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Effectiveness of Entomopathogenic Nematodes against the African White Rice Stem Borer *Maliarpha separatella* Rag

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Abstract The efficacy of three entomopathogenic nematodes (EPN) species *Steinernema carpocapsae* Weiser, *Steinernema karii* Waturu, Hunt & Reid, *Heterorhabdtids indica* Poinar, Karunaka & David and two EPN isolates collected from Coastal Kenya (*EX MOMBASA*) and Rift valley (*EX NAKURU*) was evaluated against larvae of African white rice stem borer, *Maliarpha separatella* Rag. The experiment was carried out using no- choice modified filter paper bioassay at KARI-Mwea. Whatman filter paper was substituted with white cotton cloth discs. The activity of the biological agents under study was determined at 25°C and 65% relative humidity, with concentrations of 50, 100 and 200 infective juveniles (IJs) per one *M. separatella* third instar larva. The larvae were confined in 60mm plastic petri dishes in darkness as the larvae spend their entire life inside the rice stems. The mortality rate was determined at 24, 48, 72 and 96 hours after application of the nematode suspension. Significant virulence was obtained with all the nematode species at 200 infective juveniles (IJs). There was low mortality at 50 and 100 concentration rates. All the EPNs significantly reduced *M. separatella* larvae after 48 hours in the following order *H. indica> EX NAKURU> S. carpocapsae> EX MOMBASA* >S. *karii*. The number of nematodes from the infected cadavers after 48 hours post infection was *H. indica* (553), *EX –MSA* (294), *EX-NKU* (242), *S. karii* (168) and *S. carpocapsae* (157). *S. karii* took the longest time (96 hours) to kill all the test insects. In conclusion, the study shows that EPNs are effective against *M. separatella* and could be used within an integrated pest management strategy for the pest. There is need to carry out further studies to determine effective dosages under field conditions.

Keywords Entomopathogenic Nematodes, Efficacy, African White Rice Stem Borer, *Maliarpha separatella*

1. Introduction

The control of pests and diseases including *Maliarpha separatella* at Mwea irrigation scheme is through use of pesticides following a calendar based spraying regime developed by Mwea Integrated Agricultural Development Centre (MIAD). However, use of pesticides is associated with various problems. For farmers, the most serious are the acquisition of pest resistance to the chemicals, secondary pest outbreaks, and health hazards associated with the application of chemicals. For consumers, the main problems are pesticide residues in food and environmental degradation [16]. Due to these factors, research towards developing alternative control strategies is warranted and the use of entomopathogenic nematodes (EPNs) in irrigated rice ecologies offers a viable alternative. Most of EPN formulations used in biological control of insect pests are from two Families namely Steinernematidae and Heterorhabditidae in order Rhabtida [1]. The formulations consist of third stage infective juvenile (sometimes referred to as IJ or dauer). It is non-feeding, developmentally arrested and the only EPN life stage that exists outside the host insect. The IJ seeks insect hosts, and after entering, they release an associated mutualistic bacterium, *Xenorhabdus* for Steinerernematids and *Photorhabdus* for Heterorhabditids, respectively [1]. The nematode-bacterium complex usually causes host mortality within 24- 48 hours [2]. The nematodes provide shelter to the bacteria, which, in turn, kill the insect host and provide nutrients to the nematode [1]. The bacteria

produce pigments so that insects infected by Heterorhabtidis turn brick red or maroon colour and those infected with Steinernematids turn ochre, tan or brown [9]. All nematode-infected insect cadavers have a distinct firm and rubbery consistency, and stay intact for more than a week, while the nematodes complete their life cycle [9].

In Kenya EPNs have been used to control insect pests [14], [15], [10], [19], but none have been tested against *M. separatella.* The aim of this research was to determine the efficacy of three EPN species, *Steinernema carpocapsae, Steinernema karii, Heterorhabditis indica* and two strains collected from Coastal Kenya (*EX MOMBASA*) and from Rift valley (*EX NAKURU*), against the larvae of *M. separatella*.

