INTEGRATING HOST RESISTANCE WITH TOLERANCE IN MULTIPLE CROPPING SYSTEMS FOR MANAGEMENT OF PLANT PARASITIC NEMATODES IN SUGARCANE

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DECLARATION BY THE CANDIDATE

This thesis, "Integrating Host Resistance with Tolerance in Multiple Cropping Systems for Management of Plant Parasitic Nematodes in Sugarcane" is my original work and has not been presented for a degree in any other University.

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To the loving memory of my father and mother Wilson Taparsang Kipchirchir arap Nyatogo - “Korgoren” (1918-1996) and Anna Tapsabei Chemolel nebarap Nyatogo -“Chepoterik” (1926-2015) for their belief in me.

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ABSTRACT

Sugarcane productivity in Kenya has been on the decline over the past decade due to various factors including pests and diseases. Plant parasitic nematodes (PPNs) are known to infest sugarcane fields due to poor agronomic practices that include use of traditional varieties, continuous monoculture and inadequate fertilizer use. A study consisting of glasshouse and field experiments was conducted to assess the potential of integrating host resistance, intercropping and fertilization into nematode management packages to enhance sugarcane productivity and improve food security and income generation for small-scale sugarcane farmers. Fourteen (14) sugarcane cultivars were randomly selected and screened in the glasshouse to determine their resistance status to root-knot (*Meloidogyne* spp.) and lesion (*Pratylenchus* spp.) nematodes, and compared to N14 as the standard. Ten (10) food crops commonly grown in the sugarcane zones were screened in the glasshouse to select those that suppress the lesion and/or root-knot nematodes. Four sugarcane cultivars namely KEN83-737, KEN82-216, Co945 and Co617 showed resistance against *Meloidogyne* spp. Moderate resistance was observed on varieties N14, EAK70-97, KEN98-530, CB38-22, KEN00-13, KEN82-121, KEN82-472, KEN82-493 and KEN82-62. Varieties Co421 and D8484 were susceptible. When exposed to *Pratylenchus* spp., cultivar KEN83-737 showed resistance, CB38-22, KEN82-216, KEN00-13, KEN82-121, Co617, Co945 and N14 were moderately resistant, KEN82-493, KEN98-530, KEN82-62, D8484 and EAK70-97 were moderately susceptible while KEN82-472 was classified as susceptible and Co421 highly susceptible. The intercrops amaranthus (*Amaranthus blitum*) and spiderplant (*Cleome gynandra*) were found to be suppressive to both nematode species while African nightshade (*Solanum nigrum*) and Jute mallow (*Corchorus olitorius*) were susceptible. Whereas slender leaf (*Crotalaria brevidens*) was suppressive to lesion it was susceptible to root-knot

nematodes. Intercropping of sugarcane with an appropriate food crop suppressed nematode populations and produced consistently higher sugarcane equivalent yields (SEY) than sugarcane pure stand. Intercropping KEN83-737 × spiderplant produced the highest SEY of 3.03 followed by Co421 × spiderplant at 2.81. The least SEY was that of N14 × African nightshade at 2.26. Fertilizer application enhanced tolerance of the susceptible cultivar Co421 to nematode inoculation, but this property was not demonstrated for the resistant cultivar KEN83-737. Integration of host resistance with intercropping and fertilizer application influenced nematode populations, sugarcane yields, food production and revenue generation. The lowest SEY was obtained in pure stand sugarcane plots with neither intercrop nor fertilizer. Intercropping sugarcane with spiderplant without applying fertilizer improved SEY by 1.32 times. Applying fertilizer to sugarcane pure stand improved SEY by 1.54 times. However, the highest improvement of SEY occurred when sugarcane was intercropped with spiderplant and fertilizer applied, which increased SEY by 3.22 times. The intercrop of KEN83-737 × spiderplant when fertilized with diammonium phosphate was demonstrated to be the most suitable combination for suppression of plant parasitic nematodes, higher sugarcane yields and better food production and revenue generation. This combination is recommended as it will contribute to the improvement of food security and nutrition. Further, this will contribute to better incomes at the household level, thus has the potential of contributing to the improvement of the livelihoods of the small-scale sugarcane farmers.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Sugarcane (*Saccharum* spp. hybrids) is widely cultivated in tropical and subtropical countries for the numerous benefits that it offers which include use in foodstuffs, as fiber and production of biofuels (World Sugar Statistics, 2007). Sugarcane is an important agro-industrial crop introduced in Kenya in the late 19th century that has developed into a significant player in the economy. The number of farmers growing sugarcane in Kenya is estimated at about 300,000 who rely on the crop as their main source of income (KESREF, 2010). The subsector provides direct employment to about 20,000 people. In total about six million people get their livelihoods from the sugarcane subsector (KESREF, 2009). Sugarcane is mainly grown in western and coastal counties of Kenya. The western counties include Bungoma, Busia, Homa Bay, Kakamega, Kericho, Kisumu, Migori, Nandi and Narok while the coastal county is Kwale.

Sugarcane is mainly produced as a continuous monoculture which leads to soil degradation as well as accumulation of pests and diseases associated with sugarcane (Spaull and Cadet, 1990; GoK, 2010; Nzioki and Chirchir, 2010). Common sugarcane diseases include the sugarcane smut, ratoon stunting disease (RSD), mosaic disease, pineapple disease, rust, eyespot, red rot, leaf scald and yellow leaf. On the other hand, some of the main pests afflicting sugarcane are moles, termites, early shoot borer, root borer, top borer, lady bug, sugarcane whitefly and plant parasitic nematodes (PPNs) (Ibid).

Plant parasitic nematodes associated with sugarcane are among the most damaging pests and are reported to reduce yield by 20-50% under severe infestation (Stirling and Blair, 2000). The loss

in yields is due to a reduction in the number and length of stalks. Nematode diversity in sugarcane is greater than in most other cultivated crops, with more than 310 species of 48 genera of endo- and ectoparasitic nematodes having been recorded from its roots rhizosphere (Cadet and Spaull, 2005). Reduction in yields can be addressed by use of resistant varieties, use of fertilizers and plant diversity. Although use of resistant varieties emerges as the best option in nematode management, it needs proper testing. Unlike in Brazil and South Africa, in Kenya, sugarcane cultivars developed and grown locally have not been assessed for reaction to nematodes. Sugarcane cultivars have been shown to exhibit different levels of host resistance to plant parasitic nematodes. In Brazil, Santos *et al.* (2012) tested 30 genotypes and demonstrated that though all the varieties assessed exhibited susceptibility to lesion nematodes, with reproduction factor of 1.1 to 3.8 for *P. zeae* and 1.3 to 3.7 for *P. brachyurus*. This assessment can then be followed by evaluating the potential of incorporating host-plant resistance into an integrated pest management package.

Fertilizer application is known to improve tolerance to PPNs offering a potential nematode management option (Waele and Elsen, 2007). However, the types and levels of fertilizers that imparts tolerance and specifically on sugarcane have not been determined.

Plant diversity can reduce pathogen and disease pressure compared to a single crop (Cardinale *et al.,* 2003; Sinha *et al.,* 2004). Appropriate companion crops can be used to produce food and excess sold for income thereby partially addressing food insecurity and high poverty levels. The sugarcane equivalent yields formula may be utilized to select combinations of crops with good returns. Suitable intercrops may be grown in the early months of sugarcane growth. Some of the possible intercrops are good at replenishing soils, a property that can be used to redress declining soil fertility, while others may even suppress nematode populations. The latter property may be

further enhanced by interplanting nematode-suppressive intercrops with resistant sugarcane cultivars in order to reduce accumulation of PPNs caused by prolonged monoculture.

Companion crop can contract diseases and or multiply pathogens and herbivores to which the principal crop is susceptible (Sumner *et al*., 1982; Fargette and Fauget, 1988). This could reduce the benefit of the intercrop. In addition to the reaction of intercrops to nematodes, their economic returns when combined with sugarcane and compared to each other are clear gaps that need to be addressed. Indeed currently there are no deliberate measures undertaken to manage nematodes of sugarcane in Kenya.

1.2 Statement of the problem

Sugarcane production has been on the decline in the recent past with farm level yields dropping from 74 to 55 tonnes per hectare between 2004 and 2013: a decline of 26% (KSB, 2013; Appendix 1). Among the factors leading to this decline are poor sugarcane management practices, decline in soil fertility and pests and diseases. Consequently, the low sugarcane yields have caused increased food insecurity and rising poverty levels among small-scale sugarcane farmers. To avert this situation, concerted efforts are needed to develop intervention measures aimed at increasing sugarcane productivity in Kenya.

1.3 Justification

Sugarcane is considered a low value field crop which implies minimal or no use of pesticide chemicals (including nematicides) which are also unaffordable to most small scale growers. Furthermore, according to the Montreal Protocol of 1987 (Montreal Protocol, 1987), most of the nematicides are set to be phased out by 2015 due to their negative side effects on public health and environment. Sugarcane land sizes have also been reducing due to land subdivision among heirs leading to less mechanization and diminishing economies of scale thereby leading to

reduced food production which has worsened food insecurity and nutrition of vulnerable members of the sugarcane farmers' households. Strategies must thus be developed that will manage nematodes and increase sugarcane yields while at the same time increase food production and improve revenue generation for small-scale households. This concept of managing PPNs using cultural methods (intercropping) and plant-host resistance while addressing the socio-economic and environmental (limiting chemical usage) aspects entail the classical Integrated Pest Management (IPM) strategy, a holistic solution that the study is focused on.

1.4 Objectives

1.4.1 Overall objective

To mitigate sugarcane productivity decline through integrated nematode management strategies that contribute to the improvement of the livelihoods of small-scale sugarcane farmers

1.4.2 Specific objectives

1. To determine the levels of resistance in sugarcane genotypes against lesion and root-knot nematodes
2. To identify high value intercrops that suppress nematode populations
3. To determine the effect of fertilizer on sugarcane tolerance to nematode infestation
4. To assess the influence of integrating host-plant resistance, intercropping and fertilization on yields and revenue in sugarcane production systems

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Plant parasitic nematodes associated with sugarcane and their economic importance

Nematodes are a group of multicellular thread-like organisms common in soil, fresh water and seas. Some of them are plant parasites while some are saprophagous in soil. They exhibit bilateral symmetry while the neck region has triradiate symmetry. Their body is unsegmented and has pseudocoelomate cavity. They have neither a respiratory nor a circulatory system. Their sexes are generally separate and fertilization occurs internally. The general nematode shape is vermiform (Coleman and Crossley, 1996).

It has been shown that herbivorous nematodes interfere with plant growth by impeding the uptake of nutrients and water or altering nutrient mechanisms (Blair *et al.*, 1999). The parasites suck and drain the fine hairlike roots and create knots in the smaller roots limiting the development of the root system. Galls appear all over the mass of the plant roots. Damaged roots are thus unable to absorb water and nutrients. One of the most important pathogenic nematode genus is the *Meloidogyne* in which the species *M. incognita* is most widespread and is thought to be the most serious plant parasitic nematode of tropical and subtropical regions throughout the world. The nematode occurs as a pest on a wide variety of crops infesting plant roots particularly in sandy soils where they feed and complete their life cycle. Infested roots often have distinctive swellings, called galls that damage the root's ability to take up water and nutrients. The galls can split open becoming avenues for entry of soil-borne plant pathogens. Unlike nodules in legumes, the galls are true swellings and cannot be rubbed off. If the

nematodes are not controlled they can multiply to a level where they cause severe plant damage and yield loss estimated at 80% (Cetintas and Yaiba, 2010).

The other widespread and highly pathogenic nematodes are *Pratylenchus zeae* and *P. brachyurus*. Other plant parasitic nematodes include *Xiphinema* spp. (stubby root nematodes) and *Paralongidorus* spp. (needle nematodes). The widespread but moderately or weakly pathogenic nematodes include *Tylenchorhynchus* spp. (stunt nematodes), *Helicotylenchus* spp. spiral nematodes), *Scutellonema* spp. and *Rotylenchus* spp. others are *Hoplolaimus* spp. (lance nematodes),*Criconemella* spp. (ring nematodes), *Criconema* spp., *Hemicricomoides* spp, *Ogma* spp., *Hemicychiophora* spp. (sheath nematodes) and *Rotylenchulus parus* (reniform nematodes) (Cadet and Spaull, 2005).

In sugarcane, the diversity of nematodes is greater than in most other cultivated crops, with more than 310 species belonging to 48 genera of endo- and ectoparasitic nematodes having been recorded from its roots and/or rhizosphere (Ibid). Of these, it is the species *Pratylenchus zeae* that has been cited by various authors as the most important nematode for the crop (Sundararaj and Mehta, 1994; Spaull and Cadet, 2003). Other studies have pointed out the genera *Pratylenchus* and *Meloidogyne* as the most damaging to sugarcane (Moura *et al.,* 1999; Starr and Bendezu, 2002). A study conducted in the Brazillian state of Pernambuco showed that *P. zeae* is present in all sugarcane plantations that recorded low yields (Moura and Almeida, 1981). When it was controlled, there was an impressive yield increase amounting to 41t/ha (Dinardo-Miranda *et al.,* 1988). In Kenya, 26 parasitic nematode genera have been extracted from the roots and rhizosphere of sugarcane (Kariaga, 1988; NARL, 1991; Kariaga and Nzioki, 2003; Nzioki, 2007; Chirchir *et al*., 2008).

Given the prominence of *Pratylenchus* spp. in sugarcane, it is important to consider it in this review. The nematode is commonly referred to as lesion nematode and is responsible for root lesion disease on many host plants. Members of this genus are small, vermiform nematodes. They are 0.3 to 0.9 millimeter long and virtually transparent and invisible to the naked eye. Lesion nematodes are essentially worldwide in distribution. They are migratory endoparasites that feed and reproduce in the root and feed only on the cortex (Anon, 1999). The species are distinguished primarily by the morphology of the stylets (Norton, 1978). Signs of infestation are similar in most plants and generally include necrotic lesions of the roots (Barker, 1998). Many Nematologists consider 100 specimens of *P. zeae* per 200g soil before planting or 250 specimens per 200g soil in the middle of the cropping cycle as the threshold number to cause economic loss in cane production (Stirling and Blair, 2000).

The Kenyan sugar industry is a significant contributor to agricultural gross domestic product. It provides direct employment to about 20,000 people, and is the source of income for more than half a million small-scale farmers who supply more than 85% of the sugarcane to the country's sugar millers (KSB, 2009). An estimated six million Kenyans also derive their livelihood directly or indirectly from the sugar industry which, being largely rural based, benefits the rural population and thereby significantly contribute to the attainment of one of the millennium development goals on poverty reduction (KESREF, 2009).

There has been an overall increase in acreage under sugarcane from 131,504 ha in 2004 to 154,298 ha in 2009 throughout the sugar industry, however, yields have steadily declined from 73.81 tons ha^{-1} to 65.21 tons ha^{-1} in the same period representing approximately 12% drop (KSB, 2009); while a more recent study shows farm level yields dropping from 74 to 55 tonnes per hectare between 2004 and 2013: a decline of 26 % (KSB, 2013). The decline in sugarcane

productivity has been attributed to several factors of which pests and diseases contribute significant yield losses (KSB, 2009). The most common diseases of sugarcane include sugarcane smut, ratoon stunting disease (RSD), leaf rust, sugarcane mosaic, eye spot, mid-rib red rot and pineapple disease. Termites, moles, stalk borer, scales and plant parasitic nematodes are among the main pests that parasitize sugarcane. Currently there are no deliberate measures undertaken to manage nematodes of sugarcane in Kenya.

2.2 Host resistance

Plants have developed a wide variety of mechanisms in order to protect themselves from damage. These mechanisms are both constitutive and inducible defenses (Freeman and Beattie, 2008). Constitutive defenses are continuous and include barriers meant for protection from invasion as well as adding strength and rigidity to the plants. Almost all living plant cells are able to detect invading pathogens and respond with inducible defenses. These include production of toxic chemicals, pathogen-degrading enzymes and deliberate cell suicide. Plants often wait until pathogens are detected before producing toxic chemicals or defense-related proteins because of the high energy costs and nutrient requirements associated with their production and maintenance (Ibid).

The reproductive success of plant parasitic nematodes on sugarcane is affected by several biotic factors, chief among them being the plant (Cadet and Spaull, 2005; Chirchir *et al*, 2011). Dinardo-Miranda (1994) and Mehta *et al.* (1994) have reported that there are large differences in the suitability of different cultivars as hosts to certain species of nematodes. Variable resistance of sugarcane cultivars to *M. incognita* has been observed in Hawaii, while in Brazil both resistant and tolerant cultivars have been reported against *P. zeae* (Tew *et al.,* 2005). However, in Australia, six widely grown cultivars that were tested were found to be good hosts of

Meloidogyne spp. while *Pratylenchus* spp. multiplied on all nine widely grown cultivars that were tested (Blair *et al.,* 1999). In a study by Santos *et al*., (2012), 30 genotypes of sugarcane assessed showed they were all susceptible to the nematodes *Pratylenchus zeae* and *P. brachyurus.*

Roberts (1992) has postulated that host plant resistance will become more important in managing plant parasitic nematodes and forecasts that the use of resistant cultivars will become the main nematode management tool especially with improved availability of germplasm carrying nematode resistance genes and cutting edge technology in molecular-transfer.

2.3 Tolerance

Mineral fertilizer application has been observed to improve tolerance to plant parasitic nematodes offering a potential nematode management option (Waele and Elsen, 2007). Optimum nutrient regime results in greater soil nutrient availability and plant growth, with higher leaf concentrations of nitrogen, phosphorus, potassium and magnesium (macro elements) than under deficient nutrient regime (Gaidashova *et al.,* 2008). Such a nutrient regime leads to good root development hence higher tolerance to nematodes.

It has been shown that adequate supply of nutrients has the greatest impact on sugarcane yields after the water requirements of the crop have been met (Meyer, 2011). Supply of nutrients is commonly through application of inorganic and organic fertilizers to supply respective crop nutrients. Application of inadequate fertilizers leads to a vicious cycle of soil degradation through soil fertility depletion and declined sugarcane yields (KESREF, 2002).

2.4 Intercrops

Sugarcane is normally grown as a monoculture for years often without a fallow period between uprooting of old stumps and replanting. These conditions are favourable for the development of increasing nematode populations and worsen crop damage in warmer years with regular rainfall (Spaull and Cadet, 1990). As a result, regeneration is slow and so poor that the lifespan of a crop is diminished. The damage is severe in warm moist sandy soils. The result is a gradual decrease in yield referred to as yield decline and as the loss of productive capacity of sugarcane growing soils under long term monoculture (Garside *et al*., 1997; Blair *et al*., 1999). In Australia, yield decline was thought to be a key contributor to the productivity plateau experienced in the sugar industry for the over 25 years (Stirling *et al.*, 1996). Sugarcane planted in yield decline soils develops lesions on poor and rotten root system (Lawrence, 1984). It was proved that nematodes are among the causes of yield decline since improvements were observed with soil fumigants that controlled nematodes leaving out fungal root rots (Chandler, 1984).

The undesirable effects of monocropping in sugarcane can be reduced or even eliminated by intercropping. Many recent studies have shown that correct intercropping can mitigate disease and nematode susceptibility. More recent work has shown that plant diversity could reduce pathogen and disease pressure compared to a single crop (Cardinale *et al.*, 2003; Sinha *et al.*, 2004). Cadet *et al*. (2007) reported that sugarcane cultivars interacted within the rhizosphere when intercropped because the nematode community was different. The report pointed out that damage caused by *E. saccharina* was slightly lower when intercropped.

Although intercropping has been shown to reduce parasitic nematodes in sugarcane plantations, it is not all crop species that are suitable as intercrops. Some companion crops can contract diseases and or multiply pathogens to which the main crop is susceptible thus reducing the

benefit of the intercrop (Sumner *et al*., 1982; Fargette and Fauget, 1988). According to Bridge (1987), the mechanism by which intercrops control nematodes is provision of nutrients following microbial decomposition of the crop residues which could limit the parasites by releasing humic acids and enhancing the multiplication of antagonistic micro flora. Practically, there are many examples showing that correct intercropping mitigate disease and nematode susceptibility (Sharma and Bajaj, 1988; Sinha *et al*., 2004).

A south African study showed that intercropping sugarcane with velvet bean, sugar bean and sweet potato had no effect on cane yield, but intercropping with peanut reduced both sugarcane and sucrose yield. Sugarcane velvet bean intercrop increased levels of some plant nutrients in soil and leaves of sugarcane. Further, the investigation showed intercropping with velvet bean, peanut and sweet potato increased *Meloidogyne javanica* and *Pratylenchus zeae* infestation of the sugarcane sett roots while intercropping with sugar bean reduced nematode infestation. The study concluded that intercropping can be used by small-scale growers to manage nematodes and provide nutrients to sugarcane (velvet bean) as well as offer alternative food source and/or income (sweet potatoes) (Berry *et al*., 2009).

For a resource poor small-scale sugarcane farmer, the potential of the intercrop to supply much needed food and revenue could be the determining factor in farming sustainably. In Kenya, the recommended sugarcane spacing is between 1.2 and 1.5 m (KESREF, 2010). These inter-rows are large enough to accommodate short season intercrops to optimize on land use. Studies have shown that most sugarcane growing zones in Kenya are food insecure (KESREF, 2011). Intercropping can help mitigate food insecurity. In practice however, intercropping may complicate or reduce weeding and may create competition, resulting in reduced production of

both crops aggravated by unavoidable damage during harvesting of the short cycle crop (Ofori and Stern, 1987).

There are a number of crops that can be used as companion crops in sugarcane, these can be categorized in three viz food crops, exotic and indigenous vegetables. The main staple crops grown not only in sugarcane growing zones but also in Kenya as a whole are maize and beans.

In a study conducted by Chirchir (2008) high numbers of the nematode *Scutellonema* spp. were reported when beans were intercropped with sugarcane variety N14. There seems to be little information on reaction of bean to *Pratylenchus* spp. Exotic vegetables in Kenya are dominated by kale (*Brassica oleraceae* var acephala) and cabbage (*Brassica oleraceae* var capitata). These are widely grown and are the main sources of food and income among vegetables.

