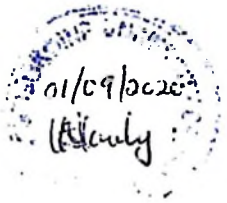
ENTOMOPATHOGENIC NEMATODES AND THEIR POTENTIAL FOR USE AGAINST BANANA WEEVIL (*Cosmopolites sordidus* Germar) IN TANZANIA



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A DESSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.

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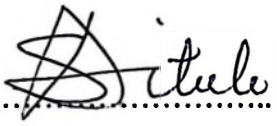
ABSTRACT

A survey on the occurrence of entomopathogenic nematodes (EPNs) was conducted in selected banana fields in three regions (Coast, Mbeya and Morogoro) of Tanzania. The main objective of the study was to isolate entomopathogenic nematodes naturally occurring in banana fields in Tanzania and to test the effectiveness of these nematodes against banana weevil (*Cosmopolites sordidus* Germar). The presence of entomopathogenic nematodes is reported for the first time in Tanzania. Entomopathogenic nematodes were found in soils sampled from banana fields in Coast region of Tanzania using the Great wax moth, (*Galleria mellonella* L.) larvae as bait. Four (4.4%) out of 90 samples contained nematodes from which nine isolates of entomopathogenic nematodes were obtained. Seven isolates were identified as *Steinernema spp* while two isolates were of *Heterorhabdits spp*. The nine isolates were studied to determine their virulence against larvae and adults of *C. Sordidus*. Nematode concentrations of 0.35, 3.5 and 7.0 infective juveniles cm^{-2} were used. All isolates were able to invade *C. sordidus* larvae but adults were rarely infected. At concentrations of 3.5 and 7.0 cm^{-2} MK7A (*Steinernema spp*) and MK7B (*Heterorhabdits spp*) caused the highest larval mortality of up to 100%. The average larval mortality ranged between 52% - 78.67% with isolates MK7C and MK7B (*Heterorhabdits spp*) being the most virulent causing an average larval mortality of 77.33% and 78.67% respectively. The potential of using EPNs as a biocontrol agent for *C. sordidus* larvae based on laboratory results has been demonstrated. However performance of these isolates under field condition must be evaluated and tested. Proper delivery systems for use in different banana production systems need to be developed and tested.

DECLARATION

I, SUMA ANDALWISYE MWAITULO, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is a result of my original work and it has not been submitted nor concurrently being submitted for a higher degree award in any other university.



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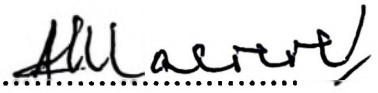

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DEDICATION

This work is dedicated to:

My parents, Mr. A.L. Mwaitulo and the late Mrs. M. Mwaitulo, for taking me to school. I will always miss my mother.

and

My husband Jimmy and my son Gamaliel for supporting me. I will always love you.

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LIST OF ABBREVIATIONS, SYMBOLS AND ACRONYMS

ANOVA	Analysis of variance
BHC	Beta- Hexachlorocyclohexane
cm	Centimetre
DDT	Dichloro-Diphenyl-Trichloroethane
DF	Degrees of freedom
DNA	Deoxyribonucleic acid
DRE	Department of Rural Economy
e.g.	for example
et al.	and others
FAO	Food and Agriculture Organisation
FSG	Food Study Group
GLM	General Linear Model
ha	hectare
i.e.	that is
IJs	Infective Juveniles
IPGRI	International Plant Genetic Resources Institute
IPM	Integrated Pest Management
kg	kilogram
LSD	Least Significance Difference
m	meter
ml	mililitres
mm	millimetre
MS	Mean Sum of square

°C	Degrees Centigrade
spp	species
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Random Fragmented Length Polymorphism
SUA	Sokoine University of Agriculture
SS	Sum of Squares
USDA	United States Department of Agriculture

CHAPTER ONE

INTRODUCTION

1.1 Background

The banana weevil *Cosmopolites sordidus* (Germar, 1824) Coleoptera: Curculionidae is an important pest of banana, plantain and ensete (Rukazambuga *et al.*, 2002). The adult weevil is black and measures 10-15mm. It is free living though most commonly found between leaf sheaths in the soil at the base of the mat or associated with crop residues (Gold and Messiaen, 2000). The weevil is nocturnally active and very susceptible to desiccation. The larvae bore in the banana corm reducing nutrient uptake and weakening the stability of the plant (Gold *et al.*, 2004). Attack in newly planted banana stands can lead to crop failure (McIntyre *et al.*, 2002). In established fields damage can lead to plant loss through premature death, toppling and snapping, delayed maturation and reduced bunch weights (Rukazambuga *et al.*, 2002).

Since the 1970s banana production has experienced major changes in all countries of the East African Highlands in particular, Tanzania and Uganda (Sikora *et al.*, 1989, Uronu and Mbwana, 2006). The occurrence of high levels of pests, diseases, declining soil nutrient status and decreased management standards have led to drastic decline in banana production (Uronu and Mbwana, 2006). In Tanzania heavy infestations of banana weevil are recorded in Kagera, Arusha, Kilimanjaro and Mbeya regions (Uronu and Mbwana, 2006). Different control strategies have been used to control banana weevils. The control strategies are likely to vary from system to system and reflect the importance and pest status of the weevil. Crop sanitation as

one control strategy does not offer complete control of banana weevil (Masanza, 2003). Resistance levels of banana weevils to organophosphate pesticides have been reported in some areas of Tanzania (Uronu and Mbwana, 2006).

In recent years the increasing awareness of pesticides pollution and its impact on human health and environment has made biological control to be considered as one of the appropriate method for insect pest management (Uronu and Mbwana, 2006). Recently there have been many advances in biocontrol of insect pests and one interesting group of organisms in this respect are entomopathogenic nematodes, which are small microscopic worms that kill only insects (Paula *et al.*, 2003).

1.2. Problem statement and justification

Entomopathogenic nematodes (EPNs) are well known as pathogenic to insects and several species have been developed as commercially available biological control agents. These nematodes currently comprising three genera; *Steinernema, Heterorhabditis* and *Neosteinernema* are widespread worldwide (Haukeland *et al.*, 2006). EPNs belonging to genera *Steinernema* and *Heterorhabditis* are biological control agents of a variety of economically important weevil pests including banana weevil, black vine weevil, citrus root weevil, sweet potato weevil and carrot weevil.

EPNs are very common in cultivated and uncultivated soils and numerous surveys have documented their occurrence throughout the world (Hominick *et al.*, 1996, Hominick, 2002, Griffin *et al.*, 2005). The level of efforts that has been applied to the

recovery of EPNs varies, with Europe being the most intensively studied continent. The only continent where EPNs have not been found is Antarctica (Griffin *et al.*, 1990).

Hominick (2002) summarizes the key references for information relating to the biogeography of EPN species. The author provides 36 references for European countries, 12 for Asia, eight for North America and the Caribbean, four for South America, two for Australia and the Pacific Ocean and one for Africa. From this report Africa is therefore a continent where fewest studies have been done as far as EPNs are concerned. Three EPNs species *S. karii, H. bacteriophora* and *H. indica* have been reported to occur naturally in Kenya, while there are no reports of identification of EPNs in Tanzania.

Although the use of EPNs in controlling banana weevil has been practiced to some extent in other countries like Australia, no studies have so far been done in Tanzania. The susceptibility of *C. sordidus* to particular strains of EPNs is variable and the assessment of a wide range of EPN strains and species would be a wise preliminary step to adopting any strain for use in other countries (Treverrow and Bedding, 1993). There is a need to evaluate if EPNs occur naturally in Tanzania, and if they can be used as bio-control agents against banana weevils as well as other insect pests. This study therefore aims at investigating the existence of EPNs and their potential use against the banana weevil in Tanzania.

1.3 Objectives

1.3.1 Overall objective

The general objective of the study was to investigate the natural occurrence of EPNs and evaluate their potential use against *C. sordidus* in Tanzania.

1.3.2 Specific objectives

The specific objectives of the study were:

a) To isolate EPNs that naturally exists in banana growing areas of Southern and Eastern zones of Tanzania

b) To test the virulence of isolated EPNs against larvae and adults of *C. sordidus*

c) To assess future potential uses of EPNs against *C. sordidus*

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of banana industry in Tanzania

Banana and plantains (*Musa spp*) generally referred to simply as bananas are the most important tropical fruit crop worldwide (Maerere *et al.*, 2001). Bananas are staple foods for rural and urban consumers in the humid tropics of East Africa. The crop is also an important source of income particularly in locations where small holders produce them in home gardens (Rugalema and Okiting'ati, 1994) on stands of less than 0.5 ha. The East Africa region accounts for 75% of African and 20% of the world's banana production (Rukazambuga *et al.*, 1998). Out of the total annual world production only 10% is for export trade (Maerere *et al.*, 2001) Hence the crop is an important component of food security in the tropical world and provides income to the farming community through local and international trade.

According to FAOstat (2007) Tanzania is the second largest banana producer in the East and Central Africa, Uganda being the first. While the major producing areas in the country are found in the high altitude zones (Kagera, Kilimanjaro, Arusha, and Mbeya regions) bananas are widely cultivated in medium and low altitude areas in Morogoro, Kigoma, Rukwa, Tanga, Coast, Mara, Mwanza and Shinyanga regions and in the Zanzibar and Pemba islands (Maerere *et al.*, 2001) It is estimated that between 235 000ha to 330 000ha are under banana production accounting for about 20% of the total major food production area (Maerere *et al.*, 2001)

In Tanzania bananas are mostly produced at the smallholder level under systems of shifting cultivation or in permanent farming systems where they are often grown in a mixture of cultivars often intercropped with other annual and perennial crops. They are also produced in intensively managed home gardens where they benefit from regular application of manure and household refuse (Rugalema and Okiting'ati, 1994). The production systems worked well in the past. However they are now unable to meet the demands of rapidly growing population particularly in the major growing regions. The cultivars grown are limited to endemic landraces dominated by the AAA East African highland bananas; the plantains (AAB) and the dessert AAA genome bananas. All these genomic groups are known to be susceptible to key pests prevalent in the East African region (IPGRI, 1998).

2.2 Constraints to banana production in Tanzania

A wide range of factors limits banana productivity. In Tanzania and Uganda a complex of socioeconomic factors, soil fertility depletion and pest constraints have resulted in declining production during the last 25 years, leading to crop disappearance in traditional growing areas (Rukazambuga *et al.*, 1998). Rising population pressure on land has led to shortened fallow periods and consequently declining soil fertility. Insect pests and disease pressure have also increased considerably leading to fast deterioration of banana fields (FSG/DRE, 1992).

