Research Article

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Natural occurrence and virulence of entomopathogenic nematodes on tea termites in barak valley of Assam, India

D.Singha,[a] B.Singha[b] and B.K. Dutta[a*]

[a]Department of ecology and environmental science, Assam university silchar-788011 Assam, India
[b]Department of zoology, G.C.College, silchar-788004 Assam, India

ABSTARCT: Survey of entomopathogenic nematodes (EPNs) have been conducted in large areas of Cachar district (Assam) for isolating potentially useful indigenous EPN strains. Out of total 110 soil samples from different habitats representing different soil systems, 9 samples were found to have shown positive results through baiting with last instars of muga silkworm (*Anthera assama*),eri silkworm (*Attacus ricini*) and greater wax moth (*Galleria mellonella*). Soil characteristics have influenced the occurrence of EPN. It has also been confirmed that last instar larvae of silkworms could be used as bait insects in isolation EPN strains like *Steinernema thermophilum*, a heat tolerant EPN described from India. In laboratory bioassays the indigenous isolate of *Heterorhabditis indica* demonstrated more virulence as compared to *S.thermophilum* against the termites causing damage to tea plantations (*Microtermes obesi* Holmgren and *Microcerotermes beesoni* Snyder).

KEYWORDS: Bait insects; Biological control; Entomopathogenic nematodes; Silkworms; Tea termites; Virulence

Introduction

Assam is a significant tea producing area in India. Termite infestation is one of the main enemy of the tea growing areas of South Assam which results in heavy economic loss. A critical field survey of Choudhury,[2] revealed that the termite species of the genera *Microtermes, Microcerotermes* and *Odontotermes* are the serious endemic termite pest associated with tea crop in this valley of Assam causing damage and economic loss. The present practice of the application of chemical pesticides has caused serious environmental hazards. Hence, it was decided to explore the possibility of using some environmentally friendly biopesticides of natural origin.

Keeping in view the diverse agroclimatic conditions in the country and their susceptibility to a wide range of insect pests, the potential of great biodiversity of insect killer nematodes occurring in the Indian soils and Barak Valley in particular deserves special attention.

Many insect antagonists are found within the phylum Nematoda, but only species within the genera *Steinernema* and *Heterorhabditida* (Rhabditida) have gained major importance as biological control agents in plant protection. Potentiality of EPN, belonging to Steinernematidae and Heterorhabditidae as biological control agents against insect pests have been well documented.[9,11,15] Presently, there are about 50 species of EPN, of which only a few species are currently being exploited for their biocontrol potentialities. Unique to EPN is their close symbiotic association with bacteria of the genera *Xenorhabdus* and *Photorhabdus* .These symbionts belong to the Enterobacteriaceae within the gamma subdivision of the purple bacteria.[4] The bactohelminthic complexes are used in the biological control of insects.[5]Termites live and forage in habitats that are moist, cool and without direct sunlight such as soil or wood materials. These environmental conditions are ideal for the survival and movement of Steinernematid and Heterorhabditid nematodes, and therefore, provide the basis for their role in the control of tea termites of Barak Valley of Assam. The potential of entomopathogenic nematodes to control tea termites has been determined by screening the species of *Steinernema thermophilum* and *Heterorhabditis indica* for pathogenicity towards two species of termites i.e. *Microtermes obesi* and *Microcerotermes beesoni.* The nematodes used in the experiment showed effectiveness against tea termites in the laboratory condition (in vitro) but do not cause colony elimination in the field. Therefore, its field application needs further investigation. Hence, the present work has been taken up to explore the possible use of indigenous EPN strains in order to exploit their insecticidal potentialities against the termite species causing damage to tea plantations.

*Author for correspondence: B.K. Dutta (e-mail: bimankdutta@rediffmail.com)

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Material and Methods

Collection and Preparation of Termites

Termites were collected from the termitarium of tea plantation area of Silcoorie Tea Estate, Cachar, Assam, India periodically. They were maintained at $25^0 \pm 2^0$C at 75-80% r.h. in the laboratory using artificial chamber. The specific identification has been confirmed from Isoptera Section, ZSI, Kolkata as *Microtermes obesi* Holmgren and *Microcerotermes beesoni* Snyder. The fungal combs were carefully broken and the different termite castes were separated using a fine hair brush. Amongst the three morphogenetic forms the active worker with uniform size were selected for treatment from the same colony. One day before testing, termite workers were taken from rearing containers, counted and transferred to petridishes lined with a filter paper. Dead and weak termites were changed with healthy termites immediately before the test.

