# FERTILIZER – ROOT KNOT NEMATODES INTERACTIONS IN HIGH TUNNEL TOMATO PRODUCTION

**DAISY CHEPKOECH NGENO**

**MASTER OF SCIENCE**

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**JOMO KENYATTA UNIVERSITY OF**

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# Fertilizer – Root Knot Nematodes Interactions in High Tunnel Tomato Production

**Daisy Chepkoech Ngeno**

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## DECLARATION

This thesis is my own original work and has not been presented for a degree in any other University.

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

**Daisy Chepkoech Ngeno**

This thesis has been submitted for examination with our approval as University supervisors.

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

**Dr. Lucy K. Murungi, PhD**

**JKUAT, Kenya**

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

**Dr. Vitalis Wekesa, PhD**

**Flamingo Horticulture (K) Ltd, Kenya**

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

**Dr. Solveig Haukeland, PhD**

**ICIPE, Kenya**

## DEDICATION

This thesis is dedicated to my dear parents Mr. Paul K. Ngeno and Mrs. Beatrice C. Ngeno for their support, understanding, prayers and encouragement during the pursuit of this degree. My sincere gratitude to them for laying down the foundation of success and being a source of inspiration, to my brother and sisters for their prayers and support and to my friends who stood by me and encouraged me during my study period at the Jomo Kenyatta University of Agriculture and Technology. May the Lord bless you all.

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## LIST OF ABBREVIATIONS AND ACRONYMNS

**RUFORUM** Regional Universities Forum for Capacity Building in Agriculture

**RKNs** Root knot nematodes

**GPS** Geographic Positioning System

**FGDs** Focus Group Discussions

**GoK** Government of Kenya

**JKUAT** Jomo Kenyatta University of Agriculture and Technology

**ICIPE** International Centre of Insect Physiologsy and Ecology

**FAO** Food and Agricultural Organisation of the United Nations

**PPNs** Plant Parasitic Nematodes

**USDA** United State Department of Agriculture

**C/N** Carbon/ Nitrogen

**HCDA** Horticultural Crops Development Authority

**MT** Metric Tonnes

**KALRO** Kenya Agricultural and Livestock Research Organization

**DEGO** Dorsal Esophageal Gland Opening

## ABSTRACT

Tomato (*Solanum lycopersicum*) is one of the important vegetables grown in Kenya. It is the second leading vegetable in terms of production and nutritional value after the potato. Tomatoes are mainly grown by small scale farmers in most arable areas with the main production areas being in Kiambu County. Production of tomatoes in Kenya has been mainly under open field conditions until recently where modified high tunnels ('greenhouse') were introduced. Tomato production in greenhouse in Kenya is hampered by pests and diseases mainly *Meloidogyne* spp., known as root knot nematodes (RKNs). RKNs are the most serious threat to utilization of the greenhouse tomato production in Kenya. The efficacy of current management strategies for RKNs is limited. Mineral nutrients are known to be important in plant-disease interaction, particularly plant-soil pathogen interaction. The challenge is that, there is limited information on how the nutrients affect the pathogens and plant's response to the pathogen infection, whether positively or negatively. Therefore, this study sought to evaluate fertilizer – RKNs interactions in high tunnel tomato production. Field surveys were conducted among small holder famers growing tomato under high tunnel 'greenhouses' in Kiambu County, Central Kenya. The field surveys include a focused group discussions which was followed by soil sampling from the high tunnels. A farmer knowledge survey was done involving focus group discussion with 32 groups of farmers in six sub-counties *viz*. Thika, Juja, Kiambu, Ruiru, Gatundu North and Gatundu South was conducted during the period July – September 2016 using a checklist with open ended questions. About 78.1% of high tunnels were in use for 1–2 years and 62.5% of farmers taking part in the study could identify symptoms caused by RKNs, which resulted in crop loss of 50%–100%. Seventy-one percent of respondents had positive attitude about high tunnel tomato production. About 82.6% had the soil in which they produced tomato in the tunnels analyzed for nutrition and presence of pathogens, but the majority (71.7%) never followed recommendations on how to amend their soils nutritionally and against the major soil-borne diseases. These findings indicate that more research and information are required so that farmers can optimize high tunnel production of tomato under tropical conditions. To determine effects of soil chemical properties on abundance of nematodes in high tunnel tomato production. Soil samples were collected from the 32 high tunnels in the six sub-counties of Kiambu County between January and November 2016. Nematodes of various genera and soil chemical properties were evaluated from composite soil samples collected from the high tunnels. Soil pH and N, P, K, Ca, Mg, Na and Cu varied significantly ($P = <0.001$) across sub-counties. Twenty-four nematode genera including 14 PPNs, 5 bacterivores, 3 fungivores and 2 predators were recovered

from soil samples. The genera *Meloidogyne*, *Alaimus*, *Aporcelaimus* and *Mononchus* were the most abundant PPNs, bacterivores, fungivores and predators, respectively, and differed significantly (P = <0.001) across sub-counties. There was a strong positive correlation between the population of *Meloidogyne* spp.(second stage juveniles counts) with soil N and P, and a weak negative correlation with soil pH, Ec, Zn and Cu existed. Fungal feeders exhibited a strong negative correlation with soil pH and Ca; predators, bacterial feeders, and PPNs had similar correlations with N, P and Ca, respectively. These findings indicate that soil chemical properties has effect on the population of nematodes and this information is useful to farmers and other stakeholders for improving farmer practices for the management of plant parasitic nematodes in high tunnel tomato production.To determine effect of NPK fertilizer application levels on population density of root knot nematodes and on tomato yield in high-tunnel tomato production. The lowest numbers of J2/100g of soil (518), galling index/root, (2) and egg masses/root (14.8) were observed in the plants treated with 10g of fertilizer. On the other hand, the highest fruit weight (yield) (g) was observed on plants treated with 10.0g of fertilizer (4148.2 g/plant) and the lowest weight of plant yield (219.6 g/plant) was observed in plants treated with no fertilizer. Optimal use of inorganic fertilizers can improve management of RKNs thus, can help to avoid over reliance and dependency on harmful toxic chemicals that can lead to environmental degradation.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background information

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetable crops grown in the world (Brickell *et al*., 2004). The crop belongs to *Solanaceae* family and it originated from South and Central America. In Kenya, tomato is one of the important vegetables grown for income and consumption due to its nutrition value including: minerals, vitamins, amino acids and fibres (Naika *et al*., 2005). It is also the second leading vegetable in Kenya in terms of production and value after potato (FAO, 2017). It mainly contains vitamin B and C, iron and phosphorus in large amounts (Naika *et al*., 2005). The development of fast food industries and tomato processing industries has been attributed to growing demand for tomato products (Tahir *et al*., 2012). However, its cultivation is limited largely due to plant parasitic nematodes (PPNs) such as root knot nematodes, *Meloidogyne* spp.

Plant parasitic nematodes (PPNs) are of considerable economic importance in agriculture worldwide (Bird *et al*., 2008). They are estimated to exceed US $100 billion/year worldwide including 10–20% yield reduction in cash crops (Chitwood 2003; Koenning *et al*., 1999)). Root knot nematodes (RKNs) (*Meloidgyne* spp.) are the most economically important plant parasitic pests worldwide. They have a wide host-range of more than 2000 plant species (Gugino *et al*., 2008). RKNs parasitize root systems of a wide variety of crops including cultivated tomatoes and capsicum. In Kenya, losses due to RKNs in tomato farms are not known but could range between 30-100%, depending upon the cropping system. These nematodes infect plants as motile second stage juvenile (J2) by piercing the cell wall using the stylet and feed only on cytoplasm of specific living plant cells resulting to formation of giant cells as a result of repeated nuclear divisions, without cytokinesis, and cortical cells proliferation and hypertrophy (Williamson & Gleason, 2003). In addition the nematode invasion provides entrance points for second-

ary pathogens such as soil-borne fungi or bacteria that can cause synergistic yield losses (Mitkowski & Abawi, 2003). Within smallholder tomato production, addressing root knot nematode problems is not straightforward for most farmers (Talwana *et al*., 2016). Limited use of quality inputs, lack of improved techniques, insufficient access to improved cultivars, poor infrastructural networks, and poor pest and disease diagnostics prevail. Farmers and agricultural staff lack the expertise to manage root knot nematode infestation. In addition, nonspecific, cryptic disease symptoms and a lack of apparent damage (Trudgill & Blok, 2001). This study explored the manipulation of the soil chemical properties using different fertilizer application rates to manage root knot nematodes for maximum tomato production in high tunnels.

## 1.2 Statement of the Problem

Diseases, weeds and pests account for an estimated 36 % crop losses globally (Oerke, 2006; Oerke *et al.*, 2012). Diseases alone account for 14 % of crop yields reduction (Agrios, 2005). Among plant diseases, soil-borne diseases associated with root knot nematodes infestations are considered the main factors in production of many crops as compared to seed-borne or air-borne and they account for 10–20% of yield losses annually (Weaver, 2014). Globally, tomatoes account for about 15 % of total vegetable production with a consumption rate of 20.5 kg/per capita/ year. Kenya is among Africa's leading producers of tomato and ranked 6th with a total production of 283,000 metric tons (FAO, 2017) accounting for 14% of the total vegetable produce and 6.72% of the total horticultural crops (GoK, 2014).

In Kenya, 40% of small and medium scale farmers depend on tomato production. Cultivation of tomatoes in Kenya by these groups of farmers is mainly under open field conditions until recently when modified high tunnels ('greenhouse') were introduced. Sustainability of profitable utilization of the high tunnel tomato production is mainly threatened by root knot nematodes (Ireri *et al.*, 2018). Heavy infestations by RKN cause substantial reduction in yield and increased susceptibility to pathogen attack, hence they are among the most damaging agricultural pests worldwide (Williamson & Hussey, 1996). In high tunnel tomato production, nematicides have been widely used to manage *Meloidogyne* spp., however, they cause serious threat to the ecosystem (Sharma & Rakesh, 2009), leading to most countries banning their use following these adverse effects. Environmental and human health concerns regarding nematicide use against *Meloidogyne* spp. has led to an increased interest to explore alternative strategies which are environmentally freciendly.

Mineral nutrients are known to be important in plant-disease interaction particularly plant-soil pathogen interaction (Spann & Schumann, 2013). Mineral fertilizers alone or in combination with manure have also been proposed for use in controlling PPN (Okada & Harada, 2007; Atandi *et al.*, 2017). However, continuous use of these fertilizers can

result in decreasing soil pH (Adamtey *et al*., 2016). The problem is that, there is limited information on how each nutrient affects plant's response to the pathogen infection, whether positively or negatively. Potentially, natural plant defence mechanisms and proper nutrients application could be utilized to develop alternative management strategies for root knot nematodes.

### 1.3 Justification of the study

Tomato is one of the most popular vegetables in the world. Tomato products like paste, juice and ketchup are widely used in kitchens all over the world. It has high contents of vitamins A and C and is widely used in various dishes (Bhowmik *et al*., 2012). Its demand has increased rapidly, with the rising affluence of the population therefore, resulting in higher development of tomato industry for production of tomato (Tahir *et al.*, 2012). In Kenya, rise of the fast food industry is also having a significant impact on the demand for tomato products. Soil-borne pathogens mainly root knot nematodes are a major threat to tomato industry in Kenya because most of the production areas are infected with these pathogens. The estimated worldwide losses caused by plant-parasitic nematodes are about US $ 125 billion annually (Chitwood, 2003). Economically and environmentally sound methods of root knot nematodes management with a real world impact on yield will be the drivers that validate the enterprise of high tunnel tomato production and result in reinvestment in the field. Mineral nutrition is an environmental factor which can be easily controlled especially in greenhouse production. The results from this study will therefore be used to inform farmers on appropriate fertilizer levels used to manage root knot nematodes to produce tomatoes in the high tunnels.

## 1.4 Objectives

### 1.4.1 General objective

To evaluate the effects of different fertilizer application rates on the population of root knot nematodes (*Meloidogyne spp*.) on the growth and yield of high tunnel tomato varieties.

### 1.4.2 Specific objectives

1. To assess knowledge, attitude and practices of high tunnel tomato farmers on root knot nematodes in Kiambu County, Kenya
2. To determine the influence of soil chemical properties on the abundance of nematode trophic groups in high tunnel tomato production
3. To determine the effect of NPK fertilizer application rates on population density of root knot nematodes, soil pH, C:N ratios and yield of different high tunnel tomato varieties

## 1.5 Hypotheses

1. Farmers' knowledge, attitude and practices do not influence population density of RKN on high tunnel tomato varieties
2. There is no influence of soil chemical characteristics on the abundance of nematode trophic groups in in high tunnel tomato production
3. There is no effect of fertilizer application rates on the population of RKNs and yield of tomato varieties
4. There is no effect of NPK fertilizer application rates on population density of root knot nematodes, soil pH, C:N ratios and yield of different high tunnel tomato varieties

# CHAPTER TWO

# REVIEW OF LITERATURE

## 2.1 Overview of Tomato (*Solanum lycopersicum*) production

Tomato (*Solanum lycopersicum*) is the most popular vegetable in the world. It is ranked fourth among other leading world vegetables. In Africa, tomato production is at 18,648,548 tones and Kenya is ranked 6th with a total production of 503,172 tonnes (FAO, 2017). It is one of the key vegetables produced by smallholder farmers as a major source of nutrition and income in sub-Saharan Africa.

In Kenya, tomato production is mainly by smallholder farmers who produce for home consumption and local market and it is the second leading vegetable in terms of production after potatoes (FAO, 2017). The crop is nutritionally important as a source of vitamin A and C as well as calcium and potassium. The tomato fruit can be cooked, processed into paste, ketchup or sauce or used as salad (Hortinews, 2016). In addition, evidence from epidemiologic studies has suggested tomato has phytochemicals; carotenoids, and lycopene in particular that prevent chronic disease such as cancer (Sharoni *et al*., 2012). The crop is mainly grown in most arable areas of the country. According to Horticultural Crops Development Authority HCDA, (2016) report, by the year 2016, the area under tomatoes was 20,111 ha and the total production for the country was 341,026 MT with a value of Kshs. 13.4 billion. The main production areas include Kirinyaga, Nyeri, Kiambu, Mwea and Gikambura (Birithia *et al.,* 2012). According to KALRO report in 2004, an estimated 75,101 tons of tomato valued at over KShs 1 billion (approximately $12 million) were produced in Central Province, outranking all the other vegetables in value.

Tomato production system is divided into two categories; the open field, which accounts for 95% and greenhouses (controlled environment), which accounts for 5% of production (Geoffrey *et al*., 2014). In Central Province, tomato production has been mainly un-

der open field conditions until the adoption of modified high tunnels (Plate 2-1) popularly known as 'greenhouses' in the last five years (Ireri *et al*., 2018).

## 2.2. Factors affecting tomato production

Tomato production is faced with many constraints such as pests and diseases. The major soil-borne diseases includes: root knot nematodes disease and bacterial wilt ae well as pests and other arthropods such as spider mites, thrips, whiteflies and African bollworm leading to high economic losses (Birithia *et al.,* 2012). Modified high tunnels production is a new technology that creates an appropriate farming environment. This production system economizes on space which makes it more important due to decreasing arable farm sizes in Kenya (Ireri e*t al*., 2018). In Kenya, high tunnels tomato production have been adopted by farmers however, the main challenge is the spread of root knot nematodes, *Meloidogyne* spp. that has caused some high tunnels to be abandoned (Hortinews, 2016).

**Plate 2.1:** Modified high tunnel constructed by National Irrigation Board through Kiambu County, Kenya

## 2.3 Root knot nematodes (*Meloidogyne* spp.)

Root knot nematodes (*Meloidogyne* spp.) are the top leading plant parasitic nematodes based on economic importance (Jones *et al*., 2013). They were first noted on plants by Berkerly in 1855, when he observed galls on roots of greenhouse grown cucumber plants

(Hartman & Sasser, 1985) and recognized them as belonging to the genus *Heterodera*. In 1884, Muller classified RKN as *H. radicicola* and in 1949 Chitwood separated root knot nematodes from cyst nematodes by reassigned these species to the genus *Meloidogyne* which was first named by Göldi in a paper published in 1887 but reprinted in 1892 (Hirschmann, 1985; Karssen, 2002).

### 2.3.1 Morphology

Use of morphological and morphometric features are key for the preliminary identification of RKNs (*Meloidogyne* spp.). The features mostly used in females are body shape, stylet length and stylet knob and perennial pattern shape. In males: head shape, stylet length, knob shape, and Dorsal Esophageal Gland Opening (DEGO)-stylet knob length. In J2s: body length, tail and hyaline tail length, DEGO-stylet length, hemizonid position and tail shape (Onkendi *et al*., 2014).

The general morphology of *Meloidogyne* species is: females are pearly white in color with rounded to pear shaped body and a protruding and or bend neck, their length range from 350 µm to 3 mm and width from 300 to 700 µm, the stylet length ranges from 10 to 25 µm. Males are motile vermiform and clearly annulated, ranges in length from 600 to 2500 µm, head composed of a head cap and head region, stylet length ranges from 13 to 33 µm DEGO is located 2 to 13 µm behind the stylet knobs. The J2 is vermiform, annulated and the length ranges from 250 to 600 µm, stylet length ranges from 9 to 26 µm and DEGO position is 2 to 12 µm behind the stylet knobs. Currently, *Meloidogyne* species are classified to belong to the Phylum Nemata, Order Tylenchida, suborder Tylenchina, superfamily Tylenchoidea, family Heteroderidae, subfamily Meloidogyninea, genus *Meloidogyne* (Abad *et al*., 2003).

