An investigation into root-knot nematode (*Meloidogyne javanica*) control in tobacco (*Nicotiana tabacum* L.) using four Kutsaga seeds varieties, monitoring the population seasonal dynamics

By

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

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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.

Chitambira Leeroy. K. …… ……..

Student No. R09941Z Signature Date

CERTIFICATION OF THESIS WORK

I, the undersigned, certify that Chitambira Lee-roy K**,** a candidate for the Bachelor of Science Horticulture Honours Degree has presented this dissertation with the title:

An investigation into root-knot nematode (*Meloidogyne javanica*) control in tobacco (*Nicotiana tabacum* L.) using four Kutsaga seeds varieties, monitoring the population seasonal dynamics

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 **21/15/2015**.

SUPERVISORS: Mr. S. Muzemu and Miss M. Takawira

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ABSTRACT

Use of Tobacco (*Nicotiana tabacum L.*) resistant varieties in plant parasitic nematode management programs has been increasing in developing countries including Zimbabwe. Resistant varieties provide both economic and sustainable benefits for smallholder farmers who dominate the tobacco industry in Zimbabwe and produce their tobacco under continuous cultivation. A field trial was conducted at the Tobacco Research Board (TRB) during the 2014-2015 growing season to evaluate the effects of varietal resistance on *Meloidogyne javanica* galling index and populations. Four Kutsaga seed varieties, KRK 26, T70, T75 and KM10 were grown on land that was continuously cultivated with susceptible variety (KM10) for 3 seasons. Treatments were laid in a split plot design in four blocks. The untreated controls were fumigated crops with ethylene dibromide. Soil sampling from plots with tobacco varieties was done and bioassays were conducted using Tomato (*Solunum lycopersicum L.*) to determine the number of egg masses produced. Significant differences were observed across all results. Results on egg masses indicated that KRK 26, T70 and T75 managed to produce egg masses index less than 2 at $P<0.01$. Destructive sampling was done and results showed significant differences at $P<0.01$ on which T70 and T75 had a gall ratting less than 2 while KRK 26 had a gall rating above 2. Fresh leaf weights obtained from destructive sampled plants indicated that T70 and T75 had higher cumulative weight gain during the season. Varietal yield functions (total and saleable) were observed and results showed that T75 followed by T75 produced higher yield at $P<0.01$. Final root galling index was assessed and results highlighted that T70 and T75 had a gall rating less than 2. Both T70 and T75 exhibited resistant characteristics on *meloidogyne javanica* and produced a better crop than KRK26.

Key words: Tobacco, Resistant varieties, *Meloidogyne javanica*, populations, galling index

DEDICATION

This work is dedicated to my sister Mrs. Aloisio and her family, Mr. and Mrs. Ticharwa and Mutero family for their encouragement, love, care and support during the course of this study.

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ABBREVIATIONS AND ACRONYMS

AFDP	African Development Bank
EDB	Ethylene Dibromide
FAO	Food and Agriculture Organisation
GDP	Gross Domestic Product
GOZ	Government of Zimbabwe
IPM	Integrated Pest Management program
MeBr	Methyl bromide
PPNs	Plant Parasitic Nematodes
RKNs	Root-Knot nematodes
spp.	Species
TIMB	Tobacco Industry Marketing Board
TMV	Tobacco Mosaic Virus
TRB	Tobacco Research Board
ZTA	Zimbabwe Tobacco Association

CHAPTER ONE INTRODUCTION

1.1 Background of study

Tobacco (*Nicotiana tabacum* L.) is an important crop in the economy of Zimbabwe (FAO, 2010; Keyser, 2002) with its production dating back to 1894 (Zimbabwe Tobacco Association (ZTA), 2015). To date, tobacco production in Zimbabwe has experienced a tremendous increase with more than 120 000 hectares of land now under tobacco. This was facilitated by the land reform program in the year 2000 in which the government of Zimbabwe distributed land to indigenous small holder farmers (ZTA, 2015). Currently the number of small holder farmers records a total of ±100 000, yielding upto 180 million kg of cured tobacco leaf in 2014 that was valued at US$685.2 million (Tobacco Industry Marketig Board (TIMB), 2015). In 2012, tobacco contributed 19.4% to national Gross Domestic Product (GDP) (Biti, 2013). Zimbabwe is the forth leading exporter of tobacco and has generated 40% of its foreign currency earnings from tobacco exports (Masvongo *et al.*, 2013). Tobacco production in Zimbabwe also contributes 30% formal employment in the agricultural sector (Kapuya *et al.*, 2010; Anseeuw *et al.*, 2012). To add on, it creates non formal employment, foreign currency and it improves the livelihoods of both tobacco farmers and the nation at large (Chivuraise *et al.*, 2011; Masvongo *et al.*, 2013). Tobacco production however, may be severely reduced if grown in medium with soil borne pathogens and diseases. Some of the constrains to optimum crop quality and yield include plant parasitic nematodes (PPNs), weeds, soil or water borne fungal pathogens (Miller, 2007).

Plant parasitic nematodes (PPNs) are known to pose a serious threat to crops including tobacco (Tahery *et al.*, 2011), and annual crop losses due to PPNs are estimated at US$125 billion worldwide (Mwangi, 2011). Most of the losses are attributed to the root-knot (*Meloidogyne spp.*) and cyst nematodes (*Heterodera* and *Globodera spp.*) (Ibrahim and Traboulsi, 2009;

Fallon *et al.*, 2002). More than 90 species of root-knot nematodes (RKNs) have been reported (Handoo *et al.*, 2005) and the most common ones being the Southern *(Meloidogyne incognita)*, Javanese *(M. javanica)* and peanut nematode (*M. arenaria*) (Rich and Thomas, 2005). Symptoms of RKNs include stunted growth, wilting and damaged roots leading to poor yields and they leave the crop susceptible to other pathogens (Priya *et al.*, 2011).

Since tobacco is susceptible to root-knot nematodes, high priority must be given each time the crop is grown (Jimmy *et al.*, 2009). In Zimbabwe, management of RKNs was done primarily using chemicals (Oka, 2010), and among the chemicals that were used include Methyl Bromide (MeBr) and Ethylene DiBromide. These were used as soil fumigants both in seedbeds and lands (Muzhandu *et al.*, 2013; Mazarura, 2004). Other nematicides which are currently used tend to be volatile, toxic, non specific to target and pose a threat to the environment (Muzhandu *et al.*, 2013) and hence their use is becoming undesirable (Abuzar, 2009). However, despite the effectiveness of chemicals, widespread concerns over their effects on the environment have made them undesirable for use. Methyl bromide has been classified as a class 1 ozone depleter and scheduled to be phased out by 2015 in developing countries (Manyumwa *et al.*, 2013) hence an intergrated pest management system becomes an important parameter in managing nematodes (Paul, 2005).

Research indicates that future control of root-knot nematodes is dependent of integrated techniques which involve cultural practices and varietal resistance to keep population under threshold levels (Jimmy *et al.*, 2009). Development of resistant varieties has been deemed the most economic and effective way of managing plant parasitic nematodes (Zasada, 2010). Through the Tobacco Research Board (TRB), Zimbabwe has engaged into a breeding program to manage plant parasitic nematodes. The program incorporated the mi-1 gene which is the source of nematode resistance (McCarter, 2008). The gene is responsible for development of a thin layer around plant root and hinders nematode penetration (McCarter, 2008). In 2013, the

Tobacco Research Board released varieties on limited release which claim to have nematode resistance and these varieties can provide a break-through on rootknot nematodes in tobacco lands.

1.2 Problem Statement

In Zimbabwe, the agricultural sector is dominated by smallholder farmers and they grow tobacco under continuous cropping (mono-cropping) since the crop gives high returns (Mapfumo, 2015). Monoculture tends to favour buildup of more nematode populations in the soil. However, the mere presence of plant parasitic nematodes does not guarantee crop damage or loss of quality as long as the populations are below economic threshold. Factors which affect plant parasitic nematode populations include soil type, multiplication rate and cultivar grown (Muzhandu *et al.,* 2013; Faske *et al.*, 2014). Methyl bromide and ethylene dibromide are still the current fumigants used to suppress nematode populations by smallholder farmers and these fumigants are scheduled to be phased in developing countries by 2015 (Mazarura, 2004; Manyumwa *et al.,* 2013). In this context alternative measures using resistant varieties becomes the future of nematode control and hence this research seeks to evaluate varietal resistance as a control measure for nematodes.

