EVALUATION OF ANTAGONISTIC PLANTS FOR ROOT-KNOT NEMATODE (*Meloidoygyne spp.*) MANAGEMENT IN TOMATO

OTIPA MIRIAM JUDITH

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT PATHOLOGY

DEPARTMENT OF CROP PROTECTION
UNIVERSITY OF NAIROBI

2003

DECLARATION

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

Otipa, M.J. _____ Date 11/9/2003

This thesis has been submitted with our approval as university supervisors

Dr. J.W. Kimenju _____ Date 10/9/2003

Dr. E.W. Mutitu _____ Date 10/09/2003

DEDICATION

To Rodney and the kids.

ACKNOWLEDGEMENTS

I sincerely appreciate my supervisors, Dr. J.W. Kimenju and Dr. E.W. Mutitu for their invaluable contributions they made towards the accomplishment of this work. I am greatly indebted to Dr. J.W. Kimenju for his brilliant ideas, consistent encouragement, enthusiastic support, numerous valuable suggestions and great interest in this work. Dr. E.W. Mutitu is acknowledged for her great suggestions and encouragements.

I wish to thank KARI for the financial support, under the World Bank Support Programme and for granting me study leave to undertake my studies. I acknowledge the technical staff and students of the Department of Crop Protection for their great encouragement and help in many ways. Douglas Miano and George Karuiki are acknowledged for reading through the thesis. Special thanks go to my dear friends and colleagues, Anne Oduor, Githinji Thiong'o and Maina Wagacha who gave me alot of moral support to endure. Iam deeply thankful to Samson Joginder who laboured in countless ways during this work. Beatrice Muteru and Joseph Kinyua's patience and creative skills in editing the manuscript are highly appreciated.

Finally my deepest gratitude go to my family who immensely encouraged and gave me a reason to go on. I thank the Almighty God for his awesome power and grace that indeed enabled me to complete the work.

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ABSTRACT

Tomato is attacked by several plant parasitic nematodes but root-knot nematodes are the most important causing considerable losses. Studies were undertaken in the greenhouse to determine the suppressiveness of a wide range of plant species to root knot (*Meloidogyne spp.*) nematodes. Plants were grown in pots and inoculated with 6000 eggs and /or juveniles. The treatments were arranged in a completely randomized design with 10 replications. After sixty days, the experiment was terminated and galling, egg mass indices and juvenile counts determined on a scale of 1-9 and the modified Baermann funnel technique, respectively. A field experiment was conducted to verify the greenhouse results in nematode infested microplots. This was arranged in a randomized complete block design with three replications. After three months the experiment was terminated and similar data taken.

Among the plants tested, *Tagetes patula, Gossypium hirsutum, Desmodium unicinatum, Chloris gayana, Zea mays, Alstromeria sp., Capscium allium, Crotalaria juncea, Arachis hypogaea, Sorghum bicolor, Tithonia diversiflora and Pennisetum purpureum* were rated as suppressive with galling and egg mass indices ranging from 0-3. High galling and egg mass indices of 7.0-9.0 were recorded on *Allium cepa*, *Statice sp.*, *Brassica oleracea* var. *capitata*, *Helianthus annuus*, *Lablab purpureus*, *Coriandum sativum* and *Vigna subterranea* while the rest of the other plants were rated moderately resistant with galling and egg mass indices ranging from 3.0-6.1. Results of an experiment conducted in the greenhouse to determine the level of root penetration of resistant plants by *Meloidogyne* juveniles showed that penetration was lower in some plants. Penetration was 95% lower in *T. patula* and 80% lower in crotalaria as compared to the control (tomato).

Damage by nematodes was significantly ($P \leq 0.05$) reduced in tomato plants planted following a crop of sweetcorn alone or in combination with *Tagetes patula, Crotalaria juncea, sorghum bicolor* and *Asparagus sp.* in the field. After the first season, nematode population density continued to decrease in all the treatments while it continued to increase throughout the two seasons under tomato monoculture. Tomato plants grown in association with *Tagetes patula,* rhodes grass and sweetcorn had lower galling indices of not more than 1.5 compared to associations with cotton, crotalaria, sorghum, asparagus, garlic, chrysanthemum, tithonia, spring onion and sesame where gall indices were higher than 2.0.

This study shows that despite the wide host range of *Meloidogyne* species, there is a wide range of economically important plants from which suitable candidate crops can be chosen and incorporated into different cropping systems. Some of the plants can be grown for advantages of soil fertility improvement through nitrogen fixization, to prevent soil erosion, quality forage and ornamental value. Extensive on-farm studies in different agroecological zones needs to be carried out and the mechanisms of nematode suppression established.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Tomato Production in Kenya

Tomato (*Lycopersicon esculentum* Mill) is one of the most widely grown and consumed vegetable in Kenya (Mwangi, 1997) and ranks third after kales and cabbages as far as production and hectare is concerned (Anon, 2000). Kenya produces approximately 255,310 metric tonnes of tomato annually (HCDA, 1990). In 1996, 13,780 hectares produced 196,210 megatonnes of tomatoes valued at Ksh 136,551M (Anon, 1996).

Tomato is grown all over the country with nearly 70% being produced by small-scale farmers mainly in Kirinyaga, Murang'a, Nyeri, Embu and Meru (Mwangi, 1997). Most produce is used within the country in salad, cooked as vegetable or used to make food products like ketchup, tomato juice, tomato paste or tomato sauce with less than 0.1% being exported (HCDA, 1990).

1.2 Production Constraints

The principal production constraints to tomato yield in Kenya include diseases, pests and poor agronomic practices (Farrell *et al.*, 1995). The major diseases of tomato in Kenya are late blight (*Phytophthora infestans),* bacterial wilt (*Ralstonia* solanacearum), root-knot nematodes (*Meloidogyne* spp.) and bacterial canker caused by *Clavibacter michiganense* subsp. *Michiganense* (Farrell *et al.*, 1995).

Tomatoes are attacked by several plant parasitic nematodes but root-knot nematodes are the most important causing considerable losses due to their wide distribution and host range (Reddy *et al.*, 1986; Valdez, 1987; Netscher and Sikora, 1990). Losses due to root-knot nematodes have been on a continuous increase in the tropics and sub-tropics (Netscher and Sikora, 1990). Root knot nematodes are widely distributed in Kenya and cause up to 80% losses in tomato (Whitehead and Kariuki, 1960; Farrel *et al.*, 1995).

The most common species of root-knot nematodes in Kenya are *Meloidogyne incognita* (Kofoid and white) Chitwood, *Meloidogyne javanica* (Treub) Chitwood and *Meloidogyne halpa* (Chitwood) (Miano, 1999). Apart from being pathogenic, plant parasitic nematodes also act as wounding agents and host modifiers resulting in reduced resistance to other plant pathogens especially those found in the soil (Mai and Abawi, 1987; Hussey and Mc Gure, 1987; France and Abawi, 1994).

1.3 Management of Root-Knot Nematodes

Several strategies have been developed for the management of root-knot nematodes but their adoption has faced some limitations (Johnson *et al.*, 1992; Mateeva *et al.*, 2000). These methods include chemical control, fallowing, cover crops, crop rotation, biological control, resistant plants and organic amendments (Sikora, 1992; Araya and Caswell-Chen, 1994; Bridge, 1996; Walker *et al.,* 1998). Cultural practices such as fallowing and crop rotation are not practical to most farming communities due to scarcity of arable land (Thomason and Caswell, 1987; Siddiqui and Alam, 1999) and are ineffective due to the broad host range of root-knot nematodes (Kerry, 1990). Use of

resistant cultivars is the cheapest and most practical method but resistant varieties are unavailable to farmers and complete reliance may result in development of resistance (Netscher and Sikora, 1990).

Use of biological control is a viable option but it takes a long time for the biological control agents to fully establish into effective populations (Becker and Schwinn, 1994). Organic amendments have a suppressive effect on nematodes through stimulation of antagonistic microorganisms or by releasing toxic by-products upon decomposition (Sayre and Starr, 1988). However, their use is limited by large quantities needed to achieve acceptable levels of nematode control (Singh and Sitaramaiah, 1970; Lung *et al.*, 1997). Nematicides are effective in nematode control (Ware 1983; Farrell *et al.*, 1995) but their use is limited by high cost and hazardous effects to environment (Hague and Gowen, 1987; Noling and Becker, 1994).

Following the banning or restricted use of chemical nematicides because of side effects on human health and the environment (Ogallo *et al.* 1999; Ibrahim and Ibrahim, 2000) considerable efforts have been directed towards development and implementation of alternative control strategies of root knot nematodes (Ploeg, 2000). Despite their wide host range, *Meloidogyne* species can be controlled by suitable antagonistic plants when interplanted with susceptible crops (Zechmeister and Sease, 1974; Reddy *et al.*, 1986; Dhanger *et al.*, 1995; Shellami and Cheija, 1997; Lung *et al.*, 1997). Use of antagonistic plants to suppress nematodes has been found to be effective by several authors (Swamy *et al.*, 1995; Zaveleta and Gomez, 1995; Bridge, 1996; Varges-Ayala *et al.*, 2000).

This has been attributed to the nematicidal root exudates that are toxic to nematodes (Uhlenbroek and Bijloo, 1957; Mohandas *et al.*, 1981; Sukul, 1992; Jacobs *et al.*, 1994). Moreover diffusates from certain non-host plants to which a given nematode is not attracted may in some way mask or neutralise the effects of the diffusates from a host plant to which the nematode would otherwise respond (Christie, 1939). In interplanting and rotation farming practices, it is possible to take advantage of the effects of these diffusates to suppress nematode population build-up (McSorley and Dickson, 1989; Caswell *et al.*, 1991; McSorley and Gallaher, 1991). The potential of antagonistic plants in root-knot nematode control is not fully exploited because most of the widely studied antagonistic plants lack market value and are perceived by farmers to be weeds.

1.4 Overall objective

This study was aimed at identifying nematode antagonistic plants with food, forage or commercial value and incorporating them into cropping systems as a component of an integrated nematode management package. The specific objectives were;

1. To screen potential antagonistic plant species on control of *Meloidogyne* species.
2. To determine the level of penetration and development of *Meloidogyne* juveniles in roots of antagonistic plants.
3. To determine the effect of growing tomato in rotation with antagonistic plants in combination with sweetcorn on root-knot nematodes.
4. To determine the effect of interplanting tomato with antagonistic plants to root knot management.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History and Origin of Tomato (*Lycopersicon esculentum L.*)

Tomato *Lycopersicon esculentum* Mill originated from the Andean region of South America (Darby, 1973) and domesticated in Mexico (Morrison, 1938). It was the Spanish and Portuguese merchants who introduced all tomato varieties to Europe and Asia from Latin America during the 16th and 18th Centuries (Morrison, 1938; Villareal, 1979). The European colonizers then introduced the tomato to Africa (Villareal, 1979). Although initially in Europe because of its erroneous reputation as a poisonous fruit, tomato has now become one of the most important vegetables worldwide (Norman, 1992).

2.2 Tomato classification

Tomato is classified under the phylum *Spermatophyta,* sub phylum *Angiospermae*, class *Dicotyledonae*, order *Solanales and* family *Solanaceae* (Rick, 1987). The genus *Lycopersicon* is divided into two subgenera; *Eulycopersicon,* the red-fruited species, and *Eriopersicon*, the green-fruited species (George and Berry, 1983). *Eulycopersicon* contains two species, *L. pimpinellifolium* and *L. esculentum. Eriopersicon* contains the species *L. cheesmanii, L. glandulosum, L. hirsutum* and *L. peruvianum* (Darby, 1973; George and Berry, 1983). All the cultivated types are in the species *L. esculentum* (Darby, 1973).

In Kenya, Kenton (2) (F1 hybrid), Kenton (1) (F1 hybrid), Hofit, M82, Beauty and Cal-J all of early maturing varieties, Roma VF and Moneymaker, medium maturing and Marglobe late maturing popular varieties grown by farmers (Anon, 1998).

