EFFECTS OF ABIOTIC FACTORS ON ENTOMOPATHOGENIC NEMATODES AND THEIR POTENTIAL AGAINST THE SWEET POTATO WEEVIL *CYLAS PUNCTICOLLIS* BOHEMAN

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DECLARATION

This is my original work which has not been presented to any university for degree award

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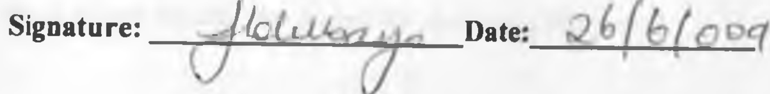

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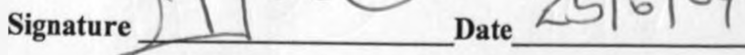

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ACRONYMS AND ABBREVIATIONS

CIP	International Potato Centre
DNA	Deoxyribonucleic acid
F.A.O	Food Agricultural Organization
FURP	Fertilizer Use Project (KARI)
GPS	Global Positioning System
ICIPE	International Centre of Physiology and Ecology
IITA	International Institute of Tropical Agriculture-
IJ	Infective juvenile
IPM	Integrated Pest Management
J3	A third stage nematode juvenile that is the active ingredient and is free living
Kakamega 4	The orange flesh sweet potato variety
KARI	Kenya Agricultural Research Institute
KEMB	Sweet potato Cultivars from KARI-EMBU
KSP	Sweet potato cultivars from KARI KATUMANI
K004	Same as Kakamega 4
LH	Lower highlands
MOA	Ministry of Agriculture
MOA&RD	Ministry of Agriculture and Rural Development
NARL	National Agricultural Research Laboratories
NFRC	National Fibre Research Centre
NHRC	National Horticultural Research Centre Thika
NK1	A nematode isolate from Upper highlands in Rift Valley
PCPs	Polychlorinated polyphenols
PCR	Polymerase chain reaction
SPK 004	Same as K004 and Kakamega 4
TA	Tropical alpine
UH	Upper highlands
UM	Upper midlands

Abstract

This study determined entompathogenic nematode distribution, effects of abiotic factors and nematode effectiveness on the sweet potato weevil *Cylas puncticollis.* A survey was conducted in the Rift Valley region of Kenya and nematodes extracted from soils using *Galleria mellonella* as baits. Nematodes occurred at 50-67% per agro ecological zone, 36% and 17% from farmlands and wild habitats respectively with steinernematids being more frequent than heterorhabditids (9:1). Nematode positive sites in soils of <1, 1-2 and 2-3% carbon were 3%, 18% and 20% respectively ($P<0.05$) while frequency in soils of pH 5-6, <4 and >7 was 25, 1 and 1% respectively. Recoveries of entomopathogenic nematodes from the altitudes 2400-2600m and 2200-2400m were 17 and 4% respectively, 75% from shores of lakes and dams and none from river banks and marshlands. *Steinernema yirgalemense*, *Steinernema weiseri*, *Steinernema karii*, *Heterorhabditis bacteriophora*, *Heterorhabditis taysearae* and a new *Steinernema* species were recovered from the region.

Steinernema karii was not responsive to soil texture when *Galleria* mortality and infectivity were used to measure the effects but *Heterorhabditis indica* was most virulent in clay loam, moderate in sandy clays and clay {80- 60) and least in sandy clay loam (40%)}. *Heterorhabditis indica* was more infective in sandy clay and clay than in sandy clay loam and clay loam soils (40, 25, 17 and 7% respectively). The highest and two lowest mean nematode establishments were significantly different ($P \leq 0.05$). Survival of *H. indica* was 100% and that of *S. yirgalemense* 70% in sandy soils compared to 57 and 27% in clay for both nematodes respectively. *Steinernema karii* survived best in clay soils (67%). The rate of survival was 22, 22 and 14% in the sand clay loam soil for *H. indica, S. karii* and *S. yirgalemense* respectively. Sweet potato weevils survived better in sandy soils than in clay soils ($P \leq 0.05$) in *H. indica* and *S. karii* treatments but at similar levels in *S. yirgalemense* treatments.

Galleria mortality increased significantly as soil pH increased from 4 to 7 (16 to 42% for both *S. karii* and *H. indica.* Infectivity was 22 and 2-7% at pH 7.1 and pH 4-6.4 respectively for *S. karii* ($P \leq 0.05$) but similar at all pH levels (3-6%) in *H. indica.* Percentage survival of *S. karii* was 24 and 20% at pH 4 and pH 6.4 respectively and that of *H. indica* was 23% at pH 6.4 and 6-11% at other levels of pH. *Steinernema karii* and *S. yirgalemense* caused higher weevil mortality at pH 4-5.4 (>1weevil /plot) than at pH 6.4(<1/plot) but mean weevil mortalities were similar in *H. indica* treatments (> 1weevil/plot). *Heterorhabditis indica* reproduced more juveniles in weevils at pH 6.4 (24ij/larva) than

at pH 5.4(4ij/larva) and S. *yirgalemense* at pH 4(54ij/larva) than pH 6.4(0) while *S. karii* reproduced poorly at all the levels of soil pH (3-4ij/larva).
In tests carried out for 12 weeks, nematodes lost virulence at 5,15 and 25^{0}C in ash and mean survival in ash was 18% for *H. indica* at 5°C compared to 5% of *S. karii and 3% of S. yirgalemense*. *Steinernema karii* survival in sand was 35% compared to 27 and 21% of *H. indica* and *S. yirgalemense* at 15°C and that of *S. yirgalemense* 30% compared to 21 and 25% of *H. indica* and *S. karii* respectively at 25^{0}C. The differences between the highest and lowest means were significant ($P<0.05$). Percentage survival of all nematodes in distilled water was 42-45%.
Heterorhabditis indica was more productive in the 5th silkworm larval instar (63,000 ij/larva) and *S yirgalemense* in *Galleria* (80,400ij/larva) while *S. karii* was equally productive in both hosts (39,000-43,000ij/larva). Mean nematode yields at 100 and 200ij/larva doses were not significantly different.

Sweet potato weevil larval mortality was 76%, 70% and 50% in petri dish bioassays with *H. indica, S. yirgalemense* and *S. karii* respectively and adult mortality was 12.5% in *H. indica* and *S. yirgalemense* and 25% in *S. karii*. Weevils at 0-10cm depths induced 75-100% vine damage and none if at >10cm. Kakamega 4 was the most tolerant variety (47% infestation), KSP20 (66%) moderately tolerant and K10 most susceptible (75%). *Steinernema carpocapsae* (13% damage) was as effective as bifenthrin (10%) and *H. indica* the most effective indigenous nematode (20% tuber infestation) compared to 40% in the control treatments. Nematodes were most effective in cool moist weather when infestations were moderate (< 40%) while nematodes in infested *Galleria* cadavers were as effective as water suspensions on sweet potato weevils.

The new findings from the study were that *Steinernema weiseri, S. yirgalemense* and *Heterorhabditis taysearae* and a new *Steinernema* species are present in Kenya. The study also confirmed the presence of *S. karii* and *Heterorhabditis bacteriophora* in the Rift Valley region of Kenya. Further the study generated knowledge on effects of soil texture, soil pH, temperature and medium of storage on entomopathogenic nematodes from a Kenyan perspective and also established that the fifth silkworm larval instar is an efficient host for entomopathogenic nematode reproduction. The study also demonstrated that indigenous entomopathogenic nematodes are comparable to pesticides for the management of sweet potato weevils when application is well timed and their efficacy enhanced when used together with appropriate insecticides and cultural practice.

CHAPTER I

1.1 INTRODUCTION

Sweet potato (*Ipomoea batatas* L) is the third most important root and tuber crop after Irish potatoes and cassava world wide. It is believed to have its origin in Latin America but has since spread to all the five continents with China leading in world production (Herman *et al.*, 2008). Nigeria and Uganda are the leading producers in Africa. Acreage under sweet potato has decreased over the last three decades but this has been accompanied by increased land use efficiency (FAO Statistics, 2005).

Over 80% of sweet potato in Kenya is grown in the Lake Basin and Western province and the rest in the Rift Valley, Eastern, Central and Coastal lowlands (MOA, 2007). Sweet potato is mainly grown for subsistence with the excess being sold in local markets to generate income. It is grown as a mono crop, an intercrop and relay crop. Several sweet potato varieties are grown including some local accessions and introductions from other regions. Sweet potato is suited to many environments and hence it's wide geographical distribution. It spreads in over 80° latitude and from sea level to 3000 m altitude. It is relatively drought resistant and is adapted to a wide range of soils (FAO Statistics, 2005). Most sweet potatoes are used for industrial starch and for animal feed in Asia in contrast with the case in Africa where tubers are mainly used as a staple food, the vine as cattle feed and very little for industrial purposes. Small scale sweet potato processing is carried out in Kenya (Gathara *et al.*, 2004; Nungo, 2004). Sweet potato is high in energy reserves and has an above average per capita production (Woolfe, 1992). Sweet potato has a high tuber yield potential but low average farm yields are realised due to several constraints which include vertebrate and invertebrate pests, shortage of disease free planting materials, poor yielding varieties and diseases (Ngunjiri *et al.*, 1993; Anginya *et al.*, 2001; Gibson and Aritua, 2002; Degras, 2003; Kihurani, 2004).

Several arthropod pests infest sweet potato, some causing direct damage and others indirect damage. Indirect damage includes predisposal of the potato to disease pathogens. Fungal and bacterial diseases enter the tubers and vine through feeding and oviposition punctures (Allard *et al.*, 1991). The white fly *Bemisia tabaci* transmits sweet potato chlorotic stunt virus while the aphids transmit the sweet potato feathery mottle virus (Gibson *et al.*, 1998). The sweet potato butterfly, the striped and spotted weevil and the tortoise beetle cause direct damage to sweet potato (Allard *et al.*, 1991).

The sweet potato weevil is the most destructive insect pest of sweet potato causing up to 73% loss (Smit *et al.*, 1995). Adult weevils defoliate leaves and oviposit on stems and tips of developing tubers. The developing larvae destroy the vascular systems in the stems and lender developing tubers unpalatable due to terpenoids which they inject into tubers (Chalfant, 1990). The options available for sweet potato weevil management include cultural practices, host plant resistance, chemical insecticides, legislation and biological control. Insecticides are usually more effective than other methods but they are associated with several detrimental effects including environmental pollution. Entomopathogenic nematodes are insect parasitic nematodes that can be formulated into commercial products to control arthropod pests. They do not pose any danger to vertebrates and plants. Sweet potato weevil is susceptible to entomopathogenic nematodes (Jansson *et al.*, 1990). Given that sweet potato weevils breed in vines, tubers and soil which are ideal habitats for entmopathogenic nematode function, there is potential of using entomopathogenic nematodes for the management of the weevils.

Nematodes in the families Heterorhabditidae and Steinernematidae have been in focus as biological control agents of pests for the last century and there has been an intensified interest in them in the last three decades. This has coincided with a greater awareness of the adverse effects of insecticides and the withdrawal of non biodegradable PCPs (Poly chlorinated polyphenols) from the markets. Nematodes

are effective on pests with one or more of their life stages in cryptic habitats and act like systemic insecticides by seeking pests out in their hidden habitats thereby causing host mortality in 48 hours (Burnell and Stock, 2000). They work mutually with bacteria in the family Enterobacteriaceae (Woodring and Kaya, 1988). Entomopathogenic nematodes have been successfully used to control arthropod pests including *Cylas formicarius* (F) and are easily isolated and reproduced and safe to vertebrates and plants (Jansson *et al.*, 1990; Mannion and Jansson, 1993; Shapiro *et al.*, 2000). Entomopathogenic nematodes can be tank mixed or used sequentially with some pesticides (Nishimatsu and Jackson, 1998; Kathyrn and Eileen, 2005). They are affected by environmental factors some of which are biotic (micro-organisms like bacteria, fungi, free living nematodes, plant parasitic nematodes and other entomopathogenic nematodes) and others abiotic (soil moisture, oxygen potential, soil texture and soil pH) (Duncan *et al.*, 2001)

Numerous entomopathogenic nematodes surveys have been conducted globally and several entomopathogenic nematodes described (Waturu, 1998; Rosa *et al.*, 2000; Mracek *et al.*, 2005). Isolates of the same species from different geographical regions differ in some functions (Burnell and Stock, 2000). The first nematode survey in Kenya isolated three distinct nematodes (Waturu, 1998). Entomopathogenic nematodes can be reproduced *in vivo* and *in vitro*. The method of choice is determined by the level of demand and capital investment (Ehlers *et al.*, 1998; Shapiro and Gaugler, 2002). The greater wax moth *Galleria mellonella* is the main host used *in vivo* reproduction of entomopathogenic nematodes. Other highly susceptible hosts can also be used depending on their availability and suitability compared to *G. mellonella*.

1.2 JUSTIFICATION

Sweet potato is an important food crop in Kenya and the sweet potato weevil the most destructive insect pest of sweet potato. The weevil reduces palatable potato yields by up to 73%. No method has so far been developed that can effectively control the weevil. The best approach is a planned combination of all appropriate methods. This study aimed at incorporating entomopathogenic nematodes in IPM for weevil management. Various soil surveys for entomopathogenic nematodes have been carried out and several nematode isolates extracted. The first survey for entomopathogenic nematodes in Kenya contrasted the nematode fauna of the central highlands with that of the coastal lowlands. The study isolated only three nematode species namely *Steinernema karii, Heterorhabditis indica and Heterorhabditis bacteriophora* which offers a very narrow genetic base as indigenous nematode options for biological control of pests. Most parts of Kenya are untapped for entomopathogenic nematode potential and this study aimed at expanding the explored area for entomopathogenic nematodes to widen the genetic base of local isolates and to determine the most frequent species as well as the factors influencing nematode occurrence and distribution. This study selected the Rift Valley region west of the explored central highlands. This is a region with diverse topographical features, soils, weather, vegetation types and farming systems. The results of the study will be useful in predicting positive nematode sites in the natural environments.

Several studies of entomopathogenic nematodes and the soil environment have been carried out as entomopathogenic nematodes have soils as their natural environment. The studies have used pests that are of economic importance in the regions of study. This study aimed at using the sweet potato weevil (*Cylas puncticollis)*, an economic pest of sweet potato in Kenya. Results of the study will be useful in the management of the weevil using entomopathogenic nematodes. Previous studies on effects of soil pH were derived from the differences in pH in the natural environment during surveys. This study used

a single soil with similar characteristics but with varying pH levels corresponding to common levels in the natural environment in Kenya. The study done on effects of soil pH in a gradient manner was expected to generate more information than what has been derived from the natural soils which differ in many characteristics besides soil pH. Nematode carrier media have been widely tested but these media are not readily available in Kenya. This study aimed at testing effects of cheaper and locally available materials on storage of entomopathogenic nematodes at temperatures that farmers can simulate in their farms. The greatest hindrance to up scaling the use of entomopathogenic nematode in pest management is their availability to farmers. Several studies have been done to improve and up scale entomopathogenic nematode production but this study was targeted at empowering the small scale farmer to manage nematode production systems using locally available materials. This is a sustainable method.

1.3 OBJECTIVES

1.3.1 Overall objective

To enhance sweet potato productivity through appropriate pest management strategies

1.3.2 Specific objectives

1. To determine the distribution and identity of entomopathogenic nematodes and factors influencing their occurrence in the Central Rift Valley region of Kenya.
2. To determine the effects of soil texture on survival and pathogenicity of *Steinernema karii* and *Heterorhabditis indica.*
3. To determine the effect of soil pH on virulence, survival and pathogenicity of the entomopathogenic nematodes *Steinernema karii* and *Heterorhabditis indica* in Kenya
4. To determine the effect of temperature and storage medium on survival of *Heterorhabditis indica, Steinernema karii and Steinernema yirgalemense.*
5. To determine the efficiency of the silkworm (*Bombyx mori*) in the multiplication of *Heterorhabditis indica, Steinernema karii and Steinernema yirgalemense*
6. To determine the effect of entomopathogenic nematodes on the sweet potato weevil (*Cylas puncticollis)*

CHAPTER 2

2 LITERATURE REVIEW

2.1 Sweet potato production

Sweet potato is the fifth most important food crop in fresh production after rice, wheat, maize and Irish potato over the world (Herman *et al.*, 2008; FAO, 2005). The most recent evidence suggest that it originated from Central America about 5000 years ago but it is now grown in all the continents with Asia producing 91% of the total world production (FAO Statistics, 2005). Sweet potato is a vegetative annual crop with vines producing 10-12 tubers. Tubers vary in shape, size and skin colour (Woolfe, 1992; Degras 2003; FAO Statistics, 2005). World production fluctuated between 121 and 147 million tons between 1994 and 2004 and between 8.9 and 9.2 million hectares over the same period and yields of 13-17 tons/ha (FAO Statistics 2005). Sweet potato production has declined over the last two decades in some developed countries but increased in most countries in Sub Saharan Africa. Uganda and Nigeria are the leading sweet potato producers in Africa with Uganda, Tanzania and Rwanda being the main producers in East Africa. Other sweet potato producers in the eastern African region include Burundi, Kenya and Ethiopia (FAO Statistics, 2005).

2.2 Climatic and soil requirements

Sweet potato is one of the most widely distributed crops in the world spreading from the tropical, subtropical and temperate regions between latitudes 48^0N and 40^0S and in different agro ecological climates between 0-3000m above sea (FAO, 2005). The ideal temperature for sweet potato production is 25^0C but it grows between 12^0C and 35^0C (FAO Statistics, 2005). Dry matter accumulates best at 20 to 30^0C. Sweet potato requires a well distributed rainfall (600-1600mm) and a dry weather period for storage root formation. The crop is suited to a wide range of soils but a well drained sandy loam soil

with clay subsoil is ideal. It requires more than 10% oxygen concentration in the initial phase and soils of pH5.6-6.6 but it can tolerate a pH as low as 4.2 (Woolfe, 1992; FAO 2005)

2.3 Uses of sweet potato and food value

Sweet potatoes are mainly used as human food (70-100%), small proportions (10-30%) as animal feed in tropical countries and negligible amounts (5-10%) for industrial purposes. In temperate Asia, 30-35% is produced for industrial purposes mainly starch and alcohol. Sweet potato is high in energy reserves ranking first among root and tuber crops including bananas. The dry matter component of the root tuber varies between cultivars region, pests, disease incidence and cultivation practice but are on average 30% (Woolfe, 1992). About 80-90% of the dry matter is carbohydrates (starch and sugars). The tuber contents on average are 3% crude protein, 17% starch and 0.3% reducing sugars (Woolfe, 1992). Tubers from one hectare contribute about 140 Kg proteins. Sweet potato is processed into several products in Kenya (Gathara *et al.*, 2004; Nungo, 2004). It is grown commercially in some parts of the Lake Basin and central highlands although it is primarily a supplementary crop for bridging the gaps of cereal shortfalls (Ngunjiri *et al.*, 1993). The orange fresh varieties have high carotene contents a precursor for vitamin A (Mcharo *et al.*, 2001; Jaarsveld *et al.*, 2005).

2.4 Sweet potato production in Kenya

Sweet potato is the third most important food crop in monetary value after maize and beans and first among root and tuber crops in Kenya (Table 2.1).

Table 2.1: Production of food crops in Kenya, 2006

Rank	Crop	Acreage	Total yields (tons)	Yields/ha (Tons/ha)	Value in (Billions Kenya shillings)
1	Maize	1,900,000	3,250,000	1.6	46.4
2	Beans	995,000	531,000	0.5	15.0
3	Sweet potato	74,937	724,646	9.7	7.5
4	Wheat	150,500	360,000	2.3	6.8
5	Irish potato	117,908	784,596	7.3	6.7
6	Cassava	68,500	656,633	9.6	4.9
7	Rice	23,100	65,000	2.8	3.3
12	Arrowroots	3144	22,846	8.0	0.5
14	Yams	755	8001	10.6	0.1

Source: Ministry of Agriculture Economic Review of Agriculture, 2007

Production ranged between 513,000 tons to 672,000 tons from 2002 to 2005 but rose by 8% to 725,000 tons in 2006. The rise in production was triggered by an increase in acreage from 61,000 to 75,000 hectares ((MOA, 2007). Over 80% of sweet potato is grown in the mid elevations (1000-1600m) in the Lake Victoria basin and western province and the rest in the Rift Valley, Central highlands, Eastern province and the Coastal lowlands (Carey *et al.,* 1999; MOA, 2007). Sweet potato is produced in small-scale farms in mono crops, intercrops or relay crops with maize, pigeon peas and cowpeas with the crop of choice depending on the region (Ngunjiri *et al.,* 1993). Some of the varieties grown in Kenya include SPK 004, KSP 11, KSP20, KEMB10, KEMB 23, and KEMB 36. SPK 004 is a red skin, orange fresh variety with high levels of carotene the vitamin A precursor while the rest are selections

from central Kenya. Orange fresh varieties are becoming important due to their vitamin A content (Mcharo *et al.*, 2001; Jaarsveld, 2005).

2.5 Sweet potato production constraints

Mole-rats, insect pests and lack of clean planting materials, poor yielding varieties, diseases, and lack of effective storage technologies are some of the major constraints to sweet potato production. Mole rats are the most widely spread vertebrate pests in Kenya (Ngunjiri *et al.*, 1993). Sweet potato is also affected by some diseases including the Alternaria leaf spot (Anginya *et al.*, 2001). Other factors such as injury and rotting diseases and sweet potato weevil infestations reduce the sweet potato shelf life after harvesting by 23-47% (Mtunda *et al.*, 2001). The sweet potato virus disease, a combination of sweet potato chlorotic stunt virus and sweet potato feathery mottle virus cause up to 90% sweet potato yield loss in Africa (Gibson and Aritua, 2002). Planting material shortages are caused by pest infestations and drought, which causes drying of plants. The rapid sweet potato propagation method is useful in overcoming the problem (Allard *et al.*, 1991). Variety adaptation studies at different agro-ecological zones have addressed the problem of low yields (Degras, 2003). Simple methods of storage that lengthen shelf life and reduce disease development in storage for instance curing the tubers have also been addressed (Woolfe, 1992; Kihurani, 2004). One of the most important limiting factors to good quality sweet potato yields remains pests.

2.6 Sweet potato insect pests

More than 300 insect and mite species attack sweet potato but not all are of economic importance (FAO Statistics, 2005). The effect is either direct or indirect. The indirect effects occur when the insect pests act as vectors or when they predispose the plant to secondary infection by diseases. For instance the white fly, *Bemisia tabaci* (Genn), is a major sweet potato insect pest whose importance is more

related to its action as a vector of the sweet potato chlorotic stunt virus while the aphid, *Myzus persicae* is the vector of sweet potato mottle virus (Gibson *et al.*, 1998; Gibson and Aritua 2002). The clear wing moth *Synanthedon dasysceles* Bradley (Lepidoptera: Sesiidae) lays eggs on vines. The larvae hatch and burrow the vines pupating at the stem base where attack induces development of galls and yield reduction. The sweet potato hornworm *Agrius convolvuli* (Lepidoptera: Sphinidae) adults feed on nectar but the larvae feed on sweet potato leaves especially the young shoots (Allard *et al.*, 1991). The adult and nymphs of different species of the tortoise beetle *Aspidomorpha* spp (Chrysomelidae: Cassidae) are defoliators (Allard *et al.*, 1991). The eggs are laid and cemented on the underside of leaves in batches. The adult beetles girdle, peel off the stems and make large holes on leaves. The brown and striped sweet potato weevil *Alcidodes dentipes* and *Alcidodes erroneous* girdle the stem while larvae develop in the stem leaving symptoms of attack similar to those of *Cylas* species but the larvae are larger. *Blosyrus* species feed on sweet potato leaves and are susceptible to the entomopathogenic fungi *Beauveria bassiana.* *Acraea acerata* Hew the sweet potato butterfly (Lepidoptera: Nymphalidae) is the 2nd most important insect pest of sweet potato in South west Uganda and the most important pest in southern Ethiopia (Smit *et al.*, 1997; Azerefegne *et al.*, 2001). Parasitoids have been found to lower butterfly infestations in Uganda (Brown *et al.*, 1995). The sweet potato butterfly is sporadic but severe in dry months in Kenya (Allard *et al.*, 1991). The eggs are laid in batches of about 130 usually on the lower leaf surface. On hatching, the larvae feed on the upper surface of the leaf in groups under protective webbing causing complete defoliation under heavy attack (Allard *et al.*, 1991). The species of sweet potato weevil *Cylas* (Coleoptera: Curculionidae) complex are the most serious pests of sweet potato.

2.7 Distribution and damage symptoms of sweet potato weevil

The sweet potato weevil species complex is the most devastating pest of sweet potato (Chalfant *et al.*, 1990). The three closely related species of *Cylas* are distinct from each other although they cause similar damage symptoms. *Cylas formicarius* has a worldwide distribution, *Cylas puncticollis* an African distribution and *Cylas brunneus* an Asian sub continent distribution (Chalfant *et al.*, 1990). *Cylas puncticollis* is the most common species in East Africa. *Cylas brunneus* and *C. puncticollis* occasionally occur together. *Cylas puncticollis* is the most important biotic factor that limits sweet potato production in Africa causing losses of 3-73% to the sweet potato when harvested at nine and a half months after planting (Smit *et al.*, 1997). It is unprofitable to harvest sweet potato beyond 20 months (Anioke and Ogbabu, 2003). The ideal time for harvesting sweet potato is at 6-7.5 months after planting (Smit *et al.*, 1997). *Cylas puncticollis* is the most commonly occurring species in Nyanza, which is the region of Kenya where most sweet potatoes are grown (Smit *et al.*, 1995). Damage to the sweet potato crop starts at the edges of the field and spreads to the centre and from above ground to the tubers. Adult weevils are mainly defoliators but can gnaw petioles and vines. The larva is the most destructive stage, boring vines and tubers as it develops in the 20cm stem and in tubers leaving frass which give tubers a bad taste. The plant reacts by swelling at the stem base causing cracking and thickening of the vines, paleness of leaves and reduced plant vigour (Allard *et al.*, 1991).

2.8 Biology of sweet potato weevil (*Cylas puncticollis*)

The eggs are laid in excavated cavities on tubers or vines. The eggs hatch in about one week to curved larvae that are 5-10 mm long and 1.5 mm wide. The spiracles are born on the first eight abdominal segments. Development of larvae takes place in stems or tubers and there are three larval instars (Belair *et al.*, 2005). Pupation takes place in the soils, the stem and the tuber in 2-3weeks depending on temperature. The pupae are brownish. Adult's life span is 97-184 days for males and 53-213 for

females with fecundity of 6-209 (Allard *et al.*, 1991; Geisthardt and Van Harten, 1992; Sathula *et al.*, 1997). Members of the *Cylas brunneus* species oviposit and develop faster than *C. puncticollis* in favourable weather conditions but the reverse occurs in less conducive weather conditions (Smit *et al.*, 1997).

2.9 Sweet potato weevil management

The options available for weevil management include cultural control, host plant resistance, chemical insecticides, pheromones and biological control (Hahns and Leuscher, 1981; Chalfant *et al.*,, 1990; Allard *et al.*, 1991; Jansson, 1992). No single method can effectively manage the sweet potato weevil. The more appropriate approach is the Integrated Pest Management method where different methods are combined in a systematic manner for optimum results.

2.9.1 Cultural control

Cultural practices involve the use of clean planting materials and clean fields located one kilometre way from infested fields, earthing and use of irrigation to prevent cracking of soils, covering cracks, ridging, intercropping, rotation, and removal of volunteers and Convolvuli hosts, destruction of trash, early planting and harvesting (Allard *et al.*, 1991; Anioke and Ogbabu, 2003). These are common practices in use by farmers and therefore acceptable.

2.9.2 Host plant resistance

No sweet potato cultivar has so far been bred or selected for total resistance to sweet potato weevil. The cultivars available only have partial resistance (Talekar, 1997). The main resistance mechanism in sweet potato to weevil attack is non-preference and avoidance/escape. Deep rooted cultivars with long

necks that are early maturing help the crop to escape serious damage (CABI, 2000). Thin-vine varieties with higher dry matter content are less susceptible to weevils (Degras, 2003).

2.9.3 Chemical insecticides

Appropriate insecticides when properly targeted at the insect pest are usually more effective than other control methods. The sweet potato weevil larva is the destructive stage. The larvae are hard to target with insecticides because they develop in vines and tubers. Systemic insecticides are more effective than contact insecticides because they can target the weevils in the vines and tubers but have the disadvantage of leaving residues in the edible potato tuber. They also reduce the natural enemies that regulate weevil populations. Early protection from weevil attack and oviposition on young seedlings can be achieved through the use of Imidan, thiodan and methyl parathion while carbaryl and endosulfan are effective on weevils attacking older vines in the field (Zehnder, 2008)

2.9.4 Pheromones

Pheromone capsules together with soapy water traps reduce weevil populations in sweet potato fields. The pheromones attract male weevils in traps while the soapy water kills them. The remnant populations have a female to male ratio of 2:1. Pheromones also disrupt weevil mating patterns (Allard *et al.*, 1991: Jansson, 1992; Yasuda, 1998). Sex pheromones are more effective on *Cylas brunneus* than *Cylas puncticollis* (Smit *et al.*, 1997).