1.3 Justification of the study

Tobacco is an important crop in Zimbabwe earning the country foreign currency and employment. Its management remain a critical problem on smallholder farmers. Many smallholder farmers grow tobacco repeatedly on the same piece of land and this has accompanied reports of resistant varieties KRK26 and T66 having been severely attacked by nematodes. The Tobacco Research Board has released two varieties (T70 and T75) under limited release to solve the nematode problem. These varieties have been improved in terms of resistance level and also they are high yielding. Therefore this research seeks to evaluate these

varieties on nematode infested soils grown continuously with susceptible tobacco and check if their yield potential will be reduced and also if they will suppress existing nematode populations. It will provide information that would pave way to sustainable nematode management for smallholder farmers who are continuously cultivating tobacco in Zimbabwe.

1.4 Main objective

The main objective is to evaluate the effects of varietal resistance on *M. javanica* virulence and populations on Kutsaga soils.

1.5 Specific objectives

a) To evaluate the effects of four Kutsaga seed varieties on *M. javanica* populations.
b) To evaluate the effects of *M. javanica* on the growth, quality and yield of four Kutsaga seed varieties.
c) To determine the damage index of *M. javanica* on four Kutsaga seed varieties.

1.6 Hypotheses

a) Four tobacco seeds varieties have no significant effect on *M. javanica* populations.
b) *M. javanica* has no significant effect on the growth, quality and yield of tobacco.
c) There are no significant differences on the damage index of four tobacco varieties

CHAPTER TWO LITERATURE REVIEW

2.1 Agriculture in Zimbabwe

The Agricultural sector in Zimbabwe is a key to economic growth contributing to 20% of the country's Gross Domestic Product (GDP) (Anseeuw *et al.,* 2012). The sector has also improved livelihoods of the country's 70% population who depend on agriculture (Robertson, 2011; Tekere *et al.,* 2015; Manyumwa *et al.,* 2013). Agriculture has contributed 30% formal employment to the economy of Zimbabwe (Kapuya *et al.,* 2010) and supplied 60 percent of the raw materials required by the industrial sector (GOZ, 2009). In Zimbabwe, agriculture has been allocated 33 million hectares of land (Tekere *et al.,* 2015). To date, the agriculture sector is dominated by smallholder farmers courtesy of the Land Reform program in 2000 which saw land ownership being transferred from white settlers to local Zimbabweans (Chivuraise *et al.,* 2011). There was a decline in agricultural produce from 2000 – 2010. In 2011, the agricultural sector rose by 4.6% and according to the statement of the Minister of finance, this was attributed by crops such as tobacco and wheat (Biti, 2013). In the same report by Biti (2013), tobacco (19.4%) was the second highest in export shipments behind minerals which accounted for (64%) and hence it is an important commodity.

2.2 Tobacco production in Zimbabwe

Tobacco (The Golden Leaf) is a major crop in Zimbabwe dominating the agriculture production (Masvongo *et al.,* 2013) and over the years it has generated foreign currency (FAO, 2010). Tobacco is mainly grown because of its sugar and nicotine rich leaves (Reddy, 2006). Tobacco (commonly known as nyoka) was traditionally grown by indigenous people of Zimbabwe since 1894. In 1897, the first commercial crop was grown in Chishawasha. By the year 2000, 92 000ha was under tobacco (ZTA, 2015). The government of Zimbabwe in 2000 launched the land reform program which distributed land from white farmers to majority black smallholder

farmers (Chivuraise *et al.,* 2011; Masvongo *et al.,* 2013). Tobacco industry was slightly affected reducing revenue from US$267million to US$73milion from 2000 to 2008 (Masvongo, *et al*., 2013) and recovered in 2009. The figure below shows tobacco harvested since 2009 to 2013;

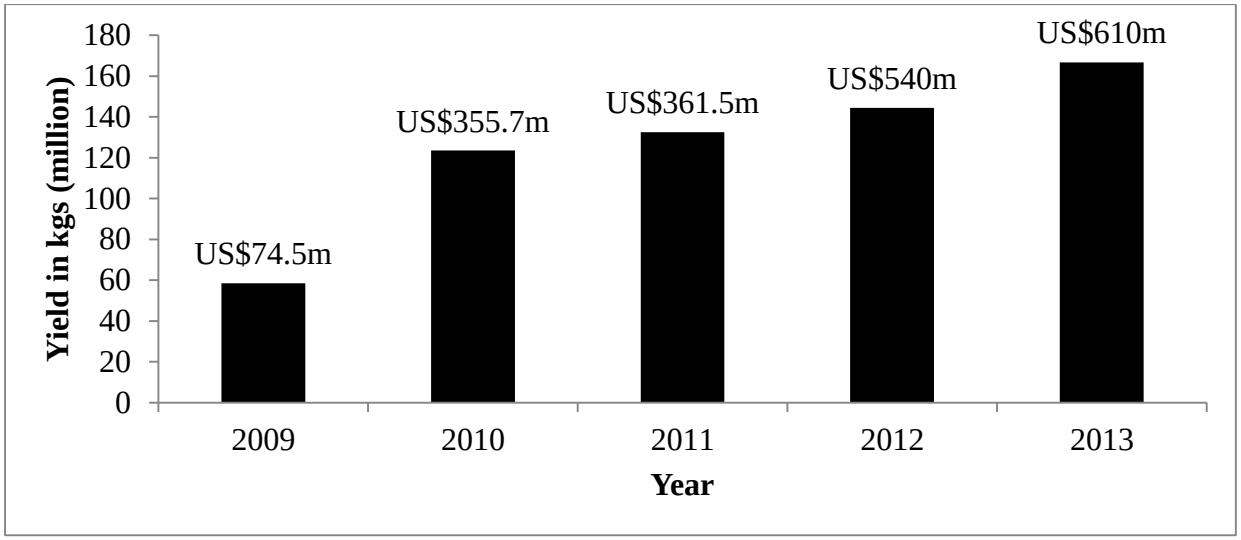

Figure 1. Tobacco yields from 2009 – 2013 seasons

(Source: ZTA, 2015)

In 2014, a 10% increase was recorded for new tobacco farmers and this resulted in a 30% rise in sales (TIMB, 2014). Approximately ±100 000 smallholder farmers were recorded in the 2013/2014 tobacco growing season and US$772.5m was generated in 2014 (ZTA, 2015). Out of the newly added tobacco farmers, about 39.5 percent are women showing an increase in women active in agriculture sector (Mutingwende, 2014). Zimbabwe is the now the fourth highest flue cured tobacco producing country and sixth in the world (Masvongo *et al.,* 2013; Chivuraise *et al.,* 2011). To date, 120 000ha is under tobacco and more yields is expected since the season was flooded with new varieties released on limited release by Kutsaga Research Station (ZTA, 2015). Some of these varieties include T70 and T75 which have been bred with 8 disease resistance potential (TRB, 2013). These varieties seek to address constrains in

production due to disease and poor varietal performance. These varieties need to be evaluated for 3 growing seasons on local conditions to ensure increase in revenue generated by tobacco.

2.3 Tobacco Crop

2.3.1 Taxonomy

Domain: *Eukarya*

Kingdom: *Plantae*

Phylum: *Magnoliophyta*

Class: *Magnoliopsida*

Order: *Solanales*

Family: *Solanaceae*

Genus: *Nicotiana*

Species: *tobaccum*

Figure 2. Tobacco crop in a field

Tobacco is a solanaceae crop and it shares the family with coffee, potato, tomato and pepper. It is a dicoty plant and has primary and vascular tissue. Crop height ranges from 1.2 – 1.8m and can have up to 25 leaves which are 60cm in size. Tobacco leaves are thick, ovate, covered with hairs and their arrangement on the stalk is spiral. The leaves contain nicotine and sugars which gives a pleasant aroma for the smoker (Reddy, 2006). Tobacco is a self-pollinator and produces 5 petals which are either white or pink. The flower can split into 2 or 4 parts at maturity.

2.3.2 Uses

Tobacco has many uses which are; manufacturing of cigarettes and medicines, used as a herb, ornamental plant and insect repellent. 98% of the tobacco produced in Zimbabwe is for exports and remaining 2% is for local cigarette companies (Sukume and Guveya, 2009).