2.3 Tomato Production Constraints

The main constraints in tomato production include diseases, pests, poor agronomic practices and soil fertility. The major diseases of tomato in Kenya are late blight (*Phytophthora infestans*), bacterial wilt (*Ralstonia solanacearum*), root-knot nematodes (*Meloidogyne* spp.) and bacterial canker (*Clavibacter michiganense* subsp m*ichiganense*) (Madumadu, 1976; Farrel *et al.,* 1995). Root-knot nematodes are widely distributed in Kenya and cause up to 80% losses (Farrell *et al*, 1995).

Other major fungal diseases are early blight *(Alternaria solani),* powdery mildew *(Leveillula taurica)*, leaf spot *(Botrytis cinerea*) and fusarium wilt *(Fusarium oxysporium)* (Rodriguez *et al.*, 1987). Viral diseases of importance on tomato are tomato mosaic virus, cucumber mosaic virus and tomato yellow leaf curl (Howard *et al.*, 1994). Major pests include American bollworms (*Helicoverpa armigera*) Red spider mites (*Tetranychus cinnabarinus*), aphids (*Aphies gossypii*, *Aphis macrosiphum* and white fly (*Bremisia tabaci*) (Farrell *et al*, 1995).

2.4 Importance of tomato

Tomatoes are consumed fresh in salads, sauces and as a flavouring ingredient in soups and meat or fish dishes. Tomatoes can be made into sweetened candies, dried fruits and even into wine (Gould, 1983). Economically, tomato is used in processed forms

such as puree's, juice, ketchup, canned whole and as diced fruits (Olkowski and Olkowski, 1996). Tomatoes are important source of vitamins A, E and C as well as potassium and beta-carotene. It is also a source of income for the small -scale farmers (Farrell *et al*, 1995). Although nutritionally they rank low they outrank other vegetables in total contribution to human nutrition because they are consumed in so many different ways.

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; subclass Sercenentea; order Tylenchida; suborder Tylenchina; Family Heteroderidae and genus *Meloidogyne* (Chitwood, 1956). There are 51 species of *Meloidogyne* (Jepson, 1987). *M. Incognita, M. javanica, M. arenaria* and M. *hapla* are of economic importance in vegetable production (Jepson, 1987).

Root knot nematodes are the most economically important plant parasitic nematodes in tropical and sub tropical agriculture (Sasser, 1980; Netscher and Sikora, 1990). They have a wide host range with over 2500 plants as hosts (Agrios, 1997). Tomato is a host of most frequently occurring species of root-knot nematodes. The nematodes of the genus *Meloidogyne* are economically important pathogens of a wide range of crops (Xu-jian Hua *et al.*, 2001)

2.5.2 Morphological Characteristics of *Meloidogyne spp.*

The males are worm-like and about 1.2 -1.5mm long by 30-60μm in diameter (Sherf and Macnab, 1986; Agrios, 1997). The mature females are pear-shaped and about 0.40-1.30mm by 0.27-0.75mm wide (Agrios, 1997). Second stage juveniles are vermiform in shape while third and fourth stage juveniles are sausage shaped and microscopic in size (Sherf and Macnab, 1986). *Meloidogyne* spp. are distinguished by use of distinct patterns in mature females which resemble finger prints of humans which are referred to as pereneal patterns (Jenkins and Taylor, 1967; Williams, 1974; Machon and Hopper, 1991).

2.6 Etiology

2.6.1 Biology

All *Meloidogyne* spp. have a similar life cycle (Agrios, 1997). When host, temperatures and surroundings are favourable, it can produce more than 2800 eggs and lay them in a sac-like gelatinous matrix (Taylor and Sasser, 1978; Sherf and Macnab 1986, Agrios 1997). A new generation may arise within 25 days. The first stage larva develops inside the egg and undergoes moulting to form second stage larva that is the infective stage (Taylor and Sasser, 1978; Agrios, 1997).

Nematode survival, egg hatching and disease severity is influenced by temperature, soil texture and structure (Netscher and Sikora, 1990). Penetration of second stage juveniles into plants is optimum at about 27° C. Root-knot Nematodes survive and reproduce

under a wide range of pH ranging from acid to alkaline (Ferris and Van Gundy, 1979; Verma *et al.,* 1998).

2.6.2. Infection Process of *Meloidogyne* spp

Infective second – stage juveniles enter the roots at the region just behind the root tip and moves intracellularly or extracellularly to the zone of differentiation (Agrios, 1997). The juveniles starts feeding from the cells next to their head by secreting saliva containing enzymes that dissolves the cell content of plant tip and push their way between or through cells until they reach the zone of differentiation where they become established (Dropkin and Nelson, 1960; Agrios, 1997).

Cells around the head of the juvenile begin to enlarge three or four days after (Sherf and Macnab, 1986; Agrios, 1997). Their nuclei divide but no cell walls are laid down. The existing walls between some cells break down and disappear and the protoplasmic contents coalesce giving rise to giant cells (Sherf and Macnab, 1986; Agrios, 1997). Each gall contains 3-6 giant cells that are maintained by a continuous stimulus from the nematode but collapses when it ceases to feed (Zhao, 2000). Enlargement of cells continue for two or three weeks until the nematode stops feeding or dies when giant cells disintergrate (Christie, 1936; Agrios, 1997).

As females enlarge and egg sacs are formed, they push outward spliting the cortex and may become exposed on the surface of the root or may remain completely covered, depending on the position of nematode in relation to the root surface (Agrios, 1997). Xylem elements are affected due to mechanical pressure from enlarging cells. Swelling

of the root results from hypertrophy and hyperplasia of the vascular parenchyma, pericycle and endodermal cells surrounding the giant cells (Agrios, 1999).

2.7 Symptomatology

The primary symptom associated with *Meloidogyne* infection is presence of galls in the root system (Wilcox and Loria, 1986; Agrios, 1997). Infected roots often branch above galls and root crumbs may be formed (Christie, 1936). Root systems of severally infected plants are reduced to a limited number of severely galled roots with completely disorganised vascular system (Netscher and Sikora, 1990) leading to reduced efficiency in absorption of water and nutrients (Agrios, 1997).

Rootlets are almost completely absent and plants wilt rapidly under warm conditions and are often stunted (Agrios, 1997). Growth is retarded and leaves may be chlorotic (Agrios, 1997) Infected seedlings result in death in the nursery but those that survive, flowering and root production is greatly reduced (Netscher and Sikora 1990; Sherf and Macnab, 1986).

2.8 Economic importance of *Meloidogyne* spp.

Root–knot nematodes are widely distributed in Kenya and cause up to 80% losses in tomato (Farrel *et al.*, 1995). Root–knot damage is associated with formation of galls that disrupts water and nutrient uptake by the plant (Agrios, 1997). When susceptible plants are infected at the seedling stage, loses are high and may result in complete destruction of the crop (Netscher and Sikora, 1990). They cause breakdown of host resistance to other pathogens (Jenkins and Coursen, 1957; Sidhu and Webster, 1977). Wounds

caused by penetrating juveniles serve as excellent sites for entry of bacterial pathogens (Valdez, 1987) and other opportunistic pathogens.

2.9 Control

Several methods for control of plant parasitic nematodes are available and have been employed with varying degrees in nematode control (Katan, 1981; Sikora, 1992; Sharma *et al.*, 1994; Abawi *et al.*, 2000; Johnson *et al.*, 2000) but cost, type of crops, nematode types, availability of arable land, abiotic and environmental considerations limit their applicability in some cases.

2.9.1 Physical Agents

Meloidogyne spp. densities drop significantly when soils are flooded for long periods but it's a costly and uneconomical means of nematode control when done artificially (Stover, 1979). Soil solarization has been used with success in the control of nematodes (Katan, 1981) but the technology is limited by cost of polythene and availability of sufficient solar energy (Netscher and Sikora, 1990; Oka and Spiegel, 1993). While fallow may be beneficial for nematode management in some situations, the lack of farm income during fallow period is a limitation (McSorley, 20001). Powers and McSorley (2000) and Abawi *et al.* (2000) observed that fallowing encouraged soil erosion by wind and water and caused a negative effect on soil structure.

2.9.2 Plant Resistance

Crop cultivars resistant to nematode infection can be the most practical and cheapest means of nematode control especially in small-scale farms (Bridge, 1996). Several tomato cultivars are known to be resistant to M*eloidogyne* spp. According to Giordano *et al.* (2000) a resistant tomato verdure was found to be resistant to most diseases including Brazilian populations of *Meloidogyne Jaavaica, M. Incognita* and *M. arenaria.* However wide spread adoption of this strategy is limited by unavailability of resistant materials to farmers and resistance breakdown after a few years of use (Escher and Sacra, 1990).

2.9.3 Chemical Control

Chemicals used in control of nematodes are either fumigants or non-fumigants (Ware, 1983). Non- fumigants are not effective against eggs of nematodes and in most cases do not kill the juveniles at recommended rates (Patel and Patel, 1999). However they give a plant a head start by delaying nematode penetration during the highly sensitive seedling stage or post planting stage of plant development. Karate was found to prevent egg-hatch and accelerated death of the infective second stage juveniles (Olubunmi and Adesiyan, 1997).

The use of nematicides is declining primarily due to their high cost, toxicity to non-target species, health considerations and environmental pollution (Hague and Gowen, 1987). According to Lambert (1979) and McKerry (1987) broad spectrum fumigants (methyl bromide, chloropicrin and vorlex) and granular nematicides (aldicarb, carbofuran and

oxamyl) have been found to be effective against root-knot nematodes. Use of nematicides is however limited because of its broad-spectrum effects that usually disrupts many beneficial soil ecological processes such as nutrients cycling and biological control (Becker *et al.,* 1988).

2.9.4 Biological Control

Biological control involves use of natural enemies of phytonematodes that act through mechanisms as parasitism, predation, competition and antibiosis in the control of root-knot nematodes (Sikora, 1992). Nematode parasites or antagonists have been incorporated in the soils for the control of root-knot nematodes on vegetables (Kerry, 1987; Badi *et al.*, 2000). Biological agents of nematodes that have shown promising results in the control of nematodes include *Verticillium chlamydosporium* (De Leij *et al.*, 1992; Sankaranarayanan *et al.*, 2000), *Peacilomyces lilacinus* (Hafeez, 2000; Khan and Goswami, 2001) and *Pasteuria penetrans* (De- Channer, 1997; Tariq and Riaz, 2000).

Verticilium chlamydosporium and *P. lilacinus* have shown promising potential as parasites of the nematode eggs (De Leij and Kerry, 1991; Al Raddad, 1995) while *Pasteuria penetrans* spores germinate on the nematode by forming germ tubes which penetrate the cuticle of the nematode and fill the body cavity thus killing the nematode (Kerry, 1987).

Rhizobacteria such as *Bacillus spp., Pseudomonas* and *Telluria chitinolytica* are known to inhibit penetration of nematodes into roots thus reducing root galling (Bowman *et al.*, 1993; Rao *et al.,* 2000; Amin, 2000). Reduction of infection and suppression of

development by plant parasitic nematodes in the plants by several *Bacillus spp.* is due to production of toxic or inhibitory metabolites (Mankau, 1995). Oostendorp and Sikora (1990) reported that presence of *Bacillus spp.* in the rhizosphere caused modification of root exudates thus affecting nematode attraction to or recognition of the host.

Efforts to acquire sustained biological control in the field has been limited by the fact that soil is a powerful buffer and the high amounts of organic matter needed for fungal establishment and spread in the soil environment limit practical application in most large scale production systems (Rodriguez-Kabana and Morgan-Jones, 1987; Kerry, 1987).

2.9.5 Organic Amendments

These are by-products and wastes from agricultural and other activities and include oil cakes, crop residues, composites, green manures, agro-industrial wastes and human excrements (Bridge, 1996; Ibrahim and Ibrahim, 2000; Vijayalakshmi *et al.*, 2000; Umar and Jada, 2000). Incorporation of organic amendments into the soil have been shown to reduce root-knot nematode densities (Muller and Gooch, 1982; Mojumder *et al.*, 2000; Jonathan *et al.*, 2000; Leon *et al.,* 2000) and it also releases nutrient and increases water holding capacity of soil thus improving plant growth and hence tolerance to nematode attack (Mohamed *et al.*, 2000).

