Potential use of Kenyan Entomopathogenic Nematodes and Neem *(Azadirachta indica)* for the Sustainable Management of Tomato Leaf Miner *(Tuta absoluta)*

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Declaration

Student Declaration

This thesis is my original work and has not been presented for a degree in any other university.

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Dedication

To my dear wife (Rachel) and my son (Marx), my parents Mr. Eustace Mutegi and Mrs. Josephine Mutegi.

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List of Abbreviations

ANOVA	Analysis of Variance
CABI	Centre for Agriculture and Biosciences International
EPNs	Entomopathogenic Nematodes
EPPO	European Plant Protection Organization
FAO	Food and Agriculture Organization of the United Nations
GOK	Government of Kenya
HCDA	Horticultural Crops Development Authority
IAPPS	International Association for the Plant Protection Sciences
ICIPE	International Centre of Insect Physiology and Ecology
IJs	Infective Juveniles
IPM	Integrated Pest Management
IPPC	International Plant Protection Convention
KALRO	Kenya Agricultural and Livestock Research Organization
LSD	Least Significant Difference
®	Registered trade mark
SC	Soluble Concentrate

Abstract

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) was reported in Kenya in 2014 and has become a devastating pest of tomato crop in both the field and in the greenhouse. The objectives of this study were; to determine the pathogenicity of *Heterorhabdities species* and *Steinernema karii* nematodes against tomato leaf miner in laboratory conditions; to evaluate the insecticidal effects of neem biopesticide against tomato leaf miner in the greenhouse and to evaluate the effectiveness of entomopathogenic nematodes and neem combined with entomopathogenic nematodes as management options for tomato leaf miner in the greenhouse conditions. Entomopathogenic nematodes (EPNs) used in the study were obtained from Kenya Agricultural and Livestock Research Organization (KALRO) entomopathogenic nematodes laboratories and *Tuta absoluta* larvae were obtained from a colony reared and maintained in a greenhouse at Kabete Campus Field Station, Nairobi. Multiplication of the EPNs was done by *in-vivo* method or the insect-bait technique with the third instar of greater wax moth *(Galleria mellonella)*. Bioassays were conducted in petri dishes where the effects of EPNs concentrations; at 100, 300and 500Ijs/ml on *Tuta absoluta* larvae exposed for 24-72 hours were evaluated. Secondly, three different concentrations namely; 20ml/20L, 40ml/20L and 60ml/20L of Nimbecidine® (Azadirachtin) were evaluated against *Tuta absoluta* populations in the greenhouse. Thirdly, the following management options; *Steinernema karii* nematodes alone applied at a rate of 1000 Ijs/ml with oil adjuvant Addit® as a wetting agent in water, *Steinernema karii* nematodes and neem (Azadirachtin 0.03%) applied sequentially after one hour at a rate of 40ml/20L were evaluated against *Tuta absoluta* populations. These were compared with Coragen® SC (20% Chlorantraniliprole) synthetic pesticide applied at the rate of 3ml/20L and control where only water was applied. A delta sticky trap supplied with *Tuta absoluta* pheromone was hanged at the centre

of the greenhouse at a height of one metre for monitoring *Tuta absoluta* numbers to enable the initiation of treatments. The results obtained showed that, the evaluated concentration rates of *Heterorhabditis species* and *Steinernema karii* at 100Ijs/ml, 300 Ijs/ml and 500 Ijs/ml significantly caused ($p < 0.05$) mortality to the *Tuta absoluta* larvae and the highest mortality was recorded at 500 Ijs/ml concentrations with an exposure period of 72 hours. *Steinernema karii* was more pathogenic compared to *Heterorhabditis species* with respect to exposure time, having shown 100% and 91.5% larval mortality, respectively. Secondly, the evaluated neem concentrations 20ml/20L, 40ml/20L and 60ml/20L significantly ($p < 0.05$) reduced *Tuta absoluta* population in the greenhouse tomato. The high concentration (60ml/20L) was more effective in reducing *Tuta absoluta* population and fruit damage compared to the lower concentrations and control. High fruit damage of 96.2% was recorded in the control compared to 30.0%, 23.4% and 20.0% for 20ml/20L, 40ml/20L and 60ml/20L dose rates, respectively. Lastly, EPNs alone and EPNs combined with neem significantly ($p < 0.05$) reduced the population of *Tuta absoluta*. The number reduction of *Tuta absoluta*by EPNs combined with neem did not differ with that which was achieved in Coragen®. Tomato fruit damage was highest in the control with 91.5% compared to 10.8%, 7.9% and 3.0% for EPNs alone, EPNs combined with neem and Coragen®, respectively. This study demonstrates the potential of EPNs alone or in combination with neem applied successively at a one hour interval, as an alternative strategy for the sustainable management of *Tuta absoluta* in the greenhouse conditions.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Tomato *(Lycopersicon esculentum* L.) a native crop of South America is one of the most widely grown vegetable in the world (FAO, 2012). In 2012, Kenya was ranked sixth in tomato production in Africa (FAO, 2012). Tomato production in Kenya is carried out in the greenhouses and in the open fields for export and local consumption and practiced by small and large scale farmers (GOK, 2014). In 2014 tomato crop was the second most important vegetable crop in the country in terms of production and value after Irish potato (HCDA, 2014). The area under tomato in 2014 was 24,074 hectares with a total production of 400,204 tons valued at Ksh. 11.8 billion (HCDA, 2014).

Tomato production plays a great role in domestic and nutritional food requirements, generation of income, foreign exchange earnings and creation of employment (GOK, 2014). Despite its great importance, tomato production in Kenya is faced with various challenges which include incidences of pests, diseases and decreasing soil fertility. The main tomato arthropod pests include; aphids, thrips, whiteflies, cutworms, red spider mites, nematodes and leaf miners. Diseases adversely affecting tomato production include; wilts, blights, leafspots and mildews (CABI, 2015). Among the pests of tomato crop, the tomato leaf miner *(Tuta absoluta)* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most destructive pest in Kenya (GOK, 2014) and it is one of the most important lepidopterous pest which is associated with tomato crops in South America (EPPO 2005; Torres *et al.,* 2001). In countries where it has been reported tomato leaf miner caused losses ranging from 50-100% in tomato crops (Cifuentes *et al.,* 2011).

Tomato leaf miner is a new pest reported in Kenya in 2014. It is very destructive causing damage to leaves, fruits and stems and its presence may reduce the quality of produce, market value and also limit the export of the product (GOK, 2014). The pest also has been reported to attack potatoes, egg plants, sweet peppers, and other cultivated plants (CABI, 2015). It also attacks weeds of the Solanaceae family for instance *Solanum nigrum*, *Datura species* among others (Derbalah *et al.,* 2012). Infestations of tomato plants occur throughout the entire crop cycle on apical buds, leaves, stems and fruits on which dark frass (caterpillar excrement) is visible (CABI, 2015). Feeding damage is caused by the four larval instars occurring throughout the whole plant (Desneux *et al.,* 2010). On the leaves, larvae feed indiscriminately on the mesophyll tissues forming wide, irregular mines which may later become necrotic (IPPC, 2014). Fruits are also attacked by the larvae, which create entry points that are used by secondary pathogens leading to fruit rot. The larvae also burrow into the stem affecting the development of the crop. Potential yield loss in tomato quality and quantity can reach up to 100% if the pest is not managed (EPPO, 2005).

Chemical control is the main method used by farmers to reduce tomato leaf miner populations. This practice can lead to serious problems such as elimination of natural enemies, environmental contamination and induced resistance in pest populations (Dahliz *et al.,* 2010). The pest requires a holistic approach through IPM programs for sustainable management to be achieved. Entomopathogenic nematodes are potential biological control agents for the tomato leaf miner which have not been fully exploited in Sub Saharan Africa. They are environmentally friendly, easy to produce in large numbers, they have low impact on non- target species and application is safe (Shapiro-Ilan *et al.,* 2006). This study aimed at evaluating the potential of

Kenyan entomopathogenic nematodes and other IPM strategies for sustainable management of tomato leaf miner.

1.2 Problem Statement

The tomato leaf miner is a new pest in Kenya affecting tomato production in open fields and in the greenhouses. According to HCDA (2014) the average tomato yield in the country was 16.6 tons per hectare which is far below the potential production compared to other countries such as Egypt (44 tons/hectare) and China (54 tons/hectare) (FAO, 2012). Invasion of tomato in the country by *Tuta absoluta* is likely to reduce yields further. Tomato cultivars are susceptible to this pest (GOK, 2014). Tomato leaf miner reproduction potential is very rapid with a lifecycle ranging from 24 days at 25^{o}C to 38 days at 19^{o}C (EPPO, 2005). Various cultural methods including crop rotation, crop removal and the selective removal and destruction of infested plant materials have been used to manage the tomato leaf miner with little success. The methods take too long to implement and others involve destruction of the crops (Desneux *et al.*, 2010; EPPO, 2005).

Chemical control is the most common method used by farmers to reduce pest populations, but a lot of environmental and health concerns are raised in addition to the development of resistance in pest numbers (Diez–Rodriguez and Omoto, 2001). Buildups of insecticide residues in tomato fruits reduce the food quality and safety thereby increasing health risks to consumers (HCDA, 2014). Reduced efficiency and control failure due to continuous application of the insecticides against *Tuta absoluta* have been reported in South America. For example, resistance to Abamectin and Deltamethrin has been reported in Argentina (Lietti *et al.*, 2005). There is need to address tomato management in Kenya with caution before it cripples tomato production in the country.

1.3 Justification

Tomato production plays a key role in diversifying the economy and agricultural sector by creating employment and providing income to many people in the country. IPM strategies may offer a solution for managing the *Tuta* pest while taking care of the environment, food safety risks and resistance to pesticide development. Use of biological control agents such as Entomopathogenic nematodes (EPNs) and other IPM strategies are some of the methods that can be used together with reduced application of chemical pesticides. The use of entomopathogenic nematodes has not been fully exploited by most farmers remaining unaware of these EPNs and how to apply them in pest management. Entomopathogenic nematodes are environmentally friendly, leave no residues as does with many synthetic pesticides and have no negative effect to non-target species (Georgis *et al.,* 2006; Shapiro-Ilan *et al.*, 2006). Despite the reported importance of EPNs in the management of below and above ground insect pests (Williams *et al.*, 2000), the pathogenicity of Kenyan EPNs against the tomato leaf miner is not documented.

1.4 Objectives of the study

1.4.1 Broad objective

The overall objective of the study was to develop sustainable IPM strategies for the management of the tomato leaf miner (*Tuta absoluta)* in the greenhouse conditions.

1.4.2 Specific objectives:

i. To determine the pathogenicity of selected native entomopathogenic nematodes against the tomato leaf miner (*Tuta absoluta)* in laboratory conditions.

ii. To determine the efficacy of neem biopesticide in the management of the tomato leaf miner *(Tuta absoluta)* under greenhouse conditions.

iii. To evaluate the effectiveness of EPNs and neem combined with EPNs for the management of the tomato leaf miner (*Tuta absoluta*) under greenhouse conditions.

1.4.3 Hypothesis

- Native EPNs will cause mortality to the tomato leaf miner *(Tuta absoluta)* in laboratory conditions.
- Neem biopesticide application will result in reduction of tomato leaf miner population under greenhouse conditions.
- Native EPNs combined with neem biopesticide will reduce tomato leaf miner population below the economic thresholds under greenhouse conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Economic importance of tomatoes

Tomato *(Lycopersicon esculentum* L.) is one of the most widely grown vegetables in the world (FAO, 2012). Kenya is among the leading producer of tomatoes in Africa and was ranked number six based on the tonnage produced in 2014 (GOK, 2014). Tomato is amongst the promising crop in horticultural expansion and development in the country as it creates employment and provides income and better livelihood for many Kenyans. It also accounted for 14% of the total vegetable produced and 6.72% of the total horticultural crops produced in 2012 (GOK, 2012). In 2014, the total production of tomatoes in Kenya was 400,204 tons with a value of Ksh.11.8 billion (HCDA, 2014).