2.3.3 Ecology

Tobacco can be grown in tropical and sub-tropical regions mostly in Africa, Asia, America and Brazil. It does well in areas that receive annual rainfall of +500mm, temperature of between 20 -30°C with 27°C being the optimum and relative humidity of 80 – 85% (Reddy, 2006). Soil requirements are deep well drained black clays (50 – 80% clay) and pH that ranges from 5.8 – 6.5 (Muzhandu *et al.,* 2013; Dale and Neilson, 2006). The diagram below shows tobacco growing regions in Zimbabwe;

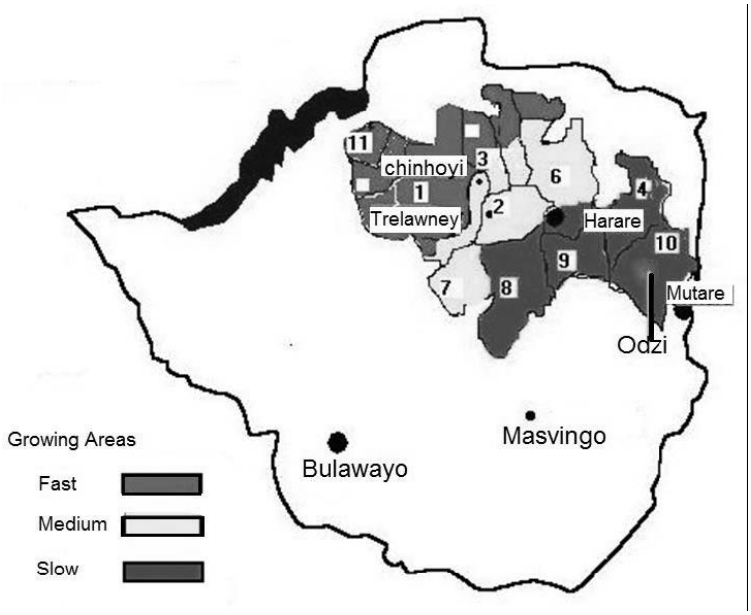

Figure 3. Tobacco growing regions in Zimbabwe

(Source: Mudzengereri, 2014)

2.3.4 Diseases and Pests

Tobacco is a high value crop but it is susceptible to a wide range of diseases which are; alternaria leaf spot, black shank, wild fire of tobacco, angular leaf spot, tobacco mosaic virus (TMV) and rook not nematodes (TRB, 2014).

2.4 Root-knot nematodes

Root knot nematodes are microscopic sedentary obligate-parasites which feed tissue (Massawe, 2010; Smiley, 2005). Females (22 – 37nm) are white and spherical with a slender neck. Males are small, thin and wormlike (887 – 1268nm). They are characterized by the presence of a stylet which they use on feeding. More than 2500 species have been identified (Gowen *et al.*, 2009).

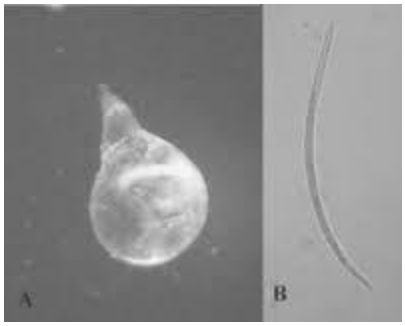

Figure 4. A female (A) and male nematode (B)

2.4.1 Meloidogyne javanica Taxonomy

Kingdom: *Animalia*

Phylum: *Nematoda*

Class: *Sercenentea*

Order: *Tylenchida*

Familly: *Meliodogynidae*

Genus: *Meloidogyne*

Species: *javanica*

(Source: Göldii, 1892)

2.4.2 Host range

More than 2000 plants are known to be susceptible to root-knot nematodes (Muzhandu *et al.,* 2013; Gowen *et al.,* 2005).Root-knot nematodes affect numerous plant species such as perennial woody ornamental and plantations (Levin, 2005), vegetables, crops (FAO, 2005) and weeds species (Gowen *et al.,* 2005). Some of the affected plants include eggplant, pepper, tomato, potatoes, carrot, cabbage, sugar cane, banana, sweet potatoes, pine apple, spinach and tobacco (James *et al.,* 2010; Massawe, 2010; Onkendi *et al.,* 2014).

2.4.3 Symptoms of root-knot nematodes

Root-knot nematodes cause above and below damage to crops prior infection. Notable symptoms include root galls (swells), chlorosis and yellowing of leaves, stunted growth and wilting. Extreme cases include plant death and distorted morphology of roots (Massawe, 2010). Overleaf are pictures showing some of the symptoms of root-knot nematode infection;

Figure 5. Stunted growth and yellowing of leaves (A) and showing plant death (B)

Figure 6. Tobacco root galls

2.4.4 Ecology and Distribution

Plant parasitic nematodes survive mostly in temperate, tropical and sub-tropical regions which are warm. They survive in the soil, dead plant material and live plant material (Gowen *et al.*, 2005). Nematodes favour sandy loams especially fine sands which have low water holding capacity (Dale and Neilson, 2006). They require temperature ranging from 25 - 30°C and temperature below 10°C is fatal for *M. javanica.* Relative soil humidity for optimum growth ranges from 40 – 60% and less than that would result in lower nematode activity. Soil pH ranges from 5.8 – 6.5. Too much fertilized soils can also affect nematode activity as they increase the electric charge of the soil (Khan *et al.,* 2012). Plant parasitic nematodes are distributed around the world and then diagram overleaf shows distribution in Africa;

Figure 7. Distribution of plant parasitic nematodes (marked in red) in Africa

(Source: http://www.infonet-biovision.org)

2.4.5 Biology and Mode of action

The life cycle of root-knot nematodes is comprised of 3 stages which are egg, larva (juveniles) and adult. The life cycle occurs in the soil, plant debris and living plant roots (Smiley, 2005). Time needed to complete the cycle ranges from 21 – 28 days at average temperature of 30°C (Massawe, 2010). Females lay eggs inside the plant roots or surface of roots and soil surface. Each female can lay up to 500 – 1500 eggs per cycle depending on prevailing environmental conditions. The eggs hatch after a process embryogenesis into a juvenile (J_1) which molts four times (J_2 – J_4) to become mature. Second juvenile (J_2) is the most problematic and infectious one (James *et al.*, 2010). The second juvenile is vermiform and depends on the host to survive. It penetrates host cell by disrupting host tissue through repeated thrusting of its stylet into the cell. Also, the juvenile can secrete enzymes that digest pectins and cellulose surrounding the host cell for easy access (Gowen *et al.*, 2005). Nematode can enter host cell through injured host roots and newly developing secondary roots which are soft. Once inside, the nematode moves through or between host cells to establishes a feeding site in the cortex. Once the feeding site is established, the nematode alters the appearance or morphology of the host cell by disintegrating cells forming a multinucleate cell (giant cell) in which it will feed for the rest of

its life (Dhandaydham *et al.*, 2008; Gowen *et al.*, 2005). Each nematode can feed on six giant cells causing extensive damage to crop. The damage on cells results inhibition of growth causing moisture stress to the crop as the roots cannot draw moisture for photosynthesis. The roots will then provide oxygen, moisture and secure habitat for the nematode. Root-knot nematodes can complete up to eight generations per year if not managed (Massawe, 2010).

