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Potential of Entomopathogenic Nematodes for the Management of *Sesamia calamistis* in Nigeria

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Abstract: *The management of agricultural insect pests with entomopathogenic nematodes (EPNs) is recognized internationally as a sustainable and environmentally friendly method of management. This study aimed at determining the efficacy of indigenous EPNs on the management of Sesamia calamistis, the stem borer of maize. Soil samples were collected within 5 × 5 m quadrants (r = 3) from seven forests and three cultivated locations within Ibadan. In the laboratory, 200 cm^3 from each quadrant per location were bulked to form a composite per location and used for the trial. The composite soil was properly mixed and moistened with water, and 500 cm^3 was measured into transparent plastic containers. Ten S. calamistis larvae were placed on the soil surface and the containers were inverted and placed in the dark. Larval mortality was recorded every 48 hrs for 10 days. Dead larvae were counted, removed and assessed for nematode infection. Descriptive statistics and ANOVA were performed on the data. Nematodes in the genus Heterorhabdidtis were identified from insect cadavers. Laval mortalities were 4- 57% from soils where they were found. Nematode populations recovered per cadaver ranged from 250 from soil of UI botanical garden to 3,350 from CRIN plantation. Nematode infection on the larvae from naturally infested soil indicates that a more targeted application from quantified laboratory cultures will likely provide good management of the stem borer species.*

Keywords: *biological control, maize, Heterorhabdidtis, Steinernema, stemborer*

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I. Introduction

Nematode parasites of insects have been known since the 17th century (1), but it was only in the 1930's that serious consideration was given to using nematodes to control an insect pest. Like other nematodes, entomopathogenic nematodes are soft bodied, non-segmented roundworms that are obligate or facultative parasites of insects. They occur naturally in soil environments and locate their host in response to various cues (2). EPNs have been found all over the world in a range of ecologically diverse habitats. The most commonly studied entomopathogenic nematodes are those that can be used in the biological control of harmful insects, the members of Heterorhabditidae and Steinernematidae (3). Species in these two families have been effectively used as biological insecticides in pest management programs (4) for insects in Lepidoptera, Coleoptera, Diptera and Hemiptera (2)

In Nigeria, some basic research have been conducted regarding EPNs mainly in the surveying for the presence of the nematodes. *Heterorhabditis bacteriophora* and *Steinernema* feltiae were the two EPNs identified from 6.5% of the positive samples taken from three states in north central Nigeria (4). In another study by Eche et al. (5), conducted in two north central states of Nigeria, 2% of the samples were positive for EPNs from which *Heterorhabditis* sp. *H. indica*, and *H. bacteriophora* were identified.

Depending on the environmental niche, EPNs have two main strategies for infecting their hosts. Those with a mobile foraging strategy (cruisers and intermediate foraging strategies) could be considered for use in above-ground habitats (foliar, epigeal habitats). Those with a sit and wait foraging strategy (ambushers) will be most effective in cryptic and soil surface habitats (6). The use of entomopathogentic nematodes is particularly attractive as a biological control method because they can tolerate and are compatible with may synthetic insecticides unlike most parasitoids. Negrisoli et al. (7) demonstrated that 12 out of the 18 insecticides approved for the management of the fall army worm in Brazil were compatible with three species of EPNs.

The Stem borer *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) is widely distributed in Nigeria where it causes damage to maize crops (8) (9). The characteristic damage of tunneled internodes and dead heart can result to yield loss of between 36-100% depending on the level of infestation (10); (11). The various strategies for managing the pest include biological control options such as entomopathogenic nematodes (12) (13). In this study, a preliminary assessment was conducted with the purpose of detecting EPNs in soils from the south west of Nigeria and evaluating mortality of *S. calamistis* in the collected samples.

II. Materials and Methods

Collection of field samples

The study was conducted in Ibadan located the south western part of Nigeria in the rain forest transition zone. Samples were collected from three cultivated and seven largely undisturbed fields in Ibadan at the Cocoa Research Institute of Nigeria (CRIN), Forestry Research Institute of Nigeria (FRIN), National Institute of Horticulture (NIHORT), International Institute of Tropical research (IITA), Agodi park, Botanical garden University of Ibadan (UI), Teak Reserve UI (Table 1). The samples representing cultivated plots were taken from experimental crop research fields of IITA, UI and NIHORT. Three sites were sampled per location and each sample site was randomly selected in the location. A 5 x 5 m quadrat was laid per site from which five samples were taken at a depth of 20 cm using a soil auger. The five samples were bulked per site in polythene bags, labeled separately then placed in an insulated box.

