USE OF ECO-FRIENDLY STRATEGIES IN SUPPRESSION OF ROOT-KNOT NEMATODES IN FRENCH BEAN (*Phaseolus vulgaris)* IN KENYA

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DECLARATION

This thesis is my original work and has not been presented for the award of a degree in any other University.

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DEDICATION

I dedicate this work to my wife Phoebe, my sons Rob, Roy and Ryan.

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Acronyms and abbreviations

COLEACP	Europe-Africa-Carribean-Pacific Liason Committee
EU	European Union
EUREP	Euro-Retailer Produce Working Group
GLOBAL GAP	Global Good Agricultural Practices
GAP	Good Agricultural Practices
HCDA	Horticultural Crops Development Authority
J2	Root-knot nematode second juvenile stage
MBTOC	Methyl Bromide Technical Options Committee
PPN	Plant Parasitic Nematode
RKN	Root-Knot Nematode

Abstract

Root-knot nematode (*Meloidogyne* spp.) is one of the major pests infecting French bean (*Phaseolus vulgaris* L.) which is an important vegetable crop for export in Kenya and a potential income earner to smallholder farmers. Essentially, the control of root-knot nematodes (RKN) involves the use of synthetic nematicides. However, due to high costs and increased safety concerns in regard to toxicity of these nematicides, research for eco-friendly and sustainable methods of controlling RKN have been on the rise. The objective of this study was to evaluate and compare the efficacy of different green manure crops, eco-friendly nematicides and conventional nematicides in suppressing RKN in French bean. Field and greenhouse experiments were carried out in a Randomized Complete Block Design and Complete Randomized Designs, respectively at Finlays Horticulture Kenya Limited, Kingfisher Farm in Naivasha to evaluate the efficacy of four green manure crops; Caliente mustard (*Sinapis alba*), Nemat (*Eruca sativa*), Sudan grass (*Sorghum sudanese*) and African Marigold (*Tagetes minuta*) compared with Metam sodium and also eco-friendly nematicides; Rigel-G (salicylic acid), Phyto protect (Sesame oil extract), Mytech (*Paeciliomyces lilacinus*) Neemraj (azidarachtin) compared to Vydate® (Oxamyl) used as a positive control in suppressing root-knot nematodes in French bean. In the field, the green manure crops were grown in micro-plots measuring 3 m by 4 m for a period of 12 weeks, then chopped and incorporated immediately back into the soil. The fields were infested with second stage juveniles (J2) of *Meloidogyne* spp. The blocks were watered and left for 14 days prior to planting with the French bean cultivar Serengeti. In the greenhouse experiment, 5 kg pots were filled with steam-sterilized soil and mixed with known weights of macerated green manure crops harvested from the field. The pots were infested with 200 second stage juveniles (J2) of

Meloidogyne spp. per pot. Trials to evaluate the efficacy of different eco-friendly nematicides and bio-control agents in suppressing RKN with the conventional nematicides in the market involved two seasons of trials conducted under field conditions. The treatments were administered according to recommended rates and stages. Damage on plants was assessed based on galling indices, crop biomass and yield whereas nematode reproductive potential was assessed based on the J2 counts. There was a significant ($P \leq 0.05$) difference in RKN population densities, galling indices and yield between the green manure treatments and the control in both field and greenhouse trials. Root-knot nematode densities and galling indices were highest in the untreated control, a clear indication that the green manure crops and eco-friendly nematicides suppressed the RKN. There was however, no significant ($P \geq 0.05$) difference between efficacy of green manure plants and Metam sodium in the greenhouse. The effective green manure crops reduced the population densities of RKN by over 90% in the greenhouse condition and by over 67% under field conditions. There was no significant difference ($P \geq 0.05$) in the nematode population densities and galling indices observed between the eco-friendly nematicides and the conventional nematicide (Vydate®). The results of this study clearly indicate that the green manure plants and eco-friendly nematicides can be fully adopted to suppress RKN in French beans as alternatives to conventional nematicides.

CHAPTER ONE

1.0 Introduction

1.1 Background

French bean is among the major horticultural crops produced in Kenya for export. The export season of French beans ranges from November to April. Most French bean producers use irrigation water for production in their intensively cultivated farms. About 50 mm of water is applied using an overhead or furrow system of irrigation weekly. The furrow system of irrigation, however, is commonly used in Kenya (Ndegwa *et al*., 1999).

French bean production is labor and capital intensive. The costing of the inputs such as fertilizers, seed, labor for land preparation and harvesting are estimated to have a total variable cost of about Kshs. 11,539.6/acre with the gross margin per acre being about Kshs. 16,530 (SNV, 2012).

The main challenges facing French bean production in Kenya include pests and diseases, regulations, standards and laws, access to inputs and equipment, knowledge, information and access to suitable financing for smallholder farmers (Monda *et al*., 2003; SNV, 2012). Farmers are aware of some bio pesticides used for management of some pests but lack information on their effectiveness for safe plant disease management.

Nematodes are important pests that cause root dysfunction by generally reducing the rooting volume, nutrient and water use efficiency (Noling, 2005). The root-knot nematodes (RKN), *Meloidogyne* spp. are the most devastating (Williams–Woodward and Davis, 2001).

The diversity of soil nematodes in agro-ecosystems and the total abundance of members of different trophic levels are largely controlled by the biophysical, chemical and hydrological conditions of the soil (Yeates and Bongers, 1999). The soil as a habitat for nematodes can therefore be changed through management practices such as monoculture, tillage, drainage, application of agrochemicals, irrigation and organic mulch (Yeates, 1999). Crop yield losses due to RKN have been on the increase in the tropics and sub-tropics (Netscher and Sikora, 1990). This has been attributed to replacement of shifting cultivation with continuous cropping systems in almost all subsistence farming, mono-cropping and narrow rotations in large scale vegetable production. It occurs mainly where irrigation is practiced and poor nematode management is practices are carried out due to the perception that nematodes are not important crop pests (Bridge, 1996).

Many of the soil amendments used as nutrient sources for crop production have been found to control plant parasitic nematodes. Such materials include green manure, cow dung, poultry droppings, dried crop residues, botanicals, camel dung, and composted agro-industrial wastes (Lord *et al*., 2011). A remarkable reduction in nematode populations both in greenhouse and field conditions with an increase in crop yield and growth has been achieved (Abubakar *et al*., 2004; Nico *et al*., 2004). An alternative management strategy that is increasingly receiving interest is bio-fumigation. The concept of bio-fumigation was described by Kirkegaard *et al*. (1993). Bio-fumigation was introduced as an alternative to Methyl bromide by the Methyl Bromide Technical Options Committee (MBTOC) under the Montreal Protocol (TEAP-MBTOC, 1997).

Many cover crops such as bio-fumigant mustards can offer additional soil conditioning benefits including improvement of soil quality and control of soil-borne diseases, pests and weeds. The cover crops of Brassicae family are very rich in sulphur, and incorporating them in the soil not only increases organic matter content but also suppresses various soil-borne diseases, pests and weeds. Bio-fumigation effect is largely related to the high concentration of glucosinolates (GLs) in many mustard species (Lord *et al*., 2011). Glucosinolates are basically the precursors of isothiocyanates (ITCs) which have broad biocidal activity. Such crops have been used extensively with varying degrees of success which has been attributed to three key factors, 1) poor incorporation which reduces the fumigant effect, 2) incomplete conversion of GLs to ITCs and 3) insufficient bio-fumigant potency (Rosangela *et al.,* 2011). More studies are needed to find out the best bio-fumigants suited for local conditions.

1.2 Problem statement

Nematodes of the genus *Meloidogyne* (RKN) are very destructive pests that have a wide host range. In Kenya, French bean is grown mainly in Mwea, Naivasha, Meru, Oloitoktok, and Mweiga-Naromoru in intensively cultivated fields. Root-knot nematode is a major pest of French bean (*Phaseolus vulgaris* L.) causing yield losses of up to 45-60 per cent (Mullin *et al*., 1991). Nematicides have been used to control these pests with remarkable results. However, there are problems of high costs and availability, particularly to resource poor farmers coupled with environmental hazards attributed to chemical nematicides of restricted classes. There is scarcity of information on available green manures that can effectively manage RKN and calls for a search for alternative options that are cheaper, readily available and sustainable with minimal negative effects on the environment.

1.3 Justification

Plant parasitic nematodes are a major problem in crop production in Kenya, limiting crop potential in a country that is often faced with food shortages. Many green manure crops have not been evaluated for their reaction to RKN. Following the withdrawal of Methyl Bromide, Metam sodium has been used as an alternative with some degree of success. However, Metam sodium has not been largely accepted due to the fact that it is quite destructive on other beneficial soil fauna such as beneficial fungi, nematodes and bacteria resulting in other opportunistic soil borne pathogens which were initially kept in check by the beneficial soil micro-organisms. Although chemical nematicides play an important role in the management of RKN in modern agriculture, they are persistent and have long-term effect on non target organisms. This has raised the need to introduce effective but safe substitutes. The use of various parts of indigenous plants as botanical extracts is important in pest management considering their environmental safety. There is therefore a continuous need to evaluate available and sustainable products for the management of RKN.

1.4 Objectives

1.4.1 General objective

To develop a sustainable strategy for the management of root-knot nematode (*Meloidogyne* spp.) in French bean (*Phaseolus vulgaris* L).

1.4.2 Specific objectives

1. To evaluate the potential of different green manure plants in suppressing RKN in French bean.
2. To compare the efficacy of different green manure crops with Metam sodium as fumigants in suppression of RKN in French bean production.

3. To compare the efficacy of different eco-friendly nematicides with Vydate® (oxamyl) against RKN in French bean.

1.4.3 Hypotheses

a) Green manure plants do not significantly suppress RKN in French bean.

b) There is no significant difference in RKN suppression between green manures and Metam sodium.

c) There is no difference in RKN control between eco-friendly nematicides and Vydate®.

CHAPTER TWO

2.0 Literature review

2.1 French bean production in Kenya

French bean (*Phaseolus vulgaris* L.) is a key export vegetable crop from Kenya to the United Kingdom and European Union (EU). Other emerging markets for Kenyan French beans are Saudi Arabia and South Africa (HCDA, 2005). French bean is grown both on large scale (50 acres) by well-established commercial farms and by smallholder farmers owning as little as a quarter of an acre. The smallholder farmers are either organized into groups or co-operatives through which they sell to the big exporters (Harou, 2011).