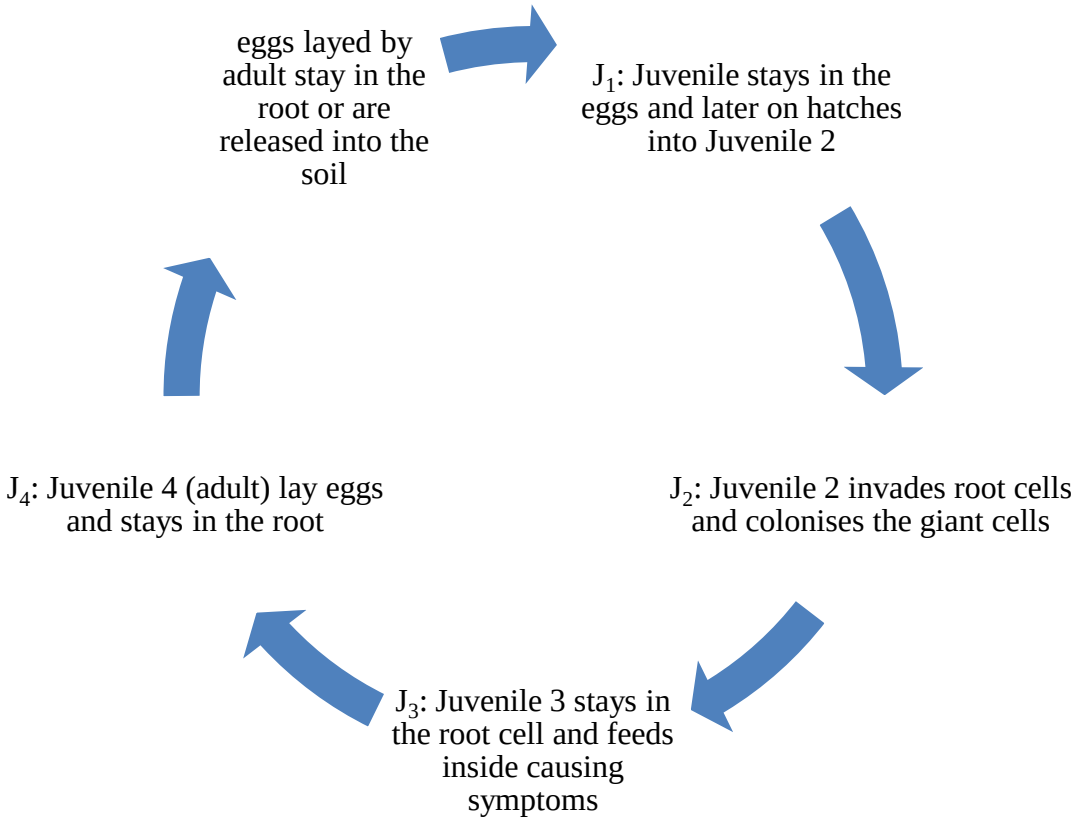

Figure 8. Root-knot nematode life cycle

2.4.6 Method of spreading

Plant parasitic nematodes are spread in infested soil, planting material, crop debris, irrigation water and unfumigated mulch. Male juveniles (J_2) can move in wet soil when looking for host but the female does not move but rather is moved through vectors and other natural ways.

Humans can also be agents of spread through use of infected footwear and faming tools. Dormant stage occurs on eggs which lie on living or dead plant material (James *et al.,* 2010).

2.5 Economic Importance

Root-knot nematodes are a major pest of field crops causing yield losses of up to US$125 billion worldwide on all crops (Mwangi, 2011). They are among the top 5 pathogens causing crop losses worldwide. About 2000 crop spies have been reported to have affected by root-knot nematodes (Muzhandu *et al.,* 2013). Losses on tobacco have been reported to be Most losses are attributed to the genus *Meloidogyne* (root-knot nematodes) and *Globodera* (cyst nematodes) (Ibrahim and Traboulsi, 2009). More than 90 *Meloidogyne spp.* have been reported to have caused crop losses (Handoo *et al*., 2005) and the most common being *M. javanica*, *M. hapla*, *M. incognita* and *M. arenarea* (Rich and Thomas, 2005; Onkendi *et al.,* 2014; Massawe, 2010) which cause 95% of the infections (Dhandaydham *et al.,* 2008). Plant parasitic nematodes induce crop injury which results in secondary infection by other pathogens. Root-knot nematodes also form disease complexes with other soil borne pathogens (FAO, 2005). Some disease complex include bacterial wilt caused by *M. javanica + R. solanacearum*, bacterial wilt caused by *M. incognita + R. solani* and vascular wilt rot caused by *M. javanica + fusarium oxysporum* (Khan *et al., 2012).*

2.6 Population densities and host relationship

The mere presence of plant parasitic nematodes in the soil does not guarantee crop damage as long as the population densities remain below damaging levels (Schomaker and Been, 2006; Khan, 2008). The threshold level is different with nematode species and is affected by factors such as environment, soil type, temperature, previous cropping, distribution, reproduction rate and the cultivar used (Fourie, 2010; Massawe, 2010). Knowledge of population densities and host relationship is important in implementing a control strategy (Schomaker and Been, 2006).

A nematode management plan must limit nematode populations below threshold levels and it should make use of prevention, monitoring and eradicating methods for effective control.

2.7 Management of Root-knot nematodes in Zimbabwe

The current nematode control program in Zimbabwe is summarized in an Integrated Pest Management (IPM) approach (Paul, 2005). This approach employs a number of control option both chemical and non-chemicals that monitors, minimizes population densities, and determines threshold levels and eradication of pathogen (Muzhandu *et al.,* 2013; James *et al.,* 2010). An IPM consists of preventative, cultural and physical, nematicides, host resistance and biological (Massawe, 2010). Integrated pest management approach aims at preventing primary attack, minimize secondary infection and achieve maximum yield at low cost James *et al.,* 2010).

An IPM makes use of both chemical and non-chemical instruments giving an economic and effective solution to disease (Karavina *et al.,* 2012). An IPM can therefore be deemed successful is crop rotations, biological control, soil amendments and resistant cultivars are used properly (Jimmy *et al*., 2009).

2.7.1 Chemical

Control of plant parasitic nematodes in Tobacco is mainly based on chemicals which is the application of inorganic formulations to interfere with pathogens (Onkendi *et al.,* 2014). Methyl bromide has been used as an effective fumigant and has been successfully used in Zimbabwe in managing plant parasitic nematodes ion seedbeds (Zasada, 2010). According to statistics provided by the Kutsaga Research Station, dependence on chemicals was between 30 – 40% of smallholder farmers (Karavina *et al.,* 2012). However, fumigants such as methyl bromide and EDB have been classified as class-1 ozone depletes and are scheduled to be phased out in developing countries by 2015 as agreed in the Montreal protocol of 1997 (Karavina *et*

al., 2012; Zasada, 2010; Muzhandu *et al.,* 2013). These chemicals are still widely used despite the ban and most of the chemical alternatives are currently not registered for use (Karavina *et al.,* 2012; Zasada, 2010). Other chemicals like methyl iodide (Karavina *et al.,* 2012), abamectins (Muzhandu *et al.,* 2013; Faske and Starr, 2007) and metham sodium (Karavina *et al.,* 2012) have been adopted for use on tobacco in recent years. These chemicals on the other hand have a broad spectrum targeting both target and non-target organisms and are a threat to the environment and humans (Paul, 2005; James *et al.,* 2010) hence their use is not sustainable (McCarter, 2008; Quarles, 2005). The use of chemicals has raised alarm due to the widespread concerns on the environment and has increased interests in exploring other options for control (Abuzar, 2009).

2.7.2 Cultural Controls

Cultural control refers to a collection of non-chemical activities or practices that are used to reduce parasite survival rate or reproduction (Karavina *et al.,* 2012).

2.7.2.1 Sanitation

Sanitation involves use of clean seeds, seedlings, mulch material, and destruction of stocks at harvest to minimize disease spread from external sources (Goven *et al.,* 2009; James *et al.,* 2010).

2.7.2.2 Organic amendments

Plant parasitic nematodes have been reported to have been controlled using organic amendments. Some of the organic amendments include use of chicken dropping, cow and goat manure, mustard, neem, marigold, dried cabbage leaves, garlic and green manure (Karavina *et al.,* 2012). Organic amendments increase the number of fungivorous and bacteriovorous nematodes which are predators of plant parasitic nematodes (Smiley, 2005; Massawe, 2010).

2.7.2.3 Rotations

Crop rotation with a non-host is one of the effective non chemical ways in managing nematodes populations in the soil. The Kutsaga Research Station recommends a four year rotation system with Tobacco, katambora rhodes grass, and fallow. Other rotations include asparagus, corn garlic and brassicas (Wang, 2004; Mwangi, 2011). However, rotations are unsustainable for smallholder farmer as they require time and larger areas (Karavina *et al.,* 2012).

2.7.3 Physical methods

2.7.3.1 Solarisation

Solarisation is a process of eradicating nematodes within the root zone (15 – 30cm) using black or clear polythine plastic (James *et al.,* 2010). The process lasts for 30 days for complete control (Karavina *et al.,* 2012). However it is costly for smallholder farmers since it require more plastic in field.

