ENTOMOPATHOGENIC NEMATODES HETEROHARBDITIS INDICA AND STEINERNEMA KARII AS CONTROL AGENTS OF LEGUME POD BORER MARUCA VITRATA SYN TESTULALIS IN COWPEA

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156/12255/09

A thesis submitted in partial fulfillment and the requirement for the award of the degree of Masters of Science (Biotechnology) in the school of Pure and Applied Science of Kenyatta University

July 2012
DECLARATION

I declare that work presented in this thesis is my original work and has not been presented for a degree in any other University or any other award.

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Signature.......................... Date.........................

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Signature.......................... Date.........................
DEDICATION

I dedicate this thesis to my dear husband Peter Ndegwa, our children Ruth and Francis for their perseverance, patience, prayers, comfort and encouragement accorded during my studies. Thanks, you made me reach this far.
ACKNOWLEDGMENTS

First and foremost I would like to acknowledge God Almighty for providing all that I needed to achieve my dreams.

I greatly appreciate my supervisors Dr. Joseph Ngeranwa, Dr. Steven Runo and Dr. Charles Waturu for the guidance they provided as I undertook my project. Your valuable time, ideas, productive discussions, instructive guidance and unlimited support during the research work are highly appreciated. I would like to express my sincere gratitude to Dr. Charles Waturu, Centre Director, Kenya Agricultural Research Institute (KARI) Thika for allowing me to carry out this project in his laboratory. His on-bench supervision, advice, guidance, assistance and personal support helped me conduct this research and complete my studies. He also gave me freedom to perform my work and only steered me to the right direction when I digressed.

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AD</td>
<td>Ana Domino</td>
</tr>
<tr>
<td>Bt</td>
<td><em>Bacillus thuringiensis</em></td>
</tr>
<tr>
<td>DJ</td>
<td>Dauer Juveniles</td>
</tr>
<tr>
<td>EPNs</td>
<td>Entomopathogenic Nematodes</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organisation</td>
</tr>
<tr>
<td>GV</td>
<td>Granular Virus</td>
</tr>
<tr>
<td>ICIPE</td>
<td>International Centre of Insect Physiology and Ecology</td>
</tr>
<tr>
<td>IJs</td>
<td>Infective Juveniles</td>
</tr>
<tr>
<td>IPM</td>
<td>Integrated Pest Management</td>
</tr>
<tr>
<td>KARI</td>
<td>Kenya Agricultural Research Institute</td>
</tr>
<tr>
<td>NPV</td>
<td>Nucleopolyhedrosis Virus</td>
</tr>
<tr>
<td>Ph</td>
<td>Potential of Hydrogen</td>
</tr>
<tr>
<td>PIP</td>
<td>Plant Incorporated Protectant</td>
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ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the major legumes largely cultivated in tropical and subtropical regions of the world. In Kenya and other parts of the world, the social and economic importance of cowpea include livestock fodder, legume vegetable food crop, green manure, nitrogen fixation, cover crop, fibre extraction, medicinal uses, shade tolerant and drought tolerant among others. In Africa, cowpea production fluctuates mainly due pests and diseases with insect pests playing the greatest role. The legume pod borer *Maruca vitrata* is particularly deadly attacking both the vegetative and reproductive parts of cowpea causing yield losses of between 20-80%. Several control options for *M. vitrata* exist including crop rotation, pesticide treatment and biological agents. Of biological agents available, entomopathogenic nematodes *Heterohabditis indica* and *Steinernema karii* have been known to prey on insect larvae but their effectiveness as agents for biological control has not been elucidated on *Maruca*. This work aimed to determine pathogenicity of entomopathogenic nematodes (EPNs) *H. indica* and *S. karii* on *M. vitrata* under laboratory and screen house conditions and determine the net effect of treatment on cowpea infested with the legume pod borer. The experiments were laid in a complete randomized design, with five treatments; zero (control), 100, 200, 300 and 400 Dauer juveniles (DJs) per insect with three replicates. The DJs concentration of 400 with the highest mortality in the laboratory was subjected to screen house trial. In the screen house trials, five *Maruca* larvae were released on each potted cowpea plant. Data on number of dead larvae and damaged flowers was recorded on 24-hour interval from the start of both laboratory and screen house experiments: 24, 48, 72, 96 and 120 hours. Cadavers were dissected to confirm nematode infection and progeny yield. There was a negative correlation between increase in nematode concentration and the total number of DJs recovered. At 300 dose of *H. indica* and *S. karii*, *M. vitrata* cadavers yielded 24000 and 17000 DJs respectively while at 400 dose the yield was14400 and 15450 DJs respectively. In all the nematode concentrations tested in the laboratory no larvae survived beyond 120 hours with both *H. indica* and *S. karii*. However, *H. indica* higher dose of 400 nematodes per larvae achieved 100% mortality within 72 hours while at the same dose *S. karii* caused mortality of 66%. The difference between the control and treatments using the EPNs was significant p<0.05 in the laboratory. In the screen house trials the treatment was significant p<0.05, though the mean larval mortality was low in the screen house (1.733) than in the laboratory (3.600). The mean flower damage in *H. indica*, *S. karii* treated plants and control was 1.667, 2.067 and 3.200 respectively. Overall, it was found that EPNs *H. indica* and *S. karii* were pathogenic to *M. vitrata* larvae in both laboratory and screen house conditions. It is concluded that, *H. indica* and *S. karii* are potential biological control for the legume pod borer in cowpea and this provides a platform for development of eco-friendly biopesticide using EPNs as viable component of integrated pest management (IPM). More studies are recommended to elucidate the effect of >400dose of the EPNs, repeated application of EPNs *H. indica* and *S. karii* on *M. vitrata* in the semi field and determination of their pathogenicity on *M. vitrata* under field conditions are necessary.
CHAPTER ONE: INTRODUCTION

1.1 Background

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most ancient human food crops since Neolithic times (Summerfield *et al.*, 1974). Lack of archaeological evidence has resulted in contradicting views supporting Africa, Asia and South America as origin of cowpea (Summerfield *et al.*, 1974; Tindall, 1983; Coetzee, 1995). Cowpea was introduced from Africa to the Indian sub-continent approximately 2000 to 3500 years ago, the same time with sorghum and millet (Allen, 1983). Before 300 BC, cowpea had reached Europe and possibly North Africa from Asia.

In the seventeenth century AD, the Spanish took the crop to the West Indies. The slave trade from West Africa resulted in the crop reaching the southern USA early in the eighteenth century. Padulosi (1997) speculated that the Transvaal region of the Republic of South Africa was the centre of speciation of *V. unguiculata*, due to the presence of most primitive wild varieties. The species moved northwards from the Transvaal to Mozambique and Tanzania where the subspecies *pubescens* evolved.

Cowpea has a wide variety of uses including livestock fodder, pulse, green manure, nitrogen fixation (Kamiti and Odee, 2010), cover crop and as a vegetable for people in developing countries, especially in sub-Saharan Africa. According to FAO, about 7.56 million tonnes of cowpea are produced worldwide annually on about 12.76 million hectares. Sub-Saharan Africa accounts for about 70% of total world production.
Cowpea has high protein content (20-25%), is very palatable and relatively free of toxins (Kareema and Taiwo, 2007). There is low production of this grain estimated at an average of 180 kg per hectare. This has been due to insect pests and diseases with the former causing the greatest yield loss (Fatokun et al., 2002; Gethi, 1990). Among the major insect pests of cowpea are the legume pod borer (*Maruca vitrata* Fabricius. *syn. Maruca testulalis* Geyer), aphids (*Aphis craccivora* Koch), flower bud thrips (*Megalurothrips sjostedti* Trybom) and pod sucking bug (*Clavigralla tomentosicollis* Stal) (Karnataka, 2010). The legume pod borer is the most important insect pest causing yield losses of up to 85%. This pest is widely distributed in the tropics and sub tropics. In Kenya, *M. vitrata* causes yield losses of between 10-80% in cowpea (Okeyo-Owour and Ochieng, 1981). Thus to realize high yield from cowpea in pure stand there is need to control insect pests (Gethi, 1990; Malick Ba et al., 2009).

There are various insect pest control measures like intercropping, use of resistant varieties, biological methods and use of insecticides. Use of insecticides is the most effective method against *M. vitrata* but they are not widely used (Singh et al., 1978). Pest tolerant cowpea varieties have been bred which have given promising alternative control against pests like *M. vitrata* (Singh, 1979; IITA, 1981). The legume pod borer is widely distributed in the tropics and subtropics. In Kenya, this pest causes yield losses of between 10-80% in pure stand cowpea crop (Okeyo-Owour and Ochieng, 1981). There is therefore an urgent need to come up with biological pesticide to overcome limitations of conventional insecticides. The current study aimed to test the pathogenicity of two entomopathogenic nematodes (EPNs) *Steinernema karii* (Waturu et
and *Heterohabditis indica* (Pionar *et al.*, 1992) species on legume pod borer. It is envisioned that data obtained from this study will provide baseline information for the development of integrated pest management (IPM) strategies that will minimize chemical pesticide use and enhance protection of the environment against pollution.

### 1.2 Problem statement and justification

In Africa, cowpea is a major food crop that forms a valuable source of protein (Fatokun *et al.*, 2002; Ndema *et al.*, 2010). Green tender leaves and immature pods are used as vegetable, while the seeds are used as pulse (Koehler and Mehta, 1972). Cowpea production fluctuates greatly due to drought, low soil fertility, pH, diseases and insect pests (Asiwe, 2010). The legume pod borer causes yield losses of up to 85% (Gethi, 1990). The best control of *M. vitrata* has been obtained with insecticides that are not common to most small scale farmers (Jackai and Singh, 1983). Insecticides pollute the environment, persist in the soil, require skill to apply and are poisonous to non-target organisms. Farmers spray with mixtures of insecticides at higher dosage and frequencies to achieve effective control. Such practices eventually raise health, economic and environment concerns among the public at large. Insecticide resistance in *M. vitrata* has also been reported (Ekesi, 1999). The use of synthetic pesticides has already proved to be negative to the human health and environment. It creates pest control problem through resistance development and by killing natural enemies of pests. This calls for alternative IPM (Ekesi *et al.*, 1996). EPNS are parasites of insects used as biopesticides which are environmentally safe and acceptable (Smart, 1995).
Some EPNs among them *H. indica* and *S. karii* have been known to prey on insect larvae. The EPNs can be applied with conventional equipment and are compatible with many agro-chemicals (Smart, 1995; Aldamario *et al.*, 2010). These nematodes are able to locate their host either actively or passively in cryptic habitats and in the soil. They do not cause indiscriminate mortality to non-target insects thus are ideal for use as biological control agents (Downes and Griffin, 1996). There are no previous reports on use of EPNs, *H. indica* and *S. karii*, in control of any development stage of *M. vitrata* in Kenya. Thus this study was conducted to evaluate these EPNs pathogenicity against the third stage of *Maruca* larvae or otherwise susceptibility of *M. vitrata* to *H. indica* and *S. karii*. The study was also necessary because EPNs infectivity varies from insect to insect and that the first hand information on dose-requirement and subsequent progeny production capability is required for all future studies.