2. Materials and Methods

The study was carried out at Kenya Agricultural Research Station, Mwea (37.36502 E, 0.62153S) entomology laboratory on 25-28 October 2010. Cultures of three entomopathogenic nematode species and two isolates were used. These were from colonies maintained in the laboratory and included *S. carpocapsae, S. karii*, *H. indica* and two EPN isolates collected from Coastal Kenya *(EX MOMBASA*) isolate [19] and from Rift valley *(EX NAKURU)* isolate [13]. Infective juveniles (IJs) of the five isolates were cultured in the last in- star of the greater wax moth, *Galleria mellonella* L. at 20-22^{0}C 60-65% relative humidity. The emerging IJs were harvested from White traps and stored in distilled water at 10^{0}C.

Maliarpha separatella third instar larvae were used for the assay. They were maintained on an artificial diet modified by the author at KARI-Katumani insectary. The colony had been established by collecting *M. separatella* eggs from rice fields at Mwea rice irrigation scheme and maintained on an artificial stem borer rearing diet.

The diet ingredients and procedure for diet preparation was adopted from the method Songa *et al* for artificial rearing of cereal stem borers [18]. Diet was modified by substituting sorghum leaf powder by rice leaf powder prepared from leaves of six weeks old rice plants which were dried and ground into a fine powder. The ingredients and the ratios used for 1.5 liter diet were: distilled water (80.1%), Brewer's yeast (2.3%), sorbic acid (0.13%), methyl-p-hydroxylbenzoate (0.2%), ascorbic acid (0.25%), vitamin E capsules (0.2%), rice leaf powder (2.5%), Bean (*Phaseolus vulgaris*) powder (8.8%), sucrose (3.5%), agar (Tech No 3) powder (1.3%), formaldehyde 40% (0.2%). and Grabacin (0.2%).

Maliarpha separatella larvae collected from infested rice fields were then introduced into diet at the rate 20 larvae per jar, where they developed up to adult moth emergence. The emerging moths were sexed and five pairs introduced into oviposition cages which were lined with wax paper. The female moths laid eggs on the wax paper. The sections of the wax paper with eggs were then cut out, put in sterilized Sterlin® plastic petridishes and incubated at 30^0 C for 24 hrs up to black head egg stage. The black heads were surface sterilized by 70% ethanol and introduced into the artificial rearing diet. They were then allowed to develop into 3rd instar larvae and used in the bioassay.

3. Entomopathogenic Nematodes Evaluation

Contact filter paper bioassays against *Maliarpha separatella* using the five entomopathogenic nematodes isolates were set up at KARI-Mwea entomology laboratory. Standard filter paper bioassays have been used before to evaluate EPNs [20] but in the present study white cotton cloth discs were used instead of the Whatman® filter paper.

Suspensions of EPNs were first agitated by blowing into the suspension by a pipette and the equivalent aliquots of nematode concentrations were drawn.

Treatments consisted of three concentrations of entomopathogenic nematodes (EPN), 50, 100 and 200 IJs and an untreated control. One ml of nematode suspension was added to each Sterlin ® 60mm plastic petridish containing a folded cotton cloth disc using a pipette, with the tip being changed after every treatment. In the control treatments 1 ml of distilled water without nematodes was added to the cotton cloth discs. One *M. separatella* 3rd instar larva was then introduced on top of cloth disc which was already moist with the nematode suspension making sure that the larvae did not drown. They were closed and incubated at temperatures of 25^{0}C±2^0 and 65-75% relative humidity. The experiment was laid out in a completely randomized design with 10 replicates for each nematode isolate at the test concentrations. Observations were done on 24, 48, 72 and 96 hours after *M. separatella* larva introduction. On each of these observations mortality was measured and *M. separatella* cadavers observed for firm rubbery consistency and colour change (brick red or maroon colour for *Heterorhabtidis* and ochre, tan or brown for *Steinernematids* [9] which indicate nematode infectivity. They were then put in 1% Ringer's salt solution and dissected under a binocular microscope in an engraved Petri dish. The number of first generation nematodes was counted using a tally counter under low power binocular microscope. Analysis of variance (ANOVA) was conducted to determine the differences in mortality rates. Least significant differences were used to separate means when found significant at p=0.05. The statistical analysis was performed using Genstat Version 12 statistical software [7]. Probit regression analysis was performed on the data to estimate effective concentration and lethal periods following the method of Finney [6].

