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Pathogenicity of Selected Native Entomopathogenic Nematodes against Tomato Leaf Miner *(Tuta Absoluta)* in Kenya

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Abstract Tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has been an important pest in Kenya since it was reported in 2014. It is adversely affecting tomato production in the country. The objective of this study was to evaluate the pathogenicity of Kenyan EPNs namely; *Heterorhabdities sp.* and *Steinernema karii* against *Tuta absoluta* larvae under laboratory conditions in petri dish bioassays. Entomopathogenic nematodes were obtained from Kenya Agricultural and Livestock Research Organization EPNs laboratories and *Tuta absoluta* larvae were obtained from a colony reared and maintained in a greenhouse at Kabete Campus Field Station, Nairobi. The effect of EPNs concentrations on *Tuta absoluta* larvae mortality exposed for 24-72 hours was evaluated. An experiment laid out in a complete randomized design with four replicates was conducted. The results showed that the evaluated concentration rates of *Heterorhabditis sp.* and *Steinernema karii* at 100, 300 and 500 Ijs/ml significantly ($p < 0.05$) caused mortality on *Tuta absoluta* larvae compared to control and that the highest mortality was recorded at 500 Ijs/ml having been exposed for 72 hours. *Steinernema karii* was more pathogenic compared to *Heterorhabditis sp.* throughout the exposure period of 24-72 hours, having achieved 100% and 91.5% larval mortality, respectively. This study demonstrates that native EPNs have a potential for management of the tomato leaf miner *(Tuta absoluta)* which can be exploited.

Keywords: *bio-control, Heterorhabdities sp., Steinernema karii, Tuta absoluta, tomato*

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1. Introduction

Tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a new pest in Kenya affecting tomato production in open fields and in greenhouses [1]. *Tuta absoluta* is a serious pest primarily in tomatoes but, is also reported in Irish potatoes and even solanaceous weeds [2]. Reproduction potential of *Tuta absoluta* is very rapid with a lifecycle ranging from 24 days at 25 ^{0}C to 38 days in 19 ^{0}C [3]. The larvae are the most destructive stage of the pest causing damage by mining on the stem, fruits and leaves producing large galleries as they feed on mesophyll tissue [4]. Potential tomato yield loss can reach up to 100% if the pest is not managed [3]. Various cultural methods including crop removal and the selective removal and destruction of infested plant materials have not been effective in the management of *Tuta absoluta* [5]. Chemical control is commonly used by farmers for pest management but can get rid of the natural enemies in the agro-ecosystem, can contaminate environment and cause pesticide resistance development in pest populations [6]. Pesticide resistance of *Tuta absoluta* to a wide range of pesticides including Bifethrin, Diflubenzuron, Permethrin, Teflubenzuron and Triflumuron has been reported [7]. There is a likelihood that a similar situation may occur in Kenya since the pest is present in tomato growing areas and farmers mainly use synthetic pesticides for management. To curb the increasing resistance of this pest to chemical pesticides, there is need to put emphasis on the importance of IPM strategies for proper management of *Tuta absoluta.* Bio-control is a component in IPM Programs which can be used to reduce over reliance on synthetic pesticides [8]. Use of EPNs as bio-control agents is an environmentally friendly approach in the management of *Tuta absoluta.* The benefits of using EPNs include; the ability to produce them in large numbers, they have low impact on non-target species, application is safe and they can be implemented in an IPM system [9,10]. The EPNs used in this study were first reported in Kenya in a survey conducted in the central highlands and coastal areas of the country where a total of 154 nematodes were isolated among them the new species *Steinernema karii* which was identified by Waturu [11] and [12]. Currently 33 nematode isolates are maintained in three laboratories at KALRO (Thika, Mwea and Kabete). Entomopathogenic nematodes are soft bodied, non-segmented roundworms that are obligate or sometimes facultative parasites of insects [13]. The objective of this study was to determine

the most pathogenic nematode species against *Tuta absoluta* larvae under laboratory conditions.

2. Materials and Methods

2.1. Study Site Selection

This study was carried out in Entomology laboratories at KALRO (Horticulture Research Institute) Thika. The institute is located 43 kilometers North East of Nairobi City Centre and lies at latitude 0° 59'South and longitude 37° 04'East and at an altitude of 1548 metres above sea level.