### 1.3 Research questions

i. Are the EPNs *H. indica* and *S. karii* pathogenic to *M. vitrata* under laboratory and semi-field conditions?

ii. What could be the net effect of EPN treatment on the cowpea plant infested with the legume pod borer?

### 1.4 Hypothesis

EPNs (*H. indica* and *S. karii*) are effective in the control of legume pod borers in cowpeas.
1.5 Objectives

1.5.1 Broad objective

To determine effectiveness of EPNs (*H. indica* and *S. karii*) in control of legume pod borer (*M. vitrata*) in cowpeas.

1.5.2 Specific objectives

i. To determine pathogenicity of EPNs *H. indica* and *S. karii* on *M. vitrata* under laboratory and screen house conditions.

ii. To determine the net effect of EPNs treatment on the cowpea plant infested with the legume pod borer.

1.6 Output and significance

This study provides the first documented report on pathogenicity of EPNs, *H. indica* and *S. karii* upon application on cowpea legume pod borer in the laboratory and screen house conditions. The pathogenicity results of the two EPNs could provide a platform for further research into the development of an eco-friendly biological pesticide. This could be an alternative strategy for controlling *M. vitrata*, a major pest in cowpea hence increased yield and food supply for the ever increasing world population.
CHAPTER TWO: LITERATURE REVIEW

2.1 The cowpea *Vigna unguiculata* (L.) Walp

Cowpea is commonly called southern pea, blackeye pea, or blackeye bean (United States), or niébé (French-speaking Africa) (James, 1999). In Kenya, it is known as Kunde (Swahili, Kipsigis), mathoroko (Kikuyu) (Annon, 2006). The plant has alternate compound leaves, cross pollinated and white to purple flowers that grow in two’s or three’s (Nkouannessi, 2005). Stems are smooth or slightly hairy sometimes tinged with purple; pods vary in colour and shape with 8-20 seeds per pod. The testa differs in texture and colour (hilum white surrounded by a dark ring) or mottled in colour (Davis *et al*., 1991; Nkouannessi, 2005).

Cowpea tolerates heat and dry conditions, but is intolerant of frost. Germination is rapid at temperatures above 18°C; colder temperatures slow germination (Davis *et al*., 1991). Cowpeas are grown under both irrigated and non-irrigated conditions. The crop responds positively to irrigation but also produce well under rain-fed conditions (Davis *et al*., 1991). Cowpea is more drought resistant than common bean making it such an important crop in many under developed parts of the world (Davis *et al*., 1991).

When irrigation is used, more vegetative growth and delay in maturity may result but the crop should not be overwatered as this suppresses growth by lowering soil temperatures (Davis *et al*., 1991). The most critical moisture requiring period is just prior to and during flowering. Cowpea performs well on a wide variety of soils and soil conditions, but performs best on well drained sandy loams or sandy soils where soil pH is in the range of 5.5 to 7.0 (Dugje *et al*., 2009).
2.1.2.1 Social-Economic importance of cowpea

Cowpea is a food and animal feed crop grown in the semi-arid tropics parts of the world and has the ability to produce nutritious leaves in only about 20 days (Adati et al., 2007). Cowpea has the ability to fix nitrogen through its root nodules (Kamiti and Odee, 2010) thus able to grow well in poor soils with more than 85% sand, less than 0.2% organic matter and low levels of phosphorus. The ability to infuse soils with nitrogen and its adaptability to different types of soil makes cowpea to be enormously valuable to Africa, where many farmers struggle with nutrient-poor soils (Adati et al., 2007; Kamiti, 2008). It is shade tolerant, and therefore, compatible as an intercrop with maize, millet, sorghum, sugarcane, and cotton.

The plant is a legume vegetable crop especially for small scale farmers in rural areas in Africa (Khadim and Mbacke, 2011). Cowpea seed is rich in proteins Moussa, et al. (2011), fats, fibre, and small amounts of vitamins thus a nutritious component in the human diet (Table 1.1) (Tindall, 1983; Davis et al., 1991). It is very palatable and relatively free of metabolites or other toxins (Quass, 1995). The dried pulse is used to make a thick soup, or ground into a meal or paste (Kay, 1979; Allen, 1983). Fresh immature pods, leaves and growing points are used as vegetables (Coetzee, 1995). Dried leaves are preserved and eaten as a meat substitute. It is also used as livestock fodder where it is used for grazing or cut and mixed with dry cereals for animal feed. It also grows, covers the ground quickly, preventing erosion and it is a green manure crop. The sale of the stems and leaves as animal feed during the dry season also provides a vital income for farmers (Adati et al., 2007).
In Nigeria special cultivars are grown for the fibre extraction from the peduncle after retting. The strong fibre is especially suitable for fishing gear, and produces a good-quality paper (Zia-ul-haq et al., 2010). The dry seeds have been used as coffee substitute. Various medicinal uses of cowpea have been reported; leaves and seeds are applied to treat swellings and skin infections, leaves are chewed to treat tooth ailments, powdered carbonized seeds are applied on insect stings. The roots are used as an antidote for snakebites and to treat epilepsy, chest pain, and constipation (Zia-ul-haq et al., 2010). The whole cowpea seed and its protein isolate are useful for remedying diet-induced hypercholesterolemia and steatosis (Frota et al., 2008). Other medicinal values are treatment of headaches, common cold, burns, epilepsy, blood in urine and seeds are diuretic (Zia-ul-haq et al., 2010).

**Table 2.1: Nutrient content of mature cowpea seed**

<table>
<thead>
<tr>
<th>Nutrient</th>
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<tbody>
<tr>
<td>Protein</td>
<td>24.8%</td>
</tr>
<tr>
<td>Fat</td>
<td>1.9%</td>
</tr>
<tr>
<td>Fiber</td>
<td>6.3%</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>63.6%</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.00074%</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.00042%</td>
</tr>
<tr>
<td>Niacin</td>
<td>0.00281%</td>
</tr>
</tbody>
</table>

(Davis et al., 1991)

**2.2 Constraints to cowpea production**

Cowpea production in Africa fluctuates greatly because of biotic and abiotic factors. Abiotic factors such as drought, low soil pH and low soil fertility are significant factors affecting production (Asiwe, 2010). Biotic factors include diseases caused by fungi, bacteria and viruses. Parasitic weeds like witch weed (*Striga gesnerioides*) and
Alectra vogelli choke the plants at all stages and nematodes prevent the roots from absorbing nutrients and water from the soil. Insect pests are among the major biotic factors causing serious damage to cowpea (Adati et al., 2007).

2.2.1 Cowpea pests

The cowpea plant is attacked by pests during every stage of its growth (Gethi, 1990). The contribution of cowpea to overall productivity of the cropping systems is reduced by a number of insect pest species (Adati et al., 2007). Damage by insect pests on cowpea can be as high as 80-100% if not effectively controlled. In the Coastal counties of Kenya, cowpea is severely attacked by several insect pests throughout its crop cycle (Kyamanywa, 1996). Some of the major and important insect pests include, aphids (Aphis craccivora), flower bud thrip (Megalurothrips sjostedji), leaf hoppers (Empoasca kerii), blister beetles (Mylabris spp.), bean fly (Ophiomyia phaseoli) and the legume pod borer (Dugje et al., 2009; Oso and Falade, 2010). Other cowpea pests are pod sucking bugs (Clavigralla totamentosicollis), cowpea storage bruchids (Callasobruchus maculatus) (Singh, 1979; Ekesi, 2000).

Cowpea aphids extract juice from the undersurface of young leaves, stem tissues of young cowpea seedlings and on young pods of mature plants. The aphids also spread the cowpea mosaic virus (Dugje et al., 2009). The flower thrips they feed on flower buds and flowers causing premature fall of flowers such that no pods are formed. Severely infested plants do not produce any flowers (Adati et al., 2007; Karnataka, 2010). The pod sucking bugs suck sap from green pods, causing them to shrivel and dry prematurely, resulting in seed loss. The blister beetles feed on cowpea flowers where
large number of beetles in a field may result in total crop loss (Dugje et al., 2009). The cowpea storage bruchid or weevil is a field–store pest. The adult beetles lay eggs on pods (field) or on the seeds (storage) and the larvae develop within seeds and eat up the cotyledon. The legume pod borer is the major pest of cowpea attacking vegetative and reproductive parts of cowpea (Okeyo-Owour and Agwaro, 1982; Dugje et al., 2009).

2.2.1.1 The biology of the legume pod borer (M. vitrata)

The legume pod borer is a major cowpea pest reported in Africa, Asia, South and North America Gethi, 1990; Egbo, 2010; Oso and Falade, 2010). It is commonly referred to as bean pod borer, legume pod borer mung moth and Maruca. It attacks 39 host plants of which 37 are leguminous which include cowpea (Vigna unguiculata), pigeon pea (Cajanus cajan), lima bean (Phaseolus lunatus), common bean (Phaseolus vulgaris) among others (Ayodele and Kumar, 2010).

The adult moth has light brown forewings with white patches, and white hind wings with an irregular brown border as shown in plate 2.1a (Dugje et al., 2009). The adults rest with their wings spread horizontally while on cowpea, they hide on the lower surface (Okeyo-Owour and Ochieng, 1981). Eggs are laid on the flower buds, flowers and the tender leaves of cowpea. The eggs are light yellow, translucent, and have faint reticulate sculpturing on the delicate cover, and measure 0.65 x 0.45 mm. In captivity, the moths lay eggs all over the host plant even on walls of cages (Singh and Jackai, 1985). The female moths lay eggs at night in batches of 10-100 eggs per female (Sharma, 1998a). The eggs hatch in 2-3 days and the emerging larvae undergo five larval instars in 8-14 days. Early instars are dull white, but the later instars are black-headed, with irregularly shaped
brown or black spots and short black hairs on the dorsal, lateral and ventral surfaces of each body segment. Mature larvae are 17-20 mm long with a head capsule that is light to dark brown in colour. The body is whitish to pale green or pale brown, with irregular brownish black spots; the spots become indistinct immediately before pupation (Plate 2.1). There is a pre-pupal period lasting 1-2 days. Pupae are 15 x 25 mm long, within a silken cocoon. The pupal stage lasts 5-15 days and the whole life cycle lasts about 18-25 days depending on climatic conditions (Okeyo-Owour and Ochieng, 1981).

Plate 2.1: *M. vitrata* adult moth (a) and larvae (b) (Adopted from: Goergen, 2010).

2.2.1.1.1 Effects of the legume pod borer on cowpea

Early generations attack peduncles and tender parts of the cowpea stems (Karnataka, 2009). The larvae cause webbing together of flowers, pods and leaves, with frass on the pods and shoots (Sharma, 1998a). After hatching, the young larvae (1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} instars) especially injure the terminal shoots and the flower buds whereas the older larvae (4\textsuperscript{th} and 5\textsuperscript{th} larval instars) particularly damage the open flowers and the pods (Plate 2.2). The larvae feed from inside a webbed mass of leaves, flowers, flower buds and pods (Liao and Lin, 2000).
Older larvae are highly mobile, feeding continuously on flowers and newly formed pods and causing severe damage throughout the reproductive cycle of the crop (Singh and Jackai, 1988). Larvae move from one flower to another, and each may consume 4-6 flowers before larval development is completed. The larvae are most active on the plant surface from 1800 to 0600 hr, with a peak period of activity from 2200 to 0600 hr. The extent of infestation is in the order: flowers > flower buds > terminal shoots > pods (Sharma, 1998a). The host plant undergoes serious loss following the damage inflicted by *M. vitrata* larvae infestation on the vegetative parts.

