MIDLANDS STATE UNIVERSITY

evaluating the effects of plant extracts *(lantana camara, tagetes minuta and amaranthus hybridus*) on root knot nematodes *(m.javanica)* in tomato seedlings (lycopersicon esculentum l)

BY

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A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honours Degree in Agronomy

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DECLARATION

I hereby declare that this dissertation has been the result of my own original efforts and investigations, and such work has not been presented elsewhere for any degree. All additional sources of information have been acknowledged by means of references.

DARRYN T CHAKURINGA

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Student No. R11659H Signature Date

CERTIFICATION OF THESIS WORK

I, the undersigned, certify that Darryn T Chakuringa**,** a candidate for the Bachelor of Science Agronomy Honours Degree has presented this dissertation with the title:

EVALUATING THE EFFECTS OF PLANT EXTRACTS *(Lantana Camara, Tagetes minuta and Amaranthus hybridus*) ON ROOT KNOT NEMATODES *(M.javanica)* IN TOMATO SEEDLINGS (Lycopersicon esculentum L)

That the dissertation is acceptable in form and content, that satisfactory knowledge of the field covered by the dissertation was demonstrated by the candidate through oral examination held on **13/05/2016**.

SUPERVISOR: MS J.T MIDZI

SIGNATURE: ……

ABSTRACT

Root Knot Nematodes are one of the major economically important pests of tomato (*Lycopersicon esculentum* L) in many regions of the world including Zimbabwe, with Meloidogyne javanica being considered the most destructive. Recent prohibition of main soil chemical fumigants for the control of Meloidogyne javanica has prompted for the search of other alternatives to manage root knot nematodes. A greenhouse experiment was carried out to evaluate the effects of aqueous plant extracts on (*Lantana camara, Tagetes minuta and Amaranthus hybridus*) with a negative and positive control where negative control had only water and positive control had a chemical nematicide (Nemacur®400 Ec (fenamophos as the active ingredient) on Root Knot Nematode (*Meloidogyne Javanica*) suppression in tomato seedlings. The experiment was arranged in a Completely Randomised Design (CRD) with five treatments and three replications. A *Meloidogyne Javanica* inoculum was obtained from Tobacco Research Board (TRB) and botanical plant leaves were obtained locally and aqueous plant extracts were prepared. Data collected were days to 50% emergence, seedling height, final nematode population density, gall density and index and root: shoot ratio. There was a significant difference ($P<0.001$) between the control, Nemacur and all the botanical treatments though *A.hybridus* showed no significant difference from control on final nematode population density, final RKN population density, number of galls and improved growth parameters of tomato seedlings. The chemical properties and compounds found in the botanicals, for example phenolic and caffeic acids might be responsible for the effects of RKN on tomato seedlings. Results suggest that application of *L.camara* and *T.minuta* leaf extracts are good alternatives to manage RKN population though they have an inhibitory suppressive effects on growth parameters.

DEDICATION

To my family and all those who push me to be a better man in life.

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Exaltation is to the Lord Almighty for his grace saw me through these challenging five years. His mercy, guidance and protection made it possible for me to persevere through these difficult years and for that I am forever grateful.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

List of Appendices

ACRONYMS AND ABBREVIATIONS

ANOVA – Analysis of Variance

RKN-Root Knot Nematode

LSD – Least Significant Difference

CM – Centimeter

TRB- Tobacco Research Board

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND AND JUSTIFICATION

Tomato (*Lycopersicon esculentum Mill*) is among the most important cash crops and vegetables grown by smallholder farmers in Zimbabwe. Production and marketing of the crop provides occupation for many people and also provides income for the smallholder farmers (Mariaton and Kwaramba1999; Zitsanza 2000). The crop is among the most important horticultural crops and is grown on over 4 million hectares of land worldwide (FAO, 2005). Tomato contains important chemical compounds that play important roles in the prevention of cancer, heart disease, cataracts and many other health problems (Beecher, 1998).

The most common varieties grown by smallholder farmers are open pollinated varieties, determinates such as Khaki and indeterminates such as Money maker (Dobson et al., 2001).The production of tomato, however is confronted with a lot of problems which include limited availability of improved planting material, high cost of labor operations such as land preparation, staking, weeding, harvesting and storability and pests and diseases. Root-knot nematodes (*Meloidogyne spp*.), according to De Lannoy (2001), are a major pest of tomato.

Root-knot nematodes are obligate plant parasites and According to Amer-Zareen *et al*. (2003), root knot nematodes have a wide host range and are considered the greatest threat to global agriculture responsible for 12, 3% yield loss of global major crops (Sasser, 1998, in Sasena, Sikora and Srivastava). *Meloidogyne spp*. attack more than 2000 host plants. Global vegetable production is affected by different nematode genus with tomato production being affected by the

genus *Meloidogyne*, the most economically important nematode in tropical and subtropical agriculture which reduces yield by 30 – 50% (RPD, 1993)

Root-knot nematodes cause extensive damage and changes in the root system and have the greatest impact on crop productivity when they attack the roots of seedlings immediately after seed germination. This damage extends from simple mechanical damage to highly evolved nematode-plant interaction caused by chemicals introduced by the nematode (Caveness and Ogunforowa, 1985).The nematode infection acts as energy sink absorbing photosynthates needed by the plant for growth and fruit production, hence crop yields are reduced and harvested produce is of poor quality and reduced storage life.

Use of chemical nematicides ,synthetic nematicides and soil fumigants have been the primary means of controlling plant-parasitic nematodes for the past five decades and has led to phytotoxicity, environmental pollution and nematodes resistance (Adegbite and Adesiyan, 2005). It is however disadvantageous because its toxic to man and animals when used improperly (Luc et al., 1990).However, the enormous economic cost of research and registration of new chemicals ,detrimental environmental effects associated with chemical control and the recent losses of methyl bromide as a multipurpose soil fumigant have spurred research into nematode control alternatives.

As registered chemical nematicides continue to become more limited in availability, Bio-control appears to offer an environmentally safe and ecologically feasible option for plant protection with great potential for promoting sustainable agriculture (Stirling, 1991). The beneficial effects of certain types of plant derived materials and microorganisms in soil have been attributed to a decrease in the population densities of plant-parasitic nematodes (Akhtar, 2000).The methods

most frequently used for managing nematodes in agriculture include rotating crops with plants that are not hosts of plant-parasitic nematodes, using resistant plants if available, applying chemical nematicides, soil solarization, use of organic amendments, trap crops, microbial bio control agents, and plants that are antagonistic to parasitic nematodes.

Plants belonging to 57 families possess nematicidal properties and it is possible to use these plants to control root knot nematodes. Researchers are developing alternative management techniques such as use of cropping systems soil amendments, organic soil amendments, biological control agents and the incorporation of plant parts or extracts (Sukul, 1992). Following this, the nematicidal potential of some botanicals have been evaluated and some found to be toxic against the root knot nematodes (Adegbite and Adesiyan, 2005).

Botanical plant extracts had an inhibitory effect on egg hatch and juvenile mortality of Meloidogyne incognita (Kofoid and White) Chitwood (Joymati et al., 1998) and other pathogens and their chemical composition is the major factor that promoted this effect. According to Gommers et al (1982), plant extracts act by producing compounds that stimulate production of oxygen radicals which block the metabolic pathways of the nematodes. Some of these extracts may include *Lantana Camara, Tagetes minuta and Amaranthus hybridus.*

Lantana Camara L. (Verbinaceae) is a perennial weed commonly found in the semi-arid regions of the world. It is one of the 10 worst weeds of the world and is a serious problem with 14 crops in 47 countries (Holm *et al*., 1979). *L. Camara* has an allelopathic potential because it contains a number of phenolic compounds (Narwal, 1994). Marigold (*Tagetes spp*),are resistant and fatal to *Meloidogyne spp* in most cases *T. minuta and T patula* are used as cover crops in rotation, green manure and source of nematode-antagonistic extracts (Chitswood,2002). Mexican marigold is an

erect annual herb often found growing in disturbed areas during early successional stages. This affinity for disturbed sites has allowed the species to colonize many areas around the world. It is used for its nematicidal and suppresses more than 14 genera of plant parasitic nematodes with root knot nematodes being the most affected.

Amaranthus spp are annual weeds widely distributed in the humid zone of the tropics including Zimbabwe. The weeds have been reported to have some pharmacological/chemical properties (Ayethan et al., 1996). Extracts of the leaves had also been used in the control of fungi and other microbes which may be hazardous to plant growth (Ayethan et al., 1996). Externally, the bruised leaves have been reported to affect microbial growth (some fungi and bacteria mainly).

This study aim at shedding light on the nematicidal potential of crude aqueous plant extracts in the management of root knot nematodes on tomato (*Lycopersicon esculentum L*) seedlings.

1.2 OVERALL OBJECTIVE

1.2.1 To evaluate the effects of aqueous plant extracts (*Tagetes minuta, Amaranthus and Lantana Camara*) on root knot nematode suppression in tomato seedlings

1.3 SPECIFIC OBJECTIVES

1.3.1 To determine the effects of aqeous plant extracts (*T.minuta, A.hybridus and L.camara*) extracts on nematode population density and average number of galls on tomato seedling roots infested with RKN (*M.javanica*)

1.3.2 To determine the effects of aqeous plant extracts (*T.minuta, A.hybridus and L.camara*) extracts on growth parameters: (days to 50 % emergence, seedling height, dry matter content, shoot: root ratio)

1.4. HYPOTHESES

1.4.1 Plant extracts (*Lantana Camara, Tagetes minuta and Amaranthus hybridus*) have no significant effect on gall density and index on seedling roots

1.4.2 Plant extracts (*Lantana Camara, Tagetes minuta and Amaranthus hybridus*) have no significant effect on growth parameters: (days to 50 % emergence, seedling height, dry matter content, shoot: root ratio)

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 ECONOMIC IMPORTANCE OF TOMATO (Lycopersicon esculentum, L)

The tomato (*Lycopersicon esculentum* Mill.) belongs to solanaceae family. The word tomato was derived from a word in the Nahuatl language, tomatl. It originated from the highlands of the west coast of South America (Smith, 1994) where its indigenous name was tomati. The specific name "*Lycopersicum*" means "Wolf peach". Other names are Love Apple, Pomo D'Oro and Garden Apple. From Mexico, tomato was taken to Europe Asia and then to Africa. Tomato is the most popular and widely consumed vegetable crop grown in outdoor fields, greenhouses and net houses of world including Zimbabwe. Climate and adaphic conditions of Zimbabwe are favorable for high production of good quality of tomatoes. (Anonymous, 2007).

