

Efficacy of native entomopathogenic nematode *Heterorhabditis bacteriophora* for ecofriendly management banana leaf and fruit scarring beetle *Nodostoma subcostatum* (Coleoptera: Chrysomelidae)

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Research Article

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Abstract

Banana leaf and fruit scarring beetle *Nodostoma subcostatum* (Coleoptera: Chrysomelidae) is one of the major economic constrain for banana growers in Assam as this pest reduces the quality and market price of banana by producing scars on the peel of the banana. The application of chemical pesticides to banana fruit is not recommended due to their residual toxicity resulting in growing interest in biological control strategy for control of the pest. In, present study an effort was made to evaluate the efficacy of four different concentrations of native isolate of *Heterorhabditis bacteriophora* against banana leaf and fruit scarring beetle under laboratory condition in Assam. Each concentration was replicated ten times with five insect per replicate. For each concentration 3rd stage infective juveniles of *H. bacteriophora* were sprayed on insect pest for 20s at 1 atm pressure and pure water used as control. The observations for mortality of the insects were recorded at 24h, 48h and 72h of inoculation and reisolation of nematodes from the dead insects were done by using White trap method till 15th days. The highest mortality rate of banana leaf and fruit scarring beetle was recorded as 100% followed by 86% and 74% after 72 h of exposure. LD₅₀ values for *H. bacteriophora*, was found as follow 296.7 IJs/ml (95% CL 174.0-506.0), 126.5 IJs/ml (95% CL 94.8-168.9) and 55.6 IJs/ml (95% CL 36.0-86.0) at 24, 48 and 72 h of exposure, respectively. Present finding determined the promising effect of native *H. bacteriophora* against the banana leaf and fruit scarring beetle and also providing an opportunity to incorporate them in Integrated Pest Management System (IPM) and thereby reducing the risk of pesticide residue on banana fruits.

Introduction

Banana leaf & fruit scarring beetle (*Nodostoma subcostatum*) (Coleoptera: Chrysomelidae) is a serious economically important insect pest of banana in the North-Eastern parts of India (Prathapan et al. 2019) which remain active during summer and *kharif* season by attacking the banana plant at young unfurl leaf stages and fruiting stages. (Sah et al. 2018). Due to the infestation of insect, scars are appeared on banana peel. The physical appearance of the fruit is one of the important criteria in highly competitive export market. External appearance of fruit and market quality are extremely influenced by the improper pre and post-harvest practices. In conventional farming system, farmer's are applying chemical pesticides such as carbaryl (0.3%), acephate (0.11%) and followed by bunch covering with polypropylene bag(Das et.al,2018;Mishra et.al,2015). This management practices has pose a serious threat to human health due to their residual effects on banana fruits. Moreover, application of insecticide not only leads to disruption of ecological balance but also do environmental hazards. Therefore, the use of bio-control agent as an alternative to chemical control measures are gaining popularity nowadays due to their compatible nature with environment and other ecological flora and fauna. In that point, EPNs are fit as an alternative to chemical control measures due to their perpetuating nature in environment and in present days their uses are gaining popularity. They entered inside the insect host through the natural opening and release the bacteria associated with their guts and kill the insect within 24-48h through septicaemia. Among them, Heterorhabditis and Steinernema are two most important groups of EPNs which are gaining popularity in managing the insect pest. They have been successfully used against banana pseudostem borer

throughout the world both in field and laboratory condition (Grewal et al., 2001; Sepúlveda-Cano et al., 2008; Treverrow and Bedding, 1993).

Therefore, present study was undertaken to evaluate the efficacy of native isolate of *Heterorhabditis bacteriophora* (Poinar, 1976) against adult beetle of *Nodostoma subcostatum* under laboratory conditions.

