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Bioefficacy of Entomopathogenic Nematodes against *Spodoptera litura* (Lepidoptera: Noctuidae) in Bhendi

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A B S T R A C T

The efficacy of entomopathogenic nematodes (EPN) against larvae of *Spodoptera litura* (Lepidoptera: Noctuidae) in bhendi was evaluated under laboratory and glasshouse conditions. Under laboratory conditions, larvae were highly susceptible to the two nematode species, *Heterorhabditis indica* and *Steinernema glaseri* (Nematoda: Rhabditidae) when used separately and the percentage mortality increased with increase the dose of nematodes. In laboratory studies, median lethal concentration and median lethal time of *H. indica* registered lowest LC_{50} of 6.81 IJ/larva and $LT_{50}$of 23.42 h/larva and for *S. glaseri* which recorded highest LC_{50} 8.45 and $LT_{50}$of 16.72 IJ /larva for *S. litura*. Median lethal time of *H. indica* was less on *S. litura* than *S. glaseri* under laboratory conditions. Under pot culture conditions larva of *H. indica* was more effective on *S. litura* than *S. glaseri* under glasshouse conditions. The highest mortality of 76.66 per cent was observed with *H. indica* @ 5×10^9 IJ/ha with the least fruit damage of 29.16 per cent in bhendi.

Introduction

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) is an obnoxious cosmopolitan pest that feeds on more than hundred host plants. This techniques have been used to combat this pest, among them chemical insecticides are well known for quick results but their serious harmful effects on the environment is also well documented.

The entomopathogenic nematodes of the genus *Steinernema* and *Heterorhabditis* (Rhabditida: Steinernematidae, Heterorhabditidae) and their symbiotic bacteria are effective biological control agents of different insect pests. They persist in the soil as non-feeding, third stage infective juveniles (IJs) which seek, infect and kill susceptible insect hosts within 24-72h. Environmental concern over the consequences of use of chemical pesticides, especially residues in field, ground water contamination, wild life kills and development of insect resistance, pest resurgence and outbreak of secondary pests have compelled for intensive research for safe, eco-friendly alternative methods for insect management. The application of entomopathogenic nematodes in biological control was traditionally used to control soil pests until a few years ago. But the research

from the last two decades indicates their marked potential against foliar pests under unique conditions. Earlier attempts have demonstrated that entomopathogenic nematodes at high concentrations, together with favourable abiotic components (high humidity and optimal temperature) can be highly effective biological control agents of insects in commercial agriculture (Laznik *et al*., 2012).

The aim of our present research was to study the efficacy of entomopathogenic nematodes against the *Spodoptera litura* to determine which species of EPN (*H. indica* and *S. glaseri*) is the most effective as related to subtropical temperature and the nematode concentration. In the present study to investigated the efficacy of entomopathogenic nematode, *H. indica* and *S. glaseri* against larval stages of *S. litura* under laboratory and pot culture conditions.

Materials and Methods

Insects rearing

The rearing of our test insect, *Spodoptera litura* was done in laboratory on its natural diet, leaves of castor plant (*Ricinuscom munis*). Hundred eggs per day of ovulation were kept in separate sterilized jars and fresh surface sterilized castor leaves were provided for the first in star larvae on emergence. Cleaning, changing of food and thinning of the culture were done on regular basis to get healthy culture.

Nematodes

The nematodes *viz*., *H. indica* and *S. glaseri* were obtained from Sugarcane Breeding Institute, Coimbatore and mass cultured in *C. cephlonica*. The larvae were reared on broken cumbu grains sterilized at 100^{o}C for 30 minutes, according to the procedure of Kaya and Gaugler (1993). The third stage juveniles (IJs) were harvested from water surrounding White's trap within 10 days of emergence from their hosts. A stock suspension of the IJs in distilled water was stored at 20^{o}C for 2 weeks before use in BOD incubator.

Laboratory conditions

Heterorhabditis indica and *S. glaseri* were used for testing virulence against *S. litura*. Dose and time mortality relationship tests were conducted in 9 cm diameter Petri dishes lined at the bottom with a Whatman No. 1 filter paper and moistened with 1ml sterile distilled water. Infective juveniles were evenly applied over the filter paper. The dosages used were 0, 50, 100, 150, 200, 250 and 300 infective juveniles per larva, with 10 larvae of *A. ipsilon* per insect per replicate and four replicates for each level.

