PREDICTION OF ROOT-KNOT NEMATODE INFESTATION USING SOIL CHARACTERISTICS IN TOMATO FIELDS IN MWEA, KIRINYAGA COUNTY, KENYA

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DECLARATION

DECLARATION BY CANDIDATE

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

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I dedicate this work to my wife Mary and daughters Peace and Harmony.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CRA	Commission on Revenue Allocation
DCCA	Detrended canonical correspondence analysis
EC	Electrical conductivity
FLN	Free living nematodes
GIS	Geographic information system
GPS	Global positioning system
IDRP	Irrigation and drainage research project
J2	Second stage juvenile
KARI	Kenya Agricultural Research Institute
KEPHIS	Kenya plant health inspectorate services
LN	Lesion nematodes
pH	Hydrogen ion concentration
PPN	Plant parasitic nematodes
RDA	Redundancy analysis
RKN	Root- knot nematodes
RPM	Rotations per minute
SSM	Site specific management

ABSTRACT

Tomato (*Solanum lycopersicum* L.) is a high value horticultural crop in Kenya. Nutritionally, the crop is rich in niacin, carotene, thiamine, and vitamin C. Mwea in Kirinyaga County is one of the major areas in Kenya where tomato is grown by smallholder farmers and is an important source of income. Tomato production in Kenya is hindered by losses due to diseases caused by pathogens that include plant parasitic nematodes. Among the plant parasitic nematodes, the root-knot nematode is the most predominant in Mwea. This study aimed at investigating the soil parameters that influence the distribution of the root-knot nematodes and map their distribution. This is important since it ensures that nematicide application is only to specific sites where these nematodes are found thereby saving on input costs as well as protecting the environment. The study consisted of surveys conducted in geo-referenced tomato production fields in seven tomato production sites in Tebere, Mwea. Soil sampling was done in a stratified random manner in both rainfed and irrigated tomato production fields in both dry and rainy seasons for determination of the spatial and temporal distribution of nematodes, respectively. Nematode extraction was done using the centrifugal floatation technique and identification done to genera level using morphological features. Soil parameters measured included soil pH, electrical conductivity, elevation and soil texture. Soil texture was determined using the hydrometer method. Detrended canonical correspondence analysis was performed to interpret and summarize major patterns of variation within the soil variable data and to estimate the ability of each soil variable to reflect variance in the entire plant parasitic nematode data set. The plant parasitic nematode incidence and diversity was determined using the Shannon-Weiner species diversity index. Fourteen genera of nematodes were identified with diversity indices ranging between 0.6 and 1.2. Root-knot nematode distribution differed significantly among different sites. Among the soil samples analyzed, 81% were positive for root-knot nematode infestation. There was no significant difference between nematode densities in the dry and rainy seasons. Rainfed fields exhibited significantly higher root-knot nematode population densities compared to irrigated fields. The study established a great variability in the soil parameters in the area. The root-knot nematode distribution pattern, density and abundance were inversely correlated with the soil pH and positively correlated with soil electrical conductivity. Based on the inverse relationship between soil pH and root-knot nematode distribution in the Mwea ecosystem, maps of nematode distribution and soil pH were developed. This enables the possibility of designing a site specific system for management of root-knot nematodes. It is recommended that farmers should avoid uniform application of nematicides in their production fields.

CHAPTER 1

1 INTRODUCTION

1.1 Background information

Agriculture is a major source of income for people living in rural areas and an important contributor to the Kenyan economy. Horticulture is ranked third as a source of foreign exchange (EPZA, 2005). Tomato (*Solanum lycopersicum,* Syn. *Lycopersicon esculentum* Mill*)* is a major cash crop and source of livelihood for many farmers in Central, Eastern, Rift Valley and Nyanza province (Wekesa, 2004) and is considered the second most important vegetable in Kenya.

Tomato farming is of great importance throughout the world. The crop is a top ranking vegetable in the East African region since it is a part of everyday diet of a significant part of the population. Tomato belongs to the family solanaceae and is an important dietary component contributing to improved nutrition and livelihood for both rural and urban populations in Kenya (Waiganjo *et al.*, 2006). It has a dietary value in respect to folate, vitamin C, vitamin E and potassium (Beecher, 1998).

Kirinyaga County is one of the major suppliers of tomatoes to urban consumers in Nairobi and other towns in Central Kenya (Waiganjo *et al.*, 2006). The major varieties grown in Mwea are Cal J, Rio Grande, and Africabest with the production regimes involving exclusive use of furrow irrigation and to a lesser extent relying on rainfall (Waiganjo *et al.*, 2006). The intensity of tomato growing in Mwea ranges from the small scale growers whose produce is consumed at the household level to those who sell a portion to the local urban markets. At another extreme are larger producers who

produce for large domestic or export markets (Foerster *et al.,* 2001). An estimated 75,101 tones of tomato valued at over KSh. 1 billion are produced in Central Province (KARI, 2005). The main areas of tomato production in Mwea are Riambogo, Gathigiriri, Ngurubani, Mbombaini, Ndindiruku, Kimbimbi, Kiumbu and Kiamanyeki.

Tomato cultivation has become a major source of household income and has created employment that has resulted in improved livelihoods (Kariuki *et al.,* 2010). It has been established that households without horticultural sales have considerably lower incomes or rely heavily on off-farm employment (Dijkstra and Magori, 1994).