![Plate 2.2](image)

**Plate 2.2:** Effect of *M. vitrata* on cowpea; (a) Larvae damaging a flower and (b) Larvae inside a pod (Adopted from: Goergen, 2010).

### 2.2.1.1.2 Control of Legume pod borer

To control cowpea insects in a sustainable manner, pest management practices such as the use of resistant cowpea varieties, mixed cropping systems, botanical and reduced risk insecticides, and biological control assisted by pest monitoring has been developed. Nevertheless, no single component is effective when used alone but specific combinations can work better (Adati *et al.*, 2007).
2.2.1.1.2.1 Natural enemies of *M. vitrata*

Natural enemies help in lowering the legume pod borer population in the field. Studies conducted in Taiwan and Benin show that, parasitoid wasp (*Apanteles taragame*) causes 92% damage on *Maruca* eggs in the field (Huang *et al*., 2003; Danon, 2010). The entomopathogenic fungi, *Fusarium* species also attack *Maruca* larvae. Predatory insects like carpenter ants, *Componotus* species and some spiders feed on both larvae and adults stages of *Maruca* (Gethi, 1990; Downham, 2003).

2.2.1.1.2.2 Cultural method

In the tropics, intercropping has been an important component of small farm agriculture. The main importance for this type of cropping system is high and more dependable returns. The high increased productivity has mainly been attributed to reduction in crop pest damage, by the principle of increasing the species diversity of an agro ecosystem (Gethi, 1990). Inter planting host and non-host plants can drastically decrease pest colonization efficiency and subsequent population density. Intercropping host and non host crops interfere with normal feeding, movement and mating behaviour of a pest. The maize and cowpea intercrop has been found to lower the effect of *M. vitrata* in cowpea (Gethi, 1990). Intercropping cowpea and maize has shown that more *M. vitrata* larvae are found on the outer cowpea rows. This is due to restricted movement caused by non-host plant which acts as physical barrier to inter or intra row migration of pest.

Trap crops in an intercropping system act as diversionary host by protecting the more susceptible or economically valuable crop from pest damage (Gethi, 1990). Cowpea attack by the legume pod borer reduces when intercropped with crops like sorghum.
Intercropping studies carried at ICIPE in Kenya over 10 years have identified sorghum and cowpea as the best combination in terms of minimizing the population of *M. vitrata* (Ekesi et al., 1996). The incorporation of cowpea cultivars tolerant *M. vitrata* in an intercropping system is an added advantage and helps to maintain high crop yield. Timely planting of cowpea helps the crop escape damage from the legume pod borer (Ekesi et al., 1996).

2.2.1.2.3 Breeding for resistance

Conventional breeding accessions of cultivated cowpea and its wild relatives has been screened for resistance (Jackai and Oghiakhe, 1989). Accessions belonging to *Vigna vexillata* which is a close relative to cowpea according to phylogenetic study of diversity in the *Vigna* species have been found to be resistant to *M. vitrata* (Jackai and Oghiakhe, 1989). A cross between cowpea and *V. vexillata* is impossible due to gene incompatibility but through Genetic Engineering development of transgenic cowpea is possible (Fatokun, 2009). The *Bacillus thuringiensis* (*Bt*) protoxins (CryIAb) have been screened on *M. vitrata* and proved to be effective (Fatokun, 2009). The protoxins have been used to design *M. vitrata* resistant cowpea in Australia and Burkina Fasso in Africa (Fatokun, 2009). The *Bt* cowpea developed in Nigeria (Langyintuo and Lowenberg-DeBoer, 2006) is a good example. The first *Maruca*-resistant cowpea seed is expected to be available for farmers in 2017 (AAF, 2012). This date will depend on approvals from regulatory agencies, the identification of effective and stable *Maruca*-resistant lines and efficient transfer of the resistance genotype to traditional varieties through conventional breeding. The *M. vitrata* resistant cowpea also need biosafety approval before it can be grown in any country.
Farmers cannot just wait for *Bt* cowpea to be developed and go through strict approval from regulatory agencies as they lose their crop to *M. vitrata*. There is therefore need for innovations to control *M. vitrata*.

### 2.2.1.2.4 Use of synthetic insecticides

The best control method of this pest has been obtained with synthetic insecticides. Eight insecticides have been tested to evaluate their efficiency in controlling the legume pod borer with deltamethrin and carbofuran being more effective than others (Liao and Lin, 2000). The highest pod yield (average 4950.9 kg/ha) was recorded in the thiodicarb treatment (Liao and Lin, 2000). At Kabete, Kiboko and Mtwa in Kenya, the efficiency of endosulfan, dimethoate, pirimiphos-methyl, fluvalinate has been assessed and proved to be effective against *M. vitrata* (Kyamanywa, 1996; Minja *et al.*, 2000).

#### 2.2.1.2.4.1 Merits and demerits of synthetic insecticides

Synthetic insecticides are effective against *M. vitrata*, fast acting and have longer shelf life. The toxicity, pollution of the environment, health hazard to human being and livestock are the major demerits of synthetic insecticides. More so, resistance of *M. vitrata* larvae to synthetic insecticides has been reported (Ekesi, 1999). Pest resistance has led to use of larger and repeated doses, increasing cost of crop production. Synthetic pesticides are also harmful to non-target beneficial organisms and due to environmental pollution they lead to unbalanced ecosystem (Oruonye *et al.*, 2010). These chemicals threaten the sustainable development for they do not degrade easily, persist in the environment for a long period and disperse easily across wide geographical areas. Human health and environmental safety are the two important issues in the long term use of pesticides (Singh *et al.*, 2010).
Thus the urgency for alternative insect control measures is obvious.

2.2.1.1.2.5 Biological control

Biopesticides are pesticides derived from animals, plants, bacteria, and certain minerals. They include microbial pesticides containing microorganisms as the active ingredient. The most common microbial pesticides are *B. thuringiensis* (Nyasani *et al.*, 2007). Plant-Incorporated-Protectants (PIPs) are pesticidal substances that plants produce from genetic material that has been added to the plant.

2.2.1.1.2.6 Biochemical compounds

Biochemical compounds are substances that control pests by non-toxic mechanisms (Downham, 2003). Such substances include lectins which are natural products with striking activities. They are able to recognize and bind simple and complex sugars. They protect plants against insects, bacteria and other harmful organisms by natural defensive molecules. Laboratory tests have shown that lectins are effective against *M. vitrata* larvae causing 60% mortality (Machuka *et al.*, 2003).

The tobacco plant extract has been proved to be lethal against the legume pod borer in the field especially when applied on cowpea at podding stage (Opolot *et al.*, 2006). Nevertheless, Neem (*Azadirachta indica*) produces and stores chemical substances with different pesticidal characteristics: for instance, insecticidal, repellant, anti-feedant, bacteriocidal, fungicidal, nematocidal among others. Neem seed extracts at 20% concentration in six applications are lethal to legume pod borer (Oparaeeke, 2007).

In Kenya, the effect of volatiles and aqueous extracts of black pepper (*Piper guineens*), neem seed, garlic bulb (*Allium sativum*) and onion bulb (*Allium cepa*) on egg viability of *M. vitrata* has been evaluated in laboratory experiments. Compared with the other
treatments, volatiles of black pepper and garlic bulb were superior in reducing hatch of freshly laid (12 hour old) eggs of *M. vitrata*. The aqueous extracts of black pepper and garlic bulb provide the highest reduction in egg hatch at all concentrations (Ekesi, 2000).

Use of volatiles from cowpea flowers and *M. vitrata* larvae on host selection behaviour of the parasitoid wasp (*A. taragame*) has been studied under laboratory conditions (Danon *et al.*, 2010). The wasp is attracted to floral volatiles produced by cowpea initially infested with *Maruca* and from which the larvae had been removed. Flowers emit parasitoid attracting volatiles in response to being infested with a herbivore insect. Host searching is an important component in parasitoid biology which influences the success of inoculative biological control (Danon *et al.*, 2010). Insect sex pheromones interfere with mating while scented plant extracts attract insect pests to traps ([http://hr4.rutgers.edu/Pdf](http://hr4.rutgers.edu/Pdf)). The female *M. vitrata* sex pheromones have been examined with the aim of developing synthetic lures (Okeyo-Owour and Agwaro, 1982). In Africa, Benin and in Asia, synthetic lures that attract significant number of male moths in the laboratory and in the field have been developed (Hassan, 2007).

### 2.2.1.2.7 Insect pathogens

Insect pathogens (Ward *et al.*, 2002) cause diseases in insect pests leading to death. This involves use of viruses like Baculovirus (NPV and GV) and protozoa (Ward *et al.*, 2002). The efficacy of Multi Nucleopolyhedrosis Virus (MaviMNpv) has been evaluated in Benin, West Africa, with very high mortality of pod borer larvae (>95%) being reported in semi natural conditions in Kano, Nigeria (Tamo, 2010). Spore and non spore forming bacteria like *B. thuringiensis* strain HD-I has been found to be highly pathogenic
to all larval instars of *M. vitrata* (Kariuki, 1987).

The ovicidal activity of entomopathogenic fungi hypomycetes to the legume pod borer has also been evaluated in Kenya. Four isolates of entomopathogenic fungi have been found to be highly pathogenic on eggs of *M. vitrata*, achieving 89-100% mortality reduction in number of emerging larvae (Ekesi *et al.*, 2002).

### 2.3 Advantages and limitations of using biological pesticides

Biopesticides are less toxic, pest specific and often cheaper. They are effective in very small quantities and decompose quickly hence low pollution problems. Biopesticides require much less data to register and are registered in less than a year, compared with an average of more than 3 years for conventional pesticides. Their disadvantages are very high specificity, which require an exact identification of the pest/pathogen and may require multiple pesticides to be used. They give variable efficacy due to biotic and abiotic factors since biopesticides are usually living organisms, which bring about pest/pathogen control by multiplying within the target insect pest/pathogen.

### 2.4 Entomopathogenic nematodes (EPNs)

Nematodes are simple roundworms, colorless, unsegmented and lack appendages. Nematodes may be free-living (beneficial in the decomposition of organic matter), parasitic (causing important diseases of plants, animals, and humans) or predaceous. The predaceous nematodes generally feed on various other nematodes (Kaya and Gaugler, 1993). The insect parasitic nematodes are also called EPNs and are very useful in controlling pests and various other harmful microorganisms (Shapiro-Ilan and Gaugler, 2002).

