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# Evaluation of Native Entomopathogenic Nematode Isolates for the Management of Selected Insect Pests in Nigeria

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## ABSTRACT

Native entomopathogenic nematodes (EPNs) were evaluated for the management of three selected pests, *Sesamia calamistis*, *Spodoptera frugiperda* and *Rhynchophorus ferrugineus* in laboratory bioassays. The EPNs were isolated from soils from various locations within Ibadan, Nigeria using the greater wax moth (*Galleria mellonella*) as insect bait. A two-factor laboratory experimental assay was laid out in a completely randomized design and replicated three times. The EPN suspension was applied on insect larvae with distilled water as control. Number of days to mortality, percentage mortality of insect larvae, and total EPN population of infective juveniles (IJs) recovered from larval cadavers were assessed and reproductive factor (RF) determined. Data were analyzed using analysis of variance, and means were separated using Tukey's Studentized Range Test at P≤0.05. The number of days to mortality for inoculated larvae of *S. calamistis*, *S. frugiperda*, *R. ferrugineus* and *G. mellonella* were 6.43, 3.57, 8.80 and 6.20, respectively. The EPN population achieved percentage mortality of 82.0%, 54.0%, 60.0% and 84.0%, respectively for *G. mellonella*, *S. calamistis*, *S. frugiperda* and *R. ferrugineus* larvae respectively. Mean numbers of EPN IJs recovered from cadaver was in the order; *R ferrugineus* (9,407.0), >*G. mellonella* (5075.08), >*S. frugiperda* (3957.23), >*S. calamistis* (742.31). From the results, EPN had the greatest fecundity in *G. mellonella* and *R. ferrugineus* showing higher ability to be recycled. This study reveals the potential of native EPNs as a biocontrol agent of insect pests and emphasizes the need for a more environment-friendly and sustainable approach to insect pest management.

***Keywords*:** Biocontrol agents, insect mortality, *Heterorhabditis* sp., *Sesamia calamistis* *Spodoptera frugiperda, Rhynchophorus ferrugineus*

## INTRODUCTION

Cultivation of plants for the production of food and fibre, needed for man's survival, is an age-long practice. However, plants and their products are attacked by defoliators, sap suckers, gall formers, stem borers, pod or fruit borers, and seed eaters either in the early stage or during the later stages of development (Rao et al., 2000; Maina et al., 2018). Insects attack and damage grains, fibers, fruits, vegetables, ornamentals and forestry resulting in yield losses that contribute to food insecurity. The damage that insects inflict on cultivated plants is highly varied, and includes direct feeding on leaves, fiber, grain and fruits, facilitating the entry of pathogens or vectoring pathogens

that cause plant diseases (Coates et al., 2015; Dhaliwal et al., 2015; Singh and Kaur, 2018). The most relevant stem boring species associated with maize production in Nigeria are the lepidopterous moths.

The African pink stem borer, *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), is one of the noxious noctuid pests of maize and most other plants of the family Poaceae (Abate et al., 2000; Okweche et al., 2015). Damage to maize varies with locations/regions within sub-Saharan Africa depending on the population of this stem borer. Crop losses and grain yield reduction may result from the damage caused to growing points leading to loss of stands (dead heart), large number of exit holes, damage to leaves (windowpane damage), stem tunnelling, and direct damage to ear shank and ears resulting in reduced cob yield (Okweche et al., 2015; Ramanujam et al., 2017). Yield losses due to lepidopterous borers in Africa vary greatly between 0% to 100%, among ecological zones, regions, and seasons. Other reports suggest a potential yield loss of 20% to 90% due to stem borers on cereals and sugarcane in Sub-Saharan Africa (Assefa and Dlamini, 2018).