The recent past has seen a general increase in French bean production in Kenya. In 2012, Muranga County was the highest producer of French bean (18,945 tonnes) earning Ksh. 601 million (Table 1). Kirinyaga County has led in production for a long time but due to the reduction in the production area, a decline in production has occurred (HCDA, 2005).

Table 1: French bean production in selected Counties of Kenya. (Source; Ministry of Agriculture)

Year	2010			2011			2012			
County	Area (Ha)	Quantity (Ton)	Value Kshs (million)	Area (Ha)	Quantity (Ton)	Value Kshs (Million)	Area (Ha)	Quantity (Ton)	Value Kshs (million)	share value
Muranga	381	2,336	78	760	2,557	92	627	18,945	601	35%
Kirinyaga	1,968	26,216	1,027	2,029	27,325	1,076	1,857	10,965	472	28%
Embu	222	1,532	33	124	1,468	190	71	804	116	7%
Meru	112	710	16	395	3,339	185	311	2,905	114	7%
Total	**4,387**	**36,108**	**1,470.4**	**4,798**	**38,945**	**1,614.2**	**4,128**	**44,139**	**1,697**	**100%**

2.2 French bean production constraints

Production of French beans faces many challenges ranging from variety selection, lack of proper extension services to poor market channels. There are a number of pests and disease constraints facing French bean growers. They include Fusarium wilt due to *Fusarium oxysporum* and root galling due to RKN including the disease complexes that are brought by the interaction of RKN and *Fusarium* and bacterial wilt. Other major pests encountered by French bean growers are whiteflies, thrips and caterpillars. The broad host range of the root-knot nematode (*Meloidogyne* spp.) has worsened the situation since it affects nearly all the possible rotational food crops grown by the small scale farmers and large exporters (Monda *et al*., 2002).

There are stringent quality requirements by French bean-importing countries. This also affects production of French bean because large amounts of French beans are rejected hence losses to farmers (Monda *et al*., 2003). Postharvest losses at farm level have been estimated to range from 1 – 20%. This occurs during sorting and grading where overgrown pods, those showing physiological defects, pest and disease damage are considered as rejects. Approximately 25% of French beans are lost during processing. Furthermore, there is a huge disparity between the domestic (Ksh. 1.6 billion) and export (Ksh. 4.4 billion) values due to the farm gate prices offered by exporters to farmers who in turn make large profit margins (HCDA, 2010).

Plant growth and yield are mainly limited by nematodes which cause root dysfunction. The nematodes generally reduce the rooting volume, nutrient and water use efficiency (Noling, 2005). The root-knot nematode, *Meloidogyne* spp. are the most devastating (Williams–Woodward and Davis, 2001). The use of nematicides is by far the common method of managing RKN. Most nematicides have negative environmental consequences. Therefore, continuous assessment of environmentally sound nematicides is important.

2.3 Nematode biology and life cycle

Nematodes are worm-like multi-cellular, bilaterally symmetrical, non-segmented organisms with well-developed reproductive and digestive systems but have primitive excretory and nervous systems (Coleman and Crossley, 1996). These organisms do not have respiratory and circulatory systems. They are bisexual and undergo four molting stages from egg to adult. Nematodes are found nearly in all agro-ecosystems where they interact directly and indirectly with plants and other micro-fauna, regulating decomposition and release of nutrients to the plants (Neher, 2010; Yeates *et al.*, 1993).

All PPN have a similar life cycle (Luc *et al.*, 2005). When the host's temperature and surroundings are unfavorable, females of *Meloidoyne* spp. produce a few eggs or may not produce eggs at all (Moens *et al*., 2009). Under less favorable conditions, such as extreme temperatures, a single female produces 300 - 500 eggs while under optimum temperatures of 27^0C it can produce more than 2800 eggs (Agrios, 2005). Females lay eggs in sac-like gelatinous matrices (Evans and Perry, 2009). A new generation can arise within 25 days but under less favorable conditions, the life cycle may be prolonged to 30 or 40 days or development may cease entirely (Agrios, 2005).

During the dormant stage, each egg takes on a thick outer covering to protect it during the inactive period. The first larval stage develops inside the egg and undergoes the first moult within the egg to become a second-stage juvenile (J2). The latter emerges from the egg into the soil where it moves until it finds a susceptible root by chemotaxis. Only the J2 are infective (Agrios, 2005). The third-stage juvenile (J3) lacks a stylet whereas the fourth-stage juvenile (J4) can be distinguished either as male or female while the final molt becomes a free-living male nematode or a parasitic adult female. The importance of temperatures in the life cycle of RKN was elucidated by Agrios (2005) where plant penetration by the J2 occurs between 10^{o}C and 35^{o}C, with 27^{o}C being the optimum temperature depending on the species.

The RKN second stage juvenile (J2) usually enters the plant through roots and modifies some root cells into feeding cells, a phenomenon necessary for the reproduction and development of the parasite. The roots of susceptible plants, once penetrated by the RKN juveniles have their cambial root cells transformed into specialized feeding cells named “Giant cells”. These are metabolically active cells induced and maintained in susceptible hosts by the feeding activities of the nematode (Hussey and Jansen, 2002) and are permanent feeding sites for the parasite throughout its life cycle (Hussey *et al.,* 1994).

2.3.1 Nematode ecology

Nematodes are generally free-living in marine, freshwater and soil environments but a large number of species are parasitic on different kinds of plants and animals (O’Halloran and Burnell, 2003). The parasitic species are of considerable agricultural, clinical and veterinary importance as pests of plants and parasites of man and livestock respectively (Ahmad and Jairajpuri, 1993). Nematodes are found at the bottom of lakes, rivers and at enormous depths in the oceans. Some species can withstand temperatures constantly below freezing point while others live in hot springs (Ferris and Melakeberhan, 2008). Some parasitic nematodes are migratory, and move in and out of root tissues, while some are sedentary and effectively don’t move at all (Flint, 1998).

In almost every soil sample, nematodes from five trophic levels namely bacteriovores, fungivores, herbivores, predators and omnivores are represented (Yeates, 1999). Due to their biological diversity and particularly feeding habits, nematodes are an integral part of the food webs in soil ecosystems (Yeates *et al*., 1993). Plant parasitic nematodes (PPN) are slender, elongated, fusiform, tapering towards both ends and circular in cross section. *Meloidogyne arenaria*, *M. incognita* and *M. javanica* are the most encountered species in the tropical regions (Semblat *et al*., 1998).

Texture is one of the most important of the soil characteristics as it influences many other properties of great significance to land use and management (Brown, 2003). This also affects nematode movement within a field e.g. nematode juveniles in sandy soils are able to move horizontally and vertically over distances of up to 75cm in 9 days (Prot, 1977). Nematode survival, emergence and disease severity are influenced by soil texture which is directly related to water holding capacity and aeration. It was however found that nematode migration decreased with increasing clay content of the soil, with no migration in soils with more than 30% clay (Prot and Van Gundy, 1981). The changes in particle-size distribution and structural change (size, shape and arrangement of the soil aggregates and voids) affect the physical behaviour of the soil and hence its exploration by soil organisms.

2.3.2 Nematode pathology

Plant parasitic nematodes (PPNs) are biotrophic parasites which obtain nutrients from the cytoplasm of living roots, stems and leaf cells for development, growth and survival (Luc *et al*., 2005). Nematodes have evolved diverse parasitic strategies and feeding relationships with their host plants (Davis *et al*., 2004). They possess a hollow and a protrusible feeding structure, the stylet and a pharynx, which has undergone morphological and physiological adaptations to maintain the feeding relationships (Lee, 2002). Depending on the species, they feed from the cytoplasm of unmodified living plant cells or have evolved to modify root cells into elaborate feeding cells as in RKN (Lee, 2002; Luc *et al*., 2005). The nematodes use their stylet to pierce and penetrate the cell wall of a plant cell, inject gland secretions through the stylet orifice into the cell and withdraw and ingest nutrients from the cytoplasm (Bilgrami and Gaugler, 2004).

Nematodes that enter the root tissue also use their stylet to pierce openings and/or inject secretions to dissolve (intracellular migration) or weaken (intercellular migration) the cell wall or middle lamella (Lee, 2002; Gaugler and Anwar, 2004). Generally, all PPNs damage plants by direct mechanical injury using the stylet during penetration and/or by secretion of enzymes into the plant cells while the nematode is feeding (Gheysen and Jones, 2006). The physical presence of endo-parasitic nematodes inside the host also affects the functioning of the host. As a result of nematode feeding, the architecture and extent of the root system is altered, so that it is less efficient at taking up nutrients and water from soil (Lee, 2002). The extent of nematode damage depends to a large extent on the inoculum density (level of infestation). Low or moderate numbers of nematodes may not cause much injury but large numbers severely damage or kill their hosts (Luc *et al*., 2005).

2.4 Plant parasitic nematodes affecting beans

Many plant parasitic nematodes have been associated with leguminous crops (Davis and Mitchum, 2005). Root-knot nematodes (*Meloidogyne spp*.), lesion nematodes (*Pratylenchus* spp.), *Tylenchus* spp., *Criconemella* spp., *Aphelenchus* spp., sheath nematodes (*Hemicycliophora* spp.), stubby root nematodes (*Trichodorus* spp.) and others are associated with beans (Kimenju *et al.,* 1999). *Meloidogyne* spp. are of considerable importance due to their prevalence and wide distribution in the warm temperate and tropical regions of the world where subsistence agricultural systems predominate (Perry and Evans, 2009).

2.4.1 Root-knot nematodes *(Meloidogyne* spp.*)*

Root-knot nematodes (RKN) belong to the kingdom Animalia, phylum Nematoda, class Nemata, subclass Sercenentea, order Tylenchida, suborder Tylenchina, family; Meloidogynidae, and genus *Meloidogyne* (Karssen *et al*,. 2006). There are more than 80 nominal species of *Meloidogyne* (Karssen, 2002), of which *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* (Chitwood, 1956) are of economic importance in bean production across the world (Bridge and Starr, 2007).

Root-knot nematode populations consist of males and females, which are easily distinguished morphologically. The males are wormlike and are about 1.20 - 1.50 mm long and 30 - 60µm in diameter (body width). Mature females are pear shaped and are about 0.40 - 1.30 mm long by 0.27 - 0.75 mm in diameter. Second-stage juveniles are vermiform in shape while third and fourth stage juveniles are sausage shaped and microscopic in size (Siddiqi and Alam, 1987).

