MANAGEMENT OF ROOT-KNOT NEMATODES AND WEEDS USING DIFFERENT AMENDMENTS IN GREENHOUSE GROWN ASCLEPIAS (*Asclepias tuberosa* L.)

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A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL FULFILMENT FOR THE REQUIREMENT OF THE MASTER OF SCIENCE DEGREE IN HORTICULTURE OF EGERTON UNIVERSITY

EGERTON UNIVERSITY

OCTOBER, 2009

DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been previously presented elsewhere for award of any degree.

Sign________________ **Date________________**

Kenneth Kinuthia Kagai

RECOMMENDATION

This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

I dedicate this work to my wife Mary Wanjiku and children Elizabeth and Kelvin Kagai for their moral support and prayers. Their patience and encouragement contributed to my success in undertaking this work.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my supervisors Dr. Joseph N. Aguyoh and Dr. Geoffrey O. Tunya of the Department of Crops, Horticulture and Soils, Egerton University for their guidance in my research work. Their input was instrumental in the completion this work. I also extend my gratitudes towards the Post Graduate School of Egerton University for the admission and guidance throughout the entire programme without which this work would not have been successful. Great thanks to the entire staff and colleagues of Crops, Horticulture and Soil Sciences Department for their encouragement and support.

Special thanks go to the Ministry of Agriculture for granting me a paid study leave during the entire duration of the programme. Their enthusiasm in following my progress throughout the course was of great importance. I wish to sincerely thank the management of James Finlay (K) Limited for allowing me to undertake this research in their farm. I would like to thank the management for supplying me with planting material, labour, green house space and laboratory facilities to do some analysis. I wish to particularly thank Mr. Elijah Getiro (Technical manager), Aggrey Motia (Flower Farm 1 Manager), Francis Maimba (Research Development Manager) and Mary Opisa (Agronomy Department). I would also like to thank Mr. Ken Onyango and Rachael for their technical assistance.

I would like to extend my gratitude towards my family, friends and colleagues for their everlasting support in career and life goals. The contribution of all and many more not mentioned here worked together for success of this work.

ABSTRACT

The research tested the potential of *Tagetes patula*, *Lantana camara*, *Tephrosia vogelii* and *Azadirachta indica* to manage root knot nematodes and weeds on greenhouse grown *Asclepias tuberosa.* Field trials were conducted at James Finlay's (K) Flowers-Kericho for two seasons under greenhouse conditions. The experiment was laid in Complete Randomised Block Design replicated three times. Chopped plant materials were applied singly as soil amendment or in combination with nemacur. *T. patula* was applied at 300 g/m^2, *A. indica* at 150 g/m^2, *L. camara* at 10% w/w, and *T. vogelii* at 10% w/w. Organic plant materials were applied at half rate in combination with nemacur at 20 g/m^2. Nemacur treatment alone was applied at a full rate of 40 g/m^2. The chopped plant materials were incorporated in the soil and left to decompose for four weeks. Nematode counts, weed density and biomass were evaluated before and after application of soil amendment treatments. *L. camara* combined with nemacur reduced root knot nematodes count by between 89.3% and 90.8%, while a single application of the same treatment reduced nematodes by 85.1%. Root galling was reduced by 79.3% when a combination of *L. camara* with nemacur was applied, while nemacur alone decreased galling by 89.6%. *A. indica* reduced root knot nematodes count by between 52.3% and 58% when applied singly and by 31.2% in combination with nemacur. *T. patula* decreased nematodes counts by between 47.7% and 55% in combination with nemacur while it reduced nematodes by 34.8% when applied singly. Nemacur or a combination of *L. camara* with nemacur led to an increase in plant height by between 22.7 cm and 26.5 cm respectively. *L. camara* with nemacur increased cumulative flower yield by between 78.6% and 83.6% compared to the untreated control. *T. patula* with nemacur reduced weed biomass by between 33.6% and 36.6% while *L. camara* alone decreased the population density of *Oxalis cornulata* weed by between 14% and 17% compared to untreated control. The improvement of nutrient status of the soil and the control of nematodes and weeds could have led to an improvement of yield characteristic of asclepias. A combination of *L. camara* with nemacur may be used in place of synthetic nematicides alone to control nematodes, weeds and improve nutrient levels. This treatment combination gave the best control of nematodes, enhanced plant growth and significantly increased flower yield compared to all other treatments. The results from this study give cutflower growers alternative options to manage root-knot nematodes and weeds under greenhouse conditions.

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ABBREVIATIONS AND ACRONYMS

AI	*Azadirachta indica*
ANOVA	Analysis of Variance
C: N	Carbon: Nitrogen Ratio
CAN	Calcium Ammonium Nitrate
CRBD	Complete Randomized Block Design
DAPL	Days after planting
DAT	Days after treatment
DMRT	Duncan Multiple Range Test
GLM	General Linear Model
HCDA	Horticultural Crops Development Authority
IPM	Integrated Pest Management
LC	*Lantana camara*
MOA	Ministry of Agriculture
N	Nitrogen
N®	Nemacur
NO TRT	No Treatment
UNEP	United Nations Environmental Programme
TV	*Tephrosia vogelii*
S1	Season 1
S2	Season 2
SAS	Statistical Analysis Software
SOM	Soil Organic Matter
TP	*Tagetes patula*
WB	World Bank
W/W	Weight by weight

CHAPTER ONE

INTRODUCTION

1.1 Back Ground Information

Horticulture in Kenya is a vibrant sub-sector making it the second foreign exchange earner after tourism. The total export earnings from horticulture in the year 2007 amounted to Ksh 65.2 billion (Ministry of Agriculture (MOA), 2008) out of which cut flowers accounted for Ksh 29.7 billion. In 2008, the sector exported 423.1 million kilogrammes that earned 73.7 billion out of which flowers accounted for Ksh 39.9 billion (Kathuri, 2009). The production of cut flowers continued to increase with a corresponding area under the crop marginally rising from 3,026 ha in 2006 to 3,063 ha in 2007 (MOA, 2008). Floriculture is the most successful sector of horticulture contributing more than 12% of Kenya's foreign exchange earnings (MOA, 2004). Kenya is the largest supplier of cut flowers in the European Union Market accounting to 58 % of all exports from Africa, Caribbean and Pacific countries (Hennock, 2002). Kenya's flower cluster emerged in the 1970's alongside fruits and vegetable production (Jensen, 2005) because they share similar characteristics in terms of growing conditions and perishable nature of products. According to World Bank (WB) estimates, the total investment into flower industry from 2002-2005 was $200- $300 million in the country (World Bank, 2005). Kenya has ideal growing conditions for flowers in Africa (Fairbanks and Lindsay, 1997) with temperature and rainfall at ideal levels year round and proximity to the equator providing 12 hours of sunlight year round (MOA, 2008).

Worldwide, commercial floriculture is characterized by high investment and strict quality demands which often imply high pesticide usage. Consumers want quality flowers-completely free of damage caused by biotic and abiotic factors. Perennial herbaceous flower production is a rapidly growing segment of the floriculture and nursery industry, with an annual gross receipt value of approximately $ 1 billion in the United States (Rhodus, 1994).

Kenya exports an assortment of flowers including roses, carnations, chrysanthemums and minor (specialty) flowers. Specialty cutflowers includes: asclepias, veronica, hypericums, statice among others. Flowers are grown in intensive cultivation including greenhouse production that contributes to accumulation of soil borne problems. The production of various flowers including asclepias is constrained by insect pests, weeds, nematodes and several diseases that build up in the soil leading to significant losses in yields and quality. Eradication of these noxious soil-borne organisms is difficult; they may render an area unsuitable for the production of susceptible flowers and hence make soil disinfestations mandatory.

Nematodes are roundworms that are found in almost all habitats (Dropkin, 1980). Beneficial species are usually referred to as free living nematodes. Other nematode species are parasitic and harmful to plants, animals, and humans. Soil provides an excellent habitat for nematodes. Thousands of nematodes may inhabit $100cm^3$ of soil (Maggenti, 1981). Plant-parasitic nematodes may live within plant roots or inhabit the rhizosphere soil around plant roots and root hairs (Agrios, 1969). Endoparasitic nematodes live and feed inside the plant's tissues, while ectoparasitic nematodes feed from the plant's root surfaces. It has been reported that a single endoparasitic nematode may reduce productivity or kill a plant while ectoparasitic nematodes feed on a plant without seriously affecting its production (Ingham et al., 1985). Few plant-parasitic species are host specific, while most generally have broad host ranges (Yeates et al., 1993). Parasitic populations are dense in warmer climates (Dropkin, 1980) and light, sandy soils harbor larger densities (Yepsen, 1984). Leaf chlorosis and wilting of *Asclepias tuberosa* followed by weak flowering or plant death has been reported in many commercial green houses (Tsror et al., 1997). Damage from nematodes not only occurs from feeding but also from interaction with other disease causing organisms that take advantage of wounds created by nematodes (Abawi and Chen, 1998). Root-Knot nematodes (*Meloidogyne spp*) have been associated with decline in production of various flowers such as roses, carnations, asclepias, and strelitizia (Reis, 1985 and Schnauber, 2003). Root lesion nematodes (*Pratylenchus spp*) affect development of asclepias, carnations, dahlia and greenhouse grown alstroemeria (Schnauber, 2003).

