EVALUATION OF ROOT- KNOT NEMATODE MANAGEMENT STRATEGIES BASED ON NEMATODE DISTRIBUTION IN TOMATO (*SOLANUM LYCOPERSICUM*) FIELDS IN MWEA, KIRINYAGA COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree or any other award in any other university.

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I dedicate this work to my supportive supervisors, friends and family.

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ABBREVIATIONS AND ACRONYMS

GPS	Global positioning system
J2	Root-knot Nematode second stage juvenile
PPN	Plant parasitic nematode
RKN	Root-knot nematode
SSM	Site specific management
SEM	Standard error of mean
GOK	Government of Kenya
KARI	Kenya Agricultural Research Institute (now Kenya Agricultural and Livestock Research Organization. – (KALRO)
KEPHIS	Kenya Plant Health Inspectorate Service
AVDRC	Asian Vegetable Research and Development Center
ICIPE	International Centre for Insect Physiology and Ecology

ABSTRACT

Plant parasitic nematodes (PPN), particularly root-knot nematodes (RKN) are a serious pest problem in smallholder tomato farms in Kenya. For sustainable food production, effective management of plant parasitic nematodes is essential. Root-knot nematode management is primarily dependent on the application of chemical nematicides and the use of resistant varieties. However nematicide application is often done uniformly regardless of the relative nematode distribution in the farms, and yet it is a well-documented fact that plant parasitic nematodes particularly RKN are irregularly distributed due to their reproductive patterns and low mobility. Therefore, identification of specific nematode infested areas within individual fields for targeted RKN management strategies may allow producers to maximize yields, lower production costs and ultimately be less detrimental to the environment. This study evaluated the use of RKN resistant varieties and the application of nematicide under varying nematode densities in Mwea, Kenya. Nematode resistant tomato varieties; Assila, Sandokan and a susceptible variety, Rio-grande in combination with varying applications of a commonly used nematicide Mocap (Ethoprophos) at the rate of 1g, 2g and 4g per plant applied at planting, were evaluated. Areas with 5 to 12 and 32 to 108 RKN per 200 cm^3 of soil were chosen to represent low and high RKN densities respectively. The General Linear Model Procedure for univariate analysis of variance analysis was used to test for significant main and interaction effects. The Tukey's HSD (honestly significant difference) test was used to separate the means. From the baseline survey, a total of 12 genera of plant parasitic nematodes were identified The most abundant plant parasitic nematode genera found were the root-knot nematodes (*Meloidogyne* spp.), followed by the lesion nematodes (*Pratylenchus* spp.) with a maximum of 110 and 116 nematodes per 200 cm^3 of soil, respectively. Mocap application at the recommended rate of 2 g per plant did not significantly ($p>0.05$) reduce the RKN population and did not eliminate the RKN under both field and greenhouse conditions. In greenhouse experiments, the addition of 4 g of Mocap per plant at planting however was shown to significantly reduce the nematode infestation and ultimately increase yields under high RKN densities with the variety Asilla recording the highest yields of 2,125.7g per plant. The variety Rio Grande recorded the lowest yields of 383.6g per plant under the same conditions. The application of Mocap at the recommended rate of 2gm per plant significantly reduced the numbers of second stage juveniles (J2s) observed at the root zones of sampled plants but did not significantly reduce ($p<0.05$) the galling index observed on the plants. The variety Assila, combined with the application of 4 gm per plant of Mocap was found to be the most cost effective management strategy for RKN under both high and low RKN densities. According to the findings of this study, it is recommended that farmers intending to plant tomato in the Mwea begin by having their soils tested for the presence of RKN and apply the nematicide Mocap at 4g per plant only on areas where there are either high or low RKN densities. It is also recommended that they plant RKN resistant varieties such as Asilla in such areas. It is is however not economical to apply these measures to areas with no RKN incidences.

CHAPTER ONE

INTRODUCTION

1.1 Background

Agriculture is the mainstay of the Kenyan economy and currently contributes 29% to GDP and employs over 80% of the rural population (Odero et al., 2015). The agricultural sector is made up of four major sub-sectors, namely, industrial crops, food crops, horticulture, livestock and fisheries. Horticultural crops contribute 38.5 % of agricultural exports and 33 % of Agricultural GDP and are therefore important for food security (GoK, 2007). The development of Kenya's agricultural potential is one of the key objectives of the Kenya Vision 2030 (GoK, 2007).

Agriculture plays an important role in the development of the economy of Mwea, Kirinyaga County of Central Province, where rice and tomatoes are the major crops. Although rice farming is the major agricultural activity in Mwea, rice farmers have also ventured into horticultural farming as well. In Mwea, tomato production is mainly produced under furrow irrigation. The two main areas of tomato production in Mwea are Kiumbu and Kiamanyeki locations.

Tomato (*Solanum lycopersicum* L.) is an important crop for majority of smallholder growers of Mwea. The major challenge in tomato production in Mwea is the reduction of yield and abandonment of land after intensive tomato production due to infestation by root-knot nematodes (*Meloidogyne* spp). This is

because tomato is the most favorable host for RKNs (Dropkin, 1980; Waiganjo *et al.,* 2006). Under furrow irrigation, farmers in Mwea have reported nematode to be second most serious limitation to tomato production after bacterial wilt while farmers in Kibirigwi area in Mwea region ranked nematodes as the most serious tomato production constraint under similar conditions (Musebe *et al.,* 2005). Effective management of RKNs is required for profitable tomato production (Kariuki *et al.,* 2006). In Kenya, the use of nematicides, biological control, cultural practices and pest-resistant varieties to reduce crop loses have been on a small-scale and often irregularly due to low level of nematodes awareness (Kimenju *et al.,* 2008). In Mwea farmers have reported low efficacy of both chemical control at the recommended rate, and cultural methods in controlling nematodes, other pests and diseases (Musebe *et al.,* 2005). Effective control methods are lacking or are poorly and ineffectively applied (Oduor-Owino *et al.,* 1995).

Problem Statement

Tomato is one of the most important vegetable in Kenya (Da Silva *et al.,* 2008). However the major tomato is plagued by constraints including diseases such as bacterial wilt, early and late blight, leaf curl, tomato spotted wilt virus, leaf spot and powdery mildew, (Varela *et al.,* 2003), insect pests and other arthropods (KARI, 2005) and nematodes, blossom end-rot and poor crop management practices, especially the lack of crop rotation (Waiganjo *et al.,* 2006). Root-knot nematodes (RKN) are among the most serious pests of vegetables in Kenya and

effective control methods are lacking or are poorly and ineffectively applied (Oduor-Owino *et al.,* 1995). Although crop rotation and other cultural practices are used to some extent to manage nematodes in tomato, nematode control is primarily dependent on the application of chemical nematicides (Radwan *et al.,* 2012). These nematicides are often applied uniformly on the cultivated fields regardless of the relative nematode distribution in the farm (Mueller *et al.,* 2010). This practice results in increased costs of production as well as adverse environmental effects. This study therefore aimed at evaluating several RKN management strategies as alternatives to large scale nematicide application and identify the one which is most cost effective strategy.

Justification

Nematode management methods depend very much on edaphic factors since edaphic factors determine the distribution of nematodes and therefore their abundance at the plant rhizosphere. Tomato growers in Mwea rely heavily on application of nematicides at planting for nematode control (Waiganjo *et al.,* 2006). Unfortunately this nematicide application is done randomly and may provide only partial nematode control, especially where densities are high (Mueller *et al.,* 2010). This study was therefore designed to evaluate the effectiveness of a combination of host resistance and chemical nematicide (ethoprophos) application rates against varying RKN densities.

1.3.1 Main objective

The main objective of this study was to evaluate the effect of different Root Knot Nematode management strategies on tomato crops under varying nematode densities and identify the most cost effective RKN management option

1.3.2 Specific objectives

i. To identify and determine the relative densities of RKN associated with tomato production in Mwea region.

ii. To determine the effects of different nematode management strategies on varying RKN densities and the effects on the yields of selected tomato varieties

iii. To identify the most cost effective nematode management strategies under varying RKN densities as an alternative to blanket nematicide application

1.4.3 Null hypotheses

i. RKN are not associated with tomato production in Mwea and are not uniformly distributed.

ii. The use of RKN resistant tomato varieties as well as applications of Ethoprophos as RKN management strategies have no effect on RKN populations or yields of tomato plants.

iii. Varying the RKN management techniques according to nematode densities cannot be used as a cost effective alternative to area-wide nematicide application.

1.4.4 Significance of study

In Mwea farmers have reported low efficacy of both chemical control at the recommended rate, and cultural methods in controlling nematodes, other pests and diseases (Musebe *et al.*, 2005). Hence effective control methods are lacking or are poorly and ineffectively applied. This study was therefore designed to evaluate the effectiveness of a combination of host resistance and chemical nematicide (Mocap) application rates against varying RKN densities.

CHAPTER TWO

LITERATURE REVIEW

2.1 The tomato plant

Tomato (*Solanum lycopersicum* L.) belongs to the genus *Solanum.*, and is grown for its edible fruit. *Solanum lycopersicum* L. was formerly known as *Lycopersicum esculentum*, the name used from 1768 to 2005. In 2005 Spooner *et al.* proposed a change back to the original nomenclature used by Linnaeus (1753). The genus *Solanum* of the family *Solanacae* is believed to have originated in the coastal strip of western South America from the equator to about latitude 30° South (Jones, 2008). The species is native to South America, especially Peru and the Galapagos Islands, having been first domesticated in Mexico. In the mid- 16^{th} century, the tomato was introduced in Europe where it was primarily used as part of herbal remedies; it was thereafter introduced into Africa (Da Silva, 2008). The crop can be grown under different conditions, but the most suitable are high altitudes, with low humidity and high luminosity. Among the diverse climatic factors, temperature merits emphasis, because higher net assimilation rate, that is, greater growth efficiency, is observed when the temperature is between 18°C and 28°C although temperature requirement of the tomato plant varies with the development of the plant (Jones *et al.*, 1991).

The tomato has diversified uses such as fresh salad, cooked foods and in processed forms like ketchup, pickles and sauce. It is highly prized for its good financial

returns and nutritional value especially for its richness in vitamins and minerals (Brown and Hutchison, 1949; Hobson *et al.,* 1979; Baloch, 1994; Da Silva *et al.,* 2008). Tomatoes contain carotenoids which are important to humans because of their nutraceutic property. The carotenoid lycopene is responsible for the red colour of the fruit, constituting 75-83 % of the total carotenoids and known to reduce the incidence of prostate cancer, heart and age related diseases (AVDRC, 2003). The β-carotene pigment is responsible for the yellowish colour and represents 3-7 % of the total weight (Dorais *et al.,* 2001).

2.2 Root-knot nematodes (RKN)

Plant parasitic nematodes are a major constraint to agricultural production worldwide (Saxena, 2004; Luc *et al.,* 2005). General symptoms of nematode infection include chlorosis, wilting, galling of roots and tubers, stunted growth, root lesion and yield loss. For sustainable food production, effective management of plant parasitic nematodes is essential. Among the plant parasitic nematodes, root-knot nematode (RKN) are the most important especially in the tropics. Of the RKN, *Meloidogyne incognita* (Kofoid and White, 1919; Chitwood, 1949) is the most widespread and probably the most serious plant parasitic nematode pest of tropical and subtropical regions throughout the world (Trudgill and Blok 2001). The genus *Meloidogyne* (Goldi, 1892) occurs as a pest on a very wide range of crops globally causing up to 5 % yield losses (Cetintas and Yarba, 2010) and an estimated annual loss of $157 billion globally (Abad et al., 2008). Root-knot

nematodes (*Meloidogyne* spp.) are capable of severely damaging a wide range of crops, in particular Vegetables, leading to dramatic yield losses mainly in tropical and sub-tropical agriculture (Sikora and Fernandez, 2005; Sahebani and Hadavi, 2008). They form synergy with plant pathogenic fungi and bacteria causing great yield loss (Rivera and Aballay, 2008). *Meloidogyne incognita* is reported to be a major limiting factor in commercial production of Vegetables in India and is responsible for 15-60 % yield loss (Sharma *et al.*, 1992; Bhatti *et al.*,. 1977; Reddy *et al.*, 1985). In Kenya, losses have been reported to be principally due to *M. incognita* and *M. javanica* (Kanyagia, 1980).

RKN have complex intimate interactions with their host plant throughout the life cycle. RKN hatch as second stage juvenile larvae (J2) from the large number of eggs released by the mature females to the surface of the root. Once hatched, the larvae can survive in the soil for a long period of time by utilizing their stored lipid reserves (Bird and Kaloshian, 2003). Their longevity in soil is dependent on prevailing soil temperatures. In a study by Tsai (2008) to test the survival of *M. incognita* second-stage juveniles under different soil temperatures, the longest survival was 380 days at 15°C and the shortest was less than 4 h at 45°C.The developmentally arrested J2 larvae penetrate the root from the elongation zone close to the meristematic zone or at the site of an emerging lateral root and then migrate intercellularly to the root apex (Wyss *et al.*, 1992; Lohar and Bird, 2003).

2.3 Symptoms of RKN

Plants infected with *Meloidogyne* spp. show typical symptoms of root galling. Some infected plants exhibit deficiency symptoms, particularly similar to that of nitrogen. Above-ground symptoms are often mistaken for nutrient deficiency, reduced growth and the presence of fewer, small, pale green or yellowish leaves that tend to wilt in warm weather but recover during cooler conditions (incipient wilting). Blossoms and fruits are few and of poor quality. Affected plants usually will continue growing and they are rarely killed prematurely, except when they occur in complexes with other disease causing pathogens like *Fusarium* spp. (Abawi and Barker, 1984; Suleman *et al.*, 1997). Characteristic symptoms of the disease appear on the underground parts of the plants. Infected roots develop the typical root-knot galls that are two to several times larger in diameter than the healthy roots (Figure 1.1 and 1.2). Several infections along the root give the root a rough, clubbed appearance. Roots infected by certain species of the nematode also develop a bushy root system. Usually, infected roots remain smaller and show necrosis and rotting, particularly late in the season. When tubers or other fleshy underground organs, such as carrots, potatoes, peanuts, and yam, are attacked, they produce small swellings over their surface, which become quite prominent and cause distortion or cracking. Roots of trees such as figs, guava, mango, papaya, pomogrenates and olives are also attacked by the RKN and develop galls roughly proportional in size to the length of time since infection (McSorley, 1981; Milne,

1982a; McSorely, 1992; Mani *et al.,* 1995; Fernandez Diaz Silveira and Ortega Herrera, 1998; Sasanelli *et al.,* 2002; Castillo *et al.,* 2003;).

Figure 2.1. Root galling in tomato roots caused by *Meloidogyne* spp. Picture taken in tomato field at Mwea, Riambogo area.

Figure 2.2. Healthy tomato roots. Picture taken at Mwea Riambogo area.

2.4 Host range of root-knot nematodes

Root-knot nematodes have a potential host range of more than 3000 species (Abad *et al.*, 2003). *Meloidogyne incognita* is also a major economic pest of food legumes in the tropics and subtropics. Many crops grown as Vegetables are susceptible to the nematode particularly tomato, aubergine, okra, cucumber, melon, carrot, gourds, lettuce and peppers. Severe Vegetable damage by RKN in Kenya has been reported, with infected plants rendered unacceptable for export (Powers *et al.*, 2005). Sasser (1990) reported the prevalence of RKN in tomatoes causing severe losses.

2.5 Root-knot nematodes management options

A number of strategies have been used to control plant parasitic nematodes with varying success. These include the use of chemical nematicides, cultural (fallowing, cover copping, crop rotation and the utilization of organic soil amendments), biological control and the use of resistant varieties (Bridge 1996; Coyne *et al.*, 2009). Concerns about environmental degradation through the use of synthetic agrochemicals (Bell, 2000) have in the recent years necessitated the search for sustainable, effective and environmentally acceptable nematode management options (Eapen *et al.*, 2009).

