pests has been tested in a wide range of laboratory assays. The most commonly used bioassays consist of Petri dish assay dose response tests (Kaya and Hara, 1980), calculation of LD_{50} value (Morris *et al.*, 1990) and penetration rate assay (Caroli *et al.*, 1996). Hence the objectives of our studies were to test the penetration and pathogenicity of five EPN species to *Chilo sacchariphagus indicus* under laboratory condition with standard bioassay techniques.

MATERIALS AND METHODS

Nematode and insect cultures

Heterorhabditis indica (isolate LN2) collected in the vicinity of Coimbatore (Poinar *et al.*, 1992), *Steinernema glaseri* obtained from Dr. R. A. Bedding, CSIRO, Tasmanian Research Laboratory, Tasmania, Australia; *S. riobrave* and *H. bacteriophora* obtained from Dr. R. U. Ehlers, Christian Albrecht's University, Kiel, Germany, were used in this study. Fifth instar larvae of wax moth, *Galleria mellonella* 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% formalin and the cultures were maintained at 11°C. The larvae of *C. sacchariphagus indicus* were collected from sugarcane field and reared in sugarcane shoots under laboratory condition. Fifth instar larvae were used for the study.

Penetration and pathogenicity studies

The penetration rate assay and dose response assay of EPN against INB were conducted in 1.5cm diameter 24 well plates (Corning, USA). Each well was padded with two filter paper discs (Whatman No.1). One INB larva was placed in the well and the IJs of respective EPN were transferred to each well in 75ml suspension. Control wells received only 0.01% formalin in distilled water. Different batches of nematodes and insets were used for each replication. The penetration rate assay was conducted as described by Caroli *et al.* (1996). About 200IJs of respective EPN were inoculated in the well plate containing INB larvae. Each treatment consists of fifty replicates. Forty-eight hours after nematode inoculation, the insect mortality was recorded and the number of penetrated nematodes was determined by dissecting the dead cadavers in Ringer's solution. In pathogenicity study, fifth instar INB larvae were exposed to different dosages (0, 10, 20, 30, 40 and 50IJs / larva) of EPN and the mortality of the INB recorded 78h after nematode inoculation. Each treatment consisted of three replications and each replication consisted of 20 INB larvae. Mortality data expressed in percentage were transformed to the arcsine of the square root and subjected to analysis of variance (ANOVA). The means were separated by DMRT (SPSS, 2002). The LD_{50} was calculated using probit analysis and all comparisons were made at $P = 0.05$ level of significance.

RESULTS AND DISCUSSION

In penetration assay, 100 per cent mortality of INB was recorded with *H. indica* and *S. glaseri*. *H. bacteriophora* and *S. riobrave* recorded 88.8 per cent mortality of INB, but all the four were significantly on par with one another. *S. feltiae* recorded 66.6 per cent mortality of INB larvae (Fig. 1a). Significant differences were observed in mean penetration of the steinernematids. The highest value (42.2%) was recorded for *S. glaseri*, which was superior to other EPN species, followed by *S. riobrave* (28.3%) (Fig. 1b). The lowest penetration was recorded for the two heterorhabtids (5.5 % for *H. indica* and 5.2% for *H. bacteriophora*). Similar results were observed by Caroli *et al.* (1996) for heterorhabditids with lower penetration than steinernematids. Even at lower penetration levels *Heterorhabditis* spp. could cause 100 per cent mortality of INB larvae in this study. The three steinernematids tested invaded INB larvae in higher numbers than heterorhabditids. The observed levels of penetration were similar to those reported

by other investigators (Caroli *et al.*, 1996; Ricci *et al.*, 1996; Epsky and Capinera, 1993). The differences in penetration by EPN in the present study may be due to infection strategies. Since the adults developing from invading IJs of heterorhabditids reproduce hermaphroditically, a single invader can potentially reproduce. Therefore, few invaders will be sufficient to establish the next generation. Steinernematids, however, are amphimictic and mating is essential for further