Siddiqui and Alam (2001) reported that root-knot nematode development on tomato was significantly inhibited by nematicidal effects of neem (*Azadirachta indica*) and that growth of tomato improved. According to Rodriguez- Kabana (1986) and Sayre and Starr (1988) presence of high organic matter stimulates the activity of indigenous soil

microorganisms some of which are antagonistic to nematodes and their decomposition results in accumulation of compounds with nematicidal effects. However, their use is limited by large quantities needed for successful control (Kerry, 1990).

2.10 Mechanisms of Nematode Suppression in Antagonistic Plants

2.10.1 Passive Resistance

These are anatomical, physiological and chemical barriers that may hinder the invasion of the nematode (Giebel, 1982). Plants may produce toxins that kill nematodes like *Asparagus officinalis* contains toxins in its leaves, stem and roots which are toxic to *Trichodorus Christie* and this leads to decline of population of this nematode around the plants (Rohde and Jenkins, 1958). The roots of *Tagetes patula* and *T. erecta* contain α terthienyil and derivative of bithienyi (Uhlenbroek and Bijloo, 1957) that reduces *Meloidogyne* and *Pratylenchus* populations (Oostenbrink *et al.*, 1957).

Marigold *(Tagetes)* suppression of soil endopathogenic nematodes is thought to be due to thiophenes heterocyclic sulfur-containing molecules abundant in this plant (Topple *et al.*, 1998). Rangaswamy *et al.* (1993) noted that *M. incognita* larvae failed to develop beyond the second stage and initiated giant cells in *Tagetes patula* roots due to hypersensitive necrotic reaction and further observed that *Tagetes patula* had least insoluble polysaccharides, proteins and nucleic acids that made it more resistant to *Meloidogyne incognita*.

When Jacobs *et al.* (1994) investigated thiophene synthesis and accumulation in germinating seedlings of both species of *Tagetes*, he found hypocotyls to be the major

thiophene accumulating organs and thiophene 5- (3- buter-1-nynl) –2,2-bithienyl and 5-buter-1-nynl) –2, 2'-bithienyl 2' bienyl) as the major compounds. He further noted that *T. patula* had higher concentration than *T. erecta* and that within *T. patula* hypocotyls, thiophene concentration were higher in the epidermis and in the vascular tissue and lower in the parenchymatic tissue of cortex and pith. He also observed that synthesis of thiopenes was high in the roots and hypocotyls and very low in leaves.

In roots of cotton cultivars resistant to *M. incognita*, a high concentration of post-infectional terpenoid aldehydes was found (Veech and McClure, 1977). Onion bulbs are resistant to diseases due to presence of protocatechnic acid and catechol that are water-soluble phenolic compounds that occur in the outer pigmented scales of onion (Meyer and Fry, 1978).

Sorghum is found to contain cyanogenic glycoside (dhurrin) in its vacuole. Following injury by nematodes, the glycosides become exposed releasing highly toxic hydrogen cyanide gas (Meyer and Fry, 1978) that is toxic to nematodes. It has been reported that the levels of hydrogen cyanide released at infection sites are sufficiently high to kill or at least inhibit the growth of penetrating hyphae (Meyer and Fry, 1978).

2.10.2. Phenolic Compounds.

Phenolic compounds are produced by plants in response to attack by nematodes and cause quick browning and formation of non-expandable necrosis in plants resistant to

migratory parasite. They cause IAA-oxidase stimulation that favours auxin decomposition.

The presence of chlorogenic acid is thought to be the cause of browning and of resistant reaction of chrysanthemum to *Aphelenchoides ritziemabosi* (Wallace, 1961). The resistance of tomatoes to *M. incognita* is attributed also to the occurrence of high concentrations of chlorogenic acid, neochlorogenic acid, caffeic acid and O-dihydrocyphenols in resistant plant leaves and roots (Bajaj and Mahajan, 1977).

The spread of some nematodes, fungal and bacterial pathogens in some plants has been effectively blocked by the presence of xylem bundle sheaths and sclerenchyma cells of leaf veins that act as post-infectional mechanism through structural barriers (Agios, 1997). Failure of females to reach maturity is a form of resistant mechanism that could be brought about by certain plants lacking essential substances for nematode development and reproduction (Giebel, 1982).

2.11 Responses of Antagonistic Plants to Root-knot Nematodes (*Meloidogyne* spp.)

Antagonistic plants are those that release root exudates that have nematicidal properties (Sukul, 1992). The most widely studied antagonistic plants in that category are *Tagetes* spp, mustard, castor, asparagus, sesame, sun hemp (*Crotalaria* spp.) and neem (*Azadiracta indica)* (Sethi Gaur, 1986; Bridge, 1996). The above plants have been found to be effective in suppressing the nematode population in soil (Swamy *et al.,* 1995). Some antagonistic plants often act as trap crops and reduce nematode populations by

allowing invasion and only partially development in the roots (Bridge, 1996). Some of the antagonistic plants may be used in cropping systems against several different *Meloidogyne* species. These include marigold (Good *et al.*, 1965), sorghum (Ibrahim *et al.*, 1998) and sun hemp (McSorley, 1999).

The main *Tagetes* species tested for root-knot nematode management is African marigold (*T.erecta*), French marigold (*T. patula*) and South America marigold *T. minuta* (Lehman, 1979). According to Sethi and Gaur (1986) and Bridge (1996), *Tagetes minuta* is used in India for drug provision, as a flavour in the food industry (Mohamed *et al.*, 2000) and as an ornamental. It is found to contain compounds that are toxic to *Meloidogyne arenaria*, *M. incognita,* M. *javanica* and other nematodes (Jacobs *et al.*, 1994; Toppel *et al.*, 1998). Kanagy and Kaya (1996) and Debprasad *et al.* (2000) found Thiophenes α terthienyl and bithienyls as active compounds in *Tagetes spp.*

Dhanger *et al.* (1995) noted that the final *Meloidogyne javanica* population was reduced up to 40.5% over the initial level owing to intercropping of *Tagetes* with eggplant. Two-months rotations of *T. erecta* and tomato also reduced populations of *Meloidogyne* spp. (Shellami and Cheifa, 1997). Reddy *et al.* (1986) observed also that rotations of marigolds resulted in reduced soil populations of root-knot and lession nematodes in all succeeding crops.

Lung *et al.* (1997) reported that *tagetes* reduced the population density of *Meloidogyne* spp by 95% after cultivation period of two months and concluded that by manipulating the tagetes planting date and spacing between plants, it is possible to achieve some

phytosanitary protection on tomato. Plant growth and yield of tomato crop increased when *T. patula* was used in *Meloidogyne spp.* control (Mateeva, 1995; and Schepman and Jansen, 1994).

Castro *et al.* (1990) also found out that incorporation of *Tagetes erecta* and its residues resulted in sufficient reduction of *Meloidogyne incognita* population, root galling by 88-96% and fruit yield increased in 72% of tomato plants. As green manure, marigolds reduced root-knot nematode populations when incorporated in infested soil (Oduor-Owino and Waudo, 1994; Zaveleta-Mejia and Gomez, 1995). In rotation systems, Doulton and Curtis (1963) reported control of root-knot nematodes in tobacco fields where *T. patula, T. erecta* and *T. minuta* proceeded tobacco. Swang *et al.* (1995) noted that nursery beds previously planted with marigold gave maximum reduction in root-knot populations.

Crotalaria spp. is a green manure crop that is widely grown in tropical environments (Singh *et al.*, 1981). Janick (1996) reported that seeds of *C. juncea* to have pyrrolizidine alkaloids, *monocrotaline*, spectabilines, riddelline, senecionine and trichodesmine which are toxic to root-knot nematodes, M. *javanica*, M. *arenaria, M. incognita.* Desaeger and Rao (1999) demonstrated that *Meloidogyne* larvae freely entered the roots of *Crotalaria* but failed to survive showing the possibility of toxic action. When planted in rotations, showy Crotalaria and hairy indigo reduced populations of root knot and lession nematodes in all the succeeding crops (Bunte and Muller, 1996; Robinson *et al.*, 1998).

As fallow crops, *Crotalaria retuse* and *Tagetes erecta* produced high reductions in population densities of *M. incognita*. Mandulu *et al.* (1994) also found *Crotalaria* to increase the yields of tomato and to suppress galling by *M. javanica* in the third season of a rotation experiment. According to Ogumo (2001) galling was reduced when tomato was intercropped with *Crotalaria sp.* Esparrago *et al.* (1999) reported that *C. spectabilis* and *C. juncea* allowed invasion of *M. javanica* and *M. arenaria* but the nematodes failed to reproduce on them.

Velvet bean (*Mucuna pruriens)* is used as an animal feed (Reddy *et al.*, 1986) and as a fertilizer as it fixes nitrogen (Sequeira, 1962). The active substance in the plant is L-3, 4-dihyroxyphenylalanine and it is a biologically active substance in animals and intermediate to many alkaloids (Fujii *et al.*, 1992). *M. Pruriens* was also found to be moderately resistant to *M. javanica* when compared with cotton and groundnut (McSorley *et al.*, 1994). According to Caamal *et al.* (2001) decomposition of velvet bean leaves in potting soil significantly reduced the development of phytopathogenic nematodes in the roots of tomato.

Reddy *et al.* (1986) observed population reductions of root knot nematode in plots where velvet bean, Crotalaria and marigolds were planted in yearly rotations. Other rotations with sesame and velvet bean have also resulted in good control of *Meloidogyne* spp. (Rodriguez-Kabana *et al.*, 1992). Herrera (1997) reported that exudates from *Mucuna deeringiana* significantly reduced the population of *M. incognita* in coffee fields.

Desmodium spp. is high quality forage (Skerman, 1977) that is widely grown in Western Kenya as green manure plant (Desaeger and Rao, 2002). Kretschmar *et al.* (1980) reported *Desmodium* spp. as being antagonistic to *M. arenaria, M. incognita, M. javanica* and *Xiphinema americanum* in greenhouse and field experiments. *D. ovalifolium* exudates were found to give greater immobilisation of second stage juveniles (J2) of *M. incognita* by Herrera (1997). Lenne (1981) found 10 accessions of *Desmodium* spp. to be resistant to *M. javanica*.

Ibrahim *et al.* (1998) observed that intercropping sesame plants with eggplant reduced the number of galls by 66% and egg masses by 77%. When sesame tissues were cultured alone or with okra they suppressed egg hatch and penetration of roots by juveniles, delayed adult development and encouraged development of males in *M. incognita* (Tanda *et al.*, 1988). Fernandez *et al.* (1992) and Varma *et al.* (1987) noted that the use of sesame as a rotational crop resulted in reduction of the infestation level of *M. incognita*, 50% higher in relation to the control with sweet potato and that the yield was increased by 3136 kg/ha. Work done by Walker (1998) indicated that all sesame accessions he worked with produced considerably fewer root-galls than tomato when inoculated with *M. incognita* race 3. Sesame (*S. orientale)* is known to suppress populations of *M. incognita* mainly due to production of root exudates containing toxic organic acids (Walker *et.al.*, 1998; Tanda and Atwal, 2000).

Asparagus is a high-value food crop and is a source of thiamine, vitamine A, B, C, calcium and iron. The lightly cooked tender young unexpanded shoots are eaten. There are references to the seed being used as a coffee substitute (Howard *et al.*, 1994).

Hasabo and Ameen (1995) found root extracts of *Asparagus scandens* to be toxic to *Rotylenchus reniformis* and 100% mortality was reached within 24 hours of exposure, and significantly reduced reniform nematode density when grown in pots together with *Cajanus cajan*.

Sorghum is the fifth most important cereal in the world after wheat, rice, maize and barley (Milliano *et al.*, 1992). It is adapted to wide range of environmental conditions and will produce significant yields under conditions that are unfavourable for most cereals (Clayton and Renvoize, 1982). Many sorghum cultivars have been reported as poor hosts of the root-knot nematodes (Kinloch and Rich, 2001). Siddiqui and Alam (2001) observed that cropping sequences containing sorghum reduced root-knot larvae and *R. reniformis* nematode population in a two-year crop rotation programmes. Some sorghum varieties have been found to be very effective in the control of *M. incognita* (Mc Sorley *et al.,* 1987; Gallaher *et al.*, 1991; Yamada, 2001).

Rapeseed (*Brassica napus*) cover crops are grown for industrial oil and are found to contain sulfur chemicals called glucosinolates (Johnson *et al.,* 1991). When incorporated as a green manure, microbial degradation of the glucosinolates produces isothiocyanates that are very similar to the active ingredients in metham sodium that is a very powerful soil fumigant (Johnson *et al.*, 1991).