Insect pests, nematodes and diseases (bacterial and fusarium wilt, early and late blight, leaf curl, tomato spotted wilt virus, leaf spot and powdery mildew) coupled with poor crop management are a threat to tomato production in Mwea. Most often, to manage plant diseases, farmers in Mwea use chemicals which pollute the environment. This is especially so for root-knot nematode (RKN) management where the application of nematicides is often uniform although it is known that RKN distribution is spatial. In a bid to reduce nematicide use and minimize environmental pollution brought about by blanket application of nematicides, a suitable soil-characteristic based site-specific system that ensures that the nematicide is applied in only areas infested with RKN needs to be developed, tested and validated.

1.2 Problem statement

Root-knot nematodes (RKN) are among the most damaging tomato pests, causing an estimated US $100 billion loss/year worldwide (Oka *et al.,* 2000). Estimates of tomato losses due to *Meloidogyne* spp. range from 24 to 38% (Infonet, 2014). Current

management strategies used to reduce losses due to nematode infestations include crop rotation and/or nematicide application. Application of nematicides is generally done uniformly across the entire field even though nematode populations are usually clumped and not uniformly distributed throughout fields. This field-wide uniform application of nematicides is due to lack of information on spatial and temporal nematode distribution and results in application to areas in a field where either RKN are not present, or nematode populations are below economic threshold levels. The results of this uniform nematicide application are potentially detrimental both economically and environmentally. To mitigate this, site-specific management (SSM) is required to enable a farmer know which part of his/her farm needs application of pesticides and the quantity required.

Costs for labour, time, and laboratory assays traditionally required to generate data necessary to develop management zones is prohibitive and in some cases, the samples do not accurately represent the spatial variability in pathogen distributions. Hence, alternative methods that rapidly and accurately show spatial and temporal distribution of plant parasitic nematodes (PPN) are needed. Recent advancements in precision farming technology make it possible to generate accurate, inexpensive geo-referenced, field-level soil textural maps with subsequent site-specific pesticide applications matching soil properties. Soil characteristics can be used in developing applications for site-specific pathogen management as it allows for rapid, detailed, and cost-effective spatial mapping of agricultural fields. This study is set out to identify soil characteristics that influence the abundance and distribution of RKN in tomato fields that can therefore be used in site-specific management (SSM). The

information obtained is essential for the development of a site-specific system for management of these nematodes.

1.3 Justification

There is a need to develop economically viable strategies of reducing losses caused by RKN. Since the cost of nematicides used to control RKN is relatively high and the nematodes are spatially aggregated. Any strategy that targets control measures to only those areas with an economically significant pathogen population will; 1) reduce pesticide usage, 2) be more environmentally acceptable, and 3) save farmers' money in the long run. Site specific management of the RKN is therefore desirable. Identification of soil characteristics which are related to RKN distribution that can be subsequently used for identification of those soils having varying densities of RKN is key and is expected to make SSM possible.

1.4 Research questions

i. Is there a significant diversity of PPN in Mwea?

ii. Do RKN population densities and abundance vary in spatial and temporal distribution in rainfed and irrigated tomato fields in Mwea?

iii. Are there soil characteristics that influence the RKN spatial and temporal distribution and density in rain-fed and irrigated tomato fields in Mwea?

1.5 Study hypothesis

i. The diversity of PPN is not significant among different sites.

ii. RKN densities and abundance do not vary significantly in spatial and temporal distribution between rain-fed and irrigated tomato fields in Mwea.

iii. There are soil characteristics that influence the RKN spatial and temporal distribution and density in rainfed and irrigated tomato fields in Mwea.

1.6 Objectives

1.6.1 General objective

To develop a soil-characteristic-based site-specific technology for rapid identification of soils in tomato fields infested by RKN.

1.6.2 Specific objectives

i. To assess the diversity, abundance and frequency of occurrence of plant parasitic nematodes in Mwea.

ii. To determine RKN spatial and temporal distribution in rain-fed and irrigated tomato fields in Mwea.

iii. To establish the relationship between soil characteristics influencing the spatial and temporal population dynamics of RKN in tomato fields in Mwea.

iv. To develop nematode-density-distribution maps for individual sampled site in Mwea.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Tomato production in Kenya

Tomato (*Solanum lycopersicum* L. [syn. *Lycopersicon esculentum* Mill.]) belongs to the family Solanacea and is one of the most widely grown vegetable food crops not only in Kenya but the whole world and is only second to potato (FAO, 2005). Although tomato production in Kenya satisfies the local demand, it is affected by seasonal scarcities (Masinde *et al.*, 2011). Tomato is traditionally marketed fresh and is the best selling vegetable crop (Borris and Brunke, 2005).

Tomato is grown in most parts of the country ranging from the Upper Humid zones to the Lower Midland zones (Jaetzold *et al.*, 2005). In Central Kenya, tomato is a vegetable crop of considerable economic importance (Waiganjo *et al.*, 2006). The fruit contains moderate quantities of vitamin C and E (Beecher, 1998).

2.2 Tomato production constraints

Tomato production faces a number of problems including diseases and pests (Maerere *et al.*, 2006). According to Bird and Kaloshian (2003), most of the crop damage is by a relatively small number of sedentary nematode genera which include root-knot (*Meloidogyne* spp.), cyst (*Globodera* and *Heterodera* spp.), and migratory nematodes (*Pratylenchus*, and *Radopholus* spp.). Root-knot nematodes (RKN) are the most damaging of these nematodes causing yield losses of up to 50% (Hollis, 1962) and therefore the need to mitigate their effects.