2.9.5 Biological control

Biological control agents which have shown potential to control the sweet potato weevil include fungal species like *Beauveria bassiana, Metarhizium anisoplae* and *Verticillium lilacinus, Aspergillus* spp and the parasitic agents such as *Cardiochiles enderleine* (Hymenoptera: Braconidae) (Lobo-Lima, 1990;

Allard *et al.,* 1991; Jansson, 1992). Despite these positive interventions on sweet potato weevil management, no method has proved effective on the weevil when applied singly. Entomopathogenic nematodes have shown acceptable potential to control the sweet potato weevil (*Cylas formicarius*) in the laboratory, greenhouse and field conditions (Mannion and Jansson, 1993).

2.10 Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae)

Entomopathogenic nematodes are plant parasitic nematodes that occur naturally in soils of all regions except the poles (Hominick, 2002). Although most research on insect parasitic nematodes has concentrated on steinernematids and heterorhabditids, other nematode families have insect parasitic species (Stock and Hunt, 2005). The Phaenositylenchidae, Merminthidae, Sphaeririidae, Tetradonematidae and Allantonematidae have been recovered attacking insects of the orders; Coleoptera, Diptera, Thysanoptera, Lepidoptera, and Hymenoptera. These do not have the mutual bacteria although the parasitic relationships are highly adapted. Their use as biological control agents of pests is limited by their inability to reproduce *in vitro* (Arthurs *et al.,* 2004). Nematode species in the families Steinernematidae and Heterorhabditidae colonise hosts and cause host mortality in 24-48 hours making them useful as biological control agents of insect hosts (Burnel and Stock, 2000). Infective nematode juveniles (J3) are motile, non-feeding and the only free living stage (Burnel and Stock, 2000). They enter the host through natural openings as well as through the cuticle region for heterorhabditids (Campbell and Gaugler, 1991; Hazir *et al.,* 2003; Koppenhofer *et al.,* 2007). The process is symbiotic with bacteria in the order enterobacteriaceae (Boemare, 2002; Nielsen and Lubeck, 2007). The bacteria use the nematode as a vector while the bacteria make the hemocoel environment appropriate for nematode growth and reproduction. The bacteria also protect the host from infection by other bacteria through the production of antibiotics and in addition also help to overcome the host immune system (Gutz *et al.,* 1981; Burnel and Stock, 2000; Hazir *et al.,* 2003).

Bacteria can cause mortality on their own but reproduction requires the presence of the nematode. The nematode can also cause mortality without the bacteria but no more development takes place (Rajagopal and Bhatnagar, 2002). The J3 has a coat that makes it resistant to environmental conditions (Campbell and Gaugler, 1993; Baur and Kaya, 2001). The J3 normally measure 400 – 1500 micrometers depending on species (Stock and Hunt, 2005).

The bacteria genus *Photorhabdus* is associated with *Heterorhabditis* while *Xenorhabdus* is associated with *Steinernema* species (Boemare, 2002; Nielsen and Lubeck, 2007). Some of the most intensively studied nematodes include *Heterorhabditis indica, Heterorhabditis bacteriophora, Steinernema carpocapsae, Steinernema glaseri* and *Steinernema feltiae* (Ciche *et al.*, 2001; Hazir *et al.*, 2003; Bornstein and Kiger, 2005). Entomopathogenic nematodes have been used successfully for the control of pests especially those with a soil phase and from cryptic habitats (Haukeland, 1993; Hazir *et al.*, 2003). Steinernematids are usually amphimictic in all generations with the exception of the *Steinernema* species from Indonesia which has hermaphrodites in the first adult generation (Griffin *et al.*, 2001). The multiplication of nematode infectives in hosts may go on up to third generation depending on food supply. The process takes 10 – 14 days for *Steinernema feltiae* in *G. mellonella*. *Heterorhabditis* juveniles first develop into a hermaphroditic generation which later reproduce a sexual generation.

2.10.1. Ecological factors affecting nematode activity

Ultra violet radiation is detrimental to entomopathogenic nematodes and cool and cryptic habitats e.g. the soil are ideal for their survival and function (Kung *et al.*, 1990; Shapiro *et al.*, 2006). Entomopathogenic nematodes are sensitive to desiccation and will infect target pests best at 25-40% humidity. Survival of the nematodes decreases with increased relative humidity (Baur *et al.*, 1995;

Hyam *et al.,* 2001). The nematodes survive at temperatures between -10 and 35^{0}C but most can not survive below 0°C and above 40 °C. Survival is best at 5-15°C and the lowest movement at 10-15°C (Hazir *et al.,* 2003). The soil usually buffers entomopathogenic nematodes from extremes of temperature (Brown and Gaugler, 1996; Hazir *et al.,* 2003). Entomopathogenic nematodes development is arrested at low temperatures. The extent of arrest depends on the nematode species (Chen *et al.,* 2003; Bornstein and Kiger, 2005). Nematodes also take time to adapt to new temperatures (Jagdale and Grewal, 2003 and 2007). Steinernematids have lower threshold temperatures than heterorhabdtids but a higher temperature range. Temperate isolates reproduce best at 15^{0}C while tropical isolates reproduce poorly at this temperature and nematode recovery and storage are species and temperature dependent (Mari *et al.,* 2000; Hazir *et al* ,2003 Mracek *et al* .,2005). Entomopathogenic nematodes are obligate aerobes with temperature determining oxygen consumption. In anaerobic conditions the infective juveniles become immobilised but this is reversed when the environments become aerobic (Qui and Bedding, 2000). The best infective juvenile yields were obtained when the nematodes were stored at 10 and 15^{0}C (Hara *et al.,* 2000). Nematode survival and virulence increases with moisture (Grant and Villani , 2003).

2.10.2 Entomopathogenic nematode interaction with chemical pesticides and fertilizers

Entomopathogenic nematodes are safe to vertebrates and plants and non target organisms in contrast with chemical insecticides which are normally toxic (Zervos, 1991; Hazir *et al.,* 2003). They are compatible with many agricultural chemical insecticides, nematicides and some natural enemies and can therefore be used alone, tank mixed with some pesticides or applied sequentially with insecticides (Kathryn and Eileen, 2005; Koppenhofer and Grewal, 2005). Some insecticides are synergists with combined application enhancing efficacy many fold but others affect nematode host finding, reproduction or survival or all of these functions (Cuthbertson *et al.,* 2003; Koppenhofer *et al.* 2003).

The insecticide tefluthrin has a synergistic effect but bifenthrin and acephate have slight negative effects above normal rates. These insecticides can therefore be used with entomopathogenic nematodes at normal rates (Kathryn and Eileen, 2005). Inorganic fertilizers are adverse to entomopathogenic nematodes but organic manures increase infectivity and recycling of entompathogenic nematodes (Bednarek and Gaugler, 1997). In view of the reports on effects of insecticides and fertilizers on entomopathogenic nematodes, the use of entomopathogenic nematodes with insecticides and fertilizers is best considered on per case basis.

2.10.3 Interaction of entomopathogenic nematodes with other organisms

Other organisms interact with entomopathogenic nematodes either synergistically or detrimentally. The entomopathogenic fungi *Hirsutella rhossiliensis,* collembolans, mites, tardigrades, predatory nematodes, the fungi *Bauveria bassiana* and *Paecilomyces fumoroseus* are some of the documented biotic factors lowering parasitism of entomopathogenic nematodes in non-sterile media (Shapiro *et al.*, 2004; Karagoz, 2007). *Paenibacillus nematophilus* spores reduce nematode dispersal in sand and agar but not the yields and quality of *Heterorhabditis megidis* (Enright and Griffin, 2004). Some organisms such as *Paenibacillus popillae, Bacillus thruringiensis* and *Metarhizium anisopliae* are synergistic to the nematodes in insect control (Koppenhofer and Kaya, 1998; Ansari *et al.*, 2004). Some entomopathogenic nematodes reduce populations of plant parasitic nematode communities but not the free living nematodes (Somasekhor *et al.*, 2002). Entomopathogenic fungi substantially reduce infectivity of steinernematids but heterorhabditid infectivity is reduced only slightly because the later retain the second juvenile coat while the former lose it (Timper and Kaya, 1989; Campbell and Gaugler, 1991). Over 2000 species of insects are susceptible to nematodes. The laboratory conditions usually favour infectivity but sometimes some hosts that are only slightly susceptible in the laboratory become more pathogenic in the field (Nguyen and Smart, 1990). The movement of the host insects

may also affect nematode dispersal. Some nematodes are cruisers attacking sedentary hosts, others are ambushers and attack mobile hosts while others use both strategies (Campbell *et al.*, 2003; Lewis *et al.*, 2006). Entomopathogenic nematodes are known to respond to cues like the carbon dioxide released by host insect cadavers (Olgaly *et al.*, 2007).

2.10.4 Entomopathogenic nematode reproduction and storage medium

The highest infectivity and optimum development occurs when nematodes are stored at 10-15^{0}C (Elsooud *et al.*, 2001). Some nematodes exhibit reduced infectivity when they are stored but others are not affected. *Heterorhabditis megidis* from storage was less infective than freshly harvested stock but there was no difference in infectivity of stored and fresh samples of *Steinernema feltiae (*Hara *et al.*, 2000). Isolates from temperate regions reproduce best at 15^{0}C and could not reproduce above 28^{0}C (Hazir *et al.*, 2001). On the other hand, the tropical isolates did not reproduce at 15^{0}C. The *in vivo* method of nematode reproduction requires low capital investment, is labour intensive and reproduces nematodes of high quality compared to the *in vitro* method which is more efficient but of high capital investment and lower nematode quality (Shapiro and McCoy, 2000; Ehlers and Shapiro-Ilan., 2005). About 95% infective juveniles are recovered in a day from *in vivo* reproduction compared to less than 81% recovery from *in vitro* reproduction (Strauch and Ehlers, 1998). Cinnamon, cloves and sponge improved nematode survival in storage but fewer nematodes survived in clay (Ehlers *et al.*, 1998). The composition of diets used in nematode reproduction whether *in vivo* or *in vitro* affects the yields of infective nematodes with higher lipids enhancing nematode juvenile yields (Moeen and Gaugler, 1998; Gil *et al.*, 2002).

2.10.5 Soil pH

The extreme ends of the pH scale are unsuitable for nematode function (Kung *et al.*, 1991). Low pH is adverse to nematode reproduction but the reduced bacteria growth prolongs infective juvenile survival (Jenssen *et al.*, 2000). Low pH also reduce host finding ability of nematodes (Mracek *et al.*, 2005). Steinernematids appear to be more suited to soil pH less than six and Heterorhabdtids to pH greater than six (Rosa *et al.*, 2000).

2.10.6 Soil texture

The soil is the natural habitat of entomopathogenic nematodes and therefore mediates entompathogenic nematodes and their hosts. How well it carries out this role depends on many factors among them the soil texture. The host must come into contact with nematodes for host mortality and infectivity to occur. Insects usually have their own defence mechanisms. Infectivity of entomopathogenic nematodes and mortality to any host is a measure of the success or failure of the defence mechanism of the host. Entomopathogenic nematodes are more effective in soils with higher sand content than loamy silts and clay soils with most entomopathogenic nematodes preferring light and organic soils (Duncan *et al.*, 2001; Susurluk *et al.*, 2001). Soil type effects were however mostly noted on dispersal and persistence of nematodes (Mracek *et al.*, 2005).

2.10.7 Nematode surveys

Several nematode surveys have been carried out in all continents with nematode recovery varying widely from 2% to 53.8% (Waturu, 1998; Mracek *et al.*, 1999; Hominick, 2002; Mracek *et al.*, 2005). The results depend on the baiting method and temperature among other factors (Mracek *et al.*, 2005). Forty *Steinernema* and sixteen *Heterorhabditis* species have been described from the surveys (Nguyen *et al.*, 2006). In Kenya, the first survey recorded a recovery rate of 24% for the central highlands and

4% for the coastal lowlands (Waturu, 1998). *Heterorhabditis bacteriophora* and *Steinernema karii* were isolated from the highlands and *Heterorhabditis indica* from the coastal lowlands. The coastal lowlands border the Indian Ocean, an environment similar to the Andaman Islands where nematode recovery was also 4% (Rosa *et al.*, 2000).

2.10. 8 Successful use of entomopathogenic nematodes as biological control agents

Entomopathogenic nematodes have host preference. S*teinernema riobrave* caused 89-100% mortality to corn earworm *Heliothis zea* but the host was not susceptible to *Steinernema carpocapsae* (Cabanillas and Raulston, 1993). Sweet potato weevil (*Cylas formicarius)* mortality ranged from 38-90% when treated with different nematode species (Mannion and Jansson, 1993). This means that the host and nematode species have to be properly matched for good performance. Entomopathogenic nematodes have also been used against several other insect pests including the vine weevil (*Ortiorynchus sulcatus),* the leaf miner *(Liriomyza spp)* and Citrus root weevil (*Diaprepes abbreviatus)* (Kakouli *et al.*, 1997; McCoy *et al* ,2002; Head and Walters, 2003).

2.10.9 Entomopathogenic nematode formulation and application

Entomopathogenic nematodes can be formulated on sponge gels or as granules, in alginate and vermiculite, activated charcoal, in heteroropolysaccharides like agarose and dextran surrounded by hydrogenated oils, in water suspensions and activated charcoal (Grewal and Peters , 2005; Shapiro *et al.,* 2006). Water dispersible granules, clay, silica, cellulose, lignin and starches at 25°C enhance nematode survival and temperature tolerance but microbial contamination has to be taken care of (Grewal, 2002). The survival of nematode varies with temperature and storage medium (El Sooud *et al.*, 2001). The most successful applications have been on soil but they can be used above ground for insects in tunnels or mines in the plant tissue (Cuthbertson *et al.,* 2003). They can be applied with

conventional chemical spray application equipment and can withstand high pressures but motorised spray equipment have the tendency of increased temperatures which are detrimental to nematodes (Lacynski *et al.*, 2007). The tendency of nematodes to settle in the tank bottom should be avoided by constant and moderate agitation of the tank. Entomopathogenic nematodes can also be applied with irrigation water in furrows but some trickle irrigation systems do not move the nematodes fast enough (Cabanillas and Raulston, 1996). The best period for application is early morning or late evening when temperatures are low and UV least. The recommended spray volume is 800-2500 litters per hectare but for low volume nozzles with outputs of many boom sprayers, corrections can be made by pre and post irrigation to assist in nematode dispersal in soil (Siegel *et al.*, 2004). Nematodes have also been tested for application from infested cadavers (Shapiro *et al* .,2001; Shapiro *et al.*, 2003)

2.11 *Bombyx mori* L. the Silkworm (Lepidoptera: Bombycidae)

The silk worm is a pest of the mulberry that has been domesticated for use in silk production in Asia and currently also in Kenya. The silk industry in Kenya started in the seventies. The farmers were trained in the techniques for cocoon production for silk and the growing of the mulberry tree for its fruits (MOARD, 2003). The industry was successful in the early part of the decade but the demand for cocoons slackened due to the trade in synthetic silk. Though the industry has been picking up with ICIPE (International Centre for Insect physiology and Ecology) intervention, the farmers have potential to produce more than the market capacity at the moment. Silkworm cocoons are rich in essential amino acids (Zang *et al.*, 1990). One silkworm rearing bed can raise 20,000 larvae in 2 months. The silkworm prefers smooth surface for egg laying. Treating the foliage with lime water at pH 9 increases silkworm larval weight (Gupta *et al.*, 1990). The silk worm is also used to isolate *Bacillus thuringiensis* from soils (Kikuta *et al.*, 1990). The silkworm is a Lepidoptera like *Galleria mellonella* and has silk producing glands (Goldsmith *et al.*, 2004). Earlier studies found that the Indian

tropical tsar silkworm *Antheraea mylitta* was susceptible to a merminthid nematode which used up the insect fats and silk glands and reproduced in the cadaver (Chaudhuri *et al.*, 1995). The relatedness of the two insects led to the speculation that the silkworm *Bombyx mori* could be a good host for entompathogenic nematode reproduction.

2.12 *Galleria mellonella* L. the Greater Wax moth (Lepidoptera: Pyrallidae)

Galleria mellonella is very susceptible to pathogens and is usually used to determine the incidence of *Bacillus thuringiensis* in soils. It is also used in trapping entomopathogenic nematodes from soils in the laboratory (Fan and Hominick, 1991). The nuclear polyhedrolysis virus of *Bombyx mori* is also infectious to *Galleria mellonella*. *Galleria mellonella* is easily raised in the laboratory using artificial diets. The life cycle is about a month but larvae for use in entomopathogenic nematology are harvested at the third stage of development, which takes about 3 weeks at 27-30^0 C.

CHAPTER 3

3 GENERAL MATERIALS AND METHODS

3.1 The Greater Wax Moth (*Galleria mellonella*) (Lepidoptera: Pyralidae)

The original culture was obtained from the Bee Keeping Station of the Ministry of Livestock Development located at Lenana off Ngong road in Nairobi. The cultures were maintained in the laboratory through artificial diets made of bee wax, yeast, honey and maize flour at the ratio of 1:2:6:7. The wax was melted and mixed with other ingredients and the cake cooked for 10 minutes. The hot contents were poured into a rectangular plastic container (12cm x30cm x 6cm) (Plate 3.1) and allowed to cool. Viable wax moth eggs were placed on the diet medium and covered shallowly with parts of the diet. The contents were incubated at 27-30^{0}C for three weeks. Third instar larvae (Plate 3.2) were harvested from the diet and used for nematode multiplication and other experiments in this study.

Plate 3.1*: Galleria* rearing boxes

Plate 3.2: Third instar *Galleria* larvae

3.2 Silkworm *Bombyx mori* (Lepidoptera: Bombycidae)

Silkworm eggs were obtained from the Sericulture Project of the Ministry of Agriculture, Thika. Eggs were incubated in 100% humidity at 25^0C under papers covered with moist towels. The eggs hatched after 10 days and were placed in trays at room temperature and 60% humidity. The silkworm stages were fed with mulberry plant parts. Mulberry leaves were chopped into tiny pieces and fed to the early larval instars (Plate 3.3).

Plate 3.3: Silk worm rearing in the laboratory.

The mulberry leaves were replaced.every day for 3 weeks. The third instars were fed on whole leaves in trays which were replaced when laminae were eaten up or when wilting was noticed. The fourth stage was fed on leaves and soft twigs (Plate 3.4) and the fifth instar (Plate 3.5) on leaves, soft and more solid twigs.

Plate 3.4 Silkworm rearing bed

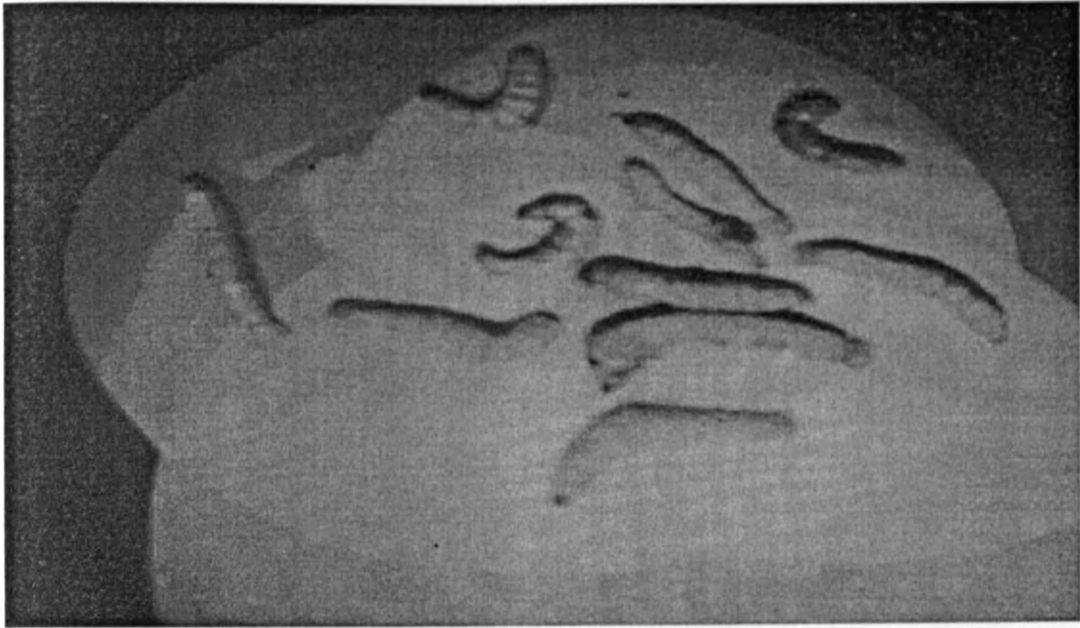

Plate 3.5 Fifth larval instars of silkworm (*Bombyx mori*)

The leaves and twigs to raise the silkworm were obtained from a mulberry plantation (Plate 3.6) established by the sericulture project at Thika.

Plate 3.6 Mulberry shrub plantation.

3.3 Nematode cultures (Steinernematidae and Heterorhabditidae)

Initial nematode suspensions of *Steinernema karii* and *Heterorhabditis indica* were obtained from National Fibre Research Centre at Mwea (KARI-NFRC). The nematodes had been isolated from soils of Central Kenya and Coastal lowlands (Waturu, 1998). *Steinernema yirgalemense* had been isolated from the upper highlands of central Rift valley in a preliminary survey. The nematodes were produced through *G. mellonella* in KARI-Kabete Entomology laboratory using the method described by Woodring and Kaya (1988). Petri dishes (9 cm x 3.5 cm) were thoroughly washed with liquid soap, rinsed with distilled water and sterilised with soft tissue paper soaked in 70% alcohol The dishes were lined with What man filter paper and five hundred (500) infective juveniles of test nematodes applied on the filter through 1ml distilled water. Five third instar *Galleria* larvae were placed on nematode treated filter papers and the contents closed firmly with covers and sealed with Parafilm. G*alleria* cadavers were transferred to other dishes after 3 days for a further 2 days for the entomopathogenic nematodes to develop. The cadavers were arranged on a filter paper placed on an inverted Petri dish, standing in another dish containing distilled water. The slanting part of the filter paper was allowed to dip into distilled water. The contents were left on the laboratory bench at 18-25^{0}C for 10 days. Emerging nematode juveniles were washed into clean beakers and more distilled water added into the harvesting bowl for continued trapping of emerging nematodes. This was repeated until very few nematodes could be recovered from the trap. The nematode suspensions were cleaned by sedimentation and decantation. Where larger numbers of entomopathogenic nematodes were required, plastic basins (45cm diameter by 14cm height and 30 x12cm height) were used in the place of the petri dish and corresponding dish containing distilled water to make a modified White trap. Muslin cloth was placed on the overturned bottom of the inner basin and allowed to dip into the distilled water in the larger basin (Plate 3.7) and filter placed on the muslin near the edges. The filter paper and muslin were

moistened with distilled water and *Galleria* cadavers arranged on the filter paper. Emerging nematodes were harvested as explained in section 3.2 and clean nematode suspensions stored at 15 ^{0}C.

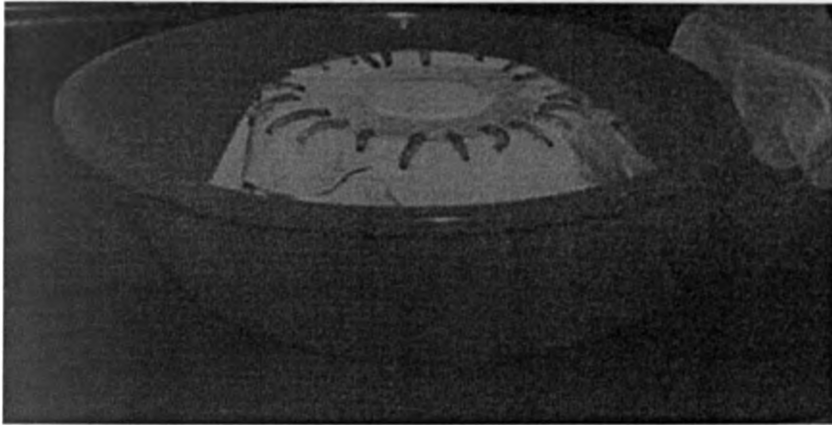

Plate 3.7: Modified White trap (Woodring & Kaya, 1988) for extraction of entomopathogenic nematodes from insect hosts.

3.4 Nematode counts and dose determination

To make nematode suspensions of given concentrations (50, 100, 200, etc), nematode suspensions were either diluted by adding given volumes of distilled water or concentrated by discarding given volumes of water. The suspension was thoroughly stirred to ensure homogeneity before drawing the ten aliquots of 1ml. Counts of nematodes in each of the ten 1ml suspensions were made under a binocular microscope. The mean count for the ten milliliters was considered to be the concentration of the suspension. This was done in all tests involving nematode quantification in this study.

3.5 Sweet potato weevils (*Cylas puncticollis*)

The initial culture was obtained from a sweet potato plot in Embakasi Division to the East of Nairobi. Multiplication of weevils was done through sweet potato tubers from Kangemi market in Kabete and Kibirigwi in Kirinyaga district. The weevils were raised in rectangular plastic containers (9 cm by 14 cm by 6 cm). The containers were lined with soft tissue paper. Three infested tubers were placed in the containers and emerging weevils removed and sexed. Five male and five female of the newly emerged weevils were put together with one clean sweet potato tuber in new plastic containers of similar dimensions as before. The tissue lining the container base was replaced every three days to keep the environment dry and to reduce fungal and mite infestation. The contents were incubated for three weeks at 27-30^{0}C. Infested tubers were used as source of weevil inoculum during this study. Infested tubers were dissected at 1 week or 2 weeks whenever larvae and pupae were required.

3.6 Sweet potato vine varieties

The vine varieties were obtained from the Biotechnology germplasm bulk maintained at Kabete Campus Field Station. The three vine varieties used in the study were Kakamega 4 (K 004), a variety with red skinned tubers of orange fresh and high vitamin A content, KSP20 a high yielding variety of red skinned tubers and white fresh which is a selection from KARI Katumani Research Centre and originally introduced from South America. The third variety was KEMB 10, a determinate local cultivar selected by the KARI Embu Root and Tuber Crop Program. KEMB 10 has thick vines, cream skinned tubers and cream fresh, is of moderate tuber yields with relatively high dry matter content.

3.7 Study Sites

The study was carried out in Kenya Agricultural Research Institute-Kabete. Soils for pH tests were collected from Nyeri at Gathuthi sub location where the soils are of very low pH (<4). Soils for the

texture test were obtained from sites with clay, sandy clay, clay loam and sandy clay loam soils as recommended by Sombroek (1982). The sites were at Kinangop in Nyandarua district (clay loam soil), Mavoloni in Machakos district (sandy clay loam), Maragua district (clay soil) and Kitengela in Kajiado district (sandy clay).

3.7.1 KARI-Kabete

Soil pH standardization and determination of percent soil carbon were done in the Soil Chemistry laboratory, soil texture determinations in Soil Physics laboratory, nematode extraction and all insect-nematode studies at the Entomology Laboratory and greenhouses at KARI-Kabete. The field experiment to evaluate the effectiveness of entomopathogenic nematodes on the sweet potato weevil and to evaluate nematode applications methods were also carried out in the KARI- Kabete experimental field. Kenya Agricultural Research Institute- Kabete is in agro ecological zone Upper midlands zone (UM2) at an altitude of 1850m above sea level. The average rainfall is 1046mm. The rainfall is bimodal with the first season receiving 800mm and the second season 400mm. The first season is from March to June and the second from October to December. The temperature ranges from 18-25^{0}C. The main crops grown are maize, vegetables, coffee and beans. The soils in the site are clay of low pH.

3.7.2 Nyeri district –Gathuthi site

Soils for the study of pH effects were sampled from a farm in Gathuthi in the Upper Midland zone (UM1) coffee tea zone. The soils of this region are acidic with pH of about 4. The site lies in the eastern windward side of Aberdare Ranges 10 Km south west of Nyeri on 37^{0} 55'E and 0^{0} 30'S at an altitude of 1710m. The mean annual rainfall is 1000- 1600 mm. The first season receives 400-800 mm in March-May and the second 340-450 mm in October to December with 60% reliability. Other crops

grown in this region include maize which requires heavy manure application and soil liming, dry beans, sweet potato for tuber and vines, napier grass and Irish potatoes. The soils are well drained and very deep.

3.7.3 Nyandarua district – Kinangop

Nyandarua district is in Central Kenya but some of its environments extend to the central Rift valley. The soil sampling site was to the south east of the Kingangop plateau with a mean rainfall of 1600 mm with one peak in April-May and the other in October. The site is at 2558 m above sea level and at 36^0 39.68'E and 0^0 45'S in the Upper Highland zone (UH2). The characteristics of the area are described in Jaetzold and Schmidt (1983). The site is about 70 Km North West of Nairobi. The crops grown include vegetables, peas, potatoes, pyrethrum and napier. The area is ideal for pasture and wheat.

3.7.4 Machakos district - Mavoloni

Machakos district is located between 37^0E and 38^0 30'E and 0^0 30'S and 3^0S. The altitude rises from 700 m in the east to 1700 m in the west and rainfall from 500 to1300 mm with 60% reliability. The first rains are 50-450 mm and come between March and May and the second 60-530 mm from October to December. The windward eastern slopes receive some rainfall between June and August due to effects of the south east trade winds. In such cases the rainfall exceeds 1000 mm in the LH2 zones. The lower UM 2 zone receives enough rainfall for coffee in the eastern slopes but the western slopes are marginal coffee zones. The soil sample site was 5 Km to the south of Matuu trading centre off the Thika Garisa road near Mavoloni trading centre at 37^0 30'E and 1^0 15'S in the LM4 zone with two short cropping seasons. This is a marginal cotton zone. The site is at 1280 m above sea level with temperatures of 21.3- 22^0C and mean annual rainfall of 700 to 850 mm with the first season receiving

220-350 mm and the second 250-350 mm. The crops grown are Katumani maize, sorghum, sunflower, millet, green grams and cowpeas.

