EFFICACY OF NEEM, TITHONIA AND TEPHROSIA LEAF EXTRACTS IN MANAGEMENT OF ROOT-KNOT NEMATODES IN FRENCH BEANS(*Phaseolus vulgaris* L.) IN THARAKA NITHI COUNTY

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DECLARATION AND RECOMMENDATIONS

Declaration

This thesis is my original work and has not been previously presented for the award of a degree in any other University.

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Recommendations

This thesis has been examined, passed and submitted with our approval as University Supervisors.

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ii

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DEDICATION

I dedicate this thesis to my family and friends and all those who assisted me in prayers.

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ABSTRACT

Agricultural production within the smallholder farming sector of East Africa is constrained by numerous factors including parasitic nematodes.Existing control measures involving applications of chemical nematicides are not viable in the medium to long term due to environmental concerns relating to their toxic residues. There is therefore a need to develop alternative control options for integrated parasitic nematode management that will promote soil eco health and reduce parasitic nematode densities. Meloidogyne spp is a major problem in Frenchbeans (*Phaseolus vulgaris* L.) productionsystems. In the search for alternatives to synthetic control of nematodes, a study was conducted at Chuka University Horticultural Demonstration Farm to determine the phytochemicalconstituents and nematicidal effects of crude extracts of Neem, Tithonia and Tephrosia on the control of root-knot nematodes in Frenchbeans. Crude extracts were subjected to phytochemical screening for the detection of various bioactive constituents. Constituents in Neem were alkaloids and saponins, In the invitro experiment the LC50 value of each extract was determined by assessing the mortality of juveniles and egg hatch (in the range of 5–95%) after 24, 48 and 72 hr for seven days. In a lath house pot and field experiments to determine the efficacy of the crude extract application on Frenchbeans root-knot nematodes, the Fenchbeans were planted on nematode infested soils and data on growth, development and yield components was collected. In the lath-house 5 kg pots were filled with steam-sterilized soil and infested with second stage juveniles of *Meloidogyne* spp.Similarly in the field experiment the planting holes were inoculated with second stage juveniles of *Meloidogyne* spp. The data collected was subjected to analysis of variance and significantly different means were separated using Tukey's Studentized Range Test at *P*≤0.05. In the phytochemical analysis Tithonia extracts showed the presence of alkaloids and flavonoids while Tephrosia only showed the presence of flavonoids. In *invitro* study, comparison between LC50 values of the extracts indicated that Neem and Tithonia at 100ml/l were the most effective on the mortality of juveniles and immobilized more than 80% of the juveniles treated. On egg hatching Neem extracts at 100ml/l were the most effective with over 90% inhibition. Among extracts treatments evaluated, crude leaves extracts obtained from Tephrosia at 100ml/l inhibited the greatest egg hatching at 0.8 eggs. Oxymyl (positive synthetic control) inhibited the greatest hatching among the treatments at 0.2 eggs. From the study the greatest egg hatching was observed in in the untreated controls at 5.5 eggs. Among the extracts evaluated, maximum mortality was recorded with Neem at 100 ml/l at 12.2 juveniles. Oxymyl inhibitory effects was recorded at 9.4 juveniles while the least inhibitory effects were observed in the untreated control at 4.3 juveniles. In both lath-house and field studies crude extract treated plots when compared to the positive control significantly had higher number of pods and pod weight. From the study it was observed that the untreated control treatment attained the least pods weight of 24.9 and 28.0 in Trials I and Trials II respectively. Neem at 50ml/l attained the highest average mean pods weight of 50.9 in both Trials I and Trials II .Oxymyl attained an average weight of 48.7 and 49.3 in Trials I and Trials II respectively. Untreated control recorded the least mean number of pods at 9.4 and 9.5 pods respectively in Trials I and Trials II. Neem at 100 ml/l attained the highest average mean number of pods at 17.4 and 17.6 pods respectively in Trials I and Trials II. (positive control) attained 15.3 and 15.5 pods respectively in Trials I and Trials II.BothLath house and field experiments indicated that the crude extracts tested had varying effects, with the majority of them reducing galling on Frenchbeans. The Neem extracts treatments had the lowest mean galling index followed by Tithonia.There was a significant (P ≤ 0.05) difference ingalling indices and yield between the leaves extract treatments and the control in both field and lath house pot Trialss. Root-knot nematode galling indices were highest in the untreated controlat 10a clear indication that the crude extracts suppressed the root-knot nematodes. There was however no significant difference between galling efficacies of crude extracts and Oxamyl in the lath house pot experiment. Once adopted, this integrated approach will result in increased yields and income to smallholder farmers. The crude extracts are affordable, easy to apply as well as environment friendly and hence sustainable over a long period of lime.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

µm	Micro meter
ANOVA	Analysis of variance
ABD	Agri-Business Development
BRC	British Retail Consortium
CIAT	International Centre for Tropical Agriculture
CV	Coefficient of Variance
DAP	Days After Planting
cm	Centimeters
DAF	Days After Flowering
DAS	Days After Sowing
DM	Dry Matter
FAO	Food and Agriculture Organization of the United Nations
GOK	Government of Kenya
MD	Man Days
LSD	Minimum Significance Difference
HI	Harvest Index
EPNs	Entomo Pathogenic Nematodes
EU	European Union
FPEAK	Fresh Produce Exporters Association of Kenya
P	Probability Level
SM	Soil Moisture
SS	Sum of Squares
WAP	Weeks After Planting
g	Grams
GAP	Good Agricultural Practices
GATT	General Agreement on Tariffs and Trade
GDP	Gross Domestic Product
Ha	Hectares
HCD	Horticultural Crops Directorate
HCl	Hydro chloric acid
Hrs	Hours
ICIPE	International Center for Insect Physiology and Ecology

IPM	Integrated Pest Management
KALRO	Kenya Agricultural and Livestock Research Organization
KEPHIS	Kenya Plant Health Inspectorate Services
Kg	Kilograms
l	Liters
LC50	Lethal dose for 50% mortality rate
LEAF	Linking Environment and Farming
m	Meters
ml	milliliter
mm	Millimeters
MOA	Ministry of Agriculture
MRLs	Maximum Residue Levels
MT	Metric ton
NTM	Non-Tariff Measures
PHI	Pre Harvest Interval
ppm	Parts Per Million
PPN	Plant Parasitic Nematodes
RGI	Root Gall Index
RKN	Root Knot Nematodes

CHAPTER ONE
INTRODUCTION

1.1.Background Information

A review of nematodes associated with crops has revealed that almost every crop is attacked by one or several nematode pests which cause economic losses in heavily infested fields (Sanoj *et al*., 2017). Globally, yield losses due to arthropods, diseases, and weeds are estimated to account for about 35% in major crops (Dougoud *et al.,* 2019). Root-knot nematodes (RKNs) are a malignant soil borne curse to vegetables, which undermine their production and few farmers are aware of them or the damage they cause (Coyne *et al.,* 2018). They Loss of yield and damage and associated with plant parasitic nematodes and plant pathogens are greater in the tropics than in the temperate regions. This is due to the favourable environmental conditions for the pathogen reproduction and dispersal ,colonization and development , lack of human activity as well as limited financial resources to combat infestations (Zaki *et al*., 2015).French beans(*Phaseolus vulgaris* L.) are among the major horticultural crops produced in Kenya for export. The export season ranges from November to April. Despite their importance, losses due to root-knot nematodes are high.The annual global losses associated to plant parasitic nematodes are estimated at 47% for most economically important crops amounting to over $80 billion annually (AGRIOS, 2017).

In the tropics the crop losses due to root knot nematodes is 42.6% compared to 38.8% in temperate regions (Daneel *et al*., 2017).The most common groups of nematodes associated with French beans are the fungivorous and omnivorous, plant parasitic, predatory and bacterivorous,. Plant parasitic nematodes (PPN) feed on plant tissue. They are known to cause serious damage to many crops in the world such as pineapple, cassava, cucumber, maize, watermelon banana, tomato, cabbage, potato and spinach among others (Onkendi *et al*., 2014). They are known to exploit all parts of vascular plants but the most economically important species infect roots (Narasimhamurthy *et al.,* 2019).

Reports from Tharaka Nithi County indicate that *Meloidogyne* species are the most predominant endoparasites occurring in 86% of the root samples (Maina *et al*., 2010). In Tharaka Nithi county *Meloidogyne* spp problem is still a serious problem in French bean production because the climate is suitable throughout the year for nematode

reproduction and survival (Kariuki *et al*., 2010).Currently control of RKNs in French beans is mainly through use of chemical nematicides. Alternative management options are required due to the recent public concern and restrictions regarding use of nematicides because of the impact they pose on environment and human health (Dougoud *et al*., 2019) .It has been observed that the increased use of synthetic fertilizers and agrosynthetics have resulted in environmental pollution, pest resistance, resurgence and poisoning of food sources for humans and livestock (Russell and Kranthi, 2009).Botanical are broad spectrum materials used in pest control because they are unique in action and they are safe to apply and can easily be processed. Locally available plant materials have been widely used in the past to protect plants from damage caused by insects (Ali *et al*., 2017).The main advantage of extracts is that they are easily produced by local farmers, cheaper and hazard free in comparison to synthethic insecticides. Higher plant species possess phytochemical compounds antagonistic to plant parasitic nematodes including root-knot nematodes. The compounds have abroad spectrum mode of action consisting mainly sesquiterpenes and monoterpenes Monoterpenes and sesquiterpenes have a good repellence effect in many instances when used as essential oils (Subramaniyan*et al*., 2017).

The secondary metabolites and essential oils from these plants have direct effects on RKNs especially on the nematode egg hatchability and juvenile stage development (Lucia, 2017).In the recent past some plant species from Asteraceae family such as Tithonia have been reported to have potential for use in the management of root-knot nematodes in the soil rhizosphere and these could offer an ecologically sound control option as a substitute to synthetic nematicides (Ogundole*et al*., 2017).Extracts of neem (*Azadirachta indica*) and Tephrosia also have nematicidal effect on root-knot nematodes (Dash *et al*., 2017).

Root-knot nematodes have been found to constrain the production of different vegetables (Waceke, 2007). In addition, farmers knowledge on the presence and the management of the nematodes remains quite low (Islam, 2017).The values of produce whose crops have been infected by *Meloidogyne* spp. are reduced by depriving them of nutrients required for their growth. The losses are huge at the seedling stage and it usually results in devastation of the crop (Huma *et al*., 2011).The impact of

Meloidogyne spp. is underestimated (Ali *et al*., 2017).There has been no comprehensive report that focuses specifically on the economic importance of *Meloidogyne* spp. (Muhammad *et al*., 2018). This study therefore aimed at investigating the role of volatile components from Neem, Tithonia and Tephrosia in the management of root-knot nematodes and to identify the active components in their extracts.

1.2. Statement of the Problem

Root-knot nematodes are a major pest in French beans causing yield losses of up to 48% annually. Control of plant-parasitic root-knot nematodes involves use of synthetic nematicides. However, apart from their high cost, indiscriminate use of synthetic nematicides in the management of root-knot nematodes has led to increased problem for phytotoxicity, environmental hazards and resistance buildup. This has necessitated a reduction in amount of synthetic nematicides used for root-knot nematodes management. Therefore, there has been an increase in the intensity of search for other efficient, ecologically sound and safe methods that can replace the synthetic methods for root-knot nematodes management. Neem, Tithonia and Tephrosia roots, leaves and seeds extracts have been used to manage various pests with remarkable results but documentation on the same is limited. It is in this context that this study sought to examine solvent leaf extracts of Neem, Tithonia and Tephrosia in the control of root-knot nematodes in French beans and to establish the active phytochemical constituents in the leaf extracts.

1.3.Objectives
1.3.1. Broad Objective
To determine the efficacy of leaf extracts in management of root-knot nematodes in French beans.

1.3.2. Specific Objectives
The specific objectives of the study were;
   i.   To determine the phyto-chemical constituents in the leaf extracts of Neem, Tithonia and Tephrosia toxic to root-knot nematodes.

ii.   To determine the *in vitro*nematicidal activity of the leaf extracts of Neem, Tithonia and Tephrosia against the root-knot nematodes

iii.   To determine the comparative effect of the leaf extracts of Neem, Tithonia, Tephrosia and Vydate (Oxamyl 10% L) on French bean growth and yield in both lath-house pot and fieldexperiment

1.4.Hypotheses

The hypotheses tested included;

H_{01}: There are no statistically significant phytochemical constituents in the leaf extracts of Neem, Tithonia and Tephrosia that are toxic to root-knot nematodes.

H_{02}: There is no statistically significant nematicidal activity in the leaf extracts of Neem, Tithonia and Tephrosia against the root-knot nematodes *in vitro*

H_{03}: There is no statistically significant difference in the comparative performance of Neem, Tithonia, Tephrosia and the commercial recommended product, Vydate (Oxamyl10%L) on root-knot nematode management in a pot experiment

1.5.Justification of the Study

Root-knot nematodes are important pests of many cultivated plants and are known to cause yield losses of over 5-48% globally and 25-50% for smallholder farmers in developing countries (Sanoj *et al*., 2017).Nematicides such dibromo-3-chloropropane (DBCP) as well as Methyl bromide have been used in the management of root-knot nematodes and have shown potent in controlling nematodes compared to non-soil fumigants. However, their toxicity has caused developed countries to phase them out as of 2003 since their introduction in the 1970's (Errico *et al.,* 2017) .These synthetic nematicides are now being phased out because of their hazardous nature, high toxicity to non-target organisms ,cost and as well as environment and ozone depletion. There is a need to venture into plant based sustainable biological avenues as alternatives to sythetic control for management of root-knot nematodes(UNEP, 2017).Plants considered antagonistic to *Meloidogyne* species such as Neem ,Tithonia and Tephrosia have been evaluated in rotation, intercrop or organic soil amendments and have been able to suppress nematode populations(Muhammad *et al*., 2018).

Metabolites produced by these plants are a potential source of new nematicidal compounds. Many of them have been found in plants including: phenols, polyacetylenes, sesquiterpenes, alkaloids, diterpenes, fatty acid, glucosinolates, isothiocyanates, and thienyls which are generally safe for the environment and humans (Okello *et al.,* 2009).These compounds can be developed for use as nematicides themselves or can serve as model compounds for the development of chemically synthesized derivatives with reduced environmental impacts and enhanced activity. The selective mode of action of plant derivatives could be exploited for safe use in integrated pest management (Wachira *et al.,*2009).Cheap methods like use of botanical extracts as soil amendments are generally not widely used by French beans growers. The major hindrance is limited documented information on their efficacy. Nematicidal bio-active products of plants, being less persistent in environment are safer for mammals and other non-target organisms (Muhammad *et al*., 2018). Botanical pesticides are more often readily available, cheaper than the synthetic ones and their crude extracts and are easy to be prepared by farmers. They reduce the chances of development of resistance.

CHAPTER TWO
LITERATURE REVIEW

2.1.Overview of French beans Production

Enhanced production of French beans can improve the livelihood of small holder farmers, as well as meet the growing consumer demand. World production of French beans was estimated at 5.8 million tonnes from 855,000 ha. China produced 2.0 million tonnes, Turkey 515,000 tonnes, the European Union 664,000 tonnes, tropical Africa about 75,000 tonnes and Northern Africa 312,000 tonnes (FAO, 2016). An important part of the tropical African production is exported to Europe: nearly 40,000 tonnes, the most important exporters being Senegal, Burkina Faso, Kenya and Zimbabwe. French beans are the third most important agricultural export product from Kenya after tea and pineapple (FAO, 2019). French bean production is practiced by small scale growers in farms averaging 0.5ha and 2.0ha. Production rate have not only been increasing but they have exceeded those for many other major food crops. The total production of French beans in 2017 was estimated at 112,666 MT valued at KES 5.04 billion (HCD, 2016).(Table 2.1).

French bean production area increased by three percent from 4,572 Ha in 2013 to 4,707 Ha in 2016 (USAID, 2019) while the output and value increased by 9 and 15 percent from 112,409 MT to 122,666 MT and Ksh 4.4 to 5.04 billion respectively and the leading Counties in production were Kirinyaga, Murang'a and Meru (Table 1).French beans are primarily grown for exports with a small quantity consumed locally. Varieties grown for processing and fresh market include Amy, Teresa, Samantha, Serengeti, Julia, Paulista and Alexandra (Adesoye, 2012).Rain-fed cultivation is possible in areas with well-distributed, medium to high annual rainfall of 900-1,200mm per annum. Up to 50mm of water per week is required (Ndegwa *et al.,* 2009). French beans can grow in different soil types ranging from sandy; loam to clay. The optimum temperature for production is about 20-25° C. French beans can however survive temperatures ranging from 14-32°C depending on the variety. Extreme temperatures result in poor flower development and poor pod set. Seedlings cannot tolerate temperatures lower than 10°C.The optimum soil pH is 6.5 to 7.5 (Ndegwa *et al*., 2009).

Table 1: Production of Frenchbeans in selected Counties

County	2014			2015			2016		
	Area (Ha)	Vol Ml	Area Ha	Area (Ha)	Vol Ml	Area Ha	Area (Ha)	Vol Ml	Area Ha
Kirinyaga	1,813	51,148	2,455	1,481	45,626	2,053	1,536	47,440	2,372
Muranga	861	3,848	1,185	885	36,810	1,268	847	34,690	1,268
Meru	326	16,615	616	367	13,328	530	407	17,030	681
Machakos	329	1,760	75	522	2,415	106	398	11,139	433
Narok	105	1,575	94	120	900	54	120	900	54
Kiambu	221	4,149	55	226	3832	45	191	3,749	47
Taita	48	1,191	42	134	3,514	147	58	1,245	43
Embu	58	746	25	43	639	34	35	490	26
Nyeri	139	428	623	148	431	9	143	525	16
Bomet	-	-	-	-	-	-	54	240	13
Makueni	74	379	16	62	376	16	97	421	13
Kajiado	88	478	17	95	580	25	81	836	13
Others	844	1,529	36	624	3,958	91	605	3,934	55
TOTAL	4,956	83,846	5,245	4,707	11,2409	4,382	4,572	12,2666	5,038

Source: HCD, 2016

2.2. Factors Affecting French beans Growth, Yield and Quality

The major factors that hinder French beans production in Kenya include; marketing constraints, insect pests, diseases and lack of resistant varieties(Kiplagat *et al*., 2016).Rejection of French beans and Price fluctuations and are the major marketing constraints that contribute to loss of income. Ndegwa *et al.* (2009) reported that export companies often buy small quantities of produce and demand for specific hygiene observance ,varieties and grades leaving farmers to sell the rest to alternative markets.The local consumption of French beans is low while the value chain is undeveloped and information is largely undocumented and often unavailable. Lack of seeds is another limiting factor to French beans production in Kenya. Ndegwa *et al.* (2009) reported that most of the commercial varieties are from temperate countries and are not adapted to the local climatic conditions. These varieties have a short harvest period with low yields of between 7-8 tonnes as compared to 13-20 tonnes/ha in other countries.

French beans productivity is also constrained by the very high cost of inputs especially the price of seeds and fertilizers. There are several diseases and pests that cause reduction in yield and produce quality. Farmers rank diseases and pests according to the crop stage attacked and damage .Bean rust is a major foliar disease of French beans (Wahome *et al*.,2013).The greatest threat to French bean production is

root knot nematodes .Wagacha (2007) reported that farmers incur losses of between 25-100 percent as a result of bean rust. Farmers also use expensive fungicides and Pesticides to control the disease. Other diseases and pests that cause significant yield losses include Fusarium wilt (Kiplagat *et al*., 2016).

Plants yield and growth are mainly limited by nematodes which cause roots dysfunction by reducing the nutrient and water use efficiency and rooting volume, (Noling *et al*, 2005).*Meloidogyne incognita* species are the most devastating nematodes .Root-knot nematodes are difficult to control because of their wide host range and high rate of reproduction with female capable of producing up to a thousand eggs (Hannah *et al*., 2017).Among the plant parasitic nematodes, root knot nematode (RKN) is the most devastating in Frenchbean growing regions.

2.3.Plant Parasitic Nematodes Affecting French beans

Field studies have indicated that *Meloidogyne* and *Pratylenchus* species are the most predominant endoparasites occurring in 86 and 61% of the root samples *Meloidogyne incognita*, lesion nematodes (*Pratylenchus penetrans*) and stubby root nematodes (*Trichodorus obtusus*) are also commonly associated with French beans (Perry *et al.,* 2009).*Meloidogyne incognita* are of most economic importance due to their prevalence and wide distribution in the warm temperate and tropical regions where subsistence agricultural systems predominate(Perry & Evans, 2009).Root-knot nematode populations consist of males and females which are easily distinguished morphologically.The life cycle consists of egg, four juvenile stages and adult the stage.