Several factors negatively affect tomato production and in the long run the cost of tomato production in Mwea. The major constraints being diseases (bacterial wilt, early and late blight, leaf curl, tomato spotted wilt virus, leaf spot, powdery mildew, insect pests and other arthropods (spider mites, thrips, white flies, African bollworm), nematodes, blossom end-rot and poor crop management especially lack of crop rotation practice (Waiganjo *et al.*, 2006).

Kavukulo *et al.* (2010) characterized several species of RKN in Mbeere district including Mwea. In Central Kenya, root-knot disease in tomato is caused by RKN particularly *M. incognita, M. javanica* and *M. hapla* which are the widely distributed nematode pests of tomato (Waiganjo *et al.*, 2006). Furthermore, RKNs can also exacerbate diseases such as vascular wilts (Gowen, 2002).

2.3 Root-knot nematodes (RKN)

Root-knot nematodes (RKNs) are parasites that must feed on roots in order to reproduce (Strand *et al.*, 1998). They are sedentary endoparasites and belong to a relatively small but important group of highly adapted obligate plant pathogens (Perry and Moens, 2006). These nematodes induce a feeding site in the tissues of its host plant in which its life cycle is strictly dependent on. They have a worldwide distribution and parasitize nearly every species of higher plants. Solanaceous crop plants, mainly tomato, may be infected at any age by the RKNs that cause root galling, stunted growth and low productivity.

The RKN second stage juvenile (J2) usually enters the plant through roots and modifies some root cells into feeding cells, a phenomenon necessary for the

reproduction and development of the parasite. The roots of susceptible plants, once penetrated by the RKN juveniles have their cambial root cells transformed into specialized feeding cells named "Giant cells". These are metabolically active cells induced and maintained in susceptible hosts by the feeding activities of the nematode (Hussey and Jansen, 2002) and are permanent feeding sites for the parasite throughout its life cycle (Hussey *et al.*, 1994).

The RKN, as its name implies, causes the development of root galls (Plate 1.) which interfere with plant water and nutrient movement as well as providing a nutrient rich food source (Strand *et al.*, 1998) which is rapidly colonized by fungi, thereby increasing wilt development and severity as a secondary infection (Noling, 1999).

Plate 1: A galled tomato root due to RKN infestation (Wendot, 2011)

2.4 Spatial and temporal distribution of nematodes

The spatial pattern of nematode distribution in a field is affected by the migratory behaviour, longevity, fecundity and oviposition behaviours of nematodes (Duncan and Phillips, 2009). In an agricultural or natural ecosystem, the spatial pattern of PPN has the horizontal distribution and the vertical distribution of the organism throughout the soil. Due to different aspects of population dynamics, active and passive redistribution and spread, both horizontal and vertical distribution of nematodes change with time (Been and Shomaker, 2006).

Climate affects nematode distribution on a geographical scale since most nematode life processes have thermic optima that also determine nematode distribution at a geographic scale (Luc *et al.,* 2005). Plants in infested areas within a field typically suffer 75–100% damage while other plants in the same field will show no symptoms. The earliest and greatest damage occur in plants under water stress (Robinson, 2008).

Cropped fields, when compared to natural areas support lower nematode diversity (Sanchez-Moreno *et al.,* 2006) hence plant feeding nematodes are generally inhibited by both tillage and intermittent fallow. The maintenance of agricultural fields under fallow conditions leads usually to a reduction in abundance of PPN (Cadet *et al.,* 2003) affecting the temporal distribution of nematodes.

Efficient dispersal causes repeated and widespread inoculations, and therefore results in practically an omnipresence of many nematode species in their potential distribution area. Polyphagy of most species and the weakness of interspecific competition allow the establishment of several species side by side and explain the

polyvalence of natural populations (Oostenbrink, 1960). The distribution ranges of RKN vary greatly throughout production fields and it is likely that certain environmental conditions favour one species over the other within localized sites.

2.5 Precision farming

In a bid to improve the economic and environmental sustainability of crop production, farmers should embrace precision farming. Precision farming employs detailed, site-specific information to precisely manage production inputs. For maximum economic production, farm inputs (seed, fertilizer, chemicals etc) should be applied only as needed and where needed for optimum output. Basically, precision farming matches inputs within a smaller area hence treating a field as a single unit. This system involves the use of right inputs at the right time and the right quantity applied in the right way (Roul, 2001). The idea behind this site specific management (SSM) is the understanding of the soil characteristics unique to a particular field. Correctly applied, precision agriculture has the potential to fundamentally alter decision making in farming (Srinivasan, 2006) and to simultaneously achieve the multiple objectives of enhancing input use, efficiency, reducing environmental pollution, increasing farm profits and product quality.

Many nematode genera and species have particular soil and climatic requirements (Guerena, 2006). Further, various edaphic factors affect the population density, dynamics and distribution of nematodes (Mcsorley, 1998). Nematode communities are also impacted by the soil bulk densities and moisture content, various nutrients, elements in the soil and soil texture - the relative amount of sand-silt-clay.

The cotton yield suppression by *M. incognita* juveniles has been observed to increase as the percentage of sand in the soil increased (Monfort *et al.,* 2007). Consequently, there also occurs an increase in crop damage with increases in RKN population density in relation to soil textural differences hence the viability of SSM of these PPNs. Coupled with this, PPN species exhibit spatially variable population densities that provide an opportunity to practice site-specific fumigation in a bid to reduce chemical usage and production costs (King and Taberna, 2009).