3.7.5 Maragua district-Maragua site

The site is located on 37^0 20'E and 0^0 40'S about 7 Km south east of Muranga and 3 Km off the Muranga–Nairobi highway at an altitude of 1400 m above sea level. The mean temperatures are 20^0C in the Upper Midland zone (UM4). Rains start in mid March. The mean annual rainfall is 900-1350 m with the first season receiving 450-500 mm and the second 250-280 mm. The soils are developed on basement systems and are excessively drained with stony out crops. Tea is grown in the upper parts of the district but the site is located in a marginal coffee zone. The crops grown include bananas, sisal, pineapples, castor, maize and fruits such as papaw and mangoes. Kale, cabbages, tomatoes, dry beans, pastures mainly Rhodes grass and French beans are grown along most valleys. Other crops grown in the region are cowpeas, dolichos, sweet potato, cassava, macadamia, chickpeas and leucana trees (as agro forestry trees).

3.7.6. Kajiado district –Kitengela

The site is located at the southern side of Kitengela township about 40 Km south east of Nairobi. The region is a range land in the drier marginal UM5 zone located at $36^0$56'E and 01^0 28.53 S. The site is in peri-urban area within the Kitengela town council at 1000 m above sea level. The rainfall is unreliable and reaches 600 mm per year. There are scattered farms growing low altitude maize, beans and vegetables. Agro forestry trees have also been established in many households. The soils at the site are sandy clay. There are two short rain seasons which rejuvenate grass and form seasonal streams. The main water sources are surface dams and boreholes.

3.7.7 Central Rift valley-Nakuru district

The survey for entomopathogenic nematodes was carried out in parts of central Rift valley within the administrative district of Nakuru between 35° 34.88'E and 36° 37.44 E and 0° 12.74'N and 0° 56.7 S. The western parts rises nearly to 3000 m and receive an annual rainfall of 1200-1400 mm with temperature ranges of 10-15°C compared to the central and eastern part which are lower (1800-1900 m above sea level) and receive less mean annual rainfall (660 mm) and have higher temperatures (22.7-26.3°C). The soils on plateaus are sandy, clay and silt clay and in some places clay with humic top soils while those on hills are calcareous loams; and those in plains around lakes imperfectly drained silt loams. Those on plains south of Longonot and Susua are greyish. Most of the bottomlands are to the north of Nakuru town. The soils in bottomlands are vertisols of varying salinity and sodicity. The site characteristics are as described in Jaetzold and Schmidt (1983) for Central and Rift valley and confirmed by observation and measurements using global positioning system (GPS) and laboratory determinations during this study in October 2005.

3.8 Statistical analysis

The survey data on pH, percent carbon and altitude, agro-ecological zones and soil texture were subjected to Chi-square analysis while laboratory and field experimental data was analysed for variance (ANOVA) and significance presented by the probability at 95% and standard error and coefficient of variation (CV%). Treatment means were separated by lowest significant difference. Differences or similarities of treatment means were shown by letters in superscripts. Similar means in a column shared letters while different letters accompanying means in a column showed that the treatment effects were significantly different between such treatments.

CHAPTER 4

4 ENTOMOPATHOGENIC NEMATODES OCCURRING IN CENTRAL RIFT VALLEY IN KENYA

4.1 INTRODUCTION

The presence of entomopathogenic nematodes in all soils except those in the Polar Regions has been established (Hominick, 2002) and their presence in Kenya confirmed (Waturu *et al.*, 1997; Waturu, 1998). A working group (COST Action 819) harmonised the taxonomy of entomopathogenic nematodes and recommended that identification of new entomopathogenic nematodes be done to species level (Hominick *et al.*, 1997). Three nematode species; *Steinernema karii, Heterorhabditis indica* and *H. bacteriophora* were isolated from the soils of the central highlands and coastal lowlands of Kenya (Waturu *et al.*, 1997; Waturu, 1998). This offers a narrow genetic base for entomopathogenic nematodes from Kenya. The objective of this study was to isolate additional entomopathogenic nematode species, determine their identity and distribution and factors influencing their occurrence in the Central Rift Valley region of Kenya.

4.2 MATERIALS AND METHODS

4.2.1 Characteristics of Central Rift Valley-Nakuru district

The survey was carried out in parts of central Rift valley within the Nakuru administrative district with characteristic as described in chapter three subsection 3.7.7. The areas surveyed in the current and previous study in Kenya are shown in figure 4.1. A preliminary survey in the area established the types of vegetation and the agro ecological zones within the area.

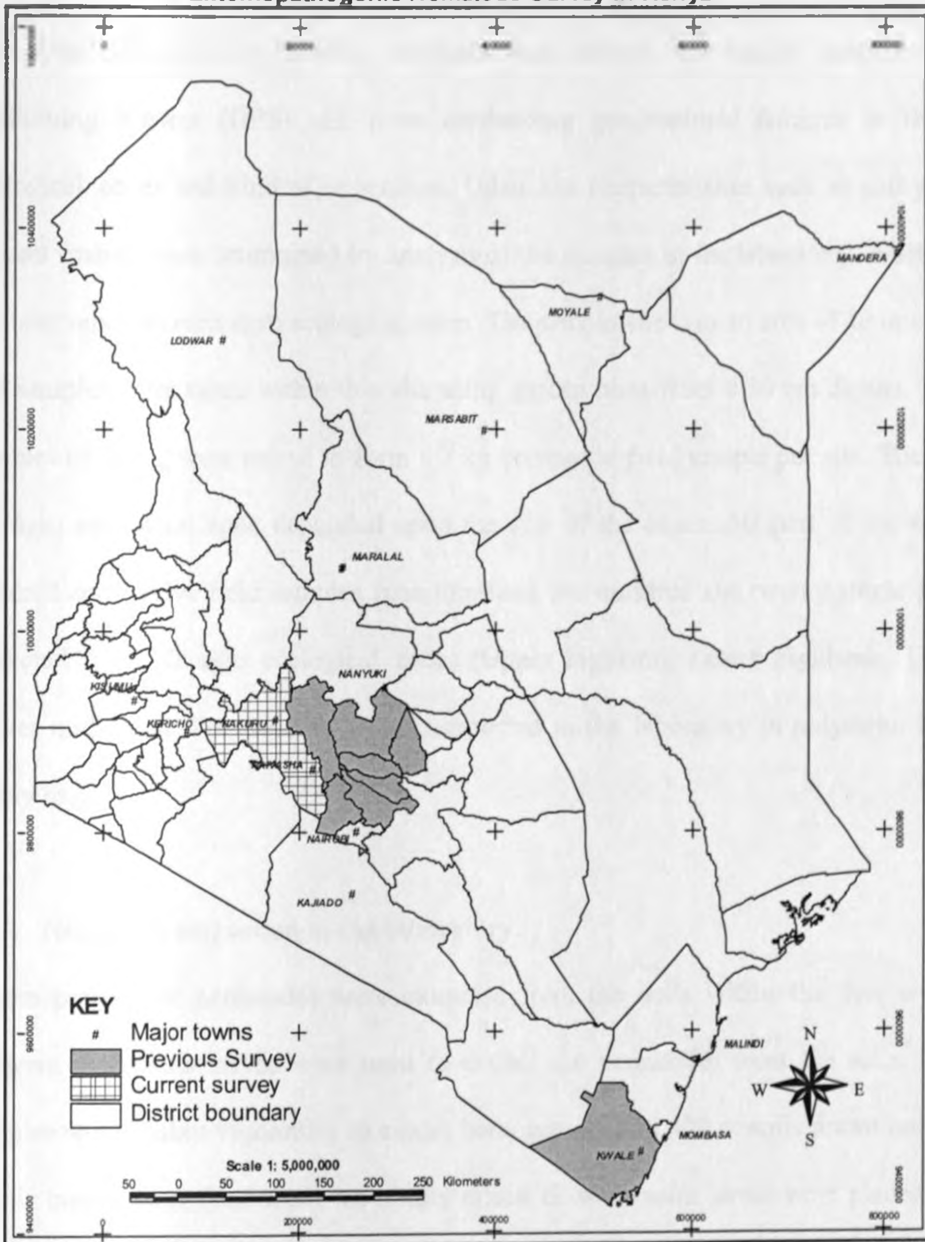

Figure 4.1: Previous and current survey for entomopathogenic nematodes in Kenya

4.2.2 Field sampling

The soil sampling survey was carried out in the second and third week of October 2005. The sample sites were identified by latitude, longitude and altitude and nearest main town using a Global Positioning System (GPS) and from outstanding geographical features in the proximity, agro-ecological zones and kind of vegetation. Other site characteristics such as soil pH, soil texture and percent carbon were determined by analysis of the samples in the laboratory. Soils were sampled at 5 km intervals into each agro ecological zone. The sample site was an area of 40 m x 40 m. Ten random soil samples were taken within this site using garden hoes from 0-30 cm depths. The ten single point samples of 300 g were mixed to form a 3 kg composite field sample per site. The number of samples per agro ecological zone depended upon the size of the accessible part of the zone. A total of one hundred and twelve field samples (one thousand one hundred and twenty single point samples) were collected from all agro ecological zones (Upper highland, Lower highlands, Upper midlands and Lower midlands). The samples were transported to the laboratory in polythene bags placed in sisal gunny bags.

4.2.3 Nematode extraction in the laboratory.

Entomopathogenic nematodes were extracted from the soils within the first week after sampling. *Galleria mellonella* larvae were used to extract the nematodes from the soils. The composite soil samples were shaken vigorously to ensure homogeneity and 400 cc soils drawn and placed in half liter plastic bowls. Four third instar laboratory raised *G. mellonella* larvae were placed on the soil surface and the contents closed with perforated lids. The contents were placed on laboratory benches for 3 days. The temperature in the laboratory ranged from 18 to 25^{0}C. *Galleria* larvae were retrieved and mortality counts taken after three days. The characteristic color of cadavers was used to determine the genus of entomopathogenic nematodes causing mortality, the brick red as an indictor for

Heterorhabditis and pale for *Steinernema* species (Woodring and Kaya, 1988). Four more *G. mellonella* larvae were placed in the soil and the contents returned to incubation for a second extraction of nematodes. The new *G. mellonella* larvae were retrieved from the soil sample after three days and a second set of data generated as before. A modification of the method of White (1927) as described in Woodring and Kaya (1988) was used to extract nematodes from *Galleria* cadavers. Each *Galleria* cadaver was placed on its trap for nematode extraction to ensure that nematodes from each site were pure cultures. The details of the method are in chapter three subsection 3.3. The cultures were observed for viability under a microscope and viable ones used to infect other *Galleria* to build up isolates cultures.

4.2.4 Determination of soil texture

Soil texture determination was done at KARI-Kabete in the Soil Physics Laboratory using the method described in Hinga et al. (1980). The soils were dried at 40-45 ^{0}C for two hours, crashed and passed through 2mm sieves. Fifty grams (50 g) of soil were placed in 500 ml plastic containers and 300 ml water added together with 50 ml of the dispersing reagent Calgon (Calgon solution, is 45 g of Sodium Hexa Metaphosphate mixed with 5 g of Sodium carbonate). The suspension was placed on a shaker overnight and then transferred to a graduated cylinder and made up to 1000 ml mark with tap water. The contents were mixed using a plunger. A graduated hydrometer was placed in the suspension immediately after stirring (about 40 seconds). The first Hydrometer reading was for the silt and clay fraction. Sedimentation was allowed to take place in the next 2 hours and another hydrometer reading taken. The second value was a measure of the clay fraction since the silt had already settled. The clay fraction, the silt fraction and the sand fraction were determined using corrections from blanks at the temperature of the suspension. The particle sizes were as follows; Clay fraction; $\leq$ 0.002 mm, Silt; 0.05-0.002 mm and sand 2 mm-0.050 mm

4.2.5. Determination of percent soil carbon

The soil was prepared as for soil texture determination. The Walkley –Black oxidation method (Nelson and Sommers, 1982) was used for the determination of percent organic carbon. One gram (1 g) soil from the grinded sample was passed through 50 meshes (0.3mm) and placed in digestion tubes into which 2 ml distilled water was added followed by 10 ml of 5% potassium dichromate solution. After wetting the soil completely with Potassium dichromate solution, 5 ml concentrated sulphuric acid were cautiously added in aliquots of 1 ml in eppendorf tubes per time interval while stirring with a vortex mixture. The contents were digested at 135^0C or 30 minutes. Curves for standard concentrations were generated on a spectrometer at 600 nm. The sample carbon was read from graphs generated by standards on the calibrated spectrometer. Samples with readings beyond the standards were repeated using 0.5 g soil instead of 1g. Barium chloride solution (50ml of 0.04%) was added to the cool samples and the samples shaken thoroughly. The contents were left overnight to form a clear supernatant. Ten percent Potassium dichromate (10 ml) and 5 ml concentrated sulphuric acid were added to the samples as before and contents heated at 150^0C for 30 minutes. Carbon levels were read from the spectrometer as before. The results were then converted to percentage carbon content.

4.2.6 Morphological characterization of entomopathogenic nematodes

Stable isolates from the various agro ecological zones of Rift Valley region were cultured through *Galleria mellonella*. *Galleria* larvae were infected with one hundred (100) nematode infective juveniles for each test isolate. Some cadavers were dissected in Ringers solution four days after infection to recover the first generation males and females and others on the seventh day for the second generation of the both sexes. The third stage nematode juveniles, adult males and females were extracted and processed for slides. The nematodes were killed in a drop of equal proportions of TAF and Ringers solution and dehydrated slowly at 60^0C and then mounted on slides in anhydrous

glycerine. The cover slips were supported by glass beads and sealed by wax or nail vanish. Morphological characters of the body parts were measured using a compound microscope with a graticule and the results analyzed and compared with the already generated information on described nematodes. The characteristics measured included total nematode body length (TBL), maximum body width (MBW), tail length (TL), anal body width (ABW), distance from the anterior end to oesophagus (ES), distance from anterior end to excretory pore (EP), distance from anterior end to nerve ring (NR), spicule length (SPL) and Gubernaculum length (GUL) for males and distance from anterior end to vulva (AV) for females.

4.2.7 Molecular characterization of entomopathogenic nematodes

DNA was extracted by the method of Nguyen (2006). Adult females were recovered from *Galleria* treated with 100 infective juveniles per larva by dissecting the *Galleria* cadavers under a dissecting microscope. The adult female nematodes were placed in sterile tubes containing 95% ethanol and contents tightly screwed and sealed with Para film to prevent evaporation of ethanol. Additionally, nematode infected cadavers were stored in vials in 95% alcohol for dissection at the time of DNA extraction. The later ensured that the nematodes were not digested by the concentrated alcohol. The contents were stored at 4^0C for a maximum of 3 months to avoid DNA deterioration. The method of Nguyen (2006) used a single female for characterization. The nematode from 95% alcohol was re-hydrated with distilled water in a petri dish for 5-12 hours and processed as a fresh sample. It was placed on a 70% ethanol swabbed microscope slide with a drop (10µ) of worm lyses buffer (50mMKCL, 10mM Tris pH 8.3, 2.5 mM$MgCL_2$, 0.45% NP40, 0.45% Tween 20, 0.01% gelatin and 60ug / ml proteinase K) and crushed with a pipette tip and transferred to a sterile 0.5 ml micro centrifuge tube on ice. The tube was frozen at -20^0 C for 10 minutes. It was then incubated at 65^0C for 1 hour followed by 10 min at 95^0C to in-activate proteinase K. The lysate was cooled on ice and

centrifuged at 12,000 revolutions per minute for 2 minutes and 5µl of supernatant used in the PCR reaction. The PCR reactions were carried out in 50µl micro centrifuge tubes. The contents for the reaction were 1.5µl 10x PCR buffer, 0.5µl each dNTP (20mM each), 0.5µl forward and 0.5µl reverse primer (1000µg/ml), 2µl Taq polymerase and 45µl double distilled water and the contents set on ice. The reactions were placed in a Techne PHC-3 thermocycler pre set at 95^{0}C and subjected to the following cycling profile; one cycle of 94^{0}C for 2 minutes followed by 40 cycles at 94^{0}C for 30 seconds, 45^{0}C for 60 seconds and 72^{0}C for 90 seconds. A final step of 72^{0}C for 5 minutes was included to ensure that all the final products were full length. The target region for rDNA amplification was the ITS1 -5.8S- ITS2 which varies among different nematode species. Repeat fragments of the region were amplified with random primers i.e. random amplified polymorphic DNA analysis (RAPD). The primers used for the PCR in this study were 18S: 5'-TTGATTACGTCCCTGCCCTTT-3' (forward) and 26S: 5'-TTTCACTCGCCGTTACTAAGG-3' (reverse) (Vrain *et al.*, 1992). Five microlitres (5µl) of the PCR product were visualized on an ethidium bromide stained agarose gel and the presence or absence of DNA demonstrated by light bands corresponding to isolates where DNA had been amplified, faint for poor amplification and no bands for isolates whose DNA had not been successfully amplified. The amplified PCR products were purified with QIAquick PCR purification kit (QIAGEN Inc Santa Clarita, California). The PCR product was then sent for core sequencing on both directions together with the primers given above (18S and 26S) and two internal primers for complete sequencing of the two D'NA strands. The two internal primers used for sequencing were KN58: 5'GTATGTTTGTTGAAGGTC-3 (forward) and KNRV:5'CACGCTCATACAACTGCTC-3'(reverse) for *Steinernema* species and H58P:5'-ACGAATTGCAGACGCTTAG-3' (forward) and H58R: 5'-GTGCGTTCAAAACTTACC-3' (reverse) for *Heterorhabditis* species (Nguyen *et al.*, 2004). The sequences generated were aligned to the published sequences of the ITS region in the GenBank http://www.ncbi.nlm.nih.gov/ of described species using the default parameters of Clustal X

(Thompson *et al.,* 1997) and optimised manually. Species were considered new if similarity was less than 95% and 97% for *Steinernema* and *Heterorhabditis* respectively.

4.2.8 Data analysis

The data on agro ecological zones, altitude, percent organic carbon, soil pH and soil texture effects on the occurrence of entomopathogenic nematodes in the natural environment was analysed by chi-square and morphometric data was analyzed for variance using the SPSS statistical program. Means were presented with their standard errors.

4.3 RESULTS

One hundred and twelve composite samples (1120 single point samples) were collected from the survey area. The percent entomopathogenic nematode recovery from one time extraction was 30% and the total from the two extractions was 50%. Recoveries from agro-ecological zones ranged from 50-67 % for broader categorization of agro-ecological zones (Upper Highlands, Lower Highlands, Upper Midlands and Lower Midlands) with most zones recording about 50 % recoveries (Table 4.1).

Table 4.1: Entomopathogenic nematodes from different agro-ecological zones

AEZ	Altitude (m)	Total samples	Percent positive samples per spp *Steinernema*	*Heterorhabditis*
Upper Highlands (UH0-UH3)	2200-3000	57	7	7
Lower Highlands (LH1-LH5)	1800-2400	36	50	3
Upper Midlands (UM3-UM6)	1800-2200	18	44	6
Chi-square $P \leq 0.05$			1.2 (NS)	2.7 (NS)

Means in the same column are not significantly different (NS)

Most positive sites for nematode occurrence were from the altitude range 2400-2600m above sea level although the effect of altitude on nematode occurrence was not significant (Table 4.2). The nematodes from higher altitudes had poor culturing ability which declined rapidly compared to those from lower altitudes. Three *Heterorhabditis* isolates from lower altitudes re-cultured after eight months while the two isolated from above 2400m could not be cultured beyond the first month.

Table 4.2: Percent entomopathogenic nematode positive sites from different altitudes

Altitude (m)	Total number of samples	Percent *Steinernema* positive samples	Percent *Heterorhabditis* positive samples
1800-2000	30	47	7
2001-2200	21	57	0
2201-2400	13	23	8
2401-2600	29	59	7
2601-2800	17	42	6
2801-3000	2	0	0
Chi-square $P \leq 0.05$		7.0	3.6

There effect of soil percent carbon on overall nematode occurrence (X^2 =14.4) and that on nematode genera occurrence was highly significant (X^2 =10) respectively within the ranges of <1 to 3-4 percent carbon. Most soils had a percent carbon of 2-3% and the least <1%. Nematode frequency was highest in soils of 2-3% organic carbon. *Steinernema* species had an overall lower mean percent carbon (2.2) than *Heterorhabditis* species (2.4) (Table 4.3).

Table 4.3: Entomopathogenic nematodes in relation to percent carbon

% carbon	Total samples	Percent *Steinernema* spp	Percent *Heterorhabditis* spp	Comments on percent carbon level
<1	3	67	33	Rare
1-2	41	46	2	Common
>2-3	50	46	2	Common
>3-4	15	60	20	Moderate
Chi-square $P \leq 0.05$		14.4	10.0	

Although the effect of soil pH on nematode occurrence was not significant, most soils samples had pH of 5.3-6.3 (Table 4.4) which was also the range with the maximum frequency of entomopathogenic nematodes.

Table 4.4: Entomopathogenic nematode occurrence in relation to soil pH

Soil pH	Total samples	Percent *Steinernema* Spp	Percent *Heterorhabditis* spp	Comments on pH level
<4	1	100	0	Rare
4-5	20	50	5	Common
>5-6	53	53	4	Common
>6-7	30	37	7	Moderate
>7	5	60	20	Occasional
Chi-square $P \leq 0.05$	2.7	NS	NS	

The soil texture classes were clay loam (60%), clay (20%), loam (10%), sandy clay loam (6%) sandy loam (3%) and sandy silty loam (1%). Entomopathogenic nematode recovery was about 50 percent for most textural classes which showed no association between nematode occurrence and soil type (Table 4.5).

Table 4.5: Entomopathogenic nematodes from various soil textural classes

Soil texture	Overall percent positive samples	Percent positive samples for	
		Steinernema spp	*Heterorhabditis spp*
Clay loam	51	48	3
Clays	50	45	5
Loam	61	46	15
Other classes of soil texture	54	45	9
X2 $P \leq 0.05$	0.5(NS)	2.9(NS)	

[1] other soil texture (sandy loam, sandy, silty loam)

Samples from coastlines of large water bodies like Lake Elementaita, Lake Naivasha and large man made dams had a higher probability of being positive for nematode occurrence than those from river banks and marshes. Seventy five percent (75%) of soil samples from near water bodies were positive for nematode occurrence with nematodes from the two genera occurring together while no sample from river banks and marshes was positive for nematode occurrence although a similar number of samples had been collected from the three kinds of environments. The types of vegetation in the survey area are detailed (Table 4.6) and the distribution of nematodes in the different vegetation group types presented (Table 4.7). The proportion of entomopathogenic nematode positive sites from crop land vegetation was higher (63% of crop type habitats sampled and 39% overall) than those from the more

stable non-cropland vegetation habitats such as pasture, forests and hedges (36% of such types and 17% overall) (Table 4.6, 4.7 and 4.8).

Table 4.6: Vegetation types

Vegetation group	Types of vegetation
Pasture:	Grasses; Star, Kikuyu, Rhodes grass, couch
	Annuals crops (pure and in mixed stands): root and tuber crops: sweet potato and Irish potato, maize, sorghums, millets, wheat, barley, oats, beans, vegetables; Kale, spinach, onions and cabbage
Perennial crops:	Tea, coffee, pyrethrum and fruit crops and fodder crops.
Forest:	Blue gums, cypress, cedar, pines, *Gravellier* and *Croton*.
Fodder crops:	Napier grass, Lucerne and *Desmodium sp*
Hedge rows:	Keiapple, Sodom apples, lantana canopies

Table 4.7: Entomopathogenic nematodes found in various kinds of vegetation

Vegetation Type	Percent positive for samples	Percent positive samples *Steinernema spp*	*Heterorhabditis spp*
Annual crops	68	64	4
Perennial crops	40	30	10
Forest	33	33	0
Hedgerows	44	39	6
Pasture	36	28	8
Chi-square $P \leq 0.05$		7.0(NS)	3.6(NS)

Table 4.8: Types of habitats sampled

Habitat	Percent over all positive samples	Percent Positive samples per vegetation type
Disturbed (crops)	39	63
Stable (non crop types)	17	36

Table 4.9: Key diagnostic character measurements of *Steinernema karii* (first generation males) from Rift valley region compared to the ones from Central Kenya (Waturu *et al.*, 1997)

Character (first generation male)	Isolate (Mean ±se and range) n=20 (µm)	Described spp (Waturu *et al* .,1997)
Total body length (TBL)	1860±56 (1470-2300)	1900
Maximum body width (MBW)	130±10 (89-140)	136
Anterior to excretion pore (EP)	99±8 (50-130)	108
Anterior to oesophagus (ES)	158±15 (70-180)	164
Anterior to nerve ring (NR)	100±5 (78-138)	102
Anal body width (ABW)	52±5 (37-74)	55
Tail length (TL)	40±2 (29-67)	37
Spicule length (SPL)	76±2 (57-90)	83
Gubernaculum length (GUL)	52±1 (42-83)	57

Table 4.10: Key diagnostic character measurements of *Steinernema karii* (first generation females) from Rift valley region compared to ones from Central Kenya (Waturu *et al.*, 1997)

Character (first generation Female)	Isolate (Mean ±se and range) n=20 (µm)	Described spp (Waturu *et al.*, 1997)
Total body length (TBL)	5600±305(3240-8900)	5900
Maximum body width (MBW)	270±7 (140-248)	298
Anterior to excretion pore (EP)	89±5 (50-125)	86
Anterior to oesophagus (ES)	129±8 (122-290)	131
Anterior to nerve ring (NR)	142±6 (90-148)	143
Anal body width (ABW)	107±8 (65-162)	104
Tail length (TL)	38±1 (25-48)	37
Anterior to vulva (AV)	2500±136 (1200-3200)	2800

Table 4.11: Key diagnostic character measurements of *Steinernema yirgalemense* (first generation males) from Rift valley region compared to ones from Ethiopia (Nguyen *et al.*, 2004)

Character (first generation Male)	Isolate (Mean ±se and range) n=20 (μm)	Described spp (Nguyen *et al.*, 2004)
Total body length (TBL)	1536±26 (1300-1729)	1572
Maximum body width (MBW)	115±6 (100-140)	118
Anterior to excretion pore (EP)	89±3 (72-108)	89
Anterior to oesophagus (ES)	162±4 (122-188)	151
Anterior to nerve ring (NR)	105±10 (45-120)	111
Anal body width (ABW)	39±5 (31-45)	40
Tail length (TL)	22±6 (15-29)	23
Spicule length (SPL)	62±9 (51-79)	68

Table 4.12 Key diagnostic character measurements of *Steinernema yirgalemense* (first generation females) from Kenya compared to the ones from Ethiopia (Nguyen *et al.*, 2004)

Character (first generation female)	Isolate (Mean ±se and range) n=20 (μm)	Described spp (Nguyen *et al.*, 2004)
Total body length (TBL)	6200±210 (4200-7000)	6144
Maximum body width (MBW)	193±20(160-220)	205
Anterior to excretion pore (EP)	104±3 (70-125)	100
Anterior to oesophagus (ES)	199±15 (110-250)	210
Anterior to nerve ring (NR)	141±18 (135-200)	150
Anal body width (ABW)	68±10 (45-100)	75
Tail length (TL)	39±8 (25-48)	46
Anterior to vulva (AV)	2070±126 (200-2800)	-

Table 4.13 Key diagnostic character measurements of *Steinernema weiseri* (first generation males) from Kenya compared with the ones from the Czech Republic (Mracek *et al.*, 2003)

Character (first generation male)	Isolate (Mean ±se and range) n=20 (μm)	Described spp (Mracek et al 2003)
Total body length (TBL)	1167±45 (810-1580)	1180
Maximum body width (MBW)	116±4 (70-140)	112
Anterior to excretion pore (EP)	93±2 (57-100)	90
Anterior to oesophagus (ES)	139±6 (35-152)	141
Anterior to nerve ring (NR)	103±6 (95-112)	99
Anal body width (ABW)	38±4 (30-45)	38
Tail length (TL)	23±3 (19-32)	25
Spicule length (SPL)	65±7 (55-71)	68
Gubernaculum length (GUL)	49±6(39-65)	53

Table 4.14 Key diagnostic character measurements of *Steinernema weiseri* (first generation females) from Kenya compared with the ones from the Czech Republic (Mracek *et al.*, 2003)

Character (first generation female)	Isolate (Mean ±se and range) n=20 (μm)	Described spp (Mracek *et al.*, 2003)
Total body length (TBL)	4520± 200(3200-5900)	4610
Maximum body width (MBW)	215±10 (200-256)	223
Anterior to excretion pore (EP)	85±11 (72-104)	80
Anterior to oesophagus (ES)	198±4(166-225)	184
Anterior to nerve ring (NR)	133±7 (102-152)	125
Anal body width (ABW)	70±9 (57-80)	63
Tail length (TL)	40±7 (29-48)	42
Anterior to vulva (AV)	2370±125 (200-2800)	-

Table 4.15: Key diagnostic character measurements of *Heterorhabditis bacteriophora* from Rift Valley region of Kenya (Hermaphrodite juveniles) compared with *H. bacteriophora* Poinar (1975)

Character (Juveniles)	Isolate (Mean ±se and range) n=20 (µm)	Described spp (Poinar, 1976)
Total body length (TBL)	530±50 (490-600)	588
Maximum body width (MBW)	17±7 (14-38)	23
Anterior to excretion pore (EP)	95±10 (68-120)	103
Tail length (TL)	78±7 (69-120)	82

Table 4.16: Key diagnostic character measurements of *Heterorhabditis taysearae* (hermaphrodite juveniles) from Kenya compared with *H. taysearae* (Shamseldean *et al* ., 1996)

Juveniles	Isolate (Mean ±se and range) n=20 (µm)	Described spp (Shamseldean *et al.*, 1996)
Total body length (TBL)	430±15 (300-520)	418
Maximum body width (MBW)	16±4 (13-25)	19
Anterior to excretion pore (EP)	88 ± 5 (60-120)	90
Tail length (TL)	60±6 (30-100)	55

The visualization of the PCR product on an ethidium bromide stained agarose gel showed either the presence or absence of DNA with light bands corresponding to isolates whose DNA had been amplified, faint bands for poor amplification and no bands for isolates whose DNA had not been successfully amplified (Plates 4.1,4.2 and 4.3). The DNA bands of Kenyan isolates in ethidium bromide in agarose gel corresponding to *Steinernema karii, Steinernema yirgalemense, Steinernema weiseri, Heterorhabditis indica, Heterorhabditis taysearae* are displayed (Plate 4.1). Sequencing was only done for DNA that was well visualised under ethidium bromide. The *Heterorhabditis taysearae*

isolate was placed in two lanes. The DNA from the first well did not produce a clear band but the second one was good (Plate 4.1)

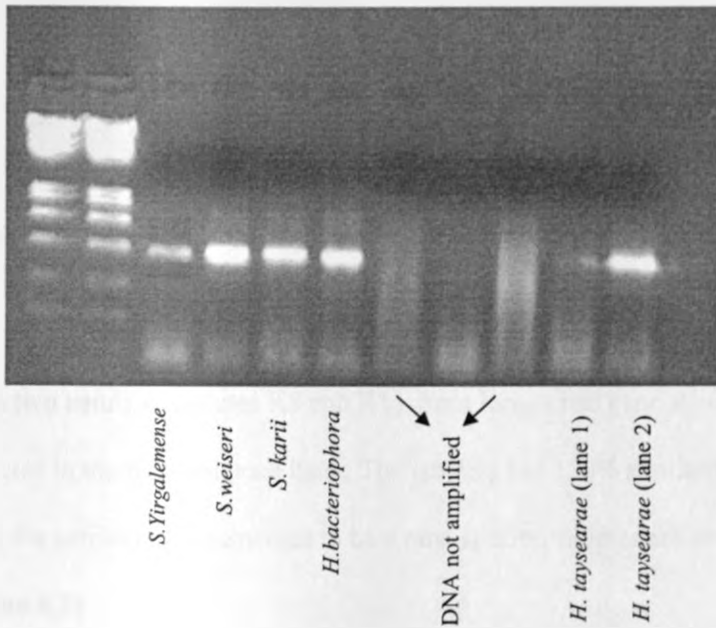

Plate 4.1: DNA visualisation on ethidium bromide stained agarose gel for entomopathogenic nematodes from Kenya

The GE band represents *H. taysearae* species from Egypt (Shamseldean *et al.*, 1996) while the LU1 is an isolate identified as *H. taysearae* from Brazil and K1 and K2a the isolate from Kenya which was sequenced and found to be 97% similar in gene structure to *H. taysearae* (Shamseldean *et al.*, 1996) (Plate 4.2)

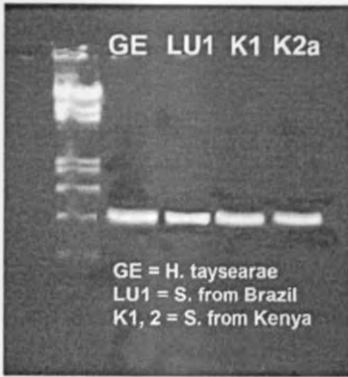

Plate 4.2: DNA bands for *H. taysearae* (Shamseldean *et al.*, 1996), *H. taysearae* from Brazil and *H. taysearae* from Kenya

The two bands of isolates K8 and K11 from Kenya had gene structures that could not match described species in the national gene bank. The isolates had 100% similarity in gene structure of the ITS region and the nematode is suspected to be a new species. Studies are on going to establish this for publication (Plate 4.3).