Cabbage is regarded as poor host of *Meloidogyne* spp. (Bello *et al*., 2004; Pattison *et al.*, 2006), however it has also been reported to host plant parasitic nematodes (Potter and Olthof, 1993; Waceke, 2007). Maina *et al.* (2011) pointed out that there are plant parasitic nematodes associated with cabbage in particular *Pratylenchus* spp. It has already been observed that *Pratylenchus* spp. are the most destructive in sugarcane hence any consideration of cabbage as a companion crop of sugarcane should take this into consideration.

Indigenous vegetables were very much neglected until concerted efforts were made in recent times to promote them and have indeed gained a commendable commercial foothold (Onyango, 2003; Abukutsa *et al.,* 2006), where they fetch better prices than their exotic counterparts (Mathenge, 2005). The vegetables play an important role in food security and nutrition especially of the underprivileged in both urban and rural areas with some playing roles in cultural heritage (Mnzava, 1997; Schippers, 2000; Onyango, 2002). There are a variety of indigenous vegetables

but some of the most popular include spiderplant (*Cleome gynandra*), African nightshade (*Solanum nigrum*), pumpkin leaves (*Cucurbita moschata*), (*Vigna ungucuilata*), vegetable amaranth (*Amaranthus blitum*), Jute mallow (*Corchorus olitorius*), slenderleaf (*Crotalaria brevidens*) and African kale (*Brassica carinata*). These can therefore be viable companion crops for sugarcane.Indigenous vegetables are largely cultivated as companion crops and as such it is necessary to understand their interaction with the main crops. But one of the most limiting factors to not only indigenous vegetables but also other crops is the presence of plant parasitic nematodes. Some studies have been conducted to find out the reaction of indigenous vegetables to plant parasitic nematodes. A study by Kimaru *et al.* (2014) showed amaranth to be tolerant to root-knot nematodes whereas spiderplant and sunhemp were moderately tolerant. Black nightshade and jute mallow were found to be highly susceptible. This closely tallies with an investigation by Myers (2004) which showed susceptibility of American black nightshade (*Solanum american* Mill) to *Meloidogyne incognita*. The American nightshade is a close relation of its African counterpart.

Schroth *et al*. (2000) has stated that cumulative effect of different allelochemicals produced in a mixed cropping system including *Crotalaria* have shown better capacity to suppress root knot nematodes in soil compared to pure stands. Several *crotalaria* species are known to be non-host to most plant parasitic nematodes and have been used to suppress soil nematodes and can be utilized as intercrop, trap crop or as soil amendment (Wang *et al.*, 2002). When intercropped with vegetables *Crotalaria* is known to suppress *Meloidogyne* populations (Desaeger and Rao, 2000).

2.4.1 Sugarcane Equivalent Yield

Sugarcane equivalent yield is a production function designed to analyze the economics of sugarcane and intercrop yields. It computes sugarcane equivalent yield, per hectare net income and resource use efficiency for each sugarcane-based intercropping system (Cobb-Douglas, 1928). Shinde *et al.* (2009) gave weightage to the prices of sugarcane and intercrops in calculating sugarcane equivalent yield as expressed in the formula:

$$\text{Sugarcane Equivalent Yield} = \frac{\sum(\text{Sugarcane yield} \times \text{Price of Sugarcane}) + (\text{Intercrop yield} \times \text{Price of Intercrop})}{\text{Price of Sugarcane}}$$

Using the formula the authors showed that sugarcane equivalent yield of sugarcane + maize combination was higher than those of chickpea and wheat combinations and attributed it to better nutrient utilization. In Punjab a similar study by Bhullar *et al.* (2006) revealed intercropping system of sugarcane with potato, *raya* and cabbage gave a response rate of 75% whereas combining it with wheat raised it to 100%. However maximum net returns were recorded in the sugarcane + cabbage intercrop (Ibid). Kanchannainwal (2009) reported that intercropping gives 15-20% higher cane yield and 0.5 more units of sugar recovery than spring planted cane. This study which named the intercrops as including pulses, oilseeds, cereals and vegetables further stated that the best combinations in terms of profit is the autumn sugarcane + winter maize(cobs) and autumn sugarcane + *rajmash.* Closer home in South Africa an agronomic package for successful intercropping of sugarcane has been developed where optimum cane and intercrop yields are produced (Parsons and Khubone, 2015).

2.5 Integrated nematode management in sugarcane

The concept of integrated pest management (IPM) is a relatively new strategy in pest control (Bird, 1987). According to Barker (2015) IPM consists of pest management strategies aimed at

favourable socio-economic and environmental consequences, it is a holistic systems approach to limit pest damage to tolerable levels through a combination of methods that include parasites and predators, antagonistic plants, botanical nematicides, host resistance, cultural practices like crop rotation and use of organic amendments, environmental modification and some pesticides if need be (Bird, 1987). The need for IPM in nematodes management in sugarcane is becoming increasingly urgent because controlling them is quite complex. Scientists have suggested use of resistant varieties as the ideal method, but sugarcane varieties resistant to the main species are scarce (Dinardo-Miranda, 2005). Roberts (1992) has postulated that host plant resistance will become more important in managing plant parasitic nematodes and forecasts that the use of resistant cultivars will become the main nematode management tool especially with improved availability of germplasm carrying nematode resistance genes and cutting edge technology in molecular-transfer.

On the other hand, using traditional varieties leads to a buildup of nematode populations, increasing the incidence and severity of the disease and causing agricultural yield to drop further (Moura and Almeida, 1981). However, management of plant parasitic nematodes in sugarcane cannot be confined to these two options. For instance fumigation of yield decline soils has been reported to increase sugarcane yield by 30% and this is attributed to recovery of root (Magarey and Croft, 1995; Magarey and Grace, 1998). The potential of intercropping as a possible cultural method for managing plant parasitic nematodes has been highlighted earlier above. Thus an optimal IPM strategy should harness a combination of these strategies (host resistance, intercropping, fertilization and if need be permitted chemicals) to mitigate the damage caused by plant parasitic nematodes on sugarcane in order to reverse the declining yields, improve food security and nutrition and at the same time raise revenue for small- scale sugarcane farmers.

CHAPTER THREE

3.0 EFFECT OF PLANT PARASITIC NEMATODES ON SUGARCANE GENOTYPES IN KENYA

3.1 INTRODUCTION

Sugarcane **(*Saccharum* spp. hybrids)** is a widely cultivated crop in the tropical and subtropical countries for its numerous benefits that include its use in foodstuffs, as fiber and production of bio-fuel (Santos *et al*., 2012). In Kenya, sugarcane is an important cash crop earning small-scale farmers approximately US$ 100 million annually (Gok, 2010). However, over the last decade there has been a steady decline of cane yields, falling from 91 ton ha^{-1} in 1996 to 63 ton ha^{-1} in 2010 (Gok, 2010; Mulwa *et al*., 2011). Probable causes for this reduction in productivity include the widespread use of low quality sugarcane varieties, poor agricultural and land management practices, and pests and diseases. Among pests and diseases plant parasitic nematodes have been reported to cause significant yield loss in sugarcane production (Gok, 2010; Nzioki and Chirchir, 2010).

Worldwide over 310 species representing 48 genera of ecto- and endoparasitic nematodes have been reported to be associated with sugarcane root rhizosphere (Cadet and Spaull, 2005; Adesiyan *et al*., 1990). Although there are many plant parasitic nematodes associated with sugarcane, studies have shown that the most damaging nematodes in sugarcane are those in the genera *Pratylenchus* and *Meloidogyne* (Moura *et al.,* 1999; Starr and Bendezu, 2002). Of these, it is the species *P. zeae* that has been cited by various authors as the most important nematode for the crop (Sundararaj and Mehta, 1994; Spaull and Cadet, 2003).

Root-knot nematodes, *Meloidogyne* spp., are also widely distributed in tropical, sub-tropical and warm temperate regions of the world, are serious pests of a broad range of food and fibre crops,

including cotton, soybean, mung bean, peanut, tomato, potato, capsicum, cucurbits, tobacco, pineapple, banana, papaya and sugarcane (Luc *et al*., 1990). In sugarcane fields, high nematode population densities of *Meloidogyne incognita* and *M. javanica* are usually found when crops are grown in light-textured soils, as these soils are ideally suited to nematode reproduction (Stirling, 2006). According to Stirling and Blair (2000), these species have been cited by various authors in different sugarcane producing regions as important pests of the crop. Cadet and Spaull (2005) have also reported that generally plant parasitic nematodes are among the most common pests that build-up over time and thus contribute to the yield decline.

Once nematodes are present in a field, it is nearly impossible to eradicate them. According to Berry *et al*. (2011), the best way to handle the infested field is to manage the nematode problem. There are several recommended practices for sugarcane farmers to manage this problem in their fields. Planting tolerant cultivars (Cook and Evans, 1987; Spaull and Cadet, 2003; Spaull *et al*., 2005) has been identified as one of the sustainable and environmental friendly approaches in management of nematodes in sugarcane fields. Several field trials have shown that certain varieties of sugarcane are more tolerant to the plant parasitic nematodes than others (Moberly and Clowes, 1981; McArthur and Spaull, 1995: Cadet and Spaull, 2003).

In recent years, the world sugar production has begun to move away from use of chemical nematicides in the management of plant parasitic nematodes towards a farming approach that includes use of conventional farming systems that are environmental friendly and sustainable in management of pests and diseases. Some of the strategies include use of intercropping between sugarcane cycles and exploring of resistance in the existing sugarcane cultivars (Spaull and Cadet, 2003). This shift in the management of plant parasitic nematodes has been driven by observations that the impact of yield decline can be reduced by use of resistant genotypes and by

the desire to cut down production costs incurred through purchase of nematicides as well as environmental concerns (Stirling *et al*., 2001).

Compared to recent advances in plant-pathogen interactions as in the case of *Arabidopsis thaliana* (Sijmons *et al*., 1991; Boiteux *et al*., 1999; Vercauteren *et al*., 2001; Gheysen and Fenoll, 2002) and *Lotus japonicus* (Lohar and Bird, 2003; Lohar *et al*., 2004), no information is available on the interaction between parasitic nematodes and sugarcane cultivars grown in Kenya. This study was therefore conducted to evaluate the relative susceptibility or resistance to root-knot nematodes (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.) among selected sugarcane cultivars grown in Kenya.

3.2 MATERIALS AND METHODS

3.2.1 Experimental site

The experiment was conducted in a glasshouse at the Field Station of the Faculty of Agriculture, Kabete Campus of the University of Nairobi.

3.2.2 Selection of sugarcane genotypes, treatments and experimental design

Stratified random sampling procedure was used to select fourteen (14) sugarcane genotypes: Old foreign introductions- Co421, Co617 and Co945; Recent foreign introductions- CB38-22 and D8484; Early local releases- EAK70-97; Recent local releases- KEN82-62, KEN82-216, KEN82-121, KEN82-472, KEN82-493 and KEN83-737; and, local pre-releases- KEN00-13 and KEN98-530. N14 was retained as the standard because of its known tolerance status (Cadet and Spaull, 2005). Potting soil, collected from sugarcane fields, was sieved to remove debris and homogenized, then mixed with sand at a ratio of 2:1 and autoclaved. The soil mixture was then placed in 5-litre plastic pots of 15cm diameter at a rate of 3kg per pot. Diammonium phosphate fertilizer was then added at a rate of 20g per pot. Single-budded setts of each cultivar that had

been subjected to hot water treatment at 50^0C for 2hours were then pre-germinated in germination boxes. Two weeks after germination a single sett was planted per pot. Topdressing was done at 30 days after planting using urea at the rate of 20g per pot.

The nematode inoculum was extracted from infested sugarcane roots by use of the modified Baermann funnel technique (Hooper *et al*., 2005). It was then multiplied on carrots and then re-extracted and maintained on maize plants. The nematode inoculum containing 2,000 juveniles was inoculated into the cane seedlings two weeks after planting. Inoculation was done by slowly dispensing 25ml of the nematode suspension into holes made in the soil around the plant and as close to the roots as possible. Control pots were inoculated with 25ml sterile water. The experiment was laid down in the glasshouse and arranged in a completely randomized design with three replications over two seasons.

3.2.3 Planting, data collection and analysis

Setts were pre-germinated in germination boxes and a single sett was planted per pot. Potting soil, collected from sugarcane fields, was sieved to remove debris and homogenized, then mixed with sand at a ratio of 2:1. The mixture was autoclaved at 121^0C and 15 bars for 3 hours after which 3 kilogrammes were placed in 5-litre pots of 15 cm diameter. At planting and thirty days after planting, each pot was fertilized with 20g diammonium phosphate and 20g urea, respectively. The nematode inoculum (*Meloidogyne* spp.) was extracted from galls with eggmasses of infested sugarcane roots by use of the modified Baermann funnel technique (Hooper *et al*., 2005). It was then reared on young tomato plants. Two weeks after transplanting, cane seedlings were inoculated with 2,000 juveniles. Inoculation was done by slowly dispensing 25ml with 80 juveniles per milliliter into holes made in the soil around the plant and as close to the roots as possible. Control pots were inoculated with25 ml sterile distilled water. The potted

plants were uprooted 120 days after planting and soil was gently shaken from the root system. Shoot height, fresh and dry shoot weight, fresh root weight and number of tillers were determined. Nematodes were extracted from 200cm^3 soil and 10g of roots (fresh weight) using the (Ibid) method and the egg mass index was assessed using the scale ranging from 1-5 as illustrated by Coyne *et al*. (2007). Data collected was subjected to analysis of variance (ANOVA) and means separated by least significant difference (LSD) using GenStat statistical package (GenStat, 2011) version 14 (VSN International).

3.3 RESULTS

3.3.1 Effect of Root-knot nematodes (*Meloidogyne* spp.) on sugarcane genotypes.

Inoculation of sugarcane with root-knot nematodes did not affect plant height and fresh and dry shoot weights for all cultivars (Table 3.1). Though infection of the cultivars by RKN did not show any effect on fresh root weight in the first season, there was significant difference ($P \leq 0.05$) on the fresh root weight of Co421 infected by the nematode as it had its fresh root weight reduced by 33% in the second season compared to the non-inoculated one (Table 3.2). However, among all the other cultivars the means of fresh root weight for inoculated plants did not differ significantly ($P \leq 0.05$) compared with the non-inoculated.

In the first season, inoculation by RKN on sugarcane had no effect on the number of tillers produced. However parasitism by RKN significantly ($P \leq 0.05$) affected prolificacy of tillering of cultivars in the second season. The tillering of Co421, KEN82-62 and KEN98-530 cultivars was reduced by 70%, 46% and 33%, respectively. On the other hand, inoculation with nematodes had no effect on the tillering of varieties KEN00-13 and KEN82-121. Uniquely, however, inoculation seemed to promote tillering for varieties EAK 70-97 and N14, with their tillering increasing by 21% and 30%, respectively.

Table 3.1. Effect of root-knot nematodes on sugarcane genotypes under glassouse conditions in experiment one.

Genotypes	Shoot height			Fresh shoot weight			Dry shoot weight			Fresh root weight			Number of tillers			NC	EMI
	In	NI	Δ%	In	NI	Δ%	In	NI	Δ%	In	NI	Δ%	In	NI	Δ%		
CB38-22	30.0	30.8	2.6	34.8	36.0	3.3	9.1	8.8	-3.4	28.1	29.4	4.4	1.3	1.7	23.5	25.0	1.7
Co421	33.2	36.2	8.3	77.4	88.2	12.2	17.9	26.8	33.2	16.8	45.3	62.9	1.7	4.3	60.5	83.3	3.0
Co617	39.5	39.8	0.8	62.2	66.0	5.8	15.7	16.1	2.5	34.8	35	0.6	4.0	4.0	0.0	16.7	1.3
Co945	23.7	24.0	1.3	36.1	42.1	14.3	9.1	12.7	28.3	31.3	31.9	1.9	2.7	2.7	0.0	12.5	1.0
D8484	40.8	47.3	13.7	103.2	100.9	-2.3	32.0	34.3	6.7	38.8	37.8	-2.6	3.7	3.0	-23.3	87.5	3.0
EAK70-97	43.5	42.2	-3.1	80.4	88.9	9.6	25.7	26.1	1.5	50.0	56.1	10.9	4.3	4.3	0.0	16.7	1.3
KEN00-13	34.5	33.3	-3.6	91.2	93.2	2.1	27.3	29.6	7.8	37.5	39.0	3.8	3.7	3.7	0.0	41.7	2.7
KEN82-121	35.5	33.7	-5.3	54.8	56.9	3.7	16.0	17.4	8.0	32.1	34.5	7.0	1.3	1.3	0.0	54.2	2.3
KEN82-216	33.8	32.8	-3.0	74.0	78.6	5.9	20.7	22.6	8.4	33.9	35.4	4.2	3.0	3.3	9.1	12.5	1.0
KEN82-472	34.7	36.2	4.1	89.2	91.1	2.1	24.0	24.2	0.8	29.3	30.8	4.9	2.0	2.3	13.0	33.3	1.7
KEN82-493	36.8	34.0	-8.2	72.0	73.1	1.5	19.9	19.3	-3.1	69.6	69.7	0.1	3.0	3.7	18.9	33.3	2.7
KEN82-62	33.3	36.4	8.5	84.0	72.7	-15.5	22.3	19.6	-13.8	57.0	59.6	4.4	2.0	5.0	60.0	20.8	1.7
KEN83-737	32.3	33.5	3.6	60.0	60.8	1.3	23.2	23.9	2.9	42.8	43.7	2.1	4.0	4.3	7.0	16.7	1.3
KEN98-530	46.3	49.7	6.8	106.3	111.2	4.4	34.7	35.5	2.3	77.0	62	-24.2	3.3	3.3	0.0	41.7	2.3
N14	33.3	33.7	1.2	75.3	77.4	2.7	29.7	30.2	1.7	47.8	47.7	-0.2	2.7	2.7	0.0	50.0	2.0
L.S.D (0.05)	10.20 ^{ns}			29.38 ^{ns}			8.34 ^{ns}			19.76 ^{ns}			1.40 ^{ns}			13.16*	0.67*
CV (%)	15.7			20.3			14.4			27.3			29.9			44.1	43.6

In = inoculated; NI = non-inoculated; Δ% = Percentage change; NC = Nematode count per 200 cm^3 soil and 10 g of roots; EMI = Egg mass index; L.S.D = least significant difference at $P \leq 0.05$; CV = coefficient of variation; *, ^{ns} = Significant, not significant respectively at $P \leq 0.05$.

Table 3.2. Effect of root-knot nematodes on sugarcane genotypes under glasshouse conditions in experiment two.

Genotypes	Shoot height			Fresh shoot Weight			Dry shoot weight			Fresh root weight			Number of tillers			NC	EMI
	In	NI	Δ%	In	NI	Δ%	In	NI	Δ%	In	NI	Δ%	In	NI	Δ%		
CB38-22	35.3	33.5	-5.4	79.2	84.1	5.8	13.8	11.5	-20.0	20.3	20.3	0.0	1.0	1.0	0.0	33.3	2.0
Co421	33.3	36.3	8.3	81.3	83.4	2.5	16.9	25.4	33.5	31.3	46.6	32.8	1.3	4.3	69.8	100.0	3.0
Co617	48.0	48.0	0.0	91.6	91.3	-0.3	18.3	19.9	8.0	42.6	43.3	1.6	3.3	3.7	10.8	16.7	1.3
Co945	22.7	25.7	11.7	38.1	34.8	-9.5	15.2	14.2	-7.0	26.8	28.0	4.3	2.3	3.0	23.3	12.5	0.7
D8484	41.2	42.8	3.7	113.7	118.8	4.3	32.1	33.6	4.5	50.7	53.8	5.8	3.7	4.0	7.5	54.2	2.0
EAK70-97	34.0	32.7	-4.0	50.7	53.6	5.4	22.9	24.7	7.3	13.5	14.4	6.3	4.0	3.3	-21.2	16.7	1.7
KEN00-13	30.0	30.3	1.0	80.9	82.3	1.7	23.2	24.2	4.1	32.8	33.5	2.1	3.7	3.7	0.0	29.2	1.3
KEN82-121	31.5	32.3	2.5	59.5	61.5	3.3	15.1	15.5	2.6	12.7	14.8	14.2	2.0	2.0	0.0	62.5	1.3
KEN82-216	35.5	34.2	-3.8	83.2	83.7	0.6	21.9	21.4	-2.3	30.1	31.0	2.9	2.7	3.3	18.2	12.5	1.3
KEN82-472	28.2	29.3	3.8	88.5	88.3	-0.2	17.4	17.8	2.2	20.4	21.6	5.6	2.0	2.7	25.9	37.5	1.3
KEN82-493	37.5	38.2	1.8	55.8	60.6	7.9	20.5	21.6	5.1	22.4	23.4	4.3	3.0	4.0	25.0	29.2	2.0
KEN82-62	32.5	33.2	2.1	80.6	80.2	-0.5	20.8	21.2	1.9	32.5	32.9	1.2	2.0	3.7	45.9	16.7	1.3
KEN83-737	47.5	48.0	1.0	120.7	123.5	2.3	22.8	23.6	3.4	43.3	44.4	2.5	3.3	4.3	23.3	4.2	0.3
KEN98-530	47.2	47.7	1.0	120.9	124.1	2.6	34.2	35.8	4.5	50.3	51.3	1.9	2.7	4.0	32.5	37.5	2.0
N14	40.3	43.3	6.9	132.3	130.9	-1.1	20.1	20.2	0.5	17.0	18.7	9.1	3.0	2.3	-30.4	50.0	2.3
L.S.D (0.05)		4.25 ^{ns}			6.27 ^{ns}			5.47 ^{ns}			5.34*			1.19*		11.51*	0.72*
CV (%)		6.5			3.9			16.4			8.4			24.5		43.6	57.4

In = inoculated; NI = non-inoculated; Δ% = Percentage change; NC = Nematode count per 200 cm^3 soil and 10 g of roots; EMI = Egg mass index; L.S.D = least significant difference at $P \leq 0.05$; CV = coefficient of variation; *, ^{ns} = Significant, not significant respectively at $P \leq 0.05$.

All the sugarcane genotypes tested were found to be host to root-knot nematode. However, the mean populations possessed by each cultivar significantly differed ($P \leq 0.05$) across the genotypes. Genotypes Co421, D8484 and KEN82-121 predominantly exhibited the highest mean populations whereas genotypes KEN83-737, KEN82-216 and Co945 had the lowest mean populations of nematodes over both seasons.