2.2. Source of EPNs

The EPNs species used in this experiment were originally collected from previous local surveys in the central highlands of Kenya and maintained in (KALRO) Horticulture Research Institute- Thika Entomopathogenic nematodes laboratories.

Multiplication of the EPNs was done by use of the in-vivo method or the insect-bait technique with *Galleria mellonella* L. (Lepidoptera: Pyralidae) as it produces good quality nematodes [10]. The nematodes were reared at 25°C in the third instar larvae of the greater wax moth, *Galleria mellonella* according to the method of Woodring [14]. *Galleria mellonella* was used because of; its susceptibility, rich nutrient source available in body, its high multiplication potential and its ability to be reared easily on semi-synthetic diet source containing maize flour (307 grams), honey (225 grams), brewer's yeast (90 grams) and bee wax (45 grams) for a single diet [15].

Twenty petri dishes (9cm diameter) were lined with whatman filter paper no. 1 and the nematodes *Steinernema karii* and *Heterorhabdities sp.* applied at the rate of 100ijs per dish in ten dishes for each nematode species. The nematodes were applied in 1ml distilled water. Two *Galleria mellonella* larvae were placed in each petri dish and incubated at 25°C in darkness. This allowed EPNs to penetrate the larvae and multiply. After 3-4 days the cadaver were removed and put into a white trap for extraction of emerging EPNs [9]. A white trap consisted of a 15 cm diameter dish and a lid of a 9 cm diameter Petri dish placed at the centre, then covered by a 15 cm diameter white muslin cloth. The emerged Ijs were harvested and washed three times in distilled water. The Ijs suspensions were stored at room temperature separately in 1 liter plastic boxes before being stored at 10°C [16].

2.3. Source of *Tuta absoluta* Larvae

The *Tuta absoluta* larvae used in this experiment were obtained from a colony reared and maintained at entomology division in tomato crops in a greenhouse in Kabete Campus Field Station, University of Nairobi which is located 15km North West of Nairobi City Centre and lies at latitude 1° 15'S and longitude 36° 44'E and at an altitude 1941 metres above sea level.

2.4. Pathogenicity bioassay under laboratory conditions

Insect mortality bioassays were conducted with two different species of Kenyan EPNs namely; *Steinernema karii* and *Heterorhabdities sp.* Four, *Tuta absoluta* larvae were placed in 9cm diameter filter paper padded petri dishes where 1ml of nematode suspension containing 100 Ijs/ml for each species were applied. The same was repeated for 300 Ijs/ml and 500 Ijs/ml treatments. Tomato leaves washed with distilled water were placed inside the petri dishes as a source of nourishment for the *Tuta absoluta* larvae. The petri dishes were sealed with perforated lids for air circulation and incubated in a dark chamber at room temperature. The experiment was laid out in a complete randomized design with four replicates and repeated twice. For the control no Ijs were added apart from distilled water to wet the filter papers before placing the *Tuta absoluta* larvae and washed tomato leaves in the petri dishes. Data on mortality was collected after 24, 48 and 72 hours from the start of the experiment. Dead larvae were individually dissected under a stereomicroscope using tweezers and needles by destroying the cadavers in petri dishes with quarter-strength Ringer solution to confirm presence of EPNs.

2.5. Statistical Analysis

Larval mortality data collected was subjected to analysis of variance (ANOVA) to assess treatment effects while the Fisher's protected least significance difference (LSD) test was used to compare treatment means. Analysis was performed using GenStat- PC v.14.1, 14th Edition [17].