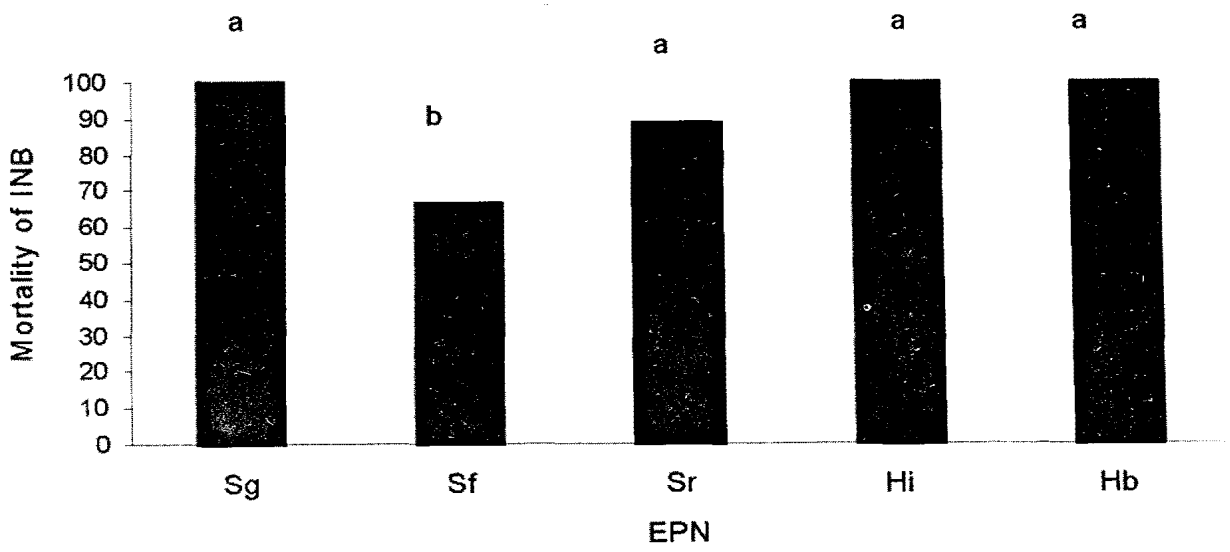

Fig. 1a. Per cent mortality of *C. sacchariphagus indicus* due to EPN at 48h exposure in penetration assay

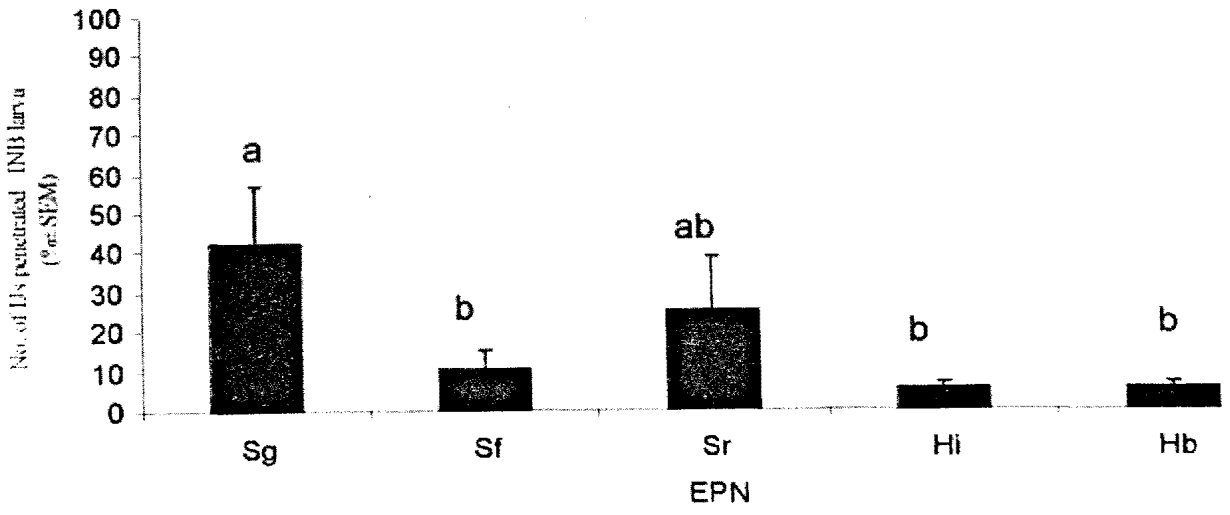

Fig. 1b. Average number of IJs of EPN penetrated in infected larvae of *C. s. indicus* at 48h exposure [Sg-*S. glaseri;* Sf-*S. feltiae*; Sr-*S. riobrave*; Hi-*H. indica*; Hb-*H. bacteriophora*. Bars with the same letters are not significantly different (DMRT, P=0.05)]