2.4.2 Management of RKN

All sectors of the vegetable industry are keen to adopt production methods based on low pesticide regimes as a result of increased health and environmental consciousness of food production techniques (FAO, 2013). There is therefore a continuous need of evaluating new products for the management of plant parasitic nematodes. The initial impact of this in Kenya is on the commercial export producers who are attempting to meet European Union standards and codes of practice through the initiative on harmonization of pesticide use. EurepGAP is an initiative of retailers belonging to the Euro-Retailer Produce Working Group (EUREP), a partnership of agricultural producers and their retail customers. EurepGap aims at meeting accepted standards and procedures for the global certification of Good Agricultural Practices (GAP) (Chandra, 2006).

Previously used chemicals like Furadan (carbosulfan) and Vydate® (oxamyl) have been withdrawn or used restrictively due to their detrimental effect on the environment, hazardous effects on mankind, and also due to pressure from the market as a result of the implementation of GlobalGAP (EUREPGAP) standards. The growers, both small and large have been left with nearly no viable alternative to use against RKN (SNV, 2012). The control of PPN is a difficult task that has mainly depended on chemical nematicides for decades and remarkable reduction of nematode population has been achieved (Akhtar and Malik, 2000). Although soil nematicides are effective and fast-acting, they are currently being reappraised with respect to the environmental hazards and human health (Wachira *et al*., 2009).

In addition, nematicides are relatively unaffordable to many small-scale farmers. Inventing alternative RKN management strategies that do not pollute the environment has been emphasized to researchers, farmers and scientists (Mashela *et al*., 2008). There are lots of alternative strategies that have been reported by researchers and this includes application of soil organic amendments such as crop residues and animal manures, heat treatment, soil solarization and crop rotation with non-hosts for managing RKN (Oka *et al*., 2007).

2.4.2.1 Cover crops

The loss of methyl bromide as a registered soil fumigation treatment created a need for alternative methods of nematode management. One possible method is the use of cover crops that exert a nematicidal effect, especially when incorporated into the soil as green manures. If incorporated, the decomposing green manure crop supplies nutrients to the next crop, and incorporated plant material may also improve water infiltration, soil tilth and add organic matter to the soil (Mwangi *et al.*, 2006). Nematicidal effects of decomposing plant amendments have long been known, and crops as diverse as Holly leaves (*Ilex aquifolium),* crotalaria (*Crotalaria spectabilis* Roth*.),* castor bean (*Ricinus communis* L.), velvet bean (*Mucuna deeringiana* L*.)* and rapeseed (*Brassica napus* L.) have been shown to suppress RKN (*Meloidogyne arenaria* and *M. chitwoodi*), with varying degrees of success (Mojtahedi *et al*., 1991).

Typically, crops high in nitrogen (N) with a low C:N ratio or those containing tannins, phenols, or glucosinolates have nematicidal activity (RodrõÂguez-KaÂbana *et al*., 1987). For green manure crops to be nematode-suppressive, residues are often added at rates ranging from 1% to 10% of total soil volume (0±15 cm soil depth) (Mian and RodrõÂguez-KaÂbana, 1982). In many previous studies, crop residues were not grown as an *in-situ* cover crop, but were instead transported to the site and incorporated (RodrõÂguez-KaÂbana *et al*., 1987). For example, rapeseed incorporated (1 kg rapeseed in the top 15 cm of 19-L micro plots) after 2 months of growth reduced populations of *M. chitwoodi* (Mojtahedi *et al*., 1991).

Many cover crops or green manures that have demonstrated nemastatic properties may be less efficacious when incorporated at rates similar to those found in forage situations. Although, cover crops such as common vetch show resistance to several species of RKN (Kimenju *et al*., 2008), the ability of such crops to suppress root-knot nematode populations has largely been documented from greenhouse evaluations (Akhtar and Malik, 2000). The ability of such crops to suppress nematode populations for a Subsequent crop in the field situations is not well documented. This is particularly true for winter cover crops such as vetch and clover, which growers often perceive as nematode suppressive (Ingels, 1998).

2.4.2.2 Use of green manures in suppressing RKN

Green manure plants play an important role in crop production as short duration leguminous plants that increase soil fertility and yields of subsequent crops especially in nitrogen depleted soils. These plants are increasingly being adopted and evaluated for soil fertility improvement in Kenya (Szott *et al*., 1999). A few of the green manure plants are thought to have resistance or even immunity which can be utilized in the management of PPN (Kimenju *et al*., 2008). Application of green manures in the soil is not only beneficial for disease management but also for improving the plant growth and productivity. On the other hand, its application leads to build-up of beneficial micro-flora that keep the plant healthy and help to reduce the PPNs around the rhizosphere (Oka *et al*., 2007). Green manures such as cabbage and cauliflower leaves, chopped pineapple leaves, dry straw of rice, rye or oats and cotton wastes are reported to reduce the incidence of RKN in the field (Pakeerathan *et al*., 2009).

Application of oil-cake of Pongamia (*Pongamia glabra*) and Margosa (*Azadiracta indica*) each at the rate of 2.5 Mt/ha was very effective in reducing root-knot of okra and tomato (Singh and Sitaramaiah, 1994). Jourand *et al*. (2004) reported that *Crotalaria virgulata* subsp. *grantiana* leaf extracts had nematicidal properties hence the leaves could be used as both green manure and natural alternative to synthetic chemicals in integrated pest management strategies. Nazli *et al*.

(2008) reported that leaf extracts of quick stick plant (*Gliricidia sepium*) has some insecticidal, nematicidal and antibacterial activity and it causes 60% mortality of second stage juvenile of *M. incognita* (Pakeerathan *et al*., 2009).

Plants of the genus *Desmodium* spp. have been widely accepted as green manures in many parts of Western Kenya (Desaeger and Rao, 2000). The plant is highly palatable and makes such excellent forage that it has been termed as the alfalfa of the tropics (Skerman, 1977). Available evidence has shown that marigolds are antagonistic to RKN (Yen *et al*., 1998). Compounds present in plants such as Sun hemp (*Crotalaria jucea*) and Mexican marigold (*Tagetes minuta*) have been confirmed to be toxic to RKN (Kimenju *et al*., 2008).

2.4.2.3 Sudan grass (*Sorghum sudanese*)

Sudan grass hybrids are unrivaled for adding organic matter to depleted soils (Clark, 2007). These tall, fast-growing, heat-loving summer annual grasses can suppress some nematode species, smother weeds and penetrate compacted subsoil if mowed once. Sudan grass hybrids followed by a legume cover crop are a top choice for renovating over farmed or compacted fields. The hybrids are crosses between forage-type sorghums and Sudan grass (Ingels, 1998). Compared with corn, they have less leaf area, more secondary roots and a waxier leaf surface, traits that help them withstand drought (Sarrantonio, 1994). Like corn, they require good fertility and usually supplemental nitrogen for best growth. Compared with Sudan grass, these hybrids are taller, coarser and more productive (Clark, 2008).

Sudan grass does best in warm climate with rich loamy soils (USDA, 1993). Forage-type sorghum plants are larger, leafier and mature later than grain sorghum plants. Compared with

Sudan grass hybrids, they are shorter, less drought tolerant, and don't re-grow as well. Still, forage sorghum as well as most forms of Sudan grass can be used in the same cover-cropping roles as Sudan grass hybrids (Clark, 2007). All sorghum- and Sudan grass-related species produce compounds that inhibit certain plants and nematodes. They are not frost tolerant, and should be planted after the soil warms in spring or in summer at least six weeks before first frost (Clark, 2007). Sudan grass cannot be considered as a green manure unless there is ample nitrogen in the soil or a long period can elapse before it is necessary to use the land (USDA, 1993)

2.4.2.4 Marigold (*Tagetes* spp.)

Growing of nematode resistant or antagonistic plants is an environmentally favorable approach closely aligned to sustainable cropping principles (Hooks *et al*., 2010). Marigold (*Tagetes* spp.) is one of the most widely studied plant genera due to its allelopathic potential against PPNs. This plant belongs to the family Compositae. Marigolds' repressive effect on nematodes has been documented for over 50 years (Steiner, 1941). Tyler, (1938) reported that 29 marigold varieties were resistant to RKN (*Meloidogyne* spp.). Early sources indicate that marigolds could prevent the population increase of 14 genera of PPNs (Steiner, 1941; Oostenbrink *et al*., 1957; Suatmadji, 1969), encompassing endoparasitic, semi-endoparasitic, and ectoparasitic nematodes (Siddiqui and Alam, 1987). *Pratylenchus* spp. and *Meloidogyne* spp. are consistently affected by marigolds (Merwin and Stiles, 1988).

Marigolds are known to control a wide range of nematodes including the RKN and *Pratylenchus* spp. They can be used as a cover crop or in a crop rotation. When grown as a monoculture in high density their nematode suppressive effect is very high (Adediran *et al*., 2005). Marigolds reduce PPN populations by several means that include acting as a non-host or a poor host, producing allelopathic compounds that are toxic or inhibit PPN development, creating an environment that favors nematode antagonistic flora or fauna (Wang *et al*., 2002) and behaving as a trap crop (Pudasaini *et al*., 2008).

Allelochemicals are released into the microenvironment by Marigolds (*Tagetes* spp.). These chemicals include several compounds such as essential oils that are biologically active (Zygadlo *et al*., 1994). These essential oils are potentially allelopathic to nematodes and other plant pathogens such as *Alternaria solani* (Gómez-Rodríguez *et al*., 2003). The allelopathic effect of marigolds responsible for nematode suppression is attributed to α-terthienyl (Gommers and Bakker, 1998). Marigolds, as a standing cover crops kill PPN and are effective following soil incorporation (Hooks *et al*., 2010). Jagdale *et al.* (1999) reported nematicidal activity of marigold root exudates but not of the homogenized extracts of marigold roots or leaves. According to Walla and Gupta (1997), marigold root extracts are more effective than extracts from aerial parts in inhibiting egg hatch of *M. javanica* while Cannayane and Rajendran (2002) found that root extracts of *T. erecta* were highly nematicidal to *M. incognita* under *in vitro* conditions.

Research data from some studies appear not to support the idea that nematicidal activity is associated mainly with functioning marigold roots. Siddiqi and Alam (1987) found that all above ground parts (flower, leaf and stem) of *T. lucida*, *T. minuta*, and *T. tenuifolia* when incorporated into the soil reduced root galls and population densities of RKN and Reniform nematode (*R. reniformis)* on tomato and eggplant. Stunt nematode (*Tylenchorhynchus brassicae*) population densities on cabbage and cauliflower in pot experiments were also reduced.