### 2.3.2 Reproduction of *Meloidogyne* spp.

Root knot nematodes, despite having considerable conserved morphological features across the genus, they exhibit a degree of reproductive plasticity unlike that among

animals (Bird *et al*., 2008; Castagnone-Sereno *et al*., 2013). Most species of economic importance are dioecious and gonochoristic (i.e. the males and females are morphologically distinct) examples include *M. hapla* and *M. incognita* that are highly damaging and polyphagus. Some species are amphimictic and reproduce solely by outcrossing and are not significant agricultural pathogens example include *M. carolinensis* (David *et al*., 2008). Many RKNs species of agricultural importance reproduce by mitotic parthenogenesis and have various degrees of polyploidy and aneuploidy (Liu *et al*., 2007). Among these the three major *Meloidogyne* species, *M. arenaria, M. incognita* and *M. javanica* reproduce by mitotic parthenogenesis (Bird *et al*., 2009). However, *M. hapla* which also is widely distributed in temperate regions, reproduce by facultative meiotic pathogenesis. In tropical regions, RKNs are considered among the most important biotic constraints to vegetables. Tropical RKNs include *Meloidogyne arenaria*, *M. incognita*, and *M. javanica* and are believed to share a cryptic hybrid origin (Castagnone-Sereno *et al*., 2013). Other nematode species that also occur in the tropics, such as *Pratylenchus* spp. (lesion nematodes) and *Rotylenchulus reniformis* (reniform nematode), but *Meloidogyne* spp. are the most important.

### 2.3.3 Life cycle of *Meloidogyne* spp.

The life cycle of RKN comprise of six stages; egg, four juvenile stages and adult. The embryonic development results into the first-stage juvenile (J1) that moults within the egg and hatch as a second-stage juvenile (J2), the infective stage (Figure 2-1). Once hatched, the J2 leaves the egg and moves through the soil to a new host plant to nearby galled roots. During this period, the J2 depends on the energy reserves stored in the intestine and their ability to invade the roots will be reduced after long periods in the soil (Moens & Perry, 2009).

Infective juveniles enter the root tip of plant through mechanical disruption of the root tissue by use of the stylet and during this process, they produce in their sub-ventral glands cell wall-degrading enzymes such as β-1,4- endoglucanases that aid in the penetration (Rosso *et al*., 1999). The J2s then migrate through the intercellular space

within the undifferentiated root cells and move towards the elongation zone of the root where it establishes a permanent feeding site and becomes sedentary. When the J2 has established a suitable feeding site, it pierces the cell walls with its stylet and the esophageal glands release secretions that are injected into the cells.

These secretions induce formation of giant cells as a result of repeated nuclear divisions, without cytokinesis, and cortical cells proliferation and hypertrophy resulting in formation of typical root galls (Cabello *et al*., 2014). Following the initiation of the feeding site and the giant cell formation, the J2 becomes flask-shaped and once inside the root tissue they molt three times into the third (J3) and fourth-stage (J4) and adult (Figure 2-1). During the J4 stage, the RKN differentiate into male and female having their reproductive organs developing into maturity. At the fourth and final molt, the adults nematodes are reveled having the three previous juveniles cuticles, the stylets reappears in both sexes, perennial pattern is observed in females and a sperm production is initiated in males (Eisenback & Triantaphyllou, 1991). The mature females deposit their eggs in a gelatinous matrix that hold them together outside the root surface. The matrix provides physical protection to the eggs and acts as a barrier to temperature fluctuations and water evaporation (Moens & Perry, 2009).

The length of the life cycle in RKN is greatly influenced by temperature (lower minimum, optimum, and maximum) and it takes approximately 25-30 days from eggs to adults (Curtis, 2007). Earlier reports indicate that cool climate species such as *M. hapla* have different temperatures for different stages of development, hatching, mobility, invasion of roots, growth, reproduction and survival than those which occur in warmer regions such as *M. incognnita, M. javanica* and *M. arenaria*. Under adverse environmental conditions, the proportion of males increases; as reproductive function implies a greater spend of energy, differentiation of females is favored when food is available. Males are vermiform and migrate out of the roots while females are globose and remain sedentary, laying several eggs into a gelatinous matrix on the surface of a galled root or inside the galls and the cycle continuous (Maleita, 2011).

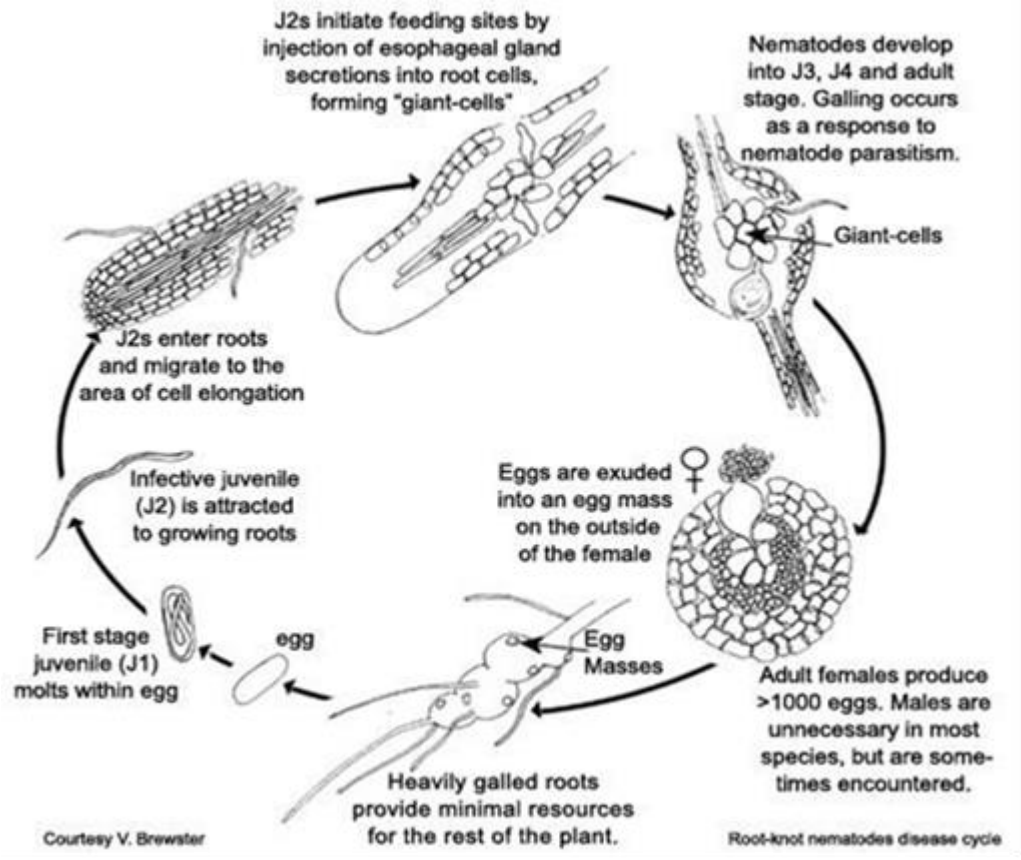


**Figure 2-1:** Basic lifecyle of root knot nematodes (*Meloidogyne* spp.), (Abawi and Brewster, 2002)

## 2.4 Management of Root Knot Nematodes

Yield loss on tomato due to RKN, *Meloidogyne* spp. range from 40 to 46% (Reddy *et al.*, 1985). Typical symptoms of plants infected with RKN include root galling, stunted growth and poor yields. They form synergies with plant pathogenic bacteria and fungi causing great yield loss (Rivera & Aballay, 2008). Edaphic factors influence nematodes distribution and abundance at the plant rhizosphere and therefore affect their management methods. Nematode management strategies used currently include crop rotation, cultural and tillage practices, use of transplants, and pre-plant nematicide treatments. These methods tend to reduce nematode populations with time. Farm specific condi-

tions, such as soil type, temperature, and moisture, determines the effectiveness of different cultural practices in nematode management (Nolling, 2014).

Identification of *Meloidogyne* spp. is the first step in deciding the most suitable control measure in crop management (Adam *et al*., 2007). Different methods have been developed in managing the effect of *Meloidogyne* spp. on crop yield (Lichtfouse, 2009) with a number of these techniques being approved for their efficacy on *Meloidogyne* spp. (Karssen *et al*., 2013).

### 2.4.1: Cultural methods

Cultural methods remain the most successful RKN control approach, as they are environmentally sustainable and friendly. The methods that have been used in the field include; crop rotation where non-host crops are rotated with the host crops. For example *M. hapla* infested vegetable field can be planted with non host crop such as corn (Bawa *et al*., 2016). According to Nicol *et al*., (2011) use of crop rotation with resistant crops and integration of fallow periods were proven effective in managing nematode infection and populations in the soil. However, rotation plan alone to reduce *Meloidogyne* spp. cannot be recommended due to their wide host range. Use of cover crops is yet another strategy, where crops are grown outside the agricultural season some of which are antagonistic to nematodes. Besides reducing nematode population, cover crops also have an added benefit as they stabilize the soil and improve its quality (Sipes & Schmitt, 2002). Thakur and Sohal (2013), reported that use of resistant plant cultivars has remained a challenge despite the fact that it is a preferred environmentally safe method to managing *Meloidogyne* damage, due to emergence of resistance breaking *Meloidogyne* spp. rendering this pest management practice ineffective (Ornat *et al*.,, 2001).

Soil flooding has also been used to reduce the density of nematodes in rice cultivation (Duncan, 1991). However, soil flooding method is not applicable in pineapple and other vegetable production due to the nature of the soil and the agronomic changes caused in

soil e.g. lack of oxygen, soil structure degradation that might alter the overall production (Collange *et al*., 2011).

Many previous studies have focused on the use of organic amendments as a control measure to *Meloidogyne* spp. showing suppressive effects (Akhtar & Malik, 2000; Waceke *et al*., 2002). Thoden *et al*., (2011) reviewed several studies in which *Meloidogyne* populations increased after the application of organic amendment. Moreover, nematode control requires a large amount of organic amendment and therefore, it is quite expensive (Noling & Becker, 1994). According to Luc *et al*., (2005) and Tariq, (2008), chemical nematicides have been widely used in managing *Meloidogyn*e spp. Despite the obvious value in these approaches, the downside is that, they require extensive planning and economic investment before successful implementation can be achieved which is not always the case. Moreover, RKNs are ployphagous thus complicating crop rotation as a management strategy.

### 2.4.2: Biologicol control

Biological control methods that have been put in place for control of nematodes includes; use of pathogenic fungi that infect eggs, rhizobacteria, endophytic fungi and obligate parasitic bacteria (Lamovšek *et al*., 2013). Most of these microorganisms have the ability to control *Meloidogyne* spp. and *Heterodera avenae* by exerting antagonistic actions through various mechanisms. Non- pathogenic bacteria control nematodes by inducing plant resistance, degrading signaling compounds to which nematodes are attracted to or by colonizing the roots thus blocking the penetration of the J2s (Lamovšek *et al*., 2013).

Fungal biocontrol agents such as *Trichoderma herzianum* have been used in screen houses for soil treatment in peat-bran formulation and effect was reduced root galling caused by *M. javanica* (Spiegel & Chet, 1998; Sharon *et al*., 2001). Other fungal that have been used to control nematodes are *Pasteura penetrans* and *Pochonia clamidosporum.* Other types of biological control agent are the rhizospheric

and endophytic fungi and bacteria, which may protect plants directly or indirectly rather than through parasitism of the nematodes by inducing resistance or inhabiting nematodes recognition sites.

Many bio-control agents of nematodes have been found and tested for the effectiveness against nematodes and have led to the development of commercial products which are cost effective as chemical nematicides. This fact has contributed to the biological control of nematodes as part of an Intergrated Pest Management (IPM) program.

### 2.4.3: Chemical control methods

Nematicides for controlling plant parasitic nematodes dates back to 1950s and were grouped as soil fumigants or non-fumigants and systemic. However, persistent use have raised concerns on their effects to human health, animal health and the environment (Danchin *et al*., 2013). As a result, effective nematicides such as dibromochloropane (DBCP), ethylene dibromide (EDB), which have been used as fumigants, have been withdrawn from the markets due to their possible effects on humans and the environment (Oka *et al*., 2000). Methyl bromide that was most effective and widely used for nematodes and other soil borne diseases and weeds has been banned from being used and was completely withdrawn from the market in 2005. Following the ban on methyl bromide other non-fumigants nematicides such as aldicarb have been put under sharp focus after being detected in ground water (Oka *et al*., 2000).

Although chemical nematicides are effective in managing *Meloidogyne* (Tariq, 2008), they are usually expensive, of limited availability, difficult to store, pollute the environment and also lose their efficacy after a prolonged use (Abawi & Widmer, 2000). Moreover, many effective nematicides are highly restricted in many countries due to their adverse effects on health and environment. Thus the development of alternative control strategies and long-term integrative approaches is urgently needed in order to replace chemical nematicides (Martin, 2003).

## 2.4 Farmers' Knowledge , Attitude and Practices on root knot nematodes

Root knot nematodes are a malignant soilborne pathogen, persistently undermining tomato production. They are a particular concern because few farmers are aware of them or the damage they cause. The limited ability to identify the nematode problems and the consequent misdiagnosis (Sikora & Fernandez, 2005) lead to unskilled and indiscriminate use of pesticides, which compromises consumer food safety, farmer health, water supplies, and the environment (Dey, 2010; Lagerkvist *et al*., 2013).

In the tropics, most farmers establish their seedling nurseries in infested fields, generating RKN-infected seedlings. The unregulated nature of seed supply systems and an inherent lack of awareness for planting material hygiene by farmers also facilitates the dissemination of nematodes to new fields (Coyne *et al*., 2018b). In order to sustainably produce and intensify tomato production in modified high tunnels, major changes to production practices are required.

## 2.5 Role of Nematodes in Soil Nutrient Cycling

Detritus and organic residues must decompose to release nutrients for plant uptake. Decomposition of organic matter in a soil food web can be divided into two energy channels, a faster bacterial channel and a slower fungal-based channel. Soil ecosystem types and nutrient forms (e.g., C:N ratios) determine the predominant decomposition channels (Ferris & Matute, 2003; Ingham, *et al.,* 1985*).* Although bacteria and fungi are the primary decomposers in the soil food web (Figure 2-2), these microbes also can immobilize inorganic nutrients in the soil (Ingham *et al*., 1985). As an extension of these decomposition channels, when the bacterivorous and fungivorous nematodes graze on these microbes, they give off carbon (iv) oxide ($CO_2$) and ammonium ions ($NH^{4+}$) and other nitrogenous compounds, affecting C and N mineralization directly (Ingham *et al*., 1985). Indirectly, nematodes can disseminate microbial propagules throughout the soil (Freckman, 1988), which advances the colonization of substrates and mineralization of nutrients. Nematode metabolites may also stimulate specific bacterial growth by releas-

ing growth-limiting nutrients (such as nitrogen and vitamins). However, overgrazing of bacterial or fungal populations by nematodes can result in a reduction of the overall activity of these decomposers. Fortunately, in the hierarchy of the soil food web, generalist predators prey on these bacterivorous and fungivorous nematodes, improving nutrient cycling and allowing more nutrients to be released (Yeates & Wardle, 1996).

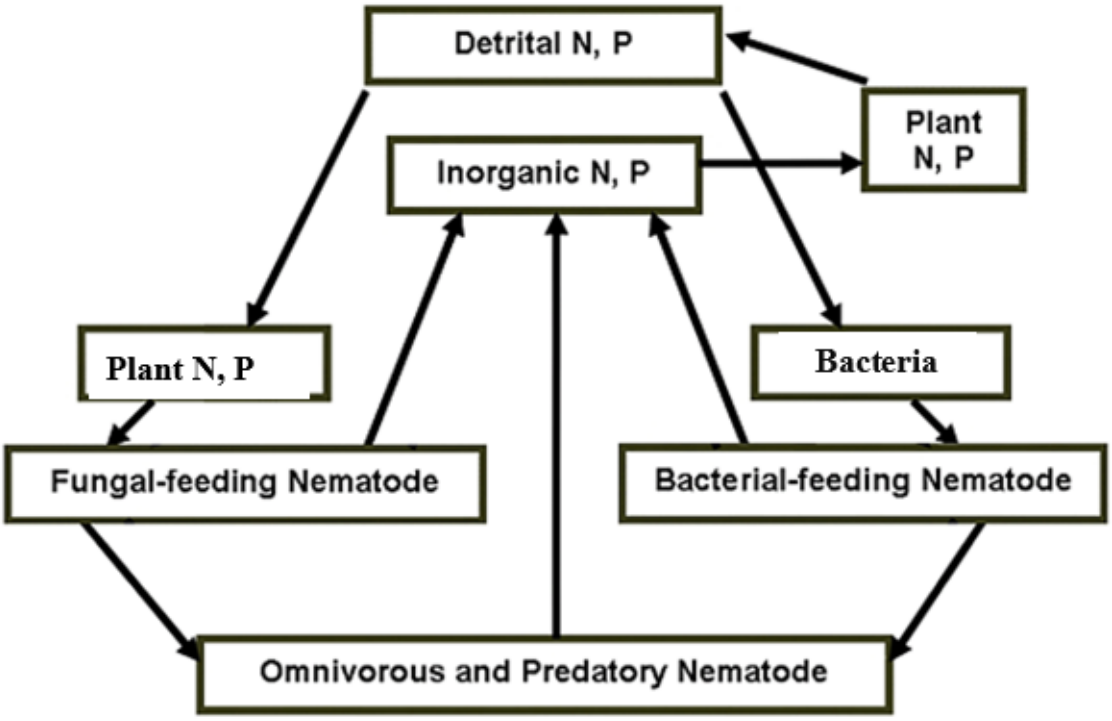


**Figure 2.2:** Roles of nematodes in organic matter decomposition (Ingham et al., 1985)

### 2.5 Plant nutrition in control of nematodes

Plant nutrients are crucial for plant growth and development and are also important in interactions between plants and diseases**.** Soil borne pathogens particularly those that infect plant roots, for example RKN reduce water and nutrient uptake**.** Hence, resulting in their deficiencies which may lead to secondary infections (Spann & Schumann, 2013). Inorganic fertilizers are often used to improve soil fertility and crop production in that they provide plants with the necessary nutrients needed to grow healthy. Furthermore, they help reduce plant stress which enables plants to withstand nematode

attack. Fertilizer application and watering plants less frequently will encourage the development of a deep root system that will reduce stress on plants and can help minimize nematode problems (Spann & Schumann, 2013).

