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REARING OF TWO ENTOMOPATHOGENIC NEMATODES, *Heterorhabditis bacteriophora* Poinar AND *Steinernema* sp. IN TERMITE (*Odontotermes obesus* Ramb.)

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ABSTRACT

***The in vivo* methods for rearing of entomopathogenic nematodes requires large quantities of insect hosts like greater wax moth, *Galleria mellonella* (L.) which involves more time and additional costs, necessitates alternative host for multiplication of entomopathogenic nematodes. The present study was aimed to assess the infectivity and multiplication of two entomopathogenic nematodes, *Heterorhabditis bacteriophora* Poinar and *Steinernema* sp. isolated from Majuli river island of Assam in subterranean termite *Odontotermes obesus* (Ramb). The laboratory tests using Petri-dishes, illustrated the effectiveness of both the nematodes against *O. obesus* at 100 IJs termite^{-1} (worker/soldier) within 24-48 h, in which the nematodes could reproduce in 5-7 days. The natural mounds (250 g) containing *O. obesus* (80-100 workers/ soldiers/nymphs) were also inoculated with *H. bacteriophora* and *Steinernema* sp. at 1000 IJs and incubated at room temperature (30±2ºC) up to 20 days for multiplication. Following the Cobb decanting and sieving method, the results exhibited the optimum yields of 30,000 and 25,000 IJs of *H. bacteriophora* and *Steinernema* sp., respectively, at 15 days beyond which the IJs starts deterioration. Under laboratory condition entomopathogenic nematodes *H. bacteriophora* and *Steinernema* sp. can be reared and mass produced for further management practices against termite.**

Key words: Entomopathogenic nematodes, *Heterorhabditis bacteriophora*, rearing, mass culture, *Steinernema* sp.

INTRODUCTION

Entomopathogenic nematodes belonging to the genera *Steinernema* and *Heterorhabditis* possess most of the characteristics of ideal biological control agent for the management of insect pests. Effective use of these nematodes requires the isolation and maintenance of nematode strains with high infectivity. *In vivo* methods contemplate an insect host for maintenance of the nematodes. Usually, immature stages of various insect orders (*i.e. Lepidoptera, Coleopter* and *Diptera*, etc.) are considered suitable hosts (David *et al*., 2012). A large number of insect-hosts may be required for this purpose demanding more time and additional costs related to the insect rearing. Usually, both steinernematids and heterorhabditids can reproduce in larvae of greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) (David *et al*., 2012). It occurs naturally in bee hives and is reared using artificial diets made of cereals, yeast and glycerol (David and Kurup, 1988). These dietary components are purchased from markets which involves additional expense and time. Choice of host species for *in vivo* production of nematode should ultimately rest on nematode yield per cost of insect (Shapiro *et al.*, 2002). However, environmental factors *viz.*, temperature, moisture and

aeration and method of nematode inoculation can affect the yield (Dolinski *et al*., 2007). Preliminary tests showed that termite (*Odontotermes obesus*, Ramb.) is susceptible to native isolates of entomopathogenic nematodes. The objective of present study was to determine the suitability of *O. obesus*, as an alternative host, to the greater wax moth (*G. mellonella*) larvae for rearing of entomopathogenic nematodes.

MATERIALS AND METHODS

Collection and preparation of termites

Odontotermes obesus mounds were collected from the sites in tea (*Camellia sinensis*) plantation ($26^0 43' 26.40'' N\ 94^0 12' 21.13'' E$) of AAU, Jorhat, Assam (India). The collected termite colonies used in the experiment were kept in large plastic containers in laboratory at room temperature (25-30°C). The frequencies of well-developed colonies, consisting of soldiers, larvae and nymphs of termites, were counted which showed an average number of 90 ± 10 in 250 g of mound. The fresh colonies were preserved in glass beaker (5.1 x 3.5 cm) for rearing of entomopathogenic nematodes. The soldiers, having the average weight of 3.2±0.6 mg, were separated, washed in sterilized water and used for infectivity test. The infectivity tests were carried out in Petri-dishes (9.0 x 1.5 cm) lined with a wet filter paper containing the 3rd instar termite workers (mean weight 3.2±0.6 mg).