The development of the first stage larvae happens within the egg where first molting takes place and this is followed by a second juvenile stage (J2) that infect plant roots after seeking the host within the soil surrounding the root (Irene *et al*.,2018). The ability of *Meloidogyne* spp. to survive is enhanced by biochemical adaptations and several physiological which includes delayed quiescence, embryogenesis, and diapause and lipid reserves that prolong viability until the J2 is attained and invades a host (Perry *et al*., 2009). The males are wormlike and are about 1.10 - 1.50 mm long and 32 – 60 μm in diameter (body width). Mature females are pear shaped and are about 0.50 - 1.40 mm long by 0.27 - 0.75 mm in diameter. Second-stage juveniles are

vermiform in shape while third and fourth stage juveniles are sausage shaped and microscopic (Wesemael *et al*., 2014).

Adult female nematodes deposit their eggs in a gelatinous protective matrix, closer to the outside of root surface. The female nematode has capacity to lay over 400 to 1500 eggs during its life cycle which lasts for 2-3 months (Tiwari *et al.*, 2009). Nematodes develop into the J3 and J4 stages as they advance feeding on the giant cells and galling manifests as a response to their feeding. They emerge as adults to lay egg (Irene *et al*., 2018).With this, they are capable of manipulating key aspects of plant biology and are able to hijack host-cellular development to establish a feeding site.

2.4. Economic Importance of Nematodes

Parasitic root-knot nematodes migrate from the soil to the roots of host plants where they use a specialized mouth piece (stylet) to pierce plant cells to establish source of nutrients for sustainability. Heavy infestations by root-knot nematodes can reduce the uptake of essential nutrients from the soil to the rest of the plant. Under such circumstances yields are reduced due to impaired water uptake and nutrient caused by distorted and reduced roots (Wesemael *et al*., 2014).

2.5. Nematode Ecology

Nematodes are generally free-living freshwater and soil environments and in marine, but a large number of species are parasitic on different kinds of plants. The parasitic species are of considerable agricultural, and veterinary importance as pests of plants and parasites of man and livestock respectively (Nderitu *et al*., 2018). Nematodes are found at rivers and at enormous depths in the oceans the bottom of lakes. Some can withstand temperatures constantly below freezing while others live in hot springs. Parasitic nematodes are migratory and move in and out of root tissues, while others are sedentary and effectively don't move at all (Irene *et al*.,2018).In almost every soil sample, nematodes from five trophic levels namely, predators and omnivores ,bacteriovores, fungivores, herbivores, are represented (Muhammad *et al*., 2018). Due to their biological diversity and particularly feeding habits, nematodes are an integral part of the food webs in soil ecosystems. Plant parasitic nematodes are elongated, fusiform, slender, tapering towards both ends and circular in cross section.

Meloidogyne arenaria, *M. incognita* and *M. javanica* are the most encountered species in the tropical regions (Kiplagat *et al*., 2016).

2.6. Root-knot Nematodes *(Meloidogyne spp.)*

Root-knot nematodes (RKN) are the members of the genus Meloidogyne, including *Meloidogyne incognita, M. javanica, M. hapla,* and *M. arenaria*. These species are considered as phytopathogenic sedentary endoparasites, can infect roots of many plant species. Root-knot nematodes belong to the kingdom Animalia, phylum Nematoda, class Nemata, subclass Sercenentea, order Tylenchida, suborder Tylenchina, family; Meloidogynidae, and genus *Meloidogyne.*

Root-knot nematode populations consist of females and males which are easily distinguished morphologically. The males are wormlike and are about 1.10 - 1.50 mm long and 30 - 60μm in diameter (body width). Mature females are pear shaped and are about 0.30 - 1.30 mm long by 0.17 - 0.65 mm in diameter. Second-stage juveniles are vermiform in shape while third and fourth stage juveniles are sausage shaped and microscopic in size (Chitwood, 2002)

2.6.1. Life cycle of Root-knot Nematodes

The basic life cycle of RKN is not much different from that of other nematodes. The eggs are retained within a gelatinous matrix in which they are embedded outside the roots or inside the galls where the infective second-stage juveniles hatch (Kimenju *et al.,* 2018).When the J2 enter plant roots, they establish a feeding site of specialized giant cells and develop and moult into third-stage juveniles (J3) and then into fourth-stage juveniles (J4) which moult either to adult males or females (Nderitu *et al*.,2018).Many RKN, including those that are of major economic importance, are parthenogenetic and males are not necessary in order for females to produce viable eggs . The female lays eggs outside the gall in a gelatinous matrix, on the root surface. A female can lay between 40 and 90 eggs per day. In the soil only the eggs, J2 and males can be found while the females and other juvenile stages remain inside the roots. The life cycle may be completed in about 30 days depending on the climatic conditions, the host and nematode species. Temperatures of 27°C, the probability of having more generations is high as the life cycle is rapid. On crops like tomato, which has a cycle of about four months to maturity, RKN will have about four generations,

an important consideration when applying management methods (Kimenju *et al.,*2018).

2.6.2.Environmental Factors Influencing Root Nematode Survival

Soil factors have a marked effect on root-knot nematodes and their host plants. Factors that are stressful to the host plants can increase root-knot nematode populations as the host plant loses its tolerance or immunity against nematodes. Soil structure, texture and temperature, soil moisture are the major environmental factors and physical and affecting nematode survival (Lambert *et al.,* 2018). Water acts as a medium for active migration that enables infection of host plants by plant parasitic nematodes. Plant root-knot nematodes may also multiply in wet soils. However, water logged soils prevent aeration and kills nematodes. Soil moisture is an important factor in prevention of nematode egg desiccation as it acts like a lubricant. Because of high aeration and mobility Soil texture influences nematode populations, highly porous and loose soils support high nematode populations. Small pore sizes of soil may hinder nematode movement especially for the migratory endoparasites while fine sand and sandy loams offer a better medium (Olabiyi *et al.,* 2018). Most nematodes are active, lay eggs and complete their life cycles at temperatures between 26-32°C on the upper soil horizon in warm temperate regions.

2.6.3.Root-knot Nematode Damage on French beans

Aboveground symptoms of severe root-knot nematodes infestation include; stunted, necrotic or wilted plants, patches of chlorotic. Infested plants that are under moisture or temperature stress may wilt earlier than other plants .Infection and feeding by the root-knot nematodes can stimulate the root tissue to swell into galls around the infection site (Irene *et al*., 2018).Root galls vary in size from pin-head to large size due to different levels of root knot infestations on the host plant and at times they may coalesce to form large secondary galls (Mohiddin & Khan, 2014). The size of the galls also depends on the host plants and nematode species involved. A clear and typical symptom of root-knot nematode infestation is 'thick-root' appearance; the egg masses or females are concealed inside the root tissue (Crow, 2017)

The infestation of RKNs results in temporal wilting of the plants due to disturbance on the uptake of nutrients, water and plant metabolic activities and this has a serious impact on yield (Murukesan , 2008).French beans with severely galled roots can have short and thick roots. Galls caused by root-knot nematodes may be confused with nodules of Nitrogen fixing Rhizobium bacteria. However, rhizobium galls are pink inside and come off the root easily when rubbed. Root-knot nematodes galls cannot be separated from the roots easily (Mathesius *et al*., 2009).

2.6.4. Root- knot Nematode Pathology

Plant parasitic nematodes (PPNs) are bio trophic parasites which obtain nutrients from the cytoplasm of living roots, stems and leaf cells for development, growth and survival .Nematodes have evolved diverse parasitic strategies and feeding relationships with their host plants .They possess a hollow and a protrusible feeding structure, the stylet and a pharynx, which has undergone morphological and physiological adaptations to maintain the feeding relationships. Depending on the species, they feed from the cytoplasm of unmodified living plant cells or have evolved to modify root cells into elaborate feeding cells as in RKN .The nematodes use their stylet to pierce and penetrate the cell wall of a plant cell, inject gland secretions through the stylet orifice into the cell and withdraw and ingest nutrients from the cytoplasm.

Nematodes that enter the root tissue also use their stylet to pierce openings and/or inject secretions to dissolve (intracellular migration) or weaken (intercellular migration) the cell wall or middle lamella. Generally, all PPNs damage plants by direct mechanical injury using the stylet during penetration and/or by secretion of enzymes into the plant cells while the nematode is feeding (Duong *et al*., 2019). The physical presence of endo-parasitic nematodes inside the host also affects the functioning of the host. As a result of nematode feeding, the architecture and extent of the root system is altered, so that it is less efficient at taking up nutrients and water from soil. The extent of nematode damage depends to a large extent on the inoculum density (level of infestation). Low or moderate numbers of nematodes may not cause much injury but large numbers severely damage or kill their hosts (Hannah *et al*., 2017).

2.6.5. Disease Development

Disease symptoms and yield losses are often associated with the pre-plant invasion levels and the environmental stresses imposed to the plant throughout the growing season. The second stage juvenile is the only infective stage. The juvenile enters the root at the region just behind the root tip and move intercellulary to the zone of cell differentiation .The juvenile settle and starts feeding from the cells next to the head by secreting saliva-containing enzyme which dissolves the cell content. Two to three days later, the cells around the head enlarge, start dividing. The wall existing between some cells breaks down and disappears and the protoplasmic content of the cells coalesce, giving rise to giant cells .Each gall contains 4-8 giant cells which are maintained by a continuous stimulus from the nematode but collapses when it ceases to feed (Agrios *et al*., 2018).

Xylem elements are affected due to mechanical pressure from enlarging cells. The swelling of the root result from hypertrophy and hyperplasia of the pericycle and endodermis cell surrounding the giant cells and vascular parenchyma, Sherf *et al*. (2017).Temperature determines the distribution and development of root-knot nematodes. The optimum temperature for *M. incognita, M. javanica* and *M. arenaria* which are prevalent ranges from 25 - 35°C.Distribution of root-knot nematodes is also determined by such soil factors as soil texture and structure. Soil texture and structure are directly related to water holding capacity and aeration, influencing nematode survival, movement and disease severity.

2.6.6.Symptoms of Root- knot Nematode Infestation

Plants infested with root- knot nematodes may show slow or stunted growth .In the field they are distributed in oval patches or elongated patches depending on the direction of cultivation. The leaves show wilting, yellowing or chlorosis and there is premature dropping of fruits and flowers and malformed fruits, infected plants are likely to wilt earlier and excessively under temperature or moisture stress leaves, defoliation, stunting and wilting (Jang *et al.,*2019). Nematicides are recommended Infestations may also occur without causing any above-ground symptoms, the typical damage caused through feeding by root-knot nematode are different sized galls formed on the plant roots or knots on roots from where the nematodes derive their

name .All species of *Meloidogyne* cause galls but they vary in shape and size. The galls obstruct water and nutrient uptake and also increase the susceptibility of the root system to invasion by disease causing fungi and bacteria. (Coyne *et al*., 2009).

2.6.7.Management of Root -knot Nematodes in French beans

Root-knot nematodes are difficult pests to control; the management strategy requires an integrated approach by implementing, prevention, cultural, chemical, biological and botanical management strategies (Jang *et al.,* 2019). Prevention from spread has been done through disinfecting contaminated tools, hands, clothing or shoes, sanitation of greenhouse structures, crates, benches, use of sterilized soil, locating seed beds away from infested fields, procuring transplants from reputable sources and use of clean irrigation water Mulrooney, 2012. Biological control agents should be released early in the crop growing cycle. 2009). There is need to develop alternative methods of control that are cheap, environmentally friendly and not harmful to humans (Priya and Pandiyan, 2019).

Botanical extracts from Neem, Tithonia and Tephrosia residuals as organic fertilizers have been evaluated for nematodes control with remarkable results. Extracts are composed of various bioactive compounds and secondary metabolites such as alkaloids, flavonoids and phenolic compounds. These substances possess synergistic effects on retarding growth of pests and suppression of pathogens hence act as insecticidal and antifungal agents (Sindhu *et al.,* 2017).Management practices can reduce nematodes population to levels that will allow producers to grow and sell high value quality produce (PIP, 2019)..

2.6.7.1. Soil Solarization

Soil solarization has been used with success in the control of nematodes and other soil-borne pathogens (Perry, 2009). Incorporation of solar heated water by drip irrigation has been found to increase its efficiency .This is restricted in application because the polythene used is expensive, solar radiation periods in some places are too short and it is not feasible on large scale farms planted with low value crops (McSorley, 2018).

2.6.7.2. Flooding

Nematode densities can drop significantly when soils are flooded for prolonged periods of time (Bridge, 2018). Flooding the soil for seven to nine months kills nematodes by reducing the amount of oxygen available for respiration and increasing concentrations of naturally occurring substances such as organic acids, methane, and hydrogen sulphide which are toxic to nematodes. Flooding leaves no toxic residues, it also conserves carbon in organic matter by slowing decomposition, increases the availability of certain micronutrients such as magnesium and iodine to crop plants, and changes the soil micro flora to favor biological pest control. As an added benefit, instead of leaving flooded fields fallow, it may be possible to grow cash crops such as rice .It may take two years to kill all the nematode egg masses. The duration of flooding for effective nematode control needs to be determined for each nematode species and it is a costly (Sherf *et al*., 2017).

2.6.7.3. Nematode-Suppressive Plants

Certain plants are able to kill or repel pests including nematodes, disrupt their lifecycle or discourage them from feeding. Some of these plants are Neem, Tithonia, Tephrosia, marigolds, castor bean, and various brassicas (powerful nematode-suppressive cover crops).Plant extracts have also been effective in killing plant-parasitic nematodes. They are useful for reducing nematode populations as well as conserving soil and often improving soil structure. In localities where carefully selected cover crops may serve as living mulches and provide multiple pest control (Sciences, 2017). Results of the effectiveness of nematode-suppressive plants refer mainly to in vitro or pot experiments and practical application of these extracts is yet to be profitable.

2.6.7.4. Chemical Control of Root-knot Nematodes

Chemical control is one of the oldest methods of controlling nematodes. Nematicides are synthetics used in the field of agriculture to mitigate the negative effects of plant parasitic nematodes on plant health and subsequently on crop productivity and quality (Muhammad *et al*., 2018). These synthetics are applied to the soil as fumigants when wet and the field is ploughed thoroughly well for better incorporation (Araya & Mario, 2016) These synthetics have a correlated effect on soil type with regards to control as they are known to respond differently to soil type.

Synthetics such as carbofuran, carbosulfan, and fenamiphos have been tried in controlling *M*. *incognita* and have shown good results as seed dressants and soil fumigants .Chemical control of root-knot nematodes has primarily been achieved through nematicides which can be fumigants or non-fumigants. Non fumigants are applied during planting and are systemic affecting the nematodes behavior. Frequent application of nematicides by farmers has rendered most nematicides in-effective and this contributes to environmental effects leading to banning of some fumigants like methyl bromide (Siddiqui *et al*., 2017).

The use of synthetic nematicides is not sustainable and has caused threat to market access for Kenya's French beans due to minimum residual levels (MRLs) (PIP, 2017). For instance, starting January 2009, the EU imposed a 10% sampling on French beans and unshelled peas from Kenya .Consequently farmers had to shift from toxic nematicides to safer nematicides. This implies higher costs of root-knot nematodes control since the new safer pesticides tend to be more expensive and often less effective in controlling pests (Okello *et al.,* 2009)

2.6.7.5. Cultural Practices in the Management of Root- knot Nematodes

Cultural control is one of the broadest methods for management of RKNs and involves cropping systems, fallowing, solarisation and use of organic amendments, intercropping, altering dates of planting, removal of infected plants and burning of crop residues. Organic soil amendments have been reported to have nematicidal properties in field vegetable. Trials thus increasing crop yields significantly (Ozores-Hampton *et al*., 2016). It works by increasing the level of nutrient supply and improves the soil structure thereby increasing nitrogen availability and consequently improving plant health (Tabarant *et al*., 2011). Other cultural practices in the control of root-knot nematodes include incorporation of cover crops as fallows in rotations although their effects on nematodes is minimal and requires prolonged application. Nematicides are slowly being phased out, alternative agronomic practices required to solve the nematode problems are encouraged as options for nematode control (Sarah *et al*., 2019).

2.6.7.6. Host Plant Resistance in the Management of Root-knot Nematodes

Crop resistance is considered the most economical way of controlling pests. The use of resistant varieties can reduce dependence on synthetic nematicides resulting in fewer inputs and reducing environmental pollution. In Kenya most of the introduced varieties have good pod characteristics but are highly susceptible to root-knot nematodes and other diseases. Ndegwa *et al.* (2009) recommended evaluation of varieties from local breeding programmes in National Trials that showed root-knot nematodes tolerance, high yield potential and good market quality. Use of cultivars that are resistant to nematode infection is thought to be the most practical and cheapest means of nematode control especially in smallholder farming systems Bridge,2018.Cultivars with resistance to different species and races of Meloidogyne have been selected in beans (*Phaseolus vulgaris*), soya beans (*Glycine max*), peas (*Pisum sativum* L.),pepper (Capsicum spp.), tomatoes (*Lycopersicon esculatum*),sweet potatoes(*Ipomea batata*)and cowpeas (*Vigna unguilata*). Resistance to *M. incognita* has been identified and incorporated in bean French bean varieties through interspecific hybridisation (Omwega *et al*.,2018). Widespread adoption of this strategy is limited by unavailability of these resistant materials to farmers, resistance breakdown after a few years and low acceptance of some of the resistant cultivars by farmers (Jang *et al.,* 2019).

2.6.7.7. Biological Control of Root-knot Nematodes

Biological control of plant parasitic nematodes is achieved by use of conservation of local antagonistic organisms or combination of the two in soil by either naturally occurring or introduction techniques. Single isolates of bacterial endophytes isolated from African marigold and *T. patula* have been tested as biocontrol agents and have shown efficacy in controlling nematodes using endo root derived bacteria (Sturz & Kimpinski, 2018).Naturally, plant growth promoting rhizobacteria play a critical role in the control of soil pathogens and is indigenous to the soil environment and rhizosphere (Siddiqui, 2017).The control of plant parasitic nematodes by biological control agents requires inundation of bio formulations in the soil for satisfactory control.

Nematophagous fungi have been suggested for control of root-knot nematodes *Paecilomyces lilacinus* which is also marketed in Kenya has been used in the control of nematodes as it has ability to effectively parasitize these plant pathogens consequently reducing the egg hatching and increasing root-knot mortality. *Pochonia chlamydosporia* a nematophagous fungus has been studied extensively as a biological agent against plant parasitic nematodes. It is one of the most studied and effective biological control agents for the nematode genera such as Globodera, Heterodera, Meloidogyne, Nacobbus and just recently Rotylenchulus (Manzanilla-Lopez *et al*., 2013).Other studies have shown that *Trichoderma harzianum* BI is a successful fungus that is capable of infesting nematode eggs and juveniles with ability to significantly reduce root-knot nematode (*M. javanica*) under greenhouse conditions (Sahebani & Hadavi, 2015). Bacteria particularly *Pseudomonas aeruginosa* was reported to have an impact on tomato growth and reduced galling (Shankar *et al*., 2017)

2.6.7.8. Bio-fumigation in the Management of Root-knot Nematodes

Bio fumigation has shown promising results as a sustainable strategy to manage plant-parasitic nematodes, soil-borne pathogens, insects and weeds (Ntalli *et al*., 2019). The concept of soil bio fumigation was initially defined as the biocidal action of volatile compounds that resulted during the decomposition of plant tissues of *Brassica* plant species incorporated into the infested soil but it was later expanded to include non-brassica plants species, other plants and agro-indus Trials residues and wastes of farm animals (Mitidieri *et al.*, 2017).Cover crops that fit well within the existing rotations is therefore an important consideration for growers. Many growers have always used annual grasses and cereals as cover crops mainly because they are cheap, quick to establish and easy to manage (Muhammad *et al*., 2018). A lot of investigations have been made about utilizing many plant extracts, residuals and agro-industrials wastes as organic soil amendments or pre planting soil bio fumigants (UNEP, 2019).