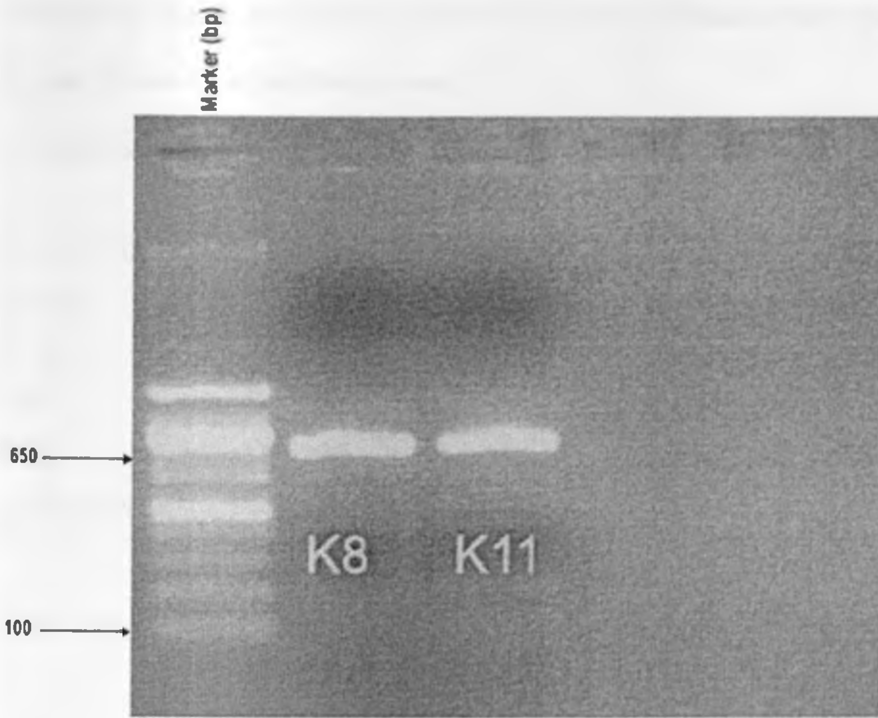

Plate 4.3: DNA bands for the new species K8 and K11

Sequences for the ITS1-5.8-ITS2 region of the rDNA (internal transcribed spacer) using the primers 18S and 26S were generated for the 11 groups of isolates made from the 33 isolated from the soils. On alignment with the sequences of described species in the national gene bank, the isolates sequences fell into six clusters as follows; cluster 1; 99% similarity with *H. bacteriophora*, cluster 2; 97% with *H. taysearae,* cluster 3; 98% for isolates identified as *S. karii*, cluster 4; 98% for those identified as *S. yirgalemense* cluster 5; 97% for the isolate identified as *S. weiseri* and the non matching cluster 6; to be confirmed as a new species (Table 4.17).

Table 4.17: Clusters of Kenyan entomopathogenic nematode isolates and their percent similarity in gene structure to described species

Kenyan isolates	Percent similarity with gene bank Sequences	Described species
K1,2,3,4,20	98	*Steinernema yirgalemense*
K5,6,13	98	*Steinernema karii*
K 10	97	*Steinernema weiseri*
K34	97	*Heterorhabditis taysearae*
K12	99	*Heterorhabditis bacteriophora*
K8, K11 (New species)	-	No match

Table 4.18; Nematodes identified from Central Rift Valley region of Kenya

Nematodes	Type of vegetation	GPS	Locality	Soil type	Percent carbon	Soil pH	AEZ	Altitude	Soil Temperature
Steinernema yirgalemense	Oats	0^{0}16.14´S 35^{0}.48.08´E	Molo	Clay loam soil	3	4.4	UH2	2630m	18.5^{0}C
Steinernema yirgalemense	Acacia trees	0 ^{0}12.35´S 35 ^{0}50.88´E	Rongai	Clay	3	6.3	LH4	1950	20^{0}C
Steinernema yirgalemense	Sweet potato	0 ^{0}27´S 36^{0} 03´E	NW shores of L Nakuru	Clay loam	3	6.1	UM5	2050	23^{0}C
Steinernema yirgalemense	Maize	0 ^{0}26.91´S 36 ^{0}15.77´E	Shores of L Elementaita	Clay loam	1.41	5.4	LH5	1818	22^{0}C
Steinernema Karii	Grass	0 ^{0}08´S 36^{0} 09´E	Bahati-Solai	Sandy clay loam	2.1	5.5	UM3	2000	25^{0}C
Steinernema karii	Grass	0^{0} 54.89´S 36^{0} 31.25´E	Mai Mahiu	Loam	0.59	6.1	LH3	2080	23^{0}C
Steinernema karii	Sodom apples	0^{0} 16´S 36^{0} 15´E	Ndondori	Sandy clay loam	2.2	5.0	LH5	2200	25^{0}C
Steinernema weiseri	Grass	0^{0} 40.1´S 36^{0} 29´E	Naivasha	Loam soil	3.3	6.4	LH5	2050	25^{0}C
Heterorhabditis bacteriophora	Grass	0 ^{0}40.1´S 36^{0}29´E	Naivasha	Loam soil	3.3	6.4	LH5	2050	25^{0}C
Feltiae related species	Onions	0 ^{0}39.9´S 36 ^{0}25.7´E	Kinangop	Clay loam	2.87	6.5	UH3	2236M	20^{0}C
Feltiae related species	Beans	0 ^{0}26.6´S 36 ^{0}1.6´E	Elementaita	Loam	1.74	7.4	UM6	1876M	19^{0}C
Steinernema sp	Beans	0 ^{0}34.9´S 35 ^{0}3.63´E	Olenguruone	Clay	3.24	4.1	UH0	2462M	19.5^{0}C
Steinernema sp	Hedges	0^{0} 21´S 35^{0} 3.63´E	Gilgil	Clay	1.57	6.8	LH5	1923M	18^{0}C
Steinernema sp	Hedges	0^{0} 44.3´S 36^{0} 1.1E	Mai mahiu	Clay loam	1.5	5.3	LH5	2050M	22^{0}C
Heterorhabditis taysearae	Beans	0^{0} 44.3´S 36^{0} 25.4´E	Northern shores of L.Naivasha	Sandy loam	0.7	7.6	LH4	1800	23^{0}C

Steinernema yirgalemense was first described from Ethiopia (Nguyen *et al.*, 2004), *Steinernema karii* from Central Kenya (Waturu *et al.*, 1997), *Steinernema weiseri* from the Czech republic (Mracek *et al.*, 2003), *Heterorhabditis taysearae* (Shamseldean *et al.*, 1996) from Egypt and a *Steinernema feltiae* related species isolated from two locations is yet to be confirmed as a new species. *Steinernema yirgalemense* was the most common nematode in the region. It was found in different kinds of vegetation, from altitudes varying from 1818-2630 m and soil pH 4.4-6.3 and from clay soil to clay loam with most sites having clay loam texture. The first site was in the western part of the survey region 7 Km south east of Molo at the edge of Marioshoni forest in the Upper Highlands zone(UH2) in an oat farm at an altitude of 2630 m above sea level and a soil pH 4.4, percent carbon 3%, clay loam soil and soil temperature of 18.5^{0}C. The second site was in the north western part of survey region at Rongai township in the Lower Highlands zone (LH4). The site was in a natural acacia trees/grass environment. The site characteristics were soil temperature 20^{0}C, soil clay texture, and soil pH 6.3, soil percent carbon of three percent and an altitude of 1950 m above sea level. The site was located at 0^{0}12.35' S and, 35^{0}50.88 E. The third site was 1km from the north western shores of Lake Nakuru about 5 km from Nakuru town in Upper Midlands zone (UM5) in a sweet potato farm at an attitude of 2050 m above sea level. The soils were clay loam of 3 percent carbon, soil pH 6.1 and soil temperature of 23^{0}C. The fourth site was in the central part of the region at the northern shores of Lake Elementaita in a maize and bean farm at an altitude of 1818 m above sea level and 0^0 26.91'S and 36^0 15.77' E in the lower highlands (LH5). The soil temperature was 22^{0}C and the soil texture clay loam of 1.41 percent carbon and pH 5.41 (Table 4.18).

Steinernema karii occurred in three sites within the central and eastern part of the survey region. One site was in the Upper Midland zone (UM3) and the other two in the Lower Highlands (LH5). The three sites had a very narrow altitude range (2000-2100 m) and were all in non crop land habitats. Soils in two of the sites were sandy clay loam while the third site had a loam soil type. Two of the sites had almost similar soil pH (5 and 5.5) and percent carbon (2.1 and 2.2) and a soil temperature of 25^0C.

Steinernema weiseri was only found in one site. It occurred in a road side pasture which was a similar environment to it first site from a road side grassland with apple trees (Mracek *et al.*, 2003). It occurred together with *Heterorhabditis bacteriophora* in a non farmland habitat. The soil pH was 6.4, the altitude 2050 m , soil texture loam and soil temperature 25^0C.

A *Steinernema* species that is phylogenetically related to *Steinernema feltiae* was found in two sites. One of the sites was in the Upper Highland zone (UH3) at an altitude of 2226 m with soils of pH 6.5 and 2.9% organic carbon and a clay loam soil texture. The other site was in the Upper Midland zone (UM6) at an altitude of 1876 m above sea level with a loam soil texture, soil pH of 7.4 and 1.7% carbon. Both sites were in crop land habitats. *Heterorhabditis taysearae* was found in only one location in a sandy soil of very low carbon content (0.7%) and slightly above neutral pH (7.6) in the Lower Highland zone (LH4) at 1800 m above sea level in a crop land habitat.

Another *Steinernema* species occurred in three sites in the Lower Highlands. The first site was in Lower Highlands zone one (LH1) while the other two were in the Lower Highlands zone five (LH5). The first site location was at 0° 21'S and 35^o36.3'E with 3.2% organic carbon and the other two sites had 1.5 and 1.6% carbon and soil pH of 6.8 and 5.3 and altitudes of 1923 m and 2050 m and temperatures of 18 and

22°C, respectively. The site which was different from the other two had a clay soil type, pH 4.1, altitude of 2462 m above sea level and a temperature of 19.5°C. Two of these isolates were extracted from soils under hedges and the third from a farmland habitat. Several attempts were made to amplify DNA and visualise it in agarose but the sequences generated could not be edited which made it hard to proceed with the identification of this isolate. The nematode isolates have remained infective to *Galleria mellonella* for more than two years.

The nematodes occurred in all agro-ecological zones of the survey area from the eastern to the western part of the zone (Figure 2).

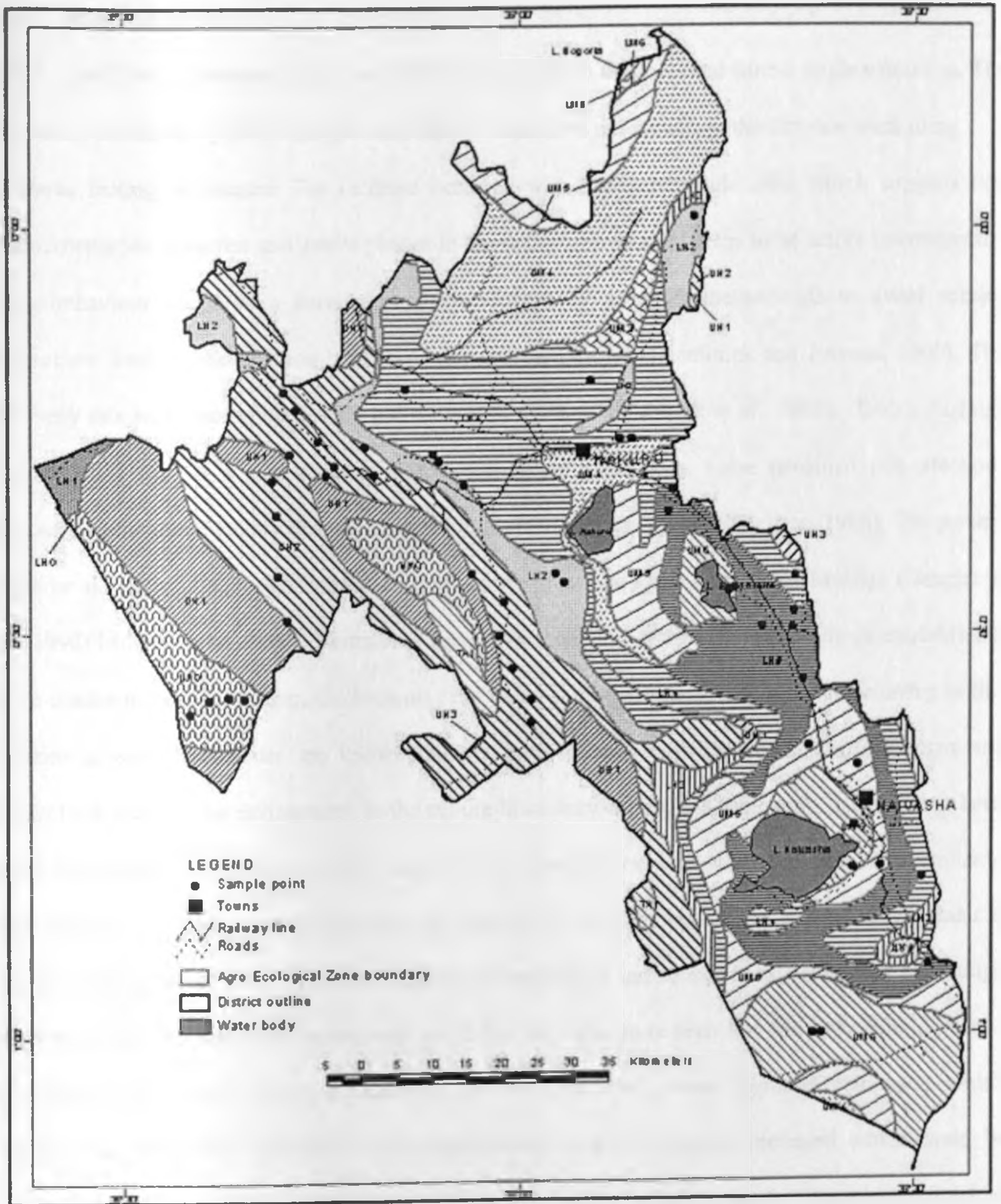

Figure 2: Entomopathogenic Nematode Occurrence in the Different Agro ecological Zones of Nakuru District

4.4 DISCUSSION

The frequency of entomopathogenic nematodes is higher than that revealed from a single extraction. The frequency increased by 20% when two extractions were done per sample in the first one week using *Galleria* baiting techniques. The increase occurred with Steinernematids only, which suggests that Steinernematids have rest and active phases in the soil which enables them to be active intermittently. This behaviour could be a survival strategy adopted by some Steinernematids to avoid species extinction. Such a phenomenon had also been observed earlier (Hominick and Briscoe, 1990). The recovery rate was also influenced by the method of extraction (Mracek *et al.*, 2005). Earlier findings had reported that in a population of entomopathogenic nematodes, some remained non-infectious although in the current study this only applied to Steinernematids species (Western, 1998). The percent positive sites from single extraction and cumulative extractions agree with earlier findings (Gaugler *et al.*, 1992; Mracek *et al.*, 2005). Nematodes present in some soil samples caused *Galleria* mortality but were unable to develop further. *Galleria* may not be an appropriate host to nematodes behaving in this manner as some nematodes are known to have natural hosts (Mracek et al., 2005; Klingen and Haukeland, 2006). The environment in the culture laboratory at KARI-Kabete may also not have been ideal for reproduction for some of these nematodes. Nematodes cultured well in environments similar to their original ones and would require time to adapt to new environments (Grewal *et al.*, 1996; Hazir *et al.*, 2001; Hyam *et al.*, 2001). The time between soil collection and nematode extraction was not enough for adaptation. The nematode populations in the soil may also have been low and only able to kill the host but not reproduce. Fan and Hominick, (1991) established a dose response relationship which showed that infectivity increased as the populations in the environment increased which could be equated to a scenario of low levels of nematodes in soils where mortality occurs with no corresponding development of juveniles in the host. It is also probable that only same sex nematodes penetrated the

host. This is a likely scenario because it was common with steinernematids and not heterorhabditids. Heterorhabditids produce asexually in the first generation but steinernematids are amphimictic in all generations with the exception of the asexual nematode from Indonesia (Griffin *et al.*, 2001). The associate bacterial cells may also have been too few to play their symbiotic role effectively as a critical number of bacterial cells is required, the number being species dependent (Burnel and Stock, 2000). The percent recovery of *Heterorhabditis* species was less (5%) than that for Steinernematids (46%) and this agrees with similar studies elsewhere (Mracek *et al.*, 1999, 2005). Weather might also have influenced nematode recovery as earlier reported (Hyam *et al.*, 2001)

The effect of altitude was not significant (X^2 =7.0, P>0.05) but the ability of nematodes to re-culture was influenced by the altitude of the area they were initially isolated from. Entomopathogenic nematodes from higher altitudes lost their viability more rapidly than those from medium and lower altitudes over an eight month period. This could probably be related to the environmental conditions prevailing in the culturing centre (KARI-Kabete; altitude 1850m and temperatures of 18-25^{0}C) which was nearly the same as the lower altitudes in the survey area. Nematodes recovered from lower altitudes may have adapted to the prevailing environment (laboratory) at the test station more easily than those from higher altitudes. The higher frequency of nematodes occurring in soils of 2-3 percent carbon and pH 5.3-6.4 suggests that these are the most suitable ranges of percent carbon and soil pH for entomopathogenic nematode survival. H*eterorhabditis* preferred a higher percent carbon (mean 2.4%) than steinernematids (mean 2.2%). Overall, nematode and nematode species occurrence were influenced by percent soil carbon (X^2=14 and X^2 =10 respectively and P $\leq$.05) which was in agreement with earlier findings (Rosa *et al.*, 2000). Since organic matter in the soil improves soil porosity and therefore aeration, the results suggest that Heterorhabditids are more sensitive to oxygen supply than

Steinernematids and could also explain partly why Steinernematids are usually more numerous in soils than Heterorhabditids.

Although the soils of the region were mainly clay and clay loam, similar percent nematode recoveries were made from all soils types with none demonstrating unique prevalence for nematode occurrence. Prevalence for particular soils was demonstrated when analysis was done per nematode species supporting the view that different nematodes behave differently in different soils (Duncan *et al.*, 2003; Koppenhofer *et al.*, 2006).

Crop lands habitats (annual and perennial crops) were more suited for nematode occurrence than non-crop lands (forests, pasture, grasslands and hedgerows) in agreement with (Mracek *et al.*, 2005). There may be many underlying reasons but plots under crops are usually more aerated than undisturbed plots which tend to be more compact. Most crop plots were small scale farms which were used for different crops in different seasons. Some pests have rest stages in the soils. The different crop pests could have been used by nematodes for continued survival. In contrast, non-cropland vegetation categories are usually mono cultural kind of vegetation with fewer pests to sustain entomopathogenic nematodes. Entomopathogenic nematodes have been recovered in the natural attacking crop pests and earlier nematode isolations from soils were made from infested insect host cadavers (Cabanillas and Raulston, 1993; Nguyen *et al.*, 2004; Mracek *et al.*, 2005; Klingen and Haukeland, 2006). Millar and Barbercheck (2002) did not find a relationship between nematode occurrence and tilled and untilled plots. Nematode recovery from soils near water bodies whether fresh (Lake Naivasha and man made dams) or salty (Lake Elementaita) was higher than those from river banks and marshlands. The percent recovery from the former was 75 and was zero for the later. This may be related to the obvious better moisture levels in

lake coastlines and the fact that deposition during heavy rains probably carries nematodes from higher sites. Marshy soils are waterlogged and poorly aerated and therefore unsuitable for entomopathogenic nematodes which are obligate aerobes.

The confirmation of the presence of five described entomopathogenic nematodes species in this region is understandable considering that *S. karii* was already isolated from the central highlands of Kenya, *S. yirgalemense* from the Ethiopian highlands while *Heterorhbditis taysearae* was first identified in Egypt (Shamseldean *et al.*, 1996; Waturu *et al.*, 1997; Nguyen *et al.*, 2004). The Great Rift Valley extends all the way to Ethiopia and Egypt and it is possible for the movement of both species along this route. *Steinernema weiseri* was isolated from a roadside environment which is a similar environment to its first site of isolation (Mracek *et al.*, 2003). It was isolated together with *Heterorhabditis megidis* in the Czech Republic while it was isolated alongside *Heterorhabditis bacteriophora* in this study.

Steinernema yirgalemense was the most frequent entomopathogenic nematode in the region. It had a wider adaptation to more agro ecological zones and environments than other nematode species in the region although it had a number of preferred habitat characteristics. The adaptability of nematodes to environments is a good indicator of potential nematodes for use in biological control of pests. Nematodes such as *Steinernema feltiae* and *Steinernema carpocapsae* are widely spread and very effective biological control agents. *Steinernema karii* is also adapted to the region but has a more restricted habitat preference than *S. yirgalemense*. *Steinernema weiseri* was sampled from only one site in the region. The environment was a natural grassland roadside pasture in the same location with *Heterorhabbditis bacteriophora*. The two *Steinernema feltiae* related isolates were found in nearby localities in the central and eastern part of the survey region but in different agro ecological zones. The

recovery from more than one site is probably an indicator that it is a potential nematode for development into a biological control product. The *Steinernema* species whose DNA could not be amplified for species identification was found in three sites which was an indicator of adaptation to different environments and a sign that the nematode has potential for use in biological control.

The overall results of this study showed that nematodes are not restricted to certain environments but that they have characteristic kinds of habitats that they prefer which was also the observation of Mracek et al. (2005). This partly explains why it is hard to get significant results for selected habitat characteristics when isolates are grouped together as different species may have contrasting preferences. The *Feltiae* related isolates were isolated from soils of near neutral and slightly alkaline pH which is contrary to the reports that *Steinernema* species occur in soils of pH<6 (Rosa *et al.*, 2000). Expansive surveys are justified because apart from determining the distribution and frequency of entomopathogenic nematodes, there is the probability of isolating additional and more virulent nematode species to add to the indigenous gene bank for further research and use in biological control.

CHAPTER 5

5 EFFECTS OF SOIL TEXTURE ON SURVIVAL AND PATHOGENICITY OF *STEINERNEMA KARII*, *HETERORHABDITIS INDICA* AND *STEINERNEMA YIRGALEMENSE*

5.1 INTRODUCTION

Entomopathogenic nematodes have shown potential as bio control agents of pests especially those with a soil phase (Georgis and Gaugler, 1991; Duncan *et al.*, 2001; Alekseev *et al.*, 2005). The soil mediates the interactions of the nematode, the host and soil factors. Living organisms like bacteria, fungi, plant parasitic nematodes and free living nematodes and other species of entomopathogenic nematodes interact with entomopathogenic nematodes in soils either positively or negatively (Somasekhor *et al.*,2002; Vestergaard *et al.*, 2003; Klingen and Haukeland, 2006). Pathogenic fungi lower steinernematid pathogenicity by over 80% but affect heterorhabditids only slightly while entomopathogenic nematodes reduce plant pathogenic nematodes but not the free living nematodes (Timper and Kaya, 1989; Somasekhor *et al.*, 2002). Host insects are known to sustain entomopathogenic nematodes while some mites feed on the nematodes (Mracek *et al.*, 2005; Karagoz *et al.*, 2007). The abiotic component of soil including soil aeration, moisture, temperature, humidity, texture and structure also affect entomopathogenic nematode function with different nematodes responding differently (Hominick *et al.*, 1997; Koppenhoffer and Fuzy 2006; Susurluk, 2001). High clay content decreases nematode movement while more lipids are utilized in aerated soils and carbohydrates in poor aeration. Nematodes survive longer when they use lipids as an energy source compared to when they use carbohydrates. The objective of this study was to determine the effects of soil texture on three locally isolated entompathogenic nematodes *Heterorhabditis indica*, *Steinernema karii* and *Steinernema yirgalemense*.

5.2 MATERIALS AND METHODS

5.2.1 Soil texture classes

Soils of different texture (clay, sandy clay, clay loam and sandy clay loam) were collected from four sites; sandy clay (Kitengela-Kajiado district), clay loam (Kinangop-Nyandarua district), sandy clay loam (Mavoloni-Machakos) and clay in Maragua district. The soil exploratory map was used for site selection (Sombroek *et al.,* 1980). The sites sampled lie between longitudes 36^0 35′E and 38^0 30′E and latitudes 0^0 30′ S and 1 ^{0} 29′S within the Upper Highlands, Upper Midlands and Lower Midland zones. The temperatures in the region varied from 15^0C in the UH2 and 23^0C in the LM4 zone. The altitude of the sample region ranged from 700 m to 2100 m above sea level and the rainfall between 500 mm and 1600 mm per year. Specific site descriptions were detailed in chapter three subsection 3.7.2-3.7.5. Soils were dug out from ten points (within an area of 10 m^2). The sampling depth was 0-30 cm. The soils were classified according to their texture as clay- 78% clay, 10% sand and 12 % silt, sandy clay- 58%, sand 30% clay and 12% silt, clay loam- 30% Clay, 38% sand and 32% silt and sandy clay loam- 46% sand, 40% clay and 14% silt. Soil texture determinations were made in the laboratory at KARI Kabete soil Physics laboratory as detailed in chapter four subsection 4.2.4.