In the soil, nematodes are attracted to their hosts by the concentration gradient formed by root exudates, which provide a recognition signal, but can also repel nematodes. However, it is not clear whether mineral nutrients play an important role in this process. Greenhouse studies have shown that applying macronutrients to sugarcane reduces the severity of the disease caused by *Meloidogyne* spp., allowing the plant to develop normally (Asano & Moura, 1995).

Among plant nutrients, nitrogen is essential for growth and yield. An abundance of nitrogen results in the production of new tissues and saps, and can extend the vegetative state and increase the number of feeding sites in the roots, encouraging nematode attack. On the other hand, a plant that is deficient in nitrogen can become debilitated, suffer slowed growth and become more susceptible (Zambolim *et al*., 2005; Ferraz *et al*., 2010). However, the form in which the nutrient is available, whether ammonium (NH4+) or nitrate (NO3-), has more effect on the severity of the nematode attack than the quantity of nitrogen available (Ferraz *et al.*, 2010).

Phosphorus is essential to plant growth and can also influence diseases caused by nematodes (Ferraz *et al*., 2010). Plants with high levels of phosphorus, release fewer root exudates and are therefore less attractive to nematodes cutting decreasing the incidence of the diseases. Furthermore, plants become more resistant when supplied with sufficient quantities of phosphorus (Zambolim *et al*., 2005), as a result of increases in protein synthesis, cell activity and production of polyphenols, peroxidase and ammonia (Wang & Bergeson, 1974). The effect of phosphorus in the control of nematodes can vary depending on the source used.

Barbosa *et al*., (2010) evaluated the use of potassium fertilizer (single and multiple doses) on populations of *H. glycines* in resistant and susceptible soybean cultivars and

observed that increasing doses of potassium reduced the number of females in the root system and the nematode reproduction factor in the susceptible cultivar. Similarly, in an experiment developed by Pinheiro *et al*., (2009), doses of potassium significantly influenced the number of cysts pot, eggs cyst females and cyst per root system and the reproduction factor of *H. glycines* in soybean. This reduction is thought to be due to the interference of the potassium in the reception of the signal by the cell membrane, reducing the number of syncytia (Barbosa *et al*., 2010)

Bednarek and Gaugler (1997) reported that addition of inorganic amendments, particularly NPK, suppressed nematode densities. Prolonged exposure to high inorganic fertilizer concentrations inhibits reproduction. However, it affected the beneficial nematodes i.e. entomopathogenic nematodes by reducing their infectivity. Oteifa (1955) reported that ammonia decreased the counts of *Meloidogyne incognita* females and egg masses produced on infected Lima beans. Proper management of diseases and pests that is mostly done through soil macro-nutrient management in cases of deficiencies can also reduce stress and help reduce damage from nematodes. Nutrient deficiencies and soil compaction can inhibit root development and increase plant sensitivity to nematode damage. Nematode damage is more severe in sandy soils than in heavy soils. There is therefore need to develop sustainable nematode management strategies to increase crop yield and crop quality while reducing reliance on nematicides.

# CHAPTER THREE

# FARMERS' KNOWLEDGE ON ROOT KNOT NEMATODES AND PRACTICES TO CONTROL THEM IN HIGH TUNNEL TOMATO PRODUCTION IN THE TROPICS


## Abstract

Sustainable production of tomato (*Solanum lycopersicum* L.) under high tunnels is threatened by root knot nematodes (*Meloidogyne* spp.). Farmers' knowledge, attitude and practices are critical in the management of root knot nematodes. Knowledge was generated about the concomitant occurrence of the root knot nematodes from January to September 2016, and knowledge and experience of farmers about practices to control them were investigated. The study involved a survey during which 32 high tunnel tomato farmer groups in 6 sub-counties of Kiambu County, Kenya, were interviewed. Data was analysed descriptively. About 78.1% of high tunnels were in use for 1–2 years and 62.5% of farmers taking part in the study could identify symptoms caused by root knot nematodes, which resulted in crop loss of 50% – 100%. Seventy-one percent of respondents had positive attitude about high tunnel tomato production. About 82.6% had the soil in which they produced tomato in the tunnels analyzed for nutrition and presence of pathogens, but the majority (71.7%) never followed recommendations on how to amend their soils nutritionally and against the major soil-borne diseases. These findings indicate that more research and information are required so that farmers can optimize high tunnel production of tomato under tropical conditions.


***Keywords***: Focus group discussions, *Meloidogyne* spp, high tunnels, smallholder farmers, tomato

## 3.1 Introduction

Tomato (*Solanum lycopersicum* L.) suffers from attack by soil-dwelling pathogens (Bolton, 2009). Tomato has an importance nutritionally as it has essential phytochemicals in human nutrition and also those that prevent chronic diseases and other additional health benefits (Sharoni *et al*., 2012). Despite its role in food and nutritional security, tomato suffers severe attack by above and below-ground pathogens and other pests (Strauss & Kluepfel, 2015). Root knot nematodes (*Meloidogyne* spp) are the most prevalent especially in smallholder farming systems (Wanjohi *et al*., 2018).

Cultivation of tomato in Kenya by 40-60% of small and medium scale farmers was mainly under open field until recently where modified high tunnels (commonly known as 'greenhouses') were introduced (Hortinews, 2016). However, sustainable and profitable greenhouse production is threatened by RKNs and bacterial wilt, which are highly prevalent (Schäfer *et al*., 2006).

Root knot nematodes are sedentary endoparasitic phytonematodes that occur worldwide (Jones, *et al*., 2013). Yield losses of between 30-100% due to RKNs infection in high value vegetables such as tomato in African farming systems may occur (Onkendi *et al*., 2014). The second stage juvenile (J2), which is the infective stage, locates and penetrates suitable host roots intercellularly to initiate nutrient sinks from vascular cells (Bird *et al*., 2009; Perry & Moens, 2011). Selected cells enlarge resulting in the formation of 'giant cells' that eventually disrupt the plant's water and nutrient uptake, hence poor growth and yield (Perry *et al*., 2009). Nematode-produced wounding also increases disease complexes from other soil pathogens (Jones & Goto, 2011). In this regard, RKNs have the potential to cause huge economic losses to important agronomic crops since smallholder farmers are often unaware of these soil dwelling pests (Coyne *et al*., 2006). Control of RKNs has become increasingly difficult due to the ban of effective, but highly toxic, nematicides such as methyl bromide (Martin, 2003).

Despite the economic importance of RKNs in tomato production, only few studies assessing the awareness of high tunnels tomato farmers regarding the root knot nematodes have been conducted in Kenya. A knowledge, attitude and Practice (KAP) study is a focused evaluation of a population that informs on what is perceived, beliefs and acted upon towards a certain situation (Yuantari *et al*., 2015). For example, KAP studies led to the development of personal protective measures against the adverse effects of pesticides (Yuantari *et al*., 2015). In this study, we sought to establish the knowledge, attitude and practices on root knot nematodes among high tunnel tomato farmers of Kiambu County, Central Kenya using a KAP study. Other pest and disease challenges in high tunnels tomato production were also established. The findings would provide information to government and other stakeholders working with smallholder farmers to enhance KAP in management of RKNs and to invest in developing sustainable RKN management strategies in order to improve the livelihoods of smallholder tomato farmers.

## 3.2 Materials and Methods

### 3.2.1 Description of the study area

The study was conducted from January to September 2016, in six sub-Counties *viz*. Thika, Juja, Ruiru, Kiambu, Gatundu South and Gatundu North of Kiambu County, Kenya (Figure 3-1), which is one of the major high tunnel tomato growing areas in Kenya (MoA, 2014). Kiambu County is subdivided into 12 sub-counties that cover an area of about 2,543.5 $km^2$. It borders Murang'a County to the north and North East, Nairobi and Kajiado Counties to the south, Machakos County to the east, Nakuru County to the west and Nyandarua to the North West. It lies between latitudes 00 25'and 10 20'South of the Equator and Longitude 360 31'and 370 15'East. Kiambu County is 40% rural and 60% urban owing to its proximity to Nairobi (20-40 km), the capital city of Kenya (CoK, 2013).

#### 3.2.1.1 Vegetation

Kiambu County is divided into four broad topographical zones *viz*, Upper Highland, Lower Highland, Upper Midland and Lower Midland Zone. Large parts of Gatundu North and Gatundu South sub-Counties are covered by forests. Other physical features include steep slopes and valleys, which are unsuitable for cultivation. The upper midland zone lies between 1,300-1,500 meters above sea level and it covers mostly parts of Juja sub-County. The landscape comprises of volcanic middle level uplands. The lower mid-land zone partly covers Thika sub-County which lies between 1,200 - 1,360 meters above sea level (CoK, 2013). Parts of Gatundu North and Gatundu South are found in the Lower Highland Zone (1,500-1,800 m asl) with the inhabitants depending on tea, dairy, cereals, legumes, potatoes and horticultural crops for their livelihoods (CoK, 2017).

#### 3.2.1.2 Soils

Kiambu County is covered by three broad categories of soils which are: high level up-land soils, plateau soils and volcanic footbridges soils. These soils are of varying fertility levels with soils from high-level uplands, which are from volcanic rocks, being very fer-tile. Their fertility is conducive for livestock keeping and growth of various cash crops and food crops such as tea, coffee, horticultural products, pyrethrum, vegetables, maize, beans, peas and potatoes (MoA, 2014). These soils are found in the highlands, mostly in Gatundu South, Gatundu North and Kiambu sub-Counties. Low fertility soils are mainly found in the middle zone and the eastern part of Kiambu County which includes parts of Juja, Thika, Ruiru, Gatundu North and Gatundu South sub-counties. The soils are sandy or clay and can support drought resistant crops such as soya beans and sunflower as well as cattle grazing (CoK, 2013).

### 3.2.1.3 Rainfall and temperature

A bi-modal type of rainfall is experienced in Kiambu County (KMD, 2017). The long rains fall between mid-March to May followed by a cold season during June to August and the short rains between mid- October to November. The annual rainfall varies with altitude, with higher areas such as Limuru receiving as high as 2,000 mm annually and lower areas of Thika sub-county receiving as low as 600 mm per annum. The average rainfall received by the county is 1,200 mm per annum. The mean temperature in the county is 26 °C with temperatures ranging from 7 °C in some parts of Gatundu North and Gatundu South sub-Counties, to 34 °C in Thika sub-county. The County's average relative humidity ranges from 54% in the dry months and 300% in the wet months (KMD, 2017).

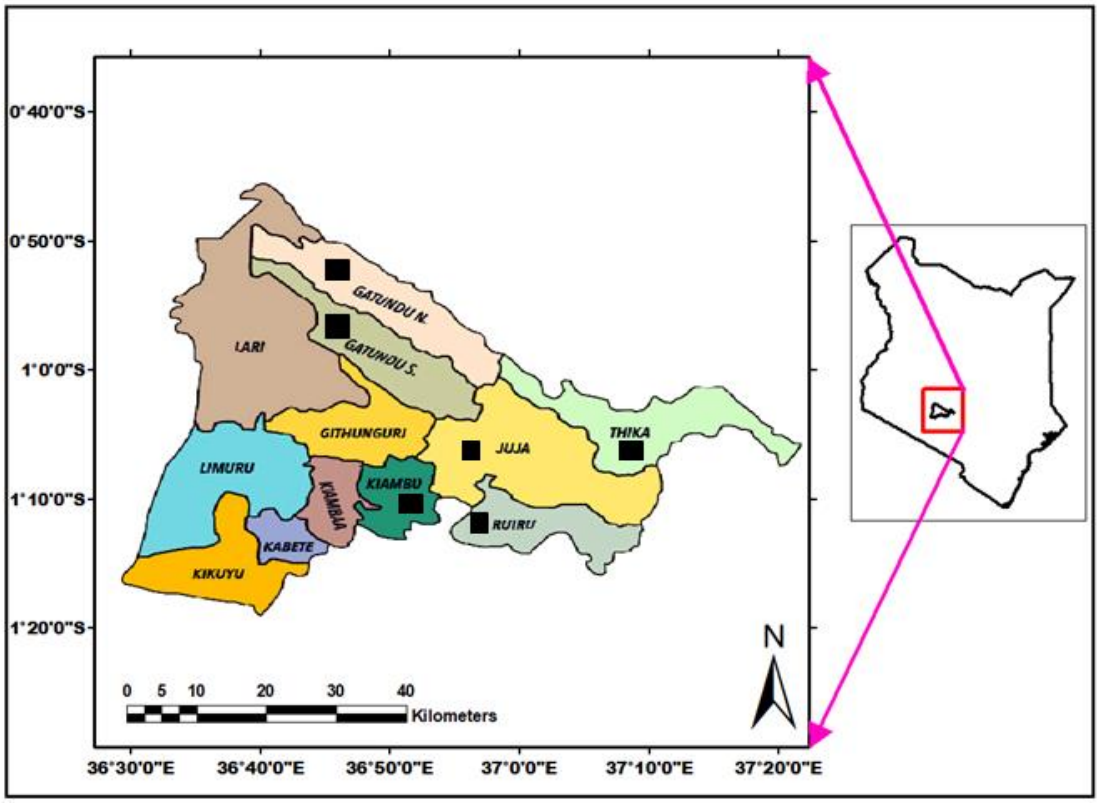


**Figure 3-1:** Map of Kiambu County (Kenya) showing the 12 sub-counties where the study was conducted in high tunnels, where tomato crops were grown in 2016

### 3.2.2 Research Design

A purposive sampling technique was used to select the sample size for conducting the knowledge, attitude and practices study among greenhouse tomato farmers in Kiambu County. Based on information from the county and sub-county crop officers, 80 high tunnels that are managed by a group of 8-30 farmers were constructed by the Kiambu County Government and the National Irrigation Board (NIB) for horticultural production in 2014. Out of these, 32 high tunnels located in the six sub counties were selected based on their previous challenges with pests and diseases in tomato production. This comprised a sample size of 40% which was a good representation of the target

population (Mugenda & Mugenda, 2003). The selected high tunnels comprised 311 respondents with 141 male and 170 female.

### 3.2.3 Data collection techniques

Data was collected using focused group discussions using a checklist with open-ended questions (Appendix I). The checklist was pre-tested among the sub-county crop officers and the enumerators (10 respondents) before administration in its final form. The checklist consisted of three sections that included farmer; (i) Knowledge, (ii) attitude, and (iii) practices in greenhouse tomato production in relation to the key target pests. The knowledge section consisted of 12 questions that were used to compute a knowledge score. For each question, a correct response was awarded one point while a wrong response or 'don't know' a zero point. The numbers of correct responses out of the 12 questions were summed up in order to calculate the knowledge score per group and subsequently respective sub-counties scores. In the attitudes section, we asked three yes/no questions on the impact of RKN towards greenhouse tomato production. Those who said yes were considered to have a positive attitude while the rest were considered to have a negative attitude. For the practices section, questions were asked using the 5W1H (When, Where, What, Which, Who and How) method to evaluate the precautionary measures the respondents took against RKN. We awarded respondents one point for each measure and a zero if a measure was not mentioned in subsequent responses. A practice score for each group was computed by summing the number of responses out of the ten questions. Respective high tunnels were located using the Global Positioning System (GPS) (Magellan, Triton windows CE core 5.0 00039_272_446_822 X11_15302)

### 3.2.4 Data analysis

For easy entry and analysis, qualitative data were assigned numbers (either 1 or 0) and entered in Microsoft excel 2010. The data was cleaned to detect any missing or invalid variable and exported to the Statistical Package for the Social Sciences (SPSS) version 22 for analysis (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0 Armonk, NY: IBM Corp). The descriptive statistics (means, frequencies and percentages) were calculated and the findings were presented in graphs, charts and tables. For knowledge analysis, the knowledge score was determined by calculating the number of correct responses and equating to the possible 12 questions regarding knowledge of the target pests and disease for individual farmer groups. Sub-county and county scores were got from summation and averaging the individual farmer group scores. Percent crop losses were calculated by establishing the crop lost as per individual farmer group and equating to the initial crops planted. For all statistical analyses, a P-value of $\leq 0.05$ was considered significant while means were separated using Tukey's HSD test.

## 3.3 Results

### 3.3.1 Characteristics of the study population

The characteristics of farmers and high tunnels in Kiambu County are given in Table 3-1. Out of the 32 sampled high tunnels, most were from Thika (25%) and Kiambu (25%) sub-counties, while the least were from Ruiru sub-county (6.3%). More female (54.7%) than male (45.3%) farmers dominated most of the high tunnels. Most high tunnels had been utilized for 1- 2 years (78.1%) while a few (6.3%) exceeded 2 years (Table 3-1). Half of the high-tunnels had been used for two cropping seasons within one year. Although there was a diversity of crops (10) grown in Kiambu County, it was evident that majority (96.9%) of the farmers grew tomato in the high tunnels for commercial purposes (78.1%) (Table 3-1).