EPNs are insect parasite nematodes known since the 17th Century but it was not until...
1930s that they were considered for use in pest control (Smart, 1995). The entomopathogenic nematodes are a nematode-bacterium complex with *Steinernema* (Family, Steinernematidae) and *Heterorhabditis* (Family, Heterorhabditidae) being the most important (Kaya and Gaugler, 1993; Cabanillas *et al.*, 1994). Genus *Heterorhabditis* was described first in 1976 by Poinar (Poinar, 1976). Steinernematid and heterorhabditid nematodes infective juveniles have a bacterial chamber carrying symbiotic bacteria in their intestines (Smart, 1995). Steinernematid carry bacteria species of genus *Xenorhabdus*, while heterorhabditid carry species of genus *Photorhabdus*. EPNs have been used to suppress carrot weevil (*Listronotus oregonensis* Lee) in USA, rice leaf folder (*Cnaphlocrosis medinalis* Goen.) and carpenter worms (*Prionoxystus robiniae* Peck.) (Georgis and Manweiler, 1994; Micklasiewicz *et al.*, 2002; Sankar *et al.*, 2009). There are some pests of which at least 80% suppression has been reported using EPNs under field conditions (Table 2.2) (Capinera, 2008).

The third EPNs juvenile stage otherwise known as Dauver juveniles (DJs) enter the insect host through body orifices or by direct penetration through the cuticle for *Heterorhabditis* species. Through the mouth or anus, the nematode penetrates the gut wall to reach the hemocoel, and if by spiracles it penetrates the tracheal wall (Grewal *et al.*, 2001). Once in the host, the DJs release the bacteria, which multiply rapidly in the hemolymph (Smart, 1995). The insect dies within 24-72 hours due to the bacteria and toxins produced by the nematodes (Georgis and Manweiler, 1994). Nematode growth and reproduction depend upon conditions established in the host cadaver by the bacterium. The bacterium further contributes anti-immune proteins to assist the nematode in overcoming host defenses, and anti-microbials that suppress colonization of the cadaver
by competing with secondary invaders (Smart, 1995). Conversely, the bacterium lacks invasive powers and is dependent upon the nematode to locate and penetrate suitable hosts. The bacteria and their metabolic by-products are fed on by the nematode in the feeding third juvenile stage (Shapiro-Ilan and Gaugler, 2002). Applications of EPNs in the Genera *Steinernema* and *Heterorhabditis* have traditionally been targeted against soil insects. Research over the last two decades highlights the potential of such agents against above-ground pests under certain circumstances (Arthurs et al., 2004).
Table 2.2: A list of some pests for which at least 80% suppression has been reported using EPNs to control pests under field conditions.

<table>
<thead>
<tr>
<th>Pest Common Name</th>
<th>Pest Scientific Name</th>
<th>Nematodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artichoke Plume Moth</td>
<td>Platyptilia cardui (Riley)</td>
<td>Sc</td>
</tr>
<tr>
<td>Banana Moth Banana Root</td>
<td>Opogonia sachari (Bojer)</td>
<td>Hb, Sc Sc, Sf Sc</td>
</tr>
<tr>
<td>Borer Black Cut Worm Black</td>
<td>Cosmopolites sordidus (Gemar)</td>
<td>Hb, Hm Hb, Sc, Sf Sc</td>
</tr>
<tr>
<td>Vine Weevil Borers</td>
<td>Agrotis ipsilon (Hufnagel)</td>
<td>Sr</td>
</tr>
<tr>
<td>Codling Moth Corn Ear worm</td>
<td>Otiorrhynchus sulcatus (F.)</td>
<td>Sc</td>
</tr>
<tr>
<td>Diamond back Moth Fungus</td>
<td>Synanthedon spp</td>
<td>Sf, Hb Hb, Sg Sc</td>
</tr>
<tr>
<td>Gnats Japanese Beetle</td>
<td>Cydia pomonella (L)</td>
<td>Sc, Sr, Ss</td>
</tr>
<tr>
<td>Leaf Miners</td>
<td>Helicoverpa zea (Boddie)</td>
<td>Sc, Sf, Sr</td>
</tr>
<tr>
<td>Mole Crickets</td>
<td>Plutella xylostella(L.) Diptera scaridae</td>
<td></td>
</tr>
<tr>
<td>Root Knot Nematodes</td>
<td>Papillia japonica (Newman)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liriomyza spp Scapteriscus spp</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meloidogyne spp</td>
<td></td>
</tr>
</tbody>
</table>

Hb-H. bacteriophora; Hm- H. marelatus; Sc-S. carpocapsae; Sf-S. feltiae; Sg-S. glaseri, Sr-S. Riobravei, Ss-S. scapterisci (Capinera, 2008).

2.4.1 Life cycle of steinernematid and heterorhabditid nematodes

Female nematodes lay eggs that hatch into first stage juveniles that molt successively to second, third, fourth stage juveniles and then to adults of second generation (Plate 2.3). Third stage juveniles are unsheathed and are referred to as dauver while second stages are the infective juvenile (Smart, 1995). Heterorhabditid and steinernematid nematodes differ in their mode of reproduction. In Heterorhabditid nematodes, the first generation individuals are produced by self-fertile hermaphrodites but subsequent generation individuals are produced by cross fertilization involving males and females. In steinernematid nematodes with an exception of one species, all generations are produced by cross fertilization involving males and females. Insects killed by most steinernematid nematodes become brown or tan, whereas insects killed by heterorhabditids become red and the tissues assume a gummy consistency. A dim luminescence given off by insects freshly killed by heterorhabditids is a foolproof diagnostic for this genus (the symbiotic bacteria...
Plate 2.3: Life Cycle of Entomopathogenic nematodes steinernema and heterorhabditis (http://www.nematodes.com/nematode) provide the luminescence. Black cadavers with associated putrefaction indicate that the host was not killed by entomopathogenic species. Nematodes found within such cadavers tend to be free-living soil saprophytes.

2.4.2 Entomopathogenic nematodes as biopesticides

Entomopathogenic nematodes in the genera Steinernema and Heterorhabditis have emerged as excellent candidates for biological control of insect pests. This is because of their broad hosts range, high virulence, safety for non-target organisms and high efficacy.
in favourable habitats. Progress achieved in liquid fermentation, formulation stability and application strategy has allowed nematode-based products to become competitive with chemical insecticides in medium and high value crops on the basis of cost/benefit ratio and ease of application (Aldamario et al., 2010). Thus there is need to experiment and improve the market potential of the nematode-based products (Georgis, 1992). The EPNs are non polluting thus environmentally safe and acceptable. They can also be applied with conventional equipment and are compatible with most pesticides. These nematodes reproduce in the insect host and thus provide new DJs to infest additional insect (Smart, 1995).

The steinermatid and heterorhabditid nematodes can be reared in vivo in insect hosts or they can be mass produced in vitro on solid medium or in liquid medium. The most commonly used insect for in vivo EPNs rearing is the greater wax moth (Galleria mellonollia), pre-pupal stage being preferred. This is because of its high susceptibility to most nematodes, ease of rearing, wide availability and ability to produce high nematode yields (Grewal et al., 2005; Divya and Sankar, 2009). In vivo production requires a low level of technology, has low startup costs, and resulting nematode quality is generally high, yet cost efficiency is low (Smart, 1995). For solid medium culture, a substrate like beef or pork kidney or liver, chicken offal among others may be used. This involves making a paste of the substrate coated onto a porous substrate such as sponge (Smart, 1995). The medium is sterilized and nematodes are added 24 hours later. The DJs are harvested after 15 days. This method is labour intensive (Smart, 1995). Alternatively in vitro solid culture, i.e., growing the nematodes on crumbled polyurethane foam, offers an intermediate level of technology and costs. In vitro liquid culture is the most cost-efficient production method but requires the largest startup capital. Liquid culture may be improved
through progress in media development, nematode recovery, and bioreactor design. A variety of formulations have been developed to facilitate nematode storage and application.

2.4.3 Entomopathogenic nematodes in Kenya

Information on indigenous EPNs in Africa is still limited, especially in sub Saharan Africa. Currently, documented studies are available only from Kenya, Egypt, Ethiopia and South Africa (IITA, 2008; Mwaniki, 2008). EPNs of the genera *Steinernema* and *Heteroharbditis* were first reported in Kenya, in a survey conducted in Central highlands and Coastal counties of Kenya where 154 nematodes isolates among them the new species *Steinernema karii* was identified (Waturu et al., 1997; Waturu, 1998). Further surveys in the Rift valley yielded 12 nematode isolates (Mwaniki, 2008). There are 33 EPNs isolates currently maintained in 3 KARI laboratories at Kabete, Mwea and Thika. There are no commercial applications of EPNs so far reported in Kenya, though research to validate their efficacy as biological pest control is still going on (Waturu et al., 2006). Further studies in Kenya reveal that the distribution, occurrence and diversity of EPNs are affected by land use and genus *Steinernema* was dominant in Embu and Taita counties. Land intensification negatively affects the occurrence and recovery frequency of *Steinernema* species in soils (Kawaka, 2011). Laboratory experiments in Kenya have confirmed the susceptibility of the sweet potato weevil larvae to both *H. indica* and *S. karii* (Waturu, 1998). The pathogenicity of *H. indica* and *S. karii* has been evaluated in the field against banana weevil (*Cosmopolites sordidus* Germar) where they were found to infect the weevils and cause mortality within the set up of the banana traps (Waturu et al., 2006). Nevertheless, semi field studies in Kenya on use of *H. indica* and *S. karii* against
Cylas puncticollis on sweet potatoes vines and tubers shows the potential of EPNs as a component of IPM (Nderitu et al., 2009). According to Nyasani et al., (2008), Diamond back moth (Plutella xylostella(L.), a major pest of Kales was found to be susceptible to H. indica, S. weiseri and S. karii in the laboratory and field conditions. Further research in Kenya has revealed pathogenicity of S. karii and H. bacteriophora against flower thrips, major pests of French beans (M. sjostedti and Frankliniella occidentalis) in the laboratory and green house experiments (Ng’ang’a et al., 2011).

The survival and success of EPNs are based on environmental condition, moisture and soil type and percentage of living nematodes actually released during the application. Nematodes should be reapplied on seven-day intervals if damage continues (Divya and Sankar, 2009). In order to ensure maximum effectiveness, it is crucial to apply them at the optimum environmental conditions needed for their better survival. Therefore, it is best to irrigate the target site, both before and after application because they need moist conditions to prevent desiccation and aid with movement to find hosts. Also, the best results are obtained when the relative humidity is high, ambient temperature is neither extremely hot nor cold, soil temperature is between 10°C to 35°C, soil is moist and direct sunlight is minimal to lower the ultra violet rays (Smart, 1995). All of these factors help prevent the nematodes from drying out and increase their survival and virulence (Divya and Sankar, 2009). However, the pathogenicity, host searching behaviour and survivability of different nematode species are varied making them suitable in biological control programs.
3.1 Study site

The study was carried out at the National Horticultural Research Centre, KARI-Thika. The Centre is located at coordinates 0° 59' S and 37° 04' E. The KARI-Thika, conducts research in fruits, vegetables, flowers, macadamia nuts, tissue culture bananas and IPM. The Centre is located 5 km from Thika Town and 43 km from Nairobi. The Centre is located at an altitude of 1548m above the sea level and receives annual rainfall of 960mm. The temperature of the area ranges between 16°C and 24°C. Soil depth is deep to shallow with good to poor drainage. The soils are classified as sandy loam to clay. These climatic conditions make the area suitable for cowpea which grows in a wide range of temperatures, rainfall and soils.