2.7.3.2 Flooding

Flooding is a process by which a field is left with water for seven months or more to kill nematodes by suffocation as it depletes the available oxygen in the soil (Karavina *et al.,* 2012). The process also increases concentrations of chemical that are toxic to plant parasitic nematodes like methane in the soil. However flooding is not recommended for smallholder farmers since it require large volumes of water (Karavina *et al.,* 2012).

2.7.3.3 Steaming

This is one of the preventive ways of controlling nematodes by subjecting the soil to temperatures of more than 70^{o}C. The process is not sustainable since it eradicates all organisms present in the root depth (Karavina *et al.,* 2012).

2.7.4 Biological control

Biological pest control is defined as the use of a living organism to kill or suppress a pest population to non-lethal densities (Stoner, 2011). It is considered the most economic and environmentally safer approach in controlling pests (Stoner, 2011). Fungi like *Trichodemaharzianum, Hirsutellarhossiliensis* and *Hirsutella*mimesotensis have been used in controlling plant parasitic nematodes (James *et al.,* 2010). Entomo-pathogenic nematodes (EPNs) belonging to the *Sternematidae and Herterorhabitidae* genus have been widely used in controlling insect pests and nematodes. The problem of biological control is that it is time consuming to establish the agent into the environment and does not eradicate pathogens (Karavina *et al.,* 2012).

2.7.5 Varietal resistance

Resistance can be defined as the ability of a crop to suppress nematode reproduction reducing the population to non-damaging levels or the ability of a crop to resist nematode penetration (William and Kumar, 2006; Massawe, 2010). Resistance in tobacco is characterized by host cell death at the feeding site of the parasite (Karavina *et al.,* 2012). Naturally, cell death is as a result of a hypersensitive response by crop prior infection (Tomczak *et al.,* 2008). Root-knot resistant varieties have been available in Zimbabwe courtesy of Kutsaga Research Station (Karavina *et al.,* 2012) and these have provided an economic and environmental safe control of nematode for smallholder farmers (Jimmy *et al.,* 2009; Zasada, 2010; Onkendi *et al.,* 2014). However, some crops suffered nematode attack even if they had resistance in fields that had higher nematode populations. In higher nematode infested areas, the crop compensate yield through a hypersensitive response at the nematode feeding site (Sikora and Bridge, 2005). Other reports indicate that resistance is broken down by higher temperatures and this would explain why some resistant cultivars produced in Zimbabwe are suffering nematode attack and hence resistance on its own may not give full nematode control (Massawe, 2010).

2.8 Developments in Breeding for resistance

Nematode resistance in crops is characterized naturally by detection of nematode by host followed by a hypersensitive response at the feeding site (William and Kumar, 2006). The host also can respond by altering the feeding site of the parasite such that the nematode experience difficulty in penetrating (McCarter, 2008). Resistance can be termed tolerant or intolerant whereby tolerance refers to the crops that show little or no plant damage prior nematode infection. Intolerant crops may show serious signs but they compensate on the yield. A successful resistance program should reduce nematode penetration, reproduction, reduce symptoms and provide economic yield (McCarter, 2008). Some sources of resistance were recorded on tomatoes using *Mi*-genes (*M. incognita*) (Roberts, 1982) and R-genes which are immune response genes (Roberts, 1982). These genes acted by first detecting pathogen then respond by a localized hypersensitive response (HR) that include cell death or altering the feeding site (William and Kumar, 2006). The Mi and R-genes have been used also on other crops such as sugar beet, potatoes and tobacco and successfully controlled *M. javanica*, *M. incognita* and *M. hapla*. In tobacco the Mi-gene has been reported to act by repeating a leucine protein around the feeding site making it difficult for the nematode to enter (Goggin, 2006). Other reports indicate that mi-genes triggers formation of giant cells around d the second juvenile head (McCarter, 2008). Massawe (2010) highlighted that the mi-genes confers resistance in a heterozygous state that result in a thin layer around the root. In Zimbabwe through the Kutsaga Research Station breeding department managed to produce root-knot nematode resistant varieties but there have been reports of varieties like KRK 26 (which is the most cultivated by smallholder farmers) being affected by nematodes (Massawe, 2010). With relative information provided by other research, resistance has been broken down by higher soil temperature and this could explain the fate of Zimbabwe since the country is experiencing severe climate changes (Onkendi *et al.,* 2014). Research by Karavina *et al.* (2012) and Sikora

and Bridge (2005) indicated that initial populations due to continuous cultivation can reduce yield since the crop would invest more on hypersensitive response than on yield. The future of nematode control in tobacco production can be centered upon breeding better varieties which put in consideration other factors that may break resistance. In Zimbabwe other tobacco varieties have been released on limited release with nematode resistance and if these show positive results on resistance under continuous cultivation may give a break through. However resistance plus other control measures such as chemicals can give the best economic and environmental nematode control (Massawe, 2010).

CHAPTER THREE METHODOLOGY

3.1 Study site

The research was carried out at Kutsaga Research Station which is located 15km east of Harare city (17^0 55′ S, 31^0 08′ E). The station lies within Agro-ecological Region IIa. It experiences a sub-tropical climate and receives an annual rainfall of 800 - 1000mm and average annual temperatures of 18 and 30^0C in winter and summer respectively (Muzhandu *et al.,* 2013). Nyamapfene (1991) classified the soils in order III of the Zimbabwean soil classification, belonging to the group 6 kaolitic soils derived from coarse sand grains from granite. Soils are deep and well drained with a pH of 5.2 based on soil tests and is ideal for tobacco.

3.2 Experimental design and treatments

The plots were laid out in a split plot design with four replications. The main plot factor was fumigation and the subplot was variety. The varietal treatments were as follows;

Table 1 Description of Kutsaga seeds that were used

KRK26	Fast to medium maturing variety most cultivated in Zimbabwe and it does not require intensive management.
T70	Medium to slow maturing, imitates KRK26 and T71, require proper fertilization and has a higher yield potential of >4000kgs/ha. Bred with nematode resistance and is under limited release.
T75	Most promising, relatively slow maturing. Exhibit nematode resistance and has a potential of >4000kgs/ha and is under limited release.
KM10	Susceptible to root-knot nematodes

3.3 Agronomic practice

This research was carried out in the tobacco field. Prior to this experiment, the field chosen was continuously cultivated with tobacco variety KM10 which is susceptible to tobacco from 2012-2014. Having this history the land was ploughed and sunflower was sown for three months to boost nematode population. Soil sampling then followed using a 20mm auger which takes samples within 20cm. A kilogram kg sample was collected from each plot and potted in the greenhouse with tomatoes which are indicator plants. After four weeks the tomato root was assessed for nematode damage using the galling scale ranging from 0 - 8; 0 being a clean plant and 8 being the most damaged (Daulton, 1961). Information obtained from bioassays indicated the presence of plant parasitic nematodes.

The land was ploughed again followed by discing and 34 plots measuring 3.6m x 16.8m were constructed. Each plot consisted of two rows sandwiched by two guard rows which blocked external factors. The main plot factor was fumigation and this was done in each plot. The other half of the plot was fumigated using Ethylene DiBromide (EDB) at the rate 21ml/m^2 two weeks before planting to prevent phytotoxicity to crop and the other half left. Clomazone was applied to control weeds in unfumigated plots. Planting stations were marked at 1.2m x 0.56m and then watered. Basal fertilizer (compound S) was applied at the rate 600kgs per ha Healthy seedling collected from the Kutsaga farm seedbed were planted and soil around was firmly pressed. Watering was done to field capacity.

Irrigation was done on a weekly basis. Weeding was done whenever weeds emerged. Top dressing was done at the rate 160kg/ha and staggered at four and eight weeks post transplanting the seedlings using cup number 2 (30g) per plant and the final at topping time (when the crop had developed 18 - 20 leaves) using the same cup size. Topping was done by removing the apical part of the crop. Other general management practices were done according to Kutsaga Tobacco Handbook production manual (Kutsaga Tobacco Handbook, 2012).