Nematode suppression can be enhanced by the activity of endophytic bacteria (Sturz and Kimpinski, 2004) and stimulation of nematode-antagonistic organisms (Oduor-Owino, 2003; Kimenju *et al*., 2004). For example, *Tagetes patula* 'Boy-O-Boy' was found to enhance activity of nematode-antagonistic microbes (Ko and Schmitt, 1996). However, incorporation of marigold into the soil did not increase the antagonistic activity sufficiently to suppress *R. reniformis* populations or enhance subsequent pineapple plant growth (Ko and Schmitt, 1996). Wang *et al*. (2002) demonstrated that *T. erecta* increased nematode-trapping fungal population densities a month after soil incorporation in a field trial. However, enhancement of nematode-trapping fungi by *T. erecta* amendments was a short-term effect and was no longer detectable shortly after pineapple planting (Wang *et al*., 2002).

Marigolds have been found to have negative effects on beneficial organisms. Oduor-Owino (1992) found that *T. patula* extracts inhibited parasitism of *M. javanica* and *M. incognita* eggs by *Fusarium solani* and *F. oxysporum* on water agar. Previously, Baker (1981) suggested that antifungal compounds are present in fresh marigold tissue. However, in a more recent study, Oduor-Owino (2003) found that amendment with composited leaves of *T. minuta* stimulated parasitism of *M. javanica* eggs by the antagonistic fungus *Paecilomyces lilacinus* in pots with field soil. Despite this increase in parasitism, the percentage of parasitized eggs was too low to cause significant suppression in root galling or nematode populations.

2.4.3 *Paecilomyces lilacinus*

This is a common soil hyphomycete found in majority of agricultural soils and it can be frequently isolated from eggs and females of the root-knot nematode (Pau *et al*., 2012). *Paecilomyces* spp. belongs to the division Eumycota, class Deuteromycetes, order Moniliales and family Moniliaceae. *P. lilacinus* shows fast hyphal growth. The conidiophores are up to 600 μm in height, and develop groups of lateral branches, from which 2-4 bottle-shaped phialides arise (Brand *et al*., 2010).

Conidiophores of the *Paecilomyces* spp. ramify in grouped branches or in an irregular form. The conidia are separated from the phialides in the form of chains. Conidia are ellipsoid, 2.5-3.0 μm long and 2.0-2.2 μm broad, lilac in color. The facultative egg parasite, *P. lilacinus,* is sometimes capable of infecting mobile nematode stages or sedentary females, but it is most aggressive against eggs (Tikhonov *et al*., 2002). The use of *P. lilacinus* as a biocontrol agent (BCA) depends on several factors such as age, virulence, viability, inoculum concentration, method of application, and environmental conditions (soil type, fertility, organic matter, fertilizers, temperature, pH, host susceptibility).

Studies have shown that *Paecilomyces lilacinus* is active against *M. incognita*. The fungus has been tested against *M. javanica* on tobacco, with or without the addition of nematicides like phenamiphos and ethoprop in microplot experiments for two years having vetch as winter culture (Brand *et al*., 2010). The fungus survived in the soil, although it was not capable of controlling nematode development, showing that the type of root system has an important role in control by *P. lilacinus* (Morton *et al*., 2004). Another *P. lilacinus* isolate was cultured in rice grains and tested under pot experimental conditions with the addition of chitin (0.1%, w/w) in tomato plants infected with *M. arenaria*. Results indicated that the combination of *P. lilacinus* and chitin were effective in the control of the *M. arenaria* (Denny, 2006).

Tomato plants infected with *M. incognita* were protected at different levels by the fungus *P. lilacinus*. The protection level against this nematode by *P. lilacinus* was positively correlated with the quantity of fungal spores applied and the period of application. The best protection against the nematode in tomato plants was achieved with 10 g and 20 g of fungus cultured in wheat, which resulted in a 3 and 4 times enhancement of tomato yield, respectively, compared to the plants infested with the nematode. The best protection achieved against the nematode was when the fungus was applied in soil 10 days before planting and during planting (Denny, 2006).

Spores of *P. lilacinus* produced in PDA were tested in micro plot experiments alone and in combination with chitin to control the nematode *M. incognita* in eggplant, tomato and chickpea. Results showed that the best treatment for the growth and development of plants affected by *M. incognita* with lower gall formation was the addition of the fungal spore suspension and chitin. When the fungus alone was applied, the treatment was less effective. However, when chitin was employed alone, root galling was higher in all plants tested indicating that chitin can be used as a substrate or food base for selective development of the BCA in the soil (Brand *et al.*, 2010).

Two hypotheses may explain the action of chitin against nematodes: chitin decomposition releases ammonia, which acts as a nematicide on J2 of RKN; or chitin may increase population of chitinolyitic microbiota, which parasitize nematode eggs and egg sacs (Mittal and Mukherji, 1995). *Paecilomyces lilacinus* was also applied alone and in combination with bone meal, horn meal and several oil cakes to control *M. javanica* in tomato plants. Results indicated that *P. lilacinus* was effective for inhibiting and parasitizing females, egg masses and eggs. Addition of organic fertilizers showed increased activity and persistence of *P. lilacinus* in soil (Mittal and Mukherji, 1995).

2.4.4 Natural plant extracts

There is increasing interest in the development and adoption of environmentally friendly tactics for managing nematodes all over the world as synthetic fumigants and other chemical nematicides become limited (Schneider *et al.*, 2003). Current alternative practices most often used include growing nematode resistant varieties and rotating with non-host crops. However, the wide host range of some nematode species and the unavailability of resistant varieties limit the use of crop rotation in several production systems. Crops and weeds may exhibit biochemical mechanisms to counteract the activity of nematodes. Numerous plant species, representing 57 families, have been shown to contain nematicidal compounds (Sukul, 1992).

2.4.4.1 Sesame oil (Phytoprotect EC)

Phytoprotect (Sesame oil) is a naturally derived nematicide for the control of parasitic nematodes on food and ornamental crops. It is manufactured by Brandt Consolidated Inc. USA and marketed in Kenya by Sineria East Africa. Phytoprotect may be applied by ground spray applications, drip irrigation, overhead irrigation systems or fertigation systems. Based on patented development work as a naturally derived control for parasitic nematodes, Phytoprotect EC is derived from extracts of specific cultivars of hybrid Sesame plants (Ehlers, 2011).

The mode of action includes nematoxic and nemastatic effects and possible disruption of nematode taxis to roots. These results account for the use of Sesame for many centuries in crop rotation for its residual nematicidal benefits to crops following Sesame. Versatile sesame oil is effective on both ecto-parasitic (live outside the plant root, and feed only on materials they can reach) and endo-parasitic nematodes (spend at least part of their life cycle inside the roots of the plants on which they feed on) (Brandt, 2008).

2.4.4.2 Neem extracts

Neem (*Azadirachta indica*) releases pre-formed nematicidal constituents into the soil. Neem products, including leaf, seed kernel, seed powders, seed extracts, oil, sawdust, and particularly oil cake, have been reported as effective for the control of several nematode species (Akhtar and Malik, 2000). Known in Asia for centuries, neem has become a topic of interest in the USA and Europe in the last 30 years. Several other plant terpenoids are also known to have nematicidal properties (Akhtar and Malik, 2000). Indian farmers, with no knowledge of the chemical constituents of neem by-products, have used them traditionally in pest control for centuries.

Neem constituents, such as nimbin, salanin, thionemone, azadirachtin and various flavonoids, have nematicidal action (Thakur *et al*., 1981). Neem oilcake has been used extensively in nematode control (Muller and Gooch, 1982). Beside the nematicidal effects, triterpene compounds in neem oil cake inhibit the nitrification process and increase available nitrogen for the same amount of fertilizer (Akhtar and Malik, 2000). Nematode control was obtained when Neem by-products and commercial products were used as seed coatings and bare-root dip treatments.

2.4.3.3 Salicylic acid (Rigel-G)

The role of salicylic acid (SA) in defense response of many crops such as tomato to RKN is conferred by the gene Mi-1 (Milligan *et al*., 1998), which is associated with a localized hypersensitive response (HR) by the cells at the site of infection (Sergio and Loffredob, 2006). RKNs enter the roots as motile second stage juveniles (J2) and migrate inter-cellularly to the vascular cylinder where they start to feed on living cells in the zone of differentiation (Williamson and Gleason, 2003). In resistant plants, localized cell death of the root tissue surrounding the nematode occurs, preventing the juvenile developing into the egg laying adult female.

The involvement of SA in salicylic acid response (SAR) and disease resistance in plants has been extensively studied. In particular, SA has been shown to play a crucial role in the induction of HR *(*Dong, 2004). Conversely, SA mediates SAR but not HR in tobacco treated with the elicitor PB90 from the cotton blight agent *Phytophthora boehmeriae* (Dong, 2004). The mechanisms by which SA may induce a defense response against pathogens have mainly been investigated in Arabidopsis and rely on NPR1 activation which promotes PR gene expression (Glazerbrook, 2005). However, enhancement of the SA signal may also occur through a signal amplification loop involving reactive oxygen species (ROS) (Glazerbrook, 2005). Rapid ROS production during the oxidative burst and an HR are defense responses that are considered hallmarks of gene-for-gene resistance.

Salicylic acid is able to inhibit the hydrogen peroxide (H_2O_2)-degrading activity of some catalase (CAT) isoenzyme, thus leading to an increase in the level of H_2O_2, which is generated by the HR-associated oxidative burst. H_2O_2 has been recognized as a diffusible signal for gene activation in HR, a trigger for hypersensitive cell death, a strong antimicrobial molecule as well as causing induction of PR-1 gene expression and conditioning SAR (Ryals *et al*., 1996). However, many reports evidenced that H_2O_2 may not be a second messenger of SA in SAR signaling (Branch *et al*., 2004).

It has already been reported that SA is somehow involved in the Mi-1-mediated defense responses to RKNs in tomato, although it is still unclear at which stage of the interaction and by which mechanism it may act. On the other hand, the ability of exogenously applied SA to induce resistance to RKNs in tomato is controversial, and, in some instances, it seems to be linked to the means of application. Generally, SA treatment seems not to significantly limit the degree of J2 infestation, although it may have an inhibiting effect on the nematode reproduction index (Molinari and Loffredo, 2006).