3.2 Cowpea establishment

Cowpea establishment for the purpose of laboratory and screen trials was done using certified seeds of early maturing variety Ken-Kunde. Seeds were obtained from Simlaw seeds company Kenya. Cowpeas flowers for use in the laboratory assay were obtained from plants grown in a plot measuring 15m x 1.5m. A teaspoonful of single super phosphate fertilizer Olubayo and Port (1997) and a handful of sheep manure were added in each planting hole and thoroughly mixed before placing the seeds. Two seeds were placed in each hole at a depth of 5cm. Watering was done every three days in a week whenever there was no rain.

Cowpea for the semi field trials were established in 20cm diameter x 24 cm long polythene sleeves in a screen house (Plate 3.1). Top soil was collected from land under
bush, put in each polythene sleeve and a teaspoonful of single super phosphate fertilizer per sleeve was applied (Olubayo and Port, 1997). The soil and the fertilizer were thoroughly mixed before placing the seeds. Two seeds were planted in each sleeve at a depth of 5cm. Watering was done every 3 days in a week using a watering can. Two weeks after crop emergence, thinning to one seedling and topdressing using calcium ammonium nitrate (CAN) at the rate of one teaspoonful per sleeve was done. Weeds were uprooted by hand. Planting in the screen house was done every 3 weeks to ensure there were ready plants for use in the experiment at any one time.

Plate 3.1: Cowpeas for screen house trials

3.3 Rearing of cowpea legume pod borer (*M. vitrata*)

The larvae of legume pod borers were collected from cowpea pods in a previously harvested cowpea field. Additional larvae were from an existing laboratory culture at the International Centre for Insect Physiology and Ecology (ICIPE) Mbita Point, Kenya. The cowpea pod borers for the experiment were raised on artificial and natural diets in the laboratory. The artificial diet formula was adopted from ICIPE Mbita Point, comprising of
brewer's yeast 20g, ascorbic acid 5.5g, sorbic acid 1.7g, methyl-4-hydroxyl 2.5g, vitamin A 0.75g soybean 175g, agar 25g, 40% formaldehyde 0.5ml, cowpea flowers flour 25g and 600ml of distilled water (Sumba and Bungu, 1983). About 200ml of cold water was topped with 300ml of hot water, added to agar and 500ml of hot water added gently while stirring the solution. All the other ingredients were added into 600ml water which had been boiled, cooled and formaldehyde 0.5ml was added into the mixture. The agar suspension was added and the mixtures blend into a smooth paste. The mixture was dispensed into 100 specimen tubes soda glass poly-stopper, 75x25mm in size quickly. The specimen tubes were then covered with sterile white cloth and let to solidify for 12 hours on a bench (Ochieng and Bungu, 1983). Using a spatula a slit was made on the solid mixture in the specimen tubes and 1-3 first instars larvae of *M. vitrata* introduced into the diet. The specimen tubes containing the larvae were clogged with sterile cotton wool and incubated at 25±2°C for the larvae to develop. Some larvae were raised on natural diet using cowpea flowers.

Cowpea flowers that were just about to open were picked from the field early in the morning and wilted before use (Ochieng and Bungu, 1983). The flowers were placed on a tray and spread on a ventilated plastic container measuring 20cm x 15cm x 8cm in size. A handful of the flowers were placed on the mesh in the container and early instars of *M. vitrata* introduced. The container was covered with a lid cut open on top but covered with a fitting net cloth for ventilation (Ochieng and Bungu, 1983). The flowers were changed every day where old flowers were replaced with fresh ones. During changing, fresh flowers were always placed at the bottom of the container on a wire mesh while the old flowers on a mesh were placed on top of the fresh ones (Ochieng and Bungu, 1983). This was repeated until the larvae were ready for use.
3.4 Maintenance of entomopathogenic nematodes

The greater wax moth (*Galleria mellonella*) larvae were let to multiply in order to maintain EPN cultures in the laboratory. This was because of its rich nutrient source available in body and ease to multiply in economical artificial diet (Divya and Sankar, 2009). Different life stages (eggs, pupae and larvae) of *G. mellonella* were used to establish a laboratory culture (Divya and Sankar, 2009). The life stages were obtained locally from bee keeping farmers honey combs and from an existing laboratory culture at KARI-Thika (Waturu, 1998). Artificial diet comprised of bee wax 45g, brewer’s yeast 95g, maize flour 307g pure and honey 225g. Honey combs were pre-melted in a pot and honey added while stirring vigorously using a wooden spoon (Waturu, 1998). Maize flour and yeast were added to the mixture of honey and bee wax and mixed thoroughly. The mixture was transferred to a ventilated 3.4 litre plastic container and allowed to cool before introducing life stages of greater wax moth. The boxes were incubated at 25±2°C for about 4-5 weeks when eggs were used to start the culture. Late instar larvae were rinsed in water heated for 20 seconds at 60°C and in cold water for 10 seconds. This method allowed for long term larvae storage at temperatures below 10°C without them pupating (Waturu, 1998).

3.4.1 Nematode culture

The nematodes *H. indica* and *S. karii* for this study were obtained from nematode suspension stock at KARI-Thika (Waturu, 1998). The nematodes were previously isolated from soils in Central highland counties and Kwale County of coastal region, Kenya. Larvae of the greater wax moth were used as baits for the nematodes (Waturu, 1998). For *in vivo* mass production of *H. indica* and *S. karii* greater wax moth were used. A suspension of DJS was drawn from a stock suspension stored at 20°C and conditioned to
room temperature for at least 1 hour. Viability of the nematode was assessed by placing part of the suspension into a petri dish and observing under binocular microscope Waturu et al., 1997. A volume of the suspension was diluted with appropriate amount of distilled water (Waturu, 1998). The diluted nematode suspension was adjusted to 200 nematodes per ml and 1 ml was added into a petri dish. Six to seven pre-pupating G. mellonella larvae were placed in each petri dish which was later covered with a lid (Plates 3.2a and b). The Petri dish was incubated at room temperature of 25 ±2°C until the death of the larvae.

Plate 3.2: Cadavers of G. mellanolla for mass production of entomopathogenic nematodes; (a) Galleria cadavers infected with H. indica and (b) Galleria cadavers infected with S. karii

3.4.2 Nematode extraction or trapping

The cadavers were then transferred into modified white traps assembled by placing an inverted plastic Petri dish in a 250ml plastic container, white cotton cloth and distilled water. The white cloth was placed in such a way that one edge touched the distilled water in the plastic container. Infected Galleria larvae were then arranged in a circular manner, close to each other on the modified White trap with the anterior part of the cadaver facing up and posterior side towards the distilled water (Plates 3.3a and b).
Plate 3.3: Extraction or trapping of nematodes from *Galleria* cadavers using filter paper. (a): *S. karii* (b): *H. indica*

The DJs emerged from the cadavers in about 7-10 days later, moved through wet white cloth and were harvested from the distilled water. The DJs were cleaned three times in distilled water. Freshly harvested DJs at a concentration of about 100 ml were then stored in 1 cm deep distilled water in 500 ml plastic containers well-labeled with isolate number and date of harvesting. The containers were stored on shelves in the dark at room temperature of 25±2°C for short term use and 20°C in an incubator for long storage.

3.5 Study design

Laboratory trials included five treatments replicated 3 times (zero, 100, 200, 300 and 400) for each EPN species. The semi field trials included two treatments replicated 3 times (zero and 400) for each EPN species. The experiments were set in a completely randomized design.

3.5.1 Determination of nematode concentration

Nematode doses were prepared from a stock suspension stored at 20°C. A known volume of nematode suspension was transferred into a 250ml beaker and shaken to
uniformity Waturu et al., 1997. Five aliquots of 1ml each of nematode suspension were drawn and counted in nematode counting dishes. The counting of nematodes in 1ml was done five times and the mean number was determined Waturu et al., 1997. To increase the concentration or number of nematodes per ml, the suspension was left to settle for 1 hour and excess water poured out. Where nematode concentration per ml was higher than the required, more distilled water was added to the stock suspension (Waturu, 1998).

3.5.2 Laboratory pathogenicity trials

Experiments were carried out to study the pathogenicity of the EPN isolates *H. indica* and *S. karii*. The dose of DJs required to kill third instars of *M. vitrata* larvae were defined in experiments in completely randomized experimental design, with five treatments; zero (control), 100, 200, 300 and 400 DJs per insect with three replicates. Five *M. vitrata* third stage larvae were placed in 9cm diameter filter paper padded petri dishes and then 1ml of nematode suspension at different concentrations was applied. Cowpea flowers were washed using sterile distilled water and placed inside the Petri dish as source of nourishment for the larvae. The petri dishes were incubated at room temperature 25±2°C for 120 hours (Plate 3.4). In the control treatment, 0.5ml of distilled water was used to wet the filter papers before placing the larvae. Cowpea flowers were also included in the control. The numbers of dead larvae were taken after 24, 48, 72, 96 and 120 hours from the start of experiment. Cadavers were selected randomly from each treatment and dissected under dissecting microscope to verify infectivity of nematodes. Data on mortality against time and concentration were recorded for all the treatments.
Plate 3.4: Laboratory experiment set up with colour coded petri dishes with nematode infected *Maruca* larvae and cowpea flowers as source of nourishment O Control, *S. karii* treated larvae and *H. indica* treated larvae

3.5.3 Screen house pathogenicity trials

Potted cowpea plants were established in a screen house. The DJs concentration of 400 per ml with the highest mortality in the laboratory trial was subjected to semi field trials. Five third- instar *M. vitrata* larvae were released onto each potted flowering cowpea plant. Sterile-distilled water was applied on the plants before and after nematode suspension application for higher nematode motility. The plants were colour coded, each colour representing individual treatment (Plate 3.5a).

For the control treatment, plants were infested with five *M. vitrata* larvae and sterile distilled water without EPNs. The EPNs suspension was sprayed on the cowpea plants using a hand pump at 6.00pm to minimize the effect of UV radiation and heat on the nematodes (Waturu *et al.*, 2006). Treated plants were caged using nets to minimize damage from birds and easy collection of dead larvae (Plate 3.5b). Data collection on number of dead larvae was done on 24 hour basis from the start of experiment: 24, 48, 72, 96 and 120 hours. Counting of damaged flowers was done at 24 hour interval for all the treatments.
3.6 Determination of pathogenicity and nematode infection

Larval death was confirmed by touching the cadaver with needle and assessing reflex movement and also by the colour of the cadavers which was determined by the symbiotic bacteria associated with the *Steinernema* and *Heterorhabditis* species (Sankar *et al.*, 2009). The cadavers were also dissected to confirm nematode infectivity. Dead *M. vitrata* larvae were washed in distilled water to remove nematodes on the body surface. To determine infection by nematodes and progeny recovery, the cadavers were dissected in Taylor and Baker’s (1978) Ringer’s solution (Waturu, 1998). The cadavers for dissection were selected randomly from each replicate in all the treatments. The cadavers were dissected under binocular dissecting microscope.