Isolation of Indigenous EPN Strain

Soil samples were collected randomly in different habitats (such as bamboo growing areas, grassland, rice growing areas etc.), at localities within Cachar district. Sampling was performed from January to December 2005. Composite soil samples (approx.500g) from each sampling site were taken using an iron sampler to obtain the top 15-20 cm depth of soil. The samples were then transported to the laboratory in separately sealed plastic bags.

Insect baiting of each soil sample was performed by placing last instar larvae of wax moth (*Galleria mellonella*), eri silk moth(*Attacus ricini*) and muga silk moth (*Anthera assama*), after keeping the soil in wide mouth plastic bottles. These were then kept at laboratory temperature for 5-7 days at which time the number of dead insect larvae was assessed. Dead insect larvae were divided in two batches – one was dissected to obtain adult nematodes of the second generation and the second batch was used for culturing on a water trap for obtaining infective juveniles(IJs).[19] Adult and IJs were fixed in TAF and identified using morphological characters (Poinar *et.al.*1992).[14] Soil samples were also used to determine the soil pH, organic carbon, texture.

Nematode Source and Preparation

Rhabditid nematode of the genera *Heterorhabditis indica*. was isolated from the local soil and the strain of *Steinernema thermophilum* was obtained from Nematology Division, IARI, New Delhi. Before the experiments were started, nematodes were propagated in last instar larvae of greater wax moth (*Galleria mellonella*) and silkworm larvae, *Anthera assama* (muga) and *Attacus ricini* (eri) found locally. Nematode infective juveniles emerging from these host larvae within 7 day from the first day of emergence were collected and kept in a tissue culture flask at 15°C.

Experimental Design

All the experimental units were kept under dark at 25°C in an incubator.

Experiment 1: Comparative infectivity of two species of nematodes against termites (*Microtermes obesi* and *Microcerotermes beesoni*).

The test was conducted in 9 cm diameter petridishes lined with a Whatman no.1 filter paper disc. Thirty termites were added to each petridish. Nematode infective juveniles (IJ) were applied to filter paper at 0, 400, 1200, 2000 per termite in three replicates. Survival of termite was checked at 2, 4, 6, 8d after exposure. Termites taken for experimentation were from the same colony.

Experiment 2: Dose- mortality response of *Steinernema thermophilum* and *Heterorhabditis indica* against *Microtermes obesi* & *Microcerotermes beesoni.*

Methods were similar to experiment 1 with 30 termites per petridish on the filter paper. Nematode infective juveniles were added to the filter paper at the rates of 0, 200, 400, 600, 900, 1200, 1500, 1800 per termite. Each treatment was replicated three times for each termite species.

Experiment 3: One – on – one Assay

Insect preparation: 72 individuals each of *Microcerotermes beesoni and Microtermes obesi* active workers of equal sizes were separated into three replicates having 24 insects of each species were used for the assay.

Arena preparation: 96-well plates were used and filter papers were placed at the bottom of 72 wells (three rows of 24 well plates for the treatment).

Setting up the assay: After proper washing in distilled water, an individual termite were transferred in each well and then individual IJ of each of *H.indica* and *S. thermophilum* nematode were transferred from the suspension in 25ml-disti lled water in each well, by micropipette.

Incubation: The plates were stored at 25°C for 48-72 hours (2-3 days) to determine the termite mortality.

The control treatments in all the experiments received water only.

Statistical analysis

Mortality data were analyzed using ANOVA for comparison among the species and between different rates and the control. Mean mortalities of different nematode species and different rates were compared by t-test. Median lethal dose causing 50 and 90 % mortality (LD_{50} and LD_{90}) and their 95% fiducial limits were determined by probit regression analysis.[8]

Results and Discussions

110 samples were collected randomly from different habitats (ranging from open grass land to tea agroecosystem soil and cropland to home garden soil associated with areca, bamboo, banana and even rice) in and around the valley, during Jan'05 to Dec'05.Out of total soil samples only 9 samples yielded Rhabditid nematodes and identified as *Heterorhabditid indica*. The EPNs are repeatedly sub cultured on 5th instar larvae of *Galleria* and 5th instar of silks worm larvae to get large number of pure population of infective juveniles (IJs) for subsequent studies.