The relative importance of constraints varies within Africa. In East Africa for example banana weevil is the key constraint followed by nematodes and black

sigatoka (Godonou, 1999). In Tanzania and Uganda, farmers have identified *C. sordidus* as an important factor contributing to banana production decline and disappearance (Gold *et al.*, 1993, 1998). Diagnostic survey results confirm farmer perceptions at many sites and suggest that *C. sordidus* is an important pest of highland cooking bananas (Sikora *et al.*, 1989, Karamura, 1993, Gold *et al.*, 1993, 1994, 1998). In Ghana the major constraints in order of their importance are nematodes, banana weevil, weeds and black sigatoka (Godonou, 1999).

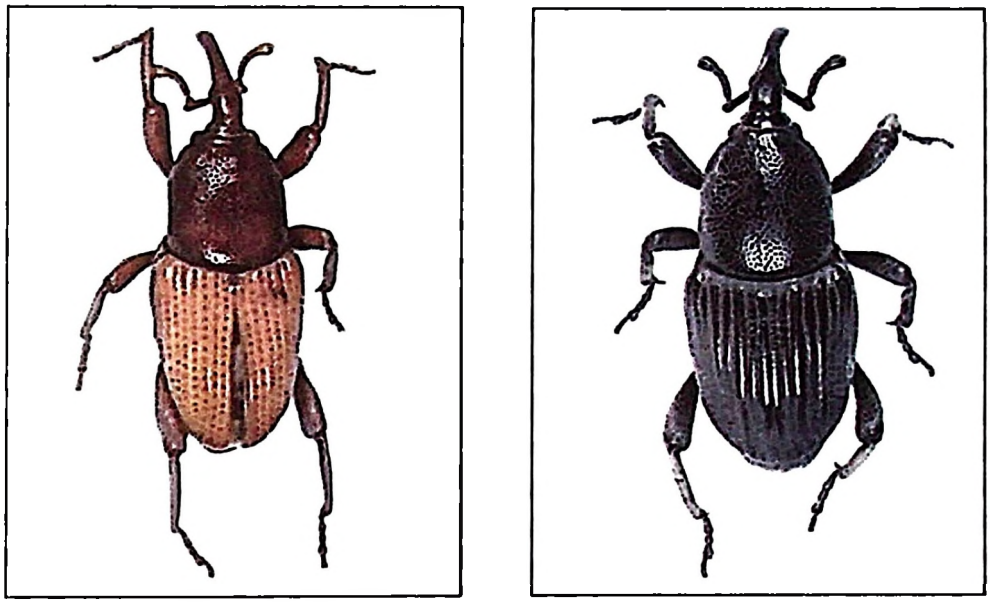
2.3 The Banana weevil (*C. sordidus*)

2.3.1 Origin, distribution and morphology

Banana weevil borer, *C. sordidus* Germar, is a native of Malaysia and Indonesia (Treverrow, 2003). According to Neuenschwander (1988) *C. sordidus* occurs in all banana growing areas and therefore is recognized as an important pest of *Musa spp* in most production areas.

The adult is about 12 mm long, hard shelled and has a pronounced snout. The newly emerged adult is red brown but turns black two or three days later (Fig. 1). The egg is elongate oval, about 2 mm long and pure white. The fully grown larva is 12 mm long. It is creamy white, stout, fleshy, legless and distinctly curved and swollen in the middle. It has a brown head with strong jaws. The pupa is white and about 12 mm long. As it develops, the shape of the adult within becomes visible (Gold *et al.*, 2001). The adults are black or dark brown 10-16mm long. They are free-living though most

commonly associated with banana mats and crop residues (Gold *et al.*, 2004). Adult weevils may live up to 4 years (Rukazambuga *et al.*, 1998).



(a) (b)

Figure 1: Dark brown (a) and Black (b) Adult banana weevil (*Cosmopolites sordidus*)

Photo: Arnstein Staverløkk

2.3.2 Biology and ecology of *C. sordidus*

Banana weevils are nocturnally active and may be sedentary for extended periods of time (Gold *et al.*, 1999). Most adults are found either in the base of leaf sheaths or in the soil around the mat, whereas some adults are found associated with cut crop residues. Marked adults have also been observed to tunnel and re-enter the rhizome (Gold *et al.*, 1998). The adults rarely fly, but commonly walk over the soil surface and vegetation (Rukazambuga *et al.*, 2002).

Larvae tunnelling in the corm and rootstock do the most harm. Weevil damage, expressed as percentage coefficient of infestation, was studied by Reddy (1989) in 22 districts in Kenya. Severe damage of more than 80% was recorded in ten districts. In Tanzania, Reddy (1989) recorded corm damage ranging from 52-95% among different cultivars. In their study Rukazambuga *et al.* (1998) found that cooking banana types were more damaged by *C. sordidus* than sweet bananas.

It is estimated that more than four generations of *C. sordidus* may occur each year. However the situation is complicated because adult weevils may live for up to 4 years (Rukazambuga *et al.*, 1998). Females can mate and oviposit a week after becoming adults and lay 2-4 eggs per week and 50-100 eggs per annum (Abera *et al.*, 1997). Female weevils can however accumulate eggs and remain inactive for extended periods of time. Both sexes are particularly attracted to stressed and damaged plants where they feed and mate. Female oviposit in and around crevices in the corm. A single cycle from egg to adult takes from 6 weeks to 6 months depending on temperature (Treverrow and Bedding, 1993).

Usually a 50% -70% survival rate of eggs within excised corm can be expected in the laboratory, but much higher mortalities of eggs and young larvae occur in the field (Abera *et al.*, 1997). Although the insect is multivoltine, fairly fecund and long lived, *C. sordidus* populations are slow to increase because oviposition is relatively infrequent. In these circumstances introduction of a significant adult mortality factor leads to adequate population control (Treverrow and Bedding, 1993).

2.4 Management of *C. sordidus*

2.4.1 Cultural control

Cultural control involves destroying the sheltering and feeding places of the adult weevils (Godonou, 1999). Harvesting of all mature pseudostems at certain intervals, rather than continually, is suggested as a preventive measure of control. This discourages the continuous breeding of the weevil, as then there will be periods in which few young suckers are present.

The use of vigorous uninfected planting material under good planting conditions in non infected fields is the most important practice to reduce infestation. It is not advisable to replant previously infested areas where old corms remain in the ground (Treverrow, 1983). Planting material should be obtained from plantations free of weevils and examined carefully by taking one or two slices from it. If larvae, pupae or tunnels are present, the material must be destroyed (Treverrow, 1983). Peeling the rhizomes free of lesions and immersing in hot water at 54°C for 10 minutes, as recommended for nematode control, is practical and effective (Stover and Simmonds, 1987).

Good husbandry practices, such as clean weeding, manuring and mulching produce vigorous banana plants which have improved weevil tolerance. The use of clean planting stock, rigid sanitation, strict quarantine measures and meticulous cultural methods are universally advocated to reduce the pest status of *C. sordidus*.

2.4.2 Trapping and pheromone control

Trapping is also an important cultural method for controlling *C. sordidus*. Pseudostem and rhizome traps are commonly used in the removal of adult weevils from infested banana fields. At Ungoye in Kenya, Reddy (1988) used split pseudostem traps and disc-on-stump traps, and the weevils were hand collected from them. The disc-on-stump traps were found to be more attractive. Reddy (1989) stated that continuous trapping over one year reduced the population of weevils by 50%. Trapping method is often of limited effectiveness against established infestation and is tedious and time consuming (Gonodou, 1999). Nevertheless trapping may be cheap and effective when used in combination with chemical or biological insecticides in an integrated weevil management programme.

Pheromones and other behaviour-modifying chemicals hold great potential as tools for pest management (Suckling, 2000). Pheromones have been used in both monitoring insect pest populations and in direct control (Tinzaara *et al.*, 2005). Pheromone traps provide an easy and efficient way of detecting insect populations in the field and in storage facilities (Phillips, 1997). Control of insect pests can be achieved by mass trapping using pheromone-baited traps that lure insects to their death (Alpizar *et al.*, 2002). The application of pheromone trapping requires optimization of the trapping method (Tinzaara *et al.*, 2005)

An aggregation pheromone has been identified for *C. sordidus*, which is specific to the weevil (Jayaraman *et al.*, 1997). A synthetic pheromone source containing a mixture of the four sordidin isomers is sold under the trade name Cosmolure+. The

pheromone has been studied in the laboratory and in the field for the management of *C. sordidus* (Tinzaara *et al.*, 2000, 2003) and attracts both male and female adult weevils (Tinzaara *et al.*, 2000). The pheromone-baited trap captures up to 18 times more weevils than a conventional split pseudostem trap (Tinzaara *et al.*, 2000) and lasts for 1 month compared to 3-5 days for pseudostem traps (Jayaraman *et al.*, 1997). Alpizar *et al.* (1999) reported that pheromone-baited traps have been effective in reducing weevil populations in Costa Rica and the Canary Islands. In Costa Rica, normal recommendations are for 4 traps/ha, changed and moved every month. Although availability of materials is not a concern in pheromone-baited traps as it is the case with pseudostem traps, high costs of pheromones may be prohibitive for subsistence farmers.

2.4.3 Chemical control

Insecticides can be used for the control of banana weevil and are mostly used by commercial banana and plantain producers. They can be incorporated into traps or applied to soil (Simmonds, 1966).

Insecticides to combat the adults are applied to the bases of the plants, including the suckers, and the surrounding soil surface for a distance of 30 cm around the plants, using knapsack sprayers, high volume spraying units or by sprinkling as dusts (Treverrow, 1983). Waterhouse and Norris (1987) found out that dipping planting material in suitable insecticides or applying granules two or three times a year provides good control of *C. sordidus*.

Persistent organochlorine such as DDT and Dieldrin were until 1970's the standard recognized chemicals used against *C. sordidus* (Nankinga, 1994). However, environmental concerns such as pollution of agricultural lands and ground water and the development of resistance by the weevils to the organochlorine chemicals necessitated a change to organophosphate and or carbamate insecticides (Mitchell, 1980). These chemicals are less persistent and less harmful to non-target organisms (Nankinga, 1994).