Tomato is among the most important vegetables grown by smallholder farmers in Zimbabwe and ranks second after leafy vegetables in terms of consumption (Mvere, 2002). It constitutes a great amount of essential nutrients for their nourishment. They contain carbohydrates, dietary fiber, proteins, vitamins (a, b, c, k, thiamine, pyridoxine and foliates), calcium, iron, magnesium, manganese, phosphorus, potassium, carotene–b, carotene-a, zeathin and lycopene. The production and marketing of the crop provides employment for a greater percentage of the rural Zimbabwean population. The crop is also a source of income for the smallholder farmers. It is produced in both rainy and dry seasons. (Sithole and Chikwenhere, 1995)

It is perhaps the most profitable crop for small-scale farmer's (Lemma *et al.,* 1992).Despite the economic importance of tomatoes in Zimbabwe, its production is facing challenges that may

include, high production transport costs, poor marketing, pests and diseases etc. Pests and diseases in tomato production had resulted in massive reduction of yield and quality. The problem of pests and diseases has been exacerbated by failure of farmers to procure pesticides to control them. (Anonymous, 2007)

The annual losses incurred by their depredations are almost incalculable. In the tropical and sub tropical climates, crop production losses due to nematodes were estimated at 18,6% (Nicol et al.,2011). In tomato yield losses by *Meloidogyne spp*. has been estimated from 20% to 33% (Sasser, 1979; Sasser and Carter, 1982; Upadhyay and Dwivedi, 1987; Sasser, 1989).

2.2 USES OF TOMATO (Lycopersicon esculentum, L)

Edible part of the tomato represents about 94% of the total weight of the fruit (De Lannoy, 2001). A 100g tomato contains 93.8g water, 1.2g protein, 4.8g carbohydrate, 7mg calcium, 0.6mg iron, 0.5mg carotene, 0.06mg thiamine, 0.04mg riboflavin, 0.6mg niacin and 23mg vitamin C (De Lannoy, 2001). It plays a vital role in maintaining health. Ripened fruit are very helpful in healing wounds because of antibiotic properties, good appetizers and suitable food for diabetic patients. Its extract has a better effect on urinary acidity as compared to orange juice. Lycomato (branded tomato extract) is used for treatment of high blood pressure. It has diversified uses such as fresh salad, cooked foods and in processed forms like ketchup, pickle and sauce. It is highly prized for its monitory gain and nutritional value especially for its richness in vitamins and minerals (Collins, 2007).

2.3 CHALLENGES IN TOMATO PRODUCTION

Among the biotic factors (fungi, bacteria, viruses and nematodes) that are obstacles in getting the high yield, root knot nematode *Meloidogyne incognita* is widespread, destructive and the most

difficult pathogen of tomato (Sasser, 1980; Jones *et al.*, 1991; Fourie and McDonald, 2000). Root-knot nematodes tremendously reduce both quality and quantity of fruit. Root knot nematodes are obligate sedentary endoparasites with wide host range encompasses more than 2000-3000 plant species (Abad *et al*., 2003; Agrios, 2005). More than 100, *Meloidogyne* species (Eisenback and Triantaphyllou, 1991) have been described; only four have been recognized as the major and widely distributed species (Eisenback *et al*., 1981). These occur in the following order; *Meloidogyne incognita* (Kofoid and white) Chitwood, 47%; *Meloidogyne javanica* (Treub) Chitwood, 40%; *Meloidogyne arenaria* (Neal) Chitwood, 7% and *Meloidogyne hapla* Chitwood, 6% (Sasser, 1980).

2.4 NEMATODE MORPHOLOGY AND CLASSIFICATION

2.4.1 What are Nematodes?

Nematodes, commonly known as eel or round worms, cylindrical organisms, with a thread-like, filiform body shape. The word nematode is derived from the Greek words 'nematos', which means thread, and 'eidos', meaning form. Mature females of some genera (*Meloidogyne*, *Heterodera* and *Nacobbus*), however, have swollen, saccate-like bodies (Agrios, 2005:838; Coyne, Nicol & Claudius-Cole, 2007:8). Nematodes are aquatic animals that inhabit oceans, freshwater rivers, lakes and marshes, body fluids of animals and humans and of particular importance in this study, the film of water present in/on plant parts and between soil particles (Agrios, 2005:830; Luc, Bridge & Sikora, 2005:2;).

Nematodes are the most abundant multi-cellular organisms on earth and vary considerably in size, generally from tenths of a millimetre up to 8 metres, the latter being *Placentonema gigantissimum*, a parasite found in the placenta of a sperm whale (Ferraz & Brown, 2002:7). Like

all animals, nematodes have a digestive system. Plant-parasitic nematodes differ from non-parasitic or free-living nematodes that feed on bacteria, algae and fungi in that they have a specialized feeding structure, the spear or stylet. This is used to pierce plant cells and in most parasitic nematodes used to inject enzymes into plant cells and tissues, liquidise cell contents and then extract it as a food source (Ferraz & Brown, 2002:).

2.4.2 Classification of Nematodes

Nematodes belong to the animal kingdom, Animalia, the sub-kingdom Eumetazoa and comprise a large phylum, Nematoda that includes plant, animal and human parasites as well as free-living or non-parasitic species (Maggenti, 1981:1-372; Kleynhans *et al*., 1996:13-15; Agrios, 2005:830).

2.4.3 Plant-Parasitic Nematodes

Plant-parasitic nematodes are grouped in two classes, the Adenophora and Secernentea. The class Secernentea comprise of two orders, Dorylaimida and Tylenchida. The latter order represents the majority of plant-parasitic nematode genera (Ferraz & Brown, 2002:8; Moens, Perry & Starr, 2009:1-17). These nematodes are usually small, eel-shaped, unsegmented roundworms of 1 mm or less except for some species belonging to the family Longidoridae which can reach lengths of up to 12 mm.

Plant-parasitic nematodes belonging to the order Tylenchida constitute about 20% of the described species within the Phylum Nematoda (Ferraz & Brown, 2002:7). Plant-parasitic nematodes are often referred to as the ‘unseen enemy’, mainly because of the fact that most of them are very small and therefore not visible to the naked eye, making it difficult for them to be identified by farmers (Ferraz & Brown, 2002:9). The majority of plant-parasitic nematodes

attack the roots of plants but generally also cause symptoms in the aerial parts of the plant. Therefore, as parasitism occurs predominantly in the parts of the plant below the soil surface, it is even more difficult to identify the real cause of the problems observed in the above ground parts of the plant (Ferraz & Brown, 2002:10).

Plant-parasitic nematodes obtain their food by injecting their feeding apparatus or stylets into plant cells of the host plants as described earlier (Agrios, 2005:827; Decreamer & Hunt, 2006:3-32). This group of nematodes are thus classified as obligate bio trophic organisms that obtain nutrients only from cytoplasm of living cells (Decreamer & Hunt, 2006:26).

Crop production and quality worldwide is limited due to infestation and parasitism by numerous plant-parasitic nematode species, including root knot nematodes (Kinloch, 1982:162; Shane & Barker, 1986:320; Sasser & Freckman, 1987:7-14; Jones *et al*., 2013:1-15). The Society of Nematology and other organizations estimate global crop losses due to plant-parasitic nematodes at $100 billion annually (Pasteuria™ bioscience, 20091; Cetintas & Yarba, 2010:222-225). In South Africa, the estimated annual loss due to plant parasitic nematodes amount to 14 %, totaling over R1.9 billion (ARC, 2011).

Plant-parasitic nematodes are frequently separated into two major groups according to their feeding habits and motility, namely ectoparasites and endoparasites which can both be either migratory or sedentary (Boerma & Hussey, 1992:242-252; Sijmons, Atkinson & Wyss, 1994:235-259; Coyne, Nicol & Claudius-Cole, 2007:3-9). Ectoparasitic nematodes usually remain outside the host tissue and they feed on epidermal plant cells, using their stylets (Boerma & Hussey, 1992:242-252). Migratory endoparasites on the other hand, enter, migrate and feed inside the host plant tissue and generally cause considerable destruction (Boerma & Hussey,

1992:242-252; Hussey & Williamson, 1998:87-108). Sedentary endoparasites have evolved specialized feeding relationships with their hosts and depend on modified host cells for the provision of nutrients in order to develop and reproduce optimally (Sijmons, Atkinson & Wyss, 1994:235-259; Hussey & Williamson, 1998:87-108).

2.4.4 Non-Parasitic Nematodes (Free-living nematodes)

Non-parasitic or free-living nematodes feed on other organisms such as viruses, bacteria, fungi, crustaceans, insects, mites and other nematodes. These nematodes do not possess a stylet, except for some genera of the Tylenchida (fungi feeding predators) that use the latter to feed on other micro-organisms and other nematodes (Kleynhans *et al*., 1996:5)

2.4.5 Root- Knot Nematodes (*Meloidogyne* species)

After their first discovery on the roots of cucumber in a glasshouse in England (Berkley, 1855:220), root-knot nematodes were soon recognised as important pathogens on numerous plants all around the world (Eisenback & Hunt, 2009:18-37). Root-knot nematodes are sedentary endoparasites. This means that the second-stage juveniles (J2) enter the plant tissue, develop a permanent feeding site, become immobile, and swell into obese bodies by developing into third- (J3) and fourth-stage (J4) juveniles and ultimately saccate-like females (Bridge & Starr, 2007:40-44). The classification of South African plant-parasitic nematodes is based on Maggenti *et al*. (1988:177-188) for the suborder Tylenchina. According to Kleynhans *et al*., (1996:13-15) and (GBIF) 1, *Meloidogyne* species, commonly known as root-knot nematodes are classified as follows:

Phylum: Nemata

Class:	Secernentea
Subclass:	Diplogasteria
Order:	Tylenchida
Suborder:	Tylenchina
Superfamily:	Tylenchoidea
Family:	Heteroderidae
Subfamily:	Meloidogyninae
Genus:	Meloidogyne

The genus *Meloidogyne* was first established in 1892 by Emil August Goeldi, a zoologist. The word, *Meloidogyne*, is of Greek origin and means 'apple-shaped female' (Moens, Perry & Starr, 2009:1-17). At present 98 species have been identified in this genus (Jones *et al*., 2013:2), four of which are un- questionably the most important, wide-spread and common species on the planet that accounts for 95 % of all root-knot nematode infestations in agriculture (Ferraz & Brown, 2002:41; Hussey & Janssen, 2002:43-70; Moens, Perry & Starr, 2009;1-17; Jones *et al*., 2013:2). These four species, *M. incognita*, *M. javanica*, *M. hapla* and *M. arenaria* probably cause more damage to agricultural crops worldwide than all the other *Meloidogyne* species combined. Generally they account for 5 % of all crop losses worldwide. *M. incognita* and

M.javanica is the economically most important species associated with agricultural crops globally (Hussey & Janssen, 2002:43-70; Moens, Perry & Starr, 2009:1-17).