Methods

The isolate *Heterorhabditis bacteriophora* (Poinar, 1976) which was already isolated from Assam and was maintained by *in-vivo* culture by using greater wax moth, *Galleria mellonella* in the laboratory of Nematology, Assam Agricultural University, Jorhat, was used for the present study. The efficacies of four different concentration of *H. bacteriophora* (50,100,150 and 200 IJs/ml) were evaluated against the banana leaf and fruit scarring beetle, *Nodostoma subcostatum*. The adult beetles of banana leaf and fruit scarring beetles were handpicked directly from young unfurl leaves of banana plant from unsprayed plot of banana maintained in the orchard, AAU and collected beetles were placed in a plastic container provided with well ventilated holes and fresh young leaves of banana as a source of food.

The experiment was set up with ten replications per concentration (5 adults per replicate). The entomopathogenic nematode (EPNs) suspension were prepared from 3rd stage juveniles and were sprayed at 1atm pressure for 20 sec (0.5ml suspension for each cavity block including 5 insects). The glass lids of cavity were kept slightly open for facilitating ventilation. After inoculation, cavity blocks were incubated at $25 \pm 2^\circ\text{C}$. Mortality of the test insect was recorded after 24h, 48h, and 72h of initial inoculation. Re-isolation of EPNs was done by using White trap method (Kaya and Stock, 1997) (Kaya and Stock, 1997) till 15 days from the day of observation started. In each observation days, dead insects were transferred to white trap and incubated at $25 \pm 2^\circ\text{C}$ in distilled water for 15 days. After 15 days the numbers of 3rd stage juveniles of *H.bacteriophora* that emerge from the treated host were counted under light microscope.

The Mortality data were corrected using Abott's formula (Abbot, 1925).

$$\text{Corrected per cent mortality} = \frac{\text{Per cent mortality in treatment} - \text{Per cent mortality in control}}{100 - \text{Per cent mortality in control}} \times 100$$

Statistical analysis

In present investigation, the mortality of insects for control treatment were corrected using Abott's formula (Abbott, 1925). The probit analysis (Finney 1964) were done for the experimental values by using IBM SPSS statistics 21 software.

Results

The result from the experiment revealed that adult stage of beetle was susceptible to the *H. bacteriophora*. The mortality of leaf and fruit scarring beetle increased with increase in nematode concentration and also with exposure period. After 24 h of exposure, the highest per cent mortality of leaf and fruit scarring beetle were recorded as 40% followed by 26% and 18% at dose 200, 150, and 100 IJs/ml respectively. While the lowest mortality percentage obtained was 8% at concentration 50 IJs/ml. After 48h of exposure, the highest per cent mortality of leaf and fruit scarring beetle were recorded as 79% followed by 58%, 30% at a concentration 200, 150, 100 IJs/ml respectively. While the lowest mortality percentage obtained was 12% at concentration 50 IJs/ml. Similarly, after 72 h of exposure, the highest per cent mortality of leaf and fruit scarring beetle was recorded as 100% followed by 86% and 74% at a concentration 200, 150 and 100 IJs/ml. While the lowest percentage of mortality obtained was 66% at 50 IJs/ml. Data presented in the (Table 1) revealed that at 50 IJs/ml, the highest mortality was recorded during 72h (66%) followed by 48h (12%) and the lowest mortality noted in 24h (8%). Similarly, at 100 IJs/ml, the highest mortality was recorded during 72h (74%) followed by 48h (30%) and the lowest mortality were recorded in 24h (18%). At 150 IJs/ml, the highest mortality was recorded during 72h (86%) followed by 48h (58%) and the lowest mortality were recorded in 24h (26%). At higher dose of 200 IJs/ml, the mortality percentage after 72h recorded as 100% followed by 48h (79%) and the lowest mortality during 24h were recorded as 40% (Fig. 1)

Table 1
Laboratory evaluation of *Heterorhabditis bacteriophora* against banana leaf and fruit scarring beetle at five different concentrations

Dosage of <i>H.bacteriophora</i> (IJs/ml)	Per cent mortality over control		
	Post treatment (Hours after exposure)		
	24	48	72
	% mortality	% mortality	% mortality
0	0 (0.00)	0 (0.00)	0 (0.00)
50	8 (16.43)	12 (19.94)	66 (45.82)
100	18 (25.10)	30 (33.36)	74 (52.45)
150	26 (30.66)	58 (49.68)	86 (63.44)
200	40 (39.23)	79 (62.77)	100 (90.00)
C.D.(P=0.05)	0.651 (4.63)	0.840 (5.26)	1.029 (5.82)