5.2.2 Effect of soil texture on nematode virulence and survival

Soils from each textural class were dried at 70°C to kill some micro organisms and mainly the natural nematode populations. On cooling, 2 Kg soils of each soil type were placed in polythene paper tubes (12 cm diameter and 28 cm depth) and thirty six such tubes prepared per soil texture class. Twelve tubes per soil texture class were treated with 6000 ij of *H. indica* per tube, another 12 with a similar concentration of *S .karii* per tube and the last 12 with *S. yirgalemense.* The first experiment was carried out using *Galleria mellonella* as the test host while the sweet potato weevil was used in the second

experiment. Both experiments were laid out in a completely randomised factorial design. Sampling was done at 2 weeks, 4 weeks, 8 weeks and 12 weeks. Parameters assessed and methods of assessment are described for each test host in subsections 5.2.3 and 5.2.4.

5.2.3. Effect of soil texture on virulence and survival of entomopathogenic nematodes using *Galleria* as the insect host

In this experiment, *Galleria mellonella* was used as the test host insect. Entomopathogenic nematodes were applied in soil tube as described in subsection 5.2.2 and *Galleria* larvae placed at five soil depths in each soil tube (0-5, 6-10, 11-15, 16-20 and 21-25 cm). The larvae were inserted at the depths in net pouches (Plates 5.1 and 5.2). The net pouches were used to restrict the highly mobile *Galleria* larvae at the set depths. The larvae were placed at the depths three days before every sampling time.

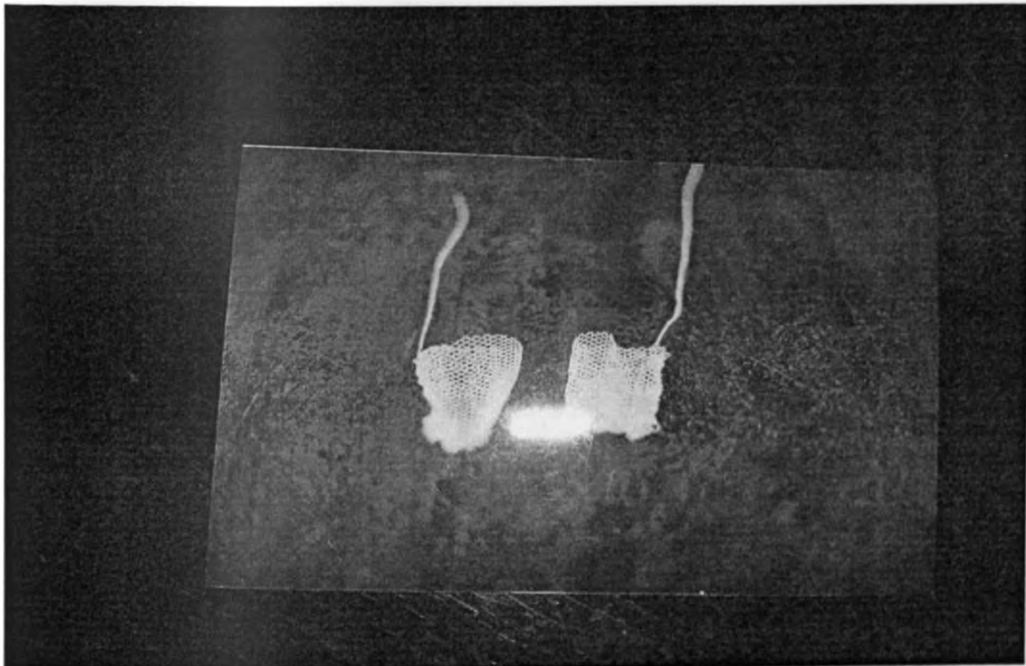

Plate 5.1: Empty *Galleria mellonella* net pouch

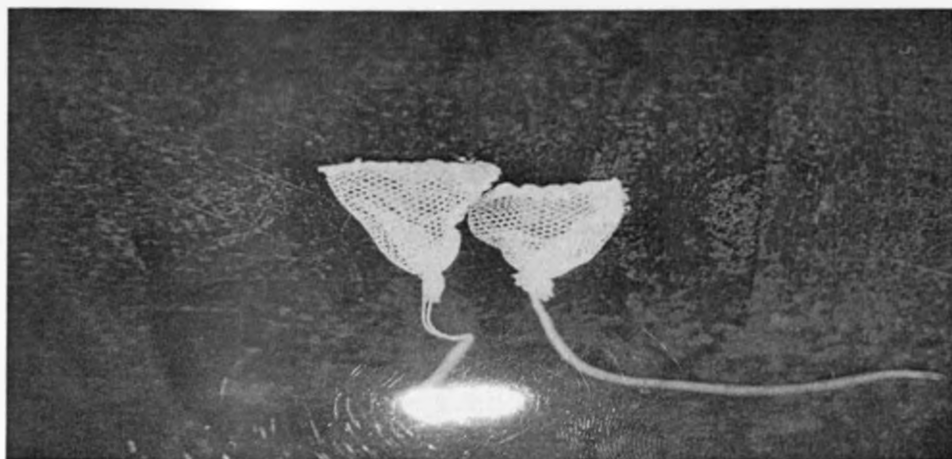

Plate5.2: *Galleria* in sealed net pouches

Galleria larvae were retrieved from the test depths at sampling and mortality records taken. New larvae were used to replace those retrieved at fortnightly intervals. The parameters assessed were *Galleria* mortality, infectivity (number of nematodes establishing in the larva) and nematodes surviving in 30 g soil at each depth. The nematodes surviving were assessed at the end of the experiment. *Galleria* cadavers were placed in own petri dishes for an extra day to allow the nematode juveniles that had established to grow to adults for ease of counting. The cadavers were then dissected in 1% Ringers solution and nematode counts made under a binocular microscope. The soils from each depth (30 g per treatment) were placed in sieves standing in a shallow 15 cm diameter dish (Plate 5.3) and distilled water added from the bottom to wet the soil (Modified Hopper, 1990). The contents were left for 48 hours to enable nematodes to move into the water dish. The sieves were removed and excess water drained and nematodes washed by sedimentation and decantation and their counts made under a microscope.

5.2.4 Effect of soil texture on virulence and survival of entomopathogenic nematodes using sweet potato weevil as the insect host.

The soil tubes preparation, nematodes application, sampling schedule and experimental design were as in chapter five subsections 5.2.2. Infested sweet potato tubers were placed at four depths instead of the five tested with *Galleria* larva. Control treatments were done where no nematode were applied but where infested tubers were placed at the respective depths. Weevil assessment on the controls (at 2 weeks) were made to generate baseline weevil populations

The parameters assessed at each sample time were number of dead weevils per treatment which was done by dissecting the tubers and counting the total dead weevils (larva + pupa + adults). The weevil cadavers were dissected in 1% Ringers solution and counts of nematode juveniles per cadaver made under a microscope. Nematodes surviving at each time interval were assessed from 30 g of soil per treatment. The 30 g samples were placed on double milk filter in sieves standing in other wider plates (Plate 5.3) and the soils wetted from below and left standing for 48 hours (Modified Hopper, 1990). The nematodes that moved into the larger plate were cleaned by dilution with water, sedimentation and decantation and counts made under a microscope. New weevil infested potato tubers were placed at the set depths to replace the retrieved ones after each sample time for three time periods (2nd, 4th and 8th week periods).

Plate 5.3: Modified Hopper (1990) method for extracting nematodes from soils.

5.3. RESULTS

5.3.1 Effect of soil texture on the virulence of *S. karii* and *H. indica* to *Galleria*

The highest *Galleria* mortality in *H. indica* treatments occurred in clay loam soil (80%) while a median mortality of 65% occurred in sandy clay and sandy clay loam soils and the lowest level of 40% in the clay soil. The three means were significantly different (Chi X^2= 18.2, $P \leq 0.05$, Table 5.1). Mean *Galleria* mortality levels were not significantly different (56-60%) in *S. karii* treatments for all soil texture classes (Chi X^2= 1.41, $P>0.05$) (Table 5.1).

Table 5.1: Mean percent *Galleria* mortality in soils of different texture treated with *Heterorhabditis indica* and *Steinernema karii*

Soil type	*Heterorhabditis indica*	*Steinernema karii*
Clay	40	56
Sandy clay	65	60
Clay loam	80	60
Sandy clay loam	65	60
Chi-square $P \leq 0.05$	18.1	1.41(NS)

Means in the column bearing NS after Chi-square value are not significantly different

The peak *S. karii* infectivity to *Galleria* occurred in the first two weeks in sandy clay soil, in the fourth week in the clay loam soils and on the eighth week in clay and sandy clay loam soils. The peak *H. indica* infectivity was at the second and fourth weeks in all soils. The effect of the interaction of soil texture and time interval was however not significantly different (Table 5.2a).

Table 5.2 a: Mean percent *Galleria* infectivity in different soils at different time intervals in *H. indica* and *S. karii* treatments

Soil texture	Time (weeks)	*S. karii*	*H. indica*
Clay	2	6	54
	4	5	45
	8	25	0
	12	5	1
Sandy clay	2	16	47
	4	9	100
	8	2	4
	12	5	1
Clay loam	2	4	12
	4	25	13
	8	3	3
	12	4	2
Sandy clay loam	2	3	29
	4	4	35
	8	27	1
	12	5	3
$P \leq 0.05$	NS	NS	NS

Means in the same column are not significantly different (NS)

Heterorhabditis indica established at a significantly higher rate in the first two to four weeks (35-49%) compared with the rate of establishment in the last four weeks (2-3%) while *Steinernema karii* established at lower rates which were not significantly different for different soil texture classes (5-14%) over the entire test period (Table 5.2b).

Table 5.2b: Mean percent *Galleria* infectivity at different time intervals in *H. indica* and *S. karii* treatments

Time in weeks	*H. indica* (Percent infectivity)	*S. karii* (Percent infectivity)
2	35[a]	7.0[a]
4	49[a]	10.0[a]
8	2[b]	14.0[a]
12	3.0[b]	5.0[a]

CV=16.5%
$P \leq 0.05$
LSD 17

Means in the same column sharing letters are not significantly different

5.3.2 Effect of soil depth on the infectivity of *Steinernema karii* and *Heterorhabditis indica* to *Galleria*

The mean percent nematode infectivity to *Galleria* differed along the depths in all soil texture classes s but the means effects of soil texture and depth interaction were not significantly different (Table 5.3a).

Table 5.3a: Mean percent nematodes establishing in *Galleria* in different soils at five depths

Soil texture	Soil depth(cm)	*H. indica*	*S. karii*
Clay	0-5	75	6
	6-10	5	4
	11-15	3	4
	16-20	29	3
	21-25	14	32
Sandy clay	0-5	56	16
	6-10	89	11
	11-15	28	5
	16-20	17	4
	21-25	9	4
Clay loam	0-5	21	16
	6-10	9	11
	11-15	1	8
	16-20	2	3
	21-25	0	3
Sandy clay loam	0-5	35	5
	6-10	34	4
	11-15	2	5
	16-20	2	7
	21-25	1	24
	$P \leq 0.05$	NS	NS

NS denotes that means in respective columns are not significantly different

The differences in *S. karii* infectivity to *Galleria* along the soil depths were insignificant but mean *H. indica* infectivity to *Galleria* was >34% at the 0-5 and 6-10 cm depths and <14% at the lower soil depths of 16-20 and 21-25 cm. The two sets of mean *H. indica* infectivity were significantly different $P \leq 0.05$ (Table 5.3b).

Table 5.3 b: Mean percent nematodes establishing in *Galleria* at five soil depths

Depth (cm)	*H. indica*	*S. karii*
0-5	50^{a}	11^{a}
6-10	34^{a}	8^{a}
11-15	10^{b}	5^{a}
16-20	13^{b}	4^{a}
21-25	6^{b}	16^{a}

CV= 16.5%
$P \leq 0.05$
LSD 17

Means in the same column sharing a letter are not significantly different

5.3.3 Effect of soil texture on *S. karii* and *H. indica* infectivity to *Galleria*

There was no difference between the mean infectivity of *S. karii* to *Galleria* in different soils but the differences in mean infectivity of *H. Indica* were significant with the highest (40%) occurring in sandy clay and clay soils (25%) and the lowest (7 and 17%) in clay loam and sandy clay loam soils respectively(Table 5.4).

Table 5.4: Mean percent nematodes establishing in *Galleria* in soils of different texture

Soil texture	*H. indica*	*S. karii*
Clay	25^{b}	10^{a}
Sandy clay	40^{a}	8^{a}
Clay loam	7^{c}	8^{a}
Sandy clay loam	17^{bc}	9^{a}

CV=16.5%
$P \leq 0.05$
LSD 11

Means in the same column sharing letters are not significantly different

5.3.4 Effect of soil texture on the survival of *H. indica, S. karii* and *S. yirgalemense*

The highest number of *H. indica* and *S. yirgalemense* survived in sandy clay soils while *S. karii* survived best in both the clay and sandy clay soils. The three nematodes were least suited for survival in sandy clay loam soils (Table 5.5).

Table 5.5: Mean percent nematodes surviving in soils of different texture

Soil texture	*H. indica*	*S. karii*	*S. yirgalemense*
Clay	56^{b}	67^{a}	42^{b}
Sandy clay	100^{a}	55^{ab}	70^{a}
Clay loam	57^{b}	46^{b}	28^{bc}
Sandy clay loam	22^{c}	22^{c}	14^{c}

CV (18%)
$P \leq 0.05$
LSD 19

Means in the same column sharing a letter are not significantly different

5.3.5 Effect of time on survival of *H. indica, S. karii* and *S. yirgalemense*

The highest nematode populations were recovered in the first four weeks in all treatments but recoveries decreased significantly on the eighth week for all nematodes and soils. There was a tendency for nematodes to be recovered in significantly higher numbers in the fourth week period compared to the first two weeks and the last two (eighth week period) for the clay loam and sandy clay loam soils. Overall, the sandy clay loam soil texture supported the least number of nematodes at all time periods (Table 5.6).

Table 5.6: Mean percent nematodes surviving in soils at different time intervals for eight weeks

Soil texture	Time(weeks)	*H. indica*	*S. karii*	*S. yirgalemense*
Clay	2	67^{a}	99^{a}	32^{b}
	4	60^{ab}	56^{b}	77^{a}
	8	41^{b}	47^{b}	18^{b}
Sandy clay	2	100^{a}	73^{a}	100^{a}
	4	100^{a}	54^{ab}	70^{b}
	8	37^{b}	38^{b}	27^{c}
Clay loam	2	45^{b}	33^{b}	17^{b}
	4	73^{a}	74^{a}	45^{a}
	8	52^{b}	22^{b}	18^{b}
Sandy clay loam	2	32^{a}	15^{b}	9^{ab}
	4	17^{a}	46^{a}	27^{a}
	8	15^{a}	17^{b}	5^{b}

CV=18%
$P \leq 0.05$
LSD 19.2

Means (in each soil texture) in the same column sharing a letter are not significantly different

5.3.6 Effect of soil depth on survival of *H. indica, S. karii and S. yirgalemense*

The top depths (0-5 and 6-10 cm) had higher nematode populations surviving than the lower depths with a gradual decline for all the treatments. The nematodes were more uniformly distributed along the depths for both *H. indica* and *S. karii* treatments while *S. yirgalemense* were higher in the 0-15 cm depths compared to the 16 to 25 cm. *Steinernema karii* and *S. yirgalemense* were distributed more uniformly along the depths in the sandy clay and clay loam soils while *H. indica* was higher at the top 0-15 cm depths than the lower 16-25 cm depths for the clay loam soil and more uniformly distributed in

the sandy clay loam soil. Overall, the sandy clay loam soil supported the least number of nematodes for all treatments (Table 5.7)

Table 5.7: Mean percent nematodes surviving in soils of different texture at five soil depths

Soil texture	Soil depth(cm)	*H. indica*	*S. karii*	*S. yirgalemense*
Clay	0-5	99^{a}	76^{ab}	79^{a}
	6-10	60^{b}	100^{a}	38^{b}
	11-15	64^{b}	42^{b}	37^{b}
	16-20	48^{b}	51^{b}	27^{b}
	21-25	11^{c}	37^{b}	30^{b}
Sandy clay	0-5	100^{a}	68^{a}	100^{a}
	6-10	99^{a}	51^{a}	81^{a}
	11-15	47^{b}	62^{a}	83^{a}
	16-20	100^{a}	42^{a}	36^{b}
	21-25	92^{a}	52^{a}	46^{b}
Clay loam	0-5	100^{a}	51^{a}	34^{a}
	6-10	96^{a}	57^{a}	32^{a}
	11-15	100^{a}	50^{a}	22^{a}
	16-20	20^{b}	40^{a}	31^{a}
	21-25	20^{b}	30^{a}	14^{a}
Sandy clay loam	0-5	33^{b}	27^{a}	16^{a}
	6-10	21^{b}	32^{a}	13^{a}
	11-15	92^{a}	17^{a}	14^{a}
	16-20	15^{b}	17^{a}	15^{a}
	21-25	25^{b}	15^{a}	11^{a}

CV=18%
$P \leq 0.05$
LSD 31

Means in the same soil texture treatment and in the same column that share a letter are not significantly different.

5.3.7 Effect of soil texture, time interval and soil depth on sweet potato weevils in *H. indica, S. karii* and *S. yirgalemense* treatments

The highest weevil populations were in the sandy clay loam soil for all treatments. Weevil infestations were also high in sandy clay and clay loam soils but significantly lower in the clay soils in *H. indica* treatments (Table 5.8). Mean number of weevils were significantly lower ($P<0.05$) in clay, sandy clay and clay loam soils than in sandy clay loam soils in *S. karii* treatments but not significantly different in all soils for *S. yirgalemense* treatments (Table 5.8).

Table 5.8: Mean number of sweet potato weevils surviving in soils treated with *H. indica, S. karii* and *S. yirgalemense*

Soil texture	*H. indica*	*S. karii*	*S. yirgalemense*
Clay	2.7^{b}	2.9^{b}	3.6^{a}
Sandy clay	4.6^{ab}	2.6^{b}	3.5^{a}
Clay loam	3.0^{ab}	2.5^{b}	3.7^{a}
Sandy clay loam	4.9^{a}	4.9^{a}	3.8^{a}
$P \leq 0.05$			
CV =38 %			
LSD 1.9			

Means in the same column sharing a letter are not significantly different ($P\leq0.05$)

Sweet potato weevil populations were low up to the eighth week but the build up was very fast after this for all the treatments. The effect of the interaction of soil texture and time interval on sweet potato weevil survival was however not significantly different (Table 5.9a).

Table 5.9a: Mean number of sweet potato weevils surviving in soils of different texture in nematode treatments at different time intervals over a twelve week period

Soil texture	Time (weeks)	*H. indica*	*S. karii*	*S. yirgalemense*
Clay	2	1	3	3
	4	1	1	2
	8	2	2	4
	12	7	6	7
Clay loam	2	2	2	3
	4	2	2	1
	8	7	1	2
	12	7	6	9
Sandy clay	2	1	2	2
	4	3	2	2
	8	2	1	3
	12	5	6	7
Sandy clay loam	2	2	3	3
	4	1	3	2
	8	4	5	1
	12	13	9	8
P ≤0.05	NS	NS	NS	NS

Means in the same column not significantly different (NS)

The effect of time interval (as a main factor) on weevil survival was significant. Weevil populations increased steadily for the first 8 weeks especially in the *H. indica* treatments but tripled in the last two weeks for all the treatments. Both the overall lowest and highest sweet potato weevil infestations were observed in the *H. indica* treatment, the former in the first two weeks and the later in the twelfth week period (Table 5.9b).

Table 5.9 b: Mean number of sweet potato weevils surviving in nematode treatments at different time intervals

Time in weeks	Nematode species		
	H. indica	*S. karii*	*S. yirgalemense*
2	1.5^{c}	2.1^{b}	2.7^{b}
4	1.9^{bc}	1.7^{b}	1.9^{b}
8	3.7^{b}	2.5^{b}	2.3^{b}
12	8.1^{a}	6.5^{a}	7.7^{a}

CV 38%
$P \leq 0.05$
LSD 1.8

Overall, the mean number of sweet potato weevils in the control treatments was highest in sandy clay loam soil with the top 0-6 cm and the lower 21-25 cm depths harbouring significantly higher weevil populations than the middle layers (7-13 and 14-20 cm). The mean number of sweet potato weevils surviving at all depths for the clay soil texture were not significantly different while significantly lower mean weevils survived in the 0-20 cm depth than the 21-25 cm depth in the sandy clay soil texture and the top layers (0- 6 cm) of the clay loam soil harboured lower mean number of weevils than lowest depth (21-25 cm) (Table 5.10).

Table 5.10: Mean number of sweet potato weevils surviving at four depths in untreated soils

	Soil texture			
Soil depth (cm)	Clay	Sandy clay	Clay loam	Sandy clay loam
0-6	8^{a}	8^{ab}	7^{b}	27^{a}
7-13	10^{a}	5^{b}	10^{ab}	12^{b}
14-20	6^{a}	8^{ab}	13^{ab}	15^{b}
21-25	6^{a}	13^{a}	14^{a}	25^{a}

CV=38%
$P \leq 0.05$
LSD 6.7

Means in the same column sharing a letter are not significantly different

5.4 DISCUSSION

The 40-80% *Galleria* mortality levels occurring in soils for both nematode species indicate that *Heterorhabditis indica* and *S. karii* can be used to control highly susceptible pests in all the kinds of soils tested but that *H. indica* is more virulent than *S. karii* in clay loam soils. *Heterorhabditis indica* was effective (49%) on *Galleria mellonella* (representing highly susceptible hosts) for 4 weeks but re-application was necessary to sustain the effects after this. *Steinernema karii* was less infective (5-14%) but the level was sustained for the entire test period (12 weeks). This suggests that *S. karii* is useful for augmenting other control methods where fewer spray applications are anticipated. The low effectiveness of *S. karii* compared to that of *H. indica* could probably be attributed to the fact that the soils were non-sterile. Such soils tend to have some microbes for instance fungi and mites which are mortality factors for some entomopathogenic nematodes especially the steinernematids which lose the second juvenile coat (Olearly *et al.*, 1998; Karagoz *et al.*, 2007).

Heterorhabditis indica was significantly more infective on *Galleria* (34-50%) at top depths (0-5 and 6-10) than the lower depths (6-13%) while the depth effects were insignificant for *S. karii*. Koppenhofer et al. (1995) found lower infectivity at the surface but his study had a narrow depth range (0-10cm) and the hosts were placed on the soil surface other than in the soil. Grewal et al. (2005) found higher mortality of *Anomala oreintalis* (the Oriental beetle) at the top than below in both *S. glaseri* and *S. longicaudum* treatments. This shows that even if a host is highly susceptible to entomopathogenic nematodes, the level of virulence in terms of infectivity will be determined by its niche along the depth and the nematode species.

The effect of soil texture was well demonstrated in *H. indica* treatments where nematode infectivity was significantly higher in the sandy clay and clay soils than in clay loam and sandy clay loam soils when

Galleria was used for assessment. Overall, *Steinernema karii* infectivity to *G. mellonella* was very low compared to the highest levels obtained in *H. indica* and did not differ significantly among soil of different texture. The behaviour of *H. indica* agrees with Shapiro et al. (2000) who found virulence highest in heavy soils (clay) but the finding from this study differ in that *H. indica* was also virulent in the lighter soil (sandy clay). The higher infectivity of *H. indica* in lighter soils is in agreement with earlier findings (Kung *et al.*, 1990; Molyneux and Bedding, 1983 ; Duncan *et al.*, 2003). The results obtained are therefore dependent on the nematode species, hosts and methods used in the study. Shapiro et al. (2000) had used the root weevil (*Diaprepes abbreviatus)* and the nematodes *Steinernema riobrave and Heterorhabditis bacteriophora.* This study used *Galleria mellonella* and the nematodes *Steinernema karii, Heterorhabditis indica* and *Steinernema yirgalemense* which are Kenyan strains while Kung et al.(1990) used *Steinernema glaseri* and *Steinernema carpocapsae* and *Galleria mellonella* as the host. This emphasises that soil texture effects are specific to nematode species, the host and the kind of nematode function tested (survival, mortality and infectivity).

This study established that the silt fraction other than aeration on its own lower nematode infectivity. Nematode infectivity to *Galleria* decreased as the percentage of silt in soils increased. Infectivity of nematodes to *Galleria* was highest in sandy clay followed by clay and sandy clay loam and lowest in clay loam (sandy clay soil had 12% silt, clay 12%, sandy clay loam 14% and clay loam 32%). Infectivity also decreased with decreasing sand content. This suggests that sand enhances nematode infectivity in *H. indica* while silt hinders the process. Koppenhoffer and Fuzy (2006) found that soil texture had no influence on infectivity of *H. bacteriophora* and *S. glaseri* but that lower infectivity of *Steinernema scarabaei* occurred in silty soils than the other soils that they tested further supporting the view that silt lowers infectivity and that effects are also influenced by nematode species.

Heterorhabditis indica and *S. yirgalemense* survived best in sandy clay soils while *S. karii* was best suited for survival in both the clay and sandy clay soils. Sandy based soils are more aerated and ideal for nematode survival but the clay soils may retain more moisture thereby enabling survival for some nematodes. This suggests that each nematode has its pattern for behaviour in soils, but survival of all nematodes was low in sandy clay loam soil. The relationship between nematode survival and infectivity was such that higher infectivity occurred where more nematodes survived corresponding to the dose response reported by Fan and Hominick (1991). *Steinernema karii* also exhibited peak infectivity over time for each soil texture. *Steinernema karii* probably hibernated in lower depths waiting for hosts and favourable conditions as some nematodes are known to have quiescence in unfavourable conditions (Koppenhoffer and Fuzy, 2006). *Heterorhabditis indica* on the other hand had higher levels of infectivity at the beginning which decreased with time. The different peaks infectivity exhibited by *S. karii* might have made the overall time effects on soils insignificant. Nematode survival declined gradually over the depths for all nematodes in the clay soil and in clay loam soil for *H. indica*, while the nematodes were uniformly distributed in the sandy clay soil. *Steinernema karii* was distributed uniformly in all soils except the clay soil texture. While it is expected that nematodes populations would decrease with the depth given that nematodes are obligate aerobes and soil has lower oxygen potential at lower depths, this only applied in the case of clay soils for each of the nematodes *H. indica*, *S. karii* and *S. yirgalemense*. *Heterorhabditis indica* tended to behave in the expected (decline with depths) in most soils than the *Steinernema* species. This could suggest that heterorhabditids are more sensitive to reduced oxygen than steinernematids. This is a likely cause of this behaviour because in some earlier part of this study, heterorhabditids were more responsive to higher percent carbon in soils than *Steinernema* species.

Significantly higher weevil populations survived in sandy clay loam than in other soil texture classes in the control treatments. Higher numbers of weevils also survived in the sandy clay soils in both *H. indica* and *S. karii* treatments. It was noted that it was in the same soil texture (sandy clay loam) that the nematodes were most effective in reducing weevil populations (75% sweet potato weevil control). In contrast, *Galleria* mortality was highest in clay loam and infectivity in the sandy clay soil. Overall, nematodes were effective on weevils in all soils up to 8 weeks. These results suggest that generalities about soil texture effects could be misleading because the nematode species, target host and nematode life function being assessed interacted to influence the results. This is in agreement with other findings (Gaugler *et al.*, 1994; Koppenhoffer and Fuzy, 2006). Results obtained from this study showed that each test nematode behaved as a cruiser as they all moved from the point of application into the tubers and attacked the weevils. *Heterorhabditis indica* is a known cruiser (Koppenhoffer *et al.*, 1995) but validation studies should be done in future for *S. karii* and *S. yirgalemense*. The mean number of dead weevils recovered per treatment probably represent only a fraction of total dead weevils because those that may have died in the earlier part of the two weeks interval may have rotted away and could not be recovered from dissection. The adults could also have emerged and moved into the soil phase where the nematode population was higher. Scouting the soils for weevils was not carried out in this study. Water was only applied to soils at the beginning of the experiment (at 10% w/v) suggesting that moistening the soils could have improved the results. Irrigation has been recommended before or after nematode application (Siegel *et al.*, 2004).

Sweet potato weevils reproduced the highest number of nematode juveniles in sandy clay loam soils at all soil depths, moderate levels in sandy clay and clay loam and the lowest in clay. If reproduction of juveniles is equated with infectivity then nematode infectivity to weevils was highest in sandy based

soils and lowest in clay based soil which is similar to the results obtained with *Galleria.* The study suggests that higher clay content depressed nematode activity on weevils while higher sand content enhanced entomopathogenic nematode activities which is in agreement with earlier findings (Choo *et al.*, 1989; Kung *et al.*, 1990; Duncan *et al.*, 2003).

CHAPTER 6

6 EFFECT OF SOIL pH ON THE VIRULENCE OF *STEINERNEMA KARII, HETERORHABDITIS INDICA* AND *STEINERNEMA YIRGALEMENSE* IN KENYA

6.1. INTRODUCTION

Entomopathogenic nematodes are affected by both biotic and abiotic factors among them soil pH. Soil pH varies from place to place depending on many factors including the rocks the soil is formed from, weather, soil erosion and soil amendments. The soil pH in most areas in Kenya vary from pH 3 to 8 with the most common being pH 5- 6 (76% of soils in Kenya have pH of soils of 5.5-6.5) (Kanyanjua *et al.*, 2002).