Development and deployment of some of these methods for management of RKN are however limited under some circumstances due to the major limitations associated with them. These include the lack of arable land, challenging

topography as well as inadequate knowledge about the actual species infesting a particular area (Mostafa, 2000). The use of cover crops to control nematodes for example, is uncertain because knowledge of nematode densities and genera present, and the susceptibility of potential cover crops are largely unknown (Abbasi *et al.,* 2005). A cultural practice such as fallowing is useful but can be restricted in adoption mainly due to scarcity of arable land and loss of production during the fallowing while flooding is limited due to topography and scarcity of water resources (Sikora *et al.,* 2005).

Historically, growers have always utilized field-wide nematode management strategies largely because of the ease of application and their inability to map areas infested with plant parasitic nematodes (Evans *et al.,* 2002). Although nematicides are generally applied field wide at a single rate, the population densities and overall distribution of plant parasitic nematodes can be highly variable and spatially aggregated in fields (Barker and Olthoff, 1976; Starr *et al.,* 1993; Wrather *et al.,* 2002; Wyse-Pester *et al.,* 2002; Monfort *et al.,* 2007).

This area-wide, single – rate application approach of nematicide is highly inefficient because some areas in these fields may have nematode population densities below economic or damage threshold levels while other areas may have severe problems. Therefore, nematicides are either over- or under-utilized in many areas throughout the field. In addition, despite the effectiveness of chemical

nematicides, they are often too expensive and are currently restricted in use since most of the effective nematicides are being phased out due to environmental and health concerns (Walker, 2004).

2.5.1 Biological control

Biological control can be achieved by treating nematode infested soil with several biological control agents (Sikora, 1997; Hallmann *et al.,* 1998). Although biological control is a viable alternative to pesticides, difficulties in mass production, imbalance in biodiversity and unaffordability by smallholder farmers limit its adoption (Kiewnick and Sikora, 2004; Tiyagi and Shamim, 2004). Integration of biological control agents and organic amendments has registered some success. For example, Rao and Reddy (1996) reported the benefits of integration of neem with fungal biocontrol agents *Paecilomyces lilacinus* and *Verticillium lecanii* for the integrated management of RKN on tomato. The integration decreased the requirement of biocontrol agents and increased their efficacy under varying nematode densities

Goswami *et al.* (2006) studied the effect of two fungal bioagents along with mustard oil cake and the nematicide carbofuran (furadan) against the root-knot nematode, *M. incognita* infecting tomato. Bio-agents, viz. *P. lilacinus* and *Trichoderma viride* alone or in combination with mustard cake and “furadan” promoted plant growth, reduced number of galls per plant, egg-masses per root

system and eggs per egg-mass. The fungal bioagents along with mustard cake and nematicide showed least nematode reproduction factor as compared to untreated infested soil.

Rangaswamy *et al*. (2000) evaluated the efficacy of *P. lilacinus* and *T. viride* and botanicals (neem and castor cakes) in suppressing the root-knot nematode, *M. incognita,* in tomatoes. *Pasteuria penetrans* alone or in combination with neem cake parasitize the nematode juveniles and adult, whereas *Trichoderma viride,* alone or in combination with either neem or castor cake was most effective in parasitizing the egg-masses of the nematode. In a field trial on assessing the efficacy of *P. penetrans* and nematicides, viz. carbofuran and phorate (a systemic insecticide) in the management of the root-knot nematode, *M. incognita*, as an integrated approach, Kumari and Sivakumar (2005) reported that the combination of carbofuran and *P. penetrans* resulted in higher rate of parasitization (83.1%) than in the *P. penetrans* alone (61.0%). They also recorded higher plant growth in the combination treatment than in all other uncombined treatments. Since root-knot nematodes are difficult to manage, it is necessary to adopt practices aimed at integrated nematode management (Ferraz *et al*., 2010; Kumar *et al*., 2006).

Pochonia chlamydosporia Zare, (syn. *Verticillium chlamydosporium)* is a facultative parasite of nematode eggs, and has been extensively investigated as a potential biological control agent for cyst and root-knot nematodes (De Leij and

Kerry, 1991; Kerry *et al.*, 1993; Rao *et al.*, 1997c, 1998a, 2003; Kerry and Bourne 1996; Kerry 2000). Varying application rates of this fungus as a biocontrol agent of RKN have been tested (Amer – Zareen, 2001). *Trichoderma viridae* has been used as an important biocontrol fungus (Papavizas, 1985) against a wide variety of nematodes, while *P. lilacinus* and *P. chlamydosporium* have long been identified as egg parasites and are associated with suppression of RKN and cyst nematodes (Jatala *et al.*, 1979; Kerry *et al.*, 1982).

Certain nematicides can easily be integrated into IPM strategies without adversely affecting their effectiveness. Biological control agents such as the bacterium *P. penetrans* may be used together with non-fumigant nematicides for nematode control (Siddiqui and Mahmood, 1999; Kariuki *et al.*, 2007a; Kariuki *et al.*, 2007b). Organophosphate (such as Ethoprop) and carbamate nematicides tend to be nematistatic in action rather than nematicidal (Desaeger, *et al.*, 2011). At low application rates they are likely to aid biological control by prolonging exposure of nematodes to antagonists or, in the case of facultative parasites, reduce nematode population densities to levels where the parasites can be effective (Carpenter, 1981)

2.5.2 Crop rotation

Crop rotation is a classical cultural approach that has enormous potential as a sustainable strategy in the management of plant parasitic nematodes (Ball-Coelho

et al., 2003). Incorporation of crops that trap or are poor hosts or antagonistic to nematodes in a rotation program have been shown to reduce the initial nematode population thus allowing the subsequent crop to establish before nematode populations build-up to economic threshold levels (Oka and Yermiyahu, 2002; Mweke *et al.*, 2008). This management method has however also exhibited some unreliable results (Sikora and Fernandez, 2005).

Different species comprising a given nematode population often differ from location to location which can seriously hinder the establishment of an effective crop rotation as the host status of each crop will differ depending on the nematode species present. An example of such a phenomenon was reported in Côte d'Ivoire where *Crotalaria* was recommended as an intercrop to control *Meloidogyne* spp. on pineapple. The intercrop proved effective in controlling RKN but increased the populations of *Pratylenchus brachyurus* to levels which were at least as harmful to the crop as *Meloidogyne* spp. (Luc *et al.*, 2005)

Root knot nematodes are also extremely polyphagous, therefore, relatively few non-host plants are available for control through crop rotation (Sikora and Fernandez, 2005). There are also many reports of *Meloidogyne* populations parasitizing plants which have been reported as non-hosts (Netscher and Taylor, 1979).

2.5.3 Use of organic soil amendments

Organic soil amendments such as organic matter from antagonistic plants *Tagetes minuta, Ricinus communis,* and *Datura stamonium* have been reported to possess nematicidal properties and increase crop yields significantly (Oduor-Owino *et al.,* 1996; Kimenju *et al.,* 2004; Akinyemi *et al.,* 2009). Organic amendments are environmentally acceptable but the large quantities required per unit area renders the strategy not practical in large scale farming enterprises. The employment of phytochemicals in food production could offer another sustainable management option due to the presence of nematicidal properties in many higher plants (Chitwood, 2002).

Amending the soil with commonly available parts and products of neem is also a common method used against RKN affecting tomatoes. Neem cake has been traditionally used by farmers for nematode control on Vegetable crops and cardamon in India and Kenya (Ahmed and Kopel, 1986 and ICIPE, 1998-99). Research has confirmed that neem cake has the potential to effectively control nematodes of *Meloidogyne* species (Bertrand and Lizot, 2000)

Plants antagonistic to nematodes are those considered to produce anti-helminthic compounds with different modes of action (Pandey *et al.,* 2003). Marigolds, (*Tagetes* species) which exude polythienyls have been proven to be nematicidal. *Tagetes erecta* for example, lowered levels of burrowing (*Radopholus similis*)

Cobb, spiral (*Helicotylenchus multicintus*) Cobb and lance (*Hoplolaimus indicus* Sher nematodes when intercropped with a highly susceptible banana crop (Wang *et al.*, 2007). Other crops such as *Mucuna pruriens* and *Tithonia diversifolia* have been used as an antagonistic plants with varying degrees of successes (McSorley and Gallaher, 1992; Rodríguez–Kábana *et al.,* 1992; Quénéhervé *et al.,* 1998; Akinyemi *et al.,* 2009).

2.5.4 Host resistance

The most effective nematicides have in the past been restricted in agriculture because of high risks to human health and the environment (Thomas, 1996) prompting the development of alternative management options. Genetic resistance of tomato to RKN is considered efficient in reducing RKN populations, thereby reducing the need for pesticides application (Medina-Filho and Tanskley, 1983; Roberts *et al.,* 1986; Danso *et al.,* 2011). Host resistance is the most practical alternative to the use of nematicides (Da Conceicao *et al.,* 2005). The most important source of the resistance is conferred by the *Mi* family of genes from the wild tomato *Lycopersicon peruvianum*, providing effective resistance against *Meloidogyne* species (Hadisoeganda and Sasser, 1982).

In tomato, 9 genes, *Mi-1* to *Mi-9*, for resistance to RKN have been reported (Peng and Tang 2001; Ammiraju *et al.,* 2003). All of them originate from wild species and only *Mi-1* has been wildly used to control the disease in cultivated tomato (Williamson 1998; Wang *et al.,* 2001; Yaghoobi *et al.,* 2005). However, *Mi-1* is

inactive above 28°C soil temperature (Williamson 1998) and doesn't always function under varying nematode densities (Philis and Vakis 1977; Jacquet *et al.,* 2005). New populations which can infect tomato lines carrying *Mi-1* gene have also been reported in Greece (Tzortzakakis and Gowen 1996; Tzortzakakis *et al.,* 2005). *Mi-2, Mi-3, Mi-4, Mi-5, Mi-6,* and *Mi-9* are heat-stable but haven't been successfully transferred from wild species to cultivated tomato due to cross incompatibility (Wu Wen-wen, *et al.,* 2009).

Applications of a single nematode management option such as the application of single chemical formulations have been shown to be either completely ineffective or minimally effective. Nematode management in the future is unlikely to rely on one type of measure, and will in addition require to be applied as needed to minimize wastage. This will necessitate the development of site specific nematode strategies that can be recommended based on nematode densities in a particular farm. Management will require the logical use of effective control methodologies in combinations that are economically acceptable to the grower (Sikora *et al.,* 2005).

2.5.5 Chemical Nematicides

Nematode control is very difficult and relies heavily on the use of soil fumigants and non-fumigant nematicides. Generally, nematicides are the most effective in the management of nematode population in a short time (Radwan *et al.,* 2012). Use of nematicides for the management of plant parasitic nematode population in soil

becomes essential when other methods like cultural practices, resistant varieties and biocontrol agents are unable to protect crops from these pests (Hague and Gowen, 1987).

Nematicides are commonly used in developed cropping systems and may directly kill nematodes or are effective by paralysing the nematodes for a variable period of time (nematistatic). Nematicides may be fumigants or non-fumigants and can be classified according to their mode of action. Fumigant nematicides mostly consist of compounds based on halogenated hydrocarbons (1,3-D and methyl bromide) and those which release methyl isothiocyanate (metham sodium and dazomet). They are mostly used during pre-planting, and most of them are liquids which enter the soil as a gas phase. In most cases the fumigants are broad-spectrum contact nematicides effective against adults, juveniles and eggs as well as other pests, diseases and weeds and have significant effects on non-target organisms, including the natural enemies of nematodes. Non-fumigant nematicides are organophosphate (such as fenamiphos, ethoprophos and fosthiazate) and carbamates (such as aldicarb, carbofuran and oxamyl), which are applied to the soil, at planting time, as granular or liquid formulations that are water soluble (Haydock *et al.*, 2006). These have either contact (nematistatic) effects, or some plant systematic activity against nematodes and insects. At low concentrations, they disrupt chemoreception and the ability of nematodes to locate their host roots while at higher concentrations, they disrupt nematode hatching and movement, but

do not kill eggs (Haydock *et al.*, 2006). At field rates, the biochemical effect is reversible (Wright, 1981). Hence, to improve the effectiveness, nematicide concentration and time of exposure must be maximized by correct timing of application and incorporation in the target zone of the soil. They mainly, protect the plant during the highly sensitive seedling or post-transplant stage of plant development.

Soil is treated before planting and the chemicals either make plants unattractive for nematodes or the nematodes are immobilised and therefore cant find their host. Good distribution through the soil is of utmost importance to the effectiveness of the nematicide as root systems are only protected when all roots are in close contact with the pesticide. Plants treated with nematistatics such as aldicarb and carbofuran (carbamates) or ethoprofos (and organophosphate), retain their ability to induce nematode eggs to hatch (such as some cyst nematodes and *Melodogyne* spp.) but juveniles become either immobilized or disoriented and cannot find their food source, the plant roots (Haydock *et al.*, 2006). It has also been observed that the application of nematicides delays the hatching process of cyst and RKN (Schomaker and Been, 1999). If this effect lasts long enough, the nematodes eventually starve. Therefore, mortality of the nematodes depends on the time that plants remain unattractive and on the time nematodes remain immobilized. Treatment of plants with nematistatics delays nematode penetration into the roots and results in a certain fraction of the root system escaping nematode attack and

thus remaining healthy. As a result, the minimum yield possible from a plant is increased by the fraction of the root system untouched by nematodes (Haydock *et al.,* 2006). Nematode penetration is postponed until the chemical is no longer effective or when the roots grow into soil layers where the nematicide is not present. However, several fumigants and nematicides have been withdrawn from the market in the last few decades due to concerns about environmental safety as well as human health (Rich *et al.,* 2004). Nevertheless, nematicides continue to be a main nematode management approach, whether used as part of an integrated management programme or as the sole control component.

Soil texture can influence nematode density and distribution both horizontally and vertically and has been suggested as a useful predictor of nematode densities and distributions that may be of value in predicting economic damage potential (Noe and Barker, 1985; Queneherve, 1988). The population density of *M. incognita* is negatively correlated with the clay or silt content of soil (Windham and Barker, 1986; Queneherve, 1988; Koenning *et al.,* 1996). Various studies have indicated that areas at risk for presence of RKN can be identified by using Electrical Conductivity (EC) and soil spectral reflectance data, both indicators of soil textural changes (Mueller *et al.,* 2010; Ortiz *et al.,* 2006 Perry *et al.,* 2006; Ortiz *et al.,* 2009; Evans *et al.,* 2002). These characteristics can then be used to produce nematode density maps that can then be used to develop and administer targeted management practices on identified risk areas (Marshall *et al.,* 1998 and Evans *et*

al., 1999). As part of this same project, it was separately shown that mapping of spatial nematode distribution by measuring the electrical conductivity (ECa) values is now possible under local conditions in tomato fields in Mwea (Wendot *et al.,* 2012). The application of variable rates of nematicides for precision plant protection against nematodes has been successfully used (Mueller *et al.,* 2010). For example site specific management has been used in the USA for the control of RKN (*M. incognita)* and the ectoparasite *Hoplolaimus columbus* (Muller *et al.,* 2002) and for root-knot and the reniform nematode (*R. reniformis)* on cotton (Wolcott *et al.,* 2004 and Davis *et al.,* 2008)

Applications of a single nematode management option such as the application of single chemical formulations have been shown to be either completely ineffective or minimally effective. Nematode management in the future is unlikely to rely on one type of measure, and will in addition need to be used where needed to minimize wastage. This will necessitate the development of site specific nematode strategies that can be recommended based on nematode densities in a particular farm. Management will require the logical use of effective control methodologies in combinations that are economically acceptable to the grower (Sikora *et al.,* 2005). In contrast, soil fumigation alone is likely to be detrimental to integrated nematode management. Soil fumigants kill large numbers of nematodes and models of infectious disease in pest populations suggest that the use of such pesticides lengthens the time required for a pathogen to control a pest (Carpenter, 1981).