### 3.3.2 Arthropod pest and disease challenges in the high tunnels

Overall, root knot nematodes (64.2%) were ranked as the most problematic pests in high tunnel tomato production in Kiambu County (Table 3-2). Root knot nematodes were mainly problematic in Gatundu South (83.3%) relative to the other sub-counties (Table 3-2). Whiteflies (100%) were ranked as the most problematic arthropod pests in high tunnels in Kiambu County, which was evident from all the sub-counties (Table 3-3). Problems associated with other arthropod pests ranged between 5-50%. Aphids (100%) were considered the most problematic in Gatundu North while the tomato leaf miner (83.3%) was the most important in Gatundu South relative to the other pests (Table 3-3).

**Table 3-1:** Characteristics of respondents and high tunnels used by farmers groups in Kiambu County, Kenya, as part of a survey study about prevalence and control of root knot nematodes

| Source | Frequency (n) | Proportion (%) | P value |
|---|---|---|---|
| *Sub-county* | | | |
| Thika | 8 | 25.0a | <0.001 |
| Juja | 5 | 15.6b | |
| Gatundu South | 6 | 18.6b | |
| Kiambu | 8 | 25.0a | |
| Ruiru | 2 | 6.3c | |
| Gatundu North | 3 | 9.4c | |
| *Respondent gender* | | | |
| Male | 141 | 45.3a[a] | 0.271 |
| Female | 170 | 54.7a | |
| *Age of high tunnels* | | | |
| <1 year | 5 | 15.6b | 0.007 |
| 1-2 years | 25 | 78.1a | |
| >2 years | 2 | 6.3c | |
| *Cropping cycles* | | | |
| 1 | 9 | 28.1b | <0.001 |
| 2 | 16 | 50.0a | |
| 3 | 5 | 15.6c | |
| >4 | 2 | 6.3d | |
| *Purpose of growing tomato* | | | |
| Sale (commercial) (a) | 25 | 78.1 a | <0.001 |
| Subsistence (b) | 0 | 0 | |
| Both (a and b) | 7 | 21.9 b | |

Values in columns followed by the same letter are not significantly different, P≤0.05; Tukey's HSD test.

**Table 3-2**: Importance of root knot nematodes relative to other diseases, according to 311 farmer respondents, in tomato producing high tunnels in 6 sub-counties of Kiambu County of Central Kenya

| | | Sub-county | | | | | | | Kiambu |
|---|---|---|---|---|---|---|---|---|---|
| Rank | Challenge | Thika (%) | Juja (%) | Gatundu South (%) | Kiambu (%) | Ruiru (%) | Gatundu North (%) | P value | County mean (%) |
| 1. | Bacterial wilt | 75.0b | 80.0ab | 100.0a | 75.0b | 100.0a | 66.7b | 0.042 | 82.8a |
| 2. | RKNs | 50.0c | 60.0ab | 83.3a | 75.0a | 50.0c | 66.7b | 0.027 | 64.2a |
| 3. | Blight | 50.0b | 60.0b | 100.0a | 37.5c | 50.0b | 33.3c | 0.019 | 55.1b |
| 4. | Fusarium wilt | 62.5a | 40.0b | 66.7a | 75.0a | 0.0c | 33.3b | 0.002 | 46.3b |
| 5. | Blossom end rot | 37.5c | 40.0c | 83.3a | 12.5d | 50.0b | 33.3c | 0.001 | 42.8 b |
| 6. | Powdery mildew | 50.0c | 80.0a | 66.7b | 25.0e | 0.0f | 33.3d | <.001 | 42.5b |
| 7. | Tomato leaf curl | 25.0c | 0.0d | 50.0a | 37.5bc | 0.0d | 0.0d | 0.018 | 18.8c |
| 8. | Root rot | 12.5c | 0.0d | 50.0a | 0.0d | 0.0d | 33.3b | 0.006 | 16.0c |
| | P value | | | | | | | | 0.007 |

Values in rows followed by the same letter are not significantly different, P≤0.05; Tukey's HSD test. RKNs = root knot nematodes

**Table 3-3:** Arthropod pest challenges in tomato producing high tunnels in Thika, Juja, Gatundu South, Gatundu North, Ruiru and Kiambu sub-counties of Kiambu County

| | Sub-counties | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Pest challenges** | **Thika (%)** | **Juja (%)** | **Gatundu South (%)** | **Kiambu (%)** | **Ruiru (%)** | **Gatundu North (%)** | **P value** | **Kiambu County Mean (%)** |
| Whiteflies | 100.0a | 100.0a | 100.0a | 100.0a | 100.0a | 100.0a | 0.731 | 100.0 a |
| Aphids | 25.0a | 20.0a | 50.0b | 25.0a | 50.0b | 100.0c | 0.013 | 45.0 b |
| Leafminers | 50.0c | 40.0bc | 83.3d | 62.5cd | 0.0a | 33.3b | 0.001 | 44.9 b |
| Mites | 50.0d | 40.0cd | 66.7e | 12.5ab | 0.0a | 33.3c | 0.007 | 33.8 c |
| Caterpillars | 25.0b | 60.0c | 66.7c | 25.0b | 0.0a | 0.0a | <.001 | 29.5 c |
| Thrips | 0.0a | 0.0a | 66.7d | 12.5b | 0.0a | 33.3c | <.001 | 18.8 cd |
| Cutworms | 0.0a | 20.0b | 33.3c | 0.0a | 0.0a | 0.0a | 0.003 | 8.9 d |
| Termites | 0.0a | 40.0b | 0.0a | 0.0a | 0.0a | 0.0a | <.001 | 6.7 d |
| M.Bugs | 12.5b | 0.0a | 16.7b | 0.0a | 0.0a | 0.0a | 0.0028 | 4.9 d |
| Crickets | 12.5b | 0.0a | 16.7b | 0.0a | 0.0a | 0.0a | 0.0028 | 4.9d |
| **P value** | | | | | | | | **0.0041** |

Values in rows followed by the same letter are not significantly different, P≤0.05; Tukey's HSD test.

M.Bugs=Mealybugs

### 3.2.3 Knowledge, attitude and practices on root knot nematodes

Knowledge of the farmers about RKNs varied between the sub-counties. The respondents had a knowledge score that indicated a moderate and high knowledge of RKNs (Figure 3-2). About 35% of farmer groups indicated that RKNs were the main contributors to the decline in tomato production in Kiambu County (Figure 3-2).

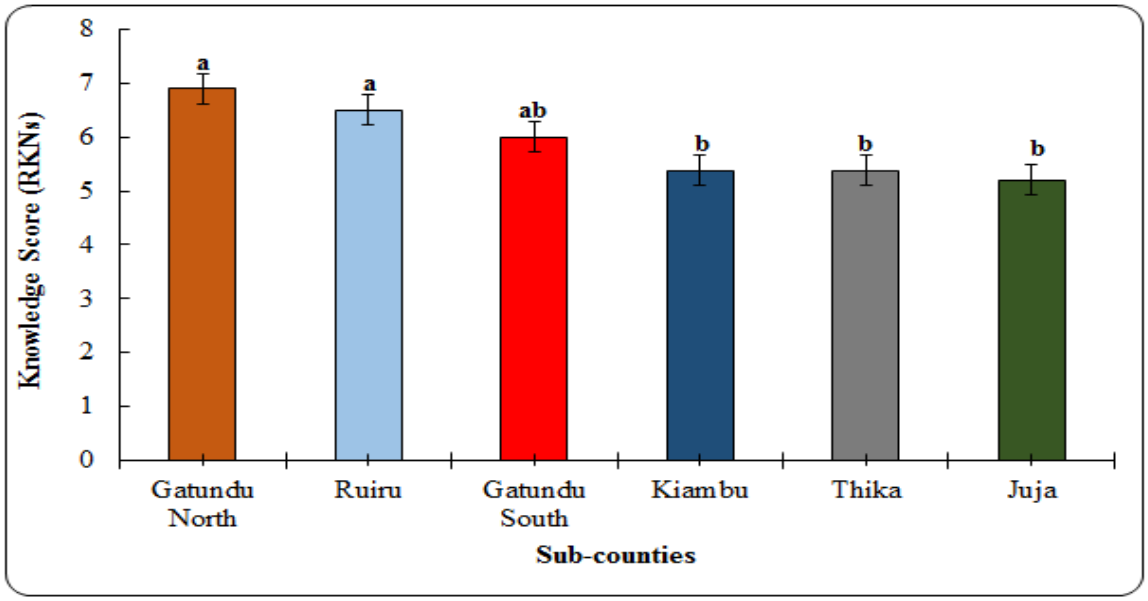


**Figure 3-2:** Knowledge score on Root Knot Nematodes (RKNs) among high tunnel tomato farmers in Gatundu North, Ruiru, Gatundu South, Thika and Juja sub-counties in Kiambu County

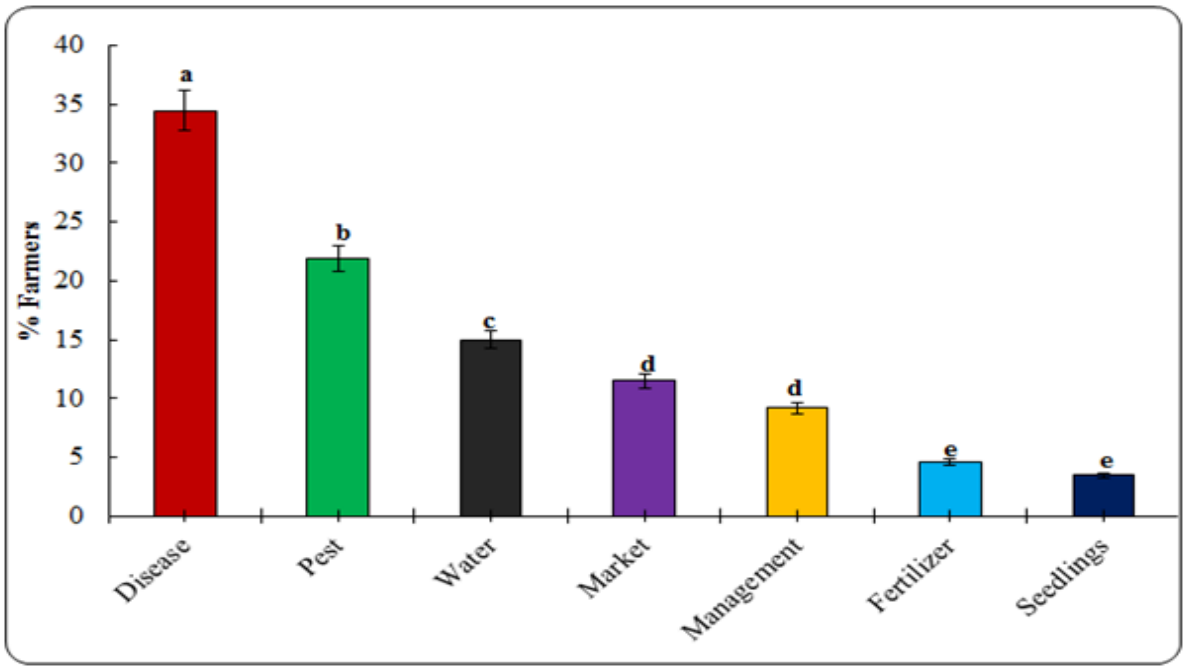


**Figure 3-3:** Perception of farmers on major contributors to tomato production decline in Kiambu County

### 3.2.4 Practices towards root knot nematodes

Tylka F1' was the most preferred tomato variety by upto 40% of farmer groups (Table 3.4). The County Government mainly provided tomato seedlings. Although most farmers took soil samples for analysis, they did not follow the recommendations. Most farmers used ≈10 g of fertilizer at planting or as top dressing per planting hole (Table 3-4). Although a diverse number of methods were used by farmers to control RKNs, uprooting and soil fumigation were the main practices used. Despite their numbers, these methods were considered not to be effective because most farmers disposed off diseased plants outside the high tunnels. Most farmers practiced crop rotation, while a minority used other tomato varieties alleged to be resistant to RKNs for rotation. Major sources of irrigation water were shallow wells and rivers (Table 3-4).

**Table 3-4:** Farmers' practices on root knot nematodes management in Kiambu County, Kenya

| | Sub-county | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Farmer practices | Thika (%) | Juja (%) | Gatundu south (%) | Kiambu (%) | Ruiru (%) | Gatundu north (%) | P value | Kiambu County (Overall mean %) |
| **Tomato variety** | | | | | | | | |
| Tylka F1 | 40.0c | 71.4a | 71.4a | 45.5c | 50.0b | 20.0d | 0.003 | 49.7a |
| Anna F1 | 10.0c | 14.3c | 28.6b | 9.1c | 0.0c | 60.0a | <0.001 | 20.3b |
| Chonto F1 | 10.0b | 0.0c | 0.0c | 18.2b | 25.0a | 20.0ab | 0.037 | 12.2c |
| Bravo F1 | 10.0c | 0.0d | 0.0d | 18.2b | 25.0a | 0.0d | 0.034 | 8.9c |
| Prostar F1 | 0.0b | 14.3a | 0.0b | 0.0b | 0.0b | 0.0b | 0.237 | 2.4c |
| Unknown | 30.0a | 0.0b | 0.0b | 9.1b | 0.0b | 0.0b | 0.04 | 6.5c |
| **Method of propagation** | | | | | | | | |
| Seed | 11.1c | 28.6b | 42.9a | 25.0ab | 0.0c | 0.0c | 0.007 | 17.9b |
| Seedlings | 88.9a | 71.4b | 57.1b | 75.0a | 100.0a | 100.0a | 0.04 | 82.1a |
| **Source of planting materials** | | | | | | | | |
| County government | 60.0a | 71.4a | 57.1a | 66.7a | 66.7a | 66.7a | 0.08 | 64.8a |
| Agrovets | 20.0b | 28.6a | 28.6a | 22.2b | 0.0c | 0.0c | 0.039 | 16.6a |
| Seed company | 0.0c | 0.0c | 14.3b | 0.0c | 33.3a | 33.3a | 0.006 | 13.5b |
| High tunnels contractor | 20.0a | 0.0c | 0.0c | 11.1b | 0.0c | 0.0c | 0.0174 | 5.2b |
| **Source of planting media** | | | | | | | | |
| High tunnel earth | 100.0a | 100.0a | 83.3a | 100a | 100.0a | 100.0a | 0.431 | 97.2a |
| Forest | 0.0b | 0.0b | 17.7a | 0.0b | 0.0b | 0.0b | 0.038 | 2.8b |
| **Media treatment** | | | | | | | | |
| No | 87.5a | 80.0ab | 66.7b | 87.5a | 100a | 100a | 0.041 | 86.9a |
| Yes | 12.5b | 20.0b | 33.3a | 12.5b | 0.0c | 0.0c | 0.033 | 13.1b |
| P value | | | | | | | | 0.001 |
| **Soil test** | | | | | | | | |
| Yes | 37.5c | 100.0a | 83.3c | 75.0bc | 100.0a | 100.0a | 0.070 | 82.6a |
| No | 62.5a | 0.0c | 16.7b | 25.0b | 0.0c | 0.0c | 0.004 | 17.4b |
| **Following recommendations** | | | | | | | | |
| No | 66.7b | 80.0a | 50.0b | 83.3a | 50.0b | 100.0a | 0.030 | 71.7a |
| Yes | 33.3a | 20.0b | 50.0a | 16.7b | 50.0a | 0.0c | 0.016 | 28.3b |
| **Fertilizer quantities used** | | | | | | | | |
| 10 g | 50.0a | 40.0a | 50.0a | 62.5b | 100c | 66.7b | 0.010 | 61.5a |
| 5 g | 50.0bc | 60.0c | 33.3b | 37.5b | 0.0a | 33.3b | 0.017 | 35.7b |
| >10 g | 0.0b | 0.0b | 16.7a | 0.0b | 0.0b | 0.0b | 0.090 | 2.8c |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **RKN control method** | | | | | | | | |
| Uprooting | 38.1a | 41.7a | 50.0a | 26.9ab | 18.2b | 14.3b | 0.013 | 31.5a |
| Soil fumigation | 23.8b | 41.7a | 30.0a | 23.1b | 18.2b | 21.4b | 0.030 | 26.4a |
| Chemical | 9.5a | 8.3a | 20.0a | 15.4a | 18.2a | 21.4a | 0.473 | 15.5b |
| Door management | 9.5a | 0.0b | 0.0b | 15.4a | 18.2a | 21.4a | 0.050 | 10.8b |
| Ashes | 4.8b | 8.3b | 0.0b | 3.8b | 18.2a | 7.1b | 0.041 | 7b |
| Side nets | 4.7a | 0.0b | 0.0b | 7.7a | 9.1a | 0.0b | 0.039 | 3.6b |
| Footbath | 9.5a | 0.0b | 0.0b | 7.6a | 0.0b | 0.0b | 0.043 | 2.9b |
| Traps | 0.0a | 0.0a | 0.0a | 0.0a | 0.0a | 14.3b | 0.028 | 2.4 b |
| **Effectiveness of method** | | | | | | | | |
| No | 62.5b | 40.0c | 33.3c | 50bc | 100.0a | 33.3a | 0.014 | 53.2a |
| Yes | 37.5c | 60.0a | 66.7a | 50ab | 0.0d | 66.7a | 0.020 | 46.8a |
| **Crop rotation** | | | | | | | | |
| Yes | 87.5a | 80.0a | 100a | 87.5a | 100.0a | 100.0a | 0.090 | 92.5a |
| No | 12.5b | 20.0a | 0.0c | 12.5b | 0.0c | 0.0c | 0.028 | 7.5b |
| **Crops for rotation** | | | | | | | | |
| Other tomato varieties | 40a | 14.3c | 27.3b | 42.9a | 50.0a | 42.9a | 0.028 | 36.2a |
| Onions | 26.7a | 28.6a | 18.2b | 14.3b | 0.0c | 28.6a | 0.017 | 19.4b |
| Spinach | 6.7c | 28.6a | 18.2b | 14.3b | 25.0a | 0.0d | 0.005 | 15.5b |
| Kale | 13.3b | 14.3b | 9.1b | 0.0c | 25.0a | 0.0c | 0.004 | 10.3c |
| Cucumber | 6.7b | 0.0c | 0.0c | 21.4a | 0.0c | 14.3b | 0.020 | 7.1d |
| Capsicum | 0.0c | 0.0c | 18.2a | 7.1b | 0.0c | 14.3b | 0.015 | 6.6d |
| Coriander | 0.0b | 14.3a | 9.1a | 0.0b | 0.0b | 0.0a | 0.021 | 3.9 e |
| **Disposal of diseased plants** | | | | | | | | |
| Piling outside the high tunnel | 25.0d | 80.0a | 57.1b | 57.1b | 50.0c | 33.3d | 0.030 | 50.4a |
| Burning | 62.5a | 0.0d | 28.6c | 28.6c | 50.0b | 33.3c | 0.014 | 33.8b |
| Burying | 12.5c | 20.0b | 14.3c | 14.3c | 0.0d | 33.3a | 0.008 | 15.7c |
| **Source of irrigation water** | | | | | | | | |
| Shallow wells | 22.2c | 0.0d | 37.5b | 66.7a | 0.0d | 60.0a | 0.010 | 31.2a |
| River | 55.5a | 33.3b | 37.5b | 22.2c | 20.0c | 0.0d | 0.004 | 28.1b |
| Rain | 11.1d | 33.3a | 25.0b | 0.0e | 20.0c | 20.0c | 0.019 | 18.2c |
| Water pans | 0.0d | 16.7b | 0.0d | 11.1c | 40.0a | 20.0b | 0.019 | 14.6c |
| Water company | 11.1b | 16.7a | 0.0c | 0.0c | 20.0a | 0.0c | 0.019 | 7.9d |

