IDENTIFICATION AND MANAGEMENT OF ROOT KNOT NEMATODES (*Meloidogyne* spp.) ATTACKING CHICKPEA (*Cicer arietinum* L.) IN NAKURU COUNTY, KENYA USING NEMATICIDES AND CULTURAL PRACTICES

KIMANI ISHMAEL MAINA

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DECLARATION AND RECOMMENDATION

DECLARATION

This is my original work and has not been submitted or presented for award of degree in any other University

Signature..... Date.....

Kimani Ishmael Maina

SM15/3675/13

RECOMMENDATION

This Thesis has been submitted for examination with our approval as supervisors for examination as per the Egerton University regulations.

Signature..... Date.....

Dr. Japhet M. Muthamia

Department of Biological Sciences,

Egerton University, Njoro

Signature..... Date.....

Prof. D.O. Otaye

Department of Biological Sciences,

Egerton University, Njoro

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DEDICATION

To my parents Mr. and Mrs. Kimani, my Aunt Ms. Esther Kahare.

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ABSTRACT

Root knot diseases are known to attack many crops especially legumes such as chickpea. Losses due to root knot nematodes (*Meloidogyne* spp.) in chickpea have been approximated at 13.7% of yield loss which translates to loss of billions of shillings annually. Four main *Meloidogyne* species; *M. hapla, M. javanica, M. arenaria* and *M. incognita* attack chickpeas. Experiments were conducted in the greenhouse to characterise, identify and control *Meloidogyne* spp. causing root knot disease in chickpea in Nakuru County. Characterization and identification were done using perineal patterns procedure on female root knot nematodes. Three treatments; poultry manure, two commercial nematicides and Sudan grass (*Sorghum sudanese*) were used in this study. Poultry manure was used at three levels; 250 g, 500 g, 750 g and control. Marshal 250 EC nematicide was used at 10 ml/ litre of water, 25 ml/ litre and 50 ml/ litre as low, recommended, high doses respectively. Nimbecidine nematicide was used at 3.5 ml/ litre of water, 7 ml/ litre and 14 ml/ litre as low, recommended, high doses respectively and control in each treatment. Completely randomized design was used in the study as a design for treatments. Thirty samples of *Meloidogyne* spp. female were used during characterisation and all had uniform perineal patterns similar to that of *M. javanica* distinguished from other species by a distinct lateral ridge separating dorsal and ventral arch. *M. javanica* was the main root knot species attacking chickpeas. There was significant difference (P=0.05) in root galling and *M. javanica* juveniles population reduction between positive control (zero grams) and other poultry manure treatments. The nematicides significantly reduced root galling and number of juveniles at recommended and high levels. Marshal 250 EC was an effective nematicide than Nimbecidine and Marshal 250 EC which recorded the lowest root galling and number of juveniles as compared to Nimbecidine. There was no significant difference (P=0.05) in results of 250 g, 500 g and 750 g poultry treatments. There was significant difference (P=0.05) between results of Sudan grass, positive and negative control. Poultry manure and Sudan grass treatments significantly reduced root galling and nematodes juvenile population. There was a relationship between root galling and juvenile number in soil, root galling and root weight per chickpea plant. The findings of this study will benefit farmers on choice of commercial nematicide to use against root knot nematodes to maximize yield of chickpea. The findings can also be used by farmers to manage root knot disease using cover crops such as Sudan grass.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACA	Agriculture crops alternatives
BeYDV	Bean yellow dwarf virus
BMI	Basal metabolic index
CRD	Completely randomized design
DF	Dietary fiber
DM	Dry matter
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization statistics
ICRISAT	International Crops Research Institute for Semi-arid Tropics
LA	Lineloic acid
LSD	Least significant difference test
RKN	Root knot nematodes
SCFA	Short chain fatty acid
USDA	United State Department of Agriculture
CpCDV	Chickpea chlorotic dwarf virus

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Chickpea (*Cicer arietinum* L.) is a legume which belongs to Fabaceae family whose seeds are rich in protein (Kumar *et al*., 2005). The plant has several common names; garbanzo bean, ceci bean, channa and Bengal gram. Chickpea is the third important legume after beans (*Phaseolus vulgaris*) and field pea (*Pisum sativum* L.) (FAOSTAT, 2008).

Chickpea is a rich source of vitamins, minerals and amino acids (Grusak, 2002). It is also a good alternative food-security legume crop in semi-arid tropics since it is more drought tolerant than other legumes currently grown. Chickpea yields have remained stagnant for the past 2-3 decades due largely to biotic and abiotic stress factors during production (Hulse, 1991; ICRISAT, 2008). In Kenya, chickpea is relatively new crop and currently expanding to new areas from semi-arid regions to the Rift Valley highlands where short rains are key factor to growth of the crop (Mulwa *et al.*, 2010). It is mainly grown towards the end of the long rainy season with receding soil moisture or during the short rain seasons (Kibe and Onyari, 2006; Mulwa *et al*., 2010).

Biotic stress factors include root knot nematodes (*Meloidogyne* spp.) (Mohiddin and Khan, 2014). *Meloidogyne* spp. are plant parasitic nematodes which infects chickpeas causing up to 13.7% yield loss (Rehman *et al*., 2012). Root knot nematodes especially *M. incognita* are the most widespread and found in every climate (Parul *et al*., 2011). Use of crop rotation remains effective in the control and managing the plant parasitic nematode populations (Noe, 1998). According to McSorley *et al.* (1994), population densities of *Meloidogyne* spp. reduced upon use of clover crops in the greenhouse and in the field. In Kenya, chickpea being relatively a new crop, estimates of losses as a result of root knot disease have not been documented.

Table 1: Diseases attacking chickpea

Disease	Causal agent	Source
Fusarium wilt	*Fusarium oxysporum* f. sp. *ciceris*	Jime'nez-Diaz *et al*. (2015)
Phytophthora root rot	*Phytophthora megasperma Drechs*. f. sp *medicaginis*	Du *et al*. (2013)
Ascochyta blight	*Ascochyta rabiei*	Moore *et al*. (2013); Kimurto *et al*. (2013)
Root knot	*Meloidogyne* spp.	Mohiddin and Khan (2014); Prakash *et al*. (2014)
Virus	Bean leaf roll (BLRV), beet western yellows (BWYV), cucumber mosaic (CMV) and Alfafa mosaic (AMV) viruses	Sharman *et al*. (2014)

1.2 Statement of the problem

Chickpea is a relatively new crop in Kenya and since its introduction it has become an important food crop. Consumption of chickpea is as important as other legumes due its nutritious seeds. However, effect of the root knot nematodes to the plant leads to damage and loss of the yield and the crop resulting to food insecurity (Rehman *et al*., 2012. Studies have been carried out in India to manage the damage of the root knot disease on chickpea in order to reduce yield and crop loss. In Kenya, no work has been documented to control root knot disease in chickpea. Root knot nematodes colonize the roots and interfere with the water and nutrient uptake stagnating growth and yield produced from chickpea (Prakash *et al*., 2014). Therefore it is important to control the root knot disease to reduce crop and yield loss.

1.3 Objectives

1.3.1 General objective

To reduce chickpea crop losses in Nakuru County by effective management of root knot disease

1.3.2 Specific objectives

i. To characterise root knot nematodes affecting chickpea in Nakuru County.

ii. To determine effectiveness of two common commercial nematicides against root knot nematode diseases affecting chickpea.
iii. To determine the effect of poultry manure in the management of root knot diseases in chickpea.
iv. To determine the effect of Sudan grass (*Sorghum sudanese*) on the *Meloidogyne* spp. population as a management strategy against root knot disease.

1.4 Hypotheses

i. There are no *Meloidogyne* spp. attacking chickpea in Nakuru County.
ii. There are no significant differences of effectiveness of commercial nematicides against root knot disease in chickpea.
iii. There is no significant effect of poultry manure in controlling of root knot nematode disease in chickpea.
iv. There is no significant effect of Sudan grass (*S. sudanese*) in management of *Meloidogyne* spp. population.

1.5 Justification of the study

Cultivation of chickpea has increased in Kenya since its introduction. This is due to its distinguished benefits compared to other legumes. Most of the soils are already infested with nematodes and among them are the root knot nematodes and this has been a large challenge to the growth and the yield produced from the chickpea. Cultivation of the legumes in nematode infested soils has resulted in overall reduction of the yield produced. Presence of root knot nematodes in the soil however has to be managed. Use of commercial nematicides, soil amendments such as composite manure and intercropping with other plants are methods used in controlling root knot diseases. Studies have shown that nematicides eliminate almost all nematodes by delaying reproduction, movement and penetration into host. Nematicides also disrupt nervous system and organic systems of nematodes resulting to paralysis and death. Manures (soil amendments) provide appropriate environment for the survival of predacious nematodes such as *Mononchus* spp. that feed on the plant parasitic nematodes. Mechanism involved when intercropping involves plants such as Sudan grass (*S. sudanese*) releasing biochemicals that kill or suppress the reproduction of nematodes, hatching of eggs is also delayed (Ogumo, 2014). Research has been done in countries like India using these methods and with success (Rehman *et al*., 2012). Therefore, there is need to evaluate their

effectiveness in controlling root knot disease in order to achieve higher yield especially for the chickpea producers.

1.6 The Scope and limitations

This study was carried out from October 2014 to August 2015 (Appendix 1). Characterisation and control of root knot disease in chickpea was done at Egerton University in Nakuru County. The research was self-funded and therefore funds were not enough to screen root knot disease in other chickpea varieties. The limitation of funds was also a challenge in movement in search of samples from all the study sites. Other limitation was insufficient literature information showing related research studies that have been carried out in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 Chickpea origin and center of diversity

Chickpea was first cultivated in the Middle East dating back in 7450 B.C. (Nut and Seeds, 2004). It originated from Turkey and was grown as far back as 7450 B.C. in Turkey and 4000 B.C. in India (ACA, 2004). FAOSTAT (2008) documented that the cultivation spread to the Asian countries reaching American continent. It is documented that the crop then spread to other parts of Asia, Middle East, and North America and later to other parts of the world (Nuts and Seeds, 2004). Today, chickpea is popular throughout China, India, Africa and Australia, and is also gaining popularity in the United States and in recent years in East Africa (Onyari *et al*., 2010). In 1994, world production was 7.9 million tons from 10.2 million hectares of which 97% was from developing countries (Nut and Seeds, 2004).

2.1.1 Plant description and ecology

Chickpea seeds are important source of carbohydrates, protein, minerals and vitamins. It is has been reported to be the most drought and heat tolerant crop suitable for low fertility soils (Kimurto *et al*., 2013). The crop is sensitive to saline, alkaline and waterlogged soils and develops slowly with an open canopy that reduces its competition against weeds (Kamithi *et al*., 2009). ACA (2004) reported that high chickpea yields of 500-600kg/acre and when grown as a rotational crop it enhances soil nitrogen and breaks diseases cycles. Mirza *et al*. (2007) reported that highest seed yield was 6.94g per plant and 1.81 tons per hectare in the varieties tested.