Nematode preparation

Two nematode species *viz*., *H. bacteriophora* Poinar and *Steinernema* sp. were isolated from soils of Majuli river island, Assam (Devi *et al*., 2016). The test nematode species were multiplied *in vivo* on greater wax moth, *G. mellonella* larvae in the laboratory at 30±2°C using the method described by Woodring and Kaya (1988). The nematode multiplications in *G. mellonella* were continued in batches and the infective juveniles (IJs) were collected up to 12 days of inoculation. Production of the IJs from *G. mellonella* larvae began at 7-9 days after IJs host exposure. Average total IJs production over a 12-day period, starting after emergence from the host was $6 \times 10^5\ g^{-1}$ host. The collected IJs were preserved in a beaker at 15°C and utilized within two days for the study.

Comparative infectivity of two nematodes against O. obesus

The infectivity test was conducted in Petri-dishes lined with Whatman No.1 filter paper. Fifty termites were placed in each Petri-dish. Infective juveniles of *H. bacteriophora* and *Steinernema* sp. were inoculated at 0 (control), 10, 50, 100,150, 200 and 250 $termite^{-1}$ in four replications. In control, the termites were not exposed to the nematodes. The Petri-dishes were wrapped with tiny perforated polyethylene bag and kept at room temperature in a dark room (30±2°C). Termite mortality was observed after 24, 48, 72 and 96 h exposure. Mortality data was transformed (arcsine of the square root) and statistical analyses were performed by two-way factorial completely randomized design by SPSS software. Significance at 5% level was considered significant. Mortality data in different doses were adjusted for the observed mortality in control and the data was then subjected to Probit analysis by using SPSS software for computation of median lethal dose for each nematode species (Hewlett and Placket, 1979).

Rearing of nematodes in O. obesus

The rearing of nematodes was carried out in mounds containing all the stages of *O. obesus* in a glass beaker. Prior to inoculation, the IJs of nematodes were surface sterilized in 0.1% formaldehyde solution for 10 min and rinsed three times in sterilized distilled water. Mounds (250 g) as substrate was inoculated with 15 mL suspension maintained at the concentrations of 500, 1000 and 1500 IJ of both the nematode species separately and kept in dark at room temperature (30±2°C) for 10,15 and 20 days to ascertain their multiplication. The experiment was conducted with four replications. Nematodes that emerged from the dead termites (progeny production) were monitored and counted

using a dissecting microscope on 10th, 15th and 20th day after inoculation by extraction of IJs following Cobbs decanting and sieving method(Cobb,1918). Progeny production data were transformed (log) and were analyzed using analysis of variance (ANOVA) for comparison among species and days of observation and control.

RESULTS AND DISCUSSION

The study revealed that *O. obesus* exposed to *H. bacteriophora* and *Steinernema* sp. exhibited increase in mortality with increase in exposure time coupled with inoculation rate. No termite mortality was recorded at 24 h exposure with *H. bacteriophora*. However, both the nematode caused 50% mortality of *O. obesus* at 100 IJs termite^{-1} at 48, 72 and 96 h exposure. Significant difference was found in causing mortality of *O. obesus* by the two nematode-species at inoculation level of 100, 150, 200 and 250 IJS termite^{-1}, irrespective of the exposure time. Of the two nematodes, *H. bacteriophora* caused higher mortality (78%) as compared to *Steinernema* sp. (74%), following by the inoculation of 200 IJstermite^{-1} at 96 h of exposure period. Irrespective of the level of inoculums, significant difference in mortality of two nematode species was observed at various exposure time. With the increase in the exposure period to 96 h, *H. bacteriophora* could induce up to 80% mortality of the tested termites at the dose of 250 IJs termite^{-1} (Table 1). The significance of interaction proved that there are real differential effects of the rate of inoculation of the two nematodal species termite^{-1} with variation in the time of exposure on the mortality of termite.