2.6.7.9. Integrated Pest Management

The most successful approaches to nematode and insect pests control relies on integrated pest management(IPM).Integrated pest management utilises options to keep pest populations below economic threshold levels. The extent of success,

however, is dependent upon having accurate damage threshold densities and readily acceptable resistant cultivars (Ntalli *et al*., 2019). A combination of management tactics or tools, including cultural practices (rotations with non-host crops and cover crops that favour the build-up of pest antagonistic), resistant cultivars and judicious chemical treatments, generally provide acceptable control of pests.

2.7.Botanical Extracts in the Management of Root-knot Nematodes

Many plant products have been well known to be nematicidal in nature .Plant extracts, their parts, products and certain other effective amendments have been reported to possess nematicidal properties. The use of such materials has merits over other methods due to their availability, low cost, being pollution free and their capacity to improve soil fertility.Studies on the identification and use of local plant materials for the control of nematodes or integrated with other methods of control are current areas of research in plant nematology (Chagas *et al*., 2017).Very often, when there is a decrease in the soil inhabiting pathogens then an increase in crop yield occurs. Basil, Marigold, Pyrethrum, Neem, Chinaberry, Tithonia, Tephrosia are the most common used plants. Other species referred to as chemo types and variants are also available (Muhammad *et al*., 2018).

Currently, there is increased interest in naturally occurring, biodegradable botanicals for pesticides, pharmaceutical and other applications (Kumar *et al*., 2017). Neem leaves have been found to be quite effective for the control of *Meloidogyne incognita* on tomato, eggplant, chilly, mulberry, *Meloidogyne javanica* on tomato, okra and chick pea, *Meloidogyne arenaria* on tomato, eggplant and okra (Uma, 2017). Ali *et al.* (2017) observed that methanolic extracts of *Lantana camara* induced significant mortality of *M. javanica* juveniles *in vitro*. Meenakshi *et al*. (2017) proved the molluscidal potential of neem in controlling the golden apple snail. The molluscidal 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 (Meenakshi *et al*., 2017)

2.7.1.Neem in the Control of Root-knot Nematodes

The Neem tree (*Azadirachta indica* A. Juss) is the most well-known plant with powerful insecticidal properties since its discovery. Different parts of the tree have been shown to exhibit antimicrobial effects against a wide variety of microorganisms (Uma, 2017).The most characteristic metabolites of this family are called limonoids, which are tetranortriterpenoides; which has considerable interest due to fascinating structural diversity and its broad biological activity. Study evidenced that plants fruits, oil, leaves, bark and other parts have important role in diseases prevention due to their rich source of antioxidant. The neem has a wide range of several therapeutic properties based on its characteristics, such as antifungal, antibacterial, antioxidant, antiviral, anti-inflammatory, analgesic, antipyretic, and immune stimulant activity Mustafa,(2016). The leaf extract is commonly used as an antibacterial agent. In addition, the neem has several applications, such as antiseptic, healing. The biological activities are attributed to the presence of many bioactive compounds in its different parts. Although various parts of the Neem plant have been used for pest control, Neem seeds have been the main source for the production of commercial Neem formulations (Arvind *et al*., 2017).

Neem constituents such as, Azadirachtin, nimbin, salanin, thionemone and various flavonoids have nematicidal action. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbicacid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol (Mariana *et al*., 2017).Neem leaf extracts at different concentration have showed remarkable root-knot nematode control with almost 87% control .The mortality of *Meloidogyne* spp. was showed to be nearly 80% after 48 hours of incubation (Duong *et al*., 2014).Neem oil cake has also been used extensively in nematode control. Besides the nematicidal effects, triterpene compounds in Neem oil cake inhibit the nitrification process and increase available nitrogen (Rajesh & David, 2017).Aqueous extracts have been found to have maximum number of phyto constituents such as saponins and flavonoid, sugar at low concentration. Medicinal plants are rich in secondary metabolites which include alkaloids, flavonoids, saponins and related active metabolites which are of great medicinal value and have been extensively used in the drug and pharmaceutical

industry (Dash *et al*., 2017).Behavioural observations of the methanolic leaf extract treated larvae revealed restlessness with persistent and aggressive anal biting behaviour indicating the probable effect of the extract on the neuromuscular system of larvae (Kumar *et al.,* 2017).Aqueous extracts of leaf, flower, fruit, bark, root and gum of neem have been reported to be highly toxic to nematodes with fruit extract showing the most lethal activity followed by leaf extract (Dash *et al*., 2017).

In a cucumber greenhouse, soil treatments with neem formulations significantly reduced the numbers of soil nematodes and plant root-knots. Soil treatment with Neem plus greatly improved the growth of cucumber plants in nematode-infested pots of neem leaf (fresh and dry. Neem leaf and garlic bulb extracts inhibited hatching of egg masses and were lethal to larva. Nematodes control is obtained when Neem by-products and commercial products were used as seed coatings and bare-root dip treatments (Mshelia *et al*., 2017). Neem leaves have been found to be quite effective in the control of *Meloidogyne incognita* on tomato, eggplant, chilli and mulberry, *Meloidogyne javanica* on tomato, okra and chick pea, *Meloidogyne arenaria* on tomato, eggplant and okra (Ntalli *et al*., 2019). Azadirachtin is a naturally occurring bio pesticide derived from the seed of the Neem tree and acts as a hormonal analogue of ecdysone with some activity against a wide range of pests (Meenakshi *et al*., 2017).

The application of Neem has also proved to be an effective barrier against banana weevils and nematodes. Nematode control was obtained when Neem by-products and commercial products were used as seed coatings and bare-root dip treatments (Ihsan *et al*., 2017). Worldwide attention on the use of Neem kernel seed extracts over several years has been biased by researchers, administrators and granting agencies. Thus, soil application of the neem-based formulations would be applicable for the control of both leaf-sucking and soil pests.

2.7.2.Tithonia in Management of Root-knot Nematodes

*Tithonia diversifolia*commonly known as the tree marigold is a herbaceous flowering plant in the Asteraceae family native to Mexico. It is a dominant plant of the Asteraceae family which suggests it produces allelosynthetics that interfere with the development of surrounding plants (Mariana *et al*., 2017). It is a shrub that is widely

distributed along farm boundaries in the humid and sub-humid tropics of Africa.Tithonia is invasive in many parts of Kenya.It is naturalized and widely distributed along the road sides and farm boundaries. The non-volatile fractions of the plant are a rich source of flavonoids and sesquiterpenoid lactones, including, diversifolin, tirotundin and hispidulin (Oyinlola, 2017).The different classes of compounds contributes to the olfactory characteristics of each of the essential oils. Phytochemical screening of extracts from the leaves of *Tithonia diversifolia* displayed the presence of Alkaloids, Saponin, Saponin glycoside, Tannin, Balsam, Cardiac glycoside and Volatile oil. Ethanol extracts of Tithonia inhibited egg hatch and juveniles mortality at different concentration rates (Akinyemi *et al.,* 2009).

Leaf extracts have confirmed to be phytotoxic and contain some allelosynthetics. Ogundale *et al.* (2017) was able to isolate growth inhibitory lactones and flavones from Tithonia. Studies showed that the leaves of Tithonia contained Saponin, Alkaloids, Saponin glycosides and Tannin. The nematicidal properties of these plants as evaluated in vitro by antimicrobial assay have revealed that aqueous extract showed growth inhibitory effects. The results showed that both aqueous and methanolic extracts of the plant parts tested positive for alkaloids, saponins, tannins, terpenoids flavonoids and phenols. These phytosynthetics have been found to be significantly highest in the leaves followed by the root and the stem (Oloruntola *et al*., 2017). Other reported uses of Tithonia includes poultry feed, soil erosion control, building materials and shelter for poultry.

Studies also identified green biomass of Tithonia as an effective source of nutrients in maize (Bernard *et al*., 2017). In addition, extracts from Tithonia plant parts reportedly protect crops from termites and contain synthetics that inhibit plant growth. Extracts from Tithonia also have medicinal value (Oloruntola *et al*., 2017).One recognized advantage of Tithonia is the ease of handling its biomass due to absence of thorns which makes Tithonia more attractive to farmers. These antagonistic plants are excellent candidates because they can be used in development of nematicides, or they can serve as model compounds for the development of chemically synthesized derivatives which enhanced activity. There is need to develop alternative methods of control that are cheap, environmentally friendly and not harmful to humans (Priya and Pandiyan, 2019).

2.7.3.Tephrosia Extract in the Control of Root-knot Nematodes

Tephrosia purpurea is a member of the Leguminosae family. It is a shrub popularly known and is well distributed in the tropical regions of Eastern Africa .The genus *Tephrosia* is a large pan tropical genus of more than 350 species any of which have important traditional uses Sindhu *et al,* (2017). It is a much branched shrub native to Africa but it is now found in Asia and other tropical regions.It is a self-generating erect or spreading. It can be found as an ingredient in traditional herbal formulations Tephrosia is an annual or perennial bushy herb it grows to a normal height of 0.3-1.3 m.Perhaps due to its pesticidal properties it has fewer pests. It is widely used as catch crop due to less labor requirement for its direct sowing. Its insecticidal and repellent properties are well known including its allelopathic activity for weed control. Whole plant may be used for its rich flavonoid and polyphenol content. Many plants from this genus have been used traditionally for the treatment of diseases.The herb is commonly grown as a green manure in paddy fields and in tobacco and rubber plantations in other countries. It grows ubiquitously in all soils, sandy, rocky and loamy. Phytochemical investigations have revealed the presence of glucosides, rotenoids, isoflavones, chalcones, flavanones, flavanols and prenylated flavonoids (Yoseph *et al*., 2017).

Bioactivity has been studied indicating that chemical constituents and extracts of the genus Tephrosia exhibit diverse bioactivities such as insecticidal, antiviral, antiprotozoal, antiplasmodial and cytotoxic activities (Yoseph *et al*., 2017). Tephrosia have been used in herbal remedies, insecticides, fish and human poisons by the various indigenous people of Kenya. Phytochemical investigation also revealed the presence of halcones, flavanones, flavanols and prenylated flavonoids, glucosides, rotenoids, isoflavones, .The group is an interesting class of compounds showing primarily fish-poisoning, insecticidal, and antimicrobial activities.The leaf extract shows more antioxidant and cytotoxic activities when compared to other parts (Ramamoorthy *et al*., 2017)

Flavones, isoflavonoids have also been isolated from the pods, the roots and leaves of *Tephrosia pentaphylla*. Three new 6-oxygenated rotenoids (dihydrostemonal,9-

demethyldihydrost emonal and 6-acetoxydihydrostemonal) were isolated and characterized (Sindhu *et al.,* 2017).Recent interest in using Tephrosia leaf extracts for the control of nematodes is not restricted to French beans root-knot nematodes. Phytochemical studies have also been restricted to root and seed extracts. Despite the existence of few studies, there is no adequate knowledge about the nematicidal potential of Tephrosia leaves. Therefore the present study aimed to evaluate and compare the in vitro and vivo nematicidal properties of ethanolic extracts of *T. purpurea* leaves.

CHAPTER THREE

MATERIALS AND METHODS

3.1.Research Site

In vitro and vivo experiments were conducted at Chuka University from January 2017 to December 2018. The area is situated approximately 186 Km from Nairobi along the Nairobi-Meru highway and lies at an approximately 0°19.9896′ South latitude, 37°38.7522′ East longitude and 1452 m elevation above the sea level. The climate is warm and temperate and experiences a bimodal rainfall of about 1000-1599 mm. The climate is considered to be Cwb according to the Köppen-Geiger climate classification. The average annual temperature is 19.5 °C .The long rainy season occurs in March to July and the short rains occur from October to December .The soils at the University farm are nitisol. Agricultural activities and agricultural student practicals are carried out at this farm.

3.2.Acquisition of Test Plants Material

Fresh shoot leaves of *Tithonia diversifolia* and *Tephrosia purpurea* were collected from open fields around Chuka University. Commercial Neem leaf formulation (Nimbecidine) for comparison with extracted Neem was purchased from a local Agro-chemical shop. Neem leaves were sourced from the coastal region where Neem Trees grow extensively. The plant species were taxonomically identified and authenticated by using the available colour pictures followed by description and identification characters. The plants were identified and authenticated by a taxonomist and voucher specimen of the plant deposited at Chuka University Chemistry laboratory with voucher No. MSC/HORT5/11/2017.

3.3.Extraction and Storage of Crude Extracts

Extracts were prepared as described by Oloruntola *et al.* (2017).20 Kgs of fresh leaves were air-dried in shade for 2 weeks then coarsely powdered with a mechanical grinder separately (Figure 1).Two hundred grams of dried powder of each plant species were weighed and dissolved in 500 ml of 95% absolute ethanol in an Erlenmeyer flask for elucidation.After 24 hours of soaking the solutions were filtered through two layers of cheese-cloth gauze and Whitman's No. 2 -filter paper before the filtrates were subjected to evaporation using a rotary flash evaporator under reduced pressure at 60

°C for 60 minutes to concentrate the extract and remove the ethanol. Extracts were stored in airtight container in refrigerator at 10 ºC. Concentrations were prepared following Muhammad *et al*. (2017) procedure. Concentration of 2.5% (25 ml/l) 5% (50ml/l) and 10 % (100 ml/l) were prepared separately by adding 2.5 ml ,5 ml and 10 ml of the extract residuals with 5 ml of acetone to enhance dissolution and made up to 100 ml by adding tap water.

Figure 1:Laboratory Phytochemical Extraction Process

3.4. Preparation of Root-knot Nematode Inoculum

Nematode eggs were extracted from heavily infested galled roots using the sodium hypoclorite (NaOCl) method. Galled roots were collected from highly infested French beans in Tharaka Nithi County. The galled roots were chopped into 1-2 cm pieces and placed in a capacity conical flask into which one litre of 0.5% sodium hypochlorite (NaOCl) was poured. The roots were shaken in the solution for 20 minutes and poured into a stack of three sieves. The retained eggs were then washed into a beaker after several rinses to remove all traces of NaOCl.

The number of eggs per milliliter of the suspension was estimated by counting under a light microscope. After counting, the suspension was transferred back to the mother container. Counting of each sample was repeated two times in the same manner. The mean number of nematodes per 10 ml was determined by averaging the counts. For extraction of juveniles roots were gently washed to remove adhering soil particles. The washed roots were cut into small bits of 2.5 cm longitudinally then placed over tissue paper spread on a wire gauge and kept in a Petri plate filled with water. Level of water was maintained in Petri plate and left undisturbed for 48 Hrs. Later, the

suspension in the Petri plate was collected and observed for nematodes using a light-binocular microscope.

3.5. Experimental Design

The treatments in this research were Tithonia (TI), Neem (NM) and Tephrosia (TE) leaf extracts each at a concentration of 2.5% (25 ml/l), 5% (50 ml/l) and 10% (100 ml/l), Vydate (Oxamyl 10 %) and Nb Commercial Neem leaf formulation (Nimbecidine) served as a standard positive control. Distilled water was used as a standard control. In all these treatments 5ml of liquid was put in the test plate.

3.5.1. Determination of Active Phyto-chemical Constituents in the Leaf Extracts

Preliminary study was carried out to determine the presence of phytosynthetics within the plant extracts using the standard laboratory procedures. Tests were done for presence of flavonoids, alkaloids, saponins and terpenoids which are effective against plant parasitic nematodes.

3.5.1.1. Test for Flavonoids

The presence of flavonoids in the extracts was determined according to procedure by Onwukaeme *et al*. (2007). NaOH test was used by adding 2 ml of dilute NaOH to 4 ml aliquot of leaf extracts followed by addition of dilute HCL.

3.5.1.2. Test for Alkaloids

Wagner test according to Chanda *et al.* (2006) was used to test for Alkaloids in the extracts. This was done by adding 5 ml of 1% aqueous HCl acid into aliquot of 2 ml of the extract and stirred in a steam bath. One ml of the filtrate was treated with two drops of Mayer's Reagent. The other 1 ml portion was treated with Wagner's reagent.

3.5.1.3. Test for Saponins

The presence of saponins in the extracts was determined with the aid of an emulsion test according to Parekh and Chanda, (2007). Aliquot of five drops of olive oil was added to 3 ml of the extracts in a test tube and the mixture shaken vigorously.

3.5.1.4. Test for Terpenoids

Procedure of Salkowski test according to Edeoga *et al.* (2005) was followed. 2 ml chloroform and 3 ml concentrated sulphuric acid (H_2SO_4) was added to 5 ml aliquot of the extract.

3.5.2. In vitro Evaluation of the Nematicidal Effect of the Plant Extracts

An *invitro* study was conducted to tests to determine the efficacy of the leaf extracts of Tithonia, Tephrosia and Neem on root-knot nematodes egg hatch and juveniles' mortality rate was carried out at the University laboratory. The experiment was laid out in a completely randomized design. Aliquots of 5ml leaf extracts and distilled water were dispensed into transparent glass blocks containing 100 root-knot nematodes eggs and 100 freshly hatched juveniles separately (Figure 2).The formula below was used to calculate the mortality.

$$Corrected\ mortality = \frac{Observed\ mortality\ in\ treatment - Observed\ mortality\ in\ control \times 100}{100 - Control\ mortality}$$

N2.5	N5	N10	Nb2.5	Nb5	Nb10	T2.5	T5	Ti10	Te2.5	Te5	Te10	Oxy	CT
Nb2.5	Nb5	Te10	T2.5	Ti5	N2.5	N5	Oxy	CT	Nb10	Ti10	Te2.5	Te5	N10
T10	Te2.5	Te5	N10	Nb2.5	Nb5	Te10	Oxy	CT	Nb10	Ti2.5	Ti5	N2.5	N5

Figure 2: Layout of *in vitro* experiment in CRD

3.5.3. Lath-house and Field experiment

The lath-house experiment was laid in a completely randomized design (CRD) and each treatment was repeated three times as shown in Figure 3 while the field experiment was laid in a completely randomized block design with each treatment repeated once in every block as shown in Figure 4. The first Trials was conducted from September 2017 to January 2018. The experiment was repeated as Trials two from February to August 2018. There were a total of 225 pots in the lath house experiment. Each of the 15 treatment had 5 potted plants which were replicated randomly three times. The field plots measured 1.5 by 1 m and a spacing of 30x15 cm was used making a total of 40 plants. In both lath-house and field experiment

inoculation with the nematodes was done at planting. Three holes were created close to the base of each plant both in the lath-house and the field experiment. Inoculation was done by pipetting 100 root-knot nematodes masses suspension using a graduated pipette and covered lightly as in Umar & Aji (2013).One week after inoculation the extracts were applied in each of the three holes at 50ml per pot/plant using a syringe. The holes were then covered lightly with soil. A final application was done four weeks after the first application.

N2.5	N5	N10	Nb2.5	Nb5	Nb10	Ti2.5	Ti5	Ti10	Te2.5	Te5	Te10	Oxy	Cwith	Cno
Nb2.5	Nb5	Te10	Ti2.5	Ti5	N2.5	N5	Oxy	Cwith	Nb10	Ti10	Te2.5	Te5	N10	Cno
Ti10	Te2.5	Te5	N10	Nb2.5	Nb5	Te10	Oxy	Cwith	Nb10	Ti2.5	Ti5	N2.5	N5	Cno

Figure 3: Layout of lath house pot experiment

3.6.Crop Maintenance in the Field

Routine field maintaince practices such as weeding and spraying against diseases was done when necessary. Weeding or physical uprooting of weeds was done anytime weeds were visible. Earthing up was done during weeding.

3.7. Data Collection

3.7.1. Determination of the Active Phyto-chemical Constituents in the Leaf Extracts

To determine the presence of phytosynthetics within the plant extracts tests were done for presence of flavonoids, alkaloids, saponins and terpenoids. A yellow precipitate in NaOH which turns colourless in addition of HCL indicated the presence of flavonoids in the extracts. A creamy white (Mayer) and reddish brown (Wagner) precipitates indicated the presence of alkaloids in the extracts. Formation of froth indicated the presence of saponins in the extracts. Formation of a reddish brown colour interface indicated the presence of terpenoids in the extracts.