3. Results

3.1. Effect of EPNs Concentrations and Exposure Time on *Tuta absoluta* Larval Mortality in Bioassay One

The two species of EPNs tested were able to affect and kill *Tuta absoluta* larvae under laboratory conditions (Figure 1). Exposure time period and EPNs concentration had a significant ($p < 0.05$) effect on the larval mortality of *Tuta absoluta. Steinernema karii* and *Heterorhabditis sp.* displayed increased pathogenicity with increase in exposure time and the number of Ijs applied (Figure 1 a, b, c). S*teinernema karii* significantly ($p < 0.05$) killed more *Tuta absoluta* larvae than *Heterorhabditis sp.* after 24 hours exposure period and in all the tested concentrations. However, there was no significant difference between *Heterorhabditis sp.* at 100 Ijs/ml concentrations and control (Figure 1 a). The two EPNs were not significantly ($p < 0.05$) different at 300 Ijs/ml concentration. At the highest nematode concentration (500 Ijs/ml) both EPNs species tested attained significantly ($p < 0.05$) higher mortality compared to control (Figure 1 a).

After 48 hours exposure period, higher larval mortality was obtained compared with mortality rate attained after 24 hours exposure period. However, at the lowest concentration (100 Ijs/ml) there was no difference

between the two EPNs tested and were not significantly ($p < 0.05$) different from control (Figure 1 b). At the 300 Ijs/ml concentrations, no significance difference was observed between *Steinernema karii* and *Heterorhabditis sp.* However, percentage mortality significantly differed ($p < 0.05$) between the two tested EPNs species and control. At the 500 Ijs/ml concentrations, *Steinernema karii* caused significantly ($p < 0.05$) higher mortality than *Heterorhabditis sp.* (Figure 1 b).

At 72 hours exposure period, no difference was observed between the two EPNs species tested at 100 Ijs/ml concentrations, but the two were significantly different ($p < 0.05$) from control (Figure 1 c). At 300 Ijs/ml and 500 Ijs/ml *Steinernema karii* caused significantly ($p < 0.05$) higher mortality compared to *Heterorhabditis sp.* Moreover, at the highest nematode concentration (500 Ijs/ml), *Steinernema karii* caused 100% mortality compared to 90.8% that of *Heterorhabditis sp.* (Figure 1 c)

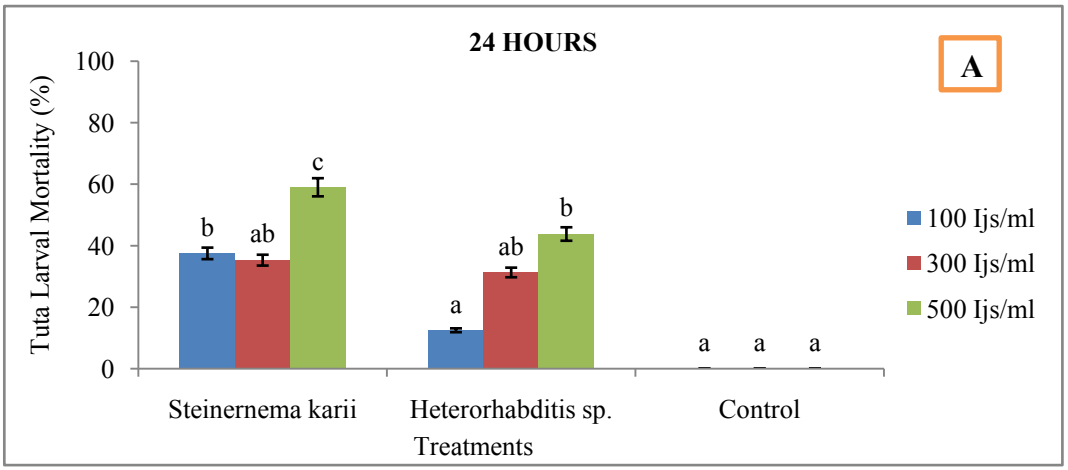

Figure 1 a. Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp.* for a period of 24 hours (A). Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$)