Weeds are detrimental to the growth of ornamental plants and they also harbour pests. In addition, they compete for water, light and nutrients. Some weeds encountered in ornamental plant production include: common chick weed (*Stellaria media*), spotted spurge (*Chamaesyce maculata*) (Miller et al., 1975), wood sorrel (*Oxalis spp*), nutsedge (*Cyperus esculentus*) and crab grass (*Digitalia spp*) (Hall and Vandiver, 1991).

Until recently, methyl bromide has been extensively used for soil borne pests and diseases control in the production of many fruit, vegetable and nursery crops (Noling and Becker, 1994). It is considered the most effective soil fumigant in controlling plant parasitic nematodes, plant pathogenic fungi, insects and weeds prior to crop planting (Malathrakis, 1999; Minito et al., 1999). However, due to its emissions to the atmosphere as well as the toxic effects to the farmers and the environment, (McKenry et al., 1994; Danse et al., 1984 and Culvert et al., 1998), its use was phased out in developed countries by the year 2005 United Nations Environment Programme (UNEP, 2004) The agricultural industry was affected because the common agricultural practice is soil fumigation with Methyl bromide after every 2-3 crops. Reports indicate that methyl bromide is highly toxic (Environmental Working Group, 1995). Methyl bromide is poisonous if absorbed by skin contact or swallowed (Lu, 1995). Repeated exposure may cause allergic disorders, systemic effects, kidney injury, upper respiratory tract damage and central nervous system depression (Wauchope et al., 1992).

The growing concern over the serious problems associated with chemical pesticides calls for sustained efforts to reduce pesticide use as much as possible and seek alternative pest control methods that are effective against target organisms, harmless to non- target organisms and are biodegradable. It is therefore important to continuously seek alternative control strategies that can either be used singly or in combination with other pesticides.

The use of soil amendments, cover crops, resistant varieties, flooding, soil solarization or bare fallowing can suppress nematodes and weed population build up (Miano, 1999). Sustainable control of nematodes and inhibition of damage to agriculture involves integration of preventive measures. Crop rotations, soil solarization and biological controls are some methods currently researched (Walz, 1999 and Gaskell et al., 2000a). However their potential has not been fully exploited due to limited knowledge by both large and small scale farmers. The use of organic and/or in-organic soil amendments has been found promising in the control of plant parasitic nematodes (Miano, 1999).

Low input technology for pest management has been reported as advantageous in developing countries. However, specific concerns have been raised because of increasing use of pesticides in third countries (Zalenzy et al., 1985).

There are a number of herbs that are found in different parts of Kenya that can be utilized to improve plant protection and increase production. Some herbs provide multiple uses that include medicine, pesticides and as organic fertilizers. Where the herbs occur in abundance they can be used as natural pest control agents. The most common herbs are lantana, tagetes, tephrosia and neem. The utilization of cultivars resistant to root-knot nematodes has been identified as a possible means of replacing methyl bromide use. However, resistance breakdown is a limiting factor (Netcher and Sikora, 1990).

Organic soil amendments with low C:N ratios and high protein or amine-type nitrogen contents have been reported to be effective against root-knot nematodes (Rodriguez-Kabana, 1986; Rodriguez and Morgan-Jones, 1987). This was attributed to increased production of toxic compounds such as ammonia. The use of soil amendments is however limited by the large quantities needed to achieve effective nematode control (Singh and Sitaramaiah, 1970; Miano, 1999). Some organic soil amendments have often resulted in phytotoxicity to plants (Rodriguez-Kabana, 1986). To overcome this problem, an optimum range of C:N ratio of 13:1 to 20:1 that balances phytotoxicity and nematicidal activity was suggested (Rodriguez-Kabana and Morgan-Jones, 1987). Most organic amendments have C:N ratios that fall outside this optimum range. Soil amendments may pose leachate problems and therefore biological or naturally occurring substances may be favorable over the use of chemicals. While some materials have been studied for growth effects and nematicidal properties, more research must be done on newly developed products. Many naturally occurring substances help reduce cost over traditional chemical methods.

Many farmers have expressed concern of lack of effective alternatives and the possibility of going out of business once methyl bromide is phased out (Pizzano, 2001). Non chemical low input pest management systems exploit practices that minimize crop loss while requiring few additional inputs. A proactive integrated pest management (IPM) option creates a conducive environment (food, shelter) for the beneficial organism and hence lowers the carrying capacity of the green houses or field for the pest.

1.2 Statement of the problem

The management of soil borne pests such root knot nematodes and weeds is difficult because they develop and stay in the soil at some stage of their life cycle. Traditionally, the treatment of choice has been soil fumigation with methyl bromide a very effective method that controls a wide range of pests. However, low input IPM strategies are only beginning to be developed in flower production. Most of the work done by researchers involves chemical control methods in vegetable crops.

There has been little research addressing proactive options and how they can be combined to complement each other. Research work on natural plant extracts to control soil borne pests is very limited. The impact of these products on the growth yield and quality of asclepias is not known. The study investigated the potential of selected plant species as pesticides when applied as soil amendments. The plant materials were used singly or in combination with a non-fumigant nematicide (nemacur) which is a possible alternative to methyl bromide. The plant materials studied included *Lantana camara, Tagetes patula, Tephrosia vogelii* and *Azadirachta indica.*

1.3 Justification

Commercial aspects of using natural pesticides have to be evaluated in the light of increasing preference for organically-grown crops and environmental concerns. Although synthetic pesticides have been used successfully for the control of nematodes and weeds, they are expensive, hazardous to human health and the environment. Bioactive products from plant materials (botanicals) can be prepared in various ways. They can be as simple pureed plant leaves, extracts of plant parts, plant teas or chemicals purified from plants. Some botanicals are broad spectrum while others are very specific. Prolonged use of synthetic pesticides may lead to pest resistance and some persist in the environment over a long period of time. Botanical are degraded quickly, are less hazardous to handle and in some cases are formulated on-farm. The selective mode of action of plant derivatives could be exploited for safe use in integrated pest management. Cheap methods like use of soil amendments are generally not widely used by cutflower growers. The major hindrance is lack of documented information on their efficacy. Attempts with chemical fumigants have given inconsistent results. Repeated use of popular chemicals such as oxamyl or nemacur has been reported to be susceptible to enhanced biodegradation. Bioactive product from plants may provide an alternative, sustainable and inexpensive means of managing cutflower pests.

1.4 General objective

To investigate whether plant materials when used singly or in combination with a non-fumigant nematicide would suppress pests without affecting growth and quality of asclepias.

1.5 Specific objectives

The specific objectives of this study were to determine;

i) The effect of *Tagetes patula*, *Lantana camara, Azadirachta indica* and *Tephrosia vogelii* on the density of weeds and root-knot nematodes within the root zone of asclepias.

ii) The effect of combining a non fumigant nematicide (Nemacur) with the selected plant materials on the density of root-knot nematodes and weeds.

iii) The influence of organic soil amendments and nemacur on the competitive ability of weeds, growth, quality and yield of asclepias.

1.6 Hypotheses

The stated hypotheses are null:

i) *Tagetes patula*, *Lantana camara*, *Azadirachta indica* and *Tephrosia vogelii* application as a soil amendment have no influence on the population density of nematodes and weeds.

ii) Combining nemacur with soil amendments has no influence on the population of the density of root knot nematodes and weeds.

iii) The soil amendments applied alone or in combination with nemacur have no influence on the growth, yield and quality of asclepias.

CHAPTER TWO

LITERATURE REVIEW

2.1 Classification and botany of asclepias

Asclepias (*Asclepias tuberosa)* belong to the family Asclepiadaceae (milkweed family), which is native to North America. The common names are butterfly weed, butterfly milkweed or pleurisy root. The plant has been grown in the United States mainly as an outdoor garden ornamental (Lyons, 1985).