2.2 Origin, distribution and host range of the tomato leaf miner *(Tuta absoluta)*

Tomato leaf miner is a tiny nocturnal moth, native to Latin America and has spread through most of Europe and Mediterranean regions (Hassan and Alzaidi, 2009). It has made its way in Africa, infesting North of Sahara in Tunisia and Egypt, Gambia and Niger in West Africa. In Sub-Saharan Africa it has infested Sudan, Ethiopia and now in Kenya, where it was reported recently (IPPO, 2014). In Kenya it was first reported in Isiolo in 2014, but now has spread to the most tomato producing parts of the country. It has been recorded in Nairobi, Meru, Kirinyaga, Njoro, Kakamega, Lamu and Loitoktok among other tomato growing regions in the country (GOK, 2014). The tomato leaf miner is now a key pest of tomato in Kenya (GOK, 2014). *Tuta absoluta* is a serious and a very destructive pest that causes 50-100% losses to tomatoes and other solanaceous crops such as Irish potato (Desneux *et al.,* 2010). Its capability to use alternative plants as

secondary hosts allows the continuous presence of this pest in many habitats, in the absence of tomato crops (Derbalah *et al.,* 2012).

2.3 Morphology and identification of *Tuta absoluta*

Tomato leaf miner adult is a tiny moth which is active at night. The moth has a grey- brown colour, is approximately 6 mm in size and has a wing span of about 10 mm (Diez–Rodriguez and Omoto, 2001). It mainly lays its eggs at night on leaves, petioles and young fruits either singly or in small batches. The eggs are creamy white to yellow in colour, measuring 0.35mm long (CABI, 2015). Eggs laid on young fruits hatch to larvae which tunnel through the fruit causing a lot of damage. Newly hatched caterpillars are approximately 0.5 mm long and have a yellowish colour (EPPO, 2005). When maturing, caterpillars turn yellow- green and a black band develops behind the head. Fully grown caterpillars are approximately 9 mm long with a pinkish colour on the back. The pupa is light brown and approximately 6 mm long (Desneux *et al.,* 2011). The larva (caterpillar) is the damaging stage. *Tuta absoluta* feeding creates a wide irregular mine and the damage should not be confused with mines made by the leaf miner fly *(Liriomyza spp.),* which are narrower and more circuitous. Dark frass (caterpillar excrement) can be seen near the entry hole (Tropea *et al.,* 2012).

2.4 Life cycle of *Tuta absoluta*

Tuta absoluta reproduces rapidly with a life cycle ranging from 24 days at 25^{o}C to 38 days at 19^{o}C (Torres *et al.,* 2001). One female may lay up to 260 eggs during her life time which are deposited on above ground plant parts such as leaves and young fruits. Eggs which are white to yellow in colour hatch within 4-5 days (Urbaneja *et al.,* 2012; Tropea *et al.,* 2012). The first instar caterpillar has cream colour with a dark or brown head. There are four larval instars stages which change in colour from green to light pink. When the larva is fully mature within 12-15 days it

pupates. Pupation may take place in the soil, on the leaf surface, or within mines on the leaf and takes 9-11 days for an adult moth to emerge (CABI, 2015). Adult moths are active during the night and hide between the leaves during the day (Diez–Rodriguez and Omoto, 2001).

2.5 Economic importance, damage and effects of *Tuta absoluta* on tomatoes

The potential yield loss in tomato quality and quantity is significant and can reach up to 100% if no control measures are taken against the pest (CABI, 2015). This moth has the potential to lower the crop yields and increase production costs. For instance, in Spain, the first year of introduction of tomato leaf miner, pesticides were applied 15 times per season and the cost of control went up by 450 Euros per hectare (Derbalah *et al.*, 2012). The *Tuta absoluta* establishment in Brazil caused disruption in tomato markets of fresh fruits (Muniappan, 2010).

All above ground parts of tomato plants in each developmental stage can be infested by *Tuta absoluta* (Tropea *et al.,* 2012). Feeding damage is caused by all larval instars and throughout the whole plant. Larvae have a strong preference for the leaves and stems but they may also be found in or under the crown of the fruit and in the fruit itself (Desneux *et al.,* 2011). The most distinctive symptoms of the presence of the species are the blotch-shaped mines (blotch mines) in leaves in which the larvae and dark, granular excrements (frass) can be found (CABI, 2015). *Tuta absoluta* larvae feed within the leaf lamina, causing severe loss of photosynthetic capability but the leaf lamina remains intact (Tropea *et al.,* 2012). Damage to the fruit may give easy access to pathogens, causing decay of the fruits resulting to total crop failure (Mallia, 2009). Feeding larvae create wide, irregular mines resulting to leaf damage. Severe defoliation leads to death of the plant (Desneux *et al.,* 2011).

2.6 Management of *Tuta absoluta* in tomatoes

2.6.1 Chemical Control

Chemical control has been the main control measure against *Tuta absoluta* (Mallia, 2009). Frequent intense application of insecticides leads to the development of insecticide resistance (Radwan and Taha, 2012). In addition, chemical control leads to serious problems such as elimination of populations of natural enemies and environmental contamination (Diez–Rodriguez and Omoto, 2001). Resistance to Abamectin and Deltamethrin has been reported in Argentina (Lietti *et al.*, 2005). *Tuta absoluta* resistance to a range of pesticides including Bifethrin, Cartap, Methamidophos and Permethrin has also been reported in Brazil (Siqueira *et al*., 2000a; Siqueira *et al*., 2000b).

Modern integrated pest management recommends effective pesticides that have low mammalian toxicity, low persistence in the environment and high degree of selectivity (Silva *et al.*, 2011). Since insecticide control currently remains an indispensable tool, the goal is to minimize the amount and impact of pesticides through the diversification of active ingredients used (Derbalah *et al.*, 2012).

2.6.2 Neem botanical pesticide

Biopesticides use in *Tuta absoluta* management has become popular in addressing risks from synthetic pesticides to the environment and human health. This has necessitated the use of bio-insecticides such as Spinosyn spinosad, *Beauveria bassiana*, *Bacillus thuringiensis* Berliner and neem (Guedes *et al.*, 2012). Botanical products are biodegradable and effective against pests without harming beneficial insects (Haseeb *et al.*, 2004). Insecticidal properties of neem *(Azadirachta indica)* have been put into use from several thousand years (Isman, 2006). Neem compounds present various effects ranging from repellency to toxicity against a wide spectrum of

insect pests including Orthoptera, Lepidoptera, Coleoptera, Hemiptera and Diptera (Shannag *et al.,* 2014; Degli *et al.,* 2013; Siddiqui *et al.,* 2009).

Neem biological properties are mediated by different group of compounds among which limonoids such as meliantriol, salanin and nimbin mainly present in the neem seeds are considered the most active components responsible for both anti-feedant and insecticidal effects (Kona *et al.,* 2014). Neem based insecticides have low negative environmental impact because of a rapid degradation in plants and in the soil (Haseeb *et al.,* 2004) and low negative effects on beneficial insects (Defago *et al.,* 2011; Isman, 2006). Moreover, azadirachtin has been proved to be non-toxic to vertebrates (Isman, 2006; Mordue and Nisbet, 2004). Neem extracts represents a valuable tool to control pest population outbreaks in the integrated pest management programs and can be used in organic farming for pest management (Boursier *et al.,* 2011).

2.6.3 Cultural Control

This is the purposeful manipulation of the crop environment to reduce the rates of pest increase and damage. Ecological management of the crop environment entails sanitation, destroying alternate hosts, intercropping and trap cropping (CABI, 2015). Various cultural methods including crop removal, selective removal and destruction of infested plant materials have been used in the control and management of tomato leaf miner (Boursier *et al.,* 2011). Also, farmers in an area make agreement on the season to grow tomatoes in order to break the cycle of the pest (Chailleux *et al.,* 2013). However, these methods have not been effective since some of them take too long to implement and others involve destruction of the crops (EPPO, 2005).

2.6.4 Sticky traps with pheromones

The decision scheme of using insecticides for the management of *Tuta absoluta* is largely based on adult captures in sexual pheromone traps, as adult catches are correlated with larval

damages and yield losses (Benvenga *et al.,* 2007). In Brazil, it was reported an action level of 45 male adult *Tuta absoluta* caught daily using pheromone traps per hectare (Benvenga *et al.,* 2007). Action threshold could also be based on the occurrence of the pest in the tomato crop with 2 females per plant or 26 larvae per plant (Bajonero *et al.,* 2008).

Mass trapping may also effectively remove sufficient males to lower overall *Tuta absoluta* population levels and reduce pest pressure. However, mass trapping would likely be most effective when used in conjunction with recommended insecticides or other control measures (Witzgall *et al.,* 2010). The mating behavior of tomato leaf miner like other moths is mediated by female sexual pheromones, which attract males. The *Tuta absoluta* female pheromone has been synthesized and is used for monitoring and mass-trapping (Cocco *et al.,* 2013). The pheromone is impregnated into rubber septa and placed in trap with a sticky paper or a water tray to kill the adults attracted to this focal point. A new lure must be replaced every 4 to 6 weeks (Lee *et al.,* 2014).

2.6.5 Biological control

This is use of living organisms to manipulate pest populations which is achieved through predation, parasitism or other natural mechanisms, but typically also involves an active human management role (CABI, 2015).Various biological control agents are used in pest management which includes predators such as spotted lady beetles, parasitoids such as braconid wasps and pathogenic micro-organisms such as *Bacillus thuringiensis* and *Beauveria bassiana* among others (Guedes *et al.,* 2012). Natural enemies play an important role in the IPM of this pest according to a recent *Tuta absoluta* symposium at (KALRO, 2014). Also various studies have been done on the use of these biological control agents in the greenhouse tomatoes in Europe and South America where this pest was reported earlier (Desneux *et al.,* 2010).

Natural enemies important for *Tuta absoluta* management include; Nesidiocoris species which is a predatory bug of the family Miridae and is indigenous in Kenya (GOK, 2014). It is commercially reared and has been widely used in Spain against *Tuta absoluta* in tomato crops (IPPO, 2014). However caution is recommended since it has been recorded as causing serious economic damage to plants if present in large numbers in tomato crop when their prey, (white flies, caterpillars and aphids) have been controlled (Molla *et al.,* 2011).

Macrolophus species is a true bug in the family Miridae. It is another biocontrol agent which preys on whitefly and also feeds on aphids, mites and eggs of moths and can easily move from one plant to another (CABI, 2015). Both *Macrolophus pygmaeus* and *M. caliginosus* have been observed feeding on young *Tuta absoluta* caterpillars (EPPO, 2005). *Macrolophus* can be a common insect in natural vegetation and has been recorded as becoming established inside tomato greenhouses in Northern Europe (Dahliz *et al.,* 2010). Macrolophus species have been observed in Kenya (GOK, 2014).

Amblyseius is a predatory mite belonging to the family Phytoseiidae and feeds on other mites such as spider mites, thrips, whitefly among others (CABI, 2015). Both *Ambylsieus swirskii* and *A. cucumeris* have been observed feeding on very young *Tuta absoluta* caterpillars. In laboratory studies, these predatory mites have been found feeding on the eggs of *Tuta absoluta* (Dahliz *et al.,* 2010). However, they are generalist predators and do not complete their life cycle on *Tuta absoluta* eggs alone. If Amblyseius is used as part of an IPM strategy for *Tuta absoluta*, they probably have to be used prophylactically as a management tool for Tuta, whitefly or thrips, respectively (Sher *et al.,* 2000).

Trichogramma species are minute wasps in the family Trichogrammatidae (CABI, 2015). They are endoparasitoids of insect eggs (Guedes *et al.,* 2012). A number of Trichogramma species

are mass reared in Europe and recent trials identified *T. achaeae* as being the most effective against *Tuta absoluta* (EPPO, 2005). However, they must be released in very high numbers to be effective. The wasps are extremely sensitive to broad-spectrum chemicals and can only be used in a spray programme that has been carefully designed to conserve Trichogramma (Dahliz *et al.,* 2013). Trichogramma parasitoids may not buildup populations on the *Tuta absoluta* tomato systems, but on combination with *M. pygmaeus* can be used to enhance biological control of the pest in tomato crops (Chailleux *et al.,* 2013).

Bacillus thuringiensis is a gram-positive soil dwelling bacteria commonly used as a biological pesticide (Desneux *et al.,* 2010). The mode of action of *B. thuringiensis* is by contact and ingestion of a lethal dose by the caterpillar (Cifuentes *et al.,* 2011). Therefore, the only life stage that is likely to be affected by *B. thuringiensis* is the first instar, free living stage (Sher *et al.,* 2000). Due to many overlapping generations of *Tuta absoluta*, this requires an intensive spray programme to provide sufficient cover of the vulnerable life stage. This raises the question of cost effectiveness of the programme (Dahliz *et al.,* 2013).