The LD_{50} values during the exposure period showed that *H. bacteriophora* was more virulent than *Steinernema* sp. The LD_{50} values for *H. bacteriophora* were 87.71 (95% FL 68.94-111.58), 72.17(95% FL 52.91-98.44) and 61.34 (95% FL 43.29-86.93) at 48, 72 and 96 h of exposures, respectively (Table 3). Compared to *H. bacteriophora*, the higher LD_{50} values of 100.66 (95% FL 79.94-126), 81.47 (95% FL 61.38-108.14) and 74.79 (95% FL 54.86-101.98) were recorded following the exposure for 48, 72 and 96 h, respectively, in case of *Steinernema* sp. (Table 2).

Laboratory bioassays for different nematode species of *Heterorhaditis* and *Steinernema* indicated varied responses against termites (Fujii, 1975; Danthanarayanan and Vitarana, 1987).

Table 1: Per cent mortality of *Odontotermes obesus* induced by nematodes at different time exposure in Petri-dish test (mean of four replicates with 50 termites per replication)

Nematode	Inoculation ratetermite^{-1}	Mortality (%) after				Mean mortality
		24 h	48 h	72 h	96 h	
Steinernema sp.	10	10 (18.36)	12 (19.72)	20 (26.25)	24 (29.27)	16.5 (23.40)
	50	10 (18.36)	24 (29.27)	32 (34.42)	34 (35.66)	25.0 (29.43)
	100	20 (26.36)	52 (46.15)	54 (47.29)	56 (48.45)	45.5 (42.06)
	150	30 (32.45)	66 (54.36)	68 (55.61)	70 (56.89)	58.5 (49.83)
	200	30 (33.03)	68 (55.58)	72 (58.07)	74 (59.41)	61.0 (51.52)
	250	40 (38.94)	76 (60.69)	78 (62.10)	78 (62.10)	68.0 (55.96)
H. bacteriophora	10	0 (0.19)	14 (21.95)	22 (27.89)	26 (30.60)	15.5 (20.16)
	50	0 (0.19)	28 (31.90)	34 (35.64)	38 (38.05)	25.0 (26.44)
	100	0 (0.19)	54 (47.29)	56 (48.46)	58 (49.62)	42.0 (36.39)
	150	0 (0.19)	68 (55.59)	70 (56.89)	72 (58.06)	52.5 (42.68)
	200	0 (0.19)	72 (58.06)	74 (59.41)	78 (62.10)	56.0 (44.94)
	250	0 (0.19)	78 (62.19)	78 (62.14)	80 (63.54)	59.0 (47.01)
Control	0	0 (0.19)	4 (11.35)	6 (14.08)	8 (16.37)	4.5 (10.49)
Mean		10.76 (12.99)	47.07 (42.62)	51.0 (45.25)	53.53 (46.93)	
$CD_{0.05}$		Dose: (2.35); Time: (1.30); Dose x Time: (4.70)				

Figures in parentheses are arc sin transformed values

Table 2: Dose response mortality of *Odontotermis obesus*

	48 h exposure		72 h exposure		96 h exposure	
	Steinernema sp.	*H. bacteriophora*	*Steinernema* sp.	*H. bacteriophora*	*Steinernema* sp.	*H. bacteriophora*
LD_{50}	100.66	87.71	81.47	72.17	74.79	61.34
(95%FL)	(79.94-126)	(68.94-111.58)	(61.38-108.14)	(52.91-98.44)	(54.86-101.98)	(43.29-86.93)
Slope	1.55	1.51	1.30	1.23	1.22	1.16
χ^2	3.69	2.58	2.78	2.68	3.54	2.81
Regression equation (Y)	1.522x + 1.943	1.481x + 2.115	1.481x + 2.115	1.261x + 2.631	1.243x + 2.648	1.241x + 2.738
R^2	0.950	0.957	0.957	0.938	0.925	0.932