There was a significant difference ($P \leq 0.05$) in nematode egg mass index among the sugar cane genotypes in first and second seasons. In both seasons, genotypes Co421 and D8484 proved to be the preferable hosts to root-knot nematodes that had the highest overall mean egg mass indices of 3.0 and 2.5, respectively. Conversely, genotypes KEN83-737 and Co945 had the least overall mean egg mass index of 0.8 over both seasons.

3.3.2 Effect of Lesion nematodes (*Pratylenchus* spp.) on sugarcane genotypes

Shoot height of all the sugarcane cultivars was significantly ($P \leq 0.05$) reduced by *Pratylenchus* spp. with the highest reductions observed on Co421 at 45.5% and KEN82-472 at 30% (Table 3.3). Reductions in plant length were 8.1 and 8.6% on cultivars KEN83-737 and EAK70-97, respectively. The fresh shoot weight of 12 varieties was significantly reduced with the highest being Co421 at 115%. There was no significant ($P \leq 0.05$) difference in the check cultivar N14. Lesion nematodes led to significantly ($P \leq 0.05$) heavier fresh shoots in cultivars CB38-22 and EAK70-97.

Cultivar Co421 had the highest reduction of its tillers by inoculation, a drop of 43.4 % followed by KEN82-216 at 32.5 %. The numbers of tillers for cultivars KEN83-737, KEN00-13 and N14 were not affected by inoculation. The only cultivar whose root weight was not significantly ($P \leq 0.05$) affected by lesion nematode was KEN83-737. The highest reductions were observed on genotypes Co421 and KEN82-216. The highest root weights after inoculation were observed on sugarcane genotypes

Co617 and N14 while the lowest were in cultivars Co421 and KEN82-216. Reproductive factor of *Pratylenchus* spp. was significantly ($P \leq 0.05$) different among sugarcane varieties with the highest being on Co421 and KEN98-530 and least on Co617 and KEN83-737. The damage caused by *Pratylenchus* spp. was significantly ($P \leq 0.05$) different among sugarcane varieties where the most severe was observed on cultivar Co421 followed by Co945. The least damage was observed on cultivar KEN83-737 (Table 3.4).

The category with majority was the moderately resistant comprising of seven genotypes namely CB38-22, KEN82-216, KEN00-13, KEN82-121, Co617, Co945 and N14 whose status is known and was confirmed by the scale (Table 3.5). There were five moderately susceptible cultivars namely KEN82-493, KEN98-530, KEN82-62, D8484 and EAK70-97. Variety KEN82-472 was rated susceptible, Co421 highly susceptible and KEN83-737 resistant.

Table 3.3. Effect of *Pratylenchus* spp. on sugarcane genotypes under glasshouse conditions in experiment one.

Sugarcane Genotypes	Shoot Height (cm)			Fresh Shoot Weight (g)			Dry Shoot Weight (g)			Number of tillers			Fresh Root Weight (g)			RF	Damage severity
	In	Ni	Δ%	In	Ni	Δ%	In	Ni	Δ%	In	Ni	Δ%	In	Ni	Δ%		
CB38-22	48.7	58.0	16.0	251.1	186.5	-34.6	35.0	54.7	36.0	2.0	2.7	25.9	83.4	99.8	16.4	1.9bc	2bcd
Co421	42.7	78.3	45.5	150.1	309.1	51.4	46.3	67.3	31.2	3.0	5.3	43.4	50.6	123.2	58.9	2.5a	4.7a
Co617	68.0	76.0	10.5	268.2	295.2	9.1	74.3	78.0	4.7	3.7	4.0	7.5	127.6	134.9	5.4	1.4fg	1.3d
Co945	56.7	64.7	12.4	254.9	286.6	11.1	61.3	65.0	5.7	6.7	7.0	4.3	58.5	65.0	10.0	1.4fg	1.3d
D8484	56.7	70.7	19.8	238.3	355.2	32.9	74.7	88.3	15.4	3.7	4.7	21.3	96.1	115.0	16.4	1.7de	2bcd
EAK70-97	74.0	81.0	8.6	317.0	274.1	-15.7	59.0	67.0	11.9	3.3	3.6	8.3	55.2	60.9	9.4	1.8cd	1.7cd
KEN00-13	70.7	78.3	9.7	268.5	322.5	16.7	72.7	74.3	2.2	4.7	4.7	0.0	107.8	124.2	13.2	1.7de	1.3d
KEN82-121	56.3	78.7	28.5	344.6	407.3	15.4	71.0	84.7	16.2	2.0	2.7	25.9	70.3	101.7	30.9	1.2gh	1.3d
KEN82-216	56.7	71.3	20.5	168.9	318.9	47.0	53.3	72.3	26.3	2.7	4.0	32.5	47.8	88.1	45.7	1.3fg	1.7cd
KEN82-472	52.0	74.0	29.7	229.0	342.3	33.1	76.7	89.0	13.8	3.7	4.3	14.0	51.3	68.6	25.2	1.9bc	3.0b
KEN82-493	48.0	58.7	18.2	143.8	179.9	20.1	21.7	29.7	26.9	3.0	4.0	25.0	105.9	120.5	12.1	1.5ef	2.7bc
KEN82-62	62.7	77.7	19.3	238.5	342.2	30.3	84.0	97.7	14.0	3.0	4.0	25.0	67.0	91.8	27.0	1.3fgh	2bcd
KEN83-737	68.3	74.3	8.1	224.0	240.6	6.9	44.3	45.7	3.1	3.0	3.0	0.0	57.0	58.6	2.7	1.0h	1.0d
KEN98-530	56.3	63.0	10.6	154.7	179.5	13.8	26.7	29.7	10.1	5.7	6.3	9.5	81.1	90.2	10.1	1.7de	2.7bc
N14	54.7	62.0	11.8	242.3	245.7	1.4	72.3	85.3	15.2	4.7	4.7	0.0	121.0	136.6	11.4	2.1b	1.3d
Significance		*			*			*			*			*		*	*
LSD(Trt)		2.2			14.1			4.3			0.4			4.0			
LSD(Var)		6.1			38.7			11.8			1.2			10.9		0.3	1.1
CV %		8.1			12.9			16.1			25.0			10.6		9.2	33.1

In = inoculated; NI = non-inoculated; Δ% = Percentage change; RF= Reproductive Factor; LSD = Least Significant Difference at $P \leq 0.05$; CV = Coefficient of Variation; means with same letters down the column are not significantly different; * = Significant at $P \leq 0.05$.

Table 3.4. Effect of *Pratylenchus* spp on sugarcane genotypes under glasshouse conditions in experiment two.

Sugarcane Genotypes	Shoot Height (cm)			Fresh Shoot Weight (g)			Dry Shoot Weight (g)			Number of tillers			Fresh Root Weight (g)			RF	Damage severity
	In	Ni	Δ%	In	Ni	Δ%	In	Ni	Δ%	In	Ni	Δ%	In	Ni	Δ%		
CB38-22	57.3	62.7	8.6	313.3	326.7	4.1	66.7	68.3	2.3	2.7	4.3	37.2	101.7	121.7	16.4	1.7cdef	1.7ef
Co421	57.3	73.3	21.8	148.3	308.3	51.9	60.0	91.7	34.6	2.3	4.3	46.5	85.0	143.3	40.7	2.9a	4.3a
Co617	54.7	59.3	7.8	236.7	280.0	15.5	98.7	96.3	-2.5	2.6	3.0	13.3	106.7	110.0	3.0	1.3gh	1.0f
Co945	47.7	59.0	19.2	186.7	191.7	2.6	46.7	48.3	3.3	4.3	4.0	-7.5	96.7	110.0	12.1	1.5efgh	1.0f
D8484	55.0	59.0	6.8	310.0	273.3	-13.4	63.3	80.0	20.9	3.3	2.6	-26.9	40.0	56.7	29.5	2.1bc	2.0ed
EAK70-97	59.0	58.0	-1.7	315.0	276.7	-13.8	76.7	56.7	-35.3	3.6	2.0	-80.0	56.7	50.0	-13.4	1.6defg	2.3cde
KEN00-13	52.0	64.0	18.8	220.0	265.0	17.0	83.3	93.3	10.7	3.0	3.3	9.1	83.3	103.3	19.4	1.8bcde	1.7ef
KEN82-121	55.7	64.3	13.4	215.0	230.0	6.5	70.0	73.3	4.5	2.0	2.6	23.1	70.0	91.6	23.6	1.4fgh	1.7ef
KEN82-216	47.7	57.7	17.3	213.3	256.7	16.9	63.3	80.0	20.9	2.3	3.3	30.3	51.7	66.7	22.5	1.3fgh	2.0de
KEN82-472	53.0	39.3	-34.9	305.0	385.0	20.8	88.3	113.3	22.1	2.6	3.3	21.2	91.7	120.0	23.6	1.9bcd	3.3b
KEN82-493	32.3	40.7	20.6	86.7	143.3	39.5	30.0	46.7	35.8	1.7	2.3	26.1	100.0	113.3	11.7	2.2b	3.0bc
KEN82-62	39.3	43.0	8.6	171.7	218.3	21.3	53.3	83.3	36.0	2.0	2.6	23.1	60.0	80.0	25.0	1.7efgh	2.7bcd
KEN83-737	77.0	78.7	2.2	280.0	266.7	-5.0	91.7	90.0	-1.9	3.3	3.3	0.0	72.0	71.7	-0.4	1.1h	1.0f
KEN98-530	59.7	65.7	9.1	241.7	366.7	34.1	65.0	80.0	18.8	3.3	3.7	10.8	78.3	80.0	2.1	1.7cdef	2.7bcd
N14	45.7	54.3	15.8	317.3	271.7	-16.8	91.7	91.7	0.0	5.0	3.6	-38.9	138.3	123.3	-12.2	1.3fgh	1.7ef
Significance		Ns			*			*			*			ns		*	*
LSD(Trt)		2.0			10.8			3.8			0.4			6.9			
LSD(Var)		5.4			29.7			10.4			1.0			18.9		0.4	0.7
CV %		8.3			10.1			12.1			28.5			18.3		14.3	19.4

In = inoculated; NI = non-inoculated; Δ% = Percentage change; Rf =Reproductive factor; LSD = Least Significant Difference at $P \leq 0.05$; CV = Coefficient of Variation; means with same letters down the column are not significantly different; *, ns = Significant, not significant respectively at $P \leq 0.05$.

Table 3.5. Classification of selected sugarcane cultivars based on their resistance to lesion nematode

Sugarcane genotype	Damage severity			Host resistance status
	First trial	Second trial	Mean	
Co421	4.7	4.3	4.5	Highly susceptible
KEN82-472	3.0	3.3	3.2	Susceptible
KEN82-493	2.7	3.0	2.9	Moderately susceptible
KEN98-530	2.7	2.7	2.7	Moderately susceptible
KEN82-62	2.0	2.7	2.4	Moderately susceptible
D8484	2.0	2.0	2.0	Moderately susceptible
EAK70-97	1.7	2.3	2.0	Moderately susceptible
CB38-22	2.0	1.7	1.9	Moderately resistant
KEN82-216	1.7	2.0	1.9	Moderately resistant
KEN00-13	1.3	1.7	1.5	Moderately resistant
KEN82-121	1.3	1.7	1.5	Moderately resistant
N14	1.3	1.7	1.5	Moderately resistant
Co617	1.3	1.0	1.2	Moderately resistant
Co945	1.3	1.0	1.2	Moderately resistant
KEN83-737	1.0	1.0	1.0	Resistant

3.4 DISCUSSION

3.4.1 Effect of Root-knot nematodes (*Meloidogyne* spp.) on sugarcane genotypes

The description of the terms ‘susceptible’ and ‘resistance’ in this study is adopted from Stirling (2006): susceptible varieties are described as those that are capable of supporting nematode reproduction, whereas resistant ones are those where multiplication is limited. Stirling (2006) adopted this scale in a study to rate susceptibility of sugarcane varieties to root-knot nematode species (*M. javanica and M. incognita*) in Australia. The results, however, provide no information on the capacity of the tested varieties to resist attack from the nematodes but rather withstand infestation, a property that is usually referred to as ‘tolerance’. Tolerance to damage is independent of resistance and relates to the ability of a host genotype to withstand or recover from the damaging effects of nematode attack and to yield well (Trudgill, 1991). The use of crop resistance approach against pest infestation such as

root-knot nematode is one of the principles of crop protection and has become important in pest management in recent years following environmental hazards caused by chemical control measures (Olowe, 1992; Mangala and Mauria, 2006). It is apparent from the results of this investigation that various sugar cane cultivars have different degrees of resistance to *M. incognita* infestations with evidence in variation of plant growth, vigor and reproduction (Stirling, 2006).

Generally, all the inoculated sugarcane cultivars showed reduced shoot height, shoot weight and root weight because all the genotypes were susceptible hosts allowing root-knot nematode to survive and parasitize cane resulting in reduced physiological processes due to deprived nutrients flow as they attack the roots affecting their uptake ability. According to Hussey (1989), root-knot nematode parasitizes the host plant by affecting on its nutrients: the infective second stage juvenile penetrates the host root near the root tip, then initiates a feeding site after which it migrates to the developing vascular cylinder. The damaged root and vascular system limits the ability of the plant to access moisture and nutrients, resulting in slower plant growth and consequently reduced crop yield (Nicol *et al*., 2011; Stirling *et al*., 2003).

In the non-inoculated sugar cane cultivars, the numbers of tillers were higher compared to the inoculated ones. This result shows that root-knot nematode reduce tillering ability in sugarcane. This trend was also observed in fresh shoot and root weights of inoculated cane. These observations agree with those reported by Stirling *et al*., 2003, Brigde *et al*., 2005 and Nicol *et al*., 2011 in their studies on rice where they demonstrated reduced growth and number of tillers in rice infested by RKN.

High tolerance to root-knot nematode was observed in KEN83-737, KEN82-216, Co945 and Co617: though they were infested by the nematode, they could withstand its effects and remained healthy unlike Co421 and D8484 whose susceptibility resulted in evident reduction in growth and high

nematode population. In addition, cultivars tolerant to the nematode maintained their high tillering ability compared to the most susceptible genotypes. Nematodes affect reproduction ability of cane since its growth vigour and reproduction is basically dependent on nutrition (Trudgill, 1991; Jacquet *et al*., 2005, Nicol *et al*., 2011, and Stirling *et al*., 2001)

KEN83-737, KEN82-216, Co945 and Co617 were more resistant to root-knot nematode and were less preferable hosts and therefore had less nematode population counts compared to Co421 and D8484 which were more susceptible thus harboring higher nematode populations. Trudgill (1991), found that less nematode populations were counted in more resistant crops compared to susceptible genotypes, a finding similar to the present study. Since the objective of every farmer is to make a profit by increasing yields and reducing cost of production incurred in the control of nematodes, they are most likely to prefer cultivars that possess higher levels of resistance or tolerance when faced with soils having high nematode infestation.

Fewer egg masses were counted in the tolerant varieties compared to the susceptible varieties possibly because the former inhibited reproduction; as a result the females of root-knot nematode in tolerant cultivars couldn't produce many eggs as compared to susceptible ones. This observation confirms the finding made by (Ibid).

The mechanism of resistance to RKN in crop plants seems to vary between crops, and among cultivars of a crop and may also manifest as either pre- or post-infection (Dhandaydham *et al*., 2008). Pre-infection resistance was clearly evident on cucumber and peanut studies conducted by Haynes and Jones (1976) and Bendezu and Starr (2003), respectively. This resistance is due to lack of nematode entry into the plant and is possibly due to the presence of pre-formed chemicals in the plant that are toxic and antagonistic to the nematodes (Huang, 1985). Additionally, post-infection

resistance mechanisms exist and are manifested after the penetration of the nematode in the host and, in some cases, are associated with a classical hypersensitive response (HR) (Dhandaydham *et al*., 2008). The HR is typically explained by the gene-for-gene-model in which a virulence gene product from the pathogen is specifically recognized by the resistance gene product of the host (Bent, 1996; 2011). The number of genes controlling resistance to RKN seemed to differ among hosts and even among varieties. For example, a single gene controls resistance in soybean cultivar 'Forrest' (Luzzi *et al*., 1994a), whereas multiple genes control resistance in soybean lines PI96354 and PI417444 (Luzzi *et al*., 1994b). Therefore, there could be multiple genes that control resistance in sugarcane but they differed in all the screened 15 test cultivars.

3.4.2 Effect of Lesion nematodes (*Pratylenchus* spp.) on sugarcane genotypes

Based on the data collected from this experiment, the results were quite varied with sugarcane varieties responding differently to *Pratylenchus* spp. This may be attributed to genotype makeup that is probably not uniform. Based on the results, it can be concluded that the status of the genotype Co421 is susceptible and KEN83-737 is resistant.

Stirling (2006) described susceptible varieties as those that support nematode reproduction, while the resistant limit it. However, Trudgil (1991) adds the concept of tolerance where genotype can yield well in the presence of nematodes or are capable of recovery. Based on this information and results obtained, a scale was developed to classify sugarcane genotypes based on their levels of resistance to *Pratylenchus* spp. (Appendix 3).

Cultivars CB38-22 and EAK70-97 exhibited heavier fresh shoots in when exposed to lesion nematodes, yet the other parameters were highly reduced leading to the two being classified as

susceptible. This uniqueness calls for further investigation of the host response of the two cultivars to lesion nematodes with a view to exploiting this apparent advantage.

3.4.3 CONCLUSIONS AND RECOMMENDATIONS

The majority of sugarcane cultivars tested showed moderate to high level of resistance to RKN. Four varieties, KEN83-737, KEN82-216, Co945 and Co617 showed a high level of resistance while nine varieties, N14, EAK70-97, KEN98-530, CB38-22, KEN00-13, KEN82-121, KEN82-472, KEN82-493 and KEN82-62 showed moderate resistance. Only two varieties, Co421 and D8484 were susceptible. This study demonstrated that sugarcane cultivars grown in Kenya possess varying levels of resistance to root-knot nematode. Therefore resistance to plant parasitic nematodes should be incorporated in the variety improvement programmes as part of an integrated pest management strategy.

This study has further demonstrated that there is a variable response amongst different sugarcane cultivars towards *Pratylenchus* spp. It has also shown that cultivars known to be high yielding and possess greater agro-ecological suitability in the Kenyan sugarcane industry are more resistant against nematodes compared to other cultivars. Based on this study it was also possible to produce a scale for classification of sugarcane cultivars based on their reaction to *Pratylenchus* spp. KEN83-737 was rated resistant while CB38-22, KEN82-216, KEN00-13, KEN82-121, Co617, Co945 and N14 were moderately resistant. KEN82-493, KEN98-530, KEN82-62, D8484 and EAK70-97 were classified as moderately susceptible, KEN82-472 susceptible and Co421 highly susceptible. Further studies should be conducted to determine the response of cultivars CB38-22 and EAK70-97 to *Pratylenchus* spp. to take advantage of their increase in shoot weight while under pressure from the lesion nematodes.

CHAPTER FOUR

4.0 EFFECT OF NEMATICIDE APPLICATION ON THE MANAGEMENT OF PLANT PARASITIC NEMATODES IN SUGARCANE PRODUCTION

4.1 INTRODUCTION

Sugarcane (*Saccharrum* spp. hybrids) is a tall perennial crop globally important due to its day to day utilities of providing for up to 60% of the global sugar needs while sugar beet provides the balance of 40% (Onwueme and Sinha, 1999; Girei and Giroh, 2012). As a C_4 crop, sugarcane has the potential of utilizing solar energy to form sucrose during its four stages of growth and development, thus is normally globally cultivated mainly within the latitudes 36.7^0 N and 31.0^0 S of the equator and up to 1600 meters above sea level in the tropical and sub-tropical regions (Anon, 2013). Sugarcane production is mainly highest in five nations worldwide, namely Brazil, Colombia, Philippines, India and South Africa (Anon, 2013). Globally sugarcane covers an area of 26 million hectares from which 1.83 billion tonnes is harvested annually (Anon, 2013). In Kenya, the sugar industry is responsible for sustaining 25% of the households amongst the population with sugarcane production contributing over 10% to the total agricultural gross domestic product (GDP). It provides a source of employment and revenue generation for most households in the sugarcane belt of western Kenyan (KSB, 2010).

Despite the important contribution by the sugar industry to the economy, the cane yields have experienced a sharp decline from 74 tons ha^{-1} to 55 tons ha^{-1} between 2004 and 2013 (KSB, 2013). The yield decline has been attributed to a number of factors among them is susceptibility of the crop to diseases (e.g. sugarcane smut, ratoon stunting disease and sugarcane mosaic) and pests which include termites, moles and plant parasitic nematodes (PPN) (KSB, 2013). Worldwide PPN have been known to cause annual yield losses of 15.3% on sugarcane (Sasser and Freckman, 1987), a crop

grown largely as a monoculture with a single cycle lasting up to 60 months in the field. This practice usually leads to build up of certain pests and diseases. Populations of PPN build up significantly as sugarcane is grown continuously on the same piece of land over a long period under monoculture. Use of nematicides by small-scale sugarcane farmers has been found to be non cost-effective due to their high pricing in addition to having detrimental environmental effects. Declining soil fertility and inadequate fertilization by the farmer is likely to have resulted in reduction of sugarcane tolerance to nematode infestation. A number of nematodes among them *Pratylenchus* spp. and *Meloidogyne* spp. have been observed to parasitize sugarcane causing a yield reduction of 1.6 million tonnes cane per annum and reducing the lengths and weights of both sugarcane shoots and roots (Cadet and Spaull, 2005; Chirchir *et al*, 2011; Dinardo-Miranda, 2005; Barbosa *et al.*, 2013).

One way of managing nematodes would be by use of resistant cultivars. Resistant cultivars have several advantages over other methods of reducing nematode populations: their use requires little or no technology and is cost-effective; they allow rotations to be shortened and best use to be made of the land; and they do not leave toxic residues. They provide an effective and economical method for managing nematodes in both high- and low-cash value cropping systems. They are environmentally compatible and do not require specialized applications, as opposed to most chemicals and, apart from preference based on agronomic or horticultural desirability, do not require an additional cost input or deficit. In less developed countries and in low-cash crop systems, plant resistance is probably the most viable solution to nematode problems (Trudgill, 1991). Studies like that by Santos *et al* (2012) have demonstrated the existence of resistance to *Pratylenchus* spp. among Brazilian sugarcane cultivars. This study was undertaken to determine the reaction of two sugarcane cultivars grown in Kenya to plant parasitic nematodes and thereby assess the potential of incorporating it in integrated nematode management packages.

