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Field and Multi-locational Study on Efficacy of Native Entomopathogenic Nematodes (*Heterorhabditis bacteriophora* Poinar) against Termite (*Odontotermes obesus* Rambur) in Assam, India

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Authors' contributions

This work was carried out in collaboration among all authors. Author GD designed the study, performed the experiment and the analyses, and wrote the first draft of the manuscript. Authors BB, Sudhansu Bhagaw, Snigdha Bhattacharjee, MB, SP managed the experiment and analyses the study and finalized the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Entomopathogenic nematodes (EPNs) are one of the important biocontrol agents against various insect pests. The effects of different doses and time of application of Entomopathogenic nematode (*Heterorhabditis bacteriophora* Poinar) were evaluated under field condition against termite (*Odontotermes obesus* Rambur). At 5.0×10^9 IJs/mound, three-time application at one month interval as soil drenching method were required to cause 60.6 % mortality of *O. obesus*. Results showed that sequential application of EPN is effective than one time application. The effective dose was further evaluated in three multi-locational trials under different agro-climatic regions of Assam. Results showed that 61.6%, 61.3% and 60.6% mortality of *O.obesus* was due to the three times application of *H.bacteriophora* at the dose of 5.0×10^9 IJs/mound in AAU-HRS Kahikuchi, AAU-SMAPRS Buralikson and AAU-ZRS Diphu, respectively. Both workers and soldiers of *O. obesus* cadavers had higher progeny production of *H.bacteriophora* with high persistence in mound soil.

Keywords: Biocontrol agent; Entomopathogenic Nematodes (EPNs); efficacy; mound; Odontotermes obesus; soil drenching method; termite.

1. INTRODUCTION

"Termites (Order Isoptera) are cellulophagous and eusocial insects found throughout the world and are a perpetual economic problem" (Eggleton, 2000)." Termite colonies contain three principal castes: workers (pseudergates), soldiers, and reproductives (king, queen, alates or swarmers). A termite mound is the most familiar form of termite nest. Termites cause huge financial losses every year in agriculture, forestry, and urban situations" (Krishna & Grimaldi, 2003). "Besides infesting wild plant species, termites cause major damage to various crops such as wheat, maize, sugarcane, paddy, cotton, groundnut, soybean and tea" (Rajagopal, 2002; Roy et al.,2020). "Economic losses due to termite in India have been estimated around 35.12 million US$" (Joshi et al., 2005). "In India 300 species of termite have been recorded and pestilence (in ecosystems) is caused by 35 species" (Verma et al., 2009). Among all the species, *Odontotermes obesus* Rambur (Blattodea: Termitidae) is commonly found species which build both subterranean and epigeal nests (Mahapatro & Chatterjee, 2018) and inflicts damage on ground timber and standing trees (Sunitha & Miranda, 2011; Rasib et al., 2014). "Most termite management practices are focused on total elimination of termite population rather than sustaining their population. Common methods for controlling these termites are the application of termiticides" (Woodrow et al., 2006; Quarcoo et al.,2010). Queen removal, breaking up termite galleries, crop rotation, and application of wood ash or burning with straw, application of plant insecticides are some of the management practices. "Among the diverse potential alternatives available for termite management, the use of microbes is gaining prominence" (Grace, 2003; Grace et al.,2009). Biocontrol agents like predators, parasitoids and pathogens have been tested to suppress termite populations (Sindhu et al., 2011). However, reproductive and nymphs of subterranean termites are present in nests near or below ground level, out of reach of some of the bio-control agents (use of predators, parasitoids and pathogens). Entomopathogenic nematodes (EPNs), *Steinernema* spp. and *Heterorhabditis* spp. can be applied as a bio-control agent against termite colonies. These EPNs have been recognized as potential bio-control agent against most of the soil dwelling pests (Gaugler & Kaya, 1990; Burnell & Stock, 2000). "Apart from being environmentally safe, the use of EPN in pest control in general, and in termite control in particular, is rapid, sustainable, cost effective, and easy to apply" (Koppenhöfer et al., 2020). Moreover, IJs can find host actively or passively and are compatible with many pesticides (Smart, 1995). "Termites live and forage in habitats that are moist, cool, and without direct sunlight. These environmental conditions are ideal for the survival and movement of entomopathogenic nematodes, and, therefore, provide the basis for the interest in their role in control of termites. The infective