2.6.6 Use of EPNs as biological control agents for insect pests

Entomopathogenic nematodes in the order Rhabditida, of the families Steinernematidae and Heterorhabditidae, are valuable biological control agents of a wide range of economically important insect pests (Grewal *et al.,* 2005). Ndiritu *et al.,* (2016) demonstrated the potential of EPNs in Steinernematidae family in the management of banana weevil larvae. Although traditionally applied to control the soil stages of insects, EPNs have proven to control some foliar pests (Arthurs *et al.,* 2004).

Entomopathogenic nematodes are used as inundatively applied biological control agents (Hazir *et al.,* 2003). The aim of inundative biological control is to reduce pest populations by

repeatedly applying large quantities of natural enemies (Mailleret and Grongnard, 2009). The benefits of using nematodes include; the ability to produce them in large numbers, lower impact on non-target species, a wide range of species makes it easy to select for specific environments, application is safe and they can be implemented in an IPM system (Shapiro-Ilan *et al.,* 2006; Georgis *et al.,* 2006; Gaugler *et al.,* 2002).

2.6.6.1 Life cycle of entomopathogenic nematodes

Both Steinernematids and Heterorhabditids develop through four immature stages before reaching maturity (Glazer and Lewis, 2000). The third stage, in which they are known as dauers or infective juveniles (Ijs), is the only free-living, non-feeding stage within the life cycle, and occurs naturally in the soil (Adams and Nguyen, 2002). Once a host is located, nematodes enter the host through natural openings (mouth, anus and spiracles), or by directly penetrating through thin layers in the cuticle (Shapiro-Ilan, 2009). Direct penetration of the host's cuticle commonly occurs in Heterorhabditids that are equipped with a dorsal tooth (Kaya *et. al.* 2006). Once inside the haemocoel of the host, nematodes release a symbiotic bacterium that multiplies rapidly within the nutrient-rich haemolymph, while producing toxins and other metabolites that kill off hosts by septicaemia or toxaemia within 24-48 hours after infection (Adams and Nguyen, 2002; Dowds and Peters, 2002).

Symbiotic bacteria associated with Steinernematids and Heterorhabditids fall in the genera, *Xenorhabdus* and *Photorhabdus*, respectively and are both gram-negative bacteria belonging to the family Enterobacteriaceae (Jagdale *et al.*, 2009; Burnell and Stock, 2000). Steinernematid Ijs retain *Xenorhabdus* symbionts within an intestinal vesicle, while *Photorhabdus* cells stick together in the anterior part of the Heterorhabditids gut (Boemare, 2002). Nematodes feed on bacteria cells,

as well as on host tissue broken down by developing bacteria, and then advance to the fourth and adult stage (Nyasani *et al.,* 2007).

The adults mate and females lay eggs that hatch and molt successively through four stages, of which the fourth stage develops into adults. The process continues as long as the insect cadaver supplies sufficient food resources (Adams and Nguyen, 2002; Ehlers, 2001). Such insect cadavers normally allow for the development of approximately two or three generations of EPNs. Once resources are depleted, the offspring develop into third stage Ijs, which stop feeding and incorporate the symbiotic bacteria before exiting the cadaver in search of a new host (Adams and Nguyen, 2002; Ehlers, 2001).

The bacteria release antibiotic substances that aid in preserving the insect cadaver and that protect it against opportunistic organisms (Shapiro-Ilan, 2009). The Ijs can survive in soil for several months by entering a near-anhydrobiotic state (Adams and Nguyen, 2002). Steinernematids and Heterorhabditids have different modes of reproduction (Boemare, 2002). First-generation Heterorhabditids are self-fertile hermaphrodites while subsequent generations consist of cross-fertilizing males and females. In Steinernematids, cross- fertilizing males and females occur in each generation (Griffin *et al.*, 2005).

2.6.6.2 Mode of action of the entomopathogenic nematodes

There exists a symbiotic relationship between EPNs and bacteria which presents one of the best biological management strategies supporting insect pest control (Lang *et al.,* 2011). Once the EPNs enter into the pests, the bacteria in their gut releases a number of virulence factors. These are toxin complexes, hydrolytic enzymes, hemolysins and anti-microbial compounds that cause mortality of insects within 48 hours (Eleftherianos *et al.*, 2010; French-constant *et al.,* 2007). This provides EPNs with nutrients needed for development and reproduction within insect cadaver. It

has been demonstrated that the bacterial symbionts are the final causal agents of the insect mortality (Campos *et al.,* 2009). The bacterial symbionts play an important role in the death of the host which provides nutrients for EPNs involved (Ciche *et al.,* 2006).

Cadavers infected with *H. bacteriophora* were protected from avian predator, the European robin due to red colour reinforced by unpalatable taste when cadavers were sampled (Fenton *et al.,* 2011). Characteristics of entomopathogenic nematodes that make them excellent biocontrol agents are the broad-host range, ability to search actively for host and kill the host within 48 hours, can easily be mass produced and applied, have long term efficacy and they are environmentally friendly (Abd El Rahman *et al.,* 2012).

2.6.6.3 Above ground foliar application of EPNs in the management of insect pests

Entomopathogenic nematodes are applied to foliage as an aqueous suspension, using ordinary agrochemical spray equipment (Grewal *et al.,* 2006; Hussaini, 2002). Nematodes are living organisms, hence the water used for application should not be heavily chlorinated, and the temperature should be within the range of 4-30°C (Wright *et al.,* 2005). Successful control of foliar pests with the aid of nematodes is challenging, because above-ground conditions are not optimal for nematode survival (Tomalak *et al.,* 2005; Mracek, 2002).

High relative humidity is essential to retard desiccation and to improve post-application EPNs survival rates. This is because nematodes require a water film to ensure their survival and to maintain mobility (Wright *et al.,* 2005). Rainy periods and tropical conditions improve nematode survival rates on foliage (Mracek, 2002). Since wind can accelerate evaporation, it is advisable to apply nematodes on non-windy days (Unruh and Lacey, 2001). Water retention reagents have been used successfully to obstruct the evaporation of application suspensions on foliage, thereby increasing the duration of nematode survival (Shapiro-Ilan *et al.,* 2006).

A significant advancement has been made with the advent of a water dispersible granule (WDG) formulation in which infective juveniles are encased in 10-20 mm diameter granules consisting of mixtures of various types of silica, clays, cellulose, lignin and starches (Bauer *et al.,* 1997). The formulation causes substantial decline of nematode respiration due to the introduction of anhydrobiosis in the granules, enabling extension of nematode shelf life in certain species thus, offering several advantages over the existing formulations (Grewal *et al.,* 2005).

2.6.6.4 Previous work with EPNs to control above ground insect pests

Nyasani *et al.,* (2007) examined the potential of using EPNs in the management of diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). The EPNs isolates evaluated namely; *Heterorhabditis species*, *Steinernema species*, *Steinernema karii*, *Heterorhabditis indica* and *Steinernema waiseri* caused mortality to the DBM larvae demonstrating EPNs potential in the management of diamondback moth (DBM) and other above ground insect pests.

Control of other pest species with EPNs on foliar surfaces has been variable (Georgis *et al.,* 2006). For example, research on *Steinernema carpocapsae* and *S. feltiae* demonstrated their potential for control of the leaf miners (Diptera: Agromyzidae); *Liriomyza trifolii* (Tomalak *et al.,* 2005; Sher *et al.,* 2000); *Liriomyza huidobrensis* (Williams and Walters, 2000); *Tuta absoluta* and other leaf miner species (Batalla-Carrera *et al.,* 2010) and banana weevil (Ndiritu *et al.,* 2016).

2.7 Integrated Pest Management as an option for *Tuta absoluta* management

IPM is a rational application of biological, biotechnological, chemical, and cultural or crop improvement measures (Dahliz *et al.,* 2013). Use of chemical plant protection products is limited and used as a last resort to keep pests below levels that may cause economically unacceptable damage or losses (EPPO, 2005). The benefits of implementing IPM include; reduced chemical pesticide input costs, reduced on-farm and off-farm negative environmental impacts and more

effective and sustainable pest management (Urbaneja *et al.*, 2012). With the invasion of the entire African continent by *Tuta absoluta* now a 'real' threat, there is an urgent need to understand the bio-ecology of the pest in its invaded range and develop environmentally sustainable, economically sound and effective integrated pest management (IPM) strategies for this pest (ICIPE, 2015).

CHAPTER THREE

Pathogenicity of selected native EPNs against tomato leaf miner (*Tuta absoluta)* in laboratory conditions

Abstract

Tomato leaf miner, *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 EPN isolates namely; *Heterorhabdities species* and *Steinernema karii* against *Tuta absoluta* larvae under laboratory conditions in petri dish bioassays. Entomopathogenic nematodes used were obtained from Kenya Agricultural and Livestock Research Organization EPNs laboratories while *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 which was laid out in a complete randomized design with four replicates was conducted. The results showed that the evaluated concentration rates of *Heterorhabditis species* and *Steinernema karii* at 100, 300 and 500 Ijs/ml significantly ($p < 0.05$) caused mortality on *Tuta absoluta* larvae compared to the 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 Kenyan EPNs have a potential for the management of the tomato leaf miner *(Tuta absoluta).*

3.1 Introduction

Tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a new pest in Kenya affecting tomato production in open fields and in the greenhouses. All cultivars of tomato are susceptible to *Tuta absoluta* infestation (GOK, 2014). 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 (Urbaneja *et al.*, 2012). Various cultural methods including crop rotation, crop removal and the selective removal and destruction of infested plant materials have not been effective in the management of *Tuta absoluta* (EPPO, 2005; Desneux *et al.*, 2010).

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 (Diez-Rodriguez and Omoto, 2001). Pesticide resistance of *Tuta absoluta* to a wide range of organophosphate and pyrethroid pesticides has been reported in South America (Derbalah *et al.*, 2012). 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.

Due to increasing resistance to chemical pesticides, there is need to put emphasis on the importance of IPM strategies for proper management of *Tuta absoluta*. 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 (Georgis *et al.*, 2006; Shapiro-Ilan *et al.*, 2006).

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 one hundred and fifty four nematodes

were isolated among them the new species *Steinernema karii* which was identified by Waturu *et al.,* (1997a) and Waturu, (1998). Currently thirty three 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 (Tofangsazi *et al.,* 2012). The objective of this study was to determine the most pathogenic nematode species against *Tuta absoluta* larvae under optimal conditions for infection.

1.2 Materials and Methods

3.2.1 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 0^0 59'South and longitude 37^0 04'East and at an altitude of 1548 metres above sea level.

3.2.2 Source of EPNs

The EPNs species used in this experiment were originally collected from the 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 (Shapiro *et al.,* 2006). The nematodes were reared at 25^{o}C in the third instar larvae of the greater wax moth, *Galleria mellonella* according to the method of Woodring and Kaya (1998). *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 (Costa *et al.,* 2007).

Twenty petri dishes (9cm diameter) were lined with whatman filter paper no. 1 and the nematodes *Steinernema karii* and *Heterorhabdities sp*. were counted under a microscope and applied using a dropper 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 (Kaya, *et. al.*, 2006). A white trap consisted of a 15 cm diameter dish and a lid of a 9cm diameter Petri dish placed at the centre, then covered by a 15 cm diameter white muslin cloth (Plate 3.1). The emerged Ijs were washed three times in distilled water. The Ijs suspensions were stored at room temperature separately in 1 litre boxes before being stored at 10^{o}C (Gulcu and Hazir, 2012).

Plate 3.1 Modified white trap for extraction of emerging EPNs

3.2.3 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 the greenhouse in Kabete Campus Field Station, University of Nairobi which is located 15km North West of Nairobi City Centre and lies at latitude1^0 15'S and longitude 36^0 44'E and at an altitude 1941 metres above sea level.

3.2.4 Pathogenicity bioassay under laboratory conditions

Insect mortality bioassays were conducted with two different species of Kenyan EPN isolates 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 using a dropper after counting under dissecting microscope. The same procedure 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 the petri dishes with quarter-strength Ringer solution to confirm presence of EPNs. Live *Tuta absoluta* larvae were greenish while EPNs infected were pale in colour (Plate 3.2 a, b).

A B

Plate 3.2: Live *Tuta absoluta* larva-Greenish (A) EPNs infected *Tuta absoluta* larva-brownish or tan (B)

Magnification x1.5 Magnification x1.5

3.2.5 Statistical Analysis

The collected larvae mortality data 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 (Payne *et al.*, 2011).

3.3 Results

3.3.1 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 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 1a, 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 the control (Figure 1 a). The two EPNs effect was different at 300 Ijs/ml concentration. At the

highest nematode concentration (500 Ijs/ml) both EPNs species attained significantly ($p < 0.05$) higher mortality compared to the control (Figure 1a).