4. Results

Probit curve showed *Heteterorhabitidis indica* as the EPN requiring the least concentration to cause 100% *M. separatella* mortality while *S. karii*, *S. carpocapsae* and *EX MOMBASA* required slightly higher than 200 IJs to kill all the test insects. The order of virulence was *H. indica> EX NAKURU> S. carpocapsae> EX MOMBASA >S. karii* (Fig 1)

All EPNs were effective against *M. separatella* within 48 hours of infection. *EX-NAKURU* had the earliest median LT50, while *S. karii* took the longest time to kill all the test insects (Figure 2)

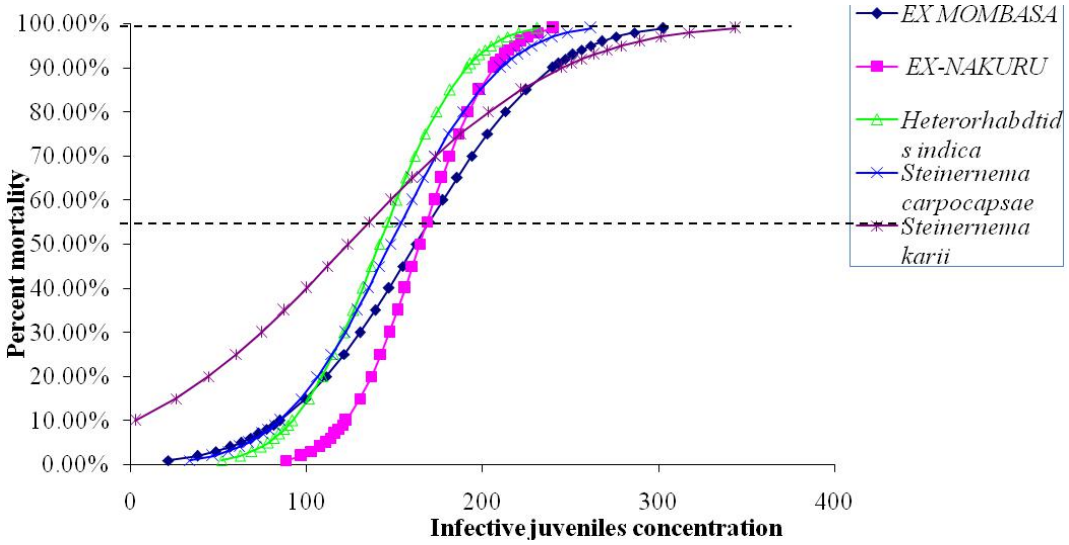

Figure 1. Median lethal concentration (LC_{50}) and LC_{90} of different EPN isolates to *M. separatella* larvae.

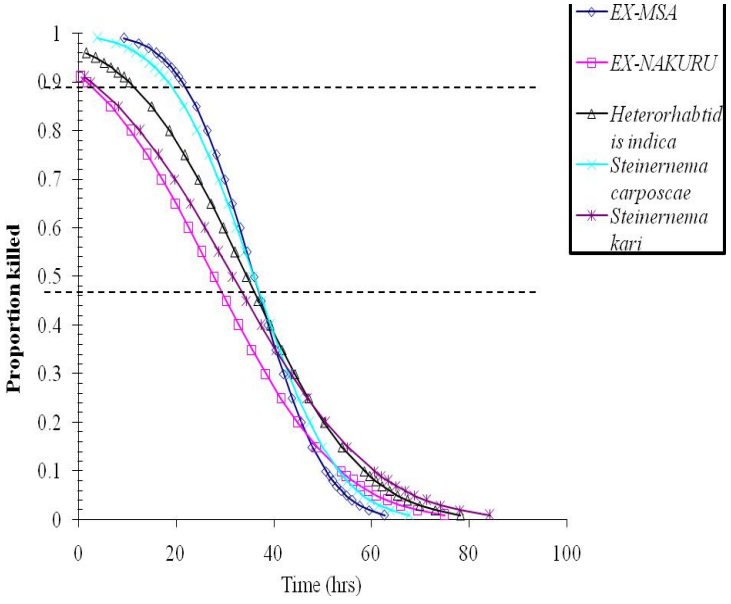

Figure 2. Median lethal time (LT 50) and LT 90 of *M. separatella* larva after infection by different EPN isolates

Heterorhabtidis indica had the highest number of first generation EPNs in *M. separatella* cadavers after 48 hours post infection followed by EX-MSA, while the least number of EPNs was in *Steinernema carpocapsae* (Table 1).