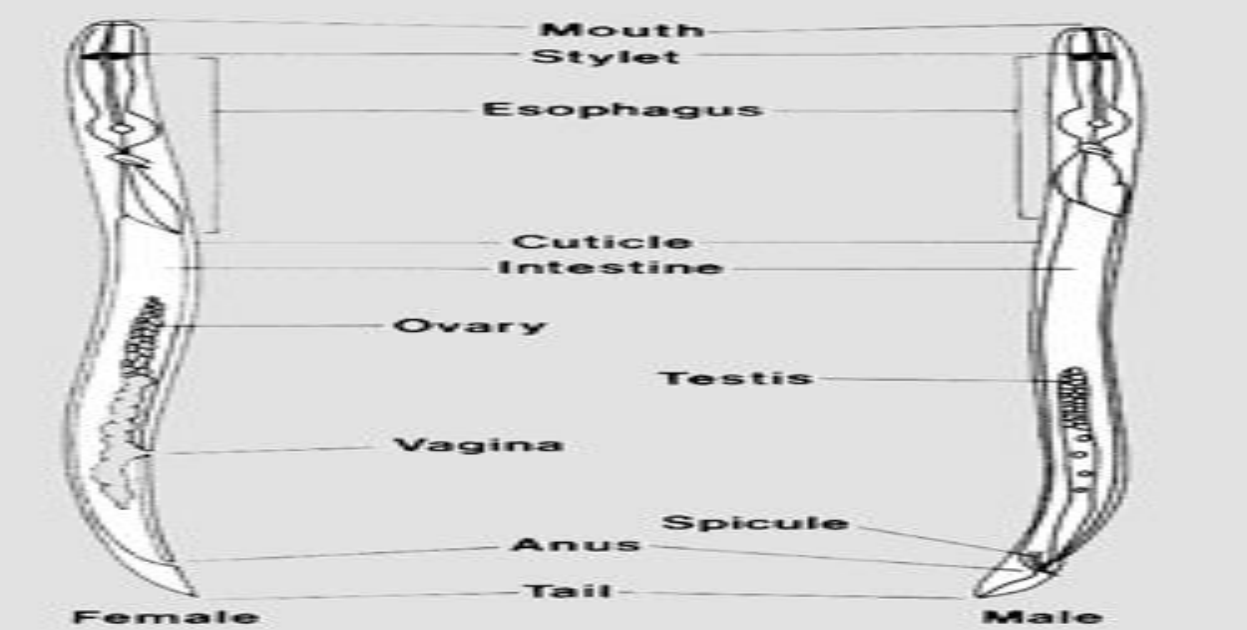


Fig 2.1 Root Knot Nematode structure (www.ipm,ucdavis.edu)

2.5 DISTRIBUTION AND ECONOMIC IMPORTANCE

Root-knot nematodes of the genus *Meloidogyne* are obligate and highly specialized plant pathogens (Kleynhans, 1991:1; Karssen & Moens, 2006:59-90; Jones *et al*., 2013:2). They constitute a major group of plant parasites and are of outstanding economic importance worldwide. Their global distribution over a wide range of climatic conditions, tropical, sub-tropical and temperate regions of the world as well as the fact that they infect nearly every plant species (monocotyledons, dicotyledons, herbaceous as well as woody plants) results in this group causing considerable crop yields and quality losses (Sasser, 1980:36-41; Mai, 1985:95-112; Starr *et al*., 2007:283-294; Moens, Perry & Starr, 2009:1-17).

M. incognita and *M.javanica* are the most important root-knot nematode species on vegetables world-wide (Lamberti, 1979:341-357) and are the most prevalent and economically important species in Southern African countries (Bridge, 1996:201-225; Coyne, Nicol & Claudius-Cole, 2007:1-82). In South Africa, these two root-knot nematode species are described as the most common parasites of plants (Kleynhans *et al.*, 1996:4) causing greater economic damage than other plant-parasitic nematodes (Van der Wal, 1999:251). *M. arenaria* occur more commonly in the subtropics but is also sporadically found in the tropics. *Meloidogyne hapla*, a species which is common in temperate regions, is occasionally found in cooler upland tropics (Sikora & Fernandez, 2005:319-392).

Several other species of *Meloidogyne* are also of economic importance, but their host range and/or geographical distribution are more restricted (Table 2.1) (Ferraz & Brown, 2002:41). Although root-knot nematodes are economically the most important group on vegetable crops there are a few other nematodes that are also important on vegetable crops. These include: *Rotylenchulus reniformis*, *Nacobbus aberrans*, *N. bolovianus*, *N. dorsalis*, *Globodera rostochiensis*, *Heterodera schactii*, *H. cruciferae*, *Cactodera*, *Ditylenchus dipsaci* and *Paratrichodorus minor* (Sikora & Fernandez, 2005:319-392).

Table 2.1.The geographical distribution of Meloidogyne species (Abid, M. (1996))

North America	South America	Africa	Europe	Asia	Australia
(1)	(1)	(1)	(1)	(1)	(1)
M.incognita	M.incognita	M.incognita	M.incognita	M.incognita	M.incognita
M.javanica	M.javanica	M.javanica	M.javanica	M.javanica	M.javanica
M.arenaria	M.arenaria	M.arenaria	M.arenaria	M.arenaria	M.arenaria
M.hapla	M.hapla	M.hapla	M.hapla	M.hapla	M.hapla
(2)	(2)	(2)	(2)	(2)	(2)
M.chitwoodi	M.exigua	M.acronea	M.naasi	M.graminicola	M.naasi
		M.ateila			
(3)	(3)	(3)	(3)	(3)	(3)
M.microtyla	M.coffeicola	M.africana	N/A	M.mali	N/A
M.graminis	M.oryzea	M.litoralis		M.camelliae	
M.naasi	M.salasi				

(1) = major pests; **(2)** = important pests and **(3)** = important pests in local areas

2.5 DAMAGE SYMPTOMS

2.5.1 Aboveground symptoms

The occurrence of patches representing poor crop performance within a field may be the result of root-knot nematode infection. Such patches may exhibit symptoms such as stunting, yellowing,

wilting, general poor appearance of the plants, reduced yields and yield quality as well as premature death of plants (Fourie, 2006:2; Coyne, Nicol & Claudius-Cole, 2007:11).

2.5.2 Belowground symptoms

Individuals from this genus induce the formation of conspicuous and characteristic galls/knots on underground parts of an infected plant and in this way made researchers and producers aware of them (Decker, 1981:248; Fourie, 2006:2). Gall formation starts soon after invasion of the roots by the J2 and apparently takes place in response to secretions of the oesophageal glands of the J2 (Kleynhans, 1991:4). Galling of roots infected with root-knot nematodes is a different response than giant cell formation and does not appear to be essential for nematode growth and development. Root-knot nematodes are instructive feeders i.e. modifying, but not destroying penetrated plant cells in order to obtain optimal food sources while other species of nematodes like *Pratylenchus* spp. (lesion nematodes) are destructive feeders. Individuals from the latter group destroy plant cells during feeding, resulting in necrosis and dying off of such cells and surrounding plant tissue (Fourie, 2006:3).

2.5.3 Life Cycle

M. incognita and *M. javanica* are both sedentary endoparasites (Kleynhans, 1991:3) that can complete most of their life cycle within roots/tubers of their host plant. Both these root-knot nematodes have four juvenile stages between the egg and adult (Moens, Perry & Starr, 2009:1-17). They can also survive on a range of weeds, particularly broadleaf species, when environmental conditions are favourable (Overman, 1991:49-52; Ntidi et al., 2012). The length of the life cycle varies considerably, since it is influenced by factors such as crop type, cultivar, variety, temperature and other biotic and abiotic factors. Both *M. incognita* and *M. javanica*

reproduce by mitotic parthenogenesis (Sikora & Fernandez, 2005:319-392) and they have a strong reproductive potential to produce multiple generations per year. The life cycles of both these nematode species are generally completed in a susceptible host in about 21 days at 26 ºC (Taylor & Sasser, 1978:9; Decker, 1981:259).

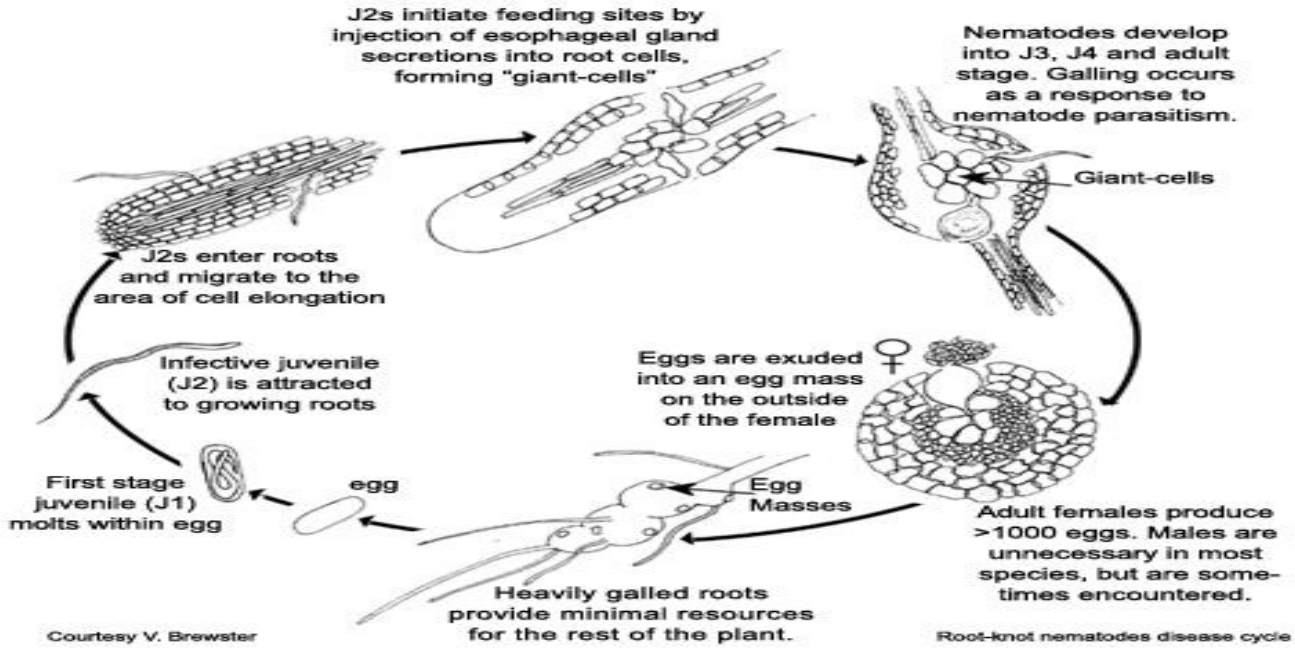
The life cycle starts with an egg which is the one-celled stage and it may occur free in the soil, embedded in the gelatinous matrix or adhered to plant tissue of the host plant. The eggs are elongated-oval in shape with rounded ends (Decker, 1981:259). A female usually deposit up to 1000 eggs. These eggs are encompassed in a gelatinous matrix, called the egg mass, which protects the eggs from dehydration on the tissue surface (Heyns, 1971:92). The egg mass usually protrudes from the root while the female remains completely or partially embedded in the root. Development within the egg continues until the first-stage juvenile (J1) is formed. This stage moults within the egg and advances to the infective J2 which hatches spontaneously from the egg when soil conditions are favourable (Jenkins & Taylor, 1967:108; Decker, 1981:260; Fourie, 2006:8). Among all the stages of development, the egg stage is most resistance to cold (Decker, 1981:259). The newly hatched J2's contain food reserves in their intestine in the form of protein and lipids which may equal one third of their body mass. These reserves sustain the J2 until a suitable host is found and penetrated (Fourie, 2006:8).