Neem2.5 %	0.5 M	Nimbecidine5%	0.5 M	Tithonia 10%	0.5 M	Neem 10%	0.5 M	Tephrosia 2.5%
				0.5 M				
CWN	0.5M	Nimbecidine2.5 %		Tephrosia10 %		Control Synthetic		Nimbecidine10 %
				0.5 M				
Neem5 %		Tithonia 2.5 %		Tephrosia 5 %		Control Only Nematodes		Tithonia 5 %
				BLOCK 1				
Nimbecidine10%		Tithonia 2.5 %		Tithonia 10%		Nimbecidine5%		Tithonia 5 %
				0.5 M				
Tithonia 10%		Neem 10%		CWN		Neem2.5 %		Neem5 %
Control Synthetic		Nimbecidine2.5 %		Tephrosia10 %		Tephrosia 2.5		Tephrosia 5 %
				BLOCK 2				
Neem2.5 %		Tithonia 5 %		Tithonia 10%		Control Synthetic		Nimbecidine5%
				0.5 M				
Tephrosia 2.5%		CWN		Nimbecidine2.5 %		Neem 10%		Control Only Nematodes
				0.5 M				
Tephrosia 5 %		Tithonia 2.5 %		Nimbecidine 10 %		Tephrosia10 %		Neem5 %

BLOCK 3

Figure 4: Layout of the FieldExperiment

KEY: CWN =Control without Nematodes

3.7.2. In vitro Evaluation of the Nematicidal Effect of the Plant Extracts

Data on the efficacy of the leaf extracts of Tithonia, Tephrosia and Neem in the control of root-knot nematodes was determined by performing egg hatch and juveniles' mortality rate.

3.7.2.1. Root-knot Nematodes Egg Hatch

Percent of hatched eggs was calculated at upto 7 days at an interval of 24 Hrs after incubation at ambient temperature using a stereomicroscope for counting following procedure of Uma (2017)

3.7.2.2. Root-knot Nematodes Juveniles Mortality Rate

To study the effect of the extracts on mortality of juveniles 5 ml of each extract was poured in sterilised Petri dish (6 cm diameter) with 100 juveniles and incubated at 26 ± 0.5 °C. Distilled water and Vydate (Oxamyl 10%) was used as control. The mortality of juveniles was assessed upto 7 days at an interval of 24Hrs (depending upon the mortality in the range 5–95% to determine the LC50 value. Juveniles were considered dead if they did not move when probed with fine needle and body become straight. The percentage of mortality in comparison with control was determined by using Abbott formula.

$$X = \frac{M_E - M_C}{100 - M_C} \times 100$$

Abbott formula

M_E: The percentage of mortality in each extract

M_C: The percentage of mortality in control

X: The percentage of mortality in comparison with control

3.7.3. Comparative Effect of the Leaf Extracts On French bean Growth and Yield

Plant data was taken on agronomic characteristics as indicated below.

3.7.3.1. Plant Height

Stem elongation was determined beginning 14, 21, 28, 35, 42, and 48 days in both Trials I and II. Ten mature plants from each treatment were selected for height measurement. The height was measured from the ground level to the tip of each plant.

3.7.3.2. Number of Leaves

Leaf production was determined beginning 14, 21,28,35, 42,48, 56 and 63 days after emergence in both Trials I and II.

3.7.3.3. Root Galling Index

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

0- Healthy root systems, no infection.

1 -Very few galls, only detected on close examination.

2- Small galls, easy to detect.

3- Numerous small galls.

4- Numerous small galls and a few big galls.

5- 25% of the root system severely galled and not functioning

6 -50% of the root system severely galled and not functioning.

7- 75% of the root system severely galled and not functioning.

8 -No healthy root, plant still green.

9- Root rotting completely galled and plant dying.

10- Plant and roots dead

3.7.3.4. Number of Pods per Plant

The harvested 10 plants per treatment beginning 55, 62, 69, 76, and 83 DAE. They were placed separately on the ground to facilitate determination of the number of pods per plant within the treatment. The pods produced per plant were counted and recorded to determine the yield per treatment plot.

3.7.3.5. Pod Weight

After counting of the pods number per plant beginning 55, 62, 69, 76, and 83 DAE all the pods from the 10 plants per treatment were combined together and placed in one "PIL®"polythene paper bag and weighed with spring balance in grams to determine the yield per treatment plot.

3.8.Data Analysis

Data on the presence, absence, the different types and the numbers of root galling was entered into Microsoft excel, arranged and cleaned. All data collected were subjected to t test and analysis of variance (ANOVA).Comparison of means was done using ANOVA.Significant means were separated using least significant difference (LSD) at $P \leq 0.05$.All means in this study were compared at 0.05 level of significance. Data obtained from the study was used to explain the efficacy of the extracts in the control of root-knot nematodes in French-beans.

The model fitted was:

$Y_{ijkl} = \mu + R_i + E_j + (RE)_{ij} + \varepsilon_{ijkl}$

Where

μ=Overall Mean

$R_i = i$ th Block Effect

$E_j = j$ th Extracts level Effects

RE_{jk} = Interaction Effects

ε_{ijkl} = Random Error Component

CHAPTER FOUR

RESULTS

4.1.Climatic Data

The experiment was conducted from September 2017 to August 2018 at Chuka University; a total of 1473 mm of rainfall was received during this period. The average temperatures ranged from18.6 ^{o}C to 23.5 ^{o}C during the experimental period (Table 2)

Table 2:Climatic Data Observations at Chuka from September 2017 to August 2018

Month	Rainfall (mm)	Average Temperature (^{o}C)	Relative Humidity (%)
2017			
Sep	151.05	19.35	71
Oct	89.95	20.55	66
Nov	147.45	19.75	76
Dec	87.15	20.05	62
Total	474.6		
2018			
Jan	1	22.1	39
Feb	17.3	22.3	44
Mar	32.6	23.5	41
Apr	288	21	69
May	182.8	20.7	70
June	167.2	19.7	73
July	88.2	18.6	77
Aug	221.3	19.7	68
Total	998.4		

Source: Tharaka Nithi Metrological Department 2017-2018

4.2.Phytochemical Constituents in the Leaf Extracts to Root-knot Nematodes

Preliminary phytochemical study was carried out to determine the presence or absence of phytosynthetics constituents in the crude extracts. Tests were done for presence of flavonoids, alkaloids, saponins which are effective against the plant parasitic nematodes. The results are indicated on (Table3)

Phytochemical Analysis of Crude Neem Extracts

The results on phyto-chemical analysis revealed that crude Neem leaf extracts contained alkaloids and Saponins. However, the result of the phyto-chemical analysis showed the absence of flavonoids in the Neem extract (Table 3).

Phytochemical Analysis of Crude Tithonia Extracts

The results on phyto-chemical analysis revealed that the crude Tithonia extracts contained alkaloids and flavonoids. However, the result of the phyto-chemical analysis showed the absence of saponins in the Tithonia extract (Table 3).

Phytochemical Analysis of Crude Tephrosia Extracts

The results on phyto-chemical analysis of crude Tithonia extracts showed that crude Tephrosia extract contain flavonoids. However, the result of the phyto-chemical analysis confirmed the absence saponins and alkaloids in the Tithonia extract (Table 3).

Table 3:Phytochemical Analysis of Neem, Tithonia and Tephrosia Crude Extract

Chemical constituents Status in plant leaf extracts			
Constituent	Tithonia	Neem	Tephrosia
Alkaloids	+	+	-
Flavonoids	+	-	+
Saponins	-	+	-

4.3.*In vitro* nematicidal activity of the leaf extracts against the root-knot nematodes

4.3.1. Effect of theExtracts onRoot-knot Nematode egg hatching

Neem, Tithonia and Tephrosia extracts treatments showed significant inhibitory effect ($P{\leq}0.05$) on egg hatching of French bean root-knot nematodes by over 98% from 2 days after incubation (DAI) and was more evidenced with 100% inhibition at 7 DAI in the in vitro studies. From the study results it was observed that the rate of root-knot nematodes egg hatching was directly proportional to exposure period and inversely proportionate to the concentration rates of the extracts as the rate of hatching was decreased with the increase in the concentration rate of the crude extracts. It was observed that the highest rate of hatching was observed at100ml/l while the lowest rate was at 25ml/l concentration in all plant extracts tested. Among extracts treatments evaluated, crude leaves extracts obtained from Tephrosia at 100ml/l inhibited the greatest egg hatching at 0.8 eggs. Oxymyl (positive synthetic control) inhibited the greatest hatching among the treatments at 0.2 eggs. From the study the greatest egg hatching was observed in the untreated controls at 5.5 eggs (Table 4).The study also

showed that *M. incognita* egg hatch inhibition increased with increase in number of days after incubation.

4.3.2. Effect of the Extracts on Nematode Juveniles (J2) Mortality

The highest mortality of root-knot nematodes (J2) was observed on the 7th day with 100ml/l concentration in all crude extracts of tested plants. The lowest root-knot nematodes (J2) mortality was observed at low concentration rates of 25ml/l in all extract treatments. From the study it was clear that the extracts were mortal (to have nematicidal action) to juveniles. Among the extracts evaluated, maximum mortality was recorded with Neem at 100ml/l at 12.2 juveniles. Oxymyl (synthetic control) inhibitory effects was recorded at 9.4 juveniles while the least inhibitory effects were observed in the untreated control at 4.3 juveniles (Table 5)

Table 4: Effects of Different Extracts on Egg Hatching

Leaf Extract	Concentration	Mean
Neem	25ml/l	4.38ab
	50ml/l	2.76abcde
	100ml/l	0.90de
Nimbecidine	25ml/l	4.09ab
	50ml/l	2.85abcde
	100ml/l	1.33cde
Tithonia	25ml/l	3.76abc
	50ml/l	3.00abcd
	100ml/l	1.28cde
Tephrosia	25ml/l	3.52abcd
	50ml/l	2.28cd
	100ml/l	0.8ed
Oxamyl (Positive control)	10ml/l	0.23e
Control (Distilled water)	No concentration	5.3a
LSD	2.72	
CV	27	

*Means with the same letter(s) are not significantly different at P≤0.05 by Tukey's test, LSD=Least Significant Difference, CV=Coefficient of Variation

4.4. Effects of the Leaf Extracts of on French bean Growth and Yield

4.4.1. Lath house plant height

From the study in the first experiment the response of Frenchbeans to selected crude extracts treatments application to *Meloidogyne spp* under lath house pot experiment differed significantly. Frenchbeans plant height among the selected crude extracts showed significant difference at 35,42,49,56 and 63 DAP (Appendices 1,2,3,4,5,6,7

and 8).The untreated control (root-knot nematode infested) attaining the lowest average mean height of 18.22 cm and 19.40 cm in Trials I and Trials II respectively. Tithonia at 25 ml/l attained the greatest average mean stem height of 21.75 cm and 23.21cm in trail I and Trials II respectively, Oxymyl (positive control) attained an average of 19.03 cm and 21.26 cm in Trials II.

Table 5: Effect of Different Extracts on Juveniles (J2) Mortality

Leaf Extract	Concentration	Mean
Neem	25ml/l	7.76cd
	50ml/l	5.61d
	100ml/l	12.28a
Nimbecidine	25ml/l	6.23cd
	50ml/l	7.19cd
	100ml/l	12.09a
Tithonia	25ml/l	7.90bcd
	50ml/l	5.71d
	100ml/l	11.42ab
Tephrosia	25ml/l	6.23cd
	50ml/l	7.00cd
	100ml/l	11.42ab
Oxamyl (Positive control)	10ml/l	7.47abc
Control (Distilled water)	No concentration	6.38cd
LSD	3.55	
CV	24	

*Means with the same letter(s) are not significantly different at P≤0.05 by Tukey's test, LSD=Least Significant Difference, CV=Coefficient of Variation.respectively.

The study showed that the plant height progressively increased from 35 to 63 DAP. From the results it was observed that the Frenchbeans plants grown in Neem crude extract amended soil were the tallest compared with the plants grown in other crude extracts treatments despite the presence of root-knot nematodes. It was clear from the study that in all treatments, higher concentration rates of 100 ml/l resulted to taller plants which attained a maximum height of 34 cm and 37.34 cm compared to 14.24 cm and 16.4 cm recorded with lower crude extract concentration rates (Table 6).

Table 6: Effects of Different Extracts on French bean Plant Height in Lath house Trials I and II

DAE	N25	N50	Nb100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	C with	LSD
14	4.33bac	4.93a	3.8bdc	4.6bac	4.5bac	4.8a.	3.2d	4.6ba	4.6ba	4.46bac	5.0a	3.7dc	4.6bac	4.6ba	4.6ba	0.88
21	17.44c	16.82de	18.58b	15.53h	16.19g	16.76fe	16.45fg	16.58fe	17.16dc	16.19a	19.59a	17.16de		16.45fg	14.10i	0.35
28	20.42bdac	20.60bdac	21.16ba	21.50a	20.13bdac	19.36dc	19.45dc	19.18dc	19.08d	20.54bdac	19.78bdac	20.54bdac	19.08d	20.54bdac	20.59bdac	1.73
35	28.5a	20.32cd	22.85bcd	20.88cd	21.20cd	26.19ba	21.31cd	22.78cd	19.84d	21.96cd	22.90bcd	20.80cd	22.66cd	20.32cd	23.64bc	3.36
42	24.33bc	21.32c	23.54bc	23.3bc	22.61c	23.55bc	21.76c	24.74bc	28.60a	21.90c	22.90c	21.81c	21.78c	27.62ab	23.44bc	4.25
49	20.48c	23.46bc	22.54bc	21.02c	22.92ab	20.91c	26.72ab	22.02bc	21.47c	21.00c	20.54c	22.15bc	22.15bc	23.02bc	24.38bac	4.88
56	31.22a	25.92a	26.75a	22.52a	25.37a	24.95a	22.92a	25.78a	26.23a	28.81a	26.35a	27.53a	26.36a	25.25a	24.95a	9.37
63	33.04ba	28.74bac	30.45bac	20.82c	28.52bac	25.82bac	23.91bc	28.41bac	25.72bac	25.72bac	26.36bac	28.18bac	26.02bac	24.26bac	25.86bac	11.8

DAE	N25	N50	Nb100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	C with	LSD
14	4.33bac	4.93a	3.8bdc	4.6bac	3.8bdc	4.8a.	3.2d	3.8bdc	4.6ba	3.8bdc	5.0a	3.8bdc	3.8bdc	4.6ba	4.6ba	0.78
21	17.34c	16.82de	17.34c	15.53h	17.34c	16.76fe	15.53h	16.58fe	17.16dc	15.53h	19.59a	17.16de	19.59a	16.47fg	17.16dc	0.35
28	20.42bdac	20.60bdac	21.16ba	20.60bdac	20.13bdac	20.60bdac	19.45dc	19.18dc	20.60bdac	20.54bdac	20.60bdac	20.54bdac	20.54bdac	20.54bdac	20.54bdac	1.63
35	28.5a	20.32cd	22.85bcd	20.88cd	21.20cd	26.19ba	20.32cd	22.78cd	19.84d	20.32cd	22.90bcd	20.80cd	22.66cd	20.32cd	20.32cd	3.36
42	24.33bc	21.32c	24.33bc	23.3bc	22.61c	23.55bc	22.61c	24.74bc	28.60a	21.90c	22.90c	21.90c	21.78c	27.62ab	21.90c	4.55
49	20.48c	23.46bc	22.54bc	21.02c	20.54c	20.91c	26.72ab	20.54c	21.47c	20.54c	20.54c	22.15bc	22.15bc	23.02bc	20.54c	4.9
56	31.22a	25.92a	26.75a	22.52a	25.37a	24.95a	22.92a	25.78a	26.23a	28.81a	26.35a	27.53a	26.36a	25.25a	22.95a	9.57
63	33.04ba	28.74bac	33.04ba	20.82c	20.82c	25.82bac	20.82c	28.41bac	25.72bac	20.82c	26.36bac	28.18bac	26.02bac	24.26bac	25.86bac	10.8

*Means with the same letter(s) along the row for DAE and the rowsfor Extracts are not significantly different at P≤0.05 by Tukey's test. KEY: N =Neem, T =Tithonia, Te =Tephrosia, Oxy =Oxamyl, CNO =Control, C with =Control with nematodes, the numbers 25, 50, and 100 represent concentration of extract in ml/l

4.4.2. Plant Height in the Field Experiment

Response of French bean to *Meloidogyne spp* under field conditions differed significantly ($P≤0.05$) among the different treatments. Frenchbeans average mean plant height among selected extracts showed significant difference at 42,49,56 and 63 DAP (Appendices 5,6,7 and 8) with the untreated controls attaining the lowest average mean height of 17.16 cm and 16.7 in Trials I and Trials II respectively. Nimbecidine at 25 ml/l attained the greatest average mean plant height at 25.2 cm and 23.8 cm in Trials I and Trials II respectively. Oxymyl (synthetic control) attained an average of 20.58 cm and 21.07 cm in Trials I and II, respectively.

4.4.3.Number of Leaves inthe Lath house

From the study, during the first Trials experiment the number of French beans leaves among different treatments differed significantly ($P≤0.05$) at 42, 49, 56 and 63 DAP (Appendices 5, 6, 7 and 8) under lath house pot experiment. The untreated severely infested controls recorded the lowest number of leaves at 19.6 in Trial I and 25.4 in Trial II. Tithonia at 50 ml/l attained the greatest number of leaves in trail I at 26.4 and 27.0 in Trial II. Oxymyl (positive control) recorded an average of 25.1 in Trial I and 25.4 in Trial II**.** Generally an increase in the extracts concentration rates significantly increased the number of leaves per plant.The study showed that the number of leaves per plant was significantly affected by the rate of root-knot nematodes control in both Trials .The results showed that the number of leaves per plant also significantly dependent on the rate of crude extract application.

4.4.4. Number of Leaves in the Field Experiment

Under the field experiment the number of leaves among the selected extracts applications showed significant differences ($P≤0.05$) beginning 35,42,49,56 and 63 DAP (Appendices 4,5,6,7 and 8). The untreated root-knot nematodes infested control attaining the least number of leaves at 19.68 and 19.61 while Tithonia at 50 ml/l attained the highest average mean number of leaves at 27.7 in Trials I while Neem at 100 ml/l attained the highest number of leaves in Trial II, Oxymyl attained an average of 24.8 and 22.3 in Trials I and II, respectively. It was also observed that Frenchbeans plant grown in Neem amended soil had the highest number of leaves compared with plants grown in other crude extract treatments.