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a key pest of maize (*Zea mays* L.) and many other crops. It is native to tropical and subtropical regions of the western hemisphere from the United States of America to Argentina. *Spodoptera frugiperda* was reported for the first time in 2016 in the African continent within the following countries; Nigeria, Sao Tomé, Benin and Togo, causing significant damage to maize hitherto (Deole and Paul, 2018). This generalist insect pest attacks many crops but exhibits a preference for members of the Poaceae family such as maize and sorghum. Larvae usually consume a large amount of foliage (defoliation) and sometimes destroy the growing point of the plant causing dead heart. The destruction of leaves, stems and/or flowers of the plant by the larval instars occurs through feeding of all instar stages. In maize, armyworm larvae damage leaves causing pinhole to shot holes and progresses into different sizes of lesions on leaves during the vegetative stage. At tasseling and silking before maturity, they may damage ears and kernels (Midega et al., 2018).

Late instar larvae can act as cutworms by entirely sectioning the stem base of maize seedlings (Goergen et al., 2016; Midega et al., 2018). Late instars of fall armyworm larvae consume large amounts of leaf tissue resulting in a ragged appearance of the leaves leaving a mass of moist brown frass. Maize damage evaluation in Ethiopia revealed that fall armyworm caused up to 30% loss at the late whorl stage even with untimely pest control (Assefa and Ayalew, 2019). When late instars act as seedling cutworms, maize losses can reach up to 100% (Fatoretto et al., 2017). Older larvae burrow into maize tassels and feed into the ears, causing extensive damage. Consequently, the impact of FAW is greatly felt in the economy of concerned regions/countries.

Red palm weevil (RPW), *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae), is a globally obnoxious tissue borer of palm trees. This polyphagous palm insect pest damage stem and core tissues of oil palm in West Africa (Mehdi et al., 2018). The damage is completely caused by the grubs which feed internally by boring the tissues of the stem and eventually kills the palm tree.

Entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) have the potential for biological control of insect pests as conferred by the symbiotic complex formed with certain bacteria (Kaya et al., 2006; El-Sadawy et al., 2020). They possess a unique combination of attributes that make them a promising alternative for pest control (Divya and Sankar, 2009; Abd-Elgawad, 2019). The uniqueness of entomopathogenic nematodes can be further buttressed by their mass

production mechanism from tissues (cadaver) of a wide variety of susceptible insects. These insect-parasitizing nematodes kill the insects by serving as vectors of bacteria which help to achieve quick kill of the target insect pests and thus have high potential in pest management (Grewal et al., 2001; El-Sadawy et al., 2020).

Steinernematid and Heterorhabditid families infect several insect pest species, yet pose no known negative effect on the environment. They can be mass-produced, formulated, and commercially utilized as bio-control agents against insect pests (Lacey et al., 2001; El-Sadawy et al., 2020). Entomopathogenic nematodes have the potential to reproduce in soil environments and are capable of maintaining an efficacious population density in soil for at least one additional season after their application has been enhanced by habitat manipulation (Riga et al., 2001; Alramadan and Mamay, 2019). In this study, the potential of EPNs was evaluated as bio-control agent against *Sesamia calamistis*, *Spodoptera frugiperda* and *Rhynchophorus ferrugineus*.

## MATERIALS AND METHODS

The laboratory assay was laid out in a completely randomized design (CRD) with three replications. The two treatments were insects' larvae species (*G. mellonella, S. calamstis, S. frugiperda, R. ferrugineus*), and entomopathogenic nematode inoculation with non-inoculated (uninfected) control. *Galleria mellonella* late instar larvae were used as a basis for comparing the three test insects.

### Source of Insects and Entomopathogenic Nematodes

*Galleria mellonella* larvae were obtained from infested honeycombs, collected from apiaries in the University of Ibadan. The larvae were maintained in Kilner jars half-filled with crushed combs. Laboratory cultured larvae of *Sesamia calamistis* were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan. The larvae were fed on artificial media in which they were maintained until used. A field population of *Spodoptera frugiperda* was collected from maize farms at Ijaye farm settlement and were fed with fresh maize leaves in Kilner jars until used. Larvae of *Rhynchophorus ferrugineus* were collected from a palm farm at Moniya, Ibadan. The larvae were kept in palm stem dust in a large aerated bucket from where they were selected for use.