4.2 MATERIALS AND METHODS

4.2.1 Site description

This study was conducted at Kenya Agricultural and Livestock Research Organization - Sugar Research Institute (KALRO-SRI) farm at Kibos (34^0 48'E - $0^0$4'S) with an elevation of 1184 meters above sea level. The site had a mean daily temperature of 23^0C with long term mean rainfall of 1464 mm per annum with eutric cambisols.

4.2.2 Sugarcane cultivars and planting

Three sugarcane cultivars were selected based on their known host resistance status to plant parasitic nematodes: KEN83-737 as resistant, Co421 susceptible and N14 tolerant. Seedcane was harvested at ten months and the stalks cut into 3-budded setts. Planting furrows were prepared each 5 m long and 1.2 m apart giving a net plot size of 30 m^2 (5 rows x 5 m x 1.2 m). Fifteen (15) setts were planted per row. Diammonium phosphate was applied at planting at the recommended rate of 100 kg ha^{-1} and urea was applied as top dressing at five months at the recommended rate of 200 kg ha^{-1}.

4.2.3 Treatments

Aldicarb (Temik® 10 G) was used for treatment in this trial and applied at two rates: recommended dose of 3 kg ha^{-1} and half the recommended dose at 1.5 kg ha^{-1}. Untreated plots served as control.

4.2.4 Experimental design

The trial was established as a split plot design and replicated three times over two blocks. Main plot received the nematicide treatment while subplot was the cultivar.

4.2.5 Data collection

Nematode populations were determined at 0, 9 and 18 months after planting (MAP). Initial nematode population was determined on newly prepared seedbed just before planting. Soil samples were

collected using a soil auger. Eight soil sub-samples were collected also at 9 and 18 MAP from the sugarcane rhizosphere at a depth of 5-20cm, mixed to form a composite sample and placed in a polythene bag and taken to the laboratory.

Germination of the setts was determined at 42 days after planting. At harvest, 10 stalks from each of the three central rows were selected at random to form the sample population of 30 stalks per plot. Using the sample, girth was determined by measuring the thickness at the mid-section of the stalk by use of veneer calipers; plant height was determined by measuring the stalk length by use of a metre rule; and, yield was determined by weighing the stalks using a tripod and weighing scale and the weight expressed as tonnes per hectare. The total plant population was determined by counting the total number of millable stalks in the three central rows using a tally counter. Nematode counts were determined at planting, nine months after planting (MAP) and at harvest. Field brix (brix % cane) was determined by use of a hand refractometer. Fifteen (15) stalks from each plot were taken for laboratory analysis to determine the juice quality parameters: Pol % cane, Fibre % cane, Pol % juice, Brix % juice, Purity % juice and commercial cane sugar calculated.

4.2.6 Processing of nematode samples

Nematodes were extracted from 200cm^3 soil obtained from each of the plots using the modified Baermann funnel technique (Hooper *et al.,* 2005). Nematodes from five gram root samples were extracted using the maceration/filtration technique described by Hooper *et al.* (2005). The nematodes were killed using gentle heat in a water bath at 50–70^{0}C and fixed using the method described by Hooper *et al.* (2005). Using a high-resolution microscope nematodes were identified up to the genus level following the key by Mai and Lyon (1975) and the counts recorded. From the preserved nematodes suspension, two ml was drawn using a pipette, placed in a counting dish under a light microscope and nematodes counted thrice with the average recorded.

4.2.7 Data analysis

Data was subjected to analysis of variance (ANOVA) and means separated by honestly significant difference (HSD) test at $P < 0.05$ and $P < 0.01$ using SAS ® Proprietary Software Release 9.2. Nematode counts were log transformed into Log x+1 to fit the assumptions of ANOVA but means reported are the actual figures collected. Multiple regression analysis was performed using PROC REG to determine the predictors of yield and commercial cane sugar using variables in their respective regression models.

4.3 RESULTS

4.3.1 Nematode species composition at the trial site

Plant parasitic nematodes extracted from the experimental site belonged to 17 genera namely *Pratylenchus, Meloidogyne, Helicotylenchus, Tylenchus, Rotylenchus, Scutellonema, Xiphinema, Trichodorus, Paratylenchus, Hirsmaniella, Ditylenchus, Hoplolaimus, Rotilenchulus, Criconema, Dolichodorus, Longidorus* and *Criconemoides.* The three most predominant nematodes were in the genera *Pratylenchus, Meloidogyne* and *Helicotylenchus* accounting for 68%, 22% and 5% of all the nematodes respectively. Members of each of the other genera accounted for less than one per cent.

4.3.2 Effect of nematicide application on sugarcane yield parameters

Different yield parameters exhibited significant differences ($P \leq 0.05$; $P \leq 0.01$) for different cultivars under varied nematicide rates of application (Table 4.1). Both plant height and millable stalks number for KEN83-737 were significantly higher compared to N14 and Co421 cultivars ($P \leq 0.01$).

Application of the nematicide at either of the two rates significantly increased the girth, plant height, total number of stalks and the yield of sugarcane. However, the recommended rate had a significantly

higher effect than the half rate ($P \leq 0.01$). There was a significant interaction between sugarcane cultivar and nematicide rate only in the improvement of the plant height.

4.3.3 Effect of nematicide application on sugarcane cultivar yield parameters

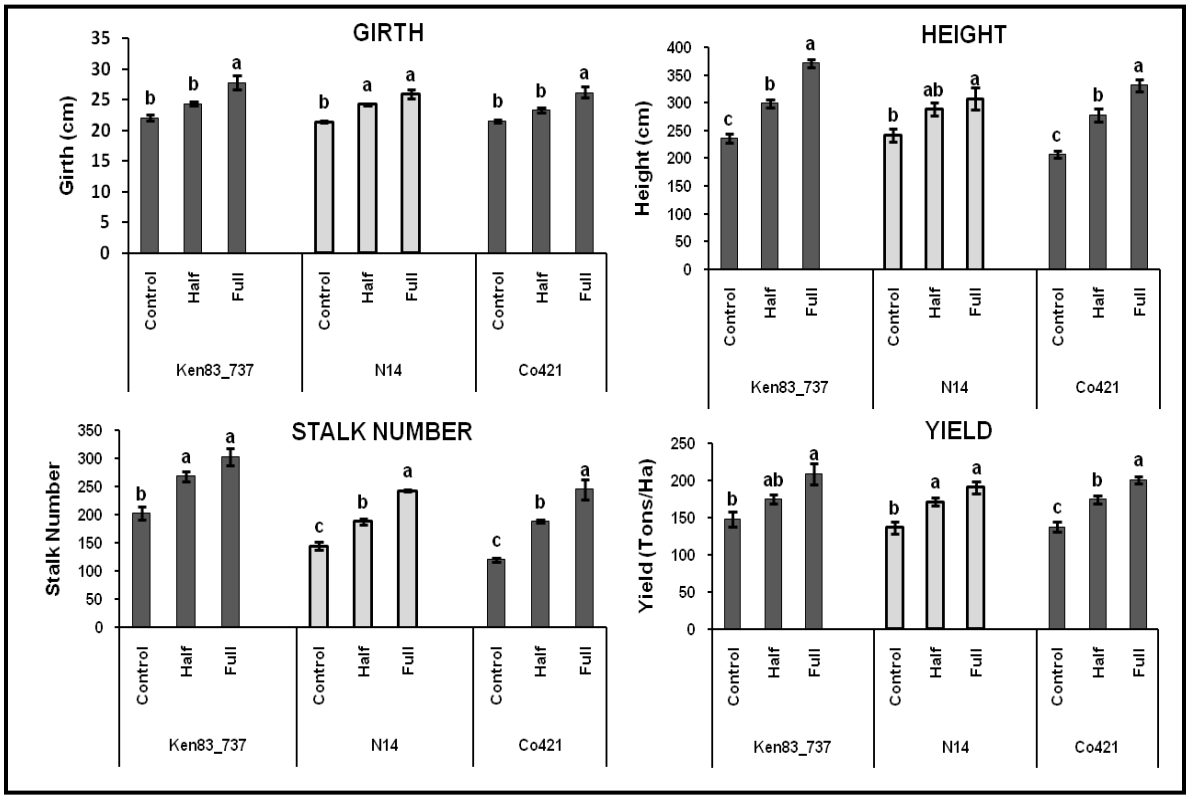
Significant ($P \leq 0.05$) interactions between sugarcane cultivars and nematicide rates were observed on the yield and its parameters (Figure 4.1). KEN83-737 and Co421 had significantly thicker stems at the higher nematicide rate compared to the lower rate and control. Whereas the stem thickness was not significantly different for the two nematicide rates for N14, both were significantly thicker than for the control.

Table 4.1. Effect of nematicide on girth, plant height, millable stalk number and yield in different sugarcane cultivars.

		Girth (mm)	Plant height (cm)	Millable stalk numbers (no.)	Yield (tonnes per ha)
Cultivar	Ken83_737	24.74	301.86 ^{a}	257.72 ^{a}	177.50
	N14	23.82	279.14 ^{ab}	191.56 ^{b}	171.17
	Co421	23.67	272.39 ^{b}	184.61 ^{b}	166.44
Nematicide Rate	Full Rate	26.62 ^{a}	336.69 ^{a}	263.44 ^{a}	200.44 ^{a}
	Half Rate	23.93 ^{b}	288.39 ^{b}	214.67 ^{b}	173.72 ^{b}
	Control	21.67 ^{C}	228.31 ^{c}	155.78 ^{c}	140.94 ^{c}
	Mean	24.07	284.46	211.30	171.70
	CV%	6.20	9.89	11.46	11.79
	H.S.D $_{(0.05)}$	0.82	22.75	19.58	16.37
f-values	Cultivar	2.71	5.42 **	49.97 **	1.35
	Nematicide Rate	49.68 **	67.00**	89.18 **	38.98**
	Cultivar*Nematicide Rate	0.72	2.94*	0.94	0.26

Means followed by different letters on the same column are significantly different at $P \leq 0.05$ (*) and at $P \leq 0.01$ (**).

Nematicide application resulted in significantly ($P \leq 0.05$) taller plants for both KEN83-737 and Co421 compared to the control. However, in sugarcane cultivar N14 the lower nematicide rate did not produce significantly longer stalks than the control. Millable stalks number for KEN83-737 was comparatively higher than both N14 and Co421 over the different rates of nematicide application. Whereas nematicide application did not produce significantly different stalk numbers over the two rates for KEN83-737, both rates had significantly higher number of millable stalks than the control. Yield of cultivar KEN83-737 yield was significantly higher under the recommended rate than the control. However, the half rate did not significantly differ from the control. Co421 had significantly different yields for all treatments with highest yields in recommended rate and lowest in the control.



Means of bars having different letters over them are significantly different at $P \leq 0.05$.

Figure 4.1. Effect of nematicide on girth, plant height, millable stalk number and yield in different sugarcane cultivars.

4.3.4 Effect of nematicide application on nematode population across different sugarcane cultivars

Interaction effects between the sugarcane cultivars and nematicide rates revealed significant differences ($P \leq 0.05$; $P \leq 0.01$) in the numbers of plant parasitic nematodes present at different times of cane growth (Table 4.2). The nematode population in KEN83-737 and N14 differed significantly in their numbers across nematicide rates only at 9 months after planting (MAP) but not at harvest. However, for Co421 the nematode numbers differed significantly across the nematicide rates at both 9 MAP and at harvest.

Table 4.2. Effect of sugarcane cultivar and nematicide application rate on nematode populations.

Sugarcane Genotypes	Nematicide treatment	P_i	P_m	P_f	Average
KEN 83-737	Full	1104.90	598.65 ^{ab}	116.02	732.87
	Half	855.98	54.71 ^{b}	188.01	570.08
	Control	1183.57	1469.50 ^{a}	1264.90	1392.61
	CV %	6.99	20.64	24.76	6.58
	H.S.D $_{(0.05)}$	0.42	1.11	1.30	0.39
	f-values	0.42	5.98 *	2.41	3.59
N14	Full	1027.95	258.30 ^{b}	262.50	618.71 ^{b}
	Half	1416.44	976.46 ^{a}	205.62	1067.84 ^{a}
	Control	1342.56	1779.11 ^{a}	1448.86	1575.90 ^{a}
	CV %	5.27	7.44	21.38	2.98
	H.S.D $_{(0.05)}$	0.33	0.44	1.17	0.18
	f-values	0.76	13.17 **	2.12	17.48 **
Co421	Full	1124.95	701.24 ^{ab}	151.25 ^{b}	828.53 ^{b}
	Half	1194.96	866.96 ^{b}	714.31 ^{ab}	1056.97 ^{b}
	Control	1238.22	2373.90 ^{a}	1909.43 ^{a}	1930.26 ^{a}
	CV %	5.31	7.37	17.06	2.76
	H.S.D $_{(0.05)}$	0.33	0.45	0.97	0.17
	f-values	0.06	5.37 *	4.45 *	16.95 **

Means followed by different letters on the same column are significantly different at $P \leq 0.05$ (*) and $P \leq 0.01$ (**). P_i, P_m and P_f are nematode population numbers at 0, 9 and 18 months after planting respectively.

The control plots generally exhibited the highest number of nematodes compared to the nematicide treated plots for all the cultivars. Indeed, in Co421 the average nematode counts was significantly higher in the control plots compared to both the half and full rate nematicide treatments, while in N14 the numbers in the control plots were only significantly higher than those in the recommended rate treatment.

4.3.5 Effect of different nematicide rates on cane quality components

The different nematicide rates significantly ($P \leq 0.05$; $P \leq 0.01$) affected cane quality components for different cane cultivars (Table 4.3). Significantly different ($P \leq 0.05$) pol % cane, pol % juice and brix juice were observed among the cultivars. Different nematicide rates and cultivars had significantly different ($P \leq 0.01$) fibre % cane and Pol % cane. No significant interaction effect was found between the cultivars and nematicide rates for the cane quality components. A significantly higher Pol % cane, Pol % juice and brix juice was measured in KEN83-737 cultivar compared to Co421 cultivar while a significantly higher fibre % cane was measured in both KEN83-737 and N14 cultivars compared to the Co421. A higher Pol % cane was measured under full nematicide rate (15.31 %) compared to the control (14.44 %).

4.3.6 Determination of the relationship between yield parameters and commercial cane sugar (CCS)

A significant ($P \leq 0.05$; $P \leq 0.01$) difference was recorded among the different yield parameters and commercial cane sugar (CCS) when applied in their respective regression models. The variables associated with sugarcane yield explained 61% of the variation observed as compared to the variables associated with commercial cane sugar that accounted for 88% of the variation (Table 4.3).

A significant ($P \leq 0.05$) relationship was recorded between stalk length and yield (Table 4.4). An increase in mean length by 0.22 cm would result in a mean yield increase of 1 tonne ha^{-1}. For

commercial cane sugar model, significant ($P \leq 0.01$; $p \leq 0.05$) relationships were determined between brix % cane, fibre % cane and pol % juice. A mean reduction of 2.21 and 0.6 brix and fibre % cane would respectively lead to a reduction in CCS 1%. On the other hand, a mean increase in pol juice of 3.8% would lead to an improvement of CCS by 1%.

Table 4.3. Effect of cultivar and nematicide application rate on cane and juice quality components.

		POL cane (%)	BRIX cane (%)	Fibre cane (%)	POL juice (%)	BRIX juice (%)	Purity juice (%)	CCS (%)
Sugarcane Cultivar	KEN83-737	15.35 [a]	16.68	15.74 [a]	19.34 [a]	20.47 [a]	94.38	14.68
	N14	14.82 [ab]	16.23	15.34 [a]	18.67 [ab]	19.94 [ab]	93.51	14.34
	Co421	14.62 [b]	16.54	13.23 [b]	18.36 [b]	19.75 [b]	92.96	14.25
Nematicide Rate	Full	15.31 [a]	16.38	14.82	18.83	19.98	94.19	14.58
	Half	15.05 [ab]	16.52	14.98	18.84	20.14	93.42	14.35
	Control	14.44 [b]	16.56	14.51	18.69	20.04	93.24	14.33
	H.S.D *(0.05)*	0.63	0.48	0.98	0.81	0.64	1.51	0.7
	C.V %	5.19	3.63	8.25	5.33	3.92	2	5.96
f - Value	Cultivar	4.3 *	2.69	22.04 **	4.44 *	4.16 *	2.62	1.27
	Nematicide Rate	5.91 **	0.46	0.7	0.12	0.21	1.29	0.48
	Cultivar*Nematicide Rate	1.58	1.3	0.78	0.72	0.96	0.55	1.03

Means followed by different letters on the same column are significantly different at $P \leq 0.05$ (*) and $P \leq 0.01$ (**). CCS = commercial cane sugar.

Table 4.4. Effect of yield parameters and nematodes on yield and commercial cane sugar.

Regression variables		Parameter estimate	*t*-value	*f*-value	R-Square
Yield Parameters Model	Girth (cm)	3.07	1.63		
	Stalk length (cm)	0.22	2.42 *		
	Stalks number	0.05	0.5		
	P_i	6.15	0.29	10.21**	0.6083
	P_m	2.04	0.32		
	P_f	0.16	0.03		
	Mean Nematodes	-30.72	-1.04		
CCS Model	Pol cane	0.01	0.17		
	Brix cane	-2.21	-6.82 **		
	Fibre cane	-0.60	-9.16 **	58.96 **	0.8827
	Pol juice	3.80	2.62 *		
	Brix juice	-1.06	-0.8		
	Purity juice	-0.54	-1.94		

Values followed by (*) and (**) are significantly different at $P \leq 0.05$ and $P \leq 0.01$ respectively. P_i, P_m and P_f are nematode population numbers at 0, 9 and 18 months after planting respectively. CCS = commercial cane sugar.

4.4 DISCUSSION

This study revealed that nematicide application and use of resistant cultivars reduced nematode populations in the plots subsequently leading to improved sugarcane yield and quality. The significantly higher mean stalk lengths for resistant and tolerant cultivars compared to the susceptible cultivar indicates that KEN83-737 and N14 actually overcame the parasitic effects of nematodes present in their rhizosphere. This was confirmed by the significantly higher mean girth, stalk length, stalks number and yield measured for all cultivars when the nematicide was applied at the higher rate. Root nematodes are known to deprive the host crop of vital nutrient access and efficient utilization leading to nutrient deficiency. Consequently this result in patchy stunted growth, reduced root mass, reduced foliar development and subsequent low yields (Coyne *et al.,* 2007). Resistance capability against plant parasitic nematodes was further demonstrated by measurements of higher means of growth parameters for resistant and tolerant cultivars in nematicide-treated

plots compared to the controls. Studies conducted by Showler and Reagan (1991) and Waraitch (1982) have shown that aldicarb do reduce nematode populations and other arthropod predators of sugarcane resulting in the improvement of sugarcane yields. The additive effect when aldicarb was applied in conjunction with use of resistant and tolerant cultivars was vital in realization of significantly higher growth parameters for KEN83-737 and N14 cultivars compared to the susceptible cultivar Co421 which also showed improved performance with increasing aldicarb application rate. The rise in performance of Co421 with increasing rate of nematicide indicates its higher level of sensitivity to varying numbers of parasitic nematodes compared to a resistant cultivar.

Nematicides applied in the soil during crop production are meant to stem crop damage to plant parasitic nematodes. Sugarcane is no exception to the effect of such nematodes and earlier studies by Birchfield (1984), Ramirez (1981) and Cadet and Spaull (2005) have indicated that sugarcane roots are parasitized by at least 14 phytoparasitc nematodes key among them *Pratylenchus*, *Meloidogyne*, *Trichodorus* and *Tylenchorhynchus* species. Mehta *et al.* (1992) reported the lesion, root-knot, sting and spiral nematodes as the major plant parasitic nematodes of sugarcane in India. In Kenya Chirchir *et al.* (2008) and Nzioki (2007) reported a significant presence of *Pratylenchus, Meloidogyne, Scutellonema* and *Trichodorus* species in the sugarcane growing zones. According to Mohan (2011) application of aldicarb and other suitable nematicides during sugarcane production averts the effect of these root parasites on sugarcane. In the current study the population of nematodes at 9 and 18 months after planting (MAP) had reduced from the initial nematode population at planting for the nematicide-treated plots. Conversely, control plots had significantly higher nematode populations compared to the nematicide-treated ones. Among the latter plots treated with higher nematicide rate had lower nematode populations compared to those treated with lower nematicide rate at both 9 and 18 MAP. This demonstrates that the higher the nematicide levels in the soil the greater the mortality of nematodes and probably the more the reduction of the reproductive factor of the nematodes. Maqbool and Hashmi (1987) and Cadet and Spaull (1985) have similarly reported increased populations of *Pratylenchus zeae* in untreated plots of sugarcane till harvest and noted gradual decline in their numbers with

increasing nematicide levels in treated pots. Qureshi *et al.* (2002) noted a significantly greater mortality rate in nematode populations when sugarcane fields were treated with nematicides Furadan, Miral and Tenekil. This study confirms these observations as it is in line with the ability of aldicarb to prohibit reproduction of nematodes. The increased mortality under the presence of a nematicide could also explain why the nematode populations in the untreated plots in our study were recorded to have increased at mid-season (9 months) and decreased (though not significantly) at harvest as the active root growth at 9 months was taking place favouring reproduction and the effects of crop senescing impeded further nematode reproduction in the rhizosphere at 18 months. Qureshi *et al.* (2002) also observed the improvement of sugarcane's agronomic outputs such as plant height, stalk weight and yields when the crop was treated with the chemical nematicides as compared to the control. In this study, generally nematode populations in the nematicide-treated plots declined from the initial population at planting to harvest. In a few instances however there was a slight increase between 9 and 18 MAP probably due to wearing off of the nematicide toxicity with time. This confirms the finding by White (1984) who observed in similar studies that aldicarb reduced in toxicity for long-term control of plant parasitic nematodes. Similarly, Waraitch (1982), Elliott *et al.* (1984), Showler and Reagan (1991) and Showler *et al.* (1998) reported in their studies that aldicarb does in fact persist strongly in the soil for 10 weeks when it consistently reduces plant parasitic nematodes.