3.4 Measurements

Systematic soil sampling using the Z-scheme was chosen for this research to reduce bias during the sampling process (Muzhandu *et al.*, 2013). Three samples were taken across, three diagonal and the across following the zigzag sampling technique. The samples were mixed in a 5L bucket and mixed thoroughly then the soil was used for bioassay in the green house. Soil samples were collected at 3 weeks after planting (wap) then resumed every 5 weeks (8, 13 and 18 wap). Soil samples were taken using a 20mm auger extracting soil within 20cm and samples collected were potted with tomato and left for four weeks. After four weeks, roots from tomato plants from bioassays were removed, washed and stained with 300ml of flaxen B solution for 5mins. This was done so that the egg masses from the roots would be more visible for counting. After staining, the roots were chopped into small particles and then added into a solution containing 1.05% sodium hypochloride (NaOCl) and shaken vigorously for 5mins. The solution was run through 200-mesh sieve and 500-mesh sieve (74 and 25µ). 1ml of the solution was then extracted and nematode counting was done under a light microscope.

1 egg mass = 500 – 1000 eggs (Coyne and Ross, 2014).

Egg masses were recorded for each plot using the scale below.

1 = 1 to 2 egg masses; **2** = 3 to 10 egg masses; **3** = 11 to 30 egg masses; **4** = 31 to 100 egg masses; **5** = More than 100 egg masses per root system (Taylor and Sasser, 1978)

Destructive sampling was also conducted at 5, 12, 16 and 20 weeks after planting. Plant selection was according to the randomization structure generated and three plants were selected in each plot. The procedure involved uprooting the whole plant and assessing the root for galling using the Daulton, (1961) scale of 0-8. After rating the plant, fresh leaves were harvested and weighed to assess the cumulative gain or loss.

Plant height as a growth parameter was measured at harvest and this was achieved by measuring all the plants per plot and then only the mean plot height was recorded. Harvesting was done and yield was recorded (total yield) and after processing (marketable yield). The remaining crop stocks was destroyed and the roots were assessed for nematode damage using the galling score which range from 0 - 8 by Daulton (1961).

Table 2 Galling Score

Galling score	Description
0	No gall
1	Traces of infection (less than 5galls)
2	More than 5 galls lees than 10 galls
3	11 to 30 galls
4	31 to 50 galls
5	51 to 75 galls (coalition of galls)
6	75 to 100 galls (root extensively damaged)
7	> 100 galls (severe root damage)
8	Root completely damaged (dead plant)

Daulton (1961)

3.5 Data Analysis

Data was collected on plant height, yield and nematode damage score were analysed for analysis of variance (ANOVA) using GenStat (14th edition). Means were separated using least significant difference (LSD) at 5% significance level.

CHAPTER FOUR RESULTS

4.1 Effects of four Kutsaga seed varieties on *Meloidogyne javanica* populations

There were significant differences ($p<0.01$) for the results from bioassays (Table 3). At week 8, unfumigated KM 10 had the highest number of egg masses (1.5) which was an 18% increase in egg masses compared with the fumigated plots. However, all the other crops had no egg masses recorded (both in fumigated and unfumigated plots). There was an increase in egg masses (31% and 41%) in both week 13 and week 18 with unfumigated KM 10 recording the highest number of egg masses (2.5 and 3.5 respectively). In week 13, both unfumigated T70 (0.25) and T75 (0.5) were comparable to the fumigated treatments which all had no egg masses. In week 18 unfumigated T70 (1) was comparable to unfumigated T75 (0.75) which was also comparable to fumigated crops. On the final sample taken at harvest, unfumigated KRK 26 (1.25), T70 (0.75) and T75 (1) were significantly different from unfumigated KM10 (3.75) which had the highest recording. However, these (unfumigated KRK 26, T70 and T75) were comparable to fumigated KM10 (1).

Table 3 *Meloidogyne javanica* mean egg masses on four Kutsaga seed varieties at weeks 8, 13, 18 and 20 after planting

Treatment	Varieties	Weeks			
		8	13	18	20
Unfumigated	KRK 26	0^{a}	1^{b}	1^{b}	1.25^{b}
	T70	0^{a}	0.25^{a}	1^{b}	0.75^{b}
	T75	0^{a}	0.5^{a}	0.75^{ab}	1^{b}
	KM 10	1.5^{b}	2.5^{c}	3.25^{c}	3.75^{c}
Fumigated	KRK 26	0^{a}	0^{a}	0^{a}	0^{a}
	T70	0^{a}	0^{a}	0^{a}	0^{a}
	T75	0^{a}	0^{a}	0^{a}	0^{a}
	KM 10	0^{a}	0^{a}	0.25^{ab}	1^{b}
Fumigation*variety	F-probability	<.001	<.001	<.001	<.001
SED	different levels fumigation	0.1443	0.2394	0.2795	0.2976
	same levels fumigation		0.2083	0.2917	0.2917
LSD	different levels fumigation	0.3019	0.5193	0.5817	0.6246
	same levels fumigation		0.4377	0.6128	0.6128
CV%		38.5	29.6	24	16.2

4.2 Effects of *Meloidogyne javanica* on the growth of four Kutsaga seed varieties

The results obtained from the final plant height showed significant differences ($p<0.01$) across varieties grown in different levels of fumigation. The highest plant height (61cm) was obtained from fumigated KRK26 followed by fumigated KM 10 which had a height of 60.75cm (Table 4). A general trend was observed from the results on which the fumigated crops had heights which were significantly different from the non-fumigated crops except for unfumigated KRK 26 (46.25cm) which was significantly different from both T70 and T75 (new crops). The least plant height result (33.5cm) was observed from T70 and KM 10 from unfumigated crops.

Table 4 Effects of *Meloidogyne javanica* on four Kutsaga seed varieties plant height

Treatment	Varieties	Plant height (cm)
unfumigated	KRK 26	46.25[c]
	T70	33.5[a]
	T75	34.75[a]
	KM 10	33.5[a]
Fumigated	KRK 26	61[d]
	T70	39[b]
	T75	39.75[b]
	KM 10	60.75[d]
Fumigation*variety	F-probability	<.001
SED	different levels fumigation	1.671
	same levels fumigation	1.66
LSD	different levels fumigation	3.498
	same levels fumigation	3.488
CV%		5.4

Significant differences ($p<0.01$) on the weights obtained from destructive sampling were noted. These differences occurred starting from weeks 12, 16 and 20 (Table 5). On week 12, KRK 26 (711.2g) from the fumigated plots had the highest weight and KM 10 (174.2g) from unfumigated plots had the lowest weight. Both T70 (600.5g) and T75 (601.2g) from unfumigated plots were comparable to the fumigated plots. On week 16, the highest weight recorded was 813g obtained from fumigated T70 whilst the least weight (189g) was recorded for unfumigated KM10. A general trend similar to that of week 12 was observed on which there was a rise in figures from the unfumigated crops to the fumigated crops. However, no significant differences were observed between unfumigated T70 (722g) and T75 (758g) and the fumigated crops. Similarly, at week 20 a linear trend was observed to that one in week 16. Both T70 (860g) and T75 (850g) from unfumigated plots were significantly different with KRK 26 (617g) and KM10 (108g) and were comparable with all the fumigated crops. Highest weight

recorded was 919g from fumigated T75 and the least weight was 108g from unfumigated KM10.

Table 5 Effects of *Meloidogyne javanica* on four Kutsaga seed varieties fresh leaf weight for weeks 12, 16 and 20 after planting

Treatment	Variety	Weeks		
		12	16	20
Unfumigated	KRK 26	517[b]	580[b]	617[b]
	T70	600.5[c]	722[c]	860[c]
	T75	601.2[c]	758[c]	850[c]
	KM 10	174.2[a]	189[a]	108[a]
Fumigated	KRK 26	711.2[d]	755[c]	818[c]
	T70	657.2[cd]	764[c]	900[c]
	T75	671[cd]	813[c]	919[c]
	KM 10	478.8[b]	705[c]	856[c]
fumigation*cultivar	F-probability	<.001	<.001	<.001
SED	different levels fumigation	29.92	53.5	51.1
	same levels fumigation	26.17	57.9	55.2
LSD	different levels fumigation	64.82	111.2	106.2
	same levels fumigation	54.98	121.7	116
CV%		6.7	12.4	10.5

4.3 Damage index of *Meloidogyne javanica* on four Kutsaga seed varieties

Significant differences ($p<0.01$) for gall ratings on destructive sampling as at weeks 12, 16 and 20 (Table 6). At week 12, no gall ratings were recorded for the fumigated crops but however, the highest gall rating was recorded for unfumigated KM 10 (5). Unfumigated T75 and KRK 26 recorded a gall rating of 1. There was an increase in gall rating (75% for KM10) in week 16 for unfumigated crops with KM 10 recording the highest gall rating of 6 followed by T75 and KRK 26 which had an average rating of 2.5. Trace infection appeared for T70 (1) and the fumigated crops had no infection. At week 20, unfumigated KM 10 recorded the highest gall rating of 7.25. There were reductions in gall ratings for unfumigated T70 (0.5) and T75 (1.5) from recordings from week 16 (that is 13-6% and 31-19% respectively). From the fumigated plots, no infection were recorded for KRK 26, T70 and T75 with only KM10 recording a trace infection of 0.5. Unfumigated T70 (0.5) was comparable to the fumigated plots.