The infective J2 migrates through the soil and penetrates relevant tissue of a suitable host plant (Ferraz & Brown, 2002:42). They generally penetrate plant roots just behind the root cap where intense meristematic activity occurs. The J2's feed ectoparasitically on the root tissue before penetrating the root and then move intercellularly to parenchyma cells within the central, vascular cylinder. The parasitic stage starts now since the J2 pierce the root cells surrounding its

head using its stylet and injects growth regulating substances from its dorsal oesophageal gland into these cells through the stylet (Ferraz & Brown, 2002:44).

Resulting from the latter process is the induction of a group of so called giant cells. Giant cells are transformed parenchyma cells within the central, vascular cylinder that are essential for root-knot nematode growth, development and reproduction. The nuclei of these elongated cells multiply, while the cytoplasm becomes dense and cell walls thicken. The cytoplasm of giant cells contains much more protein than those of normal cells. These giant cells are maintained by repeated injection of secretions from the oesophageal glands. The juvenile eventually moults to form the J3, which lacks a stylet and does not feed. After a short time this larva moulds to the J4 which also does not feed. Finally it moults into the adult, pear-shaped female (Ferraz & Brown, 2002:44; Fourie, 2006:8). The mature root-knot nematode female is embedded inside the roots or other plant tissue and is generally visible as a swelling of such plant tissue (Ferraz & Brown, 2002:44). Continuous feeding by root-knot nematode females adversely affects normal physiological processes of the host plant, which include hampering of water and nutrient uptake and transport (Sikora & Fernandez, 2005:321).

Fig 2.2 Root Knot Nematode life cycle (www.cabi.org 2017)



2.6 RKN CONTROL

Control and management of plant-parasitic nematodes are essentially preventive and are aimed at maintaining or increasing food and other crop production through suppression of the nematode numbers to below the population density levels at which economic damage begins, i.e. the damage threshold (Kleynhans *et al.*, 1996:7). Control strategies aimed at reducing plant-parasitic nematodes effectively are categorised into two major groups, namely classical (chemical) and cultural control (Bridge, 1996:202; Ferraz & Brown, 2002:162).

2.6.1 CHEMICAL CONTROL

Control by nematicides is the most rapid and effective way of protecting plants against nematodes (Kleynhans *et al.*, 1996:6). Nematicides have been used extensively since the 1900's (Ferraz & Brown, 2002:173) as the major control strategy to reduce nematode numbers in high value crops such as vegetables (Netscher & Sikora, 1993:237-283) and a range of other crops

(Luc, Sikora & Bridge, 1993:530). Despite the high toxicity of some chemicals to mammals and birds, the harmful effects on natural parasites and predators of nematodes, food contamination and environmental pollution, high overhead costs and difficulties with application of these chemicals, the use thereof will always play an important role in protecting crops from plant-parasitic nematodes. The same applies in terms of nematicide use with regard to regulatory and quarantine procedures (Johnson, 1985:249-301; Ferraz & Brown, 2002:173-176).

2.6.2 CULTURAL CONTROL

The high costs and potential health and environmental hazards of agricultural chemicals are turning nematode control options towards non-chemical or cultural methods. The use of cultural control methods to manage root-knot nematodes is the most environmentally sustainable and potentially the most successful methods for limiting root-knot nematode damage. These control strategies are applied in both commercial and subsistence agricultural systems. None of the available methods on their own provides complete and effective control but they all have some suppressive effect on plant-parasitic nematode population densities (Kleynhans *et al*., 1996:7). According to Madulu, Trudgill and Philips (1994:438-455) it is in particular small-scale farmers in developing countries that use a combination of these practices to combat nematode pests.

2.6.2.1 Crop Rotation

Crop rotation can only be successful if knowledge of the nematode pests involved and their host range is known. Crop rotation entails growing the main crop with such long intervals between the cultivation of host crops that the population density of the principal nematode parasite of the crop decreases to a non-damaging level (Brown, 1987:351-382; Kleynhans *et al*., 1996:7). Crop

rotation is more suited to low-value annual and short-term perennial crops, and can be very effective against nematode species with narrow host ranges (Kleynhans *et al*., 1996:7)

2.6.2.2 Fallowing

On lands kept free of vegetation and the soil turned frequently during the dry season, nematodes are subjected to starvation, mechanical injury and the desiccating effects of the sun, wind and climate (Kleynhans *et al*., 1996:7; Ferraz & Brown, 2002:180). Removal of all plant roots and/or other nematode-infected plant tissue is essential as these may harbor endoparasitic species. Fallowing is not effective for species with resistant stages in their life cycles (Kleynhans *et al*., 1996:7). Fallowing has to be economical and acceptable to the grower, therefore, it is most effective when other control techniques are used simultaneously (Kinloch & Dunavin, 1993:806-808).

2.6.2.3 Flooding

Flooding of fields can be a successful method of nematode control since anaerobic conditions are created. It is only successful if soil is flooded for a period of at least six months. This control strategy is normally used in areas where water is abundant and fields are level (Johnson & Fassuliotis, 1984:323-372). This method is not economically feasible for sustainable subsistence agriculture as abundant water supply is often not available in resource poor areas (Ferraz & Brown, 2002:180). Thames and Stoner (1953:187-192) demonstrated that constant flooding of rice fields in the Philippines for three months gave acceptable control of root-knot nematodes for two succeeding vegetable crops.

2.6.2.4 Trap Cropping

Endoparasitic nematode species may be controlled by cultivating a quick growing, highly susceptible host crop on an infested land and uprooting and destroying the crop before the nematode can complete its life cycle. Disadvantages of this method include practical difficulties with the timely and effective removal of all trap crop roots, sacrifice of income from both the main crop and the trap crop, and cost involved in raising a crop that cannot be marketed (Kleynhans *et al*., 1996:7).

2.6.2.5 Soil Amendments

Plant and animal wastes are incorporated in the soil principally to benefit crop growth through improvement of the soil structure and the provision of plant nutrients (Sikora, 1992:245-270). Decomposition of organic matter also promotes the build-up of organisms such as nematophagous fungi and predatory nematodes that will have some suppressive effect on plant-parasitic nematode populations. The use of organic amendments such as, coffee husks, oil cakes, neem products, marigold residues, leaves, crustacean skeletons, sawdust, urea, sugarcane bagasse and chicken manure amongst others have been used with some success (Singh & Sitaramaiah, 1966:349-355 and 1967:668-672; Sikora, Singh & Sitaramaiah, 1973:123-127; Muller & Gooch, 1982:319-326; Sikora, 1992:245-270; 27 McSorley, 2011:69-81). When used for green manuring, residues of nematode–antagonistic plants such as *Ricinus communis* (Kleynhans *et al*., 1996:8) and *Tagetes* species (Chitwood, 2002:221-249) release substances that are toxic to the nematodes or have a nematostatic effect.

2.6.2.6 Heat

The lethal temperature for control of plant parasitic nematodes is considered to be 45°C. Nematodes that occur in plant bulbs, tubers, corms and rootstocks can be controlled by immersion of the plant material in water heated to temperatures that will kill the nematodes without harming the planting material (Kleynhans *et al*, 1996:8). Heating the soil either with dry or steam heat has been used for many years in protected cultivation to manage root-knot nematodes, but the high cost of heating oil has limited its use drastically. Soil solarization with plastic mulches, which leads to the development of lethal temperatures in the soil is, however, being used as a cost-effective strategy to control root-knot nematodes and other soil borne diseases (Katan, 1981:211-236).

2.6.3 GENETIC HOST PLANT RESTANCE

Agricultural scientists consistently identify genetic host plant resistance as the highest research priority for nematode pest management. The advantages and benefits of breeding or finding crop plants resistant to injurious parasitic nematodes, and growing them on infested land, are many. Resistant crops provide an effective and economical method for managing nematodes in both high- and low-cash value cropping systems (HPR, 2010)1. The term “host” is used in nematology to indicate a plant species on which plant-parasitic nematode species can feed and reproduce optimally, while the term “resistance” refers to the ability of the host plant to oppose or use force against the nematode in order to prevent the advance of the normal development and reproduction of the nematode.

There are two types of genetic resistance namely, vertical or qualitative resistance which is only effective against one species/race and horizontal or quantitative resistance which is effective

against multiple species/races (Trudgill, 1991:167-192). Genetic resistance can be classified in either pre infectional resistance (before the nematode penetrates the root surface) or post-infectional resistance (after the nematode penetrates the plant root, with the latter being the most common form of resistance (Fourie, 2006:21).

Host plant resistance mechanisms involved in post-infectional resistance include: Non-preference (antixenosis), antibiosis and tolerance (Painter, 1951:358-367). Non-preference is a property exhibited by a host plant that denotes a nematode's response to plants that lack characteristics to serve as host. The nematode will therefore avoid a plant or have a negative reaction to the plant during its search for food, penetration sites or shelter (Painter, 1951:358-367; Cook & Evans, 1987:179-231). Antibiosis on the other hand, includes all adverse effects exerted by the host plant on the nematode's biology, e.g. its survival, development and reproduction. Tolerance includes all responses by the host plant that result in the ability to withstand nematode infection and to support nematode populations and crop yield which otherwise severely damage susceptible plants (Roberts, 2002:23-41).

2.7 EFFECTS OF BOTANICALS ON NEMATODE SUPPRESSION

Botanicals (plant-based pesticidal chemicals) have found favour as alternatives to pesticides in recent times. Some of these botanicals are already being exploited commercially in insect pest management (Agnihotri *et al*., 1999). Different plant species are being tested to identify the sources of nematicidal substances and many of them have shown promising results in the control of plant parasitic nematodes outside the country (Abdi, 1996).

