RESEARCH ARTICLE

Evaluation of Indigenous Entomopathogenic Nematodes from Tea Soil of Assam as Potential Biocontrol Agents against Termite and Cockchafer Grub in Tea

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Abstract

This study aimed to isolate native entomopathogenic nematodes (EPN) in the tea growing areas of Assam and to determine their potential for control of Termite and Cockchafer grub Soil samples were collected from three tea estates of jorhat, Assam for EPN isolation. A total of 30 soil samples were tested for the study. Larva of meal moth, *Corcyra cephalonica* was use as insect bait. Out of 30 samples 9 were found to be positive for EPNs. The infected host larvae were separated for extraction using "white trap method". All the isolates were evaluated to ascertain their pathogenicity against Termite and cockchafer grub. From the experiment *the* isolated strain @ 50IJ, 75IJ and 100 IJ was found to be highly pathogenic to Termite and cockchafer grub, which recorded 94–100% mortality in termite and 100% in all concentrations against cockchafer grub. After infection to prove the cause of mortality EPN could be recovered from the termite and cockchafer cadavers.

Keywords: *Corcyra cephalonica,* Cadavers, Entomopathogenic nematodes, Pathogenicity.

International Journal of Tea Science (2022); DOI: 10.20425/ijts1612

INTRODUCTION

Tea being a perennial crop is being infested by an array of leaf damaging pests, which accounts for huge crop loss. Apart from these foliage pests, the live wood eating (*Microcerotermes* sp) and dead wood eating (*Odontotermes* spp.) termites cause considerable damage to tea plants especially in Cachar and North Bank areas of Assam and Dooars in North Bengal and affect the productivity to the tune of 10–15%, resulting in the capital loss on both young and mature tea. The VP cuttings are often damaged badly in the nursery beds. The live wood eating termites have their nests located underground at a depth of 5 to 10 cm and they attack the tissues under the cover of earth runs. The activity of live wood eating termites is confined to the live wood of tea which differs from the other group called the scavenging termites which restrict their attack to the dead wood caused by borers or, pruning cuts, scars, dead snags, cankers etc. Out of several species of Cockchafer grubs *Holotrichia impressa* is the most destructive in Dooars and Darjeeling. In Assam particularly in the North Bank of the Brahmaputra the grubs of *Sophrops plagiatula* was found to be mainly responsible for damage. Larva eats away the bark in the collar region just below the soil surface either in a ring or in patches. The damage is healed up by callus growth if the damage is not severe. The extensive callus growth just above the damage portion may lead to the death of the plant when debarking is done in a girdle. The damage done by cockchafer is very similar to that of manure damage, but in the later case the bark remains intact. Although several methods have been adopted for the management of these two soil born pest, but the work on the management of termite and cockchafer in tea is limited.

Entomopathogenic nematodes (EPNs) are having potentiality as promising biological control agent and can be an alternative tool of controlling many soil born pests.[2] There are mainly three genus considered as EPNs: *Steinernema*, *Heterorhabiditis* and *Neosteinernema*. They are actually the vectors of particular bacteria, which are entomopathogenic in nature.[1] Moreover recently a new genus *Oscheius* was discovered as EPN.[3] Studies showed that EPN of genus *Oscheius* also contribute a good enough as biological control agent. Most of the species of this genus showing the nature of facultativeparasite.[4] EPN of this genus have symbiotic relationship with entomopathogenic bacteria, but it is not carrying them inside its body like other obligate EPNs do, rather on its body surface Entomopathogenic nematodes (EPNs) of the families Steinernematidae and Heterorhabditidae are lethal pathogens of insects. These pathogens contribute to the regulation of natural populations of insects, but the main interest in them is an inundatively applied biocontrol agent.[5] Their success in this role can be attributed to the unique partnership between a host-seeking nematode and a lethal insect pathogenic bacterium. Because of their biocontrol potential, considerable attention has been directed over the past few decades to genus *Heterorhabditis* and *Steinernema* and their expectative bacterial partners, *Photorhabdus* and *Xenorhabdus*.[6] Keeping these in mind and also in view of the restrictions on the use of pesticides for the management of tea pests, an attempt has been made to make a survey, isolation, identification of indigenous entomopathogenic nematode and evaluate its infectiveness against two major soil born pest of tea i.e., Termite and Cockchafer in laboratory condition.