This study demonstrated that if any of the yield parameters (girth, height, millable stalk population) can be used to predict the final yield with an accuracy level of 61%. If the mean stalk length increases by 0.22 cm, it will likely lead to an increase in yield of 1 kg ha^{-1}. Similarly, commercial cane sugar (CCS) can be predicted with an accuracy level of 88% using the sugarcane quality parameters that are measured during its growth (pol % cane and fibre % cane) and after harvest (pol % juice) all of which are interrelated in their determination. In particular, a 4% increase in pol % juice will highly likely lead to an increase of CCS by 1%. However, a 1 percent reduction in fibre % cane will highly likely lead to a 1% increase in the CCS. These results have confirmed the observations made by Zorilla (2007) who reported that application of the nematicides Apache lOG (Cadusafos) and Furadan 3G (Carbofuran) reduced nematode populations and

consequently increased cane yields and sugar yield and quality. In this study, the combined application of nematicides and the host resistance status of the selected cane cultivars could possibly have caused the reduction in the nematode populations thereby minimizing their effect on the cane. This consequently led to an increase in cane yields and quality and increased sugar yield. Studies conducted by Qureshi *et al.* (2002), Reyees (1988) and Mehta and Sundararaj (1995) have also shown improvement of sugarcane agronomic parameters that in turn lead to realization of higher sugarcane yield and increased sucrose content when chemical nematicides are applied in soils during cane production. In this study, the resistant and tolerant varieties KEN83-737 and N14 reduced the nematode populations between 0 - 9 months by an average of 67% and 49% respectively when combined with nematicide application, whereas the susceptible variety Co421 reduced them by an average of 32%. The nematode populations increased between 0 – 9 months in the control plots by 24%, 33% and 92% for KEN83-737, N14 and Co421 respectively. The reduction in nematode population consequently resulted in an increase of cane yield, girth and stalk length, millable stalks number and pol % cane by an average of 34%, 18%, 37 %, 53% and 6% respectively.

4.4.1 CONCLUSION

This study has demonstrated that sugarcane cultivars possessing host-plant resistance or tolerance to plant parasitic nematodes have an inherent ability to reduce the nematode population in the rhizosphere. This reduction was associated with improvement in cane yield of 34% and cane quality of 6%, while increasing the girth by 18%, plant height 37% and millable stalks number by 53%. This study further revealed that the resistant cultivar restricted the growth of nematode populations. The nematode population in the control plots at 9 MAP had grown by 24%, 49% and 92% for KEN83-737, N14 and Co421 respectively. Resistant and tolerant cultivars may therefore be incorporated in developing integrated nematode management practices to enhance sugarcane productivity.

CHAPTER FIVE

5.0 EFFECT OF SELECTED INTERCROPS ON NEMATODE POPULATIONS AND THEIR INFLUENCE ON SUGARCANE EQUIVALENT YIELDS

5.1 INTRODUCTION

In Kenya, vegetable production is a key source of food and income and a variety of these are commonly cultivated at small-scale and large-scale levels (Ministry of Agriculture, 2007). There is an untapped economic and nutritional value in these crops in the African context and the Kenyan agricultural sector (Onyango, 2003). Kimaru *et al*. (2014) and Mbugua *et al*. (2005) have documented the high demand that is created by the low supply to the market for the Kenyan population who prefer the indigenous leafy vegetables as a food source. In as much as these food crops are easy to cultivate, have short maturity periods and require minimal input costs, their optimal production is also limited by soil borne pathogens such as nematodes (Kimaru *et al*., 2014). In addition to the nutritional value, the medicinal potential has also been documented by Manoko and Van der Weerden (2004).

Vegetables can be used as intercrops with sugarcane in the early stages of growth and thus improve food security and increase income. However, not all vegetable species are suitable for various reasons but perhaps a more harmful effect may arise from using species that may be hosts of pests and diseases of the main crop (Sumner *et al.*, 1982; Fargette and Fauget, 1988). It is therefore important to evaluate the suitability of potential intercrops. In sugarcane, vegetables that may be good hosts of plant parasitic nematodes should be avoided. Therefore, the determination of the occurrence and level of differential host response to lesion and root-knot nematodes is required to determine the suitability for these vegetables as potential intercrops with sugarcane. *Meloidogyne* spp. has been documented to cause production constraints in various African Leafy Vegetables (ALV) (Fontem and Schippers, 2004; Nchore *et al*., 2012b) where the pathogens stem growth through gall formation that strain the nutrient and water uptake channels of the plant. In particular, Castillo *et al*., (2008) has documented amaranths, black nightshades and other ALV's as hosts of *Meloidogyne* spp.

A higher income and healthier soils can be achieved if intercropping is done in such a manner that parasitic nematodes are restricted while yields of both crops are high. Several combinations can be tested using the Cobb-Douglas (1928) production function that measures sugarcane equivalent yields. This study was conducted to evaluate the different intercropping combinations of sugarcane to determine appropriate intercrops that suppress populations of PPNs while improving food security and revenue generation of the small holder sugarcane farmers.

5.2 MATERIALS AND METHODS

The study was conducted in a glasshouse and in the field.

5.2.1 Glasshouse trial

5.2.1.1 Experimental site

The site is as described in section 3.2.1.

5.2.1.2 Selection of intercrops, treatment and experimental design

Ten food crops commonly grown by small- scale sugarcane farmers namely spiderplant (*Cleome gynandra*), African nightshade (ANS), (*Solanum nigrum*), (*Vigna ungucuilata*), vegetable amaranth (*Amaranthus blitum*), Jews mallow (*Corchorus olitorius*), slenderleaf (*Crotalaria brevidens*), kale (*Brassica carinata*), cabbage (*Brassica oleracea*), common bean (*Phaseolus vulgaris*) and maize (*Zea mais*) were selected for this study.

Potting soil was sieved to remove debris, homogenized and mixed with river sand at a ratio of 2:1. The mixture was autoclaved after which 2 Kg was placed in 3 L plastic pots of 10 cm diameter. Each crop was sown in a pot following respective recommended cultural practices. At planting and after 30 days, pots were fertilized by applying 20 g diammonium phosphate (DAP) and 20 g calcium ammonium nitrate (CAN) for maize and amaranth and 50 % N, P and K for sweet potato. Legumes received no fertilizer at topdressing.

Meloidogyne spp. nematodes were extracted from galls of infested sugarcane roots by use of the modified Baermann funnel technique (Hooper *et al*., 2005). The nematodes were then multiplied and maintained in

young tomato plants. Two weeks after planting, the crops were inoculated with 2000 juveniles. Inoculation was done by slowly dispensing 25 ml of the previously prepared nematode suspension into holes made in the soil around the plant and as close to the roots as possible. Control pots were inoculated with 25 ml distilled water. The treatments were arranged in a completely randomized design with three replications and the experiment conducted over two seasons.

Pratylenchus spp. was extracted from infested sugarcane roots using the modified Baermann funnel technique described by Hooper *et al.* (2005). The nematodes were multiplied aseptically on carrot discs as described by Stapleton *et al.* (2002). The culture was then grown and maintained on young maize crop. Two weeks after planting the crops were inoculated with 2000 juveniles in aliquots of 25 ml. Inoculation was done by slowly dispensing 25 ml of the previously prepared nematode suspension into holes made in the soil around the plant and as close to the roots as possible. Control pots were inoculated with 25 ml distilled water. The treatments were arranged in a completely randomized design with three replications and the experiment conducted over two seasons.

5.2.1.3 Data collection and analysis

The plants were uprooted 120 days after planting and soil gently shaken from the root system. The shoot height was determined by measuring the length of the primary shoot from the soil base to the top leaf collar by use of a meter ruler. The fresh and dry shoot weights together with fresh root weight were measured by use of a weighing balance. Nematodes were extracted from 200 cm^3 soil and 10 g roots (fresh weight) by the Hooper *et al.* (2005) method. Galling and egg mass indices for RKN were assessed using the scale ranging 1-5 as illustrated by Coyne *et al*. (2007). Data was subjected to analysis of variance (ANOVA) and means separated by Tukey's Least Significant Difference (L. S. D) test at $P \leq 0.05$ using SAS ® Proprietary Software Release 9.2.

5.2.2 Field trial

5.2.2.1 Experimental site

The site is as described in section 4.2.1.

5.2.2.2 Treatment, experimental design and data collection

Two sugarcane cultivars, KEN83-737and Co421, categorized in chapter 6 as resistant and susceptible respectively were selected with N14 retained as a standard because of its known tolerance status (Cadet and Spaull, 2005). Spiderplant and African nightshade, categorized respectively as resistant and susceptible in chapter 6 were similarly selected. Sugarcane was planted using 3-4 eye-budded setts in furrows each 10 m long. Each plot had 5 rows of cane at a spacing of 1.2 m apart, hence giving a plot size of $60m^2$ which were then separated by a 2m path. The intercrops were sown between the sugarcane rows at the same time as sugarcane planting adopting their respective recommended agronomic practices. Control plots had no intercrop. Diammonium phosphate was applied at planting in the cane furrows at 100 kg ha^{-1} and urea as topdressing was applied at three months after planting at 200 kg ha^{-1}. The treatments were arranged in a randomized complete block design (RCBD) with three replications.

Nematodes were extracted from the rhizosphere at 0, 9 and 18 months after planting (MAP) using the modified Baermann funnel technique described by Hooper *et al.* (2005). Initial nematode population was determined on newly prepared seedbed just before planting. Soil samples were collected using a soil auger. Eight soil sub-samples were collected at 9 and 18 MAP from the sugarcane rhizosphere at a depth of 5-20cm, mixed to form a composite sample and about 500 g placed in a polythene bag and taken to the laboratory. At harvest, 10 stalks from each of the three central rows were selected at random to form the sample population of 30 stalks per plot. Using the sample, girth was determined by measuring the thickness at the mid-section of the stalk by use of veneer calipers; the stalks were measured by use of a metre rule to determine stalk length; and, the millable stalks number was determined by counting the total number of stalks in the three

central rows using a tally counter. Sugarcane yields were determined by weighing the sample stalks using a tripod and weighing scale and the weight expressed as tonnes per hectare. The first harvest of spiderplant and African nightshade occurred at 30 days after planting (DAP) and continued subsequently after every 10 days till the final harvest at 100 DAP. Their individual yields were determined by weighing the harvested shoot material using a weighing balance and then totaling the weights from all the harvests and the final sum expressed as tonnes per hectare. The prevailing price of sugarcane at harvest was adopted from the nearest sugarcane milling factory while that of spiderplant and African nightshade was adopted from the nearest open air market.

5.2.2.3 Processing of nematodes

Nematodes were processed as described in section 3.2.2.

5.2.2.4 Data analysis

Sugarcane and intercrop yields were subjected to economic functional analyses using the Cobb-Douglas production function to determine their respective sugarcane equivalent yields, per hectare net income and resource use efficiency for each sugarcane-based intercropping system (Cobb-Douglas, 1928) according to the formula used by Shinde *et al.* (2009) whereby:

$$\text{Sugarcane Equivalent Yield} = \frac{\Sigma\ (\text{Sugarcane yield} \times \text{Price of sugarcane}) + (\text{Intercrop yield} \times \text{Price of intercrop})}{\text{Price of Sugarcane}}$$

Nematode counts were log transformed into Log x+1 to fit the assumptions of ANOVA but means reported are actual figures collected. Data was subjected to analysis of variance (ANOVA) and means separated by Tukey's Least Significant Difference (L. S. D) test at $P < 0.05$ using SAS ® Proprietary Software Release 9.2.

5.3 RESULTS

5.3.1 Response of intercrops to infestation by root - knot (*Meloidogyn*e spp.) nematodes

The intercrops had significantly different ($P \leq 0.05$) growth and disease infection parameters between inoculated and control plants in season 1 (Table 5.1). Inoculation led to significant ($P \leq 0.05$) reductions in shoot height of all the ten intercrops except cabbage. The greatest reduction in shoot height was observed on African Nightshade (ANS) and while the least was on Spiderplant, amaranth and maize. This was replicated in season 2 where the highest reductions in shoot height were observed in ANS, Jute mallow, beans and slender leaf while the least were on spiderplant and kale (Table 5.2).

There were significant differences ($P \leq 0.05$) in all the other growth and disease infection parameters- fresh and dry shoot weights, dry root weight, nematode counts, galling index (GI) and egg mass index (EMI)- between inoculated and control plants for both seasons 1 and 2 (Table 5.1 and 5.2). Jute mallow, ANS and beans had the highest reduction of fresh shoot weight while the lowest was recorded for spiderplant, maize and amaranth. Dry shoot weight followed the same trend with ANS, Jute mallow, bean and Slenderleaf having the highest reduction while spiderplant, maize and amaranthus recorded the lowest reduction. Root-knot nematode counts and galling index were significantly highest in the ANS, cowpea, Jute mallow and slender leaf while the lowest were recorded by spider plant, maize and amaranthus. Egg mass index was significantly higher in the ANS and second highest but equal for bean, cowpea and Jute mallow. Slender leaf recorded the third highest significantly different EMI.

In season 2 all the growth and disease infection parameters were similarly significantly different ($P \leq 0.05$) between inoculated and control plants and generally depicted the same trends as those of season 1.

Different intercrops responded differently to inoculation by root- knot nematode. The nematode counts, galling index and egg mass index in the two seasons differed significantly at $P \leq 0.05$ across the intercrops. The highest significant difference for both RKN counts, galling index and egg mass index between the treated and control plants was observed for African nightshade, cowpeas, Jute mallow and slender leaf intercrops. The lowest measurements for the RKN counts were recorded in maize, spider plant, amaranthus

and cabbage while the lowest galling index amongst the intercrops was recorded in spider plant, amaranthus, maize and cabbage. The highest significantly different egg mass index amongst the treated intercrops was in beans, African nightshade, cowpeas and Co421 mallow while the lowest significantly different egg mass index was recorded in maize, spider plant, amaranthus and cabbage.

5.3.2 Response of intercrops to infestation by lesion (*Pratylenchus* spp.) nematodes

There were significant differences ($P \leq 0.05$) between inoculated and control plants on their growth and infection parameters in season 1(Table 5.3). The shoot heights, fresh and dry shoot weights for all intercrops were reduced by inoculation. There was however no significant difference ($P \leq 0.05$) observed in the root weights of cabbage, kale, slenderleaf and spiderplant between the inoculated and non-inoculated plants. Jute mallow, ANS and maize had the highest percentage reduction of shoot height whereas spiderplant, amaranthus and slenderleaf had the lowest. ANS and had the highest percentage reduction in fresh shoot weight while spiderplant, slenderleaf and amaranthus had the lowest. Highest significant reductions in dry shoot and root weights were observed for and ANS while the lowest were recorded by spiderplant, slenderleaf, amaranthus and maize. Lesion nematode counts and damage severance were significantly higher in the ANS, and bean while lowest in spider plant, amaranthus and slenderleaf.

There were also significant differences ($P \leq 0.05$) between the treated and control plants on their growth and infestation parameters for some intercrops in season 2 (Table 5.4). The shoot heights of ANS, bean, Jute mallow, kale and maize were significantly reduced by inoculation. The highest percentage reduction was observed in ANS while the lowest was in kale. There was no significant difference in the shoot heights of amaranthus, cabbage, slenderleaf and spiderplant between their treated and control plants. ANS, cabbage, kale and maize had their fresh shoot weights significantly ($P \leq 0.05$) reduced with ANS having the highest percentage reduction and kale the lowest. There was no significant ($P \leq 0.05$) effect on the fresh shoot

Table 5.1. Effect of root-knot nematodes on sugarcane intercrops under glasshouse conditions in experiment one.

Intercrop	Shoot Height (cm)			Fresh Shoot Weight (g)			Dry Shoot Weight (g)			Nematode counts	Galling Index	Egg Mass Index
	Ni	In	Δ%	Ni	In	Δ%	Ni	In	Δ%	In	In	In
African Nightshade	62.33	32.33	48.1	96.97	63.53	34.5	24.55	15.77	35.8	788.33	7.33	3.67
Amaranthus	54.67	47.33	13.4	145.97	136.00	6.8	37.47	33.70	10.1	185.00	2.33	1.67
Beans	45.67	32.83	28.1	47.37	34.30	27.6	14.40	10.68	25.8	546.67	6.00	3.33
Cabbage	9.17	7.67	16.4	243.73	216.40	11.2	31.20	27.64	11.4	380.00	2.67	1.67
Cowpea	26.00	15.17	41.7	46.33	31.93	31.1	14.06	9.74	30.7	703.33	7.00	3.33
Co421 Mallow	56.00	37.83	32.4	56.43	38.03	32.6	24.57	18.11	26.3	650.00	6.67	3.33
Kales	26.50	21.50	18.9	137.07	117.47	14.3	32.98	28.42	13.8	456.67	3.33	2.33
Maize	105.83	91.17	13.9	200.27	190.27	5.0	58.79	54.14	7.9	135.00	2.33	1.33
Slender Leaf	44.00	34.33	22.0	39.00	28.87	26.0	10.61	8.00	24.6	631.67	5.33	3.00
Spider Plant	51.17	44.83	12.4	140.57	134.57	4.3	35.60	33.17	6.8	125.00	2.00	1.33
LSD(0.05)	3.25*			13.66*			2.34*			17.46*	0.24*	0.20*
CV %	14.70			24.40			17.15			14.54	20.69	31.00

Δ% = Percentage change; L.S.D followed by (*****) indicates treatment means that are significantly different at $p < 0.05$ where Ni and In indicates non –inoculated (control) and inoculated treatments.

Table 5.2. Effect of root-knot nematodes on sugarcane intercrops under glassouse conditions in experiment two.

Intercrop	Shoot Height (cm)			Fresh Shoot Weight (g)			Dry Shoot Weight (g)			Nematode counts	Galling Index	Egg Mass Index
	Ni	In	Δ%	Ni	In	Δ%	Ni	In	Δ%	In	In	In
African Nightshade	48.67	27.67	43.1	82.67	81.30	1.7	22.00	13.30	39.5	770.00	7.30	3.33
Amaranthus	57.67	51.00	11.6	122.67	112.00	8.7	35.33	31.70	10.3	182.00	2.30	2.00
Beans	49.00	33.67	31.3	41.33	31.30	24.3	17.00	12.70	25.3	530.00	5.70	4.00
Cabbage	8.00	6.67	16.6	219.67	192.00	12.6	26.00	22.70	12.7	377.00	2.70	2.00
Cowpea	29.00	18.00	37.9	41.00	27.00	34.1	11.67	7.33	37.2	708.00	6.30	3.00
Co421 Mallow	51.00	35.33	30.7	62.33	38.00	39.0	28.33	20.0	29.4	668.00	6.30	2.67
Kales	26.67	22.33	16.3	135.67	107.00	21.1	26.00	20.70	20.4	470.00	3.70	2.67
Maize	105.33	96.00	8.9	183.00	161.00	12.0	57.00	49.00	14.0	110.00	2.30	1.67
Slender Leaf	46.00	31.00	32.6	38.00	26.30	30.8	9.67	6.33	34.5	633.00	5.70	2.67
Spider Plant	48.00	42.67	11.1	131.00	118.00	9.9	36.33	32.30	11.1	127.00	2.00	1.67
LSD(0.05)	1.60*			7.10*			1.38*			15.00*	0.30*	0.23*
CV %	7.34			13.93			10.88			12.60	29.0	34.85

Δ% = Percentage change; L.S.D followed by (*****) indicates treatment means that are significantly different at $p < 0.05$ where Ni and In indicates non –inoculated (control) and inoculated treatments.

weights of amaranthus, slenderleaf and spiderplant. Lesion nematode counts were significantly highest in the ANS, cowpeas, Jute mallow and maize but significantly lowest in the spider plants. The damage severance caused on roots by the lesion was significantly highest in the ANS, bean and Jute mallow while lowest for spider plant.

The variability in the levels of lesion nematode counts and damage severance differed significantly ($P \leq 0.05$) across the intercrops in both seasons. The highest numbers for lesion nematode counts and damage severance were observed in the ANS, cowpeas, bean and Jute mallow while the lowest measurements for both parameters were recorded in spider plant, amaranthus, slender leaf and maize.

5.3.3 Effect of intercrops on sugarcane and nematode populations

The millable stalks number of sugarcane varied significantly ($P \leq 0.05$) when they were interplanted with indigenous vegetables (Table 5.5). Intercropping sugarcane with spiderplant led to higher millable stalks number than in pure stand with the highest being when sugarcane variety N14 was used. The reverse was true when African nightshade was used as it lead to less millable stalks number than pure stands.

Sugarcane stalk length differed significantly ($P \leq 0.05$) when intercropped with vegetables that are resistant and susceptible to plant parasitic nematodes (Table 5.5). When sugarcane varieties were intercropped with spiderplant longer stalks were produced, however, varieties KEN 83-737 and Co421 produced longer ones than N14. Intercropping sugarcane with African nightshade led to shorter stalk length in the three sugarcane varieties as compared to when grown in pure stand.

Table 5.3. Effect of intercrops on *Pratylenchus* spp. nematode numbers and sugarcane growth parameters in experiment one.

Intercrop	Shoot Height (cm)			Fresh Shoot Weight (g)			Dry Shoot Weight (g)			Fresh Root Weight (g)			Nematode counts	Damage severance
	Ni	In	Δ%	Ni	In	Δ%	Ni	In	Δ%	Ni	In	Δ%	In	In
African nightshade	40.67	25.33	37.7	79.33	57.67	27.3	9.10	6.27	31.1	20.33	10.33	49.2	506.67	3.67
Amaranthus	48.33	45.00	6.9	59.00	50.67	14.1	13.67	11.57	15.4	51.67	47.67	7.7	110.00	1.67
Beans	41.33	32.00	22.6	38.67	31.00	19.8	12.77	9.77	23.5	14.33	8.67	39.5	321.67	3.00
Cabbage	10.00	8.33	16.7	139.30	115.33	17.2	21.93	18.10	17.5	10.67	9.17	14.1	241.67	2.67
Cowpea	23.00	20.00	13.0	45.67	34.67	24.1	6.37	4.33	32.0	8.33	3.67	55.9	410.00	3.33
Co421 Mallow	45.67	30.00	34.3	65.33	52.33	19.9	18.93	13.73	27.5	7.67	4.17	45.6	224.33	3.00
Kales	25.00	20.67	17.3	151.70	122.33	19.4	19.60	15.57	20.6	12.00	10.17	15.3	280.00	2.67
Maize	97.33	66.33	31.9	160.70	131.33	18.3	44.97	31.67	29.6	52.33	45.67	12.7	186.67	2.67
Slender Leaf	29.00	26.67	8.0	39.33	34.00	13.6	12.80	10.90	14.8	17.00	14.67	13.7	153.33	2.00
Spider Plant	41.33	38.67	6.4	89.00	79.00	11.2	15.27	13.13	14.0	11.33	10.20	10.0	95.00	1.33
L. S. D $_{(0.05)}$	0.79*			3.26*			1.00*			2.86*			16.37*	0.21*
CV %	4.20			7.93			12.41			29.59			24.80	31.40

Δ% = Percentage change; L.S.D followed by (*****) indicates treatment means that are significantly different at $p < 0.05$ where Ni and In indicates non –inoculated (control) and inoculated treatments.