2.2 Varieties of chickpea

There are two main varieties of chickpeas; Desi and Kabuli. Desi and Kabuli are more popular and most cultivated. Desi variety is small, with darker seeds and a rough coat, pink flowers mainly grown for its seeds and it accounts for 80-85% of the total chickpea grown in Asia and Africa (Kamithi *et al*., 2009). It is cultivated in India and much of the Indian Sub-continent, Ethiopia, Mexico and Iran (Mansfeld's database, 2008). Kabuli variety is associated with Kabul in Afghanistan. They are lighter coloured white flowers, with larger seeds mainly grown to provide salads and vegetable oils. It's grown in Southern Europe, West Asia, Northern Africa, South America and Indian Sub-continent (Kimurto *et al*., 2013; Kamithi *et al*., 2009).

2.3 Economic importance of chickpea

Chickpea is source of proteins and carbohydrates which constitute 80% of the total dry seed mass (Grusak, 2002). Research indicates that chickpea is cholesterol free and a source of dietary fiber, vitamin and minerals to the consumers (USDA, 2010). It is consumed in different forms in many countries where it is grown (Muehlbauer and Tullu, 1997). In the Indian subcontinent for example, chickpea cotyledons are used as flour for making paste which makes snacks (Chavan *et al.,* 1986). In Asia and Africa it is consumed as stew, salads or in roasted, boiled or fermented forms (Hulse, 1991). Chickpea serves health benefits more than the nutritional ones, it comprises of components which improve health such as dyspepsia and relieve for diabetes (Jukanti *et al*., 2012).

2.3.1 Carbohydrates

Carbohydrate content differs in Desi and Kabuli, for instance, Kabuli has more soluble sugars than the Desi type (Hulse, 1991). The starch content varies from 41-50% dry weight of the entire carbohydrates (Jambunathan and Singh, 1980). The total starch content is 525g/kg of dry matter (DM) with higher amount of amylose (Guillon and Champ, 2002).

2.3.2 Proteins

Chickpea has higher protein content which is important in supplementing protein malnutrition in developing countries of Africa and Asia (Iqbal *et al.*, 2006). Kabuli has higher protein digestibility of than Desi variety according to Sanchez-Vioque *et al.* (1999).

2.3.3 Fat and fatty acids

Total fat content in raw chickpea seeds varies from 2.70 to 6.48% (Alajaji and El-Adawy, 2006). Shad *et al*. (2009) reported lower values (about 2.05 g/100 g) for crude fat content in Desi chickpea varieties. Fat content of 3.40–8.83 and 2.90–7.42% in Kabuli and Desi chickpea seeds respectively (Wood and Grusak, 2007). Fatty acids are also present in chickpea with linoleic acid (LA) being in higher amount in Kabuli than in Desi varieties and linoleic acid being dominant source of fatty acid having the highest fraction (51.2% LA) in chickpea than other edible lentils such as peas and beans (Wang and Daun, 2004).

Other components such as minerals include zinc, iron, magnesium and calcium which are present in the chickpea diet (FAO, 2002; USDA, 2010). Desi have higher amounts of calcium than Kabuli varieties though in addition there are no significant differences between the two types for the other minerals (Ibanez *et al.*, 1998).

2.3.4 Vitamins

Vitamins are important in the nutritional health, chickpea supplements provision of vitamins when consumed with other foods especially cereals (Singh and Diwakar, 1993). They are sources of riboflavin (B2), pantothenic acid (B5) folic acid, niacin and pyridoxine (B6) (Lebiedzinska and Szefer, 2006).

2.3.5 Health benefits

In addition to the nutritional benefits to the body, chickpeas serve health benefits. Foods rich in dietary fibre (DF) are associated with low basal metabolic index (BMI) (Howarth *et al.,* 2001). Chickpea has DF and low glycemic index (GI), it is therefore important in reduction of weight hence obesity reduction. Short chain fatty acids (SCFA) include butyrate, is produced after chickpeas consumption suppresses cell proliferation (Cummings *et al.,* 1981). Butyrate also inhibits DNA compaction and gene expression by histone deacetylase suppression (Mathers, 2002). Consumption of fibre foods leads to reduced levels of plasma cholesterol. Foods which are rich in saponins to reduce cholesterol (16 to 24%) (Thompson, 1993). The mechanism of cholesterol reduction involves inhibition of the synthesis of fatty acids in the liver by the fiber components such as butyrate and SCFA hence reduced cholesterol (Crujeiras *et al.,* 2007).

2.4 Chickpea production in Kenya

Global chickpea production by 2006-2009 was 9.6 million metric tons with an average yield of 849 kg/ha. (FAO, 2011). In Kenya, Kimurto *et al*. (2013) reported that chickpea is a relatively new crop grown by small scale farmers in Eastern and Rift Valley regions. Its cultivation has been recorded in dry highlands and dry lowlands where rainfall ranges between 250-550 mm per annum (Kibe and Onyari, 2007; Onyari *et al*., 2010). Kenya's chickpea production was reported to be 55,000 tons according to ICRISAT (2008) statistics. In Nakuru, cultivation is done in Naivasha and Egerton-Njoro (Kimurto *et al*., 2013; Mulwa *et al*., 2010). Drought resistant chickpea are also found in Kenya, the crop is a bonus crop as it is planted after and besides the main crop such as maize is harvested (Kimurto *et al*., 2004).

2.5 Diseases of chickpea

According to Agrios (2005) and Jime'nez-Diaz *et al*. (2015) there are a number of fungal, bacterial and viral diseases that attack chickpeas, these include ascochyta blight, black

root rot, damping off, downy mildew, powdery mildew, rust, bacterial blight, bacterial wilt, and stunt virus, root knot and root lesion diseases, among others.

2.5.1 Ascochyta blight

The major constraint to yield improvement in chickpea is Ascochyta blight (AB), a necrotrophic disease caused by *Ascochyta rabiei L.* (Pande *et al*., 2005). Kaiser *et al*. (1998) reported that Ascochyta blight epidemic resulted in complete yield loss in India and Pakistan. In Kenya, the infection was more severe at Egerton-Njoro than ATC-Koibatek because Njoro is located in higher altitude than ATC-Koibatek which favoured rapid disease development in wet conditions (Kimurto *et al*., 2013).

The fungus thrives at cool temperatures of about 20°C. It is both asexual and sexual state pathogen (Harveson *et al*., 2011). The disease has a wide host range from field pea, vetch, common bean and cowpea after artificial inoculation. It is seen as blighted patches during flowering and podding time (Kaiser, 1991). Initial symptoms may be unnoticed but become quite evident upon flowering, first, symptoms are seen as small necrotic specks on young and new leaves and stems (Kaiser and Muehlbauer, 1984). As a result of cool and moist conditions the specks coalesce to form large necrotic lesions on young leaves and buds. Lesion on petals and stems elongate and eventually girding stems breakage (Harveson *et al*., 2011).

2.5.2 Bacterial blight

According to reports by Khan and Siddiqui (2005), the disease is caused by a bacterium *Xanthomonas campestris* pv *cassiae*. It is a minor disease and only reported in India. The disease is characterized by leaves drying up and shed. Post emergence damping off usually occurs leading to death of the seedlings within 3- 4 days. Water soaked lesions appear on radicles and on leaves causing a dark brown appearance, soft rot of infected tissues also occur. In fully grown chickpea plant, lesions turn into dark brown spots with chlorotic halos, the chlorotic halos coalesce and cause chlorosis of a leaflet resulting into a leaf blight symptoms (Rangaswami and Prasad, 1960; Nene *et al*., 2012). The infection has not been documented in Kenya.

2.5.3 Chickpea stunt virus disease

It is caused by a Mastervirus which belongs to family Geminiviridae (Mumtaz *et al*., 2011). Reports indicate that two viruses are involved; chickpea chlorotic dwarf virus (CpCDV) and Bean yellow dwarf virus (BeYDV) (Liu *et al*., 1999; Nahid *et al*., 2008; Mumtaz *et al*., 2011). Geminiviridae family comprises viruses with single stranded and circular DNA and the disease is transmitted by means of an insect vector with large host range (King *et al*., 2011).

The disease is characterized by reddening of the leaves of desi-type and yellowing in Kabuli-type of chickpea, stunting, internode shortening and phloem browning are observed (Nene *et al*., 1989). Other symptoms include yellowing, chlorosis, leaf reddening and tip wilting as reported in Najar *et al*. (2011). The disease results in plant decline and poor performance leading to premature death and reduced production (Horn *et al*., 1996). It has been reported to cause chickpea stunt disease and found to cause 80-95% yield losses in chickpeas in India (Kotasthane and Gupta, 1978; Reddy *et al*., 1979). Chickpea stunt disease has also been reported in South Africa (Liu *et al*., 1999). In Kenya information related to the disease has not been documented since chickpea is a new crop.

2.6 Nematode diseases

Nematode of chickpea diseases include; root knot disease, root lesion disease, pearly root disease caused by cyst nematodes and dirty root disease caused by reniform nematode (Agrios, 2005). Root knot nematodes accounts for 13.7% yield loss (Rehman *et al*., 2012).

2.6.1 Root knot nematode

Root knot nematodes (RKN) belong to genus *Meloidogyne* and are sedentary endoparasites that induce root-knot symptoms and cause serious agricultural damage (Trudgill and Blok, 2001) with over 100 species (Karssen *et al*., 2013). *M. javanica, M. arenaria, M. incognita* and *M. hapla* are four major species which accounts for 95% of all crops loss (Agrios, 2005) and 13.7% of chickpeas yield loss due to root knot diseases. (Rehman *et al*., 2012).The species are characterised on the basis of their perineal patterns, the morphology which is located at the posterior body region of adult females (De Ley and Blaxter, 2002). The posterior region comprises the vulva, anus, lateral lines, phasmids, tail and surrounding cuticular striae (De Ley and Blaxter, 2004), these parts differ in *Meloidogyne* spp. and useful for identification as summarized in Table 2.

Table 2: Taxonomic characters of perineal patterns of the four common root knot nematodes

Species	**Dorsal arch**	**Lateral field**	**Striae**	**Tail terminus**
M. incognita (Koffoid and White)	high, squarish	lateral ridges absent, marked by breaks and forks in striae	coarse, smooth to wavy, sometimes zigzaggy	often with distinct whorls
M. javanica (Treub)	low, rounded	distinct lateral ridges	coarse to slightly wavy	often with distinct whorls
M. arenaria (Neal)	low, rounded, indented near lateral fields	lateral ridges absent, marked by short, irregular and forked striae	coarse to slightly wavy	usually without distinct whorls
M. hapla (Chitwood)	low, rounded	lateral ridges absent	fine, smooth to slightly wavy	whorls absent , marked by subcuticular punctations

Source: Eisenback, (1985).

2.6.2 Pathogenesis

Meloidogyne spp. is microscopic adapted for parasitism by development of a stylet and secretory gland cells in the esophagus (Davis *et al*., 2004). Stylet is useful in piercing host cell walls to access cell contents for ingestion, it also delivers effector produced by nematode gland cells (Davis *et al*., 2008; Mitchum *et al*., 2013) in order to modify host cells for source of nutrients. Effectors are defined by Hogenhout *et al*. (2009) as pathogen proteins released in small molecules that alter structure and function of the host cells. They are synthesized in gland cells and directed to the secretory pathway by N-terminal signal peptides where they are packaged in membrane enclosed spherical granules. The granules are transported to cytoplasmic extensions where the effector proteins are released via exocytosis (Mitchum *et al*., 2013). Kim *et al*. (2006) reported that exocytosis pathway is regulated by an external stimulus.