CHAPTER THREE

MATERIALS AND METHODS

3.1 The study area

Kirinyaga County is one of the largest tomato producing areas in Kenya. The county covers about 1,437 Km^2, and is located at latitude 1.73 east and longitude 37.48 south (fig.3.1) There are two permanent rivers, namely Thiba and Nyamindi, which facilitate the irrigated farming of rice and horticultural crops in the lower parts of the county. The region has annual average rainfall ranging from 800-2200 mm. In most of the areas, the soils are deep and moderately to highly fertile. The average annual temperature range is 9.7-21.6°C. Among the most important horticultural crops are tomato, French bean, onions, banana, mango, pawpaw and avocado (Waiganjo *et al.*, 2006). The county has five Sub Counties namely; Kirinyaga East, Kirinyaga West, Mwea East, Mwea West and Kirinyaga Central (Fig. 3.1). The Sub Counties are further sub-divided into 12 Divisions, 30 Locations and 81 Sub-Locations. (County Government of Kirinyaga, 2014). Mwea is one of the major areas in Kirinyaga County where tomato is grown by smallholder farmers and is an important source of income to the locals. The main agro ecological zone in Mwea division is the Low Midland Zone (LMZ) and the climate enables two short cropping seasons (Koening *et al.*, 2008). The field study was conducted in the Riambogo area of Mwea East division within Kirinyaga County and was based around Kiamanyeki village of Tebere location in Mwea constituency. Mwea East division which covers an area of 512.8 km^2 is located 307 km south of Isiolo and

81 km south east of the capital Nairobi and has an average elevation of 1,368 meters above sea level with impervious, heavy black cotton soil (Koening *et al.*, 2008).

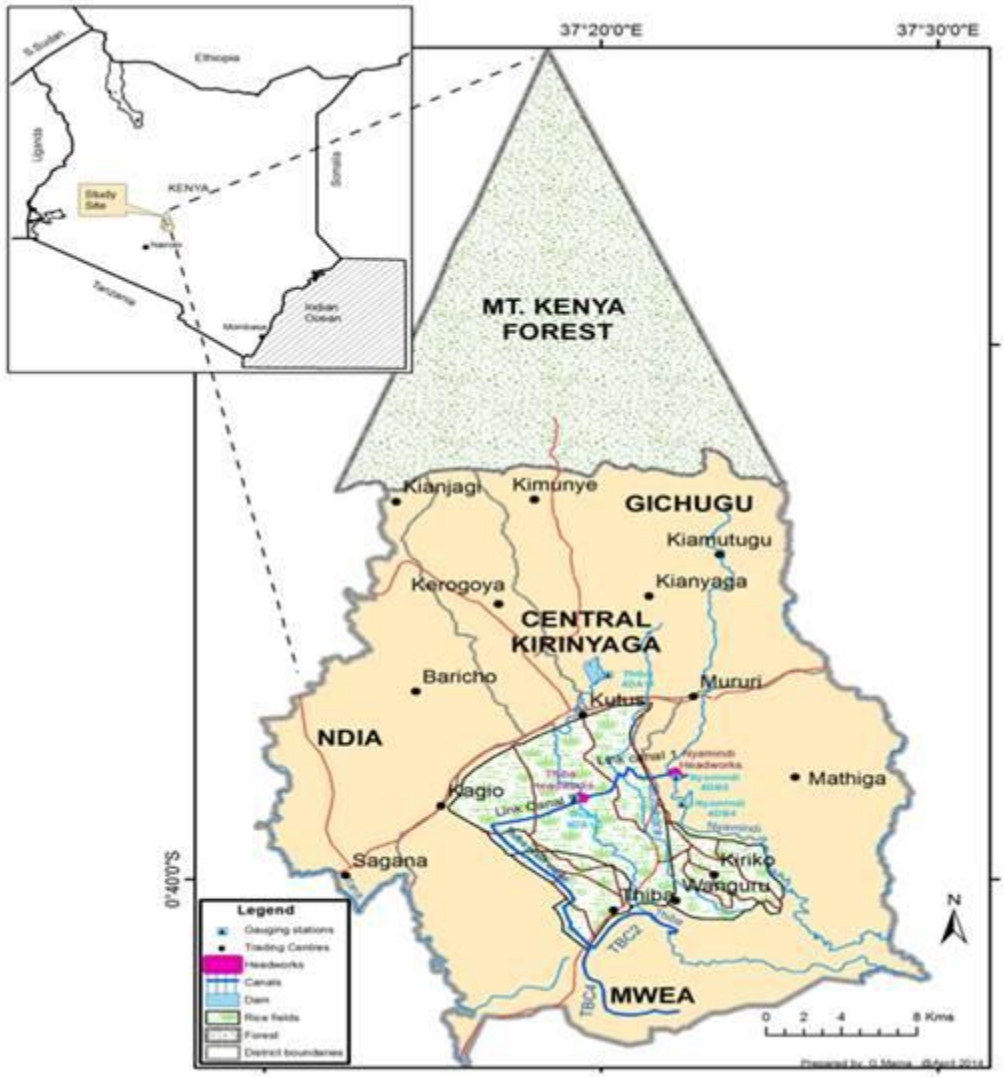


Figure 3.1. Map of Kenya showing the general location of Kirinyaga County. Source: Imbenzi *et al.* 2015

3.2 Field experiments.

3.2.1 Baseline survey

A baseline survey of RKN and other plant parasitic nematode population levels in tomato fields in Riambogo region of Mwea was carried out by sampling soil and plants from tomato fields in the area to determine spatial density of PPN. Soil and plant samples were taken systematically (along a line transect every 5 M) along the entire lengths of selected fields. The sampling points were coded sequentially to allow for subsequent geo-referencing and determination of areas with differing nematode densities. Soil samples of approximately 1kg in weight, were obtained from a depth of 10-30 cm from the surface using a garden trowel and placed in suitable labeled plastic sample bags. Plant samples with their roots intact were collected separately and taken to the laboratory and processed. Plant parasitic nematodes were extracted from both the soil and root samples, enumerated and identified. All the sampling points were geo-referenced and the areas with high and low RKN densities identified. Equally, the areas with no RKN infestation were also geo-referenced.

3.2.2 Nematode extraction from soil

The soil extraction procedure for the field experiments was adopted from Dropkin (1980). Each soil sample was mixed thoroughly and nematodes extracted from a 200 cm^3 soil composite sample using the centrifugal-floatation method as described by Jenkins (1964). This involved separation of nematodes from the soil sample by

suspending the soil sample in water, mixing soil samples thoroughly and gently tumbling them in a clean plastic bucket. Five hundred (500) ml of water was then poured in the plastic bucket followed by gentle mixing of the soil with tap water, stirring by hand to free nematodes from soil and re-suspending them in 5 l of water. The mixture was left to settle for 1 min and the water was decanted through a 2 mm sieve into a second bucket to eliminate plant debris and heavy soil particles. The water in the second bucket (containing the nematodes) was re-suspended by stirring. The bucket was left to stand for about 10 seconds and the contents poured through a set of sieves arranged in order from 500 μm, 125 μm, 90 μm and finally nematodes collected on 38 μm sieves by backwashing into a 250-ml beaker and concentrating to 50-60 ml in a 100 ml beaker. The contents were then poured equally into four 15-ml centrifuge tubes. Each tube was filled to within 0.5 cm of the top with fresh water and centrifuged for 7 min at 1750 rpm. This concentrated nematodes and soil particles in a pellet at the bottom of the tube. The supernatant was decanted and the tubes were refilled halfway with sucrose solution (450 g/l water) having a specific gravity of 1.18. Nematodes were then re-suspended in the sucrose solution by thorough mixing. This was followed by centrifugation for 3 min at 1750 rpm. The nematodes were then collected by pouring the supernatant through a 38 μm sieve and backwashing into a 100 ml beaker.

3.2.3 Determination of nematode densities

The nematode-water suspension for each extracted sample was concentrated to equal volumes of between 5 ml to 8 ml. The nematode suspension was stirred by blowing air through it using a pipette for homogeneity and a 1ml aliquot pipetted into a counting dish mounted on an inverted stereo microscope set at x10 magnification and the individual nematodes counted and subsequently identified. Identification keys (Hussey, 1990; Hunt *et al.*, 2005 and Karssen, 2006) were used for morphological identification. Four counts were made for each sample and the mean counts obtained. The total number of nematodes in the suspension which translated to the nematode density in the soil sample also calculated (Campos and Campos, 2005). Based on these counts, the density of nematodes in the field was determined.

3.2.4 Seedling establishment for field and greenhouse experiments

Seedlings of the three varieties to be used in the field experiments were germinated in raised nurseries measuring 3 x 1.5 m. Seeds were sown at a spacing of 15cm x 5cm in rows made in the finely tilled soil. They were then covered with a thin layer (about 1 cm) of fine soil and watered regularly for four weeks after which they were ready for transplanting. During the nursery preparation, Ridomil Gold ® (4 % w/w metalaxyl-M and 64 % w/w mancozeb) was applied into the soil at the recommended rate to prevent dieback of the seedlings caused by soilborne pathogens.

Seedlings used in the greenhouse experiments were directly sown singly into the individual pots. The pots were filled with about 2,000 cm^3 of forest soil sterilized in bulk by passing steam at 80°C through it for 30 minutes.

3.2.5 Field experiments to evaluate different nematode management strategies based on the varying densities of RKNs

Field experiments in Riambogo area in Mwea district were conducted to evaluate different nematode management strategies based on the varying densities of RKNs established as described in section 3.2.3. The experiments were conducted in areas with high, low density and no RKN as determined during the survey. A highly susceptible variety (Rio Grande) and two RKN resistant tomato varieties (Sandokan and Assila) with or without 2g of a commonly used nematicide, Mocap (Ethoprophos) were used. In total there were six treatments used as follows :- i) Resistant Tomato (Assila) + Mocap 2 g, ii) Resistant Tomato (Assila) + No Mocap , iii) Resistant Tomato (Sandokan) + Mocap 2 g, iv) Resistant Tomato (Sandokan) + No Mocap , v) Susceptible Tomato(Rio Grande) + Mocap 2 g and finally, vi) Susceptible Tomato (Rio Grande) + No Mocap. Each treatment was repeated in each of the 3 blocks characterized by high, low and no RKN densities. Three (3) week-old tomato seedlings (established as described in section 3.2.5) of each tomato variety were transplanted at the rate of twelve (12) plants of each tomato variety were planted per treatment in each of the three blocks. Diamonium Phosphate (DAP) 18:46:0 fertilizer at the rate of 50 kg/acre was applied at

planting. The treatments were arranged in a randomized complete block design (RCBD) replicated three times. -The experiment was repeated twice and data collected as described in section 3.3 below.

Additional experiments were also conducted to determine the effects of selected nematode management strategies on the RKN populations and galling index. Field experiments were conducted to determine the effects of application of Mocap, which is (a commonly used nematicide in the area), at the recommended rate (2 g per plant) on the population of RKN. Due to the popularity of Mocap among the farmers in the Riambogo area at the time, it was considered in this experiment as a farmer's practice.

Field experiments were conducted in two areas identified to be infested with RKN during the baseline survey, at both a high and low density. A highly susceptible tomato variety (Rio Grande) was used. Ten (10) of the plants in each treatment were uprooted after every 30 days until harvest and data of galling index obtained. Soil samples were also collected from these treatments and nematode extraction and counting performed.

3.3 Greenhouse experiments

Greenhouse experiments were conducted at the KEPHIS Plant Quarantine and Biosecurity Station in Muguga to test different nematode management strategies based on the varying densities of RKNs. The greenhouse experiments were set up

with steam-pasteurized soil, designed to eliminate the unintended effects of extraneous factors such as the presence of other diseases such as *Fusarium* wilt and insect pests which are common under varying nematode densities in the area.

For the greenhouse experiments, four plants each of a highly susceptible variety (Rio-Grande) and two resistant tomato varieties (Sandokan and Assila) and 3 rates of Mocap application (1, 2 and 4g per plant) were used. This was (repeated for two seasons) and the experiments were set up in a completely randomised design (CRD). Seeds were sown in plastic pots (25 cm diameter) filled with steam pasteurized soil (treated at 72^{o}C for 30 minutes) and grown under an insect proof screenhouse. Twelve plants (12) of each variety were artificially inoculated with high nematode densities (4,000 J2s per plant) low (1,000 J2s per plant) or left un-inoculated plants being used as controls. During RKN inoculation, four to six holes were made using a sterile metallic rod up to the middle of the pots around the plants and the required numbers (1,000 and 4,000 J2s) of juveniles pipetted Into the holes which were then covered with soil to prevent drying. These pots were watered regularly and carefully to prevent flooding, leaching and drying. The potted nematode cultures were maintained in the greenhouse where temperatures ranged from 22-28^{o}C. Thereafter, treatments were applied as described in Table 3.1.

Table 3.1. Treatment units (combinations) used in the greenhouse experiments

	Density	Mocap	Variety
1.	High RKN	1 gm	Rio Grande
2.			Sandokan
3.			Assila
4.		2 g	Rio Grande
5.			Sandokan
6.			Assila
7.		4 g	Rio Grande
8.			Sandokan
9.			Assila
10.		None	Rio Grande
11.			Sandokan
12.			Assila
13.	Low RKN	1 gm	Rio Grande
14.			Assila
15.			Sandokan
16.		2 g	Rio Grande
17.			Assila
18.			Sandokan
19.		4 g	Rio Grande
20.			Sandokan
21.			Assila
22.		None	Rio Grande
23.			Sandokan
24.			Assila
25.	No RKN	1 gm	Rio Grande
26.			Sandokan
27.			Assila
28.		2 g	Rio Grande
29.			Assila
30.			Sandokan
31.		4 g	Rio Grande
32.			Sandokan
33.			Assila
34.		None	Rio Grande
35.			Assila
36.			Sandokan

Crop yield data was collected at the end of the experiment where the total number and weight of the fruits was taken at harvest. The initial and final nematode populations were also determined by obtaining soil samples in each of the respective treatment areas before and after the experiment. After 75 days, the tomato crop was harvested and data on fruit number, shoot and root weight, and galling index collected. Second stage juveniles RKN were then extracted from composite samples of 200 cm^3 of soil from each of treatments and their numbers tabulated and analysed.

3.3.1 Determining egg mass index

Approximately 75 days after inoculation, plants were uprooted and washed clean of soil. The root systems were placed in 1000-ml beakers containing 300 ml of 0.05 % (v/v) Phloxine B solution for 3–5 min to stain egg masses bright red to allow for visual determination of the number of egg masses per root system (Holbrook *et al.,* 1983; Daykin and Hussey, 1985). Each plant was indexed for root galls and egg mass indices using a scale of 1-9 where 1=no egg mass, 2=1-5, 3=6-10, 4=11-20, 5=21-30, 6=31- 50, 7=51-70, 8=71-100, 9= >100 egg masses per root system (Sharma *et al.,* 1994).

The greenhouse experiments were arranged in a CRD containing 36 treatment combinations, each replicated 3 times as shown in Table 3.1. The experiments

were repeated twice with the first one carried out from January to April 2012 and the second one running from June 2012 to September 2012.