Because of its attractive orange-red inflorence and long sturdy stems, it has been developed recently as a cut flower crop in Europe and Israel (Ecker and Barzilay, 1993). The plant is propagated sexually by seeds (Borland, 1997) and vegetatively by tuberous root cuttings (Albrecht and Lehmann, 1991). It is self incompatible for pollination and plants derived from seeds are genetically heterogeneous, and this is expressed in growth habit and flower colour pattern (Wyatt, 1980). It is an absolute long day (LD) plant that requires warm temperature during growth and flowering. For winter production it is grown in heated greenhouses and provided with supplementary light at night (Halevy, 1999).

Asclepias tuberosa (butterfly weed) is a 0.6 m tall herbaceous perennial plant that dies back in winter and regenerates from underground tubers in spring. The brilliant orange or red flower clusters are formed during midsummer. These are followed by attractive green pods that open and release silky parachuted seeds that drift away on autumn winds.

The butterfly weed is unique among the milkweed family because the sap is not milky and the leaves are not opposite. The leaves can be up to 13 cm in length. Many flowers have an inner whorl of petals (corolla) and an outer whorl of petals called the calyx. These are the showy, colourful parts of a typical flower. The milkweed is special because they have a third whorl above the corolla called the corona. The flowers have 5 regular parts and are up to 1 cm wide. Individual flowers of the self-incompatible milkweed are available to pollinators for 7 or 8 days; however, there is a large overlap in flowering time (4 to 5 weeks) within and among inflorescences on a single plant and therefore the possibility of high levels of self-pollination. Pollinator activity closely follows availability of flowers, although the more rapid decline of the former may indicate switching of pollinators to other plant species flowering simultaneously (Wyatt, 2006). Propagation of the flower is by seed or tubers that can be divided before a new flush emerges. The showy butterfly weed is planted at home in mixed borders meadows and natural areas. The flower is also used as bouquet filler during flower arrangement.

The butterfly weed performs well at an optimum soil pH of 6.2 and organic matter content of 2%. The soils should be well drained and deep to allow the expansion of the tuberous roots (Campbell, 1983).

2.2 Integrated pest management strategies

Pest control strategies that solely rely on chemical control have been the subject of widespread criticism (Black and Sweetmore, 1994). However, the shift of focus towards integrated pest management (IPM) strategies can be regarded as a response to concerns raised over the various shortcomings of pest control based purely on chemical methods. These strategies seek to build upon complementaries between different control measures. These include:-

- Use of cultural practices such as soil solarization, organic amendments, cover crops, mulches, selection of planting dates and crop rotation (Liebman and Ohno, 1998; Walz, 1999 and Gaskell et al., 2000a).
- Breeding and use of plant varieties that are resistant to diseases and pests (Finckh, 2008).
- Selective use of pesticides (chemical, botanical and microbial).
- Biological control mechanisms such as conserving natural enemies, manipulation of their populations or introducing exotic organisms.
- Local control over issues such as management practices and quarantines (Hartz et al., 1993; Ramirez-Villapudua and Munnecke, 1988; McSorley and Gallaher, 1991 and Csinos et al., 2000).

Chemicals derived from plants (Gommers, 1981) and industrial by products (Rodriguez – Kabana et al., 1984) have been or are being tested for nematicidal efficacy by numerous scientists. A number of plants contain compounds that are toxic to certain soil borne pests. For example neem (*Azadirachta indica*) has been reported to have a variety of biologically active ingredients with different modes of action. Neem resists more than 200 species of insects, mites and nematodes (Akhtar and Mahmood, 1994). Furthermore, plant products are also harmless to most predators, human beings and other animals.

2.3 Use of organic amendments

Organic soil amendments are commonly used in agricultural systems to recycle nutrients and energy as well as to improve soil conditions for plant growth (Hadar et al., 1992; Muchovej and Pacovsky, 1997). Some organic amendments suppress soil-borne plant pathogens and/or the diseases they cause, and several also control plant-parasitic nematodes (Rodriguez-Kabana, 1986; Ali et al*.,* 2001). Since organic amendments require high rates of application to be effective against plant parasitic nematodes, these organic materials must be cheap and of local origin if they are to be exploited practically.

The use of organic amendments for the control of nematodes has been known for a long time (Miano, 1999). Since then, many different types of organic amendments have been applied to a variety of crops to reduce plant parasitic nematodes (Rodriguez-Kabana, 1986). Many of these amendments have been used by farmers though not necessarily with nematodes in mind (Bridge, 1996). The amendments are mainly by-products and wastes from agricultural and plant composts, green manures, agro-industrial wastes and human excrements (Singh and Sitaramaih, 1970; Rodriguez –Kabana, 1986; and Bridge, 1996).

The intensity of root galls in Okra and tomatoes caused by *Meloidogyne javanica* reduced significantly with the incorporation of compost in the soil. When oil cakes of marghosaw were applied to the soil, a significant reduction of root-knot nematodes in tomatoes was reported with the use of poultry manure (Chindo and Khan, 1990)

Although it has been shown that organic amendments have the potential in controlling plant parasitic nematodes, research on their use has been relegated to secondary importance. This has been due to availability of effective fumigant nematicides (Rodriguez-kabana and Morgan-Jones, 1987; Noling and Becker, 1994).

2.4 Mechanisms of action leading to pest suppression in soil amended with organic materials

Organic amendments stimulate build up of saprophytic micro-organisms some of which are antagonistic to plant parasitic nematodes. Population of actinomycetes, algae, bacteria, fungi and organisms such as microbivorous nematodes are known to increase rapidly in soils rich in organic matter (Rodriguez-Kabana, 1986). The magnitude of microbial stimulation and the qualitative nature of responding microflora have been linked to the nature of the organic matter added, reflecting abundance and kind of substrate on which various micro-organisms feed (Rodriguez-Kabana and Morgan-jones, 1987).

Proliferation of micro-organisms results in increased enzymatic activities in the amended soils and release products that may be nematocidal (Patrick, 1965). Decomposition by products such as ammonia and organic acids have been found to be toxic to plant parasitic nematodes in the soil. Decomposition residues of plant tissues release simple organic acids such as acetic, propionic and butyric acids. Some of these compounds remain for several weeks in concentrations sufficient to kill phytonematodes but are not toxic to free living species. Patrick, (1965) showed that fatty acids released by decomposition of rye are highly toxic to nematode larvae.

Amended soils with fresh or composted organic matter also alter the physical, chemical and biological properties of the soil (Singh and Sitaramaiah, 1970). The soil temperature, pH oxygen, nitrogen status of the soil changed (Brady and Weil, 2002). This may result in unfavorable environment for nematode activity. The resulting changes can lead to increased host resistance, good root development and unfavorable nematode osmotic relations (Singh and Sitaramaiah, 1970). Soil amendments will increase soil fertility and water holding capacity thereby increasing plant vigour and their tolerance to nematodes.

Most organic materials with namaticidal effect have low C: N ratio and high protein or amine contents (Rodriguez Kabana and Morgan-Jones, 1987). A direct relationship between the nitrogen content in the amendment and suppression of phytonematode has been established. The nitrogen in the amendment stimulates a specialized soil micro flora capable of decomposing materials resulting in accumulation of ammonia. However, high ammonia amounts leads to phytotoxicity due to accumulation of nitrates and ammoniacol nitrogen in the soil (Rodriguez-Kabana et al., 1986).

Soil amendments containing, 2-7% (w/w) nitrogen were effective when incorporated at 4-10 t/ha (Rodriguez-Kabana, 1986). It was reported that poultry manure was effective in the control of *Meloidogyne spp* of nematodes when applied at 2 tons/ha (Chindo and Khan, 1990). Several plants have been found to help control nematodes when grown for several months in the soil. The plants reduce nematode injury to susceptible crops grown in the next season. These plants include; *Tagetes spp*(*Tagetes minuta, T. erecta T.patula* and *T. tenufolia*), *lantana camara* *and* pangola grass. Organic amendments improve both tolerance of the host plant and reduce nematode population (Dunn, 2006).

2.5 Nemato-toxic Potential in Plants

Many plant products have been well known to be nematicidal in nature. During the past few decades, it has been realized that there are beneficial effects of adding certain waste materials of plant origin to the soil because it decreased the levels of soil inhabiting plant parasitic nematodes (Badra et al., 1979; Akhtar and Alam, 1993a). This is attributed to the alteration in soil physical and ecological state by the materials or to the breakdown or decomposition by products. The addition of organic matter also stimulates populations of actinomycetes, bacteria and fungi which may be antagonistic to nematodes (Godoy et al., 1983). Very often, when there is a decrease in the soil inhabiting pathogens then an increase in crop yield occurs.

