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MANAGEMENT OF ROOT-KNOT NEMATODES (*Meloidogyne* spp) IN OKRA (*Abelmoschus esculentus* (L) moench) USING NEMATODE SUPPRESSIVE CROPS AND ORGANIC SOIL AMENDMENTS

MWEKE ALLAN NDUA

A thesis submitted in partial fulfilment of requirements for the award of Master of Science degree in crop protection at the University of Nairobi Department of Plant Science and Crop Protection, Faculty of Agriculture, College of Agriculture and Veterinary Sciences

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DECLARATION

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

Mweke. A. N
University of Nairobi
Department of Plant Science and Crop protection
P.O Box 30197
Nairobi

Signed-- Date---09-08-2007---

This thesis has been submitted for examination with our approval as University supervisors:

Dr. J. W. Kimenju
University of Nairobi
Department of Plant Science and Crop Protection
P.O Box 30197
Nairobi

Signed-- Date---9/8/07---

Prof. E. W. Mutitu
University of Nairobi
Department of Plant Science and Crop Protection
P.O Box 30197
Nairobi

Signed-- Date---13/08/07---

DEDICATION

To Teresia, Jossineter and Cynthia.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF APPENDICES

ABSTRACT

This study was undertaken to develop an intergraded root- knot nematode management strategy in okra by using crop rotation of nematode suppressive crops and incorporation of organic amendment in to the soil. Several cereals and legume crops were screened in a greenhouse and okra was used as a control. The greenhouse experiment revealed that maize, sorghum, millet and guwar were suppressive to root-knot nematode. Pigeon pea was moderately susceptible while cowpeas and green grams were susceptible.

After the greenhouse experiment, three maize varieties (babycorn, sweetcorn and pioneer-Ph3253) and guwar were selected for a repeat experiment under field conditions in rotation with okra. Results showed that guwar and sweetcorn were the most suppressive followed by maize cv Pioneer (Ph3253) and Babycorn.

Based on the results of greenhouse experiments, four crops namely guwar, sweet corn, baby corn and maize cv Pioneer (Ph3253) were selected for their effectiveness in suppressing root-knot nematodes in rotations with okra. The findings from this study demonstrate that one season rotation of these suppressive crops with okra was not enough to reduce nematodes to levels below economic damage threshold because nematode populations quickly build up when okra was planted immediately after these crops. Rotation of sweetcorn followed by okra appeared to be more effective in reducing nematodes build up as it recorded least population build up followed by guwar, maize cv Pioneer (3253) and Baby corn in that order.

Farmyard manure was incorporated into the soil before planting okra after these selected crops to determine the effect of combining rotation of suppressive crops and organic amendment in root- knot nematode management.

There was clear evidence that combining organic amendment and crop rotation incorporating nematodes suppressive crops was more effective in reducing nematode population in the soil than rotation alone. Reduction of the *Meloidogyne* population in the soil was higher in soils where guwar was planted followed by okra indicating that this is a good combination in root-knot nematode management. Among the rotation cycles tested was sweet corn in the first season followed by okra in the next season.

This study shows that integrated management approach in root knot nematodes management is the way forward because it is more effective and sustainable in the long run. Organic manure (farmyard manure) incorporated in guwar and rotated with sweet corn can be recommended to farmers as rotation crops because of their performance in this study. Also because they mature fast and have ready market. Once adopted, this integrated approach will result in increased yields and income to smallholder farmers. It is affordable, easy to apply as well as environment friendly and hence sustainable over a long period of time. Farmers will reap multiple benefits.

CHAPTER ONE

1.0 INTRODUCTION

Okra (*Abelmoschus esculentus* (L) Moench) is an important crop in the tropics and sub-tropics and is one of the export vegetables grown in Kenya's warm lowlands. In Kenya, smallholder farmers supply about 80% of okra to exporters (Varela and Seif, 2004). The crop is cultivated for its immature fruits (pods) and as an export crop is the third foreign exchange earner after French beans and snow peas. It is a native of West Africa (MOALD M., 1993, HCDA, 1996). The most suitable growing areas in Kenya include Kibwezi, Yatta, Perkerra, Mitunguu, Makuyu, Lower Nyanza, Kerio Valley and Coastal regions of Taveta and Rombo (MOA RD, 2000).

Okra is a tender vegetable that cannot tolerate low temperatures for long. Frosts are deadly to the plant and optimum temperature for its growth is 24-30°C while the maximum is 35°C. Although okra is highly resistant to drought, it still requires considerable amount of water for optimum growth and optimal yields. Pests and diseases are major production constraints and cause serious yield losses. The most economically important pests and diseases include red common bugs, aphids, red spidermites, thrips and root knot nematodes (*Meloidogyne* spp). Black leaf mold, yellow vein mosaic virus (transmitted by white flies), powdery mildew and leaf spots characterized by brown spots on leaves are among the most important diseases that attack okra. Nematodes can cause yield losses of 10-50% in severely infested fields (Berkerlaar, 2001).

Okra is known to be highly susceptible to root knot nematodes damage (Sikora,1992; Noling, 2002). Nematode infected plants are usually stunted, show signs of nutrient deficiency and appear unhealthy with elongated round swellings on both large and small roots. With severe infestations yields loss ranges between 10-50% (Berkerlaar, 2001).

1.1 Management of Root - Knot Nematodes

A number of strategies have been developed for the management of root - knot nematodes but their adoption is restricted due to some limitations (Johnson *et al.*,1992; Mateeva *et al.*, 2000). These strategies include chemical control, fallowing, cover cropping, crop rotation, biological control, flooding, host resistance and use of organic amendments (Berkerlaar, 2001; Noling , 2002).

Cover crops grown for forage or soil improvement may negatively impact on nematodes. Certain cover crops are grown deliberately to suppress nematodes (Rodriguez-Kabana *et al.*, 1989, McSorley, 1998). A number of cover crops like castor (*Ricinus communis*), oat (*Avena sativa*), velvet bean (*Mucuna pruriens*), Sorghum (*Sorghum bicolor*), *Crotalaria spectabilis*, sunnhemp (*C. juncea*) among others are known to be suppressive to more than one *Meloidogyne* spp(Hagan *et al.*, 2002; McSorley *et al.*, 1998), However the effects from suppressive rotation cover crops are typically short lived when they are rotated with a susceptible crop (McSorley, 1999).
Therefore, effective use of cover crops for nematode management requires knowledge of the nematodes present and the susceptibility of the crops to be used (Rodriguez-Kabana *et al.*, 1989, McSorley, 1999).

Management of root knot nematodes by suppressive mechanisms mainly involves use of cover crops and green manures which improve soil fertility by releasing nutrients upon decay (Luna, 1998; McSorley, 1998; Sardanelli *et al.*, 2002). The time that is required for cover crops or green manures to fully decay varies and can take up to an entire year. Both cover crops and green manures increase the bacteria and fungi and other beneficial microbial populations in the soil which can aid in reducing root knot nematodes (Barker *et al.*, 1985).

Soil amendments such as compost and mulches with high organic matter increase the number of micro-organisms antagonistic to nematodes (Luna, 1998). Other suppressive mechanisms include crop rotation, clean fallow and resistant varieties (McSorley, 1998). Cultural practices such as fallowing and crop rotation are limited in adoption due to scarcity of arable land and are ineffective due to the broad host range of root knot nematodes (Thomason and Caswell, 1987; Siddiqui and Alam, 1999). The use of resistant cultivars is the cheapest and the most practical method but they are largely unavailable to farmers (Netscher and Sikora, 1990). Use of biological control is limited by difficulties in mass production and imbalance in biodiversity and un-affordability by smallholder farmers (Becker and schwinn, 1994).

Organic amendments have a suppressive effect on nematodes through stimulation of antagonistic microbes or by releasing toxic by-products upon decomposition (Sayre and Starr, 1988; McSorley, 1998). The soil amendments also greatly contribute to soil fertility and increased water holding capacity resulting in improved plant growth and hence tolerance to nematodes (Sikora, 1992). The amendments supply nutrients after decomposition and also increase the level of biological activity and diversity in the soil (Berkerlaar, 2001). Increase in biodiversity within the soil habitat may play an important role in the natural regulation of plant parasitic nematodes, keeping their densities below damage thresholds.(Sikora, 1992, Siddiqui and Alam, 2001). Organic amendments have a strong stimulatory effect on saprophytic fungi and bacteria, predaceous nematodes and predatory mites that are natural enemies of plant parasitic nematodes (Sikora, 1987). Decomposition by-products such as ammonia and fatty acids have been found to be ten times more toxic to *Meloidogyne incognita* than to free living nematode *Panagrellus redivivus* (Rodriguez-Kabana, 1986;Sayre and Starr, 1988).