3.4 Determining the relative cost of different RKN management options under varying nematode densities

Relative costs of different management options were tabulated and compared with costs that are normally incurred by farmers under the same conditions. Relative costs of the various inputs (such as weeding, planting, watering, pest control and fertiliser) were recorded and compared for all the treatments and the most cost effective management option identified. Benefit-cost analysis (BCA) which is a technique for evaluating a project or investment by comparing the economic benefits with the economic costs of the activity was used. The BCR was computed using the formula below. A plant population of 10,000 tomato plants were used to compute the costs and gross margins per acre.

$$\text{Benefit Cost Ratio (BCR)} = \frac{\text{Benefit or gross margin / acre (Ksh)}}{\text{Cost / acre (Ksh)}}$$

3.4.1 Data collection

Data on initial and final nematode densities, fruit numbers, fresh shoot weight and root weight were taken at the end of the trials. The data was collected from 8 randomly selected plants per treatment in the field trials and four plants in the greenhouse trials. The experiments were both terminated 75 days after transplanting and the fresh weights of tomato fruits, tomato shoot heights, dry weights of shoot and root, and number of galls per gram root weight were

assessed. This was repeated for two seasons. Damage assessment was done using a galling index (GI) scale of 1-9, (Sharma *et al.*, 1994).

3.4.2 Data analysis

The General Linear Model Procedure for univariate analysis of variance analysis was used to test for significant main and interaction effects. Data of all dependent variables were subjected to the Kolmogorov–Smirnov test for normality (Stephens, 1974) using SPSS version 20. The data were analyzed by a one-way ANOVA (Analysis of Variance*)* test after confirming that the data was normally distributed. The significance level (alpha) was set at the 0.05 level. When significant differences were found, the Tukey's HSD (honestly significant difference) test (Jaccard *et al.*, 1984, Field A., 2009) was used to separate the means for the various treatments. The significance level for the Tukey's HSD was also set at the 0.05 level. All statistical data analysis was performed using IBM - SPSS/PASW (Predictive Analytics Software) version 20.

CHAPTER FOUR

RESULTS

4.1 Plant parasitic nematodes associated with tomato production in Riambogo area of Mwea

A total of 12 genera of plant parasitic nematodes were identified using morphological characteristics. These included *Meloidogyne* spp., *Hoplolaimus* spp., *Criconemella* spp., *Helicotylenchus* spp., *Pratylenchus* spp., *Psylenchus* spp., *Tylenchorynchus* spp., *Belonolaimus* spp., *Tylenchulus* spp., and *Tylenchus* spp. The most abundant plant parasitic nematode genera found were the root-knot nematodes (*Meloidogyne* spp.), followed by the lesion nematodes (*Pratylenchus* spp.) with a maximum of 110 and 116 nematodes per 200 $cm^3$of soil, respectively. Consequently the areas coded SP-1 to SP-4 in the selected sites were chosen for the experiments with high RKN densities while the areas coded SP-9 to SP-12 and N-6 to N-9 were chosen for the experiments with low and no RKN densities, respectively as shown in Table 4.1 and 4.3.

Table 4.1 Nematode genera identified from soil samples obtained from Riambogo area in Mwea.

Sample No	GPS Coordinates		Plant Parasitic Nematode Genus												
	South	East	Non Parasitic	*Meloidogyne spp*	*Hoplolaimus spp*	*Criconemella spp*	*Helicotylenchus spp*	*Pratylenchus spp*	*Psylenchus spp*	*Tylenchoryhnchus spp*	*Belonolaimus spp*	*Tylenchulus spp*	*Aphelenchoides spp*	*Tylenchus spp*	*Aphelenchoides spp*
Sp*-1	00.71986	037.40170	227	32	52	8	-	-	4	-	-	-	-	-	-
Sp-2	00.71979	037.40169	730	110	-	5	-	-	10	-	-	-	-	-	-
Sp-3	00.71970	037.40171	280	104	32	-	-	-	-	-	-	-	-	-	-
Sp-4	00.71961	037.40171	27	108	-	12	-	-	-	-	-	-	-	-	-
Sp-5	00.71959	037.40167	96	40	32	-	-	-	-	-	-	-	-	-	-
Sp-6	00.71969	037.40165	40	8	-	-	-	-	-	-	-	-	-	-	-
Sp-7	00.71976	037.40161	10	5	10	-	-	-	-	-	-	-	-	-	-
Sp-8	00.71985	037.40159	83	4	-	-	-	-	-	-	-	-	-	-	-
Sp-9	00.71982	037.40155	52	5	-	7	-	11	7	-	-	-	-	-	-
Sp-10	00.71975	037.40158	18	6	-	-	-	-	-	-	-	-	-	-	-
Sp-11	00.71967	037.40162	9	10	-	-	-	-	-	-	-	-	-	-	-
Sp-12	00.71959	037.40162	17	12	-	-	-	42	-	-	-	-	-	-	-
N*-1	00.71891	037.40223	156	18	22	34	8	8	-	12	-	-	-	-	16
N-2	00.71903	037.40217	276	9	-	3	39	20	3	-	5	-	3	18	-
N-3	00.71915	037.40216	66	-	6	41	50	23	-	-	-	3	-	-	-

Sample No	GPS Coordinates		Plant Parasitic Nematode Genus												
	South	East	Non Parasitic	*Meloidogyne spp*	*Hoplolaimus spp*	*Criconemella spp*	*Helicotylenchus spp*	*Pratylenchus spp*	*Psylenchus spp*	*Tylenchoryhnchus spp*	*Belonolaimus spp*	*Tylenchulus spp*	*Aphelenchoides spp*	*Tylenchus spp*	*Aphelenchoides spp*
N-4	00.71924	037.40212	100	18	-	34	26	18	-	14	-	-	-	-	26
N-5	00.71941	037.40213	245	25	-	97	27	20	-	-	-	14	-	-	-
N-6	00.71956	037.40212	64	-	-	50	6	10	-	-	-	-	-	-	-
N-7	00.71969	037.40210	92	-	-	56	7	11	-	-	11	11	-	-	-
N-8	00.71984	037.40211	179	-	16	68	5	14	7	12	4	9	1	9	9
N-9	00.71994	037.40208	66	-	-	-	-	6	-	5	-	6	-	-	8
N-10	00.72007	037.40207	66	3	13	31	10	6	4	6	15	6	-	-	-
N-11	00.72021	037.40207	99	12	18	21	8	6	-	14	-	-	-	-	17
N-12	00.72031	037.40204	110	9	32	18	11	14	-	-	-	-	-	-	-
N-13	00.72039	037.40206	1-9	3	15	18	14	14	-	-	-	-	-	-	-
N-14	00.72053	037.40206	133	21	21	25	9	7	-	16	-	-	-	-	19
N-15	00.72070	037.40204	189	5	11	18	-	-	-	-	-	-	-	-	-
R*-1	00.72225	037.40141	58	8	-	-	16	64	-	-	-	-	-	-	-
R-2	00.72223	037.40141	41	-	-	-	24	114	-	-	-	-	-	-	-
R-3	00.72240	037.40141	49	-	-	-	-	84	-	-	-	-	-	-	-
R-4	00.72246	037.40141	26	-	-	-	5	72	-	-	-	-	-	-	-
R-5	00.72261	037.40141	43	-	-	-	-	80	-	-	-	-	-	-	-

Sample No	GPS Coordinates		Plant Parasitic Nematode Genus												
	South	East	Non Parasitic	*Meloidogyne spp*	*Hoplolaimus spp*	*Criconemella spp*	*Helicotylenchus spp*	*Pratylenchus spp*	*Psylenchus spp*	*Tylenchoryhnchus spp*	*Belonolaimus spp*	*Tylenchulus spp*	*Aphelenchoides spp*	*Tylenchus spp*	*Aphelenchoides spp*
R-6	00.72253	037.40141	63	4	-	-	16	116	-	-	-	-	-	-	-
R-7	00.72268	037.40140	95	-	-	-	6	114	-	-	-	-	-	-	-
R-8	00.72275	037.40140	49	-	-	-	7	35	-	-	7	-	-	-	-

Shaded areas represent sections chosen for the various field experiments. *The description of the sampling points are as shown in table 4.3 below. The elevation for all the samples was 1,120 masl.

Table 4.2. Common names of Plant Parasitic nematodes observed in Riambogo Area of Mwea

	Genus name	**Common Name and hosts**
1.	*Belonolaimus*,	Sting nematode of cereals, legumes, cucurbits, etc.
2.	*Tylenchorhynchus*,	Stunt nematode of tobacco, corn, cotton, etc.
3.	*Pratylenchus*,	Lesion nematode of almost all crop plants and trees
4.	*Hoplolaimus*,	Lance nematode of corn, sugarcane, cotton, alfalfa, etc.
5.	*Heliocotylenchus*,	Spiral nematode of various plants
6.	*Scutellonema*,	Dry rot nematode of yam, cassava, etc.
7.	*Meloidogyne*,	Root-knot nematode of almost all crop plants
8.	*Criconema* and *Criconemoides*,	Ring nematode of woody plants, cause of peach tree short life
9.	*Hemicycliophora*,	Sheath nematode of various plants
10.	*Tylenchulus*,	Citrus nematode of citrus, grapes, olive, lilac, etc.
11.	*Aphelenchoides*,	Foliar nematode of chrysanthemum, strawberry, begonia, rice, coconut, etc.
12.	*Trichodorus*,	Stubby-root nematode of sugar beet, potato, cereals, and apple

Source: Luc *et al.*, 2005

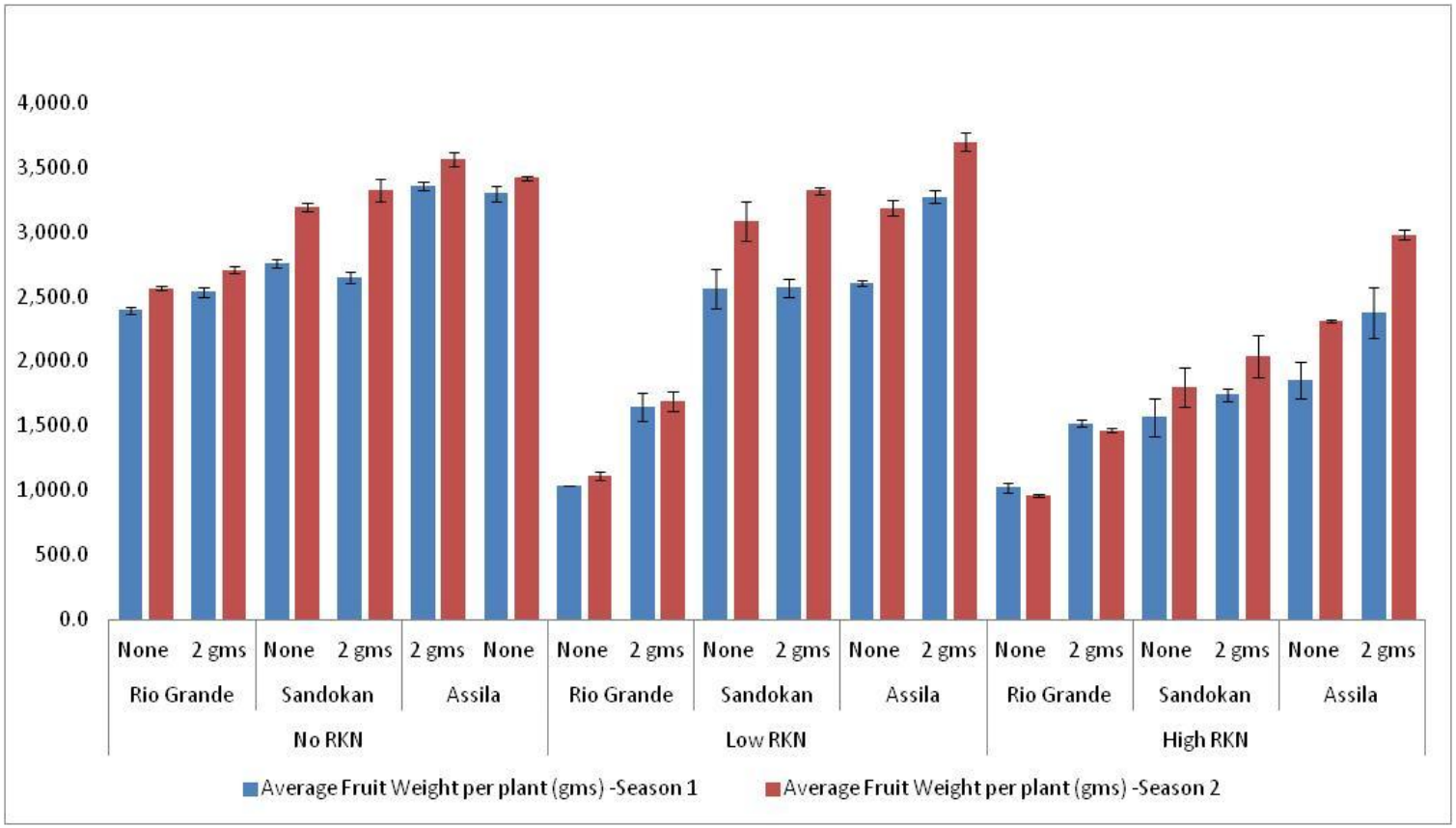
Table 4.3. Descriptions of soil sampling points in Riambogo area in Mwea.

Sample No	**Description**
Sp-1 to Sp-12	This portion (sp-1 to sp-12) had been planted with spinach the previous season which was reported to have been heavily galled at harvest.
N-1 to N-15	Portion N-1 to N-15 had laid fallow for at least the previous two seasons. This portion was also prone to seasonal flooding
R-1 to R-8	Portion was next to the Thiba River and had laid fallow for the previous one season, before which the area had been grown with Tomatoes. This portion was also prone to seasonal flooding

4.2 Effect of treatments on the fruit weight per plant under varying nematode densities

The results on effect of treatments on the fruit weight per plant are presented in Fig. 4.1. Overall, there was a significant difference between the average fruit weights in all three varieties ($F_{(2, 51)} = 16.57$, $P < 0.05$) across all nematode densities with the varieties Assila and Sandokan recording the highest yield in all the areas. There was a significant difference ($p \leq 0.05$) between the fruit weights of all three varieties grown in RKN free soils with the RKN resistant variety, Assila showing the highest average yields (3,356.0) g with application of 2 g Mocap. There was however no significant difference ($p=0.68$) between the treatments with 2 g of Mocap and those without Mocap application among each individual variety