According to Mojtahedi *et al.* (1991), Potter *et al.* (1999) and (Chen *et al.*, 1999) some rapeseed varieties were found to be effective in suppressing *Meloidogyne incognita* and *M. javanica.* Mojtahedi *et al.* (1991) also noted that some varieties supported high

populations of *M. incognita* and *M.hapla*. Later Potter *et al.* (1999) noted that rapeseed varieties that contained more than a certain threshold level of 2-phenylethyl glucosinolate showed reduced susceptibility to *Pratylenchus neglectus*.

Rhodes grass (*Chloris gayana*) is an excellent quality forage crop that is grown as a livestock feed. Daulton and Curtis (1963) noted that it reduced numbers of root-knot nematodes. Caswell *et al.* (1991) found *Chloris gayana* having some potential in the reduction of reniform nematode populations especially in between cropping systems. He further noted that it was immune to penetration by these nematodes and that it reduced nematode populations better than in the fallowing systems.

Sweetcorn is a vegetable that is greatly used in the Western World. Luna (1998) developed some rotations using super sweet corn and other crops and found them to be more effective against root-knot nematodes. Mustard has been found to be a trap crop and is thought to be an alternative to nematicides for control of nematodes (Krall *et al.*, 2000).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Inoculum preparation

Root-knot nematodes were multiplied on tomato cv. Moneymaker in a greenhouse. Nematode inoculum was obtained from galled tomato roots using the technique described by Hussey and Baker (1973) and modified by Sikora and Cireco (1990). Galled roots were washed free of adhering soil particles using tap water. The roots were then cut into 1 cm segments and macerated in 100ml of water in a blender for 15 seconds at high speed, twice.

The macerate was then vigorously shaken in 0.5% sodium hypochlorite solution for three minutes then poured into a bucket containing about ten litres of water. This was passed through 2mm sieves to remove the plant debris. The eggs and juvenile suspension was then filtered using 0.25mm – aperture sieves. The eggs were rinsed free of sodium hyphochlorite and transferred into a 1000ml conical flask to which 500ml sterile water was added and egg suspension continuously aerated using an aquarium pump. Second-stage juveniles were obtained in about 5 – 10 days and used as inoculum.

3.2 Screening of potential antagonistic plants to *Meloidogyne* species

3.2.1 Greenhouse experiment

Thirty-six plant species as shown in Table 1 were chosen and evaluated to determine their reaction to root knot nematodes under greenhouse conditions (Table 1). Tomato cv. Moneymaker and *Tagetes minuta* was included as negative and positive controls, respectively. Pots measuring 21 cm in diameter were filled with 5 kg heat sterilized loam: sand mixed in the ratio 2: 1(v/v). Three seeds of each test plant were sown in each pot and thinning done after emergence to leave one seedling per pot.

Ten days after emergence of seedlings, 6000 eggs and/ or juveniles were suspended in 10ml of tap water, were pipetted into indentations made around the base of the plants in each pot and soil pushed back to cover the roots. Treatments were arranged in a completely randomized design with ten replications. Plants were watered when necessary and fertilized once every two weeks using 5g of calcium ammonium nitrate (CAN) per pot. The experiment was terminated eight weeks after inoculation.

Table I. Plants screened for suppressive effects against root-knot nematodes

Common name	Scientific name	Use(s)
Coriander	*Coriandum sativum*	Vegetable
Lablab	*Lablab purpureus*	Vegetable
Cabbage	*Brassica oleracea* var. *capitata*	Vegetable
Chinese cabbage	*Brassica oleracea* var. *chinensis*	Vegetable
Capsicum	*Capsicum annuum.*	Vegetable
Lettuce	*Lactuca sativa*	Vegetable
Leekswiss	*Allium ampeloprasum*	Vegetable
Asparagus	*Asparagus officinalis*	Vegetable
Spring onion	*Allium cepa*	Vegetable
Broccoli	*Brassica oleracea* var. *botrytis*	Vegetable
Sweetcorn	*Zea mays*	Vegetable
Red onion	*Allium cepa*	Vegetable
Bambara nuts	*Vigna subterranea*	Vegetable
Garlic	*Allium sativum*	Vegetable
Mustard	*Brassica oleracea* var. *alba*	Fodder/green manure
Cotton	*Gossypium hirsutum*	Fibre
Rhodes grass	*Chloris gayana*	Fodder
Napier grass	*Pennisetum purpureum*	Fodder
Desmodium	*Desmodium unicinartum*	Fodder
Mucuna	*Mucuna prupriens*	Green manure/fodder
Crotalaria	*Crotalaria juncea.*	Green manure/fodder/ vegetable
Tiithonia	*Tithonia diversifolia*	Fodder/green manure
Sorghum	*Sorghum bicolor*	Cereal
Rapeseed	*Brassica napus*	Oil crop
Sunflower	*Helianthus annuus*	Oil seed
Sesame	*Sesamum indicum*	Oil seed
Peanut	*Arachis hypogaea*	Oil seed
Statice	*Statice sp.*	Ornamental
Marigold	*Tagetes patula*	Ornamental
Alstroemeria	*Alstroemeria sp.*	Ornamental
Ornithogolum	*Ornithogolum arabicum*	Ornamental
Tuberose	*Tuberose sp.*	Ornamental
Onnis	*Onnis sp.*	Ornamental
Chrysanthemum	*Chrysanthemum indicum*	Ornamental

The plants were uprooted, roots washed free of adhering soil using tap water and galling indices rated using the scale of 0-10 by Bridge and Page (1980) where, 0 = healthy root system, 1 = very few galls only detected upon close examination, 2 = small galls easy to detect, 3 = numerous small galls, 4 = numerous small galls and a few big ones, 5 = 25% of the root system severely galled, 6 = 50% of the root system severely galled, 7 = 75% of the root system severely galled, 8 = no healthy root but plant still green, 9 = completely galled root system and plant dying, 10 = plants and roots dead. Plants with scores ranging from 0-3 were rated as resistant while those with scores ranging from 4-6 and from 7-10 were rated as moderately resistant and susceptible, respectively. Egg masses were stained using phloxine B (Holbrook *et al.*, 1983) and quantified using a scale of 1-9 where 1=no egg masses, 2=1-5, 3=6-10, 4=11-20, 5=21-30, 6=31-50, 7=51-70, 8=71-100, 9= >100 egg masses per root system (Sharma *et al.*, 1994).

Second-stage juveniles were extracted from 200cm^3 soil samples using the modified Baermann funnel technique with extraction dishes (Hopper, 1990). Soil was spread on a double layer of milk filters supported by a sieve placed in a shallow 15 cm – diameter dish. Water was gently added into the dish until it just touched the soil so that the soil layer looked wet. This was left to stand for 2 days to allow nematodes to move from the soil suspension, through milk filters, into the water in the dish.

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

beaker. One ml of the nematode suspension was pipetted into a counting slide and counting done under a light microscope. Counting was repeated four times and the average calculated. The experiment was repeated once.

3.2.2 Field Experiment

A field experiment was conducted to determine the effect of several antagonistic plants selected as the effective ones based on findings from the greenhouse experiments on supresiveness to root-knot nematodes. The test plants selected were *Tagetes patula*, C*rotalaria juncea, Sorghum bicolor, Desmodium sp., Alstroemeria sp., Zea mays* and *Gossypium hirsutum*, with tomato being included as a negative control. The plants were grown in nematode infested micro-plots measuring 1 m by 2m. Each microplot had 4 rows with 5 plants per row. The experimental design was randomised complete block design with three replications.

Initial inoculum in the soil was determined by randomly taking samples from each micro-plot and extracting second-stage *Meloidogyne* juveniles using the modified Baermann funnel technique (Hooper, 1990). Before planting, 5g of DAP was added into each planting hole. Weeds were controlled regularly and plants were irrigated when necessary.

After three months, ten randomly selected plants were carefully uprooted from each pot and assessment for root-knot nematode damage done as in section 3.2. Soil samples were collected from ten different rhizospheres in each plot for *Meloidogyne* juvenile

population assessment. The experiment was repeated once. The macerate was then vigorously shaken.

3.3 Penetration and development of *Meloidogyne* juvenile in roots of antagonistic plants

A greenhouse experiment was conducted to determine the number of *Meloidogyne* juveniles that penetrated roots of plants considered to be antagonistic to nematodes in the screening experiment. The test plants were crotalaria, desmodium, cotton and peanut with tomato and *Tagetes patula* as positive and negative controls, respectively.

Seeds were pre-germinated on Whatman No.4 filter papers in petri dishes and and one seedling transplanted into each cone containing 250cm.^{3} sterilized sand. The cones were perforated at the bottom and a nylon mesh used to cover the holes to prevent loss of sand. Second-stage *Meloidogyne* juveniles were obtained from galled tomato roots using the method described by Omwega *et al.* (1988) by washing roots free of soil using tap water, immersed in sterile tap water and then aerated using an aquarium pump. Second stage juveniles were obtained in about seven days.

Ten days after transplanting the test plants, a 10 ml. nematode suspension containing ca 400 Juveniles (J2) was added into the root zone of each plant. Treatments were arranged in a completely randomised design with five replications. Roots were harvested at 7, 21, 35 and 49 days after inoculation. The plants were carefully removed from the cones, roots washed free of sand and weighed. The staining procedure described by Byrd *et al.* (1983) was used.

The roots were chopped into 1-2 cm. segments and immersed in a beaker with 50 m.l tap water and 20ml of 5.25% NaOCl added to give a final concentration of 1.5% NaOCl. The root tissues were allowed to remain in this solution for 4 minutes with occasional agitation. The roots were then rinsed in running water for 30-45 seconds and allowed to stand in tap water for 15 minutes to remove traces of NaOCl. The material was then drained and transferred to a beaker containing 30ml of water to which 1 ml of stain (3.5g acid fuchsin, 250 ml acetic acid and 750 ml distilled water) was added and heated to boiling for about 30 seconds. This was allowed to cool to room temperature and excess stain removed by rinsing in running water.

The root material was then placed in 20-30ml glycerin acidified with 3-4 drops of 5N HCl, heated to boiling and allowed to cool to room temperature. The root segments were then pressed between glass slides and the nematodes in the roots counted at 40x magnification. Second-stage juveniles (J2) were extracted from the sand using the sieving and filtration technique (Hooper, 1990) and enumerated. The experiment was repeated once following the procedure described above.

3.4 Effect of growing tomato in rotation with antagonistic plants in combination with sweetcorn on *Meloidogyne spp.* in an infested field

This experiment was conducted to determine the effectiveness of undersowing sweetcorn with *Crotalaria sp, Asparagus sp, S. bicolor, T. patula* and *A. sativum* on root-knot nematode suppression in a nematode infested field. Plots measuring 4m x4m were sown with sweetcorn with antagonistic plants sown in single rows between the

sweetcorn rows. The experimental design was randomized complete block with five replications.

The initial *Meloidogyne* juvenile (J2) population (Pi) in the field was determined by taking samples randomly from each plot. Before planting sweet corn and antagonistic plants, 5 grams of diammonium phosphate (DAP) fertilizer was added into each planting hole. Weeds were controlled regularly and overhead irrigation was done when necessary. After three months, ten sweetcorn plants were randomly selected, uprooted and washed free of soil. Data on dry shoot weights and weight of cobs were recorded. Soil samples were taken from ten different points in each plot for nematode juvenile population assessment. Plots were then tilled and one-month-old tomato cv. Moneymaker seedlings were transplanted into them.

The experiment was terminated 60 days after transplanting by gently uprooting 10 randomly selected tomato plants from each plot. The roots were uprooted, washed free of adhering soil and indexed for galling, egg masses. The juvenile counts were determined as described in section 3.2 above. Dry shoot weights of the ten plants were also taken. The experiment was repeated once following the same procedure.