Pot culture experiment

Two pot culture experiments were carried out under glass house conditions for testing the bioefficacy of entomopathogenic nematodes against *S. litura* in *Abelmoschuses culentus*(cv. Parbhani Kranti). Seeds of bhendi were surface sterilized in 0.1 per cent mercuric chloride for a minute, washed in distilled water and the seeds @ 3 per pot, were sown in earthen pots of 5kg capacity, containing pot mixture. After germination, the plants were thinned to four/pot. The plants were properly maintained with regular irrigation. After 40 days, plants reached the fruiting stage. Laboratory reared 4th instar larvae of *S. litura* were collected and starved for about 3 h and fed with bhendi leaves to increase host suitability of larvae. The larvae were released on 40 day old plants at 4 per plant and the nematode treatments were given as *H. indica* @ 1.25, 2.5 and 5×10^{9} IJ/ha and *S. glaseri*@ 1.25, 2.5 and 5×10^{9} IJ/ha.

The treatments were replicated thrice in a Completely Randomized Design (CRD).

Antidesicant, glycerine @ 0.1 per cent (1ml/1) and UV protectant, Tween 20 @ 0.05 per cent (0.5ml/1)were added to the spray fluid (25 ml/pot) containing nematodes. Spraying of nematodes spray fluid was done at 1h before sunset with a hand atomizer @ 25 ml/ plant. Insect mortality counts were taken every 24 h up to 72 h after spraying. The dead larvae were dissected in 0.1 per cent saline water and examined under microscope to confirm the death of larvae due to nematodes. Damaged leaves due to the larvae were also recorded in all the treatments.

Statistical analysis

The observations recorded were statistically analysed for the experiments. Means of all experiments were used to compare the efficacy of treatments. Per cent insect mortality data were analysed by multifactor ANOVA followed by Duncan's multiple range test (P>0.05) for separation of means.

Results and Discussion

Laboratory experiment

The virulence of two species of EPNs, *viz*., *H. indica* and *S. glaseri* were tested against larvae of *S. litura* under laboratory conditions. *H. indica* was more virulent to larvae of *S. litura*. The nematode *H. indica* caused lowest $LC_{50}$of 6.81IJ/larva and $LT_{50}$of 23.42 h/larva were observed for *S. litura*. The nematode species *S.glaseri* caused 50 per cent larval mortality of *S. litura* after 8.45 h/ larva and 16.72 IJ/larva respectively, which consumed more time and high dose for causing maximum mortality period (Tables 1 and 2).

The virulence was determined in the present study by median lethal concentration (LC_{50}) and Median lethal time (LT_{50}) of *H. indica* and *S. glaseri* against insect pest of *S. litura*. The findings showed that LC_{50} and LD_{50} of *H. indica* was low for *S. litura* and high for *S. glaseri*. Umamaheswari *et al*., (2004) and Saravanapriya and Subramanian (2007) reported that *H. indica* as highly virulent against *S. litura* (LC_{50}-3.5 and 7 IJ/larva) and caused 50 per cent mortality in a minimum time of 34.53 h/larva which confirm the present findings.

Divya *et al.,* (2010) and King (1994) reported early instar larvae of *H. armigera, S. litura* and *G. mellonella* were more susceptible to *H. indica*, which confirm the present findings. The LT_{50} of *H. indica* to cause 50 per cent mortality of *S. litura* larvae in a minimum period was 23.42 h/ larva. Similarly, Kalia *et al*., (2014) reported that the LT_{50} values of *S. thermophilum* were 41.40 h with *S. litura* and 33.6 h with *G. mellonella*.

Pot culture experiment

In pot culture experiment conducted under glass house conditions, *H. indica* and *S. glaseri* were found effective at all the dosages tested *viz*., 1.25×10^9, 2.5×10^9 and 5×10^9 IJ/ha. With respect to time, increased mortality was observed with increased exposure time after treatment.

At 24 h of exposure period, the highest larval mortality of 26.66 per cent was caused by *H. indica* @ 5×10^9 IJ/ha followed by *S. glaseri* which caused a lowest larval mortality of 6.66 per cent @ 1.25×10^9 IJ/ha. Similarly at 48 h of exposure period the same trend was observed for the two nematode species at different intervals. A larval mortality of 48.33 per cent was caused by *H. indica* @ 5×10^9 IJ/ha and 11.66 per cent of mortality was caused by *S. glaseri* at 1.25×10^9 IJ/ha respectively (Table 3).