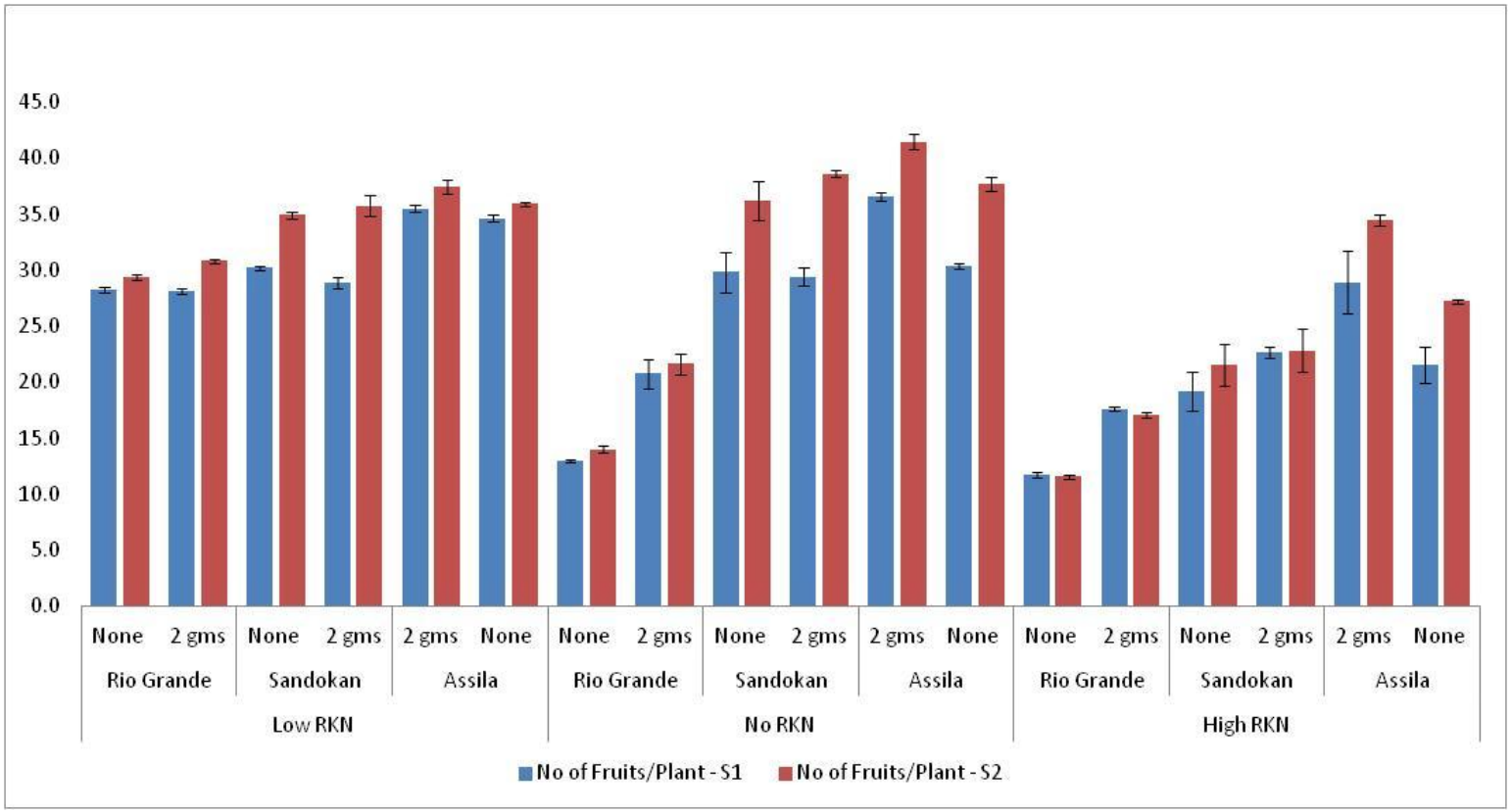
Fig 4.1 Chart showing effects of treatments on average fruit weight for the three varieties (field season 1 and 2)



4.3 Effects of treatments on the number of fruit per plant under varying nematode densities

Significant differences were observed in the number of fruit per plant within all the treatments ($F_{(2, 51)} = 11.980$, $P < 0.05$) and between the varieties. Assila recorded the highest number (35 (0.84) and 41(1.17) in season 1 and 2 respectively under low nematode densities with application of 2 g Mocap (Fig 4.2). The variety Sandokan recorded the second highest number (30.2 (0.65) and 38.6 (1.91) in season 1 and 2, respectively under low nematode densities, with and without application of 2 g Mocap. The variety Rio Grande recorded the least number of fruits under high, low and no RKN and both with and without application of 2 g Mocap. Generally application of 2 g of Mocap resulted in the highest number of fruit in the three varieties under all nematode densities.

Figure 4.2 Chart showing effects of treatments on the average number of fruit per plant (field season 1 and 2).



4.4 Effects of treatments on the galling index

There was no significant difference between the galling index of the treatments planted under both low and high nematode densities among all the varieties. However the galling indices differed significantly from those of plants grown in nematode free soils $F_{(2, 51)}=125.03$, $P<0.05$ among all varieties. Plants grown in nematode-free soils showed no visible galling and therefore scored the least galling index of 1, while the galling index of those plants grown in both high and low RKN densities were given galling index scores ranging from 2 to 9.

There was no significant difference between the galling index of the treatments with pre-plant application of 2g Mocap and those without Mocap in all nematode densities. This implies that the application of Mocap did not significantly affect the infestation rate of the tomato varieties by RKN (Table 4.4). The galling and RKN root infection observed are as shown in figure 2.1.

Table 4.4. Effects of treatments on the galling index (field seasons 1 & 2)

Density	Variety	Mocap	Season 1 mean	SE Season 1	Season 2 mean	SE Season 1
No RKN	**Rio Grande**	None	1.00 [a]	0.00	1.0 [a]	0.00
	Sandokan	None	1.00 [a]	0.00	1.0 [a]	0.00
	Assila	None	1.00 [a]	0.00	1.0 [a]	0.00
Low RKN	**Rio Grande**	None	5.67 [bc]	0.56	6.3 [bc]	0.28
	Sandokan	None	7.33 [bc]	0.29	6.8 [bc]	0.62
	Assila	None	6.67 [bc]	0.43	6.7 [bc]	0.45
High RKN	**Rio Grande**	None	8.00 [c]	0.64	7.9 [c]	0.64
	Sandokan	None	7.33 [bc]	0.43	7.9 [c]	0.19
	Assila	None	6.67 [bc]	0.38	7.2 [bc]	0.27
No RKN	**Rio Grande**	2 g	1.00 [a]	0.00	1.0 [a]	0.00
	Sandokan	2 g	1.00 [a]	0.00	1.0 [a]	0.00
	Assila	2 g	1.00 [a]	0.00	1.0 [a]	0.00
Low RKN	**Rio Grande**	2 g	5.00 [bc]	1.13	4.6 [b]	1.25
	Sandokan	2 g	5.67 [bc]	0.91	5.5 [bc]	0.87
	Assila	2 g	4.67 [b]	0.33	4.6 [b]	0.33
High RKN	**Rio Grande**	2 g	5.33 [bc]	1.29	5.8 [bc]	0.69
	Sandokan	2 g	7.00 [bc]	0.07	6.3 [bc]	0.46
	Assila	2 g	6.33 [bc]	1.25	5.2 [bc]	1.12

Means bearing same letters in the same column are not significantly different from each other at $p \leq 0.05$
Figures are averages of galling index from three replicates
Mocap applied at a rate of 0g and 2g per plant
SE represents the Standard Error of means

4.5 Effects of Mocap application on the numbers of *Meloidogyne* spp. J2s

There was a significant difference between the average numbers of J2s extracted in different RKN densities. The maximum numbers of J2s measured at 90 days were 13 and 68 respectively for soils with low initial RKN numbers. The average numbers of J2s extracted from each of the treatments are shown in Table 4.5.

Table 4.5. Effects of application of nematicide (2g Mocap) on the number of *Meloidogyne* spp. J2s

RKN density	Rate of Mocap	Days after planting	Mean No J2s / 200 cm^3 soil	SEM
High RKN	2 g	30 days	104 ^{de}	0.6
		60 days	113 ^{e}	0.0
		90 days	205 ^{f}	0.3
	None	30 days	59 ^{bc}	0.3
		60 days	105 ^{de}	0.6
		90 days	180 ^{f}	0.3
Low RKN	2 g	30 days	3 ^{a}	0.0
		60 days	7 ^{a}	0.3
		90 days	13 ^{a}	0.9
	None	30 days	7 ^{a}	0.3
		60 days	18 ^{ab}	0.3
		90 days	68 ^{cd}	0.6

Means bearing same letters are not significantly different from each other at $p \leq 0.05$
Figures are averages of J2 counts from three replicates
SEM represents the Standard Error of means

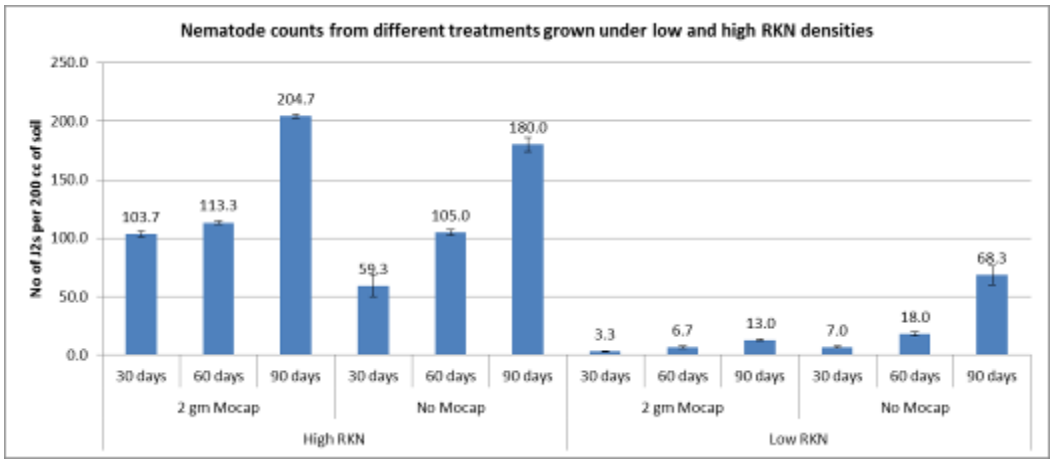


Figure 4.3 Effects of Mocap application on the numbers of *Meloidogyne* spp. j2s.

There was a significant difference ($P<0.05$) between the average galling index of the tomato variety Rio Grande in different RKN densities. There was however no significant difference ($P<0.05$) in the final galling index between tomatoes grown with and without 2g Mocap in high RKN density. These treatments recorded a final galling index of 8.7 both with and without Mocap. The application of Mocap however significantly reduced the galling index (to 5.7) of treatments planted under low RKN; this was however not the case under high RKN where application of Mocap did not significantly reduce the galling index. The average galling index for each of the treatments are shown in Table 4.6.

Table 4.6 Effects of treatments on the average galling index.

RKN density	Mocap	Days after planting	Mean	
High RKN	2 g Mocap	30 days	3.0±0.6	a
		60 days	6.0±0.1	bc
		90 days	8.7±0.3	d
	No Mocap	30 days	5.7±0.3	b
		60 days	7.0±0.6	bc
		90 days	8.7±0.3	d
Low RKN	2 g Mocap	30 days	2.0±0.1	a
		60 days	3.3±0.3	a
		90 days	5.7±0.9	b
	No Mocap	30 days	2.7±0.3	a
		60 days	5.7±0.3	b
		90 days	8.0±0.6	cd

Means bearing same letters in are not significantly different from each other at $p \leq 0.05$
Figures are averages of J2 counts from three replicates

4.6 The effects of different nematicide (Mocap) applications on the numbers of fruit per plant under greenhouse conditions

Significant differences ($P<0.05$) between varieties were observed in the number of fruit per plant in all densities of RKN ($F_{(2, 141)} = 10.9$, $P < 0.05$) with the varieties Assila and Sandokan recording the highest number. Both hybrid tomato varieties (Assila and Sandokan) showed no significant differences in the number of fruits per plant when grown under either low RKN or RKN free soils with and without Mocap.

Both hybrids showed significantly higher fruit numbers than those of the variety Rio Grande under all nematode densities with application of 4g Mocap. Rio Grande recorded the fewest fruits under high nematode densities when grown without Mocap. The average fruit numbers of all treatments are shown in Figure 4.3.

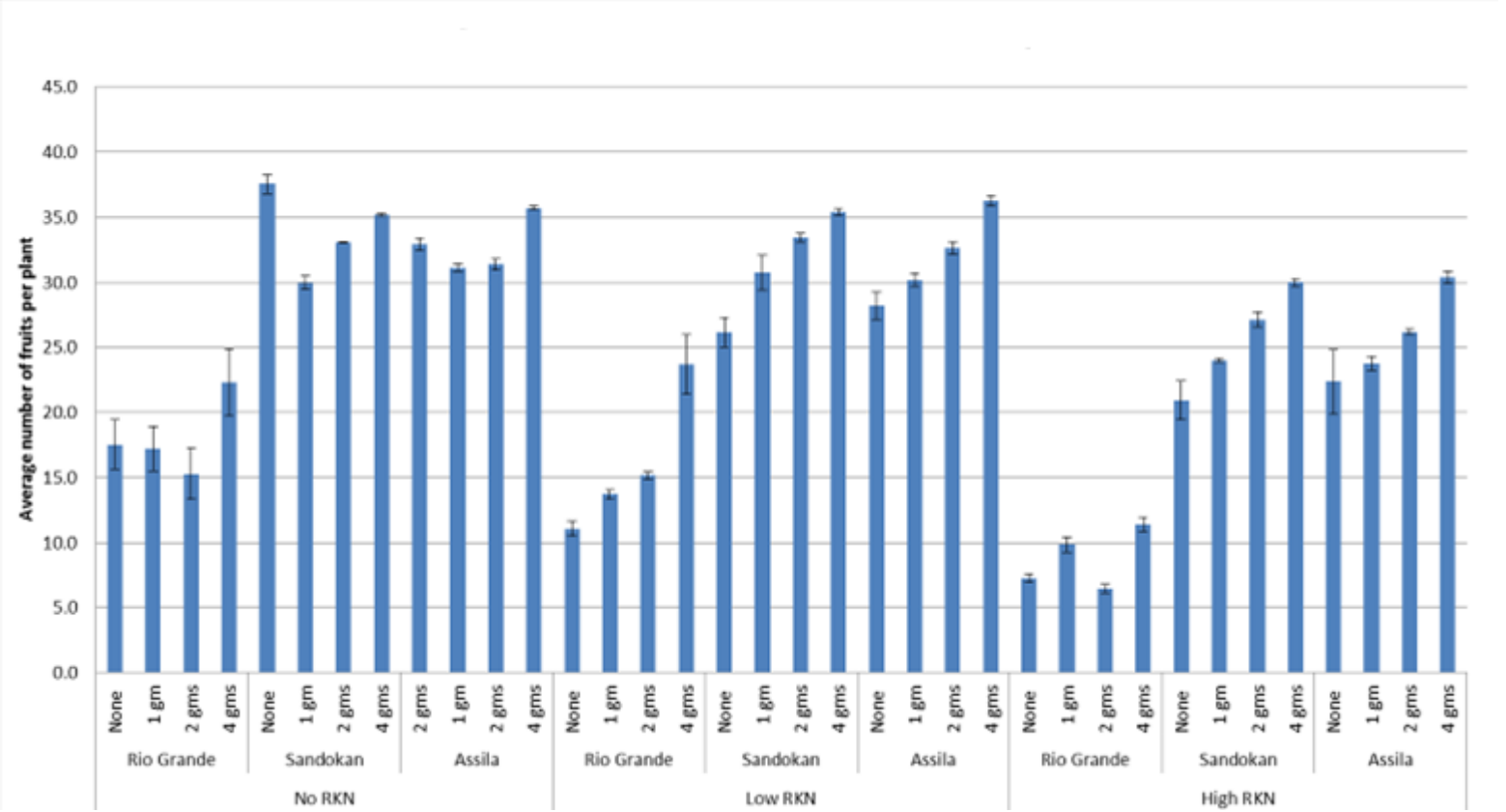


Figure 4.3 Effect of treatments on the average number of fruit per plant in the greenhouse experiments.

4.7 Effects of different nematicide (Mocap) applications on the weight of fruit per plant under greenhouse conditions

Significant differences were observed in the number of fruit per plant within all the treatments ($F_{(2,141)} = 52.7$, $P < 0.05$) with the varieties Assila and Sandokan recording the highest weights in all the nematode densities where 4 g Mocap was applied. The two hybrid tomato varieties (Assila and Sandokan) showed no significant differences in the weights of fruits per plant when grown under either low RKN or RKN free soils and with application of both 2 and 4 g Mocap (Fig 4.4). All three varieties did not show any differences in the fruit weights when grown in nematode free soil.