2.7.1 Marigold

Marigold (Tagetes spp.) is one of the most widely studied plant genera due to its allelopathic potential against populations. Marigolds' repressive impact on nematodes has been documented for over 50 years (Steiner, 1941). Tyler (1938) reported that 29 marigold varieties were resistant to root-knot nematodes (Meloidogyne spp.). Early literature also indicated that marigolds could prevent the population increase of 14 genera of nematode populations (Steiner, 1941; Oostenbrink et al., 1957; Suatmadji, 1969), encompassing endoparasitic, semi-endoparasitic, and ectoparasitic nematodes (Siddiqui and Alam, 1987a). Of the 14 genera, Pratylenchus and Meloidogyne are most consistently affected by marigolds (Suatmadji, 1969).

In an experiment carried out at the department of Agronomy, Faculty of Agricultural sciences, Ludoku Akintola University of Technology, Ogbomoso, Nigeria, marigold root extracts treatment recorded a reduction of root knot nematode population in the soil with corresponding increase in plant height, leaf and fruit yield over the control treatment.(Plant Pathology Journal 7(1):45-49,2008)

2.7.2 Lantana Camara

Lantana camara Linn is a significant weed of which there are some 650 varieties in over 60 countries. It is established and expanding in many regions of the world. *Lantana* (from the Latin *lento*, to bend) probably derives from the ancient Latin name of the genus *Viburnum* which it resembles a little in foliage and inflorescence. *Lantana camara* is a notorious, noxious and invasive weed belonging to verbenaceae family. *Lantana camara* is one of the ten worst weeds of the world, which is a native of tropical and subtropical America. The species was introduced

in India from Sri Lanka in 1809. *Lantana* was introduced to India at the National Botanical Gardens, Calcutta in 1807 as an ornamental plant.

Lantana camara L's morphological variation and it occurrence all over the warmer parts of the world many different names have been reported for various forms of *L. camara*. Ecosystems threatened by *Lantana camara* include frontal dune and nearby community types such as mangroves, sedge and health land, wood lands associated with melaleucas, banksias and casuarinas, as well open wood land and forest communities (Casado, 1995; Rajbansi and Inubushi, 1997)

Leaves contain enzymes like oxidase, catalase, amylase and lipase, glucosidase, secondary metabolites as tannins, sugar, resin, sesquiterpines, caryophylline, phelandrene, aldehydes, alcohols, lantadene-A, lantadene-B, triterpenoid, lancamarene, and lantadene. The impact of root leachates of *Lantana camara* L., a tropical weed, against *Meloidogyne javanica*, the root-knot nematode, was tested under laboratory and pot conditions. Concentrated and diluted root leachate caused substantial mortality of *M. javanica* juveniles. Significant suppression of the nematode was achieved when soil was treated with a full-strength concentration of the leachate. Whilst this high concentration retarded plant height and shoot fresh weight, more diluted concentrations actually enhanced plant growth.

To establish whether this inhibition of plant growth from the leachate was the result of depleted nitrogen levels in the soil due to the leachate, soil treated with such leachates was given urea as an additional nitrogen source. Urea not only enhanced nematode suppression activity of the root leachates but also increased seedling emergence and growth of mungbean. Application of the *L.camara* root leachates in combination with *Pseudomonas aeruginosa*, a plant growth-

promoting rhizobacterium, significantly reduced nematode population densities in roots and subsequent root-knot infection, and enhanced plant growth. The root leachate of *L. camara* was found to contain phenolic compounds, including *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, ferulic acid and a quercetin glycoside, 7-glucoside. It also contained weak enzymic hydrogen cyanide. (Begum et al. 2000)

2.7.3 Amaranthus hybridus

Amaranthaceae (Amaranthus family).With the recent inclusion of the former Chenopodiaceae, the Amaranthaceae now contains about 160 genera and 2400 species of mostly herbs or shrubs of the tropical and subtropical regions of the world. The leaves contain various chemical which include phenols and caffeic acid that could be detrimental to a lot of pathogens. Not much work has been done on Amaranthus hybridus. (Afouda et al, 2008) Evaluated *Amaranthus sp*. and *Vernonia amygdalina,* and Soil amendments with Poultry Manure for the Management of Root-knot Nematodes on Eggplant. Phytoparasitica 36, 368-376.

Amaranthus spp are annual weeds widely distributed in the humid zone of the tropics including Zimbabwe. The weeds have been reported to have some pharmacological properties (Ayethan et al., 1996).

2.8 MECHANISMS OF NEMATODE SUPPRESSION BY BOTANICALS

2.8.1 Marigold

Marigold may reduce RKN populations by several means, including (1) acting as a non-host or a poor host, (2) producing allelopathic compounds that are toxic or inhibit population development, (3) creating an environment that favors nematode antagonistic flora or fauna

(Wang et al., 2001); or (4) behaving as a trap crop (Pudasaini et al., 2008).These mechanisms may occur separately or in combination resulting in lower RKN numbers

2.8.2 Lantana camara

There are reports that certain plants possess nematicidal properties (Gommers, 1972;Nandal and Bhatti, 1983) and their potential for use in nematode control programs has been proved effective (Siddiqui and Alam, 1988). Application of such plant parts or extracts to nematode infested soils affect nematodes directly and stimulates soil microbes that reduce nematode population (Nandal and Bhatti, 1986; Zaki and Bhatti, 1990; Akhtar and Alam, 1990; Rodriguez, 1991).Keeping in view the importance of the biological control, it was planned to investigate the biological control of root knot nematodes through screening, by the use of plant extracts

2.8.3 Amaranthus hybridus

Amaranthus hybridus is said to contain chemical compounds that affect pathogen life. *Amaranthus hybridus* is rich in phenolic compounds whose biological activities are well established which is detrimental to fungal life cycles and other pathogens. The study of the biological activities confirmed the phytochemical results regarding anti-radical, antioxidant, and goute-related enzyme inhibition activities (Fernand 2012)

2.9 OBJECTIVE OF STUDY

Natural products with nematicidal potential have been identified by testing the effect of plant extracts (from leaves, stems, fruits and seeds), oil extracts, plant exudates and plant volatiles on nematodes that infect plants. Application of chopped plant parts and plant extracts to soil were shown to be nematicidal to root-knot nematodes and to reduce infection of plants. Many naturally occurring compounds are known to possess nematicidal activity (Chitwood, 2002).

Plythienyls in *Tagetes* spp. (Kyo *et al*., 1990), isothiocynates and glucosinolates from Brassicaceae (Brown and Morra, 1997), polyacetylenes from Asteraceae (Kogiso *et al*., 1976), alkaloids (Matsuda *et al*., 1989), phenolics (Evans *et al*., 1984) and pentacyclic triterpenoids from *Lantana camara* , *Tagetes minuta* and *Amaranthus hybridus* (Qamar *et al*., 2005) have been reported to possess anti pathogenic and nematicidal effects. Therefore, natural products seem to provide a viable solution to the environmental problems caused by synthetic pesticides and may be more readily available and less costly in developing countries including Zimbabwe for eco-friendly nematode management option. So there is need for these avenues to be explored since they are promising and may hold great essence to smallholder farmer who are usually resource limited.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 DESCRIPTION OF STUDY SITE

A greenhouse pot experiment was carried out at Midlands State University which is (19°45' S and 29°84' E) and is located in Natural Farming Region III of Zimbabwe. The site is located at 10km southeast of Gweru Central Business District. It is elevated at 1428m above sea level with an average mean temperature of 18^{o}C. The site is characterized by sandy loams soils belonging to the fersialitic group. The soil is dominated by kaolinite clay minerals according to (Nyamapfeni, 1991).

3.2 EXPERIMENTAL DESIGN

The experiment follows a Completely Randomized Design (CRD) with 5 treatments, replicated 3 times. Variety used was money-maker and allocation of treatment to plots was done randomly.

Table 3.1.Table of treatments and variety

Treatment	Treatment description
1	Nemacur (Positive control)
2	Water (Negative control)
3	Lantana Camara (33% w/v)
4	Amaranthus hybridus (33% w/v)
5	Tagetes minuta (10% w/v)

NB-difference in concentrations used is because of difference in chemical composition

3.3 EXPERIMENTAL PROCEDURE

3.3.1 Counting of *M.javanica* eggs to be used as inoculum

The *M.javanica* inoculum obtained from Tobacco Research Board (TRB) was well agitated by bubbling using a pipette. 0.5mls of the inoculum was placed in a small counting dish with square grids and counting was done thrice and averaged. A dissection microscope was used for identification of stages and counting of *M.Javanica*.

3.3.2 Soil sterilization and filling of pots

Sandy loam soil with organic matter/manure was collected from Midlands State University Farm, placed in an oven for 2hrs at 200 degrees Celsius and the soil was covered with foil paper till use. The pots were filled with sterilized soil and watered to allow it to settle in.

3.3.3 Collection of plant leaves and aqueous plant extract preparation

For the purpose of isolation of leaf extracts three wild plants were selected. The plants were collected in an around Midlands State University campus. The taxonomic identification of the specimens was performed based on various morphological characters.

The three plants selected were L.*camara, A.hybridus* and *T.minuta.* Extracts were prepared from leaves of the three *L.camara, T.minuta* and *A.hybridus* plants. The fresh leaves were thoroughly washed in running tap water and sterile distilled water and ground to obtain extracts of each plant species. 40g of *L.camara* crushed leaves was soaked in 400ml distilled water for 24hours, 25g crushed leaves of *T.minuta* was soaked in 250mls of distilled water for 24 hours and 50g of *A.hybridus* was soaked in 150mls distilled water for 24hrs. The material was then sieved and filtered and the filtrate was kept in a refrigerator till use.

3.3.4 Variety selection

The variety used for this trial was the standard variety on the market and available to the majority of the farmers. Money maker, a variety that is susceptible to nematode infestation which can adapt to most tomato growing areas in Zimbabwe.

3.3.5 Inoculation of soil in pots with M.javanica

Two days before planting, 2mls of inoculum was placed in each pot rendering 512 eggs and J2 *M.javanica* juveniles. Watering was done to promote nematode life and enable them to adapt to the new environment.

3.3.6 Planting of seeds and application of treatments

25mls of aqueous plant extracts was placed in each pot (Khan et al, 2008). Nemacur® 400 EC (fenamiphos), a commercially available nematicide was used in plots for positive control and untreated, inoculated plots for negative control were also included Three seeds were planted per station in a depth of between 5-15 mm and were covered with a thin layer of soil. Watering was also done.

3.4 DATA COLLECTION

Data collection was done at four weeks (30 days) after planting since seedlings would have reached early transplanting maturity.