3.5 Effect of interplanting tomatoes with antagonistic plants on root-knot nematodes

3.5.1 Greenhouse experiment

A greenhouse experiment was established to determine the effect of rhizosphere interactions between nematode antagonistic plants and a susceptible tomato cultivar on root-knot nematodes. Twelve antagonistic plants, *Tagetes spp.*, Crotalaria, sweetcorn,

rhodes, cotton, sorghum, asparagus, garlic, chrysanthemum, sesame, Tithonia and spring onion were selected based on their ability to suppress nematodes as determined in the screening experiment. Tomato monocrop and nematicide (carbofuran) treatments were included as negative and positive controls, respectively.

Pots measuring 21 cm in diameter were filled with 5 kg of heat sterilized loam and sand, mixed in the ratio of 2:1. Fertilizer (DAP) was added at a rate of 5 g per pot before planting. A one-month-old tomato seedling was transplanted into each pot and seeds of antagonistic plants sown into the same pot after ten days. The treatments were arranged in a completely randomised design with ten replications. Nematode inoculum comprising of 6000 eggs and juveniles were added around the root zone of the plants in each pot. The experiment was terminated 60 days after inoculation.

Plants were gently uprooted and washed free of adhering soil. Galling and egg mass indices, juvenile counts and dry shoot weights were assessed as in section 3.2 above. The experiment was repeated once.

3.5.2 Field Experiment

A field infested with root-knot nematodes was selected and the initial nematode population determined. Nematode antagonistic plants, *T. patula*, sorghum, crotalaria, spring onion and asparagus that were rated as suppressive to nematodes in the screening experiment were selected. A sole tomato crop was included as a control.

Plots measuring 3 by 4 m were sown with tomato cv. Moneymaker. Two weeks after planting tomato, four seeds of each antagonistic plant were sown around the tomato seedling. Weeding to remove other plant competitors was done regularly and overhead irrigation done when necessary.

The experiment was terminated three months after transplanting tomatoes and the tomato plants carefully uprooted and washed free of soil. Egg masses were stained using phloxine B as described by Holbrook *et al.* (1983). Galling, egg mass indices, juvenile counts and dry shoot weights were assessed as in section 3.2. The experiment was repeated once.

3.6 Data analysis

All data collected were analysed using GENSTAT version. 5 Release 3.2 and means of significantly different treatments separated using the least significant difference test (LSD) at P=0.05.

CHAPTER FOUR

4.0 RESULTS

4.1 Screening of potential antagonistic plants to *Meloidogyne* species

4.1.1 Greenhouse experiment

There were significant ($p \leq 0.05$) differences in galling, egg masses and juvenile counts among the plants tested (Table 2). Galling and egg mass indices ranged from 6.6 - 9 in tomato, rapeseed, lablab, coriander, spring onion, cabbage cv. Gloria, sunflower, statice and bambara nuts. These plants were rated as susceptible. Ornithogolum, tuberose, onnis, leekswiss, chrysanthemum, garlic, velvetbean, chinese cabbage, asparagus, broccoli, lettuce, sesame and red onion were rated as moderately resistant with galling and egg mass indices ranging from 3 – 6. *Tagetes patula*, desmodium, rhodes grass, alstroemeria, cotton, crotalaria, napier, sorghum, peanut, sweet corn, capsicum and tithonia were resistant with galling and egg mass indices ranging from 1-3.

No egg masses were observed on roots of desmodium, rhodes grass and alstroemeria. Few egg masses (<10) were observed on sweetcorn, cotton, capsicum and nappier grass roots. Tomato cv. Moneymaker had the highest number of egg masses but not significantly (P=0.05) different from cabbage cv. Gloria, rape seed, sunflower, lablab, bambara nuts and corriander (Table 2). The highest number of *Meloidogyne* juveniles was recovered from soils grown with tomato and the lowest counts from soils grown with peanut (Table 2).

Table 2. Galling indices, egg mass indices and numbers of *Meloidogyne* juveniles (J_2) on different plants species grown in soil infested with root-knot (*Meloidogyne* spp.) nematodes

Plant (treatment)	Galling indices	Egg mass indices	Juvenile counts/200 cm soil	Reaction
Tagetes *(Tagetes patula)* (control)	1.0	1.0	229	Resistant
Tagetes (*Tagetes minuta*)	1.0	1.0	235	"
Desmodium *(Desmodium uncinatum)*	1.0	1.0	299	"
Rhodes (*Chloris gayana*)	1.0	1.0	299	"
Alstroemeria *(Alstroemeria sp.)*	1.0	1.0	182	"
Cotton (*Gossypium hirsutum*)	1.4	1.4	255	"
Crotalaria *(Crotalaria juncea)*	1.5	3.4	239	"
Napier grass *(Pennisetum purpureum)*	1.6	1.6	621	"
Sorghum *(Sorghum bicolor)*	1.8	4.4	314	"
Peanut *(Arachis hypogaea)*	1.8	1.6	100	"
Sweetcorn *(Zea mays)*	1.9	2.0	219	"
Capsicum *(Capsicum annuum)*	2.2	2.1	260	"
Tithonia *(Tithonia diversifolia)*	2.9	3.0	405	"
Garlic *(Allium sativum)*	3.1	3.4	373	
Velvet bean (*Mucuna pruriens*)	3.8	3.6	370	Moderate resistant "
Lettuce *(Lactuca sativa)*	3.9	3.9	248	"
Leekswiss *(Allium ampeloprasum)*	4.1	4.4	703	"
Sesame (*Sesamum indicum*)	4.4	4.3	966	"
Red onion *(Allium cepa)*	4.5	4.3	346	"
Onnis *(Onnis sp.)*	4.6	5.0	520	"
Chinese cabbage *(Brassica oleracea* var. *Chinensis)*	4.6	4.5	847	"
Asparagus *(Asparagus officinalis)*	4.9	5.8	756	"
Broccoli *(Brassica oleracea* var. *botrytis)*	5.1	5.1	195	"
Ornithogolum (*Ornithogolum arabicum*)	5.6	5.9	652	"
Tuberose *(Tuberose sp.)*	5.9	5.6	238	"
Chrysanthemum *(Chrysanthemum indicum)*	6.1	6.0	246	"
Mustard (*Brassica oleracea* var. *alba*)	6.6	6.6	944	
Statice *(Statice sp.)*	7.2	7.0	330	Susceptible
Spring onion *(Allium cepa)*	7.2	7.3	342	"
Rapeseed (*Brassica napus*)	8.0	7.5	438	"
Cabbage Gloria *(Brassica oleracea* var. *gloria)*	8.4	8.3	380	"
Sunflower *(Helianthus annuus*)	8.4	8.4	547	"
Lablab (*Lablab purpureus*)	8.5	8.4	738	"
Coriander *(Coriandum sativum)*	9.0	9.0	370	"
Bambara nuts *(Vigna subterranea)*	9.0	9.0	381	"
Tomato (*Lycopersicon esculentum*)- Control	9.0	9.0	1457	"
LSD (P = 0.05)	0.6	1.3	103.6	
CV%	26	28	23.9	

4.1.2 Field microplot screening of antagonistic plants

Results of the microplot experiment were similar to those observed in the greenhouse. There were significant ($P \leq 0.05$) differences in galling and egg mass indices among the plants tested (Table 3). Galling ranged from 1.2 to 6.8 with rhodes grass having the lowest (1.2) and tomato the highest (6.8).

The test plants had galling indices that ranged between 1.2 and 2.4. They were rated resistant compared to tomato (control) that was susceptible with galling indices of 6.8. The egg masses followed a trend similar to that observed on galling. The egg mass indices ranged from 1.4 to 2.9 among the test plants while the tomato had the highest of 7.3.

There were significant ($P \leq 0.05$) differences in juvenile (J2) populations between treatments and the control (Table 3). *Meloidogyne* juvenile counts were exceptionally high (1630) in plots where tomato was grown and lowest (373) in plots grown with Tagetes (Table 3).

Table 3: Galling indices, egg mass indices and number of *Meloidogyne* juveniles (J2) on several antagonistic plants in nematode infested microplots

Plant (treatment)	Galling indices	Egg mass indices	J2 counts/200cm^3
Rhodes (*Chloris gayana*)	1.2	1.4	831
Cotton (*Gossypium hirsutum*)	1.3	1.2	671
Tagetes (*Tagets patula*)	1.3	1.4	373
Alstroemeria (*Alstroemeria sp.*)	1.5	1.7	502
Desmodium (*Desmodium uncinatum*)	1.6	1.8	399
Sweetcorn *(Zea mays)*	1.8	2.0	954
Sorghum *(Sorghum bicolor)*	2.4	2.9	829
Tomato *(Lycopersicon esculentum)*	6.8	7.3	1630
LSD (P=0.05)	0.60	0.73	746
CV%	47.2	52.2	48
SE	1.1	1.3	426

4.2 Penetration and development of *Meloidogyne* species in roots of antagonistic plants

Meloidogyne juvenile numbers in roots differed ($P \leq 0.05$) significantly among the plants tested (Table 4). Nematode juvenile numbers were significantly ($P \leq 0.05$) higher in tomato roots than in the other plants. Few (≤ 10) nematodes were detected in crotalaria, desmodium, cotton and peanut roots seven days after inoculation. No nematodes were detected in *Tagetes patula* roots 7 days after inoculation.

Third and fourth-stage juveniles (swollen juvenile stages) were first detected in tomato roots, 21 days after inoculation. After the same period, no swollen juvenile stages were recorded in Crotalaria, peanut and *Tagetes*. No mature females were detected in crotalaria, tagetes and desmodium roots. The highest number of swollen juveniles 78 and eggs 22 were detected in tomato roots 35 days after inoculation. Few mature females (≤ 5/root system) were detected in cotton and peanut roots.

Egg production per female were more in tomato roots than in the rest of the plants (Table 4). No nematode eggs were detected on *Tagetes* roots. Juvenile numbers in the potting medium (sand) were lower in cones sown with tomato at 7 days after infestation, compared to the other plants. The population declined further at 21 days after infestation and then started increasing. A continuous decline in juvenile numbers in sand was observed in cones under Tagetes, Desmodium, Crotalaria and cotton.

Table 4: **Root penetration and early development of *Meloidogyne* juveniles in roots of resistant plants at 7, 21, 35 and 49 days after inoculation (DAI)***

Plant	DAI	Vermiform Juveniles in roots	Swollen juveniles in roots	Mature females in roots	Eggs in roots	J2 counts in sand
Tomato	7	53	0	0	0	115
	21	117	12	0	0	100
	35	18	78	31	22	285
	49	4	9	19	16	390
Crotalaria	7	1	0	0	0	340
	21	4	0	0	0	280
	35	3	1	0	0	165
	49	7	0	0	7	234
Tagetes	7	0	0	0	0	320
	21	1	0	0	0	358
	35	2	1	0	0	160
	49	0	0	0	0	90
Rhodes	7	7	0	0	0	160
	21	9	1	0	1	145
	35	2	1	0	2	325
	49	13	5	7	12	265
Cotton	7	10	0	0	0	350
	21	2	1	0	0	290
	35	2	0	0	1	120
	49	6	3	1	1	165
Desmodium	7	5	0	0	0	385
	21	0	1	0	0	230
	35	2	2	0	0	105
	49	1	1	0	3	75
Peanut	7	4	0	0	0	380
	21	3	0	0	0	100
	35	0	0	0	0	105
	49	3	1	2	3	155
LSD (P=0.05)		2.3	1.9	1.4	1.2	6
CV%		59	75	66	50	27
SE		1.6	1.3	0.9	0.8	3.9

*DA1-Days after inoculation

The original data was transformed = $\sqrt{X+1}$ and analysed

4.3 Effect of growing tomato in rotation with sweetcorn undersown with antagonistic plants on root-knot nematodes

4.3.1 Plant damage

The effect of growing tomato in rotation with sweetcorn undersown with antagonistic plants on root-knot nematode differed significantly ($P \leq 0.05$) among the treatments (Table 5). Galling was lowest on tomato plants grown in rotation with sweetcorn undersown with *Tagetes patula* and highest under tomato monoculture. Galling indices ranged from 1.9 to 3.0 on tomatoes grown in rotation with sweetcorn alone or in combination with nematode antagonistic plants. The egg masses on tomato grown in rotation with different rotational treatments followed a trend similar to the one observed on galling. Tomato grown in rotation with sweetcorn undersown with *Tagetes patula* had the lowest egg mass indices of 2.9. This score was significantly ($P \leq 0.05$) different from that on tomato grown under monoculture.