The diverse range of microclimates in tomato production sites, the varying tomato production systems (rainfed and irrigated) and RKN management strategies applied by producers provide a good test measure for the ability to manage RKNs site specifically. Horizontal, vertical distribution and density of nematodes can be influenced by soil texture which has been suggested as a useful predictor of nematode densities and distributions that may be of value in predicting the economic damage potential (Georgis and Poinar, 1983; Noer and Barker, 1985). For instance, the population density of *M. incognita* is reported to be negatively correlated with the clay or silt content in the soil (Windham and Barker, 1986; Queneherve, 1988; Koenning *et al*., 1996). It was observed that damage to cotton was greater in coarse than in fine-textured soils (Monfort *et al.,* 2007). Koenning *et al* (2004) suggested that this was likely because nematode reproduction was suppressed at higher silt or clay contents.

2.6 Effect of soil parameters on nematode population density

The physical aspect of an environment, and in particular the soil environment, provides such a complicated situation that a practicable solution to even a precisely

defined ecological question may be impossible using current scientific methods (Smiles, 2001). The soil system is composed of a comparatively stable matrix of solid material with individual particles ranging in size from a few nanometres to several millimetres. The particles in this system are clustered in aggregates and the voids within and between the aggregates contain air and aqueous solutions. The water content constantly changes, the total volume of the system may change, and the degree and type of aggregation may change within season and management.

The soil ecosystem is a result of several intricate interactions between a physical and chemical matrix of highly variable composition and living communities composed of essentially all forms of life (Tate, 2000). The relationship between soil physical and chemical properties and RKN population densities can be considered direct or indirect. Cadet *et al.* (2004) showed that repellence was exhibited by *M. javanica* and *M. incognita* towards some mineral salts (NaCl, $NaNO_3$, KCl, KNO_3, $CaCl_2$, Ca $(NO_3)_2$, $MgCl_2$, $MgSO_4$, $FeCl_2$ and $FeSO_4$).

The presence of a host plant is crucial for the presence of a particular PPN. Furthermore, the survival and reproduction of PPNs is determined by the physical and chemical characteristics of the soil environment (Norton, 1979; Castro *et al.,* 1990). As such, there are soils or regions within the soils that are unsuitable for particular nematode species and which, thus, contain fewer individuals or different nematode communities than more favourable environments (Cadet and Thioulouse, 1998). Soil texture, temperature, moisture, organic matter, pH, plant susceptibility and microorganisms are some of the ecological factors that affect nematode density and distribution in the soil (Norton, 1989).

Nematodes depend on the conditions of the soil and are comparable to an agronomic factor, such as organic matter. Therefore, their management is achievable at the field scale as the agronomic factors (Yeates, 1984). Popovici and Ciobanu (2000) noted that the variations in the composition of nematode communities in grasslands of Romania could be explained by relevant environmental variables such as soil pH, total Nitrogen, humus content, exchangeable bases and soil type but no single factor could be selected as being of overriding importance.

Nematodes are functionally diverse and ubiquitous and respond readily to environmental changes (Sanchez-Moreno *et al.,* 2006) and there is a tight relationship between soil characteristics and abundance of nematodes in different functional guilds that are used to develop soil assessment criteria. In both natural and experimental conditions, nematode assemblages are used to assess the effects of pollution (Korthals *et al.,* 1996), and they are further used as indicators of enrichment and disturbance (Bongers, 1990).

The relative importance of abiotic versus biological processes in regulating community structure appears to change dramatically between communities along environmental gradients (Schlosser, 1987). Screening patterns of species abundance or distribution and determination of the factors responsible for these patterns is the common goal in studies of community ecology (Hinch *et al.,* 1991). Many of the patterns in species presence or absence have been shown to be carried by biogeographic variation and physical or chemical gradients related to anthropogenic pressures (Tonn and Magnuson, 1982; Rahel, 1983).

Mornfort *et al.* (2007) noted that crop damage increased with increases in RKN population density, and the damage potential changed in relation to soil textural differences. RKN are usually suppressed in clay soils, with few exceptions, among which, is *M. artiellia* (Greco and Vito, 2009) but are typically found in sandy or sandy loam soils. Other factors such as pH and electrical conductivity may affect build-up of nematode population densities but, generally if they allow satisfactory plant growth, they will not suppress populations of nematodes significantly (Perry and Moens, 2006).

In order to better understand and manage ecosystems, it is important to study the relationship between environmental factors and organisms existing therein. Among different environmental factors, soil is of relatively high importance in species occurrence. It is a function of climate, organisms, topography, parent material and time (Hoveizeh, 1997). Zalewski and Naiman (1985) considered that abiotic factors are of primary importance in many situations, but when the environment becomes stable and predictable, the role of biotic factors gradually increases.

2.6.1 Significance of soil texture

Texture is one of the most important of the soil characteristics as it influences many other properties of great significance to land use and management (Brown, 2003). Nematode survival, emergence and disease severity are influenced by soil texture which is directly related to water holding capacity and aeration. This also affects nematode movement within fields e.g. nematode juveniles in sandy soils are able to move horizontally and vertically over distances of up to 75cm in 9 days (Prot, 1977). It was however found that migration decreased with increasing clay content of the

soil, with no migration in soils with more than 30% clay (Prot and Van Gundy, 1981). The changes in particle-size distribution and structural change (size, shape and arrangement of the soil aggregates and voids) affect the physical behaviour of the soil and hence its exploration by soil organisms.