3.4.1 Final nematode population density at transplant stage

Initial juvenile and egg population was recorded and after experiment, the final population was assessed and the difference was recorded as change in root knot nematode population. RKN were then extracted using the maceration method where the roots were chopped and weighed to 10g

and placed in an electrical macerator for 15 seconds. After maceration the mixture was passed through a series of mesh sieves of sizes 150, and 38µm which allow nematode retention.

Residue in larger sieves was backwashed, collected and centrifuged at 4000 rpm for five minutes. A second centrifugation of nematode residue was done in a sucrose solution at 4000 rpm for two minutes after thorough shaking. This mixture was then tilted 38° to the horizontal in the 38µm sieve after the sucrose solution had dissolved thoroughly. Serial samples of the liquid were collected for observation under a microscope.

Soil of volume 100 cm3 was collected from the pots, placed in a bucket, filled with water and allowed to settle. This mixture was then poured down a series of sieves of diameters sizes 250µm, 150µm and 38µm in descending order, which captures soil and dirt but allows RKN through the upper two. The residue in the 38µm diameter sieve that contains RKN was tilted at a 30° angle and gently washed back with a stream of water and collected first into a beaker, then a centrifuge tube. Centrifugation was done at 4000 rpm for 5 minutes, and gentle discarding of the supernatant fluid leaving about 5ml to avoid nematode loss. Further addition of residue to sucrose solution preceded a 2 minute centrifugation at 4000 rpm. The nematodes were counted in a rectangular counting dish. Sucrose solution was made by adding distilled water to 684g sucrose granules to give a liter.

3.4.2 Number of Galls

The number of gall on each seedling was counted with the aid of a hand lens that helped in magnifying to give a better and clearer vision.

3.4.3 Days to 50 % emergence

The number of days taken for 50 %of the seedlings to emerge was recorded and averaged.

3.4.4 Seedling height

A 30 centimeter rule was used to measure the plant height. Stalk height was obtained by measuring the distance from the ground level to the topped part of the plant. Seedlings from each treatment were used as samples. Stalk height was taken at the fourth week after planting.

3.4.5 Dry matter weight

Fresh transplants were taken and washed with water to remove dirt. They were then oven dried at 37 degrees Celsius for 48 hours and weighed on an electronic balance and the mass for each treatment was recorded.

3.4.6 Shoot: root ratio

Seedlings from each treatment were taken, dried separately and shoots and roots were separated by cutting at soil level mark on the plant. Mass of each shoot and root was measured using a sensitive electron balance and mean root and shoot was calculated.

3.6 Data analysis

Analysis of variance was done using Genstat 14th edition and means will be separated using LSD at 5% significance level and square root transformation was used on count data (final nematode population density and number of galls)

CHAPTER 4

RESULTS

4.1 Effects of aqueous plant extracts on Final Nematode Population Density

There was a significant difference (P<0.001) in nematode population density control by aqueous plant extracts and Nemacur compared to the control. There was also a statistical difference between *A.hybridus, L.camara, T.minuta* Nemacur and the control which had an average of 631, 227, 198.7, 0 and 810.3 respectively.

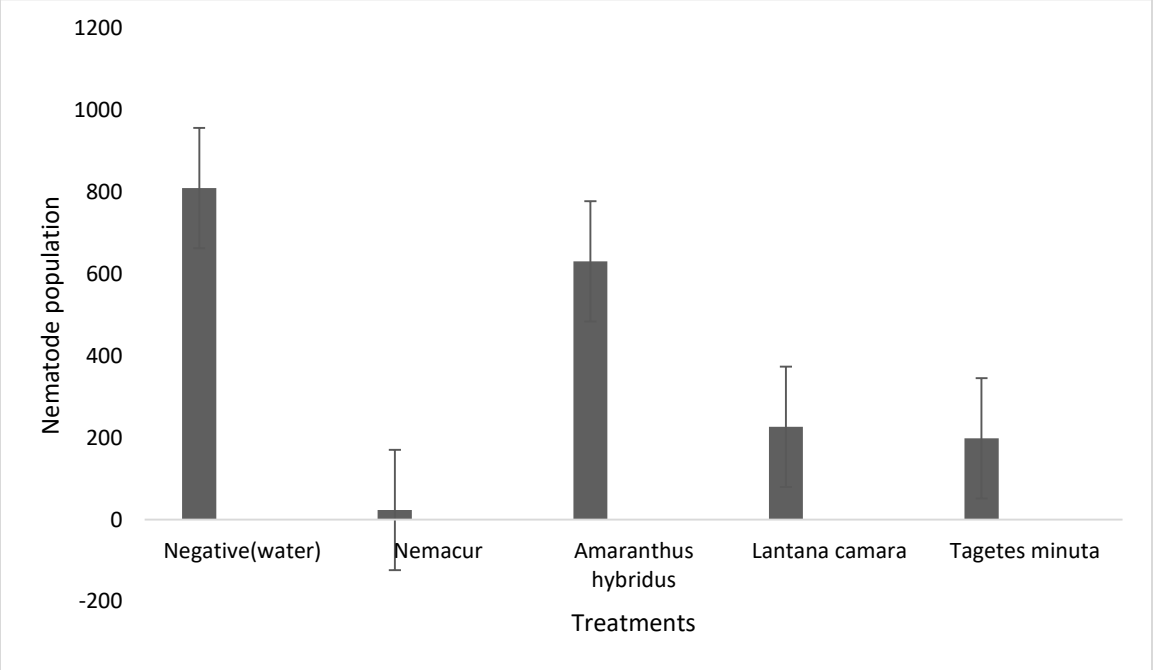


Fig 4.1 Mean final nematode population

4.2 Effects of aqueous plant extracts on days to 50% emergence

There was a significant difference (P<0.001) in days to 50% emergence between *A.hybridus* Nemacur and the control which showed a normal emergence time. However, there was no significant difference between *T.minuta* and *L.Camara* which showed a significant effect on the

number of days to 50% emergence. (Fig 2). There was no statistical difference between the control, Nemacur, and *A.hybridus* which generally had the same number days to emergence which is 8.67, 8.33 and 8.33 respectively. There was however a statistical difference between *L.camara, T.minuta* and the control on the number of days to 50% emergence and the average number of days is 9.67 and 9.33 respectively.

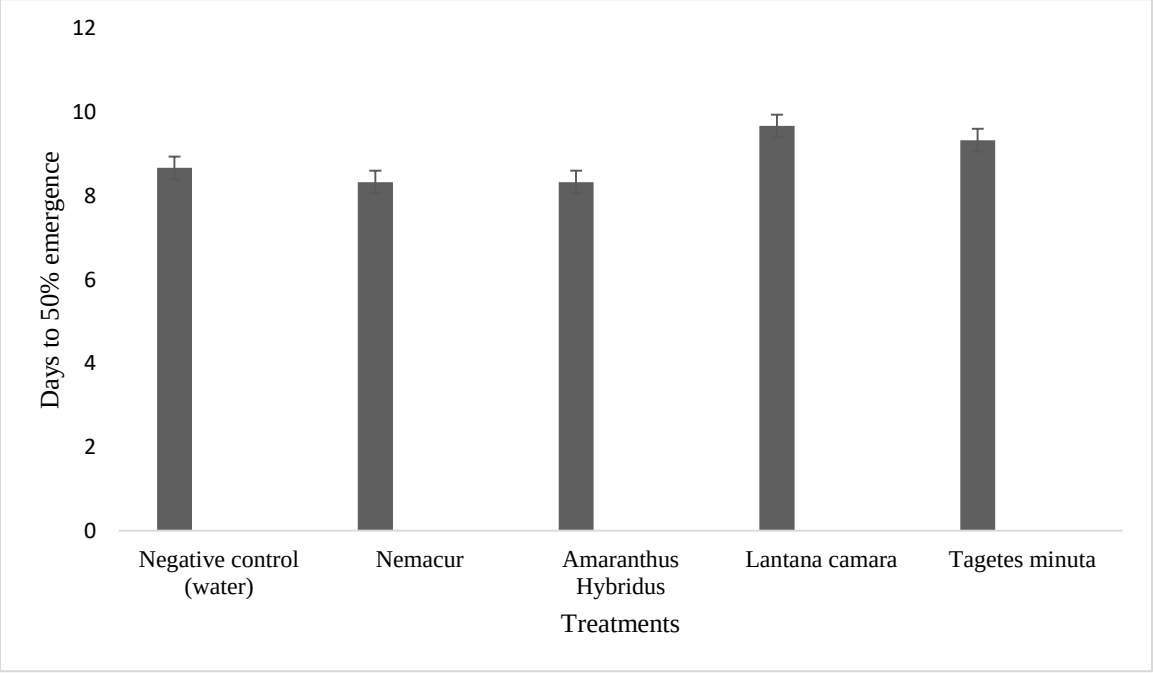


Fig 4.2 Mean number of days to 50% emergence

4.3 Effects of aqueous plant extracts on seedling height

There was a significant difference (P<0.001) between Nemacur, *T.minuta* and the control and there was no significant difference between the control, *L.camara* and *A.hybridus.* There was no statistical difference between the control, *A.hybridus* and *L.camara* which had a mean seedling height of 6.87, 5.97 and 7.33 respectively but there was a statistical difference between the control, *T.minuta* and Nemacur which gave an average of 5.97, 9.7 and 9.83. (Fig 4.3)

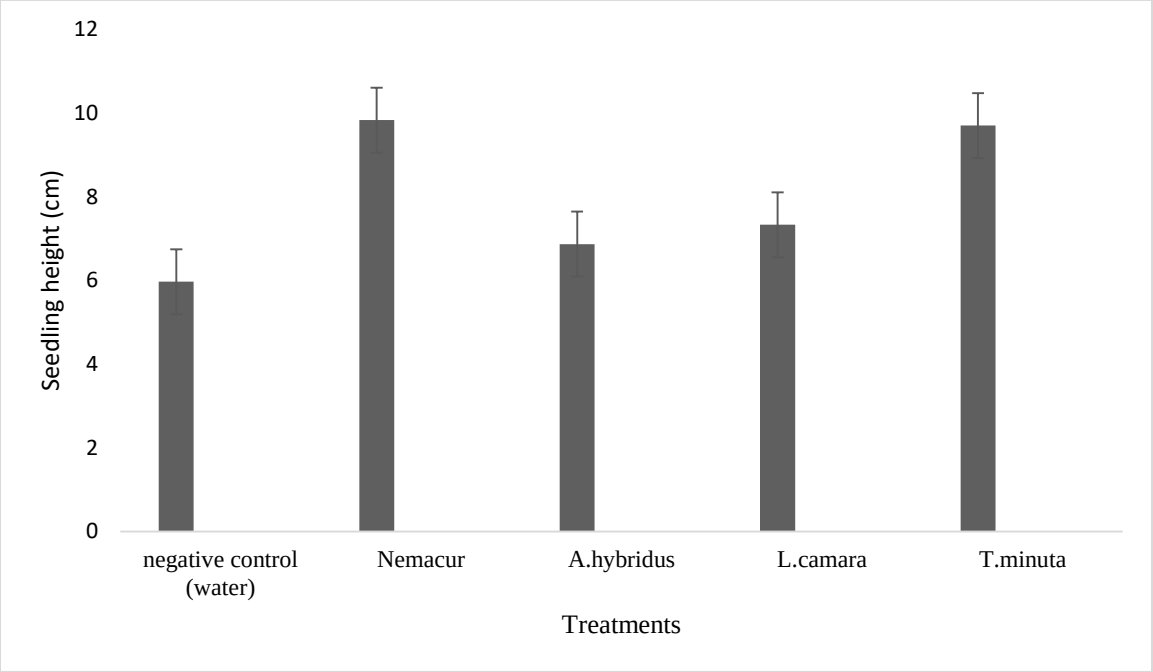


Fig 4.3 Mean seedling height

4.4 Effects of aqueous plant extracts on Dry matter content

There was a significance difference (P<0.001) between Nemacur, *L.camara* and *T.minuta* and the control and there is no significant difference between the control and *A.hybridus* in average dry matter content. There was a statistical difference between all the botanical plant extracts and the control in average dry matter content but no statistical difference between Nemacur, *L.camara* and *T.minuta* which had 0.27g, 0.2g and 0.24g respectively.