The data showed in (Table2) revealed that the LD₅₀ values for *H. bacteriophora* against leaf and fruit scarring beetle were 296.7 IJs/ml (95% CL 174.0-506.0), 126.5 IJs/ml (95% CL 94.8-168.9) and 55.6 IJs/ml (95% CL 36.0-86.0) at 24, 48 and 72 h of exposure, respectively.

Table 2
The calculated LC₅₀ value (IJs/ml) of *H. bacteriophora* against adult banana leaf and fruit scarring beetle after 24, 48 and 72h of exposure period

Exposure time	Slope	LD ₅₀ (IJs/ml) 95% CL	χ ²
24h	(-4.66) + 1.89x	296.7 (174.0-506.0)	0.82
48h	(-7.06) + 3.36x	126.5 (94.8-168.9)	0.38
72h	(-3.98) + 2.28x	55.6 (36.0-86.0)	0.06

The mean number of re-isolated 3rd stage juveniles of *H.bacteriophora* per individual from adult beetle of banana leaf and fruit scarring beetle which was treated with four different concentration of *H.bacteriophora* is given in Fig. 2. The highest mean numbers of 3rd stage juveniles were isolated from 200 IJs/ml water concentration of *H.bacteriophora* and lowest mean number was isolated from 50 IJs/ml water concentrations respectively. There was no difference among the other concentration for mean number of reisolated 3rd stage juveniles from adult beetle of banana leaf and fruit scarring beetle. The re-isolation of 3rd stage juveniles of entomopathogenic nematodes from the host after reproducing inside the host is very important in terms of maintaining the EPNs population in soil and thus attaining the sustainable pest management practice.

Discussion

In this study, it was evident that tested native *H. bacteriophora* were pathogenic to adult banana leaf & fruit scarring beetle which is a serious pest of banana in the North-eastern parts of India (Prathapan et al., 2019) and they caused different mortality rates under laboratory conditions. From the study it was observed that infectivity of adult beetle increased with increase in nematode concentration and also with exposure period. Numerous studies have been conducted in different parts of the world to use EPNs against number of lepidopteron and coleopteran insects under laboratory and field condition. Sharifi et al. (2014) evaluated *H. bacteriophora* for their potential efficacy against the larvae of rosaceae longhorned beetle, *Osphranteria coerulescens* and the highest mortality of rosaceae longhorned beetle (42.5-87.8%) was observed after 72h of exposure at dose of 5 IJs/larva. The LC₅₀ value obtained for *H. bacteriophora* after 24h, 48h and 72h as 43.1, 15.7, 9.0 IJs/larva. The efficacy of three native Turkish strains of entomopathogenic nematodes, *Heterorhabditis bacteriophora* (ZET35), *Steinernema feltiae* (ZET31), and *Steinernema websteri* (AS-1) were evaluated against pre-pupae and adults of alder leaf beetle (*Agelastica alni*) which is another member of Chrysomalidae family. Out of three strains, *H. bacteriophora* was found to cause highest mortality to pre-pupae and adults of *A. alni* at 1000 IJs/ ml at 25°C and the LC₅₀ value was obtained as 6220.4 IJs/ml for adult beetle Bayramoglu et al. (2015). Similarly, the infectivity and reproductive capacity of native *H. bacteriophora* (Strain Veli i.e.Villa Elisa) was determined against the strawberry sap beetle *Lobiopa insularis* by Eliceche et al. (2017). They found that infectivity percentage of adult sap beetle within 21-25 days as $1.7 \pm 4.1\%$ when sap beetles were treated with concentration of 10,000 IJs /container of *H. bacteriophora*. Akpinar et al. (2020) demonstrate that native Turkish isolate FLH-4-H: *Heterorhabditis bacteriophora* had a promising efficacy against scarab beetle, *Epicometis hirta* (Coleoptera: Scarabaeidae). The mortality percentages of the adult beetles were recorded at dose of 190 IJs/ml and 380 IJs/ml on 4th, 8th and 12th days after treatment. The highest mortality obtained on 12th day at 380 IJs/adult beetle.