Collins *et al.* (1991) has provided an overview of insecticide resistance to banana weevil. Such resistance has been documented in Australia (Swaine and Corcoran, 1980, Collins *et al.*, 1991), Africa (Bujulu *et al.*, 1983) and Latin America (Mello *et al.*, 1979) for a range of chemicals including cyclodienes (BHC and dieldrin), organophosphates (chlorpyrifos, ethoprophos, pirimiphos-ethyl and prothiophos) and carbamates (carbofuran). Cross-resistance has also been demonstrated (Collins *et al.*, 1991).

The high cost and scarcity of synthetic insecticides have limited farmers' ability to acquire and use them (Godonou, 1999). Farmers who can afford to buy chemicals tend to apply inadequate doses (under-dosing) a situation that renders them ineffective and enhances probability of pest developing resistance to chemicals (Nankinga, 1994). Chemical control methods are therefore environmentally and socio-economically unsound in the context of the resource-poor farmers who mainly grow the crop in Africa.

2.4.4 Biological control

2.4.4.1 Classical biological control

Possibilities and considerations for classical biological control of banana weevil have been reviewed by Greathead (1986), Greathead *et al.* (1986), Waterhouse and Norris (1987), Neuenschwander (1988), Kermarrec *et al.* (1993). A partial list of arthropod natural enemies has been provided by Schmitt (1993). Approaches include the use of endemics, exotics (classical biological control), secondary host association and microbial control.

According to Waterhouse and Norris (1987), though weevils as a group seem to be poor candidates for biological control, the establishment of *Plaesius javanus* and *Plaesius laevigatus* in Fiji appears to have reduced the pest status of *C. sordidus* there. Although several beetle predators have established successfully in a number of instances, no significant reductions of the pest have been achieved (Koppenhöfer and Schmutterer, 1993). A major reason for some of these failures might have been that the pest's biology was not taken into consideration sufficiently, and neither the biology of the predators, nor their quantitative impact had been studied. The greatest potential for the biological control of *C.sordidus* now seems to lie in the use of indigenous or exotic entomopathogens (Nankinga, 1994, Gonodou, 1999).

2.4.4.2 Biological control using entomopathogens

Pathogens used for insect and mites control include viruses, bacteria, fungi, protozoa and nematodes. For the control of *C. sordidus*, entomopathogenic nematodes

(Treverrow *et al.*, 1991) and entomopathogenic fungi (Nankinga, 1994, Gonodou, 1999) are considered the most promising candidates.

In greenhouse trials, the nematodes *Steinernema carpocapsae*, *S. glaseri* and *S. bibionis* reduced the number of tunnels made by larvae in plantain corms at rates of 400, 4000 and 40 000 nematodes per 4-month-old plant. At the higher two rates, the nematodes caused 100% larval mortality (Figueroa, 1990). Two large-scale field trials in New South Wales, Australia using the nematodes *S. carpocapsae* A11 and *S. carpocapsae* NC513 gave acceptable levels of larval *C. sordidus* control (Treverrow *et al.*, 1991).

According to Koppenhöfer and Schmutterer (1993), fungi have shown useful efficacy as control agents of this pest and might become substitutes for insecticides in the future (Delattre and Jean-Bart, 1978, Castineiras *et al.*, 1991). However, in many tropical countries where bananas are an important staple food, distribution and application of biocontrol agents are still restricted by lack of facilities. They may also be too expensive for the resource-limited subsistence farmers who represent the majority of the banana producers.

2.5 Entomopathogenic nematodes (EPNs)

2.5.1 Biology of EPNs

Entomopathogenic nematodes are lethal obligatory parasites of insects (Liu, 2000). They are ubiquitously distributed and comprise the families Heterorhabditidae and Steinermatidae. These nematodes are characterized by their ability to carry specific

pathogenic bacteria. Heterorhabditidae nematodes carry *Photorhabdus* bacteria while Steinermatidae nematode carries *Xenorhabdus* bacteria. These bacteria are released into the insect hemocoel after penetration into the insect hosts has been achieved by the infective stage of the nematode (Griffin *et al.*, 2005). The Heterorhabditidae is monotypic, represented by the genus *Heterorhabditis*. The Steinermatidae comprises two genera; *Steinernema* and *Neosteinernema*. These nematodes because they serve as vectors of bacteria are termed entomopathogenic, reinforcing the link between insect nematology and insect pathology (Liu *et al.*, 2000).

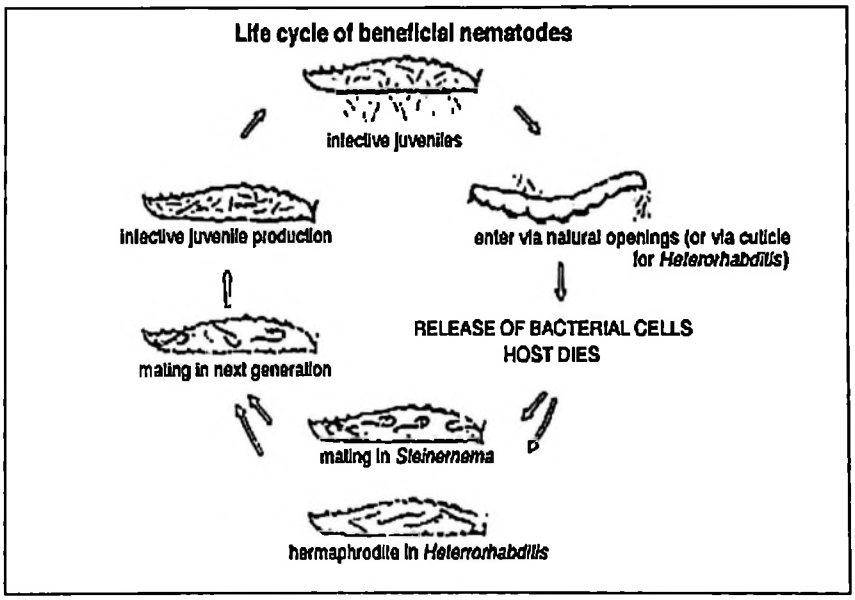


Figure 2: Life cycle of entomopathogenic nematodes

Source: Weeden *et al.*, 2008

2.5.2 Properties of EPNs as biological control agents

Entomopathogenic nematodes (EPNs) are increasingly attracting attention as biological control agents particularly of insects for which part of the life cycle is in soil or cryptic habitats. Entomopathogenic nematodes possess many attributes of an

excellent biological control agent. They are environmentally safe and acceptable (Kaya and Gaugler, 1993), can be produced in large quantities with artificial media (Bedding, 1984); successfully packaged and transported (Smart, 1995) and are easily applied with standard spraying equipment or irrigation systems (Liu *et al.*, 2000). Most species have a broad host range and are highly virulent killing their host rapidly. More than 30 entomopathogenic nematode species exist and hundreds of isolates have been collected from every continent. Many of these isolates have been tested for biological control of insect pests (Liu *et al.*, 2000).

In the soil the free living infective juvenile nematode penetrates host insects through natural body openings such as the mouth and spiracles (Bornstein-Forst *et al.*, 2005). Once inside the host species-specific, symbiotic bacteria are released from the infective juvenile gut into the insect hemocoel and through the synergistic actions of both nematodes and bacterial virulence factors, the host is killed within 24 - 48 hours post infection (Boemare, 2002). The bacteria are also responsible for antibiotic production and for providing nutrition for feeding and development. The host carcass subsequently becomes a nutritional rich environment for EPNs mating and progeny development, which includes the egg and four juvenile stages (Adams and Nguyen, 2002).

Often 2-3 generations are completed within the hoist cadaver. When resources within cadaver are depleted a new generation of IJs is produced which leaves the cadaver to search for new hosts (Kaya and Gaugler, 1993) (Fig. 3).

Figure 3: Nematodes emerging from a Wax Moth (*Galleria mellonella*) cadaver

Source: http://en.wikipedia.org/wiki/Entomopathogenic_nematode

2.5.3 Habitat preferences and distribution of EPNs

All organisms have specific niche requirements that will be satisfied only in particular habitats. Habitat preferences are likely to reflect the distribution of suitable host (Hominick, 2002). EPNs are very common in cultivated and uncultivated soils and numerous surveys have documented their occurrence throughout the world (Griffin *et al.*, 2005). The level of effort that has been applied to the recovery of EPNs varies with Europe being the most intensively studied continent (Hominick, 2002). The distribution of EPNs on a global scale like that of other taxa is probably strongly influenced by climate and chance dispersal events including those associated with human activities. Soil texture, vegetation and availability of suitable hosts are amongst the factors that have been implicated in affecting local distribution pattern (Griffin *et al.*, 2005).

There is growing evidence of nematode species preferences for certain habitats (Griffin *et al.*, 2005). The extensive and intensive surveys in Germany have revealed habitat preferences for several *Steinernematids* (Sturhan and Liskova, 1999). *S. affine*

prevails in cultivated fields and grasslands while species *S. intermedium* and *S. kraussei* are mainly forest species (Stock *et al.*, 1999). The association with sandy coastal soils remains the most robust correlation between habitat and presence of *Heterorhabditis* (Hominick, 2002). For example *H. megidis* and *H. indica* are almost exclusively found in sandy soils resulting in a mainly coastal distribution (Hara *et al.*, 1991; Amarasinghe *et al.*, 1994; Griffin *et al.*, 1994, 2000, 2005). As a broad generalization, prevalence of Steinernematids seems to be highest in woodlands, which support the largest Steinernematids biodiversity (Stock *et al.*, 1999). Soil type is essential for the existence of some *Heterorhabditis* species but is apparently of less importance for Steinernematids (Hominick, 2002).

2.5.4 EPNs as biological control agents

Wide spread resistance to conventional insecticides coupled with a reduction in the number of products registered in many countries for control of insect pests make the development of effective alternative management options a high priority (Ehlers, 2005). Availability of commercial EPNs has sparkled extraordinary interest and created immense opportunities to test them against a wide variety of insect pests. Excellent results have been obtained against the black vine weevil, *Otiorhynchus sulcatus* (Kaya and Gaugler, 1993). Much of the efficacy data have been previously summarized with the general consensus that EPNs are most efficacious against insects in cryptic and soil habitats (Stock and Hunt, 2005).

Foliar applications of nematodes have been used successfully for the control of pests such leaf miners on chrysanthemum and lettuce (Williams and Walters, 2000). In the

Caribbean, local and exotic strains of entomopathogenic nematode of the Heterorhabditidae and Steinernematidae have been tried against *C. sordidus* adults and larvae. Kermarrec *et al.* (1993) reports that EPNs are very effective against the larval stages, but less effective against the adults, which unfortunately are the underground targets of the intended control.