### Isolation of Native Entomopathogenic Nematodes (EPNs) from collected soil samples

### Sample Collection

Entomopathogenic nematodes were directly baited with the late instars of the greater was moth (Bedding and Akhurst, 1974), from soil samples obtained during the rainy season (July and August) from locations within Ibadan. The locations include uncultivated field; Agodi gardens (N 7°24'23.5368", E 3°54'12.9852"), Botanical garden University of Ibadan (UI) (7°27'25.1"N 3°53'41.8"E), Cocoa Research Institute of Nigeria (CRIN) (7°13'29.2"N 3°52'02.6"E), Forestry Research Institute of Nigeria (FRIN) (7°23'32.4"N 3°51'37.6"E), International Institute of Tropical Agriculture (IITA) (7°30'08.0"N 3°54'34.9"E), National Institute of Horticulture (NIHORT) (7°24'11.9"N 3°50'57.6"E), Teak reserve UI (7°27'27.3"N 3°53'55.0"E), and uncultivated field types; IITA, NIHORT, Teaching and Research Farm UI. Samples

were obtained by random sampling of fields from three quadrats (Župunski et al., 2017) and five samples per quadrat.

The EPNs were isolated from these soil samples using the larvae of the greater wax moth (*Galleria mellonella*), obtained from the Apiary Unit of the Department, as bait. *G. mellonella* larvae are most commonly used to rear nematodes in *in-vivo* protocol because of their commercial availability and high susceptibility to most EPN species Grewal et al. (2001).

Soil samples were sieved then moistened and 300 $cm^3$ of soil was placed in a clear rectangular plastic container (6 cm x 4.5 cm x 2 cm) into which 5 larvae of *G. mellonella* were introduced. Soil samples were incubated in the dark and observed every 24 hrs to obtain infected and dead larvae which were removed and observed using a zoom stereomicroscope to confirm the cause of death as internally borne EPNs. Each dead *G. mellonella* was then placed in a White trap set-up (White, 1927; Rahoo et al., 2017) to collect infective juveniles of the nematodes. The native population was preliminarily identified as *Heterorhabitis* sp. and is further undergoing species identification. The identification was based on cadaver colour and morphological characteristics of infective juveniles and adult males.

**Multiplication of Entomopathogenic Nematodes' Infective Juveniles**

*Galleria. mellonella* larvae were infected with IJs of harvested EPN from soil samples. The larval cadavers recovered after periodic inoculation yielded a mass population of entomopathogenic nematodes through White-trap extraction, that served as inoculum for the laboratory bioassay.

**Laboratory Bioassay**

Five larvae of each test insect were placed into Petri-plates (12 cm diameter) lined with filter paper moistened with distilled water and each larva of *G. mellonella, S. calamistis,* and *S. frugiperda,* was inoculated with 50 IJs of EPN suspension in 1 ml of water. Due to the large size of *R. ferrugineus,* one larva was introduced singly on each filter paper-lined Petri-dish and inoculated with 140 IJs of EPN suspension per larva. The choice of IJ numbers used in the inoculation of each insect species was based on the relative sizes of the treated insect larvae. Five samples represented a replicate and three replications were made per treatment with EPN inoculation and the respective control (without EPN). The Petri-dishes were covered and labelled accordingly. *Gallaria mellonella*, was used as the standard insect for comparison with other test insects since it is widely accepted that entomopathogenic nematodes successfully yield high progeny per larval cadaver of *G. mellonella* (Grewal et al., 2001). The larvae in the Petri dishes were incubated in the dark at room temperature (26 ± 2 ℃) to allow larval infection. All dead larvae were transferred singly to a White trap for nematode extraction. Water suspension of emerged EPN juveniles from white traps was harvested every 2-3 days over five weeks. The trial was repeated once following the same procedures.

**Data Collection and Analysis**

Data collected were number of days to mortality, percentage larval mortality, EPN population per week and the reproductive factor (RF) [Total population ($P_f$) of EPN/ Initial population ($P_i$) of EPN]. All data were analyzed using Analysis of Variance (ANOVA), SAS 9.3 software and means were separated using Tukey's Studentized Range Test at $P \leq 0.05$.