Through the present investigation, we confirm that the 5th instar larvae of silk worms (*Anthera assama* and *Attacus ricini*) can be included in the host range of EPN reported by Ganguly *et.al.*,[9] as because some soil samples of this region could produce mortality to the larvae and then subsequently a large population of EPN IJs have been recovered from the cadaver larvae.

Variation in habitat and soil type has been found to influence the distribution of entomopathogenic nematodes with the porous well drained loamy soil with low organic matter content favouring more than sandy and clayey soils[1] and the findings of the present random survey is very closely coincide to the normal occurrence of EPN in other parts of India and abroad (Table 1 & 2). *Heterorhabditis indicus* was found in Kanyakumari district, described by Poiner *et. al.*[17] from Coimbatore, India. The only other species of the genus *H.bacteriophora* was reported from Tamil Nadu by Sivakumar *et. al.*[18] The present report has been the only from this part of India. However, a few samples require confirmation of its identification.

Comparative infectivity of two species of nematodes against termites (*Microtermes obesi* and *Microcerotermes beesoni*).

In experiment 1, *M.obesi* exposed to two entomopathogenic nematodes (EPNs) exhibited increased mortality until 8d after exposure. Termite mortality at 8d varied significantly between the two EPN species. *Heterorhabditis sp.* was

Table 1: Distribution among habitats of positive samples for EPN

Habitat	No. of samples tested	Positive samples		
		Heterorhabditid indica	Unidentified	Total
Rice growing area	25	2	0	2
Bamboo growing area	20	2	0	2
Coconut growing area	15	0	1	1
Arecanut growing area	15	0	1	1
Vegetable growing area	15	1	0	1
Uncultivated area	20	1	1	2
Total	110	6	3	9

Table 2: Distribution by soil characteristics of positive samples for EPNs

Nematode	Soil texture	% of positive soil sample	Organic C (%) (Mean ±S.E)	pH (Mean ±S.E)
Heterorhabditis indica	Sandy	1.82	1.04 ±0.15	6.3 ±0.7
	Sandy loam	2.73	1.3 ±0.08	5.8 ±0.6
	Loamy	0.91	1.42 ±0.09	5.5 ±0.2
Unidentified	Sandy	0.91	1.02 ±0.07	6.1 ±0.7
	Sandy loam	1.82	1.45 ±0.09	5.6 ±0.2

found to be more effective than the *S. thermophilum* at all the rates of nematodes per termite (Table.3). Both the EPN species showed increasing mortality of termites with the increase in rate of nematodes per termite.

The mortality of *M. beesoni* caused by EPN species also ceased to increase after inoculation with the nematodes. Both the EPN species showed increasing mortality of termites with the increase in rate of nematodes per termite(Table.4). The *Heterorhabditis sp.* was found to be more virulent as compared to the *S. thermophilum.*

Dose-mortality relationship among *Heterorhabditis sp.* and *S.thermophilum* and termites (*M.obesi* and *M. beesoni*)

In petridish tests, *M.obesi* exposed to *S.thermophilum* & *H. indica* suffered increased mortality as nematode number increased .For *H .indica* the LD_{50} was 368, much less from that of *S.thermophilum* 9131(Table.5).

In case of *M.beesoni* for *H. indica* the LD_{50} was 356; much less than that of *S. thermophilum*, 8730. This signifies that both the termite sp. were more susceptible to *H.indica* as compared to *S. thermophilum*.The LD_{50} and LD_{90} values show that *H.indica.* was more virulent than *S. thermophilum* for both the termite sp.

One on one Assay

In this experiment there was no mortality of termites after 24 hr. But after 48 hr the mortality increased abruptly. Then there was lesser rise in mortality after 72 hr.(Table.6) The zero mortality after 24hr might be due to high mobility of termites and limited mobility of nematodes that delays the entrance of nematode through

Table 3: Percent mortality of *Microtermes obesi* induced by EPNs 8d after exposure in pettridish test (Means ±S.E.)