There were significant ($P \leq 0.05$) differences in juvenile (J2) populations among the treatments (Table 5). The lowest juvenile population was recovered from plots planted with sweetcorn undersown with *Tagetes patula* while the highest was recovered from plots under tomato monoculture. Shoot weights of tomato were significantly ($P \leq 0.05$) different among the treatments. The lowest shoot weight (10.5) was recorded under tomato monoculture and the highest (21.60) on tomato grown in rotation with *Tagetes patula* indicating that it promoted tomato growth.

Table 5. ***Galling indices, egg mass indices, yield of sweetcorn cobs, dry weight of stalks, number of*** ***Meloidogyne*** **juveniles (J2), and shoot weight of tomato plants grown in rotation with sweetcorn undersown with antagonistic plants.**

Treatment		Galling indices	Eggmass indices	Dry weight of stalk in gms	Dry shoot weight of tomato	Yield of sweetcorn in gms	J2 count /200cm3	J2 count /200 cm^3	Dry shoot weight
Season I	**Season II**								
Sweetcorn + *Tagetes patula*	Tomato	1.9	2.9	22.9	21.60	79	240	240	21.60
Sweetcorn + *Crotalaria juncea*	Tomato	2.4	4.9	33.1	19.50	197	444	444	19.50
Sweetcorn + *sorghum bicolor*	Tomato	2.8	5.5	24.4	15.50	110	437	437	15.50
Sweetcorn + *Asparagus sp.*	Tomato	2.7	5.1	28.6	20.0	122	371	371	20.0
Sweetcorn + *Allium sativum*	Tomato	3.0	5.3	25.3	18.3	122	300	300	18.3
Sweetcorn alone	Tomato	2.7	4.5	28.5	19.0	160	363	363	19.0
Tomato alone	Tomato	7.4	8.2	-	10.5	-	906	906	10.5
SE		0.97	1.27	7.8	5.97	55.5	134	134	5.97
CV%		29.7	24.4	28.6	34.0	42.2	30	30	34.0
LSD (P=0.05)		0.43	0.56	11.7	2.63	83.7	175	175	2.63

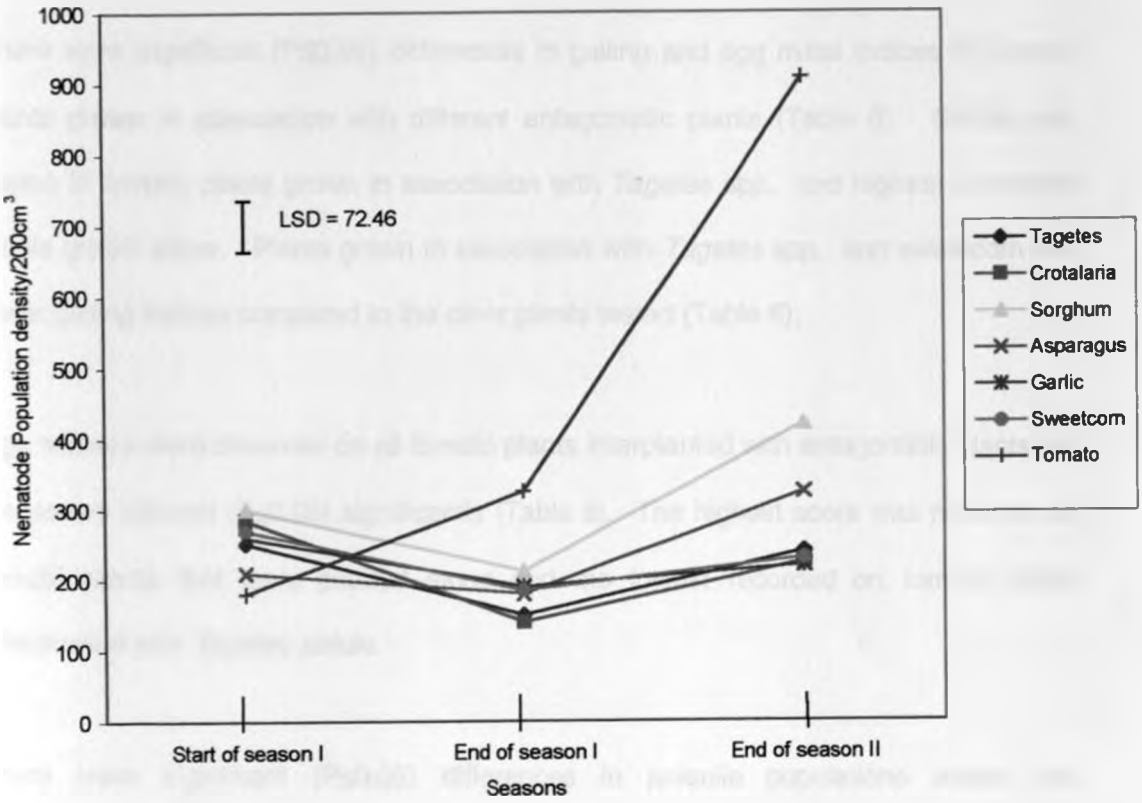
There were significant (P≤0.05) differences in the yield of sweetcorn among different rotational treatments (Table 5). The lowest sweetcorn yield was recorded in plots where sweetcorn was underswon with *Tagetes patula* while the highest was observed in plots undersown with *Crotalaria juncea*. The dry weight of sweetcorn stalks followed a similar trend (Table 5).

4.3.2 Nematode population changes

Generally nematode populations in plots planted with sweetcorn alone or sweetcorn undersown with *Tagetes* sp., Crotalaria, sorghum, asparagus or garlic were grown continued to decrease after season 1 compared to tomato monoculture (Fig. 1). However, at the harvest of the tomato crop, nematode population increases were observed in all the plots.

The highest increase was obtained from plots where tomato was rotated with sweetcorn and undersown with *Crotalaria juncea* while the lowest was in those plots of tomato rotated with sweetcorn undersown with *Tagetes patula* (fig.1). There was a continuous nematode population increase in tomato monoculture while the highest reduction in nematode population was noted in rotations using sweetcorn undersown with *Tagetes patula*.

Fig 1. Nematode population changes in plots where sweetcorn, was undersown with different antagonistic plants and rotated with Tomato



4.4 Effect of interplanting tomatoes with antagonistic plants on root-knot nematodes

4.4.1 Greenhouse experiment

There were significant ($P \leq 0.05$) differences in galling and egg mass indices on tomato plants grown in association with different antagonistic plants (Table 6). Galling was lowest in tomato plants grown in association with *Tagetes* spp. and highest on tomato plants grown alone. Plants grown in association with *Tagetes* spp. and sweetcorn had lower galling indices compared to the other plants tested (Table 6).

Egg masses were observed on all tomato plants interplanted with antagonistic plants but the scores differed ($P \leq 0.05$) significantly (Table 6). The highest score was recorded on tomato plants that were planted alone and the lowest recorded on tomato plants interplanted with *Tagetes patula.*

There were significant ($P \leq 0.05$) differences in juvenile populations among the treatments. The lowest juvenile count was recovered from pots that had tomato interplanted with *Tagetes patula* and the highest in pots that had tomato grown alone (Table 6). Shoot weight was significantly ($P \leq 0.05$) higher in tomato plants interplanted with asparagus, garlic, chrysanthemum, sesame, cotton and spring onion than the tomato alone (Table 6). The highest shoot weight was recorded on tomato plants interplanted with sesame (Table 6). Shoot weight was lower in tomato interplanted with *T. patula* compared to all other treatments.

Table 6. **Galling indices, egg mass indices and number of *Meloidogyne* juveniles on tomato interplanted with antagonistic plants in soil infested with root-knot nematodes in a greenhouse**

Treatment combinations	Galling Indices	Egg mass Indices	J2 counts /200cm^3	Dry shoot weight in grammes
Tagetes patula/ Tomato	1.4	2.6	64.0	0.32
Sweetcorn / Tomato	1.4	4.8	117.0	0.83
Rhodes / Tomato	1.5	4.9	83.0	1.02
Cotton / Tomato	2.1	6.8	404.0	16.7
Carbofuran / Tomato	2.2	5.5	134.0	20.5
Sorghum / Tomato	2.2	5.6	141.0	7.5
Crotalaria / Tomato	2.2	7.8	100.0	18.4
Asparagus / Tomato	2.6	6.9	149.0	24.5
Garlic / Tomato	3.3	7.6	157.0	20.7
Chrysanthemum / Tomato	3.6	6.9	193.0	22.8
Sesame / Tomato	3.6	8.3	138.0	38.0
Tithonia / Tomato	4.1	6.5	191.0	19.4
Spring onion / Tomato	5.8	7.6	279.0	20.5
Tomato alone	8.5	9.0	871.0	8.5
L.S.D. (P=0.05)	0.6	8.6	193.0	2.4

4.4.2 Field Experiment

There were significant ($P \leq 0.05$) differences in galling and egg masses on tomato interplanted with different antagonistic plants (Table 7). Galling and egg mass indices were lower in tomato grown in association with nematode-suppressive plants than in tomato grown alone. Generally, galling and egg mass indices were highest in the plots where tomato was grown alone and lowest where tomato was interplanted with *T. patula*. Galling and egg mass indices were significantly lower in tomato interplanted with *T. patula* than with the other antagonistic plants tested. Juvenile population differed significantly ($P \leq 0.05$) among the treatments (Table 7). The lowest juvenile population was recovered from soils where tomato was interplanted with *T. patula* and highest in soils where tomato was grown alone.

The changes in nematode numbers in plots sown with different plant combinations varied greatly (Table 7). A decline in nematode numbers was observed in plots where tomato was interplanted with *T. patula* and *Sorghum bicolor*. Tomato interplanted with *Crotalaria juncea, Asparagus officinalis* and *Allium cepa* resulted in nematode increase. The highest increase of 338% in nematode numbers was observed in plots where tomato was grown alone while a slight increase of 12% was recorded where tomato was interplanted with *Crotalaria juncea*. Dry shoot weight was significantly ($P \leq 0.05$) higher in tomato plants interplanted with *crotalaria, asparagus* spp. and spring onion than the control (Table 7). The highest shoot weight was recorded on tomato plants interplanted with spring onion and lowest in tomato interplanted with *T. patula* compared to all the other treatments (Table 7).

Table 7. **Galling and egg mass indices, numbers of *Meloidogyne* juveniles and dry shoot weights of tomato plants interplanted with antagonistic plants in a nematode infested field.**

Interplant combinations	Galling indices	Egg mass indices	Shoot weights in (g)	Initial (J2) counts	Final (J2) counts	% Change in (J2) population
Tagetes patula/ Tomato	1.6	2.7	9.7	376	165	- 56%
Sorghum bicolor/Tomato	2.5	5.1	30.7	258	213	- 17%
Crotalaria juncea / Tomato	3.0	5.2	65.4	284	317	+ 12%
Asparagus sp. /Tomato	3.0	4.6	77.3	171	308	+ 80%
Spring onion/Tomato	2.7	5.0	101.6	206	293	+42%
Tomato alone (control)	8.1	8.1	38.0	190	833	+338%
LSD (P=0.05)	0.8	0.6	21.1		135.9	
Cv%	14.6	7.7	23.8		25.4	
SE	0.51	0.39	13.97		90.0	

CHAPTER FIVE

5.0 DISCUSSION

5.1 Reaction of potential rotation and cover crops to *Meloidogyne* species

This study showed that marigold *(Tagetes patula* and *T. minuta),* sun hemp *(Crotalaria juncea),* cotton (*Gossypium hirsutum*), desmodium (*Desmodium unicinartium*), rhodes grass (*Chloris gayana*), sorghum (Sorghum *bicolor*), sweetcorn (*Zea mays*), alstroemeria (*Alstoemeria* sp.), capsicum (*Allium annuum*) and peanuts (*Arachis hypogaea*) were suppressive to root-knot nematodes under the greenhouse and field conditions. These findings are in agreement with those of previous studies (Sukul, 1992; Oduor-Owino and Waudo, 1994;Rich and Rahi, 1995; Asmus and Ferraz, 1998; Mc Sorley, 1999; Ploeg and Maris, 1999). Strong suppression of root-knot nematodes by *Tagetes spp.* is widely reported (Mc Sorley and Fredrick, 1999; Kagundu, 2001). Swamy *et al. (*1995) observed that nursery beds previously planted with marigold gave maximum reduction in root-knot nematode population in soil and increased germination of seeds and production of more healthy tomato seedlings.