Since plant pathogenic nematodes are obligate parasites, the absence of susceptible hosts from the soil for extended period results in population decline due to starvation and inability to reproduce. Crop rotation is based only on particular non-host plants (Berkerlaar, 2001). Combining rotation with nematode suppressive cover crops offers some potential for nematode management (Luna, 1998). Several plants are known to be antagonistic to nematodes because they produce exudates that possess nematicidal properties (Noling and Becker, 1994). The most widely reported are *Tagetes spp*, mustard, asparagus, sesame, sunnhemp (*Crotolaria* sp.) and neem. Species of *Crotolaria* and *Tagetes* depress populations of certain nematodes more rapidly than is recorded under total absence of plants. In addition to nematode suppression some of the antagonistic plants provide other benefits to crop production. For instance, sorghum suppresses root knot nematode populations and restores large amounts of soil organic matter (Noling and Becker, 1994)

Integrated management of nematodes by combining two or more management strategies offers a sustainable solution because it helps to reduce the population to levels that do not cause damage to crops and allows for economic production. Therefore, combining rotation of nematode suppressive crops with organic soil amendment will provide an affordable and sustainable intervention to small-holder farmers.

1.2 Overall Objective

The overall objective of this study was to develop an integrated strategy of managing root- knot nematodes in okra.

1.3 Specific Objectives

1. To identify potential rotation crop varieties suppressive to *Meloidogyne* species.
2. To evaluate the potential of rotating okra with nematode suppressive crops in root-knot nematode management.
3. To determine the effect of combining organic amendments and crop rotation with nematode suppressive crops on root - knot nematodes management in okra.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin, history and classification of Okra

Okra *(Abelmoschus esculentus* (L) Moench) originated around the Ethiopian region in Africa and along the Nile basin in Egypt (MOALD M, 1993). The crop then spread through North Africa from Nile basin and onto Eastern Mediterranean, Asia Minor, India and to the new world from New Orleans. By 1731, the crop was grown as far North as Philadelphia. Today, the major centers for okra production are in the South Eastern United States, Texas, Georgia, Florida, California, Tennessee and Alabama because of sensitivity of the crop to cold temperatures. Okra belongs to the family *Malvaceae* and is also commonly referred to as lady's finger in its (MOARD 2000).

2.2 Climatic and soil requirements

Okra is a warm season crop and thrives best during long warm growing seasons and it is very sensitive to frost (MOALDM, 1993). It is adapted to high temperatures throughout the growing period and the optimum temperature for growth and high quality pods ranges from 24-30°C and does well in altitude ranging from 0-1600m above sea level (MOA RD, 2000). Maximum tolerable temperature is 35°C (MOARD, 1993).

The crop is tolerant to a range of rainfall from 1500-1800mm p.a. The optimum water requirement for this crop is 400mm that is evenly distributed during the growing period. Supplementary irrigation is required throughout growing period if rainfall is inadequate so as to maintain vigorous growth (HCDA, 1996). Mature seeds contain 20% edible oil which can be used in the manufacture of margarine (MOAL M, 1993). Okra yields 8-10 tons per hectare.

Most soils are suitable for growing okra provided they are not water logged and the desirable pH is 6 to 7.3 (MOA & RD, 2000). Where soil pH is very high (up to pH 10), it is recommended to lower it by application of sulphur at the rate of 50kg/ha for every 0.5pH unit above 7. In Kenya, the main okra growing areas include Kibwezi, Yatta, Makuyu, Mitunguu, Lower Nyanza, Kerio Valley, Coastal areas of Taveta and Rombo (MOARD, 2000).

2.3 Importance of Okra

Okra is cultivated for its immature pods and is the leading Asian vegetable exported from Kenya and the third most important foreign exchange earner after French beans and snow peas (HCDA, 1996, MOARD, 2000). The pods contain high amounts of vitamin A and C, and traces of vitamin B and proteins. They are also a good source of calcium, phosphorous and iron The leaves contain proteins and significant amounts of vitamin B_2 (MOARD, 2000). Tender pods are boiled and used as vegetables or sliced and fried or used to make soup or mixed with other vegetables. Tender pods may also be served raw with salt, oil, pepper and lemon as a tasty salad (MOARD, 2000). It is also a good source of calcium and proteins comparing favorably with those in poultry eggs, and soya beans (Schippers, 2000). Okra seed flour is used as an additive to maize flour in Egypt while the tender fruits are used for policing (massaging aching muscles) (HCDA, 1996; MO ARD, 2000).

The most common variety is Pusa sawani which has long, dark green, smooth five ridge fruits and the yield levels are 8-15 tonnes/ha (MOARD, 2000). Other varieties are Prabhani kranti, Ex-india, Red wonder, Clemson spineless, Green emerald, Dwarf long, Pod Green and white velvet whose yields ranges between 9-12 tonnes/ha (MOARD, 2000).

2.4 Production Constraints

Okra does not tolerate low temperatures for long and frosts are deadly to the crop. Optimum temperature is 24-30°C while the maximum is 35°C (MOALD, 1993; MOARD, 2000). Pests and diseases are the major production constraints and cause serious yield losses (MOALD M, 1993). The most important pests include red common bugs, aphids, red spidermites, thrips, fruit borers and root- knot nematodes (*Meloidogyne* spp.) (Williams, 1974, MOARD, 2000).

Insect pests of okra fall into two categories that is foliage feeders and pod feeders. The foliage feeders only cause economic damage or loss of yield when pest numbers are high or when plants are young or stressed. The extent of the damage also depends on the health of the plants. Among the foliage feeders are flea beetles, blister beetles, caterpillars such as loopers, while the pod feeders include corn ear worms and stink bugs important diseases of okra include, black leaf mold caused by *Corticium rolfsii*, yellow vein mosaic (transmitted by white flies), powdery mildew, leaf spots, collar and stem rot caused by *Rhizoctonia solani* and *Fusarium* spp (MOARD, 2000).

2.5 ROOT - KNOT NEMATODES (*Meloidogyne* spp)

2.5.1 Classification and distribution

Root knot nematodes belong to the Kingdom: *Animalia*, phylum; *Nematoda*, Class *Nemata;* Sub class *Secernentea*, Order *Tylenchida*; Sub order *Tylenchina*; Family; *Heteroderidae* and Genus *Meloidogyne* (Chitwood, 1956) of which *Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla* are of economic importance in okra production (Jepson, 1987). Root - knot nematodes are present in all parts of the world (Sasser, 1980, Netscher and Sikora, 1990) and they have a wide host range with over 2500 plants listed as hosts (Agrios,1997). Among the plant parasitic nematodes, the genus *Meloidogyne* consists of economically important plant pathogens of a wide range of crops (Xu-jian Hua *et al.*, 2001).

2.5.2 Morphological Characteristics

The adult male and female root knot nematodes are easily distinguishable morphologically. The males are worm-like and about 1.2 – 1.5mm long by 30 – 36 μm in diameter while the females are pear-shaped and 0.4 – 1.3mm long by 0.27 – 0.71mm wide (Sherf and Macnab, 1986; Agrios 1997).They are endoparasitic and mature females are sedentary (Loius, 1982) while the second stage juveniles are vermiform in shape and the third and the forth stage juveniles are sausage shaped. All the stages are microscopic in size (Sherf and Macnab, 1986). The species within the genus *Meloidogyne* are usually distinguished using distinct patterns at the posterior end of mature females which resemble fingerprints and are referred to as perineal patterns (Jenkins and Taylor, 1967, Williams, 1974; Machon and Hooper, 1991).

2.5.3 Biology

All the species in the genus *Meloidogyne* have similar life cycles (Agrios, 1997) but optimum temperatures differ in different species (Netscher and Sikora, 1990). Under suitable conditions of host and temperatures, a single female can produce approximately 2800 eggs in a sac-like gelanatinous matrix produced by the nematode (Taylor and Sasser, 1978; Sherf and Macnab, 1986; Agrios, 1997). If conditions are favorable the life cycle is completed in about 25 days. The first stage Juvenile develops inside the egg and after undergoing the first molt within the egg, it develops into second stage juvenile which is the only infective stage(Taylor and Sasser, 1978). The eggs hatch to release 2nd stage juveniles and penetration of the host is optimum at 27°C. Egg hatching, emergence, survival and disease severity is affected by soil texture, structure, water holding capacity and aeration as well as pH (Taylor *et al.*, 1982). According to Ferris and Van Gundy (1979) *Meloidogyne* species survive and reproduce best at pH ranges from 4 – 8 while emergence is best at pH 6.4 – 7.0 while a pH below 5.2 inhibits their development (Wallace, 1966).

2.5.4 Epidemiology

Root - knot nematodes is the common name for *Meloidogyne* spp.which cause galls on many plant roots (Louis, 1982). They have endoparasitic mode of parasitism and mature females are sedentary (Louis, 1982). Root - knot nematodes are a severe constraint in agricultural production in tropical and sub-tropical areas and their effects are aggravated when coupled with abiotic factors such as drought, heat, poor crop management and low soil fertility (Sikora *et al.*, 1990) The ability of root - knot nematodes to move on their own is limited, but they can be spread by water or soil adhering on farm implements or otherwise introduced into uninfested areas via infected planting materials (Allen *et al.*, 1996). Their spread is also aided by man's activities such as movement of infested soil and infected plant debris, irrigation and wind.