According to Rosso *et al*. (2012), plant parasitic nematode effectors focus on their role in promoting susceptible parasitic interactions with their hosts, these effectors condition the

host defense response during parasitism. Secreted effectors mimic endogenous plant signaling peptides as reported in Kikuchi *et al*. (2011).

2.6.3 Life and disease cycles of *Meloidogyne* spp.

The lifecycle starts with female producing approximately 500 eggs. The first and second juvenile stages take place inside the egg. Second juvenile emerges from the egg into the soil (Agrios, 2005). The infective second juvenile stage (J2) penetrates the root tip and moves intercellularly until it reaches the vascular cylinder (Rosso *et al*., 2005; Huang *et al*., 2013). J2 induces root knot, disrupts hosts physiology through their reproduction and feeding resulting to crop yield reduction and quality damage (Dang *et al*., 2011; Naz *et al*., 2012).

Many juveniles also hatch inside the roots and re-infect the same root by migrating inside the root to a new feeding site (Mulk**,** 1976). Females remain within the galled roots, and eggs are deposited in egg masses inside the root cortex. Up to 50 egg-laying females can be found in a single gall, indicating that infection can be extremely high (Bridge *et al*., 2005). A second moult takes place and a third juvenile emerges in which male and female can be distinguished Fourth moult takes place and which is the last moult, male comes out from the root and drops into the soil (Agrios, 2005), the cycle is completed in 25 days in optimum temperature (27° C) and takes longer in higher and lower temperature.

2.6.4 Symptoms

Symptoms due to the root knot infestation are manifested in above and below ground parts of the plants. Above ground symptoms include yellowing, stunting and wilting. Underground symptoms are more typical and include galls, bushy roots, rotting, necrosis, cracking and distortion (Barker *et al*., 1985; Agrios, 2005).

2.7 Control of root knot nematodes

Crop losses caused by *Meloidogyne* spp. can be managed by use of commercial nematicides, crop rotation (Crow *et al*., 2001), soil amendments (Rehman *et al*., 2012), biocontrol, host resistance, manipulation of planting time and use of indigenous plants e.g. Sudan grass and *Azadirachta indica* (Muthamia, 2004; Ogumo, 2014).

2.7.1 Crop rotation

Crop rotation is regarded as a cultural practice of managing root knot nematodes, this method has been used to reduce *Meloidogyne* spp. populations, in order to employ this

method identity of root knot nematode and the host range should be understood (Ripoll *et al*., 2003; Onkendi *et al*., 2014). Growth challenges related to human population such as land scarcity make crop rotation impractical in certain areas. Crop rotation may also affect the soil ecosystem by changing microbial and nematode structure (Wang *et al*., 2004).

2.7.2 Use of Chemical formulations

This involves use of different formulations of nematicides to interfere with the reproduction or kill of root knot nematodes in the soil (Onkendi *et al*., 2014). Nematicides are the most effective method of controlling high levels of root knot nematodes in the farms these include Adicarb, Dazomet, Metasodium, Oxamyl and 1, 3 dichloropropene, however, some nematicides containing methyl bromide among other harmful compounds have been banned in various countries (Muthamia, 2004). Sirias (2011) reported that nematicides reduce high root knot nematodes populations and they can be applied as pre-plant, fumigants or contact nematicides (Strajnar and Sirca, 2011).

2.7.3 Use of Sudan grass (*S. sudanese*) to manage nematodes

These are tall, fast-growing, heat-loving summer annual grasses with the ability to suppress some nematode species, smother weeds and penetrate compacted subsoil if mowed once (Clark, 2007). Sudan grass does best in warm climate with rich loamy soils (USDA, 1993). Forage-type sorghum plants are larger, leafier and mature later than grain sorghum plants and when compared with Sudan grass hybrids, they are shorter, less drought tolerant, and don't re-grow as well (Ogumo, 2014). Still, forage sorghum and most forms of Sudan grass can be used in the same cover-cropping roles as Sudan grass hybrids (Clark, 2007).

All sorghum and Sudan grass-related species produce compounds known as cyanoglucoside dhurrin that suppress certain plants and nematodes (De Nicola *et al*., 2012). They are not frost tolerant, and should be planted after the soil warms in spring or in summer at least six weeks before first frost (Clark, 2007). Sudan grass cannot be considered as a green manure unless there is ample nitrogen in the soil or a long period can elapse before it is necessary to use the land (Widmer and Abawi, 2002). Sudan grass hybrids followed by a legume cover crop are a top choice for renovating over farmed or compacted fields (USDA, 1993). The hybrids are crosses between forage-type sorghums and Sudan grass (Ingels, 1998). They have less leaf area, more secondary roots and a waxier leaf surface, traits that

help them withstand drought like corn, and they require good fertility and usually supplemental nitrogen for best growth (Ogumo, 2014).

2.7.4 Biological management

Nematophagous fungi and bacteria have been the subject of many European studies on nematode control (Viaene *et al*., 2006). Kiewnick and Sikora (2006) demonstrated that a single pre-plant application of the fungus *Paecilomyces lilacinus* strain 251 could control *M. incognita* on tomato. A one-off application of *P. chlamydosporia* was able to slow down the build-up of *M. javanica* for at least 5-7 months in tomato and lettuce rotations in a glasshouse (Van Damme *et al*., 2005). Endophytic fungi that grow within plant tissues without causing disease can play a protective role against parasitic nematodes (Riegel and Noe, 2000). *Pasteuria penetrans* is a bacterial parasite of plant parasitic nematodes, including *Meloidogyne* species, and can significantly reduce their numbers in some cropping systems (Trudgill *et al*., 2000). Biological control agents generally provide too little control to be effective alone and their successful use in sustainable management strategies will depend on their integration with other control measures (Viaene *et al*., 2006).

Several studies conducted to manage root knot nematodes using *Trichoderma* spp. showed reduction of galling indices and less nematode penetration in the roots of tomatoes (Sharon *et al*., 2001). Research indicate that there was significant reduction of egg production in *M. arenaria* attacking maize (Windham *et al*., 1989).

2.7.5 Host resistance

Plant resistance is the environmentally safest method to control root-knot nematodes (Agrios, 2005). Resistance against *Meloidogyne* spp. has been reported in many food crops but it's not often used but resistance-breaking populations of *M. incognita* and *M. javanica* (Wesemael and Moens, 2009). There is resistance for *M. chitwoodi* and *M. fallax* in bean cultivars. Genes resistant to root knot nematodes are incorporated in plant genes using methods such as gene electrophoresis and cross breeding between resistant and susceptible chickpea varieties and use of host resistance effects to the environment (De Ley and Blaxter, 2004).

2.7.6 Use of soil amendments

Use of soil amendments is ecofriendly to other important microbes as well as plants (Rehman, 2012). Soil amending involves application of neem, organic manure, oil cakes,

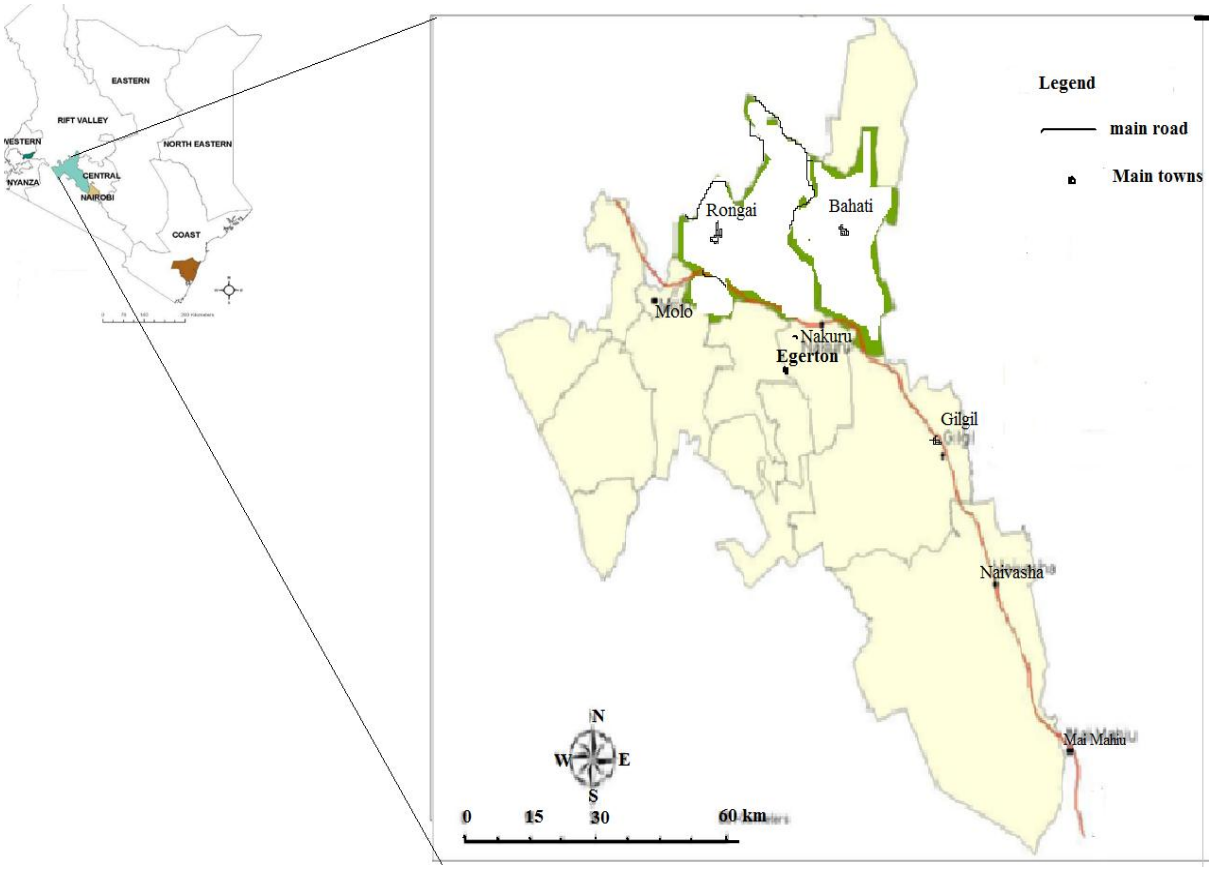
plant latex and saw dust and these are known to have nematicidal property (Siddiqui *et al*., 1990; Yadav *et al*., 2006). Reduction and suppression of nematode intensity and infection in chickpea, is due to the compounds such as phenols and acidic compounds released during decomposition of organic amendments (Hooks *et al*., 2010; Rehman *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of study area

The study area, shown in figure 1 is located at Nakuru County in Kenya. The study sites included Egerton University, Gilgil and Naivasha. Nakuru County stands at an altitude of 1800 meters above sea level with an average annual rainfall of 1000 mm and temperatures range of 17°- 22°C (Walubengo, 2010).



Source: Okello *et al*. (2010)

Figure 1: Map of study area showing study sites

3.2 Sample collection of *Meloidogyne* spp.

Heavily infected roots of chickpea plants were collected from the sites (Field 3, Field 7, Gilgil, Naivasha and Department of Biological Sciences garden) by random selection in the garden. The infected plants were uprooted and samples were put in labeled polythene bags for nematode extraction. The samples were preserved at 5°C in the refrigerator.