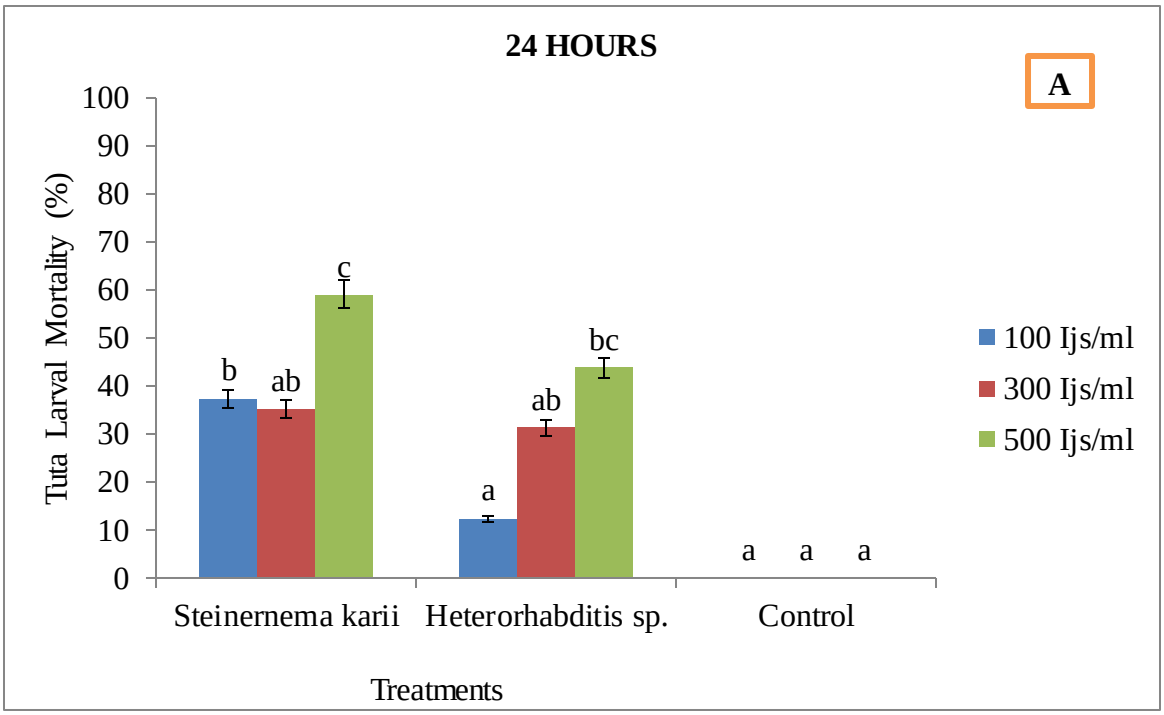


Figure 1 a: Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp.* for a period of 24 hours (A).
Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$).

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

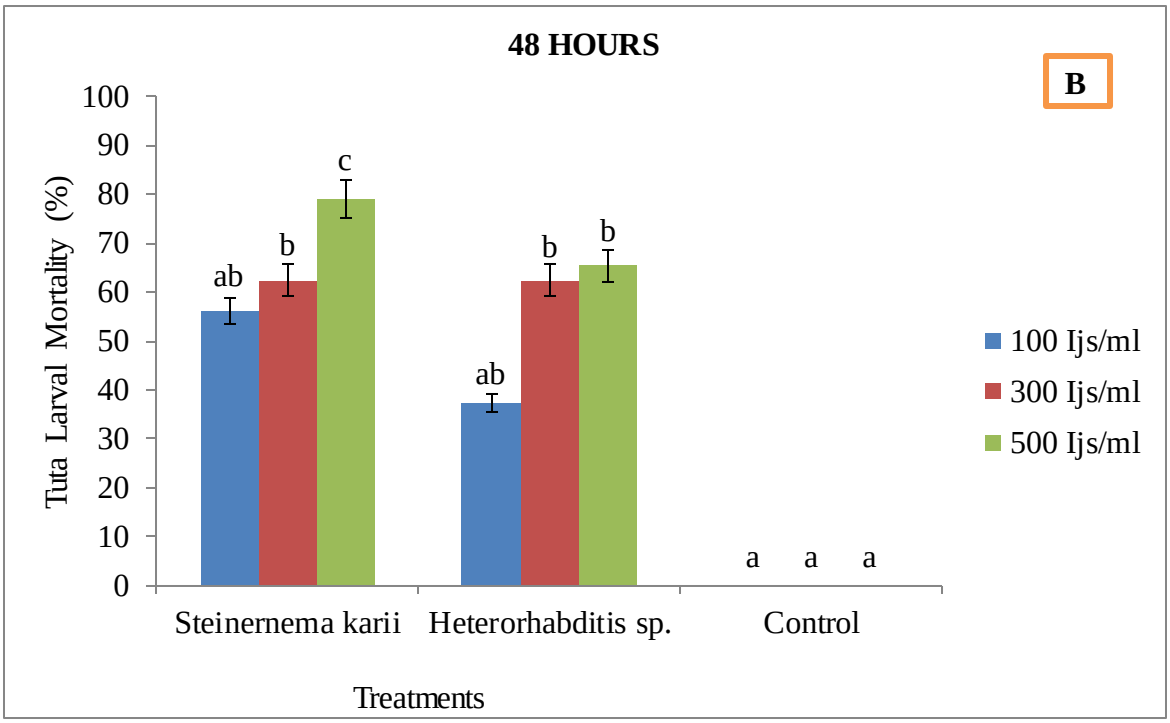


Figure 1 b: Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp.* for a period of 48 hours (B).
Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$).

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

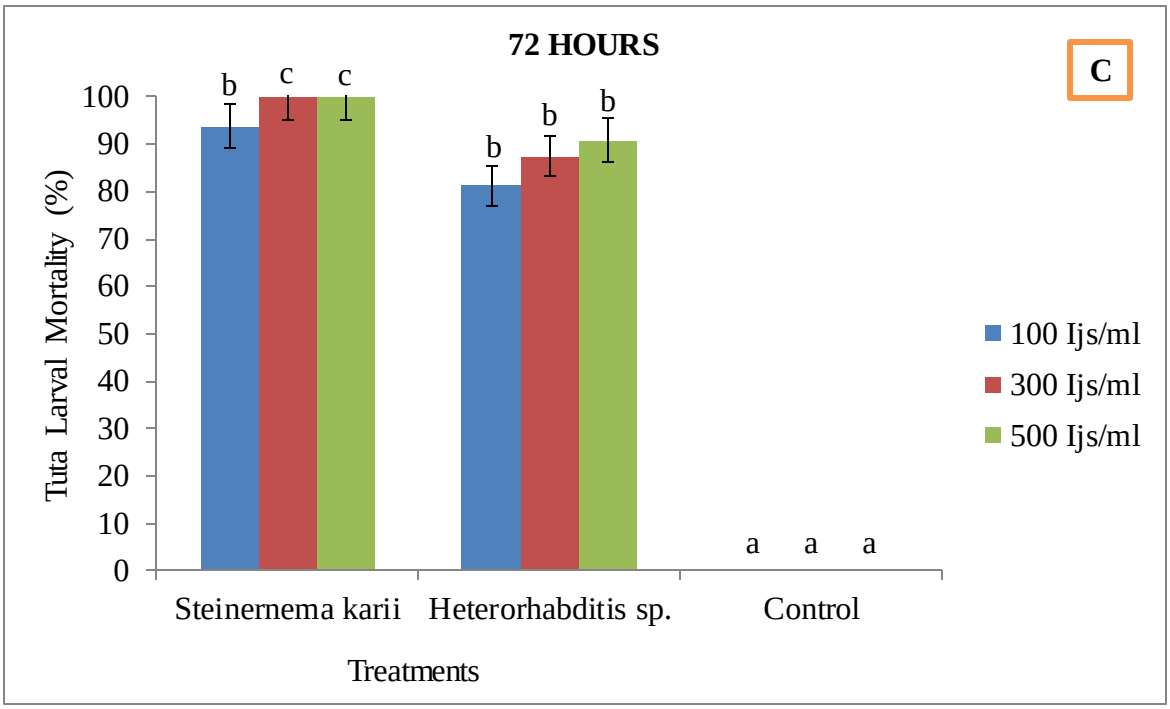


Figure 1 c: Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp.* for a period of 72 hours (C).
Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$).

3.3.2 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 the increase in exposure time and the applied number of Ijs (Figure 2 a, b, c), 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 the achieved larval mortality 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).

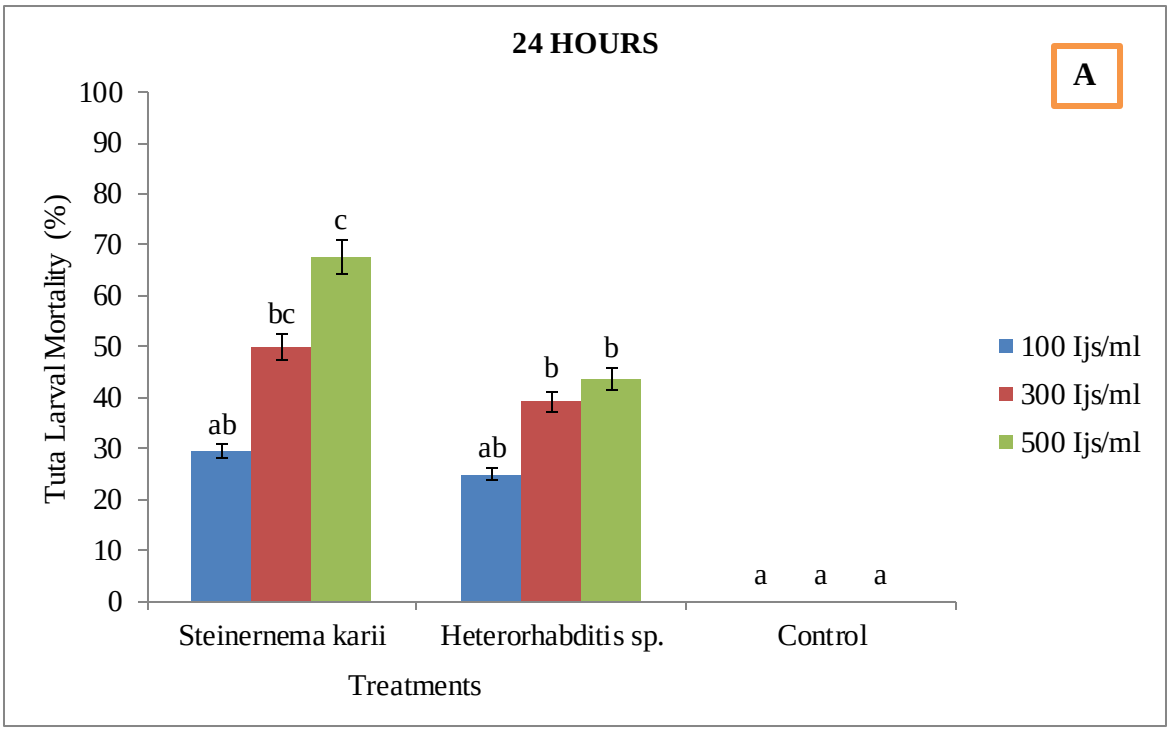


Figure 2 a: Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp*. for a period of 24 hours (A).
Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$).