In our study, it was observed that four different concentration of *H.bacteriophora* can cause mortality of 8%-100% on banana leaf and fruit scarring beetle in different exposure time period under laboratory condition. The infectivity of the insect increases with increase in EPNs concentrations and exposure time.

The highest number of 3rd stage juveniles of EPNs from dead insect was isolated from higher concentration of *H.bacteriophora*. The pathogenicity and reproductive capacity of EPNs are very important in terms of persistence and perpetuation in areas where they are applied and this is one of the important attribute of biocontrol agents (Susurluk and Ehlers 2008;Susurluk *et al.* 2009).Apart from this, EPNs have many superior attributes such as easy in vivo or in vitro commercial production, easy to apply, perpetuating nature in environment, compatibility with many chemicals, safe to environment and human being, host searching behaviour, pathogenicity and survivability which make them good biocontrol agent (Canhilal 2011).Moreover, due to their sensitivity to moisture, UV rays, seasonal fluctuation has limit their application in fields. Therefore, further researches are needed to increase their chance to establish them as good biocontrol agents and to include them as a component of Integrated Pest Management System.

From our study, it was observed that native *H.bacteriophora* effective in controlling banana leaf and fruit scarring beetle. Though this is a preliminary study conducted under laboratory condition but the results revealed the importance of *H.bacteriophora* as biocontrol agent. Present study is relevant in organic agriculture system where emphasis are given to minimise the application of pesticides and adopting the natural and geographically suitable biocontrol agents thereby reducing the risk to human being and environmental hazard. Further studies are needed on field application of *H.bacteriophora* and effect of various biotic and abiotic factors on their infectivity. So that in near future they can be incorporated as a strategy of Integrated Pest Management System.

Declarations

Authors' contributions

Study conception and experimental design were prepared by NB. Experiment, Data collection and analysis was performed by MG. The manuscript was written by NB. The authors read and approved the final manuscript.