The species *Tagetes minuta, T. erecta T.patula* and *T. tenufolia* are the most common. Other species referred to as chemotypes and variants are available. While initial studies pertain to chemical and biochemical constituents of the plant, plant chemistry and biology in general, more interest has been in the bioactivity of various plant extracts and their isolates. Currently, there is increased interest in naturally occurring, biodegradable botanicals for pesticide, pharmaceutical and other applications. Bioactive extracts of different Tagetes parts exhibit nematocidal, fungicidal and insecticidal activity.

2.6 Effect of Marigolds (*Tagetes spp*) on root-knot nematodes *Meloidogyne spp*

Root nematodes (*Meloidogyne spp*) are economically the most important plant parasitic nematodes in Tropical and subtropical agriculture (Sasser, 1979). The control of these nematodes is dependent primarily on nematicides and resistant crop cultivars. However, as the use of nematicides is being severely restricted and resistant cultivars are available for only a limited number of crops (Roberts, 1990), development of alternative control strategies is urgently required.

Marigold (*Tagetes spp*) has long been known to poses nematicidal activity and has been confirmed for *M. arenaria, M. hapla, M. incognita* and *M. javanica* (Daulton and Curtis, 1963; Hackney and Dickerson, 1975). However results from these studies were often equivocal and inconsistent.

The suppression of lesion nematodes (*Pratylenchus ssp*) by marigold has also been reported (Hackney and Dickerson, 1975; Hutchinson, 1962; McKenry, 1988; Suatmadji, 1969); and appears less variable than the root-knot nematodes. Marigold produces a substance called alpha-terthienyl, which can aid in the reduction of root-knot nematodes and other disease promoting organisms, such as fungi, bacteria, insects, and some viruses (Hethelyi et al., 1986; Soule, 1993).

2.7 Bioactive Components of Tagetes

The major category of phytochemicals of interest includes terpenoids, flavonoids, alkoloids, polyacetylens and fatty acids (Akhtar and Mahmood, 1994). Petals of the lemon variety of the common African marigold contains thienyls quantity varying from 15-21 mg/kg of fresh petals extracted by methanol (Vasudevan et al., 1997). Other varieties of *Tagetes erecta* grown simultaneously did not exhibit antibiotic activity. An extensive study of constituents of tagetes species in relation to thienyls, the recovery of several bithienyls and terthienyl from fresh ground roots of *Tagetes erecta* and *Tagetes patula* have been extracted by ether.

Despite numerous reports on suppression of lesion and root-knot nematodes, few studies have investigated the effects of marigolds on nematode infestation or yields of subsequent crops. Fewer studies have also characterised differences among marigold cultivars with regard to suppression of *Meloidogyne* species. In order to evaluate the usefulness of marigold in an IPM system, field studies incorporating effects of marigold on *Meloidogyne* population development, yield response and economic outcome are required.

A study using *Tagetes minuta*, reported a significant increase in tomato growth, fruit yield and decreased galling after marigold was grown compared to fallow in a field infested with *M. javanica* (Oduor-Owino and Waudo, 1994).

Water extracts of marigold are nematicidal and in some cases phytotoxic to established crops (Westerdahl et al., 1993).Water extracts of marigold applied at 1137 litres/ha/application or 18 grams/litre of plant material was reported to have some nematicidal activity (McKenry, 1991).

2.8 Potential Phytochemicals

The nematicidal potential of different plants has been described. Natural nematicidal chemicals discovered so far includes alkaloids, diterpenes, triterpenes, polyacetylenes, phenolics, thienyls derivatives and sulphur containing plants. African marigold, *lantana camara* contain some of these compounds (Akhtar and Mahmood, 1994).

The insecticidal and repellent effects of *Lantana camara* and *Tephrosia vogelii* have been documented on maize grain borers (Ogendo et al., 2003).

2.9 Neem seed kernel extract

Three natural ingredients of neem – azadirachtin, salanin and meliantriol make better and effective pesticides ever known (Thakar et al., 1981). Azadirachtin is a naturally occurring biopesticide derived from the seed of the neem tree and acts as a hormonal analogue of ecdysone with some activity against a wide range of pests (Raemaekers, 2001). The potential role of neem seed extract depends on a thorough characterization of the extracts use in the commercial setting. Using the leaf miner *Liriomyza trifolii* as a model green house pest (Schmutterer and Ascher, 1986) demonstrated that the rate of death of an insect depended on whether it was applied as a soil drench or as a foliar spray. The application of neem has proved to be an effective barrier against banana weevils and nematodes. Neem pytotoxicity occurred when the powder was applied at 4 tons/ha while 60-100 kg/ha of neem seed powder was effective against banana pests (Musabyimana et al., 2000). In india, Nair (1981), and Reddy et al., (1997) effectively used 400kg/ha of neem seed powder against nematodes attacking tomato crops.

Worldwide attention on the use of neem kernel seed extract over several years has been biased by researchers, administrators and granting agencies. They have approached studies on neem with the opinion that the future of the extract is limited solely to underdeveloped or developing countries. With renewed restriction in use of environmentally harmful and toxic chemicals, certainly the extract has a real promise as an alternative pesticide in many countries where the seed can be grown, harvested and processed locally.

2.10 Integrated weed management

In recent years, concerns over environment effects, economic costs, and long term efficacy of conventional weed management systems have led to research supporting the development of ecologically based management (Benbrook, 1996). One approach to improve weed management and reduce reliance on herbicide technology involves the integration of soil, crop and weed management (Liebman and Davis, 2000).

Organic Agriculture literature (Liebman and Gallandt, 1997; Bond and Grundy, 2000; Liebman and Davis, 2000; and Lotter, 2003) indicates that weeds are best controlled through systemic management. Systemic weed management involves the whole cropping system (Liebman and Davis, 2000) and integrates a wide range of cultural practices to promote biological diversity through rotations, cover cropping, organic amendments to stimulate soil biological activity, and the careful use of selected biological control techniques, naturally occurring plant extracts, and minerals (Lampkin, 1990). Organic soil amendments such as legume green manures, cover crops, animal manures, and composts are fundamental components of low external-input cropping systems that may be valuable for weed management (Liebman and Davis, 2000). Traditionally, the most common uses of organic soil amendments has been as a means of increasing soil fertility and improving soil physical characteristics. Dyck and Liebman, 1994 found that the use of crimson clover (*Trifolium incarnatum* L.) green manure as a nitrogen source decreased lambsquaters (*Chepnopodium album L.)* biomas by 65% and increased sweet corn biomas by 131% compared to a treatment in which a similar amount of nitrogen was supplied in the form of ammonium nitrate fertilizer at planting.

Organic farmers have identified the following soil fertility and/or soil tilth management issues of greatest concern on their farms: building and maintaining organic matter levels, developing soil biological activity, reducing soil compaction, balancing soil pH, and balancing soil nutrients (Walz, 1999). Soil nutrient availability can be improved through a combination of crop rotation, tillage, cultural practices, and soil amendment.

Organic matter constitutes the active or-living component of the soil, affecting physical and chemical properties (Swiader et al., 1992). Organic matter affects plant growth, promotes development of soil aggregates, improving drainage, soil tilth, and structure.

Organic matter can be added to the soil by various methods using green manure crops, cover crops, crop residues, animal manures, mulches, and composts (Precheur et al., 2004). However, the use of fresh organic matter in close proximity to planting of vegetables may cause problems that include burning from rapid decomposition, formation of excessively aerated layers and pockets, which interfere with water movement, locking up of available nitrogen by decomposition bacteria, mechanical interference to plowing and cultivation, and formation of toxic organic compounds, under certain anaerobic conditions (Precheur et al., 2004; Swiader et al., 1992). Soil organic matter (SOM) is linked to desirable soil physical, chemical, and biological properties and is closely associated with soil productivity (Stevenson, 1982; Tate, 1987). Changes in biologically active SOM are linked to a transition effect. The transition from conventional to organic production normally involves a period of suppressed yield followed by a return to yield similar to conventional production (Brusko, 1989; Liebhardt et al., 1989). This transition effect has been attributed in part to changes in the soils biological, chemical, and physical properties governing nutrient cycling, plant growth and development (Wander et al., 1994).

Soil fertility affects weeds, but not much work has been done on the manipulation of soil fertility to manage weed populations. Fertilizer is added to improve crop yield, but often weeds are more competitive with crops at higher nutrient levels. At low weed densities, added fertilizers, particularly nitrogen, will increase crop yield and make the crop a more vigorous competitor. At high weed densities, added nutrients favor weed over crop growth (DiTomaso, 1995). The primary plant nutrients are nitrogen, phosphorus and potassium. Weeds have a large nutrient requirement and will absorb as much or more than crops. Nitrogen is the first nutrient to become limiting between weed and crop competition. The nitrate ion is highly mobile and not held strongly in soil.