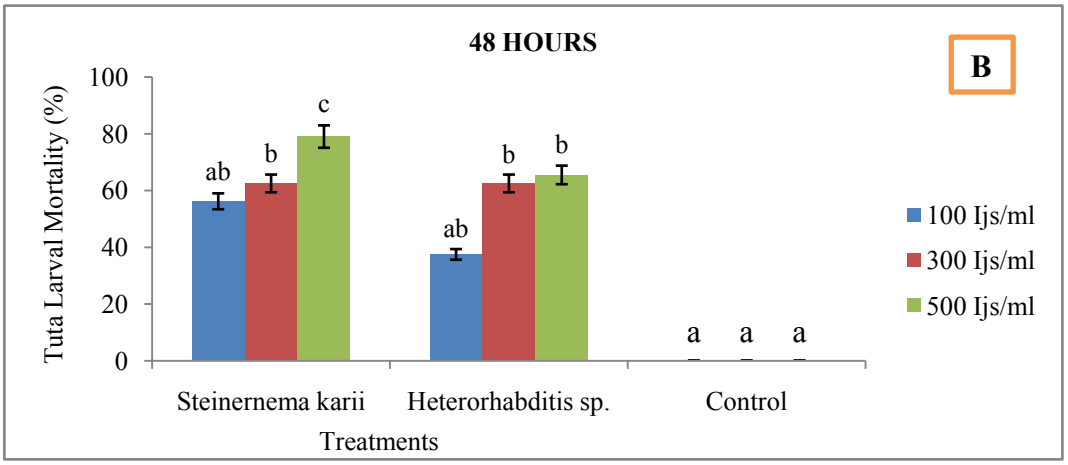

Figure 1 b. Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp.* for a period of 48 hours (B). Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$)

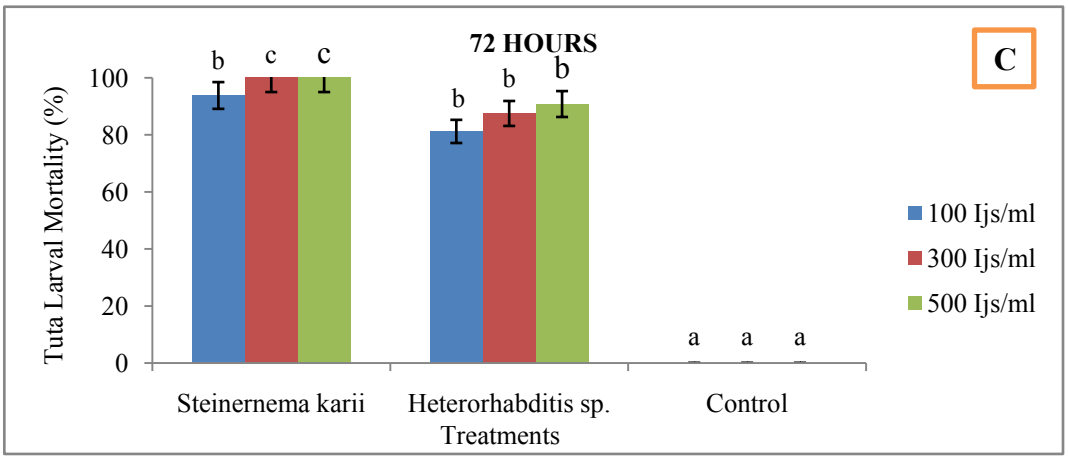

Figure 1 c. Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp.* for a period of 72 hours (C). Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$)

3.2. Effect of EPNs Concentrations and Exposure Time on *Tuta absoluta* Larval Mortality in Bioassay Two

In bioassay two, EPNs concentrations and exposure period (24-72 hours) had a significant ($p < 0.05$) effect on *Tuta absoluta* larvae mortality. *Steinernema karii* and *Heterorhabditis sp.* displayed increased virulence with increase in exposure time and the number of Ijs applied (Figure 2. a, b, c), and displayed a similar trend as that observed in bioassay one. At 24 hours exposure period, *Steinernema karii* attained higher mortality than *Heterorhabditis sp.* but, there was no difference in mortality achieved by the two EPNs tested at 100 Ijs/ml concentrations. *Steinernema karii* significantly ($p < 0.05$) killed more *Tuta absoluta* at 500Ijs/ml compared to 300 Ijs/ml while, there was no difference in percent kill for *Heterorhabditis sp* at both concentrations (Figure 2 a).