2.5 Biofumigation

Bio-fumigation, a term used more broadly was originally coined to describe pests and disease suppression by glucosinolate-containing plants arising from the biocidal properties of the glucosinolate hydrolysis. The term has been defined by several researchers as a process that occurs when volatile compounds with pesticidal properties are released into the soil during decomposition of plant material or animal by-products (Bello *et al*., 2000). Biofumigation described a phenomenon which had been observed for centuries and studied for decades but just received renewed interest fuelled by the need to seek alternative pest control options to widely used soil fumigants such as Methyl bromide and the desire to reduce dependence on synthetic pesticides (Messenger and Braun, 2000).

The vegetable industry relies so much on the top 15cm of soil. However, intensive production practices and reduced fallow periods reduce the resilience of soils leaving them less productive and prone to soil erosion. This may call for more inputs to maximize crop performance. Incorporating cover crops back into the soil can help recycle nutrients, build up soil organic matter and improve soil structure (ADAS, 2006). Identifying cover crops that fit well within the existing rotations is therefore an important consideration for many growers, as is their profitability. Many growers have always used annual grasses and cereals as cover crops, mainly because they are cheap, quick to establish and easy to manage (Johnstone *et al*., 2009). In addition, some of these grasses and cereals have been utilized by the farmers as animal feeds. Other cover crops usually used as green manures are from the legume family, and they represent a means of integrating fertilizer (N) in soils low in organic matter. Green manure decomposition time is usually short due to its high moisture content (Bierman and Rosen, 2005).

2.5 1 Plants as bio-fumigants

The main options for control of phytoparasitic nematodes include chemical nematicides, crop rotation and resistant cultivars when available. The broad host range of *Meloidogyne* species makes crop rotation difficult. Fumigant nematicides, although effective, have negative side effects that have led to their ban or restricted use. Resistance breaking populations of *Meloidogyne* are further challenging the use of resistant cultivars (Robertson *et al*., 2006).

Intensive production practices and extended fallow periods can reduce the resilience of soils, leaving them less productive and more prone to erosion. More inputs may be required to maximize crop performance in these systems. Incorporating cover crops back into the soil can help cycle nutrients, build soil organic matter and improve soil structure. Identifying cover crop options that fit well within existing rotations is therefore an important consideration for many growers, as is their profitability (Johnstone *et al*., 2009).

Among the large variety of organic materials from animal and vegetable origin that have been tried as biofumigants, agricultural by-products especially crop residues, are increasingly becoming of interest. Although crop residues are usually considered as “waste” materials without any value, and even as contamination sources, their incorporation into the soil contributes to the nutrient and organic matter recycling into the system, decreasing losses in organic matter and energy, as well as the costs needed to compensate those losses (Andrews, 2006). Besides, crop residue secondary metabolites which are generally produced inside plant tissues and released during the decomposition process, may be introduced into the soil (Gamliel *et al*., 2000).

Studies have found out that when crop by-products are used as biofumigant materials, the compounds released are mainly aldehydes and isothiocyanates, which have biocidal activity and have been related to the control of PPN (Riegel and Noe, 2000). Earlier, it was found that agricultural and agro-industrial residues such as orange juice, industry wastes, tomato, pepper, strawberry and cucumber crop by-products (alone and combined with sheep or commercial manure), showed promise for nematode control (Piedra Buena *et al*., 2006).

CHAPTER THREE

3.0 Materials and methods

3.1 Objective 1; Evaluation of green manure plants in suppressing RKN in French bean

3.1.1Study site

Both greenhouse and field experiments were conducted at Dudutech Research and Development Station, Finlay's Kingfisher Farm, Naivasha, longitude 36.2^{o} E latitude 0.8.498^{o} S. Naivasha is situated about 100 kilometres northwest of Nairobi. Average regional temperatures range between 7.3 and 22.7 ^{0}C; annual rainfall ranges from 156 mm to 1134 mm per month.

3.1.2 Root-knot nematode bulking and growing of green manure crops

The French bean crop was planted in rows in the experimental field previously cropped with French bean with a history of RKN infestation. Sampling was done and it was determined to have about 200 second stage juveniles (J2's) in 100 cm^3 soil. The beans were grown for a period of 8 weeks in order to build up the nematode population ahead of the trials. Subsequently, green manure plants namely Caliente mustard (*Sinapis alba*), Nemat (*Eruca Sativa*), African marigold (*Tagetes minuta)* and Sudan grass (*Sorghum sudanense*) (Plate 1) were planted on the periphery of the blocks measuring 3 m by 4 m for use in the experiments.

3.1.3 Field experiment

3.1.3.1 Preparation and application of green manures

Eight weeks after planting the green manures, the plants were incorporated into the soil by working in directly. Two weeks later, the French bean crop (Serengeti variety) was planted in plot with the four treatments (Caliente mustard, Nemat, African marigold and Sudan grass) being arranged in a randomized complete block design (RCBD) replicated five times. Drip irrigation was done three times a week while diammonium phosphate (DAP) fertilizer was added at a rate of 10g/plant at planting time and calcium ammonium nitrate (CAN) fertilizer was applied at the rate of 5g/plant twice at two and six weeks after germination.

Plate 1: Green manure plants before incorporation into soil. a) Sudan grass, and b) Caliente and Marigold on the background.

3.1.3.2 Assessment of French bean reaction to green manures

Assessment for various parameters was done 50 days after sowing. Data collected included nematode densities, galling indices and dry biomass. Five plants from each block were randomly selected and the roots washed free of soil for galling index determination. The plants were then dried in an oven to constant dry weights. A composite soil sample which consisted of five cores was obtained from each treatment from which nematodes were extracted from 200 cm^3 of soil using the modified Baermann technique described by Hooper (1990). The galling index was determined by counting the galls using a scale of 0-10 adopted from Bridge (1980) where; 0 = No galls on roots, 1 = Few small galls difficult to find, 2 = Small galls clearly visible, main root clean, 3 = Some larger galls visible, main roots clean, 4 = Larger galls predominate but main root clean, 5 = 50% of roots infested, galling on parts of main roots, reduced root system, 6 = Galling on main roots, 7 = Majority of main roots galled, 8 = All main root galled, few clean roots visible, 9 = All roots severely galled, plant usually dying and 10 = All roots severely galled, no root system, plant already dead. The galls were distinguished from the nitrogen nodules since nodules could be rubbed off while the nematode galls could not be removed.

3.1.4 Greenhouse experiments

3.1.4.1 Preparation and application of green manures

Eight weeks after planting the green manures, several plants of each green manure plant species were randomly selected and carefully uprooted at the start of flowering. The uprooted plants were separated into shoots and roots. The roots were dipped in a bucket of water to soak and the soil rinsed off. The roots and shoots were then oven-dried at 70^{0}C to constant weight.

The oven dried roots and shoots were macerated together and introduced into nematode-infested soil at the rate of 5% w/w so as to constitute a total of 5kg of medium per pot. The soil-green manure mixture was mixed thoroughly to homogeneity. The mixture was left in the greenhouse for a period of 14 days after which French bean cultivar Serengeti was planted at a seed rate of 4 seeds per pot. The four treatments (Caliente mustard, Nemat, African marigold and Sudan grass) were arranged in a completely randomized design (CRD) replicated five times. Drip irrigation was done three times a week while Di-ammonium phosphate (DAP) fertilizer was added at a rate of 10g/plant at planting time and calcium ammonium nitrate (CAN) fertilizer was applied at the rate of 5g/pot twice at two and six weeks after germination. Assessment was done as outlined in 3.1.3.2 above.

3.1.5 Nematode analysis

A composite soil sample of 200 cm^3 was taken from each plot in the field and from each pot in the green house from a depth of 5-10 cm at the beginning and end of the season. Root-knot nematodes were extracted from each of the 200 cm^3 of soil samples at Dudutech Laboratory using the modified Baermann method and enumerated in a 5 ml aliquot of nematode suspension. The results were expressed in nematode population densities per 200 cm^3 of soil.

3.2 Objective 2; Comparison of the efficacy of green manures with Metam-sodium in suppression of RKN in French bean

3.2.1 Experimental design

The experiment comprised of six treatments namely 1) Caliente mustard, 2) Nemat (*Eruca sativa*), 3) Sudan grass (*Sorghum sudanese*), 4) American marigold (*Tagetes minuta*), 5) Metam sodium and 6) Untreated control (soil infested with RKN only). The initial population of the RKN was determined prior to treatment and found to be 200 RKN per 200 cm^3 of soil. The French bean cultivar Serengeti was planted at the rate of 4 seeds per pot in pots filled with 5 kg soil infested with RKN and amended with the green manures as described in section 3.1.3. Treatments were arranged in a completely randomized design (CRD) with five replicates. Drip irrigation was done three times a week while Di-ammonium phosphate (DAP) fertilizer was added at a rate of 10g/plant at planting time and calcium ammonium nitrate (CAN) fertilizer was applied at the rate of 5g/pot twice at two and six weeks after germination. Assessment of French bean reaction to green manures and nematode analyses were carried out as outlined in section 3.1.4 and 3.1.5.

3.3 Objective 3; Evaluation of the efficacy of different eco-friendly nematicides in comparison to Vydate® (oxamyl) against RKN in French bean

3.3.1 Experimental design

The experiment consisted of six treatments namely; Rigel-G (*salicylic acid*), Phyto Protect (Sesame oil extract), Mytech (*Paecilomyces lilacinus*), Neemraj 0.3% (*Azadirachtin*), Vydate® (*Oxamyl*) as a positive control and an untreated control.

The treatments were arranged in a randomized complete block design (RCBD) in blocks measuring 3 m by 4 m replicated five times for two seasons. Watering was done on a daily basis in line with the normal agronomic processes for French beans as used in the farm. Rigel-G (*salicylic acid*) was applied at a rate of 2.5 mls/L, Phyto Protect (Sesame oil extract) at a rate of 10L/ha at planting, repeated at the rate of 6 L/ha in week 2, 6 L/ha^{-1} in week 4, 5 L/ha^{-1} in week 6 and finally 5L/ha^{-1} in week 8, Mytech (*Paecilomyces lilacinus*) at the rate of 125 $gmha^{-1}$ 14 days before planting, repeated 4 and 6 weeks after germination at the same rate, Neemraj 0.3% (*Azadirachtin*) at the rate of 3L/ha applied at planting, Vydate® (*Oxamyl*) was applied at a rate of 6 L/ha^{-1} soon after crop germination

The crop came into maturity at 9 weeks whereby harvesting started and continued for two weeks. Pod yields from different treatments were measured and recorded accordingly. At 12 weeks, the experiment was terminated and crops uprooted. The roots were assessed for galling which was scored as outlined in 3.1.4. Soil samples were subsequently taken to the laboratory for nematode analysis as outlined in 3.1.5.