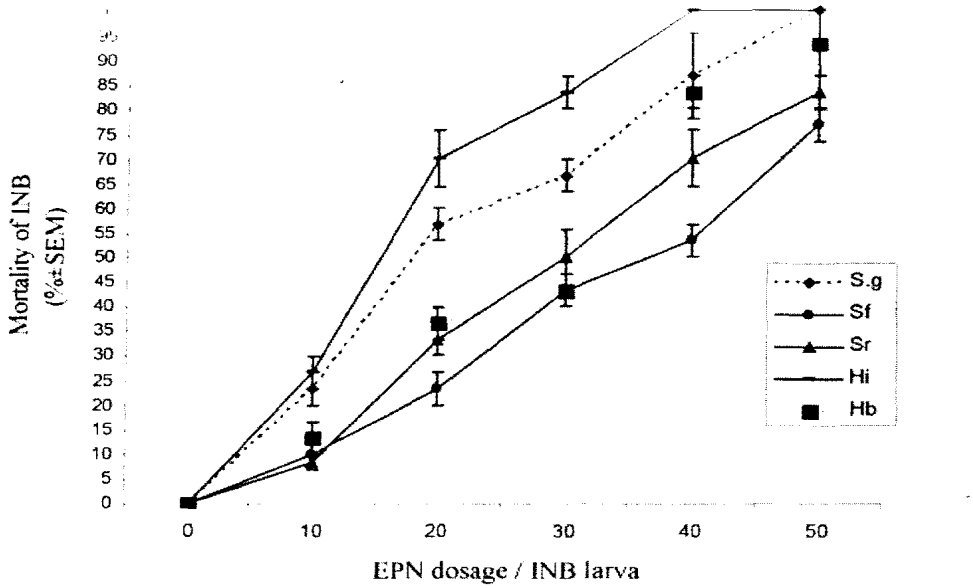

Fig. 2. Per cent mortality of *C. sacchariphagus* indicus larvae following 72h exposure to different concentrations of IJs of *H. indica* (Hi), *H. bacteriophora* (Hb), *S. glaseri* (Sg), *S. riobrave* (Sr) and *S. feltiae* (Sf)

Table 1. LD_{50} of different entomopathogenic nematodes against larvae of *Chilo sacchariphagus indicus*

EPN species	LD_{50} (No. IJs / INB larva)	95% confidence limit
Heterorhabditis bacteriophora	24.4	12.0–39.5
H. indica (isolate LN2)	14.7	9.5–18.9
Steinernema glaseri	17.9	9.6–24.7
S. feltiae	33.1	26.3–44.7
S. riobrave	27.5	25.1–29.9

reproduction (Poinar, 1990), thus an invasion of high numbers of individuals increases the probability for mating and further reproduction.

In pathogenicity study with different dosages of EPN against INB larvae, mortality of *C. sacchariphagus indicus* increased with increase in dosage (Fig. 2). INB recorded 100 per cent mortality with 40 and 50IJs larva^{-1} in the case of *H. indica*, whereas *S. glaseri* required only 50IJs larva^{-1}. The lowest mortality (10 to 26.6%) of INB was observed at a dosage of 10IJs larva^{-1} for all EPN. The LD_{50} values were calculated from different dosage levels for all EPN tested (Table 1). *H. indica* was found to be superior among all other EPN species with LD_{50} of 14.7IJs larva^{-1}. The LD_{50} values for other EPNs were 17.9IJs larva^{-1} for *S. glaseri*, 24.4IJs larva^{-1} for *H. bacteriophora*, 27.5IJs larva^{-1} for *S. riobrave* and 35.1IJs larva^{-1} for *S. feltiae*.

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