Movement of phosphorus is slow and occurs over shorter distances. Competition for these nutrients often occurs after plants are mature and have extensive root development (Zimdahl, 1999).

2.11 Non-chemical Weed Control

Without the aid of herbicides, weed management is one of the greatest challenges for organic growers. Research has devoted little attention to the threat of weeds in organic crop production (Barberi, 2002). As a result, many producers are reluctant to undertake organic transition because of uncertainty over weed population dynamics and management (Ngouajio and McGiffen, 2002). Integrated weed management that relies on multiple weed control methods provide a holistic approach to managing weeds in organic systems (Barberi, 2002). Furthermore, an increase in the understanding of weed biology and ecology underpins long-term improvements in sustainable weed control (Bond and Grundy, 2000). Growers interested in non chemical weed control require more information to meet this challenge.

Cover crop rotations, soil amendments and strategic use of tillage offer an effective approach to non-chemical weed control. In the long term, management strategies to reduce the weed seed bank is a practice to decrease weed pressure (Teasdale et al., 2003). Tillage can be an effective method of weed control; however soil inversion buries new weed seeds and brings buried seed to the surface (Carter and Ivany, 2006). Furthermore, both living and mulched (dead) cover crops physically obstruct weed growth (Creamer et al., 1996; Schonbeck et al., 1991; Teasdale, 1993). In addition, cover crops produce allelochemicals for weed suppression (Einhellig et al., 1993; Ekelme et al., 2003; Khanh et al., 2005). The combination of mulched cover crops, soil amendments and tillage where necessary suppresses weeds and reduces weed seed bank.

2.12 Allelopathic weed suppression

The current trend in agriculture production is to find a biological solution to reduce the perceived hazardous impacts from herbicides and insecticides (Khanh et al., 2005). Plant allelopathy may be an effective tool to help resolve this critical issue. The detrimental effect of allelopathy can be useful for weed and pest control (Rice, 1984). Much research has documented the potential of allelopathic plants to reduce pathogens and affect weed emergence. An explanation of the allelopathy mechanism has been attempted and the influence from a complex of phytochemicals synthesized in plants as secondary metabolites has been proposed. The allelochemicals can be broadly classified as plant phenolics and terpenoids, which show great chemical diversity and are involved in a number of metabolic and ecological processes (Xuan et al., 2005). The most common effects of allelochemicals may occur through leaching, volatilization, root exudation, and the death and decay of the fallen plant parts either via biotic or abiotic means (Anaya, 1990). Allelopathic inhibition of weeds is complex and can involve interaction of different classes of chemicals such as phenolic compounds, flavaniods, terpenoids, alkanloids, steroids, carbohydrates and amino acids. Organic amendments may also suppress weeds by releasing phytotoxins such as phenolic compound during decomposition (Ohno et al., 2000) and short chain fatty acids (Ozores-Hampton et al., 1999). These compounds have been linked to allelopathic inhibition of weed growth (Liebman and Ohno, 1998; Ohno et al., 2000) and increases in the pathogenicity of soil borne diseases (Dabney et al., 1996). *Lantana camara* roots and shoots when incorporated into the soil reduce germination and growth of weeds such as the milkweed vine (Kruse and Stranberg, 2000). *Tephrosia spp* is used to suppress weeds when used as dry fallow in rice fields (Becker and Johnson, 1998).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

The research work was conducted at James Finlay's flowers (K) Limited, Kericho District in Kenya. The site lies approximately at Latitude 0° 23'S and Longitude 35° 17'E. The farm is in the Agro-ecological zone LH_I at an altitude of 1950 m above sea level. The mean annual rainfall is 1400 mm. The mean temperature ranges between 14.8°-16.4° C. The soils are moderate to high in fertility. They are well drained, extremely dark redish brown, friable and slightly smeary clay with acid humic top soil (ando-humic NITOSOLS) (Jaetzold and Schimdt, 1993).

3.2 Weather conditions during research

The temperatures remained consistently high during the first planting season (week 1 to week 15) compared to the second season (week 20 to week 35) as shown in table 1. The research was conducted during the month of June to October 2006 (first season) and November to March 2007 (second season). The relative humidity was initially high during the first season decreasing by 8 units towards the end of the season, but initially low at the beginning of the second season increasing by 5 units.

Table 1. Temperature and Relative humidity during the growing period

Week	Maximum Temp °C	Minimum Temp °C	% Relative Humidity
1- 4	25	11	75
5- 8	25	11	74
9-12	25	11	70
13-15	26	11	67
16-19	26	11	70
20-23	27	11	66
24-27	23	12	75
28-31	24	12	75
32-35	26	12	74

Maximum, minimum temperature and Relative humidity values were the daily averages taken at 4 week intervals.

3.3 Field production and culture of *Asclepias tuberosa*

Finlay's flowers obtained planting seed from Genesis Seeds Ltd, a breeder in Israel. The seeds were sown in ground beds in the greenhouse. The ground beds were treated with chopped plant materials (organic soil amendments), a granular nematicide (nemacur) or a combination of both. The size of each ground bed was 30m long and 1m width and was separated from other beds by a 50 cm path. The flower seeds were planted at a spacing of 20 cm by 20 cm on netting material at a population density of 125 plants/m^2.

The plants were irrigated by two micro irrigation tubes placed at 30.5 cm a part on the beds. Seedlings were fertilized using a feeding programme starting 4 weeks after planting. The plants were supplied with the following nutrients during different growth phases by fertigation.

i) Establishment: N-60, P-20, K-38, Ca-39, Mg-25, Fe-660, B-205, and Mn-200ppm
ii) Vegetative phase; N-105, P-20, K-126, Ca-70, Mg-34, Fe-660, B-205 and Mn 200ppm.
iii) Production phase; N-100, P-20, K-109, Ca-66, Mg-32, Fe-660, B-205 and Mn 200ppm.

Asclepias is a qualitative long day plant and requires 15-16 hours of light to flower (Halevy, 1999). Cyclic lighting was done using normal 100-150 watt bulbs to provide 15 watts per square meter. The bulbs were placed 2.5 m above the canopy of the crop. Two layers of support wire were used to hold the crop with the first one placed 20 cm above the ground followed by another one at 40 cm above the ground to ensure flowering stem remained upright. Drip irrigation and watering was maintained on a constant schedule throughout the production cycle.

3.4 Treatment application

The treatments consisted of the following soil amendments applied alone or in combination with nemacur.

Treatment number	Treatment/Treatment combination
1	Control- Untreated (No TRT)
2	*Tagetes patula* (TP) 300gm/m^2
3	*Tagetes patula* + Nemacur (TP+N) 75 gm/m^2 + 20 gm/m^2
4	*Lantana camara* (LC) 10% w/w
5	*Lantana camara* + Nemacur (LC+N) 5% w/w + 20 gm/m^2
6	*Azadiracta indica* (AI) 150 gm/m^2
7	*Azadiracta indica* + Nemacur (AI+N) 75 gm/m^2 + 20 gm/m^2
8	*Tephrosia vogelii* (TV) 10% w/w
9	*Tephrosia vogelii* + Nemacur (TV+N) 5% w/w + 20 gm/m^2
10	Nemacur (N®) 40 gm/m^2

The experiment was set up in a greenhouse naturally infested with a mixed nematode population of *Meloidogyne javanica*, *Pratylenchus penetrans* and *Meloidogyne incognita.* Fumigation or contact nematicides had not been used in the greenhouse for one year. The experiment was laid down in a complete randomized block design (CRBD). The plots measured 2.4 m by one metre separated by a 50 cm path. There were three replications. Blocks were separated by a one meter path.

Organic soil amendments were incorporated in the soil five weeks before planting of flower seeds to allow decomposition to take place (Asirifi et al., 1994). The differents plant materials were chopped into small pieces of about 2 cm and mixed with moist soil. Nemacur was applied during planting. The organic soil amendments were mixed with soil at 300 gm/m^2 for *T. patula* (Chindo and Khan, 1990). Succulent leaves of neem were applied at 150 gm/m^2 (Musabyimana et al., 2000; Sharma et al., 1996). *L. camara* and *T. vogelii* were incorporated in the soil at 10% w/w (Ogendo et al., 2003). Where the plant materials were combined with nemacur, rates of 5% w/w were used.