3.2.1 Extraction of root knot nematodes from root galls for characterisation and identification

The extraction was done using the method described by Hussey and Barker (1973). Galled roots of chickpeas were washed and galls cut open using a scalpel and a dissecting needle to tease out adult female nematodes in a petri dish containing water. *Meloidogyne* females' perineal patterns were cut using a method described by Taylor and Netsch (1974). Cuticles of the female nematodes were ruptured by cutting and gently pushing out body tissues. Thirty samples of cuticles were then placed in 45% lactic acid in a petri dish, lactic acid aided in facilitating removal of body tissues and allowed to stand for half an hour. After tissues removal, the cuticle was transferred to a drop of glycerin where it was carefully trimmed so as to be only slightly larger than the perineal pattern. The piece of cuticle with the perineal pattern was transferred to a drop of glycerin on a slide. Observations were made on a compound microscope for identification as described by Taylor and Netsch (1974).

3.3 Inoculum preparation

Inoculum preparation was done at Egerton University, Department of Biological Sciences greenhouse, from galled chickpeas obtained from Field 3, Field 7, Gilgil, Naivasha and Department of Biological Sciences garden. Egg sacs containing female nematodes were extracted from the roots as described by Hussey and Barker (1973) and incubated for 3 days to hatch into second stage juveniles (J2). The juveniles were inoculated in potted chickpea seedlings in the greenhouse and this served as source of inoculum for perineal patterns.

3.3.1 Procedure for inoculation

Meloidogyne spp. juveniles were used as inoculum and inoculation followed as described by Hussey and Barker (1973). Six chickpea seeds were planted in each pot containing clay and loam soils, thinning was done to leave one seedling after germination. A depression of 3 cm was made around each pot and inoculation was made by dispensing nematode larvae (7 ml of the suspension which had one hundred juveniles) into the depression. The control was treated with distilled water.

3.4 Determination of the effectiveness of common commercial nematicides against *Meloidogyne* spp.

The experiment was carried out as described by Widhi and Trivedi (2011). Two commonly used nematicides (Marshal 250 EC and Nimbecidine) were used. Each nematicide

was used in 3 doses (recommended, lower and higher doses) and trenched in sterilized soil in the pots. 10 ml/litre of water, 25 ml/litre of water and 50 ml/litre of water per pot for Marshal 250 EC as low, recommended and higher doses respectively and 3.5 ml/litre, 7 ml/litre and 14 ml/litre as low, recommended and high doses respectively for Nimbecidine.. Six chickpea seeds were then sowed in each pot, two weeks after germination thinning was done to leave one seedling. Freshly hatched juveniles, J2 (7 ml of the suspension which had one hundred juveniles) were inoculated as described by Hussey and Barker (1973). Pots were arranged in a completely randomized design with 5 replicates at each level and a control. The control was treated with distilled water only. After 60 days, chickpea plants were uprooted and washed gently. Evaluation was done on fresh root and dry root by measuring the weight (in grams) of the root of treated and untreated chickpea plants. Number juveniles per 100 g of soil were extracted from the soil using Baermann's funnel method and juveniles were counted from a counting slide with the aid of a dissecting microscope. Roots galling and root knot index was assessed on a scale of 0-5 (Sasser *et al*., 1984) (Rehman *et al.,* 2012). Screening of root knot nematodes was scored with a galling index: 0=no galling, 1=trace infections (few galls), 2=galled roots, 3=25-50% galling, 4=50-75% galling, and 5=75% of galled roots.

3.5 Determination of the effect of poultry manure on the control of root knot nematodes

The effect of farmyard manure (poultry manure) was carried out as described by Rehman *et al.* (2012). Soil was sterilized by autoclaving at 121^0C for 15 min, 250 g as low, 500 g and 750 g as high levels of poultry manure treatment. Treatments were thoroughly mixed with soil in pots. Each pot of 15cm diameter consisted of poultry manure, clay and loam soils in the ratio of 2:7:7 in 250 g level, ratio of 2:3:3 in 500 g and 6:5:5 in 750 g respectively Pots were arranged in a completely randomized design with 5 replicates per level of treatment. Positive control was treated with root knot nematodes only while negative control was treated with distilled water only. Six chickpea seeds of desi variety were surface sterilized in 1% sodium hypochlorite, washed thoroughly with distilled water and sowed in pots containing soil. Two weeks after germination, thinning was done by uprooting to leave one seedling for inoculation. Seven millilitres of the juveniles (J2) suspension which had one hundred juveniles was used to inoculate. After 60 days, chickpea plants were uprooted and washed gently. Evaluation was done on fresh root and dry root by measuring the weight (in grams) of the root of treated and untreated chickpea plants. Number juveniles per 100 g of

soil were extracted from the soil using Baermann's funnel method and juveniles were counted from a counting slide with the aid of a dissecting microscope. Roots galling and root knot index was assessed on a scale of 0-5 (Sasser *et al*., 1984) (Rehman *et al.,* 2012). Screening of root knot nematodes was scored with a galling index: 0=no galling, 1=trace infections (few galls), 2=galled roots, 3=25-50% galling, 4=50-75% galling, and 5=75% of galled roots.

3.6 Determination of the effect of Sudan grass (*S. sudanese*) in *Meloidogyne* spp. population

The effect of Sudan grass on root knot nematodes was done as described by Crow *et al.* (2001) and Rehiayani and Hafez (1998). Pots were arranged in a completely randomized design with eight replicates and a control. Chickpea seed was sown in each pot containing autoclaved soil and two weeks after germination; six Sudan grass seeds were sown around chickpea seedling. Each pot was inoculated with 7ml of juveniles, J2 population equivalent to one hundred juveniles and watering was done regularly. Positive control was treated with root knot nematodes only while negative control was treated with distilled water only. After 60 days, chickpea plants were uprooted and washed gently. Evaluation was done on fresh root and dry root by measuring the weight (in grams) of the root of treated and untreated chickpea plants. Number juveniles per 100 g of soil were extracted from the soil using Baermann's funnel method and juveniles were counted from a counting slide with the aid of a dissecting microscope. Roots galling and root knot index was assessed on a scale of 0-5 (Sasser *et al*., 1984) (Rehman *et al.,* 2012). Screening of root knot nematodes was scored with a galling index: 0=no galling, 1=trace infections (few galls), 2=galled roots, 3=25-50% galling, 4=50-75% galling, and 5=75% of galled roots.

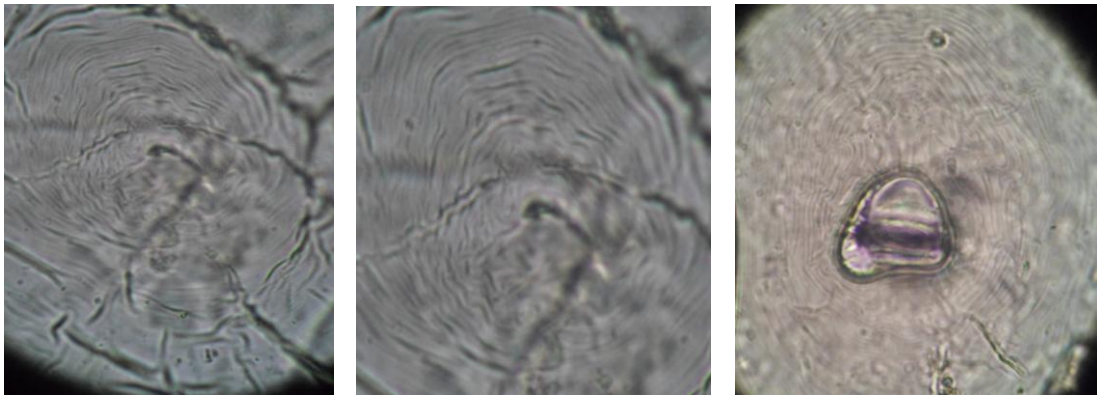
Data was analysed using SAS 9.3 statistics package using one-way anova. Least significance difference (LSD) was used in mean separation.

CHAPTER FOUR

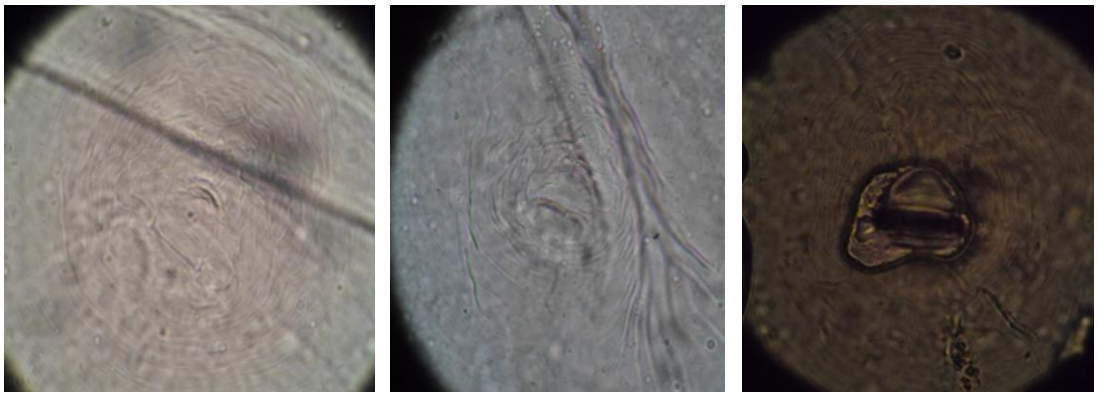
RESULTS

4.1 Characterisation and identification of *Meloidogyne* spp. attacking chickpea in Nakuru County

Perineal patterns of root knot nematodes collected from all study sites had a uniform pattern as depicted in Plate 1. The perineal patterns had distinct lateral ridges which divide the dorsal and ventral striae, ridges ran the entire width of the pattern (Plate 2). The striae were smooth to slightly wavy and some bent towards the vulval edges. The dorsal arch contained a whorl in the terminal area. The perineal patterns had uniform taxonomic features that are characteristic of *M. javanica*. *M. javanica* was present in all the samples collected while none of other species (*M. hapla*, *M. incognita* and *M. arenaria*) was isolated from the five sites.



(a) (b) (c)



(d) (e) (f)

Plate 1: Perineal patterns of *M. javanica*

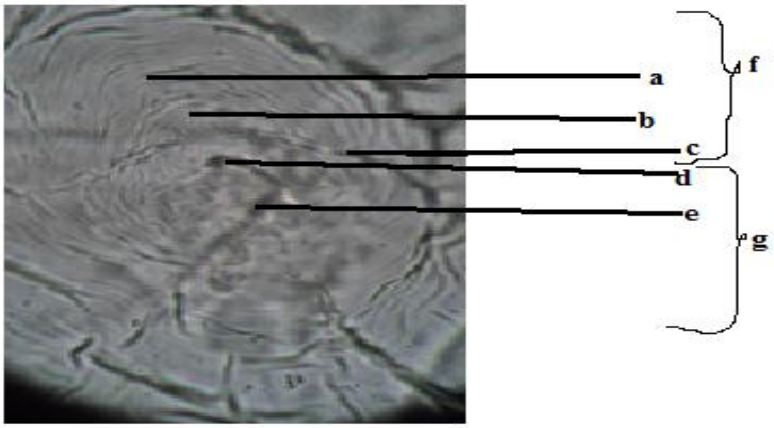
Key

(a) and (b) - Perineal patterns of root knot nematodes samples from the department of Biological Sciences garden.

(c) - Perineal patterns of root knot nematodes samples from Naivasha.

(d) and (e) - Perineal patterns of root knot nematodes samples from Field 3 and 7 of Egerton University.