## RESULTS AND DISCUSSION

By 10 days post-inoculation, no mortality was observed in all non- infected insect larvae in the Petri-dishes with a few larvae pupating in case of *S. calamistis* while those inoculated with EPN died within 3 – 9 days after inoculation (Fig. 1). The initial levels of inoculum and body tissue of the insects' larvae both play a complementary role in influencing the incidence of early mortality (Krishna, 2005). This was confirmed from the results obtained where relatively large-sized larvae of *R. ferrugineus* died later than other tested insects, (*G. mellonella*, *S. calamistis*, *S. frugiperda)*, with smaller larval size.

The results show that EPN had a significant impact on the insect larva types by achieving a percentage mortality of 56.00%, 60.00%, 82.00% and 84.00%, respectively for *S. calamistis*, *S. frugiperda*, *G. mellonella*, and *R. ferrugineus* (Fig. 2). There were no significant differences in percentage mortality among the inoculated larvae and also among the control (non-infected larvae). Mortality of more than 60% was not possible for *S. frugiperda* either due to cannibalism amongst insect larvae of the same experimental unit or migration out of the enclosure. This observation is in line with the report from Fatoretto et al. (2017) that the *S. frugiperda* larvae movement is density-dependent and influenced by strong cannibalism.

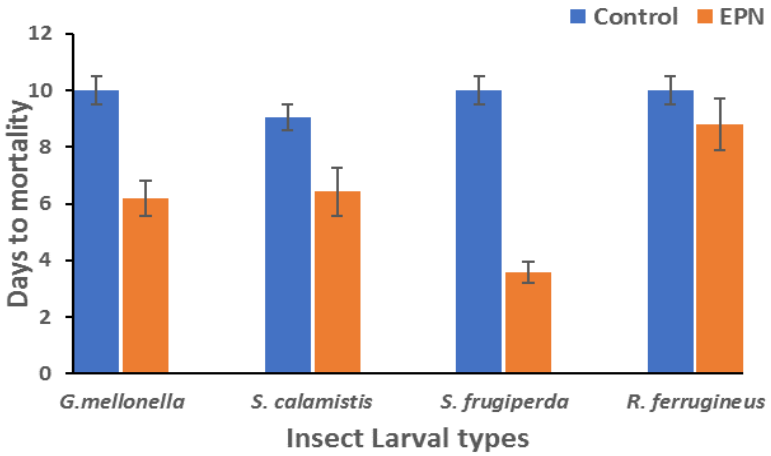


**Figure 1**: Number of days to mortality of insect larvae infected with native populations of EPNs. Bars represent Standard error

Some of the *S. calamistis* larvae pupated during the study, this may have accounted for the lower mortality (56%) recorded for them. Similarly, Garcia-del-Pino et al. (2013) reported no pupal mortality in their trial with tomato leaf miner. White trap extraction from the pupae in this study, in addition, yielded no EPN. Yet the findings are different from those of Kurtz et al. (2009) who observed mortality in the studied pupae. Studies have primarily revealed the infective ability of entomopathogenic nematodes (EPN) in the management of insect pests. They can achieve relatively quick kill of the target insect pests upon inoculation with number of active sufficient level of infective juveniles capable of initiating infection and thus leading to mortality of insect pests (Grewal et al., 2001; Divya and Sankar, 2009). It is evident from this study, that the

native EPNs isolated from soils were effective in causing death of all the test insects to varying degrees.

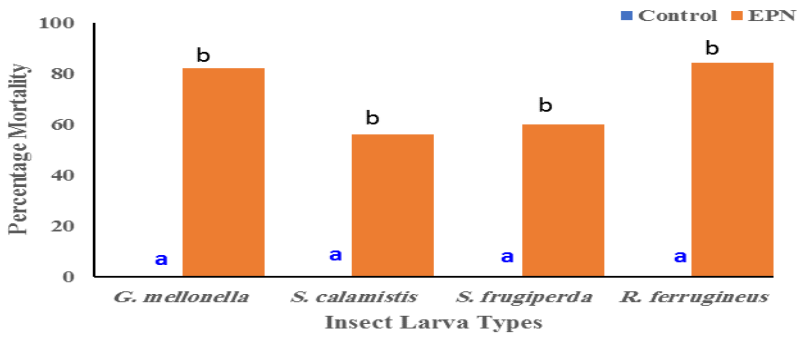


**Figure 2:** Mortality (%) of insect larvae infected with EPN compared to non-infected larvae (control) 10 days after inoculation. Bars with the same letters are not significantly different using Tukeys' studentized multiple range test at p≤0.05)