juveniles (IJs) of EPNs are soil dwelling and obligate parasites of insects" (Kaya & Gaugler, 1993). "Once IJs locate a possible host in the soil environment, they penetrate the host hemolymph through natural openings such as mouth, anus, and spiracle or directly through the integument" (Lewis et al., 2006). "Having penetrated the host, the nematodes release the bacteria (*Xenorhabdus* spp. in *Steinernema* spp. and *Photorhabdus* spp. in *Heterorhabditis* spp.) into the host hemolymph that cause septicemia and death of the insect. The nematode feeds on the proliferating bacteria and two or three cycles of reproduction occur in the host prior to emergence of infective stages" (Adams et al., 2006). "Certain species of nematodes although effective in laboratory control is often quite variable under field conditions" (Wang et al., 2002; Gutema et al.,2025). This is because soil moisture and soil type appear to limit the nematode's ability to move in the soil and locate termites (Poinar & Georgis, 1989). Reports of the use of entomopathogenic nematodes to control under field condition are very few or have been limited to laboratory conditions (Devi et al., 2018; Bhairavi et al., 2021). Therefore, field studies were conducted for two consecutive years to check the efficacy of a native isolate of EPNs, *Heterorhabditis bacteriophora* Poinar and the effective dose was evaluated in three multi-locational trials.

2. MATERIALS AND METHODS

2.1 Source of Entomopathogenic Nematodes

EPN, *H. bacteriophora* Poinar previously recovered from the District Majuli were used in this study (Devi et al.,2016). "The EPNs were reared *in vivo* on the last instar larvae of *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) under laboratory conditions. The larvae of *G.mellonella* were obtained from the Department of Entomology, AAU, Jorhat. The last larval stage of this insect was used to maintain and propagate the nematodes throughout the entire period of time in this study. *G.mellonella* were multiplied in glass jars at 28± 2°C, 60% RH, with an artificial diet" described by Metwally et al., (2012). The harvested IJs were kept at 10-12°C for experiments for less than a week before they were applied in the experiment. Before use, they were allowed to warm up to room temperature (25 ±1°C) for 2 h. Also, their viability for motion was confirmed using dissecting microscope.

2.2 Field Trial

Field trials were conducted to evaluate the efficacy of *H.bacteriophora* against *O.obesus* during 2021-2022 and 2022-2023 in various agro-ecosystems located in the district Jorhat. The multi-locational trials were conducted with the best treatment with *H.bacteriophora* along with control in three districts of Assam having different agro-climatic condition. These are AAU-HRS Kahikuchi (district Kamrup), AAU-ZRS Diphu (district Karbi Anglong) and AAU-SMAPRS Buralikson (district Golaghat) during 2023-2024.

The agricultural fields were naturally infected with the termite pest (*Odontotermis obesus* Rambur). No natural colonization of the termite nests by EPNs was detected after baiting (*G. mellonella*) soil samples from the nests. Experiments were performed on *O.obesus* with mounds or a central nest structure. In preliminary assays, it was observed that *O.obesus* colonies were able to reconstruct an aboveground nest within one month after its demolition. So, the aboveground nests were first pulled down before the application of treatment regardless of the nest size. *H. bacteriophora* were used in the field trials carried out in the rainy season. Treatment (EPN) was applied over the demolished surface. Six treatments were compared:T_1:2.5×10^9IJs/mound (One time application), T_2:2.5×10^9 IJs/mound (Two times , one month interval) ,T_3:2.5×10^9 IJs/mound (Three times, one month interval) ,T_4:5.0×10^9 IJs/mound (One time application) ,T_5:5.0×10^9 IJs/mound (Two times ,one month interval) ,T_6:5.0×10^9IJs/mound (Three times, one month interval) and (T_7) untreated control. The nematodes were applied with different doses using a manual sprayer as a soil drench. Sterilized water alone was added to the untreated control. The trial was conducted in a randomized complete block design with three replications. Soil drenching was done with the required dose of EPNs.

Three parameters *viz*. Mortality of different life stages of termites, Progeny production of nematode inside their host, nematode persistence in the nests were recorded.