(f) - Perineal patterns of root knot nematodes samples from Gilgil.



LEGEND

a- arch
b- tail tip
c- lateral line
d- anus
e- vulva
f- dorsal side
g- ventral side

Plate 2: Features in the perineal patterns

4.2 Effectiveness of two common commercial nematicides against root knot nematode disease in chickpea

In Marshal 250 EC, fresh and dry root weight ranged from 0.436 to 0.828 and 0.054 to 0.072 respectively, with low and high treatment level associated with highest and lowest fresh root weight means respectively, low and recommended treatment level associated with highest and lowest dry root weight respectively. There was no significant difference between fresh and dry root weight in all treatment levels. Means of root galls, gall index and number of *M. javanica* juveniles per 100 g soil significantly differed (P=0.05) from all treatment levels with low treatment level associated with the highest number. There was no significant difference between recommended and high treatment levels mean root galling, gall index and number of M. *javanica* juveniles per 100 g soil. Root gall index means differed significantly (P=0.05) between recommended and high levels with recommended and high level recording highest and with lowest index respectively (Table 3; Figure 2 and 3; Appendix 7 and 8).

Table 3: Fresh root, dry root weight, galls, gall indices and number of *M. javanica* juveniles/100 g soil in Marshal 250 EC treatment eight weeks after inoculation

Treatment level [z]	Fresh weight	Dry weight	Galls	Gall index	Juveniles
Low	0.828**a**[*]	0.072**a**	9.8**a**	2.4**a**	14.4**a**
Recommended	0.514**a**	0.054**a**	4**b**	1.4**ab**	2.4**b**
High	0.436**a**	0.062**a**	0.6**b**	0.2**bc**	0.4**b**
Control	0.68**a**	0.064**a**	0**b**	0**c**	0**b**

[a*] In the column, means followed by the same letter are not significant different from each other at P=0.05 according to Least Significant Difference (LSD) test.

[z] there were five treatments in each level

In Nimbecidine, means of fresh root weight, galls and number of *M. javanica* juveniles per 100 g soil showed no significant difference in all treatment levels. However, mean root gall index significantly reduced (P=0.05) at recommended level recording the lowest index (0.4) as compared to low treatment level which recorded highest index. Number of *M. javanica* juveniles per 100 g soil ranged from 2.2 to 9.8 with the lowest and highest numbers

associated with low and high treatment levels respectively (Table 4; Figure 2 and 3; Appendix 6).

Table 4: Fresh root, dry root weight, galls, gall indices and number of *M. javanica* juveniles/100 g soil in Nimbecidine treatment eight weeks after inoculation

Treatment level[z]	Fresh weight	Dry weight	Galls	Gall index	Juveniles
Low	0.858**a***	0.116**a**	12.8**a**	2**a**	9.8**a**
Recommended	0.592**a**	0.044**b**	1.4**a**	0.4**ab**	2.2**a**
High	0.688**a**	0.056**b**	9.4**a**	1.8**ab**	9**a**
Control	0.746**a**	0.074**ab**	0**a**	0**b**	0**a**

[a*] In the column, means followed by the same letter are not significant different from each other at P=0.05 according to Least Significant Difference (LSD) test.

[z] there were five treatments in each level

Both nematicide treatments showed significant difference (P=0.05) between means of low treatment level and means of all other treatment levels (recommended and high). Low and recommended levels recorded highest and lowest fresh root weight, dry root weight, galling, gall index and number of *M. javanica* juveniles per 100 g soil respectively. Root gall indices ranged from 1 to 2.3 with lowest and highest indices associated with low treatment level and other treatments levels (recommended and high levels) respectively. There was no significant difference between fresh root weight, dry weight, gall index and number of *M. javanica* juveniles per 100g soil means at recommended and high levels level (Table 5; Plate 3).

Table 5: Root knot disease parameters in Marshal 250 EC and Nimbecidine treatments eight weeks after inoculation

Treatment levels[z]	Fresh weight	Dry weight	Galls	Gall index	Juveniles
Low	0.843**a***	0.133**a**	16.4**a**	2.3**a**	16.3**a**
Recommended	0.553**b**	0.058**b**	4**b**	1**b**	4.7**b**
High	0.562**ab**	0.059**b**	5a**b**	1**b**	3.6**b**
Control	0.713**ab**	0.088**ab**	0**b**	0**b**	0**b**

[a*] In the column, means followed by the same letter are not significant different from each other at P=0.05 according to Least Significant Difference (LSD) test.

[z] there were five treatments in each treatment level

Both nematicides, Marshal 250 EC and Nimbecidine treatments showed no significant difference on the means of all parameters evaluated. However, Nimbecidine recorded higher means of galls, gall index and the number of *M. javanica* juveniles in 100g soil. Gall indices and number of *M. javanica* juveniles per 100g soil in Marshal 250 EC and Nimbecidine ranged between 1.30 to 1.35 and 4.60 to 5.55 respectively. Marshal 250 EC and Nimbecidine were associated with lowest and highest means respectively (Table 6).

Table 6: Comparison of Marshal 250 EC and Nimbecidine treatments in root knot disease parameters evaluated eight weeks after inoculation into chickpea

Treatment	Fresh weight	Dry weight	Galls	Gall index	Juveniles/100 g soil
Marshal 250 EC	0.6145**a***	0.063**a**	3.90**a**	1.30**a**	4.60**a**
Nimbecidine	0.721**a**	0.0725**a**	6.20**a**	1.35**a**	5.55**a**

[a*] In the column, means followed by the same letter are not significant different from each other at P=0.05 according to Least Significant Difference (LSD) test.

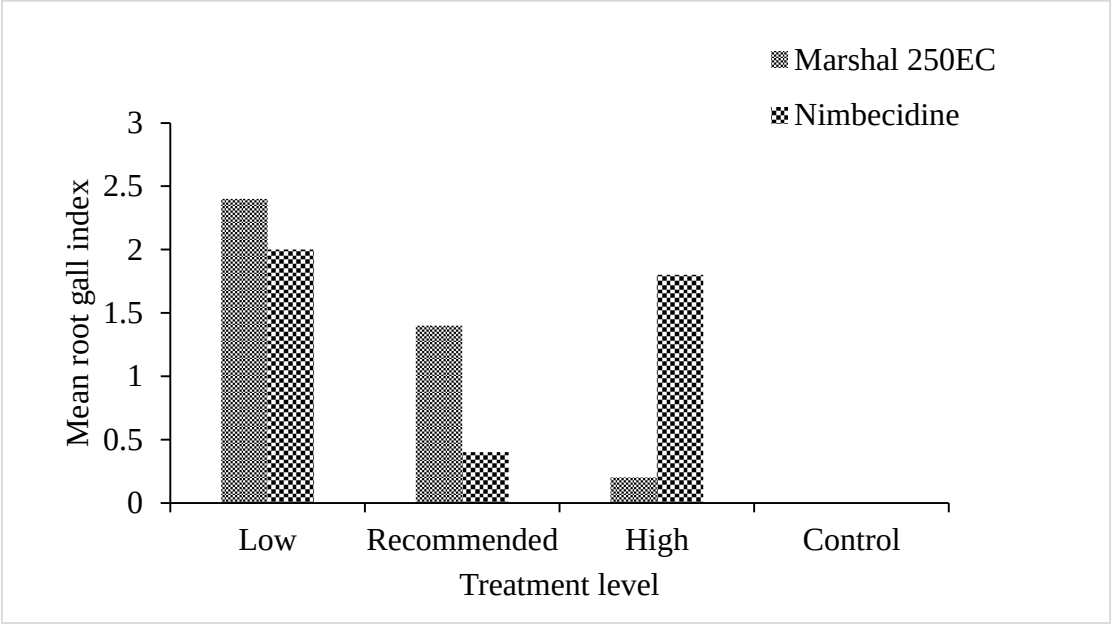


Figure 2: Mean root gall index for each level of nematicide treatment

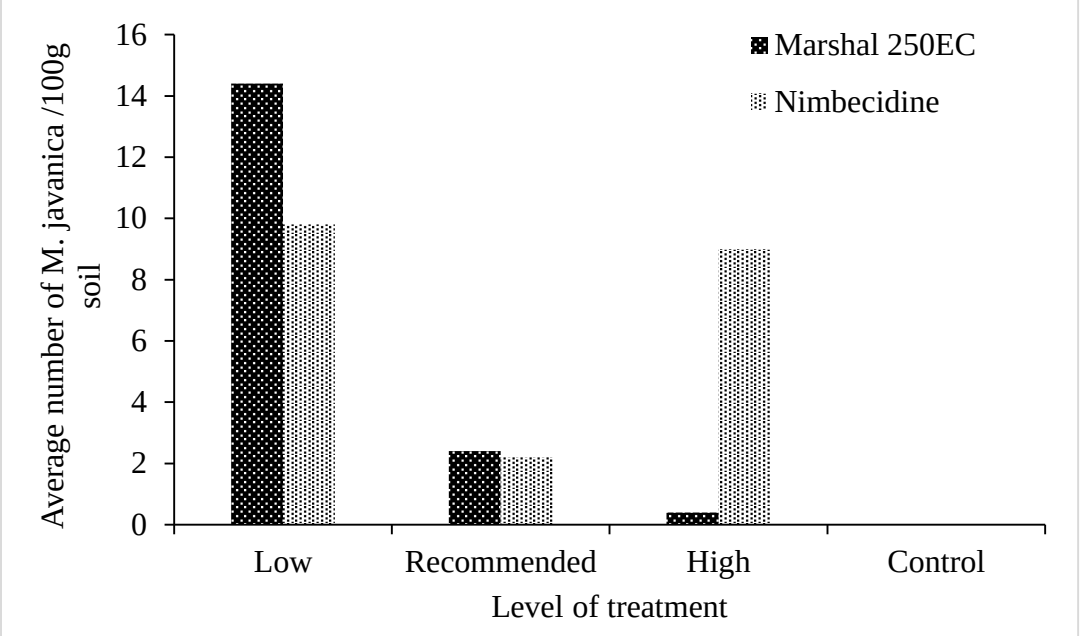


Figure 3: Average number of *M. javanica* juveniles/100 g of soil for each level of nematicide treatment

4.3 Effect of poultry manure in the control of root knot nematode diseases in chickpea

The range of root fresh weight 8 weeks after inoculation was between 0.462 and 2.026 with zero grams treatment having the highest root weight while 250 g and 500 g treatments having the lowest fresh weight respectively. There was significant difference (P=0.05) between means of zero grams treatment and other treatments 250 g, 500 g and 750 g. There was no significant difference (P=0.05) between means of treatments 250 g, 500 g and 750 g. Root dry weight ranged from0.074 and 0.198 with zero grams treatment having the highest root weight while 250 g and 500 g treatments having the lowest dry weight respectively. There was significant difference (P=0.05) between means of 0g treatment and other treatments 250 g and 500 g but no significance difference between means of treatments zero grams and 750 g and between 250 g and 500g treatments (Table 8). Gall indices ranged from 0 to 5 with zero grams treatment and control with highest and lowest index respectively. There was significant difference (P=0.05) between 0g and other treatments; 250 g, 500 g and 750 g. No significant difference between 250 g, 500 g and 750 g treatments. There was relationship between galling indices and root weight. Roots with highest galling indices had highest root fresh and dry weight while roots with lowest galling had lowest root fresh and dry weight respectively (Table 7; Figure 5; Plate 4; Appendix 2).