3.4 Data collection

In the field, data of nematode densities was collected before planting, at mid-season and at the end of the experiment to determine nematode densities. Each plot was sampled and analysis done to determine the nematode population. Crop yield, dry plant biomass (root and shoot weights) and galling indices were determined 12 weeks after planting in the field experiment and 8 weeks after planting in the green house trials. In both the field and greenhouse, 5 plants per plot, respectively were randomly selected, uprooted gently and washed free of any adhering soil from the roots for gall index scoring.

3.5 Data analysis.

All data were subjected to Analysis of Variance (ANOVA) using the generalized linear model (GLM) procedure of Statistical Analysis Systems (SAS) for means to check for any differences in treatments applied both in CRD and RCBD. The means obtained were separated using Student-Newman-Kuel's (S-N-K) test at 95% confidence level.

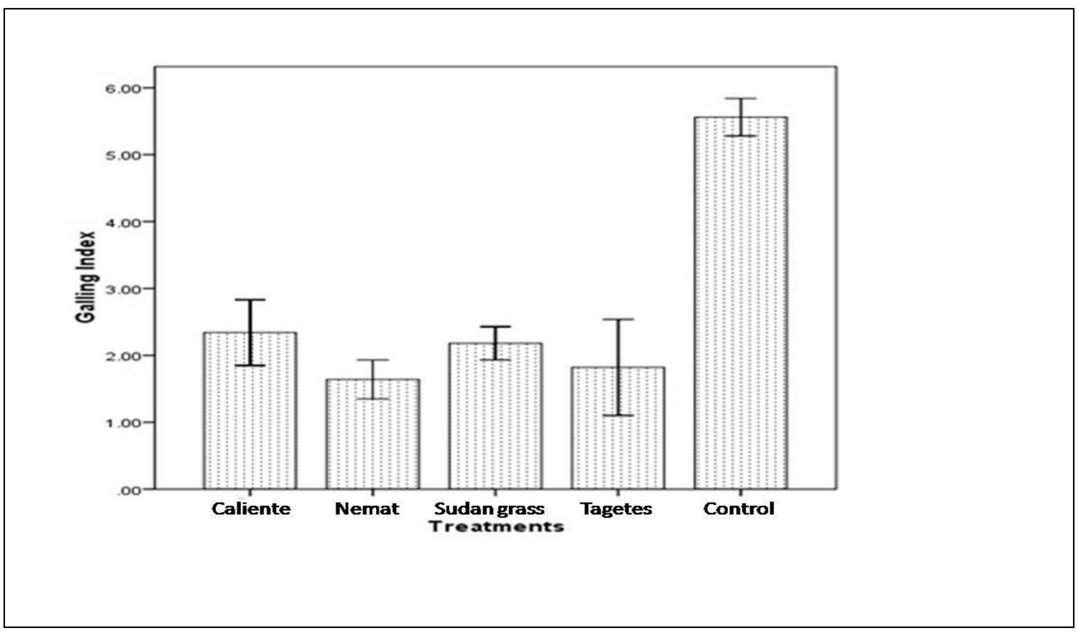
CHAPTER FOUR

4.0 Results

4.1 Effect of green manure plants on root-knot nematode (RKN) in French bean

Green manure plants suppressed RKN significantly compared to untreated control in both greenhouse and field experiments (Fig. 4.1). A significantly higher mean galling index was observed in French bean roots from untreated plots compared to those treated with green manures. The green manure Nemat (*Eruca sativa*) had the lowest mean galling index (1.64) followed by *Tagetes* spp. (1.8). Considerable reduction in galling was also observed in French bean roots from plants treated with Caliente and Sudan grass. The reduction was comparable to that of Nemat and Tagetes (Fig. 4.1).

Figure 4.1: Galling index in French bean roots in different green manure treatments.



The nematode densities and galling indices at the end of the season in the untreated control were significantly higher ($P \leq 0.05$) compared to the RKN densities and galling indices in the green manure-treated plots (Table 4.1). Nematode suppression that occurred following treatment with all the green manures was not significantly different ($P \geq 0.05$) among them.

There was no significant difference ($P \geq 0.05$) in biomass between the green manure-treated and the untreated control but the yields in the green manure treatments were relatively higher than the untreated control except for Tagetes which gave a yield that was significantly ($P \leq 0.05$) high. Furthermore, French bean plants treated with Tagetes yielded significantly higher ($P \leq 0.05$) than Caliente, Nemat and Sudan grass (Table 4.1).

Table 4.1: Effect of green manures on Root-knot nematode (RKN) population densities, biomass and yield of French bean plants under greenhouse conditions.

Green manure plants	RKN in 200 cc soil	Biomass of French bean (g)	Yield (Kg/ ha)
Caliente	22.0 ±4.47[a]	52.0[a]	9.4[ab]
Nemat	12.0 ±5.70[a]	65.7[a]	8.6[ab]
Sudan grass	20.0 ±3.16[a]	64.8[a]	9.2[ab]
Tagetes	13.0 ±8.37[a]	73.5[a]	12.2[b]
Control	1200 ±501.31[b]	61.0[a]	7.5[a]

Values having same superscripts in a column are not significantly different at 95% confidence level.
Key: GHSE- Greengouse; t ha^{-1}- Tonnes per hectare.

4.2 Effect of different green manure plants and alternative fumigant Metam sodium on RKN in French bean

4.2.1 Greenhouse experiments

There were no significant differences ($P \geq 0.05$) observed in RKN second stage Juvenile (J_2) population densities, galling indices and biomass of French bean plants treated with different green manure plants and Metam sodium (Table 4.2). Higher ($P \leq 0.05$) RKN population densities and galling indices were observed in the control (520 J2s/200cc soil and galling index of 5.6, respectively) whereas the lowest nematode population densities were observed in the pots treated with Nemat (42 J2s/200cc soil and galling index of 1.6).

High RKN densities and galling indices were observed in the untreated control which was significantly higher ($P \leq 0.05$) than other treatments. However, there was no significant differences ($P \geq 0.05$) in crop biomass among all the treatments (Table 4.2).

Table 4.2: Effect of different green manure plants on root-knot nematode (RKN) infestation on French bean in comparison to Metam Sodium under greenhouse conditions.

Green manure plant / type of treatment	Mean RKN (in 200 cc soil)	Mean Galling index	Mean dry crop Biomass (g)
Caliente	1.72[a]	0.52[a]	1.72[a]
Nemat	1.63[a]	0.41[a]	1.82[a]
Sudan grass	1.70[a]	0.51[a]	1.82[a]
Tagetes	1.64[a]	0.45[a]	1.87[a]
Metam sodium	1.67[a]	0.46[a]	1.72[a]
Control	2.72[b]	0.82[b]	1.79[a]

Transformation by log(x+1). Values having the same superscripts along a column are not significantly different at 95% confidence level.

4.2.2 Seasonal population dynamics of RKN in the greenhouse

There was a decline in RKN population among the tested biofumigants. The RKN populations reduced at mid-season and a further decrease occurred at the end of the season for all treatments except for the untreated plot where the opposite trend was observed (Fig. 4.2).

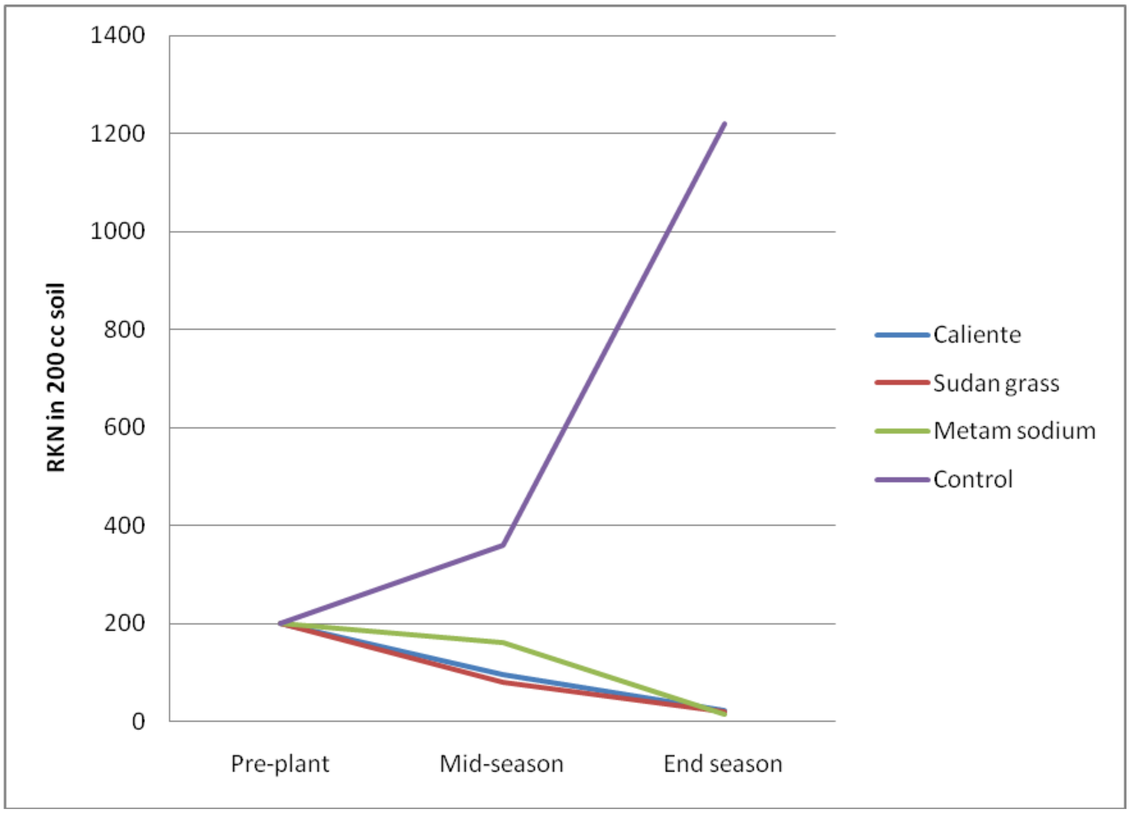


Fig. 4.2: Population dynamics of root-knot nematodes (RKN) throughout the growing season in the greenhouse.

4.2.3 Field experiments

Root-knot nematode population densities among the treatments; Caliente, Nemat, Sudan grass, *Tagetes* spp. and the fumigant Metam sodium did not differ significantly ($P \geq 0.05$) from each other but differed significantly ($P \leq 0.05$) with the untreated control (Table 4.3). The mean galling index observed following treatment with Nemat was the least (1.26) but did not differ significantly ($P \geq 0.05$) from other green manures. However, higher galling indices were observed following treatment with Metam sodium and the untreated control which were significantly different ($P \leq 0.05$) from the galling indices observed following treatment with the green manures (Table 4.3).