After 48 hours exposure period, the two EPNs species tested attained significantly ($p < 0.05$) higher larvae mortality than control. At 100 Ijs/ml and 300 Ijs/ml, *Steinernema karii* showed numerically higher mortality than *Heterorhabditis sp* but, no significant different was observed between the two concentrations. At 500 Ijs/ml *Steinernema karii* caused higher mortality than *Heterorhabditis sp.* and was significantly ($p < 0.05$) different from the lower Ijs concentrations tested (Figure 2 b). During the same period *Heterorhabditis sp.* at all the concentrations did not differ in the percent mortality achieved although the 500 Ijs/ml had the highest kill.

At the highest exposure time (72 hours), the two EPNs species tested attained the highest mortality of *Tuta absoluta* larvae which was significantly different ($p < 0.05$) from control (Figure 2 c). At the highest EPNs concentration (500 Ijs/ml) *Steinernema karii* significantly ($p < 0.05$) caused a high mortality (100%) compared to lower concentrations (100 Ijs/ml and 300 Ijs/ml) At all the concentrations tested *Heterorhabditis sp.* achieved a percentage kill that was not significantly different among them. However, 500 Ijs/ml achieved the highest mortality at 91.5% (Figure 2 c).

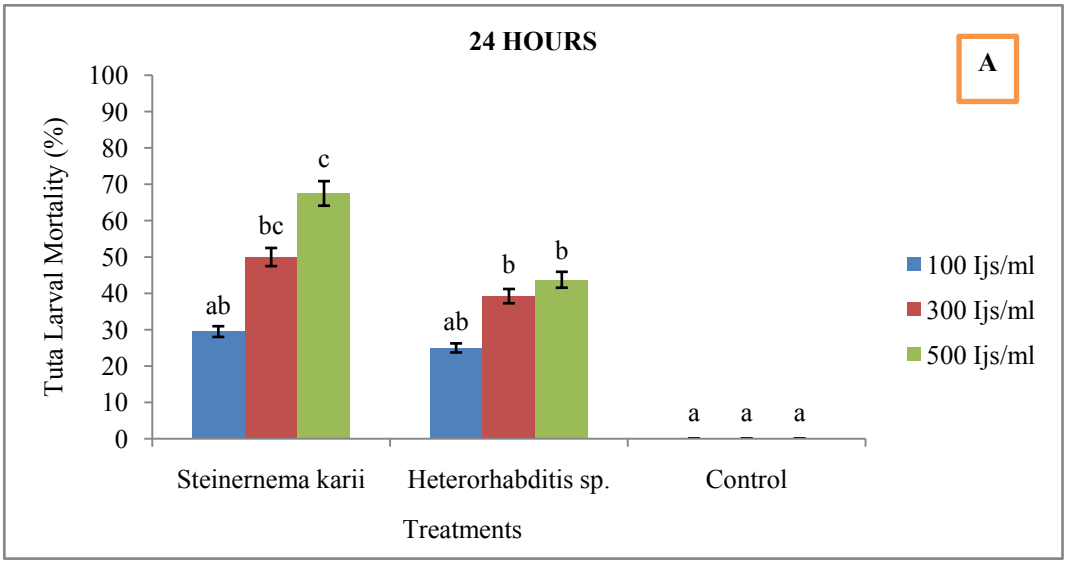

Figure 2 a. Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp.* for a period of 24 hours (A). Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$)