The biocontrol potential of nematodes is not restricted to insects only. *Phasmarhabditis hermaphrodita* (Schneider), a member of the family Rhabditidae is known to suppress several slug species and has recently been developed as a biological molluscicide (Stock and Hunt, 2005).

2.5.5 Effects of EPNs on non-target organisms

Not only do entomopathogenic nematodes affect their host insects, they can also change the species composition of the soil community (Somaseker *et al.*, 2002). Many familiar animals like earthworms and insect grubs live in the soil, but smaller invertebrates such as mites, collembolans and nematodes are also common. Aside from EPNs, the soil ecosystem includes predatory, bacteriovorous, fungivorous and plant parasitic nematode species (Strong *et al.*, 1996).

Since EPNs are applied in agricultural systems at a rate of 250 000 individuals per square meter, the potential for unintended consequences on the soil ecosystem appears large. EPNs have not had an adverse effect on mite and collembolan populations (Georgis *et al.*, 1991), yet there is strong evidence that they affect the species diversity of other nematodes. In a golf course ecosystem, the application of *H.*

bacteriophora, an introduced nematode, significantly reduced the abundance, species richness, maturity, and diversity of the nematode community (Somaseker *et al.*, 2002). EPNs had no effect on free-living nematodes. However, there was a reduction in the number of genera and abundance of plant-parasitic nematodes, which often remain enclosed within growths on the plant root (Somaseker *et al.*, 2002).

2.5.6 Extraction and Identification of EPNs

Isolation of entomopathogenic nematodes from soil can be accomplished by standard extraction techniques for soil nematodes. The extraction process only succeeds in recovery of numerous nematodes and for entomopathogenic nematodes only the free living infective juveniles are extracted which need to be separated and identified. This is a laborious and difficult procedure and it can therefore facilitate processing of only a few samples (Hominick, 2002).

The "trap" insect method developed by Bedding and Akhurst (1975) is unique for extraction of entomopathogenic nematodes (Kaya and Stock, 1997). However even a susceptible insect host recovers only a portion of the nematode population. Negative assays may reflect either absence of nematodes or lack of infectivity of the resident population at the time of sampling (Hominick *et al.*, 1996). Extraction efficiency depends on a number of factors including the method, soil type, nematode species, sample size and the laboratory personnel (Kaya and Stock, 1997). The great advantage of this method is that live nematodes can be isolated for further cultivation and identification.

For several decades nematode identification has been done using morphological characters. In most cases this method has been limited to nematode identification to genus level. Nematode identification, even limited to the genus level, is a difficult process, which is not mastered by all plant nematologists (Fortuner, 1989). Because of the amount of data related to identification (100 to 150 morphological criteria, differentiating 125 to 200 plant nematode genera), it is necessary to use an identification aid when identifying an unfamiliar form (Siddiqi, 1997). Dichotomous keys are the traditional identification aids in nematology (Fortuner, 1989). Species identification utilizes characters/features of many kinds including morpho-anatomical, physiological, ecological, ethological, embryological and cytogenetical. By far the most useful and widely used characters are the morpho-anatomical (Siddiqi, 1997).

Traditional identification aids have been used successfully for a hundred years. However it has been shown that their usefulness is greatly reduced when the user has no preconceived idea of the identity of the specimens. Morphological units may actually consist of assemblages of cryptic species or ecotypic variants of a species. It is essential that biochemical and molecular-based approaches link the biochemical/molecular phenotype to these morphological units (Stock and Hunt, 2005).

The relative paucity of morphological traits and their limited utility in identification and/or diagnosis of many nematode groups have resulted in the exploration of alternative tools such as biochemical and molecular methods (Stock and Hunt, 2005).

Many of these approaches have provided interesting and important insights into biodiversity and evolution, particularly for parasitic nematodes such as Steinernematidae and Heterorhabditidae (Stock *et al.*, 2001). A wide range of molecular approaches has been used and/or adopted for diagnostics/identification of nematodes. However three methods (RAPD, RFLP and DNA sequencing are being used most extensively (Stock and Hunt, 2005).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Selection and description of the study area

This study was conducted in three regions of Pwani (Coast), Mbeya and Morogoro in Tanzania (Fig. 4). The regions represent different agro-ecological zones as per classification by USDA (2005) shown in table1. In each region three villages were selected for soil sampling.The villages were Mkenge, Mwalusembe, Kimanzichana (Pwani), Kiwira, Kyimo, Bujela (Mbeya), Tangeni, Kiroka and Pangawe (Morogoro)

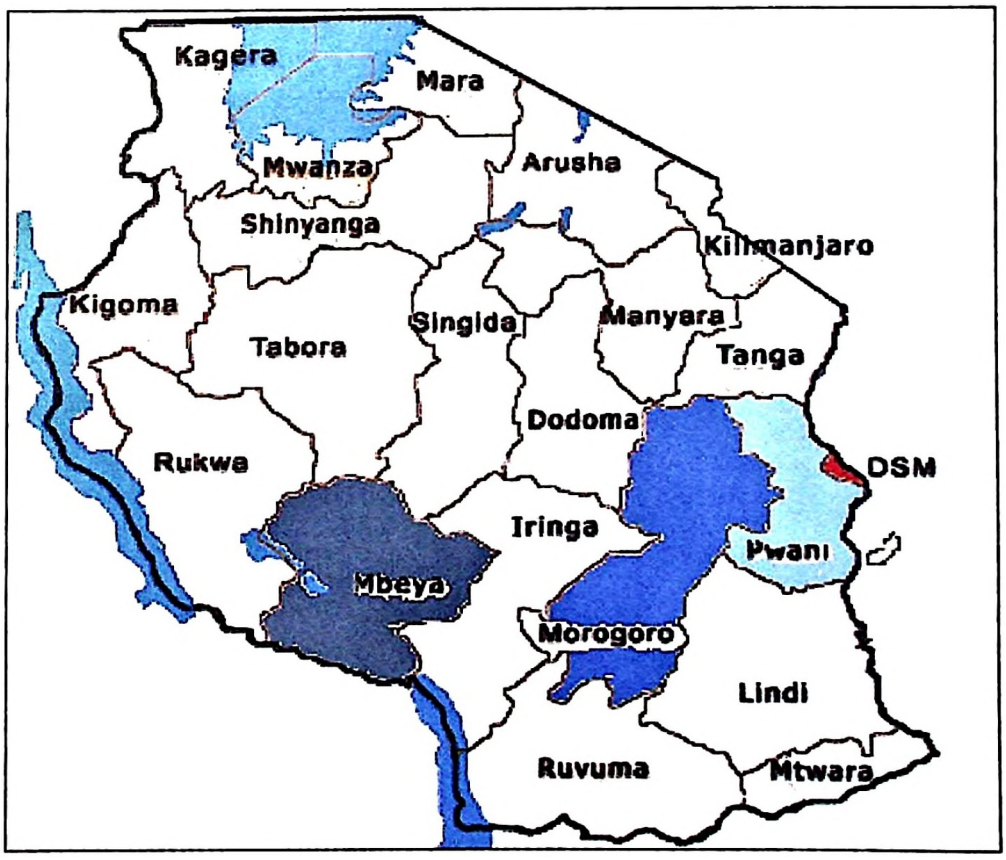


Figure 4: Map of Tanzania showing study regions.

Source: Local Government information (2008)

Table 1: Agro-ecological zones of Tanzania

Zone	Sub-zone and area	Soils and topography	Altitude	Rainfall (mm/yr)	Growing season
COAST	North: Tanga (except Lushoto) Pwani and Dar-es salaam	infertile sands on gently , rolling uplands, alluvial soils in Rufiji fertile clays on uplands and river flood plains	under 300m	bimodal 750-1200mm	October, December and March toJune
SEMI-ARID LANDS	SouthEastern: Morogoro (except Kilombero & Wami basin and Uluguru mountains) also Lindi and SW Mtwara	Flat or undulating plans with rocky hills. Moderately fertile loams and clays in Morogoro and infertile sands in centre	200-600m	unimodal 600-800mm	December to March
SOUTHERN AND WESTERN HIGHLANDS	Southern: a broad ridge from N. Morogoro to N. lake Nyasa covering part of Iringa and Mbeya	Southern: Undulating plains to dissected hills and mountains. Modelately fertile clay soils with volcanic soils in Mbeya	1200-1500m	Unimodal, reliable local rains shadows. 800-1400mm	December to April

Source: USDA (2005

3.2 Soil sampling

From each location or village, a banana field was selected and an area of approximately $100m^2$ (10m×10m) was sampled. Soil samples were taken inside banana fields to a depth of at least 15cm using a hand shovel. The sampling was conducted by taking several shovels along a "W" in each sampling area. The sub samples were mixed to get one sample. A sample weight of 1.5 kg was taken per sampling unit and kept separately in plastic bags. Ten soil samples were taken per village making a total of 90 samples for nine villages. Immediately after sampling, soil samples were kept in cool boxes for transportation to the laboratory. In the laboratory soil samples were kept in a cooling incubator at 14^0C before nematode extraction began.

3.3 Extracting nematodes using insect trapping technique

3.3.1 Rearing and conditioning of Great wax moth

Great wax moth (*Galleria* mellonella Linnaeus) used in this study were initially collected from abandoned beehives in the SUA apiary and reared in the laboratory following the method/procedure described by Maney (2006). Larvae of *G. mellonella* were heat treated in order to prevent them from cocooning. The procedure described by Woodring and Kaya (1988) was followed. The larvae were heat treated by putting them into hot water ($56\ ^0C$) for 15 seconds. Thereafter the larvae were strained from the hot water and cool tap water was run over them for 30 sec. The larvae were then dried on tissue papers and kept in a plastic container with perforated rid. After three hours all larva became active again. Bigger and fat active larvae were selected for soil baiting (Fig. 5).

3.3.2 Baiting of the soil

EPNs were extracted from all soil samples following the trap insect technique as described by Woodring and Kaya (1988). Soils were placed in 500ml plastic containers with perforated lids (Fig. 6). Heat-treated last instar *G. mellonella* larvae (Fig. 5) were used as bait at a rate of five larvae per container. The containers were incubated at room temperature 28^0C-30^0C. Bait larvae were examined for mortality after 6 days thereafter at every three to four days for three weeks.

Each dead larva was treated individually to avoid contamination in case of different causes of death. Dead larvae that exhibited signs of infection with entomopathogenic nematodes (Woodring and Kaya, 1988) were placed in modified white traps (Fig.7). The dead larvae on the white trap were checked for emergence of EPNs after one week and thereafter daily. Nematodes emerging from an individual insect larva were collected and considered as one isolate. These nematodes were later used for re-infection on *G. mellonella* larvae for multiplication.