Table 7:Effects of Different Extracts on French bean Plant Height in a field Experiment Trials I and II

	Extracts Concentration Rate															
DAE	N25	N50	Nb100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	C with	LSD
14	6.43bdac	6.36bdc	6.21de	6.36bdc	6.6a	6.6bd	6.36bdc	6.44bdac	6.36bdc	6.57bac	6.62a	6.62a	6.33dc	6.62a	6.02e	0.27
21	17.3c	16.6feg	18.48b	15.5i	16.6feg	16.6feg	16.7fe	19.37a	16.5de	16.89e	16.9de	19.49a	17.2dc	16.37hg	14.5j	0.32
28	21.5b	19.7dgfe	19.5gfe	20.57dc	19.67dgfe	21.2bc	20.4dce	19.83dgfe	22.1ba	20.4dce	22.5a	19.24gf	19.24gf	20.1gf	17.23h	0.98
35	23.13cb	20.39feg	20.68fedg	26.6a	21.23fcebg	22.8cbd	19.4g	20.05fceb	22.9cbd	20.8fcedg	22.64cebd	19.64g	23.4b	28.35a	20.10fg	2.25
42	22.95cd	21.00ced	21.69ced	28.93a	22.98cb	24.59b	20.05e	23.41cb	23.40cb	21.71ceb	23.24cb	20.19ed	25.19ed	29.77a	21.50ced	2.62
49	22.95cd	21.25cd	23.58bcd	32.32a	25.28bc	26.12bc	23.88bcd	22.19ed	23.89bcd	19.86d	23.89bcd	19.86d	28.45ba	25.34bc	23.73bcd	5.1
56	25.04edc	23.82ed	26.27edc	37.58a	28.18bdc	31.55bac	20.37e	28.73bdc	25.99edc	22.28ed	26.27edc	21.88ed	34.52ba	25.12edc	26.73edc	7.4
63	24.18bdc	23.34dc	26.89bac	33.74a	28.43bac	31.48ba	19.36d	28.86bac	25.96bdc	22.74dc	26.50bc	24.18dc	31.57ba	23.33dc	26.36dc	7.07
DAE	N25	N50	Nb100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	C with	LSD
14	6.43bdac	6.36bdc	6.21de	6.6a	6.6a	6.6bd	6.41bdac	6.44bdac	6.39bdac	6.57bac	6.62a	6.3dc	6.33dc	6.43bdac	6.02e	0.25
21	17.3c	16.6feg	17.3c	15.5i	16.6feg	16.6feg	16.7fe	19.37a	16.5de	17.3c	16.9de	17.3c	17.2dc	16.37hg	14.5j	0.32
28	21.5b	19.7dgfe	21.5b	20.57dc	19.67dgfe	21.2bc	20.4dce	21.2bc	22.1ba	20.4dce	22.5a	19.24gf	19.24gf	19.24gf	17.23h	0.8
35	22.13cb	20.39feg	20.68fedg	23.13cb	21.23fcebg	22.8cbd	23.13cb	20.05fceb	22.9cbd	23.13cb	22.64cebd	19.64g	23.13cb	28.35a	20.10fg	2.15
42	22.81cbd	21.00ced	2.81cbd	28.93a	27.81cbd	24.59b	20.05e	23.41cb	23.40cb	23.41cb	23.24cb	23.41cb	25.19ed	29.77a	20.05e	2.32
49	22.95cd	21.25cd	23.58bcd	32.32a	25.28bc	26.12bc	23.88bcd	22.19ed	23.89bcd	19.86d	23.89bcd	19.86d	28.45ba	25.34bc	23.73bcd	5.2
56	25.04edc	23.82ed	25.04edc	37.58a	25.04edc	31.55bac	25.04edc	28.73bdc	25.04edc	22.28ed	26.27edc	22.28ed	34.52ba	22.28ed	22.28ed	7.4
63	28.43bac	23.34dc	26.89bac	33.74a	28.43bac	26.89bac	19.36d	26.89bac	25.96bdc	22.74dc	26.50bc	24.18dc	26.89bac	23.33dc	26.36dc	7.07

*Means with the same letter(s) along the row for DAE and the rows for Extracts are not significantly different at P≤0.05 by Tukey's test. KEY: N =Neem, T =Tithonia, Te =Tephrosia, Oxy =Oxamyl, CNO =Control, C with =Control with nematodes, the numbers 25, 50, and 100 represent concentration of extract in ml/l

Table 8: Effects of Different Extracts on Lath house Number of Leaves Trials I and II

Extracts Concentration Rate

DAE	N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	CWith	LSD
14	4.33bac	4.93a	3.8bdc	4.6bac	4.53bac	4.8a	4.8a	3.2d	4.66ba	4.66ba	4.4bac	5.0a	3.7dc	4.6bac	4.66bac	0.88
21	24bac	22.6bdac	20.8dec	21.2dec	20.4de	22.3bdac	22.06bdc	25.7a	22.2bdc	21.4dec	21.4dec	20.8dec	24.9ba	23.13bdac	18.2e	3.52
28	30.6a	26.6dc	23.9e	28.2bc	25.0de	24.9de	23.8e	17.4f	25.0de	24.7e	29.9ba	27.9c	27..0c	24.4e	7.3f	1.77
35	24.0e	26.0d	35.0b	15.2g	24.2e	21.4f	24.6ed	37.3a	28.0c	21.3e	33.86b	24.53ed	28.0c	29.3c	15.0g	1.59
42	32.1a	28.1a	31.9a	33.0a	30.0a	35.8a	34.2a	34.6a	31.2a	28.4a	35.1a	31.2a	30.3a	32.4a	28.0a	8.6
49	34.73a	27.4bc	28.2bac	33.9ba	29.4bac	29.2bac	29.6bac	30.2bac	25.9c	30.4bac	25.93c	29.73bac	30.6bac	34.73a	34.2a	6.6
56	29.6de	28.6de	32.0bdc	23.9e	31.0dc	34.bdac	30.2dc	39.3a	39.6a	39.6a	29.4de	32.46bdc	29.4de	37.66ba	30.46dc	5.7

DAE	N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	CWith	LSD
14	4.33bac	4.93a	4.33bac	4.6bac	4.53bac	4.8a	4.8a	3.2d	4.8a	4.66ba	4.4bac	4.8a	3.7dc	4.8a	4.66bac	0.78
21	24bac	24bac	20.8dec	21.2dec	24bac	22.3bdac	22.06bdc	22.3bdac	22.2bdc	22.3bdac	21.4dec	20.8dec	22.3bdac	23.13bdac	18.2e	3.32
28	30.6a	26.6dc	30.6a	28.2bc	30.6a	24.9de	23.8e	17.4f	25.0de	30.6a	29.9ba	27.9c	27.9c	24.4e	7.3f	1.77
35	24.0e	26.0d	35.0b	26.0d	24.2e	26.0d	24.6ed	37.3a	28.0c	21.3e	33.86b	24.53ed	33.86b	29.3c	15.0g	1.54
42	32.1a	28.1a	31.9a	32.1a	30.0a	32.1a	34.2a	32.1a	31.2a	28.4a	32.1a	31.2a	30.3a	28.4a	28.0a	8.2
49	34.73a	27.4bc	34.73a	33.9ba	29.4bac	29.2bac	29.6bac	34.73a	25.9c	30.4bac	25.93c	29.73bac	34.73a	34.73a	34.73a	6.6
56	29.6de	28.6de	32.0bdc	28.6de	31.0dc	34.bdac	30.2dc	39.3a	39.6a	39.6a	28.6de	32.46bdc	28.6de	37.66ba	30.46dc	4.7
63	31.0a	31.1a	36.2a	31.0a	32.2a	33.2a	32.33a	34.1a	26.2a	29.2a	28.2a	33.2a	29.2a	33.2a	25.2b	7.2
63	31.0a	31.1a	36.2a	32.2a	32.2a	33.2a	32.33a	34.1a	26.2a	29.2a	28.2a	27.3a	29.2a	30.2a	25.2b	8.2

*Means with the same letter(s) along the row for DAP and the rows for extracts are not significantly different at P≤0.05 by Tukey's test. KEY: N =Neem, T =Tithonia, Te =Tephrosia, Oxy =Oxamyl, CNO =Control, C with =Control with nematodes, the numbers 25, 50, and 100 represent concentration of extract in ml/l

Table 9: Effects of Different Extracts on Field Number of Leaves Trials I and II

Trials I

DAE	N25	N50	N100	Nb25	Nb50	Nb 100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	Cwith	LSD
						Extracts Concentration Rate										
14	4.6a	4.8a	4.87a	4.6a	5.0a	3.86dc	4.56ba	4.56ba	4.9a	4.8a	3.36de	4.6a	4.78a	4.96a	3.0e	0.57
21	26.83a	15.13f	24.1b	23.63cb	21.53d	19.16e	22.36cbd	20.76ed	27.13a	22.46cbd	20.90ed	21.53d	26.83a	21.00ed	21.76cd	2.24
28	21.54b	19.79dgfe	19.58gfe	20.57dc	19.67dgfe	21.28bc	20.42dce	19.83dgef	22.13ba	20.41dce	22.50a	19.24gf	19.07g	20.12dfe	17.23h	0.90
35	36.93a	28.96c	22.06fg	33.0b	25.30de	16.63h	24.4fe	27.40fe	22.1fg	24.40fe	27.23dc	32.06b	16.76h	24.1fe	23.8feg	2.66
42	28.43b	35.93a	29.23b	28.20b	32.26ba	29.26b	29.90b	27.07b	27.23b	29.90b	28.93b	32.00ba	31.26ba	29.63b	27.50b	5.22
49	27.46c	37.36aq	30.8bc	29.76c	29.5c	34.8ba	30.90bc	23.03d	27.56c	31.50bc	28.26c	36.36a	34.8ba	36.13a	28.30c	4.08
56	28.16g	39.66a	31.56dfge	31.70dfge	30.1fge	33.56dce	33.56dce	24.06h	31.40dfge	34.26dc	28.90fg	39.0ba	35.53bc	40.23a	24.06h	3.81
63	28.53fg	39.46a	32.0fdec	31.76fdec	31.83fdec	31.20fde	34.2bdec	25.2g	31.63fdec	34.96bdac	29.43feg	38.46ba	36.03bac	28.53fg	39.16a	4.82

DAE	N25	N50	N100	Nb25	Nb50	Nb 100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	Cwith	LSD
14	3.86dc	4.8a	4.87a	4.6a	4.56ba	3.86dc	4.56ba	4.56ba	4.9a	4.8a	3.36de	4.6a	4.9a	4.96a	3.0e	0.57
21	26.83a	15.13f	24.1b	23.63cb	21.53d	19.16e	22.36cbd	20.76ed	27.13a	22.46cbd	20.90ed	21.53d	26.83a	21.00ed	21.76cd	2.24
28	21.54b	19.79dgfe	20.42dce	20.57dc	19.67dgfe	21.28bc	20.42dce	19.24gf	22.13ba	20.41dce	22.50a	19.24gf	19.07g	19.58gfe	17.23h	0.90
35	36.93a	28.96c	22.06fg	28.96c	25.30de	16.63h	24.4fe	27.40fe	22.1fg	24.40fe	24.4fe	32.06b	16.76h	24.1fe	16.76h	2.66
42	28.43b	35.93a	28.43b	28.20b	32.26ba	29.26b	28.20b	27.07b	28.20b	29.90b	28.20b	32.00ba	31.26ba	31.26ba	27.50b	5.22
49	27.46c	37.36aq	30.8bc	29.76c	29.5c	34.8ba	30.90bc	23.03d	34.8ba	31.50bc	28.26c	36.36a	34.8ba	28.26c	28.30c	4.08
56	28.16g	39.66a	31.56dfge	28.16g	30.1fge	33.56dce	30.1fge	24.06h	31.40dfge	34.26dc	31.40dfge	39.0ba	35.53bc	35.53bc	24.06h	3.81
63	28.53fg	39.46a	32.0fdec	31.76fdec	39.46a	31.20fde	34.2bdec	39.16a	34.2bdec	34.96bdac	29.43feg	38.46ba	29.43feg	28.53fg	39.16a	4.82

*Means with the same letter(s) along the row for DAP and the rows for Extracts are not significantly different at P≤0.05 by Tukey's test. KEY: N =Neem, T =Tithonia, Te =Tephrosia, Oxy =Oxamyl, CNO =Control, C with =Control with nematodes, the numbers 25, 50, and 100 represent concentration of extract in ml/l

The results of this study indicates that the extracts application suppressed French bean root-knot nematodes significantly compared to the untreated control in lath house pot experiment

Figure 5: Severely infested plants

4.4.5. Root Galling in a Field Experiment

The results of these study indicated that root-knot nematodes galling indices among the extracts showed were significantly different ($P{\leq}0.05$) [Appendix 25] with the untreated control recording the highest root galling index at 9 and 10 galls respectively in Trials I and II, respectively. The highest reduction in root galling index was recorded under the plots treated with Neem at both 50 ml/l and 100ml/l at 1 gall in both Trials I and II. 3.3 and 3.1 galls were observed in Oxymyl (positive synthetic control) treated plots in Trials I and II, respectively

4.4.6. Number of Pods in Lath house Potted Plants

It was observed that French beans number of pods among different crude extract treatments differed significantly($P{\leq}0.05$) at 55, 63, 69 and 76 DAE (Appendices 26,27,28 and 29).The results of this study showed that crude extracts treated Frenchbeans plants produced significantly higher French bean number of pods compared to untreated controls infested with root-knot nematodes. From the lath house study the untreated control attained the least number of pods at 9.5 and 9.7 pods respectively in Trials I and II.

Table 10: Effects of Different Extracts on Root galling Indices

Trial I (lath house)

						Extracts Concentration Rate									
N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	CWith	LSD
*1c	1.6c	1c	1.8c	1.7c	2.7c	2.4c	2.8c	2.8c	4.6bc	3.4bc	1.9c	1.8c	1.1.c	9a	3.2

Trial II

*1.5c	1.6c	1.5c	1.9c	1.9c	2.8c	2.4c	2.8c	2.8c	4.9bc	3.4bc	2.1c	1.8c	1.4.c	10a	2.9

Trial I (Field Experiment)

						Extracts Concentration Rate									
N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	CWith	LSD
2.4bc*	2.1bc	2.1bc	1.1c	1.0c	1.5c	1.1c	3.13bc	4.8b	3.0bc	3.2bc	2.7bc	3.3bc	3.4cb	8.8a	2.9

Trial II

2.1bc	1.2b	1.2b	1.4bc	1.2c	1.4c	1.7c	2.4bc	2.3b	2.5bc	2.4b	2.2bc	3.0b	3.0b	9.2a	2.6

*Means with the same letter(s) along the row for DAP and the rows for Extracts are not significantly different at P≤0.05 by Tukey's test. KEY: N =Neem, T =Tithonia, Te =Tephrosia, Oxy =Oxamyl, CNO =Control, C with =Control with nematodes, the numbers 25, 50, and 100 represent concentration of extract in ml/l.

Neem at 100 ml/l recorded the greatest number of pods at 18.1 and 18.5 pods respectively in Trials I and II, Oxymyl (positive control attained an average of 16.1 and 16.4 pods respectively in Trials I and II. Frenchbeans pod yield from plots treated with extracts were higher than the untreated control showing the potential of increasing yields with the adoption of the extracts.

4.4.7. Number of Pods in a Field Experiment

In the field the number of pods under selected extracts treatments to *Meloidogyne* spp differed significantly (*P≤0.05*)   among the different treatment (Appendices 26,27,28,29 and 30) .The extract produced significantly high French bean number of pods, which was statistically significant compared to untreated control. Untreated control recorded the least mean number of pods at 9.4 and 9.5 pods respectively in Trials I and II. Neem at 100 ml/l attained the highest average mean number of pods at 17.4 and 17.6 pods respectively in Trials I and II. Oxymyl (positive control) attained 15.3 and 15.5 pods respectively in Trials I and Trials II

4.4.8. Pods Weight in a Lath house Potted Plants

From the study it was observed that Frenchbeans pods weight differed significantly among the different extracts treatments at 69, 76 and 83 DAP under lath house experiment (Appendices 32, 33 and 35) .The results of this study showed that French-

beans plants treated with the extract produced significantly higher French bean pods weight when compared to the untreated root-knot nematode infested plants. From the study it was observed that the untreated control treatment attained the least pods weight of 24.9 and 28.0 in Trials I and II, respectively. Neem at 50 ml/l attained the highest average mean pods weight of 50.9 in both Trials I and Trials II. Oxymyl (positive control) attained an average weight of 48.7 and 49.3 in Trials I and II, respectively.

Neem, Tithonia and Oxymyl compared in suppression of which resulted in increase in French bean pod weight. This can be attributed to better control of the root knot nematodes especially in early stages therefore allowing the French bean crop to grow with vigor resulting in French bean pod weight increase. In both Trials, Oxamyl gave lower French bean pod weight.

Table 11: Effects of Different Extracts on Lath house Number of Pods Trials I and II

	Extracts Concentration Rate															
DAE	N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	Cwit	LSD
55	22.2bdac	24.6a	23.6cab	24.6a	23.3bac	21.1bdec	22.6bdac	19.7de	20.4bec	21.0bdec	18.4e	22.2bdec	20.46cbec	24.26a	15.1f	3.09
62	21.7a	22.3a	20.4a	21.3a	20.7a	22.1a	20.1a	24.2a	22.6a	22.8a	20.6a	20.86a	20.2a	21.1a	16.3b	4.81
69	16.0ba	17.0a	10.4bac	14.2ba	15.6ba	14.2ba	16.2ba	16.8a	16.4ba	15.8ba	15.8ba	7.93bc	13.2bac	10.4bac	4.93c	8.61
76	11.2ba	13.7ba	18.3ba	11.46ba	17.2ba	15.6ba	16.7ba	15.7ba	19.1a	17.3ba	18.2ba	17.3ba	17.9ba	15.6ba	10.2b	8.39
83	8.67a	8.06a	8.8a	7.93a	7.26ba	7.2ba	4.8ba	4.2ba	5.8ba	9.8a	7.8a	7.7a	7.13ba	6.26ba	1.4b	5.9
DAE	N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	Cwit	LSD
55	22.2bdac	24.6a	23.6cab	24.6a	23.3bac	21.1bdec	22.6bdac	19.7de	20.4bec	21.0bdec	18.4e	22.2bdec	20.46cbec	24.26a	15.1f	3.09
62	22.7a	22.3a	21.4a	21.3a	20.7a	21.1a	21.1a	24.2a	22.6a	21.8a	21.6a	20.86a	20.2a	22.1a	16.3b	4.61
69	16.1ba	16.0a	1014bac	14.2ba	15.6ba	13.2ba	16.4ba	16.8a	16.4ba	15.8ba	12.8ba	7.95bc	12.2bac	11.4bac	4.83c	8.41
76	11.1ba	13.7ba	18.4ba	11.56ba	17.1ba	15.6ba	15.7ba	15.8ba	19.1a	17.4ba	18.2ba	17.3ba	17.7ba	15.4ba	9.2b	8.69
83	8.57a	8.16a	8.8a	7.93a	7.26ba	7.4ba	4.9ba	4.2ba	5.9ba	9.6a	7.8a	7.4a	7.13ba	6.26ba	1.4b	6.9

*Means with the same letter(s) along the row for DAP and the rows for Extracts are not significantly different at P≤0.05 by Tukey's test. KEY: N =Neem, T =Tithonia, Te =Tephrosia, Oxy =Oxamyl, CNO =Control, C with =Control with nematodes, the numbers 25, 50, and 100 represent concentration of extract in ml/l

Table 12: Effects of Different Extracts on Field Number of Pod Trials I and II

Extracts Concentration Rates

DAE	N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	CWith	LSD
55	22.dce	20.3fe	21.6fde	22.6bdac	21.7dec	23.5bac	24.36a	24.53a	24.13ab	22.30bdac	23.06bdac	21.66fdec	19.9f	21.63fdec	14.33g	1.87
62	20.76ba	21.06ba	20.33ba	21.46a	17.4b	21.6ba	20.1ba	21.63a	21.73a	21.73a	21.76a	21.76a	19.80ba	19.66ba	11.1c	4.05
69	13.6ba	14.1ba	14.4ab	14.76ba	13.6ba	11.06bc	13.3ba	15.3ba	12.8bac	16.8a	17.2a	17.4a	15.6ba	13.5ba	7.26c	5.79
76	11.5dc	17.6ba	13.3bc	17.0bac	15.6bac	18.3ba	17.7ba	16.7bac	16.7bac	19.4a	13.3bc	18.7ba	15.8bac	15.16bac	6.7d	5.47
83	7.5a	8.1a	9.8a	6.9a	8.5a	6.4a	6.6a	6.9a	7.7a	8.3a	7.3a	8.0a	5.7a	7.6a	5.2a	4.73

DAE	N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	CWith	LSD
55	23.dce*	22.3fe	21.6fde	23.6bdac	21.7dec	24.5bac	24.36a	24.53a	21.13ab	24.30bdac	23.16bdac	21.26fdec	19.7f	21.53fdec	14.53g	1.97
62	20.16ba	20.06ba	21.33ba	21.56a	16.4b	22.6ba	20.1ba	21.63a	21.73a	22.73a	20.76a	22.76a	19.90ba	19.66ba	11.1c	4.15
69	13.6ba	14.1ba	14.4ab	14.76ba	13.6ba	11.06bc	13.3ba	15.3ba	12.8bac	16.8a	17.2a	17.4a	15.6ba	13.5ba	7.26c	5.79
76	10.5dc	16.6ba	13.3bc	17.3bac	15.8bac	17.3ba	17.4ba	16.7bac	16.7bac	19.4a	13.2bc	18.6ba	15.8bac	15.26bac	6.5d	5.37
83	7.3a	8.3a	9.1a	6.9a	8.1a	6.4a	6.1a	6.1a	7.7a	8.3a	7.3a	8.0a	5.7a	7.6a	5.1a	4.23

*Means with the same letter(s) along the row for DAP and the rows for Extracts are not significantly different at P≤0.05 by Tukey's test. KEY: N =Neem, T =Tithonia, Te =Tephrosia, Oxy =Oxamyl, CNO =Control, C with =Control with nematodes, the numbers 25, 50, and 100 represent concentration of extract in ml/l

Table 13: Effects of Different Extracts on Lath house Pods Weight Trials I and II

DAE	N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	CWith	LSD
55	59.46a	63.1a	62.6a	52.6ab	57.5a	60.5a	62.4a	59.5a	43.8ba	54.6ba	57.4ba	62.0a	62.2a	55.8a	35.5b	20.2
62	63.1a	63.7a	63.1a	63.5a	56.4a	65.6a	55.1a	57.2a	58.9a	59.7a	54.6a	54.8a	57.6a	58.2a	31.5b	22.15
69	51.7ba	51.8ba	44.7ba	5654a	53.6ba	46.8ba	52.4ba	55.5ba	58.8a	53.4a	49.7ba	28.7bc	43.8ba	46.8ba	15.9c	27.15
76	43.5ba	48.2a	52.7a	22.7b	50.6a	46.4ba	45.2ba	44.8ba	42.4a	49.8a	46.4a	52.6a	45.3ba	50.3a	29.1ab	22.5
83	32.2a	26.9a	28.7a	23.9ba	29.0a	23.5ba	16.8ba	17.7ba	23.4ba	23.7a	23.4ba	26.72a	21.3ba	27.34a	20.3ba	20.1

DAE	N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	CWith	LSD
55	59.36a	64.1a	61.6a	55.6ab	59.5a	61.5a	63.4a	58.5a	44.8ba	56.6ba	56.4ba	62.0a	62.2a	57.8a	35.5b	21.2
62	64.1a	63.7a	64.1a	63.5a	57.4a	65.6a	54.1a	58.2a	58.9a	59.7a	57.6a	55.8a	57.6a	58.2a	30.5b	23.05
69	50.7ba	52.8ba	45.7ba	56.4a	53.6ba	46.8ba	51.4ba	55.5ba	59.8a	54.4a	49.7ba	28.6bc	43.5ba	46.3ba	15.5c	27.05
76	46.5ba	48.2a	53.7a	22.9b	50.6a	46.4ba	46.2ba	44.9ba	48.4a	49.8a	48.4a	52.6a	45.3ba	53.3a	30.1ab	24.4
83	31.2a	25.9a	27.7a	22.9ba	29.0a	23.5ba	15.8ba	17.7ba	21.4ba	26.7a	21.4ba	26.72a	20.3ba	26.34a	19.3ba	21.1

*Means with the same letter(s) along the row for DAP and the rows for Extracts are not significantly different at P≤0.05 by Tukey's test. KEY: N =Neem, T =Tithonia, Te =Tephrosia, Oxy =Oxamyl, CNO =Control, C with =Control with nematodes, the numbers 25, 50, and 100 represent concentration of extract in ml/l

Although Tephrosia would be expected to boost French bean pod weight, it seems their effect was overwhelmed by the significantly high RKN populations. Generally French-beans pod weight from plots treated with the extracts were higher than the untreated control showing the potential of increasing pod weight.