Table 1. Site description of locations for entomopathogentic nematode collection

Location	Description	Location coordinates
Agodi Zoological Garden	Forest reserve	7° 28'56.252"N 3° 54'52.595"E
Cocoa Research Institute of Nigeria	cocoa plantation	7 ° 13'28.743"N 3° 52'23.398"E
Forestry Research Institute of Nigeria	Forest reserve	7 ° 23'28.56"N 3 ° 51'46.135"E
International Institute of Tropical Agriculture	cassava plot	7 ° 29'48.751"N 3 ° 54'13.271"E
International Institute of Tropical Agriculture	Forest trail	7 ° 29'38.737"N 3 ° 53'18.39"E
National Horticultural Research Institute	Vegetable plot	7 ° 23'52.527"N 3 ° 52'4.045"E
National Horticultural Research Institute	Regenerated forest	7 ° 23'52.527"N 3 ° 52'4.045"E
University of Ibadan Botanical Garden	lawn	7 ° 22'39.128"N 3 ° 56'49.342"E
University of Ibadan Teaching Research Farm	Maize plot	7 ° 22'39.128"N 3 ° 56'49.342"E
University of Ibadan Teak Forest	Teak plantation	7 ° 27'0.426" 3 ° 53'48.9797"E

Nematode extraction and population estimation

In the laboratory, 200 cm^3 was measured from the samples collected per location and thoroughly mixed in a plastic tray while intermittently spaying with tap water to moisten the soil (14). From the total of 600 cm^3 of soil, 100 cm^3 was measured out for nematode extraction while the remaining 500 cm^3 soil were collected in 16 x 12 x 8 cm plastic containers. Nematodes were extracted from the soil using the extraction tray method (15). The extract from the set up was poured every 48 hours for 6 days. The three collections (nematode suspensions) from each sample were bulked and concentrated by sieving with a 38 µm sieve. The nematodes retained in the sieve were collected in 100 ml plastic cups in 50 ml of water. A syringe was used to homogenize the suspension by pumping action and 2 ml was drawn from the homogenized suspension into a counting slide. Entomopathogenic nematodes (infective stages) were identified and counted from each suspension while observing under a microscope using morphological characters (16).

Mortality test on *Sesamia calamistis*

Larvae of *Sesamia calamistis* were obtained from the Entomology Unit of the International Institute of Tropical Agriculture. The 500 cm^3 of moistened soil in the plastic containers (previously described) were left for 48 hrs in the dark. After this period 10 larvae of 3rd insta *S. clamistis* were placed in the containers. The containers were inverted such that the larvae were below the soil (Plate 1). The containers were placed in a larger plastic container and covered with a dark cloth. Larvae were observed every 48 hrs for 10 days. Dead larvae were counted and removed from the containers. Each dead larva was placed in a petri dish and teased to observe for the presence on EPNs. Previous identification was confirmed using infective juveniles and males because morphology of females and hermaphrodites vary with the nematode generation in the insect (17). The following ratios were calculated based on the measurements taken; A (total length divided by width), B (total length divided by distance from head to base of pharynx), C (tota1length divided by length of tail), D (distance from head to excretory pore, divided by distance from head to base of pharnyx), and E (distance from head to excretory pore divided by length of tail) (16) (18) for 10 infective juveniles and males each.

The EPN found in the insect cadavers were counted. The nematode counts data were transformed using $\sqrt{x + 0.5}$. Descriptive statistics as well as analysis of variance was performed on the data as appropriate. Significant means were compared using least significant difference (LSD) and $\alpha = 0.05$.

Plate 1. Experimental set up showing inverted plastic containers containing soil samples baited with the insect larvae.

III. Results

Nematodes found in the soil

Generally, entomopathogenic nematode populations found in the soil samples collected from cultivated plots were fewer than those found in uncultivated sites (Table 2). The highest populations ($p \leq 0.05$) of EPNs were found in CRIN, UI Teak Plantation and FRIN plantation with more than one nematode per cm^3 of soil. Not unexpectedly, the populations of plant-parasitic nematodes in the cultivated soil samples were high (> 1 nematode per cm^3 soil); similar populations of plant-parasitic nematodes were found in uncultivated soils from CRIN and FRIN.