Figure 5: Conditioned *Galleria mellonella* larvae ready for baiting

Figure 6: Bottles containing soils baited with *Galleria mellonella* larvae

3.3.1 Nematode identification

Nematode identification for this study was based only on the appearance of infected *G. mellonella* larvae as described by Wooding and Kaya (1988). Insects infected by steinernematids are often flaccid and change colour to orange, yellow or brown while those infected with heterorhabditids become flaccid and change colour to brownish red to brick red that faintly luminesces in the dark. Also live nematodes were isolated from infected insects and cultured to confirm the identity.

3.3.2 In vivo multiplication of EPNs

A beaker containing nematode suspension was placed on a stirring magnetic bar in order to have uniform distribution of nematodes. Then 1ml of nematode suspension was drawn from the beaker and placed in a counting plate. Nematodes were counted under stereo microscope, whereby 5 counts were done for each isolate and the average number of nematodes per ml was recorded for every isolate. The nematode

suspension for each isolate was adjusted such that the concentration was 200nematode/ml.

Two filter paper of diameter 55mm were placed on the lids of 60mm diameter Petri dishes. 1.5 ml of distilled water was added to moisten the filter paper. Ten heat treated *G. mellonella* larvae were placed in petri dishes where 1ml of nematode suspension was added for infection. After 4 days the dead insect larvae were placed in a modified white trap (Woodring and Kaya, 1988) for collection of emigrating nematodes from the dead host cadaver.

Figure 7: Modified white trap for collecting entomopathogenic nematodes

Nematode harvesting started immediately when infective juveniles (IJs) emerged from the insect cadavers. The IJs were harvested by removing the petri dish with dead larvae then the IJs were poured into a beaker. More water was added in the beaker containing IJs. Infective juveniles settled at the bottom after 30 minutes and

water was decanted and fresh water was added to the beaker. This was repeated three times.

Harvested IJs nematodes were stored in bottles at 14^0C. The bottles containing nematode were placed in a flat position with amount of water no more than 1cm deep to allow air flow.

3.4 Multiplication rate of nematode isolates in *G. mellonella* larvae

To examine the multiplication rate of nematode isolates, five *G. mellonella* larvae were placed in 60mm petri dishes containing moist filter paper. Infective juvenile nematodes were applied on the insect larvae at a rate of 30 nematodes/insect larva. After 4 days dead *G. mellonella* larvae were individually placed in a nematode emergence trap as described in Kaya and Stock (1997). After three weeks emigrating infective juveniles in the suspension were transferred into small beakers which were then filled to 100ml with sterile water. Two 100µl alquots were taken from the beaker and the number of nematodes contained in 100µl was determined under stereo microscope. The mean number of nematodes in these two alquots was multiplied by 100 to estimate the total number of infective juveniles that had exited the host cadaver. This provided a measure of multiplication. The multiplication data were averaged for each nematode isolate.

3.5 Screening for nematode virulence against larvae and adults *C. sordidus*

Banana weevils used in this study were initially trapped from the banana experimental plots at Sokoine University of Agriculture (SUA) and reared in an outdoor rearing cage where both adults and larvae were harvested from. The new EPN isolates were screened in small-volume arena, i.e. 143cm^2 cylinders (diameter 4.5cm and height 9cm) filled to 6cm with sterile sand adjusted to 15% water content. Two experiments were set whereby adults and larvae of *C. sordidus* were used as experimental units.

Each cylinder was supplied with five larvae/ adult banana weevil and a piece of fresh banana corm (~2cm^3) as food source for banana weevil. Nematode concentration of 50 (0.35cm^{-2}), 500 (3.5cm^{-2}) and 1000 (7.0cm^{-2}) infective juvenile in 1ml distilled water were applied on the centre of each cylinder. Five replicates were made for each nematode concentration, plus a control group where only water was added. The experiments were set as split plot experiments arranged in complete randomized block design. The main plot were three nematode concentrations randomized in five replicates and the sub plots were the nine nematode isolates randomized in the main plots.

After 4 days, banana weevils were checked for mortality and number of dead insects/ larvae was recorded. Dead insect/larvae were individually dissected under microscope to check if they died due to nematode infection. Number of dead insects due to nematode infection was recorded as percent infection and number of nematode per insect cadaver were recorded as invasion rate. For each nematode isolate, a test was

conducted to check if the nematodes were able to reproduce within *C. sordidus* larvae.

3.6 Data analysis

Data on propagation rate and virulence of new EPN isolates were subjected to analysis of variance (ANOVA) using the General linear model (GLM) procedure of the MINITAB 15. Means were separated using Tukey's multiple range tests. Regression analysis was conducted to determine the relationship between mortality percentage and nematode concentration.

CHAPTER FOUR

RESULTS

4.1 Isolation of EPNs from soil

Entomopathogenic nematodes were present in four samples (4.4%) out of 90 soil samples collected (Table 2). Three positive samples were from Mkenge village and one was from Mwalusembe village both from Coast region. Nine isolates (the progeny from a single *G. mellonella* larva was considered one isolate) of entomopathogenic nematodes were isolated from the four soil samples. Dead *G. mellonella* larvae which appeared flaccid and yellowish or brown in colour were identified to be of *Steinernema* spp and those which appeared brownish red were considered *Heterorhabditis spp* (Woodring and Kaya, 1988). From the appearance of dead *G. mellonella* larvae seven isolates designated by site and sample number as MK1A, MK1B, MK4A, MK4B, MK7A, MW8A & MW8B were of *Steinernema spp* and two isolates designated as MK7B & MK7C were of *Heterorhabditis spp.* These isolates are kept in the plant protection laboratory in the Department of Crop Science and Production at SUA.

Nematodes were recovered from loamy sand (LS) and sandy loam (SL) soils (Table 3). EPNs were recovered mostly in more acidic soils from low altitude (below 300masl). No nematodes were recovered from heavy soils such as sand clay loam (SCL) and sand clay (SC) which were the characteristics of most soil sampled from the selected villages in Morogoro region (Appendix 2).

Some of the dead larvae in soil samples mostly from Mbeya region were infected with entomopathogenic fungi (Appendix 1). The dead larvae due to entomopathogenic fungi were usually black with hard body. When these larvae were left in water agar, fungi growth appeared. The entomopathogenic fungi were identified to be of *Metarhizium spp* and *Beauveria spp.*

Table 2: Number of soil samples positive for EPNs and number of isolates recovered from three regions

Region	Villages	Number of samples	Number of samples with nematodes	Number of nematode isolates recovered
Mbeya	Bujela	10	0	0
	Katabe	10	0	0
	Kyimo	10	0	0
Pwani	Mkenge	10	3	7
	Mwalusembe	10	1	2
	Kimanzichana	10	0	0
Morogoro	Pangawe	10	0	0
	Tangeni	10	0	0
	Kiroka	10	0	0
TOTAL		90	4	9

Table 3: Soil characteristics of the positive soil samples

Village name	Sample number	SOIL pH (H_2O)	EC (mS/cm)	%Clay	%Silt	%Sand	Textural Class
Mkenge	MK1	6.12	0.31	13	3	84	LS
Mkenge	MK4	5.90	0.10	13	3	84	LS
Mkenge	MK7	5.86	0.11	15	3	82	SL
Mwalusembe	MW8	6.25	0.04	9	4	87	LS

4.2 Multiplication rate of nematode isolates in *G. mellonella* larvae

Significant differences in multiplication rate was observed among nine nematode isolates ($F_{(8,36)}$=20.75, P<0.001). Mean IJs production of entomopathogenic nematodes within *G. mellonella* larvae is summarized in figure 8. High numbers of infective juveniles per host larva were found in *Heterorhabditis spp* MK7C (6860 ± 610) and MK7B (6760±296) as well as *Steinernema spp* MK7A (6420±376) (Fig. 8). These isolates were from the same soil sample. A moderate number of infective juveniles were produced by (*Steinernema spp*) MK4A (5200±700). In MK4B and MK1B the mean harvest was 5000±644 and 4260±716 respectively (Fig. 8). These results did not differ significantly although the isolates were from different soil samples. MK1A (*Steinernema spp*) produced significantly the fewest IJs per host larva (Fig. 8).

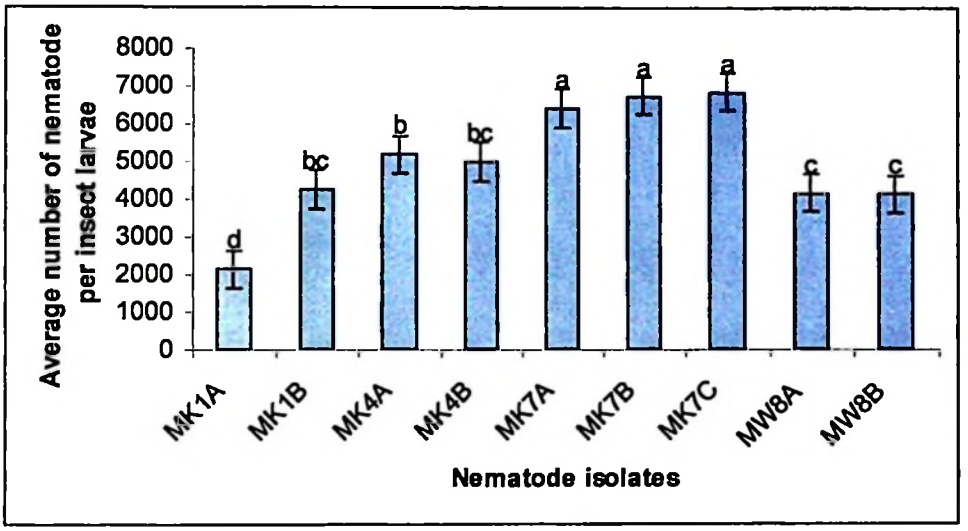


Figure 8: Mean number of infective juveniles per individual larvae of *G. mellonella.*

(Different letters on bars indicate significant differences at $P < 0.05$)

4.3 Virulence of nematode isolates against adult *C. sordidus*

Regardless of the dosage, adult *C. sordidus* were rarely infected. Only four adult weevils out of all treatment units were infected. Isolates MK7B and MK7C (*Heterorhabdits spp)* and MW8B (*Steinernema spp*) infected 1, 2, 1 adult *C. sordidus* respectively. Natural mortality of adult *C. sordidus* banana weevil in both test and control did not occur.