Table 5.4. Effect of intercrops on *Pratylenchus* spp. nematode numbers and sugarcane growth parameters in experiment two.

	Shoot Height (cm)			Fresh Shoot Weight (g)			Dry Shoot Weight (g)			Fresh Root Weight (g)			Nematode counts	Damage severance
	Ni	In	Δ%	Ni	In	Δ%	Ni	In	Δ%	Ni	In	Δ%	In	In
African nightshade	94.30	60.67	35.7	130.20	85.60	34.3	14.33	9.37	34.6	24.43	15.40	37.0	451.67	3.67
Amaranthus	89.00	88.33	0.8	115.97	109.0	6.0	26.87	25.13	6.5	34.17	30.70	10.2	103.33	1.33
Beans	43.00	34.00	20.9	39.30	29.70	24.4	13.00	9.37	27.9	14.13	10.17	28.0	286.67	3.00
Cabbage	15.00	13.33	11.1	291.77	239.0	18.1	45.80	34.63	24.4	13.60	11.53	15.2	163.33	2.33
Cowpea	47.67	38.00	20.3	87.97	59.20	32.7	12.13	7.33	39.6	14.80	10.27	30.6	346.67	3.00
Co421 Mallow	53.67	36.33	32.3	51.43	37.90	26.3	14.90	9.93	33.4	12.17	8.57	29.6	266.67	3.00
Kales	34.00	28.00	17.6	255.80	228.0	10.9	33.00	29.20	11.5	12.30	10.57	14.1	170.00	2.00
Maize	259.0	175.00	32.4	429.73	347.0	19.3	120.33	83.67	30.5	59.70	50.10	16.1	181.67	2.67
Slender Leaf	37.33	32.67	12.5	29.57	43.60	-47.4	9.73	13.50	-38.7	17.03	15.37	9.7	123.33	1.67
Spider Plant	70.00	68.33	2.4	153.20	142.0	7.3	26.07	23.47	10.0	12.30	11.60	5.7	71.67	1.00
L. S. D $_{(0.05)}$	5.78*			16.65*			3.55*			0.71*			14.69*	0.19*
CV %	16.80			21.95			24.16			7.02			26.01	30.86

Δ% = Percentage change; L.S.D followed by (*****) indicates treatment means that are significantly different at $p < 0.05$ where Ni and In indicates non –inoculated (control) and inoculated treatments.

Table 5.5. Effect of plant parasitic nematodes on sugarcane intercropped with different crops.

Intercrop	**Sugarcane variety**								
	Millable stalk number			Stalk length			Nematode numbers		
	KEN 83-737	Co421	N14	KEN 83-737	Co421	N14	KEN 83-737	Co421	N14
Spider plant	237.0	251.3	253.7	297.2	294.2	271.8	213.3	345.0	743.3
African Nightshade	192.0	178.3	145.7	267.3	251.8	234.1	318.3	503.3	1358.3
Control	213.7	219.0	206.0	273.9	267.5	272.7	368.3	470.0	1005.0
LSD		15.9			9.4			101.4	
CV%		7.6			3.5			17.2	

Sugarcane intercrops led to significant ($P \leq 0.05$) differences in sugarcane yields. Intercropping sugarcane with spiderplant led to higher tonnage than in a pure stand (Table 5.6). African nightshade did not lead to a significant ($P \leq 0.05$) difference in tonnes of cane produced as compared to a pure stand. Significant ($P \leq 0.05$) differences were observed on cane yield of selected sugarcane varieties in a field infested with plant parasitic nematodes (Table 5.6). Variety KEN 83-737 yielded significantly ($P \leq 0.05$) higher compared to varieties Co421 and N14. Significant ($P \leq 0.05$) differences were observed on cane girth of selected sugarcane varieties in a field infested with plant parasitic nematodes (Table 5.6). Variety KEN 83-737 and N14 produced significantly ($P \leq 0.05$) thicker cane compared to variety Co421.

Significant ($P \leq 0.05$) interaction was observed between intercrops and sugarcane varieties on the final nematode count. Intercropping sugarcane variety N14 with African Nightshade led to the highest numbers of plant parasitic nematodes, higher than even in pure stand. Smaller number of the parasites was observed when all the sugarcane varieties were intercropped with Spiderplant. The plant parasitic nematode counts in intercropped fields were significantly ($P \leq 0.05$) less than in pure stand at nine months. These counts were however not significantly different from each other among the intercrops.

5.3.4 Sugarcane Equivalent Yield

The different combinations of sugarcane equivalent yield were significantly different ($P \leq 0.0001$) with the highest combination being that of KEN83-737 × Spiderplant followed by Co421 × Spiderplant (Table 5.7).

Table 5.6. Effect of selected intercrops on sugarcane yield and girth.

Variable		Cane yield in tonnes per hectare	Girth in cm
Sugarcane cultivar	KEN83-737	141.6a	2.7a
	N14	122.4b	2.7a
	Co421	115.6	2.5b
Intercrop	Spiderplant	135.9a	
	African nightshade	127.6ab	

Means with same letters down the column are not significantly different.

Table 5.7. Sugarcane equivalent yields for different intercrop combinations.

Sugarcane intercrop combination	**Sugarcane equivalent yields**	**Tukey's grouping**
KEN83-737 × spiderplant	303.1	A
Co421 × spiderplant	280.9	AB
KEN83-737 × ANS	275.3	AB
N14 × spiderplant	262.8	AB
Co421 × ANS	241.2	AB
N14 × ANS	225.5	B
KEN83-737	137.7	C
N14	117.7	C
Co421	93.3	C

Means with same letters down the column are not significantly different.

The pure stand for each of the three sugarcane varieties produced the least sugarcane equivalent yield and did not differ from each other.

5.4 DISCUSSION

5.4.1 Effect of selected sugarcane intercrops on plant parasitic nematodes

This study proved that *Pratylenchus* spp. and *Meloidogyne* spp. have varied infection and disease causation on different intercrops. Spiderplant and amaranthus were observed to be the most resistant vegetables to both *Meloidogyne* spp. and *Pratylenchus* spp. The potential to utilize them as intercrops would have immense contribution in reducing sugarcane production losses in fields highly infested with these nematodes. The shoot heights of spider plant and amaranthus were higher due to reduced nutrient uptake to support shoot and root development. These findings concur with past studies conducted by Nchore *et al.* (2012b) and Kimaru *et al.* (2013, 2014) who observed similar root damage by these nematodes while working with African Indigenous Leafy Vegetables (AILVs) in Kenya. Maize, slender leaf and kales were observed to be moderately resistant to both pathogens.

Cowpea and ANS were the most susceptible to RKN and lesion nematodes. The susceptibility of the ANS, cowpea, Jute mallow and bean was confirmed by reduced shoot heights, fresh and dry shoot and root weights which resulted from reduced plant growth. The high galling and egg mass indices in the root system of intercrops lead to significant reduction in nutrient uptake and water absorption. These symptoms have also been reported in similar studies by Kimaru *et al.* (2013) who observed symptoms similar to those of nutrient- deprived plants when the AILV's were treated with RKN. Atkins *et al.* (2004) and McSorley *et al.* (2004) have also reported similar findings in and Co421 mallow. High lesion formation, gall formation and egg mass production is

a key indicator that ANS and are favorable hosts to the lesion and root knot nematodes as they promote survival of these pathogens in their root systems and this also directly increased the nematode counts in the rhizosphere. Similarly Kimaru (2013) and Wesemael and Moens (2008) have reported in their studies the symptomatic effects attributed to RKN infection on susceptible varieties such as cowpea, ANS and Co421 mallow.

Studies also conducted by Caveness and Ogunfowora (1985) have listed more than 50 species of plant parasitic nematodes to be invasive to cowpea with RKN being the most destructive to crop production, particularly *Meloidogyne incognita* and *M. javanica* as the key pathogens (Sarmah and Sinha, 1995). Studies conducted in West Africa have documented the prevalence of *Pratylenchus* spp. as a key parasite in the production of cowpea (Sarr *et al*., 1989b). The lesion nematode has been shown to cause infections in mixed populations with RKN leading to decrease in growth and production (Sarr and Baujard, 1988).

5.4.2 Effect of intercrops on sugarcane, nematode populations and sugarcane equivalent yields

Spiderplant and African Nightshade are the top two most popular indigenous vegetables (or African leafy vegetables) in western Kenya (Abukutsa, 2007). Most of these areas cover the western sugar belt so that it is safe to infer that the two crops are top candidates as companion crops of sugarcane. These crops are also widespread at the coast (Mathenge, 2005) where sugarcane is also being re-introduced as a cash crop. Companion crops of sugarcane are supposed to have a short life cycle because sugarcane can accommodate them the first seven months before shading the ground. The two vegetables in question meet short life cycle threshold as they may be ready for harvest after three to four weeks (Abukutsa, 2007) making it possible to

grow several cycles within the seven month window and thus generating the much needed income for the mainly resource poor farmers.

Results obtained showed the sugarcane variety KEN83-737 to be superior in nearly all measured parameters compared to the standard N14 and susceptible Co421. The variables included production of significantly longer and thicker canes leading to the highest yields thus confirming its status as resistant to plant parasitic nematodes (Chirchir *et al*., 2008). Large numbers of the nematodes were found in Co421 fields while comparatively smaller numbers were in the rhizosphere of KEN 83-737, agreeing with a study by Chirchir *et al.* (2008).

When spiderplant and African nightshade were intercropped with sugarcane in the presence of plant parasitic nematodes, they produced sharply contrasting results. Spiderplant led to favourable results including a higher number of millable stalks that were longer and subsequently more tonnage.

The numbers of plant parasitic nematodes counted in intercropped fields were less than in pure stand midway through the trial. These numbers were however not significantly different from each other among the intercrops. Intercropping with African nightshade led to shorter stalk length in the three sugarcane varieties as compared to when grown in pure stand and subsequently lower yields. Variety N14 intercropped with African nightshade led to the highest numbers of plant parasitic nematodes, higher than even in pure stand. A smaller number of nematodes was observed when all the sugarcane varieties were intercropped with spiderplant. These findings point to the African nightshade as being a good host of plant parasitic nematodes with whatever combination of sugarcane variety and should therefore be avoided in nematode infested fields.

The two combinations that differed were sugarcane variety KEN8373 × Spiderplant and N14 × African nightshade. However African nightshade has been found to be seriously limited by the presence of plant parasitic nematodes and is seemingly a good host of the same. So although its combination with sugarcane may make good returns, this is outweighed by its potentially detrimental effects on soil.

The top two highest sugarcane equivalent yields involved spiderplant as the intercrop, a fact that may be attributed to the high price of the vegetable. Combining spiderplant with the resistant sugarcane cultivar KEN83-737 and the susceptible Co421 produced high sugarcane equivalent yields. The pure stands for each of the three sugarcane varieties produced the least sugarcane equivalent yields which did not significantly differ ($P \leq 0.05$) from each other.

The choice of sugarcane intercrops depends on the socio-cultural and environmental conditions. Previous studies on sugarcane equivalent yields have been based on intercrops that are quite different from what obtains in Kenya. Such intercrops as *rajmash*, winter maize (Kanchannainwal, 2009), wheat and chickpea (Shinde *et al.*, 2009), *raya*, wheat, potato (Bhullar *et al.*, 2006) have been subjected to these studies but these are not crops grown in sugarcane growing zones of Kenya. Hence it was necessary to subject Kenyan food crops to sugarcane equivalent yields study.

Combining the findings of host response of sugarcane and intercrops to plant parasitic nematodes with those of sugarcane equivalent yields leads to choosing of a combination that will promote both the root health due to sustainable soil conditions as well as improve food security and income. Such a combination in this case should avoid the susceptible sugarcane variety Co421 and African nightshade as an intercrop which has been found to be vulnerable too. Therefore

suitable combinations are KEN83-737 × spiderplant and N14 × spiderplant. Further studies are necessary to come up with combinations to fit different localities, seasons and markets.

5.4.3 CONCLUSION

This study has demonstrated that there is differential infection of the tested intercrops by *Pratylenchus* spp. and *Meloidogyne* spp. Spiderplant, amaranthus and slenderleaf indicated the highest resistance to both pathogens while African nightshade, cowpea and Jute mallow had the highest susceptibility.

Sugarcane variety Co421 is susceptible to plant parasitic nematodes and should therefore be avoided. It is recommended that the resistant variety KEN83-737 and the moderately tolerant N14 be used in combinations with intercrops. Among the intercrops tested spiderplant is recommended because it is suppressive to root-knot and lesion nematodes. The African nightshade should be avoided as an intercrop because it is not only susceptible but also encourages a rapid build-up of populations of parasitic nematodes. Suitable combinations arising from the study and giving satisfactory sugarcane equivalent yields are KEN83-737 × spiderplant and N14 × spiderplant. These are the combinations found to favour sustainable soil conditions as well as having the potential to improve food security and household income. Further studies to include other food crops are necessary to determine more combinations that would fit different localities, seasons and markets.

CHAPTER SIX

6.0 EFFECT OF FERTILIZER ON TOLERANCE OF SUGARCANE TO INFESTATION BY PLANT PARASITIC NEMATODES

6.1 INTRODUCTION

Plant mineral nutrition studies have shown that favourable conditions and uptake of essential and trace elements directly affect plant growth and development. Plant pathogenic nematodes interfere with plant growth by impeding the uptake of nutrients, water or altering nutrient mechanisms (Blair *et al.,* 1999). Optimum nutrient regime results in greater soil nutrient availability and plant growth, with higher leaf concentrations of the macro elements (N, P, K and Mg) than under deficient nutrient regime (Gaidashova *et al.,* 2008). Mineral fertilizer application has been observed to improve tolerance to plant parasitic nematodes offering a potential nematode management option: An optimum nutrient regime provides high nutrient levels and subsequent plant growth which improves root development hence higher tolerance to nematodes (Waele and Elsen, 2007).

Meyer (2011) has shown that adequate supply of nutrients has the greatest impact on sugarcane yields after the water requirements of the crop have been met. The nutrients are usually applied in the form of organic and inorganic fertilizers. When fertilizers are not adequately supplied, soil degradation occurs that leads to low sugarcane yields (Wawire *et al*., 2006). Sugarcane cultivars differ in their resistance (or susceptibility) to plant parasitic nematodes, and also differ in terms of their response to fertilizers. This study was established to investigate the effect of fertilizer application on tolerance of selected sugarcane cultivars grown in Kenya to *Pratylenchus* spp. nematodes.

6.1 MATERIALS AND METHODS

6.1.1 Site description

The site is as described in section 3.2.1.

6.1.2 Treatments, experimental design and data collection and analysis

Three sugarcane cultivars; KEN83-737, D8484 and Co421 categorized as resistant, moderately resistant and susceptible, respectively were selected for this study. Single-budded setts of the cultivars were treated and planted as described in section 3.2.2. Diammonium phosphate (DAP) was applied at planting at three rates of 10g, 20g and 40g per pot (with 20g per pot being the usual recommended application rate). At 30 days after planting calcium ammonium nitrate (CAN) was applied at three rates of 10g, 20g and 40g per pot (where 20g per pot is the usual recommended application rate) respectively matching the DAP rates. Control pots did not receive any fertilizer. Single pre-germinated sugarcane seedlings were then transplanted each into a planting pot. Nematodes were extracted as described in section 3.2.2.

The experiment was conducted in a split-split plot design and arranged in a RCBD in the glasshouse with three replications over two seasons. Sugarcane cultivar was placed in the main plot, inoculation treatment in the sub-plot and fertilizer rate in the sub-sub plot. Data collection and analysis was done as described in section 3.2.3.

6.2 RESULTS

Fresh shoot weight of the cultivars differed significantly ($P \leq 0.05$) as a result of interaction between nematode inoculation and fertilizer rates (Table 6.1). In season 1, inoculation of KEN83-737 and D8484 did not give any significant differences ($P \leq 0.05$) between the shoot weights of treated and control plants for all the tested fertilizer rates. However, Co421 inoculated

plants had significantly reduced ($P \leq 0.05$) shoot weights compared to the non-inoculated at fertilizer rates of 0, 10 and 20 g but not at 40 g DAP per pot. This reduction decreased with an increase in fertilizer rate. Thus the decrease was highest at fertilizer rate of 0 g and lowest for 20 g per pot. In season 2, inoculation of KEN 83-737 significantly reduced ($P \leq 0.05$) shoot weight for plants that received no fertilizer while those that received any amount of fertilizer had no significant difference. Inoculation of both D8484 and Co421 produced significantly reduced ($P \leq 0.05$) shoot weights for inoculated plants compared to the non-inoculated for all the fertilizer rates. This reduction however decreased with an increase in fertilizer rate: thus the highest reduction occurred with no fertilizer application (0 g per pot) while the least reduction occurred at fertilizer rate of 40 g per pot.

Inoculation produced significant ($P \leq 0.05$) reduction of the shoot length for sugarcane cultivars at all the fertilizer rates tested (Figure 6.1). This reduction decreased with an increase in fertilizer rate. Results for both seasons followed a similar trend. Co421 had significantly ($P \leq 0.05$) shorter shoots compared to both KEN 83-737 and D8484 in both seasons 1 and 2 (Figure 6.2). Cultivar KEN 83-737 had significantly longer shoots than D8484 in season 1 but not in season 2.

There were significant differences ($P \leq 0.05$) between numbers of tillers produced by sugarcane inoculated and non-inoculated under different fertilizer application rates. In season 1 there were no significant differences ($P \leq 0.05$) at the higher rates of 20 and 40g DAP per pot regardless of inoculation (Figure 6.3). However, in season 2 inoculation caused a significant ($P \leq 0.05$) reduction in the number of tillers at all fertilizer rates. For both seasons the highest numbers of tillers were recorded at fertilizer rates of 20 and 40 g DAP per pot without inoculation while the least was for both controls that were neither inoculated nor had fertilizer applied. KEN83-737

and D8484 produced significantly ($P \leq 0.05$) more tillers than Co421 but did not differ from each other (Figure 6.4).

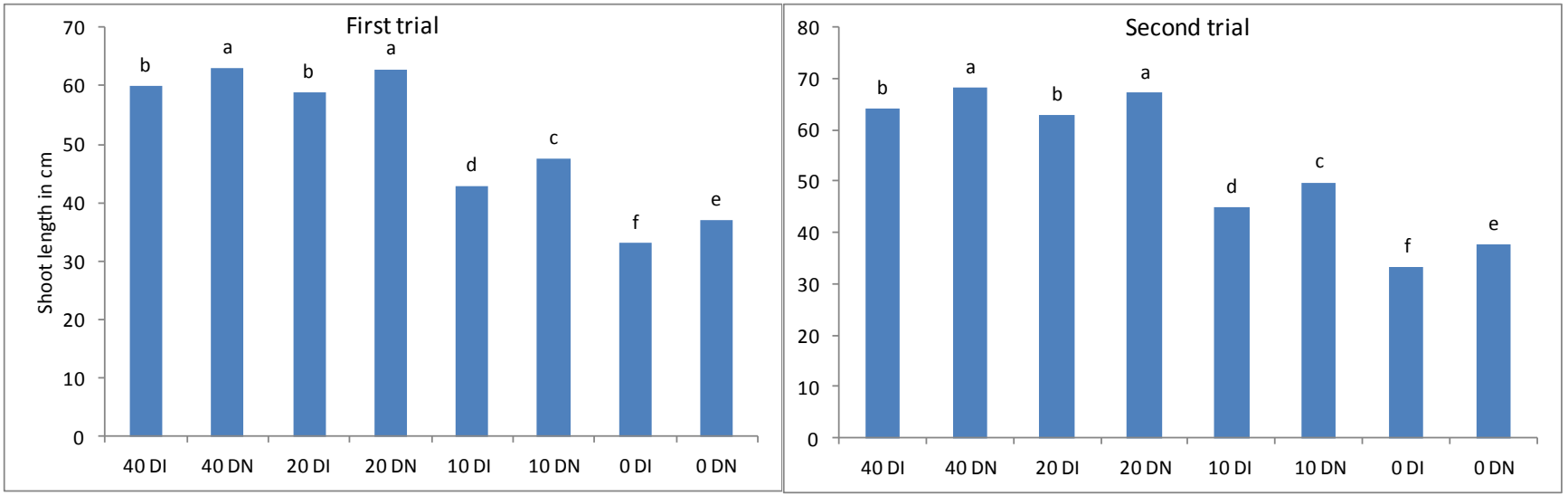
Significant differences ($P \leq 0.05$) were observed in root weights of sugarcane inoculated and non-inoculated under different fertilizer regimes. In both seasons 1 and 2 there were no significant differences ($P \leq 0.05$) at the higher rates of 20 and 40g DAP whether inoculated or not (Figure 6.5).

Table 6.1. Effect of fertilizer on fresh shoot weight of selected sugarcane genotypes.