Fujii (1975) reported the infectivity of *S. carpocapsae* against *Coptotermes formosanus* at 3069 IJs termite^{-1}. Similarly, Danthanarayanan and Vitarana (1987) reported that the infectivity of *Heterorhabditis* sp. against *Glyptotermes dilatatus* at 3670 IJs termite^{-1}. Epsky and Capinera (1988) found that *H. indica* was more effective than *Steinernema* sp. against *Reticulitermes tibialis* where LD_{50} value of *H. indica* was 1.5 x 10^4 termite^{-1}. Wang *et al*. (2002) evaluated four entomo-pathogenic nematodes, *viz*., *H. indica, H. bacteriophora, S. carpocapsae* and *S. riobrave* against *R. flavipes* and *C. formosanus*. The virulence of *H. indica* and *H. bacteriophora* in terms of LD_{50} value against *R. flavipes* were reported to be 296 (95% FL 231-353) and 494 (95% FL 357-625), respectively, termite^{-1}. Rathour *et al*. (2014) observed 100% mortality of *Odontotermes obesus* within 24-48 h by *S. thermophilum* and *S. glaseri* at10 IJs termite^{-1} soldier in laboratory bioassay.

In current study, it was observed that infective juveniles were emerged through the cuticle of dead termite at 5-6 days after inoculation with *Steinernema* sp. whereas at 6-7 days with *H. bacteriophora*. There were no significant differences in progeny production between the inoculum level of 1000 and 1500 in 250 g of mounds at 10th and 15th days of observation (Table 3). At the same level of inoculum (1000 IJs 250 g^{-1}), the average number of IJs of *Steinernema* sp. and *H. bacteriophora* produced from *O. obesus* were 20520 and 22560, respectively, on 10th day and 25000 and 30000, respectively, on 15th day. The propagation potential of nematode inside termite revealed that number of infective juveniles collected termite^{-1} were maximum in *H. bacteriophora*. However, further incubation beyond 15 days, the IJs production were reduced and deteriorated. Wang *et al*. (2002) observed that average numbers of IJs produced from *R. flavipes* and *C. formosanus* (based on 11 and 8 workers) were 289±50 and 642±93 worker^{-1}. Lenz (2005) reported the potentiality of EPNs for the management of subterranean termites because of their ability to actively search, infect and kill the host insect. However, consistency in *in vivo* productivity of EPN is influenced by host insect larval biomass, fecundity, optimized host life cycles, rearing conditions (temperature, RH, nutrition, etc.) and diet composition.

Table 3: Infective juvenile production of *Steinernema* sp. and *Heterorhabditis bacteriophora* in *Odontotermes obesus* at different nematode inoculation rate and time (mean of 4 replicates)

Nematode	Rate of inoculation (IJs 250 g^{-1})	Number of IJs 250 g^{-1} termite colony		
		10th day	15th day	20th day
*Steinernema*sp.	500	14350 (4.15)^{c}	19855 (4.29)^{c}	5125 (3.70)^{c}
	1000	20520 (4.31)^{b}	25000 (4.39)^{b}	6400 (3.80)^{b}
	1500	21845 (4.34)^{b}	25970 (4.41)^{b}	6937 (3.84)^{b}
H.bacteriophora	500	19275 (4.29)^{b}	24030 (4.38)^{b}	5862 (3.77)^{bc}
	1000	22560 (4.35)^{ab}	30000 (4.47)^{a}	6887 (3.83)^{b}
	1500	24939 (4.40)^{a}	30922 (4.49)^{a}	8562 (3.93)^{a}
Control	0	0 (0.00)^{d}	0 (0.00)^{d}	0 (0.00)^{d}
SEm±		(0.034)	(0.017)	(0.038)
$CD_{0.05}$		(0.07)	(0.04)	(0.09)

The values in parentheses are log transformed values; The values denoted by same alphabet are not significantly different ($P_{0.05}$) from each other.