Nematode species	Control	Rate (nematodes per termite)		
		400	1200	2000
Steinernema thermophilum	11.1± 1.18^{a}	14.4±0.88^{bA}	27.7±0.72^{bA}	47.7±0.66^{cA}
Heterorhabditis indica	10.0 ± 1.18^{a}	41.1±1.71^{bB}	62.2±1.31^{cB}	86.6±2.84^{dB}

Means within a row followed by different lowercase letters are significantly different (P<0.05)
Means within a column followed by different uppercase letters are significantly different (P<0.05)

Table 4: Percent mortality of *Microcerotermes beesoni* induced by EPNs 8d after exposure in petridish test (Means ±S.E.)

Nematode species	Control	Rate (nematodes per termite)		
		400	1200	2000
Steinernema thermophilum	7.77± 1.18^{a}	13.3±1.61^{bA}	26.6±1.26^{bA}	48.8±0.65^{cA}
Heterorhabditis indica	8.88 ± 1.18^{a}	45.5±0.63^{bB}	60.0±1.13^{bB}	87.7±1.01^{cB}

Means within a row followed by different lowercase letters are significantly different (P<0.05)
Means within a column followed by different uppercase letters are significantly different (P<0.05)

Table 5: Dose-mortality relationship between nematodes and termites

Termite sp.	Nematode species	Lethal dose (Mean & 95% fiducial limit) (nematodes per termite)		χ^2
		LD_{50}	LD_{90}	
Microtermesobesi	Steinernema thermophilum	9131 (6801 – 2.1 x10^4)	18122 (5611 – 1.8 x 10^6)	9.2 (P<0.1)
	Heterorhabditis indica	368 (189 – 534)	617 (480 – 978)	12.7 (P<0.01)
Microcerotermes beesoni	Steinernema thermophilum	8730 (5912 – 1.4 x 10^4)	16230 (4870- 2.4 x 10^6)	5.9 (P<0.2)
	Heterorhabditis indica.	356 (174 – 526)	683 (552- 1145)	14.2 (P<0.01)

Table 6: One on one assay of nematodes on termites

Termite sp.	Nematode species	Rate of mortality (Means ±S.E.)		
		24 hr	48 hr	72hr
Microtermes obesi	Steinernema thermophilum	0±0	56.4 ± 0.54[b]	83.3 ± 1.25[b]
	Heterorhabditis indica	0±0	91.6 ± 1.69[a]	95.3 ±1.35[a]
Microceotermes beesoni	*Steinernema thermophilum*	0±0	59.2 ± 0.54[b]	80.5 ± 1.17[b]
	Heterorhabditis indica	0±0	88.8 ± 1.46[a]	92.5 ± 0.32[a]

Means followed by different letters are significantly different (P<0.05)

the termite. The *H.indica* showed the same trend like the earlier experiments i.e. more virulent to the termites than the *S.thermophilum*. In control the mortality of termites was less than 15%.

The experiments showed that different nematode species had very different levels of infectivity against termites. *H.indica* was more effective compared to *S.thermophilum*. Its LD_{50} had very low value compared to that of *S.thermophilum* but both have low LD_{50} as compared with that of *S.carpocapsae* against *Reticulitermes tibialis* (Banks) (1.5 x 10^4 nematodes/termite).[7] In a field study on *Heterorhabditis sp* effective control was achieved against a dry wood termite *G.dilatatus* to protect tea bushes in Sri Lanka.[3] The study also confirmed the higher virulence of symbiotic bacteria *Photorhabdus* sp. in the intestine of *Heterorhabditis* sp. than *Xenorhabdus indica* in the gut of *Steinernema thermophilum*.[10]

The high number of termites in a colony and the wide foraging range are obstacles for nematodes to eliminate termite colonies. The limited mobility of nematodes & low rate of production in dead termites make it unlikely that nematodes will reach and maintain a large density enough to eliminate a termite colony in the field. There is a lot to learn about nematode biology, ecology and relationships with their hosts. Termites stressed by sublethal doses of chemical or pathogens probably are more susceptible to EPNs. A combination of nematodes with other biocontrol agents or chemicals may improve their control over termites. It is known that imidacloprid improves the effect of nematodes against scarab species (Koppenhöfer *et.al.*2000).More study on bactohelminthic biology, screening for more infective nematode species, strains or application techniques should provide valuable new information on the possible use of nematodes in termite control.

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