Table 7: Relationship between gall indices, fresh and dry root weights eight weeks after poultry manure treatment

Treatment[z]	Mean gall indices	Mean root fresh weight (g)	Mean root dry weight
0g	5**a**[*]	2.026**a**[*]	0.198**a**[*]
250g	1.4**b**	0.474**b**	0.112**b**
500g	1**b**	0.462**b**	0.074**b**
750g	1.2**b**	0.830**b**	0.192**a**
Control	0**c**	0.818**b**	0.088**b**

[a*] In the column, means followed by the same letter are not significant different from each other at P=0.05 according to Least Significant Difference (LSD) test.

[z] there were five treatments in each treatment

zero grams (positive control)

Eight weeks after inoculation, zero grams and 750 g treatments had the highest and lowest mean number of *M. javanica* juveniles ranging from 6.6-252.4 respectively. There was significant difference (P=0.05) in the means of 0g treatment and other treatments, 250 g, 500 g, 750 g and control. There was no significant difference in the means of 250 g, 500 g, 750 g and control. This shows a relationship between root galling indices and nematode population. The treatment with highest and lowest root gall indices had the highest and lowest *M. javanica* juvenile population respectively (Table 8; Figure 4).

Table 8: Number of *M. javanica* juveniles per 100 g of soil, eight weeks after poultry manure treatment

Treatment	Mean number of *M. javanica* juveniles/100 g soil
0g	252.4**a***
250g	40.8**b**
500g	17.4**b**
750g	6.6**b**
Control	0**b**

[a*] In the column, means followed by the same letter are not significant different from each other at P=0.05 according to Least Significant Difference (LSD) test.

zero grams (positive control)

The root galling indices by the eight weeks after inoculation was between 1.2 and 5 with zero grams treatment with the highest galling and 250 g, 500 g, and 750 g treatments associated with lowest galling. There was a significant difference (P=0.05) between means of zero grams treatment and other treatments 250 g, 500 g and 750 g. There was no significant difference (P=0.05) between the means of treatments 250 g, 500 g and 750 g (Table 9; Plate 4; Appendix 3).

Table 9: Root gall indices eight weeks after inoculation and poultry manure treatment

Treatment	Mean gall indices
0g	5**a***
250g	1.4**b**
500g	1**b**
750g	1.2**b**
Control	0**c**

[a*] In the column, means followed by the same letter are not significant different from each other at P=0.05 according to Least Significant Difference (LSD) test.

zero grams (positive control)

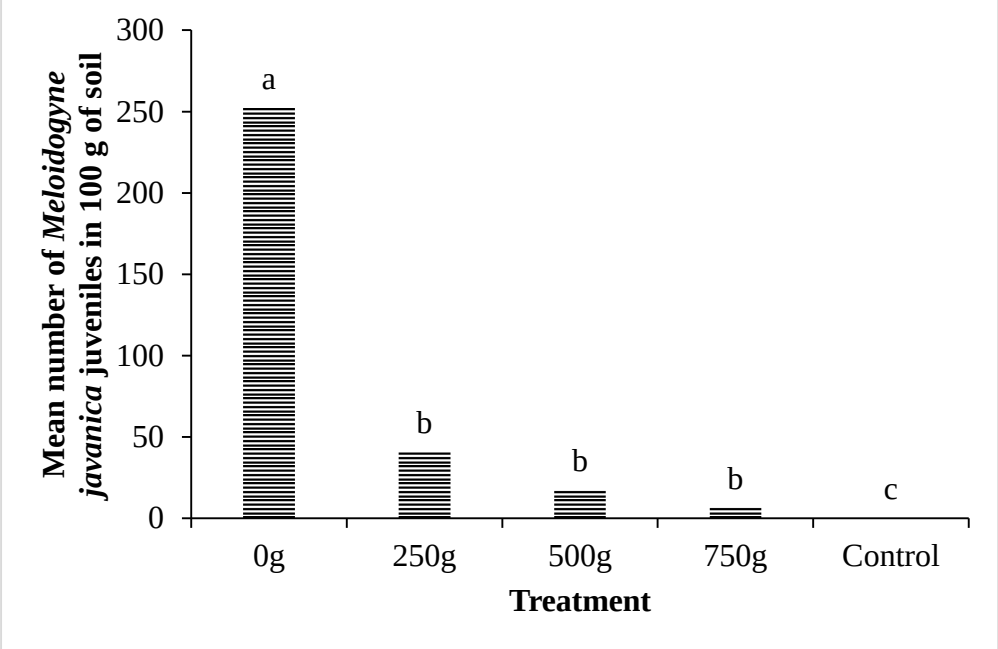


Figure 4: Mean number of *M. javanica* juveniles/100 g of soil in each level of poultry manure treatment

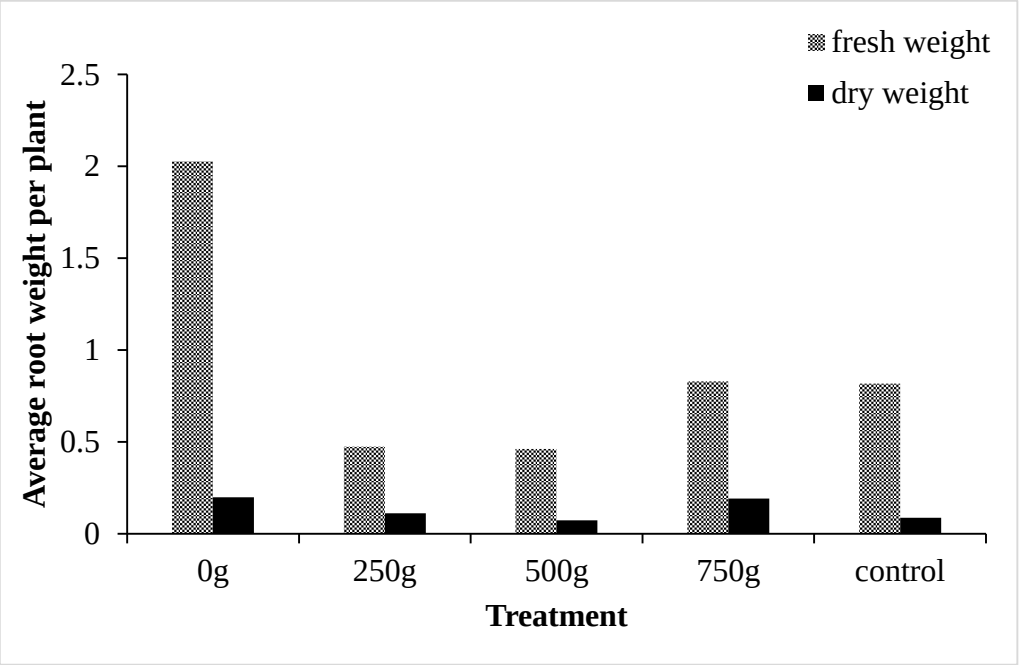


Figure 5: Average fresh root and dry root weight per plant for each level of poultry manure treatment

zero grams (positive control)

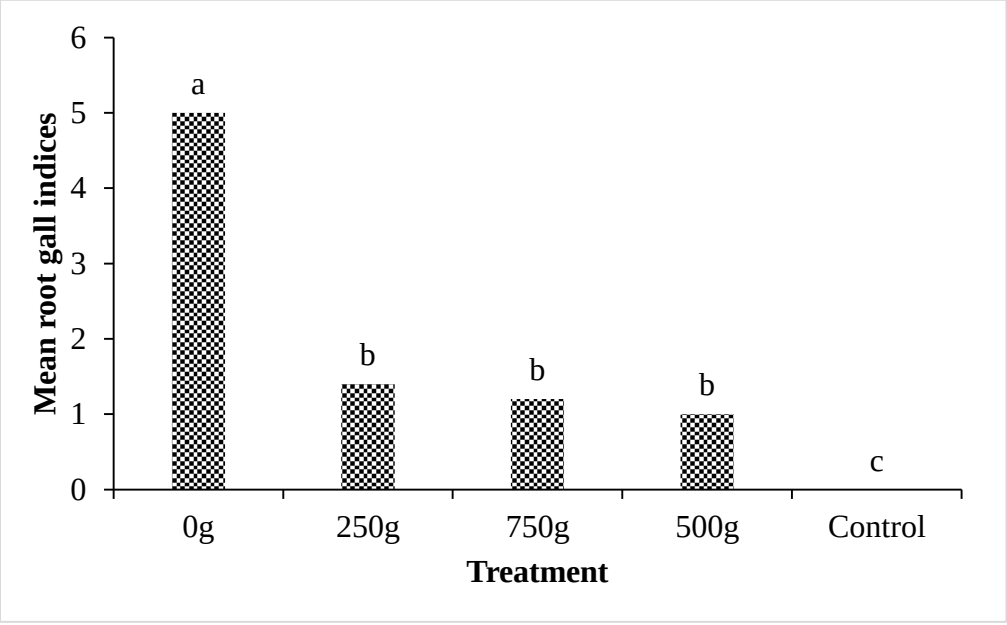


Figure 6: Mean root gall indices per plant in each level of poultry manure treatment

zero grams (positive control)

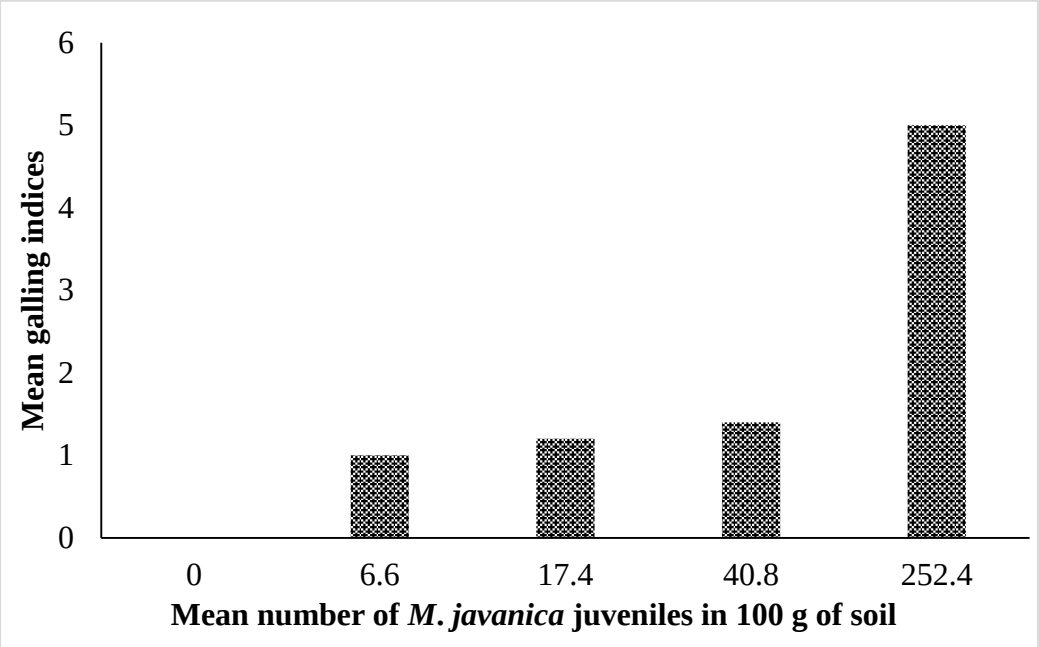


Figure 7: Relationship between root galling indices and the number of *M. javanica* juveniles in 100 g of soil on poultry manure treatment

4.4 Effect of Sudan grass (*S. sudanese*) in the *Meloidogyne* spp. population as a management strategy against root knot disease

Eight weeks after inoculation with root knot nematodes, the mean gall index ranged between 5 and 0, treated control with the highest gall index and untreated control with the least. There was a significant difference (P=0.05) of gall index in all the treatments. The mean fresh and dry root weight ranged between 0.5263-1.7888 and 0.0525-0.175 respectively. There was no significant difference between treated control and Sudan grass treatments in both fresh and dry root weights. There was significant difference (P=0.05) between treated control, Sudan grass treatments and untreated control in both fresh and dry root weight (Table 10; Figure 8; Appendix 4).