2.6.2 Significance of soil pH

Knowing the soil pH makes it possible to choose to grow crops e.g. plants with neutrophilic cells or acidophilic plants and also provides useful information enabling the right choice of corrective measures like application of fertilizers or amendment to increase or decrease the pH and fertility of cultivated soils (Pansu and Gautheyrou, 2003).

Plant parasitic nematodes also have different soil pH preferences. For instance, *Trichodorus similis* which shows a high tolerance to low acid soils (pH 5–6). The soil pH is typically buffered by ammonium, carbonates, sulphates and phosphates often bound to or comprising soil particles (Perry and Moens, 2006). Therefore, plant roots and soil microflora change their concentrations and end up varying markedly with depth and distance from roots.

2.6.3 Significance of soil electrical conductivity

A strong positive correlation between soil chemical properties and soil electrical conductivity (EC) has been observed and therefore, low levels of soil chemical properties can be related to low EC. Previous studies have shown that low EC has been associated with coarse textured soil at the coastal plain of Georgia (Perry *et al.*, 2007). The RKN population density increases in areas of coarse textured soils and low

EC. Electrical conductivity is an unpredictable and slightly structured variable, with a strong variability even at very short distances. Therefore, a high density of sampling is required to predict EC.

2.7 Site specific management of root-knot nematodes

The management of RKN has for a long time been achieved by the use of nematicides. Although crop rotation and other cultural practices are used to some extent by farmers, these practices have not been exhausted to their outmost potential. With the identification of specific areas within individual fields with varying nematode densities, farmers are able to target these areas for nematode management, particularly using nematicides.

In regard to soils, every field is unique (Overstreet *et al.*, 2008). Therefore, the potential for site-specific nematicide application may become more widely adopted with understanding of the relationship among nematode populations, soil texture, pH and electrical conductivity. Nematodes are not uniformly distributed within fields, and there may be substantial acreage in most fields where nematodes either are not present, or are below the economic threshold levels (Khalilian *et al.*, 2001). The spatially aggregated nature of nematodes, the relatively high cost of fumigant nematicides, the fact that some growers use multiple types of nematicides on the same crop and the relatively high crop value makes site specific fumigation appealing from an economic stand point (King and Taberna, 2009).

Site-specific application of nematicides, as well as other inputs is made possible by precision farming technology. Unfortunately, for SSM to succeed an affordable

nematode distribution map within a field should be available as the basis for making management decisions (Wyse-Pester *et al.,* 2002).

To optimally develop a SSM plan, information concerning the spatial distribution of various soil physical and chemical properties within a field is needed along with correlated plant yield relations (Rhoades *et al.*, 1999). These are required to apply management that accounts for the variability in disease prevalence, crop and yield differences that exist within a field. Precision farming therefore uses information technologies to segment a field into smaller units and determine each unit's individual characteristics. Production inputs are then applied in the precise locations at quantities that are needed for optimum yield.

2.8 Mapping nematode distribution

When a Global Positioning System (GPS) is combined with Geographic Information System (GIS), precision farming is possible (Hanna and Culpeppe, 1998). Site-specific farming allows farmers to monitor pest and disease prevalence in their farms and manage them in a completely controlled manner. In dealing with crop pests and diseases which are major hindrances to agriculture mainly in the developing world, site specific farming becomes a valuable tool which aids in determining susceptibilities within units and therefore the best measures to be applied in dealing with the crop diseases in the field.

In a bid to optimize inputs, reduce wastes and generate the maximum possible yields, geographic technology, which involves the use of GPS, remote sensing for data collection and GIS, which is now used generically for any computer-based capability

in the manipulation of geographical data for data processing and analysis is utilized (Bernhadsen, 2002).

Geographic information systems, being able to store relationships between features, in addition to feature locations and attributes, is one of the most important sources of power and flexibility of this technology. Furthermore, stored data may be processed in a GIS for presentation in the form of maps, tables, or special formats. With the ability to map locations using GIS, one is able to find places that have certain features and identify where to apply mitigation measures. Emergence of patterns is observed following a careful look at the distribution of features on maps. Furthermore, site-specific application of nematicides may become more widely adopted as the understanding of these relationships among nematode populations, soil texture, and yield response becomes clearer (Overstreet *et al.*, 2008).

CHAPTER 3

3 MATERIALS AND METHODS

3.1 The study area

The study was conducted in Mwea, Kirinyaga County (Fig. 1). Mwea, which covers about 1437 $km^{2,}$ is one of the largest tomato producing areas in Central Kenya. The area is about 100 km North East of Nairobi, located at latitude -1.7333 and longitude 37.4833. The average elevation is 1368 meters above sea level (Koening *et al.,* 2008) with an annual rainfall of 800-900 mm. The climate in Mwea enables two short cropping seasons (Koening *et al.,* 2008) but since irrigation water is readily available, farmers are currently able to produce crops throughout the year (pers. Obs.).

3.2 Soil sampling

Tomato production sites were identified by making a reconnaissance visit to the area. The soil was sampled in a stratified random manner (Yates and Finney, 1942) in order to obtain a representative sample (Kent and Coker, 1992). The number of samples obtained per site was determined by the four factors; soil types where determination of the soil types was taken into account. However, since the existing soil type in the area was found to be vertisols which was uniform across the sites, season, production system and the number of tomato fields in individual sites took precedence, with a minimum of 10 samples per site. Sampling was done in both irrigated and rainfed farms in both dry and rainy seasons between May and December 2011.