The dissected *M. vitrata* cadavers were allowed to stand on the bench for at least 35 minutes for the nematodes to move from the dissected tissues into Ringer’s solution (Waturu, 1998). The recovered DJs were put in a beaker and diluted using distilled water to make 150 ml of nematode suspension. Using a graduated pipette, 1ml of the suspension
was obtained from the stock suspension, placed on a nematode counting dish and the number of nematode progeny recovered was determined under the microscope. The counting was repeated 3 times and the average calculated in all the treatments.

3.7 Data analysis

The laboratory and screen house trials data on mortality were subjected to Analysis of Variance (ANOVA), Student to test and non parametric correlation using the SPSS version 12.0 statistical software. Means were separated at 5% significance level. Percent mortality was determined by dividing the mean of R1, R2 and R3 *M. vitrata* cadavers by the total number of larvae and expressed as a percentage. Data were summarized in tables and graphs.
CHAPTER FOUR: RESULTS

4.1 Pathogenicity of EPNs on *M. vitrata*

The pathogenicity of *H. indica* and *S. karii* on *M. vitrata* larvae was determined by death of the test pest (Plate 4.1a). The nematode infected *M. vitrata* cadavers did not break open on pressing them and assumed their shape on releasing pressure. The cadavers assumed spongy form confirming that they died as a result of nematode infection. The *H. indica* infected cadavers appeared brick red while those that died due to *S. karii* assumed gray colour (Plate 4.1 b and c).

**Plate 4.1:** (a) Some *M. vitrata* cadavers in the laboratory set up with flowers as source of nourishment, (b) *S. karii* infected *Maruca* cadavers and (c) *H. indica* infected *Maruca* cadavers.
4.1.1 Dauver juveniles’ recovery

On dissecting the *M. vitrata* cadavers, adult nematodes and DJs (progeny) were observed. The number of the DJs recovered is summarized in Figure 4.1. Emergence of both EPNs was observed from the dead larvae (Plate 4.2). The yield of DJs increased with increase in EPNs concentration up to 300 for *H. indica* and 200 DJs for *S. karii* after which notable decrease in yield was observed. For the *S. karii* the lowest yield was 15450 DJs at the highest concentration of 400 DJs used.

At concentration of 100, *S. karii* yielded 17250 DJs. The highest *S. karii* DJs count was 29610 at concentration of 200. The *H. indica* yielded 12000 DJs at the lowest concentration of 100 used and a maximum of 24000 DJs at concentration of 300. The *S. karii* generally yielded more except at concentration of 300 doses where it yielded 17000 DJs a decrease in yield from the previous yield. At the concentration of 400, *H. indica* yielded 14400 DJs higher than at the dose of 100 DJs unlike *S. karii* whose yield at the highest concentration of 400 DJs were lower than at the lowest concentration of 100 DJs. There was a negative correlation (-0.146) between increased concentration and the total number of DJs recovered from the cadavers as indicated in the Appendix 1.
Plate 4.2: Dauver juveniles and adult nematode escaping from macerated *M. vitrata* cadaver. Magnification x40

4.2 The effects of *H. indica* and *S. karii* on the *Maruca* under laboratory conditions

The laboratory results for the mortality of *M. vitrata* larvae induced by *S. karii* and *H. indica* applied at four different doses are summarized in Figure 4.2. No mortality was observed in the control. For all the doses mortality of *M. vitrata* larvae due to *H. indica* was higher compared to mortality due to *S. karii*. Mortalities of 100% were obtained for the highest exposure time for all the nematode concentrations tested thus no larvae survived beyond the 120 hours in both *H. indica* and *S. karii*.

It was also observed that at 48 hour exposure time, 50% *Maruca* larvae mortality was achieved in 200, 300 and 400 concentrations of *H. indica* but only 27-46% mortality was achieved due to *S. karii* at the same time and concentration. Consequently, *H. indica* highest concentrations of 400 nematodes per larvae achieved 100% mortality within 72 hours while at the same of *S. karii* mortality was 66%.
There was significant difference (p < 0.05) between larval mortalities due *H. indica* and *S. karii* (Appendix 2). The nematode *H. indica* had a higher mortality mean of 4.133±0.7118 than *S. karii* with 3.066±0.7409 in the laboratory. The difference between the control and treatments using the EPNs was significant (p < 0.05). At 400 nematode concentrations the mortality pooled mean mortality mean of 3.600±0.5159 for both nematodes. There was significant difference (p< 0.05) effect of both nematodes and the control (Appendix 2).

![Average DJs recovered per cadaver](image)

**Figure 4.1:** Mean number of EPNs DJs recovered from *M. vitrata* cadavers.
Figure 4.2: The effect of *H. indica* and *S. karii* on *M. vitrata* larvae at different concentrations (A 100, B 200, C 300 and D 400) of the nematodes.
4.3 The effects of *H. indica* and *S. karii* on *M. vitrata* under screen house conditions

The *M. vitrata* cadavers were found on leaves and flowers of treated cowpea plants as shown in Plates 4.3a and b. Results for the mortality of *M. vitrata* larvae due to *H. indica* and *S. karii* are summarized in Figure 4.3. From the laboratory results, the 400 concentration was observed to cause the highest *M. vitrata* mortality and there was need to elucidate whether the same results would be realized in the screen house using potted flowering cowpea plants.

The screen house temperature at the time of trials ranged between 28°C and 31°C. In the first 24 hours, no *M. vitrata* mortality was recorded for the two EPN isolates. At 72 hours, both the EPNs performed the same and mortality of 47% was recorded. For the *H. indica* treated plants, 50% mortality was achieved in 96 hours while by 120 hours only 47% mortality had been achieved for *S. karii* treated plants and there was no more mortality observed. The two EPNs failed to achieve 100% mortality by 120 hours. However, mortality in screen house also increased with time up to the 96 hour after which mortality stagnated. The mean mortality due to *H. indica* was 1.8667±0.5011 higher than mortality due to *S. karii* which was 1.6000±0.4761.

There was no significant difference (p>0.05) in larval mortality due to *H. indica* and *S. karii* at 400 concentration and control in the screen house (Appendix 3). The mean larval mortality was (1.7333±0.3289) for both species and the control had a mean of 0.6667±0.2981. There was no significant difference (p>0.05) between both nematode pooled means and the control, while the difference (p<0.05) was significant in the laboratory
trials at 400 concentration (Appendix 4 and 5).

Plate 4.3: Dead *M. vitrata* on cowpea plants in the screen house; (a) *H. indica* infected cadaver and (b) *S. karii* infected cadaver

Figure 4.3: The effect of treatment on *M. vitrata* larvae infected with 400 concentration of *H. indica* and *S. karii* in the screen house trials
Figure 4.4: The effects of *H. indica* and *S. karii* on *M. vitrata* larvae in the laboratory and screen house conditions at 400 nematode concentration.

4.4 The net effect of treatment on cowpea flowers in the screen house trials

Damaged flowers were observed on cowpea plants in the screen house as in Plate 4.4. Flowering cowpea plants were treated with two nematode species and percent flower damage recorded as shown in Figure 4.5. At 24 hour period there was 33%, 7% and 12% flower damage registered in the control, *H. indica* and *S. karii* respectively. There was an increase in mortality with increase in exposure time. At 48 hours, 50% of the flowers had been destroyed in the untreated plants, while the damage was only 26% and 33% for *H. indica* and *S. karii* treated plants respectively.

After 120 hours there was 92% flower damage in the control while *H. indica* and *S. karii* was 47% and 60 % respectively. The *H. indica* was more pathogenic in the semi field trials than *S. karii* (Table 4.1. The mean flower damage in the control, *H. indica* and *S. karii* was 3.2000±0.5228, 1.6667±0.3801 and 2.0667±0.4137 respectively but the differences were significant (p< 0.05) between the control and *H. indica* only (Appendix 6 A, B and C).
Table 4.1: The net effect of treatment using *H. indica* and *S. karri* on flowering cowpea plants infested with *M. vitrata* larvae in the screen house trials (mean ± SE).

<table>
<thead>
<tr>
<th>Screen house trials</th>
<th>Treatment</th>
<th>Mean mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>3.2000±0.5228</td>
</tr>
<tr>
<td>Flower damage</td>
<td><em>H. indica</em></td>
<td>1.6667±0.3801</td>
</tr>
<tr>
<td></td>
<td><em>S. karri</em></td>
<td>2.0667±0.4137</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ±SEM for three determinations per dose; *a p < 0.05* is considered significant by ANOVA and post ANOVA test (SNK).

Plate 4.4: Damaging effect of *M. vitrata* larvae on cowpea flower in the screen house.
Figure 4.5: Flower damage by *M. vitrata* larvae exposed to 400 concentration of *H. indica* and *S. karii* and observed over a period of 120 hours at 24-hour interval under screen house condition.

4.5 Effect of time on larval mortality at 400 concentration in the laboratory and screen house trials for both nematode species

The experiments were conducted for a period of 120 hours and data on mortality taken at 24-hour interval in laboratory and screen house trials. It was observed that at the exposure time of 24 hours, the mean mortality in all the treatments was low where larval mean mortality in the laboratory was 0.6667 and at 72 hours the mean mortality was 2.7778±1.4699, an increase of 2.1111 which translated to 76% increase in mortality. At the maximum exposure time of 120 hours, the mean mortality was 3.3333±1.6667, thus a mean difference of 2.6667 from the lowest exposure time of 24 hours. This was 80% increase in mortality from the lowest to highest exposure time. In the screen house tests, at the lowest exposure time of 24
hours, the mean larval mortality was 0.000 while at 72 hours it was $1.7778 \pm 0.5556$ a mortality increase of $1.7778$ which was 100% increase. At the maximum time of 120 hours the mean mortality was $2.1111 \pm 0.4006$ hence a mean difference of $2.1111$ between mean mortality at the lowest and highest exposure time. The effect of time on mortality was significant both in the laboratory ($P < 0.05$) and in the screen house ($P < 0.05$) respectively (Appendix 7). The results are summarized in Table 4.2.

### Table 4.2: Effect of time on mean larval mortality in the laboratory and screen house conditions at concentration of 400 for both $H. indica$ and $S. karii$ (Mean ± SE).

<table>
<thead>
<tr>
<th>Exposure time in hours</th>
<th>Laboratory Trials</th>
<th>Screen house trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.6667 ± 0.3849</td>
<td>0.0000 ± 0.0000</td>
</tr>
<tr>
<td>48</td>
<td>2.2222 ± 1.2522</td>
<td>0.8889 ± 0.4843</td>
</tr>
<tr>
<td>72</td>
<td>2.7778 ± 1.4699</td>
<td>1.7778 ± 0.5556</td>
</tr>
<tr>
<td>96</td>
<td>3.0000 ± 1.5275</td>
<td>2.1111 ± 0.4006</td>
</tr>
<tr>
<td>120</td>
<td>3.3333 ± 1.6667</td>
<td>2.1111 ± 0.4006</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ±SEM for three determinations per every timing; $^a$ p < 0.05 is considered significant by ANOVA and post ANOVA test (SNK).