The infective stage of root knot nematodes is juvenile stage two (J_2) which enters the root just behind the root tip. The juvenile feeds from cells around its head and secretes saliva into plant cells (Dropkin and Nelson, 1960). The juveniles become sedentary and cells around the head region begin to enlarge (Sherf and Macnab, 1986). Cell nuclei divide but no cell walls are laid and the existing walls between some of the cells breakdown and disappear (Taylor, 1976). These sedentary endoparasites results in transformation of root cells whose function is to supply nutrients to the nematodes continuously enabling them to produce large number of eggs (Luc *et al* .,1990). This also stimulates multiplication of cells leading to characteristic galls. The protoplasmic contents of several cells coalesce giving rise to giant cells. The enlargement of cells ceases when nematodes stop feeding and die (Dropkin, 1988). Xylem element may be crushed by the mechanical pressure exerted by the enlarging cells. Swelling of the roots also results from hypertrophy and hyperplasia of the vascular parenchyma, pericycle and the epidermal cells surrounding the giant cells (Wilcox and Loria, 1986).

2.5.5 Symptomatology

The presence of galls in roots is the most characteristic symptom associated with damage by root knot nematodes (Wilcox and Loria, 1986; Agrios, 1997) . Root systems of severely infected plants are reduced to a limited number of severely galled roots (Nescher and Sikora, 1990). Root- knot nematodes also damage plant roots by devitalizing root tips and either stopping their growth or causing excessive root production. Infected plants tend to produce branches above galls and root crumbs may be formed (Christie, 1936). Severely infected plants are chlorotic, stunted and necrotic at leaf margin and show excessive wilting during periods of mild moisture stress (Melakeberham *et al.*, 1985). Young plants are more susceptible to damage by root- knot nematodes (Noling, 2002).

Meloidogyne hapla causes excessive branching by continually attacking cells just behind the root growing point thus stopping root growth while *Meloidogyne incognita* induces bigger galls, more severe stunting, yellowing and wilting symptoms (Miano, 1999). Death of infected plants is associated more often with *M. incognita* than *M. hapla*. The above ground symptoms however, are similar to those caused by many other root diseases and environmental factors and may include stunting, chlorosis of leaves, necrosis of leaf margins and excessive wilting during periods of mild moisture and or temperature stress (Melakeberham *et al.*, 1985). These symptoms result in impaired absorption of nutrients and water by plants. (Wilcox and Loria, 1986).

2.5.6 Economic importance of *Meloidogyne* spp

Root- knot nematodes are widely distributed in Kenya and cause up to 20% losses yield (White head and Kariuki, 1960). In Kenya the estimated area under okra in 2001 was 814 ha, fetching about US$4,413,773 (Miano, 1999; Valera and Seif, 2004). Nematodes infestations can cause 20% loss in yield which translate to 1620 tones per year with a value of $882,754 (Valera and Seif,2004).

In Kenya root - knot nematodes results in loss of about 97 million Kenya shillings annually (HCDA, 2003). According to Sasser and Kirby (1979), estimations of vegetable crop losses ranged 17-20% on egg plant, 18-33% on melon and 24-30% on tomato. Though figures for okra are not stated, the range is within those stated because it is in the same family with egg plants and it's susceptibility is rated within the same range with tomatoes. The root- knot nematodes are important not only as independent agents causing disease in plants but are also associated with fungal, bacterial and viral diseases of plants (Male *et al.*, 1981; Valdez, 1987). They cause breakdown of host resistance to other pathogens (Jenkins and Coursen, 1957; Sidhu and Webster, 1977). The root-knot nematode (*Meloidogyne spp.*) are polyphagus plant parasitic nematode attacking roots of over 2000 different hosts (Orion, 1996; Siddiqui and Alam, 1999) and in many cases acts as initiators or synergists in fungal and bacterial disease complexes (Male *et al.*, 1981).

The tolerance limit of the population at which damage is first observed are given for a number of vegetables (Barker *et al.*, 1985). The wide variation in tolerance limits reflects the great difference in plant response to nematode infection as well as the influence on soil type and environmental conditions on disease development and severity (Ferris *et al.*, 1986).

Table 1: Tolerance limit of some vegetables to *Meloidogyne* sp

Crop	*Meloidogyne incognita**
Bell pepper	65
Cabbage	150-1000
Cantaloupe	20
Chilli pepper	39
Egg plant	5.4
Lettuce	60
Tomato	2-100
Water melon	2-50

*Number of juveniles/100cm^3

Source: Ferris *et al.*, 1986

2.6 Management of Root knot nematodes

Several methods of controlling plant parasitic nematodes have been developed and employed with varying degrees of success (Johnson *et al.*, 1992; Sikora, 1992; Sharma *et al.*, 1994, Abawi *et al.*, 2000). They include crop rotation with less susceptibleor resistant varieties, cultural and tillage practices, use of transplants and pre-plant nematicide treatments biological control, use of physical agents among others (Noling, 2002). However, acceptability of the available methods depends on the cost of application, type of crops, nematode types, availability of arable land and abiotic and environmental considerations (Johnson *et al.*, 1992; Sharma *et al.*, 1994).

2.6.1 Physical control

Heat treatment is mainly practiced for treating soil pots, nurseries and greenhouses. (Sardanelli *et al*, 2002). Most plant pathogens, insect pests and weed seeds are destroyed when soil is heated at a temperature of 60^0c for 30 minutes. Soil is generally heated by use of steam which penetrates the soil and imparts large quantities of heat (Katan, 1981; Berkelaar, 2001). Nematode infested planting materials can be disinfected by hot water treatment. However, this is only applicable where small quantities of planting materials have been infested and can be effective for seedling if the fields are free from infection.

Soil solarization has been successfully used to reduce populations of soil borne plant pathogenic fungi, bacteria and nematodes (Nescher and Sikora, 1990), but adoption is limited by the cost, loss of production during period of solarization and its applicability in areas only receiving adequate sunshine (Netscher and Sikora, 1990; Oka *et al.*,1993). Its other advantages include increasing the range and effectiveness of soil inhabiting antagonists that compete with inhibiting microorganisms causing soil borne diseases.

It also improves plant growth vigour, yield and soil condition and reduces soil salinity (Valera and Seif, 2004).

2.6.2 Cultural practices

Sanitation, one of the basic principles of disease control, plays an important role in preventing introduction of pathogens into the farm. Infested roots remaining in the field after harvest serve as a reservoir of plant parasitic nematodes (IFAS, 1989). Farms or greenhouses that are free from nematodes can retain their status if efforts are made to exclude the pests (McSorley, 2002). Farmers could avoid introducing nematodes with contaminated nursery stock, soil, tools, farm machinery and water (Sardaneli *et al.*, 2002). However, complete exclusion of nematodes may be impossible since they can be carried in soil, blown by the wind or in surface water (IFAS, 1989). Additionally nematodes swept from infested fields can gain access into irrigation water and be disseminated through the irrigation pipeline.

Flooding has been shown to be effective in suppressing *Meloidogyne* spp. densities especially when done over long periods (Stover, 1979). However this is un economical means of nematode control when done artificially. Effectiveness of flooding in nematodes management can be improved when combined with alternate drying of the soil (IFAS, 1989). Clean fallowing and deep ploughing gives immediate impact on reduction of root knot nematode population densities (Kinloch and Rich, 2001). Most nematodes are found in the upper layers of the soil (5-30cm). Deep ploughing buries inoculum so deep that infections are less severe in the subsequent crop (Berkelaar, 2001). Ploughing also exposes nematode eggs and juveniles to lethal radiation and desiccation. Eggs of *M .javanica* survive for only two hours in a relative humidity of 50% and exposure to UV radiation is lethal to eggs and juvenile stages of the nematode (Sardanelli *et al.*, 2002). However, uncontrolled weed growth and loss of farm income and soil erosion during fallowing period limits its applicability (Abawi *et al.*, 2000; McSorley, 2001).

2.6.3 Plant Resistance

Resistant varieties can prevent root knot nematodes reproduction thus reducing populations significantly (McSorley, 2002). A few cultivars are now available that are resistant to certain plant parasitic nematodes. They have no requirements for special application techniques or equipment as in the case of chemical management. They have almost equal costs to non-resistant cultivars and are the most practical and cheapest means of nematode control especially in small-scale farms (Bridge, 1996; McSorley, 2002). Host resistance can be successfully combined with other suppressive mechanisms such as crop rotation and soil amendments with impressive results. Their acceptance and adoption by farmers is limited due to unavailability, high costs involved whenever available and breakdown of resistance after a few years of use (Netscher and Sikora, 1990).

2:6.4 Organic Soil Amendments

This approach to nematode control has been documented for a long time (Linford *et al.*, 1938; Singh and Sitaramaiah, 1970; Bridge, 1996). According to Berkerlaar (2001), nematodes problems are worse in soils with low amounts of organic matter content than in soils with high amounts. Organic soil amendments should ideally be by-products and wastes from industrial and other activities and include oil cakes, sawdust, plant composites, green manures and agro-industrial wastes (Singh and Sitaramaih, 1970; Ibrahim and Ibrahim, 2000; Umar and Jada, 2000). Incorporation of organic matter into the soil may suppress nematodes through several mechanisms (Sikora, 1992; Berkerlaar, 2001). Organic matter may support higher populations of natural pests of nematodes such as bacteria and/or fungi, it may also release toxic compounds to nematodes during decomposition and may increase soil nutrients and water levels thus enabling plants to escape damage by nematodes (Mohamed *et al.*, 2000 ;Berkerlaar 2001,). According to Miano (1999), chicken manure and pyrethrum have been found to be most effective.