4.4.9. Pods Weight in a Field Experiment

In the field experiment French beans pods weight under extracts treatments differed significantly($P \leq 0.05$) [Appendices 31,32,33 and 34].The study showed that the untreated control treatment severely infested by the root-knot nematodes attained the least pods weight of 28.3 and 32.6 in Trials I and II, respectively. Nimbecidine at 100ml/l treatments attained the highest pod weight of 60.5 in Trials I while Tephrosia treatments at 100 ml/l attained the highest pods weight of 62.2.Oxymyl (positive control) attained pod weight of 58.2 and 59.0 in Trials I and II, respectively .In both Trialss, Oxamyl gave lower French bean pod weight. Although Tephrosia would be expected to boost French bean pod weight, it seems their effect was overwhelmed by the significantly high root-knot nematodes populations. Generally Frenchbeans pod weight from plots treated with the extracts were higher than the untreated control showing the potential of increasing pod weight.

Table 14: Effects of Different Extracts on Field Pods Weight Trials I and II

							Extracts Concentration Rate									
DAE	N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	CWith	LSD
55	67.0a*	73.4a	71.1a	72.0a	70.52a	74.1a	73.2a	70.9a	71.1a	67.7a	69.2a	74.0a	69.2a	69.2a	29.9b	18.5
62	69.2a	71.0a	73.3a	73.4a	62.6a	72.6a	67.4a	66.8a	73.0a	72.5a	69.0a	65.2a	69.2a	68.9a	32.4b	19.0
69	54.3a	48.1a	47.3a	49.7a	50.0a	46.2a	46.9a	51.9a	44.3a	52.9a	51.5a	53.1a	48.2a	53.82a	26.7b	16.4
76	57.1a	63.3a	65.5a	63.7a	64.2a	66.5a	63.2a	63.3a	58.09a	62.0a	60.1a	64.2a	64.3a	57.7a	20.2b	16.7
83	35.2ba	37.5ba	42.1ba	37.5ba	41.4ba	43.16a	38.0ba	40.4ba	39.1ba	41.3ba	39.4ba	42.0ba	40.5ba	39.2ba	27.82ba	20.5

DAE	N25	N50	N100	Nb25	Nb50	Nb100	T25	T50	T100	Te25	Te50	Te100	Oxy	CNO	CWith	LSD
55	68.0a*	73.4a	72.1a	72.0a	71.52a	74.1a	73.2a	71.9a	71.1a	67.7a	69.2a	75.0a	69.2a	67.2a	28.9b	17.5
62	68.2a	70.0a	71.3a	71.4a	62.6a	72.6a	62.4a	66.8a	73.0a	71.5a	70.0a	65.2a	67.2a	67.9a	32.4b	18.1
69	52.3a	45.1a	47.3a	49.7a	51.0a	46.2a	47.9a	51.9a	43.3a	52.9a	52.5a	52.1a	48.2a	53.82a	26.7b	16.5
76	51.1a	62.3a	61.5a	63.7a	64.2a	63.5a	63.2a	62.3a	58.09a	61.0a	61.1a	64.2a	63.3a	57.7a	21.2b	16.7
83	34.2ba	36.5ba	43.1ba	37.5ba	41.4ba	42.16a	37.0ba	40.8ba	39.5ba	42.3ba	38.4ba	41.0ba	40.5ba	38.2ba	25.8ba	21.5

*Means with the same letter(s) along the row for DAP and the rows for Extracts are not significantly different at P≤0.05 by Tukey's test. KEY: N =Neem, T =Tithonia, Te =Tephrosia, Oxy =Oxamyl, CNO =Control, C with =Control with nematodes, the numbers 25, 50, and 100 represent concentration of extract in ml/l

CHAPTER FIVE

DISCUSSION

5.1. Phyto-chemical Constituents in the Leaf Extracts of Neem, Tithonia and Tephrosia

Application of botanical extracts has been used in pest control strategies probably because they contain insecticidal effects. Preliminary qualitative phytochemical analysis performed in this study revealed the presence or combination of alkaloids, Terpenoids and Saponin in the crude leaf extracts of Neem, Tithonia and Tephrosia. Mishra (2018) indicated that Neem, Tithonia and Tephrosia formulations contains phenols, amino acids, aldehydes and fatty acids which are antagonistic to root-knot nematodes and other soil borne pest. The crude extracts and metabolites of these plants have been found to exhibit various bioactivities including, Nematicidal properties, antimicrobial, insecticidal, phytotoxic among others (Susmitha *et al*., 2013).

Mariana *et al.* (2018) reported that the most characteristic metabolites in Neem are called limonoids which has considerable interest due to its broad biological activity and fascinating structural diversity. Mariana *et al*. (2018) indicated that flavonoids are purified from neem fresh leaves which confirms earlier findings by Dash *et al*. (2018) that extracts from neem are rich in secondary metabolites and Phytoconstituents such as saponins and flavonoid and reducing sugar. Investigation by Dalwadi *et al*. (2018) on the aerial extract of Tephrosia yielded the saponin and prenylated flavonoids and Phytochemical studies by Rahman *et al.* (2018) on Tithonia plants indicated that plants of this genus had led to the isolation of compounds including lignins, flavonoids, terpenoids , steroids and Nitro group containing compounds

5.2. *In vitro* Nematicidal Activity of the Leaf Extracts of Neem, Tithonia and Tephrosia

5.2.1. Effects of Different Extracts on Root-knot Nematode EggHatch

After the analysis for the phytochemical properties the Neem, Tithonia and Tephrosia crude extracts were tested againstFrench beans root-knot nematodes *Meloidogyne incognita* egg hatch. The results showed that Neem, Tithonia and Tephrosia crude extracts treatments significantly affected the root-knot nematode egg hatch capacity. The results indicated that the extracts from Neem and Tithonia especially at the

highest concentration (100 ml/l) exhibited the greatest activity on egg hatchability after 36 h of the exposure time in invitro conditions. All extracts gave hatching capacity values less than the control as a nematicidal index. The mortality was non-significantly similar to Oxymyl (positive control).This shows the capacity of the extracts to reduce the hatchability of the root knot nematodes was similar to that of Oxymyl a synthetic nematicide.

Sturtz *et al*. (2018) conducted a survey onthe nematicidal activity of Neem, Tithonia and Tephrosia compounds based on 24-h LC50 values and reported that there ispotent nematicidalactivity of these extracts against the root-knot nematodes egg hatch. In this study the capacity to reduce the hatchability of the nematodes was similar with the synthetic nematicide used as the control. During the phytochemical analysis presence or combination of alkaloids, Terpenoids and Saponin in the crude leaf extracts of Neem, Tithonia and Tephrosia were observed. These compounds observed in the extracts were probably responsible for the activity observed. From the study it was observed that the rate of egg hatching was inversely proportionate to concentration of extracts and directly proportionate to exposure period. This indicates that the root knot nematode hatchability decreased with increase in concentration of the extracts. Similar findings were reported by Wahome *et al*. (2018) who observed that extracts from these plants increased mortality percentage of juveniles and inhibited nematode egg hatch when compared with control.

5.2.2. Effects of Extracts and Synthetic Nematicides on Root-knot Nematode Mortality

The crude extracts of Neem, Tithonia and Tephrosia were evaluated against *Meloidogyne incognita* J2mortality through exposing the juveniles to the extract in *invitro* conditions.. All the crude extracts evaluated showed significant activity against the Frenchbeans root-knot nematode juveniles (J2) mortality. The results indicated that the crude extracts exhibited highest activityon the J2 mortality after 24 h of the exposure time in *invitro* conditions. All extracts resulted to LC50 values less than the control as a nematicidal index. However, the mortality was not significantly different from the results of the Oxymyl which was the positive standard control. Resha *et al*.(2018) evaluated the efficacy of crude extracts from Neem on their activity against the root-knot nematodes and reported that there was highest decrease in the number of

root-knot nematodes J2 mortality and mobility inhibition when juveniles were exposed to the crude extract at higher concentration of 100 ml/l. The results of this study showed that the inhibitory effects of Neem, Tithonia and Tephrosia on the juveniles nematode mortality was dependent on the concentration rate where , the activity of the root-knot nematode (J2) decreased by increasing the rate of crude extract concentration. From the study Neem extracts at 100ml/l were the most effective which indicates that the activity of the root-knot nematode (J2) probably depended on the source and the concentration of the extract. This results corraborates with earlier findings by Mishra (2018) who reported that the neem formulations are most effective in the control of root knot nematode when compared to other botanicals extracts

Lambert *et al.* (2018) reported that aldehydes, fatty acids, phenols, amino acids are released from botanical extracts and are antagonistic to root-knot nematodes. It was observed that the rate of juvenile mortality was directly proportional concentration of extracts and exposure period. The highest mortality rate of the juveniles larvae was observed at 7[th]day in all crude extracts while lowest mortality of the J2 being observed at low concentration rates of 25ml/l in all extracts evaluated. From the study it was observed that mortality was nil in control at the beginning of the experiment.

Similar findings were reported by Abdul *et al.* (2018)who indicated that crude extract of leaves from Neem, Tithonia and Tephrosia are toxic against the 2nd stage juveniles of root knot nematode. The findings are in agreement with those by Odeyemi *et al.* (2018) who showed the inhibitory effects from crude leaf extracts from Tithonia. The nematicidal effect of Neem, Tithonia and Tephrosia crude 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. The results of this study support the preliminary scientific knowledge of local farmers and validation for the use of these extracts for nematicidal activity and other pesticidal activities to promote proper sustainable use of these plant resources.

5.3. Comparative Effect of the Leaf Extracts on French bean Growth and Yield

5.3.1. Effects of Different Extracts onFrench bean Growth Parameters

The yield of Frenchbeans, growth and development and are highly influenced by the well-being of Frenchbeans from germination to end of the harvesting season. In the present study, application of Neem, Tithonia and Tephrosia crude extracts significantly increased the growth parameters of French bean which included the plant height, number of leaves, the number of branches and yield attributes like the number of pods and the pod weight. From the results of the study the increase in growth, yield and other attributes can be attributed to the better control of the root-knot nematodes through utilization of plant extracts in root knot nematode management.

The highest French bean yield was noticed with application of Neem and Tithonia at 100ml/l.The processes involved in French-beans production like plant height, number of pods, and number of branches help in determining the French beans yield. French bean yield have been recognized as differing in sensitivity to root-knot nematode infestation. The nematodes activity is normally concentrated on the roots. This probably hinders the access of the plant to growth resources like nutrients and water. Presence of root knot nematodes probably resulted to water stress and other physiological parameters during the growing stages hence the greatest reductions in French bean pod yield where no extracts were applied. The number of pods formed and the pod weight is related to available water and nutrition (USAID, 2018). Al-Hazmi *et al*. (2018) reported that the number pods and pod weight is determined by the soil conditions and environmental during the growth stage of Frenchbeans.

This explains why the root-knot nematodes and their consequent maintenance in French bean production are very crucial in determining number of pods and pod weight. The number of pods per plant also depends on the number of stems per plant. Olsen (2017) reported that there is a general relationship between plant height, numbers of branches, plant height, pod number and pod weight whereby an increase in plant height, number of branches and number of leaves often results to an increase in the number of pod weight in French beans. It is possible in this the untreated plants were severely infested by root-knot nematodes and thus experienced severe water and

nutrition stress, which resulted to fewer number of stems, number of branches, pods number and lower pod weight.

It has been reported that improvement in yield in Frenchbeans may be due to higher availability of soil moisture, which helps in better nutrient uptake by the crop which in turn results in assimilation of photosynthate towards the sink .Plant height increased with the increase in concentration of all tested plant extracts and the increase was significant at all concentrations rates. Nematode inoculated plants drenched with the Neem extracts attained the maximum height followed by plants treated with Tithonia at 100ml/l.

The results obtained from this study showed that Neem, Tithonia and Tephrosia extracts had significant effect in the suppression of French bean root-knot nematodes under both *invitro*, lath house pot experiments and under field conditions. Therefore, it was evident that the botanical extracts can be used in the management of French bean parasitic root-knot nematodes. In the Lath house pot experiments French bean treated with Neem, Tithonia and Tephrosia extracts at 100ml/l significantly increased the plant height, number of branches, number of pods and pod weight and number of root galls when compared with the un-inoculated plants that received no treatment.

5.3.2. Effects of Different Extracts on French bean Yield

The presence of the nematode on the Frenchbeans plants significantly affected the Frenchbean yield. Crude plant extract inoculated plants had 50% higher yields than plants which were not inoculated with the extracts. Yield in terms of pod number and pod weight from French beans treated with the crude plant extracts were significantly better than yields from plants treated with Oxamyl. The findings of this study also compares with those of Sidhu *et al.* (2017) who reported that application of botanical extracts increases yields and may help manage root-knot nematode populations in infested fields. Probably Neem, Tithonia and Tephrosia extracts above their ability to suppress plant parasitic nematodes by changing the soil physical properties and probably they also changed the chemical properties and enriched the soil.

Changes in the soil properties probably negatively affected the multiplication and growth of the nematodes in the French beans. The findings of this research are also in agreement with those of Aiyadurai *et al*. (2019) who suggested that root-knot nematodes can be controlled by application of botanicals. Nutrition and water and are important components in French bean production and their unavailability and limited supply is one of the major abiotic factors that adversely affect French-beans production in many ways. Nutrition is the basic need of all plants as the nutrients are not only required for better development and plant growth but they are helpful to alleviate stresses like drought stress and different kinds of abiotic stress.

Recent trends indicate that fertility of soils are globally declining and productivity is declining due to intensive use of soils without consideration of proper soil management practices and use of synthetic synthetics (Muhammad *et al*., 2018). Studies by Sikora *et al*. (2018) indicated that root-knot nematodes inhibits photosynthetic carbon fixation which directly inhibit plant metabolism. It is possible that French-beans plants treated with higher crude extracts level compared to low extract levels and untreated plants experienced better and high metabolism and consequently better growth .From the study it was noted that the crude extracts significantly .increased the pod number and pod weight of French bean plants per plant when compared to the untreated control. This results of these study compares with those by Stirling *et al.* (2017) who reported that crude extracts from Neem leaf extracts at 100 ml/l and Tithonia 100 ml/l were most effective and increased the yield significantly.

 From the study it was noted that the increase in French bean yield was more when compared with the chemical nematicides. Nimbecidine leaf extracts at all levels of concentration followed the neem in improving the French beans yield. The least yield was obtained from the severely infested untreated control. In all botanical extracts application, yields of French bean per plant increased with the increase in concentration. On average tephrosia leaf extracts, were the least effective in increasing the yield of French bean per plants.

The importance of organic production, which avoids synthetic nematicides applications, increased the research on botanical pesticides with potential use for rnematode management (Mishra *et al*., 2018). In this study application of plant extract was found to reduce root-knot nematode galling indices on French beans root system and thus the final nematode population density in the soil was significantly lowered over the untreated treatments. With the increase in level of botanical extract concentration, a corresponding significant reduction was observed in the number of galls and nematode population over untreated control. Root-knot infestation stunted all untreated plants and reduced leaf production as well as French bean yield. The effects of the tested botanical extracts against infestation of root-knot nematode and yield of French beans plant were different, in some cases. The differences in the toxicity of different botanical extracts could be due to the differences in the synthetic compositions and concentrations of extracts components. Crude extract treated plots when compared to the positive control (Oxamyl) a synthetic nematicides controls yielded significantly heavier pod weight at harvest. Also extract treatments gave a 100% increase in both pod number and pod weight over the untreated control in which most of the plants withered away and died. These yield benefits can be attributed to increased root knot nematode control in the soil by the crude extracts amendments.

Botanical extracts have been reported to improve plant tolerance to root-knot nematode damage and in turn promote better yields (Pavaraj *et al*., 2018).Although Oxamyl suppressed RKN population satisfactorily, lower results were obtained when compared to the crude extracts treatment application observed in terms of crop yield. The lower results in Oxamyl when compared to crude extracts could be attributed to the fact that Oxamyl is a broad-spectrum fumigant and could have eliminated the beneficial micro-organisms that would have been useful in maintaining the soil biology and checking the nematodes population.

5.3.3.Effects of Different Extracts on Root galling Indices

Root-knot index is a means for detecting the infestation of *Meloidogyne* species in the roots and the severity caused. In this study, all the Plant extracts were highly effective in reducing root-knot index when compared with untreated plants. The extracts

reduced root galling significantly and were at par with Oxamyl (synthetic nematicides). The study showed that among the crude extracts treatment evaluated, there were variations in root-knot nematode damages. Low galling indices in extract treated control treatments indicated low damages on the roots in French beans, this confirms earlier studies that these extracts are potent in managing the root-knot (Mcsorley, 2018). Root gall index appeared to be a useful parameter in evaluating the efficacy of the crude extracts formulation on *Meloidogyne indica*.

Thestudy revealed that the botanical extracts evaluated improved plant status, exerted significant control on the root-knot nematodes and reduced the root galling indices inFrench beans. It was observed that French bean performance was best in plants treated with Neem and closely followed by those treated with Nimbecidine. These results agrees with those obtained results are in agreement with findings by Pavaraj *et al*. (2018) and Mahmood (2017)who reported that leaf extracts of Neem, Tithonia and Tephrosia significantly reduce root-knot nematodes.

5.3.4. Effect of Different Extracts and synthetics (Oxamyl) on Root knot Nematodes

Studies by Maina *et al. (*2018) reported that botanical extracts release chemical constituent's compounds which are nematicidal, antiviral, insecticidal and cytotoxic in nature. The presence of these synthetics constituents inhibits the hatching of nematode eggs. Siddiqui *et al*. (2018) reported that *Meloidogyne* spp. juveniles are unable to fully develop in the presence of crude botanical extracts.

The low galling indices observed following treatment with Neem and Tithonia agrees with reports by Renco *et al*. (2018) who observed that only a few of the nematodes are able to infest the plants treated with extracts and consequently the root knot nematode juveniles does not reach maturity resulting to low gall index. The present study indicates that Neem, Tithonia and Tephrosia compared in suppression of root-knot nematodes .The study showed that both Neem and Tithonia gave consistent performance in the suppression of root-knot nematodes both in the lath house and in the field conditions. Oxymyl had a significant reduction in nematode counts as was also observed by Kimenju *et al*. (2018) who reported reduction in root-knot nematodes densities following treatment with Oxymyl.