3.5 Source of plant materials used in preparation of soil amendments

Flowering twigs of *L. camara* and *T. vogelii* were collected from uncultivated farm boundaries or in cultivated areas where they grow vigoursly and colonize land in highland regions of Kenya. *T. patula* was obtained from planted bedding plants while neem twigs were harvested from established trees in the Kerio Valley region, Marakwet District.

3.6 Determination of the organic matter and ash content of the plant material

Organic matter and ash content were determined using the method by Bremmer, 1982 (Appendix 3). This method uses the ash content of the organic plant material.The sample is ignited slowly in a muffle furnace to a final temperature of 550^{o} C. The loss in weight represents the moisture and organic content of the sample, while the residue represents the ash (Anderson and Ingram, 1993).

3.6.1 Determination of the C:N ratio of the organic amendments

Critical C:N ratio was determined by method described by Black, 1965 (Appendix 4). Organic carbon was determined by the sulphuric acid and aqueous potassium dichromate mixture.

3.6.2 Determination of the Nitrogen Content

The organic material and soil Nitrogen content was determined using Micro-Kjeldah technique as described by Steyermark et al., 1958 and Bremmer, 1982 (Distillation and titration method-Appendix 2). The nitrogen content of the soil was determined six weeks after incorporation of the plant material into the soil.

3.6.3 Soil chemical analysis

The soil analysis established moderate acidity (Table 2). The bases of sodium (Na) were adequately supplied while potassium (K) was low. The micronutrient manganese (Mn) was fairly adequate during the first season but excessive during the second season. Calcium (Ca), magnesium (Mg) and phosphorus (P) were excessive in the soil. Nitrogen (N) and carbon (C) were moderately supplied in the soil. The trace elements copper (Cu), iron (Fe) and zinc (Zn) were adequate in the soil in both seasons (Brandy and Weil, 2002).

Table 2. Fertility evaluation analysis before application of treatments

Parameter	Season 1	Season 2
pH	5.81	5.80
Sodium (Na) %	0.58	0.42
Potassium (K) %	1.7	1.88
Calcium (Ca) m %	44.80	36.00
Magnesium (Mg) %	4.02	3.25
Manganese (Mn) %	1.42	2.36
Phosphorus (P) %	30.18	15.43
Nitrogen (N) %	0.80	0.95
Carbon (C) %	2.01	1.99
Copper (Cu) ppm	3.82	3.03
Iron (Fe) ppm	49.87	44.82
Zinc (Zn) ppm	26.65	28.56

Soil analysis results of samples taken at 30 cm depth.

3.7 Nematode sampling and extraction

3.7.1 Asclepias root extraction

Five plants were systematically sampled from each plot when the flowers were in full bloom. The plants were uprooted taking care not to damage the root system i.e. fine roots. The roots were then removed and placed in moist polythene bags. The roots were washed, sliced into 3 cm lengths and mixed thoroughly. A 10 gm random sample of fine root tissue was taken and placed in a 250 ml Elenmeyer flask, washed with distilled water and shaken for three days on an Eberbach platform shaker. The distilled water surrounding each root sample was decanted onto a sieve and then backwashed onto a wet strength tissue in a modified Baermann funnel. Following a 48 hours period of incubation, nematodes were drawn off in 20 ml water and the water volume standardized to 25 ml. The sample was stirred for 5 seconds before a 5 ml aliquot was pipetted into clear polystyrene 60 x 15 mm tissue culture dish for nematode counting and identification. Nematodes were counted in the 5 ml sub sample and multiplied by 5 to give the number of nematodes in 10 g of roots. The extraction method was adopted from Alexander and Waldenmaier (2002).

3.7.2 Soil extraction

Soil samples were taken prior to planting and at harvest. Fifteen soil cores, 15 to 20 cm deep were randomly collected from each plot with a standard 2.0-cm internal diameter sampling tube, approximately 10 cm from the plant stems. The soil cores from each plot were combined and mixed thoroughly. A 250 cm^3 sub sample was diluted in 3 litres of water, stirred for 30 seconds, allowed to settle for 60 seconds and decanted through a 595 µm pore sieve placed over a 45 µm pore sieve. Contents of the 45 µm pore sieve was backwashed into a wet strength tissue in a modified Baermann funnel. After 48 hours at room temperature, nematodes were drawn off in 20 ml water and the water volume standardized to 25 ml. The sample was stirred for 5 seconds before pipetting a 5ml aliquot into clear polystyrene 60 x 15 mm tissue culture dish for nematode counting and identification. The number was multiplied by 5 to calculate the number of nematodes per 250 cm^3 soil sample (Alexander and Waldenmaier, 2002).

3.8 Evaluation of Vegetative, flowering characteristics, yields and quality

Data collected included the number of days from the date of planting to seedlings emergence and establishment. Flower stems were harvested when 70-75 % of the flowers in the terminal inflorescence had opened.

Plant height in centimeters was measured 30, 60, 90 and 120 days after planting. The stems were cut 5 cm above the soil and the following data were recorded: the number of days from planting to harvesting, the total cut stem height, cut stem weight, stem base diameter and inflorescence diameter. The stems were classified into five grades which were: Grade 1 (60 cm and above), Grade 2 (50-59 cm), Grade 3 (40-49 cm), Grade 4 (30-39 cm) and Grade 5 (20-29 cm). The numbers of flowers in an inflorescence per stem were counted and the size of the flower head measured in centimeters.

3.9 Galling index

Sampling of infected and damaged plants was done for nematodes. A rating scheme of 0-10 developed by (Zeck, 1971) for evaluation of root-knot nematodes infestation, root infection and galling was used to quantify the level of damage by nematodes as follows:

0	Healthy root systems, no infection.
1	Very few galls, only detected on close examination.
2	Small galls, easy to detect.
3	Numerous small galls.
4	Numerous small galls and a few big galls.
5	25% of the root system severely galled and not functioning
6	50% of the root system severely galled and not functioning.
7	75% of the root system severely galled and not functioning.
8	No healthy root, plant still green.
9	Root rotting completely galled and plant dying.
10	Plant and roots dead.

3.10 Weed density and biomass

A quadrat with an area of 1 m^2 was used to randomly determine the distribution and identify weeds under different treatments. The following weeds were identified; *Oxalis corniculata* and *Stellaria media*. Weed biomass was determined by clipping the shoots at the soil surface and weighing them to obtain the fresh weight. The shoots were then dried at 60° C for 120 hours to a constant weight and then weighed as described by Warembourg and Paul (1973).

3.11 Plant biomass

Above ground biomass of ten flower stems was determined by clipping the shoots at the soil surface. Plant roots then were carefully removed from the soil. Fresh weights of both shoot material (stems and leaves) and root material for plant were measured using model N02120 Ohaus digital balance. For dry weight, the plant shoot and root materials were placed in separate brown paper bags and marked separately for each plant. The shoots and roots were dried at 60°C for 120 hours and then weighed as described by Warembourg and Paul (1973).

3.12 Statistical Analysis

The data obtained was subjected to Analysis of Variance (ANOVA) at P $\leq$ 0.05 using PROC GLM (SAS version 8, 1999). Significantly different means were separated using Duncan's Multiple Range Test at 5% level of significance. The univariate procedure of SAS was used to check that the data were normally distributed before analysis. Data transformation was done for root galling index while all the other data met the assumptions of ANOVA and did not require transformation.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 The influence of soil amendments and nemacur in the management of root-knot nematodes

4.1.1 Counts of root-knot nematodes before and after soil treatment

The degree of control of root-knot nematodes was relative to the type of soil amendment used and the amount of nemacur used in the combination (Table 3). Nemacur applied alone at 40 g/m^2 reduced root-knot nematode count by between 79.8% and 82.6% compared to the control 35 Days after treatment (DAT). Although the application of *Lantana camara* generally suppressed root-knot nematodes, a combination with nemacur was more effective. *L. camara* alone achieved a control of 54% to 58%, but the combination of *L. camara* and nemacur significantly reduced nematode count by up to 73.4% compared to the control.

Table 3. Count of root-knot nematode (second juvenile stage) in $250cm^3$ of soil sampled from different plots before treatment and after bed treatment (35 DAT)

	Root knot nematode counts/250 cm^3			
	No. Before Bed Treatment		No. after Bed Treatment (35 DAT)	
Treatment	Season 1	Season 2	Season 1	Season 2
NO TRT	34.00 ab	29.67 abc	36.33 a	34.67 a
TP	28.67 b	23. 00 c	23.67 d	22.67 cd
TP+ N®	38.33 a	28.67 abc	19.00 e	18.67 de
LC	33.33 ab	28.33 abc	15.33 f	16.00 e
LC+ N®	32.67 ab	30.00 abc	9.67 g	10.00 f
AI	29.00 b	29.67 abc	17.33 ef	20.00 cde
AI+ N®	32.67 ab	35.33 a	25.00 cd	23.33 bc
TV	31.33 ab	27.67 c	29.33 b	27.33 b
TV+ N®	33.00 ab	32.33 ab	27.33 bc	27.33 b
N®	31.00 ab	28.67 abc	6.33 h	7.00 f

[1]Means within a column followed by different letters are significantly different at $P \leq 5\%$ level of significance according to Duncan's Multiple Range Test.