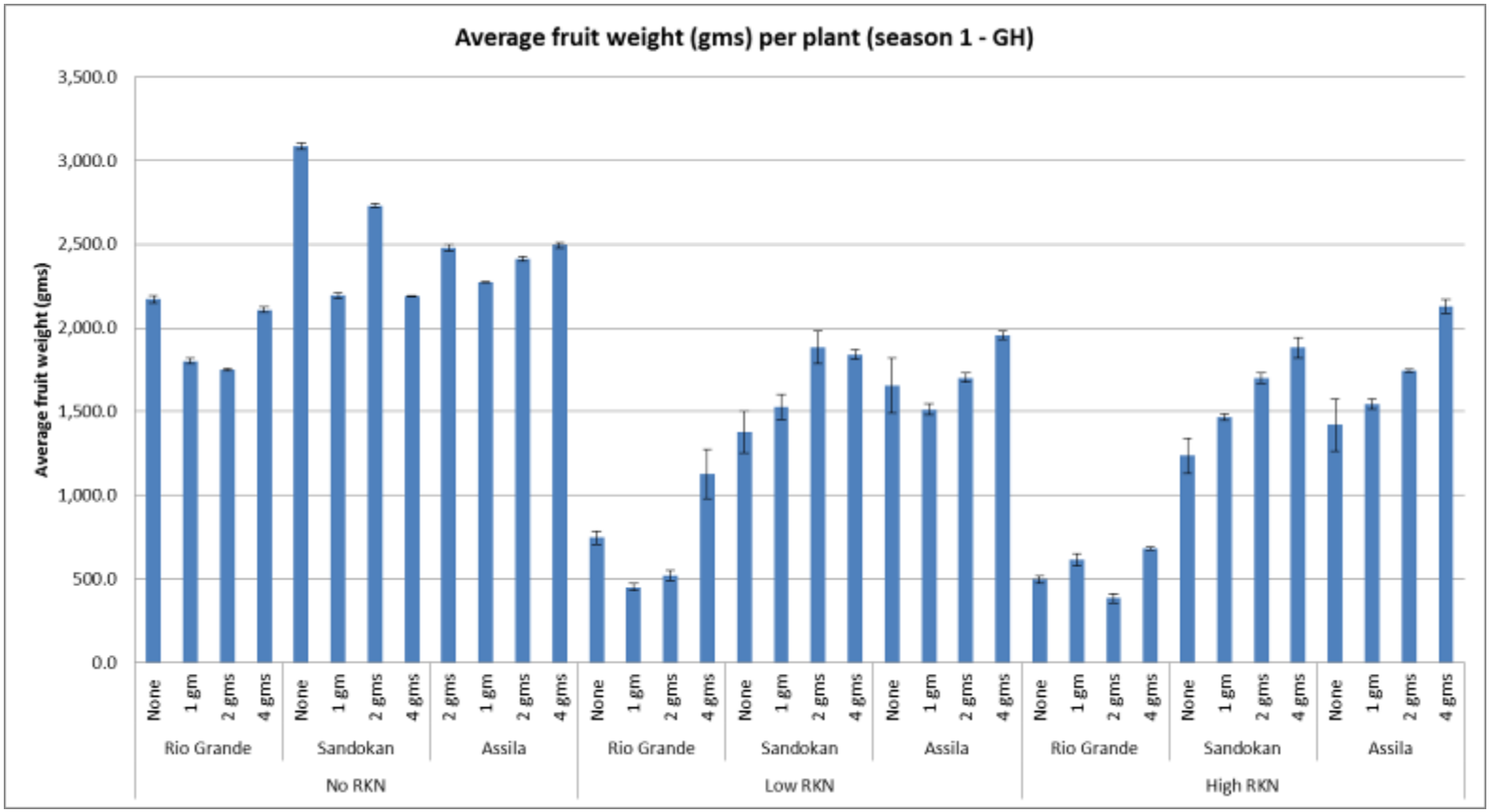


Figure 4.4 Effects of the various treatments on the average fruit weight per plant in the greenhouse experiment.

4.8 Effects of different nematicide (Mocap) applications on the the galling index per plant under greenhouse conditions

Significant differences were observed in the galling index per plant within all the treatments in both seasons ($F_{(2,141)} = 205.7$, $P < 0.05$) and ($F_{(2,141)} = 203.7$, $P < 0.05$) respectively with the varieties Assila and Sandokan recording the highest galling index under all treatments. Both hybrid tomato varieties (Assila and Sandokan) showed no significant differences in the galling index when grown under either low RKN or RKN free soils. However both hybrids showed significantly higher galling index than those of the variety Rio Grande under all nematode densities (Table 4.7).

Table 4.7 Effects of treatments on the average galling index in the greenhouse experiment (season 1 & 2)

Density	Variety	Mocap	Average Galling Index Season 1	SEM Season 1	Average Galling Index Season 2	SEM Season 2
High RKN	Assila	1 gm	7.1 gh	0.1	8 fgh	0.4
	Rio Grande	1 gm	7.6 gh	0.4	8.8 h	0.2
	Sandokan	1 gm	6.5 efg	0	7.8 fgh	0.5
	Assila	2 g	7 gh	0	7.3 fgh	0.3
	Rio Grande	2 g	6.5 efg	0.5	7 efgh	0.4
	Sandokan	2 g	5.5 def	0	6.1 def	0.4
	Assila	4 g	4 bc	0	3.4 bc	0.3
	Rio Grande	4 g	3.1 b	0.3	5 bcd	0.4
	Sandokan	4 g	3.2 b	0.6	3.4 bc	0.6
	Assila	None	8.4 h	0.2	8.9 h	0.1
	Rio Grande	None	7.7 gh	0.4	8.8 h	0.3
	Sandokan	None	7.4 gh	0.4	8.5 gh	0.3
Low RKN	Assila	1 gm	7.3 gh	0.3	7.4 fgh	0.4
	Rio Grande	1 gm	6.4 efg	0.6	6.3 def	0.3
	Sandokan	1 gm	6.6 efg	0.1	6.6 defg	0.2
	Assila	2 g	7.1 gh	0.1	6.6 def	0.6
	Rio Grande	2 g	5.3 cde	0.6	5.1 cde	0.5
	Sandokan	2 g	5.8 def	0.3	6.6 defg	0.6
	Assila	4 g	3.8 b	0.2	3.1 b	0.4
	Rio Grande	4 g	2.9 b	0.4	3.3 bc	0.4
	Sandokan	4 g	4.1 bcd	0.1	5.3 cde	0.5
	Assila	None	8 h	0	8 fgh	0.4
	Rio Grande	None	7 fgh	0.7	7.3 fgh	0.5
	Sandokan	None	6.9 fgh	0.3	7.4 fgh	0.7
No RKN	All varieties	0 - 4 g	1 a	0	1 a	0

Means bearing same letters in the same column are not significantly different from each other at $p \leq 0.05$

Figure 4.5 Root-knot nematode female stained in 0.05 % (v/v) phloxine B solution

Figure 4.6 Heavily galled roots of tomato observed during the experiments

4.9 Effects of treatments on the benefit cost ratio per acre of production

Costs of the different management options were calculated and compared with costs normally experienced by farmers under the same conditions. Relative costs of the various inputs (such as weeding, planting, watering, pest control and fertiliser) were recorded as shown in Table 4.8. Benefit-cost analysis was

performed which showed that the variety Rio Grande had the lowest benefit cost ratio (CBR) under high RKN densities while the variety Assila showed the highest (5.6 and 6.2 in the 1st and 2nd season respectively) when planted in RKN free soils. Under high RKN densities, planting the variety Assila with an application of 2 g Mocap showed the highest CBR of 2.9 in the first season and 3.7 in the second season (Table 4.11).

Table 4.8 Effects of treatments on the benefit cost ratio per acre of production in the field experiment (season 1 & 2)

Cost Benefit ratio (Benefit / Cost)

Density	Variety	Mocap	Benefit / Cost Season 1		Benefit / Cost Season 2	
High RKN	Assila	2 g	2.9	abcdef	3.7	bcd
	Sandokan	2 g	1.9	abc	2.9	abc
	Rio Grande	2 g	1.7	ab	1.6	ab
	Assila	None	2.7	abcde	3.7	bcd
	Sandokan	None	2.3	abcd	3	abc
	Rio Grande	None	1.3	a	1.2	a
Low RKN	Assila	2 g	4.1	efghi	4.3	cde
	Sandokan	2 g	3.8	defgh	3.3	abcd
	Rio Grande	2 g	2	abc	1.9	ab
	Assila	None	5.2	hi	4.2	cde
	Sandokan	None	4.9	ghi	4.3	cde
	Rio Grande	None	1.4	a	1.3	a
No RKN	Assila	2 g	4.5	fghi	4.6	cde
	Rio Grande	2 g	3.5	cdefg	3.4	abcd
	Sandokan	2 g	3.4	bcdefg	5.5	de
	Assila	None	5.6	i	6.2	e
	Sandokan	None	4.7	ghi	6.1	e
	Rio Grande	None	4.4	efghi	4.8	cde

Means bearing same letters are not significantly different from each other at $p \leq 0.05$
Figures are averages cost benefit ratios from three replicates

Table 4.9 Cost of production per acre in the field experiments, used in the calculation of benefit cost ratios

Production costs and output (1 acre at a plant population of about 10,000)				**Assila + 2gm Mocap**	**Sandokan + 2gm Mocap**	**Rio-grande + 2gm Mocap**	**Assila + No Mocap**	**Sandokan + No Mocap**	**Rio-grande + No Mocap**
Inputs	**Units**	**No of Units**	**Unit cost Kshs**	**Total Kshs**	**Total Kshs**	**Total Kshs**	**Total Kshs**	**Total Kshs**	**Total Kshs**
Land Preparation ploughing	Acre	1	2,500.0	2,500.0	2,500.0	2,500.0	2,500.0	2,500.0	2,500.0
Purchase of seeds	No of seeds / g	10,000	1.0	10,000.0	7,800.0	1,500.0	10,000.0	7,800.0	1,500.0
Planting Fertilizer DAP	50kg bag	1	4,000.0	4,000.0	4,000.0	4,000.0	4,000.0	4,000.0	4,000.0
Planting	Man-Days	8	200.0	1,600.0	1,600.0	1,600.0	1,600.0	1,600.0	1,600.0
Weeding (twice)	Man-Days	64	200.0	12,800.0	12,800.0	12,800.0	12,800.0	12,800.0	12,800.0
Fungicide (Ridomil)	Kilograms	1	1,500.0	1,500.0	1,500.0	1,500.0	1,500.0	1,500.0	1,500.0
Insecticide (Alfa Tata)	litres	1	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Nematicide (Mocap 2gm / plant)	Kg	20	880.0	17,600.0	17,600.0	17,600.0	-	-	-
Application of pesticide labour	Man-Days	12	200.0	2,400.0	2,400.0	2,400.0	2,400.0	2,400.0	2,400.0
trellising string	Units	20	25.0	500.0	500.0	500.0	500.0	500.0	500.0
trellising Labour	Man-Days	8	200.0	1,600.0	1,600.0	1,600.0	1,600.0	1,600.0	1,600.0
Fuel (for irrigation)	litres	120	100.0	12,000.0	12,000.0	12,000.0	12,000.0	12,000.0	12,000.0
Labour (for irrigation)	Man-Days	36	250.0	9,000.0	9,000.0	9,000.0	9,000.0	9,000.0	9,000.0
Harvesting	Man-Days	5	200.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0	1,000.0
Caretaker (6,000 ksh per month)		3	6,000.0	18,000.0	18,000.0	18,000.0	18,000.0	18,000.0	18,000.0
Total input cost				**95,500.0**	**93,300.0**	**87,000.0**	**77,900.0**	**75,700.0**	**69,400.0**

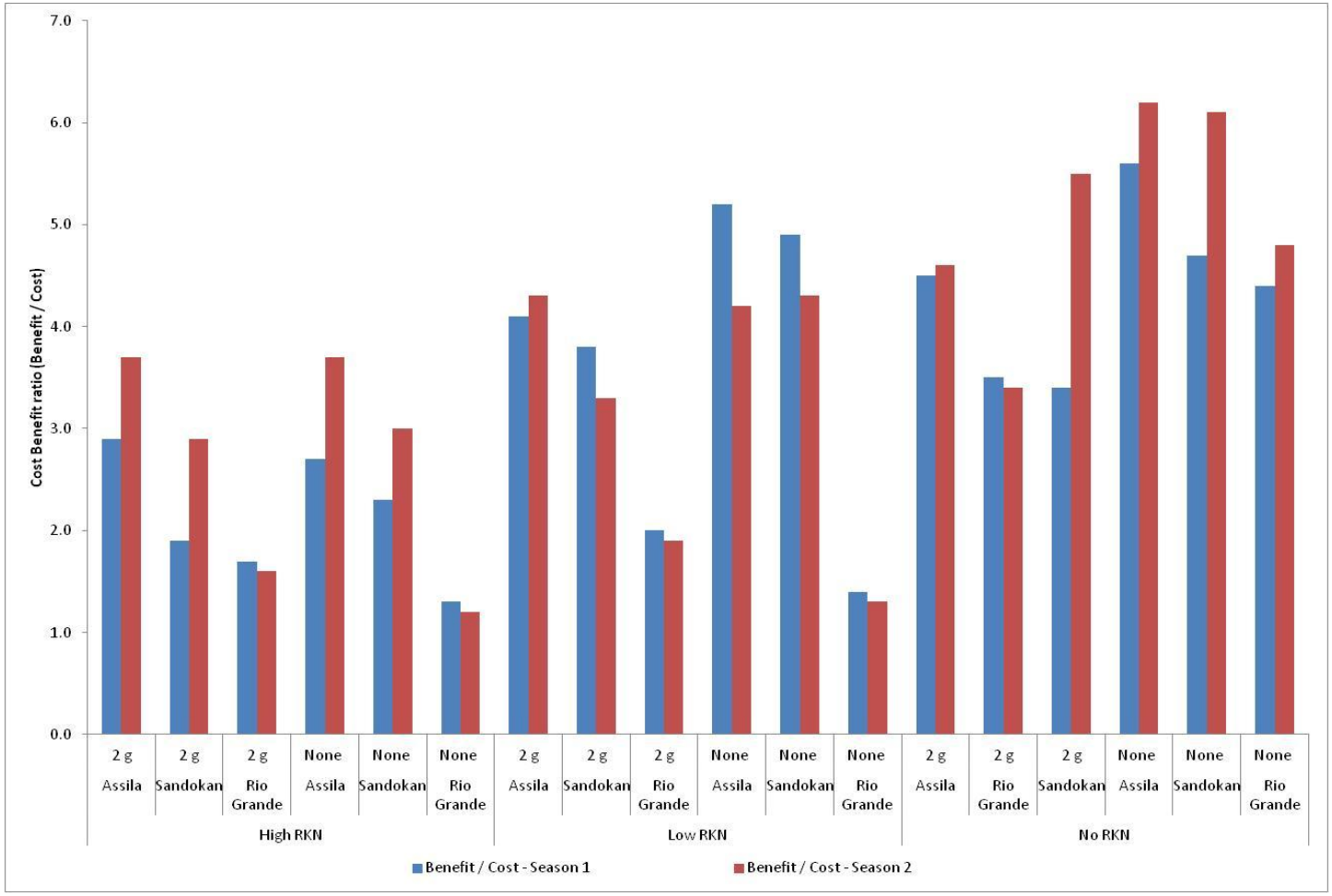


Figure 4.7 Effects of treatments on the benefit cost ratio per acre of production in the field experiment (season 1 and 2)

CHAPTER FIVE

5 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Baseline survey of plant parasitic nematodes

From the baseline survey carried out, it is now evident that the most abundant plant parasitic nematode genera found in the area are the root-knot nematodes (*Meloidogyne* spp.), and lesion nematodes (*Pratylenchus* spp.) with an average of 110 (8.40) and 116 (15.70) nematodes per 200 cm^3 of soil respectively. Mwea area is largely a tomato growing area, thus presence of RKN was expected RKNs (Dropkin, 1980; Waiganjo *et al.*, 2006).During the period of the study, it was observed that most of the farmers obtain seedlings for planting from nearby central nurseries. This practice could be responsible for the introduction of a wide range of nematode species into the tomato farms in the region. This is also compounded by the observation that all the tomato farms utilize water from the nearby Thiba River for irrigation, without first treating the water. In studies by Di Vito *et al.*, (1991), the relationship between initial population densities of *M. incognita* race 1 and yield of susceptible and resistant tomato showed a tolerance limit of 0.55 eggs and juveniles/cm^3 soil for both types of tomato. The RKN populations in the sampled areas in Riambogo area were near or at this threshold level therefore making management of this nematode a priority for farmers.