Table 6 Damage index of *Meloidogyne javanica* on four Kutsaga seed varieties from destructive sampling done at weeks 12, 16 and 20 weeks after planting

Treatment	Variety	Weeks		
		12	16	20
Unfumigated	KRK 26	1^{b}	2.25^{c}	2.25^{b}
	T70	0^{a}	1^{b}	0.5^{a}
	T75	1^{b}	2.5^{bc}	1.5^{b}
	KM 10	5^{c}	6^{d}	7.25^{c}
Fumigated	KRK 26	0^{a}	0^{a}	0^{a}
	T70	0^{a}	0^{a}	0^{a}
	T75	0^{a}	0^{a}	0^{a}
	KM 10	0^{a}	0^{a}	0.5^{a}
Fumigation*variety	F-probability	<.001	<.001	<.001
SED	different levels fumigation	0.2041	0.3461	0.3062
	same levels fumigation		0.3359	0.2887
LSD	different levels fumigation	0.4269	0.728	0.6484
	same levels fumigation		0.7058	0.6065
CV%		33	32.3	27.2

Significant differences ($p<0.01$) were noted for final gall rating done at harvesting (Table 7). KM 10 had an outstanding recording of 6 (75 percent increase). From the unfumigated plots, only T70 (0.5) was comparable to the fumigated plots. T70, T75 and KRK 26 had gall ratings below 2. No infections were recorded for fumigated KRK 26, T70 and T75, only KM 10 had a trace infection of 0.5.

Table 7 *Meloidogyne javanica* final gall rating at harvesting

Treatment	Variety	Final gall rating
Unfumigated	KRK 26	1.75[b]
	T70	0.5[a]
	T75	1.5[b]
	KM 10	6[c]
Fumigated	KRK 26	0[a]
	T70	0[a]
	T75	0[a]
	KM 10	0.5[a]
Fumigation*variety	F-probability	<.001
SED	different levels fumigation	0.3385
	same levels fumigation	0.3656
LSD	different levels fumigation	0.7041
	same levels fumigation	0.7681
CV%		40.4

4.4 Effect of *Meloidogyne javanica* on yield of four Kutsaga seed varieties

Results for both total yield at harvest and saleable showed significant differences at ($p<0.01$) (Table 8). Highest total yield obtained was recorded from fumigated T75 (964.5g) and the least total yield was 118.2g obtained from unfumigated KM 10. Both results for unfumigated T70 and T75 (909.9g and 899.8g respectively) indicated that there were comparable to the fumigated treatments although there were significantly different with unfumigated KM 10 and KRK 26. After processing, the results followed the same trend on which the highest and lowest saleable yields were obtained from fumigated T75 (941g) and unfumigated KM 10 (98.5g) respectively. KRK 26, T70, T75 and KM10 lost 7, 3, 3 and 17% weight respectively after processing. Both unfumigated T70 (875g) and T75 (873g) were comparable to the fumigated crops.

Table 8 Effects of *Meloidogyne javanica* on yield of tobacco varieties at harvest and saleable

Treatment	Variety	Total harvest yield(g)	Total saleable yield (g)
Unfumigated	KRK 26	651.8^{b}	604.5^{b}
	T70	909.8^{cd}	875^{cd}
	T75	899.8^{cd}	873^{cd}
	KM 10	118.2^{a}	98.5^{a}
Fumigated	KRK 26	866.8^{c}	829.2^{c}
	T70	958^{cd}	930.2^{cd}
	T75	964.5^{cd}	941^{cd}
	KM 10	883.2^{c}	853.2^{c}
Fumigation*varieties	F-probability	<.001	<.001
SED	different levels fumigation	33.42	33.21
	same levels fumigation	35.8	35.49
LSD	different levels fumigation	69.5	69.07
	same levels fumigation	75.2	74.56
CV%		6.5	6.7

CHAPTER FIVE DISCUSSION

5.1 Effects of four tobacco seeds varieties on *Meloidogyne javanica* populations

The results indicated that the four varieties had significant increase or reduction on the nematode populations. KRK 26, T70 and T75 from the unfumigated plots managed to reduce the egg mass index number to below 2 which is economic threshold level according to Kankam and Adomako (2014). Egg masses are produced when adult female lay eggs inside the roots of the plant forming layers. Larger indexes of egg masses can be achieved if the nematode populations are high hence the fact that the egg mass indexes were lower than 2, it means that the population of nematodes used for bioassays had lower populations. As highlighted by Onkendi *et al.,* (2014), tobacco varieties in Zimbabwe which have nematode resistance have been bred using the Mi-1 and thus the case of KRK 26, T70 and T75. The above three crops (KRK 26, T70 and T75) could have used a mechanism of resistance to reduce nematode entry and this has been reported in some researches by Massawe (2010) and Karavina *et al.,* (2012) which indicated that crops bred with the mi-1 resistant genes can reduce or prevent nematodes from entering the root cell hence low egg masses. KM10 however produced higher indexes of egg masses as it does not have the mi-1 genes present in KRK 26, T70 and T75.

5.2 Effect of *Meloidogyne javanica* on the weight, quality and yield of tobacco

The results indicated that *M. javanica* had no significant effect on the growth (weight), quality and yield of T70 and T75. Both T70 and T75 were comparable to the fumigated crops as shown in the progressive weight gain, total yield and saleable yield. As outlined in the previous section (5.1) T70 and T75 managed to reduce nematode entry and by so doing, the crops could have accessed nutrients and water without disturbance. Dhandaydham *et al.,* (2008) explained this when he said that nematodes interfere with nutrient and water uptake so any prevention of entry

would result in high yields. Yield can be linked to the function of the rate of photosynthesis and nutrient uptake hence without damaged root crops can maximise on nutrient and water uptake. This could have been what made T70 and T75 to have higher yields even in the presence of plant parasitic nematodes. However T70 and T75 did not attain maximum plant heights in the unfumigated plots. This may be linked to the results of a research by Sikora and Bridge (2006) which highlighted that crops that exhibit nematode resistance can compensate on stature and thicker roots in order to retain a high yield value. The research also outlined that the crop can invest more energy to the site of infection so as to trigger a hypersensitive response that result in cell death at site of entry. This could have been a case of T70 and T75 since their overall result showed higher yield returns. KRK 26 was not comparable to T70 and T75 and this could have been so since they had damage indexes above 2 as explained in section 5.3.

5.3 Effect of *Meloidogyne javanica* on the damage index of four tobacco varieties

The results show that there were significant differences on the galling index of the 4 varieties. T70 and T75 had their gall ratings less than 2 which were significantly different from KRK 26 and KM 10. The results also showed that infestation on T70 and T75 occurred in the late stages of the growing cycle. These results can be linked to nematode resistance which is defined by William and Kurma (2006) as the ability of a crop to reduce or prevent nematode penetration into the root. The fact that there was less gall produced would mean that there was limited pathogen entry into the host root and this could have been the case of T70 and T75. Karavina *et al.,* (2012) also supports the same phenomenon by that crops with nematode resistance induce cell death at the point of entry there by inhibiting pathogen entry. The results of his research also showed similar characteristics with T70 and T71. Onkendi *et al.,* (2014) highlighted that tobacco varieties in Zimbabwe that exhibit nematode resistance were bred using the mi-1 gene isolated from tomatoes (quoted from Roberts, 1982) and this can be linked

to the case of T70 and T75 which have this gene. The presence of the mi-1 gene might also have delayed pathogen entry by a process of repeating a leucine protein on the point of entry as supported by Goblin (2006). However, KRK 26 in the unfumigated plots was affected by nematodes as it had a gall rating above 2. This can be linked to the results of the finding by Massawe (2010) which highlighted that KRK 26s resistance can be broken by high temperatures in the soil. Temperature could have played a row in weakening KRK 26's resistance since the season experienced some dry spells.