## 4.6 Effect of time on larval mortality at different concentration in the laboratory trials for both nematode species

The larval mean mortality increased with time in all the concentrations in both nematode species in the laboratory. It was observed that from 24 to 48 hours exposure time $M. vitrata$ larval mortality mean increased by 72% in all concentrations (Figure 4.6). From 48 to 120 hours larval mortality increased by 50%, and between 24 and 120 hours (minimum and maximum exposure time) the mortality rise was 87%. The effect of time on larval mortality was significant ($p < 0.05$) in both nematode species at different concentrations in the laboratory trials (Appendix 9).
4.7 Effect of concentration on larval mortality in the laboratory

The *M. vitrata* larvae were exposed to different concentrations of *H. indica* and *S. karii* in the laboratory. It was observed that at the minimum concentration of 100, the mean mortality was $2.8000\pm0.6070$ and at the highest concentration of 400 nematodes, it was $3.6000\pm0.5159$. This was 22% increase in mortality from the lowest dose of 100 to the maximum dose of 400 as shown in Table 4.3. There were significant difference ($p < 0.05$) between the control (untreated) and the nematode treated groups (Appendix 8). All the concentrations achieved 100% larval mortality at the maximum exposure time of 120 hours (Figure 4.7).
Table 4.3: The effect of nematode concentrations on larval mortality in the laboratory (Mean ± SEM).

<table>
<thead>
<tr>
<th>Nematode Dose</th>
<th>Laboratory trial Mean mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0000±0.0000</td>
</tr>
<tr>
<td>100</td>
<td>2.8000±0.6070\textsuperscript{a}</td>
</tr>
<tr>
<td>200</td>
<td>2.9000±0.5422\textsuperscript{a}</td>
</tr>
<tr>
<td>300</td>
<td>3.3667±0.5079\textsuperscript{a}</td>
</tr>
<tr>
<td>400</td>
<td>3.6000±0.5159\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ±SEM for three determinations per dose; \textsuperscript{a} p<0.05 is considered significant by ANOVA and post ANOVA test (SNK).

Figure 4.7: The effect of nematode concentration on larval mortality in the laboratory trials.

It was observed that there was no interaction between nematode concentration and time the larvae were exposed to treatment. The interaction between time and nematode concentration was of no significance (p > 0.05) on \textit{M. vitrata} larvae (Appendix 10).
CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In this study *H. Indica* and *S. karii* were found to be pathogenic to 3rd instar *M. vitrata* larvae while mortality was recorded in all the EPNs treatments in the laboratory and screen house conditions. The *M. vitrata* cadavers infected with *H. indica* assumed brick red while those with *S. karii* assumed gray colour indicating that mortality was indeed caused by the nematodes. The observations are in line with Sankar (2009) who investigated pathogenicity of *H. indica* and *S. asiaticum* against rice leaf folder larvae and observed brick red and grey black colours. These colours are known to be produced by the symbiotic bacteria *Photorhabdus* and *Xenorhabdus* associated with *Heterorhabditis* and *Steinernema* respectively (Smart, 1995).

Emergence of both species of EPNs were observed from dissected *M. vitrata* cadavers which are an indication that *H. indica* and *S. karii* overcame host resistance and thus were able to reproduce in *M. vitrata* cadavers. The finding is in agreement with Sudheer and Prabhu (2008) who got similar result from *H. indica* and *Steinernema* species in red hairy caterpillar. They suggested that production of proteolytic enzymes by the symbiotic bacteria in the nematodes helps to overcome the problem of host resistance thus making it possible for nematodes to reproduce in insect cadavers.

The number of DJs recovered from *M. vitrata* cadavers infested with EPNs varied with *S. karii* yielding more DJs than *H. indica* in all the treatments. These results indicate progeny relationship between the yield and EPNs species. This is in agreement with Nyasani et al. (2008) who observed that *S. karii* are more persistent and has a high recycling ability.
(ability to initiate a new life cycle) in a susceptible insect pest. The high recycling ability of *Steinernema* spp. can be explained by their mode of reproduction where *Steinernema* spp. is amphimitic (that is, it undergoes sexual reproduction) which tends to yield more progeny. The *H. indica* is a hermaphrodite thus tends to yield less progeny (Waturu, 1998).

Nematode reproduction increased with DJs concentration but further increase in *H. indica* and *S. karii* concentration led to decrease in progeny recovery thus a negative correlation. It was observed that from 300 and 200 DJs doses for *H. indica* and *S. karii* respectively, there was decrease in progeny recovery. This may reflect the effect of small size of 3rd instar *Maruca* larvae which limited space for the number of DJs the larvae could accommodate compared to *G. mellonella* used for *in vivo* EPNs production. Also it is speculated that the small larvae size impacted negatively on nourishment of the progeny due to limited nutrients in the cadavers. Another reason for decrease in progeny yield could have been due to DJs density and progeny relationship. The high density of DJs penetrating the larvae led to competition for entry points hence decreased the number of DJs that gained entry and establishment in the larvae. According to Waturu (1998), pest size and nematode dose influence progeny of *S. karii* where *C. partellus* (large) yielded more than *C. puncticollis* (small). The results are also consistent with Flanders *et al.* (1996) and Boff *et al.* (2000) reports that progeny production by the host pest varies according to the host size and nematode dose. This also conforms to Gupta *et al.* (2008) who reported gradual increase in progeny production of *S. carpocapsae* per larvae of cabbage butterfly (*Pieris brassicae*), after which a sudden decrease was noticed.

Furthermore, Dowds and Peters (2002) have shown that nematode juveniles encounter
various behavioral and mechanical forms of resistance during entry into the insect hosts. These behaviours include rubbing with an abrasive raster situated on the ventral end of the abdomen, brushing with prolegs or mouthparts and scraping and chewing motions with their mandibles (Koppenhöfer et al., 2000). This could have caused injury and death of DJs at the point of infection. The *M. vitrata* larvae used in the study had been raised on artificial diet and not on the pest host plant. This could also have had negative effect on progeny yield. Nutrition has effect on virulence and fitness of entomopathogenic nematodes with some diet supplements having positive effects on nematode quality, whereas others have negative or neutral effects (Shapiro-Ilan et al., 2008).

The mortality of *M. vitrata* due to *H. indica* and *S. karii* under laboratory conditions differed greatly. Higher mortality was recorded in the *H. indica* treatments compared to *S. karii*. There was no mortality in the control experiment. This suggests that is a relationship between mortality and nematode species, a factor to consider when adopting EPNs as biological control agents for good results. This finding is consistent with Griffin et al. (2005) report which asserts that insect pest mortality depends on EPN species. *H. indica* was previously reported to be the most promising EPN against cabbage butterfly (*P. brassicae*) and of brinjal fruit borer (*Leucinodes orbonalis*) larvae (Hussaini et al. (2002; Lalramliana and Yadav. (2009). Earlier studies also suggest that *Heterorhabditis* are more pathogenic than *Steinernema* in pests like root weevils (Bedding et al., 1983). In the control of sweet potato weevil (*Cylas punicollis*) using EPNs, (Nderitu et al. (2009), noted that *H. indica* caused higher mortality than *S. karii* in the field. This is also in agreement with Kulkarni et al. (2011), where *H. indica* was more pathogenic against teak skeletonizer (*Eutectona machaeralis*) in the screen house conditions. It is also suggested that the high pathogenicity of *H. indica* was due to its high mobility and ability to
recognize the host pest better than *S. karii*. According to Griffin *et al.* (2005), the dispersal behavior and capabilities after application of EPNs vary among species with *Heterorhabditis* nematodes tending to migrate farther than *Steinernema* nematodes hence are better in host seeking.

Higher pathogenicity of *H. indica* could be attributed to the presence of mural tooth which helps the nematode to penetrate the soft segments of the larvae while *S. karii* which lacks the tooth enters through natural openings of the insects. Presence of hairs on *M. vitrata* body further prevented the access of nematodes directly suggesting why *S. karii* caused less mortality in *M. vitrata*. Sudheer and Prabhu, (2008) observed that, *H. indica* was more pathogenic *Steinernema* species in red hairy caterpillar in groundnut.

Pathogenicity to *M. vitrata* from *H. indica* and *S. karii* to was far much lower in the screen house experiments than in the laboratory. Fetoh (2009), in his study found that larvae of greasy cutworm (*Agrotis ipsilion*) in the screen house conditions were less susceptible to the effect of the nematodes than in the laboratory. This discrepancy was attributed to the differences in the laboratory and semi field conditions. Physical parameters like distance between *M. vitrata* larvae and nematodes, plant surface area, nematode pest contact, temperature, ultra violet rays and biological factors like EPNs natural enemies could have contributed to decreased *M. vitrata* mortality in the screen house conditions. Woodring and Kaya (1988), suggested that infectivity of *Steinernema* and *Heterorhabditis* nematodes vary greatly due to some environmental factors or requirements which could be physical or biotic parameters. For instance, the DJs in the screen house had to locate the insect host and gain entry into the haemocoel where they covered longer distance on the plant, a situation absent in the laboratory condition.
In the laboratory trials, the *M. vitrata* larvae were in confinement of a petri dish which increased nematode-larvae contact. This close contact ensured less distance coverage by the EPNs to locate their host pest increasing larval mortality in the laboratory trials. The larvae were very mobile immediately on being placed on the cowpea plant where this mobility could have reduced the chance of EPNS locating their host easily lowering larval mortality in the screen house trials. More so, the surface area of the cowpea plant reduced nematode larvae contact, so the EPNs were expected to cover a longer distance to reach their target host.

The cowpea plants could not provide high confinement levels as in case of a petri dish. The difference between laboratory and screen house results could also be due to vagaries of the environment. For instance, nematodes are susceptible to desiccation, so incase of high environmental temperatures they tend to die hence lowering their infectivity. It is speculated that *H. indica* was more tolerant to high temperatures in the screen house. According to Mahar *et al.* (2007), temperature influences nematode mobility, reproduction and development. The nematode mobility reduces at low temperature and nematodes have optimal temperature for reproduction and infection (Chen *et al.*, 2003). This is also supported by Jagdale *et al.* (2007) who reported that *H. indica* is a heat tolerant nematode that works better against many insect pests when the temperature is between 25°C and 29°C thus has great potential for use as a biological control agent to manage greenhouse pests in greenhouse industry. According to Mason and Hominick (1995), effect of temperature is thought to be related to the geographic origins of the species.

In the screen house, unlike in the laboratory, the EPNs could also have been affected by Ultra violet radiations from the sun and their natural enemies since sterile soil was not used, thus lowering the number of nematodes to attacking the larvae and this was not the case in the
laboratory. There was also a chance of having lost dead larvae to natural predators such larvae could never recovered. Furthermore, the cowpea plants provided *M. vitrata* with natural habitat thus increasing their survival rate unlike in laboratory set up. In the present study, the application of the EPNs on cowpea was done once and it would be interesting to investigate the effect of repeated doses on larval survival.