The application of *L. camara* generally suppressed root-knot nematodes both in the first and second season. The combination of *L. camara* (5% w/w) and nemacur (20 g/m^2) was more effective than *L. camara* alone at 10% w/w. This could be due to the effect of chopped *L. camara* on soil properties (texture and moisture retention). Nematode reproduction and distribution has been found to differ with soil texture (Mateille et at., 1995).

In one study, it was found that amendment of soil with *L. camara* caused soil suppressiveness to *Meloidogyne javanica* and produced marked changes to the fungal communities in the soil and endorhiza. It is likely that those fungal species, especially the endophytes that were reduced or specifically promoted by *L. camara* had a role in suppressing *M. javanica.* However, a role of toxic compounds produced by *L. camara* itself cannot be ruled out. *L. camara* is reported to produce unknown compounds of a polar nature that caused juvenile mortality of *M. javanica* (Ali et al., 2001). In that same study, *L. camara* added to the soil generally caused a strong reduction in nematode population density and root-knot nematodes of mungbean.

Lantana was selected based on both field observations and historical references on the toxic nature of this species. Lantana has been observed to successfully inhabit citrus groves heavily infested with nematode populations. It is possible that root exudates and decomposing litter suppress the nematodes. Morton (1971) detailed the toxic characteristics of *L. camara*: "Long recognized as highly toxic to grazing animals; has caused death in children when a large quantity of unripe berries was eaten." Lantana produces allelopathic substances in its roots and shoots, potentially increasing its competitive ability (Sahid and Sagau, 1993). It strongly resists herbivory, contributing to its pest-plant status as a noxious weed outside its natural range (Janzen, 1983).

It is possible that soil amendment with lantana had increased surface area which allowed for better contact between the root-knot nematode and nemacur. The number of root-knot nematodes recovered from lantana amendment alone was less than those in the combination with nemacur probably due to rapid multiplication in organic matter. Nematode abundance is high in high input organic system and lowest in the popular conventional farming systems (Freckman and Ettema, 1993), where organic matter content is undoubtedly low.

Nemacur had the same synergetic effect on the efficacy of *Tagetes patula* and *Azadirachta indica* as observed on *L. camara*. While the application of *T. patula* (300 g/m^2) alone reduced root-knot nematode count by between 34.7% and 34.8%, a combination of nemacur with *L. camara* reduced the root-knot nematode population by between 34.7% and

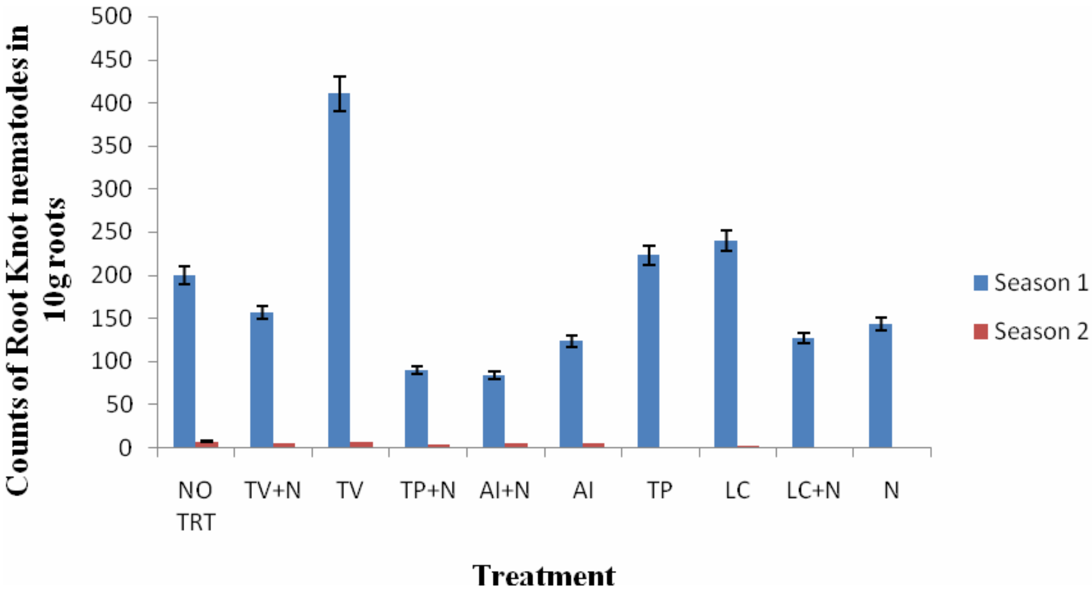
34.8%, a combination of nemacur with *L. camara* reduced the root-knot nematode population by between 47.7% and 46.1% (Table 3). The application of *A. indica* alone reduced root-knot nematodes by between 42.3% and 52.2% compared to the control. The trends from shoot evaluations were similar in both *T. patula and A. indica* amended soils. The shoot portions of several selected plant species including poinsettia (*Euphorbia pulcherrima*) and spotted spurge have been found to provide more mortality than root portions of the same species. These results are similar to those previously published about some of these species dealing mainly with insect pests (Prakash and Rao, 1997). Most observations on these plants noted that their pesticidal characteristics occur in the shoot and had activity against insect species.

Organic amendments probably improve tolerance of host and apparently reduce nematode populations (Dunn, 2006). In another study, *Brassica juncea* biofumigants applied at 3 and 4 kg/m^2 were effective in suppressing root knot nematodes and *Fusarium oxysporum f. sp. rosae* in greenhouse grown rose flowers (Oloo, 2006). In the present study, *L. camara, T. patula* and *A. indica* all showed root knot nematode suppression 35 days after treatment.

4.1.2 Counts of Root-knot nematodes at flowering stage of *A. tuberosa*

In the first season, nemacur 40g/m^2, *L. camara* (5% w/w) + nemacur, (20gm^2), *L. camara*, (10% w/w), *T. patula* (300g/m^2) and *A. indica* (150g/m^2) reduced root-knot nematode population by between 69% and 90% compared to the control (Fig. 1).

The application of *T. patula* (150g/m^2) + Nemacur (20g/m^2), *T. vogelii* (10 %w/w) and *T. vogelii* (5%w/w) + Nemacur (20g/m^2) reduced root-knot nematodes by between 67% and 74% at the flowering stage of *A. tuberosa* compared to the control. There was a significant reduction in the number of root-knot nematodes in plots where *L. camara* was applied compared with the control. However a combination of *L. camara* and nemacur gave better results than *L. camara* alone. The results compare with the findings by Shaukat and Siddiqui, (2001) who concluded that addition of *L. camara* to the soil at 1% (w/w) significantly reduced *Meloidogyne javanica* population density in the soil and in the roots of mungbean, as well as subsequent root-knot infestation of mungbean. This confirms that suppression of nematodes was induced by organic amendment. The findings are also in agreement with the work undertaken by Ogendo et al., (2003) who reported a 75% reduction of maize grain weevils by *L. camara* and *T. vogelii*.



No TRT-Control, TP-*Tagetes patula*, LC-*Lantana camara*, AI-*Azadirachta indica,* TV-*Tephrosia vogelii,* N®-Nemacur.

Fig. 1. Influence of soil amendments and nemacur on the number of root-knot nematodes in 10 g roots at the flowering stage of *Asclepias tuberosa*.

Lantana leaves contain poisonous triterpines and lantadenes A and B that cause death of horses, cattle, sheep, goats, and rabbits by causing liver failure and other organs (Morton 1994; Munyua et al.,1990). Marigold roots release the chemical alpha-terthienyl, one of the most toxic naturally occurring compounds found to date (Gommers and Bakker, 1988). This compound is nematicidal, insecticidal, antiviral, and cytotoxic (Arnason et al., 1989; Marles et al., 1992). The presence of alpha-terthienyl inhibits the hatching of nematode eggs (Siddiqui and Alam, 1988).