4.4 Virulence of nematode isolates against *C. sordidus* larvae

The average mortality rates of *C. sordidus* larvae caused by different nematode isolates were compared among three different nematode concentrations (Fig. 9). Significant differences in percent mortality were observed among isolates ($F_{8,96}$ = 18.53; $P<0.0001$) (Appendix 5). For the nine isolates compared, *Heterorhabdits spp* MK7B and MK7C were the most virulent of all isolates, causing the highest

mortality rate in overall average i.e. 79% (±31) and 77% (±21) respectively (Fig. 9). These were isolates from the same soil sample. On the other hand isolates (*Steinernema spp)* MK4A and MK4B also from the same soil sample caused significantly lower average mortality than all other isolates (Fig. 9).

Significant differences in percent mortality also occurred among nematode concentrations ($F_{2,96}$ = 230.34; P<0.0001) (Appendix 5). At low nematode concentration (0.35cm^{-2}) all isolates caused mortality less than 50% with MK4A and MK4b causing lower mortality than all other isolates (Fig. 10). *Heterorhabdits spp* MK7C was the most virulent causing a mortality of 52% at low concentration. Highest percent mortality was caused by isolate MK7B (*Heterorhabdits spp*) and MK7A (*Steinernema spp*) whereby the percent mortality reached 100% at nematode concentration of 3.5cm^{-2} and 7cm^{-2} respectively (Fig. 10). Generally virulence increased with increasing nematode concentration for all isolates except for isolate MK4A (*Steinernema spp*) and MK7B (*Heterorhabdits spp*) in which the virulence decreased at the highest concentration.

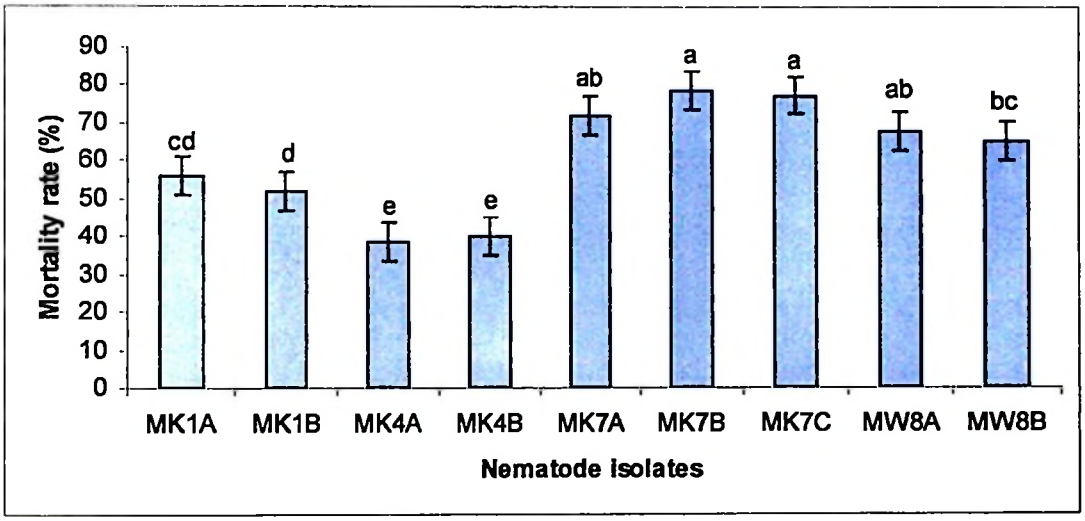


Figure 9: Average mortality rate (%) of banana weevil larvae caused by nine entomopathogenic nematode isolates.

(Different letters on bars indicate significant differences at $p < 0.05$)

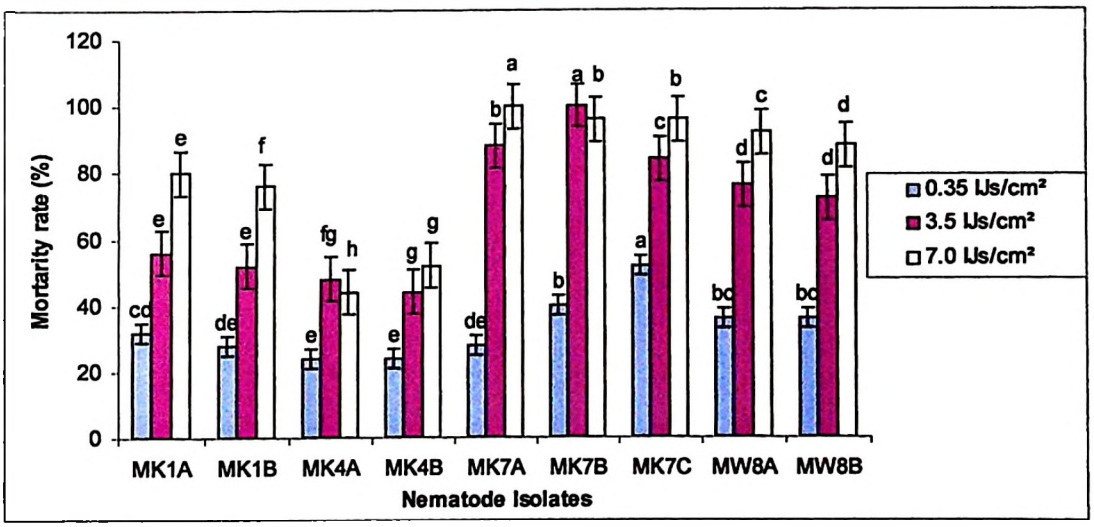


Figure 10: Mortality rate (%) of banana weevil larvae caused by nine nematode isolates at different nematode concentrations

(Different letters on bars indicate significant differences at $P < 0.05$).

Regression analysis

The p-value in the regression ANOVA table (Table 6) indicates that the relationship between mortality percentage and nematode concentration is statistically significant at α-level of 0.05. This is also shown by the p-value for the estimated coefficient of dosage, which is 0.000 (Appendix 5). The R^2 value shows that dosage explains 45.9% of the variance in percent mortality. From figure 11 it is shown that for isolates MK1A and MK1B (*Steinernema spp*) the relationship between nematode concentration and mortality rate was linear whereby increase in nematode concentration increased the mortality rate. On the other hand some nematode isolates have shown quadratic relationship whereby the mortality percentage increased with increase in nematode concentration but there is a point whereby a maximum mortality percentage is reached and increase in nematode concentration leads to decrease in mortality percentage e.g., MK4A and MK7B.

Table 4: Regression analysis for percent mortality of *C. sordidus* at different nematode concentration

Source	DF	SS	MS	F	P
Regression	1	49 938	49 938	113.04	0.000
Residual Error	133	58 756	442		
Total	134	108 693			

S = 21.0184 R-Sq = 45.9% R-Sq (adj) = 45.5%

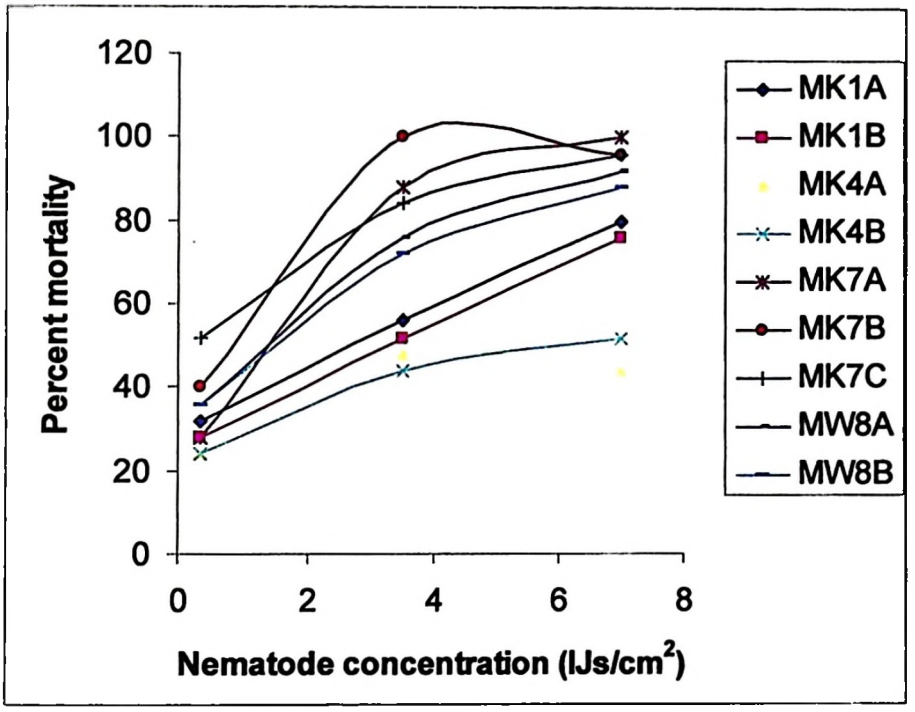


Figure 11: Regression fitted lines for percent mortality of *C. sordidus* caused by nine entomopathogenic nematode isolates at different nematode concentrations

4.5 Invasion rate of nematode isolates in *C. sordidus* larvae

In figure 12 the overall invasion rate of all nematode isolates are compared. Number of nematodes that penetrated into the larvae of banana weevil within four days after exposure differed significantly among the nematode isolates ($F_{8,96}$ =4.6; P<0.001 and the dosage ($F_{2,96}$ = 96.57; P<0.0001) (Table 7). *Steinernema spp* MW8B and MK1A had significantly higher invasion rate followed by MK4B (Fig. 12). Fewer IJs of *Heterorhabditis spp* MK7B and MK7C penetrated the haemocoel of the host larvae (Fig. 12).