Values in rows followed by the same letter are not significantly different, $P \leq 0.05$; Tukey's HSD test

### 3.3 Discussion

The results showed that nearly all the groups interviewed had and/or were growing tomato in the high tunnels. This was not unusual as the crop was ranked first in a prioritization of vegetable crop value chains in Kenya (FAO, 2017). However, we found most of these high tunnels had been in existent for only 1-2 years indicating that farmers were yet to realize the full potential of producing tomato in these structures relative to the open field.

Most farmer groups reported RKNs as the most problematic in high tunnel tomato production which is consistent with previous reports (Ireri *et al*., 2018). Despite this general perception, there was an indication of a moderate level of knowledge on the RKN based on overall scores. The low level of knowledge for RKNs shown in our study could be due to farmer's inability to identify damage symptoms caused by RKNs which happen below-ground before the aerial symptoms become visible (Mitkowski & Abawi, 2003). In addition, some farmer groups could also have forgotten some information following that they had diversified in growing other crops within the high tunnels. This is an indication that continued farmer education is necessary and should be intensified throughout the tomato production season.

Despite individual farmer groups experiencing crop losses of between 50-100% and average individual sub county losses of between 45 – 65%, there was a high interest in producing tomato in high tunnels. Most of the farmers were interested to know more about the diseases and prevention methods. There is need to organize farmer field schools as a means to reach farmers who may not get information regarding RKNs and other pest and disease challenges in tomato production in the occasional and poorly coordinated extension services.

Farmers have poor practices towards prevention of root knot nematodes. For example, they did not obtain clean planting materials, did not sterilize the soil and disposed diseased plants outside the high tunnel among others. For example: previous reports have

shown that crop rotation with alternate crops has been shown to suppress nematode populations (Guerena, 2006). Studies using these field tested practices need to be conducted in high tunnels where this study was done to establish their impact on RKNs.

In conclusion, RKNs are the most problematic in high tunnel tomato production in Kiambu County. Despite the high interest in producing tomato in high tunnels, farmers have poor farming practices that intensify the severity of the pathogens. Therefore there is need for continuous farmer education and intensified extension services by well-trained personnel to increase adoption of good farming practices. This will impact positively on tomato production and proper utilization of the high investment high-tunnels.

## CHAPTER FOUR

# SOIL CHEMICAL PROPERTIES INFLUENCE ABUNDANCE OF NEMATODE TROPHIC GROUPS IN HIGH TUNNEL TOMATO PRODUCTION


## Abstract

Plant Parasitic Nematodes (PPNs) are a serious soil-borne pests of tomato in high tunnel (commonly known as 'greenhouse') production in Kenya. The objective of this study was to determine the associations between soil chemical properties and abundance of nematodes in high tunnel tomato production in Kiambu County, Kenya. Soil samples were collected from 32 high tunnels in six sub-counties *viz.* Gatundu North, Gatundu South, Juja, Thika, Ruiru and Kiambu between January and November 2016. Nematodes of various genera, and soil chemical properties were evaluated from composite soil samples collected from the 32 high tunnels using validated scientific procedures. Soil analysis results showed that the composition of soil elements; N, P, K, Ca, Mg, Na and Cu varied significantly ($P = <0.001$) between the sub-counties. Although significantly high levels of N were found in Gatundu North (0.6%), significantly lower levels were found in Juja (0.1%), Ruiru (0.2%) and Thika (0.2%) sub-counties. Twenty four nematode genera that were grouped into four trophic groups were recovered from soil samples. Of these, 14 represented phytoparasitic genera, five bacterivores, three fungivores and two predators. The genus *Meloidgyne* was the most abundant phytoparasitic nematode across the six sub-counties with significantly high populations in Gatundu North. A weak negative correlation with the soil pH, EC, Zn and Cu existed. Farmers should use good agricultural practices such as optimal fertilizer application rates for efficient nematode management in tomato.



**Key words**: high tunnels; plant parasitic nematodes; small holder farmers

## 4.1 Introduction

The PPNs of economic importance can be grouped into relatively restricted specialized groups that either cause direct damage to their host or act as disease vectors. Most affect crops through feeding on plant roots and/or other below-ground plant parts, whilst a minority are aerial feeders (Nicol *et al*., 2011). In addition to direct feeding and migration damage, nematode feeding facilitates subsequent infection by secondary pathogens, such as fungi and bacteria (Powell, 1971).

Free-living nematodes, however, are the most abundant metazoans in the soil, constituting an important component of the soil fauna which significantly impact nutrient cycling and primary productivity in diverse ecosystems (Liu *et al*., 2006). Increased microbial activity in the soil leads to an increased proportion of fungal and bacterial feeders (Bongers & Ferris, 1999). This is important for the decomposition of soil organic matter and mineralization of plant nutrients (Ingham *et al*., 1985; Hunt *et al*., 1987). The nematode diversity in high tunnels production system offers many possibilities for use as biological indicators of agricultural practices, soil characteristics and the degree of conservation of soils, especially in continuous cropping of the same soil (Liu *et al*., 2011). Previous reports showed an adverse trend between free-living nematode populations and the second stage juveniles (J2s) of *Meloidgyne* spp (root knot nematodes; RKNs) in different continuously cropped soils (Wu & Shi 2011). In high tunnel tomato production, RKNs are the most prevalent (Ireri *et al*., 2018).

Soil chemical characteristics are known to influence the abundance and diversity of soil pathogens (Spann & Schumann, 2010). For example, when ammonium nitrogen fertilizer such as calcium ammonium nitrate, is applied, the positive charge on the ammonium ion ($NH_4+$) allows it to be adsorbed by plant roots, resulting in the release of positively charged hydrogen ions into the surrounding that lowers the soil pH (Barak *et al*., 1997). Consequently, diseases that are more common in acidic soils increase in severity. In Kenya and its sub-region, intensification of agriculture, combined with poor agronomic practices have led to an increase in PPNs and other soil pathogens (Wachira

*et al*., 2009). For instance, in monoculture cropping systems, the high selection pressure results in the substantial buildup of PPNs, especially RKNs (Kimenju *et al*., 2008). Despite the importance of root knot nematodes in tomato farming in Kenya, little is known about the impact of soil chemical characteristics in high tunnel tomato production system. In this study, data from six sub-counties in Kiambu County, Central Kenya, demonstrating that soil chemical characteristics correlate with the abundance and distribution of RKNs in high tunnels earmarked for tomato production is presented.

## 4.2 Materials and Methods

### 4.2.1. Description of the study area

The study was carried out during January to November 2016 in six sub-counties *viz*. Thika, Juja, Ruiru, Kiambu, Gatundu South and Gatundu North of Kiambu County, Kenya , which is one of the major high tunnel tomato growing areas in Kenya (MoA, 2014). Kiambu County is subdivided into 12 sub-counties that cover an area of about 2,543.5 $km^2$. It borders Murang'a County to the north and North East, Nairobi and Kaji-ado Counties to the south, Machakos County to the east, Nakuru County to the west and Nyandarua to the North West. It lies between latitudes 00 25' and 10 20' South of the Equator and Longitude 360 31' and 370 15' East. Kiambu County is 40% rural and 60% urban owing to its proximity to Nairobi (20-40 km), the capital city of Kenya (CoK, 2013). Kiambu County is divided into four broad topographical zones *viz*, Upper High-land, Lower Highland, Upper Midland and Lower Midland zones. Other physical fea-tures include steep slopes and valleys, which are unsuitable for cultivation (CoK, 2017). It is also covered by three broad categories of soils which are: high level upland soils, plateau soils and volcanic footbridge soils with varying fertility levels (MoA, 2014). The mean temperature in the county is 26 °C and a relative humidity ranging from 54-300% (KMD, 2017).

### 4.2.2. Soil sampling

Soil samples were collected from 32 high tunnels in the Kiambu County, in which tomato crops were grown. Seven tunnels were sampled in Thika, five in Juja, two in Ruiru, 10 in Kiambu, five in Gatundu South and three in Gatundu North. These sub-counties were identified by researchers during focused group discussions. The location of each high tunnel was identified using a GPS device (Magellan, triton windows CE core 5.0 00039_272_446_822 X11_15302, Integritytech, Illinois, USA) (Table 4-1). A systematic pattern which entailed dividing each high tunnel into four quadrants was used to collect soil samples. Five sub-samples per each quadrant were collected with a soil auger to a depth of 15 cm in a cross-diagonal pattern (Coyne *et al*., 2018a). The five sub-samples were mixed in a plastic basin to make a composite sample of ~1 kg and placed in a labelled plastic bag. A similar procedure was repeated across the quadrants with four samples (comprised 20 sub-samples) collected from each high tunnel and constituting a total of 128 samples for the 32 high tunnels. The soil samples were placed in cool boxes and transported to the Soil Science laboratory at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Juja, Kenya (latitude 0° 10′ 48″ S, longitude 37° 07′ 12″ E, altitude 1525 m a.s.l.) and stored at 10 °C for 1-2 weeks for nematode and soil chemical analyses. For nematode identification and quantification, the samples were transported in cool boxes to the Nematode laboratory at the International Centre of Insect Physiology and Ecology (ICIPE), Duduville Campus, Kasarani, Nairobi, Kenya (S01°13.140'; E036°53.440'). Soil chemical properties were analyzed at JKUAT.

**Table 4.1:** Location of high tunnels in six sub-counties of Kiambu County, their location coordinates and main tomato varieties grown

| Sub-county | Name of high tunnel | Latitude | Longitude | Common tomato variety |
|---|---|---|---|---|
| **Gatundu North** | Gitwe United | 0° 57' 03.1" S | 36° 53' 29.2" E | Anna F1 |
| | Kihururu | 0° 57' 29.1" S | 36° 54' 04.2" E | Anna F1 & Chonto F1 |
| | Ngaraka | 0° 53' 25.2" S | 36° 52' 39.6" E | Tylka F1 & Anna F1 |
| **Gatundu South** | Kahuguini Dairy Group | 1° 02' 42.2" S | 36° 55' 12.2" E | Tylka F1 |
| | Kianyoni Dairy | 0° 57' 20.1" S | 36° 46' 04.0" E | Tylka F1 |
| | Kimunyu Self-Help Group | 1° 02' 56.6" S | 37° 56' 42.7" E | Anna F1 |
| | Mwirutiri Self-Help Group | 1° 04' 06.9" S | 36° 53' 23.4" E | Tylka F1 |
| | New Gitwe | 0° 56' 48.1" S | 36° 48' 47.9" E | Tylka F1 & Anna F1 |
| **Juja** | Focal Area Group | 1° 09' 54.3" S | 37° 05' 54.8" E | Tylka F1 & Prostar F1 |
| | Jokumo Kihuria Group | 1° 03' 27.0" S | 37° 00' 29.1" E | Tylka F1 |
| | Juja Botanical | 1° 06' 43.5" S | 37° 00' 46.5" E | Tylka F1 |
| | Mirimaini Primary | 1° 04' 24.8" S | 36° 59' 30.3" E | Tylka F1 |
| | Mwinjoyo | 1° 09' 40.7" S | 37° 07' 38.7" E | Tylka F1 & Anna F1 |
| **Kiambu** | By Grace | 1° 17' 03,4" S | 36° 81' 71,2" E | Anna F1 |
| | Agricultural Booster | 1° 17' 29.1" S | 36° 80' 12.8" E | Anna F1 |
| | By Faith | 1° 09' 28.2" S | 36° 50' 27.7' E | Tylka F1 |
| | Gikirthia | 1° 17' 18.7" S | 36° 84' 59.1" E | Cheong Gang |
| | Horticulture Investors | 1° 07' 13.1" S | 36° 49' 42.8" E | Chonto F1 |
| | Kahuguini | 1° 02' 42.5"S | 36° 55' 12.6" E | Anna F1 |

| | | | | |
|---|---|---|---|---|
| | Kanene GH | 0° 57' 23.9" S | 36° 46' 03.5" E | Tylka F1 |
| | Kilimo Biashara | 1° 09' 22.3" S | 36° 48' 55.6" E | Tylka F1 |
| | Urban Farming | 1° 79' 03.2" S | 36° 83' 78.1' 'E | Tylka F1 |
| | Wendi Mwega | 1° 88' 79.7" S | 36° 33' 45.3" E | Tylka F1 |
| **Ruiru** | Membeley Park | 1° 09' 32.8" S | 36° 55' 22.2" E | Tylka F1, Chonto F1 & Bravo |
| | Ruiru Baptist | 1° 08' 52.0" S | 36° 57' 48.0" E | Tylka F1 |
| | | | | Cheong Gang |
| **Thika** | Digital Women | 1° 07' 49.3" S | 37° 08' 39.9" E | |
| | Gatuanyaga Youth Focus | 1° 02' 52.9"S | 37° 10' 29.4" E | Tylka F1, Bravo & Chonto F1 |
| | Kirigu Men | 1° 06' 21.7" S | 37° 20' 26.1" E | Tylka F1 |
| | Thogoto Men’s Group | 1° 06' 06.2" S | 37° 19' 45.4" E | Tylka F1 |
| | Upendo Men And Women Group | 1° 03' 29.0" S | 37° 15' 17.3" E | Cheong Gang |
| | Ushirikiano Booster Group | 1° 06' 07.5" S | 37° 09' 13.6" E | Cheong Gang |
| | Vision Farmer Group | 1° 04' 29.1" S | 37° 05' 38.7" E | Tylka F1 |

### 4.2.3. Soil analysis

The soil samples from respective high tunnels per sub-county were air dried on the bench in the laboratory (25 ± 2 °C) for three days and their chemical properties determined using the following protocols: total nitrogen (N) by Kjedahl's method (Kjeldahl, 1883; McGeehan & Naylor, 1988); available phosphorus (P) using the Double Acid Extractable P ($HCl–H_2SO_4$) method (Mehlich, 1953; Olsen, 1954); potassium (K), calcium (Ca) and magnesium (Mg) using the ammonium acetate extraction method (Normandin *et al*., 1998; Jones, 1999); iron (Fe), copper (Cu) and zinc (Zn) using the EDTA extraction method (Lindsay & Norvell, 1978); electrical conductivity (EC) by the four electrode method (Nadler & Frenkel, 1980) and the soil pH using an electric pH meter (Conkling & Blanchar, 1988).

### 4.2.4. Nematode quantification and identification

Nematodes were extracted from 100 ml soil of each composite sample using the modified Baermann's technique (Coyne *et al.*, 2018a). They were fixed for identification using the Seinhorst's technique (Seinhorst, 1962). Briefly, 50 ml falcon centrifuge tubes containing nematodes were immersed in heated water (55 °C) for 2 min to kill them. Two drops of formalin glycerol (obtained by mixing 10 ml of 40% formaldehyde, 1 ml of glycerol and 89 ml of distilled water) were then added into the tube and stored at 20 °C to allow the fixed nematodes to adequately settle at the bottom of the vial. Nematodes were identified to genus level based on morphological features of at least 100 nematodes studied per sample using a compound microscope (Carl Zeiss Primo Star iLED, Carl Zeiss Promenade 10, Jena, Germany) (Hunt *et al*., 2005; KSU, 2015) (Plate 4-1). Identified nematodes were assigned to trophic groups as PPNs (Mai & Lyon, 1975), bacterial feeders (Overgaard, 1949), fungal feeders (Thorne & Swanger, 1936) and predators (Small, 1987) following methods previously described by Yeates *et al*. (1993), and KSU (2015) (Plate 4-1).