Table 2. Total number of entomopathogenic nematodes and plant-parasitic nematodes estimated from soil samples (100 cm^3)

Location	Total number of EPNs	Total number of PPN
IITA (Cultivated)	11.1d	199.8b
NIHORT (Cultivated)	14.8d	325.6a
UI T&R Farm (Cultivated)	22.2d	140.6b
NIHORT (Uncultivated)	51.8d	33.3c
IITA (Uncultivated)	55.5d	59.2c
UI Botanical Garden (Uncultivated)	55.5d	77.7c
Agodi Garden (Uncultivated)	85.1cd	44.4c
FRIN (Uncultivated)	133.2c	103.6bc
UI Teak Forest (Uncultivated)	362.6b	22.2c
CRIN (Uncultivated)	606.8a	347.8a
LSD	104.3	63.8

Table 3. Morphometric indices of infective juveniles and males of entomopathogenic nematodes isolated from larvae cadavers

	Heterorhabditis spp.	
Morphometric measurements	Infective juveniles	Males
A	22.7±0.9	17.8±1.5
B	4.5±0.1	8.1±2.0
C	6.7±0.2	26±0.3
D	78.2±2.2	92.9±0.5
E	111.9±3.1	297.1±9.9
SL		43.7±2.8
ABD		16.8±1.5

A (total length divided by width), B (total length divided by distance from head to base of pharynx), C (tota1length divided by length of tail), D (distance from head to excretory pore, divided by distance from head to base of pharnyx), and E (distance from head to excretory pore divided by length of tail), SL: Spicule length ABD (Anal body diameter).

Mortality test on *Sesamia calamistis*

Nematodes in the genus *Heterorhabditis* were identified from insect cadavers (Table 3). Stages observed in the dead cadavers were second stage juveniles (J2), infective juveniles (IJ3), females, males and hermaphrodites (Plate 2 a-e). Laval mortality was highest in CRIN uncultivated soil samples with 57.1%

followed by soil collected from uncultivated NIHORT plots, UI Teak forest and UI Botanical garden with 50.8, 50.6 and 50.4% respectively (Figure 1). No larvae died in soil collected from cultivated plots at the UI Teaching and Research farm and the FRIN forest, while mortality was low in NIHORT cultivated soil.

When dead larvae were teased, no nematodes were found in the cadavers picked from soil collected at FRIN, IITA (cultivated), NIHORT (cultivated) and UI Teaching and Research farm (Table 4). The highest ($P \leq 0.05$) population of EPN were recovered from larvae picked from CRIN uncultivated soil followed by UI Teak forest. Populations in the latter were however not significantly higher than was counted in cadavers from uncultivated soil from NIHORT and IITA. The recreational gardens had the lowest nematode populations recovered from larvae of 250 and 400 respectively from UI and Agodi gardens.

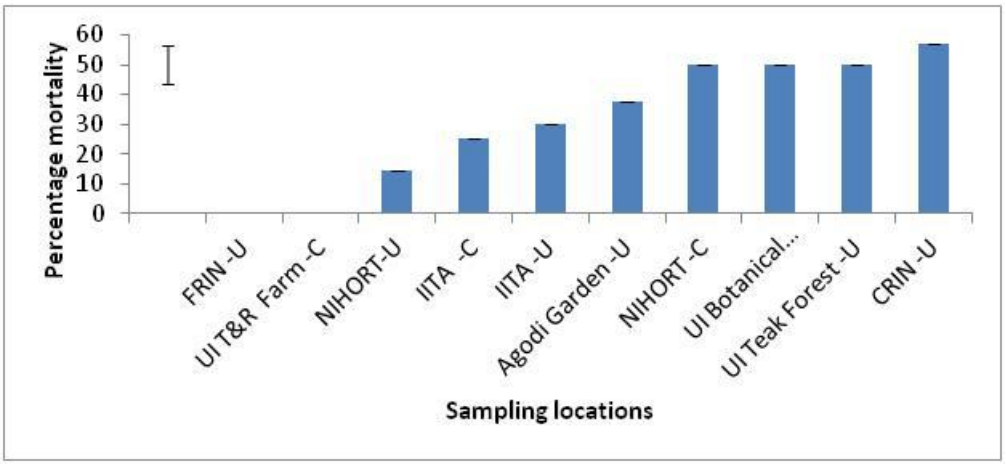

Figure 1. larva mortality of *Sesamia calamistis* exposed to indigenous entomopathogenic nematode populations in soil