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

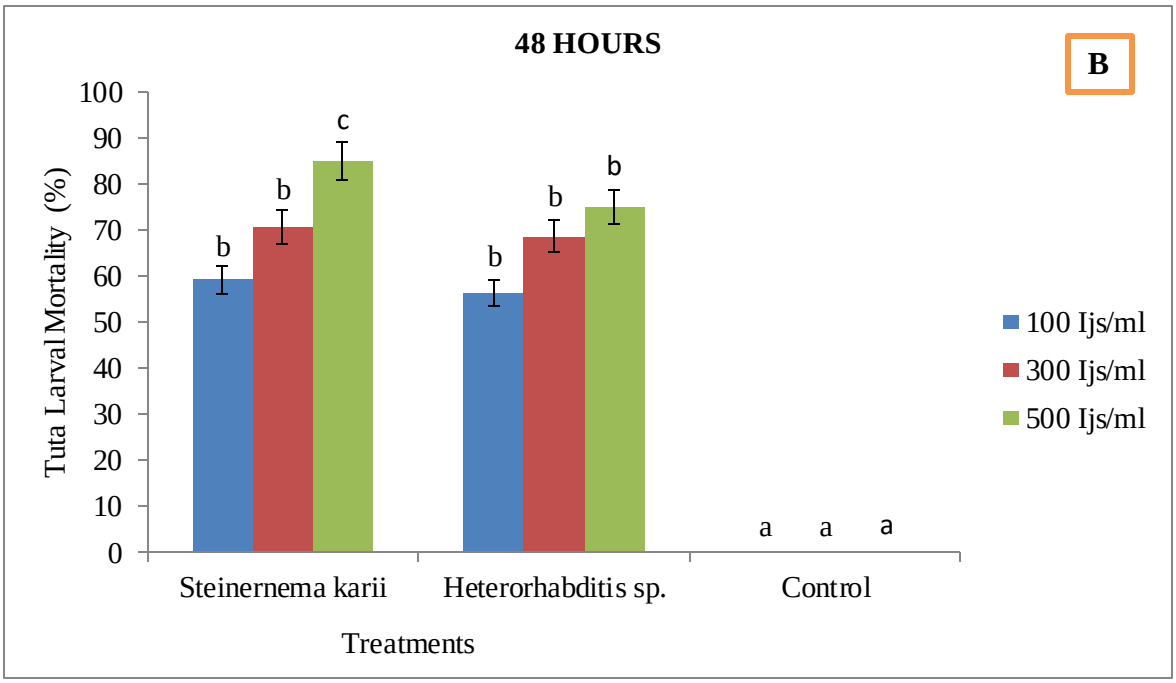


Figure 2 b: Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp*. for a period of 48 hours (B).
Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$).

At the highest exposure time (72 hours), the two tested EPNs species attained the highest mortality of *Tuta absoluta* larvae which was significantly different ($p < 0.05$) from the 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 different among them. However, 500 Ijs/ml achieved the highest mortality of 91.5% (Figure 2 c).

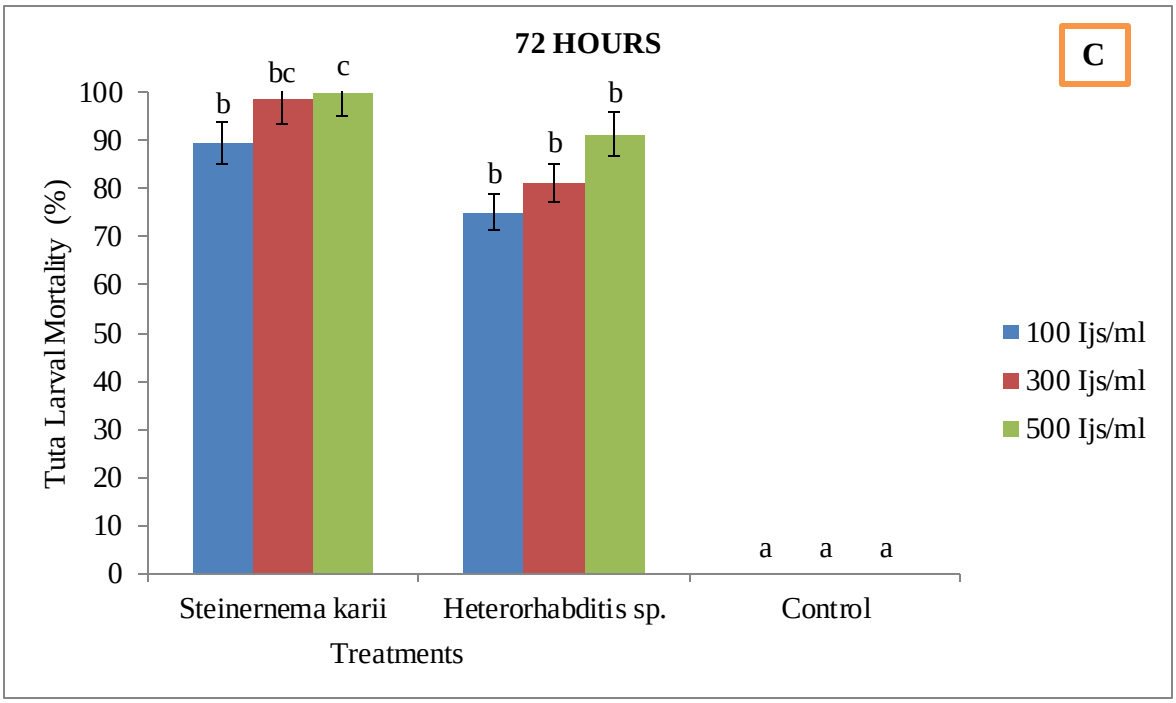


Figure 2 c: Mortality (%) of *Tuta absoluta* larvae following application of entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis sp*. for a period of 72 hours (C).
Data are expressed as mean ± SEM. The same letter above the error bars indicates no significance differences ($p < 0.05$).

3.4 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 *et al.* (2010).

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 (Dillman *et al.*, 2012). Once inside the host nematodes release bacteria which kill larvae through production of toxins and hydrolytic enzymes. The enzymes, digest internal contents

of host and in turn provide nourishment to EPNs (Jagdale *et al.*, 2009). This agrees with the study by Ndiritu *et al.* (2016) who reported that increasing the dosage of EPNs 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). This showed that the EPNs were able to search and infect the larvae after which they released bacteria which caused toxicity which led to mortality of the larvae at high populations and longer exposure. *Steinernema karii* was more virulent probably due to the large population of the symbiotic bacteria cells which were carried by dauers in their gut, causing high pathogenicity as reported by Koppernhoofer and fuzzy (2003). Research by Emelianoff *et al.* (2007) revealed that *Steinernema scapterisci* contains low amount of bacterial cells compared to *Steinernema carpocapsae* that has a large number of bacterial cells, which cause 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 and Kapinera, (1993) and Caroli *et al.* (1996). This agrees with a study by Lacey and Georgis (2012) 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 48-72hours.

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 the large number of bacteria species associated with Steinernematids, hence their pathogenicity compared to Heterorhabditids. This agrees with a study by Stock and Goodrich-Blair (2012) and Nguyen (2010) 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 (Woodring and Kaya, 1998).

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 *et al.* (2006). According to Grewal *et al.* (2006) *Steinernema feltiae* has adopted both ambushing and cruising strategies for host finding thus making it more virulent than *Heterorhabditis sp.*

The findings in this study compares with those of Van Niekerk and Malan (2012) who on conducting various bioassays to determine the potential of South African EPNs isolates in controlling the citrus mealybug *Planococcus citri* (Risso), reported that the pest was most susceptible to *Steinernema yirgalemense* (97% mortality) compared to the *Heterorhabditis zealandica* (91% mortality). *Steinernema yirgalemense* was more pathogenic and was faster at locating and infecting *P. citri* (Van Niekerk and Malan, 2012). This study therefore, demonstrates the virulence and infectivity potential of the Kenyan EPN isolates against tomato leaf miner. Moreover, the EPNs have the ability to infect and kill *Tuta absoluta* larvae within 48-72 hours.

3.5 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 the Kenyan EPNs that could be exploited for tomato leaf miner management.

CHAPTER FOUR

Efficacy of neem biopesticide in the management of tomato leaf miner *(Tuta absoluta)* in the greenhouse conditions

Abstract

The *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most devastating new pest affecting tomato crops in Kenya. This experiment which was repeated twice, evaluated the insecticidal effect of neem (Azadirachtin 0.03%) against *Tuta absoluta* at various dose rates namely: 20ml/20L, 40ml/20L and 60ml/20L under greenhouse conditions. Neem was compared to the control where only water was sprayed. The experiment was laid out in a complete randomized design with four replications. The results reveal that neem significantly ($p < 0.05$) lowered *Tuta absoluta* population on the treated plants compared to the untreated ones. The mean *Tuta absoluta* population reduction at 40ml/20L and 60ml/20L was significantly ($p < 0.05$) higher than that at 20ml/20L, the lowest dose rate. Mean population reduction at 40ml/20L and 60ml/20L dose rates did not differ significantly. Among the evaluated neem dose rates, 60ml/20L was most effective in reducing *Tuta absoluta* population in the greenhouse compared to other treatment. High fruit damage of over 90% was recorded in the control treatments compared to 30.0%, 23.4% and 20.0% for 20ml/20L, 40ml/20L and 60ml/20L dose rates, respectively. The results demonstrate the potential of neem in reducing *Tuta absoluta* number which can be exploited in pest resistance mitigation program for sustainable management.

4.1 Introduction

The tomato leaf miner *Tuta absoluta* (Meyrick) has been a key pest in Kenya since 2014 and is adversely affecting tomato production in open field and in the greenhouses (GOK, 2014). It is a serious pest primarily in tomatoes but has also been reported in Irish potatoes and in solanaceous weeds (CABI, 2015). Potential yield loss in tomato quality and quantity can reach up to 100% if the pest is not managed (EPPO, 2005). Many control programs have been investigated in South American countries which were affected by *Tuta absoluta*, with chemical control being reported as first line of treatment (Lietti *et al.*, 2005; Siqueira, *et al.*, 2000a). However, due to the development of resistance, chemical control has shown limited efficacy even after using different types of pesticides and increasing the application frequencies (Kona *et al.*, 2014). Increasing concern about the risks from synthetic insecticides to the environment and human health has given way for a search of less hazardous chemicals or the biologically based products (Isman, 2006). Due to increasing resistance to chemical pesticides, there is need for emphasis on the importance of IPM programme in a resistant management strategy. Neem is a botanical product that can be used for the management of *Tuta absoluta*. According to Al-Zaidi (2009) and Mohamed and Siam (2011) pheromone traps also have the potential to control *Tuta absoluta*. Various studies have reported the use of biological control agents in the greenhouse tomatoes in Europe and South America where this pest was reported earlier (Desneux *et al.*, 2010).

Neem *(Azadirachta indica)* has insecticidal properties which have been put into use for several thousands of years since it is biodegradable and effective against pests without harming beneficial insects (Haseeb *et al.*, 2004). Its biological properties are mediated by compounds such as limonoids, particularly Azadirachtin mainly present in the neem seeds. The limonoids are considered to be the most active components responsible of both anti-feedant and insecticidal

effects on insect pests (Isman, 2006). Neem compounds present various effects ranging from repellency to toxicity against a wide spectrum of insect pests (Shannag *et al.,* 2014; Degli *et al.,* 2013; Siddiqui *et al.,* 2009; Isman, 2006).

Neem based insecticides have low negative environmental impact because of a rapid degradation in plants and in the soil (Isman, 2006) and low effects on beneficial insects (Defago *et al.,* 2011; Haseeb *et al.,* 2004). According to Mordue and Nisbet (2004) and Isman (2006) Azadirachtin is non-toxic to vertebrates. Therefore, neem botanical pesticide represents a valuable tool to control population outbreaks in integrated pest management programs (Boursier *et al.,* 2011). This experiment was initiated to determine the insecticidal effects of neem biopesticide in the management of tomato leaf miner (*Tuta absoluta*) under greenhouse conditions.

4.2 Materials and Methods

4.2.1 Study site selection

This experiment was carried out 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 1^0 15'S and longitude 36^0 44`E and at altitude 1941 meters above sea level.

4.2.2 Neem biopesticide experiment under greenhouse conditions

Tomato seedlings were raised in a nursery bed measuring 1 x 2M and after one month they were transplanted onto polythene pots measuring 20cm diameter and 26cm height containing three kilograms of soil, where normal agronomic practices were carried out to nurture the plants till harvesting. The plants were nurtured in the greenhouse measuring (6M width x16M length), with a temperature range of 15-34 ^{0}C and a relative humidity (RH) range of 75.4-85.8%. Infestation of the crop by *Tuta absoluta* occurred naturally. The experiment was laid out in a complete

randomized design with four replicates and was repeated twice. The treatments consisted of different concentrations of Nimbecidine® at 20ml/20L, 40ml/20L and 60ml/20L which were compared with a control where only water was applied. Nimbecidine®, (Azadirachtin 0.03%) is a commercial biopesticide product in Kenya. Larval count was done on four leaves per plant on twelve tomato plants randomly selected per treatment on weekly basis. A delta sticky trap supplied with *Tuta absoluta* female pheromone was hanged at the centre of the greenhouse at a height of one metre for monitoring *Tuta absoluta* population to enable the initiation of treatments. Treatment application was done using a knapsack sprayer after a threshold level of 3 *Tuta absoluta* adult moths were caught in the pheromone trap (Bajonero *et al.,* 2008). According to damage descriptor by Fernandez and Montagne (1990), the *Tuta absoluta* larvae attacks fruits by making galleries, penetrating mainly at the base or near the peduncle insertion zone. The damage observed included: puncture marks where the larvae entered the fruit, exit holes and dried frass produced by last larvae as they pupate especially under the calyx. Therefore, fruits harvested with *Tuta absoluta* marks were recorded as damaged fruits, while those without were recorded as undamaged fruits. Harvesting was done per plot. Percent of damaged tomato fruits was calculated using the formula: - (Weight of damaged fruits/total weight of harvested fruits)*100 (* = is the multiplication sign).