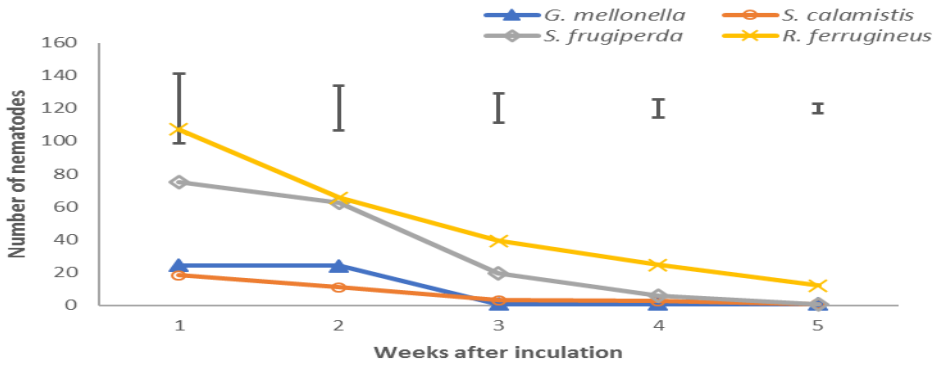


**Figure 3.** Weekly nematode harvest from insect cadavers over a five-week period after inoculation. Values of nematode numbers are square-root transformed; Bars represent standard error of means for each nematode per week

Generally, the number of nematodes harvested from the dead insect larvae declined throughout observation period (Fig. 3). By three weeks after inoculation no nematodes were recoverable from dead larvae of both *G. mellonella* and *S. calamistis* however, larvae were obtained in *R. ferrugineus* at five weeks after inoculation (Fig 3). This demonstrates the ability of the nematodes to be available for reinfection of more larvae in the community for up to three, four and five weeks respectively, in *S. calamistis*, *S. frugiperda* and *R. ferrugineus*, but with majority of the nematode being dispersed at one week after inoculation. Smits (1996) observed persistence of EPN for 2 to 6 weeks after field application. Furthermore, the findings of Ebssa and Koppenhöfer (2011) demonstrate that EPNs can persist to effectively manage the black cut worm in turf grass on golf fields. Extended duration of IJs ability to infect insect host is a very important trait in the biological control of pests.

With respect to EPN production of IJs from insect cadavers, *R. ferrugineus* had a significantly higher population of EPN 9407.00, > *G. mellonella* 5075.08, > *S.*

*frugiperda* 3957.23, > *S. calamistis* 742.31 (Table 1). There was no significant difference between the total EPN population generated from cadavers of both *G. mellonella* and *S. frugiperda*. However, *G. mellonella* had total EPN numbers that differed significantly from each of the EPNs from individual cadavers of *S. calamistis* and *R. ferrugineus*, with *R. ferrugineus* cadavers having the highest EPN yield (Table 1). Larvae in the non EPN control dishes did not yield harvestable EPN through to the end of the extraction process.