Lesion nematodes (*Pratylenchus* spp.), were widely distributed especially in the areas that had been left fallow with grasses for at least two seasons. These nematodes were however not isolated from soil sampled in areas where tomatoes and other horticultural crops had previously been grown. Lesion nematodes are a group of migratory plant pathogenic nematodes which attack many crops especially cereals but not of economic importance to tomato or other horticultural crops (Uehara *et al.,* 2001; Vidhyasekaran, 2004). For example, host range studies by Endo (1959) showed that corn, crabgrass (*Digitaria sanguinalis* (L.) Scop.), millet, sorghum, rye, soybean and Sudan grass were very good or good hosts of the *Pratylenchus* species *P. zeae*, while alfalfa, barley, bean, clover, lettuce, oat, tomato, vetch and watermelon were non-hosts of *Pratylenchus* spp. This genera is an important pest of maize crops which is also used as a rotation crop in the Mwea region but does not attack tomato (Rich *et al.,* 1977; Johnson, 1998). However, Potter and Olthof (1977) reported that a particular species of *Pratylenchus*; *P. penetrans,* caused significant losses in tomatoes even at population densities of two nematodes/cm^3 of soil.

In similar studies, the growth of tomato plants was reduced by 20-66 % after 2 months' infection with *P. penetrans* populations ranging from 8-55 nematodes per 100g of soil at planting and the severity of root necrosis increased with nematode populations (Miller, 1975). Literature indicates that the presence of *P. penetrans* could seriously affect the yields and vigor of tomato plants especially in association with other nematodes. For

example, in studies by Estores, (1971) on tomato, it was found that the population level of *P. penetrans* on its own was about four times greater than in combination with *M. incognita*, and *M. incognita* alone reproduced twice as fast as it did in the presence of *P. penetrans*. Root invasion by *P. penetrans* was also significantly inhibited by the presence of *M. incognita.* The lack of infestation by this nematode in soils which had grown tomatoes suggests that the species of *Pratylenchus* present in Riambogo area may not be *P. penetrans* but one which is non-parasitic to tomatoes. Further characterization of this nematode from mwea therefore necessary to determine the particular species of *Pratylenchus* are involved, since some have been reported to attack tomato and cause significant losses especialy in association with RKN.

The research also showed the uneven distribution of both root-knot nematodes and lesion nematodes in the sampled areas. This was consistent with previous surveys which showed the overall distribution of RKN, Reniform nematodes, and Columbia lance nematode (CLN); *Hoplolaimus columbus,* can be highly variable and spatially aggregated in fields (Barker and Olthoff 1976,; Starr *et al.,* 1993; Wrather *et al.,* 2002; Wyse-Pester *et al.,* 2002; Monfort *et al.,* 2007). The spatial patterns of nematodes in nature are patchy or aggregated at almost any resolution at which they have been studied (Goodell and Ferris, 1980, 1981; Duncan *et al.,* 1994a, 1995; Been and Schomaker, 1998). For example, it has been reported that in cotton fields, the distributions of *M. incognita* within fields are uneven and scattered (Blasingame, 1994; Thomas and

Kirkpatrick, 2001). Infested areas are oblong in the direction of cultivation and are often 7–13 m long and 3–10 m wide. Infested areas within a field typically suffered 75–100 % damage while other areas in the same field showed no symptoms (Thomas and Kirkpatrick, 2001).

Site-specific management of RKN is now possible after results from a recent study indicated that there is a relationship between the soil electrical conductivity and RKN populations (Wendot, 2012). High RKN populations were detected in locations with high soil EC values. It was also established from that study that the spatial patterns of soil EC and RKN population densities are very similar, therefore making mapping of their distribution possible (Wendot, 2012.), Although nematicides are generally applied field wide at a single rate, which is wasteful because some areas in these fields may have nematode population densities below economic or damage threshold levels or none at all, while other areas may have severe nematode infestations. Therefore, nematicides end up either over- or under-utilized in many areas throughout the field.

Historically, growers have utilized field-wide nematode management strategies because of their inability to locate and identify areas of differing nematode densities to allow delivery of nematicides in a site-specific manner within fields (Evans *et al.*, 2002). Site-specific application of nematicides offers an opportunity to improve nematode management efficiency. A system that could identify areas within fields with potentially

high or low nematode population densities, providing guidance for more efficient, targeted sampling, combined with the technology to deliver a nematicide or planting a resistant variety in a site-specific manner would significantly improve nematode management efficiency, profitability, and environmental stewardship.

5.1.2 Effects of treatments on number and weight of fruits per plant

There was a significant difference ($p \leq 0.05$) between the fruit weights of all three varieties grown in RKN free soils with the nematode resistant variety Assila showing the highest average. This variety had fruit yields that did not differ significantly with the treatments planted in RKN free soils. This observation indicates that the variety of tomato played a more important role in increasing yields than the addition of Mocap at the recommended rate under varying nematode densities in Mwea. The data also showed that there was no significant difference ($p \leq 0.05$) between the fruit weights of varieties planted with Mocap at the recommended rate, and those planted without Mocap under both field and greenhouse conditions.

There was a significant difference ($p \leq 0.05$) between the fruit weights of all three varieties' grown in RKN free soils with the RKN resistant variety, Assila showing the highest average yields (3,356.0 (120) g with application of 2 g Mocap under varying nematode densities as well as in greenhouse conditions with 4g Mocap.The variety Sandokan recorded the second highest fruit weight per plant under the same conditions.

The two hybrid tomato varieties (Assila and Sandokan) showed no significant differences in the weights of fruits per plant when grown under either low RKN or RKN free soils and with application of both 2 and 4 g Mocap (Table 4.8). All the three varieties showed no significant differences ($P<0.05$) in the fruit weights when grown in nematode free soil. This suggests that the farmers could choose to plant any of the varieties under RKN free soils but should choose one of the nematode resistant varieties under either low or high RKN densities of infestations preferably with 4g Mocap under high RKN densities. This is a further indication that, depending on the nematode infestation levels, the choice of which RKN resistant variety is very important.

Significant differences were also observed in the number of fruit per plant within all the treatments grown under field and greenhouse respectively although variety Assila recorded the highest number and Sandokan the second highest under all nematode densities and Mocap applications. In both the field and greenhouse experiments, the two hybrid tomato varieties (Assila and Sandokan) showed no significant differences in the number of fruits per plant when grown under either low RKN or RKN free soils. This was also noted both with and without Mocap application. These results also indicate that the farmers could choose to grow either of these two hybrid varieties once they determine that their soils have a relatively low RKN density (8-10 RKN per 200 cm^3 soil).

Similar experiments have shown that RKN resistant tomato varieties are a viable cost effective management strategy for the RKN. Experiments to determine the effectiveness and profitability of resistant tomato in suppressing populations of *M. javanica* in a plastic house with a natural infestation of the nematode concluded that growing of the resistant variety as opposed to susceptible tomato varieties in non-fumigated soil increased profits by 3,000,016 euros ha^{-1} (Sorribas, *et al.,* 2005). The results of this study are also in agreement with those of Roberts, (2002); Rich and Olson, (1999) and Ornat *et al.,* (1997), who observed that yield is not significantly reduced in commercially available resistant tomato varieties when they are planted in nematode infested soils as compared to RKN susceptible ones. They attributed this to the coupling of tolerance with resistance in the varieties used. Plant resistance has been reported to be the single most important control measure that is able to suppress or retard invasion by a potential pathogen (Roberts, 2002).

Cultivation of nematode-resistant varieties has a dual purpose, to avoid crop damage by nematodes, and to reduce the buildup of nematode population levels. As such, nematode resistant varieties can reduce the nematode levels before a subsequent nematode susceptible crop is planted (Colyer *et al.,* 1998; Hanna, 2000; Ornat *et al.,* 1997). Host plant resistance is particularly useful for organic farming or integrated production since these systems do not allow, or restrict, the use of chemical control, respectively (Netscher and Sikora,1990). Resistance in tomato is conferred by the single dominant

gene Mi, which was introgressed from the wild relative of tomato *Lycopersicon peruvianu* M. (Smith, 1944; Medina-Filho and Stevens, 1980) and is present in all RKN resistant commercial varieties.

Mi mediated resistance triggers a hypersensitive response leading to cell death soon after nematodes initiate feeding near the vascular bundle (Dropkin, 1969a). The *Mi*-resistance gene confers resistance, but not immunity, to *M. incognita*, *M. javanica* and *M. arenaria* (Roberts and Thomason, 1989). However, expression of resistance is affected by some factors such as soil temperature, species and populations of RKN, , and tomato genetic background. The efficient use of resistance to manage RKNs must take into consideration the following factors. First, soil temperatures higher than 28^{o}C suppress resistance expression (Dropkin, 1969b; Williamson and Hussey, 1996). Secondly, it has also been found that resistant tomatoes have a high level of resistance to populations of *M. incognita* and *M. arenaria*, but are less resistant to *M. javanica* (Ornat *et al.*, 2001b). In addition, some *Meloidogyne* populations can overcome resistance. The main RKN species affecting Vegetables in Kenya has been found to be *M. incognita* and *M. javanica* (Kanyagia, 1980). It may therefore be necessary to combine plant resistance / tolerance with other management techniques if resistance durability is to be assured and to ensure effectiveness of a site specific management strategy.

5.1.3 Effects of MMocap on galling index and average numbers of *Meloidogyne* spp. J2s

In both the greenhouse and field experiments, there was no significant difference between the galling index on pre-plant application of 2gm Mocap and those without Mocap. This implies that the application of Mocap did not significantly affect the infection rate of the tomato varieties. The data on both galling index and number of J2s showed that the tomato variety had a significantly larger effect on fruit yields than application of Mocap at the recommended rate of 2g per plant. However when 4 g per plant of Mocap was applied under greenhouse conditions, the galling index was significantly reduced to between 3 and 5 under both low and high nematode densities. This results show that doubling the level of Mocap application to 4g per plant can reduce the effects of RKN infection in plants and ultimately increase yields.

There was a significant difference in the average numbers of *Meloidogyne* spp. J2s under all the treatments observed over a period of 90 days in the field grown tomatoes. In addition there was also a significant difference between the average galling index of all the treatments over the same period of time. However none of the treatments were able to completely eliminate the RKNs from the soil. Many factors could have contributed to this including the mode of action of Mocap as well as the continued use of flooding irrigation used in the experiments. It was noted that RKN are still able to invade and multiply in the resistant varieties as is evidenced by the galling indexes of the two varieties, both with and without Mocap applications.

The above scenario is often observed in the field with resistant varieties and is termed as virulence; defined as the ability of nematodes to reproduce on a host plant that possesses one or more resistance genes (Netscher, 1976; Ornat *et al.*, 2001). It occurs naturally in *Meloidogyne* populations on tomato, even without previous exposure to or selection by the *Mi* resistance gene (Prot, 1984; Ornat *et al.*, 2001). Although the *Mi-1* gene should block nematode development at an early stage of the interaction, it does not confer total immunity, and occurrence of and variation in RKN reproduction on resistant tomato genotypes has also been documented (Tzortzakakis *et al.*, 1999; Lopez-Perez *et al.*, 2006). In addition it has also been reported that RKN biotypes virulent against the *Mi-1* gene (i.e. able to reproduce on *Mi-1*-resistant tomatoes) are present in many of the tomato growing areas in the world (Castagnone-Sereno 2002). The apparent lack of effectiveness of Mocap(Ethoprophos) in the experiments may also have been due to the prevailing use of flooding irrigation. This may have been the case in the experiments in Riambogo area of Mwea where irrigation by flooding is a common practice (Wendot *et al.'*, 2012).

A study by Rahi, *et al.*, (1992) suggests that the continued presence of water in the soil may adversely affect the efficacy of Mocap. Ethoprop is more soluble than other nematicides such as fenamiphos and can be rendered ineffective with as little as 2.5 cm of precipitation (Rahi, *et al.*, 1992). In trials comparing Ethoprop and Fenamiphos, it

was found that the latter was not affected by 5 cm simulated rainfall applied 1 or 3 days after fenamiphos application as opposed to Mocap (Johnson, *et al.,* 1991). They therefore concluded that Ethoprop required more frequent applications or a higher dosage to be as effective as fenamiphos. The above was confirmed in the greenhouse experiments in this study where addition of upto 4 g of Mocap effectively reduced the galling index and significantly increased fruit yields. The method of application in the field trials was by pre plant incorporation in the first 10 cm of soil at the root zone of transplanted tomato seedlings. This was followed by planting under furrow irrigation where the furrows with the transplants were flooded. This method of application probably contributed to the relative ineffectiveness of the recommended rate of Mocap (2g/plant) in significantly reducing the galling index or increasing the average fruit yields of the treatments.

Ingham *et al.,* (1991) found that all post-planting applications of Mocap were ineffective, and none significantly reduced the percentage of tubers infected, the infection index, or the percentage of culled tubers. However, pre plant applications of Mocap had better results. Santo *et al.,* (1988) also found post-plant water incorporation of Mocap less effective for suppression of *M. chitwoodi* than was pre-plant physical incorporation. In the experiments by Ingham *et al.,* (1991) on potato, the failure of post-plant Mocap applications at a rate of 2g/plant to control tuber infection may be related to application method, because in their trials, all post-plant treatments were surface-applied

and water-incorporated. Downward movement of Mocap applied to loam and sandy soils is restricted to a few centimeters, even with 35.3 cm of rainfall (Smelt *et al.*, 1977), and there is little chemical movement below the depth of physical incorporation (Smelt *et al.,* 1976). Similarly, Brodie (1971) observed that complete control of *Meloidogyne* spp. extended only 5 cm below depth of incorporation in pots watered daily for 6 weeks.Mocap They also found that surface drying lowered water content several centimeters deep and reversed the direction of diffusion. These drying patterns exist in many tomato production areas in Mwea where day temperatures are high; resulting in the drying of soil layers near the surface and may further restrict the downward movement of Mocap. This property of Mocap is however attractive because it decreases the probability that the compound will be leached into groundwater, but emphasizes the need for physical incorporation.

The field application of Mocap at the recommended rate of 2g Mocap per plant did not significantly affect either the number or weight of the fruit of the tomato varieties. The results suggested that the differences in tomato yields among the treatments was attributed to the choice of variety used rather than in the application of Mocap, with the variety Assila consistently showing higher yields than both Sandokan and Rio-Grande under both high and low RKN densities. At low nematode densities, the variety Assila showed the highest yields, and in addition showed no significant difference in yield between the un-infected plot and the infected plots with application of two grams of

Mocap. In the greenhouse experiments, the effect of Mocap at the rate of 4 g per plant was not significantly different from the uninoculated plants for all varieties suggesting that a single application of Mocap at double the recommended rate was effective in controlling RKN especially at high densities. Performance of Mocap in different studies may also be affected by historical use because soils treated with this nematicide can experience enhanced biodegradation and reduced effectiveness (Mojtahedi *et al.*, 1991). Many nurseries in the Mwea region have been using granular Mocap applications during tomato nursery setup and its continued use may have an effect of enhancing biodegradation of the active ingredient by microorganisms especially after the seedlings are transplanted in the new fields.

For many non-fumigant nematicides (carbamates and organophosphates), the biochemical effects at field rates can be reversed, and nematode recovery may occur if concentration and exposure time are too low (Nelmes, 1970; Opperman and Chang 1991; Faske and Starr, 2006). Carbamates and organophosphates are therefore often called 'nematistatics' instead of nematicides (Desaeger *et al.,* 2011). These products do not always kill nematodes, but at times appear to disorient, paralyze, or confuse nematodes and so prevent infestation of roots (Opperman, 1998). If the product is present for a long enough period of time, the nematodes will eventually starve to death. In practical application, the product decomposes in soil over a period of several weeks (Mojtahedi *et al.*, 1991). This can result in nematodes regaining their abilities to feed on

and penetrate roots. However, although technically, and under laboratory conditions, nematodes may recover, in real life they are probably too weak to locate a host root and would likely die of starvation (Haydock *et al.,* 2006).