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How to cite this article: Rahman, A. Evaluation of Indigenous Entomopathogenic Nematodes from Tea Soil of Assam as Potential Biocontrol Agents against Termite and Cockchafer Grub in Tea. International Journal of Tea Science 2022, 16(1):5-9.

Source of support: Nil

Conflict of interest: None

Received: 07/09/2022; **Revised:** 13/10/2022; **Accepted:** 25/12/2022

Figure 1: Insect baiting for extraction of EPN

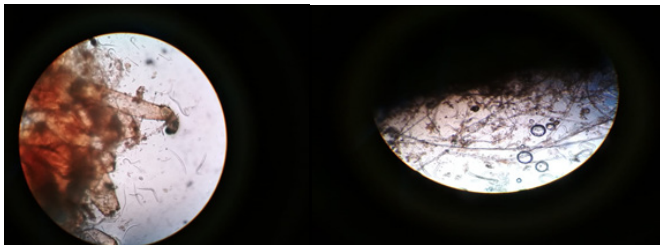

Figure 2: Recovery of EPN from termite cadaver after infection

METHODOLOGY

Insect trap method (Bedding and Akhurst, 1975 Isolation of EPN

Thirty soil samples were collected from a depth of at least 15 cm of three estates during the period July 2018 to September 2018. Samples were keept in a cooler (8–15°C) during transport. The collected samples were brought to laboratory. Removed any debris (*i.e.*, rocks, pieces of wood or bark, leaves, *etc.*) collected with samples to avoid contamination with saprobic microorganisms. Water is added with spray bottle to slowly increase the moisture level of the soil and facilitate the movement of nematodes. From each sample approximately 250 gm of moist soil were kept in a 20 cm petridis with a lid. Thirty host larvae of **meal moth,** *Corcyra cephalonica* as insect bait were released on each petri dish and covered with a thin cloth. Petri dishes were observed every 2–3 days and dead insects were removed. Additional healthy insect larva was added to each petridish for further baiting of the soil sample. Cadavers with a brown or ochre coloration are usually parasitized are removed and rinsed in sterile water. Now the cadavers were placed in a modified White trap for recovery of juvenile nematode progeny (Figure 1).

Entomopathogenic Nematode Emergence from Cadavers

The results of the present studies indicated that the initiation of emergence of infective juveniles of the entomopathogenic nematodes from cadavers of termite and cockchafer started from the 8th day of inoculation. The duration of nematode emergence was 21-26 *(23)* (Figure 2).

Nematode Recovery from Infected Cadavers with Modified white Trap

A 50 (or 60) mm diameter Petri dish was placed inside a larger dish (100 mm). One single circular filter paper (Whatman) was placed inside the smaller dish. Cadavers were placed on the filter paper of the smaller dish. Outer (larger) petri dish filled sterile distilled water and large petri dish was covered with the lid. Trap kept as such for 10–25 days for migration of juveniles from cadaver to water. Now water with IJs is harvested by removing the larger dish of the trap and collecting water with nematodes into a beaker. Nematodes were allowed to decant at the bottom of the beaker for few minutes. Water is poured carefully, making sure nematodes remain in the bottom of the beaker. Stored flask with nematode suspension in a incubator between 10–20°C. This method is widely used by EPN workers the world over and gives good results even with very low population of IJs. Soil samples (200–500 cc) are placed in different containers with lids (having small holes for aeration). IJs are extracted from dead larvae by using "White Trap" method (Figure 3).[7]

Maintanance of EPN culture on alternate host,

Corcyra *cephalonica*: Host larva *Corcyra cephalonica* were released on the petridish lined with filter paper. The filter paper kept moist by adding distilled water. EPN extract was added to filter paper and allowed it to stand. After 48 hours the host larvae were seen got infected and the cadavers were kept for further study as stock culture.

Laboratory bioassays on the pathogenicity of entomopathogenic nematode on *Scavanging termite* (*Odontotermes sp.) and Cockchafer grub*

The termite and cockchafer is found throughout the tea plantation of Assam and is a perennial
problem on tea.EPN of genus rhabditis can be used effectively to manage these two underground pest.

Test for host range

The efficacy of EPNs against different insects was evaluated by simple bioassay procedures: Petri-dish method (25).

Pathogenicity test

Petri dish bioassay method

In the laboratory bioassay, Workers of termite and 4th instar grub of cockchafer were used for evaluation for both the experimental insects it was found to develop infection within 24-48 hours following their release. These findings are quite encouraging and it may be due to the fact that *experimental* insects and nematodes were confined to a small area and contact between the two was assured. Our results support the findings of (24) who have reported 100% mortality of 4th instar larvae of *A. ipsilon* by exotic strain of *H. bacteriophora* within 72 hours after their topical applications in Kashmir.