**Table 1**: Total entomopathogenic nematodes from insects' larval cadavers and reproductive factor (RF) of entomopathogenic nematodes in larval types

| Insect species | Total EPN Population | | Reproductive factor | |
|---|---|---|---|---|
| | Infected | Control | Infected | Control |
| *Galleria mellonella* | 71.43(5075.08)b | 0.71 (0.00)a* | 101.50a | 0.00a* |
| *Sesamia. calamistis* | 38.99(742.31)c | 0.71 (0.00)a* | 14.85c | 0.00a* |
| *Sposoptera frugiperda* | 62.14(3957.23)b | 0.71 (0.00)a* | 79.59a | 0.00a* |
| *Rhynchophorus ferrugineus* | 143.68(9407.00)a | 0.71 (0.00)a* | 67.24a | 0.00a* |

Mean values with the same letters within the column are not significantly different according to Tukey's Studentized Range Test at P > 0.05. Values are transformed means with actual mean values in parenthesis. * denotes significant difference between treatments in a row for each parameter. Reproductive factor = total population divided by initial population.

The initial numbers of IJs used to infect the test insects can influence the final yield of emerged IJs from the larval cadavers (Krishna, 2005), as observed with the significantly lower EPN yield from standard insect, *G. mellonella* inoculated with a lower level of inoculum, in contrast with the maximum EPN yield from *R. ferrugineus*. At the same time, *Sesamia calamistis* yielded low EPN population upon inoculation with a relatively low level of inoculum, the reason could be attributed to the small size of the larvae in addition to the older stage of instars based on the appearance of pupae shortly after inoculation. The highest populations of EPNs were recovered from infected *R. ferrugineus* because EPNs continued to recycle in them due to their larger size than other tested insect larvae. These findings imply that density of the initial inoculum, nature of host, age of larval instar and size of larvae can influence the yield of infective juveniles from the cadavers of infected larvae. This impacts on the ability of the EPN population to be sustained in the system in order to enhance the longevity of the management effect of the EPNs on the infected insect pests.

The greatest reproductive factor (RF), a measure of fecundity, of EPN was found in *G. mellonella,* followed by *S. frugiperda,* and *R. ferrugineus*, (Table 1). *S. calamistis*, displayed the lowest ($P \leq 0.05$) level EPN reproductive factor in dead larvae when compared to the EPN reproductive factor in all other insects' larval cadavers. Meanwhile, *Galleria* ranked in the category of highest EPN yielding larvae (significantly high reproductive factor) in the short-term period of extraction of harvestable nematode IJs. This conforms with the report of Grewal et al. (2001);

solidifying its use as the standard larvae for EPN inoculum production. It being not significantly different from the fecundity of EPN in *S. frugiperda* and *R. ferrugineus* implies that these two insect hosts can encourage the persistence of the pathogenic nematodes in the environment.

Entomopathogenic nematodes possess a unique combination of attributes that make them a promising alternative to synthetic chemical insecticides for pest control (Divya and Sankar, 2009; Abd-Elgawad, 2019). These nematodes have exceptional potential for biological control of insects as conferred by the symbiotic complex formed with bacteria of the genus *Xenorhabdus* associated with the *Steinernema* species and *Photorhabdus* with the *Heterorhabditis* species (Grewal et al., 2001; Kaya et al., 20006; Ardpairin et al., 2020) to achieve their successful parasitism and quick-kill effect on their insect host. This ability was evidenced in the high mortality observed on the test insects. The uniqueness of entomopathogenic nematodes can be further buttressed by their mass production mechanism from tissues (cadaver) of susceptible insects. The infective juveniles of these EPNs are motile, virulent with high reproductive potential and possess the ability to disperse from the dead host to infect new healthy ones (Krishna, 2005; Ramakuwela, 2014; Assefa and Ayalew, 2019). This explains the rapid multiplication of the offspring (infective juveniles) in the parasitized larval cadavers and their subsequent release from the cadaver's dead tissues.

From the present research, it can be concluded that the native/indigenous Nigerian population of entomopathogenic nematodes are potentially effective as a bio-control agent against lepidopterous insect pests *Sesamia calamistis* and *Spodoptera frugiperda* insect larvae and a coleopteran destructive palm pest *Rhynchoporus ferrugineus* larvae. Production of several generations of EPNs in insect cadaver is possible, when there is sufficient food (large-tissue cadaver), as observed for *R. ferrugineus*, to nourish the developing infective juveniles. However, further studies should be conducted to evaluate the efficacy of entomopathogenic nematodes on insect pests of different crops under screen house and field conditions.

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