This could probably contribute to the observations in the trials where Mocap application at the recommended rate of 2 g per plant did not significantly affect the fruit yield of the tested varieties. The addition of 4 g of Mocap per plant at planting however was shown to have significant effect in reducing the nematode infestation and ultimately increasing yields under greenhouse conditions. However this rate of Mocap application may be considered too high (double the recommended rate) and may not be environmentally friendly given the high mammalian toxicity of organophospahtes. Non-fumigants nematicides such as Mocap can also deteriorate in their efficacy after repeated applications due to microbial degradation (Mojtahedi*, et al.,* 1991 and Giannakou*, et al.,* 2004). Consequently, identification and testing of several active compounds is recommended. This would especially be important since alternation of active ingredients is often required to prolong their efficacy and be better integrated in site specific nematode management strategies (Sikora *et al.,* 2005).

The application of Mocap at the recommended rate significantly reduced the numbers of J2s observed at the root zones of sampled plants but did not significantly reduce the galling index of the plants. These results agree with previous observations by Ingham *et*

al., (2000), who in a study to evaluate the control of *M. chitwoodi* with fumigants and non-fumigant nematicides, found that Mocap did not control *M. chitwoodi* populations or tuber infection when used alone. However, in combination with metam sodium, Mocap reduced numbers of *M. chitwoodi* more than metam sodium alone and provided adequate tuber protection. Pinkerton *et al.,* (1986) also found that Mocap had no effect on soil population densities of *M. chitwoodi* but reduced culls of potato from 100 % in non-treated plots to 28 % when used alone, and from 85 % in 1,3-D plots to <3 % when used in combination with 1,3-D. Santo and Wilson (1990) found no effect of Mocap on either soil population densities of *M. chitwoodi* or tuber infection.

In a study by Radwan and others (2012) to test the efficiency of several nematicides in reducing the nematode infection of tomato, it was found that oxamyl followed by Mocap and cadusafos were able to suppress *M. incognita* (Radwan *et al.,* 2012). These results were in conformity with Stephan *et al., (*1998) and Meher *et al., (*2010) who reported that Mocap and Cadusafos were effective in reducing nematode populations and increased the yields on tomato and aubergine although Cadusafos was superior to Mocap. They also found that both cadusafos and Mocap suppressed *M. javanica* gall formation (Radwan *et al.,* 2012). The results also showed that the use of resistant tomato varieties was effective in managing the effects of RKN infection. The yields in both resistant varieties, Sandokan and Assila were higher under all treatments and RKN densities.

The results of these experiments are in agreement with results from previous workers which showed the effectiveness of Mocap and host plant resistance in plant parasitic nematodes management. A study by Cadet and Daly in 1996 found that coating yam seed tubers with liquid Mocap controlled populations of *Scutellonema bradys* (the yam nematode) in yam tissues. Although this did not result in yield improvement, it greatly reduced the infestation and storage rot of tubers in the field (Cadet and Daly, 1996). Other studies showed that Phenamiphos at 1 % a.i./vine followed by carbofuran and Mocap was effective in controlling nematodes in Malaysia (Leong, 1986) and in Sri Lanka (Ratnasoma *et al.*, 1991). A study by Gourd and others in 1993 showed that both Mocap and fenamiphos are equally effective invitro in reducing penetration of host roots by RKN and were similarly effective against *R. reniformis* (Gourd, *et al.*, 1993). However, in other field evaluations on pineapple, Mocap has not matched the efficacy of fenamiphos (Melton,. 1991). They concluded that the differences in performance between the two post-plant nematicides may lie in their chemical properties. In 1987 several studies by Rinehold and Witt, (1989) and Williamson and Kriesel, (1987) on potato showed that applications of metam sodium, Mocap, aldicarb, and 1,3-D applied at rates 68 %, 66 %, 29 %, and 2 %, respectively, of the potato acreage within the Columbia Basin area of Oregon did not adequately control *M. chitwoodi*. The studies showed that even at these application rates, infections with the nematode often reached

unaccepTable levels on long-season crops during warm summers (Rinehold and Witt, 1989; Williamson and Kriesel, 1989).

5.1.4 Effects of treatments on relative costs of nematode management strategies

A Benefit-cost Ratio (BCR) is the ratio of the benefits of a project or proposal, expressed in monetary terms, relative to its costs, also expressed in monetary terms. As a general rule, the higher the BCR the better the investment. (Boardman, 1996). Benefit-cost analysis for all treatments showed that the variety Rio Grande had the lowest cost benefit ratio (CBR) under high RKN densities while the variety Assila showed the highest cost (5.6 and 6.2 in the 1^{st} and 2^{nd} season respectively) when planted in RKN free soils. Under high RKN densities, planting the variety Asilla with an application of 2 g Mocap showed the highest CBR of 2.9 in the first season and 3.7 in the second season.

Under low RKN, Assilla variety planted with 2 g Mocap recorded a CBR of 4.1 and 4.3 in the first and second seasons respectively. This is comparable to the variety Sandokan which had a ratio of 3.8 and 4.3 during the two seasons. The variety Rio Grande had significantly less CBR than the two other varieties under all nematode densities and would be a relatively poor choice for the region especially when grown in areas infested by RKN.

The cost benefit ratio indicates how much a farmer is likely to benefit from his investment; for example, under high RKN densities, he would get back a maximum of 3.7 times the investment if he chooses to plant the variety Asilla and apply 2 g Mocap and only 1.6 times the investment if he chose to use the variety Rio-Grande under the same conditions. These options would cost him about Ksh 97,500 and 87.000 respectively but he would clearly stand to benefit the most if they chose the latter option. These results are in agreement with similar RKN management strategies, which have utilized host plant resistance and been found to be cost effective. For example in a study by Barrett *et al.,* (2012), it was found that the use of RKN resistant tomato varieties as rootstocks was relatively more cost effective. They showed that under severe RKN pressure, such grafting may be an economically feasible pest control measure to help maintain a profitable production given that the risk of economic crop losses due to RKN outweighed the higher cost of RKN resistant grafted transplants.

Another study by Sorribas, *et al.,* (2005) found that *Mi*- gene mediated resistance in tomatoes can be an effective and economic alternative to methyl bromide in plastic-houses infested with RKN. Sorribas *et al.,* (2005) documented its value in the production of tomato in glasshouses where the soil beds were infested with *M. javanica*. Growing three successive crops of a variety with *Mi1.2* increased gross returns by €30,000 ha^{-1} (US$37,500 ha^{-1}) compared with three crops of a susceptible cultivar. The resistant

variety also increased returns by €10,000 ha^{-1} (US$12,500 ha^{-1}) relative to susceptible varieties when the soil was treated with methyl bromide before planting the first crop.

Identification of specific areas within individual fields for nematicide application may allow producers to reduce the amount of nematicide or other management options applied for nematode control and lower production costs (Evans *et al.,* 1999). Precision farming technology now makes site-specific application of nematicides, as well as other inputs, possible (Wendot 2012). Once such affordable and effective map of management zones has been constructed for a target field, the zones become the units for targeting nematode sampling and subsequent RKN management. Appropriate management options such as nematicide or resistant tomato variety are then focused only to zones with specific range of nematode population (Evans *et al.,* 2002; Wrather *et al.,* 2002). Tomato growers in Mwea rely heavily on application of nematicides at planting for nematode control (Waiganjo *et al.,* 2006) and this technology could be of benefit to them if adopted.

5.2 CONCLUSION

This study has demonstrated that the use of RKN resistant varieties has the potential for use as an economical, cost effective method for site specific management of RKNs in tomato production. This is especially so under high RKN densities.

Field-wide application of a uniform nematicide rate results in chemical application to areas without RKN or where nematode densities are below an economic threshold, or the application of sub-effective levels of nematicides in areas with high nematode densities.

Mocap application at the recommended rate of 2 g per plant demonstrated little value in reducing the RKN population and certainly did not eliminate the RKN under both field and greenhouse conditions. However the application of 4g per plant of Mocap in areas where of high RKN densities (above 32 RKN per 200/cm^3 of soil) is effective in significantly reducing the effects of RKN on tomato yields.

This study has also shown the significance of using resistant tomato varieties in integrated site-specific management which can be used to project where nematodes are likely to occur within an area or field. Host plant resistance is particularly useful for organic farming or integrated production since these systems do not allow, or restrict, the use of chemical control, respectively.

RKNs were shown to colonize and multiply in the three varieties equally. However the data shows that the two hybrid varieties, which are currently marketed as RKN resistant varieties, may in fact be only RKN tolerant varieties. These two hybrids showed significantly more yields and better benefit cost ratios under all nematode densities than

the open pollinated variety, Rio-Grande which is commonly planted in Mwea and would therefore be the variety of choice in the area, especially under heavy RKN infestation.

5.3 RECOMMENDATION

There is need to characterize the *Pratylenchus* spp. found on tomato in Mwea.

As this research has demonstrated, farmers can easily adopt the use of nematode tolerant tomato varieties as well as targeted applications of Mocap as a viable and cost effective method of RKN management. This will ensure sustainable yields for tomato farmers in the Mwea and other tomato growing regions. This can be coupled with the use of nematode density maps, which has now been made possible and can enable targeted application of nematicide as well as aid the choice of tomato variety for planting.

Farmers in Riambogo area of Mwea are also encouraged to adopt farming practices that eliminate sources of RKN infestation as well as manage the infestations already on their farm. They are encouraged to source for disease-free seedlings from nematodes since there is a possibility of getting RKN inoculums from commercial nurseries.

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Appendix 1

ANOVA tables and Normality Plots

Table 1.1. Tests of Normality by Kolmogorov-Smirnov[a] test

Variable	KS Statistic	df	Sig.
Shoot Weight	.114	53	.083
Root Weight	.093	53	.200*
Number of Fruits Per Plant	.125	53	.039
Galling Index	.223	53	.000
Average Number of Large Fruit	.056	53	.200*
Average Number of Medium Fruit	.095	53	.200*
Average Number of Small Fruit	.129	53	.028
Average Fruit Weight Per Plant	.119	54	.055
Average Weight Large Fruit Per Plant	.068	53	.200*
Average Weight Medium Fruit Per Plant	.105	53	.200*
Average Weight Small Fruit Per Plant	.118	53	.065

*. This is a lower bound of the true significance
a. Lilliefors Significance Correction

Figure 1.1: Normal probability (Q-Q) plots and histograms showing normal distributions of Average Galling Index

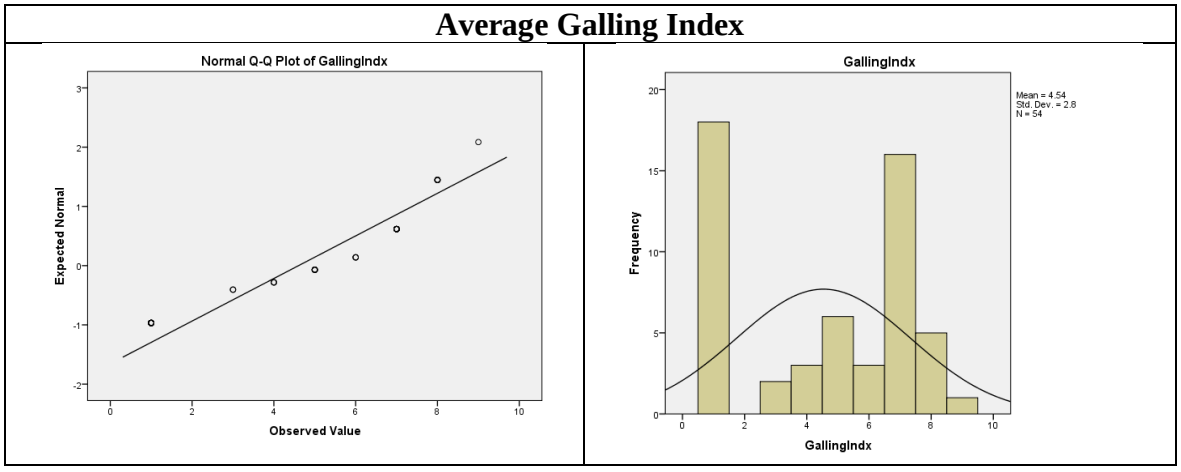

Figure 1.2: Normal probability (Q-Q) plots and histograms showing normal distributions of Shoot, Root and Fruit weight per plant

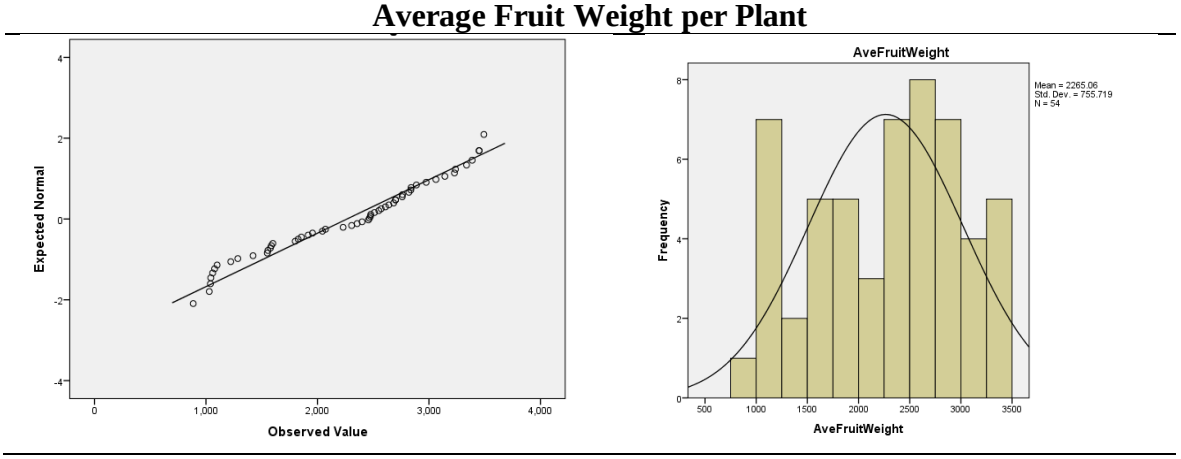

Table 1.2 Analysis of variance for the average fruit weight per plant

Anova: Single Factor **fruit weight**

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Low RKN	18	41089.3254	2282.7403	626344.0866
No RKN	18	50966.75345	2831.486303	157848.5794
High RKN	18	30256.67749	1680.926527	295004.9114

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11922538.74	2	5961269.368	16.57139386	2.85173E-06	3.178799292
Within Groups	18346358.82	51	359732.5258			
Total	30268897.55	53				

Table 1.3 Analysis of variance for the average number of fruit per plant

Anova: Single Factor **No of fruits/plant**

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Low RKN	18	479.7357143	26.65198413	70.61083874
No RKN	18	556.4452381	30.91362434	10.38993668
High RKN	18	365.0629509	20.28127505	48.05311093

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1030.766177	2	515.3830883	11.98064862	5.42867E-05	3.178799292
Within Groups	2193.916068	51	43.01796212			
Total	3224.682244	53				

Table 1.4 Analysis of variance for the average galling index

Anova: Single Factor **galling Index**

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Low RKN	18	104.4119048	5.800661376	2.089687356
No RKN	18	18	1	0
High RKN	18	122.4194444	6.801080247	2.063606939

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	346.1983176	2	173.0991588	125.0326703	2.17365E-20	3.178799292
Within Groups	70.60600303	51	1.384431432			
Total	416.8043206	53				