Soil sampling was done by the procedure adopted from Dropkin (1980). A soil auger was used to extract soil from depths of 5-30 cm after scrapping off the top 5 cm soil. Soil sub-samples taken from each plot were mixed and a composite sample of 1 kg

drawn. The composite sample was used for determination of RKN population densities, soil electrical conductivity, soil pH, and texture.

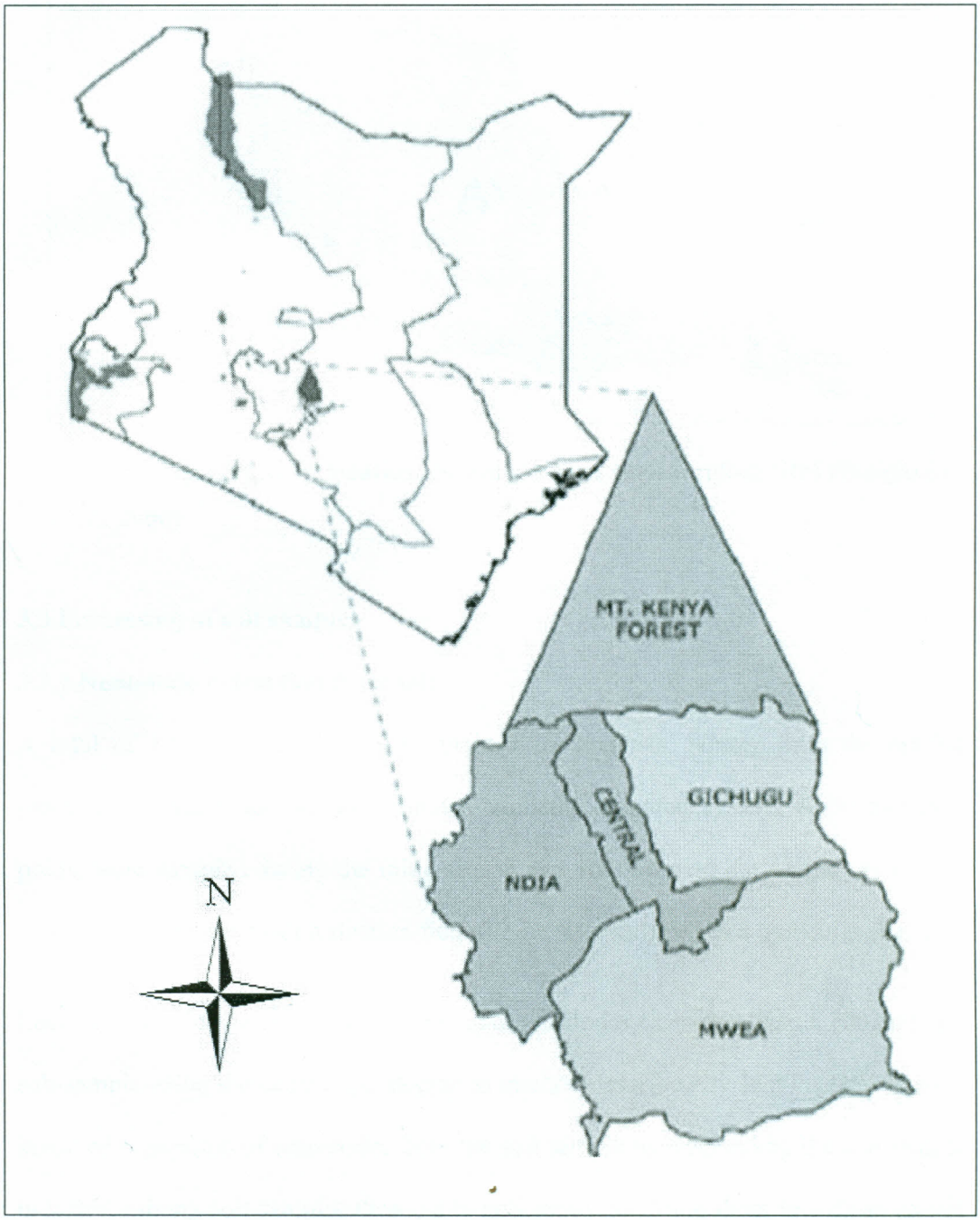


Figure 1: Map of Kenya showing the location of Kirinyaga County and Mwea division (Waiganjo *et al.*, 2006).

Plate 2: Map of Tebere location, Mwea showing the sampling sites (Waiganjo *et al.*, 2006)

3.3 Processing of soil samples

3.3.1 Nematode extraction from soil

A total of 170 soil samples were obtained for analysis. Ninety from the rainfed production system and 80 soil samples from the irrigated system, Sixty one (61) points were sampled during the rainy season and 109 sampled during the dry season guided by the number of production fields in the sites sampled in a particular season.