The dry crop biomasses observed in all treatments applied were not significantly different ($P \geq 0.05$) from each other. However, significant differences ($P \leq 0.05$) were observed in the pod yield. The least harvestable pod yield was observed in the control plots (6.30 kg/plot) and the highest pod yield was observed in the *Tagetes* spp. treated plots at 10.17 kg. There was a significant pod yield difference ($P \leq 0.05$) between *Tagetes* spp. treated plots and other treatments. The pod yield observed among Caliente, Nemat, Sudan grass and Metam sodium treatments were not significant ($P \geq 0.05$) (Table 4.3).

Table 4.3: Effect of bio-fumigants on root-knot nematode (RKN) population densities, galling indices, dry crop biomass and yield under field conditions.

Green manure /type of treatment	RKN density in 200 cc soil Log(x+1)	Mean root galling index	Mean dry crop Biomass (g)	Pod yield (Kg/plot)
Caliente	1.32^{a}	1.96^{a}	171.26^{a}	7.83^{a}
Nemat	1.71^{a}	1.26^{a}	219.08^{a}	7.17^{a}
Sudan grass	1.61^{a}	1.76^{a}	209.46^{a}	7.67^{a}
Tagetes	1.23^{a}	1.44^{a}	244.96^{a}	10.17^{b}
Metam sodium	1.15^{a}	2.86^{b}	195.92^{a}	8.84^{a}
Control	2.28^{b}	4.40^{c}	216.54^{a}	6.30^{c}
LSD $_{(P=0.05)}$	0.00	0.00	0.437	0.053

Values having same superscripts in a column are not significantly different at 95% confidence level.

4.2.2.1 Seasonal population dynamics of root-knot nematodes (RKN) in the Field

A reduction of RKN in response to biofumigation was observed: Caliente and Metam sodium showed similar declining trends throughout the growing seasons. The reduction in RKN population density in the mid-season was followed by a further reduction at harvest was observed (Fig 4.3). Nemat, Sudan grass and Tagetes displayed a different trend, whereby a reduction in the population density of RKN in the mid-season was followed by an increase in the RKN density at the end of the experiment. The untreated control had the highest RKN population densities at the end of the season (Fig. 4.3).

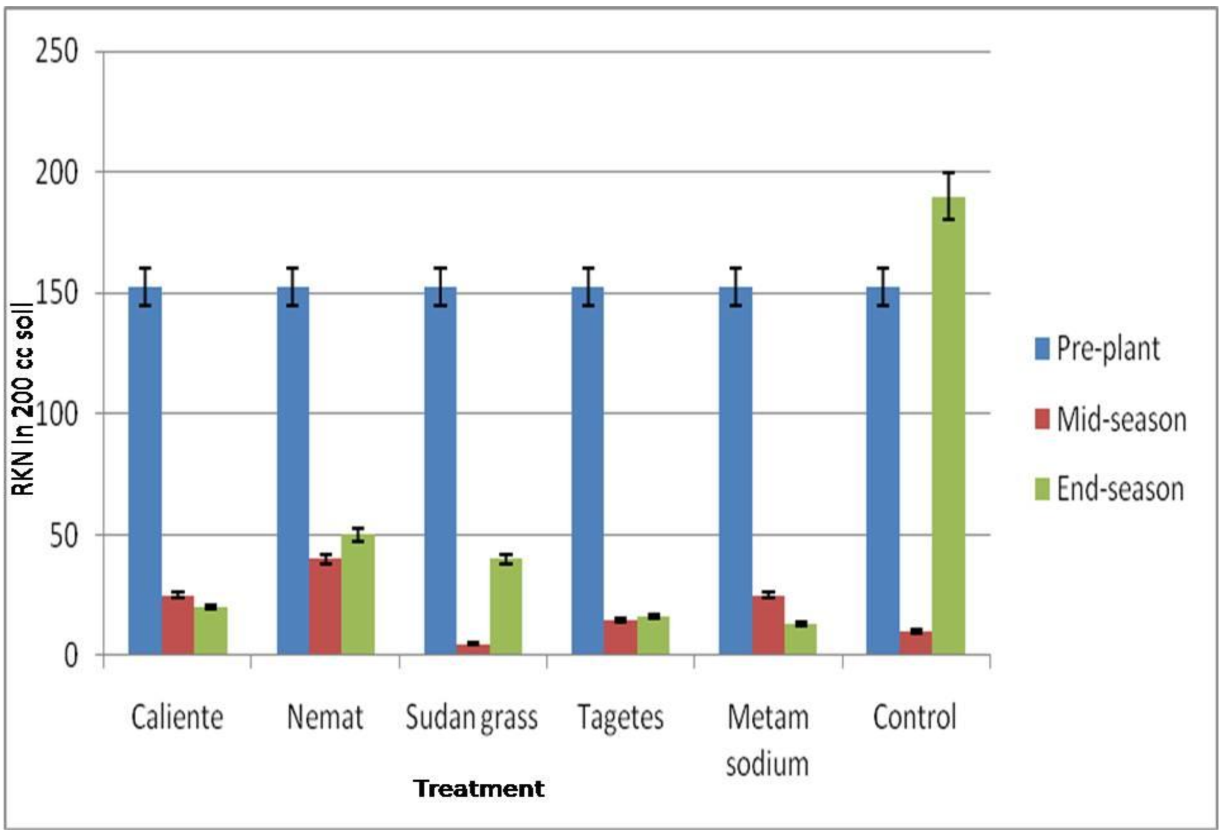


Fig. 4.3: Population trend of root-knot nematodes (RKN) through the growing season in the field.

4.3 Effect of eco-friendly nematicides and bio-control agents in suppressing root-knot nematodes (RKN)

In both the first and the second season, eco-friendly nematicides had a significant ($P \leq 0.05$) reduction of RKN J2 population densities compared to the control (Table 4.4). The control had the highest mean RKN densities (240 RKN/200 cc soil) and a galling index of 3.77 while Vydate and Neemraj had the least mean RKN density (40 RKN/200 cc soil) in the first season.

Similar results were observed in the second season with control having the highest RKN J2 population densities (285 RKN/200 cc soil) and a galling index of 3.89 and Vydate had the lowest (23 RKN/200 cc soil) (Table 4.4).

The highest galling index among the eco-friendly nematicides was observed in Phytoprotect treatments at 2.91 in season one and Neemraj at 2.61 in season 2 (Table 4.4). Rigel G and Mytech had comparable galling indices in season one. Subsequently, treatment with Vydate® resulted in the highest percent reduction in root galling (74.16%) while the least reduction was observed following treatment with Phytoprotect (Table 4.4).

Table 4.4: Effect of eco-friendly nematicides on root-knot nematode (RKN) densities and galling indices in French beans under field conditions.

Nematicide	Mean RKN in 200 cc soil		% Reduction in RKN	Mean Galling index		% Reduction in Galling index
	Season 1	Season 2		Season 1	Season 2	
Salicylic acid	50 [a]	28 [a]	62.96	2.51 [a]	2.00 [a]	68.54
Sesame oil	65 [a]	33 [a]	51.85	2.91 [ab]	2.30 [a]	62.92
P. lilacinus	42 [a]	24 [a]	68.89	2.52 [a]	2.44 [a]	73.03
Azadirachtin sp.	40 [a]	24 [a]	70.37	2.88 [ab]	2.61 [a]	73.03
Oxamyl	40 [a]	23 [a]	70.37	2.09 [a]	2.04 [a]	74.16
Control	240 [b]	285 [b]		3.77 [b]	3.89 [b]	

Values having same superscripts in a column are not significantly different at 95% confidence level (P=0.05).

The resulting crop biomass was highest in the plots treated with Vydate® in both seasons (265.07 and 231.93 grams, respectively) these represented the highest increases in crop biomass for the two seasons (43.32% and 30.02%, respectively). The lowest crop biomass was recorded in the untreated control plots in the two seasons (150.24 and 162.30g). However, there was no significant differences ($P \geq 0.05$) in crop biomass between the eco-friendly nematicides and the untreated control (Table 4.5). In the second season, there was no evidence of differences in crop biomass between the eco-friendly treated plots and the untreated control (Table 4.5).

Plate 2: (a)Wilting French beans due to root-knot nematode infection and (b) root galling in infected plants.

Table 4.5: Effect of eco-friendly manures on crop biomass and its percentage increase under field conditions.

Nematicide	Season 1 Crop Biomass		Season 2 Crop Biomass	
	Mean	% Increase	Mean	% Increase
Rigel G	181.97[a]	17.44	169.43[a]	4.21
Phytoprotect	174.26[a]	13.78	165.88[a]	2.16
Mytech	205.15[a]	26.77	185.29[a]	12.41
Neemraj	184.25[a]	18.46	176.60[a]	8.10
Vydate®	265.07[a]	43.32	231.93[a]	30.02
Control	150.24[a]		162.30[a]	

Values having same superscripts in a column are not significantly different at 95% confidence level.

CHAPTER FIVE

5.0 Discussion

5.1 Effect of green manure crops on root-knot nematode (RKN) population densities

Green manure crops have a significant potential in the suppression of RKN (*Meloidogyne* spp.) under both greenhouse and field conditions. This study shows evidence that green manures can be used for the management of RKN. These findings compare with those by Morris and Walker (2002), Wang *et al*. (2003) and Kimenju e*t al*. (2008).

Green manure crops have the ability to suppress nematode diseases by changing the soil physical and chemical properties and by enriching the soil with beneficial microflora. The findings of this study are in agreement with Wang and Chao (1995) who suggested that RKN can be controlled by application of green leaf manures to the RKN infested soil. The suppression of RKN may also result from a combination of processes amongst which is the decomposition of organic matter by many soil microbes (fungi, actinomycetes, and bacteria) and release of nematicidal compounds by the plants. The availability of decomposing matter increases the populations of the natural enemies of PPN leading to an enhanced natural nematode control.

Organic soil amendments, which reduce nematode populations, have high nitrogen contents relative to carbon (Lazarovits *et al*., 2000). In addition to the build-up of microbial antagonists of nematodes, the presence of the nitrogenous compound ammonia released by green manures plays an important role in the reduction of nematode populations in treated soils. Anhydrous and aqueous ammonia, urea and other ammonium compounds have been used for nematode control (Smiley *et al*., 1970; Rodriquez-Kabana *et al*., 1987).