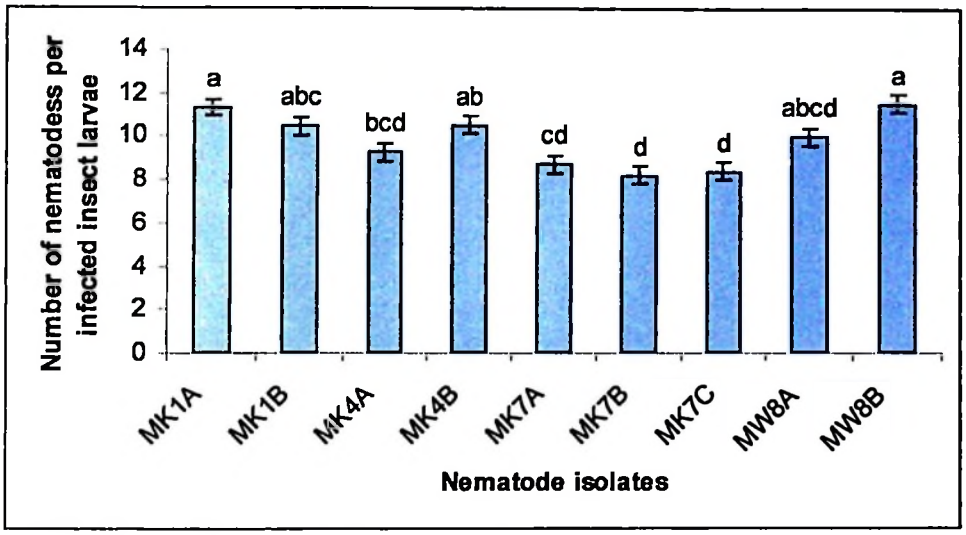


Figure 12: Mean Invasion rate of entomopathogenic nematode isolates to *Galleria mellonella.*

(Means with the same letters are not significantly different at p≤ 0.05)

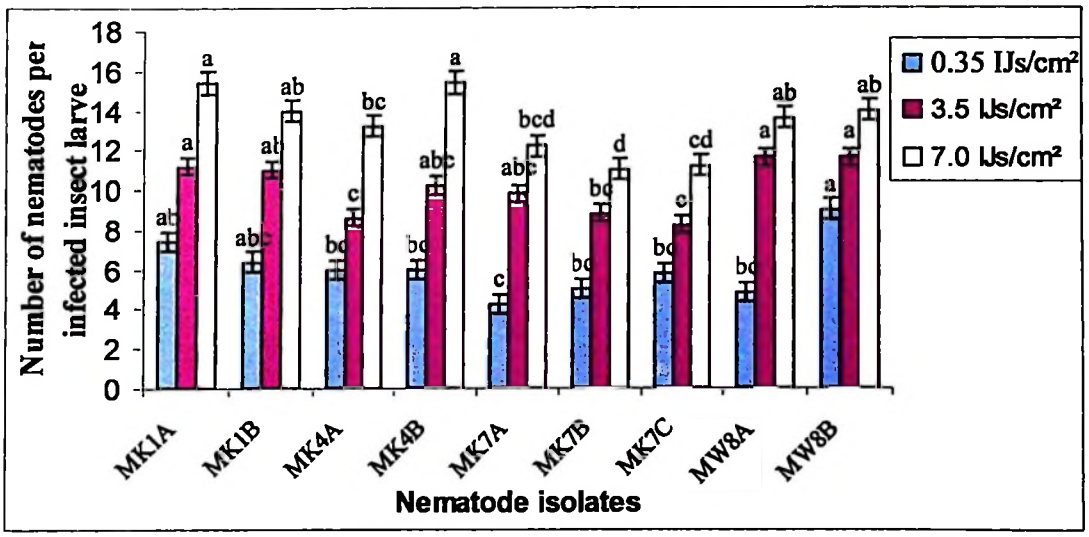


Figure 13: Mean number of nematode infective juveniles penetrated banana weevil larvae after exposure to different nematode concentrations.

(Means with the same letters are not significantly different at p≤0.05)

At low concentration the mean recovery of IJs per infected host was higher in isolate (*Steinernema spp*) MW8B (9 IJs insect^{-1}) and was lowest in (*Steinernema spp*) MK7A (4.2IJs insect^{-1}) $P \leq 0.0001$). Highest mean recovery per insect host were observed at the highest nematode concentration (7IJscm^{-2}) in all nematode isolates with mean IJs per insect cadaver ranging from 11-15. In general the number of IJs penetrating the host larvae was increasing significantly with increasing nematode concentration (Fig. 13).

CHAPTER FIVE

DISCUSSION

A total of nine isolates of Entomopathogenic nematodes (7 *Steinernema spp* and 2 *Heterorhabditis spp*) were isolated from three (4.4%) among 90 collected soil samples. This is the first isolation of entomopathogenic nematodes in Tanzania. In this study *Steinernema spp* were recovered more often than *Heterorhabditis spp.* Hominick (2002) reported that *Steinernema spp.* are recovered more often simply because there are many more species of *Steinernema* than *Heterorhabditis* and they therefore occupy more niche. Another reason is the difference in life history of the two genera which also contributes to the unequal recovery. A single *Heterorhabditis* infective juvenile is sufficient to multiply after invasion while at least two *Steinernema spp,* a male and female must invade before reproduction can occur (Downes and Griffin, 1996). This study therefore supports the earlier observations that *Steinernema spp* are expected to be more abundant in the environment and therefore higher possibilities of being recovered.

Results in this study indicate the prevalence of EPNs in the coastal low altitude (0-300masl) compared to medium and high altitude areas. The overall prevalence in this study is similar to other studies where EPNs were more frequently recovered in coastal areas compared to other areas. Hominick (2002) comments that coastal zones provide excellent sources for EPNs.

EPNs were recovered in light soils (loamy sand and sandy loam) in this study. This is in agreement with other studies whereby *S. carpocapsae, S. feltiae and S. kraussei*

were recovered from sandy loam soils (Smard *et al.*, 2007). Mrábek *et al.* (2005) also indicated that most EPNs strongly prefer light soils. This justifies the reason why no EPNs in this study were isolated in soil samples from Morogoro region where most of the soils were heavy clay types. However, soil texture is probably more important for infective juvenile survival and movement than for the nematode prevalence (Mrábek *et al.*, 2005). This means that EPNs may prevail in certain areas but may fail to survive and move due to heavy soils which are also associated with poor air circulation.

The highlands (above 1000 masl) are characterized by high rainfall and mostly sandy clay loam soils. In these areas no EPNs were isolated indicating their absence. However entomopathogenic fungi were found to be the cause of high percent death of *G. mellonella* baited in these soil samples. Some of these fungi were identified as *Metarhizium spp* and *Beauveria spp.* The occurrence of entomopathogenic fungi could be suppressing the occurrence of entomopathogenic nematodes. Barbercheck and Kaya (1991) observed that fungi and nematodes rarely co-produce progeny in infected hosts. This is because the symbiotic bacteria and fungi compete with each other. The first to infect the host, inhibiting the development of the second (Acevedo *et al.*, 2007; Ansari *et al.*, 2004). Shapiro *et al.* (2004) suggested that the negative interactions between entomopathogens may be a result of toxins produced by both. These effects could therefore account for the absence of EPNs in soils in Mbeya region.

The entomopathogenic isolates tested against larvae and adults of *C. sordidus* in this study, showed the ability to infest the larval stage of the banana weevil whereas adults were generally not attacked. Despite the lack of success in using EPNs against adult *C.sordidus,* all nine nematode isolates tested were able to infest the banana weevil larvae. The mortality in *C.sordidus* larvae reached up to 52% at low concentration of $0.35cm^{-2}$ and up to 100% at concentrations of $3.5cm^{-2}$ and $7cm^{-2}$. In a study conducted by Treverrow and Bedding (1993) in Australia, 85% infection of *C.sordidus* larvae by *S. carpocapsae* BW was reached in the laboratory testing. Many studies on virulence of EPNs on different species of weevil have shown that *Heterorhabditis* spp. are more infective than *Steinernema spp.* (Treverrow and Bedding, 1993; Dolinski *et al.*, 2006). The same trend has been observed in this study whereby the most virulent isolates MK7B and MK7C were both of *Heterorhabditis* spp. A high efficacy of an entomopathogenic nematode isolate is one important characteristics required for the successful biological control of a pest using nematodes. Nevertheless commercial feasibility with respect to the potential for mass propagation is also important.

Previous studies have shown that adults of some weevil species are especially susceptible to EPNs and this includes the banana weevil (Treverrow *et al.*, 1991). Another study conducted by Parniski *et al.* (1990) has shown those adult banana weevils are highly resistant to all species of EPNs. Treverrow and bedding (1993) suggest that the resistance is almost certainly due to the difficulty of nematodes entering the host rather than establishment once infection is successful. Paula *et al.* (2003) found that both adults and larvae of banana weevil were susceptible to attack

by infective juveniles of *S. carpocapsae* and *H. bacteriophora.* The great capability of *Heterorhabditis* compared to *Steinernema* in killing banana weevil larva as observed in this study might be explained by various host finding, host recognition and host defence. *Heterorhabditis* IJs have an interior "tooth-like structure" that could enable enhanced penetration of larval cuticle (Bedding and Molyneux, 1982, Dolinski *et al.*, 2006). This capability might be especially important for virulence against banana weevil larvae since there are some evidence based on histopathological studies that IJs enter the larvae mainly through the cuticle and less often through the anus (Dolinski *et al.*, 2006). Insect defence strategies against EPNs can be morphological, behavioral or immunological and can be highly specific to particular EPN species (Wang *et al.*, 1994). The results of this study suggest that *C. sordidus* adults have defence strategies that are effective against the EPN isolates that were tested. Further studies are necessary to determine the nature of these defences.

Results in this study indicate that the variation in invasion rate was not so dramatic but significance differences were observed within both nematode isolates. Gaugler *et al.* (1989) found similar variation amongst geographically distant strains of *S. carpocapsae*. Interestingly in this study, significant difference in invasion rate was observed even in the isolates originating from the same collection sites. These results might suggest that the isolates were of different species or strains. However, Tomalak (2004) observed variation in invasion rate between new isolates of *S. feltiae* originating from collection sites located within a distance of only few kilometers. The observation by Tomalak (2004) suggests that variation in invasion rate can be observed between EPNs of different spp but also between strains of the same EPN

species. Isolate MK7C (*Heterorhabdis* spp) produced the highest number of IJs compared to other isolates. However the number of IJs produced by isolates from the same soil sample, i.e. MK7A, MK7B and MK7C were not significantly different. This may indicate that the isolates from the same soil sample were similar since even their virulence was not statistically different. The same was observed for Isolates MW8A and MW8B which were also from the same soil sample.

The Heterorhabditid nematode MK7B appeared to be the most promising candidate for biological control of banana weevil larvae. This isolate caused the highest mortality in banana weevil larvae in the sand assay both at lower and higher concentration (52%-100%). The virulence was increasing as nematode concentration was increasing.