CHAPTER SIX
CONCLUSION AND RECOMMENDATION

6.1.Conclusion

These study provides empirical data confirming the usage of botanicals extracts for improved Frenchbean production as a food security crop in Kenya, although the botanical extracts are in high demand in medicinal practices, they appear to have a higher potential in the control of *M. incognita* and have shown to be favorable alternatives to synthetic nematicides pesticides in French bean production. The application of organic amendments has in the past been recognized in the improvement of soil health and management of parasitic nematodes .Due to environmental benefits associate with botanicals extracts they have been considered in integrated nematode management with other inorganic methods. Although many research have shown reduced parasitic nematode populations with the use of organic amendments, others have shown increased populations with the effectiveness of these amendments (Renco, 2018).

Introduction of crude botanical extracts as an option in the management of parasitic nematodes has become a major component in productivity and sustainable management of soil health and. Various botanical have variable effects on the biological activities in soil and more so against pathogens .Soil nematodes are affected by botanicals added in the form of extracts, compost and other soil amendments. Different botanicals extracts used in this study showed variable degrees in the suppression of root-knot populations in the soil. Among the extracts and other synthetics tested, the best for a practical control application would be those exerting effective and efficient control on root-knot nematodes.

This study established that root-knot suppression between Neem, Tithonia, Tephrosia and Oxamyl did not differ. The botanicals that showed low control can still be considered in future researches for their nematicidal and insecticidal potential on Frenchbean root-knot nematodes. From the study, it is evident that continued use of the botanical extracts nematicides would result in better control levels for root knot nematodes. Eco-friendly nematicides used in the experiment led to root-knot nematodes suppression which did not differ significantly from Oxamyl. This study

illustrates that agricultural utilization of botanical phytosynthetics, although currently under Trials and development in many situations, would offers tremendous potential in the control of root-knot nematodes in Frenchbeans. Since these crude extracts were found to control the root-knot nematodes there is a diverse range of important extracts from which suitable candidates can be selected. Studies should be undertaken to examine the mode of action involved in these crude extracts such as, production of toxic substances, physical barriers and post- inflectional compounds that these extracts produce against the root-knot nematodes. Use of antagonistic plants in nematodes control should be evaluated in comparison to other control strategies like botanical amendments to establish their effects

Studies should be undertaken to determine the effects of these crude extracts antagonistic plants to other damaging nematodes that could be present in the same fields. From the study, it is evident that continued use of the botanical nematicides would result in acceptable control levels for root knot nematodes. Botanical extracts used in the experiment led to root-knot nematode suppression which did not differ significantly from Vydate (synthetic nematicides). This study illustrates that utilization of botanicals .Although currently under development and Trials in many situations, would offers great potential in the control of root-knot nematodes in Frenchbeans. Although the plants in this study are locally used in medicinal practices they could serve as alternatives in a sustainable organic farming system to meet the demand in food production.

6.2.Recommendations

The following of recommendations can be made from the study

  i.   Since the extracts of Neem, Tithonia and Tephrosia were effective in root-knot nematode suppression, they can be recommended for incorporation into integrated root-knot nematode management in French bean production.

 ii.   Since Neem, Tithonia and Tephrosia botanical extracts were comparable to Synthetic nematicides in root-knot nematode management, they can be considered for use as an alternative.

iii.   More studies on the integrationof Neem, Tithonia and Tephrosia botanical extracts and other control agents on the activities of plant parasitic nematodes and other soil borne pest in crop production systems are highly recommended.

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Appendix 1: ANOVA table for Plant Height 14 DAE

Lath house

Trial I						Trial II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	0.08240000	0.04120000	0.46	0.6331	2	0.09240000	0.04120000	0.46	0.4331
Extract	14	1.86106667	0.13293333	1.48	0.1229	14	1.96106667	0.13293333	1.48	0.1329
Block*Extract	28	3.11493333	0.11124762	1.24	0.2039	28	3.11493333	0.21124762	1.24	0.2239
R-Square	0.238128					0.338128				
CV	4.696927					4.796927				
Error	180	16.1840000	0.08991111			180	13.18400000	0.09991111		
Corrected Total	224	21.24000				224	21.24000			
Field										
`Block	2	0.39853333	0.19926667	2.40	0.0121	2	0.39853333	0.19926667	2.40	0.0121
Extract	14	11.000666	0.78576190	9.46	<.0001	14	19.000666	0.93576190	9.76	<.0001
Block*Extract	28	2.71280000	0.09688571	1.17	0.2581	28	2.72280000	0.09688271	2.17	0.2581
R-Square	0.295570					0.295570				
CV	4.495699					4.495699				
Error	33.63300	0.08304444	0.08304444			33.63300	0.08304444	0.08304444		
Corrected Total	449	47.7450000				449	47.7450000			

Appendix 2: ANOVA table for Plant Height 21 DAE

Lath house

Trial I						Trial II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	0.3330667	0.1665333	2.09	0.1269	2	0.4330667	0.3665333	2.19	0.4269
Extract	14	404.6456000	28.9032571	362.40	<.0001	14	406.6456000	28.9032571	382.40	<.0001
Block*Extract	28	1.6749333	0.0598190	0.75	0.8141	28	1.6749333	0.1698190	0.75	0.8141

70

	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
R-Square	0.965901					0.865901				
CV	1.658116					1.658116				
Error	180	14.3560000				180	14.9560000			
Corrected Total	224	421.0096000				224	422.0096000			
Field										
Block	2	0.2025333	0.1012667	0.75	0.4727	2	0.3025333	0.1212667	0.65	0.3727
Extract	14	713.2718667	50.9479905	377.71	0001	14	714.2718667	51.9479905	378.71	0001
Block*Extract	28	4.1568000	0.1484571	1.10	0.3332	28	4.2568000	0.2484571	1.20	0.2332
R-Square	0.929261					0.939261				
CV	2.159812					2.259812				
Error	405					405				
Corrected Total	449					449				

Appendix 3: ANOVA table for Plant Height 28 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	5.9331556	2.8165778	1.48	0.2300	2	5.6331556	2.9165778	1.68	0.5300
Extract	14	112.4219556	8.0301397	4.22	<.0001	14	112.4219556	8.0301397	4.22	<.0001
Block*Extract	28	326.1655111	14.5773397	6.09	<.0001	28	326.1655111	11.5773397	6.09	<.0001
R-Square	0.563812					0.563812				
CV	6.833403					6.933403				
Error	180	344.1200000				20.17511				
Corrected Total	224	785.3406222								
Field										
Block	2	7.2925778	3.6462889	3.49	0.0314	2	7.1925778	3.7462889	3.89	0.0214
Extract	14	724.3985778	51.7427556	49.52	<.0001	14	754.3985778	53.8427556	49.12	<.0001
Block*Extract	28	246.0594222	8.7878365	8.41	<.0001	28	245.0594222	8.6878365	8.81	<.0001
R-Square	0.697909					0.697909				
CV	5.053571					5.053571				
Error	405					405				

Corrected Total	449					449				

Appendix 4: ANOVA table for Plant Height 35 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	5.6331556	2.9165778	1.48	0.2300	2	5.6331556	2.9165778	1.68	0.1300
Extract	14	112.4219556	8.0301397	4.22	<.0001	14	112.4219556	8.0301397	4.22	<.0001
Block*Extract	28	324.1655111	11.5773397	6.09	<.0001	28	324.1655111	11.5773397	6.09	<.0001
R-Square	0.963812					0.563812				
CV	6.833403					6.933403				
Error	180	342.7200000				180	342.3200000				
Corrected Total	224	784.3406222				224	794.3406222				
Field										
Block	2	77.091733	38.545867	5.86	0.0031	2	79.091733	39.545867	5.56	0.0021
Extract	14	2602.842800	185.917343	28.27	<.0001	14	2902.842800	183.917343	27.27	<.0001
Block*Extract	28	89.012267	3.179010	0.48	0.9890	28	87.012267	3.189010	0.38	0.7890
R-Square	0.509673					0.409673				
CV	11.50029					11.90029				
Error	405					405				
Corrected Total	449					449				

Appendix 5: ANOVA table for Plant Height 42 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	96.045067	49.022533	4.58	0.00	2	99.045067	49.022533	4.28	0.015
Extract	14	91434.42560	57.049981	5.75	<.0001	14	91435.4256	67.649981	5.85	<.0001
Block*Extract	28	39.699733	5R.265200	4.37	<.0001	28	39.699733	51.165200	4.47	<.0001
R-Square	0.545148					0.545148				
CV	14.37400					14.37400				
Error	180	2062.692000				180	2065.692000				

Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Corrected Total	224	4534.862400				224	3534.862400			
Field										
Block	2	541.705244	270.852622	30.52	<.0001	2	544.705244	272.852622	31.52	<.0001
Extract	14	3376.774978	241.198213	27.18	<.0001	14	3370.774978	252.198213	21.18	<.0001
Block*Extract	28	2238.666756	79.952384	9.01	<.0001	28	2238.666756	79.752384	8.01	<.0001
R-Square	0.631443					0.831443				
CV	12.75178					12.95178				
Error	405					405				
Corrected Total	449					449				

Appendix 6: ANOVA table for Plant Height 49 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	332.960000	150.480000	3.17	0.0043	2	302.960000	150.480000	3.17	0.0443
Extract	14	1250.640000	69.331429	1.97	0.0322	14	1250.640000	86.331429	1.87	0.0322
Block*Extract	28	2175.440000	77.622857	1.72	0.0118	28	2173.440000	74.622857	1.62	0.0318
R-Square	0.302386					0.202386				
CV	21.52669					21.52669				
Error	180	8596.40000				180	8598.40000				
Corrected Total	224	4534.862400				224	3534.862400				
Field										
Block	2	2215.1509	1107.575467	32.57	<.0001	2	2255.1509	1147.575467	38.57	<.0001
Extract	14	4408.603200	314.900229	9.26	<.0001	14	4438.603200	314.900229	9.26	<.0001
Block*Extract	28	2971.889067	106.138895	3.12	<.0001	28	2991.889067	109.138895	5.12	<.0001
R-Square	0.410604					0.310604				
CV	23.99780					25.99780				
Error	405					405				
Corrected Total	449					449				

Appendix 7: ANOVA table for Plant Height 56 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	375.665156	182.832578	3.48	0.0400	2	365.665156	182.832578	3.28	0.0400
Extract	14	990.899822	68.635702	1.53	0.057	14	960.899822	68.635702	1.23	0.2567
Block*Extract	28	3583.709511	124.418197	2.23	0.0009	28	3483.709511	124.418197	2.23	0.0009
R-Square	0.523887					0.323887				
CV	27.64996					28.64996				
Error	180	10041.42000	56.78567			180	10041.42000	55.78567		
Corrected Total	224	14855.69449				224	14851.69449			
Field										
Block	2	2064.641378	1032.320689	14.56	<.0001	2	2164.641378	1232.320689	12.56	<.0001
Extract	14	9162.494978	654.463927	9.23	<.0001	14	9162.594978	674.463927	9.43	<.0001
Block*Extract	28	8512.012622	304.000451	4.29	<.0001	28	8552.212622	324.000451	4.39	<.0001
R-Square	0.407389					0.507389				
CV	31.23204					33.23204				
Error	405					405				
Corrected Total	449					449				

Appendix 8: ANOVA table for Plant Height 63 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	1571.594756	889.797378	10.25	<.0001	180	1417.74400	79.7596		
Extract	14	3080.311289	260.022235	1.79	0.0009	224	2632.77262			
Block*Extract	28	7604.122578	284.290092	3.27	<.0001	2	1771.594756	885.797378	11.25	<.0001
R-Square	0.559579					14	3080.311289	220.022235	2.79	0.0009
CV	33.25639					28	7204.122578	257.290092	3.27	<.0001
Error	0.459579					0.459579				
Corrected Total	32.25639					32.25639				
Field										

Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	35.336311	17.668156	0.27	0.7605	2	37.336311	18.668156	0.17	0.4605
Extract	14	6195.444978	442.531784	6.86	<.0001	14	6185.444978	449.531784	6.96	<.0001
Block*Extract	28	7176.145022	256.290894	3.97	<.0001	28	7178.145022	250.290894	3.47	<.0001
R-Square	0.339223					0.439223				
CV	30.30640					31.30640				
Error	405					405				
Corrected Total	449					449				

Appendix 9: ANOVA table for Number of Branches 14 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	0.43555556	0.21777778	0.70	0.5004	2	0.63555556	0.31777778	0.90	0.4004
Extract	14	29.44888889	2.10349206	6.71	<.0001	14	29.54888889	2.30349206	6.91	<.0001
Block*Extract	28	79.96444444	2.85587302	9.11	<.0001	28	79.86444444	2.95587302	9.41	<.0001
R-Square	0.660750					0.660750				
CV	12.43301					13.43301				
Error	180	56.4000000	0.3133333			180	56.4000000	0.3133333		
Corrected Total	224	166.2488889				224	166.2488889			
Field										
Block	2	0.6977778	0.3488889	1.81	0.1643	2	0.7977778	0.3588889	1.71	0.1343
Extract	14	233.2311111	16.6593651	86.61	<.0001	14	235.2311	16.9593651	87.61	<.0001
Block*Extract	28	6.2355556	0.2226984	1.16	0.2674	28	6.4355556	0.3226984	1.26	0.1674
R-Square	0.755081					0.775081				
CV	9.679134					9.879134				
Error	405					405				
Corrected Total	449					449				

Appendix 10: ANOVA table for Number of Branches 21 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	3.546667	1.973333	0.67	0.5150	2	3.346667	1.773333	0.67	0.5550
Extract	14	3598.960000	257.068571	96.56	<.0001	14	3599.960000	257.068571	96.56	<.0001
Block*Extract	28	48.453333	1.830476	0.65	0.9111	28	49.453333	1.730476	0.65	0.9111
R-Square	0.883975					0.983975				
CV	7.367392					7.567392				
Error	180	479.200000	2.662222			180	479.200000	2.662222		
Corrected Total	224	4130.160000				224	4130.160000			
Field										
Block	2	2.791111	1.395556	0.40	0.6686	2	2.891111	1.495556	0.30	0.5686
Extract	14	6222.457778	444.461270	128.34	<.0001	14	6322.457778	444.761270	126.34	<.0001
Block*Extract	28	36.142222	1.290794	0.37	0.9988	28	36.342222	1.490794	0.27	0.7988
R-Square	0.816988					0.916988				
CV	8.457247					8.557247				
Error	405	1402.600000				405	1422.600000			
Corrected Total	449	7663.991111				449	7463.991111			

Appendix 11: ANOVA table for Number of Branches 28 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	46.942222	23.471111	5.29	0.0059	2	48.842222	25.471111	5.89	0.0059
Extract	14	1013.395556	72.385397	16.30	<.0001	14	1023.395556	75.385397	16.30	<.0001
Block*Extract	28	167.857778	5.994921	1.35	0.1251	28	169.857778	5.994921	1.55	0.0151
R-Square	0.605800					0.605800				
CV	8.851838					8.951838				
Error	180	799.200000	4.440000			180	795.200000	4.940000		
Corrected Total	224	2027.395556				224	2058.395556			
Field										

Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	5.613333	2.806667	0.68	0.5089	2	5.513333	2.906667	0.58	0.4089
Extract	14	2210.800000	157.914286	38.07	<.0001	14	2110.800000	159.914286	37.07	<.0001
Block*Extract	28	40.186667	1.435238	0.35	0.9994	28	42.186667	1.335238	0.45	0.8994
R-Square	0.573250					0.773250				
CV	8.474229					8.574229				
Error	405					405				
Corrected Total	449					449				

Appendix 12: ANOVA table for Number of Branches 35 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	0.808889	0.704444	0.55	0.7796	2	0.908889	0.604444	0.25	0.6796
Extract	14	8782.862222	660.204444	372.32	<.0001	14	8782.862222	690.204444	392.32	<.0001
Block*Extract	28	45.591111	1.628254	1.00	0.5669	28	45.791111	1.928254	1.00	0.4669
R-Square	0.867632					0.967632				
CV	4.923116					4.723116				
Error	180	222.000000	1.922222			180	299.000000	1.922222		
Corrected Total	224	9421.262222				224	9081.262222			
Field										
Block	2	18.613333	9.306667	1.52	0.2207	2	19.613333	9.706667	1.72	0.1207
Extract	14	3126.786667	223.341905	36.40	<.0001	14	3226.786667	225.341905	38.40	<.0001
Block*Extract	28	1850.920000	66.104286	10.77	<.0001	28	1950.920000	67.104286	11.77	<.0001
R-Square	0.667813					0.767813				
CV	10.14416					11.14416				
Error	405					405				
Corrected Total	449					449				

Appendix 13: ANOVA table for Number of Branches 42 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	3.562222	2.731111	0.15	0.0031	2	3.662222	1.631111	0.25	0.0331
Extract	14	1559.528889	110.923492		<.0001	14	1551.528889	110.823492		<.0001
Block*Extract	28	657.737778	23.483492	5.36	<.0001	28	657.537778	23.483492	7.36	<.0001
R-Square	0.924020					0.624020				
CV	11.34807					11.34807				
Error	180	1533.200000	7.406667			180	1433.200000	7.406667		
Corrected Total	224	3545.928889				224	3555.928889			
Field										
Block	2	8.804444	4.402222	0.36	0.6969	2	8.904445	4.802822	0.56	0.4969
Extract	14	2025.977778	144.712698	11.88	<.0001	14	2125.987778	145.712698	12.88	<.0001
Block*Extract	28	806.595556	28.806984	2.37	<.0001	28	809.595556	28.806984	2.47	<.0001
R-Square	0.365484					0.465484				
CV	14.46793					15.46793				
Error	405					405				
Corrected Total	449					449				

Appendix 14: ANOVA table for Number of Branches 49 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	9.9288889	4.9644444	0.47	0.6280	2	9.9288889	4.9644444	0.47	0.2280
Extract	14	471.7155556	33.6939683	3.17	0.0002	14	471.7155556	29.6939683	5.17	0.0002
Block*Extract	28	673.2711111	24.0453968	2.26	0.0007	28	697.2711111	29.0453968	5.76	0.0007
R-Square	0.376131					0.876131				
CV	13.52256					14.52256				
Error	180	1955.600000	11.442222			180	1915.600000	10.642222		
Corrected Total	224					224				
Field										

Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	9.880000	4.940000	0.40	0.6679	2	9.780000	4.340000	0.40	0.4675
Extract	14	1597.533333	114.109524	9.33	<.0001	14	1547.933333	11.149524	7.33	<.0001
Block*Extract	28	1588.986667	56.749524	4.64	<.0001	28	1558.986667	59.449524	4.94	<.0001
R-Square	0.392269					0.492269				
CV	14.46940					14.86940				
Error	405					405				
Corrected Total	449					449				

Appendix 15: ANOVA table for Number of Branches 56 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	5.395556	2.697778	0.49	0.6129	2	5.3195556	2.397778	0.49	0.6129
Extract	14	1481.582222	105.827302	19.26	<.0001	14	1481.582222	115.827302	18.26	<.0001
Block*Extract	28	428.471111	15.302540	2.78	<.0001	28	488.471111	19.302540	2.78	<.0001
R-Square	0.659442					0.859442				
CV	9.809532					9.709532				
Error	180	989.200000	5.495556			180	996.200000	4.895556		
Corrected Total	224	2904.648889				224	271.648889			
Field										
Block	2	1.853333	0.926667	0.10	0.9022	2	1.753333	0.986667	0.50	0.7022
Extract	14	2637.333333	188.380952	20.92	<.0001	14	2637.333333	180.380952	20.72	<.0001
Block*Extract	28	206.213333	7.364762	0.82	<.0001	28	216.213333	7.564762	0.92	<.0001
R-Square	0.438293					0.538293				
CV	12.53757					12.83757				
Error	405					405				
Corrected Total	449					449				

Appendix 16: ANOVA table for Number of Branches 63 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	0.595556	0.297778	0.05	0.9546	2	0.595556	0.297778	0.05	0.9546
Extract	14	2660.195556	190.013968	29.68	<.0001	14	2660.195556	190.013968	29.68	<.0001
Block*Extract	28	354.071111	12.645397	1.98	0.0043	28	354.071111	12.645397	1.98	0.0043
R-Square	0.723464					0.723464				
CV	10.48644					10.48644				
Error	180	1152.400000	6.402222			180	1152.400000	6.402222		
Corrected Total	224	4167.262222				224	4167.262222			
Field										
Block	2	559.960000	279.980000	9.34	0.0001	2	554.960000	274.880000	9.64	0.0001
Extract	14	6910.533333	493.609524	16.47	0.0001	14	6810.833333	493.709524	15.47	0.0001
Block*Extract	28	3932.106667	140.432381	4.69	0.0001	28	3942.806667	134.432381	4.79	0.0001
R-Square	0.484434					0.584434				
CV	16.58768					16.98768				
Error	405					405				
Corrected Total	449					449				