Different concentrations of IJ were used to determine pathogenicity of the test nematodes against host. Petri dishes lined with what man filter paper were taken for the experiment. Topical application of juvenile nematode was done at various concentrations on the wet filter paper on the Petri dish. On those

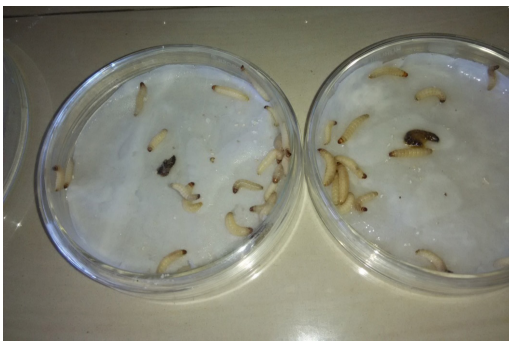

Figure 3: EPN laboratory culture on *Corcyra cephalonica*

Table 1: Pathogencity evaluation of local isolate of EPN in Laboratory condition Against Termite

Treatment	*Dose*	*% mortality after*		
		12 hours	*24 hours*	*36 hours*
EPN	50IJ	15.6	62.3	94.6
EPN	75 IJ	21.3	70.3	97.6
EPN	100 IJ	30.0	81.6	100.0
Control	Blank	0.0	0.0	0.0

Table 2: Pathogenicity evaluation of local isolate of EPN in Laboratory condition against Cockchafer grub

Treatment	*Dose*	*% mortality after*		
		12 hours	*24 hours*	*36 hours*
EPN	50IJ	0	66.6	100
EPN	75 IJ	26.6	86.6	100
EPN	100 IJ	46.6	100.0	100
Control	Blank	0.0	0.0	0.0

treated Petri dishes experimental insects (Termite and Cockchafer) were released for infection. The inoculated insects were kept at room temperature with mean temperature of 22.10°C and 54.3% Relative humidity. The data on mortality of insects at 12, 24, 48, 72 and 96 hours after the application of the test nematodes was recorded.

RESULT

For pathogenicity evaluation against termite three different dosages were used i.e., 50 infective juvenile, 75 infective juvenile, 100 infective juvenile. Result revealed that after 36 hours of treatment application the infection rate recorded were 94.6, 97.6 and 100% respectively (Table 1) (Figure 4).

For pathogenicity evaluation against cockchafer grub three different dosages were used i.e., 50 infective juvenile, 75 infective juvenile, 100 infective juvenile. Result revealed that after 36 hours of treatment application 100% infection was recorded for all used dosages of EPN (Table 2) (Figure 5).

DISCUSSION

Entomopathogenic nematodes have been found with various densities in most terrestrial habitats. In this study we aimed to found out, native isolates of EPN, which is virulent to tea pests. From the survey a genus of Rhabditid family are found abundantly inhabiting in tea soil, which is having potentiality of infection to the two major soils born pest of tea. The percentage of positive samples for nematodes obtained in this study was approximately 30%. The current study represents the first survey and systematic survey of indigenous EPN species in a disturbed habitat of tea plantation area of North eastern region, which has a variety of different climatic regions with high temperatures and humidity. With large agricultural areas, climate and altitudes that also result in a high diversity of insects, it is reasonable to suppose that the tea plantation in North Eastern region is an undisturbed niche for a number of different species and strains of EPNs.[8-10]

During the survey of tea fields, one positive EPN isolates were recovered from 30 soil samples collected from 3 tea estates of jorhat region in assam. The occurrence of EPNs in the study area of our survey was relatively high (30%). We hypothesize that the