According to Ali *et al.* (1995) *Tagetes* species were more effective in reducing damage by *Meloidogyne* species on tomato roots than carbofuran. The possible explanations for the effectiveness of marigold in the management of root-knot nematode could be secretion of toxin α terthienyl and derivatives of bithienyle that kill the nematodes (Uhlenbroek and Bijloo, 1957).

Desmodium was found to be resistant to root-knot nematodes. This finding concurs with that of Good *et al.* (1965) who reported that *Desmodium spp.* were antagonistic to *Meloidogyne spp.* and *Xiphinema americanum* under greenhouse and field conditions. This is a high quality forage crop and thus more acceptable as a rotation crop than *Crotalaria* spp. or *Tagetes minuta* which are generally regarded as weeds.

There was minimal reproduction of root-knot nematodes on sorghum indicating that it is suppressive to the nematodes. This study confirms earlier findings by other authors (Clayton and Renvoize, 1982; Kinloch and Rich, 2001). Yamada *et al.* (2002) found some sorghum varieties to be very effective in the control of *M. incognita*. This could be attributed to the glycosides that are found in its vacuole that become exposed when injured by nematodes leading to the secretion of hydrogen cyanide (Meyer and Fry, 1978) that is toxic to nematodes. This cereal can be incorporated in different farming systems to control these nematodes especially in areas with low rainfall, as it is well adapted to a wide range of environmental conditions.

The present study also demonstrated that cotton suppressed root-knot nematode reproduction. *Meloidogyne* species are serious pests of cotton but existence of varieties that are highly resistant to the nematodes is well established (Ogallo *et al.*, 1999). Veech and McClure (1977) reported that roots of nematode resistant cotton cultivars contained a high post-infectional concentration of terpernoid aldehydes that are toxic and become exposed when they are injured which could have been the reason why root knot nematode reproduction was suppressed.

Antagonistic plants may also reduce nematode populations by acting as trap crops (Bridge, 1996). Nematodes invade roots of such plants but their development and reproduction is inhibited. For instance, Dasaeger and Rao (1999) reported that juveniles of *Meloidogyne* species entered roots of resistant plants like crotalaria but failed to multiply. In addition, roots of some plants may not be a food source for certain nematodes, thereby reducing their numbers by starvation. Some of these plants are good crops that can be incorporated in rotation of different cropping systems since some of them are nitrogen fixers; good quality forage and some have ornamental value.

Information on the reaction of nappier grass, alstroemeria and sweetcorn to root knot nematodes was not readily available. Napier grass is an important forage crop that is very common in majority of the farms in high potential areas and can easily be included in cropping systems to control root knot nematodes. One of the constraints that floriculture farmers face is root knot nematode infestation. Flower growers should be encouraged to incorporate into their cropping systems alstroemeria flowers that were found in this study to be suppressive to these nematodes.

There was moderate nematode damage on roots of garlic, velvetbean, lettuce, leekswiss, sesame, red onion, onnis, chinese cabbage, asparagus, broccoli, ornithogolum, tuberose and chrysanthemum. Information on the host suitability of most of these crops to root knot nematodes is missing or not readily available. However, some of these findings disagree with those of other authors (Ibrahim *et al.*, 1998; Walker, 1998; Hasabo and Ameen, 1995) who found sesame, asparagus and velvet bean to suppress root knot nematodes. This shows that resistant varieties exist among

these cultivars. The moderate galling exhibited on these plants indicate that these crops support root-knot nematode reproduction to a certain extent and should be introduced with a lot of caution especially if they are to be planted in the same field with the susceptible plants.

Damage by root-knot nematodes on mustard, statice, spring onion, rapeseed, cabbage cv. Gloria, sunflower, dolichos, corriander and bambara nuts was not significantly different from tomato. This indicates that the crops support nematode population build up in the soil and should be avoided as much as possible in the multiple cropping systems. Some of these results are a confirmation of earlier findings by Mojtahedi *et al.* (1991) who reported that some rapeseed varieties supported *Meloidogyne* species. However the results also contradict a report by Krall *et al.* (2000), which shows that some mustard varieties can be used in the control root knot nematodes.

5.2 Penetration and development of *Meloidogyne* species in roots of antagonistic plants

This study revealed that penetration of second-stage *Meloidogyne* juveniles into roots of some plants was inhibited. Araya and Caswell-Chen (1994) noted that two *Crotalaria* genotypes were highly resistant to *M. javanica* as fewer juveniles (J_2) penetrated into the roots compared to a susceptible host, *Lycopersicon esculentum.* Mc Sorley (1999) also observed that the number of juveniles that hatched from eggs per root system were low in both *Crotalaria spp.and Tagetes spp.*

Similar findings have been reported on a wide range of plants, indicating that lack of root penetration by nematodes may be a resistance mechanism (Rodriguez-Kabana, 1992; Sharma and Trivendi, 1992; Araya and Caswell-chen, 1994). According to Barker and Trivend (1999), *Crotalaria spectabilis* acts as a trap crop for *Meloidogyne spp.* through prevention of juveniles from maturing and reproducing once they enter the roots.

Out of the few juveniles that entered into roots of tagetes and desmodium roots, none of them developed into mature females. Slow or complete lack of root knot nematode development and reproduction in antagonistic plants such as *Tagetes spp.* and crotalaria has been reported (Lawrence and Clarke, 1986; Reynolds *et al.*, 2000). According to Rangaswang *et al.* (1993*), M. incognita* larvae failed to develop on tagetes even after 45 days due to hypersensitive necrotic reaction (Heijbroek, 1996).

The actual mechanisms that led to reduced penetration of nematodes into roots of the plants tested was not determined but It is possible that the roots of resistant plants secreted toxins that inhibited nematode penetration or repelled those that invaded (Araya and Caswell-Chen, 1994; Diogo *et al.*, 2000). In addition, roots of some plants may simply not be a good source of food for certain nematodes thereby reducing their numbers by starvation (Windham and Williams, 1994).

It is known that a greater proportion of *Meloidogyne* juveniles develop into males when conditions are unfavourable for the nematodes (Tanda *et al.*, 1988). Although this phenomenon was not tested in this study, it is possible that the juveniles that penetrated into *Tagetes* and *Desmodium* roots developed into males that are not parasitic on plants

and thus moved out. The low number of juveniles that penetrated into roots of *Crotalaria, Desmodium*, cotton, peanut and *Tagetes* may account for the reduction in nematode population in fields where the plants are grown.

This study also established that the nematode's life cycle from juvenile to egg was completed within 22-35 days and 36-49 days in tomato and antagonistic plants, respectively. This indicates a delay in one or more processes between inoculation, through juvenile development to egg laying by mature females. Root-knot nematodes are known to complete their life cycles, from egg to egg, in 21 days when conditions are favourable (Agrios, 1997) and this demonstrated that some mechanisms operating in antagonistic plants delayed the completion of the life cycle of the nematodes.

5.3 Effect of growing tomato in rotation with sweetcorn in combination with nematode antagonistic plants

Undersowing sweetcorn with nematode antagonistic plants suppressed galling by root-knot nematodes resulting in vigorous growth of a subsequent tomato crop. This conforms to other related studies (Reddy *et al.*, 1986; Dhanger *et al.*, 1995; Lung *et al.*, 1997; Shellami and Cheija, 1997; Khan and Sharma, 1999; Korthals *et al.*, 2000).

Increase in nematode population density was slowest in tomato plots previously under sweetcorn and *Tagetes patula*. These findings are consistent with earlier reports by Siddiqui and Alam (1988), Mc Sorley and Frederick (1999) and Ball *et al.* (2001). In a similar rotation experiment involving *Tagetes spp.* and tobacco, Reynolds *et al.* (2000) observed reduction in nematode population. Ploeg (2000) also observed reduction in

galling and final nematode population with marigold (Tagetes) and an increase when tomato followed tomato. This could be attributed to continued nematode suppression after removal of marigold. *Tagetes* species have attracted a lot of attention as fallow crops that can be incorporated into crop rotation systems.

Tomato plants grown in rotation with sweetcorn undersown with *Tagetes patula* had higher shoot weight than tomato grown after tomato. Similar findings have been reported by Mateeva (1995), Schepman and Jansen (1994) and Swamy *et al.* (1995). According to Yamada (2001), practical rotation methods using antagonistic plants like Marigold have been developed to control nematode injury. This means that the toxic effects of Marigold reported by McKenry (1991) were observed in the current study.

Undersowing sweetcorn with *Crotalaria juncea* resulted in reduced nematode populations and minimal damage on the succeeding tomato crop. These results confirm earlier reports by Amus and Ferraz (1998) and Rich and Rahi (1995). According to Madulu *et al. (*1994), *Crotalaria sp.* was found to increase the yields of tomato and to suppress galling by *M. javanica* just as much as *Tagetes erecta.*

Wang-Koonhui *et al.*, (2000) reported that *R. reniformis* densities were reduced when *Crotalaria spp.*, yellow mustard and marigold were grown as intercycles for three months. Due to its resistance to a broad range of root knot nematodes, *Crotalaria spp.* is a suitable green manure and vegetable crop that should be used in fields that are heavily infested with mixed populations of root-knot nematode species.

There was an increase in yield of sweet corn when it was underswown with crotalaria showing that nitrogen fixation was taking place that led to improved growth of the crop. Crotalaria is therefore a good rotation crop as it both reduces root knot nematode population density and improves the yield of the companion or succeeding crop.

Sorghum has shown high potential as a rotation crop for root-knot nematode management (Mc Sorley *et al.*, 1987; Mc Sorley and Gallaher, 1991; Kinloch and Rich, 2001). This study revealed the similar findings. Yamada *et al.*, (2001) reported that some sorghum varieties were suppressive against *M. incognita* and *M. arenaria*. According to Siddiqui and Alam (2001), cropping sequences, in a 2-year crop rotation programme that contained sorghum were found to be beneficial in reducing the root-knot nematode larvae and reniform nematode populations.

Most probably it is because grain sorghum contains dhurrin, a precursor for hydrogen cyanide, which if released during decomposition of the crop can be harmful to nematodes in the soil (Johnson *et al.*, 1996). This is a suitable rotational crop especially in the dry land areas that can be incorporated into the cropping systems in order to boost the food security in those areas. Farmers should be encouraged to grow sweet corn as it can be utilized to control nematodes in fields that are heavily infested and its yields are reportedly not affected by *Meloidogyne spp.* (Vawdrey and Stirling, 1996).

Although nematode numbers may be suppressed by crop rotation, they build up quickly when a susceptible crop is grown (Mc Sorley, 2001). This was evident in this study where nematode population densities picked up in all the plots after the removal of

antagonistic plants. The root-knot nematode density progressively increased in the plots with continuous cropping of tomato. This means that crops that are susceptible to root-knot nematodes should be grown selectively and where possible alternated with resistant varieties.

5.4 Effect of interplanting tomatoes with antagonistic plants on root-knot nematodes

Damage by nematodes was suppressed in tomato plants interplanted wit Tagetes, sweet corn, rhodes grass, cotton, sorghum, crotalaria and garlic. The strong suppression of *M. incognita* and *M. javanica* by *Tagetes patula* has been observed elsewhere as reported by Mc Sorley and Frederick (1999), Reynolds *et al. (*2000) and Mateeva *et al.* (2000). *Tagetes* species can easily be used as interplant in nematode infested fields as, its root exudates are toxic to the nematodes (Mojumder *et al.*, 2000; Naidu *et al.*, 2000; Mohamed *et al.*, 2000).

Introduction of *Tagetes patula* suppressed *Meloidogyne* species on tomato but reduced tomato growth significantly. This means that it might not be a viable choice as a companion to a crop that loses on the competition. The same case applies to sweet corn, rhodes grass and sorghum.