Appendix 17: ANOVA table for Number of Leaves 14 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	0.43555556	0.21777778	0.70	0.5004	2	0.63555556	0.31777778	0.90	0.4004
Extract	14	29.44888889	2.10349206	6.71	<.0001	14	29.54888889	2.30349206	6.91	<.0001
Block*Extract	28	79.96444444	2.85587302	9.11	<.0001	28	79.86444444	2.95587302	9.41	<.0001
R-Square	0.660750					0.660750				
CV	12.43301					13.43301				
Error	180	56.4000000	0.3133333			180	56.5000000	0.3433333		
Corrected Total	224	166.2488889				224	166.2488889			
Field										

Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	0.9733333	0.4966667	1.23	0.3234	2	0.7733333	0.48667	1.13	0.2234
Extract	14	155.5200000	11.1085714	25.84	<.0001	14	155.5200000	11.1085714	26.84	<.0001
Block*Extract	28	4.6266667	0.1652381	0.38	0.9984	28	4.8266667	0.1552381	0.28	0.7984
R-Square	0.480640	0.4866667				0.490640	0.4066667			
CV	14.74475	11.1085714				13.74475	11.1085714			
Error	405	174.1000000				405	172.1000000			
Corrected Total	449	335.2200000				449	336.2200000			

Appendix 18: ANOVA table for Number of Leaves 21 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	5.342222	2.671111	0.34	0.7126	2	5.342222	2.971111	0.34	0.2126
Extract	14	735.848889	52.560635	6.68	<.0001	14	737.848889	52.560635	6.98	<.0001
Block*Extract	28	1022.257778	36.509206	4.64	<.0001	28	1028.257778	38.509206	4.94	<.0001
R-Square	0.554570					0.654570				
CV	12.69428					12.99428				
Error	180	1416.400000	7.868889			180	1419.400000	7.968889		
Corrected Total	224	3179.848889				224	3179.848889			
Field										
Block	2	14.217778	7.108889	1.37	0.2561	2	14.317778	7.208889	1.27	0.1561
Extract	14	3518.231111	251.302222	48.32	<.0001	14	3718.531111	252.302222	45.32	<.0001
Block*Extract	28	216.782222	7.742222	1.49	0.0545	28	218.782222	7.842222	1.29	0.0345
R-Square	0.640300					0.570300				
CV	10.38250					11.38254				
Error	405					405				
Corrected Total	449					449				

Appendix 19: ANOVA table for Number of Leaves 28 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	2159.222222	1079.111111	542.57	<.0001	2	2158.222222	1179.111111	562.57	
Extract	14	2096.915556	148.779683	75.31	<.0001	14	2066.915556	129.779683	73.31	
Block*Extract	28	2915.777778	104.134921	52.36	<.0001	28	2915.777778	114.134921	52.36	
R-Square	0.952450					0.852450				
CV	5.458678					5.758678				
Error	180	358.000000	1.788889			180	358.000000	1.988889		
Corrected Total	224	7528.915556				224	7528.915556			
Field										
Block	2	3490.417778	1745.208889	386.66	<.0001	2	3590.417778	1845.208889	396.66	<.0001
Extract	14	4398.364444	314.168889	69.61	<.0001	14	4598.364444	324.168889	67.61	<.0001
Block*Extract	28	5405.048889	193.037460	42.77	<.0001	28	5505.048889	197.037460	41.77	<.0001
R-Square	0.879115					0.979115				
CV	8.223236					8.523236				
Error	405					405				
Corrected Total	449					449				

Appendix 20: ANOVA table for Number of Leaves 35 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	2158.222222	1079.111111	542.57	<.0001	2	2258.222222	1089.111111	544.57	<.0001
Extract	14	2096.915556	149.779683	75.31	<.0001	14	2076.615556	129.779683	76.31	<.0001
Block*Extract	28	2915.777778	104.134921	52.36	<.0001	28	2915.777778	121.134921	53.36	<.0001
R-Square	0.952450					0.652450				
CV	5.758678					5958678				
Error	180	358.000000	1.788889			180	368.000000	1.988889		
Corrected Total	224	7528.915556				224	7588.915556			
Field										

	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	5.25778	2.62889	0.29	0.5499	2	5.5778	2.82889	0.39	0.4499
Extract	14	13349.36444	953.52603	104.47	<.0001	14	13379.36444	993.52603	105.47	<.0001
Block*Extract	28	818.14222	29.21937	3.20	<.0001	28	828.14222	29.81937	3.70	<.0001
R-Square	0.793136					0.893136				
CV	11.66055					12.66055				
Error	405					405				
Corrected Total	449					449				

Appendix 21: ANOVA table for Number of Leaves 42 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	332.960000	150.480000	3.17	0.0043	2	302.960000	150.480000	3.17	0.0443
Extract	14	1250.640000	69.331429	1.97	0.0322	14	1250.640000	86.331429	1.87	0.0322
Block*Extract	28	2175.440000	77.622857	1.72	0.0118	28	2173.440000	74.622857	1.62	0.0318
R-Square	0.302386					0.202386				
CV	21.52669					21.52669				
Error	180	8596.40000				180	8598.40000			
Corrected Total	224	12395.44000				224	12325.44000			
Field										
Block	2	1329.751111	663.375556	18.87	<.0001	2	1329.751111	663.375556	18.87	<.0001
Extract	14	2185.924444	4001.982222	4.62	<.0001	14	2285.924444	4001.982222	4.92	<.0001
Block*Extract	28	4271.988222	1818.14222	4.07	<.0001	28	4071.982222	1818.142	4.07	<.0001
R-Square	0.298256					0.248156				
CV	19.730856					19.930856				
Error	405					405				
Corrected Total	449					449				

Appendix 22: ANOVA table for Number of Leaves 49 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	21.662222	10.831111	0.39	0.6765	2	24.662222	10.831111	0.69	0.5765
Extract	14	1549.982222	110.713016	4.00	<.0001	14	1649.982222	118.713016	4.00	<.0001
Block*Extract	28	2509.671111	89.631111	3.24	<.0001	28	2509.671111	88.631111	3.24	<.0001
R-Square	0.450510					0.450510				
CV	17.14347					17.14347				
Error	180	4978.000000				180	4778.000000			
Corrected Total	224	9159.315556								
Field										
Block	2	48.084444	24.042222	1.14	0.3204	2	49.084444	24.042222	1.44	0.4204
Extract	14	6551.097778	467.935556	22.22	<.0001	14	6591.097778	467.935556	21.22	<.0001
Block*Extract	28	3408.315556	121.725556	5.78	<.0001	28	3108.315556	171.725556	8.78	<.0001
R-Square	0.539834					0.939834				
CV	14.92887					14.92887				
Error	405					405				
Corrected Total	449					449				

Appendix 23: ANOVA table for Number of Leaves 56 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	16.115556	8.257778	0.69	0.3785	2	16.115556	8.457778	0.79	0.2785
Extract	14	3882.062222	277.290159	13.37	<.0001	14	3882.062222	277.290159	13.37	<.0001
Block*Extract	28	2224.951111	80.176825	3.87	<.0001	28	2244.951111	80.176825	3.87	<.0001
R-Square	0.422081					0.622081				
CV	14.06138					14.06138				
Error	180	3732.000000	23.733333			180	3732.000000	20.733333		
Corrected Total	224	9873.128889				224	9875.128889			

Field

Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Source	2	128.991111	64.495556	3.45	0.0328	2	118.991111	62.495556	3.55	0.0328
Block	14	8401.697778	600.121270	32.07	<.0001	14	8201.697778	610.121270	32.17	<.0001
Extract	28	4320.142222	154.290794	8.25	<.0001	28	4320.142222	144.290794	7.15	<.0001
Block*Extract	0.5435					0.529048				
R-Square	13.3435					12.20239				
CV	17.16138					11.6138				
Error	405					405				
Corrected	449					449				

Appendix 24: ANOVA table for Number of Leaves 63 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	454.408889	227.204444	5.32	0.0057	2	434.408889	247.204444	5.42	0.0057
Extract	14	899.662222	64.261587	1.50	0.1133	14	879.662222	64.261587	1.60	0.0133
Block*Extract	28	3440.257778	122.866349	2.88	<.0001	28	3470.257778	113.866349	2.98	<.0001
R-Square	0.384015					0.384015				
CV	19.55963					18.55963				
Error	180	7690.40000	42.72444			180	7680.40000	43.72444		
Corrected Total	224	12484.72889				224	12484.72889			
Field										
Block	2	559.960000	279.980000	9.34	0.0001	2	599.960000	273.980000	9.54	0.0001
Extract	14	6910.533333	493.609524	16.47	0.0001	14	6980.533333	495.309524	19.27	0.0001
Block*Extract	28	3932.106667	140.432381	4.69	0.0001	28	3937.106667	140.332381	4.39	0.0001
R-Square	0.484434					0.584434				
CV	16.58768					16.88768				
Error	405					405				
Corrected Total	449					449				

Appendix 25: ANOVA table for Root Galling Indices

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	25.920000	12.960000	1.63	0.1992	2	27.920000	13.960000	1.63	0.1992
Extract	14	2797.426667	184.173333	19.14	<.0001	14	2298.426667	164.173333	19.14	<.0001
Block*Extract	28	436.213333	16.579048	3.82	0.0109	28	436.213333	15.579048	1.82	0.0109
R-Square	0.641537					0.641537				
CV	84.14957					84.14957				
Error	180	1543.600000	7.675556			180	1543.600000	8.575556		
Corrected Total	224	4305.160000				224	4306.160000			
Field										
Block	2	27.920000	13.960000	1.63	0.1992	2	26.920000	13.160000	1.73	0.2992
Extract	14	2298.426667	164.173333	19.14	<.0001	14	2398.426667	162.173333	19.24	<.0001
Block*Extract	28	436.213333	15.579048	1.82	0.0101	28	416.213333	15.779048	1.92	0.0105
R-Square	0.641537					0.541537				
CV	84.14957					81.14957				
Error	180	1443.600000	8.575556			180	1453.600000	8.875556		
Corrected Total	224	4306.160000				224	4316.260000			

Appendix 26: ANOVA table for Number of Pods 62 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	104.222222	52.111111	3.55	0.0308	2	114.222222	53.111111	3.55	0.0008
Extract	14	2157.422222	154.101587	10.49	<.0001	14	2257.422222	154.101587	11.49	<.0001
Block*Extract	28	514.177778	18.363492	1.25	0.1935	28	524.177778	19.363492	1.25	0.0935
R-Square	0.512123					0.512123				
CV	18.52636					17.52636				
Error	180	2644.400000	14.691111			180	2544.400000	13.691111		
Corrected Total	224	5420.222222				224	5220.222222			
Field										
Block	2	896.497778	448.248889	21.20	<.0001	2	895.497778	443.248889	28.20	<.0001
Extract	14	3049.524444	217.823175	10.30	<.0001	14	3048.524444	212.823175	18.30	<.0001
Block*Extract	28	1325.635556	47.344127	2.24	<.0001	28	1323.635556	44.744127	2.14	<.0001
R-Square	0.381069					0.481069				
CV	22.96938					24.96938				
Error	405					405				
Corrected Total	449					449				

Appendix 27: ANOVA table for Number of Pods 69 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	1964.480000	982.240000	20.87	<.0001	2	1974.480000	985.240000	20.87	<.0001
Extract	14	2792.640000	199.474286	4.24	<.0001	14	2792.640000	197.74286	4.24	<.0001
Block*Extract	28	2247.920000	82.282857	1.71	0.0205	28	2247.920000	80.282857	1.71	0.0205
R-Square	0.452585					0.452585				
CV	50.10350					50.10350				
Error	180	8472.80000	47.07111			180	8472.80000	47.07111		
Corrected Total	224	15477.84000								
Field										

Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	1812.90918	906.45459	1.93	0.1464	2	1617.90918	926.45459	1.93	0.1264
Extract	14	42826.61727	3059.04409	6.52	<.0001	14	43826.61727	3059.04409	6.12	<.0001
Block*Extract	28	10353.35702	369.76275	0.79	0.7740	28	11323.35702	359.76275	0.69	0.4740
R-Square	0.224345					0.224345				
CV	32.28157					32.28157				
Error	405					405				
Corrected Total	449					449				

Appendix 28: ANOVA table for Number of Pods 76 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	377.768889	188.884444	4.53	0.0161	2	337.768889	198.884444	3.23	0.0161
Extract	14	1660.595556	128.613968	2.65	0.0015	14	1860.595556	118.613968	1.65	0.0015
Block*Extract	28	884.097778	31.574921	0.91	0.8609	28	894.097778	21.574921	0.71	0.8609
R-Square	0.266501					0.366501				
CV	42.44024					32.44024				
Error	180	8043.60000	44.68667			180	8043.60000	44.68667		
Corrected Total	224	10966.06222				224	10966.06222			
Field										
Block	2	1298.964444	642.482222	16.72	<.0001	2	1288.764444	641.482222	12.72	<.0001
Extract	14	4538.591111	324.042222	8.41	<.0001	14	4516.591111	314.032222	8.11	<.0001
Block*Extract	28	1691.635556	61.415556	1.57	<.0001	28	1621.635556	63.455556	1.07	<.0001
R-Square	0.325048					0.425048				
CV	39.80723					39.70723				
Error	405					405				
Corrected Total	449					449				

Appendix 29: ANOVA table for Number of Pods 83 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	9.7266667	4.9533333	0.22	0.8063	2	9.6066667	4.9533333	0.42	0.6063
Extract	14	942.0000000	67.2857143	2.99	<.0004	14	942.0000000	69.4857143	2.69	<.0004
Block*Extract	28	461.0933333	16.4676190	0.73	0.8351	28	491.0933333	17.4676190	0.73	0.4351
R-Square	0.258471					0.458471				
CV	69.10617					69.10617				
Error	180	4073.200000	22.517778			180	4253.200000	25.517778		
Corrected Total	224	5466.000000				224	5566.000000			
Field										
Block	2	818.5911111	409.2955556	14.14	<.0001	2	828.5911111	429.2955556	13.14	<.0001
Extract	14	564.1777778	40.2984127	1.39	0.1532	14	534.1777778	41.2984127	1.19	0.1532
Block*Extract	28	734.8088889	26.2431746	0.91	0.6057	28	732.8388889	27.2431746	0.81	0.3057
R-Square	0.153007					0.253007				
CV	72.48416					73.48416				
Error	405					405				
Corrected Total	449					449				

Appendix 30: ANOVA table for Pods Weight 55 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	19765.05369	8892.52684	31.00	<.0001	2	17765.05369	8887.52684	21.00	<.0001
Extract	14	12233.49565	873.82112	3.05	<.0003	14	12235.49565	873.82112	3.05	<.0003
Block*Extract	28	9268.86902	337.45961	1.44	0.0 942	28	8168.86902	357.45961	1.84	0.1942
R-Square	0.431647					0.431647				
CV	39.50924					29.50924				
Error	180	51571.95400	286.51086			180	51573.95400	296.51086		
Corrected Total	224	90739.37236				224	90759.37236			

Field

Block	2	9770.62136	4885.31068	11.00	<.0001	2	9970.62136	4685.31068	12.00	<.0001
Extract	14	49269.21018	3519.22930	7.13	<.0001	14	45269.21018	3419.22930	8.93	<.0001
Block*Extract	28	2837.33786	101.33349	0.23	<.0001	28	2987.33786	111.33349	0.13	<.0001
R-Square	0.256016					0.356016				
CV	30.94343					31.94343				
Error	405					405				
Corrected Total	449					449				

Appendix 31: ANOVA table for Pods Weight 62 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	3154.17978	1577.08989	4.68	0.0105	2	3234.17978	1567.08989	3.28	0.0105
Extract	14	14671.23158	1047.94511	3.11	<.0002	14	13671.23158	1247.94511	2.11	<.0002
Block*Extract	28	6177.08453	229.89588	0.65	0.9094	28	6757.08453	239.89588	0.65	0.1094
R-Square	0.283221					0.283221				
CV	32.65317					31.65317				
Error	180	60695.13460	337.19519			2	3234.17978	1567.08989	3.28	0.0105
Corrected Total	224	84677.63049				14	13671.23158	1247.94511	2.11	<.0002
Field										
Block	2	1812.90918	906.45459	1.93	0.1464	2	1832.90918	926.45459	1.33	0.0464
Extract	14	42826.61727	3059.04409	6.52	<.0001	14	43826.61727	3259.04409	6.72	<.0001
Block*Extract	28	10353.35702	369.76275	0.79	0.7740	28	14353.35702	369.76275	0.89	0.0740
R-Square	224345					234345				
CV	32.28157					33.28157				
Error	405					405				
Corrected Total	449					449				

Appendix 32: ANOVA table for Pods Weight 69 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	23599.72886	11993.86443	21.35	<.0001	2	9162.54400	4581.27200	12.09	<.0001
Extract	14	27973.43304	1971.24522	3.25	<.0001	14	14947.19866	1067.65705	2.82	<.0008
Block*Extract	28	23462.51304	782.16118	1.65	0.0176	28	7328.62905	261.73675	0.69	0.8764
R-Square	0.364873					0.315461				
CV	56.42327					42.45491				
Error	180	73529.8779	561.0549			180	68220.10004	379.00056		
Corrected Total	224	146093.5528				224	99658.47176			
Field										
Block	2	4665.54522	2332.77261	6.95	0.0011	2	4465.54522	2552.77261	6.4	0.0011
Extract	14	18873.48825	1348.10630	4.01	<.0001	14	19873.48825	1248.10630	4.01	<.0001
Block*Extract	28	7994.08120	285.50290	0.85	0.6886	28	7974.04120	245.50290	1.55	0.1886
R-Square	0.188212					0.788212				
CV	37.88268					34.88268				
Error	405					405				
Corrected Total	449					449				

Appendix 33: ANOVA table for Pods Weight 76 DAE

Lath house

Trials I						Trials II				
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	7916.618515	3918.309257	13.78	<.0001	2	7816.618515	3908.309257	13.78	<.0001
Extract	14	9345.201803	669.371557	2.35	<.0051	14	9343.201803	667.371557	2.35	<.0051
Block*Extract	28	6290.339299	237.797832	0.72	0.4300	28	6490.339299	231.797832	0.82	0.7300
R-Square	0.316571					0.316571				
CV	74.59041					42.45491				
Error	180	51027.02884	283.65016			180	69220.10004	379.00056		

Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Corrected Total	224	74707.18846				224	99658.47176			
Field										
Block	2	2387.24638	1243.62319	3.74	0.0146	2	2687.24638	1243.62319	3.74	0.0146
Extract	14	54123.51176	3865.96513	12.76	<.0001	14	54143.51176	3165.96513	10.76	<.0001
Block*Extract	28	9938.46356	340.65941	0.95	0.3440	28	9538.46356	320.65941	0.25	0.2440
R-Square	0.313154					0.213154				
CV	31.69583					32.69583				
Error	405					405				
Corrected Total	449					449				

Appendix 34: ANOVA table for Pods Weight 83 DAE

Lath house

		Trials I						Trials II			
Source	DF	SS	Mean Square	F Value	Pr > F	DF	SS	Mean Square	F Value	Pr > F
Block	2	7916.618515	3918.309257	13.78	<.0001	2	7816.618515	3908.309257	13.78	<.0001
Extract	14	9345.201803	669.371557	2.35	<.0051	14	9373.201803	667.871557	2.35	<.0051
Block*Extract	28	6290.339299	237.797832	0.72	0.4300	28	6490.339299	233.797832	0.82	0.7300
R-Square	0.316571					0.316571				
CV	74.59041					74.59041				
Error	180	51027.02884	283.65016			180	51057.02884	283.65016		
Corrected Total	224	74707.18846				224	74707.18846			
Field										
Block	2	11687.39244	5843.69622	10.72	<.0001	2	11287.39244	5853.69622	10.12	<.0001
Extract	14	10257.50499	732.67893	1.34	0.1781	14	10257.50499	712.67893	1.14	0.0781
Block*Extract	28	19310.01188	689.64328	1.27	0.1686	28	17310.01188	649.64328	1.27	0.1086
R-Square	0.157473					0.257473				
CV	60.38364					60.38364				
Error	405					405				
Corrected Total	449					449				