Table 10**:** Root gall indices, fresh and dry roots weight eight weeks after inoculation with *M. javanica* juveniles in Sudan grass treatment

Treatment [z]	Mean gall index	Mean fresh root weight	Mean root dry weight
INC	5**a**	1.7888**a**	0.175**a**
Sudan grass	2.625**b**	0.8663**a**	0.165**a**
UNC	0**c**	0.5263**b**	0.0525**b**

[a*] In the column, means followed by the same letter are not significant different from each other at P=0.05 according to Least Significant Difference (LSD) test.

[z] there were eight replicates in each treatment
INC- positive control, UNC- negative control

Eight weeks after inoculation with root knot nematodes, the mean gall index ranged between 0 and 5, positive control with the highest gall index and negative control with the least. There was significant difference (P=0.05) in all three treatments. The average number of *Meloidogyne javanica* in 100g of soil ranged 0 to 83.5, with treated control with highest and untreated control with lowest. There was a significant difference (P=0.05) in all three treatments. There was a relationship between gall indices and number of juveniles in the soil, highest gall index was associated with high number of *M. javanica* juveniles in soil while least gall index related to least number of *M. javanica* juveniles in soil (Table 11; Figure 9; Plate 5; Appendix 5)

Table 11: Relationship between root gall index and juveniles in 100 g soil eight weeks of Sudan grass treatment

Treatment [z]	Mean gall index	Average number of juveniles per 100g soil
INC	5**a**	83.5**a**
Sudan grass	2.625**b**	32.875**b**
UNC	0**c**	0**c**

[a*] In the column, means followed by the same letter are not significant different from each other at P=0.05 according to Least Significant Difference (LSD) test.

[z] there were eight replicates in each treatment

INC- positive control, UNC- negative control

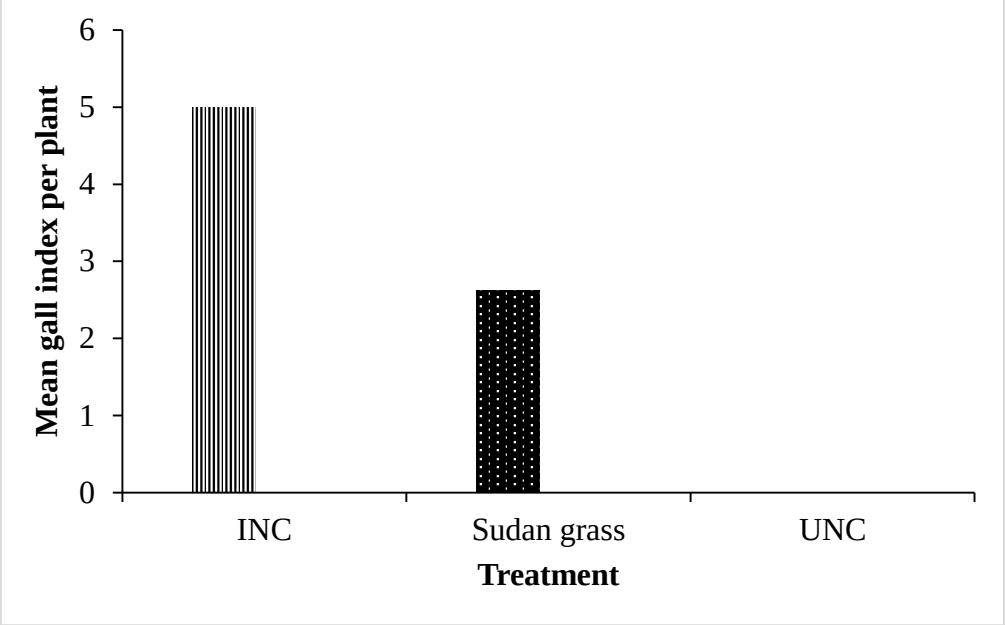


Figure 8: Mean root gall index per plant in Sudan grass treatments

INC- positive control, UNC- negative control

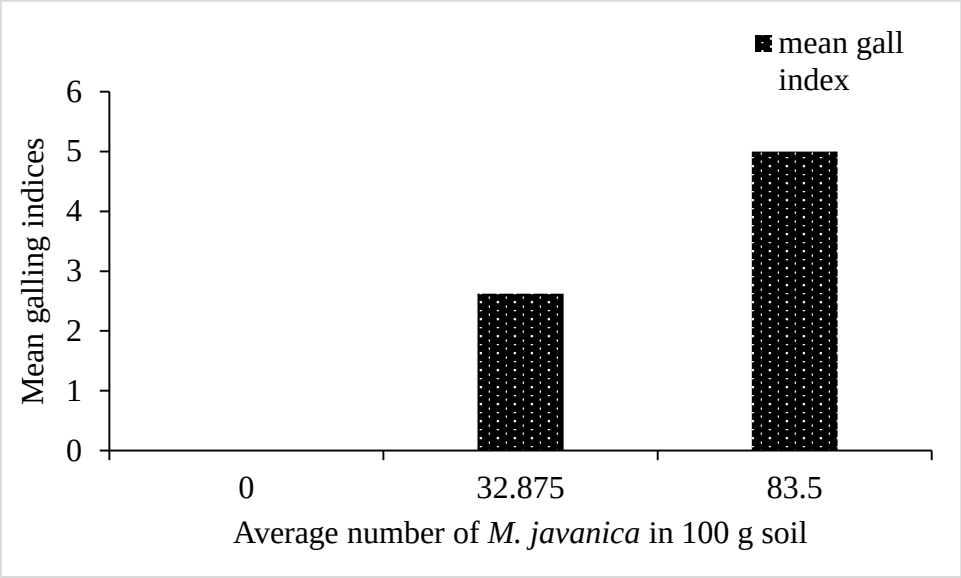


Figure 9: Relationship between root galling indices per plant and average number of *M. javanica* in 100 g soil in Sudan grass treatment


A

B

Plate 3: Uninoculated (A) and inoculated (B) chickpea plant


C

D

Plate 4: Heavily galled (C) and non-galled chickpea roots (D)

Plate 5: Chickpea plant cropped with Sudan grass

CHAPTER FIVE

DISCUSSIONS

5.1 Characterisation and identification of *Meloidogyne* spp. attacking chickpea in Nakuru County

The perineal patterns were found to have distinct lateral ridges that run the entire width of the pattern and divide the dorsal and ventral striae unlike in *M. incognita, M. hapla* and *M. arenaria* which do not have distinct lateral lines (Muthamia, 2004). They also have low and rounded dorsal arch unlike *M. incognita* whose dorsal arch is high and squarish (Abad *et al*., 2008). The striae were coarse, smooth to slightly wavy and bend towards the vulva which is unlike *M. hapla* which has fine dorsal and ventral striae that meet at an angle as was reported by Eisenback (1985).

There was consistency in the perineal patterns of the root knot nematodes extracted from chickpea grown in Nakuru indicating the occurrence of a single species. The features were characteristic of *M. javanica*. In Kenya, CABI (2002) *M. javanica* was associated with the damage of broad beans, tomato and cabbage and also attack cowpeas and pigeon peas in Mbeere, Mwea, Gachoka and Siakago areas (Waceke *et al*., 2013). Ngundo and Taylor (1974) reported infestation of *M. javanica* and *M. incognita* in beans in Thika. This is evident that *M. javanica* is present in Kenyan soils.

Ansari *et al.* (2012) reported that local chickpea cultivars produced low yield in *M. javanica* infested fields, however Sharma and Sharma (1988) reported that *M. javanica* is the second species predominant in chickpea losses in India. Susceptibility of chickpea cultivars to *M. javanica* was reported to be high (Sharma *et al*., 1993). Maheshwari *et al*. (1997) indicated that inoculation of *M. javanica* juveniles prior to *Fusarium oxysporum* f. sp. *ciceri* caused greater wilt incidence in susceptible cultivars and induced vascular discoloration in roots of resistant cultivars of chickpea. Inoculation of *M. javanica* and *Rhizoctonia bataticola* in chickpea seedlings reduce plant growth with tap root devoid of lateral roots and appearing dark and rotting (Ali *et al*., 2003).

5.2 Effectiveness of two common commercial nematicides against root knot nematode diseases in chickpea

Both Marshal 250 EC and Nimbecidine were effective against *M. javanica* at recommended levels. This was necessitated by toxic threshold of nematicides. High root galling, gall indices and large number of juveniles recorded at low level of Marshal 250 EC and Nimbecidine treatments reveal that the concentration of the active ingredient having not reached injurious threshold to the nematodes, this injurious threshold is given credibility by significant adverse effects at recommended and high levels of both nematicides. Significant adverse effects and reduction of root galling, gall indices and number of juveniles reveal that root knot disease in chickpea was greatly reduced.

Lack of significant differences in recommended and high levels of both nematicide treatment indicate that the active ingredients reached the threshold against nematodes but it also implies that uniform host parasite physiological and biochemical reactions, Muthamia (2004) found similar observations in *M. incognita*.

Marshal 250 EC recorded a higher reduction of root galling and, gall index and juvenile population than Nimbecidine, this can be explained that most of juveniles were killed before they could initiate infection or the active ingredient in Marshal 250 EC was more efficient against nematodes than that of Nimbecidine. Active ingredient in Marshal 250 EC is carbofulsan and in Nimbecidine are neem oil and Azadirachta. In Azadirachta and neem oil the compound limonoid triterpenoid azadirachtin is insecticidal, antifeedant and a motility inhibitor (Mordue *et al*., 1993), azadirachtin is one of the active components in neem against phytoparasitic nematodes. Studies have demonstrated that neem oil is nematicidal. Muthamia (2004) found that *Azadirachta indica* (neem plant) extract significantly reduced *M. incognita* juveniles at 500 µl, most juveniles died due to high toxicity which they could not withstand.

Nematicides mode of action against nematodes is by acetychorine esterase inhibition (Opperman and Chang, 1990). Recommended doses usually paralyse soil nematodes, they become nematostatic (McGarvey *et al*., 1984; Opperman and Chang, 1990) and eventually die. Higher doses above recommended kill nematodes. Once nematodes have absorbed lethal doses of nematicide, they become immobile and with time they die (Oka *et al*., 2009), hatching is also inhibited at lethal dosage. Organophosphate and carbamate nematicides affect the mobility of nematodes (Wright, 1981) and the most likely explanation of the reduction in infection is a reduction in mobility resulting to a decreased probability of contact

between nematodes and chickpea roots. This explains the reduction root galling and gall indices at recommended and high level of treatments.