Table 1. The mean number of first generation Entomopathogenic nematodes in M. separatella host cadavers after 48 hours post infection.

Isolate	Number of EPNs per *M. separatella* cadaver (Mean±SE)
Steinernema carpocapsae	157.23±0.64
Steinernema karii	168.19±0.81
Heterorhabdtidis indica	552.93±0.74
EX-NKU	242.00±0.90
EX-MSA	293.56±0.66
P	0.33
CV (%)	48.7

5. Discussion

The results of this study indicated that *Heterorhabtidis indica* nematode was effective against the African white rice stem borer *M. separatella* at higher concentrations (200 IJs/larva) and caused host mortality within 24 to 48 hours after infection. These results are in agreement with most studies on EPNs which indicate that they kill insect pests within one to two days [4] . There were significant differences in infectivity among the test entomopathogenic isolates. These results are consistent with the findings of Ellis and co-authors who found from screening bioassays of 10 nematode isolates against small hive beetle, *Aethina tumida* that nematode efficacy varied significantly among the tested nematode species [5]. The efficacy was also affected by the number of the applied infective juveniles (IJs). Nyasani and co-workers found significant differences in the efficacy of different entomopathogenic nematodes against diamond back moth [15]. Past studies indicate that EPNs are effective when well matched with host arthropod pests [8] and that different species of entomopathogenic nematodes vary in the range of the insects they attack and their environmental needs[17] . The high mortality caused by *H. indica* which also had the highest multiplication rate in *M. separatella* host cadavers is in agreement with the findings of Shapiro-Ilan and Gaugler who demonstrated that invasion and reproduction in hosts by different nematode species varied quantitatively [17]. Menti and co-workers reported that *Heterorhabditis spp* often infect at lower rates than *Steinernema spp* but comparative mortality is often similar or higher as was found out in this study [12]. The discrepancy in infectivity between Heterorhabditids and Steinernematids may be attributed to differences in the time of establishment of the symbiotic bacteria in the insect host.

The most effective entompathogenic nematode was *H. indica*. It is heat tolerant, infecting insects at 30°C or higher. The nematode produces high yields in vivo and in vitro, but shelf life is generally shorter than most of the other nematode species. Nderitu and co-workers also found this EPN to have significant efficacy against *Cylas puncticollis* in sweet potato fields at Kibwezi in Kenya [14]. Similarly, Mahar and others in a study to control nymphs of desert locust *Schistocerca gregaria* found that Heterorhabditids (*H. indica* and *H. bacteriophora*) were more effective as compared to Steinernematids (*S. carpocapsae*, *S. feltiae*) at 30 degrees centigrade [11] and that the highest concentration of each isolate (200 IJs per ml) proved to be most appropriate for maximum insect death. In the current study most of the nematodes had high chances of penetrating the host and this is supported by reports by Declan and co-authors who when screening *S. feltiae* and *S. carpocapsae* against *Plectrodera scalator* reported that filter paper bioassays killed 50-58% of larvae but the mortality in diet cup bioassay was less than 10% [3].

The period of exposure and concentration were important factors for the activity of the nematodes. This can possibly be explained by the fact that the number of EPNs penetrating the host is influenced by the number of invasive nematodes which earlier managed to penetrate it. The results of this research show that application of entomopathogenic nematodes is a possible way for controlling *M. separatella* but there is need to optimize environmental factors to improve efficacy under field conditions.

6. Conclusions

- *Heterorhabtidis indica* caused the highest mortality and also had the highest multiplication rate in *M. separatella* host cadavers.
- Application of *Heterorhabtidis indica* was able to control *M. separatella*
- *Heterorhabdtids indica* nematode was effective against African white rice stem borer *Maliarpha separatella* at higher concentrations (200 IJs/larva) and was heat tolerant, infecting insects at 30°C or higher

7. Recommendations

Heterorhabdtids indica which was the species with the highest number of penetrated nematodes in host cadavers can be used for *M. separatella* management. However, there is need to optimize environmental factors and use the most appropriate method for application of this species

under field conditions.

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