4.2.3 Statistical Analysis

The larval count data collected was square-root transformed before analysis. The data was then subjected to analysis of variance (ANOVA) to assess treatment effects while the Fischer's protected least significance difference (LSD) test was used to compare treatment means. Similar analysis was done on tomato fruit yield data. The analysis was done using GenStat-PC v.14.1, 14th Edition (Payne *et al.,* 2011).

4.3 Results

4.3.1 Evaluation of insecticidal effects of neem against tomato leaf miner *(Tuta absoluta)* larvae in the greenhouse conditions

During the experimental period, *Tuta absoluta* naturally affected the tomatoes which were established in the greenhouse. *Tuta absoluta* population generally decreased after neem applications began up to the fourth week before the numbers started to increase in the fifth week. Meanwhile, the *Tuta absoluta* population in the control continuously increased to reach a mean high of (7.2) at the seventh week. Neem (Azadirachtin 0.03%) at different dose rates tested significantly reduced *Tuta absoluta* population compared to the control. Neem application significantly ($p < 0.05$) lowered *Tuta absoluta* populations on treated plants compared to the untreated ones. In season one neem significantly ($p < 0.05$) reduced *Tuta absoluta* larvae populations on the tomato plants. Control had the highest mean *Tuta absoluta* numbers (3.9) while the mean larval count achieved by the highest dose rate (60ml/20L) was the least (1.4). The mean number count in the 60ml/20L dose rate (1.4) did not differ significantly from that counted in the 40ml/20L dose rate (1.5). Mean *Tuta absoluta* numbers in 40ml/20L and 60ml/20L was significantly ($p < 0.05$) lower than that counted on the control (Table 4.1).

Table 4.1: Mean population of *Tuta absoluta* larvae per four leaves of a tomato plant after neem (Azadirachtin 0.03%) treatment in season one during (December 2015 to February 2016)

		Sampling Period in Weeks								
Treatment	Rate/20L	1	2	3	4	5	6	7	8	Mean
Low dose	20mls	1.4	1.3	1.2	1.1	1.7	2.1	3.0	2.1	1.7b
Medium Dose	40mls	1.5	1.2	1.1	1.0	1.3	1.8	2.5	1.9	1.5a
High dose	60mls	1.4	1.2	1.0	1.0	1.7	1.6	2.0	1.5	1.4a
Control	Water	1.3	2.1	2.3	2.6	3.2	5.6	7.2	7.1	3.9c
L.S.D.										0.1
%CV										26.4

Means followed by the same letter in the column were not significantly different at ($p < 0.05$) Transformation Formula is = Square root of (X+0.5), where X is the data to be transformed.

In season two, *Tuta absoluta* numbers were higher than those recorded in season one. Nevertheless, a similar trend like that in season one was observed (Table 4.2). There was a general reduction of *Tuta absoluta* larvae numbers as neem was applied up to fourth week in all the dose rates tested before the numbers started to increase in the fifth week but at a slow rate. Meanwhile the numbers counted in control continuously increased reaching a mean high of (7.3) at the seventh week (Table 4.2). Neem applications significantly ($p < 0.05$) reduced *Tuta absoluta* larvae populations on tomato plants. Control had the highest mean *Tuta absoluta* numbers (4.2) while the highest dose rate (60ml/20L) had the least mean *Tuta absoluta* population (1.4). The mean population on the 60ml/20L dose rate was not significantly different from that which was recorded on plants treated with 40ml/20L dose (1.5). Mean *Tuta absoluta* populations on 40ml/20L and 60ml/20L dose rates treated plants was significantly ($p < 0.05$) lower than that which was recorded on 20ml/20L dose rate plants (Table 4.2).

Table 4.2: Mean population of *Tuta absoluta* larvae per four leaves of a tomato plant after neem (Azadirachtin 0.03%) treatment in season two during (March to May, 2016)

		Sampling Period in Weeks								
Treatment	Rate/20L	1	2	3	4	5	6	7	8	Mean
Low dose	20ml	1.6	1.5	1.2	1.3	1.9	2.4	2.5	2.1	1.8b
Medium Dose	40ml	1.6	1.3	1.1	1.2	1.4	2.0	1.9	1.7	1.5a
High dose	60ml	1.5	1.4	1.1	1.0	1.5	1.6	1.8	1.6	1.4a
Control	Water	1.7	2.3	2.5	2.6	3.7	6.6	7.3	7.0	4.2c
L.S.D.										0.1
%CV										25.4

Means followed by the same letter in the column were not significantly different at ($p < 0.05$)
Transformation Formula is = Square root of (X+0.5), where X is the data to be transformed.

In the first season, high tomato damage of over 90% was recorded in the control compared to 30.0%, 23.4% and 20.0% for 20ml/20L, 40ml/20L and 60ml/20L dose rates, respectively. Control had the lowest total harvested yield (Table 4.3).

Table 4.3: Effect of neem (Azadirachtin 0.03%) treatments on tomato yield in season one

	Average Fruit Weight (tons/hectare)			
Dose Rates	Damaged	Not Damaged	Total	% Damaged Fruits
20ml/20L	2.2	5.2	7.4	30.0c
40ml/20L	2.2	7.2	9.4	23.4b
60ml/20L	2.0	8.1	10.1	20.0b
Control	2.6	0.2	2.8	92.9a
LSD				0.1
%CV				9.0

Means followed by the same letter in the column were not significantly different at ($p < 0.05$)
Percent damaged tomato fruits = (Weight of damaged fruits/total weight of harvested fruits)*100
(* = Is the multiplication sign).

In the second season, high fruit damage of 96% was recorded in the control plots compared to 31.5%, 22.1% and 20.2% for 20ml/20L, 40ml/20L and 60ml/20L dose rates, respectively. The control had the least harvested (2.6) tons/hectare tomato yield (Table 4.4).

Table 4.4: Effect of neem (Azadirachtin 0.03%) treatments on tomato yield in season two

	Average Fruit Weight (tons/hectare)			
Dose Rates	Damaged	Not Damaged	Total	% Damaged Fruits
20ml/20L	2.3	5.0	7.3	31.5c
40ml/20L	2.1	7.4	9.5	22.1b
60ml/20L	2.0	7.9	9.9	20.2b
Control	2.5	0.1	2.6	96.2a
LSD				0.1
%CV				9.1

Means followed by the same letter in the column were not significantly different at ($p < 0.05$)
Percent damaged tomato fruits = (weight of damaged fruits/total weight of harvested fruits)*100
(* = Is the multiplication sign).

4.4 Discussion

The results obtained from this study reveal that it is possible to reduce the *Tuta absoluta* larvae population by the application of neem biopesticides. *Tuta absoluta* population reduction can be attributed to an active ingredient in neem (Azadirachtin) which has an anti-appetizing, anti-feedant, disgusting and sterile properties that inhibit molting, growth and larval development (Patil and Goud, 2003). The evaluated neem concentrations caused significant reduction in *Tuta absoluta* population, with the highest reduction achieved at 60ml/20L dose rate and the lowest at 20ml/20L dose rate. These results are not surprising given that the toxicity of insecticides of plant extracts has been shown to vary with dose and duration of exposure (Bouchikhi *et al.*, 2010). Neem was most effective in reducing *Tuta absoluta* population when 60ml/20L dose rate was applied probably because it offered a high concentration of Azadirachtin.

These findings are similar to those of a study by Charleston *et al.* (2006), who reported that botanical extracts derived from *Melia azedarach* and *Azadirachta indica* reduced population of *Plutella xylostella*. According to Goncalves-Gervasio and Vendramim (2007) neem seeds extract

induced high *Tuta absoluta* larval mortality under laboratory conditions. However, the results suggest that in the greenhouse tomato cultivation treatment with neem alone is not enough to successfully reduce tomato leaf miner *(Tuta absoluta)* damages, as fruits in all the treatments had varying percentage damage. These results are similar to those by Blue *et al.* (2012), who reported that in open field cultivation treatment with *Azadirachtin* alone was not enough to successfully reduce *Tuta absoluta* damages. Control (where only water was applied) had the highest percentage of fruit damage and a low number of fruits compared to the neem treated plants. This implies that the total number of fruits and the undamaged fruits were affected by increasing infestation densities of *Tuta absoluta*. This is probably the reason why the control had the least total yield of the harvested tomato. The high fruit damage percentages could have been a result of *Tuta absoluta* consumption of fruit while still in developing stages. Velez (1997) explained that sometimes the larvae bore the ovary of the tomato flower promoting the fall of the buds and flowers resulting to low yields. However, the diminished number of fruit produced by the plants exposed to large densities of *Tuta absoluta* in the control treatments can also be in response to foliar area damage, as suggested by Marcano (1995) who explained that the average number of fruit per plant is affected by the percentage of defoliation in tomato plants.

4.5 Conclusion

This study has demonstrated that among the evaluated neem (Azadirachtin 0.03%) dose rates, 60ml/20L and 40ml/20L were effective in reducing *Tuta absoluta* population and percent fruit damage. Neem has the potential of reducing *Tuta absoluta* population on tomato.

CHAPTER FIVE

Evaluation of EPNs alone and EPNs combined with neem as management options for tomato leaf miner (*Tuta absoluta*) under greenhouse conditions

Abstract

The tomato leaf miner *Tuta absoluta* (Meyrick) is one of the most devastating pests affecting tomato crop in Kenya. This study was carried out to evaluate management options for sustainable management of *Tuta absoluta* with the aim of reducing over reliance on synthetic pesticides used for tomato production in greenhouse conditions. The management options evaluated included; EPNs alone and EPNs combined with neem. The treatments consisted of EPNs alone and EPNs combined with neem compared to Coragen® SC (20% Chlorantraniliprole) as a standard synthetic pesticide and the control where only water was applied. A sticky pheromone trap was used for monitoring *Tuta absoluta* adults to guide the initiation of the treatments. The experiment was laid out in a complete randomized design with four replicates and repeated twice. The results showed that all the treatment used significantly ($p < 0.05$) reduced *Tuta absoluta* population on tomato plants compared to the control. *Steinernema karii* combined with neem significantly ($p < 0.05$) reduced numbers of *Tuta absoluta* and, the mean number was different from that of the Coragen® treated tomato plants. EPNs alone significantly ($p < 0.05$) reduced *Tuta absoluta* population compared to control but the mean population reduction was not comparable to that of EPNs and neem or Coragen®. Tomato fruit damage was the highest in control treatment with 91.7% compared to 10.2%, 7.4% and 2.9% for EPNs alone, EPNs combined with neem and Coragen®, respectively. The results demonstrate the potential of EPNs alone and EPNs combined with neem

(Azadirachtin 0.03%) as alternative tomato leaf miner management options for the sustainable management of *Tuta absoluta* in the greenhouse conditions.

5.1 Introduction

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) which is native to South America was recently introduced to Kenya, and has become a major pest of both field and greenhouse tomatoes (GOK, 2014). *Tuta absoluta* larvae is 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 (CABI, 2015). Larvae can destroy up to 100% of the leaf surface and damage 50-100% of fruits in severely attacked fields (EPPO 2005). The extensive insecticide use can cause on the one hand several undesired side-effects on human and environment safety and on the other hand resistance development in *Tuta absoluta* (Haseeb *et al.,* 2004). In this view, there is need for the use of environmentally-friendly *Tuta absoluta* management strategies in order to reduce the use of synthetic pesticides and consequently, improve food safety and environment quality (Isman, 2006). Botanical products, biophysical products and biological methods fit within the environmental friendly strategies, being biodegradable and effective against pests without harming beneficial insects (Hasseeb *et al.,* 2004).

The application of EPNs is widespread in most parts of the world and can be multiplied in large quantities at low costs (Shapiro-Ilan *et al.,* 2006). They are also potent and effective against target insect pests which make them worthy of attention by researchers, especially those interested in the preservation of the environment, responding to demanding calls for preservation of the environment from excessive use of chemical pesticides (Georgis *et al.,* 2006; Shapiro-Ilan *et al.,* 2006; Gaugler *et al.,* 2002). Extensive studies have been undertaken in the field for biological

control of insect pests using several biocontrol agents such as entomopathogenic nematodes (Arthurs *et al.*, 2004).