According to Sikora, (1992); Oka *et al.*, (1993) and Bridge, (1996) the incorporation of organic soil amendments into the soil increases nutrient levels and improves water holding capacity of the soil thus improving plant growth and hence tolerance to nematode attack. Berkerlaar (2001) reported that organic soil amendments incorporation into the soil results in increased soil nutrient and water levels resulting in plants partially overcoming the effect of having a damaged root system. Organic soil amendments increase the level of biological activity within the soil resulting in increased biodiversity which plays a role in natural regulation of plant parasitic nematodes keeping their densities below damage thresholds (Sikora, 1992; Siddique and Alam, 2001). Increased level of organic matter in the soil increases soil antagonistic potential and has strong stimulatory effects on saprophytic fungal and bacterial antagonists of nematodes as well as predatory mites and predacious nematodes (Sikora, 1987). Decomposing by-products were found to be ten times more toxic to *Meloidogyne incognita* than to free living nematode *Panagrellus redivivus* (Rodriquez-Kabana, 1986; Sayre and Starr, 1988).

However, use of this method is limited due to large quantities needed for successful control (Kerry, 1990).

2.6.5 Crop Rotation

Crop rotation has long been used as an important non-chemical practice for nematode management (McSorley, 1999).The crops used for rotation could either be cover, trap or antagonistic to nematodes (Luna, 1998).Several mechanisms of nematode suppressive crops have been reported and include antagonism, improved fertility for subsequent crops, trapping, nematicidity, nemastaticity and starvation (Meyer and Fry, 1978 ; Giebel, 1982; Toppel *et al.*, 1998; Hagan *et al.*, 2002). Trap crops may be useful when conventional crop rotation have apparently failed to control nematodes (Sardanelli *et al.*, 2002; McSorley, 2002).It is important that crops being considered for rotation be tested for host status to local populations before rotation schemes are recommended because plants considered good host for one nematode species in one part of the world are not necessarily hosts to all populations of that species (Hagan *et al.*, 2002).

For effective use of crop rotation in nematode management, information on nematodes present in the field, host range of species present, possible rotation crops and expected rate of population increase and decrease, availability of resistant varieties, growing season in terms of planting and harvesting time as well as damage threshold is very important (McSorley, 1998 and 2002; Sardanelli *et al.*, 2002). Crop rotation is most frequently considered for annual crops as well as perennials which can be profitably grown for several years (Orion, 1996). Drought tolerant crops such as sorghum, maize, green grams and cowpeas should be incorporated in the rotation programme. Although the crops are economically less attractive that vegetables, their contribution to nematode management deserves consideration

(Orion, 1996)

A crop rotation program should be planned several years in advance because if the expected rate of population increase is known, it is possible to forecast population at harvest (McKay Blythe, 2000). This allows consideration of other possible options such as harvesting the crop early when nematode numbers might be lower which then permits shorter rotation period (McKay Blythe, 2000; McSorley, 2002). However, this may not be applicable to smallholder farmers.

26.6 Antagonistic plants

Antagonistic plants are those that release root exudates that have nematicidal properties (Sukul, 1992). Some of the nematode suppressive antagonistic plants include marigolds (*Tagetes minuta*),

chrysanthemum(*Chrysanthemum morifolium*), caster bean (*Ricinus communis*), partridge pea (*Cassia fasciculate*), *Crotalaria* spp., velvet bean (*Mucuna pruriens*), common vetch (*Vicia sativia*), rape seed (*Brassica napus*) and Jackbean (*Canavalia ensiformis).*

2.6.7 Chemical Control

The use of chemicals becomes economic when other methods of control become inadequate to suppress nematodes sufficiently but they are expensive and not applicable where low value crops are involved (Hague and Gowen, 1987).Chemicals used in nematodes control are either fumigants or non-fumigants (Ware, 1983).Non fumigants are not effective against eggs of nematodes and most cannot kill the juveniles at recommended rates (Netscher and Sikora, 1990). All of the non-fumigant nematicides (aldicarb, carbofuran and oxamyl) currently registered for use are applied to the soil except vydate (Noling, 2002). This approach is also limited by the fact that the range of products registered in and acceptable to all the European Union (EU) for the management of pests and diseases in okra is very narrow (Valera and Seif; 2004).These products are not registered or available locally for use in okra or are prohibitively expensive to smallholder farmers.

Fumigants such as methyl bromide, chloropicrin and vortex effectively reduce nematode populations and increases vegetable production. However these fumigants also kill other non-target organisms and are poisonous to people even at low concentrations (Berkerlaar, 2001; Noling, 2002). The nematodes that escape treatment can resume feeding when the chemical breaks down in the soil to build high populations within a short time (Sardanelli *et al.*, 2002). The use of nematicides is declining particularly due to their high cost, toxicity to non-target species and environmental pollution (Hague and Gowen, 1987)

2.6.8 Biological Control

This involves reduction of nematode populations by use of natural enemies which include parasites, predators and antagonists of adult nematodes, juveniles and eggs (Dropkin, 1988). Nematode parasites or antagonists have been incorporated into the soil for the control of root knot nematodes on vegetables (Kerry, 1987; Badi *et al.*, 2000). Some species of fungi such as *Arthobotrys* spp and *Monacrosporium* can trap and kill nematodes (Berkerlaar, 2001; Hafeez, 2000; Khan and Goswami, 2001), while *Paceilomyces lliacinus* and *Verticilium chlamylosporium* parasitize nematode eggs (De Leiji *et al.*, 1992; Al Raddad, 1995; Berkerlaar, 2001). *Pasteuria penetrans* is an obligate bacterial parasite of some plant parasitic nematodes including *Melodlogyne* spp and is often found attached to juveniles. Nematodes infected with *pasteuria* are not able to grow and reproduce efficiently (De channer, 1997; Tariq and Riaz, 2000) Although the efficacy of *P. penetrans* has been demonstrated in the field, it is very difficult to culture it in the laboratory and they are not yet available commercially and hence its use as a substitute or supplement to nematicides is still far (Berkerlaar ,2001).

Plant health promoting rhizobacteria such as *Bacillus* spp, *Pseudomonas* spp and *Telluria chitinolytica* are a promising group of micro organisms that may have some effect in reducing damage by nematodes (Sikora and Greco, 1990, Sikora, 1992). These have been shown to inhibit penetration of nematodes in roots thus reducing galling (Bowman *et al.*, 1993; Rao *et al.*, 2000; Amin, 2000). Most of these bacteria belong to the fluorescent *pseudomonas* sp and can be applied as seed dressings or through drip irrigation (Zavaleta-Meija and Van Gundy, 1982; Becker *et a.*, 1988). This method is slow and generally unacceptable to farmers mainly because under field conditions soil is a powerful buffer and therefore an additional organism may not have an immediate measurable effect (Mankau, 1981; Rodriguez-Kabana and Morgan Jones, 1987).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Sites

Greenhouse and field experiments were carried out in University of Nairobi irrigation project farm at Kibwezi. Soils are loamy with a temperatures ranging from 25 - 30°C, mean annual rainfall of 600mm, and the altitude is 700m above sea level (Orion, 1996). The area lies at latitude 2°21.5'S and longitude 38° 025'E (MacMillan Atlas, 1995). The experiments were conducted from August 2003 to April 2004.

3.2 Reaction of potential drought tolerant nematode suppressive crops to *Meloidogyne* species - greenhouse experiment

This experiment was carried out to determine reaction of different crop varieties to *Meloidogyne* spp under greenhouse conditions. Twenty-one different varieties consisting of cereals and legumes were selected and evaluated to determine their potential for nematode suppressiveness under greenhouse conditions (Table 3.1). Some sorghum and millet seeds were obtained from ICRISAT field office in Kiboko, Makueni district while the rest were obtained from different seed companies in Kenya

Ballast (Volcanic ash) and top forest soil were mixed in the ratio 1:3, sterilized by heating for three hours at a temperature of about 50^0C and then put in 5kg pots. Pre-plant fertilizer (DAP) was added at the rate of 5g per pot, watered and then four seeds sawn in each pot. Thinning was done to leave two seedlings per pot.

Soil was infested with 6000 eggs/ juveniles of *Meloidogyne* species suspended in 10mls of water. The inoculum was pipetted into 2cm deep indentations made around the bases of plants in each pot and then covered with soil ten days after emergence of seedlings. Light watering was done for the first one week to avoid washing down the inoculum below the root zone. The pots were arranged in a completely randomized design with seven replications of each crop variety. The experiment was terminated 60 days after soil infestation and repeated once.

Table 3.1: Crop plants screened for their potential in nematode suppression in Kibwezi irrigation project farm from August 2003 to April 2004

Crop	Variety	Use
Maize	Katumani	Cereal
	DLC1	Cereal
	DH01	Cereal
	Pioneer (Ph3253)	Cereal
Sweetcorn	Endeavour F_1	Vegetable
Babycorn	Manor F_1	Vegetable
Sorghum	Kari Mtama 1	Cereal
	Seredo	Cereal
	Serena	Cereal
	Gadamhanam	Cereal
Pearl millet	ICMV221	Cereal
	KATPM1	Cereal
Greengram	KS20	Vegetable/Pulse
	Ranress	Vegetable/Pulse
Cowpea	K80	Vegetable/Pulse
	KK1	Vegetable/Pulse
Pigeonpea	Landrace	Pulse
	ICPL 87091	Pulse
Guwar	Pusa naubahar	Vegetable
Okra	Pusa sawani	Vegetable
Okra	Indiana	Vegetable

3.3 Inoculum Preparation

Second stage *Meloidogyne* juveniles and eggs were obtained from galled roots of infested okra plants using the maceration extraction method described by Hussey and Barker (1973) and modified by Sikora and Greco (1990).