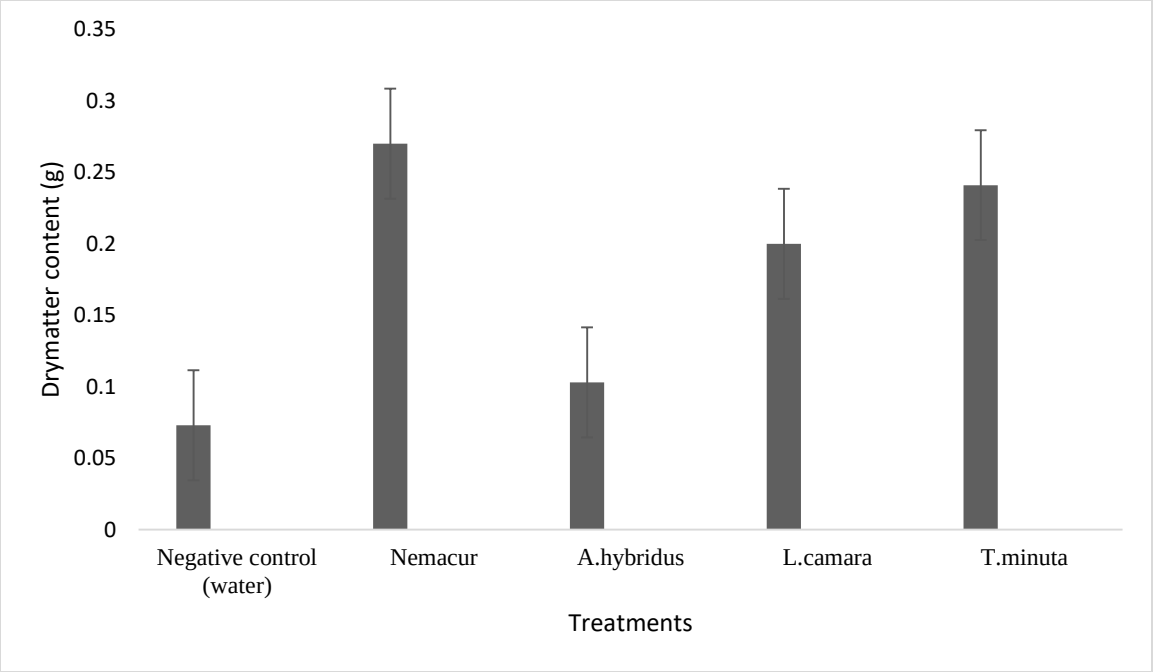


Fig 4.4 Mean Dry matter content

4.5 Effects of aqueous plant extracts on number of galls

There was a significant difference (P<0.001) between Nemacur, *L.camara, T.minuta* and the control on the average number galls per seedling. There was no significant difference between *A.hybridus* and the negative control on the average number of galls per seedling. There was a statistical difference between Nemacur, *A.hybridus, L.camara, T.minuta* and the control on the average number of galls per seedling with average numbers of 0, 4.67, 2.33, 2 and 9.33 respectively.

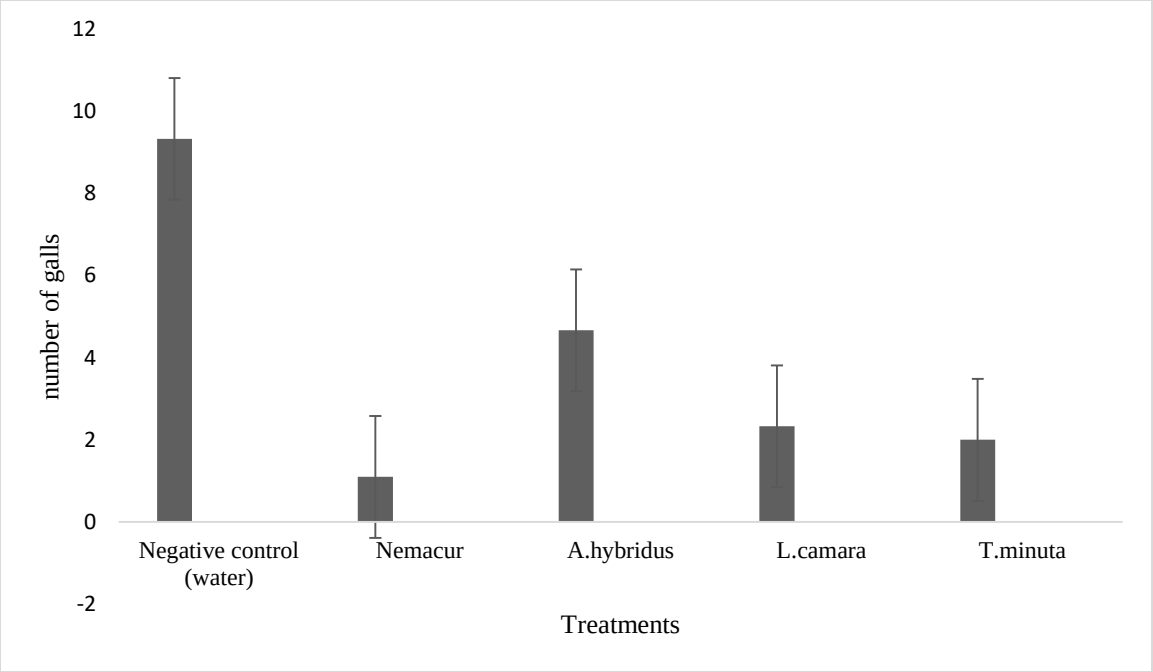


Fig 4.5 Mean number of galls

4.6 Effects of aqueous plant extracts on root: shoot ratio

There was a significant difference (P<0.001) between Nemacur, *L.camara, T.minuta* and the control on root: shoot ratio. There is no significant difference between *A.hybridus* and the control on root: shoot ratio. There was a statistical difference between Nemacur, *L.camara, T.minuta* and the control on root: shoot ratio which had measurements of 3.2, 2.1, 2.9 and 1.3 respectively. There was no statistical difference between *A.hybridus* which had 1.5 and the control on root: shoot ratio.

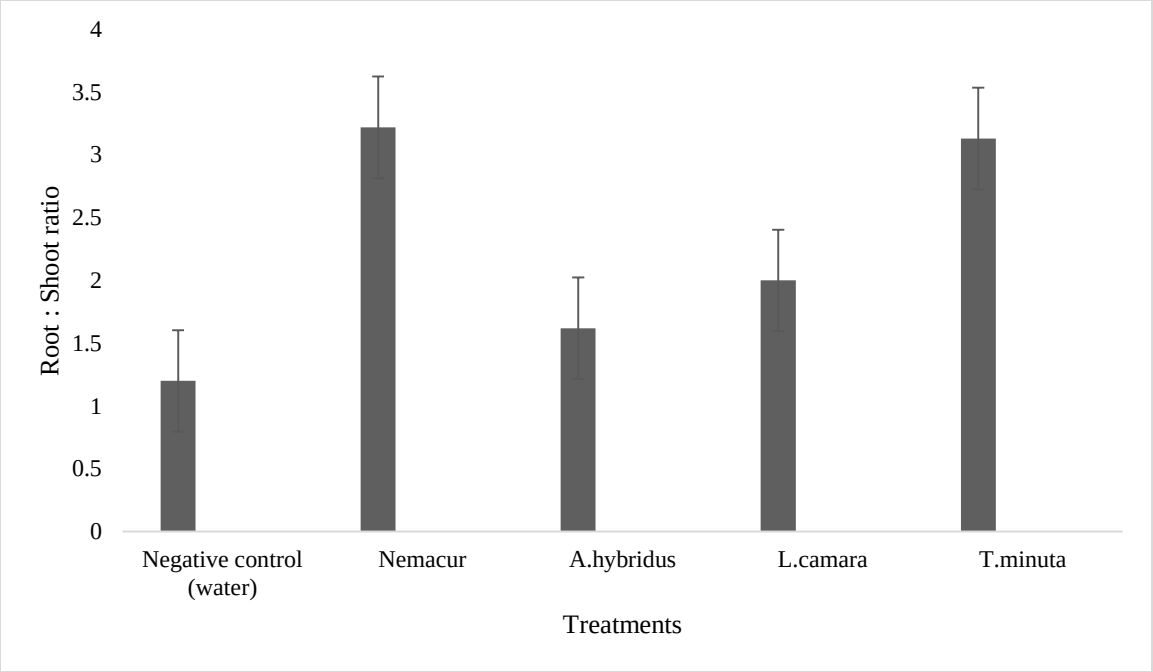


Fig 4.6 Mean Root: Shoot ratio

CHAPTER 5

DISCUSSION

5.1 Effects of aqueous plant extracts on Final Nematode Population Density

The results in Figure 1 indicate that Nemacur inhibited RKN survival and completely eradicated RKN population which would have led to the deprivation of plant nutrients and affect growth parameters findings suggested by Ploeg and Phillips (2001), and reinforced by Luc *et al.* (2005). Nemacur's active ingredient, fenamiphos is a systemic organophosphorous chemical that offers contact activity and inhibits the enzyme cholinesterase (an enzyme important in the transmission of neurosignals) and interferes with RKN nervous system (Bayer Environmental Science, 2003; Makhteshim Agricultural Industries Ltd, 2003).