The percent flower damage by *M. vitrata* on treated plants was lower compared to the untreated control plants and the difference was significant, though the difference in larval mortality was not significant in the screen house conditions. The EPNs infection may have made *M. vitrata* less active hence reduced mobility and feeding resulting in decreased flower damage. Thus to increase pest mortality, time and method of application, pest most vulnerable stage, natural nematode enemies and dosage should be considered.

As observed from the laboratory and screen results, the period of time a pest is exposed to the EPNs is an important factor influencing percentage pest mortality. In this regard, linear increase in the percentage mortality of *M. vitrata* larvae with increase in exposure time was observed. This observation is consistent with previous experiments on use of *H. indica* and *S. karii* for the control of Diamond back moth (*P. xylostella*) in cabbages under semi field conditions by (Nyasani *et al.* 2008).

In this study it was further observed that increased EPNs concentration was associated with increased pest mortality. This is in agreement with findings of Kulkarni *et al.* (2011) who reported *H. indica* dose-dependent relationship against tree pest teak skeletonizer (*Eutectona machaeralis*). Increase in EPNs concentration raised larval mortality thus a positive correlation. It is speculated in this study that high EPNs dose increased probability of *Maruca* infection by the nematodes. Similar studies with EPNs *Steinernema* and *Heterohabditis* species indicated that nematode concentration had direct impact on the pest
mortality. The findings are also in agreement with a report by Lalramliana and Yadav, (2009) who observed that the effects of EPNs against cabbage butterfly larvae (Pieris brassicae) are dose dependent.
5:2 Conclusions

In the present study, the two EPNs species, *H. indica* and *S. karii* were found to be pathogenic and infective to the 3rd larval stage of *M. vitrata* in the laboratory and screen house. However both nematode species were found to have negligible impact on *M. vitrata* larvae mortality in the screen house conditions irrespective of subjecting the pest to the high dose 400 dose of nematode per ml observed to cause highest mortality in the laboratory trials. At the same time no significant difference was obtained between the nematode treated and untreated (control) in the semi field study. Based on the findings of this study, therefore, one can conclude that:

1. The entomopathogenic nematodes *H. indica* and *S. karii* are pathogenic to *M. vitrata* under laboratory and semi field conditions and *H. indica* was more pathogenic than *S. karii* at a statistically significant level. The pathogenicity of these nematodes provides insight in understanding the effect of nematode concentration and time on pest mortality.

2. The pathogenicity of the two EPN species *M. vitrata* larvae under laboratory and screen house conditions was significantly different which is attributed to differences between laboratory and screen house conditions with the former being more favourable to the infectivity of the nematodes.

3. The EPNs *H. indica* and *S. Karii* have the potential for use as biological pesticides for control of *M. vitrata* in cowpea but there is need to carry out further research as recommended below in order to increase the mortality rates of *M. vitrata* larval stages. Such information can be useful in developing nematode based biopesticide forming part of integrated pest management.
5.3 Recommendations

1. The results of this study form a valuable resource for further research on pathogenicity of *H. indica* and *S. karii* at higher concentration (>400) on *M. vitrata* 3rd larvae in the laboratory and screen house conditions. This would help determine the concentration causing highest mortality within shortest time possible which can be further utilized to reduce damage of cowpea by the pest.

2. Further studies need be carried out to elucidate pathogenicity of *H. indica* and *S. karii* on *M. vitrata* egg and other larval stages. This would provide information on the stage to target with nematodes. In addition if pathogenicity on *M. vitrata* egg stage could be established, then it would be the best time to control the pest in order to reduce crop damage.

3. Based on findings from this study it is fundamental to evaluate if *H. indica* and *S. karii* can cause death in *M. vitrata* in the field. This is because cowpea is mainly grown under field conditions. Field results will provide a platform for the development of the two EPNs as biological control agents of *M. vitrata* in cowpeas under field conditions.

4. The results of single application of 400 doses of the two nematodes tested in the screen house in this study caused mortality in *M. vitrata*. Further work to assess effect of split or repeated application of EPNs *H. indica* and *S. karii* throughout the cropping period in the screen house and possibly in the field are necessary.
5. The pathogenicity of other EPNs on various developmental stages of *M. vitrata* is also recommended in the laboratory, screen house and field conditions. This would provide a basis for establishing EPN species that could be more or less pathogenic than *H. indica* and *S. karii* investigated in this study and thus add unto EPNs as potential agents of biological control.
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APPENDICES

Appendix 1: Relationship between mean number of nematodes that established in *Maruca* cadavers at different doses of *H. indica* and *S. karii*.

<table>
<thead>
<tr>
<th>TMT</th>
<th>Correlation Coefficient</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMT</td>
<td>1.000</td>
<td>-0.146</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.</td>
<td>0.729</td>
</tr>
<tr>
<td>N</td>
<td>60</td>
<td>8</td>
</tr>
<tr>
<td>Yield</td>
<td>Correlation Coefficient</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.146</td>
<td>1.000</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.729</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

Appendix 2: Mean larval mortality due to *H. indica* and *S. karii* treatment at different nematode dose in the laboratory.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory trial</td>
<td>Control</td>
<td>5</td>
<td>0.0000</td>
<td>0.00000</td>
<td>0.00000</td>
</tr>
<tr>
<td></td>
<td><em>H. indica</em></td>
<td>5</td>
<td>4.1333</td>
<td>1.59164</td>
<td>0.71181</td>
</tr>
<tr>
<td></td>
<td><em>S. karii</em></td>
<td>5</td>
<td>3.0667</td>
<td>1.65664</td>
<td>0.74087</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix 3: Screen house larval mean mortality due to *H. indica* and *S. karii* treatment at a dose of 400.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screen house</td>
<td>Control</td>
<td>5</td>
<td>0.6667</td>
<td>0.66667</td>
<td>0.29814</td>
</tr>
<tr>
<td></td>
<td><em>H. indica</em></td>
<td>5</td>
<td>1.8667</td>
<td>1.12052</td>
<td>0.50111</td>
</tr>
<tr>
<td></td>
<td><em>S. karii</em></td>
<td>5</td>
<td>1.6000</td>
<td>1.06458</td>
<td>0.47610</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td>0.165</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix 4: Comparison between the effects of treatments on *Maruca* larvae in the laboratory and Screen house at concentration of 400.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Concentration</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td>0</td>
<td>5</td>
<td>0.0000</td>
<td>0.00000</td>
<td>0.00000</td>
<td>0.000</td>
</tr>
<tr>
<td>Both species</td>
<td>400</td>
<td>10</td>
<td>3.6000</td>
<td>1.63148</td>
<td>0.51592</td>
<td></td>
</tr>
<tr>
<td>Screen house</td>
<td>0</td>
<td>5</td>
<td>0.6667</td>
<td>0.66667</td>
<td>0.29814</td>
<td></td>
</tr>
<tr>
<td>Both species</td>
<td>400</td>
<td>10</td>
<td>1.7333</td>
<td>1.03994</td>
<td>0.32886</td>
<td>0.059</td>
</tr>
</tbody>
</table>
**Appendix 5:** Analysis of variance of treatment in the laboratory and screen house at nematode concentrations of 400.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory trials</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>43.200</td>
<td>1</td>
<td>43.200</td>
<td>23.443</td>
<td>0.000</td>
</tr>
<tr>
<td>Within Groups</td>
<td>23.956</td>
<td>13</td>
<td>1.843</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>67.15</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screen house</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>3.793</td>
<td>1</td>
<td>3.793</td>
<td>4.283</td>
<td>0.059</td>
</tr>
<tr>
<td>Within Groups</td>
<td>11.511</td>
<td>13</td>
<td>0.885</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15.305</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Appendix 6:** The net effect of treatment on flower damage  
A: *H. indica* and *S. karii.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower damage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. indica</em></td>
<td>5</td>
<td>1.6667</td>
<td>0.84984</td>
<td>0.38006</td>
<td>0.038</td>
<td>0.850</td>
</tr>
<tr>
<td><em>S. karii</em></td>
<td>5</td>
<td>2.0667</td>
<td>0.92496</td>
<td>0.41366</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B: Control and *H. indica*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower damage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>3.2000</td>
<td>1.16905</td>
<td>0.52281</td>
<td>0.569</td>
<td>0.472</td>
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<tr>
<td><em>H. indica</em></td>
<td>5</td>
<td>1.6667</td>
<td>0.84984</td>
<td>0.38006</td>
<td></td>
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</table>
### Appendix 7: Effect of time on larval mortality in the laboratory and screen house conditions at 400 concentration for both nematode species

<table>
<thead>
<tr>
<th>Trials</th>
<th>Exposure time in hours</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory</td>
<td>24</td>
<td>3</td>
<td>0.6667</td>
<td>0.66667</td>
<td>0.38490</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>3</td>
<td>2.2222</td>
<td>2.16880</td>
<td>1.25216</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>3</td>
<td>2.7778</td>
<td>2.54588</td>
<td>1.46986</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>3</td>
<td>3.0000</td>
<td>2.64575</td>
<td>1.52753</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>3</td>
<td>3.3333</td>
<td>2.88675</td>
<td>1.66667</td>
</tr>
<tr>
<td>P-value</td>
<td>0.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screen house</td>
<td>24</td>
<td>3</td>
<td>0.0000</td>
<td>0.00000</td>
<td>0.00000</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>3</td>
<td>0.8889</td>
<td>0.83887</td>
<td>0.48432</td>
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<tr>
<td></td>
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<td>0.69389</td>
<td>0.40062</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>3</td>
<td>2.1111</td>
<td>0.69389</td>
<td>0.40062</td>
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<td>P-value</td>
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</table>

### Appendix 8: The effect of nematode concentrations on larval mortality in the laboratory

<table>
<thead>
<tr>
<th>Nematode Dose</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
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<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>0.0000</td>
<td>0.00000</td>
<td>0.00000</td>
</tr>
<tr>
<td>100</td>
<td>10</td>
<td>2.8000</td>
<td>1.91936</td>
<td>0.60696</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
<td>2.9000</td>
<td>1.71450</td>
<td>0.54217</td>
</tr>
<tr>
<td>300</td>
<td>10</td>
<td>3.3667</td>
<td>1.60593</td>
<td>0.50784</td>
</tr>
<tr>
<td>400</td>
<td>10</td>
<td>3.6000</td>
<td>1.63148</td>
<td>0.51592</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.004</td>
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</tr>
</tbody>
</table>
### Appendix 9: Effect of time on larval mortality at different concentration in the laboratory trials for both nematode species

<table>
<thead>
<tr>
<th>Time of exposure</th>
<th>Mean mortality</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.5926</td>
<td>0.54716</td>
<td>0.18239</td>
</tr>
<tr>
<td>48</td>
<td>2.1852</td>
<td>1.32404</td>
<td>0.44135</td>
</tr>
<tr>
<td>72</td>
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<td>1.45721</td>
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### Appendix 10: Interaction between time and nematode concentration on larval mortality.

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<th>Mean Square</th>
<th>F</th>
<th>P-value</th>
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<td>5.991</td>
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<td>15.255</td>
<td>25.662</td>
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<td>12.225</td>
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