Galled roots of okra collected from Kibwezi irrigation farm were washed free of adhering soil particles using tap water. The roots were cut into one cm long segments and macerated in 100ml of water using a warring blender for15 seconds at high speed. The macerate was then vigorously shaken in 0.5% sodium hypochlorite solution for three minutes. The eggs/juveniles were collected on a 45μm-aperture sieve by washing them down through a 2mm sieve. The eggs were rinsed free of sodium hypochlorite and transferred into a 1000ml flask to which 50ml sterile water was added and egg suspension continuously aerated using an aquarium pump. The second stage juveniles that were obtained were used as inoculum.

3.4 Damage Assessment

Damage assessment was done 60 days after soil inoculation of twenty one different varieties in the greenhouse. The plants were uprooted and washed free of adhering soil before assessment of damage. Galling index (GI) and egg mass index (EMI) were assessed using gall and egg mass index on a scale of 1 to 9 where: 1 = no galls/egg masses; 2 = 1 –5 galls/egg masses; 3 = 6 – 10 galls/egg masses; 4 = 11 – 20 galls/egg masses; 5 = 21 – 30 galls/egg masses; 6 = 31 – 50 galls/egg masses; 7 = 51 – 70 galls/egg masses; 8 = 71 – 100 galls/egg masses; 9 = > 100 galls/egg masses was used (Sharma *et al.*, 1994). Phloxine B stain was used to stain the egg masses as described by Holbrook *et al.*, (1983)

Second stage juveniles (J_2) were extracted from 200cm^3 soil samples obtained from the pots and the field using the modified Bearmann funnel technique, using extraction dishes (Hooper, 1990).The soil was spread on a double layer of milk filters supported by a sieve. The sieves were then placed in a shallow 15cm-diameter dish and water added to a level where it just touched the base of the sieve and the soil layer looked wet. The dishes were then covered and left for 24 hours to allow nematodes to move from the soil suspension, through the milk filters into the water.

The sieves were then carefully removed and the nematode suspension concentrated by draining off excess water by passing it through a series of 45μm – aperture sieves. The juveniles were collected from each sieve by back washing the residues into a beaker.

One mililitre aliquots of a well agitated juvenile suspension was then pipetted into a counting slide and observed under a microscope. Counting was repeated four times and average calculated.

3.5 Field Experiment

3.5.1 Effect of rotating okra with nematode suppressive crops

The field experiment was conducted to determine the effect of rotating okra with nematodes suppressive crops as well as combining rotation of selected nematode suppressive crops and incorporation of organic soil amendment (farmyard manure) into the soil in the control of root knot nematodes in okra. Four crops namely sweetcorn, babycorn, maize cv. Pioneer (Ph3253) and guwar were selected after screening in the greenhouse based on the nematode suppressive potential and availability of market for the suppressive crops since okra is a high value export vegetable. The experiment was carried out in two seasons where in the first season the nematode suppressive crops were grown in plots measuring 6m square at their recommended spacing (sweetcorn-30cmx 90cm, babycorn- 60cm x 15cm, pioneer (ph3253)-90cm x30cm and okra- 50cm x 30cm inter and intra rows) and nurtured to maturity. In the second season a split experiment was done. Okra was planted in the plots previously sown with the suppressive crops where half of the plots were incorporated with farmyard manure as organic soil amendment before okra was sown. The plots measured 2.5m x 6m and each experiment was repeated once

In the first season experimental layout was complete randomized block design with four replications of each variety while in the second seasons the layout was split plot design. Initial nematode population (initial inoculum (p_i)) was determined before planting by taking five samples randomly from each plot and extracting nematodes using the modified Bearmann funnel technique (Hooper, 1990) Counting was repeated four times and average determined. The crops were left to grow to maturity and then the experiment was terminated. Prior to terminating the experiment, ten plants were selected per plot and uprooted. Soil samples were taken from the depth of about 5cm – 20cm in the rhizosphere. Data on yield was determined and given in tonnes/ha.

The roots were washed to remove any adhering soil particles and galling index and egg mass index determined. Phloxine B was used to stain roots for egg mass index determination (Holbrook *et al.*, 1983).

The roots were washed to remove any adhering soil particles and galling index and egg mass index determined. Phloxine B was used to stain roots for egg mass index determination (Holbrook *et al.*, 1983). The final nematodes population in the soil at the end of the season (p_f) was determined. Damage by nematodes was quantified using a scale of 1 – 9 as described above (Sharma *et al.*, 1994).

3.5.2 Effect of combining organic soil amendment (farmyard Manure) and crop rotation with nematode suppressive crops on root-knot nematodes control in okra

A field experiment was conducted using sweetcorn, babycorn, maize cv. Pioneer (Ph3253) and guwar as the potential nematode suppressive crops that were selected in an earlier greenhouse experiment. Soil samples were collected from ten randomly selected plots initially grown with okra. Nematodes were extracted using the modified Bearmann funnel technique (Hooper, 1990). The nematodes were examined under the microscope, counting done four times and average determined. This was noted as the initial nematode population (p_i).

Each plot measuring 6msquare was split into two plots measuring 2.5m x 6m. Farm yard manure was applied into half of the plots at the rate of 2kgs/m^2 before sowing okra at the spacing of 50cm x 30cm.The other half of the plots were sown with okra without incorporating farmyard manure. The plots that were previously sown with okra were used as control. The crops were grown to maturity and yields determined. The final nematode population (p_f) and damage assessment were determined using the methods and scales described above. The experimental design employed here was split plot design with four replications of each treatment and the experiment was repeated once.

CHAPTER FOUR

4.0 RESULTS

4.1 Reaction of potential rotation drought tolerant crops *Meloidogyne* species greenhouse experiment

The galling indices, egg mass indices and juvenile counts were significantly different ($P \leq 0.05$) among the drought tolerant crops tested (Table 4.1). The highest galling index recorded was 7.3 (average for season I&II- Indiana) followed by okra pusa sawani (6.3 average) while greengrams (KS20) was the third most susceptible among the crops that were screened averaging 5.7.Cowpea cv. KK1 and greengram Ranress were rated as highly susceptible to *Meloidogyne* spp With an average galling index range of 5.4-5.7. Cowpea cv.K80 was rated moderately susceptible with an average galling index of 4.8.

The least susceptible crops among those tested included sorghum, which had galling indices ranging from 1.4 – 1.8 for maize, 2.2-2.6 for millet , 1.8-2.0 for sorghum , 1.4 for pigeon peas, 1.4 for guwar , 1.4 for babycorn , sweetcorn 1.6 and 1.6 for pioneer (Ph3253) The trend in eggmass indices was similar to that in the galling indices with okra (Indiana) recording the highest average of 8.5 followed by pusa sawani (okra) with an average of 8.4. Sweetcorn recorded the lowest average 1.7. The highest number of juveniles 757(average) were recovered from soils grown with okra (Indiana), followed by cowpea (KK1) with an average of 730. The lowest number was recovered from soils grown with sorghum cv. Kari mtama1 with an average of 147 and cv. Seredo (163). Most of the maize and millet varieties were averaged 160-220 in terms of juvenile counts.

Table 4.1: Galling indices, egg mass indices and numbers of *Meloidogyne* juveniles (J_2) recovered from soils grown with different crops under greenhouse conditions (1st & 2nd season)

Crop, variety and species	Use	GI		EMI		J_2/200cm3		Reaction
		I	II	I	II	I	II	
Maize-Katumani *(Zea Mays)*	Cereal	1.4	1.4	2.8	3.4	193	239	Res
Maize-DLC1 "	,,	1.6	1.6	3.0	2.8	148	214	Res
Maize- DH01 "	Cereal	1.6	1.6	2.2	2.6	144	176	Res
Maize – Pioneer(Ph3253) "	,,	1.6	1.6	1.6	2.6	186	164	Res
Maize-Sweetcorn (*Zea mays saccharata*)	Veg.	1.8	1.8	1.8	1.6	204	205	Res
Maize-Babycorn (*Zea mays scarni*)	Veg.	1.4	1.4	3.8	2.2	222	173	Res
Sorghum – Serena (*Sorghum bicolor*)	Cereal	2.0	2.0	2.0	2.0	155	146	Res
Sorghum – Seredo (*S. bicolor*)	Cereal	1.8	1.8	2.0	1.8	161	166	Res
Sorghum – Kari mtama1 (*S. bicolor*)	,,	2.0	2.0	2.2	2.0	147	147	Res
Sorghum – Gadam (*S. bicolor*)	,,	1.8	1.8	2.4	1.8	167	222	Res
Millet – ICMV 221 (*Pennisetum glacum*)	,,	2.2	2.2	3.6	2.2	203	171	Res
Millet – KATPM 1(*P. glacum*)	Pulse/veg.	2.6	2.6	3.0	2.6	183	179	Res
Pigeon peas – Land race(*Cajanas cajan*)	"	1.4	1.4	2.6	2.6	218	161	Res
Pigeon peas- ICPL 870/9(*C.cajan*)	,,	1.4	1.4	3.2	2.4	237	165	Res
Guwar – Pusa Naubahar (*Cyamopsis tetragonoloba*)	Veg.	1.4	1.4	2.6	2.2	218	227	Res
Cow peas- K80 – (*Vigna unguiculata*)	Pulse/veg	4.0	5.6	5.4	5.8	406	429	M.Res
Cowpeas- KK1(*Vigna unguicuilata*)	"	5.6	5.8	6.2	7.8	900	560	Sus
Green grams – Ranress (*vigna aurens*)	Pulse/veg	5.6	5.2	5.2	6.2	237	660	Sus
Green grams – KS20 (*Vigna. aurens*)	Pulse/veg	6.2	5.2	4.0	6.0	144	563	Sus
								Sus
Control	Veg	7.4	6.4	8.2	8.6	697	699	Sus
Okra – Pusa sawani(*Hibiscus esculentus*)	Veg.	7.2	7.4	8.6	8.4	774	740	Sus
Okra – Indiana (*H.esculentus*)								
LSD (P $\leq$ 0.05)		1.6	1.0	1.5	1.2	219.5	124.0	