Nemacur suppressed root-knot nematodes to low levels compared to the plots with no treatment in both seasons (Fig 1.). The findings in this study indicated that nemacur was highly effective against root-knot nematodes. Fumigant nematicides provided good control of nematode population when application was followed by application of a non fumigant nematicide such as Cadusafos or Oxamyl (Giannakou et al., 2002). It has been pointed out that fumigant nematicides do not provide season long control of nematodes. There is synergistic effect when fumigants are applied in combination with other chemicals (Giannakou et al., 2002). The use of organic phosphate or carbamate nematicides for control of root-knot nematodes on tobacco were shown to be very promising. Since they were most effective when applied to the soil surface and incorporated, making them better suited than volatile nematicides for application in mixtures with other pesticides such as herbicides and fungicides (Broodie and Good, 1973).

Generally, there were fewer root knot nematodes during the second season compared to the first season (Figure 1). This could be attributed to the prevailing weather conditions as shown in table 1. During the second season (week 20-35), temperatures and relative humidity were higher compared to the first season (week 1-15). Nematode densities are subject to conspicuous seasonal fluctuations (Buth & de Wolf 1985). Some species are only found in certain seasons. In one study, Ploeg and Stapleton (2001) reported that temperature and amendment of soil with broccoli residues had synergistic effect on the infestation of melon plants by *Meloidogyne* i*ncognita* and *M. javanica.* An increase of temperature from 20°C to 25, 30 or 35°C dramatically reduced infestation and galling compared to that in unamended soils.

4.1.3 Galling index

The root-knot galling index of asclepias generally varied according to the type of organic soil amendment used and the amount of nemacur used in the combination (Table 4). During the first and second seasons, *A. indica* (150 g/m^2), *T. patula* (150 g/m^2) + nemacur (20 g/m^2), *T. patula* (300 g/m^2), *L. camara* (5% w/w) + nemacur (20 g/m$^{2)}$ and nemacur (40 g/m^2) significantly reduced galling of asclepias roots compared to the control. The reduction in galling index ranged from 0.0 to 0.58 compared to the control.

Nemacur alone or in combination with *L. camara* caused the greatest reduction in the galling index in both seasons. Galling was reduced up to 79% in both seasons. In one study, *L. camara* added to the soil caused a strong reduction in nematode population density and root-knot of mungbean (Ali et al., 2001).

Nemacur generally reduced galling on asclepias roots 60 days after planting (DAPL). The results of this study are consistent with a study on root-knot nematode control and tobacco yields in plots infested with *Meloidogyne incognita* (Broodie and Good, 1973). Treatments with the nonvolatile nematicides, Aldicarb, Mocap, or Nemacur gave greater nematode control than those on similar plots treated with volatile nematicides such as tetrachlorothiophene.

In current the study, *Azadirachta indica* (neem) alone (applied at 150 g/m^2) suppressed nematodes better compared to the control. The results of the current study are consistent with the finding by Sharma et al., (1996) who found that neem cake applied at 1500 kg/ha reduced nematodes on tomatoes.

Table 4. Influence of Soil amendments and nemacur on gall index.

	Gall index (0 – 10) 60 DAPL	
Treatment	Season1	Season 2
NO TRT	0.98 a	0.98 a
TP	0.56 c	0.56 c
TP+ N®	0.58 c	0.32 d
LC	0.73 b	0.80 ab
LC+ N®	0.30 d	0.30 d
AI	0.69 bc	0.73 bc
AI+ N®	0.88 a	0.90 ab
TV	0.90 a	0.97 a
TV+ N®	0.93 a	0.80 ab
N®	0.00 e	0.16 d

[1]Means within a column followed by different letters are significantly different at $P \leq 5\%$ level of significance according to Duncan's Multiple Range Test.
Gall index 0 = no damage, 1 = few galls, 2-7 = small galls to big galls, 8 = no healthy roots, 9 = root rotting, completely galled and plant dying, 10 = plant and roots dead.
Data transformation (Log) was carried out before analysis because the mean and the standard deviation were not normall and independently distributed.

Musabyimana et al., 2000 established that 60-100 kg/ha of neem seed powder was very effective against banana weevils and nematodes. Regardless of the treatment or the treatment combination in which neem was applied, there was a significant reduction in nematode population in comparison with untreated control (Table 4). However, the lowest nematode population was recorded when neem had been applied alone. This gave a gall index of 0.69 to 0.73compared to an index of 0.88 to 0.90 when it was applied in combination with nemacur.

Recently, much effort has been devoted to neem extracts as an alternative to synthetic pesticides. Neem extracts have been evaluated against various insect pests (Saxena, 1989 and Schmutterer, 1990), and nematodes (Mojumder, 1995 and Sharma et al., 1996). The efficacy of neem seed powder and neem cake against banana parasitic nematodes was demonstrated in screen house and outdoor tests (Musabyimana and Saxena, 1999).

Tagetes patula (300 g/m^2) alone or *T. patula* (150 g/m^2) in combination with nemacur (20 g/m^2) reduced root galling significantly. In another study it was reported, sixty days after the start of the experiment the highest numbers of the second juvenile stage nematodes were extracted from tomato roots, and the fewest from *Tagetes spp*. Galls were seen only on roots of tomato and Scarlet Sophie, with average gall indices of 3.0 and 2.6, respectively (Ploeg and Maris, 1999). *T. patula* may not eradicate nematodes. However, it could provide a continuous suppression effect on nematode populations where it is grown every season before the actual crop is planted. This would suppress nematode populations over time as it happens in the presence of susceptible crops like vegetables and bedding plants (McSorley et al., 1994).

4.1.4 Counts of Saprophytic nematodes before treatment and at planting time

In first season, before treatment there were variations on the number of saprophytic nematodes in the different plots. However, during the second season there were no variations between the treatments and the control (Table 5). At the time of planting in the first season, the number of saprophytic nematodes recovered for all the treatments were significantly lower compared to the control. A combination of *L. camara* (5% w/w) + nemacur (20 g/m^2) or nemacur alone (40 g/m^2) gave the least nematode count by 75.2% at planting. However, during the second season treatments did not affect nematode count.

Table 5. Count of Saprophytic nematodes in 250cm^3 soil sampled from different plots before treatment and during planting

	Before Bed treatment no. / 250 cm^3		During planting no. / 250 cm^3	
Treatment	Season 1	Season 2	Season 1	Season 2
NO TRT	44.00 ab	41.33 a	40.33 a	38.33 a
TP	38.67 b	42.67 a	28.67 c	36.67 a
TP+ N®	48.00 a	44.67 a	36.67 ab	36.67 a
LC	40.67 ab	44.00 a	33.67 bc	40.67 a
LC+ N®	49.00 a	48.00 a	10.00 d	38.33a
AI	44.67 ab	39.33 a	29.00 c	40.67 a
AI+ N®	46.00 ab	49.00 a	32.00 bc	36.67 a
TV	38.67 b	43.33 a	30.67 bc	43.33 a
TV+ N®	40.67 ab	44.67 a	32.67 bc	44.00 a
N®	42.67 ab	44.33 a	10.33 d	44.67 a

[1]Means within a column followed by different letters are significantly different at P ≤ 5% level of significance according to Duncan's Multiple Range Test.

4.1.5 Counts of saprophytic nematodes in the soil at the flowering stage

At the flowering stage (60 DAPL) in the first season, *Tagete patula* (300 g/m^2), *Azadirachta indica* (150 g/m^2), *Lantana camara* (10% w/w), *Tephrosia vogelii* (10% w/w) and combinations of the plant materials with nemacur (20% g/m^2) suppressed saprophytic nematodes numbers by between 42.7% and 83.7% (Table 6). Generally, number of saprophytic nematodes recovered in the second season was low compared to the first season. However, during the second season, there was no significant difference between the treatments. In the first season, treatments with nemacur and *L. camara* + nemacur had the lowest nematode numbers. Therefore, a lower dose of nemacur in combination with *L. camara* was as effective as the recommended dosage of nemacur alone. This was probably due to synergistic effect of the active ingredients in both materials.

Table 6. Counts of saprophytic nematodes in 250cm^3 soil sampled from treatment plots at flowering stage

	Flowering stage no. / 250 cm^3(60 DAPL)	
Treatment	Season 1	Season 2
NO TRT	143.00 a[1]	62.67 a
TP	36.67 ed	19.33 b
TP+ N®	49.33 d	29.00 b
LC	42.00 d	20.33 b
LC+ N®	23.00 e	21.33 b
AI	47.00 d	19.67 b
AI+ N®	82.00 cb	30.67 b
TV	94.67 b	22.33 b
TV+ N®	77.33 c	32.22 b
N®	23.33 e	24.00 b

[1]Means within a column followed by different letters are significantly different at $P \leq 5\%$ level of significance according to Duncan's Multiple Range Test.

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