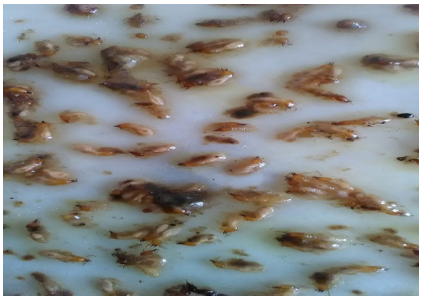

Figure 4: EPN infected termites

Figure 5: EPN infected Cockchafer grubs

region might have more recovery rate for EPNs for this region, which may be little low due to several factors. First, only *Corcyra cephalonica was* used as insect bait, which is much smaller in size and it may not be an appropriate host for all EPNs.[11] Second, only room temperature was used for baiting the soil samples during EPN isolation. Third, a high rate of chemical pesticides is being used to control pests in tea growing areas.[12] Another reason may be the sample size. A larger sample size covering more areas may increase the species diversity and the number of positive samples. It is well known that chemical pesticides can dramatically affect EPNs both directly and indirectly by reducing host population and nematode abundance.[13] However, we should mention that the low recovery rate of EPN is not unusual, as other works have also suggested similar low rates of recovery.[15] For instance, occurrence of EPNs in Slovenian soils in only 2.5% of soil samples.[14]

From the literature it is observed that the use of entomopathogenic nematodes in biological control of insect pest was initiated some years ago, which is still traditionally confined only with suppressing soil-inhabiting insect pests.[16] The research findings proved that EPN have also potential to suppress above-ground insect pests, but it is with certain conditions.[17,18] Due to certain factors efficacy of entomopathogenic nematodes in suppression of aboveground insect pests is low. The factors are primarily due to inappropriate (insufficient) moisture, exposure to high temperature and ultraviolet radiation. These factors play a crucial and important role in the survival of nematodes.[5] For this reason nematodes are found less efficient against above-ground outdoor insect pests, though the previous laboratory tests showed much higher efficiency.[19] To put nematodes on plants we use some equipment, which is generally made for spraying plant protection products, manuring and irrigation. Generally to serve our purpose of application of different agrochemicals backpack manual or tractor sprayers, sprinklers and also planes are in use. While spraying EPN formulation infective juvenile larvae of EPN can be passed through spray tubes with diameter of at least 500 µm, which is capable to withstand pressure up to 2000 kPa.[5] EPN infective juveniles (IJs)

can also tolerate short-term exposure (2-24 hours.) to many agro-chemicals, thus EPN can be tank-mixed and applied together.[20,21] Tank mixing of EPN-chemicals could offer a cost-effective alternative means to foliar integrated pest management (IPM) systems. As EPN's are very much sensitive to ultraviolet radiation, due to this reason EPN should better applied to above ground plant parts in the evening, early in the morning or in a cloudy weather, when the radiation is not so intense.[5] Nematode survival and efficacy on foliage can be enhanced to great extent by addition of various adjuvants to the spray fluid, which have antidesiccant (e.g. glycerol, various polymers) or UV-protective (brighteners) actions[26] although more works needs to be done yet to enhance survival of EPN after application. There is a great potential of EPN for using against foliar pests in IPM programmes, in combination with other biocontrol agents[22] or selective chemicals.[27] EPNs are considered exceptionally safe biological agents.[28] Because their mode of action is very specific, their environmental risk is considerably lower than that of chemical agents for plant protection. Since the first use of EPNs for suppressing *Popillia japonica* Newman in the USA[29] until now, no case of environmental damage due to these biological agents has been documented. The use of nematodes is safe for users. EPNs and their bacteria are not harmful for mammals and plants.[28]

CONCLUSION

The use of nematodes parasitic to insects to manage insect pests has growing popularity. Currently, the problem is that, many insect pests have developed resistance to certain insecticides, new pests have arisen to replace the previous one. The effectiveness of biocontrol agents such as predators, parasites and pathogens has been reduced by use of hard chemical pesticides and there is increased concern about pesticide safety and environmental quality. In this context these beneficial organisms, particularly EPN can be an important component of an integrated pest management (IPM) program for tea pests. Establishment of entomopathogenic nematodes for the management of tea pests would bring several benefits. Firmly establishing insect parasitic nematodes will promote the sustained use of agriculture and develop a better understanding of biodiversity. In our investigations, the Rhabditid nematode species is so far the most promising biocontrol agent, which are isolated from the local tea soils, further studies to establish EPN utilities to the planters recommendation, mass production technology and identifying suitable formulations technologies are in active process. As a conclusion, nematodes of the genera *Rhabditidae* are found in tea soils of Jorhat Assam. These are found to be pathogenic to two major soils born pest i.e., Termite and Cockchafer grub, which are facultative EPNs population available in the tea soils.

ACKNOWLEDGEMENT

The authors would like to thanks the Director, Tocklai Tea Research Institute for permitting to carry out the study as in house project of the Entomology Department. Thanks, are also due to TRA Scientific Advisory committee to approve the project.

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