In this study the potential of EPNs in controlling *C. sordidus* larvae was demonstrated. The result suggests that up to 100% of *C. sordidus* larvae may be infected by new EPN isolates in the laboratory. The results have an important implication for the use of the nematodes for the control of *C. sordidus* larvae as the nematodes were able to reproduce within the infected insect larvae. It is known that the capacity to disperse in addition to host factors, pathogen virulence, infectivity and persistence is a key factor in the ability of entomopathogens to develop epizootics (Tinzaara *et al.*, 2004). Since this study was carried out in the laboratory, investigation to see if nematodes reproduced within *C. sordidus* larvae can persist in the fields is needed. However the challenge will be the delivery system of nematodes

in the field since *C. sordidus* larvae are found tunnelling inside the banana pseudostems thus may not be easily accessed by the nematodes.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The present study has shown that EPNs both *(Heterorhabditis* spp. and *Steinernema* spp.) occur naturally in some parts of Coast region of Tanzania. EPNs are recovered preferentially in acidic sandy loam and loamy sand soils. All isolates of new EPNs have shown the ability to infest *C. sordidus* larvae in the laboratory. However the percent infection differed with nematode isolates and concentration. The infestation by these nematode isolates on adult *C. sordidus* was negligible.

In all cases isolates MK7B and MK7C both of *Heterorhabdits spp* isolated from Mkenge village are identified as the most promising EPNs for further studies in biological control of *C. sordidus*.

This study so far indicates the potential of using EPNs as a biocontrol agent on *C. sordidus* larvae based on laboratory tests. Field trials need to be carried out to confirm these findings whereby suitable delivery systems of the EPNs in the field need to be established.

6.2 Recommendations

As a follow-up of this study, it is important to conduct more studies on:

- Identification to species level of the isolated entomopathogenic nematodes
- Field testing of the available EPNs isolates against *C.sordidus*

- Potential uses of EPNs in controlling *C.sordidus* in different banana growing systems
- Potential delivery systems of EPNs in different banana production systems

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APPENDICES

Appendix 1: Number of dead *Galleria mellonella* larvae in baited soil samples and their cause of death after three weeks of incubation

Region	Village	Number of insect used as bait in 10 samples	Number of dead insects and their cause
MBEYA	Bujela	50	2**
	Katabe	50	6**
	Kyimo	50	6**
COAST	Mkenge	50	7*
	Mwalusembe	50	2*
	Kimanzichana	50	0
MOROGORO	Pangawe	50	2***
	Tangeni	50	0
	Kiroka	50	0

* *Galleria* bait infected with Entomopathogenic nematodes, ** *Galleria* bait infected with entomopathogenic fungi, *** *Galleria* bait with unknown infection

Appendix 2: Soil characteristics of 90 soil samples

Sample Number	SOIL PH (H_2O)	EC (mS/cm)	%Clay	%Silt	%Sand	Textural Class
KY1	6.54	0.18	29	19	52	SCL
KY2	6.99	0.12	31	19	50	SC
KY3	6.97	0.22	29	19	52	SCL
KY4	6.76	0.13	31	19	50	SC
KY5	6.52	0.15	27	21	52	SCL
KY6	6.47	0.12	27	21	52	SCL
KY7	6.37	0.20	29	19	52	SCL
KY8	6.41	0.22	33	19	48	SCL
KY9	6.33	0.13	27	21	52	SCL
KY10	6.74	0.14	35	17	48	SCL
MK1	6.12	0.31	13	3	84	LS
MK2	5.77	0.04	13	3	84	LS
MK3	5.14	0.08	15	3	82	SL
MK4	5.90	0.10	13	3	84	LS
MK5	5.77	0.06	13	3	84	LS
MK6	6.03	0.08	13	3	84	LS
MK7	5.86	0.11	15	3	82	SL
MK8	5.41	0.07	15	3	82	SL
MK9	5.69	0.06	17	1	82	SL
MK10	5.83	0.05	17	3	80	SL
KM1	6.17	0.07	29	1	70	SCL

KM2	6.50	0.08	25	3	72	SCL
KM3	6.65	0.10	25	3	72	SCL
KM4	7.20	0.15	25	3	72	SCL
KM5	6.10	0.05	29	3	68	SCL
KM6	6.56	0.06	25	1	74	SCL
KM7	6.42	0.05	25	1	74	SCL
KM8	6.37	0.08	23	3	74	SCL
KM9	6.75	0.09	27	3	70	SCL
KM10	6.14	0.07	29	3	68	SCL
BJ1	6.45	0.19	33	20	47	SCL
BJ2	6.56	0.34	39	22	39	CL
BJ3	6.38	0.28	41	22	37	C
BJ4	7.28	0.25	31	22	47	SCL
BJ5	6.40	0.11	35	20	45	SCL
BJ6	6.56	0.26	23	20	57	SCL
BJ7	6.09	0.34	31	26	43	CL
BJ8	7.40	0.24	31	22	47	SCL
BJ9	6.76	0.43	41	22	37	CL
BJ10	6.34	0.27	43	20	37	CL
MW1	5.69	0.19	29	2	69	SCL
MW2	6.38	0.05	11	2	87	LS
MW3	6.73	0.06	13	4	83	LS
MW4	5.58	0.03	13	4	83	LS

MW5	6.16	0.09	11	4	85	LS
MW6	6.01	0.08	17	6	77	SL
MW7	6.94	0.10	9	4	87	LS
MW8	6.25	0.04	9	4	87	LS
MW9	6.35	0.04	23	6	71	SCL
MW10	6.39	0.15	21	22	57	SCL
KT1	0.18	0.18	21	22	57	SCL
KT2	0.17	0.17	23	20	57	SCL
KT3	0.26	0.26	23	18	59	SCL
KT4	0.27	0.27	25	20	55	SCL
KT5	0.25	0.25	25	20	55	SCL
KT6	0.17	0.17	23	20	57	SCL
KT7	0.14	0.14	23	20	57	SCL
KT8	0.10	0.10	23	20	57	SCL
KT9	0.16	0.16	25	18	57	SCL
KT10	0.15	0.15	25	18	57	SCL
TN1	6.68	0.16	27	10	63	SCL
TN2	6.45	0.12	31	8	61	SCL
TN3	6.41	0.10	27	10	63	SCL
TN4	6.48	0.09	27	10	63	SCL
TN5	6.45	0.10	27	10	63	SCL
TN6	6.46	0.10	27	8	65	SCL
TN7	6.52	0.08	27	10	63	SCL

TN8	6.45	0.09	27	10	63	SCL
TN9	6.55	0.15	29	8	63	SCL
TN10	6.54	0.16	27	10	63	SCL
KR1	7.05	0.36	23	10	67	SCL
KR2	7.06	0.32	25	8	67	SCL
KR3	7.11	0.30	23	12	65	SCL
KR4	7.08	0.34	23	12	65	SCL
KR5	7.07	0.32	23	12	65	SCL
KR6	7.15	0.29	21	10	69	SCL
KR7	7.13	0.32	21	10	69	SCL
KR8	7.06	0.29	21	10	69	SCL
KR9	7.09	0.33	23	10	67	SCL
KR10	7.12	0.29	23	10	67	SCL
PN1	6.47	0.36	43	8	49	SC
PN2	6.48	0.35	39	8	53	SC
PN3	6.41	0.35	37	10	53	SC
PN4	6.43	0.33	43	8	49	SC
PN5	6.50	0.27	41	10	49	SC
PN6	6.48	0.34	39	10	51	SCL
PN7	6.53	0.27	41	10	49	SC
PN8	6.40	0.35	39	12	49	SC
PN9	6.36	0.37	37	12	51	SCL
PN10	6.43	0.36	37	12	51	SCL

Appendix 3: ANOVA table for multiplication rate of nine nematode isolates

Source	Df	Ss	Ms	f	p
Isolate	8	93763111	11720389	20.75	0.000
Error	36	20336000	564889		
Total	44	114099111			

Appendix 4: Mean number of infective juveniles per *G.mellonella* larvae

Rank	Isolates	N	Mean of infective juveniles	StDev
9	MK1A	5	2140[d]	650.4
6	MK1B	5	4260[bc]	716.2
4	MK4A	5	5200[b]	700
5	MK4B	5	5000[bc]	644.2
3	MK7A	5	6420a	376.8
2	MK7B	5	6760[a]	296.6
1	MK7C	5	6860[a]	610.7
7	MW8A	5	4180[c]	1348
8	MW8B	5	4141[c]	907.2

Different letters indicate significant differences at $P < 0.05$

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Source	Df	Ss	Ms	f	p
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2	MK7B	5	6760[a]	296.6
1	MK7C	5	6860[a]	610.7
7	MW8A	5	4180[c]	1348
8	MW8B	5	4141[c]	907.2

Different letters indicate significant differences at $P < 0.05$

Appendix 5: ANOVA table for infection rate of nematode isolates at different nematode concentrations.

Source	Df	Ss	Ms	F	P
Blocks	4	960	240.0	1.47	0.220
Dosage	2	54257.8	27128.9	230.34	0.000
Isolates	8	27200	3400.0	15.11	0.000
Blocks × dosage	8	942.2	117.8	0.72	0.670
Blocks × isolates	32	7200.0	225.0	1.38	0.135
dosage × isolates	16	7715.6	482.2	2.96	0.001
Error	64	10417.8	162.8		
Total	134	108693.3			

Appendix 6: Mean of mortality rate of banana weevil larvae by nematodes

Rank	Isolates	N	Mean mortality rate	StDev
6	MK1A	15	56.00^{cd}	25.30
7	MK1B	15	52.00^{d}	24.84
9	MK4A	15	38.67^{e}	15.98
8	MK4B	15	40.00^{e}	16.90
3	MK7A	15	72.00^{ab}	34.48
1	MK7B	15	78.67^{a}	30.67
2	MK7C	15	77.33^{a}	21.20
4	MW8A	15	68.00^{ab}	28.08
5	MW8B	15	65.33^{bc}	25.60

Different letters indicate significant differences at $P < 0.05$

Appendix 7: ANOVA table for invasion rate of nematode isolates at different nematode concentrations

Source	DF	SS	MS	F	P
Blocks	4	20.04	5.011	1.08	0.375
Dosage	2	1193.17	596.585	96.57	0.000
Isolates	8	181.48	22.685	4.23	0.002
Blocks ×Dosage	8	49.42	6.178	1.33	0.245
Blocks ×Isolates	32	171.56	5.361	1.15	0.308
Dosage ×Isolates	16	77.36	4.835	1.04	0.429
Error	64	297.38	4.647		
Total	134	1990.42			

Appendix 8: Estimated coefficient of regression variable

Predictor	Coef	SE Coef	T	P
Constant	13.778	4.786	2.88	0.005
DOSAGE	23.556	2.216	10.63	0.000