Some crops are sensitive to small changes in pH. Sweet potato is best suited for pH 5.6-6.6 (FAO, 2005) and the sweet potato weevil, which is the major pest of the potato breeds in vines, tubers and soil which are cryptic habitats (Chalfant *et al.*, 1990; Smit *et al.*, 1997). Such habitats are ideal for effectiveness of entomopathogenic nematodes (Georgis and Gaugler, 1991; Duncan *et al.*, 2003). Various soil characteristics including soil texture, structure and pH affect nematode function. Entomopathogenic nematodes are active between pH 4 and 8 but activity is low before and after this range (Kung *et al.*, 1990). Low pH reduces entomopathogenic nematode emergence during production (Jenssen *et al.*, 2000). Steinernematids are more suited to pH< 6 while heterorhabditids prefer pH >6 (Rosa *et al.*, 2000). The objective of this study was to determine the effects of varying the levels of soil pH on Kenyan strains of the entomopathogenic nematodes *Steinernema karii, Heterorhabditis indica* and *Steinernema yirgalemense* and their effectiveness on the sweet potato weevil *Cylas puncticollis*.

6.2 MATERIALS AND METHODS

6.2.1 pH standardization

The soils were collected from Gathuthi in Nyeri district with site characteristics as described in chapter three subsection 3.7.3. The soil pH was below 4. The soil was sterilized by heating at $150^{0}C$ for two hours to kill micro organisms including natural nematode populations and on cooling standardized to different levels of pH using agricultural lime (Kanyanjua *et al* .,2002). Agricultural lime was prepared by mixing Magnesium carbonate with Calcium carbonate at the ratio of 1:4. Two grams of lime were used per kilogram of soil to raise pH by one unit. The soil and lime were thoroughly mixed and wetted to field capacity, placed in black polythene bags and incubated in the laboratory for three weeks for the soil reactions to reach equilibrium. The pH of each sample was determined by the equal soil to water method (w/v) Hinga et al. (1980). Twenty grams (20 g) of each soil sample were mixed with 20ml of distilled water and contents stirred thoroughly. The contents were left standing for two hours and stirred again. A pH-meter was placed in the suspension for the pH reading.

6.2.2 Effect of soil pH on entomopathogenic nematode virulence

A completely randomised designed factorial experiment was carried out in the Entomology laboratory at KARI-Kabete. The test factors were soil pH, time interval and soil depth on the nematodes *S. karii* and *H. indica* using *G. mellonella*. *Steinernema yirgalemense* was included in the treatments in the second test using sweet potato weevil as the test host. Three hundred grams (300 g) of each of the pH standardized soil samples were drawn into 60 plastic tubes of diameter 6 cm x 28 cm depths. The soil columns were sealed at the bottom and placed in basins. Half of the soil tubes had one thousand (1000) infective nematodes juveniles of *Steinernema karii* applied per tube and the other half 1000ijs of *H. indica* per soil tube in 1 ml of distilled water. The rates were based on 35ij per cm^2. One pre weighed *G.*

mellonella larva was placed at each of the three depths (0-5 cm, 11-15 cm, and 21-25 cm) 3 days before the expiry of each sampling time (3, 7, 14, 30, 60 and 90 days). The *Galleria* larvae in net pouches were inserted at the respective depths through 0.5 cm apertures (plates 5.1 and 5.2). The apertures were sealed with sale tape after larvae placement (Plate 6.1). The contents were incubated at 25° C.

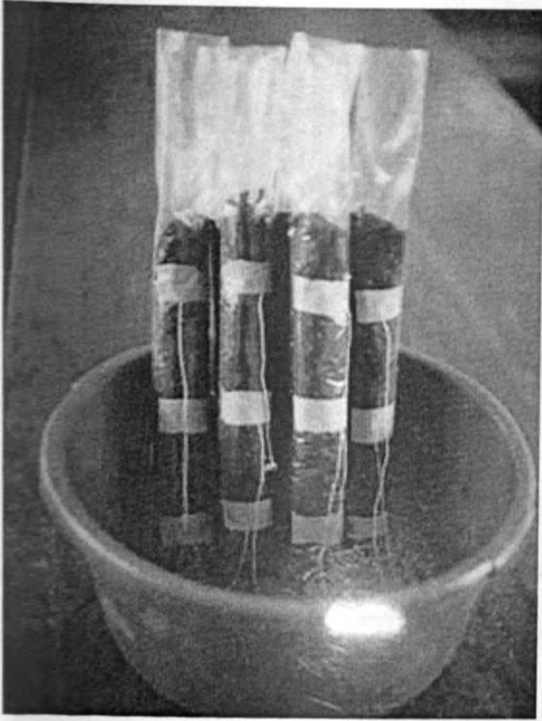

Plate 6.1 *Galleria mellonella* at three depths in soils of different pH

The soils used in the sweet potato weevil experiments were standardized into different levels of pH as in subsection 6.2.1 but placed in larger soil tubes (12cm diameter and 28cm depth). Infested sweet potato tubers (about 4 cm x 4 cm) were placed in the soil tubes at four depths (0-6 cm, 7-13 cm, 14-20 cm and 21-27 cm). The experimental design was completely randomised factorial design with three replicates. Six thousand infective juveniles (6000ij) of *H. indica, S. karii* and *S. yirgalemense* were applied in 10ml of water for each (soil texture class, soil depth and time interval) treatment. Weevil infested tubers were

incubated at the respective depths for 2 weeks when the tubers were removed and dissected to extract and take counts of both the dead and live weevils per instar. Forty eight tubes were scored per sample time which gave 192 observations per sample time (48 x 4 depths). The dead weevils were dissected under a binocular microscope and nematode juveniles per treatment counted.

6.3 RESULTS

6.3.1 Effects of soil pH on entomopathogenic nematode virulence to *Galleria*

Overall, nematode induced *Galleria* mortality increased as soil pH increased from 4 to 7.1 in both *S. karii* and *H. indica* treatments. The range was higher for *S. karii* than for *H. indica*. The increase was consistent for *S. karii* but not regular for *H. indica* (Table 6.1).

Table 6.1: Mean percent nematode induced *Galleria* mortality at five pH levels in soils treated with *H. indica and S. karii*

Soil pH	Nematode species	
	S. karii	*H. indica*
4.0	15.6^{c}	24.4^{ab}
4.5	28.9^{b}	31.1^{ab}
5.4	28.9^{b}	20^{b}
6.4	44.4^{a}	22.2^{b}
7.1	42.2^{a}	35.6^{a}

CV= 10%
$P \leq 0.05$
LSD 11.3

Means in the same column sharing a letter are not significantly different

6.3.2 Effect of time interval on nematode induced *Galleria* mortality in soils

Galleria mortality was generally highest in the first seven days at all levels of soil pH for both nematode treatments but the effect of the interaction of soil pH and time interval on nematode induced *Galleria* mortality was not significant (Table 6.2a).

Table 6.2a: Mean percent nematode induced *Galleria* mortality at different time periods in soils of different pH

Soil pH	Time (days)	*H. indica*	*S. karii*
4.0	3	56	33
	7	33	33
	14	0	0
	30	0	11
	60	0	11
	90	0	11
4.5	3	56	44
	7	33	33
	14	11	11
	30	11	0
	60	44	56
	90	0	22
5.4	3	0	22
	7	33	67
	14	44	22
	30	0	11
	60	22	22
	90	0	44
6.4	3	0	0
	7	44	67
	14	44	100
	30	11	11
	60	22	44
	90	0	11
7.1	3	33	0
	7	67	100
	14	78	11
	30	0	44
	60	22	22
	90	0	26
	$P \leq 0.05$	NS	NS

Means in the same columns are not significantly different (NS)

Galleria mortality occurred at all time intervals for both *S. karii* and *H. indica* nematode treatments but was highest on the 7th day in *S. karii* treatments and on both the 7th and 14th day in *H. indica* treatments. The effect of time on nematode induced *Galleria* mortality was significant (6.2b).

Table 6.2b: Mean percent *Galleria* mortality at different time periods in soils treated with *S. karii* and *H. indica*

Time in days	Nematode species	
	S. karii	*H. indica*
3	26.7^{b}	24^{b}
7	60^{a}	42.2^{a}
14	28.9^{b}	42.2^{a}
60	13.3^{c}	2^{c}
90	31.1^{b}	22.2^{b}
CV=10% $P \leq 0.05$ LSD 11.3		

Means in the same column sharing a letter are not significantly different

Galleria mortality was highest at the 0-5 cm depth at pH 4.0 for both *S. karii* and *H. indica* treatments and more uniform across the soil depths in soils of pH 4.5 to 7.1 for both nematodes. The effect of the interaction of soil pH and depth on nematode induced *Galleria* mortality was however not significant (Table 6.3a).

Table 6.3a: Mean percent *Galleria* mortality at three soil depths in soils of different pH treated with *S. karii* and *H. indica*

Soil pH	Soil depth	Nematodes	
		S. karii	*H. indica*
4	0-5	33.3	53
	11-15	0	0
	21-25	0	13.3
4.5	0-5	26.7	53
	11-15	40	27
	21-25	20	13
5.4	0-5	53	40
	11-15	13	0
	21-25	20	13
6.4	0-5	46.7	10
	11-15	46.7	5
	21-25	40	1
7.1	0-5	40	40
	11-15	20	33
	21-25	66.7	33
	$P \leq 0.05$	NS	NS

Means in the same column are not significantly different (NS)

6.3.3 Effect of soil depth on nematode induced *Galleria* mortality in soils

Effect of depth (as a main factor) was significant on nematode induced *Galleria* mortality. *Galleria* mortality was highest (≥40%) at 0-5cm depth in both nematode treatments and significantly lower (17-31%) at the middle and bottom depths for both nematode treatments (Table 6.3b).

Table 6.3b: Mean percent *Galleria* mortality at three soil depths in soils treated with *S. karii* and *H. indica*

Soil depth (cm)	Nematode	
	S. karii	*H. indica*
0-5	40^{a}	45^{a}
11-15	25^{b}	17^{b}
21-25	31^{b}	17^{b}

CV=10%
$P \leq 0.05$
LSD 7.1

Means in the same column sharing a letter are not significantly different

6.3.4 Effect of soil pH on nematode infectivity to *Galleria*

Galleria infectivity decreased from pH 4.0 to 5.4 and then increased from pH 6.4 to 7.1 in *S. karii* treatments. The mean infectivity at pH 7.1 was significantly higher than means at other levels of soil pH for *S. karii*. The pH effect was not significant in *H. indica* treatments (Table 6.4).

Table 6.4: Mean percent nematodes establishing in *Galleria* at five levels of soil pH

Soil pH	*S. karii*	*H. indica*
4.0	4^{bc}	3^{a}
4.5	3^{bc}	6^{a}
5.4	2^{c}	5^{a}
6.4	7^{b}	5^{a}
7.1	22^{a}	4^{a}

CV=10%
$P \leq 0.05$
LSD 4.4

Means in the same column sharing a letter are not significantly different

6.3.5 Effect of time on nematode infectivity to *Galleria*

Infectivity of *S. karii* to *Galleria* was lowest from pH 4 to 5.4 and highest from pH 6.4 to 7.1 for the first fourteen days while the levels of *H. indica* establishment were only higher in the first three days and very low after that for all levels of soil pH. The nematodes did not establish in *Galleria* beyond 30 days in any treatment. The interaction effects of soil pH and time interval on nematode infectivity to Galleria were not significant (Table 6.5a).

Table 6.5a: Mean percent *Galleria* infectivity to *S. karii* and *H. indica* at different time periods in soils of different pH

Soil pH	Time (day)	*S. karii*	*H. indica*
4.0	3	12	3
	7	9	11
	14	3	2
	30	2	0
	60	0	0
	90	0	0
4.5	3	7	24
	7	7	3
	14	4	4
	30	0	1
	60	0	0
	90	0	0
5.4	3	1	17
	7	4	3
	14	3	10
	30	4	8
	60	0	0
	90	0	0
6.4	3	8	14
	7	3	0
	14	28	13
	30	4	2
	60	0	0
	90	0	0
7.1	3	32	5
	7	6	11
	14	91	4
	30	O	1
	60	0	0
	90	0	0
	$P \leq 0.05$	NS	NS

Means in the same column are not significantly different (NS)

The effect of time (as a main factor) on nematode infectivity to *Galleria* was significant. The highest infectivity of *S. karii* was on the fourteenth day and that of *H. indica* on the third day. Both nematodes were not infective after thirty days (Table 6.5b).

Table: 6.5b: Mean percent *Galleria* infectivity to *S. karii* and *H. indica* at different time intervals

Time in days	*S. karii*	*H. indica*
3	12.0 [b]	12 [a]
7	6 [c]	6 [b]
14	29 [a]	5 [b]
30	2 [cd]	2 [bc]
60	0 [d]	0 [c]
90	0 [d]	0 [c]
CV 10.5%		
$P \leq 0.05$		
LSD 5.6		

Means in the same column sharing a letter are not significantly different

6.3.6 Effect of soil depth on nematode infectivity to *Galleria*

The infectivity of both *S. karii and H. indica* to *Galleria* was highest at the top 0-5 cm depth for each level of soil pH but the effect of the interaction of soil pH and depth on nematode infectivity was not significant (Table 6.6a).

Table 6.6a: **Mean percent of nematodes establishing in *Galleria* in soils of different pH at three soil depths**

Soil pH	Soil depth (cm)	Nematodes	
		S. karii	*H indica*
4.0	0-5	13	8
	11-15	0	0
	21-25	0	0
4.5	0-5	8	16
	11-15	1	0
	21-25	0	1
5.4	0-5	5	14
	11-15	0	1
	21-25	1	0
6.4	0-5	11	10
	11-15	9	5
	21-25	1	1
7.1	0-5	54	9
	11-15	4	1
	21-25	7	0
	$P \leq 0.05$	NS	NS

Means in the same column are not significantly different (NS)

The effect of soil depth (as a main factor) on *Galleria* infectivity was significant. The highest infectivity occurred at the top 0-5 cm layer (15%) compared to (2%) for both the middle (11-15 cm) and bottom (21-25cm) depths. *Heterorhaditis indica* was generally less infective than *S. karii* (Table 6.6b).

Table 6.6 b: Mean percent of nematodes establishing in *Galleria* at three soil depths

Soil depth (cm)	*S. karii*	*H. indica*
0-5	18^{a}	11^{a}
11-15	3^{b}	2^{b}
21-25	2^{b}	0^{b}
CV=10%		
$P \leq 0.05$		
LSD 5.6		

Means in the same column sharing a letter are not significantly different

6.3.7 Effect of soil pH on nematode survival

The highest mean number of *S. karii* survived at both the pH 4.0 (24.02%) and pH 6.4 (20.3%) while the highest mean number of *H. indica* infective juveniles survived at pH 6.4 (23.1%) only. The mean nematodes surviving at other levels at pH (4.5, 5.4 and 7.1) were not significantly different (Table 6.7).

Table 6.7: Mean percent of nematodes surviving at five levels of soil pH

Soil pH	*S. karii*	*H. indica*
4.0	24.0[a]	6.4[b]
4.5	11.3[b]	8.2[b]
5.4	10.8[b]	8.3[b]
6.4	20.3[a]	23.1[a]
7.1	6.2[b]	9.0[b]

CV=30%
$P \leq 0.05$
LSD 8.7

Means in the same column sharing a letter are not significantly different

6.3.8 Effect of soil depth on nematode survival

Both *S. karii* and *H. indica* survived best at the top 0-5 cm depth at all levels of soil pH. The different means for survival were however not significantly different for the soil pH and depth interaction (Table 6.8a).

Table 6.8 a: Mean percent of nematodes surviving in soils of different pH at three soil depths

Soil pH	Soil depth (cm)	Nematodes	
		S. karii	*H. indica*
4.0	0-5	63	16
	11-15	4	3
	21-25	4	3
4.5	0-5	30	22
	11-15	3	2
	21-25	1	1
5.4	0-5	32	20
	11-15	1	2
	21-25	0	1
6.4	0-5	50	64
	11-15	9	7
	21-25	2	1
7.1	0-5	16	21
	11-15	2	2
	21-25	0	0
	$P \leq 0.05$	NS	NS

Means in the same column are not significantly different (NS)

The effect of soil depth (as a main factor) on nematode survival was significant. *Steinernema karii* populations were significantly higher at the top and medium depth (0-5 and 11-15 cm) compared to those at the bottom depth (21-25 cm) while *H. indica* populations were highest at the top depth and lowest at the medium and lowest depths (11-15 and 21-25 cm). The two sets of means were significantly different for each nematode. Overall, the populations of *S. karii* were higher than those of *H. indica* at all soil depths (Table 6.8 b).

Table 6.8b: Mean percent of nematodes surviving at three soil depths

Soil depth(cm)	*S. karii*	*H. indica*
0-5	38.3 [a]	29.8 [a]
11-15	38.4 [a]	2.6 [b]
21-25	1.4 [b]	0.6 [b]

CV=30%
$P \leq 0.05$
LSD 5.6

Means in the same column sharing a letter are not significantly different

6.3.9 Effect of soil pH on nematode induced sweet potato weevil mortality

The mean numbers of dead sweet potato weevils were not significantly different for the different levels of soil pH for both *H. indica* and *S. karii* treatments *while the* sweet potato weevil mortality decreased significantly with increasing soil pH in *S. yirgalemense* treatments (Table 6.9).

Table 6.9: Mean number of dead sweet potato weevils at different levels of soil pH

Soil pH	*H. indica*	*S. karii*	*S. yirgalemense*
4.0	1.4 [a]	1.3 [a]	2.7 [a]
4.5	1.2 [a]	1.3 [a]	1.5 [b]
5.4	1.7 [a]	0.8 [a]	1.5 [b]
6.4	1.3 [a]	0.9 [a]	0.5 [b]

CV=30%
$P \leq 0.05$
LSD 1

Means in the same column sharing a letter are not significantly different

6.3.10 Effect of time interval on nematode induced sweet potato weevil mortality

The highest weevil mortality occurred in the first two weeks for all nematode treatments. *Heterorhabditis indica* and *S. karii* maintained a moderate level of weevil mortality for 4 weeks while *S.*

yirgalemense was effective for eight weeks. The lowest weevil mortality occurred in the twelfth week period for all treatments (Table 6.10).

Table 6.10: Mean number of dead sweet potato weevils at different time intervals over a twelve week period.

Time in weeks	*H. indica*	*S. karii*	*S. yirgalemense*
2	3.3^{a}	1.9^{a}	2.4^{a}
4	1.4^{b}	1.1^{ab}	1.1^{b}
8	0.3^{c}	0.8^{b}	1.4^{b}
12	0.5^{c}	0.5^{b}	0.7^{b}

CV=30%
$P \leq 0.05$
LSD 0.8

Means in the same column sharing a letter are not significantly different

6.3.11 Effect of soil pH on nematode induced sweet potato weevil larva mortality

Mean dead sweet potato weevil larvae were highest at pH 5.4 and 4.0 and lowest at pH 4.5 and 6.4 in *S. yirgalemense* treatments and highest at pH 4.5 and lowest at pH 5.4 in *S. karii* treatments and highest at pH 6.4 in *H. indica* treatments. The mean dead weevils were lower and not significantly different for other levels of pH in *H. indica* treatments (Table 6.11).

Table 6.11: Mean number of dead sweet potato weevil larva at different levels of soil pH

Soil pH	*H. indica*	*S. karii*	*S. yirgalemense*
4.0	0.2^{b}	0.1^{b}	0.3^{b}
4.5	0.2^{b}	0.3^{a}	0.1^{c}
5.4	0.2^{b}	0.0^{c}	0.4^{a}
6.4	0.3^{a}	0.1^{b}	0.1^{c}

CV=25%
$P \leq 0.05$
LSD 0.02

Means in the same column sharing a letter are not significantly different

The mortality of sweet potato weevil larvae was higher in the first four weeks compared to other time periods at all levels of soil pH for all nematode treatments but the effect of soil pH and time period interactions on sweet potato weevil larval mortality was not significant (Table 6.12a).

Table 6.12.a: Mean number of dead sweet potato weevil larvae in soils of different pH at different time intervals

Soil pH	Time (weeks)	*H. indica*	*S. karii*	*S. yirgalemense*
4.0	2	0.7	0.2	1.0
	4	0.1	0.1	0.3
	8	0.1	0.1	0.1
	12	0.1	0	0
4.5	2	0.3	0.5	0.5
	4	0.6	0.7	0
	8	0	0	0
	12	0	0	0
5.4	2	0.4	0	1.2
	4	0.3	0.1	0.3
	8	0	0.1	0
	12	0	0	0.2
6.4	2	0.5	0	0.3
	4	0.6	0.1	0.2
	8	0	0	0
	12	0	0.2	0
	$P \leq 0.05$	NS	NS	NS

Means in the same column are not significantly different (NS)

The highest larval mortality occurred in the first four weeks in all nematode treatments but none occurred beyond four weeks (Table 6.12b).

Table 6.12b: Mean number of dead sweet potato weevil larvae at different time intervals

Time in weeks	*H. indica*	*S. karii*	*S. yirgalemense*
2	0.5^a	0.2^a	0.7^a
4	0.4^a	0.2^a	0.2^b
8	0^b	0^b	0^c
12	0^b	0^b	0^c
CV=25%			
$P \leq 0.05$			
LSD 0.1			

Means in the same column sharing a letter are not significantly different

Sweet potato weevil pupa mortality mainly occurred in the first four weeks for all levels of soil pH. The mean mortalities were not significantly different for the interactions of soil pH and time period (Table 6.13a).

Table 6.13.a: Mean number of dead sweet potato weevil pupae in soils of different pH at different time intervals

Soil pH	Time (weeks)	*H. indica*	*S. karii*	*S. yirgalemense*
4.0	2	0.4	0.3	0.6
	4	0.2	0	0
	8	0	0.7	0.2
	12	0	0	0
4.5	2	0.3	0.7	0.1
	4	0.5	0	0
	8	0	0	0
	12	0	0	0
5.4	2	0.1	0.1	0.2
	4	0	0	0
	8	0	0	0
	12	0	0	0.1
6.4	2	0.2	0.1	0.1
	4	0	0	0
	8	0	0	0
	12	0	0	0
	$P \leq 0.05$	NS	NS	NS

Means in the same column are not significantly different (NS)

Sweet potato weevil pupa mortality occurred in the first four week period for *H. indica* and *S. karii* treatments and up to the eighth week for *S. yirgalemense* treatments (Table 6.13b).

Table 6.13b: Mean number of dead sweet potato weevil pupae at different time intervals

Time in weeks	*H. indica*	*S. karii*	*S. yirgalemense*
2	0.2 [a]	0.2 [a]	0.2 [a]
4	0.2 [a]	0.2 [a]	0.1 [ab]
8	0 [b]	0 [b]	0.1 [ab]
12	0 [b]	0 [b]	0 [b]
CV=28%			
$P \leq 0.05$			
LSD 0.1			

Means in the same column and sharing a letter are not significantly different

6.3.12 Effect of soil pH on nematode reproduction in sweet potato weevils

Heterorhabditis indica reproduced most infective juveniles in sweet potato weevils (l+p+a) at soil pH 6.4 and 4.5 and the least at 5.4 while *S. yirgalemense* reproduced the highest number of juveniles at pH 4 and the least at pH 4.5 and 6.4. *Steinernema karii* reproduced poorly at all the levels of pH. The differences between the mean juveniles reproduced in different treatments were not significant (Table 6.14).

Table 6.14: Mean number of nematode juveniles reproduced in sweet potato weevils (l+p+a) at different levels of soil pH

Soil pH	*H. indica*	*S. karii*	*S. yirgalemense*
4.0	13	4	54
4.5	20	4	5
5.4	4	4	29
6.4	24	3	0
$P \leq 0.05$	NS	NS	NS

Means in the same column are not significantly different(NS)

6.3.13 Effect of time interval on nematode reproduction in sweet potato weevil

Nematode juveniles were only reproduced in sweet potato weevils in the first two weeks in all treatments. *Steinernema yirgalemense* was the highest yielding nematode in sweet potato weevils while *H. indica* yielded moderately and *S. karii* the lowest number of juveniles (Table 6.15).

Table 6.15: Mean number of nematode juveniles reproduced in sweet potato (l+p+a) weevils at different time intervals

Time in weeks	*H. indica*	*S. karii*	*S. yirgalemense*
2	60^a	14^a	88^a
4	1^b	0^a	0^b
8	0^b	0^a	0^b
12	0^b	0^a	0^b
CV=52%			
$P \leq 0.05$			
LSD 35			

Means in the same column sharing a letter are not significantly different

The entomopathogenic nematodes mainly reproduced infective juveniles in the sweet potato weevil larvae in the first four weeks but the effects of the interactions of soil pH and time interval on nematodes reproduction were not significant (Table 6.16a).

Table 6.16a: Mean number of nematode juveniles reproduced in weevil larvae at different time intervals in soils of different pH

Soil pH	Time (weeks)	*H. indica*	*S. karii*	*S. yirgalemense*
4.0	2	23	5	118
	4	1	3	6
	8	0	2	1
	12	1	0	0
4.5	2	13	6	9
	4	28	31	0
	8	0	0	0
	12	0	0	0
5.4	2	6	0	107
	4	39	1	4
	8	0	2	0
	12	0	0	84
6.4	2	21	0	5
	4	42	5	4
	8	0	0	0
	12	0	1	0
	$P \leq 0.05$	NS	NS	NS

Means in the same column are not significantly different (NS)

The time effect on nematode reproduction in weevil larvae was not significant for both *H. indica* and *S. karii* but *S. Yirgalemense* reproduced significantly higher numbers of juveniles in the first two weeks than other time periods (Table 6.16 b).

Table 6.16b: Mean number of nematode juveniles reproduced in weevil larvae at different time intervals

Time in weeks	*H. indica*	*S. karii*	*S. yirgalemense*
2	16[a]	3[a]	60[a]
4	27[a]	10[a]	3[b]
8	0[a]	1[a]	0[b]
12	0[a]	0[a]	21[b]

CV 21%
$P \leq 0.05$
LSD 31

Means in the same column sharing a letter are not significantly different

Heterorhabditis indica yielded best in weevil pupa compared with *S. karii* and *S. yirgalemense* both of which yielded poorly over all the time intervals. Nematodes were not reproduced after the first 2 weeks (Table 6.17).

Table 6.17: Mean number of nematode juveniles reproduced in sweet potato weevil pupae at different time intervals

Time in weeks	*H. indica*	*S. karii*	*S. yirgalemense*
2	44[a]	6[a]	1[a]
4	0[b]	1[a]	1[a]
8	0[b]	0[a]	0[a]
12	0[b]	0[a]	0[a]

CV =42%
$P \leq 0.05$
LSD 10

Means in the same column sharing a letter are not significantly different

Steinernema karii yielded the highest number of nematode juveniles in adult weevils at four weeks while *H. indica* and *S. yirgalemense* yielded moderate numbers at both the two weeks and four week period (Table 6.18).

Table 6.18: Mean number of nematode juvenile reproduced in sweet potato weevil adults at different time intervals

Time in weeks	*H. indica*	*S. karii*	*S. yirgalemense*
2	19^{a}	4^{b}	19^{a}
4	10^{ab}	24^{a}	15^{a}
8	0^{b}	0^{b}	1^{b}
12	0^{b}	0^{b}	0^{b}

CV= 25%
$P \leq 0.05$
LSD 10

Means in the same column sharing a letter are not significantly different

6.4 DISCUSSION

Although entomopathogenic nematodes were functional at all levels of soil pH between 4 and 7, they were more suited to pH 6.4-7.1 when effects were measured with *Galleria* mortality. The peak mortality for *S. karii* was at pH 6.4 and that for *H. indica at* pH 7.1. *Steinernema karii* was more virulent at the lower soil pH than the heterorhabditid which is in agreement with Rosa et al. (2000) who classified pH 6 as borderline with heterorhabditids being more suited for pH above six and steinernematids below six. That view was not supported when nematode infectivity to *Galleria* and surviving nematodes in soils were used as the measure for virulence .*Steinernema karii* was most infective at pH 7.1 and nematode recoveries were highest at pH 6.4 for both nematodes while *H. indica* had very low infectivity of *Galleria* at all levels of soil pH which suggests that the results depend on the nematode species and the function assessed. *Galleria* mortality was highest for the first 14 days after nematode application with a sharp decline in the performance of both *H. indica* and *S. karii* at sixty days. *Heterorhabdtis indica* was more effective than *Steinenerma karii* at 14 days but *S. karii* maintained a moderate effectiveness over a

prolonged period probably explaining why steinernematids are more frequent in soils. The depth effects were significant (P≤0.05). The hosts at the top (0-5 cm) depth had double the chances of nematode induced mortality compared to those at 11-15 and 21-25 cm depths suggesting that nematodes go to lower depths in search of hosts but that host mortality below 10 cm is half the level of the mortality observed above the 10 cm depth. This supports the view that ecology of pests is also a determinant factor in effectiveness of entomopathogenic nematodes (Koppenhoffer *et al.,* 1995).