Entomopathogenic nematodes are safe to the environment and do not cause any harmful effects, either to humans or farm animals and beneficial insects (Ehlers, 2001). Entomopathogenic nematodes can be combined with other compatible agricultural chemicals and control agents for various purposes (Laznic and Trdan, 2013). First, nematodes and other control agents may be applied simultaneously or within a short time at least one hour interval of each other to control different pest species or stages of a pest. For convenience, nematodes may also be tank-mixed with other compatible control agents that is, combined in the tank of the application equipment, thus increasing the chances of interactions due to the higher concentration of both agents (Grewal *et al.*, 1998). Entomopathogenic nematodes may be combined with other compatible control agents to achieve better control of a single pest through additive or, preferably synergistic effects on pest mortality (De Nardo and Grewal, 2003).

Furthermore, in an IPM programme, pesticide treatments using either chemical or biologically-based insecticides may not be compatible with biological control agents such as parasitoids and/or predators, since some active ingredients are harmful to some bio-control agents of *Tuta absoluta* (Biondi *et al.*, 2013). *Tuta absoluta* management should be geared towards an IPM progamme in a resistance management strategy through provision of alternative measures. The use of conventional selective pesticides is also advisable. This may be achieved by using eco-friendly plant extracts with bio-insecticide properties like neem (Tome *et al*., 2013), mass trapping using tomato leaf miner's sex pheromone which when integrated may provide environmentally safe and adequate control of this pest (Cabello *et al.*, 2012; Chailleux *et al.*, 2012). This study was conducted to evaluate the effectiveness of EPNs alone and EPNs combined with neem as a

sustainable management option for tomato leaf miner (*Tuta absoluta*) under greenhouse conditions.

5.2 Materials and Methods

5.2.1 Study site selection

This experiment was carried out 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 1^0 15'S and longitude 36^0 44'E and at an altitude 1941 meters above sea level.

5.2.2 Greenhouse experiment to evaluate the effectiveness of EPNs combined with neem in the sustainable management of tomato leaf miner

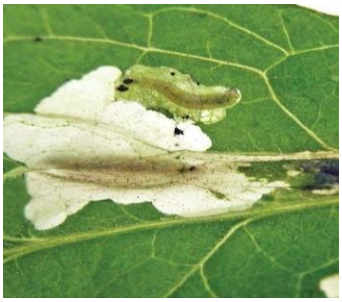
Experiments were conducted to evaluate the effectiveness of *Steinernema karii* alone, *S. karii* combined with neem (Azadirachtin 0.03%), compared to Coragen® (Chlorantraniliprole) in an integrated management of tomato leaf miner *(Tuta absoluta)* in greenhouse conditions. Tomato seedlings were raised in a nursery bed measuring 1 x 2M. After a month of care the seedlings were transplanted into polythene pots filled with three kilograms of soil, where normal agronomic practices were carried out to nurture the plants till harvesting. The plants were nurtured in a greenhouse measuring (6M width x16M length), with a temperature range of 16.5-34^0C and a relative humidity (RH) range of 75.4-85.8%. Infestation of the crop by *Tuta absoluta* occurred naturally. The experiment was laid out in a complete randomized design with four replicates and repeated twice. The experiment had four treatments namely; EPNs alone applied at a rate of 1000 Ijs/ml of water with oil adjuvant Addit® (Koppert) as wetting agent, EPNs with oil adjuvant in water followed by Nimbecidine® (Azadirachtin0.03%) application at a rate of 40ml/20L after a short duration of 1 hour, Synthetic pesticide Coragen® SC (20% Chlorantraniliprole) at the rate

of 3ml/20L and control where only water was applied. A delta sticky trap supplied with *Tuta absoluta* pheromone was hanged at the centre of the greenhouse at a height of one metre for monitoring *Tuta absoluta* numbers to enable the initiation of treatments. Coragen® was used for comparison purposes with other treatments in this experiment.

All the treatments started when three *Tuta absoluta* adults were caught in the trap. The action threshold used was based on occurrence of the three *Tuta absoluta* adult moths on the trap as reported by Bajonero *et al.* (2008). Treatment was carried out using a knapsack sprayer on a regular basis. *Tuta* larval count was done on four leaves per plant on twelve tomato plants randomly selected from each treatment. According to damage descriptor by Fernandez and Montagne (1990) the *Tuta absoluta* larvae attacks tomato fruits by making galleries, penetrating mainly at the base or near the peduncle insertion zone and indiscriminate, wide irregular mines on leaves (Plate 5.2). The damage observed included: puncture marks where the larvae entered the fruit, exit holes (Plate 5.1 a) and dried frass produced by last larvae as they pupate especially under the calyx. Therefore, fruits harvested with *Tuta absoluta* marks were recorded as damaged fruits, while those without (Plate 5.1 b) were recorded as undamaged fruits. Harvesting was done per plot. Percent of damaged tomato fruits was calculated using the formula: - (Weight of damaged fruits/total weight of harvested fruits)*100 (* = is the multiplication sign).

A B

Plate 5.1: Fruit damaged by *Tuta absoluta* larvae (A) Healthy fruit (B)



A B

Plate 5.2: *T. absoluta* larvae damage on tomato plants (A) *T. absoluta* larvae in leaf mine (B)

5.2.3 Statistical Analysis

The data collected was square-root transformed before analysis. The transformed data 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. Similar analysis was done on tomato fruits yield data. The data was analyzed using GenStat-PC v.14.1, 14th Edition (Payne *et al.,* 2011).

5.3 Results

5.3.1 Evaluation of EPNs alone and EPNs combined with neem as management options for tomato leaf miner *(Tuta absoluta)* in the greenhouse conditions

Results obtained in this study reveal that all the evaluated treatments namely; EPNs alone, EPNs combined with neem and Coragen® significantly ($p < 0.05$) reduced *Tuta absoluta* population compared to the control in season one. Mean *Tuta absoluta* population were sustained at an almost constant number within the treated plots throughout the sampling period as opposed to the control where they continuously increased to reach a high of (7.9) by the eighth week of sampling. Coragen®, the standard chemical had the least mean number of *Tuta absoluta* which was not significantly different from that of EPNs combined with neem. The mean population of *Tuta absoluta* in EPNs alone treated plants was low but significantly different ($p < 0.05$) from that recorded in Coragen® and EPNs combined with neem plants. Plants sprayed with water only (control) had the highest *Tuta absoluta* mean numbers (5.7) which was significantly different ($p < 0.05$) from the other treatments (Table 5.1).

Table 5.1: Mean population of *Tuta absoluta* larvae recorded in four leaves per tomato plant after treatment application in season one (June to August, 2016)

	Sampling Period in Weeks								
Treatment	1	2	3	4	5	6	7	8	Mean
EPNs alone	1.8	1.8	1.6	1.7	1.5	1.4	1.3	1.3	1.6b
EPNs + neem	1.6	1.5	1.3	1.2	1.2	1.1	1.0	1.0	1.2a
Coragen	1.6	1.4	1.2	1.1	1.1	1.0	1.0	1.0	1.1a
Control	1.8	3.9	5.6	6.0	6.4	6.7	7.4	7.9	5.7c
L.S.D.									0.1
%CV									1.1

Means followed by the same letter in the column were not significantly different at ($p < 0.05$)
Transformation Formula is = Square root of (X+0.5), where X is the data to be transformed.

There was a significant ($p < 0.05$) reduction in *Tuta absoluta* populations in the three treatments evaluated compared to the control in season two. The mean population of the three treatments were low during the sampling period compared to that of control which continued to increase to reach a peak of (8.0) by the eighth week of sampling. Plants sprayed with water only (control) had the highest *Tuta absoluta* mean numbers (5.9) which was significantly different ($p < 0.05$) from other treatments. Coragen®, the standard chemical, had the least mean population of *Tuta absoluta* which was not significantly different from that of EPNs combined with neem. The mean population of *Tuta absoluta* in EPNs alone treated plants was low closely following that of the Coragen® and neem combined with EPNs. However, it was significantly different ($p < 0.05$) from the mean population which was recorded in Coragen® and Neem combined with EPNs treated plants (Table 5.2).

Table 5.2: Mean population of *Tuta absoluta* larvae recorded in four leaves per tomato plant after treatment application in season two (September to November, 2016)

	Sampling Period in Weeks								
Treatment	1	2	3	4	5	6	7	8	Mean
EPNs alone	1.8	1.7	1.5	1.5	1.4	1.3	1.3	1.2	1.5b
EPNs + neem	1.6	1.4	1.3	1.3	1.2	1.1	1.1	1.0	1.3a
Coragen	1.5	1.3	1.2	1.2	1.1	1.1	1.0	1.0	1.1a
Control	1.8	3.5	5.8	6.6	6.9	7.1	7.5	8.0	5.9c
L.S.D.									0.1
%CV									1.4

Means followed by the same letter in the column were not significantly different at ($p < 0.05$)
Transformation Formula is = Square root of (X+0.5), where X is the data to be transformed.

In season one, high fruit damage of 91.5% was recorded in control compared to 10.8%, 7.9% and 3.0% for EPNs alone, EPNs combined with neem and Coragen® treatments, respectively. Control had the lowest total weight (3.0) tons/ hectare of the tomato fruits harvested (Table 5.3).

Table 5.3: Effect of selected IPM options on tomato yield in season one

	Average Fruit Weight (tons/hectare)			
Treatments	Damaged	Not Damaged	Total	% Damaged Fruits
EPNS alone	0.8	6.6	7.4	10.8c
EPNS+Neem	0.7	8.8	9.5	7.9c
Coragen	0.3	9.6	9.9	3.0b
Control	2.7	0.3	3.0	91.5a
%CV				16.8
LSD				0.1

Means followed by the same letter in the column were not significantly different at ($p < 0.05$) Percent damaged tomato fruits = (weight of damaged fruits/total weight of harvested fruits)*100
(* = Is multiplication sign)

In season two, high tomato fruit damage of 87.1% was recorded in control compared to 11.0%, 6.3% and 2.0% for EPNs alone, EPNs combined with neem and Coragen® treatments, respectively. Again the control had the least harvested yield (3.1) tons/hectare of tomato fruits (Table 5.4).

Table 5.4: Effect of selected IPM options on tomato yield in season two

	Average Fruit Weight (tons/hectare)			
Treatments	Damaged	Not Damaged	Total	% Damaged Fruits
EPNS alone	0.8	6.5	7.3	11.0c
EPNS+Neem	0.6	8.9	9.5	6.3bc
Coragen	0.2	9.7	9.9	2.0b
Control	2.7	0.4	3.1	87.1a
%CV				17.1
LSD				0.1

Means followed by the same letter in the column were not significantly different at ($p < 0.05$)
Percent damaged tomato fruits = (weight of damaged fruits/total weight of harvested fruits)*100
(*= Is multiplication sign)

5.4 Discussion

With the invasion of Kenya by *Tuta absoluta* which is now a real threat to tomato production, there is need for developing a holistic approach to environmentally sustainable, economically sound and effective strategies for the management of this pest (ICIPE, 2015). IPM is the rational application of biological, biotechnological, cultural or crop improvements and chemical measures in pest management. Limited use of synthetic chemical plant protection products is practiced. Synthetic insecticides are mostly used as a last resort to keep pests below levels that may cause economically unacceptable damage or losses (EPPO, 2005). In this study EPNs as biological agents, neem botanical pesticide and Coragen® synthetic pesticide were evaluated as management options for *Tuta absoluta*. Results of this study revealed that it is possible to reduce *Tuta absoluta* population by the application of *Steinernema karii* nematodes and *S. karii* combined with neem in the greenhouse conditions.

The ability of *Steinernema karii* to greatly reduce the pest numbers, demonstrates that *Tuta absoluta* would allow the survival of the infective juveniles (Ijs) long enough to find and infect the larvae on the surface of the leaf as well as penetrate the galleries through entry holes to infect the larvae. Inside the galleries Ijs are protected from adverse environmental conditions thus enhancing their effectiveness in infecting the larvae. Other researchers like Schroer and Ehlers (2005) have reported similar results, and they found that nematodes invaded the diamond back moth larvae *(Plutella xylostella)* within one hour after foliar application of *Steinernema carpocapsae* and obtained a mean survival of 90% Ijs for greater than three hours. Kim *et al.* (2006) reported a twelve hour survival time period of Ijs of *Steinernema carpocapsae* in foliar application on Chinese cabbage leaves under greenhouse conditions. The study has shown that foliar applications of EPNs, pest habitat in the leaves determine the efficacy of the nematodes. In cryptic foliage (leaf

mines) the nematodes are more effective than in the exposed foliage (pests on leaf surface). Hence, the effectiveness of these EPNs can also be attributed to the fact that *Tuta absoluta* makes galleries in leaves which provide nematodes an excellent habitat which is cryptic to avoid harmful environmental factors (desiccation and ultraviolet light) and hence parasitize the insect target (Batalla-Carrera *et al.*, 2010).