Root-knot nematode reduction was highest following treatment of French bean plots with Nemat (*Eruca sativa*) (79% reduction) followed by *Tagetes erecta* and Metam sodium in the greenhouse experiment and Caliente (66.67% reduction) in the field. The two brassicas; Nemat (*Eruca sativa*) and Caliente (*Sinapis alba*) have demonstrated their ability to suppress RKN. This agrees with earlier studies by Lord *et al*. (2011) that indicate members of the brassica family can control RKN by producing glucosinolates which when hydrolysed in the soil in the presence of moisture will form isothiocyanates (ITC) responsible for nematode suppression. Halbrendt (1999) also reported reduction of *Xiphinema americanum* populations in temperate orchards following incorporation of brassicas in the soil. In a recent study by Pakeerathan *et al.* (2009), incorporation of the fresh foliage of wild-grown Chrysanthemum (*Chrysanthemum coronarium* L.) into a *M. javanica*-infested soil at concentrations ≥40g/kg reduced root galls of tomato plants and increased plant weights. The nematicidal compound(s) contained in the plant or the mode of action against the RKNs are not yet known.

The performance of *Tagetes minuta* in suppressing RKN in this study is in agreement with studies by Rahman (2012) who reported that French marigolds (*T. patula*) and African marigolds (*T. erecta*) were two plant species devoid of RKN infection. Marigold roots have been reported to release the chemical alpha-terthienyl which is nematicidal, insecticidal, antiviral and cytotoxic. The presence of alpha-terthienyl inhibits the hatching of nematode eggs (Siddiqui and Alam, 1987). According to Ploeg and Maris (1999), *Meloidogyne* spp. juveniles were unable to fully develop in the roots of *T. erecta*. In addition, the low juvenile counts observed following treatment with *Tagetes* spp. agrees with Steiner (1941) who observed that only a few of the nematodes able to penetrate marigold roots reached maturity, indicating that during the *T. erecta* growing phase, a number of juveniles did not make it to maturity thus resulting to low juvenile counts. In this study, *T. erecta* gave consistent performance in the suppression of RKN both in the greenhouse and in the field.

5.2 Effect of green manure crops on biomass and yield of French bean

The green manures tested in this study (*Eruca sativa*, *Sinapis alba*, *Sorghum sudanese* and *Tagetes minuta*) produced significantly high French bean biomass weight, which was statistically significant ($P \leq 0.05$) compared to untreated control. This could be attributed to decomposition of green manures where the necessary nutrients such as N, P and K were released to the plants hence altering the soil physical and chemical properties. This resulted to French bean biomass increase observed.

Although Metam sodium suppressed RKN population satisfactorily, poor results were observed in terms of crop biomass. This could be attributed to the fact that Metam sodium is a broad-spectrum fumigant in terms of activity and thus eliminated all the beneficial soil micro-organisms that would have been useful in checking the nematode population and also in maintaining the soil biology. The findings of this study agree with Ingham *et al*. (2000) who observed reduction in RKN densities following treatment with metam sodium. The effectiveness of metam sodium treatment was affected by the warm growing season which permit additional nematode population increase.

French bean pod yield from plots treated with green manures were higher than the untreated control showing the potential of increasing yields with the adoption of green manure crops. Pod yields from plots treated with *Tagetes* spp. were the highest compared to other treatments showing the great potential that *Tagetes* spp. has after reducing RKN populations. The amount of nitrogen that is available to the succeeding crop from green manure crops is usually in the range of 40-60% of the total amount of nitrogen that is contained within the green manure crop (Sullivan *et al*., 2003). The available N may have led to the increased yield in the plots treated with green manure crops.

5.3 Effect of eco-friendly nematicides on root-knot nematode (RKN) population densities and galling of roots

All eco-friendly nematicides tested in this study reduced RKN juvenile indicating the importance of the eco-friendly nematicides in management of RKN in French bean production. The reduction was comparable between Vydate and eco-friendly nematicides. Lopez-Perez *et al*. (2011) reported minor galling following treatment with Vydate® which also reduced nematode reproduction. This confirms that use of eco-friendly nematicides is effective in managing RKN.

Treatment of experimental plots with Phytoprotect (Sesame oil extract) resulted in reduction of RKN population densities. However, this reduction was comparable to the standard control (Vydate®) since this essential oils have nemastatic effects on RKN. A few essential oils and their components have been evaluated for nematicidal effects (Coventry and Allan, 2001). In a study by Oka *et al*. (2000), essential oils from 25 spices and aromatic plants were evaluated for their nematicidal effect on the RKN *M. javanica*. Twelve of the 27 essential oils immobilized more than 80% of *M. javanica* juveniles at a concentration of 1ml/ litre^{-1} *in vitro*, further inhibiting egg hatching.

Essential oils of caraway (*Carum carvi* L), fennel (*Foeniculum vulgare* Mill), applemint (*Mentha rotundifolia* Hudson), and spearmint (*Mentha spicata* L) showed the highest nematicidal activity *in vitro* among the tested oils. These oils and those from oregano (*Origanum vulgare* L), Syrian oregano (*Origanum syriacum* Lour) and wild thyme (*Coridothymus capitatus* (L) Reichb) mixed in nematode-infected sandy soil at concentrations of $\geq 100 mgkg^{-1}$ reduced the root galling of cucumber seedlings in pot experiments. The main components of these essential oils, carvacrol in the oils of Oregano and Syrian Oregano, *t*-anethole from Fennel oil, thymol from the oils of Oregano, Syrian Oregano and wild thyme, and carvone in caraway oil, were found to immobilize the juveniles and inhibit hatching at $\geq 125 \mu l^{-1}$ *in vitro*. Most of these components, mixed in the sandy soil at concentrations of 75 and $150 mgkg^{-1}$ reduced root galling of cucumber seedlings (Oka *et al*., 2000) agreeing with the findings of this study that plant extracts are effective in reducing impacts of RKN infection.

Mytech (*Paecilomyces* lilacinus) performed much better than both Rigel and Phytoprotect in reducing RKN populations.This can be attributed to the conducive environment and the appropriate application done. Kerry (1997) found that *P. lilacinus* gave variable results in a range of conditions and that it required relatively high soil temperatures to be effective. The variable results obtained from this study could probably be due to the prevailing soil temperatures in the field during the time of the experiment. Other eco-friendly chemicals like Rigel G and Neemraj also gave results which were significantly different from the control. However, they varied in their suppression of RKN. Many factors may be attributed to this difference. Physiological characteristics such as the permeability of nematode cuticles may favor the penetration of certain compounds. Bio-chemical differences between different nematode species may also have affected the degradation or detoxification of the compounds, therefore reducing activity of the compounds (Kerry, 1997).

5.4 Effect of eco-friendly nematicides on crop biomass

Mytech (*P. lilacinus*) and Vydate® compared in suppression of RKN which resulted in increase in crop biomass. This can be attributed to better control of the RKN especially in early stages and therefore allowing the French bean crop to grow with vigor resulting in biomass increase. In both seasons, Vydate® gave the highest crop biomass. This may be due to the fact that it kills both beneficial and harmful microflora and microfauna but its suppression of RKN seems to have given the French bean crop a better start and hence an increase in crop biomass resulting to an increased yield. Neemraj (*Azadirachtin* spp.) and Phytoprotect (Sesame oil extract) gave variable results with respect to crop biomass. They performed poorly than the positive control (Vydate®) in both seasons. The biomass observed following all the treatments in both seasons was not different from each other. Although Neemraj and Phytoprotect are natural products and would be expected to boost crop biomass, it seems their effect was overwhelmed by the significantly high RKN populations.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The introduction of green manures as an option in the management of plant parasitic nematodes has become a major component in the overall sustainable management of soil health and productivity. The addition and maintenance of high levels of organic matter, especially the active fraction greatly improves the physical, chemical and biological properties of the soil, therefore increasing productivity. Various green manures have variable effects on the general biological activities in soil and more so against specific pathogens and the diseases they cause. Soil nematodes including PPN are affected by organic materials added in the form of rotational cover crop debris, green manures, compost and other soil amendments.

Different green manures used in this study showed variable degrees in the suppression of RKN populations in the soil. Among the green manures and other chemicals tested, the best candidates for a practical control application would be those exerting effective control on RKN. This study established that RKN suppression between green manure crops and Metam sodium did not differ. The green manures that showed lesser control can still be considered in future studies for their nematicidal potential on RKN and other PPN.

From the study, it is evident that continued use of the eco-friendly nematicides would result in acceptable control levels for root knot nematodes. Eco-friendly nematicides used in the experiment led to RKN suppression which did not differ significantly from Vydate®. This study illustrates that agricultural utilization of phytochemicals, although currently under trial and development in many situations, offers tremendous potential in the control of RKN.

6.2 Recommendations

- Since green manure crops are effective in RKN suppression, green manure crops are recommended for incorporation into integrated nematode management in French bean production.

- Since green manure plants are comparable to metam sodium in RKN management, they can be used as an alternative. However, several repeated studies need to be carried out to affirm this.

- The increase in yield following treatment with *Tagetes* spp. calls for more studies on the same to elucidate the effect of *Tagetes* spp. on French bean yield.

- More studies on the effect of green manure addition on the activities of PPNs and other soil-borne pathogens in crop production systems are highly recommended to obtain more green manures suitable for different conditions.

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Appendices

Appendix 1: Analysis of variance for greenhouse juvenile count in 200 cc soil

Source of variation	d.f.	s.s.	m.s.	F	Sig.
VAR	5	6034424	1206884.833	70.861	0.000
Residual	24	408760.0	17031.667		
Total	**29**	**6443184**			

Appendix 2: Analysis of variance for greenhouse French bean root galling index

Source of variation	d.f.	s.s.	m.s.	F.	Sig
VAR	5	55.107	11.021	61.629	0.000
Residual	24	4.292	0.179		
Total	**29**	**59.399**			

Appendix 3: Analysis of variance for greenhouse French bean biomass

Source of variation	d.f.	s.s.	m.s.	F	Sig
VAR	5	1791.008	358.202	2.198	0.000
Residual	24	3910.449	162.935		
Total	**29**	**5701.456**			

Appendix 4: Analysis of variance for field French bean biomass

Source of variation	d.f.	s.s.	m.s.	F.	Sig
VAR	5	155227.286	3045.457	1.004	0.437
Residual	24	72806.184	3033.591		
Total	**29**	**88033.470**			