Infectivity was generally low and mainly occurred up to 14 days and not beyond 30 days for both nematodes even when the nematodes were still present in the soil. Nematodes lost ability to attack hosts beyond four weeks. This supports the view that the choice of the function the nematode decides to take (survival or infection) is a state dependent approach (Fenton and Rands, 2004). The nematodes probably depleted their food reserves thereby losing energy to seek hosts. The results on infectivity with depth show that nematodes are mainly suited for infection of hosts at the upper soil depth (0-5cm) and that even highly susceptible pest in lower soil depths will rarely reproduce entomopathogenic nematodes. This suggests that pests suited for lower depths in the soil will not contribute significantly to entompathogenic nematodes survival and recycling additionally supporting the view that pest ecology is an important factor in nematode survival and recycling. Infectivity was higher at pH 6.4-7.1 for *S. karii* but not significantly different for the different pH levels in *H .indica* treatments indicating that near neutral pH are more ideal for *S karii* and emphasising the fact that each nematode responds differently to soil pH for different life functions. The results on nematode recovery also suggest that each nematode has its optimum soil pH for survival. The highest nematode recovery was at pH 6.4 for both nematodes with a sharp drop in recoveries above this pH. The drop in percent recovery for both nematodes was drastic. *Steinernema karii* had two recovery peaks (pH 4.0 and 6.4) compared to *H. indica* which had

only one at pH 6.4. The differences between the highest and lowest means were significant ($P \leq 0.05$). These trends suggest that *H. indica* survives better at near neutral pH but that *S. karii* was suited at both the lower and upper pH level. The results also demonstrate that both entomopathogenic nematodes were not suited for survival beyond the neutral pH.

Sweet potato weevil mortality was highest at pH 5.4 in *H. indica* and at both the pH 4.0 and 4.5 in *S. karii* treatments although the means over the different levels of pH were not significantly different for the two nematodes. Sweet potato weevil mortality was highest at pH 4.0 and decreased significantly as pH increased in *S. yirgalemense* treatments. This showed that the two steinernematids were more virulent at lower pH and *H. indica* at a higher pH (though only weakly responsive to pH) when weevil mortality was used to assess pH effects partly agreeing with the pH 6 borderline theory of Rosa et al. (2000) but showing that host and nematode species have to be put into perspective. *Heterorhabditis indica* reproduced more juveniles in weevils at pH 6.4 than at pH 4.5 while *S. yirgalemense* was more productive at pH 4 and 5.4 but *S. karii* reproduced poorly at all pH levels. The results show that *H. indica* and *S. yirgalemense* respond to pH changes as far as reproduction is concerned but that *S. karii* was not responsive. The results demonstrate yet again that each nematode species has its ideal pH for each life function. The three nematodes caused the highest weevil mortality in the first four weeks and additionally up to the eighth week in *S. yirgalemense* treatments which suggest that the application intervals should be four weeks for *S. karii* and *H. indica* and up to eight weeks for *S. yirgalemense*. The depth effect was insignificant showing that the three nematodes searched out the weevils at all depths.

The study demonstrated that effectiveness of entomopathogenic nematodes on *Galleria* (a highly susceptible host) can be assessed accurately by either of the parameters; host mortality, infectivity or

nematode recoveries but not with a host like the sweet potato weevil because the measurements varied from one parameter to the other (weevil mortality and juvenile reproduction). This is probably because sweet potato weevil behaves like three different host insects with each instar having its ideal conditions for defence or succumbing to invading nematodes. The depth effects were insignificant in the sweet potato tests but highly significant in *Galleria* tests. Weevils probably produce stronger attractants than *Galleria*. Entomopathogenic nematodes are known to follow cues like carbon dioxide, faeces and other volatiles from hosts (Campbell and Kaya, 2000; Hazir *et al.*, 2003). This further stresses the view that the kind of pest, nematode type and the environment are important factors in the use of entomopathogenic nematodes (Koppenhofer *et al.*, 1995).

CHAPTER 7

7 EFFECT OF TEMPERATURE AND STORAGE MEDIUM ON THE VIRULENCE AND SURVIVAL OF *STEINERNEMA KARII*, *STEINERNEMA YIRGALEMENSE* AND *HETERORHABDITIS INDICA*

7.1 INTRODUCTION

Entomopathogenic nematodes survival, reproduction and retention of virulence in storage and after field application is greatly determined by medium of storage and temperature among other factors (Mari *et al.*, 2000 ; Hazir *et al.*, 2001; Mracek *et al.*,2005). A good carrier material enhances effectiveness of the product, is easy to handle and miscible with the pest control product. It must also be stored at the temperature which maintains it as effective as possible. Nematodes have been stored in sponge, vermiculite, alginate gels, wet table powder, water-dispersible granules (WG) and water suspensions (Ehlers *et al.*, 1998; Grewal and Peters, 2005). Nematode distribution and oxygenation is ensured by frequent agitation of the sprayer but spray quantities and depth must be checked to maintain bearable pressures during transit and application (Woodring and Kaya, 1988). Ash which is easily available has been used in small scale vegetable farms to control cut worms and in maize whorls to control stem borers (Waturu, 1998). The objective of this study was to determine the suitability of ash and moist sand as storage media for Kenyan strains of *Heterorhabditis indica*, *Steinernema karii* and *Steinernema yirgalemense* at different temperatures.

7.2 MATERIALS AND METHODS

Sand was obtained from KARI-Kabete and sterilized at 150^0C for 2 hours to kill micro organisms while ash was obtained by burning dry banana pseudo stems from the KARI Kabete tissue culture banana

plantation and water was distilled in the Entomology laboratory. The ash and sand were passed through 2 mm sieves.

Ash and sand were moistened to 10% (v/v). The moistened ash was put in half litre plastic containers to the 300 ml mark (equivalent to 100 g) in twenty seven containers. This was also done for moistened sand and distilled water. The contents were rearranged in three groups of twenty seven in such a manner that each group consisted of nine ash, nine sand and nine water treatments. Three thousand infective juveniles (3000ij) of *Heterorhabditis indica* were applied to three replicates of ash, three of sand and three of water and 3000ij of *S. karii* to another three replicates of ash, three of sand and three of water and finally 3000ij of *S yirgalemense* to the last three replicates of ash, sand and water. The nematodes were applied in 3 ml of water. The contents were thoroughly mixed to ensure uniformity in nematode distribution in the medium. A similar procedure was repeated for the second set of twenty seven containers and again for the third set of twenty seven. The first set of nine treatments (in three replicates) was incubated at 5°C, the other at 15^0C and the third at 25^0C (Table 7.1). The experiment was laid out in a completely randomised design.

Table 7.1: Table of treatments

Treatments	Medium	Nematode treatment	Temperature of incubation		
			5 °C	15 °C	25°C
1-3	Sand	*Heterorhabditis indica*	1	2	3
4-6		*Steinernema karii*	4	5	6
7-9		*Steinernema carpocapsae*	7	8	9
10-12	ash	*Heterorhabditis indica*	10	11	12
13-15		*Steinernema karii*	13	14	15
16-18		*Steinernema carpocapsae*	16	17	18
19-21	Water	*Heterorhabditis indica*	19	20	21
22-24		*Steinernema karii*	22	23	24
25-27		*Steinernema yirgalemense*	25	26	27

Three *Galleria mellonella* larvae were placed in each treatment three days before the expiry of each incubation time at the 3rd, 7th, 14th, 30th, 60th and 90th day. The water treatment was sampled up to the 30th day only. The larvae from the sand and ash treatments were retrieved at sampling time when mortality records and infectivity determinations were made. The number of dead larvae was recorded and cadavers dissected in 1% Ringers solution for the counts of nematodes penetrated per larva. The counts were done under a binocular microscope as a measure of infectivity. Thirty millilitres (30ml) of ash and sand were drawn per treatment at each sample time and the modified Hopper (1990) method used to extract the nematodes from each medium (Plate 5.3) as described in chapter five subsection 5.2.4. The nematodes counts were done under a binocular microscope as a measure of surviving nematodes.

7.3 RESULTS

Galleria mortality in the 5°C treatments was caused by freezing and not by nematode infection. *Galleria* larvae also remained alive in al ash treatments at 15 and 25°C. Nematodes surviving were recovered from ash treatments in moderate numbers through the modified method of Hopper (1990). Overall, mean nematodes from ash were less than those recovered from sand in all treatments although the means were not significantly different (Table 7.2 a).

Table 7.2a: Mean number of nematodes surviving in ash and sand at different temperatures

Medium	Temperature	*H. indica*	*S. karii*	*S. yirgalemense*
Ash	5	3.6	0.7	0.4
	15	5.5	3.2	3.4
	25	4.7	5.8	7.0
Sand	5	6.7	2.4	1.7
	15	10.8	17.7	9.3
	25	8.2	9.4	11.6
	$P \leq 0.05$	NS	NS	NS

NS denotes that means in the same column are not significantly different

More *H. indica* was recovered at 5^0 C than *S. karii* and *S. yirgalemense* while most of *S. karii* was recovered at 15^0C and the highest numbers of *S. yirgalemense* at 25^0 C (Table 7.2).

Table 7.2b: Mean number of nematodes surviving at different temperatures

Temperature ^{0}C	*H. indica*	*S. karii*	*S. yirgalemense*
5	5.2^{b}	1.6^{c}	1.0^{c}
15	8.1^{a}	10.4^{a}	6.4^{b}
25	6.4^{ab}	7.6^{b}	9.3^{a}
CV=28%			
$P \leq 0.05$			
LSD 1.8			

Means in the same column sharing a letter are not significantly different

Nematodes recovered decreased with time with the greatest rate of decline occurring at 7 and 60 days for all nematodes (Table 7.3).

Table 7.3: Mean number of nematodes surviving at different time intervals

Time in days	Nematode species		
	H. indica	*S. karii*	*S. yirgalemense*
3	24.0^{a}	22.3^{a}	13.6^{a}
7	6^{b}	5.1^{b}	8.3^{b}
14	6.0^{b}	4.9^{b}	3.7^{c}
30	1.8^{bc}	5.1^{b}	6.4^{bc}
60	0.3^{c}	0.6^{c}	1.7^{d}
90	1.3^{c}	1.3^{bc}	0.4^{d}

CV 28%
$P \leq 0.05$
LSD 4.3

Means in the same column sharing a letter are not significantly different

All the nematodes species survived better in sand than in ash (Table 7.4).

Table 7.4: Mean number of nematodes surviving in ash and sand

Medium	*H. indica*	*S. karii*	*S. yirgalemense*
Ash	4.6^{b}	3.3^{b}	3.6^{b}
Sand	8.6^{a}	9.9^{a}	7.6^{a}

CV= 28%
$P \leq 0.05$
LSD 2

Means in the same column sharing a letter are not significantly different

Nematodes survival in water decreased with time. The rate of decrease was higher for *H. indica* and *S. karii* than for *S. yirgalemense* in the first seven days. The survival of *Steinernema yirgalemense*

remained at 73% for the three and seven day period compared to 82% and 60% of *S. karii* and 73% and 60% for *H. indica* over the same time period (Table 7.5).

Table 7.5: Mean number of nematodes surviving in water

Time in days	*H. indica*	*S. karii*	*S. yirgalemense*
3	218[a]	246[a]	219[a]
7	176[b]	175[b]	216[a]
14	77[c]	79[c]	75[b]
30 days	37[d]	12[d]	29[c]

CV=36%

$P \leq 0.05$

LSD 19

Means in the same column sharing a letter are not significantly different

7.4 DISCUSSION

Ash was detrimental to entomopathogenic nematode function. Nematodes lost their virulence and had their survival adversely affected in ash. Cutworms remained alive in ashes but could not attack Kale seedlings in an earlier study (Mwaniki *et al.*, 2000). This suggests that ash components inactivate both the pests and bio control agents. Ash was therefore incompatible with entomopathogenic nematodes as a carrier material. Water retained 40% of the nematodes overall in the one month test period while moist sand retained 33% of infective nematodes. Water was therefore the best medium for storage over the test period but where it was not appropriate, sand could be used for storage of nematodes. Overall, the effect of temperature of storage was significant on nematode virulence (infectivity and mortality of *Galleria*) and survival ($P \leq 0.05$). The $5^{0}C$ temperature was un-suitable for nematode storage with the most affected being *S. karii* and *S. yirgalemense.* Bornstein et al. (2005) reported that cold temperature treatment arrested nematode development. Different nematode species stored best at different temperatures. The

15^0C temperature was best suited for storage of both *H. indica* and *S. karii* but not for *S. yirgalemense*. Other studies found 10-15^{0}C optimal for nematode storage (Mari *et al.*, 2000; Elsooud *et al.*, 2001; Mracek *et al.*, 2005). This study found that each nematode species has an optimum temperature and medium for storage and these finding are in agreement with Hazir et al. (2001) who found that isolates from different geographical regions had different ideal temperatures for storage and reproduction. Growth and reproduction of *Heterorhabditis* species ceases below 15^{0}C and above 35^{0}C (Ryder and Griffin, 2002).

The rate of survival for the three nematodes (*H. indica, S. karii and S. yirgalemense)* declined rapidly at 7 days and 30 days ($P \leq 0.05$) suggesting that the nematodes should be cultured once a month and reapplied at the same interval in the field to maintain them in virulent form. Moist sand was best for *H. indica* storage at 5^{0}C and 25^{0}C while *S. karii* stored best at 15^0 C in moist sand and *S. yirgalemense* at 25^{0}C in water and moist sand. This emphasises that the ideal medium for storage depends on temperature and nematode species. Chen et al. (2003) also found some relationship between nematode species and temperatures between 10^{0}C and 20^{0}C.

CHAPTER 8

8 EFFICIENCY OF THE SILKWORM (*BOMBYX MORI*) IN THE REPRODUCTION OF *HETERORHABDITIS INDICA*, *STEINERNEMA KARII* AND *STEINERNEMA YIRGALEMENSE*

8.1 INTRODUCTION

Entomopathogenic nematodes will only be widely used as pest control products when they become available on demand by the different clients (commercial growers and small scale farmers). Small scale farmers will benefit from *in vivo* production of nematodes using cheap materials and ideally those from their farms while large scale commercial farms' nematode needs can be met by the capital investment mass propagation methods using fermentation chambers (Ehlers *et al.*, 1998). The later have been developed fairly well (Ehlers *et al.*, 1998; Shapiro *et al.*, 2000). The start up costs for *in vitro* methods is beyond the scope of small scale farmers. The hosts used in *vivo* methods must be susceptible, have high reproductive potential, not prone to become crop pests, reared easily and using cheap materials. *In vivo* nematode production is labour intensive and produces good quality nematodes (Shapiro *et al.*, 2000).

Diets used to raise nematode hosts have effects on juvenile yields with antibiotics like nipagin lowering host susceptibility by 50-250% (Kermarrec and Mauleon, 1989; Nunchanart *et al.*, 2002). *Galleria mellonella* is the conventional host for *in vivo* multiplication of entomopathogenic nematodes. Galleria occurs naturally in bee hives and is reared using artificial diets made of cereals, wax, yeast and glycerol. These dietary components are purchased from markets and are therefore an additional expense (Costa *et al.*, 2007). The silkworm is a Lepidopteran that is related to *Galleria* but that feeds on mulberry leaves and twigs (Goldsmith *et al.*, 2004). The mulberry tree has been domesticated for over four centuries in the orient and over three decades in Kenya for silk production (MOA&RD, 2003). Preliminary tests showed that the silk worm is highly susceptible to entomopathogenic nematodes and a potential host for

multiplication of entomopathogenic nematodes. The objective of this study was to determine the comparative efficiency of silkworm larval instars over the third larval instar of *Galleria* in the reproduction of *Heterorhabditis indica*, *Steinernema karii* and *Steinernema yirgalemense* at two infective dose levels per larva.

8.2 MATERIALS AND METHODS

Steinernema karii infective juveniles were applied on petri dishes (9 x 3.5) lined with filter paper at the rate of 100ij/larva per dish to fifty dishes and at 200ij/larva to another fifty dishes. The nematodes were applied in 1ml distilled water and given 30 minutes to distribute on the filter. The fifty petri dishes treated with 100ij of *S. karii* per dish were allocated to five treatments in ten replicates. The treatments were third instar *G. mellonella*, 5th, 4th, 3rd and 2nd stage silk worm larval instar. The same (insect hosts) treatments were similarly allocated to the 200 ij per dish treatment. The procedure was repeated with *H. indica* and finally with *S. yirgalemense*. The petri dishes and respective contents were sealed with Para film and incubated at room temperature (18-25°C) for three days. The experiment was laid out in a completely randomised designed. *Galleria* cadavers from each treatment were placed in own White traps (Woodring & Kaya, 1988) for extraction of emerging entomopathogenic nematodes. Nematodes were harvested on alternate days between the 9th day and the 15th day or until less than ten nematodes were produced per harvest whichever occurred earlier. The nematodes were cleaned by sedimentation and decantation. Ten 1 ml aliquots were drawn from the nematode suspensions and infective juvenile counts made under a binocular microscope. The concentration of the suspension was the mean number of nematodes from ten counts for each harvest per treatment. The total nematodes per treatment per sampling period were derived from multiplying the concentration per ml by the volume of the

suspension in millilitres. The overall total per nematode treatment was the sum of all nematodes harvested at all sampling times.

8.3 RESULTS

Galleria and silkworm mortality occurred between 24 and 72 hours in all treatments. Infected silkworm larvae were floppy and retained their cream colour while *Galleria* infected with *S. karii* and *S. yirgalemense* maintained the pale colour and those infected with *H. indica* the brick red colour characteristic of heterorhabditid infected *Galleria* (Woodring and Kaya, 1988). Younger larval instars died faster than older ones in all treatments. The lower dose of *Steinernema karii* and *S. yirgalemense* tended to kill larvae of all ages faster than the higher dose while the rate of larval mortality in *H. indica* treatments was higher for the 200ij/larva dose for the 4th and 5th silk worm instars and *Galleria* (Table 8.1). The silk worm treatments were harvested thrice but it took more than five harvest times for *Galleria* cadavers to be depleted.

Table 8.1: Mean percent insect host mortality twenty four hours after nematode application

Insect host	*S. karii*		*H. indica*		*S. yirgalemense*	
	100ij/larva	200ij/larva	100ij/larva	200ij/larva	100ij/larva	200ij/larva
Galleria mellonella	0^{b}	0^{a}	10^{b}	100^{a}	100^{a}	0^{b}
Silk worm stage 5	0^{b}	0^{a}	90^{a}	80^{a}	10^{b}	0^{b}
Silkworm Stage 4	20^{b}	0^{a}	0^{b}	90^{a}	10^{b}	0^{b}
Silkworm stage 3	100^{a}	0^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Silkworm stage 2	100^{a}	0^{a}	100^{a}	100^{a}	100^{a}	100^{a}
CV=5.1%						
$P \leq 0.05$						
LSD	20		52		20	

Means in the same column sharing a letter are not significantly different

Steinernema yirgalemense did not reproduce juveniles in the second silkworm larval instar while only ten to twenty percent of the replicates of *S. karii* and *H. indica* treatments reproduced in this larval instar for both the 100 and 200 ij per larva doses. Except for the second silkworm larval instar, all the replicates of *H. indica* treatments were productive (Table 8.2).

Table 8.2: Mean percent replicates reproducing entomopathogenic nematodes

Nematode	Insect instar	Percent productive replicates	
		100ij/larva	200ij/larva
Steinernema yirgalemense	3rd instar *Galleria* larva	90^{a}	60^{a}
	Silkworm stage 2	0^{c}	0^{b}
	Silkworm stage 3	70^{ab}	10^{b}
	Silkworm stage 4	80^{a}	80^{a}
	Silkworm stage 5	50^{b}	70^{a}
Steinernema karii	Galleria stage 3	60^{b}	60^{b}
	Silk worm stage 2	20^{c}	10^{c}
	Silkworm stage 3	80^{ab}	80^{ab}
	Silkworm stage 4	90^{a}	70^{b}
	Silkworm stage 5	90^{a}	100^{a}
Heterorhabditis indica	Galleria stage 3	100^{a}	100^{a}
	Silkworm stage 2	20^{b}	10^{b}
	Silkworm Stage 3	100^{a}	100^{a}
	Silkworm stage 4	100^{a}	100^{a}
	Silkworm stage 5	100^{a}	100^{a}

CV= 15%
$P \leq 0.05$
LSD 24

Means (per nematode treatment) in the same columns that share a letter are not significantly different

Steinernema yirgalemense was the highest yielding nematode in *G. mellonella* while *H. indica* yielded the highest number of infective juveniles in the 5th silkworm larval instar and *S. karii* reproduced moderate yields in the 4th and 5th silkworm instars and the 3rd *Galleria* instar which were not significantly different among themselves (Table 8.3).

Table 8.3: Mean number of nematode juvenile reproduced per insect host

Insect host	*S. yirgalemense*	*S. karii*	*H. indica*
Galleria mellonella	60,680 ^{a}	43,780^{a}	43,470^{b}
Bombyx mori 2nd instar	3^{b}	60^{b}	99^{c}
Bombyx mori 3rd instar	583^{b}	7170^{b}	6620^{c}
Bombyx mori 4th instar	4430^{b}	35,620^{a}	10,920^{c}
Bombyx mori 5th instar	8945^{b}	38,970^{a}	63,170 ^{a}

CV=30%

$P \leq 0.05$

LSD 13810

Means in the same columns sharing a letter are not significantly different

The nematode yields were higher at the 100ij/larva than the 200ij/larva with the exception of *S. yirgalemense* in the 5th silkworm larval instar where the yields per infective juvenile applied were higher in the 200ij dose per larva treatment. Overall, the mean nematode juvenile yields per host were not significantly different between the two doses (Table 8.4).

Table 8.4: Mean number of nematode juveniles reproduced per dose of applied nematodes

Nematode	Insect host	Dose(ij/larva	Yields (infective juveniles)	Yields/juvenile applied
Steinernema yirgalemense	*Galleria mellonella*	100	80380[a]	804
		200	40880[b]	204
	Silkworm stage 2	100	10[c]	0
		200	0[c]	0
	Silkworm stage 3	100	1100[c]	11
		200	20[c]	0
	Silkworm stage 4	100	2560[c]	26
		200	60[c]	0
	Silkworm stage 5	100	3160[c]	32
		200	14740[c]	74
Steinernema karii	*Galleria mellonella*	100	45580[a]	456
		200	41690[a]	208
	Silkworm stage 2	100	110[b]	1
		200	0[b]	0
	Silkworm stage 3	100	4400[b]	44
		200	9930[b]	45
	Silkworm stage 4	100	52950[a]	530
		200	18230[b]	91
	Silkworm stage 5	100	39400[a]	394
		200	38530[a]	193
Heterorhabditis indica	*Galleria mellonella*	100	50910[ab]	509
		200	40980[b]	205
	Silkworm stage 2	100	100[c]	1
		200	90[c]	1
	Silkworm stage 3	100	5160[c]	52
		200	8090[c]	41
	Silkworm stage 4	100	12420[c]	62
		200	9420[c]	47
	Silkworm stage 5	100	61760[a]	617
		200	63,000[a]	315

CV=30%
$P \leq 0.05$
LSD 18,400

Means (per nematode treatment) in the same column sharing a letter are not significantly different

8.4. DISCUSSION

The fifth silkworm larval instar compared very well with *Galleria* in reproducing entompathogenic nematodes juveniles. *Steinernema yirgalemense* yielded best in *Galleria* at both the lower and upper dose and *S. karii* moderately in *Galleria*, the fourth and fifth silkworm instar. *Heterorhabditis indica* yielded best in the fifth silkworm instar. *Galleria* and silk worm are Lepidopterans which share the silk producing gene (Goldsmith *et al.*, 2004). The good performance of the silkworm could probably be attributed to the high levels of amino acids in silkworm and body size (mean body weight of 5th silkworm larval instar was 1.6g compared to *Galleria* 0.2g (Zang *et al.*, 1990: Flanders *et al.*, 1996). The fifth silkworm instar was fatty and probably most of the fats were lipids. Diets rich in lipids increased juvenile yields in both *in vivo* and *in vitro* reproduction (Moeen *et al.*, 1998; Gil *et al.*, 2002). Nematodes with small juveniles were also more productive than large nematodes (Zervos, 1991). This may explain the higher yields of *H. indica* whose juveniles are small in size but *S. yirgalemense* is a large nematode and yet the highest yielding nematode species in *Galleria*. Incubation temperature during reproduction is important (Moeen *et al*, 1998). Earlier on while testing different media for nematode storage, *Steinernema karii* and *H. indica* survived better and were more infective at 15^{0}C while *S. yirgalemense* stored better at 25^{0}C. The laboratory temperatures during the study ranged between 21 and 25^{0}C which could be ideal for the *S. yirgalemense* and *Galleria* match but not for the silkworm-*S. yirgalemense* match.

The 5th larval instar takes 12 days to pupate which is enough time for farmers to make decisions on whether to use the larva for producing nematodes or cocoons (pupae) for silk production depending on demand. These results suggest that for optimum nematode yields, the nematodes should be screened for effectiveness on important pests and selected nematodes matched with appropriate hosts for propagation.

The establishment of the silkworm as an alternative host for entomopathogenic nematodes reproduction is an important finding because the silkworm is easily raised by farmers in most farming districts of Kenya (MOA&LD, 2003). The raising of the silkworm requires some quarantine measures which farmers have mastered over the years. The activity will be sustainable as it fits with farmer practice and nematode propagation is an easy task that farmers can take up. The insect host to be selected will be determined by the cost and nematode species to be reproduced.

CHAPTER 9

9 THE EFFECT OF *HETERORHABDITIS INDICA*, *STEINERNEMA KARII*, *STEINERNEMA CARPOCAPSAE* AND *STEINERNEMA YIRGALEMENSE* ON THE SWEET POTATO WEEVIL *CYLAS PUNCTICOLLIS*

9.1 INTRODUCTION

Sweet potato is an important food crop in Kenya whose potential for maximum yields is limited by many factors including sweet potato weevil (Ngunjiri *et al.*, 1993; Smit *et al.*, 1997). The weevil is the major insect pest of sweet potato. The main methods for sweet potato weevil management include cultural control and chemical insecticides (Allard *et al.*, 1991). Insecticides are the most effective methods but they cause environmental pollution and are expensive and not always available in affordable quantities and are hazardous to operators. The other methods include quarantine, cultural control, use of botanical extracts and biological control. Entomopathogenic nematodes are biological control agents with potential to manage weevils. The weevil species, *Cylas formicarius* is known to be susceptible to entomopathogenic nematodes (Mannion and Jansson, 1993). The objective of this study was to determine the effectiveness of Kenyan entomopathogenic nematodes in the management of the sweet potato weevil species *Cylas puncticollis* Boheman.

9.2 MATERIALS AND METHODS

The trial was carried out at the Kenya Agricultural Research Centre Kabete with characteristics as described in chapter three subsections 3.7.1. The study was laboratory, greenhouse and field based. It was carried out between 2003 and 2006. The materials used in this study; sweet potato vine varieties, sweet potato weevils, nematode cultures and *Galleria* for raising the nematodes as well as soils were obtained as described in chapter three subsections 3.1-3.3 and 3.6.

9.2.1 Laboratory bioassay

Adult weevils and larva were placed singly in petri dishes (twenty replicates per instar) lined with filter paper and treated with 50 ijs (infective nematode juveniles) in 1ml distilled water. The nematodes tested in this study were *S. karii, H. indica* and *S. yirgalemense*. The test organisms were incubated at 18-25^{0}C for 2 days after which observations of weevil mortality were made. The experiments were arranged in a completely randomised design.

9.2.2 Greenhouse experiment.

Two experiments were carried out in the greenhouse. The first one was designed to test for weevil movement from lower depths to growing vines on the surface and the second the nematode movement from top depths (point of application) to lower depths and their effectiveness on sweet potato weevils at different soil depths. Both experiments were laid out in a completely randomised design. Soils from the KARI-Kabete experimental field were heated to 70^{0}C and maintained at this temperature for 2 hours to kill natural nematode populations. On cooling, the soils were evenly moistened with distilled water at 10% v/w. The soils were then put into 30 cm x 28 cm polythene paper bags and three Kemb10 vines planted in each soil bag. The plants were watered regularly until they established. In the first experiment, single weevil infested sweet potato tubers were placed at 0-5 cm, 6-10 cm, 11-15 cm and 16-20 cm depths. The treatments were replicated four times and incidence of weevil damage on vines made once a week for 12 weeks. Soils for the second experiment were prepared as before and placed in bags of 12cm x 28 cm. One infested sweet potato tuber was placed at each depth as follows; 0-6, 7-13, 14-20 and 21-25 cm. *Steinernema karii* infective juveniles were applied at the rate of 16,000 ij per soil bag for each treatment (16 soil bags; 4 depth treatments replicated 4 times) and similarly *H. indica* and

lastly *S. yirgalemense*. The tubers were removed after one month and dissected to score weevil mortality and incidence of nematode reproduction in weevil cadavers.