Therefore, setting up the experiment in the greenhouse and the cryptic habitat of *Tuta absoluta* in tomato plants, afforded the Ijs some protection from unfavourable environmental conditions at the target site. Another reason for the effectiveness of the EPNs used in this study is the use of oil adjuvant Addit® in water. The adjuvant minimized desiccation and allowed free movement of nematodes towards their hosts. Desiccation is one of the obstacles to EPNs efficacy in foliar applications as it limits their persistence (Glazer *et al.*, 1992). The findings of this study agree with those of Bauer *et al.* (1997); Mason *et al.* (1998) and Piggott *et al.* (2000) who showed that, Ijs persistence on foliage is improved by the use of adjuvants such as Silwet® L77 and Tween® 20. Similarly, the improvement of EPNs in foliar/cryptic habitats has been reported with the addition of adjuvants such as glycerin and polymers (Broadbent and Olthof, 1995).

A study by Piggott *et al.* (2000) reported a reduced desiccation rate for *Steinernema feltiae* on the surface of leaves treated with a formulation containing a polymer humectant. Foliar spray of EPNs was mainly targeted at larval stages of *Tuta absoluta* and proved to be effective in reducing pest populations. This concurs with a study by Batalla-Carrera *et al.* (2010), who reported that larva is the most susceptible stage to the EPNs, and foliar application of these nematodes is necessary to achieve a successful control of *Tuta absoluta*. It was also observed that the application of EPNs on soil would control last instar larval stage, when they slide down from the leaves to pupate, as well as emerging adults from the buried pupae (Batalla-Carrera *et al.*, 2010).

Entomopathogenic nematodes may be combined with other compatible control agents such as *Bacillus thuringiensis*, neem and other botanical insecticides or half recommended dose rates of neonicotinoids like Imidacloprid insecticide to achieve better control of a single pest through additive or, preferably synergistic effects on pest mortality (Koppernhoofer and Fuzy, 2003). Various research which have been carried out have shown that neem does not affect the survival, virulence or infectivity of EPNs when combined together (Piggott *et al.,* 2000). Research carried out by Laznic and Trdan (2013) on the compatibility of Azadirachtin with EPNs revealed that the mortality rates for Ijs were comparable with the control (water only) treatment. Similar results were obtained by Grewal *et al.* (1998) which showed that *Steinernema feltiae* was compatible with Azadirachtin. Moreover, a study by Kulkarni *et al*. (2013) confirmed that the relationships between entomopathogenic nematodes and botanical insecticides such as neem at recommended doses indicated their compatibility and allowed higher nematode survival up to 72 hours after combination where, the survival rate of *Steinernema carpocapsae* after combining with 2.0% Neemgold® (neem) was 92.4%.

The present study revealed that there was no significant difference between EPNs combined with neem and Coragen® treatments in *Tuta absoluta* population reductions. Coragen was used for comparison purposes. The effectiveness of EPNs combined with neem is attributed to pathogenicity of EPNs coupled with neem (Azadirachtin) insecticidal properties. The effectiveness of EPNs combined with neem compared to EPNs alone is attributed to joint approach in the control of *Tuta absoluta* with EPNs targeting the larvae, while neem (Azadirachtin) has insecticidal, insect repellant, antifeedant and ovicidal properties (Pathak and Tiwari, 2010). The united attack by EPNs combined with neem on the pest could have brought about the synergistic effect which resulted to decreased *Tuta absoluta* numbers (Laznic and Trdan, 2013).

Previous studies of the combined neem and a nematode species against white grub showed that their combination was better than the use of nematodes alone (Koppernhoofer and Fuzy, 2003). The installed delta trap with sticky plate and pheromone was for monitoring to guide the commencement of treatments after the alert of the presence of *Tuta absoluta* adult moths. This is because the decision scheme of using insecticides for the management of *Tuta absoluta* is largely based on adult captures in sexual pheromone traps (Benvenga *et al.,* 2007). The action threshold used was based on the occurrence of the three *Tuta absoluta* adult moths on the trap (Bajonero *et al.,* 2008). This study revealed that the control treatments had the highest percentage of fruit damage, compared to other treatments. According to Velez (1997) the larvae sometimes bore the ovary of the tomato flower promoting the fall of the buds and flowers resulting to low yields. This can be attributed to increased *Tuta absoluta* larvae populations in the untreated tomato plants, while EPNs alone, EPNs combined with neem and Coragen had reduced larvae numbers in the treated plants which resulted to less damage on fruits.

5.5 Conclusion

EPNs combined with neem (Azadirachtin 0.03%), applied at one hour interval were able to reduce the population of tomato leaf miner (*Tuta absoluta*) under greenhouse conditions. EPNs with Addit® adjuvant in water were equally able to reduce *Tuta* population on tomato under greenhouse conditions.

CHAPTER SIX

General Discussion, Conclusions and Recommendations

6.1 General Discussion

The use of entomopathogenic nematodes as inundative bioinsecticides against the above-ground pests have been exploited for several decades as biological tools against many important insect pests in the world (Georgis *et al.*, 2006). The study intended to determine pathogenicity of the selected native entomopathogenic nematodes against tomato leaf miner in laboratory conditions. The study established that the Kenyan EPNs namely; *Heterorhabditis sp*. and *Steinernema karii* at 100, 300 and 500 Ijs/ml caused mortality of *Tuta absoluta* larvae, with 500 Ijs/ml concentration and exposure time of 72 hours achieving the highest mortality. The *S. karii* was found to be more pathogenic compared to *Heterorhabditis sp* in both concentration levels and exposure time assays. The findings concur with a research by Caroli *et al.* (1996) which showed that Steinernematids have higher invasion efficiency of their hosts than Heterorhabditids.

A study by Koppernhoofer and Fuzy (2003) reported that differences in the pathogenicity of infective juveniles can be attributed to their ability to actively search for host, their effect on the host immune system, the pathogenicity of the symbiotic bacteria and the number of bacterial cells carried by dauers in their gut. This explains why in this particular study, the test nematodes caused different mortality rates. Moreover, a report by Koppernhoofer and Kaya (1999) indicated that *Steinernema sp*. dauers can carry different amounts of bacterial cells in their intestines. The bacteria released by nematodes is the one which kills larvae through the production of toxins and hydrolytic enzymes, thus high numbers of bacteria leads to high mortality hence the higher concentration of Ijs showed high pathogenicity differences in the EPN species, and immune response of the host (Griffin *et al.*, 2005). The results agree with those of Mwaitulo *et al.* (2011)

and Waturu *et al.* (1997b) who reported that the banana weevil mortality increased with the increasing entomopathogenic nematode dosage. No mortality was reported in the control experiments, thus an indication that the mortality was caused by EPNs infection which was confirmed through dissection of the cadaver to check for the presence of Ijs in the haemocoel.

The study also evaluated insecticidal effects of neem (Azadirachtin 0.03%) in the management of tomato leaf miner (*Tuta absoluta*) in the greenhouse conditions. The study revealed that neem (Azadirachtin 0.03%) application dose rates of; 20ml/20L, 40ml/20L and 60ml/20L lowered *Tuta absoluta* populations on the treated plants. Among the evaluated neem concentrations, 60ml/20L was found to be more effective in *Tuta absoluta* population reduction and protecting the fruits resulting in very low percentage of damaged fruits. The results are similar to those by Bouchikhi *et al.* (2010) who reported that the toxicity of botanical extracts as insecticides varies with dose and duration of exposure. Neem biopesticides in the insect pest management have become popular as an alternative to synthetic chemicals whose extensive use interferes with the health of humans and the environment affects non-target organisms and may lead to the development of pesticide resistance (Lietti *et al.*, 2005). Several researchers have confirmed that most of the botanical insecticides are environment-friendly because they have less toxicity to human and other non-target organisms (Dadang *et al.*, 2009; Isman, 2008).

In case of experiments in chapter four and five control had the highest percentage of fruit damage and low yield of fruits. This implies that the total number of fruits and the undamaged fruits were affected by increasing infestation densities of *Tuta absoluta*. The high fruit damage percentages were probably a result of *Tuta absoluta* consumption of developing fruit protected in the sepals. According to a study by Velez (1997) sometimes the larvae bore the ovary of the tomato flower promoting the fall of the buds and flowers resulting into low yields.

In addition the study evaluated the effectiveness of EPNs alone and EPNs combined with neem as the management options for the tomato leaf miner (*Tuta absoluta*) compared to Coragen®, a standard chemical, in greenhouse conditions. The results revealed that all the tested treatments, reduced *Tuta absoluta* numbers. The reduction of *Tuta absoluta* numbers by EPNs combined with neem was comparable to that of Coragen®. This shows that EPNs are capable of infecting and killing *Tuta absoluta* larvae inside the galleries in tomato plants. This may be attributed to cruiser and the intermediate foraging strategy which is employed by EPNs as they search for their host (Grewal *et al.,* 2005). This finding concurs with that of Arthurs *et al.* (2004) which showed a significant trend of efficacy of EPNs increasing with the degree of habitat concealment for instance, pests inside leaf mines and pruning wounds in trees. The same author, Arthurs *et al.* (2004) reported that relationship between nematode efficacy and degree of habitat concealment supports the hypothesis that among foliar pests, those occupying cryptic habitats are preferred targets because infective stage juveniles are to some degree protected in the target site. According to Witzgall *et al.* (2008) migration of *Tuta absoluta* larvae between leaves when leaving the tunnel and making a new one in another leaf generates large entry holes to the larval galleries that can easily be used by nematodes to penetrate the leaf, thus avoiding desiccation and ultraviolet light, and finally infect the larvae.

Entomopathogenic nematodes alone achieved a lower kill compared to the EPNs combined with neem. This agrees with earlier studies which reported that, to enhance nematode infection against insect pests, combinations of nematodes with other control agents can be synergistic and provide better control than each agent alone (Koppernhoofer and Fuzy 2003; Koppernhoofer and Kaya, 1999). A study by Unruh and Lacey (2001) reported that nematode activity would be promoted by the maintenance of surface moisture and elevated humidity following treatment

applications. As a complementary factor, environmental mediation may explain the apparent better performance of nematodes applied in protected environment (notably greenhouses) compared with field crops. In the study where water only was applied, the highest percentage of fruit damage and low total weight of the harvested fruits were achieved. This agrees with the fact that high densities of larvae bore the ovary of the tomato flower promoting the fall of the buds and the flowers, thus resulting to low yields (Velez, 1997).

6.2 Conclusions

Kenyan EPNs namely; *Heterorhabditis species* and *Steinernema karii* can cause mortality to *Tuta absoluta* larvae and *Steinernema karii* was more pathogenic than *Heterorhabditis species.* High concentrations such as 500Ijs/ml are more efficient in causing larval mortality than the lower ones of 100Ijs/ml and 300Ijs/ml.

Neem (Azadirachtin 0.03%) as a plant based extracts can reduce *Tuta absoluta* populations on tomatoes applied at a rate of 40mls in 20 litres of water in the greenhouse conditions.

Neem (Azadirachtin 0.03%) combined with *Steinernema karii* nematodes formulated in a wetting agent (Addit®) in water effectively reduced *Tuta absoluta* population in the greenhouse conditions. The EPNs are applied first and after one hour, neem should be applied. Kenyan EPNs combined with neem can offer an alternative management of tomato leaf miner in an IPM program under greenhouse conditions. EPNs alone in a wetting agent (Addit®) carried in water also reduced *Tuta absoluta* population and hence can be used in protected environments.

6.3 Recommendations

- Research should be carried out to evaluate the survival and virulence of Kenyan EPNs when combined with synthetic pesticides against *Tuta absoluta* in order to come up with well guided and complete strategies for IPM programs.
- Further research work is needed to evaluate appropriate concentrations of Kenyan EPNs against tomato leaf miner (*Tuta absoluta)* under greenhouse and open field farmer conditions.
- There is need to determine the appropriate formulation components and application techniques that enhance the efficacy of Kenyan EPNs for control and management of *Tuta absoluta*.

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