Fertilizer rate (g DAP per pot)	Experiment 1									Experiment 2								
	KEN83-737			D8484			Co421			KEN83-737			D8484			Co421		
	In	Ni	Δ%	In	Ni	Δ%	In	Ni	Δ%	In	Ni	Δ%	In	Ni	Δ%	In	Ni	Δ%
40	76.0	79.1	3.9	92.0	96.7	4.9	79	84.6	6.6	81.6	84.8	3.8	98.7	103.8	4.9	85.4	89.8	4.9
20	80.2	82.6	2.9	97.1	103	5.7	79.6	88.8	10.4	82.5	86.1	4.2	96.4	101.8	5.3	83.3	91.7	9.2
10	62.5	66.1	5.4	87.6	93.7	6.5	54.2	72.4	25.1	67.9	71.4	4.9	89.6	97.4	8.0	59.3	80.6	26.4
0	63.8	67.9	6.0	73.4	78.6	6.6	47.2	64.5	26.8	56.6	60.9	7.1	76	82.8	8.2	53.6	74.1	27.7
$LSD_{0.05}$(trt)					6.2									4.1				
$LSD_{0.05}$(var)					3.8									2.5				
CV(%)					8.3									5.3				

In=Inoculated; Ni=Non-inoculated; Δ%=Percentage change

Root weight at the lower rate of 10g DAP was significantly ($P \leq 0.05$) reduced by inoculation and so were the non fertilized sugarcane plants. Results from the two seasons followed similar trend. Varietal responses were similar to those of tillering where varieties KEN83-737 and D8484 produced significantly ($P \leq 0.05$) heavier roots than Co421 but did not differ from each other (Figure 6.6). The numbers of plant parasitic nematodes varied significantly ($P \leq 0.05$) in the rhizospheres of different sugarcane cultivars. The largest population of the parasites was found in the rhizosphere of Co421 while the least number was recorded for KEN83-737.



40 DI – 40g Diammonium Phosphate per pot and inoculated; 40 DN – 40g Diammonium Phosphate per pot and not inoculated ; the same repeats in other rates

Figure 6.1. Effect of fertilizer on shoot length of three sugarcane cultivars in the first trial.

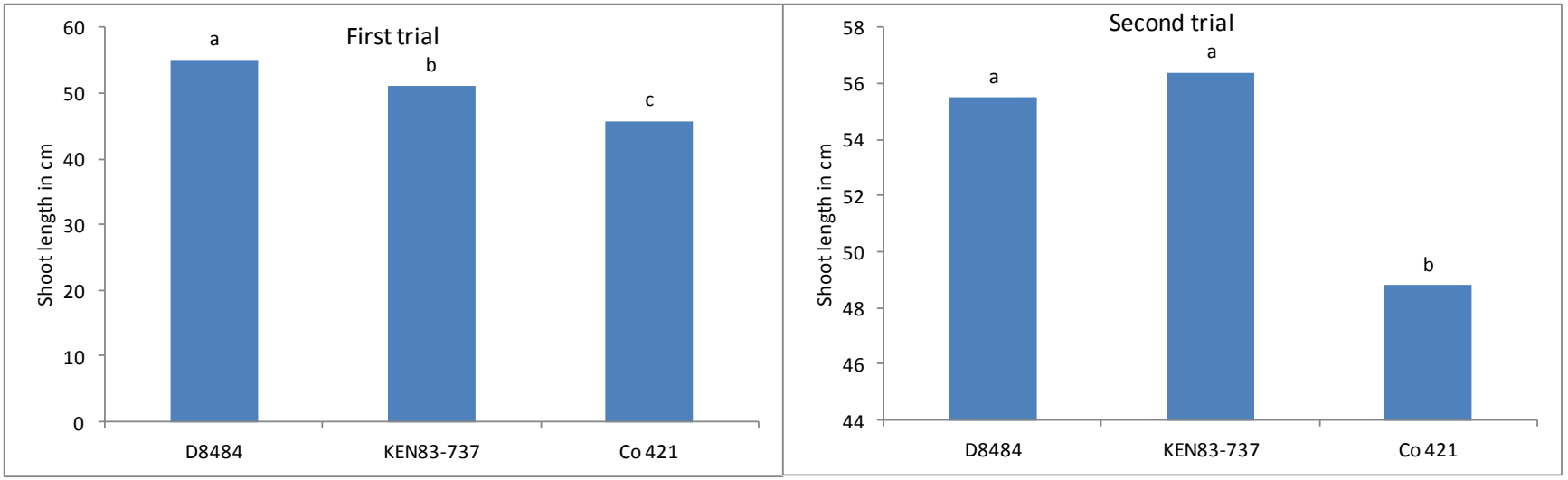
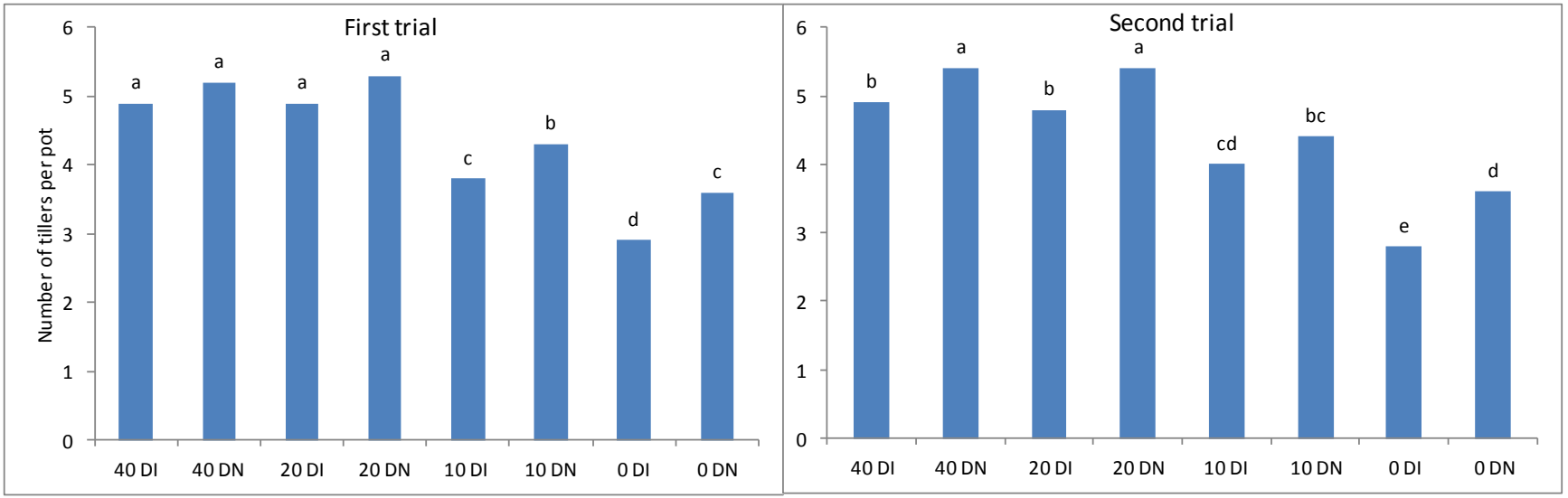


Figure 6.2. Effect of fertilizer on shoot length of sugarcane cultivars in the first and second trials.



40 DI – 40g Diammonium Phosphate per pot and inoculated; 40 DN – 40g Diammonium Phosphate per pot and not inoculated; the same repeats in other rates

Figure 6.3. Effect of fertilizer and plant parasitic nematodes on tillering of sugarcane cultivars in a glasshouse trial.

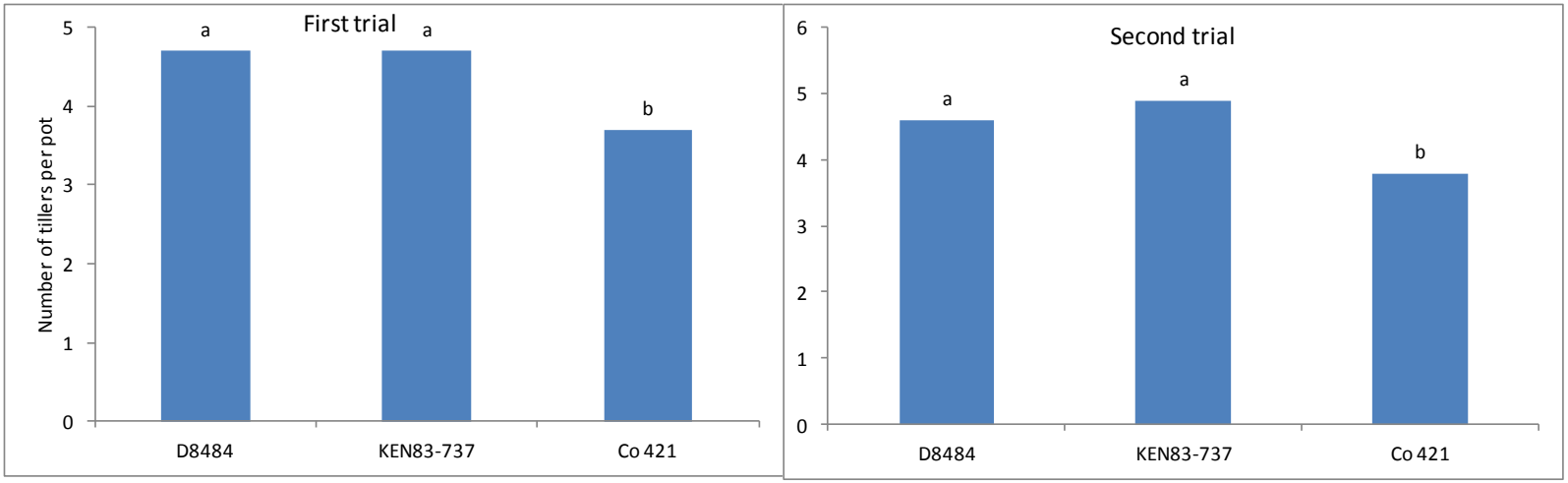
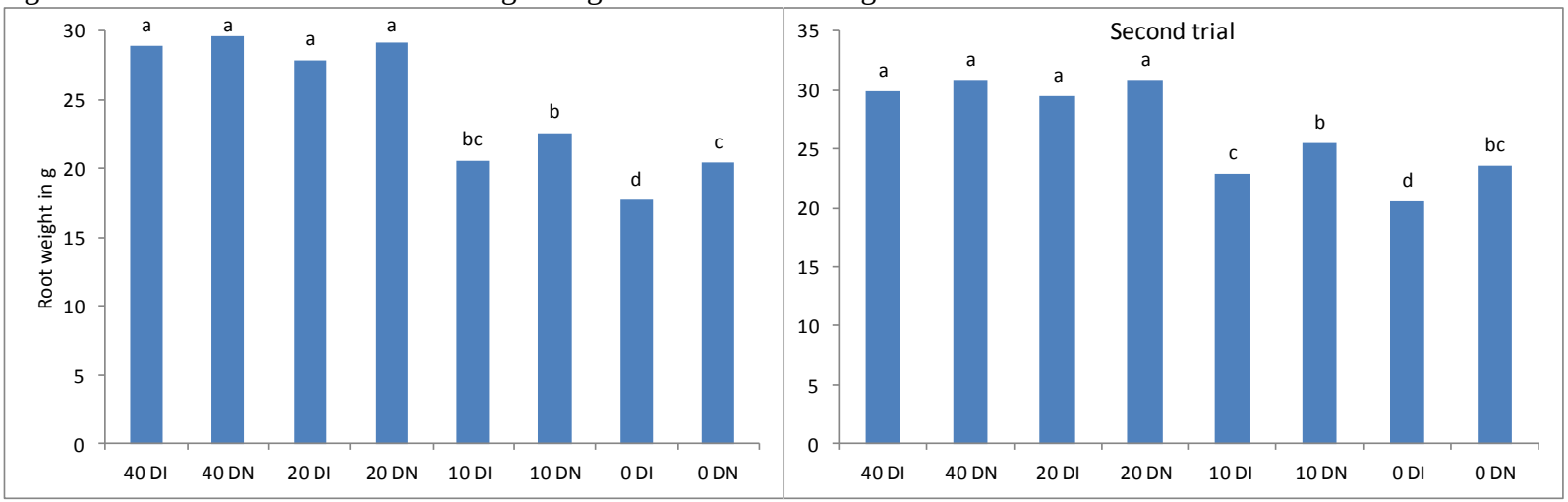


Figure 6.4. Effect of fertilizer on tillering of sugarcane varieties in a glasshouse trial.



40 DI – 40g Diammonium Phosphate per pot and inoculated; 40 DN – 40g Diammonium Phosphate per pot and not inoculated; the same repeats in other rates

Figure 6.5. Effect of fertilizer and plant parasitic nematodes on root weight of the three sugarcane cultivars (average) in a glasshouse trial.

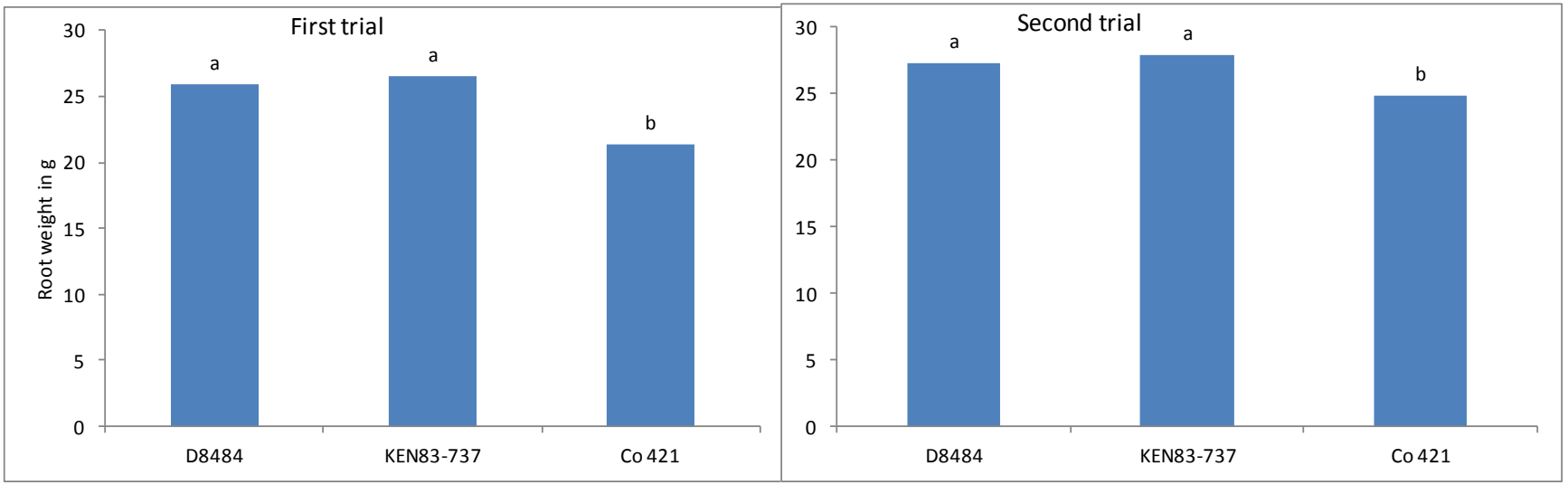


Figure 6.6. Effect of fertilizer on root weight of sugarcane varieties in a glasshouse trial.

6.3 DISCUSSION

This study demonstrated that inoculation of sugarcane with *Pratylenchus* spp. led to a reduction in yield components - shoot length, shoot weight, numbers of tillers and root weight. Similar findings have been observed in earlier studies (Blair *et al.,* 1999; Moura *et al.,* 1999; Starr and Bendezu, 2002; Cetintas and Yaiba, 2010). DAP at the rate of 20g per pot was observed producing similar results to the higher rate of 40g per pot and in some instances even gave better results (Table 6.1; Figures 6.1, 6.3, 6.5). Thus the fertilizer rate of 20g DAP per pot confirms what Gaidashova *et al.* (2008) called the optimum nutrient regime. Meyer (2011) has averred that it is crucially important to ensure that the optimum nutrient regime is reached because nutrients are second only to water with regard to the impact they have on sugarcane yields. The high rate of 40g DAP per pot is certainly in the region of diminishing returns and therefore an unnecessary additional cost.

Inoculation of the resistant cultivar KEN83-737 and the moderately resistant D8484 did not lead to significant differences in shoot weights at any of the tested fertilizer rates. However, differences were observed when the susceptible cultivar Co421 was inoculated at fertilizer rates of 10 and 20g DAP per pot. In season 2 however, both D8484 and Co421 produced significantly reduced shoot weights at all fertilizer rates. This reduction however decreased with increase in fertilizer rate with the highest reduction occurring at no fertilizer application and the least reduction at fertilizer rate of 40g DAP per pot.

Results from the two seasons produced similar trends for root weight. Cultivar KEN83-737 had the numbers of nematodes in its rhizosphere thus confirming its resistant status. The largest

population of the parasites was found in the rhizosphere of variety Co421 followed by D8484 the susceptible and moderately resistant cultivars, respectively.

Although large populations of plant parasitic nematodes were found in the rhizospheres of varieties Co421 and D8484, it is these same varieties that responded best to fertilization. Waele and Elsen (2007) observed that fertilizer application improves tolerance to plant parasitic nematodes. What is emerging here is that the tolerance is imparted on the susceptible and the moderately susceptible cultivars but not the resistant. This means that susceptible varieties can be grown in infested soils and be managed through fertilizer application. The rate at which this fertilizer is applied has to be at optimal levels to derive this benefit, a regime that gives the best root development for tolerance to plant parasitic nematodes (Gaidashova *et al.,* 2008) and avoid soil degradation (KESREF, 2002).

6.3.1 Conclusions and recommendations

Fertilizer application on inoculated plants of the resistant cultivar KEN 83-737 produced the least response of its yield components. However, addition of fertilizer to inoculated plants of the susceptible cultivar Co421 produced the highest response of all its yield parameters. Fertilizer application to inoculated plants of Co421 decreased the reduction caused on the yield components by nematode infestation. The effect of nematodes on the yield components was reduced by fertilizer application. This reduction increased with an increase in fertilizer application rate, clearly demonstrating the contribution of fertilization to lowering the impact of nematode damage on the susceptible cultivar. Fertilization therefore probably enhanced the tolerance of Co421 to nematode infestation. This interaction however was not demonstrable for the resistant cultivar KEN83-737. Fertilizer application in sugarcane growing is recommended particularly on soils infested by plant parasitic nematodes. Fertilizer application in sugarcane

growing is recommended especially where susceptible cultivars are used on soils infested by plant parasitic nematodes. The main fertilizer in sugarcane growing areas is Diammonium Phosphate which should be applied at the rate of 20g per stool.

CHAPTER SEVEN

7.0 INTEGRATING HOST RESISTANCE INTERCROPPING AND FERTILIZATION FOR NEMATODE MANAGEMENT IN SUGARCANE PRODUCTION SYSTEMS

7.1 INTRODUCTION

Plant parasitic nematodes (PPNs) are difficult pests to manage in sugarcane production because their effects are largely insidious causing them to be generally ignored. Chemical control in management of not only nematodes but most pests and diseases in general is increasingly coming into scrutiny due to its negative impacts on human health and environment. This has led to concerted global efforts to look for alternative management techniques that are more sustainable and deadlines have been given to discontinue the use of some of the more effective chemicals in favour of Integrated Pest Management (IPM) techniques.

Some of the methods adopted in IPM strategies include use of host resistance, cultural practices, parasites and predators (Bird, 1987). Use of host resistance in managing PPNs in sugarcane has been cited as the main technique for the future (Roberts, 1992; Dinardo-Miranda, 2005). Sugarcane cultivars possessing resistance traits in Kenya have in recent years been released (Chirchir *et al*, 2011) and whereas it is a big positive step, it may be enhanced if it is combined with suitable cultural practices including intercropping with suitable companion crops and judicious use of fertilizers. This study was conducted to demonstrate and document the effect of adopting IPM combination packages that utilize resistant cultivars, appropriate intercrops and recommended fertilizer regimes to improve sugarcane productivity, increase food security and generate extra revenue for small scale sugarcane farmers.

7.2 MATERIALS AND METHODS

7.2.1 Site description

The site is as described in section 4.2.1.

7.2.2 Treatments, experimental design and data collection

Sugarcane cultivars were selected as described in section 4.2.2. Spiderplant earlier categorized in section 5.4.3 was selected. Sugarcane was planted using 3-4 eye-budded setts in furrows each 4 m long. Each plot had 7 rows of cane at a spacing of 1.2 m apart, hence giving a plot size of 33.6 m^2 which were then separated by a 2 m path. Spiderplant was sown as an intercrop between the sugarcane rows and both crops planted at the same time. Plots with no intercrop acted as controls. Diammonium phosphate (DAP) was applied at planting separately for sugarcane and spiderplant each at 100 kg ha^{-1}. Only sugarcane received urea at 200 kg ha^{-1}as topdressing three months after planting (MAP). Control plots did not receive fertilizer. The treatments were arranged in a split-split plot design with sugarcane cultivar in the main plot, intercrop sub-plot and fertilizer in the sub-sub-plot. The experiment was replicated thrice. Soil and root samples were collected from each plot using a soil auger at 0, 9 and 18 MAP. Initial nematode population was determined on newly prepared seedbed just before planting. Eight soil sub-samples were also collected at 9 and 18 MAP from the sugarcane root rhizosphere at a depth of 5-20 cm, mixed to form a composite sample and about 500 g placed in a polythene bag and taken to the laboratory. At harvest, 5 stalks from each of the three central rows were selected at random to form the sample population of 15 stalks per plot.

Girth, stalk length, millable stalk numbers and sugarcane yields were obtained as described in section 4.2.5. The first harvest of spiderplant occurred at 30 days after planting (DAP) and

continued subsequently after every 10 days till the final harvest at 100 DAP. Their individual yields were determined by weighing the harvested shoot material using a weighing balance and then totaling the weights from all the harvests and the final sum expressed as tonnes per hectare. The prevailing price of sugarcane at harvest was adopted from the nearby Kibos sugarcane milling factory while that of spiderplant was adopted from the nearest open air market.

7.2.3 Processing of nematodes

Nematodes were processed in a similar manner as in section 3.2.3.

7.2.4 Data analysis

Data were analysed as described in section 5.2.2.4.

7.3 RESULTS

Sugarcane cultivar, intercrop and fertilizer application led to significant differences ($P \leq 0.05$) in cane yield, quality and yield components among the cultivars (Table 7.1). Cultivar KEN83-737, presence of intercrop and fertilizer application produced the highest yields compared to the other combinations. Presence of either intercrop or fertilizer gave significantly higher ($P \leq 0.05$) values for yield and yield components except girth which was unaffected. The interaction of intercrop and fertilizer produced significant differences ($P \leq 0.05$) in sugarcane equivalent yields (SEY) (Table 7.2). The highest value was observed in the presence of both intercrop and fertilizer while the least was in non-fertilized pure stand of sugarcane. Sugarcane cultivars produced significantly different ($P \leq 0.05$) sugarcane equivalent yields. Cultivars KEN83-737, D8484 and Co421 recorded SEY of 155.8, 131.7 and 99.3, respectively. The yield of spiderplant was significantly influenced ($P \leq 0.05$) by fertilizer application resulting in a mean yield of 5094 compared to 869 without fertilizer. Thus fertilizer application increased its yield by 5.86 times.