CHAPTER SIX CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This research has shown that plant parasitic nematodes can be managed by cultivation of resistant varieties. The resistant varieties tested in this research (T70 and T75) were successful in suppressing root-knot nematodes and exhibited resistance characteristics. The number of egg masses produced from the inoculum extracted from the soil samples taken was below index 2 which is the economic one. Root-knot nematodes did not reduce the quality, yield and growth for both T70 and T75. The research showed also that cultivation of T70 and T75 varieties ion tobacco lands that are continuously grown with tobacco can be fruitful and successful. The two hybrids (T70 and T75) also proved to produce high yields than KRK 26 and KM10.

However, the research indicated that KRK 26 is a low resistance crop and can suffer nematode attack under continuous cultivation. Its growth, quality and yield have been affected negatively when grown in unfumigated lands. Also, the research highlighted that KRK 26 perform better than KM 10 when grown under continuous cultivation but not as successful as T70 and T75.

6.2 Recommendations and further study

- The resistant varieties used for this research (T70 and T75) can be grown on nematode infested soils under continuous cultivation in order to reduce populations since they have shown to reduce egg masses produced from soil samples taken from their plot. KRK26 should be grown with supplement nematicides as they have shown no to reduce nematode populations significantly.
- T70 and T75 can be used to optimise growth and yield of tobacco grown in nematode infested soils since they managed to produce higher yield. They can be classified as

resistant crops since they managed to thrive well in nematode infested soil and produced higher yields.

- T70 and T75 have shown resistance by inhibiting or limiting nematode damage on the crop and can therefore be grown on soils with higher nematode populations to reduce the damage on the crop. KRK 26 and KM10 should be grown with nematicides to reduce the damage on the crop as they have responded negatively to nematode attack.
- However, there is need to test these varieties for another season to check if they will produce the same results. More so, factors affecting resistance should be evaluated so as to produce varieties that will last longer.

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APPENDICES

Appendix 1 Analysis of variance for egg masses at 8 weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.12500	0.04167	1.00	
Fumigation	1	1.12500	1.12500	27.00	0.014
Residual	3	0.12500	0.04167	1.00	
Variety	3	3.37500	1.12500	27.00	<.001
Fumigation*variety	3	3.37500	1.12500	27.00	<.001
Residual	18	0.75000	0.04167		
Total	31	8.87500			

Appendix 2 Analysis of variance for egg masses at 13 weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.59375	0.19792	1.00	
Fumigation	1	9.03125	9.03125	45.63	0.007
Residual	3	0.59375	0.19792	2.28	
Variety	3	6.09375	2.03125	23.40	<.001
Fumigation*variety	3	6.09375	2.03125	23.40	<.001
Residual	18	1.56250	0.08681		
Total	31	23.96875			

Appendix 3 Analysis of variance for egg masses at 18 weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.8438	0.2812	2.45	
Fumigation	1	16.5312	16.5312	144.27	0.001
Residual	3	0.3438	0.1146	0.67	
Variety	3	10.0938	3.3646	19.78	<.001
Fumigation*variety	3	6.5938	2.1979	12.92	<.001
Residual	18	3.0625	0.1701		
Total	31	37.4688			

Appendix 4 Analysis of variance for egg masses at 20 weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	0.5938	0.1979	1.00	
Fumigation	1	16.5312	16.5312	83.53	0.003
Residual	3	0.5938	0.1979	1.16	
Variety	3	21.3438	7.1146	41.82	<.001
Fumigation*variety	3	4.8438	1.6146	9.49	<.001
Residual	18	3.0625	0.1701		
Total	31	46.9688			

Appendix 5 Analysis of variance for tobacco plant height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	3	8.375	2.792	0.48	
Fumigation	1	1378.125	1378.125	237.95	<.001
Residual	3	17.375	5.792	1.05	
Variety	3	1658.125	552.708	100.24	<.001
Fumigation*variety	3	652.625	217.542	39.45	<.001
Residual	18	99.250	5.514		
Total	31	3813.875			

Appendix 6 Analysis of variance for fresh leaf weight at 12 weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Block stratum	3	4576.	1525.	0.50	
Fumigation	1	195469.	195469.	63.98	0.004
Residual	3	9166.	3055.	2.23	
Variety	3	541561.	180520.	131.82	<.001
Fumigation*variety	3	81609.	27203.	19.86	<.001
Residual	18	24651.	1369.		
Total	31	857032.			

Appendix 7 Analysis of variance for fresh leaf weight at 16 weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	3	48555.	16185.	5.86	
Fumigation	1	310275.	310275.	112.43	0.002
Residual	3	8279.	2760.	0.41	
Variety	3	544833.	181611.	27.08	<.001
Fumigation*variety	3	293973.	97991.	14.61	<.001
Residual	18	120723.	6707.		
Total	31	1326637.			

Appendix 8 Analysis of variance for fresh leaf weight at 20 weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	3	9675.	3225.	1.26	
Fumigation	1	558624.	558624.	218.12	<.001
Residual	3	7683.	2561.	0.42	
Variety	3	860937.	286979.	47.06	<.001
Fumigation*variety	3	652569.	217523.	35.67	<.001
Residual	18	109775.	6099.		
Total	31	2199264.			

Appendix 9 Analysis of variance for galling index destructive sampling at 12 weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	3	0.25000	0.08333	1.00	
Fumigation	1	24.50000	24.50000	294.00	<.001
Residual	3	0.25000	0.08333	1.00	
Variety	3	29.50000	9.83333	118.00	<.001
Fumigation*variety	3	29.50000	9.83333	118.00	<.001
Residual	18	1.50000	0.08333		
Total	31	85.50000			

Appendix 10 Analysis of variance for galling index destructive sampling at 16 weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	3	0.8438	0.2812	1.00	
Fumigation	1	69.0312	69.0312	245.44	<.001
Residual	3	0.8438	0.2812	1.25	
Variety	3	27.5938	9.1979	40.75	<.001
Fumigation*variety	3	27.5938	9.1979	40.75	<.001
Residual	18	4.0625	0.2257		
Total	31	129.9688			

Appendix 11 Analysis of variance for galling index destructive sampling at 20 weeks

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	3	0.7500	0.2500	1.00	
Fumigation	1	60.5000	60.5000	242.00	<.001
Residual	3	0.7500	0.2500	1.50	
Variety	3	63.2500	21.0833	126.50	<.001
Fumigation*variety	3	45.7500	15.2500	91.50	<.001
Residual	18	3.0000	0.1667		
Total	31	174.0000			

Appendix 12 Analysis of variance for final gall rating at harvest

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	3	0.5938	0.1979	1.73	
Fumigation	1	42.7812	42.7812	373.36	<.001
Residual	3	0.3438	0.1146	0.43	
Variety	3	43.0938	14.3646	53.73	<.001
Fumigation*variety	3	28.8438	9.6146	35.96	<.001
Residual	18	4.8125	0.2674		
Total	31	120.4688			

Appendix 13 Analysis of variance total harvested tobacco leaf yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	3	6562.	2187.	1.75	
Fumigation	1	597324.	597324.	478.66	<.001
Residual	3	3744.	1248.	0.49	
Variety	3	1001773.	333924.	130.30	<.001
Fumigation*variety	3	678617.	226206.	88.27	<.001
Residual	18	46128.	2563.		
Total	31	2334148.			

Appendix 14 Analysis of variance total saleable tobacco leaf yield

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Blocks stratum	3	5334.	1778.	1.40	
Fumigation	1	608029.	608029.	479.51	<.001
Residual	3	3804.	1268.	0.50	
Variety	3	993470.	331157.	131.45	<.001
Fumigation*variety	3	647645.	215882.	85.69	<.001
Residual	18	45346.	2519.		
Total	31	2303628.			