Each soil sample was mixed thoroughly and nematodes extracted from a 200 cm^3 soil sub-sample using the centrifugal-floatation method described by Jenkins (1964). This involved separation of nematodes from the soil sample by suspending the soil sample in water, mixing soil samples thoroughly and gently tumbling them in a clean plastic bucket. 500 ml of water was then poured in the plastic bucket followed by gently mixing of the soil with tap water, stirring by hand to free nematodes from soil and re-suspending them in 5 litres of water. The mixture was left to settle for 1 min and the

muddy water was decanted through a 2mm sieve into a second bucket to eliminate trash and heavy soil particles. The nematodes in the second bucket were re-suspended by stirring the water. The bucket was left to stand for about 10 s and the contents were poured through a 500μm, 125μm, 90μm sieve and nematodes collected on a 45μm and 38μm sieves by backwashing the sieve and collecting the mixture into a 250-ml beaker and concentrating to 50-60 ml in a 100 ml beaker. The contents were poured equally into four 15-ml centrifuge tubes. Each tube was filled to within 0.5 cm of the top with fresh water and centrifuged for 7 min at 1750 rpm. This concentrated nematodes and soil particles in a pellet at the bottom of the tube. The supernatant was decanted and the tubes were refilled halfway to the top with sucrose solution (450g/l water) having a specific gravity of 1.18. Nematodes were then re-suspended in the sucrose solution by thoroughly mixing with a stirring rod and the centrifuge loaded followed by centrifugation for 3 min at 1750 rpm. The nematodes were then collected by pouring the supernatant through a 38μm sieve and backwashing into a 100 ml beaker.

3.3.2 Determination of nematode densities

The nematode-water suspension for each sample was concentrated to equal volumes of 5 ml. The nematode suspension was stirred by blowing air through it using a pipette for homogeneity and a 1ml aliquot pipetted into a counting dish mounted on an inverted stereomicroscope and the number of nematodes counted. Subsequently, nematode identification was done using the morphological features. Four counts were made for each sample and the mean counts were then calculated. The total number of nematodes in the suspension which translated to the nematode density in the soil sample was also calculated.

3.3.3 Determination of textural class

The soil particle size distribution for each soil sample was determined at the National Agricultural Research Laboratories (NARL) Soil Physics Laboratory using the physical analytical tests. Three fractions (sand, silt and clay) of soil were determined for each sample using the hydrometer method (Bouyoucos, 1962).

Fifty (50) grams of the soil was placed in a 500 ml plastic shaking bottle and 50 ml of calgon (45 g sodium hexametaphosphate, 5 g sodium carbonate in 1 litre of water), the dispersing agent and 300 ml tap water added then corked and shaken overnight using a mechanical shaker. Hydrometer readings were taken 10 seconds after plunging the soil-water mixture in a 1000 ml sedimentation cylinder and the second reading taken 2 hrs later. The first reading was used to calculate the percentage of sand particles in the soil as per equation 1 and the second hydrometer reading was used to calculate the percentage clay content in the soil as per equation 2. The difference of which gave the percentage silt content as illustrated in equation 3.

$$\% \text{ Sand} = 100 - ((R_1 \div 50) \times 100) \quad \text{(Equation 1)}$$

Where R_1 = First reading

$$\% \text{ Clay} = (R_2 \div 50) \times 100 \quad \text{(Equation 2)}$$

Where R_2 = Second reading

$$\% \text{ Silt} = 100 - (\% \text{ sand} + \% \text{ Clay}) \quad \text{(Equation 3)}$$

Soil textural class was determined using United States Department of Agriculture (USDA) textural triangle (Figure 2) which specifies the textural category by the quantities of the different soil particle separates it contains (Soil Survey Staff, 1975).

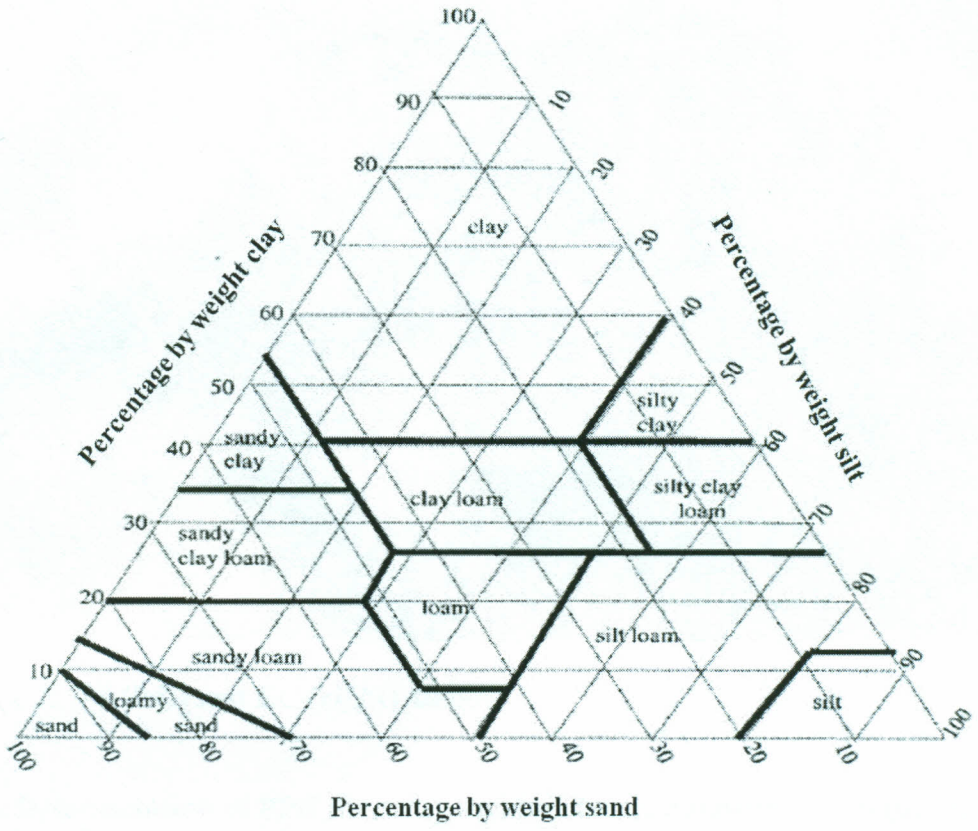


Figure 2: The USDA soil textural triangle (Soil Survey staff, 1975)