High root fresh and dry weights recorded at low dosage levels of both treatments are explained by moderately high root galling which initiates formation of lateral roots and hence increased root weights.

5.3 Determination of the effect of poultry manure on the control of root knot nematodes

All the treatments were effective against *M. javanica* populations in the soil. The significance difference in the mean number of juveniles at different treatments confirms nematicidal effect of poultry manure. At 250 g, 500 g and 750 g treatments injurious dosage to the nematode juveniles was reached and many juveniles could not withstand and neither could the eggs hatch. Organic soil amendments improve soil fertility and structure, change the soils physical properties which in turn may have adverse effects on nematode behaviours such as hatching, survival and movements, hence reducing the nematodes population (Rodriguez-Kabana, 1986; Rodriguez-Kabana *et al*., 1987; Widmer and Abawi, 2000). Toxic compounds accumulate in soil as a result of the amendments and which have lethal effects on nematodes (Noweer and Hasabo, 2005).

Similar observations were made by Ismail *et al*. (2012) on *Meloidogyne* spp. population reduction upon organic soil amendment application in the soil. Organic soil amendments facilitate decomposition of specific proteins or materials that affect nematode cuticle structures and release of organic acids, ammonia and nitrates during decomposition, such compounds are toxic to soil nematodes and so, they play a nematicidal role (Abadir *et al*., 1996; Oka, 2010). Findings from Darwish *et al*. (2002) show that organic amendments improve physiological and biochemical processes leading to increased nutrient uptake and plant growth. Results from Kaplan *et al*. (1992); Kaplan and Noe (1993); Jaffe *et al*. (1998) and Timm *et al*. (2001) also found out that organic soil amendments and especially chicken manure stimulate build-up of nematode-destroying microbes and fungi such as *Arthrobotrys oligospora*, *A. superba* and *Monacrosporium cionopagum* and related nematode-destroying structures in the soil and indirectly decreasing the population of plant parasitic nematodes. Organic amendments supply the needed food sources to the nematode-trapping fungi which results in their enhancement (Riegel *et al*. 1996; Riegel and Noe, 2000).

Increased fresh and dry root weight in zero gram treatment (untreated and inoculated) than rest of treatments is as a result of increased nematode populations which led to increased

galling. Root knot galling initiate abnormal cell growth and production of lateral roots which contribute to the increased root weight. Lack of amendment in zero gram treatment made juveniles infest in roots causing massive galls and lateral roots. Fresh and dry root weight, root galling and gall indices decreased with increase in poultry manure treatments respectively. These observations were in agreement with those of Agwu and Ezigbo (2005) and Amulu *et al*. (2015) on the effect of *M. incognita* in okra in Nigeria and on the management of *M. incognita* in eggplant (Abadir *et al*., 1996; Karmani *et al*., 2011).

Root galling, gall indices and number of *M. javanica* juveniles were significantly reduced by the 250g, 500g and 750g treatments. This confirms that poultry manure promotes physiological, biochemical processes and growth hence reduce nematode gall formation, this observation agrees with those of Karmani *et al*. (2011) whereby poultry manure was most effective amendment against *M. incognita* in eggplant and most effective soil amendment in root knot nematode suppression in *Phaseolus vulgalis* (Kimenju *et al*., 2004). Similar observations were made by Owino *et al*. (1993), gall ratings number of *M. javanica* juveniles and egg masses were reduced in poultry manure amended soils suggesting that the treatment was toxic to *M. javanica*. Shiferaw *et al*. (2014) and Kankam *et al*. (2015) and reported root galling and root knot nematode reduction in carrot and in tomatoes respectively using poultry litter, poultry litter in combination with rapeseed cake suppressed *Meloidogyne* spp. remarkably. Other mechanisms involved in suppression of soil nematodes are: release of pre-existing nematicidal compounds in soil amendments, enhancement or introduction of antagonistic microorganisms, increase in plant tolerance and resistance, and changes in soil physiology that are unsuitable for nematode behaviour (Oka, 2010).

5.4 Determination the effect of Sudan grass (*S. sudanese*) on the *Meloidogyne* spp. population as a management strategy against root knot disease

The results demonstrated that there was significant reduction of gall indices and *M. javanica* population in the soil. When compared to the inoculated control, *M. javanica* heavily infested the roots of chickpea resulting into many root galls hence high gall index. The number of juveniles in the soil was dependent on number of eggs in root galls, as eggs in the galls hatched juveniles drop into the soil. Sudan grass roots showed that they have nematicidal effect and therefore reduce the population of juveniles by suppressing reproduction or by death; therefore this explains why Sudan grass roots are poor hosts of soil nematodes.

Suppression of *M. javanica* by Sudan grass was primarily attributed to chemical mechanisms, this is because exposure of *M. javanica* juveniles to Sudan grass reduced root gall index and number of juveniles in the soil (Viaene and Abawi, 1998). The hypothesis that cyanide was the chemical compound responsible for suppression was initially based upon what is known biochemically about Sudan grass, and Widmer and Abawi (2002) reported suppression of *M. hapla* by Sudan grass using the hypothesis. Cyanide is known to be toxic to different organisms and is present within Sudan grass root tissue as a cyanogenic glucoside. Cyanide appears to have adverse effects to both egg development and hatching (Widmer and Abawi 2000).

Epidermal cells of roots of Sudan grass contain cyanogenic glucoside dhurrin which degrade into hydrogen cyanide. Hydrogen cyanide (HCN) is known for its toxicity to nematodes (De Nicola *et al*., 2012). Cyanogenic glucoside dhurrin is degraded through a cyanohydrin intermediate after enzymatic hydrolysis catalysed by endogenous beta-D-glucoside glucohydrolase and alpha-hydroxynitrilase via a process known as cyanogenesis (Conn, 1991). Once root tissues are damaged, dhurrin is hydrolysed by endogenous dhurrinase found in the intermediate p-hydroxy-(S)-mandelonitrile which is unstable compound and which releases HCN. HCN is toxic to nematodes (Vetter, 2000; Widmer and Abawi 2000, 2002). Similar observations were made by Widmer and Abawi (2000), *M. incognita* juveniles were reduced as a result of dhurrin degradation, dhurrin hydrolysis prevented hatching of *M. incognita* eggs. When *M. hapla* eggs and juveniles were exposed to 0.1 ppm of cyanide, root penetration was reduced by 4% and the same concentration of cyanide reduced *M. hapla* infection by 48% (Viaene and Abawi, 1998).

Sudan grass has high dhurrin content and thus suppressed the nematodes. For efficient suppression, Widmer and Abawi (2002) suggested that Sudan grass should be used as green manure at 1-2 months since there is high HCN content in young Sudan grass plants and it is known to decrease with age of the plant. Sudan grass extracts also reduced the population of *M. hapla* and this was associated with the presence of cyanide in the extracts, dhurrin was involved in the mode of action of Sudan grass on *M. hapla* (Widmer and Abawi, 2000).

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Meloidogyne javanica is the prevalent root knot nematode associated with chickpeas in Nakuru County. Marshal 250 EC was more effective nematicide than Nimbecidine this is due to efficiency of active ingredient in Marshal 250 EC (Carbofuran) in comparison to that of Nimbecidine (Neem oil and Azadirachtin). Both Marshal 250 EC and Nimbecidine showed low and highest root galling and number of juveniles. Both nematicides significantly reduced root galling and the number of juveniles at recommended and high levels. There were significant differences between different levels of poultry manure treatments. Low level of poultry manure (250 g) was associated with highest root galling and number of juveniles. High level (750 g) was associated with the least root galling and number of juveniles per 100 g of soil. Poultry manure treatments with high and low galling index recorded highest and lowest root fresh and dry weight respectively. Sudan grass significantly reduced root galling and juvenile population in the soil. Sudan grass roots showed adverse effects to root knot nematodes in the soil and thus can be used as an effective nematicide.

6.2 Recommendations

According to the study desi variety of chickpea is susceptible to root knot nematodes, therefore it is recommended that there is need to carry out a resistance and susceptibility study to other chickpea varieties and cultivars which are economically important known to be affected by root knot disease. Since poultry manure and Sudan grass have nematicidal properties, it is recommended that further research be done to other legumes susceptible to root knot disease. Further study should be done on Sudan grass green manure to control other *Meloidogyne* spp. attacking chickpea. Other cover crops with nematicidal properties should also be studied in chickpea. Molecular characterization and second juvenile (J2) morphology should also be studied in order to identify of root knot nematodes. Agricultural officers and farmers will manage root knot disease in cultural crops using information from this study.

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APPENDICES

Appendix 1: Work plan

Time / Activities	2014					2015						
	Aug	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**July**
Proposal preparation												
Sample collection												
Identification of *Meloidogyne* spp.												
Determination of effect of nematicides, farmyard manure and Sudan grass												
Data collection and analysis												
Thesis writing												

Appendix 2: Analysis of Variance for root dry weight in chickpeas 8 weeks of growth in poultry manure

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.07493	0.00937	3.99	0.0089
Error	16	0.03758	0.00235		
Corrected Total	24	0.1125			

Coefficient of Variation=36.49196 LSD value=0.065 at alpha=0.05

Appendix 3: Analysis of Variance for root gall indices in chickpeas 8 weeks of growth in poultry manure

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	73.28	9.16	83.27	<.0001
Error	16	1.76	0.11		
Corrected Total	24	75.04			

Coefficient of Variation=19.2827LSD value=0.4447 at alpha=0.05

Appendix 4: Analysis of Variance for root gall indices in chickpea 8 weeks of growth in Sudan grass

Source	Degrees of freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	104.7083333	11.6342593	17.61	<.0001
Error	14	9.2500000	0.6607143		
Corrected Total	23	113.9583333			

Coefficient of Variation= 31.98072 LSD= 0.8717 at Alpha 0.05

Appendix 5: Analysis of Variance for *M. javanica* juveniles in 100g soil in chickpeas 8 weeks of growth in Sudan grass

Source	Degrees of freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	30980.37500	3442.26389	17.67	<.0001
Error	14	2727.58333	194.82738		
Corrected Total	23	33707.95833			

Coefficient of Variation= 35.98210 LSD= 14.969 at Alpha 0.05

Appendix 6: Analysis of Variance for root galling in chickpeas 8 weeks of growth in Sudan grass

Source	Degrees of freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	21004.04167	2333.78241	15.95	<.0001
Error	14	2047.91667	146.27976		
Corrected Total	23	23051.95833			

Coefficient of Variation= 42.87604 LSD=12.97 at Alpha 0.05

Appendix 7: Analysis of Variance for fresh root weight per chickpea plant in Nimbecidine

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.05000630	0.00714376	0.97	0.4925
Error	12	0.08819814	0.00734984		
Corrected Total	19	0.13820443			

Coefficient of Variation= 37.65451 LSD= 0.1181 Alpha 0.05

Appendix 8: Analysis of Variance for fresh root weight per chickpea plant in Marshal 250EC

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.04091573	0.00584510	1.06	0.4400
Error	12	0.06586579	0.00548882		
Corrected Total	19	0.10678152			

Coefficient of Variation=36.77141 LSD=0.1021 Alpha 0.05

Appendix 9: Analysis of Variance for dry root weight per chickpea plant in Marshal 250EC

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.00083066	0.00011867	1.35	0.3079
Error	12	0.00105278	0.00008773		
Corrected Total	19	0.00188345			

Coefficient of Variation=35.44652 LSD=0.0129 Alpha 0.05