2.3 Mortality of Different Life Stages of Termites

Seven days after application of last treatment, the top nests made by *O.obesus* were broken to collect the dead insects. Samples of 250 g

each were collected from four corners as well as from the middle of the nest. Samples were collected in plastic containers, mixed properly, and transferred to the laboratory. From the mixer, 250g of samples were taken for observation. Dead individuals of worker and soldiers were separated and counted. Dead insects were dissected for presence of nematodes.

2.4 Progeny Production of Nematode Inside their Host

To assess progeny production, the dead insects were rinsed and transferred to White traps in 2.2-cm diam. plates lined with a filter paper, individually and incubated at room temperature (25±1°C) for 10 days. The total number of emerging IJs from each insect was determined.

2.5 Nematode Persistence

Nematode persistence in the nest area was assessed by randomly taking soil samples composed of 3 cores (0-15 cm depth) from each treated nest 30 days after last treatment (application). The three soil core samples were individually baited with 10 last instar *G. mellonella* larvae and kept at room temperature (25±1°C) for one week. Then, dead larvae (%) were recorded daily from the fifth day to the seventh. Cadavers were dissected to confirm EPN infection.

2.6 Statistical Analysis

Prior to analysis, all data were corrected for the mortality rate of the control group using Abbott's formula (Abbott, 1925; Fleming & Retnakam, 1985). To stabilize the variance of means, mortality percentages were arcsine transformed and subjected to one way ANOVA (OPSTAT) to test for significant differences among treatment means (Sheoran et al., 1998). The 5% level of probability was used in all statistical tests. Combined ANOVA for all the observations was obtained by pooling individual ANOVA in two years field data after significant difference was observed.

3. RESULTS AND DISCUSSION

Pool analysis of data of two years of field experiments, it was observed that *H.bacteriophora* caused a significant mortality of *O.obesus* as compared to control (Table 1). Insect mortality was found to be increased with increasing doses and frequency of application. There was a significant difference with other treatments in mean mortality (60.6 %) of *O.obesus* when application was done at the dose of 5.0×10^9 IJs/mound three times at one month interval. High virulence and ability to search and locate host in cryptic habitats of *H.bacteriophora* is one important characteristic required for the successful biological control of a pest.

Amarasinghe and Hominick (1993) reported "complete control within 60-95 days when *Heterorhabditis* sp. was applied at high concentrations (8000 IJs/ml) directly into the galleries of *Glyptotermes dilatatus* Bugnion & Popoff in tea plantations". "Similarly, *G.dilatatus* was successfully controlled within 2-3 months in tea plantations on Sri Lanka with *Heterorhabditis* sp. with a dose of 4000 and 8000 ml nematode suspension in doses of 40 and 30 ml per tea bush, respectively" (Danthanarayana & Vitarana,1987). "A field study testing the efficacy of *Steinernema carpocapsae* (Weiser) at $1x10^7$ per m^2 in pasture land against foraging workers of *Reticulitermes tibialis*documented that the entire colony of termites should be treated rather than feeding sites" (Epsky & Capinera,1988). "*Coptotermes formosanus* Shiraki (97.9% mortality) with *S. feltiae* (Filipjev) Wouts, Mráček, Gerdin & Bedding at the concentration of 1,500,000 IJs per nest demonstrated the effectiveness in field colonies" (Wu et al.,1991). Wilson-Rich et al., (2007) showed that "*S.carpocapsae* cause dose dependent mortality of the dampwood termite (*Zootermopsis angusticollis* Hagen) where both nymphs and soldiers significantly alter the frequency and duration of several behavioral acts during and after exposure to *S. carpocapsae.* In our experiments it was observed that there was a significant difference in the treatments for the mortality of workers (10.6-31.9 %) as well as soldiers (8.6-28.0%) of *O.obesus*." "Similar laboratory results showed that after 4 days of exposure of infectives juveniles of *H.indica* Poinar, Karunakar & David to *Coptotermes vastator* worker and soldiers, worker mortality was more than soldiers" (Mankowski et al., 2005). Wagutu et al., (2017) also demonstrated that *C.formosanus* workers were more susceptible than soldiers to *S.karii* Waturu, Hunt & Reid.