**Plate 4.1:** Nematode identification using compound microscope

### 4.2.5 Statistical analysis

To determine the soil chemical characteristics across the six sub-counties, data were subjected to ANOVA in the package "vegan" under R version 3.2.3 (R-Development-Core-Team, 2017). Means were separated using Fisher's Least Significant Difference (LSD) test at $P = \leq 0.05$. Data on nematode abundance was transformed using natural logarithm [ln (x+ 1)] prior to analysis to stabilize the variance and normalize the data (Gomez & Wiley, 1976). The transformed data were subjected to analysis of variance to evaluate nematode abundance across the sub-counties. To compare nematode data across the six sub-counties, nematode genera counts were expressed as relative proportions of their feeding type. Means were separated using Fisher's least significant difference (LSD) test at $P = \leq 0.05$ using the package "agricolae" (De Mendiburu, 2015). To evaluate the degree of linear association between soil chemical characteristics and nematode population, correlation analyses were carried out by estimating and testing of the significance of simple linear correlation coefficient (r) (Gomez & Wiley, 1976). All analyses were implemented using R version 3.2.3 (R-Development-Core-Team, 2017).

## 4.3 Results

### 4.3.1 Chemical characteristics of soils from high tunnels in Kiambu County

The soil pH was slightly acidic to neutral (5.3-7.0), and differed significantly (P = <0.001) between the six sub-counties. However, the EC, which was within the normal range, was not significantly (P = 0.144) different across the sub-counties (Table 4-2). Except Zn, Fe and Mn, other elements i.e. N, P, K, Ca, Mg, Na and Cu differed significantly (P = <0.001) between the sub-counties (Table 4-2). Although high levels of N were recorded in soils from Gatundu North (0.6%), significantly low levels were found in Juja, Ruiru and Thika sub-counties. Moreover, P was significantly low across the sub-counties, except in Gatundu North that was within the normal range (Table 4-2). Notably, Mg was three times higher in Gatundu North relative to the other sub-counties and above the normal range across all the sub-counties (Table 4-2).

### 4.3.2 Correlations between soil chemical characteristics, nematode trophic groups and *Meloidogyne* spp.

We found mainly weak correlations between the nematode trophic groups with the soil chemical characteristics (Table 4-3). However, a few strong correlations were found. For instance, fungal feeders had a strong negative correlation with the soil pH and Ca, while predators, bacterial feeders and PPNs had a similar correlation with N, P and Ca respectively (Table 4-3). In addition, PPNs and fungal feeders had a strong positive correlation with N, P and Mg (Table 4-3). Whereas there were weak positive and negative correlations between *Meloidogyne* spp. populations with the soil pH, EC, K, Na, Zn, Fe, Cu and Mn, we found a strong positive correlation with the soil N and P (Table 4-3). Notably, Ca had strong and weak negative correlations with *Meloidogyne* spp. (Table 4-3).

### 4.3.3 Abundance of nematodes in Kiambu County

There were 24 genera of nematodes from soils collected in the 32 high tunnels in six sub-counties of Kiambu County. Out of these, 14 were plant parasitic, five were bacterial feeders, three were fungal feeders, and two were predatory nematodes (Table 4-4). Of the PPNs, the genus *Meloidogyne* was the most abundant across the six sub-counties with significantly (P = < 0.001) higher populations in Gatundu North than in the other sub-counties (Table 4-4). Among the bacterial feeders, the genus *Alaimus* was the most abundant in >50% of the sub-counties with significantly (P = <0.001) higher populations in Juja and Ruiru relative to the other sub-counties (Table 4-4). Of the fungal feeders and predators, the genera *Aporcelaimus* and *Mononchus* were significantly abundant in Gatundu North and Ruiru sub-counties respectively.

**Table 4-1:** Chemical characteristics of soils collected in high tunnels in Gatundu North, Gatundu South, Juja, Kiambu, Ruiru and Thika sub-counties

| Characteristic | Normal Range | Sub-county | | | | | | P value |
|---|---|---|---|---|---|---|---|---|
| | | Gatundu North | Gatundu South | Juja | Kiambu | Ruiru | Thika | |
| Soil pH | 5.5-6.8 | 5.3a | 5.6a | 7.0b | 6.6b | 6.6b | 6.7b | <.001 |
| Soil Ec (µS/cm) | <0.8 | 0.4a | 0.7a | 0.5a | 0.6a | 0.4a | 0.6a | 0.144 |
| N (%) | >0.25 | 0.6c | 0.3a | 0.1a | 0.4b | 0.2a | 0.2a | <.001 |
| P (mg/kg) | 0.01-0.3 | 0.01e | 0.004c | 0.002a | 0.005d | 0.002a | 0.003b | <.001 |
| K (cmol (+)/kg ) | 0-2 | 0.6d | 0.4b | 0.3a | 0.6d | 0.5c | 0.5c | <.001 |
| Ca (mg/kg) | 2-200 | 254c | 120a | 338d | 204b | 293.0c | 186b | <.001 |
| Mg ( mg/kg) | 1-120 | 884b | 192a | 168a | 315a | 169.0a | 187a | <.001 |
| Na (mg/kg) | 20-250 | 11.6b | 10.1ab | 8.3a | 11.2b | 10.9ab | 8.3a | <.001 |
| Zn (mg/kg) | 2-1600 | 303.1a | 444.6a | 415.8a | 383.0a | 486.6a | 320.3a | 0.561 |
| Fe (mg/kg) | 2000-100000 | 4291.0a | 2828.0a | 2540.0a | 3874.00a | 555.00a | 4832.00a | 0.336 |
| Cu (mg/kg) | 2-960 | 19.2a | 15.8a | 21.6a | 20.5a | 25.1b | 19.2a | <.001 |
| Mn (mg/kg) | 37-4600 | 3122.0a | 3039.0a | 3029.0a | 3038.0a | 547.0a | 2807.0a | 0.723 |

Means followed by the same letter within rows are not significantly different; Student Newman Keuls test, at $\alpha = 0.05$. EC = Electrical conductivity; N = Nitrogen; P = Phosphorus; K= Potassium; Ca =Calcium; Mg = Magnesium; Na = Sodium; Zn = Zinc; Fe = Iron; Cu = Copper; Mn = Manganese; µS = micro Siemens; cmol (+)/kg = centimoles of positive charge per kilogram of soil

**Table 4-2:** Correlation co-efficient (r) of the abundance of nematode trophic groups *Meloidogyne* spp. relative to the soil chemical properties

| Soil chemical parameters | Plant parasitic nematodes | Bacterial feeders | Fungal feeders | Predators | *Meloidogyne* spp. |
|---|---|---|---|---|---|
| Soil pH | -0.3765 | 0.2469 | -0.5882** | 0.2837 | -0.3681 |
| Soil Ec | -0.1269 | -0.2 | 0.1259 | -0.3247 | -0.1573 |
| Nitrogen | 0.6291** | -0.4306 | 0.7793** | -0.5052 | 0.6256** |
| Phosphorus | 0.5857** | -0.5262 | 0.6986** | -0.6334** | 0.5963** |
| Potassium | 0.3497 | -0.4032 | 0.3848 | -0.332 | 0.3918 |
| Calcium | -0.573** | 0.2724 | -0.5289 | 0.2493 | -0.6043** |
| Magnesium | 0.5756** | -0.3458 | 0.7096** | -0.2092 | 0.5804** |
| Sodium | 0.2173 | -0.3748 | 0.4105 | -0.2721 | 0.1899 |
| Zinc | -0.1042 | -0.1484 | -0.036 | 0.0023 | -0.1158 |
| Iron | 0.2035 | -0.1387 | 0.3149 | -0.3037 | 0.1728 |
| Copper | -0.1716 | 0.1316 | -0.12 | 0.1471 | -0.152 |
| Manganese | 0.2583 | 0.0754 | 0.2921 | -0.2677 | 0.3238 |

** = significant correlation at 0.01 probability level

**Table 4-3:** Population densities of nematode genera, assigned to different trophic groups, in rhizosphere soil from tomato grown in high tunnels

| Trophic group & genus | Gatundu North | Gatundu South | Juja | Kiambu | Ruiru | Thika | p-value |
|---|---|---|---|---|---|---|---|
| **Plant parasitic** | | | | | | | |
| *Meloidogyne* | 2225.0 ± 61.3c | 905.0 ± 37.2ab | 796.5 ± 30.4a | 1267.5 ± 61.4b | 575.0 ± 23.0a | 1007.1 ± 53.6ab | <0.001 |
| *Pratylenchus* | 42.5 ± 4.5a | 37.0 ± 2.6a | 23.3 ± 3.7a | 37.8 ± 4.0a | 17.5 ± 3.1a | 26.1 ± 3.2a | 0.431 |
| *Tylenchus* | 13.3 ± 1.9a | 16.0 ± 1.9a | 13.5 ± 2.3a | 22.3 ± 3.0a | 0. 0 a | 10.0 ± 1.4a | 0.211 |
| *Filenchus* | 8.3 ± 1.1a | 40.0 ± 2.8c | 18.5 ± 1.8ab | 8.8 ± 1.4a | 12.5 ± 1.2ab | 31.4 ± 2.3bc | <0.001 |
| *Radopholus* | 12.5 ± 1.6a | 10.0 ± 1.2a | 1.5 ± 0.4a | 5.0 ± 1.1a | 0. 0a | 8.6 ± 1.2a | 0.06 |
| *Hoplolaimus* | 2.5 ± 0.5a | 2.5 ± 0.6a | 6.0 ± 2.0a | 8.3 ± 1.8a | 20.0 ± 2.8a | 8.6 ± 1.8a | 0.332 |
| *Rotylenchus* | 8.3 ± 1.2ab | 8.0 ± 1.9ab | 0. 0a | 7.3 ± 1.4a | 0. 0a | 0. 0ab | 0.067 |
| *Helicotylenchus* | 25.0 ± 2.6a | 24.0 ± 3.5a | 33.0 ± 3.3a | 43.0 ± 5.6a | 18.8 ± 1.5a | 13.9 ± 1.5a | 0.137 |
| *Tylenchorynchus* | 14.2 ± 2.3ab | 8.0 ± 0.7a | 27.5 ± 3.3ab | 39.5 ± 5.1ab | 18.8 ± 2.0ab | 12.9 ± 2.3ab | 0.028 |
| *Trichodorus* | 12.5 ± 1.9ab | 0. 0 a | 3.5 ± 1.2a | 18.0 ± 2.7b | 1.3 ± 2.0ab | 2.9 ± 0.8a | 0.008 |
| *Xiphinema* | 10.8 ± 1.8ab | 19.0 ± 1.7ab | 17.0 ± 2.9ab | 7.3 ± 1.4a | 33.8 ± 3.2b | 20.4 ± 2.1ab | 0.038 |
| *Longidorus* | 44.2 ± 3.9b | 20.5 ± 1.7ab | 18.0 ± 1.8ab | 12.8 ± 2.2a | 11.3 ± 1.0a | 14.6 ± 1.5a | 0.005 |
| *Ditylenchus* | 3.0 ± 0.0a | 2.0 ± 0.0a | 21.9 ± 0.1a | 9.4 ± 0.1ab | 9.7 ± 0.1ab | 11.3 ± 0.1ab | <.001 |
| *Aphelenchoides* | 12.0 ± 0.1ab | 10.8 ± 0.1ab | 3.8 ± 0.1a | 18.3 ± 0.1b | 5.5 ± 0.1ab | 1.1 ± 0.0a | <.001 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Bacterial feeders** | | | | | | | |
| *Alaimus* | 11.7 ± 0.5a | 43.6 ± 0.1a | 121.0 ± 0.1c | 36.3 ± 0.1a | 90.6 ± 0.1b | 19.3 ± 0.1a | <.001 |
| *Acrobeles* | 5.0 ± 0.1a | 11.4 ± 0.1abc | 7.1 ± 0.1ab | 6.0 ± 0.1ab | 14.4 ± 0.1abc | 15.5 ± 0.1ac | 0.018 |
| *Leptolaimus* | 5.0 ± 0.1ab | 5.6 ± 0.1bc | 33.6 ± 0.1d | 13.3 ± 0.1c | 21.5 ± 0.1c | 1.7 ± 0.1a | <.001 |
| *Plectus* | 12.1 ± 0.1a | 35.1 ± 0.1ab | 4.3 ± 0.0a | 22.5 ± 0.1ab | 15.0 ± 0.1a | 191.9 ± 0.3a | 0.041 |
| *Desmodora* | 27.0 ± 0.1a | 2.5 ± 0.0a | 2.6 ± 0.0a | 11.8 ± 0.1ab | 3.0 ± 0.0a | 16.9 ± 0.1ab | 0.002 |
| **Fungal feeders** | | | | | | | |
| *Dorylaimus* | 19.6 ± 0.1b | 0.0a | 17.8 ± 0.1b | 13.5 ± 0.1b | 26.0 ± 0.1c | 7.0 ± 0.0ab | <0.001 |
| *Aphelenchus* | 23.4 ± 0.1b | 16.1 ± 0.1b | 2.6 ± 0.0a | 18.9 ± 0.1b | 0.0a | 3.4 ± 0.0a | <0.001 |
| *Aporcelaimus* | 29.8 ± 0.1b | 9.5 ± 0.1a | 7.0 ± 0.0a | 5.1 ± 0.0a | 4.4 ± 0.0a | 5.0 ± 0.0a | 0.001 |
| **Predators** | | | | | | | |
| *Mononchus* | 2.6 ± 0.0a | 1.7 ± 0.0a | 18.3 ± 0.1ab | 8.4 ± 0.1a | 35.2 ± 0.1b | 5.7 ± 0.1a | 0.001 |
| *Discolaimus* | 7.5 ± 0.1a | 5.5 ± 0.1a | 8.9 ± 0.1ab | 3.8 ± 0.0a | 8.8 ± 0.1a | 14.8 ± 0.1ab | 0.014 |

Means followed by the same letter within rows are not significantly different; Student Newman Keuls test, at $\alpha = 0$.

## 4.4 Discussion

The results showed that soil chemical properties differed across the sub-counties which influenced nematodes populations. Whereas Gatundu North sub-county supported high populations of *Meloidogyne* spp. the pest was significantly low in Gatundu South, Thika, Kiambu, Ruiru and Juja sub-counties. The soil pH in Gatundu North was slightly acidic relative to the other sub-counties which consequently led to high *Meloidogyne* spp populations. These results agree with those of Wang *et al*., 2009 who reported that an increased soil acidity of pH 4.5-5.4 in the root zone led to a faster reproduction an of *Meloidogyne* spp leading to high crop losses. Acidic soils have been found to increase the population of soil microbial communities, survival and reproduction of root knot nematodes (Kesba & Al-Shalaby, 2008). In high potential areas with high rainfall, infestations with bacterial wilt and root knot nematodes are common due to low soil pH hence farmers encounter heavy crop losses since RKNs are soil-borne.

When the soil chemical characteristics were correlated with the nematode trophic groups, *Meloidogyne* spp. positive and negative relationships existed suggesting their role in pathogen-host interactions (Desaeger & Rao, 2000; Wang *et al*., 2004). Despite the fact that the soil pH had weak positive and negative correlations with the abundance of plant parasitic, bacterial feeders and predators results suggest their role in influencing nematode diversity (Zhong & Cai, 2007; Ingham *et al*., 1985). For instance, soil pH had a strong negative correlation with the fungal feeders indicating that a decrease in the former may lead to an increase in the latter and vice versa. Our results suggest that soil pH may alter the soil microbiota by affecting the soil microbial activities (Rocha *et al*., 2006). Previous studies reported that continuous use of mineral fertilizers such as NPK and CAN decreased the soil pH (Adamtey *et al*., 2016), consequently affecting the soil microbes and nematodes population and diversity (Zhong *et al.*, 2010). Differences in soil chemical properties and their subsequent effect on nematode populations could be attributed to changes in farmer practices and varying environmental conditions in the high tunnels which were, however, not measured in this study.

In addition, there was a strong positive correlation between plant parasitic nematodes, fungal feeders and *Meloidogyne* spp. with N, P and Mg suggesting that any increase in these mineral elements may lead to a similar effect in the nematode trophic groups. Our results concur with previous studies that showed that an increased N levels consequently led to high populations of plant parasitic nematodes (de Melo Santana *et al*., 2013). In addition, P in forms such as potassium phosphate has been found to increase the hatchability of *Meloidogyne exigua* (Salgado *et al*., 2007). Micronutrients, for example Mg that showed a positive correlation with PPNs in our study, have also been reported to reduce production of some plant metabolites that protect plants from nematode attack, thus increasing their prevalence (Fancelli, 2008). Previous reports indicated that fungal feeders are associated with mycorrhiza that facilitate the recycling of nutrients in the soil by forming a carbon sink (Teotia *et al*., 2017). We envisage that an increase in N and P fertilizers does not impact negatively on the abundance of fungal feeders that facilitate the availability of these nutrients to plants. However, further research is required to corroborate this scenario in Kiambu County.

There was a strong negative correlation between plant parasitic nematodes, fungal feeders and *Meloidgyne* spp. with calcium (Ca) indicating that any increase in this element in the soil lowers population of these nematode trophic groups. This concurs with previous studies that application of calcium based components in the soil reduced root galling, egg masses and growth of juveniles and susceptibility of plants to nematode attack (Mohamed & Youssef, 2009). The fact that *Meloidogyne* spp. populations were both high in Gatundu North relative to other sub-counties suggests that disease complexes exist in high tunnel tomato farming (Agrios, 2005). It appeared that *Meloidogyne* spp. is highly abundant in Gatundu North sub-county.