Bar = standard error of means; C = cultivated land, U = uncultivated land

Table 4. Number of nematodes recovered from *Sesamia calamistis* cadavers

Location	Mean Nematode population/larvae
Agodi Garden (Uncultivated)	20.0(400)
CRIN (Uncultivated)	57.9(3350)
FRIN (Uncultivated)	0.7(0)
IITA (Cultivated)	0.7(0)
IITA (Uncultivated)	37.4(1400)
NIHORT (Cultivated)	0.7(0)
NIHORT (Uncultivated)	33.2(1100)
UI Botanical Garden	15.8(250)
UI Teaching Research Farm	0.7(0)
UI Teak Forest	44.2(1950)
LSD	13.4

Transformed ($\sqrt{x+0.5}$) means presented with real means in parenthesis.

Plate 2. Stages of *Heterorhabditis* spp. isolated from *Sesamaia calamistic* cadavers

IV. Discussion

Majority of the samples were positive for the presence of entomopathigenic nematodes based on gross morphology of the soil inhabiting juveniles. The populations of EPNs appeared to be higher in soils that were generally undisturbed compared to plots that are regularly cropped or cultivated. It is possible the cultural practices including application of various pesticides in such fields may be responsible for the lower populations of EPNs. Entomopathogenic nematodes were also reported to be more frequently found in less disturbed plant communities than in arable crop fields (19). No EPNs were found in arable crop fields in Benin while sampling for EPNs (20). As observed in this study, it is not unusual for plant-parasitic nematodes to be found in higher populations in cropped fields as they usually would be found in places where their host is abundant.

Based on the morphometric data collected the genus of EPN associated with the field that were sampled was *Heterorhabditis*. Recovery of nematodes from the majority of the cadavers was less than 1000. This is generally lower than expected from nematode infected larvae which is usually in several thousands. This is not to say there are no other or many EPNs from the soil samples collected. While identifying from the samples, only representative specimens were picked per sample, it is therefore possible that not all counts for EPNs under low magnification were of the same genus or species. It is also possible that the EPNs were unable to cause infection on the specific insect used either due to low populations or due to host preference. It is a known fact that the most susceptible insect is the greater wax moth and even with that, it has been reported that the wax moth baiting method may not detect all EPN even in positive samples (21).

It was observed in the study that there were missing larvae, that is neither the live nor dead larvae were seen in the soil. In a few cases there was pupation however it is suspected that majority of the missing larvae were due to cannibalism. Cannibalism has been reported in stem borers including *Sesamia* sp. in cases where there was overcrowding and limitation in food sources (22). It is possible that some infected and weak larvae may have been consumed by the more aggressive ones thus reducing the recovery of more EPNs from potential cadavers.

Larvae mortality was 50% and above in 50 % of the soils sampled, and the samples with high mortality corresponded with the samples with high recovery rate of the nematodes. There are several reports that demonstrate the ability of EPNs to cause mortality and reduction of insect populations which results in an increase in crop productivity. Mortality of four lepidopterous pests of cabbage was 50% and 78% in 6 and 12 hrs respectively in *in vitro* tests with *Steinernema* spp. (23). Wang and Li (24) reported that *S. carpocapsae* DD-136 caused 89.4% mortality of *Pieris rapae* in 72 hours in field trials. (25) demonstrated the effective reduction of termites with applications of entomopathogenic nematodes. EPNs are particularly useful since they are compatible with many insecticides and other management options (7).

Though preliminary, this is the first demonstration of management of a pest using a local population of EPN in Nigeria. The results prove that some insect pests can be effectively managed with EPNs. The effectiveness of the field populations of EPNs, which is usually low, demonstrates that better results can be achieved with the generation of cultures of the identified nematodes for the management of stem borers and other emerging pests such as the fall army worm. Further research can also be conducted to widen the scope of the survey and also to identify collected EPNs to species level in order to further determine which species are most effective in managing specific insect pests.

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