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Code availability

Not applicable

Compliance with ethical standards

Conflict of interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

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Figures

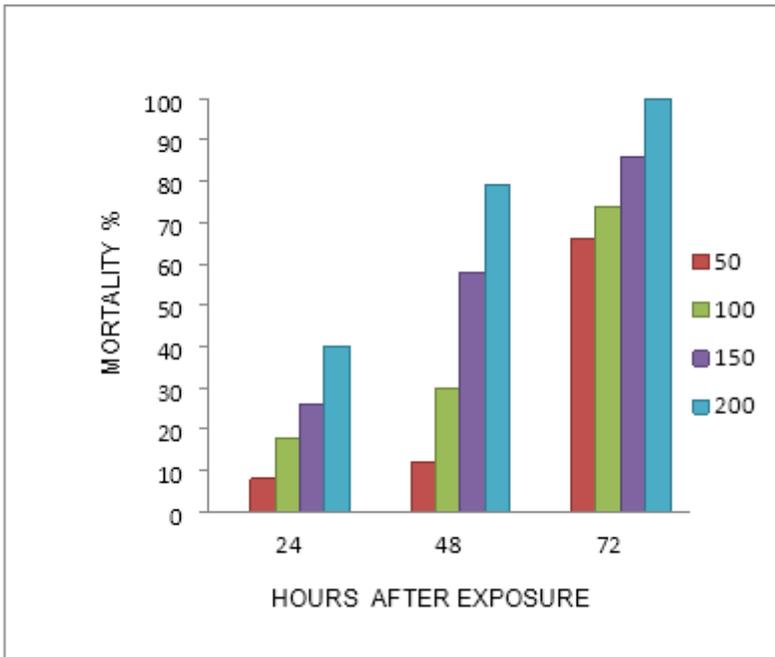


Figure 1

Percent mortality of banana leaf and fruit scarring beetle exposed to

H. bacteriophora at five different concentrations

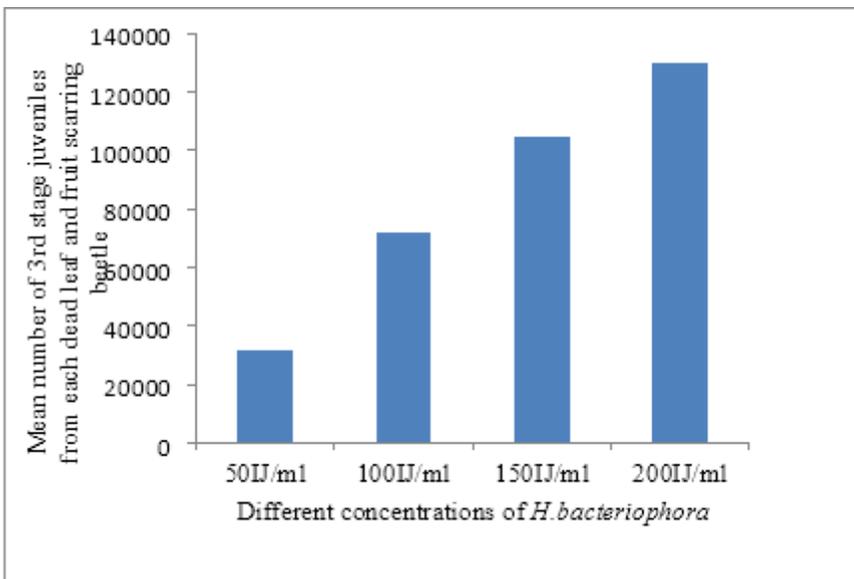


Figure 2

Mean number of re-isolated 3rd stage juveniles from banana leaf and fruit scarring beetle at different concentration of *H. bacteriophora*

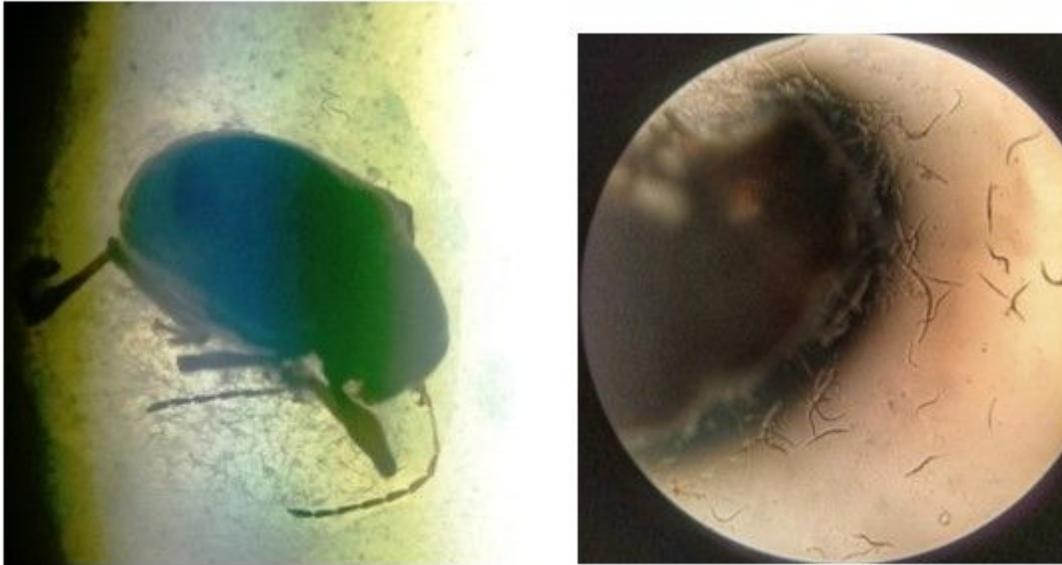


Figure 3

Emergence of 3rd stage infective juveniles of *H. bacteriophora* from infected banana leaf and fruit scarring beetle (Observe under 40x)