Res=resistant. M.Res=moderately resistant. Sus=susceptible. Veg=vegetable
I= season one.
II= season two

4.2 Reaction of selected nematode suppressive crops to *meloidogyne* species in rotation with okra under field conditions

In the first experiment (season I) reaction of selected nematode suppressive crops to *Meloidogyne* spp under field conditions differed significantly ($P \leq 0.05$). Galling indices among selected nematode suppressive crops namely; babycorn, guwar, Sweetcorn and maize cv. Pioneer (Ph3253) showed significant difference with maize cv. Pioneer(Ph3253) recording the highest galling index at 1.7 and guwar the least 1.3 (Table 4.2). The highest overall galling index (8.2) was recorded on okra. The decline in juvenile numbers also differed significantly ($P \leq 0.05$) among the suppressive crops with the highest reduction in population recorded under the plots grown with guwar (44%) and the least observed in plots grown with baby corn (21%). However, plots grown with okra (control) recorded an increase in population of the juveniles (98%).

In the repeated experiment (season II) the galling indices showed significant difference ($P \leq 0.05$) and the trend in the first experiment was maintained. Among the test crops maize cv.pioneer (Ph3253) recorded the highest galling index at 1.8 and guwar the least at1.2. The juvenile numbers differed significantly, with the highest decline being recorded in plots grown with sweet corn (59%) and the least in those grown with baby corn (23%). Juvenile numbers increased by 83% in plots under okra.

Table 4.2: Galling indices, juvenile counts and percentage change in juvenile population in soils grown with selected nematode suppressive crops under field conditions (1st &2nd seasons)

	GI		P_i/200cm³		P_f/200cm³		P_f/p_i		%change in nematode population	
Treatment	I	II	I	II	I	II	I	II	I	II
Sweet corn	1.6	1.5	270	268	180	109	0.67	0.41	-33	-59
Guwar	1.3	1.2	334	358	185	195	0.55	0.54	-44	-45
Maize (pioneer)	1.7	1.8	309	329	234	205	0.75	0.62	-24	-39
Baby corn	1.5	1.6	249	346	196	265	0.79	0.77	-21	-23
Okra (control)	8.2	8.1	234	236	464	464	1.98	1.96	+98	+83
LSD (P = 0.05)	0.4	0.39	78.7	86.4	70.9	69.7	0.12	0.1	12.4	14.0

P_i = Initial juvenile population at the beginning of the season.
P_f = Final juvenile population at the end of the season.
I= Season one
II=Season two

4.3 Effect of rotating okra with nematode suppressive crops on root -knot nematode control under field Conditions

In the first season, galling indices, juvenile counts and yield differed significantly ($P \leq 0.05$) among the plots previously sown with different nematode suppressive crops. The highest galling indices 8.6 were recorded in control plots while the least (3.5) was recorded in plots sown with sweet corn (Table 4.3). Highest juvenile counts were also recorded in control plots 939 which also represented the highest increase in the nematode population 27%. Differences in yield were significant ($P \leq 0.05$) with the highest recorded in plots sown with guwar (5.3 tonnes/ha) followed by maize cv.pioneer (Ph3253) – 5.2 tonnes /ha while the least yield was recorded in the control plots (2.5 tonnes /ha)

This trend was also observed under the second season where galling indices, juvenile counts and yields showed significance differences ($P \leq 0.05$). However plots under sweet corn in the second season recorded a 15% decline in juvenile population while population increases were recorded under all the other treatments.

Table 4.3: Galling indices, juvenile counts and yields of okra in plots previously sown with selected nematode suppressive crops- 1st & 2nd season

Treatment	GI		P_i / 200 cm^3		P_f / 200 cm^3		P_f/P_i		%Change in nematode population		Yield(Tonnes/ha)	
(Previous crop)	I	II	I	II	I	II	I	II	I	II	I	II
Babycorn	4.2	4.0	134	191	200	248	1.49	1.2	+33	+29.8	4.4	4.8
Guwar	3.8	3.6	162	130	178	145	1.09	1.1	+9.8	+11.5	4.8	5.3
Sweetcorn	3.5	3.0	158	210	162	170	1.02	0.84	+2.7	-15	4.0	5.0
Pioneer	4.5	4.2	210	179	235	208	1.11	1.16	+11	+16	4.5	5.2
Control (Okra)	8.6	8.5	178	225	939	1150	5.2	5.1	+427	+411	2.5	3.0
LSD (P=0.05)	0.68	0.64	47.6	52.3	96.8	108.4	0.27	0.25	27.3	27.2	1.1	1.2

P_i =Initial juvenile population at the beginning of the season.
P_f = Final juvenile population at the end of the season.
I=Season one
II=Season two
(+)=Increase in juvenile population

4.4 Effect of combining organic amendment (Farmyard manure) and crop rotation incorporating nematode suppressive crops on nematodes population and yields of okra under field conditions

There were significant differences ($P \leq 0.05$) in galling indices, percentage change in juvenile population and yields (Table 4.4). In the first season galling indices ranged from 3.6 for plots sown with sweet corn followed by okra plus farmyard manure to 2.5 in those sown with guwar followed by okra with incorporation of farmyard manure. Control plots recorded the highest galling indices (74%). The highest decline in juvenile population was observed in plots initially sown with guwar while the least was recorded in the plots previously under babycorn

(46%).

Yields also showed significant difference ($P \leq 0.05$) with the highest (5.3tonnes/ha) being recorded in plots previously under sweetcorn followed by okra plus organic amendment. Lowest yields were observed in control plots (1.1tonnes/ha). The trend was similar in the second season. Galling indices showed significant difference ($P \leq 0.05$) where the range was 2.8 and 8.8.

Table 4.4: Galling indices, percentage change in juvenile population and yields of okra in plots previously sown with selected nematode suppressive crops followed by okra plus organic amendment (farmyard manure)-1st & 2nd season

Treatment (Previous crop)	GI I	GI II	P_i/200cm3 I	P_i/200cm3 II	P_f/200cm3 I	P_f/200cm3 II	P_f/P_i I	P_f/P_i II	%Change in population I	%Change in population II	yield (Tonnes/ha I	yield (Tonnes/ha II
Babycorn	3.4	3.0	235	134	126	68	0.53	0.50	- 46	-49	4.9	4.8
Guwar	2.5	2.8	113	162	29	55	0.25	0.33	-74	-68	5.2	3.9
Maize (Pioneer)	3.2	3.2	215	158	108	88	0.50	0.55	-49	-44	4.8	4.3
Sweetcorn	3.6	3.5	195	210	89	111	0.45	0.52	-54	-52	5.30	4.9
Control Okra, okra	8.8	8.8	205	172	1135	939	5.53	5.45	+453	+445	3.0	2.1
LSD (P=0.05)	0.60	0.59	53.4	47.2	83.9	71.1	0.19	0.19	38.1	37.0	1.2	1.0

P_i = Initial Juvenile population at the beginning of the season

P_f= Final juvenile population at the end of the season

I=Season one

II= season two

(+)=Increase in juvenile population

(-)=Decrease in juvenile population

CHAPTER FIVE

5.0 DISCUSSION

5.1 Reaction of drought tolerant crops with potential for rotation to *Meloidogyne* spp under greenhouse conditions

This study has shown that among the susceptible crops, there are variations in nematode damage and some of the less damaged varieties can be further evaluated for rotation especially where the nematode build up in the soil is likely to be slow due to environmental factors. The high galling indices indicating damage on the root of okra, cowpeas and green grams confirms earlier studies that these crops are susceptible to root knot nematodes (Mcsorley, 2002). This study has demonstrated that shows that these crops are good hosts of root knot nematodes and should not be used in rotation with other susceptible crops. However, planting them in nematode infested soils and uprooting them before their maturity can be used as a mechanism of nematode management since they can act as trap crops but this needs a careful consideration. However, there were significant differences in the change in nematode population among different varieties of the crops reported as susceptible, for example cowpea (*Vigna unguiculata*) variety KK1 (Kenya Kunde 1) was more severely damaged than variety K80. Similar observations were made in green grams where KS20 variety was more damaged than variety Ranress and the case was true in okra

Strong suppression of nematodes by sorghum, maize, millet and guwar in this study was in agreement with previous studies (Orion, 1996; Hagan *et al*., 2002). There was minimal reproduction of root knot nematodes in sorghum, millet and maize and confirms earlier findings (Luna 1998; Berkelaar, 2001; Kinloch and Rich, 2001). According to Yamada *et al* (2002) some sorghum varieties are more effective in suppressing nematodes than others and this finding was confirmed in the study where varieties Seredo and Gadam were more suppressive to nematodes than and Serena and Kari mtama1. Suppressiveness of sorghum to nematodes could be attributed to the glycosides that are found in its vacuole that become exposed when injured by nematodes leading to the release of highly toxic hydrogen cyanide that kills nematodes (Meyer and Fry, 1978).