There was minimal damage on tomato interplanted with Crotalaria. These findings were consistent with earlier reports by several authors (Richi and Rahi, 1995; Wang *et al.*, 2000; Robinson and Cook, 2001; Dasaeger and Rao, 2001; Kagundu, 2001). Species of Crotalaria have been found to produce nematoxins that prevent the nematodes from feeding on the roots resulting in nematode death. Crotalaria that is used as a green

manure crop and is antagonistic to nematodes (Ohara *et al.*, 2000) could be recommended for use in the interplant cropping systems (Bringel and Silva, 2000; Wang *et al*, 2000).

Tomato plants interplanted with sorghum recorded lower galling. This finding is consistent with reports by other authors (Kinloch and Rich, 2001; Yamada *et al.*, 2002) who found some sorghum species to be effective against *Meloidogyne* species. However, an interplant with sorghum suppressed tomato growth. This could have been due to the dense sorghum root system that interfered with tomato growth. This implies that even if sorghum reduces nematode population in the soil it might be uneconomical to use it in association with tomato.

An intermediate level of galling was observed on tomato interplanted with spring onion. This finding is inconsistent with that of Mateeva *et al* (2000) who reported that *Allium sativum* and *Allium cepa* were effective against root-knot nematodes on tomato. Damage was also noted on the spring onion indicating that nematodes reproduced on its roots and this shows that it is not a suitable as an interplant crop in nematode control. This study established that spring onion varieties were susceptible to root-knot nematodes and therefore farmers should be discouraged in planting this crop together with tomato as is a common practice.

There was an increase in nematode population recovered from soils planted with spring onion interplanted with tomato. This could be because the nematodes found both hosts

suitable and so reproduced and multiplied. This makes spring onion an unsuitable candidate crop for interplanting with tomatoes as it leads to nematode population build up that can be disastrous to the companion crops. However, this crop can be utilized as a trap crop if it is removed before nematodes mature and cause damage to the companion crop. Chrysanthemum and sesame resulted in considerable damage when interplanted with tomatoes because they supported root-knot nematode reproduction that led to an increase of root knot infestation on tomato. They are not suitable to farmers as interplants with other crops.

Use of antagonistic plants in nematode management is limited because most of recommended plants have no or low market value compared to the preferred crops (Bridge, 1996). With the exception of *Crotalaria* and *Tagetes,* some direct returns can be obtained by growing the plants evaluated in this study. Apart from the direct returns, other characteristics such as improvement of soil fertility, potential for soil erosion control, allelopathic effects on subsequent crops and susceptibility to other plant pathogens should be considered when selecting nematode-antagonistic plants for incorporation into various cropping systems.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 CONCLUSION

The crops that were found to be suppressive to root-knot nematodes included forage crops (desmodium, rhodes grass and napier grass), a fibre crop (cotton), ornamental plants *(alstromeria spp.* and *Tagetes patula)*, an oil seed crop (peanut), a cereal (sorghum), green manure plants (crotalaria and tithonia) and vegetables (sweetcorn and capscum). Therefore, there is a diverse range of economically important plants from which suitable candidates can be selected for use under different farming/cropping systems.

Tomato, rape, lablab, corriander, spring onion, cabbage cv gloria, sunflower, statice and bambara nuts were found to be susceptible to root-knot nematodes and thus pose serious problems in cropping systems where *M. incoginta* or *M. javanica* are predominant and should be introduced with a lot of caution.

Ornithogolum, tuberose, leekswise, mustard, chrysanthemum, garlic, chinese cabbage asparagus, broccoli, lettuce, sesame and red onion were intermediate hosts that supported limited nematode multiplication. Therefore, care should be taken when incorporating them into cropping systems. Very few *Meloidogyne* juveniles penetrated and reproduced in *Tagetes patula*, *Crotalaria sp.*, *Desmodium sp.*, cotton and peanut.

Growing of tomatoes in rotation with *Tagetes patula* and *Crotalaria juncea* did not favour nematode multiplication and resulted in reduced nematode population compared to tomato monoculture. These plants can be used in the rotational and interplanting cropping systems for root-knot management. However, this study revealed that the yield of sweetcorn when undersown with *T. patula* was very low compared to the other treatments.

6.2. RECOMMENDATIONS

Observations made from this study were based on greenhouse and microplot experiments. On-farm studies are required to verify these findings and establish the acceptability of selected crops as rotational or interplants for root-knot nematode management.

Studies should be undertaken to explore the mechanisms of resistance involved in these plants such as, physical barriers like xylem bundle sheaths, production of toxic substances and post- infectional substances that these plants produce when attacked by nematodes.

More screening work should be done particularly on vegetables to establish their host suitability for root knot nematodes and farmers advised accordingly since the crops are commonly grown in isolated plots in river valley bottoms where build up of plant diseases and pests is eminent.

Use of antagonistic plants in nematode control should be evaluated in comparison to other control strategies like organic amendments to establish their effect on belowground biodiversity.

Investigations should be done to determine the effect of these antagonistic plants to other potentially damaging nematodes that could be present in the same fields.

CHAPTER SEVEN

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CHAPTER 8

APPENDICES

Appendix 1. ANOVA for nematode galling indices for potential rotation and cover crops in the greenhouse

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Treatments	35	1996.3715	57.0391	37.3469	<0.000
Residual	252	384.875	1.5273		
Total	287	1234.7804			

Appendix 2. ANOVA for nematode egg mass indices for potential rotation and cover crops in the greenhouse

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Treatments	35	1961.3715	56.0397	34.5807	<0.000
Residual	252	408.375	1.6205		
Total	287				

Appendix 3. ANOVA for juvenile numbers from soils grown with potential rotation and cover crops in the greenhouse

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Treatments	35	21127839	603653	54.50	<0.001
Residual	252	2713466	11075		
Total	287	24057288			

Appendix 4. ANOVA for nematode egg mass indices for potential rotation and cover crops in nematode infested field

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	2	12.484	1.78	1.08	
Rep. *Units* Stratum					
Treatments	7	700.462	100.06	60.7	<.001
Residual	14	289.796	1.647		
Total	23	1001.529			

Appendix 5. ANOVA for nematode galling indices for potential rotation and cover crops in nematode infested field

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	2	9.91	1.416	1.27	
Rep. *Units* Stratum					
Treatments	7	595.911	85.130	76.63	<0.001
Residual	14	196.630	1.111		
Total	23	802.453			

Appendix 6. ANOVA for juvenile numbers from soils grown with potential rotation and cover crops in nematode infested field

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	2	109060	54530	0.30	
Rep. *Units* Stratum					
Treatments	7	6770239	967177	5.32	0.004
Residual	14	2546589	181899		
Total	23	9425888			

Appendix 7. ANOVA for swollen *Meloidogyne* juvenile that penetrated and developed in roots of resistant plants

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
DAI. Stratum	3	7.141	2.380	1.48	
DAI. *Units* Stratum					
Plant	6	29.479	4.913	3.05	0.031
Residual	18	28.978	1.610		
Total	27	65.598			

Appendix 8. ANOVA for mature *Meloidogyne* juveniles that penetrated and developed in resistant plant roots

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
DAI. Stratum	3	4.6321	1.5440	1.78	
DAI*. Units* Stratum					
Plant	6	13.1295	2.1882	2.52	0.060
Residual	18	15.6277	0.8682		
Total	27	33.3894			

Appendix 9. ANOVA for vermiform *Meloidogyne* juvenile that penetrated and developed in resistant plant roots

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
DAI. Stratum	3	6.308	2.103	0.88	
DAI*. Units* Stratum					
Plant	6	66.344	11.057	4.60	0.005
Residual	18	43.223	2.401		
Total	27	115.875			

Appendix 10. ANOVA for *Meloidogyne* juveniles that were recovered from the sand

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
DAI. Stratum	3	56.41	18.80	1.20	
DAI*. Units* Stratum					
Plant	6	21.02	3.50	0.22	0.964
Residual	18	282.56	15.70		
Total	27	359.99			

Appendix 11. ANOVA for *Meloidogyne* eggs that were found in roots for resistant plants

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
DAI. Stratum	3	9.3270	3.1090	5.08	
DAI*. Units* Stratum					
Plant	6	8.6038	1.4340	2.34	0.076
Residual	18	11.0235	0.6124		
Total	27	28.9542			

Appendix 12. ANOVA for nematode galling indices for tomatoes grown in rotation with sweetcorn undersown with antagonistic plants in the field

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	4	11.0821	1.5832	1.70	
Rep. *Units* Stratum					
Treatments	6	916.8714	136.1452	146.00	<0.001
Residual	24	248.0429	0.9325		
Total	34	1075.9964			

Appendix 13. ANOVA for nematode egg mass indices for tomatoes grown in rotation with sweetcorn undersown with antagonistic plants in the field

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	4	7.186	1.027	0.64	
Rep. *Units* Stratum					
Treatments	6	608.786	101.464	63.12	<0.001
Residual	24	427.614	1.608		
Total	34	1043.586			

Appendix 14. Anova for nematode juvenile numbers from soils where tomatoes were grown in rotation with sweetcorn undersown with antagonistic plants in the field

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	4	145268	36317	2.00	<0.001
Rep. *Units* Stratum					
Treatments	6	1392296	232049	12.78	
Residual	24	435644	18152		
Total	34	1973208			

Appendix 15. ANOVA for dry shoot weights of tomatoes grown in rotation with sweetcorn undersown with antagonistic plants in the field.

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	4	178.24	25.46	0.71	
Rep. *Units* Stratum					
Treatments	6	3215.74	643.15	18.05	<0.001
Residual	24	8089.08	35.63		
Total	34	11483.06			

Appendix 16. ANOVA for yield of sweetcorn undersown with antagonistic plants in the field.

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	3	5889	1963	0.64	
Rep. *Units* Stratum					
Treatments	5	33781	6756	2.19	0.110
Residual	15	46210	3081		
Total	23	85880			

Appendix17. ANOVA for dry weight of sweetcorn stalks undersown with antagonistic plants in the field

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	3	44.70	14.90	0.25	
Rep. *Units* Stratum					
Treatments	5	273.39	54.68	0.91	0.502
Residual	15	904.48	60.30		
Total	23	1222.57			

Appendix 18. ANOVA for nematode galling indices for tomato interplanted with antagonistic plants in the greenhouse

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Treatments	13	320.96	24.69	52.89	0.000***
Residual	98	45.75	0.47		
Total	111	366.71			

Appendix 19: ANOVA for dry shoot weight of tomato interplanted with antagonistic plants in the greenhouse

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Treatments	13	12082.461	929.420	156.81	<.001
Residual	98	539.349	5.927		
Total	111	12691.665			

Appendix 20. ANOVA for nematode egg mass indices for tomato interplanted with antagonistic plants in the greenhouse

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Treatments	13	1280.71	98.52	1.30	0.2258ns
Residual	98	7425.25	75.77		
Total	111	8705.96			

Appendix 21. ANOVA for juvenile numbers from soils where tomato were interplanted with antagonistic plants in the greenhouse

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Treatments	13	4477244.43	344403.42	9.05	0.000***
Error	98	3725932.62	38019.72		
Total	111	8203177.0			

Appendix 22. ANOVA for nematode galling indices for tomato interplanted with antagonistic plants in nematode infested field

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	3	0.8846	0.2949	1.12	
Rep. *Units* Stratum					
Treatments	5	107.9821	21.5964	82.16	<0.001
Residual	15	3.9429	0.2629		
Total	23	112.8096			

Appendix 23. ANOVA for nematode egg mass indices for tomato interplanted with antagonistic plants in nematode infested field

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	3	1.1079	0.3693	2.38	
Rep. *Units* Stratum					
Treatments	5	60.2671	12.0534	77.78	<.001
Residual	15	2.3246	0.1550		
Total	23	63.6996			

Appendix 24. ANOVA for dry shoot weight of tomato interplanted with antagonistic plants in the field

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	3	590.0	196.7	1.01	
Rep. *Units* Stratum					
Treatments	5	22017.0	4403.4	22.55	<0.001
Residual	15	2928.5	195.2		
Total	23	25535.5			

Appendix 25. ANOVA for juvenile numbers from soils tomato plants were interplanted with antagonistic plants in the field

Source of variation	d.f	s.s.	m.s.	v.r.	F.pr.
Rep. Stratum	3	10926	3642	0.45	
Rep. *Units* Stratum					
Treatments	5	1167609	233522	28.72	<.001
Residual	15	121975	8132		
Total	23	1300509			