9.2.3 The effect of *Steinernema karii* and *Heterorhabditis indica* on the management of sweet potato weevils

A randomized complete block designed experiment with three replicates was conducted at KARI-Kabete experimental field for three seasons. Sixteen rows of each vine variety (Kemb10, KSP20 and K004) were planted in 12x3 m plots per replicate in the first season. The replicates were spaced at 2 m and the rows at 1m while plant to plant spacing was 0.3 m. Each main plot (Variety) was subdivided into 4 plots with 4 rows of 10 hills each. Four treatments were assigned at random to each main plot per replicate. The treatments were *Steinenema karii* and *Heterorhaditis indica* at 5.0 million juveniles per plot applied in 2 litres water, bifenthrin at the rate of 1 ml/2 litres water per plot and control where no pest control product was applied. Spraying was done by Knapsack with frequent agitation to ensure continued uniformity of the nematode spray suspension. Treatments were applied once a month starting at tuber initiation while sampling was done twice a month one day before spraying and 3 days after. Number of insect pests and natural enemies and respective species were assessed from ten plants of the middle row per plot. Each plant was thoroughly inspected for all insects. Total number of insect pests per treatment and respective species were scored. Insect pests causing severe damage were sampled and treated with entomopathogenic nematodes (*H. indica, S. karii* and *S. yirgalemense*) at 50 ij per larva/ pupa/ adult or nymph and insect mortality assessed. Destructive sampling was done at harvest to measure the total tuber yields, percent vine and tuber damage caused by sweet potato weevil as described in Sutherland et al. (1996). Three tubers per plot were placed in rectangular plastic containers (9x 10x 6 cm) lined with white tissue and contents incubated at 27-30^{0}C for a month. Weevils emerging during incubation were

counted. Similarly, three 15 cm crowns per plot were incubated and emerging weevil counts made after a month. Results from the first season showed that Kemb10 was more susceptible to weevils than K004 and KSP20 and was therefore selected for later season tests. *Steinernema carpocapsae* (EX UK strain) was included in the list of treatments in the later seasons for comparative purposes. The later season experiments were laid out in a completely randomised block design, replicated three times and treatment application, insect pests and natural enemy assessments in the field and at harvest and post harvest carried out as described earlier in this subsection.

9.2.4 Effect of nematode infested *Galleria* cadavers on the sweet potato weevil and appropriate nematode application methods

A 48 x 20 m plot was marked out and KEMB 10 vines planted in parallel blocks of 5 m by 36 m laid out in a randomized block design with 3 replicates. The blocks were spaced at 2 m. Each block consisted of 12 plots of 3 x 5 m with three rows. Each row had 16 hills. The rows were spaced at 1 m and hills at 30 cm. Plots were separated by one untreated row. The treatments were; *H. indica*, *S. karii* and *S. carpocapsae* evaluated against the weevil either in water suspensions or infested cadavers at monthly and fortnightly intervals, bifenthrin applied at twice a month and control where no treatment was applied. Entomopathogenic nematodes in water suspensions were applied at 4.5 m ij per plot in 2 liters water and four *Galleria* cadavers infected at the rate of 100ij per larvae were applied (4 days after infection) at the base of each hill and rolled in soil to keep them firm. The cadavers were covered lightly with soil around the plant base. Water was applied at the rate of 2 liters per plot immediately after nematode application and sampling carried out from ten plants of the middle row of each plot. Sampling details were as described in subsection 9.2.3. The best treatments (*H. indica* cadaver once and twice, *H*

indica water suspension twice) were tested in the second season together with control and the bifenthrin. Treatments application and sampling were done as before.

9.3 RESULTS

9.3.1 Laboratory bioassay

Weevil larvae were susceptible to the three entomopathogenic nematodes. The larvae were more susceptible than adults. *Heterorhabditis indica* and *S. yirgalemense* caused higher weevil larval mortality than *S. karii* while *S. karii* was more effective on adults than *H. indica* and *S. karii* (Table 9.1).

Table 9.1: Percent sweet potato weevil mortality in bioassay tests.

Nematode species	% Larval mortality	% adult mortality
Heterorhabditis indica	76^{a}	12.5^{b}
Steinernema yirgalemense	71^{a}	12.5^{b}
Steinernema karii	48^{b}	25^{a}
CV= 4.6%		
$P \leq 0.05$		
LSD	6.5	3

Means in the same column sharing a letter are not significantly different

9.3.2 Effect of soil depth on weevils in the greenhouse

Weevils moved from infested tubers at 0-5 cm and 6-10 cm to the surface. Those from 0-5 cm and 6-10 cm depths induced 100% and 75% vine damage respectively but weevils placed at 11-15 and 16-20 cm did not induce damage on the growing vines. Nematodes applied at the surface were effective on weevils in tubers at all soil depths buried (0 to 25 cm) (Table 9.2). The three nematodes also reproduced in weevil cadavers within tubers at all depths.

Table 9.2: Mean number of sweet potato weevils at different soil depths

Soil depth (cm)	*H. indica*	*S. karii*	*S. yirgalemense*	Control
0-6	4	4	4	8[c]
7-13	4	3	5	11[b]
14-20	5	4	3	9[c]
21-25	3	2	3	20[a]
$P \leq 0.05$				
CV =8.3%				
LSD	NS	NS	NS	1.7

Means in columns with NS are not significantly different

9.3.3 Insect pests and natural enemy incidence in the sweet potato experiment.

Twenty six species of insect pests and eight natural enemies were sampled on the sweet potato experiment. Eight insect species were severe, ten moderate and eight insignificant while three species of natural enemies were abundant, four common and one rare (Table 9.3).

Table 9.3: Incidence of insect pests and natural enemies in the sweet potato field experiment at KARI-Kabete in 2003.

ORDER	FAMILY	GENUS	SPECIES	SEVERITY
Coleoptera	Curculionidae	*Cylas*	*Puncticollis*	Severe
Coleoptera	Curculionidae	*Alcidodes*	*Dentipes*	Common
Coleoptera	Curculionidae	*Alcidodes*	*Erroneous orientalis*	Severe
Coleoptera	Tenebrionidae	*Gonocephalum*	*Simplex*	Rare
Coleoptera	Cassididae	*Aspidomorpha*	*Tecta*	Common
Coleoptera	Cassididae	*Aspidomorpha*	*Tigrma*	Severe
Coleoptera	Cassididae	*Aspidomorpha*	*Concinna*	Common
Coleoptera	Meloidae	*Coryna*	*Apicicornis*	Common
Coleoptera	Curculionidae	*Sphrigodes*	*Subdenudatus*	Severe
Coleoptera	Curculionidae	*Systates*	*Pollinosus*	Common
Coleoptera	Curculionidae	*Conchyloctenia*	*Pavummaculata*	Common
Coleoptera	Coccinellidae	*Alesia ,muls*	*Aurora*	Common*
Coleoptera	Coccinellidae	*Chellomenes*	*Lunata*	Common*
Coleoptera	Curculionidae	*Blosyrus*	*Obliquatus*	Common
Coleoptera	Curculionidae	*Brachycerus*	*Vugulosus*	Severe
Coleoptera	Anthicidae	*Formicomus*	*Tubercucifer*	Numerous*
Coleoptera	Halticidae	*Phyllotreta*	*Mashonana*	Rare
Coleoptera	Coccinellidae	*Adalis Muls*	*Intermedia*	Numerous*
Coleoptera	Tenthredinidae	*Gonocephalum*	*Simple*	Severe
Hemiptera	Coreidae	*Cletus*	*Fuscescens*	Common
Hemiptera	Cydnidae	*Cydnus*	*Sp notni*	Severe
Hemiptera	Lygaeidae	*Aulacopeltus*	*Armatipes*	Severe
Hemiptera	Coreidae	*Cletus*	*Ochvaceus*	Severe
Hemiptera	Coreidae	*Stictopleurus*	*Scutellaris*	Common
Hemiptera	Tingidae	*Urentius*	*Nanus*	Common
Lepidoptera	Sphngidae	Protoparce	*Convolvuli*	Rare
Lepidotera	Agrotidae	*Agrotis*	*Iisilon*	Rare
Dictyoptera	Mantidae	*Sphodromantis*	*Spp*	Rare*
Dictyoptera	Trombidiinae	*Trombidium*	*Spp-*	Common*
Hymenoptera	Formicidae	*Technomymex*	*Albines*	Numerous*
Hymenoptera	Braconidae	*Disophrys*	*Iridipennis*	Common*
Orthoptera	Gryllidae	*Liograyluss*	*Bimaculatus*	Rare
Orthoptera	Agridiidae	*Acrotylus*	*Variegates*	Rare
Isoptera	Termitidae	*Odontotermes*	*Spp*	Rare

Key

Scores	Infestation levels
0<1	Rare
1- 3	common
>3-5	Moderate
>5-7	Severe/abundant

* Natural enemies

The natural enemies belonged to the Hymenoptera and Coleoptera orders. Some of the severe insect pests were *Aspidomorpha* spp (Tortoise beetle), *Cletus* spp (sucking bugs) and the *Systase* spp (Dusty brown beetle) while infestations were moderate for *Blosyrus spp* (Rough sweet potato weevil) and *Alcidodes* spp (striped and spotted sweet potato weevil) infestations were moderate. Adult *Aspidomorpha* spp mortality was low in bioassay tests with entomopathogenic nematodes (25 and 30% for *H. indica* and *S. karii* respectively) and nymphal mortality moderate (60 and 69% for *H. indica* and *S. karii* respectively) at 3 days. Mortality of both instars was 100% after five days exposure. *Alcidodes spp, Aspidomorpha spp, Blosyrus spp,* and flea beetles were fewer in nematode and chemical treatments than in the control even though most of the treatment means were not significantly different (Table 9.4).

Table 9.4: Mean number of insect pests per treatment in the first season, 2003

Treatments	Sucking bugs	*Alcidodes*	Systates weevil	Flea beetles	*Blosyrus spp*	*Aspidomorpha spp*
Control	3^{a}	3^{a}	6	1	3	8^{a}
S. karii	2^{ab}	1^{b}	4	0	3	4^{ab}
H. indica	2^{ab}	0^{b}	2	0	1	6^{a}
Bifenthrin	1^{b}	1^{b}	4	0	1	1^{b}
CV=67%						
$P \leq 0.05$						
LSD	1.3	1	NS	NS	NS	4.1

Means in a column and sharing a letter not significantly different and (NS) denotes that means in respective columns are not significantly different

9.3.4. Susceptibility of vine varieties to sweet potato weevils

Mean field sweet potato weevil infestations, percent tuber damage at harvest and mean weevils emerging from tubers in the store were lowest in K004 treatments, moderate in KSP20 and highest in KEMB10 (Tables 9.5, 9.6 and 9.7). Mean weevils emerging from infested tubers were highest from the control treatments and overall lowest in K004. Nematode and bifenthrin treatments were generally effective in lowering weevil development and subsequent emergence from tubers in store (Table 9.7).

Table 9.5: Mean number of sweet potato weevils in the field in the first season, 2003

	Sweet potato varieties		
Treatments	K10	K004	KSP20
Control	18^{a}	6^{a}	13^{a}
S. karii	14^{a}	3^{a}	12^{a}
H. indica	15^{a}	2^{a}	9^{ab}
Bifenthrin	5^{b}	2^{a}	3^{b}

CV=43%
$P \leq 0.05$
LSD 6.8

Means in the same column sharing a letter are not significantly different

Table 9.6: Mean percent tuber damage in the first season, 2003

Treatments	Sweet potato varieties		
	KEMB10	K004	KSP20
Control	94^{a}	64^{a}	89^{a}
S. karii	93^{a}	70^{a}	69^{a}
H. indica	73^{a}	49^{a}	92^{a}
Bifenthrin	41^{b}	6^{b}	14^{b}

CV=15%
$P \leq 0.05$
LSD 31

Means in the same column sharing a letter are not significantly different

Table 9.7: Mean number of sweet potato weevils emerging from tubers in the store in the first season, 2003

	Sweet potato varieties		
Treatments	K10	K004	KSP20
Control	16[a]	5[a]	6[a]
S. karii	3[b]	0[a]	1[a]
H. indica	4[b]	0[a]	2[a]
Bifenthrin	5[b]	0[a]	5[a]
CV=15%			
$P \leq 0.05$			
LSD 6.1			

Means in the same column sharing a letter are not significantly different

9.3.5 Effect of *S. karii* and *H. indica* on the management of the sweet potato weevils in the second season, 2004

All treatments *(Steinernema carpocapsae, H. indica, S. karii and* bifenthrin*)* were significantly better than the control treatments in suppressing sweet potato weevil infestations in the field, percent weevil damage on tubers and weevil development in tubers in the store in the second season trial. *Heterorhabditis indica* was consistently better than *S. karii* on all parameters in both seasons (Tables 9.8 and Table 9.9).

Table 9.8: Mean number of sweet potato weevils and mean percent weevil damage on tubers and vines in the second season, 2004

Treatments	% vine damage	% tuber damage	Field weevil infestations	Tuber yields	Mean weevils emerging from Tubers	Mean weevils emerging from vines
S. karii	43	40[a]	3[bc]	3	1[b]	1[b]
S. carpocapsae	18	13[b]	2[c]	3	1[b]	1[b]
H. indica	27	20[b]	2[c]	6	1[b]	0[b]
Bifenthrin	11	10[b]	4[b]	3	2[b]	5[b]
Control	43	41[a]	9[a]	3	18[a]	36[a]
CV =19.3% $P \leq 0.05$ LSD	NS	17.5	1.6	NS	3.1	18.3

Means in the same column sharing a letter are not significantly different and NS denotes column of means that are not significantly different

Treatment means were only significantly better than control on percent tuber damage at harvest in the 3rd season, 2005 (Table 9.9).

Table 9.9: Mean number of sweet potato weevils and mean percent weevil damage on tubers and vines in the third season, 2005.

Treatments	% vine damage	% tuber damage	Mean tuber yields	Mean number of weevils emerging from Tubers	Mean number of weevils emerging from Vines
Steinernema karii	23	7[b]	3.7	1	4
Steinernema carpocapsae	23	0[b]	3.5	0	2
Control	40	20[a]	2.4	2	8
Heterorhabditis indica	23	7[b]	5	1	3
Bifenthrin	13	0[b]	4.7	0	0
CV = 29% $P \leq 0.05$ LSD	NS	12.5	NS	NS	NS

Means within a column are not significantly different if they share a letter and NS denotes that means in that column are not significantly different

9.3.6 Effectiveness of nematode application methods on sweet potato weevil

Vine damage was highest in the control and lowest in the bifenthrin treatment. All the treatments were significantly better than the control. *Steinernema carpocapsae* infested *Galleria* cadavers were as effective in reducing tuber damage as bifenthrin. All the other treatments were also significantly more effective in reducing weevil tuber damage and weevil development and subsequent emergence from tubers in the store compared to the control treatments. Overall, *Heterorhabditis indica* was more effective than *S. karii* (Table 9.10) and was therefore tested in the second season.

Table 9.10: Mean number of sweet potato weevils and mean percent % weevil damage on tubers and vines for different nematode application methods in the first season, 2005

Treatment	Mean Vine damage	Mean tuber damage	Mean tuber yields	Mean weevils emerging from	
				Tubers	Vines
H. indica cadaver once	33^{de}	3^{fg}	2.5	0^{d}	0
H. indica water once	40^{bc}	7^{e}	3.5	3^{c}	1
S. karii cadaver once	23^{g}	5^{ef}	2.2	1^{d}	5
S. karii water once	37^{cd}	30^{b}	2.8	1^{d}	2
Bifenthrin	13^{h}	0^{g}	2.8	5^{b}	1
Control	53^{a}	47^{a}	1.9	9^{a}	6
H. indica cadaver twice	43^{b}	8^{e}	3.6	5^{b}	4
H. indica water twice	37^{cd}	13^{d}	2.1	0^{d}	0
S. karii cadaver twice	43^{b}	3^{fg}	3.5	1^{d}	2
S. karii water twice	30^{ef}	12^{d}	3.5	0^{d}	2
S. carpocapsae cadaver once	37^{cd}	0^{g}	2.8	4^{bc}	0
S. carpocapsae water once	27^{fg}	17^{c}	1.9	1^{d}	0
CV	17%	17%	NS	43%	NS
$P \leq 0.05$	-	-		-	
LSD	5.9	3.6		1.8	

Means within a column are not significantly different if they share a letter and NS denotes that means in the column are not significantly different

Field sweet potato weevil infestations were very low in the second season trial (2006) of the different nematode application methods (means ranged from zero to one weevil per treatment). Means of percent tuber damage were also not significantly different among the treatments (the mean percent damage 3-5% per treatment) while percent vine damage ranged from 3 to 6% for the nematodes and bifenthrin treatments compared to 11% for the control treatments. The treatment means were not significantly different ($P \leq 0.05$). The mean weevils emerging from tubers from nematode and bifenthrin treatments were one weevil per treatment compared to four weevils from the control treatment. The means were not significantly different ($P \leq 0.05$) (Table 9.11).

Table 9.11: Mean number of sweet potato weevils and mean percent weevil damage on tubers and Vines for different nematode application methods in the second season, 2006

Treatments	Mean number of weevils in the field	Mean percent tuber damage	Mean percent vine damage	Mean number of weevils emerging from tubers
H. indica cadaver twice	0	5	3	1
H. indica cadaver once	0	5	4	1
H. indica water twice	0	3	3	1
H. indica water once	1	5	6	2
Control	1	5	11	4
Bifenthrin	1	5	4	1
$P \leq 0.05$	NS	NS	NS	NS

NS denotes that means in respective columns are not significantly different

9.4 DISCUSSION

Sweet potato weevil larvae in the study were more susceptible to entompathogenic nematodes than the adults in agreement with earlier findings (Mannion and Jansson, 1993; Waturu, 1998). *Heterorhabditis indica* was more effective against sweet potato weevils than *S. karii* in all tests in the current study. Earlier studies found a *Heterorhabditis* species, HP88 more effective than *Steinernema* spp and chemical insecticides on *Cylas formicarius* (Mannion and Jansson, 1993; Ekanayake *et al.*, 2001; Kinoshita and Yamanaka, 1998). The good performance of the *Heterorhabditis* species (HP88) may be attributed to the fact that heterorhabditids survive better than stenernematids in non sterile soils (Timper and Kaya, 1989) but may however not be generalised to cover all heterorhabditids because *S. carpocapsae* was more effective than *H. indica* on *Cylas puncticollis* in the current study. This suggests that nematodes need to be matched with target pests and appropriate environments for optimum effects. The study also showed that burying weevil infested tubers below 10 cm was effective in disrupting weevil activity at the surface thereby reducing early season weevil infestation on vines. The study

however demonstrated that weevils continued to breed below 10 cm and that future deep digging would bring weevil generations to the surface predisposing new sweet potato vines to attack. Entomopathogenic nematodes moved from the surface through the soil depths in search of weevils, effectively lowering weevil populations at all depths between 0 and 25 cm. This may have happened because entomopathogenic nematodes survived and were functional at lower soil depths (Koppenhofer *et al.*, 1995; Gouge *et al.*, 2000). This suggests that infested potato debris buried below 10 cm together with appropriate entomopathogenic nematode application would effectively manage sweet potato weevils.

Significantly fewer sweet potato weevil infestations were observed in chemical treatments in the first season experiment compared to nematode and the control treatments. Bifenthrin and the nematode treatments reduced tuber damage at harvest and weevils emerging in the store. The first season experienced a severe drought. This suggests that entomopathogenic nematodes though poor performers on foliage pests especially in drought conditions, are effective in sheltered habitats e.g. in soils. The heavy foliar infestations could be reduced by the use of appropriate chemical pesticides such as bifenthrin and nematodes used to maintain the low infestations. This is practical in commercial farms where non synthetic insecticides such as bifenthrin and other effective and environmentally friendly pyrethroids can be economical. Use of pesticides and entomopathogenic nematodes in a sequential manner had synergistic effects on the control of chafer grubs (Kathryn and Eileen, 2005). Moisture is one of the main factors that affect nematode function and wetting the soil before and after nematode application is recommended to enhance nematode effectiveness (Gouge *et al.*, 2000; Grant and Villani, 2003; Siegel *et al.*, 2004).

The sweet potato varieties KEMB10, KSP20 and K004 are well distributed in potato farming districts of Kenya. KEMB 10 is selected for high dry matter and, Kakamega 004 for high Vitamin A and KSP 20 for high tuber yields. K004 was the most tolerant variety to sweet potato weevil attack in this study and had moderate yields while KSP20 gave the highest yields and had moderate weevil tolerance and KEMB 10 was the most susceptible. The varieties K004 and KSP20 have thin vines compared to KEMB 10. Thick sweet potato vines and high tuber dry matter are positively correlated to weevil infestation (Degras, 2003; Were *et al.*, 2003).

The experiment also demonstrated that in severe drought, weevil infestation levels can surpass the 73% reported by Smit et al. (1997). The soil temperature could have reduced the effectiveness of entomopathogenic nematodes as it was over 18°C during the season. Siegel et al. (2004) reported that temperatures above 18°C were unsuitable for entomopathogenic nematode function in soils. The potato tuber damage level was variety dependent and ranged from 64 to 94 % for the varieties K004 and Kemb 10 respectively. The use of entomopathogenic nematodes against the sweet potato weevil brought about additional benefit in terms of reducing some other sweet potato pests. It was notable that despite the severe drought and minimum nematode contact with foliar pests, the nematode effects on the foliar pests were evident.

Steinernema carpocapsae was more effective on sweet potato weevils during the growth period than *H. indica* and *S. karii*. *Steinernema carpocapsae* is an ambusher (sit and wait foraging strategy) which may prefer the plant litter habitat (Campbell and Gaugler (1993). Sweet potato vines were part of such a habitat during the growing season. *Steinernema carpocapsae* was also as effective as bifenthrin in reducing weevil damage on tubers but *H. indica* and *S. karii* were also significantly more effective than the control in the second and third seasons ($P<0.05$). This could have happened because the weather

was cool and moist during these later seasons. Cool and moist weather are ideal for nematode function. Weevil populations were moderate in the third season (mean 40%) in the control plots. This demonstrated that nematodes were effective when weevil infestations were moderate and therefore appropriate options for maintaining low weevil infestations. The results also showed that nematodes can be as effective as pesticides in ideal conditions taking into account the performance of *S carpocapsae.* This suggests that nematode effectiveness on hosts should not be generalised as different nematode species manifest different effects on different pests which is in agreement with Mannion and Jansson (1993).

The study showed that the benefits of entomopathogenic nematodes on sweet potato weevil are weather dependent and that their effects are enhanced by combining them with cultural practice and appropriate insecticides. Effective non synthetic insecticides are useful in lowering heavy weevil infestations. The cultural control practice of trash burying at soil depths >10 cm retains weevil populations at a safe distance from growing vines and predisposes the weevil to entomopathogenic nematode infection. Entomopathogenic nematodes should be applied in early mornings and late afternoons with additional water. Siegel et al. (2004) recommended 800-2500 litres per hectare.

All methods of nematode application were more effective than control when tuber damage was used to measure effects ($P \leq 0.05$). The different methods of nematode application were also effective in reducing weevil development and subsequent emergence from tubers ($P \leq 0.05$). The best treatment overall was the application of *H. indica* infested cadavers at the rate of once and twice a month and *H. indica* in water suspensions twice a month. Entomopathogenic nematodes were more effective in suppressing weevil development in tubers than in vines both in the field and in store. This may be due to the short nematode/weevil contact time in vines compared to the better contact achieved in the soil and

whose effects continued in store. This suggests that high vine damage in nematode treated plots may not correspond to equally high damage on tubers.

Nematodes in *Galleria* cadavers applied either once or twice a month were as effective as water suspensions and the chemical pesticide in suppressing weevil development in tubers. Nematodes were especially effective when weevil infestations were about 40%. The second season infestations were low and treatment means not significantly different probably because the crop grew in abundant water. Nematodes applied as water suspensions and cadavers nevertheless suppressed weevil infestations compared with the control. The differences between the treatment means for nematode infested cadavers and water suspensions were not significant. The results were similar to earlier studies where S*teinernema riobravis* in infested sweet potato weevil cadavers was effective in controlling *Cylas formicarius (*Denny *et al.,* 2005). The mean weevils from the different nematode application methods; fortnightly and monthly intervals for both the cadaver and water suspensions were not significantly different. This could be because the infective nematode juvenile (J3) takes about 10-14 days to be reproduced and then 14 days of peak effectiveness as observed from previous experiments leading to an overlap of J3 availability in the soils at the once and twice a month cadaver application. It was also found that weevil infestations were not severe where water supply was regular. The usefulness of host cadavers in sweet potato weevil control makes the nematode technology easier for adoption by small scale farmers.

CHAPTER 10

10 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

10.1 General discussion

This study focused on broadening the genetic base of entomopathogenic nematodes from Kenya, determination of the influence of abiotic factors on the performance of entomopathogenic nematodes as biological control agents on a Kenyan perspective, enhancing their availability as tools in crop protection and evaluating their effectiveness on sweet potato weevils.

The study surveyed the Central Rift Valley region of Kenya and reports for the first time the occurrence of *Steinernema weiseri, S. yirgalemense,* and *H. taysearae* in Kenya and *S. karii* and *H. bacteriophora* in this region. Studies to confirm the sixth nematode (a *Steinernema* species) phylogenetically related to the *Feltiae* group are on going.

Further the study demonstrated and validated the ubiquitous nature of entomopathogenic nematodes whereby in every kind of soil sampled, vegetation type, agro ecological zone, and altitude and farming system there were some positive sites for entomopathogenic nematodes (Hominick, 2002). Some habitats had a more frequent distribution of entomopathogenic nematodes than others. The study demonstrated that Steinernematids were more numerous than heterorhabditids and this in agreement with surveys from continental Europe (Hazir *et al.*, 2003; Mracek *et al.*, 2005).

The study also established that although entomopathogenic nematodes thrive and function at wide ranges of pH, they prefer a near neutral soil pH for optimum function. This implies that when nematodes are used on farms, their optimal function would be at near neutral soil pH depending on the function, nematode species and the host insect targeted. Extreme ends of soil pH had been reported as unsuitable

for entomopathogenic nematode survival (Kung *et al.*, 1990). Further, the study showed that heterorhabditids have preferences for higher organic carbon than steinernematids agreeing with other studies (Rosa *et al.*, 2000).

The study also demonstrated that soil texture had varying effects on different entomopathogenic nematodes with regard to host mortality, nematode infectivity, reproduction and nematode survival which responded differently for each nematode species (Kung *et al.*, 1990; Koppenhoffer and Fuzy, 2006). For instance, the Kenyan strain of *H. indica* was more tolerant to cold temperatures, *S. karii to* moderate temperature and *S. yirgalemense* to warmer temperature which further confirms that different nematode strains have different optimum temperature ranges for different functions (Klingen and Haukeland , 2006; Mari *et al* .,2000).

The study further demonstrated that the fourth and fifth silk worm larval instars are useful as alternative hosts for nematode reproduction and that the level of reproduction was dependent on matching appropriate nematodes with appropriate hosts.

The study also showed that thin sweet potato vine varieties which have tubers of low dry matter are more tolerant to the common weevil (*Cylas puncticollis*) than thick vine varieties with higher dry matter thus supporting previous views (Degras, 2003; Were *et al* ,.2003). The study further demonstrated that indigenous Kenyan strains of nematodes when used in combination with effective insecticides and appropriate cultural control methods can effectively manage the sweet potato weevil. Finally, the study demonstrated that entomopathogenic nematodes are as effective in controlling weevils when applied in infested cadavers as in water suspensions.

10.2 Conclusions

The key findings of the study were the first report *of S. weiseri, S .yirgalemense, H. taysearae* and a new steinernematid in Kenya and *H. bacteriophora* and *S. karii* in the Rift Valley region of Kenya. The study also identified specific habitats that are preferred by nematodes for survival and therefore recovery for instance farms; shores of lakes and dams, soils of 2-3% carbon and soils of pH 5-6. The other finding was that the silkworm can be used effectively for the multiplication of entomopathogenic nematodes and that Kenyan strains of entomopathogenic nematodes can be used in IPM for the management of the sweet potato weevil both in aqueous suspensions and in *Galleria* infested cadavers.

10.3 Recommendations from the study

- Nematode application intervals should be 7-30 days depending on the nematode species, the host, and the soil texture and pH.
- Regular conventional tillage should be carried out in crop fields where nematodes are applied. This will loosen the soil thereby increasing aeration for optimum nematode effectiveness against soil pests.
- The addition of manure to soils will raise soil carbon leading to increased soil aeration thereby enhancing nematode effectiveness where heterorhabditids are used for pest control. Manure application will also raise soil pH in acidic soils therefore improving nematode effectiveness especially for heterorhabditids.
- Nematodes can be stored in moist sand but only after matching the nematode with ideal temperature of storage. Nematode application should be accompanied by water application/irrigation to enhance their effectiveness in soils.
- Laboratory screening of entomopathogenic nematodes for effectiveness on major insect pests prior to field application will give the trends to expect in the field for most pests.

- An integrated pest management strategy should be designed combining entomopathogenic nematodes with other viable stategies for optimal results.

10.4 Recommendations for future work

- More surveys for entomopathogenic nematodes should be carried out to expand the part of Kenya covered and further broaden nematode genetic base from Kenya. There is a possibility of isolating more virulent species. More types of media should be tested for effectiveness as carriers of entomopathogenic nematodes at different temperatures.
- Infested host cadavers should be tested for effectiveness on other pests of economic importance as cadavers are easier to use than water suspensions for small scale farmers.
- Cost benefit analysis studies should be conducted for appropriate methods of producing entomopathogenic nematodes and the most optimal patented
- A centre for preservation of identified indigenous entomopathogenic nematode species should be established for their safe keeping and for ease of retrieval by stakeholders.

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12 APPENDICES

Appendix 1: Graded levels of soil acidity from 65 sites in Kenya

Degree of acidity	pH range
Extremely acidic	<4.5
Strongly acidic	4.5-5.0
Moderately acidic	5.0-6.0
Slightly acidic	6.0-6.5
Near neutral	6.5-7.0

Appendix 11: Distribution of FURP sites in various pH ranges

pH	No of sites	%
< 4.5	3	5
4.5-5.0	6	10
5.0-5.56	14	24
5.5-6.0	15	26
6.0-6.5	15	26
6.5-7.0	2	4
>7.0	3	5
Total	58	100

Source: (Kanyanjua *et al.*, 2002)