This suppression could also be attributed to the production of a compound called dhurrin, which is produced by Sudan grass (a wild grass closely related to sorghum) and which breaks down to produce hydrogen cyanide upon decomposition in the soil. These attributes makes sorghum a good rotation crop for the management of root knot nematodes especially in low rainfall areas. The only limitation when used in rotation with okra is its low market value compared to okra, which is a high value export crop. It can, however, be considered where the nematodes build up is high and options of rotation crops limited due to environmental factors

Low reproduction of root- knot nematodes under maize indicates that it is a poor host and is in agreement with previous studies (Otipa, 2002). According to Orion (1996), growing drought tolerant cereals such as sorghum and corn which are poor hosts, after nematode susceptible crops, decreases nematode population level drastically. Sorghum and maize therefore offers opportunities as good rotation crops for the management of root knot nematodes. However, careful evaluations are needed to determine the spectrum of nematode species in a given field as they may not offer good results when *pratylenchus* species are present unless varieties specifically bred for resistance to the lesion nematodes are used. *Meloidogyne* reproduction potential was significantly different for maize varieties and types and this confirms earlier findings that sweet corn and maize cultivars are tolerant to nematode attack (McSorley, 2002).

Reaction of pearl millet to root knot nematode was significantly different across the two varieties tested with KATPM1 recording a slightly higher damage compared to ICMV 221. This observation is similar to earlier findings (Vaishav and Sethi, 1978; McSorley 2002). According to McSorley (2002), reaction of pearl millet to root knot nematodes varied with nematode species present in the soil. Therefore, although pearl millet may offer an alternative rotation crop as a nematode suppressive crop, careful evaluation of the root knot nematodes present in a particular field is needed to ensure that good results are obtained since it is a poor host to certain *Meloidogyne* species and at the same time offering resistance to others. Lack of good market for the crop as well as its labour intensive production system presents some limitation in providing opportunities for rotation in the management of root knot nematodes.

Slight damage on roots of pigeon peas and low reproduction potential of root knot nematodes indicates that some pigen peas varieties are moderately resistant to *Meloidogyne*. This is consistent with earlier reports (Acosta, *et al*., 1986; Patel *et al*., 1987). Therefore, before pigeon peas are incorporated into rotation with root-knot nematode susceptible crops like okra, careful evaluation needs to be done to identify the resistant cultivars for use. Further, attention should be paid to those varieties, which produce fruits within 4 to 6 months so as to provide alternative sources of income to farmers for their acceptability.

Strong suppression of root-knot nematodes, as indicated by almost undetectable damage on roots and low reproduction by the nematodes in the plots under guwar was recorded. Information on guwar and nematodes is scarce though its suppression can be partly attributed to its nitrogen fixation as a legume thus providing additional nutrition which enables the crop to grow fast and escape nematodes damage The crop is very popular with farmers around Kibwezi and is used for rotation with nematodes susceptible crops such as tomato and okra grown under irrigation. The popularity of the crop is also partly due to the fact that it is a high value export crop which provides income during rotation hence its acceptability.

5.2 Reaction of selected nematode suppressive crops for rotation with okra to *Meloidogyne* species under field conditions

The highest decline in *Meloidogyne* juvenile numbers was observed in soils grown with guwar, sweetcorn, pioneer maize (Ph3253) and babycorn in that order. Although, information on the reaction of guwar to *Meloidogyne* spp. was not available, this crop showed good results in nematode suppressiveness. It provides a good rotation due to its ability to fix nitrogen into the soil. Its suitability for rotation is enhanced by the fact that it is a high value export crop and provides the much needed income during the rotation period with okra. The reaction of maize (baby corn, pioneer(Ph3253) and sweet corn) is in agreement with previous findings (Hagan *et al*., 2002 ; McSorley 2002) . Orion, (1996) reported that roots of sweetcorn were found free of root knot nematodes despite the fact that the crop was continuously grown in fields highly infested with root- knot nematodes.

These crops provide good rotation alternatives to nematode susceptible crops like okra. Though these grain crops are less attractive economically, their contribution to sustainable vegetable crops in the future is their most significant property The intensity of damage by root knot nematodes in okra confirms earlier findings that okra is highly susceptible to root knot nematodes (MOA LD, 2000, Hagan *et al* .,2002).

Baby corn and sweet corn have been frequently incorporated into rotation programs in the management of root- knot nematodes partly because of their high value and ready availability of export and local market. These crops also mature relatively fast (90 days for babycorn and 75days for sweetcorn) and hence can provide a high turnover within a short time. These attributes have made them popular with farmers as rotation alternatives to export and local market oriented vegetables such as tomato and okra that are highly susceptible to root knot nematodes especially under irrigation production systems. Maize cv. Pioneer (Ph3253) is also a good rotation crop because it fetches good prices when marketed green and its returns can easily be compared to those of sweet corn and slightly better than baby corn. However, proper timing of the market is crucial to ensure that maximum returns are realized

5.3 Effects of rotating okra with nematode suppressive crops under field conditions

When okra was planted after selected nematode suppressive crops, there was a build up of root knot nematode population to levels beyond tolerance limits of most susceptible crops. This study confirms earlier findings that one season rotation is not adequate to manage root knot nematodes (Luna, 1998, Berkelaar, 2001). McSorley (2002) observed that effects from suppressive crops are typically short-lived, with nematodes recovering following a season of susceptible crops. According to Luna (1998), root knot nematodes susceptible crops should be grown after two years of rotation with non-host crops or nematodes suppressive crops.

Effective use of nematodes suppressive crops for management of root knot nematodes requires knowledge of the nematodes present and the susceptibility of any crops to be used. The highest increase in the nematode population was recorded in plots previously sown with babycorn (+33%) while the least increment was in plots previously under sweetcorn (+2.7%). When the experiment was repeated there was an interesting observation where the nematodes population actually declined in plots previously sown with sweet corn though the highest increase in the population of root knot nematodes was again in plots sown previously with baby corn. This study was in agreement with earlier observations (Johnson, 1975, Berkelaar, 2001).

According to Berkelaar (2001), some root -knot nematode species are very host specific and are only able to Parasitize one plant species or variety while others can infect a number of different species. The ability of baby corn, sweet corn and maize cv. Pioneer (Ph3253) to suppress nematodes differed and sweet corn appeared to perform better implying that effectiveness of rotation crops in nematode suppression is very cultivar specific.

These observations are therefore in agreement with earlier findings (Norton *et al.*, 1985). According to Orion (1996), sweet maize (sweet corn) cultivars are generally tolerant to nematode attack. Sweetcorn appeared to trap the *Meloidogyne* juveniles thus reducing their reproduction potential while other maize cultivars appeared to be non-hosts although this was not scientifically proven hence there is need for more work to be done to evaluate this possibility.

Guwar (legume) performed very well in nematode suppression and it was second to sweet corn in terms of performance. Although data on this crop was difficult to find, its ability to suppress root knot nematodes could be attributed to the microflora in its rhizosphere. The bacteria that colonize the rhizosphere have been categorized into two groups namely plant growth promoting rhizobacteria (PGRR) and plant health promoting rhizobacteria (PHPR) depending on their mode of action (Sikora, 1991). These rhizobacteria have considerable potential to improve plant health through protection against soil borne pests.

The rhizobacteria have been shown to possess antagonistic activity towards cyst and root knot nematodes (Oostendorp and Sikora, 1986). According to Becker *et al* (1988), the rhizobacteria also have other mechanisms that affect nematodes in various ways like interfering with hatching ability of the eggs, reduced attraction to roots and interference with host recognition. There is need for further evaluation of the characteristics of the crop (guwar) to determine the mechanisms that lead to nematode suppression.

Evaluation of yields indicated that the highest levels of yields were observed under plots earlier sown with guwar. This further shows that okra sown immediately after guwar might have benefited from increased levels of nutrients attributed to the ability of guwar to fix nitrogen into the soil. Although sweet corn recorded the least increment in *Meloidogyne* juveniles, yields of okra sown after sweet corn were slightly lower compared to okra following guwar. Use of nematodes suppressive crops in rotation cycles with okra offers an exciting potential for nematode management.