From present study it appears that *H. bacteriophora* and *Steinernema* sp. have the potential to continue their infestation and progeny production in termite (*Odontotermes obesus*). Under laboratory conditions entomopathogenic nematodes can be reared and mass produced for use in management practices against termite. It may be concluded that EPN can be successfully cultured on large scale and used under field condition to provide protection to the crops from insects.

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REFERENCES

Cobb, N.A.1918. *Estimating the Nema Population of the Soil.* Agric. Tech. Circ. Bur. Pl. Ind. U.S. Dep. Agric., No.1. 48 pp.

Danthanarayana, W. and Vitarana, S. 1987. Control of the live-wood tea termite *Glyptotermes dilatatus* using *Heterorhabditis* sp. (Nemat.). *Agricultural Ecosystem and Environment*, **19**: 333-342.

David, H. and Kurup, N.K. 1988. Techniques for mass production of *Sturmiopsis inferens* Tns. pp. 87-92. **In**: *Biocontrol Technology for Sugarcane Pest Management.* Sugarcane Breeding Institute, Coimbatore, India.

David, I., Shapiro, I., Richou, H. and Claudia, D. 2012. Entomopathogenic nematode production and application technology. *Journal of Nematology*, **44**: 206-217.

Devi, G., Mishra, H., Bhattacharyya, B. and Nath, D. 2016. Occurrence of entomopathogenic nematode (Rhabditida: Heterorhabditidae: Steinernematidae) in white grub infested areas of Majuli, Assam, India. *Journal of Biopesticides*, **9**: 148-156.

Dolinski, C., Del Valle, E.E., Burla, R.S. and Machado, I.R. 2007. Biological traits of two native Brazilian entomopathogenic nematodes (Heterorhabditidae: Rhabditida). *Nematologia Brasileira*, **31**: 180-185.

Epsky, N.D. and Capinera, J.L. 1988. Efficacy of the entomogenous nematode *Steinernema feltiae* against a subterranean termite, *Reticulitermes tibialis* (Isoptera: Rhinotermitidae). *Journal of Economic Entomology*, **81**: 1313-1317.

Fujii, J.K. 1975. *Effects of an Entomogenous Nematode Neoaplectana carpocapsae Weiser, on the Formosan Subterranean Termite, Coptotermes formosanus,* Ph.D. Dissertation, University of Hawaii, Honolulu, USA.

Hewlett, P.S. and Plackett, R.L. 1979. *An Introduction to the Interpretation of Quantal Responses in Biology.* University Park Press, New York, USA.

Rathour, K.S., Ganguly, S., Anupama, Das, T.K., Singh, P., Kumar, A. and Somavanshi, V.S. 2014. Biological management of subterranean termites (*Odontotermes obesus*) infesting wheat and pearl millet crops by entomopathogenic nematodes. *Indian Journal of Nematology*, **44**: 47-100.

Shapiro-Ilan, D.I., Gaugler, R., Tedders, W.L., Brown, I. and Lewis, E.E. 2002. Optimization of inoculation for *in vivo* production of entomopathogenic nematodes. *Journal of Nematology*, **34**: 343-350.

Wang, C., Powell, J.E. and Nguyen, K. 2002. Laboratory evaluation of four entomopathogenic nematodes for control of subterranean termites (Isoptera: Rhinotermitidae). *Environmental Entomology*, **31**: 381-387.

Woodring, J.L. and Kaya, H.K. 1988. *Steinernema and Heterorhabditis Nematodes: A Handbook of Biology and Techniques.* Southern Cooperative Series Bulletin 331, Arkansas Agricultural Experiment Station, Fayetteville, Arkansas, USA.