L.camara and *T.minuta* extracts significantly suppressed root-knot nematode over untreated control thereby reducing the final population density considerably and *A.hybridus* showed no significant effect in suppressing and reducing population of nematodes but rather there was an increase in RKN population. This may be due to the fact that *A.hybridus* may vary in chemical composition and since these are water extracts, only polar compounds were extracted and the non-polar's effects failed to take effect. *L.camara* and *T.minuta* extracts' ability to affect nematodes may be because the nematicidal effect of the tested extracts may possibly be attributed to their high contents of certain oxygenated compounds which are characterized by their lipophilic properties that enable them to dissolve the cytoplasmic membrane of nematode cells and their functional groups interfering with the enzyme protein structure (Knoblock *et al.*, 1989). The mechanisms of plant extracts action may include denaturing and degrading of

proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation (Konstantopoulou *et al*., 1994).

The observed nematoxic effects of indigenous medicinal plants extract can be attributed to the presence of nematicidal saponins, flavonoids, tepernoids, acids and tannins that were seriously injurious to Meloidogyne javanica eggs. Saponins, flavonoids, tepernoids, acids and tannins were present in many botanical nematicides and are probably responsible for the toxicity of indigenous medicinal plants extracts to *M.javanica*. The compounds provide plant defense and provide resistance against nematode attack because flavonoids produce auxins with allellopathy effects. Flavonoids biosynthesis to isoflavonoid phytoalexins that confer resistance to nematodes. Phenolic hydroxyl group is also known to negatively affect nematodes activities. This observation agrees with various researchers who reported the effectiveness of flavonoid, tannin and saponin in reducing *M.javanica* population and enhancing egg-hatch inhibition of *M. javanica* in their various experiments. (Oyedunmade et al., (2001, 2004), Oyedunmade (2004))

In other researches, *L.camara* and *T.minuta* extracts recorded a reduction of root knot nematode population with corresponding increase in plant height, leaf and fruit yield over the control treatment. (Plant Pathology Journal 7(1):45-49, 2008)

5.2 Effects of aqueous plant extracts on days to 50% emergence

Results show that *T.minuta, L.Camara* had a significant effect on the number of days to 50% emergence whereas the control (water), Nemacur and *A.hybridus* showed no significant effect on the number of days to 50% emergence (Figure 4.2). *L.Camara* took the longest to reach 50% emergence followed by *T.minuta.* Negative control (water), Nemacur and *A.hybridus* took generally the same number of days to reach 50% emergence. The reason why *L.camara* and

T.minuta treatments took longer to emerge is because of the fact that they possess allelopathic chemicals which might have inhibited or suppressed germination, growth, development or metabolism of crops due to secretion of allelochemicals to the rhizosphere of neighboring crop plants (Qasem JR, 2006). Various phenolic compounds inhibited cell division. Many investigators have suggested phenolics as the cause of inhibition of metabolic process during germination. Possible damage of plasma membrane as a result of seed pretreatment with the leaf extracts (Hussain et al., 2011). Maiti et al. (Maiti et al., 2010)

5.3 Effects of aqueous plant extracts on seedling height

The results of this study show that all botanical extracts applied on had a significant effect on seedling height over the negative control (Figure 4.3). Nemacur was the most effective in increasing seedling height followed by treatment with *T.minuta and L.camara. A.hybridus* and control (water) showed the least effects on seedling height with increase showing no significant difference. Though *T.minuta* and *L.camara* and took longer to emerge, they improved on growth pattern and gave the second and third best results after Nemacur respectively over control.

This may be attributed to by the fact that there was good root establishment in these two treatments amongst the botanicals and also, reduced RKN activity giving rise to a favourable growth environment thought the two had an inhibitory effect on germination. *A.hybridus* had a poor seedling height close to that of the control and this may be due to mechanical damage associated with feeding or invasion of *M.javanica* which caused withdrawal of nutrients and impaired other physiological aspects. Generally damage reduced the rate of root extension which decreased the uptake of nutrients and water (Anwar and Din, 1986). Top growth of plant was affected due to the impaired water relations. This is probably due to developing giant cell which

interfere the nutrient uptake and developing xylem. Secondary effects include reduced photosynthetic efficiency with reduction in light interception and carbohydrate synthesis.

5.4 Effects of aqueous plant extracts on Dry matter content

The results show a significant difference in average dry matter content with botanicals and Nemacur over control which had very low dry matter content of about 0.07g. Nemacur was the most effective in increasing average dry matter content resulting in the highest shoot dry weight followed by *T.minuta*, *L.camara A.hybridus* and lastly the control (Figure 4). All the botanical extracts significantly increased the tomato seedling average dry matter content. This may be possibly due to the fact that by virtue of reducing *M.javanica* to population by botanicals, it provided a conducive environment for good plant growth and proper assimilate utilization since nematode presence hinders uptake of water and nutrient by the plants giving rise to improved average dry matter content.

5.5 Effects of aqueous plant extracts on number of galls

The extent of galling on roots (root-knot index) is a means for detecting the infestation of *M.javanica* in the roots and the damage or severity caused. In this study, botanical aqueous plant extracts were effective in reducing galling when compared with untreated plants and *A.hybridus* which showed no significant difference (Fig 4.5). Nemacur®400 Ec (fenamophos) use eradicated the inoculated nematode population completely. Nemacur, *L.camara* and *T.minuta* reduced galling significantly though Nemacur completely eradicated nematode population. *A.hybridus* showed no significant difference over control in reducing galling. Statistical analysis indicated that there was a direct relationship between root gall and production of egg masses and nematode population density. Plant generates more roots to overcome the limitations due to nematode

damage hence reducing root to galls per root system ratio (Trudgill, 1992). The reduced number of galls in botanicals maybe due to the fact that there was a reduced number of final nematode population with application of *L.camara* and *T.minuta* and Nemacur. This also increased plant growth generally hence enabling the seedlings to tolerate nematode attack. *A.hybridus* and the control had no significant difference in reducing the number of galls. This may possibly be because of the fact that they had a poor growth pattern which means that the seedlings were prone to nematode attack

5.6 Effects of aqueous plant extracts on root: shoot ratio

Nemacur®400 Ec (fenamophos) was the most effective resulting in the highest root: shoot ratio followed by *T.minuta, L.camara, A.hybridus* and control gave the least ratio (Figure 6). Nemacur®400 Ec (fenamophos), *T.minuta* and *L.camara* showed a significant effect on seedling root: shoot ratio over control and *A.hybridus* showed no significant difference. Statistically, Nemacur and *T.minuta* showed the greatest effect and *T.minuta* was the leading botanical in promoting good root: shoot ratio. It is crucial for a seedling of good quality to possess a good root: shoot ratio (Wondirad and Kifle, 2000).

Nematodes are bio-trophic pathogens which withdraw contents from cells, reduce water uptake and photosynthesis rate which in turn reduce leaf expansion and total photosynthesis. All this alter partitioning of photosynthates which ultimately changes in the root: shoot ratio. (Jones, J.D.G, Dangl, J.L 2006). The effect of each treatment on nematode population is directly related to the root: shoot ratio because Nemacur®400 Ec was the most effective with the highest ratio followed by *T,minuta, L.camara, A.hybridus* and negative control with the least. This is mainly

because there were no nematodes in the Nemacur®400 Ec treatment with the least seen in *T.minuta ,L.camara, A.hybridus* and negative control.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, the use botanical plant extracts may provide one of the most efficient, cheap methods of nematode control that are necessary and environmentally safe to smallholder farmers and produce end users. Other researchers (Oka *et al.*, 2000; Afouda, 2008) have reported successes in using various plant extracts in nematode management. Therefore, the use of indigenous plant extracts should be considered in integrated disease management strategies. It is suggested that further trials be conducted in the field on the basis of the promising results from these studies.

The investigation indicated that Nemacur (positive control) is the most effective in the suppression of nematodes and gave the highest increase in dry matter, root: shoot ratio, seedling height. A reduction in gall density and gall index in tomato seedlings was recorded. The nematicide suppressed root knot to the greatest extent. Out of different botanicals tested, *L.camara* and *T.minuta* leaf extract reduced mean gall density, mean final nematode population density. They also increased mean days to 50% emergence, mean seedling height, mean dry matter content and mean root: shoot ratio. *A.hybridus* had no effect on final nematode density and gall density and generally all growth parameters. In consideration of the alternative hypotheses, botanical plant extracts had a significant effect on the growth parameters of tomato seedlings except for *A.hybridus* that had little significance. *L.camara* retarded the number of days to 50% emergence and *T.minuta* enabled 50% emergence to occur a day after the seeds sown in control had emerged.

6.2 Recommendations

Although greenhouse pot experiments are sometimes warranted to remove external factors and to obtain a better assessment, more research under field conditions is desirable. Greenhouse temperatures might be too high for the extracts to fully express their nematicidal potential. Further, the extended effect of botanicals on naturally occurring free-living nematodes, nematode antagonists, and other beneficial soil organisms in tandem can only be assessed in a field environment. This information is needed to obtain a better assessment of the economic cost and practical feasibility of incorporating botanical extracts into an overall integrated pest management program.

Testing the activity of plant extracts with other solvents, time and method of application as a further research work could help in coming up effective control and management methods.

Farmers should use Tagetes minuta in the management of RKN since it has proven to be the best of all the botanicals used.

For the effectiveness of the botanicals, there is need for let the seedlings reach maturity and assess other parameters such as yield.

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APPENDICES

A 1: ANOVA for the effects of aqueous plant extracts on final Nematode Population

Analysis of variance

Variate: final_nematode_count

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	1345982.3	336495.6	620.46	<.001
Residual	10	5423.3	542.3		
Total	14	1351405.6			

A 2: ANOVA for the effects of aqueous plant extracts on days to 50% emergence

Analysis of variance

Variate: days_to_50%_emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	4.4000	1.1000	3.30	0.057
Residual	10	3.3333	0.3333		
Total	14	7.7333			

A 3: ANOVA for the effects of aqueous plant extracts on seedling height

Analysis of variance

Variate: seedling_height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	36.2893	9.0723	80.52	<.001
Residual	10	1.1267	0.1127		
Total	14	37.4160			

A 4: ANOVA for the effects of aqueous plant extracts on Dry matter content

Analysis of variance

Variate: dry_matter_content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	0.0813677	0.0203419	84.55	<.001
Residual	10	0.0024060	0.0002406		
Total	14	0.0837737			

A 5: ANOVA for the effects of aqueous plant extracts on number of galls

Analysis of variance

Variate: Number_of_galls

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	153.333	38.333	31.94	<.001
Residual	10	12.000	1.200		
Total	14	165.333			

A 6: ANOVA for the effects of aqueous plant extracts on root: shoot ratio

Analysis of variance

Variate: root_shoot_ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	9.87777	2.46944	83.26	<.001
Residual	10	0.29660	0.02966		
Total	14	10.17437			