In conclusion, these findings suggest that soil chemical characteristics influence root knot nematodes in high tunnels and thus will be used by farmer stakeholders to design effective nematode prevention strategies which would in turn impact positively on the livelihoods of small holder tomato high tunnel farmers.

# CHAPTER FIVE

## EFFECT OF NPK FERTILIZER APPLICATION RATES ON POPULATION OF ROOT KNOT NEMATODES, SOIL PH , C:N RATIOS AND YIELD OF HIGH TUNNEL TOMATO VARIETIES


### Abstract

Tomato (*Solanum lycopersicum*) is one of the most important vegetables grown for income and consumption in Kenya. Cultivation has mainly been under open field conditions until recently where modified high tunnels (commonly known as 'greenhouses') were introduced in the country. Sustainability of profitable utilization of the high tunnel tomato production is mainly threatened by root knot nematodes (RKN), the *Meloidogyne* spp. There is evidence that mineral fertilizers alone or in combination with manure have potential in controlling RKN. However, continuous use of these fertilizers can result in decreasing soil pH consequently affecting crop growth and yield. This study sought to determine effect of NPK 17:17:17 fertilizer application levels on the population density of root knot nematodes and the yield of commonly grown in high-tunnel tomato varieties. The least number of nematodes/100g of soil (518), galling index/root (2), and egg masses/root (14.8) were observed in the plants treated with 10 g of NPK fertilizer relative to the control, 5g and 20 g. Similarly, the highest fruit weight (4148.2 g/plant was observed on plants treated with 10g of fertilizer relative to the control (219.6 g/plant). The impact of the fertilizer on the soil chemical characteristics will be discussed. In conclusion, application of optimum amounts of inorganic fertilizers can improve RKN management thus, can help to minimize the impact of harmful chemicals that can lead to environmental degeradation



**Key words:** Root knot nematodes, fertilizer, population, yield, management

## 5.1 Introduction

Several control strategies, such as host plant resistance, rotation with non-hosts, destruction of residual crop roots, and use of nematicides, have been reported to be used to control root knot nematodes (Chitwood, 2002). Of the existing methods, fumigant nematicides such as methyl bromide were most promising, but due to their ozone depleting properties their use has been completely phased-out (Perry *et al*., 2009) necessitating alternative ecofriendly control strategies. Mineral nutrients are known to be important in plant-disease interaction particularly plant-soil pathogen interaction (Spann & Schumann, 2010). Bednarek & Gaugler (1997) reported that addition of inorganic amendments, particularly NPK, suppressed nematode densities. They confirmed that prolonged exposure to high inorganic fertilizer concentrations inhibited their reproduction. However, it affected the beneficial nematodes i.e. entomopathogenic nematodes by reducing their infectivity. Oteifa (1955) reported that ammonia decreased the counts of *Meloidgyne incognita* females and egg masses produced on infected lima beans. The problem statement is that, there is limited information on how each nutrient affects plant's response to the pathogen infection, whether positively or negatively. Potentially, natural plant defense mechanisms and proper nutrients application could be utilized to develop alternative management strategies for RKNs. A greenhouse experiment was carried out to determine the impact of fertilizer application rates on the population of the dominant *Meloidogyne* spp. and on growth of greenhouse tomato varieties.

## 5.2 Materials and Methods

### 5.2.1 Greenhouse experiment

Two pre-dorminant indeterminate tomato varieties namely Tylka F1 and Anna F1 grown by the farmers were used in the study. The seeds of these varieties were obtained from Syngenta and Monsanto seed companies respectively. NPK 17:17:17 fertilizer obtained from MEA Fertilizer Company was used. Seeds of the two tomato varieties namely Tylka F1 and Anna F1 were purchased and the seedlings established in the greenhouse

by raising them in plastic plugs containing sterilized (autoclaved) coco peat substrate. The seedlings were watered every two days with nutrient solution (standard Hoagland solution) having a mixture of both macro and micronutrients (Lambert *et al*., 1992). After 3 weeks the seedlings were be transplanted.

### 5.2.2 Experimental Design

The greenhouse experiment was laid out as a factorial experiment in a complete randomized design (CRD) with five levels (0, 2.5, 5, 10 and 20 g per per pot) of fertilizer application rates and the two tomato varieties (Tylka F1 and Anna F1) replicated three times. Each treatment had 20 plants. Fertilizer application levels were randomized within the tomato varieties. Controls included similar number of the treatments receiving no fertilizer (0 g). The experinment was repeated for two seasons.

### 5.2.3 Treatments

Tomato plants were treated with different NPK 17:17:17 fertilizer application rates. The plants received treatments of fertilizers 2 weeks after transplanting. The fertilizer application rates used are; 0, 2.5, 5, 10 and 20 g per hole/plant. Control plants did not receive any fertilizer treatments. Each fertilizer treatment was inoculated with 1000 second stage juveniles of *Meloidogyne javanica* 1 week after fertilizer application.

### 5.2.4 Source of Root knot nematodes

Root knot nematodes (*Meloidogyne javanica)* populations were maintained in the greenhouse at 23 -27$^{\circ}$C and 60-70% RH on potted Cal J tomato plants at International Centre of Insect Physiology and Ecology (ICIPE) Nairobi, Kenya. The plants were watered with nutrient solution to provide macro- and micro-nutrients (Lambert *et al*., 1992). Galled roots of tomato plants from the greenhouse were picked and washed with running water to remove soil and sand debris. The roots were immersed in an aqueous solution of phloxine B (0.15g/lit) for 20 minutes to stain the egg masses then washed with tap

water to remove the excess stain (Coyne *et al*., 2018a). Under a dissecting microscope egg masses, were removed singly and placed into distilled water in a multiple well tray and incubated in the dark at 23-27°C for 3 days after which the hatched J2s were used for inoculation.

### 5.2.5 Assessment of nematodes

Assessment of the root knot nematodes development in the different treatments included, galling index, number of eggs/10g root, number of egg masses/10g root and no. of J2s (second stage juveniles) /100g soil. Plant and soil sampling was done after 30 and 60 days of nematode inoculation.

Plants were uprooted and their roots washed with tap water to avoid the soil particles. Number of galls, number of egg masses, as well as number of J2s /100 g soil, were determined. Nematode infected roots (Plate 5-1) were dipped into phloxine-B staining solution of 0.15 g/l for 20 min. to staining and count egg-masses according to Daykin & Hussey, (1985).

**Plate 5-1**: Galled roots due to nematode attack

The amount of root damage (galling index) was estimated visually using the scoring procedure modified from Bridge & Page (1980); 0-10 non parametric scale (0 =0% number of knots on the roots; 1=10% few small knots, difficult to find; 2= 20% small knots only but clearly visible, main roots clean; 3= 30% some large knots visible, main roots clean; 4=40% large knots predominate but main roots clean; 5- 50% of roots affected, knotting on some main roots, reduced root system; 6= 60% knotting on the main roots; 7= 70%, majority of main roots knotted; 8=80% all main roots including tap root knotted., few clean roots visible; 9=90% all roots severely knotted, plant usually dying; 10=100% all roots severely knotted, no root system (Bridge & SamPage, 1980) (Appendix II).

### 5.3 Carbon/ Nitrogen ratio determination

Plants were sampled for total carbon and nitrogen analysis. After carefully lifting out each plants and the surrounding medium with a hand shovel, each plant were divided into four compartments; leaves, shoots, stem and roots. These plant samples were then placed in labeled khaki paper bags and transported to the laboratory for analysis. Total organic carbon (TOC) determined using modified Walkley- Black titration method according to Sahrawat (1982) which involves oven drying the plant samples at 70$^{\circ}$C, oxidation of the organic matter of the dried plant sample is done using Potassium dichromate reagent by heat of dilution. The excess of chromate left after C oxidation was titrated with standard ferrous sulfate solution. Titration of each of the digested sample was done to determine total carbon. Total nitrogen (N) was determined using modified Kjedahl's method according to Eastin (1978). Kjedahl's method quantitatively determines $NH_4$ and protein N in plant tissues based on wet oxidation of organic matter using $H_2SO_4$ and a digestion catalyst (Isaac & Johnson, 1976). The samples were oven dried at 70$^{\circ}$C and then digested using digestion mixture at 36$^{\circ}$C. Distillation of digested samples was done to determine total nitrogen.

## 5.4 Statistical analysis

Nematode population data were transformed using natural logarithm [ln (x+ 1)] prior to analysis to stabilize the variance and normalize the data (Gomez & Wiley, 1976). The transformed data were subjected to analysis of variance to evaluate the effect of fertilizer application rates on the population of *Meloidogyne* spp. under greenhouse conditions. The transformed data were subjected to analysis of variance (ANOVA). These statistics were performed in R software version 3.2.3 (R Core Team, 2017) and means were separated using Fisher's least significant difference (LSD) test at $p = \leq 0.05$ using the package "agricolae" (De Mendiburu, 2015).

## 5.5 Results

### 5.5.1 Effect of NPK fertilizer application rates on nematode population

The analysis revealed significant difference in galling index among the different treatments ($P \leq 0.001$) (Table 5-1) after 30 days of inoculation. Galling index was lower on plants treated with 10.0g of NPK 17:17:17 fertilizer (Anna F1= 0.7 ± 0.3, Tylka F1= 1.0 ± 0.3) compared to the control (0g) (Anna F1= 5.0 ± 0.3, Tylka F1= 5.7 ± 0.1). The analysis showed significance differences in the number of egg masses among the different treatments ($P \leq 0.01$) (Figure 5-1) after 30 days of nematode inoculation. The number of egg masses per 10 g root sample on plants treated with 2.5g and 20g of NPK fertilizer was similar to the control (0g) (Anna F1= 38.0 ± 2.0) (Table 5-1).

After 60 days of root knot nematodes inoculation, analysis revealed significant difference in galling index, no. of J2s/ 100g of soil, no. of egg masses/ root and no. of eggs/ 10g root among the different NPK application rates ($P \leq 0.001$) (Table 5-1). The number of second stage juveniles (J2s), egg-masses/root and eggs/10g in tomato plants treated with 10g of NPK fertilizer was significantly ($P \leq 0.05$) greater compared to treated with 0g, 2.5g and 20.0g (Table 5-1).

### 5.5.2 Effect of NPK fertilizer application rates on tomato root fresh weight

Fresh root weight of control (without fertilizer) (Anna F1= 13.6 ± 0.28 and Tylka F1= 6.1 ± 0.32) was significantly ($P \leq 0.05$) lower than all other treatments (Table 5-2). In addition, fresh root weights of plants treated with 10.0g of NPK fertizer was significantly ($P \leq 0.05$) greater compared to all other treatments. However, fresh root weights of plants in which 2.5g of NPK fertilizer was applied (Anna F1= 30.5 ± 0.86 and Tylka F1= 25.4 ± 0.69) was similar to those applied with 20.0g (Anna F1= 38.8 ± 3.44 and Tylka F1= 21.3 ± 0.46) (Table 5-2).

### 5.5.3 Effect of NPK fertilizer application rates on soil pH

There was significantly lower pH (4.3 ± 0.1) in soils treated with high (20.0g) amounts of NPK and significantly higher pH (7.4 ± 0.1) unlike untreated soils with NPK fertilizer (Figure 5-1).

### 5.5.4 Effect of NPK fertilizer application rates on C:N ratio of tomato leaves

The analyses of variance of carbon/ nitrogen ratio in tomato leaves showed significant variances amongst the NPK application rates ($P< 0.001$) in the two tomato varieties (Table 5-3). Notably, the plants treated with 10g of the NPK fertilizer showed significantly low carbon/nitrogen ratio compared those treated with 0g, 2.5g and 20g of NPK fertilizer but were similar to those treated with 5g of NPK fertilizer as showed in Table 5-3.

### 5.5.5 Effect of NPK fertilizer application rates on tomato yield

Results showed that 10g of NPK fertilizer in 2kg soil increases tomato yield in the two tomato varieties (Fig 5-2). Analyomato plants treated with 10g of NPK fertilizer produced significantly greater fruit weight per plant (yield) (Anna F1= 4,733.64g, Tylka F1=3,562.74g) compared to the other plants treated with 0g, 2.5g, 5g and 20g of NPK fertilizer (Figure 5-2).

**Table 5-1:** Effect of different NPK fertilizer rates on root knot nematodes population in two tomato varieties at 30 and 60 days after inoculation

| | **30 Days after inoculation** | | **60 Days after inoculation** | |
|---|---|---|---|---|
| **(a) Galling index / root** | | | | |
| **Treatments** | **Anna F1** | **Tylka F1** | **Anna F1** | **Tylka F1** |
| 0g | 5.0±0.3c | 5.7±0.1c | 7.7±0.1c | 8.0±0.3c |
| 2.5g | 3.3±0.5b | 4.3±0.1b | 6.3±0.1c | 8.0±0.3c |
| 5.0g | 2.0±0.4ab | 1.0±0.1a | 3.3±0.1b | 4.0±0.7b |
| 10.0g | 0.7±0.3a | 1.0±0.3a | 2.0±0.3a | 2.3±0.1a |
| 20.0g | 5.0±0.1c | 5.0±0.3b | 7.3±0.1c | 6.7±0.1c |
| P- value | <0.001 | <0.001 | <0.001 | <0.001 |
| **(b) No. of J2s/ 100g of soil** | | | | |
| **Treatments** | **Anna F1** | **Tylka F1** | **Anna F1** | **Tylka F1** |
| 2.5g | 121.7±17.0b | 185.0±13.2b | 1165.7±59.70bc | 1464.3±93.70b |
| 5.0g | 176.0±2.30b | 206.0±10.7b | 997.70±140.5b | 1441.3±42.10b |
| 10.0g | 27.30±3.80a | 58.70±7.10a | 170.70±34.50a | 1481.7±118.4b |
| 20.0g | 159.7±22.3b | 316.3±62.6c | 1026.7±81.70bc | 342.00±19.30a |
| P- value | <0.001 | <0.001 | <0.001 | <0.001 |
| **(c) No. of egg masses/ root** | | | | |
| **Treatments** | **Anna F1** | **Tylka F1** | **Anna F1** | **Tylka F1** |
| 0g | 38.0±2.0c | 60.0±1.0d | 73.7±1.8c | 97.0±3.0c |
| 2.5g | 32.3±0.8c | 38.7±1.4b | 62.7±1.9c | 59.3±4.3b |
| 5.0g | 26.0±1.3b | 30.3±2.6b | 45.0±1.2b | 67.7±1.1b |
| 10.0g | 2.00±0.9a | 12.3±1.3a | 3.30±1.5a | 64.3±4.9b |
| 20.0g | 38.7±2.2c | 45.3±2.6c | 75.3±3.8c | 11.3±0.3a |
| P- value | <0.001 | <0.001 | <0.001 | <0.001 |
| **(d) No. of eggs/ 10g root** | | | | |
| **Treatments** | **Anna F1** | **Tylka F1** | **Anna F1** | **Tylka F1** |
| 0g | 9630.00±156.5e | 12193.3±564.5c | 17576.7±195.7d | 21463.3±573.7d |
| 2.5g | 6300.00±180.7c | 7116.70±209.9c | 13700.0±880.7c | 10306.7±190.7b |
| 5.0g | 4020.00±189.3b | 6620.00±115.0b | 7290.00±481.6b | 16586.7±285.0c |
| 10.0g | 1626.70±54.50a | 1686.70±144.6a | 370.00±175.7a | 15216.7±778.1c |
| 20.0g | 7700.00±180.7d | 10936.7±453.8c | 14793.3±438.1c | 3810.00±111.7a |
| **P- value** | <0.001 | <0.001 | <0.001 | <0.001 |

Means followed by the same letter within rows are not significantly different; Student New-man Keuls test, at $\alpha = 0.05$

**Table 5-2:** Effect of NPK fertilizer rates on root fresh weights (g) of two tomato varieties

| | Tomato varieties | |
|---|---|---|
| **Treatments** | **Anna F1** | **Tylka F1** |
| **0g** | 13.6 ± 0.28a | 6.1 ± 0.32a |
| **2.5g** | 30.5 ± 0.86c | 25.4 ± 0.69b |
| **5g** | 58.3 ± 1.63d | 43.7 ± 0.45c |
| **10g** | 75.8 ± 1.36e | 68.3 ± 0.14d |
| **20g** | 38.8 ± 3.44b | 21.3 ± 0.46b |
| **P-value** | <0.001 | <0.001 |

Means followed by the same letter within rows are not significantly different; Student New-man Keuls test, at $\alpha = 0.05$

**Table 5-3:** Effect of NPK fertilizer application rates on carbon/nitrogen ratio on tomato leaves of the two tomato varieties

| **Treatments** | **Anna F1** | **Tylka F1** |
|---|---|---|
| **0g** | 15.9 ± 0.27c | 14.3 ± 0.41c |
| **2.5g** | 7.4 ± 0.28b | 8.1 ± 0.26b |
| **5g** | 5.7 ± 0.34ab | 4.7 ± 0.17ab |
| **10g** | 3.3 ± 0.12a | 2.8 ± 0.23a |
| **20g** | 13.1 ± 0.19c | 13.8 ± 0.59c |
| **P-value** | <0.012 | <0.001 |

Means followed by the same letter within rows are not significantly different; Student Newman Keuls test, at $\alpha = 0.05$