Table.1 Mortality of *Odontotermes obesus* by *Heterorhabditis bacteriophora* (Pooled, 2021-2022 & 2022-2023)

Treatments	Observations (Av. of 3 replications)		
	Mortality (%) of caste of termites		
	Worker	Soldier	Total
T1:2.5×10^9 IJs/mound (One time application)	10.65^e	8.65^d	19.75^e
T2:2.5×10^9 IJs/mound (Two times, one month interval)	12.25^{de}	10.15^{cd}	22.90^e
T3:2.5×10^9 IJs/mound (Three times, one month interval)	14.95^{cd}	11.85^c	27.35^d
T4:5.0×10^9 IJs/mound (One time application)	18.35^c	15.05^b	34.10^c
T5:5.0×10^9 IJs/mound (Two times, one month interval)	23.55^b	18.85^b	43.35^b
T6:5.0×10^9 IJs/mound (Three times, one month interval)	31.90^a	28.00^a	60.60^a
T7: Control	2.00^f	2.00^f	4.00^f
CD (0.05)	3.52	2.96	4.30

Table 2. Progeny production and persistence of *Heterorhabditis bacteriophora* against *Odontotermes obesus* ((Pooled, 2021-2022 & 2022-2023)

Treatments	Observations (Av. of 3 replications)		
	Progeny production of nematode inside their host (mean± SE)		Persistence of the nematodes in the nests Insect (*Galleria mellonella*) mortality (%)
	Worker	Soldier	
T1:2.5×10^9 IJs/mound (One time application)	1260-1500	1240-1500	6.20^e
T2:2.5×10^9 IJs/mound (Two times, one month interval)	1230-1530	1250-1530	11.50^{de}
T3:2.5×10^9 IJs/mound (Three times, one month interval)	1240-1580	1280-1580	20.30^{cd}
T4:5.0×10^9 IJs/mound (One time application)	1242-1560	1260-1580	22.92^b
T5:5.0×10^9 IJs/mound (Two times, one month interval)	1240-1690	1280-1720	31.56^{ab}
T6:5.0×10^9 IJs/mound (Three times, one month interval)	1282-1850	1280-1860	39.80^a
T7: Control			4.3^e
CD (0.05)			8.20

Perusal of data from the Table 2, it was observed that, *H.bacteriophora* reproduced well in workers and soldiers of *O.obesus.* The level of progeny production did not varied significantly between doses. However, it was found to be higher progeny production of *H.bacteriophora* (1282-1850 and 1280-1860) at higher dose (5.0×10^9 IJs/mound) with three time application. The larger size of either worker or soldiers of *O.obesus* enabled the higher number of IJ /insect whereas the small soldiers and workers of *O.obesus* produced the fewest number of IJ /insect. This is in agreement with findings of Blinova & Ivanova (1990) and Flanders et al*.* (1996), who demonstrated that IJ yield is proportional to host size. "*H.bacteriophora* penetrated and successfully established in soldiers as well as workers. Progeny production or multiplication is an essential character for EPN populations to increase their chance for getting established in the insect environment" (Phan et al*.*, 2005; Griffin, 2012). In samples collected at 30 days after last application of EPNs, mortality of *G.mellonella* larvae (6.2-39.8%) was observed due to the presence of *H.bacteriophora* in soil samples (Table 2). Koppenhofer et al., (1997) and Susurluk and Ehlers (2008) stated that the number of infected larvae found by sampling was related to the number of nematodes that were present in the soil after application of nematodes.

On farm multi-locational trials constitute technology verification experiments wherein bioefficacy of native EPN species, *H.bacteriophora* were tested in different agro-ecological conditions. Results showed that 61.6% mortality of *O.obesus* was due to the three times soil drenching of *H.bacteriophora* at the dose of 5.0×10^9 IJs/mound in AAU-HRS Kahikuchi (Table 3; Fig. 1). Mauldin and Beal (1989), and Georgis et al., (2006) also insisted on sequential application instead of simultaneous application of EPN for better efficacy of EPNs. Progeny production was as high as 1478±4.70 with higher field persistence (Table 3). Al-Zaidawiet al. (2020) demonstrated 43.6 % mortality of subterranean termite, *Microcerotermes diversus* Silvestri by application of native strain of *H. bacteriophora* under field condition. In AAU-ZRS Diphu, *O.obesus* mortality was 60.6% with 1478±3.65 progeny production and high persistence (Table 4; Fig. 2). Similar results obtained from AAU-SMAPRS Buralikson where *O.obesus* mortality was 61.3% with 1478±3.65 progeny production and high persistence (Table 5; Fig. 3). Following a bait station with naturally infested *D. sissoo* plants,