However, the results did not indicate any interaction between sugarcane cultivar and intercrop for this yield.

Sugarcane cultivar and presence of either intercrop or fertilizer significantly ($P \leq 0.05$) affected nematode numbers (Figure 7.1). Large populations were found in the rhizospheres of Co421 compared to KEN83-737 and D8484. The presence of either intercrop or fertilizer caused significant reduction in the numbers of nematodes.

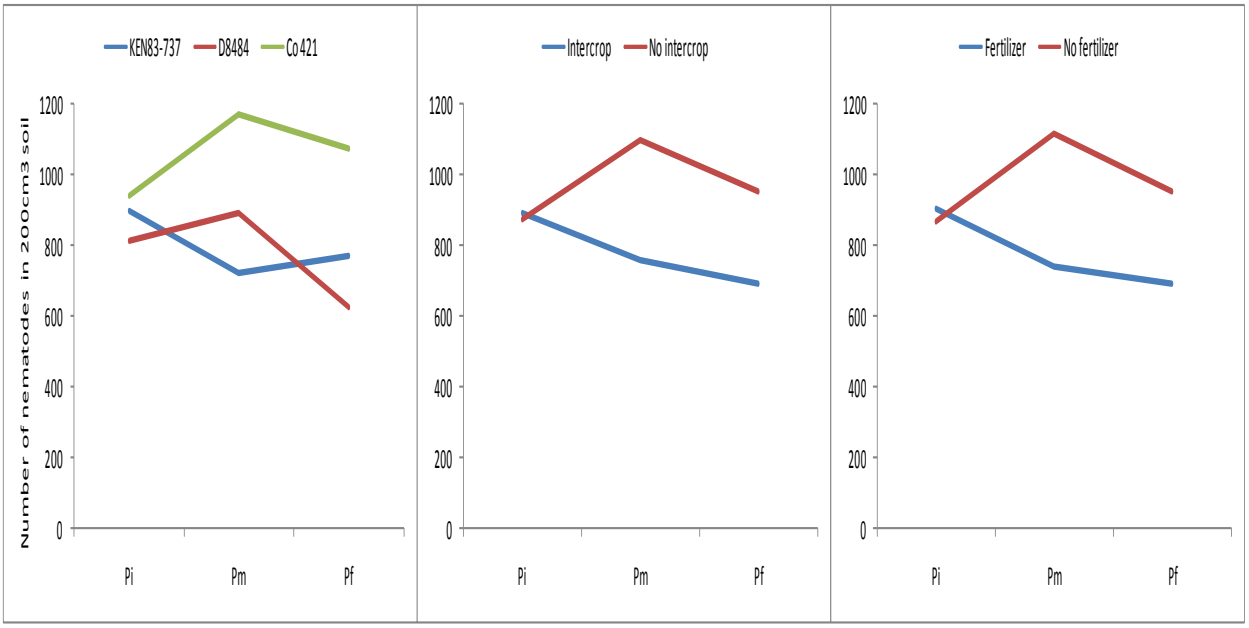
Table 7.1. Effect of sugarcane cultivar, intercrop and fertilizer on cane yield, stalk length and weight, girth and millable stalks.

Factor		Cane yield in tonnes per hectare	Stalk length	Girth	Stalk weight	Millable stalk number
Cultivar	KEN83-737	124.1a	1267.3a	2.8a	13.9a	157.8a
	D8484	101.5b	1147.6ab	2.3b	12.8b	66.2b
	Co421	69.5c	997.4b	2.1c	12.1b	132.7a
Intercrop	Present	107.5a	1250.2a	2.4a	13.9a	133.1a
	Control	89.2b	1024.7b	2.4a	11.9b	104.7b
Fertilizer	Present	115.2a	1310.4a	2.4a	14.8a	130.5a
	Control	81.6b	964.5b	2.4a	11.1b	107.3a

Means with same letters down the column are not significantly different.

Table 7.2. Effect of intercrops and fertilizer on sugarcane equivalent yields.

		Fertilizer	
		Present	Absent
Intercrop	Present	226.7	92.8
	Absent	108.1	70.3
LSD $_{(0.05)}$		9.5	
CV %		10.7	



Pi, Pm and Pf mean initial, median and final nematode populations respectively.

Figure 7.1. Effect of cultivar, intercrop and fertilizer application on nematode populations.

7.4 DISCUSSION

The IPM package adopted in the present study combined the use of host resistance (provided by resistant sugarcane cultivar), a cultural method (intercropping by use of spiderplant) with improvement of tolerance (provided by fertilizer application) to demonstrate its influence on

sugarcane yield. The selection of KEN83-737, spiderplant and appropriate fertilizer rate was based on studies in the earlier chapters. Intercropping of KEN83-737 with spiderplant produced the highest sugarcane equivalent yield (SEY) compared to the other combinations.

This study has demonstrated the influence of incorporating a resistant cultivar and intercrop with fertilizer application in sugarcane production to manage nematodes and improve yields and increase food production. Absence of intercrop caused a drop in sugarcane yield and millable stalks number of 17 and 21%, respectively. On the other hand, lack of fertilizer application caused a reduction in cane yield, stalk length, stalk weight and millable stalks number of 29, 26, 25 and 18%, respectively. If these losses are averted by intercropping and fertilizer application then more food and income can become available to the small scale farmers some of whom are faced with food insecurity and high poverty levels due to low income generation.

An earlier study did recommend suitable combinations as being KEN83-737 × Spiderplant and N14 × Spiderplant on account of their sugarcane equivalent yields. The former combination was selected for the present study and combined with fertilizer to determine their effect on sugarcane equivalent yields. The lowest yield of 70.3 (SEY of 1) was obtained in pure stand plots with neither intercrop nor fertilizer. Intercropping sugarcane with spiderplant without applying fertilizer improved SEY by 1.32 times. Applying fertilizer to the pure stand cane improved SEY by 1.54 times. However, the highest improvement of SEY occurred when sugarcane was intercropped with spiderplant and fertilizer applied which caused an increase in SEY by 3.22 times.

This study has demonstrated through the nematode population trends that using the susceptible cultivar Co421 could lead to buildup of nematode populations which Moura and Almeida (1981) have reported could eventually lead to lower yields.

7.4.1 Conclusions and recommendations

Intercropping of sugarcane with an appropriate food crop together with fertilizer application is necessary for higher yields, food production and income generation. Host resistance against plant parasitic nematodes in resistant and moderately resistant cultivars (KEN83-737 and D8484 respectively) is an important property in developing IPM strategies for sustainable nematode management. Co421, a susceptible cultivar, will most probably cause a buildup of nematode populations and its production in soils infested with PPNs should be discouraged. The intercrop of KEN83-737 × spiderplant with fertilizer applied was shown to be the most appropriate for suppression of PPNs, high cane and intercrop yields, food production and income generation. Thus this combination will contribute to the improvement of household food security and better incomes and hence may improve the socio-economic status of the small- scale farmers. The combination may be recommended for soils infested with plant parasitic nematodes.

CHAPTER EIGHT

8.0 GENERAL DISCUSSION CONCLUSIONS AND RECOMMENDATIONS

8.1 GENERAL DISCUSSION

Adoption of good agricultural practices (GAP) and other new technologies among small holder farmers who are not well educated is on average a slow process that often stalls on occasions and eventually remains among a minority. However, where these are shown to lead to better returns especially in the now highly commercialized global village, it is increasingly taking less effort to disseminate such technologies. It is with this in mind that this study was conceptualized to try and address the declining sugarcane yields in Kenya. Improved methods of production are essential if high yields are to be realized from soils that have been under cultivation for generations and fields that are reducing in size as population grows.

The principle objective of the study was to assess the potential of integrating host resistance, intercropping and fertilization for nematode management in sugarcane production systems. Thus the process began by screening fifteen randomly selected local cultivars for host resistance. These were compared to the effects produced by nematicides application in reducing nematode populations and ultimately improving sugarcane yield and quality parameters.

The reaction of sugarcane genotypes to two principle nematode species *Meloidogyne* spp. and *Pratylenchus* spp. was extensively explored giving rise to clear classifications. The genotypes that showed resistance to *Meloidogyne* spp. were four, KEN83-737, KEN82-216, Co945 and Co617. The moderately resistant were the majority at nine, these were N14, EAK70-97, KEN98-530, CB38-22, KEN00-13, KEN82-121, KEN82-472, KEN82-493 and KEN82-62. The susceptible varieties were Co421 and D8484. On the basis of reaction to *Pratylenchus* spp. the

fifteen genotypes were classified into five groups; the moderately resistant cultivars were CB38-22, KEN82-216, KEN00-13, KEN82-121, Co617, Co945 and N14. The moderately susceptible were KEN82-493, KEN98-530, KEN82-62, D8484 and EAK70-97. Variety KEN82-472 was found susceptible, Co421 highly susceptible and KEN83-737 resistant. There were some unique observations that merit mention. There was positive correlation between growth of genotypes D8484 and EAK70-97 and the *Pratylenchus* spp. counts. This was quite a point that could indicate that these varieties should be recommended for fields infested by the nematode. However, this is not indicative enough and should be investigated further in the field. In soils infested by *Pratylenchus* spp. high yields were harvested from varieties Co 945 and KEN00-13, while KEN82-472, D8484 and EAK70-97 performed well too in the presence high numbers of this nematode. What is emerging is the need to test soils from individual fields for nematode identity in order to recommend suitable cultivars accordingly. For instance, soils infested with lesion nematodes should be planted to genotypes KEN82-472, D8484 and EAK70-97. The susceptible sugarcane cultivar to *Pratylenchus* spp. was KEN82-493.

The reaction of possible companion crops of sugarcane to both *Meloidogyne* spp. and *Pratylenchus* spp. were found to be largely similar. A category of suppressive crops to both nematodes comprised spiderplant, amaranthus and cabbage. However, while slender leaf was found to be suppressive to *Pratylenchus* spp. it was susceptible to *Meloidogyne* spp. On the other hand, African nightshade and Jute mallow exhibited a high level of susceptibility to both nematode species. Among the legumes, both common bean and cowpea showed high susceptibility to *Meloidogyne* spp. and *Pratylenchus* spp.

Kenya's staple food crops are maize and beans and this includes even the sugarcane growing regions, therefore any effort that can improve their production is likely to have a direct impact on

food security in the area thus making them have good potential as sugarcane intercrops. Maize showed that it can be a good companion crop but beans is a good host of plant parasitic nematodes and can aggravate the nematode problem in sugarcane if interplanted, therefore it should be avoided. These findings are pointing at the need to develop a catalogue of sugarcane companion crops based on the findings of soil tests showing the species of nematodes in particular fields.

At the heart of this study was to seek economically viable combinations of sugarcane and intercrop in trying to address sugarcane production constraints thereby increasing yields, incomes and reducing poverty. This concept was well captured using the Cobb-Douglas production function that computes sugarcane equivalent yields of various combinations. Companion crops suitably rated according to their reaction to nematodes were then tried in different combinations to get those with high values of sugarcane equivalent yields. These analyses gave two suitable combinations namely KEN83-737 × spiderplant and N14 × spiderplant.

Fertilizer application in sugarcane production among small holder farmers is limited because of lack of knowhow and limited resources to purchase. This study clearly established the importance of fertilizer in increasing yield. Whereas the resistant genotype requires fertilizer for growth and yield, the susceptible was found to require it for the same in addition to imparting tolerance hence a higher rate is required.

When host resistance, intercropping and use of fertilizer are used independently, the results may be different as compared to when they are used in combination. It was therefore necessary to combine these methods and observe practically what obtains of them. The different combinations

resulted in KEN83-737 × spiderplant showing best potential; however it is necessary to carry out more studies to build a longer list that can be useful to different soil types and across localities.

8.2 GENERAL CONCLUSIONS AND RECOMMENDATIONS

The process of screening the fifteen selected sugarcane cultivars showed that some genotypes possess host resistance against plant parasitic nematodes. The investigation has demonstrated that sugarcane cultivars possessing host-plant resistance or tolerance to plant parasitic nematodes have an inherent ability to reduce the nematode population in the rhizosphere. Further it has been established that the resistant varieties restricted the buildup of nematode numbers.

Sugarcane varieties that showed resistance to *Meloidogyne* spp. were KEN83-737, KEN82-216, Co945 and Co617, whereas nine varieties, N14, EAK70-97, KEN98-530, CB38-22, KEN00-13, KEN82-121, KEN82-472, KEN82-493 and KEN82-62 showed moderate resistance. The remaining two varieties, Co421 and D8484 were susceptible to root-knot nematode. The fifteen sugarcane genotypes were classified into five according to their reaction to lesion nematode; the moderately resistant including CB38-22, KEN82-216, KEN00-13, KEN82-121, Co617, Co945 and N14. The moderately susceptible were KEN82-493, KEN98-530, KEN82-62, D8484 and EAK70-97. The susceptible and highly susceptible varieties were KEN82-472 and Co421, respectively. Cultivar KEN83-737 was found to be resistant. The tendency of genotypes D8484 and EAK70-97 to produce heavier shoots under infestation of lesion nematodes merits further investigation.

There is need to test soils from individual fields to identify nematodes in order to recommend suitable cultivars accordingly. The concept of soil testing is new and hard to sell to small grower farmers but there is need to promote it for the sake of overcoming plant parasitic nematodes.

Three categories of companion crops of sugarcane in relation to *Meloidogyne* spp. were established. These were the suppressive to the herbivore, spiderplant, amaranthus and cabbage; the moderately suppressive were kale and maize; and the susceptible; African Nightshade, Jute mallow, slender leaf and cowpea. These categories were remarkably similar to the ones arising from reaction to *Pratylenchus* spp. These were as follows; suppressive were Spiderplant, amaranthus, slender leaf and cabbage while the susceptible were African Nightshade, Jute mallow and bean.

When the intercrops were combined with selected sugarcane genotypes and returns compared using sugarcane equivalent yields, KEN83-737 × spiderplant and N14 × spiderplant showed optimum values which coupled with reaction to nematodes make them suitable combinations for adoption by farmers. However, there was need to include fertilizer to improve tolerance in the susceptible variety. The susceptible cultivar Co421 responded well to a high rate of fertilizer application (40g DAP per pot) while the moderately susceptible D8484 and the resistant KEN83-737 required 20g DAP per pot to achieve optimum growth. Fertilizer application in sugarcane growing is recommended, and in particular where susceptible cultivars are used on soils infested by plant parasitic nematodes. The main fertilizer in sugarcane growing areas is Diammonium Phosphate which should be applied at the rate of 20g per stool.

The combination of KEN83-737 × spiderplant with fertilizer was found to be the more favourable in this study. It is a combination that is potentially beneficial economically to farmers and to soils especially in fields that are infested by plant parasitic nematodes and exhausted of nutrients. More combinations ought to be tested to increase the choices available because there are differences between locations and even individual fields.

It is recommended that a catalogue of sugarcane companion crops be developed based on species of nematodes present in soil and host resistance status of both the main crop and its companion. Soil testing should be promoted among small holder farmers so that the catalogue developed can make sense. There is need to refine the scale so as to clearly distinguish the five categories of sugarcane genotypes based on their reaction to plant parasitic nematodes, these are resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible.

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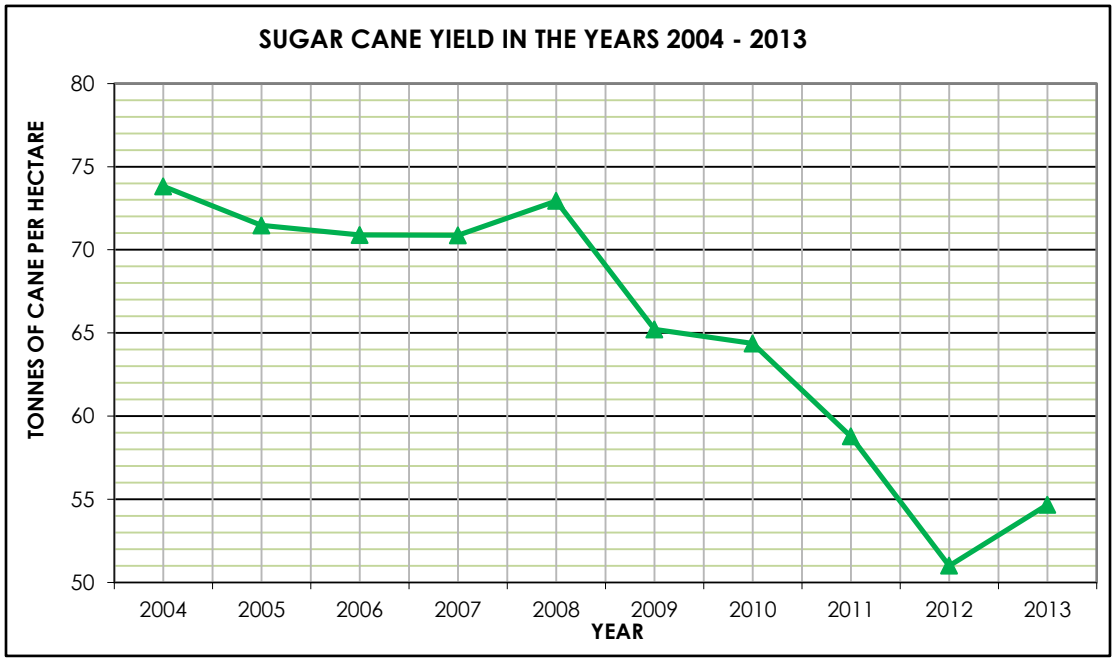
APPENDICES

Appendix 1. Sugarcane yields from 2004 - 2013.



Appendix 2. Summary analysis output for chapter 3.

Variable	Season 1				Season 2		
	Degrees of freedom	Mean square	Error mean square	F - value	Mean square	Error mean square	F-value
Shoot height	31	106.1	38.6	2.74	146.4	6.7	21.85
Fresh shoot weight	31	1207.4	318.5	3.79	2092.7	14.6	143.7
Dry shoot weight	31	157.8	25.1	6.29	103.7	11.2	9.2
Tiller count	31	3.1	0.7	4.15	2.6	0.5	4.8
Fresh root weight	31	594.9	145.7	4.08	453.1	10.5	43.3
Nematode count	31	1745.4	64.8	26.9	1695.7	49.7	34.1
Egg mass index	31	3.4	0.2	20.07	2.5	0.2	12.8

Appendix 3. Scale used to classify sugarcane cultivars based on their reaction to *Pratylenchus* spp.

Resistance status	Damage severity Score
Resistant	≤ 1.0
Moderately resistant	1.1 – 1.9
Moderately susceptible	2.0 – 2.9
Susceptible	3.0 – 3.9
Highly susceptible	≥ 4.0

Appendix 4. Summary analysis output for chapter 3.

Variable	Season 1				Season 2		
	Degrees of freedom	Mean square	Error mean square	F - value	Mean square	Error mean square	F-value
Shoot height	31	647.8	121.0	5.35	305.8	22.1	13.81
Fresh shoot weight	31	21439.1	5018.5	4.27	11857.0	2013.3	5.89
Dry shoot weight	31	1301.2	258.2	5.04	897.2	123.8	7.24
Tiller count	31	5.99	1.48	4.04	3.21	0.94	3.4
Fresh root weight	31	2150.9	804.6	2.67	2137.8	627.3	3.41
Nematode count	31	1919.39	227.7	8.45	2190.5	461.4	4.75
Final population	31	1199621.9	141940.1	8.45	1369048.6	288379.8	4.75
Reproductive factor	31	2.1	0.26	8.04	2.4	0.5	4.79

Appendix 5. Summary analysis output for chapter 5.

Variable	Season 1				Season 2		
	Degrees of freedom	Mean square	Error mean square	F - value	Mean square	Error mean square	F- value
Shoot height	21	1088.9	2.0	546.1	9242.4	112.7	82.0
Fresh shoot weight	21	4976.9	37.1	134.0	36642.6	1059.6	34.6
Dry shoot weight	21	234.2	3.7	63.2	2127.5	46.9	45.4
Fresh root weight	21	723.5	31.3	23.1	528.5	1.9	284.7
Damage severity	21	5.5	0.2	33.0	1.9	0.2	8.4
Nematode count	21	67970.1	974.0	69.8	35522.0	1445.3	24.6

Appendix 6. Summary analysis output for chapter 5.

Variable	Degrees of freedom	Mean square	Error mean square	F – value
Tonnes cane per hectare	10	615.0	148.2	4.2
Fresh leaf weight of Spiderplant	10	31.0	0.3	91.5
Fresh leaf weight of African Nightshade	10	17.7	0.5	35.2
Dry leaf weight of African Nightshade	10	0.94	0.9	10.1
Millable stalk number	10	2992.6	254.0	11.8
Stalk length	10	931.5	88.8	10.5
Median nematode count (9 months after planting)	10	752488.0	165999.0	

Appendix 7. Summary analysis output for chapter 6.

Variable	Season 1				Season 2		
	Degrees of freedom	Mean square	Error mean square	F - value	Mean square	Error mean square	F-value
Shoot length	25	414.0	9.2	44.9	521.9	6.5	80.1
Shoot weight	25	542.9	42.1	12.9	568.9	18.4	30.8
Tiller counts	25	2.9	0.3	10.0	3.2	0.3	10.1
Root weight	25	77.2	4.8	16.2	47.9	5.3	9.0
Nematode count	13	222934.0	3464.3	64.4	234969.4	2448.5	96.0

Appendix 8. Summary analysis output for chapter 7.

Variable	Degrees of freedom	Mean square	Error mean square	F -value
Tonnes cane per hectare	11	2921.8	146.3	20.0
Sugarcane equivalent yields	11	13065.0	189.1	69.1
Brix	11	5.7	0.4	13.0
Stalk length	11	230560.7	68001.2	3.39
Stalk weight	11	17.5	0.9	20.4
Girth	11	0.3	0.01	34.3
Millable stalk number	11	7073.9	1394.7	5.1
Initial nematode count	11	66135.1	65219.3	1.01
Median nematode count	11	337751.9	11912.7	28.4
Final nematode count	11	321342.9	12627.4	25.45