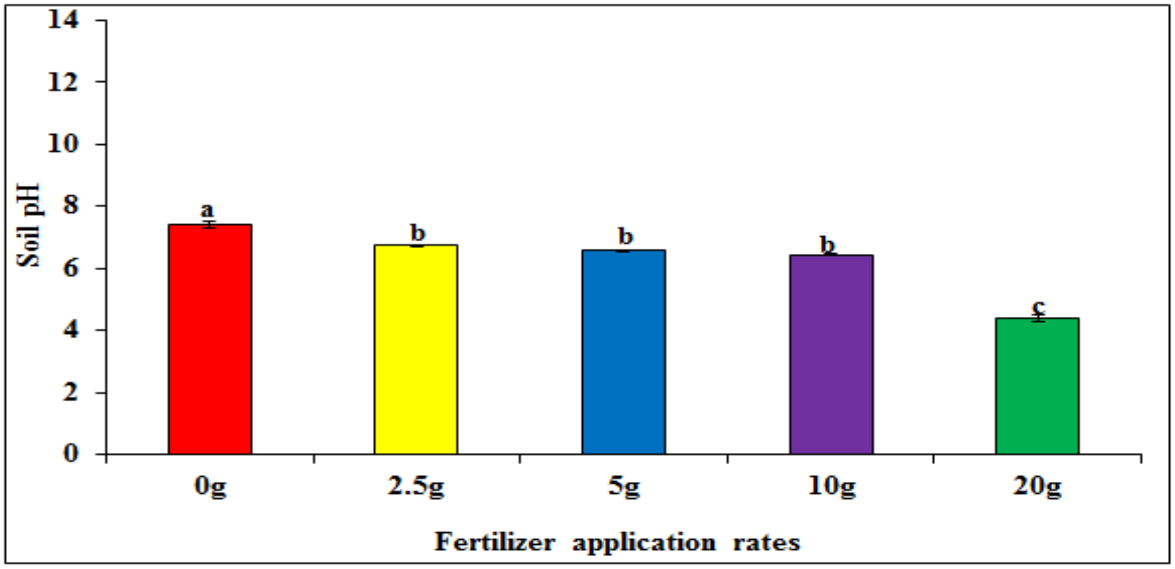


**Figure 5-1:** Effect of NPK fertilizer application rates (0g, 2.5g, 5g 10g and 20g) on soil pH

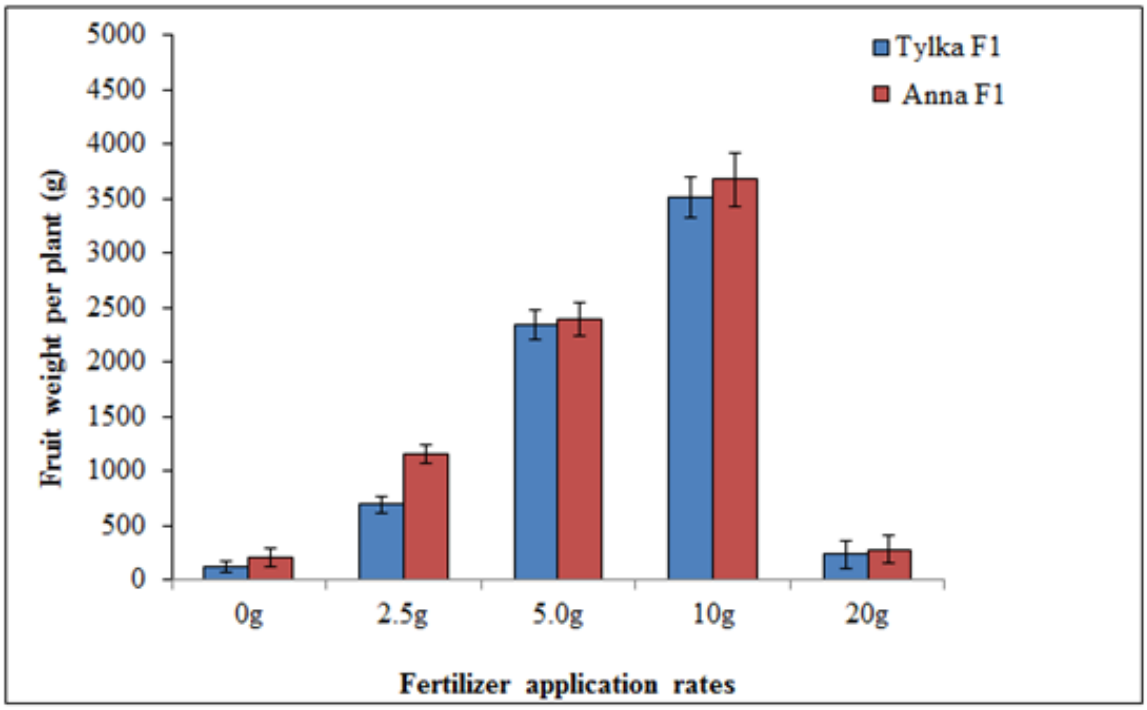


**Figure 5-2:** Effect of NPK fertilizer application rates on the tomato yield (fruit weight in grams per plant)

## 5.6 Discussion

Results showed the lowest numbers of J2/100g of soil, galling index/root and egg masses/root were observed in the plants treated with 10g of fertilizer after 30 and 60 days. The application of fertilizers affects the incidence of diseases and pests induced by the plants' nutritional status and indirectly produce dense stands and alterations in the interception of light and moisture within the crop (Agrios, 2005). The numerous effects of plant nutritional status and fertilizer use on diseases and pests are directly relevant to the control of these organisms by chemical means. The application of mineral fertilizer replaces or reduces the need for chemical control in some cases, whereas in others it can increase this need. For instance, chemical control is essential in the presence of high levels of nitrogen (Marschner, 2012). Applying fertilizer can, partially, offset nematode-induced damage by stimulating plant development (Ferraz *et al*., 2010). The general rule is that, if a nutrient is essential to a plant species, it should be supplied in balanced proportion to other essential nutrients, since deficiency can aggravate disease, especially in short-cycle crops (Zambolim *et al*., 2001).

To determine the effect of NPK fertilizer application rates on tomato root fresh weight, results showed that the fresh root weights of plants in which 10.0g of fertilizer was applied was significantly ($P \leq 0.05$) greater compared to all other treatments. This could be explained by the fact that application of mineral fertilizer boost the nutritional status of the plant leading to root growth and development. Some studies show that nematodes cause a drop in root system activity and growth (Caillaud *et al*., 2008). To determine the effect of NPK fertilizer application rates on carbon/nitrogen ratio of tomato leaves, results revealed that plants treated with 10g of the NPK fertilizer showed significantly low carbon/nitrogen ratio compared those treated with 0g, 2.5g and 20g of NPK fertilizer but were similar to those treated with 5g of NPK fertilizer. Among plant nutrients, nitrogen is essential for growth and yield. An abundance of nitrogen results in the production of new tissues and saps, and can extend the vegetative state and increase the number of feeding sites in the roots, encouraging nematode attack.

On the other hand, a plant that is deficient in nitrogen can become debilitated, suffer slowed growth and become more susceptible (Zambolim *et al*., 2001; Ferraz *et al*., 2010). However, the form in which the nutrient is available, whether ammonium ($NH_4^+$) or nitrate ($NO_3^-$), has more effect on the severity of the nematode attack than the quantity of nitrogen available (Ferraz *et al*., 2010). On the other hand, highest fruit weight (yield) (g) was observed on plants treated with 10.0g of fertilizer (4148.2 g/plant) and the lowest weight of plant yield (219.6 g/plant) was observed in plants treated with no fertilizer. In conclusion, proper fertilizer application reduces the *Meloidogyne* sp. population in high tunnel tomato production. 10g of NPK fertilizer in 2kg soil increases tomato yield.

# CHAPTER SIX

# GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Discussion

The study showed that root knot nematode is a pest of economic importance encountered by farmers producing tomatoes in high tunnels in six sub-counties of Kiambu County. Additional pest faced by farmers include whiteflies thrips, aphids, leaf miners and caterpillars. Diseases namely bacterial wilt (*Ralstonia solanaceareum*), blights (*Phytopthora infestans* and *Alternaria solani* ), Fusarium wilt ( *Fusarium oxysporum*), blossom end root, root rots and tomato yellow leaf curl virus were reported to be the major contributor to the decline in tomato yield. There was a variation in the knowledge and attitude about root knot nematodes across the six study sites suggesting different management measures adopted by farmers. Even though most farmers had produced tomatoes in high tunnels by up to two years, continuous cropping of a maximum of four cropping cycles in a year without replacement of the media increased the prevalence of soil-borne pests and diseases hence yield decline. Farmer's knowledge on management of crop pests has been reported to be important since pests reduce the quality of the yield and spread of diseases (Okonya & Kroschel, 2016). Pests such as aphids have been reported to spread viral diseases and reduce the quality of marketable tomato fruits (Van der Waals *et al*., 2016). Continuous cropping with root knot nematodes host plants using the same media in high tunnels by farmers exacerbates the prevalence of nematodes and soil borne diseases such as bacterial wilt, *Fusarium* wilt and root rots all leading to reduction in yield. Farmers should be enlightened on recommended tomato production and root knot nematode management practices so as to increase yield.

There was a significant variation in soil nutrients namely N, P, K, Ca, Mg, Na and Cu across the six sub-counties of Kiambu County and nitrogen was the most variant nutrient. This can be attributed to differences in agro-ecological zones, soil types and farming practices among farmers in the study sites (Jaetzold *et al*., 2007). Up to twenty four

nematode genera grouped into four trophic groups namely phyto-parasitic genera, bacterivores, fungivores and predators were found to infest soil samples collected from farmers producing tomatoes in high tunnels. *Meloidogyne* spp, was the predominant genera with significantly high populations. There was also a weak negative correlation between soil pH, electrical conductivity, zinc and copper. A weak negative correlation between the chemical properties of soil, Zn and Cu might have been caused by poor farming practices such as increased monocropping and high applications of nitrogenous fertilizers with hydrogen and ammonium ions. Nitrogenous fertilizers alter the soil pH making some micro nutrients unavailable. Studies have shown that ammonium ions ($NH_4$+) increases soil acidity reducing mineralisation of fertilizers (Barak *et al*., 1997). In addition, increased application of N,P and K fertilizers increases the population of plant parasitic nematodes (de Melo Santana *et al*., 2013). Due to a variation in soil chemical characteristics in the study sites, there was diversity in nematode genera and a variation in the correlation of soil nutrients with nematode trophic groups. This necessiates enlightening of farmers on good farming practices used in management of nematodes .

The study showed a significant effect of different rates of nitrogenous fertilizer on galling index and nematode egg masses unlike a slight variation on soil pH. Low galling indices and fewer egg masses in soils treated with higher rates of nitrogenous fertilizer can be attributed to improved and optimal nutrition of the plant enabling it withstand nematode attack. Similar findings were reported by (Agrios, 2005) and (Ferraz & de Felício, 2010) showed that good nutrition increases defence mechanisms of crops against disease and pest attack whilst promoting growth and development. Significant effects of application of different rates of nitrogenous fertilizers were also observed on yield of tomatoes. Significantly higher yields were obtained in plots treated with 10g of fertilizer. This is explained by the fact that this rate was optimal for plant growth unlike 20g. Previous studies have reported that application of mineral nutrients in optimal amounts reduces damage by crop pests and diseases especially in short duration crops such as tomato (Zambolim *et al*., 2001). These findings therefore suggest that for efficient management of nematodes in tomatoes, farmers should use optimal rates of nitrogenous fertilizers de-

termined by soil nutrient and pH analysis so as to provide optimal nutrition to crops and maximise yields.

## 6.2 Conclusions

Root knot nematodes are the most problematic in high tunnel tomato production in Kiambu County. Despite the high interest in producing tomato in high tunnels, farmers have poor farming practices that intensify the severity of the pathogen. Therefore there is need for continuous farmer education and intensified extension services by well-trained personnel to increase adoption of good farming practices. This will impact positively on tomato production and proper utilization of the high investment high-tunnels.

Significantly high occurrence of plant parasitic nematodes was observed among the high tunnel tomato production in Kiambu County. This is as a result of continuous cultivation over the infected soils. These findings also suggest that soil chemical characteristics influence soil pathogen in high tunnels and thus will be used by farmer stakeholders to design effective nematode management strategies which would in turn impact positively on the livelihoods of small holder tomato high tunnel farmer.These findings therefore suggest that for effective management of root knot nematodes in tomatoes, farmers should use optimal rates of fertilizers (10g of NPK 17:17:17 fertilizer in 2kg soil equivalent to 180kgha-1 NPK 17:17:17) determined by soil nutrient and pH analysis so as to provide optimal nutrition in tomato production and maximise yields.

### 6.3 Recommendations

From this study, the following are recommendations to improving productivity of tomatoes in high tunnels.

i. Additional research should be carried out to determine plant chemical defense components such as volatile chemistry as influenced by fertilizer application
ii. Additional research should be carried out to identify and characterize nematodes to the generic level so as to develop efficient management practices
iii. There is need to test additional organic and inorganic fertilizer types and rates to establish their impact on nematode- plant interactions

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## APPENDICES

**Appendix I:** Checklist of focus group discussion with open-ended questions

**Checklist for a focus group discussion on farmer knowledge attitude and practices on management of pests and diseases in high tunnel 'greenhouse 'tomato production in Kiambu County**

**A) Introduction**

i. How many members do you have? Total: ……. Male …….Female ……

ii. When did the group start practicing greenhouse farming?

**B) Knowledge of the problem**

i. How long have you been using the greenhouse
ii. During the time you have been using the greenhouse, which vegetables have you grown?
iii. How many seasons have you grown each crop?
iv. Are the vegetables for sale or for home consumption?
v. If for sale, are they profitable?
vi. How profitable are they compared to open field production?
vii. What are the main pest and disease challenges in growing the vegetables?
viii. Have you observed the following problems (*show pictures of Bacterial wilt symptoms & RKN damage on roots and patches in the field*) in tomato?
ix. What do you associate these (above) symptoms with?
x. At which stage do you mostly observe the above symptoms in a crop's life?
xi. Approximately, what proportion of the crop population is affected?
xii. Do you have a local name for these problems? Which one?
xiii. Has anyone of you participated in a group discussion or survey before to discuss RKN and bacterial wilt problems in your farms?
xiv. What did you discuss?

**C) Attitude towards the problem**

i. Which factors do you consider as the main contributors to the decline in greenhouse tomato production?

ii. Do you consider the above issues (*RKN and Bacterial wilt*) among major factors leading to decline in crop production?

iii. How?

iv. Do you think any greenhouse practices affect the level of any of the problems above? Which one and how?

v. Is there any method that you can suggest, as a control of the problem(s) (RKN and bacterial wilt)? Which one and how?

vi. How much is the estimated yield loss in tomato and other crops due to RKN and bacterial wilt?

vii. Has this affected your interest in growing tomato

How?

**D) Practices**

i. How do you grow tomato for maximum production?

ii. Which variety of tomato do you plant?

iii. Where do you acquire your seeds/seedlings from?

iv. How long does tomato take from planting to the first harvest?

v. Do you uproot tomato and plant another crop (tomato or other crop) immediately? If not how long? And if yes, which other crop after tomato?

vi. Which type of planting media do you use?

vii. Where do you obtain it from?

viii. How do you prepare the media before planting?

ix. Do you test your soil before planting?
x. Where and what test?
xi. If you don't, why?
xii. How often do you test after the first test?
xiii. Do you follow the test recommendations?
xiv. Which fertilizer type do you apply?
xv. How do you apply these fertilizers?
xvi. At what stage of plant growth do you apply the fertilizer?
xvii. How many times do you apply each fertilizer?
xviii. How much fertilizer do you apply; a) per stem/hole b) the whole greenhouse
xix. Which methods do use to you control the pests and diseases in the greenhouse?
xx. How do you control the above problems (*show pictures of Bacterial wilt symptoms & RKN damage on roots and patches in the field)*?
xxi. What is the cost implication of each of these methods?
xxii. How do you apply these methods?
xxiii. How effective are these methods in reducing the above problems?
xxiv. Do you find any differences in terms of weather conditions in the severity of problems?
xxv. Under which conditions is the bacterial wilt and RKN problems most severe?
xxvi. Do you practice crop rotation in the greenhouse?
xxvii. If yes which crops?
xviii. If no why?
xxix. How dispose diseased plants?
xxx. Where do you get your irrigation water from?
xxxi. What type of irrigation do you use?

**E) Wrap-up session**

i. Other than the ministry of Agriculture are there any Organizations or actors you are working with concerning the same pest and disease problems.
ii. What kind of support have they given you?
iii. Which pest and/or disease would you prefer to have the greatest focus for interven-

tion?

Why?

**F) Participants gender (by observation)**

i. Male
ii. Female

**Appendix II:** Diagrammatic root knots scoring chart by Bridge and SamPage 1980

0-No knots on roots

1-Few small knots

2- Small knots but clearly visible.Main roots clean

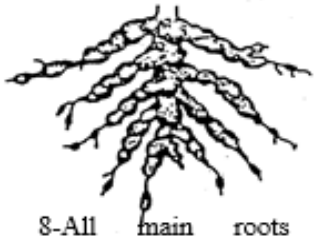
3-Some larger knots visible.Main roots clean

4-Larger knots predominate but main roots clean

5-50% of roots affected. Knotting on some main roots.Reduced rooting

6- Knotting on some main roots

7-Majority of main roots knotted



8-All main roots knotted including tap root. Few clean roots visible

9-All roots severely knotted.Plant usually dying.

10-All roots severely knotted. No root system. Plant usually dead