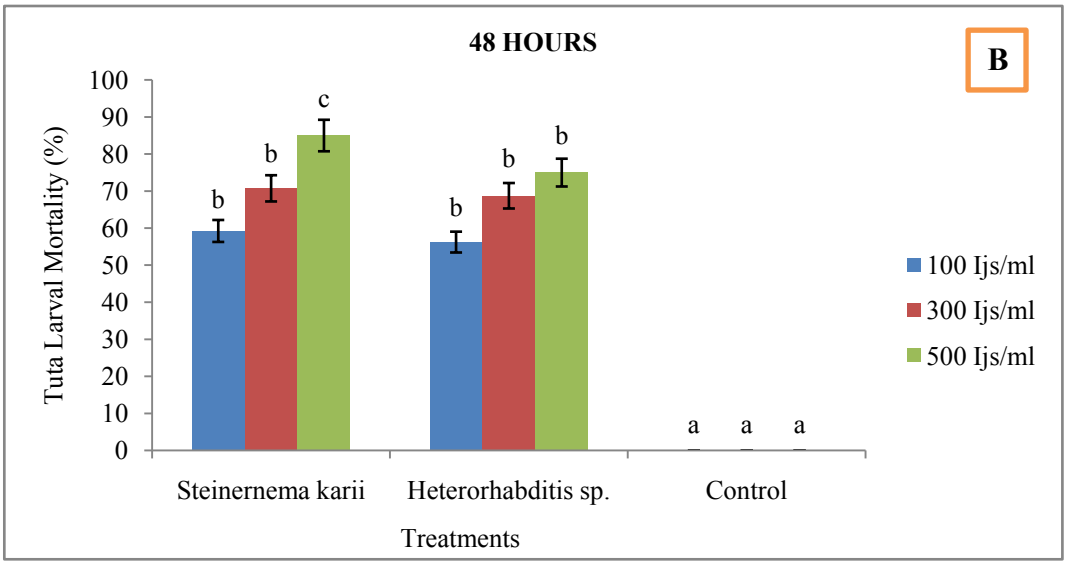

Figure 2 b. Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp.* for a period of 48 hours (B). Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$)

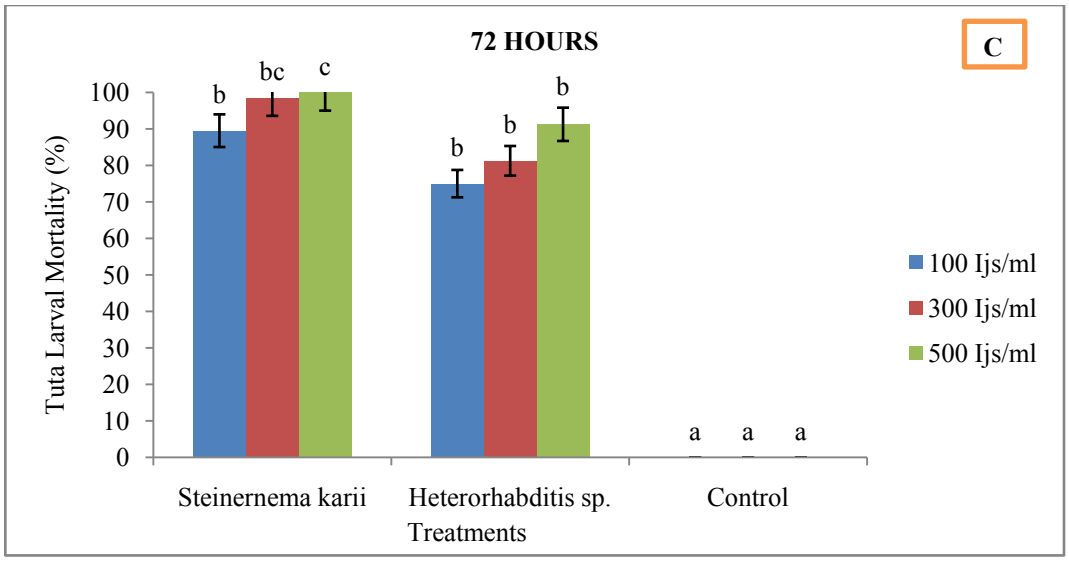

Figure 2 c. Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp.* for a period of 72 hours (C). Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$)