Fig. 1. Termite mound after application of *H.bacteriophora in* AAU-HRS Kahikuchi

Table 3. Progeny production and persistence of *Heterorhabditis bacteriophora* and mortality per cent of *Odontotermes obesus* at AAU-HRS Kahikuchi

Treatments	Observations (Av. of 3 replications)					
	Mortality (%) of caste of termites			Progeny production of nematode inside their host (mean± SE)		Persistence of the nematodes in the nests Insect mortality (%) (*Galleria mellonella*)
	Worker	Soldier	Total	Worker	Soldier	
T1:5.0×10^9 IJs/mound (Three times, one month interval)	33.0	28.6	61.6	1272-1580 (1460±3.60)	1290-1592 (1478±4.70)	43.8
T0: Control	2.0	2.0	4.0			

Table 4. Progeny production and persistence of *Heterorhabditis bacteriophora* and mortality per cent of *Odontotermes obesus* at AAU-ZRS Diphu

Treatments	Observations (Av. of 3 replications)					
	Mortality (%) of caste of termites			Progeny production of nematode inside their host (mean± SE)		Persistence of the nematodes in the nests Insect mortality (%) (*Galleria mellonella*)
	Worker	Soldier	Total	Worker	Soldier	
T1:5.0×10^9 IJs/mound (Three times, one month interval)	33.3	27.3	60.6	1282-1590 (1478±3.65)	1280-1580 (1460±4.72)	45.0
T0: Control	2.0	1.0	3.0			

Table 5. Progeny production and persistence of *Heterorhabditis bacteriophora* and mortality per cent of *Odontotermes obesus* at AAU-SMAPRS Buralikson

Treatments	Observations (Av. of 3 replications)					
	Mortality (%) of caste of termites			Progeny production of nematode inside their host (mean± SE)		Persistence of the nematodes in the nests Insect mortality (%) (*Galleria mellonella*)
	Worker	Soldier	Total	Worker	Soldier	
T1:5.0×10^9 IJs/mound (Three times, one month interval)	35.3	26.0	61.3	1272-1594 (1478±3.65)	1290-1586 (1470±4.75)	48.0
T0: Control	2.0	2.0	4.0			

Fig. 2. Termite mound after application of *H.bacteriophora in* Diphu

Fig. 3. Termite mound after application of *H.bacteriophora in* AAU-SMAPRS Buralikson

O.obesus mortality caused by *S. Carpocapsae* (58.46%), *H. bacteriophora* (45.45%) and *H. indica* (32.39%) at a concentration of1000 IJs/cm^2 (Aslam et al., 2023). "Successful nematode establishment in the larvae implies a potential for recycling of EPNs in the host environment, thereby increasing the control potential" (Zadji et al.,2014). Baimey etal., (2015) applied *H. sonorensis* (Azohoue2) infected *G. mellonella* larvae to the underground populations of *Macrotermes bellicosus* with 63.2% mortality where soil temperature was as high as 35°C. "Termite workers and soldiers might come across EPNs in soil when foraging, or when de-winged reproductive burrow into soil to establish initial colonies" (Baïmey et al., 2017; Labaude & Griffin,2018).

4. CONCLUSION

Based on the field results as well as on farm multi-locational trial it is confirmed that the native isolate of EPNs, *H. bacteriophora* is virulent to *O.obesus* and possess the characteristics needed to create an epizootic within the mound i.e., to self-replicate, disperse and reach secondary cycling within the termites. Three time application of *H. bacteriophora* at one month

interval could able to cause mortality as high as 61.6% with successful persistence may support the hypothesis that biological control for termites only works with inundative methods where most of the nest is accessible for treatments and sequential instead of simultaneous application of EPN is effective.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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