Table.1 LC50 Values calculated from dosage response assays conducted with different nematodes species and larvae of *S. litura*

Nematode species	Incubation period (h)	LC_{50}	Fiducial limit (95 %)	
			UL	LL
H. indica	24	8.41	6.23	8.23
	48	8.76	7.12	9.65
	72	6.81	4.98	9.32
	96	6.12	4.96	10.16
S. glaseri	24	11.6	8.63	14.56
	48	10.89	7.89	13.41
	72	8.45	6.05	11.81
	96	7.23	5.42	6.87

Table.2 LT50 Values calculated from dosage response assays conducted with different nematodes species and larvae of *S. litura*

Nematode species	Incubation period (h)	LT_{50}	Fiducial limit (95 %)	
			LL	UL
H. indica	24	25.34	21.54	23.45
	48	24.53	21.31	22.79
	72	23.42	19.98	27.44
	96	22.41	18.41	25.89
S. glaseri	24	18.12	14.56	23.45
	48	17.56	14.23	22.89
	72	16.27	13.51	19.60
	96	15.89	12.56	18.23

Table.3 Bioefficacy of entomopathogenic nematodes against larvae of *S. litura* on bhendi under pot culture conditions

Treatments	Per cent insect mortality (hr after treatment)			Per cent leaves damage
	24	48	72	
T_1- *H. indica* @ 1.25×10^9 IJs/ha	6.66^{d} (14.75)	18.33^{d} (25.30)	60.00^{c} (50.79)	58.33^{bc} (45.83)
T_2- *H. indica* @ 2.5×10^9 IJs/ha	16.66^{b} (24.04)	31.66^{c} (34.23)	68.33^{b} (55.77)	45.83^{c} (42.58)
T_3- *H. indica* @ 5×10^9 IJs/ha	26.66^{a} (31.07)	48.33^{a} (44.04)	76.66^{a} (61.14)	29.16^{bd} (32.58)
T_4- *S. glaseri* @ 1.25×10^9 IJs/ha	6.66^{d} (14.75)	11.66^{e} (19.88)	31.66^{e} (34.23)	66.66^{b} (54.82)
T_5- *S. glaseri* @ 2.5×10^9 IJs/ha	11.66^{c} (19.88)	21.66^{c} (27.71)	43.33^{d} (41.16)	58.33^{c} (54.82)
T_6- *S. glaseri* @ 5×10^9 IJs/ha	21.66^{ab} (27.71)	40.00^{d} (39.21)	66.66^{b} (54.75)	45.83^{c} (42.58)
T_7- Control	0 (0.28)	0 (0.28)	0 (0.28)	83.33^{a} (66.19)
CD (p=0.05)	4.11	3.59	3.28	7.79

Subsequently at 72 h of exposure period, the highest mortality of 76.66 per cent was caused by *H. indica* followed by *S. glaseri* which caused a larval mortality of 66.66 per cent @ 5×10^9 IJ/ha. The lowest mortality of 31.66 per cent was caused by *S. glaseri* @ 1.25×10^9 IJ/ha. Percentage of damaged leaves with bore holes decreased with increased dosages of nematodes. *H. indica* was more effective than *S. glaseri*. *H. indica* @ 5×10^9 IJ/ha recorded the least leaves damage (29.16%) which was on par with *S. glaseri* @ 5×10^9 IJ/ha (45.83 %). The highest leaves damage of 83.33 % was observed in untreated control plants.

The pot culture experiment with bhendi revealed that *H. indica* and *S. glaseri* at the dosage range of 1.25 to 5×10^9 caused mortality of *S. litura* ranging from 60.00 to76.66 per cent and 31.66 to 66.66 per cent for both *H. indica* and *S. glaseri* respectively. Highest mortality of the host due to both nematodes was observed at the highest dosage level of 5×10^9/IJ/ha. Earlier reports by Narayanan and Gopalakrishnan (1987), Choo *et al*., (1989) and Sezhian *et al*., (1996) established the biocontrol potential of *S. carpocapsae* on *S. litura* where increased dosage levels caused high insect mortality.

In the present study, *H. indica* was found to be more effective than *S. glaseri*. This was also reported by Subramanian (2000) whereas *H. indica* caused higher mortality of *Liriomyza trifolii* than *S. glaseri*. Divya *et al*., (2010) reported the bioefficacy of per cent larval mortality of *H. armigera* and *S. litura* on cotton plant influenced by *H. indica* was significantly more on final instar larvae at 60h.

In the present study, the least leaves damage was recorded as with *H. indica* and *S. glaseri* at the highest dosage level of 5×10^9/IJ/ha. The higher concentration proved to be more efficient in the present research which demonstrated their efficacy also at lower concentrations. *S. glaseri* caused dosage mortality response against different stage of four lepidopteran insects (Saravanapriya and Subramanian, 2007).

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