3. Discussion

In this study it is evident that *Heterorhabditis sp.* and *Steinernema karii* were able to kill *Tuta absoluta* larvae at various concentrations namely 100, 300, and 500 Ijs/ml. *Tuta absoluta* larvae mortality increased with increase in concentrations. In this case the highest concentration tested (500 Ijs/ml) achieved the highest mortality under laboratory conditions. This mortality increase with increase in concentration can be attributed to large population of symbiotic bacteria released by EPNs when they penetrate the larvae as reported by Eleftherianos [18]. Steinernematid Ijs retain *Xenorhabdus* symbionts within an intestinal vesicle, while *Photorhabdus* cells stick together in the anterior part of the Heterorhabditids gut and releases them upon invasion of an insect host [19]. Once inside the host nematodes releases bacteria which kills the larvae through production of toxins and hydrolytic enzymes, digesting internal contents of host and in turn provide nourishment to EPNs [20]. This agrees with the study by Nderitu [21] who reported that increasing the dosage of EPNs usually increased the larval mortality rate in the management of banana weevil *(Cosmopolites sordidus)*. Exposure time periods 24, 48 and 72 hours had an effect on the mortality of *Tuta absoluta* larvae. The highest larvae mortality was recorded after 72 hours of exposure while using the highest concentration of infective juveniles (500 Ijs/ml). It is probable the longer the time period and the more the Ijs available, the EPNs were able to search and infect the larvae after which they released bacteria which caused toxicity which led to mortality. *Steinernema karii* was more virulent probably due to large population of the symbiotic bacteria cells carried by dauers in their gut, causing high pathogenicity as reported by Koppernhoofer [22]. Research by Emelianoff [23] revealed that *Steinernema scapterisci* contains low amount of bacterial cells compared to *Steinernema carpocapsae* that has a large number of bacterial cells, causing higher pathogenicity. The high virulence of *Steinernema karii* compared to *Heterorhabditis sp.* can also be attributed to their efficiency in the invasion of their hosts as reported by Epsky [24] and Caroli [25]. This agrees with a study by Lacey [9] who reported that, besides the high mortality caused by EPNs in *Tuta absoluta* larvae, an additional beneficial trait is their speed to kill, usually within 48-72 hours. Among the evaluated EPNs against *Tuta absoluta* larvae *Steinernema karii* was more pathogenic compared to *Heterorhabditis sp.* in all the exposure time period and concentrations tested, having achieved 100% and 91.5% mortality, respectively. This can be attributed to large number of bacteria species associated with Steinernematids enhancing their pathogenicity compared to Heterorhabditids. This agrees with a study by Stock [26] and Nguyen [27] who reported that there are three species of bacteria recognized within the *Photorhabdus* genus that colonizes *heterorhabditis* nematode host of which there are eighteen recognized species compared to twenty two species of *Xenorhabdus* that colonize one or more, of the more than seventy known species of *Steinernema* nematodes. The ideal laboratory conditions in this study made it easier for EPNs to interact with their host. Despite this *Steinernema karii* caused higher *Tuta absoluta* larval mortality than *Heterorhabditis sp.* in all the exposure time periods and concentrations tested. This can be attributed to the ability to locate and infect the host by probably adopting the crushing and ambushing strategies for host finding as reported by Lewis [28]. According to Grewal [29] *Steinernema feltiae* has adopted both ambushing and cruising strategies for host finding making it more virulent than *Heterorhabditis sp.* The findings in this study compares with those of Van Niekerk [30] who on conducting various bioassays to determine the potential of South African EPNs isolates in controlling *Planococcus citri* (Risso) the citrus mealybug, reported *P. citri* was most susceptible to *Steinernema yirgalemense* (97% mortality) compared to *Heterorhabditis zealandica* (91% mortality). *Steinernema yirgalemense* was more pathogenic and was faster at locating and infecting *P. citri*. [30].

4. Conclusion

Under the laboratory conditions, *Steinernema karii* and *Heterorhabditis sp.* were able to infect and kill *Tuta absoluta* larvae with the highest mortality recorded at 500

Ijs/ml concentrations after an exposure period of 72 hours. *Steinernema karii* was more pathogenic and infective compared to *Heterorhabditis sp.* The results from this study demonstrate the potential of Kenyan EPNs that could be exploited for tomato leaf miner management. More research should be carried out to evaluate appropriate concentrations and efficacy of Kenyan EPNs against tomato leaf miner (*Tuta absoluta)* in greenhouse and open field farmer conditions.

Acknowledgements

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Statement of competing interests

The authors had no competing interests.

List of Abbreviations

ANOVA Analysis of Variance
CABI Centre for Agriculture and Biosciences International
EPNs Entomopathogenic Nematodes
GOK Government of Kenya
ICIPE International Centre of Insect Physiology and Ecology
IPPC International Plant Protection Convention
IJs Juveniles
IPM Integrated Pest Management
KALRO Kenya Agricultural and Livestock Research Organization
LSD Least Significant Difference.

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