5.4 Effect of combing organic amendment (farmyard manure) and crop rotation with nematode suppressive crops on root- knot nematodes control in okra

This study revealed that combining organic amendment and one season rotation with nematode suppressive crops reduced nematode populations in the soils significantly. Muller and Gooch (1982) noted that incorporation of organic material into the soil reduces root knot nematode densities. According to McSorley (2002), incorporation of organic amendments such as manure, compost and mulches with high organic matter in to the soil increases chances of development of root knot nematodes antagonistic organisms in the soil. Berkelaar (2001) reported that incorporation of organic matter into the soil may achieve three things. It may support higher populations of natural pests of nematodes such as bacteria and/or fungi. It may also release compounds that are toxic to nematodes as it decays. It may also increase soil nutrient and water levels, enabling the crops to partially overcome the effect of having a damaged root system. Sardanelli *et al* (2002) reported that management tactics rarely destroy all plant parasitic nematodes, but rather reduce the population(s) to levels below which economic damage occurs. Available literature on combination of rotation of nematode suppressive crops and organic amendments is a pointer on the superiority of the integrated approach in root knot nematodes management

The most common integrated approach is use of nematode suppressive crops in rotation with resistant vegetables. Although use of nematode suppressive crops is inferior to fumigation or soil solarization, their performance can be improved by combining them with other methods, such as organic soil amendment and crop rotation (McSorley, 2002).

The findings in this study show that combining organic amendments and nematode suppressive crops is more effective in management of nematodes than use of crop rotation or incorporation of organic amendments alone. Since of either approach alone result in build of nematodes population to threshold level after one season. This confirms earlier findings that for effective management of nematodes through crop rotation, long periods of rotations are needed and organic amendments may take up to an entire year to decompose (Mcsorley, 2002).

Highest decline in nematode population was observed in soils planted with guwar followed by application of farmyard manure before planting okra in the next season (71%) . Combination of two or more methods of nematodes management is the way to go in integrated management systems, which provides more sustainable and long term solutions to the wide spread nematodes problems in farming systems. However the methods selected must be compatible, affordable and easy to use for farmers to adopt them.

5. 5 CONCLUSIONS AND RECOMMENDATIONS

5. 5.1 CONCLUSION

The crops that were found to be suppressive to nematodes included several cultivars of maize (*Zea Mays*), Sorghum (*Sorghum bicolor*), Pearl millet (*Pennisetum glacum*) among the cereals. Among the legumes guwar (*Cyamopsis tetragonoloba*) were resistant while pigeon pea (*cajanus cajan*) was moderately resistant. This array of tested crops offers an acceptable choice of potential crops for rotation with susceptible crops like okra but the choice of either of these crops should be guided by ready availability of market for them. Cowpeas and greengrams were confirmed as susceptible to root knot nematodes except cowpea cv K80 which was moderately susceptible and should be used with care as a rotation crop in nematode management. Existence of some resistant cultivars among them is an advantage but they should first be evaluated for identification of the resistant cultivars for use in rotation aimed at managing root knot nematodes.

Combination of rotation of nematodes suppressive crops and organic amendment offered an exciting and interesting potential in the integrated approach of nematode management. This is because it eliminated use of lengthy rotation periods normally recommended for example 2 –4 years, which leads to loss or reduction of income during this period and bulk use of organic amendments which is expensive and cumbersome according to experiments, carried out elsewhere. The integrated approach could further be enhanced by use of nematode resistant crop varieties (a few are available) in combination with crop rotation and organic soil amendments. This integrated approach is superior to single approach strategies and is more sustainable and therefore shows that integrated approach is the way forward.

5. 5.2 RECOMMENDATIONS

Observations of this study were based on screening done on a few cereals and pulses (legumes).

There is need to screen more crops especially vegetables to determine their reaction to root knot nematodes so as to advise farmers accordingly. Further studies should be undertaken to determine the mechanisms involved in nematode suppression by some of the crops like guwar. Knowledge gathered could be used in selection of potential rotation crops. Other strategies of nematodes management like organic amendments and biological control should be investigated with the aim of incorporating them into integrated packages for nematode management.

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5. 5. 4 LIST OF APPENDICES

Appendix 1. Nutritive value of Okra /100g edible portion

	Fruit	Leaves
Dry matter (g)	10.4	10
Energy (Kcal)	31	33
Protein (g)	1.8	2.0
Calcium (mg)	90	70
Phosphorous (mg)*	56	
Magnesium (mg)*	43	
Iron (mg)	1.0	10
Carotene (mg)	0.1	0.99
Thiamine (mg)	0.07	0.10
Riboflavin (mg)	0.08	0.10
Niacin (mg)	08	1.0
Vitamin C (mg)	18	25

Source: Grubben (1977) Note: *from Hamon and Charrier (1997)

Table 2. A summary of Okra export volumes for the past 5 years

Product	Year	Total Weight	Total Value
Okra	1999	2,757,514.00	122,319,093.00
	2000	2,603,474.00	126,433,345.00
	2001	2,281,137.00	344,274,276.00
	2002	2,382,309.00	332,509,121.00
	2003	1,840,894.00	330,576,449.80

Source HCDA Annual report 2003

Appendix 3 ANOVA for nematode juvenile counts for potential rotation nematode suppressive crops in the greenhouse 1st season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	20	294.533	14.727	10.46	<001
Residual	32	87.250	1.407		
Total	104	382.133			

Appendix 4: ANOVA for galling indices for potential rotation nematode suppressive crops in the green house 1st season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	20	422.857	21.143	12.55	<.001
Residual	62	104.467	1.685		
Total	104	528.057			

Appendix 5: ANOVA for egg mass indices for potential rotation nematode suppressive crops in the greenhouse – 1st season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	20	385.7905	19.2895	20.99	<.001
Residual	84	77.2000	0.9190		
Total	104	462.9905			

Appendix 6 : ANOVA for nematode juvenile counts for potential rotation nematode suppressive corps in the greenhouse – 2nd season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	20	277.773	13.888	14.27	<.001
Residual	84	186.873	9.732		
Total	104	821.209			

Appendix 7 : ANOVA for galling indices for potential rotation nematode suppressive crops in the green house – 2nd season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	20	273.7333	13.6867	18.91	<.001
Residual	84	60.8000	0.7238		
Total	104	334.5333			

Appendix 8: ANOVA for egg mass indices for selected nematode suppressive crops under field conditions – 2nd season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	20	365.1078	18.3289	20.88	<.001
Residual	84	76.2310	0.8905		
Total	104	460.9804			

Appendix 9: Annova for initial nematode population count (p_i) for selected nematode suppressive crops under field conditions –1st season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Blocks stratum	3	7094	2365	0.22	
Treatments	4	44518	11129	1.03	<.001
Residual	12	129462	10789		
Total	19				

Appendix 10 : ANOVA for final nematode population for nematode (p_f) of selected nematode suppressive crops under field conditions – 1st season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Blocks stratum	3	27324	9108	1.97	
Treatments	4	231808	57592	12.56	<.001
Residual	12	55382	4615		
Total	19				

Appendix 11 : ANOVA for initial nematode population count (p_i) for selected nematode suppressive crops under field conditions – 2nd season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Blocks stratum	3	48545	16182	0.74	
Treatments	4	283538	70884	3.23	<0.001
Residual	12	263642	21970		
Total	19				

Appendix 12 : ANOVA for initial nematode population (p_i) for okra minus organic amendment(farmyard manure) grown after selected nematode suppressive crops – 1st season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	4	278195	69549	10.44	<0.001
Residual	15	99925	6662		
Total	19				

Appendix 13 : ANOVA for final nematode population (p_f) for okra minus organic amendment(farmyard manure) grown after selected nematode suppressive crops – 1st season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	4	1865195	466299	28.2	<0.001
Residual	15	247818	16521		
Total	19				

Appendix 14 : ANOVA for intial nematode population (P_i) for okra minus organic amendment(farm yard manure) grown after selected nematode suppressive crops – 2nd season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	4	296058	74014	4.9	<.001
Residual	15	226488	15099		
Total	19				

Appendix 15 : ANOVA for final nematode population (p_f) for okra minus organic amendment(farmyard manure) grown after selected nematode suppressive crops – 2nd season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	4	1788005	447001	18.99	<0.001
Residual	15	353119	23541		
Total	19				

Appendix 16: ANOVA for initial nematode population (p_i) for okra plus organic amendment(farmyard manure) grown after different nematode suppressive crops – 1st season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	4	278571	69643	10.09	<0.001
Residual	14	96592	6899		
Total	18				

Appendix 17 : ANOVA for final nematode population (p_f) for okra plus organic amendment(farmyard manure) grown after different nematode suppressive crops 1st season

Source of variation	d.f.	s.s.	m.s.	v.r.	F.pr.
Treatments	4	2418047	6045	60.89	<0.001
Residual	14	138984	9927		
Total	18				

Appendix 18 : ANOVA for initial nematode population (p_i) for okra plus organic amendment(farmyard manure) grown after selected nematode suppressive crops – 2nd season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	4	296058	74014	4.90	<0.001
Residual	15	226488	15099		
Total	19				

Appendix 19 : Annova for final nematode population(p_f) for okra plus organic amendment(farmyard manure) grown aften selectedt nematode suppressive crops – 2nd season

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Treatments	4	2365382	591346	28.62	<0.001
Residual	15	309938	20662		
Total	19				