3.3.4 Determination of soil pH and electrical conductivity

Soil pH for each soil sample in a 1:2.5 soil water suspension was determined using a calibrated Fieldscout pH meter (Spectrum technologies, Inc). The soil Electrical conductivity (EC) for the same soil sample was subsequently determined using a calibrated Fieldscout EC meter (Spectrum technologies, Inc) (Plate 3) in a saturated soil paste.

Plate 3: pH (left) and EC (right) meters

3.4. Determination of PPN diversity and incidence, abundance and the spatial and temporal distribution of RKN

Surveys were conducted in several geo-referenced points with different soil types and production regimes in tomato production fields which included Kiumbu, Gathigiriri, Mbombaini, Riambogo, Kiamanyeki, Ndindiruku and Ngurubani (Plate 2). They were considered to be 'problem fields' by the concerned tomato producers based on stunted tomato plants and declining yields.

Soil samples were taken from both irrigated and rainfed tomato production fields during dry (June-September) and rainy seasons (October-December) for determination of the spatial and temporal distribution of nematodes, respectively. Soil samples were processed as described in section 3.3. The nematode-water suspension for each sample was concentrated to equal volumes of 5ml. The nematode suspension was stirred by blowing air through it using a pipette for homogeneity and a 1ml

aliquot pipetted into a counting dish mounted on an inverted stereomicroscope and counted.

Plant parasitic nematode diversity was calculated using the Shannon-Weiner species diversity index (Kent and Coker, 2001). Nematode incidence was assessed by the formula;

$$\{(\text{Number of samples with RKN} \div \text{total number of samples}) \times 100\}$$

For determination of nematode abundance, four counts were done for each sample and the mean counts were then calculated. The total numbers of nematodes in the suspension were translated into the nematode density in the 200 cm^3 soil sample.

3.5 Determination of RKN spatial and temporal distribution in rain-fed and irrigated tomato fields in Mwea

To assess the RKN spatial and temporal distribution in Mwea, soil sampling was done in various sampling units from both rain-fed and irrigated tomato fields as outlined in section 3.2. The geographic locations of the sampling plots were determined during sampling using a Garmin global positioning system receiver (Garmin, inc., Olathe, Kansas, USA). Nematode densities were obtained from these soil samples as outlined in section 3.3.2 and soil physicochemical properties of each sampling unit were obtained as described in section 3.3.3 and 3.3.4.

3.6 Determination of the relationship between RKN and soil characteristics and establishing factors influencing RKN spatial and temporal population dynamics

Data from the experiments described above was used to identify the characteristics influencing RKN spatial and temporal population dynamics. The relationships between the soil pH, elevation, texture and/or electrical conductivity and nematode densities were established using a Detrended canonical correspondence analysis (DCCA) where soil characteristic data were interpolated with corresponding nematode densities. These established the extent to which each soil characteristic relates to the distribution of different nematode genera.

3.7 Development of nematode-density-distribution maps for individual site

The tomato fields were divided into geo-referenced blocks using a differential global positioning system (GPS) receiver. The data from the blocks were examined in a homogeneous scale based on their geographic location within the field. The soil pH was used to categorize fields.

Mapping of soil pH and nematode distribution density was done using arc-GIS 10 ArcMap software (Esri, Redlands, CA, USA) and quantum GIS (QGIS) software using soil pH and RKN abundance data.

3.8 Statistical analysis

A combination of univariate and multivariate methods was used to analyze data. Description of environmental factors' effects at community level was achieved using a multivariate method proposed by Van den Brink *et al.* (1984) based on redundancy

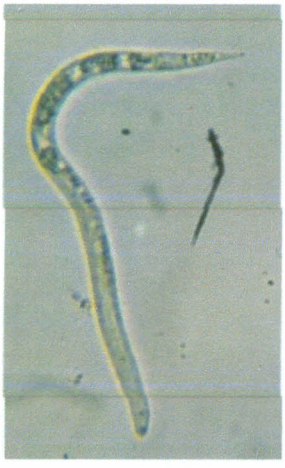
analysis (RDA). These multivariate methods summarized all information on the investigated populations simultaneously. Differences between groups of sites and within groups of sites (*i.e* all rainfed *vs.* irrigated plots and rainy season *vs.* dry season samples) and total densities were analyzed by a one way ANOVA in STATISTICA 6.0 (Statsoft, Inc.).

CHAPTER 4

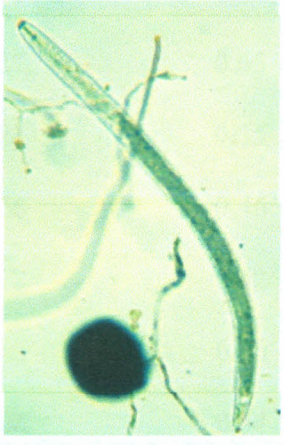
4 RESULTS

4.1 Plant parasitic nematode diversity in Mwea tomato production fields

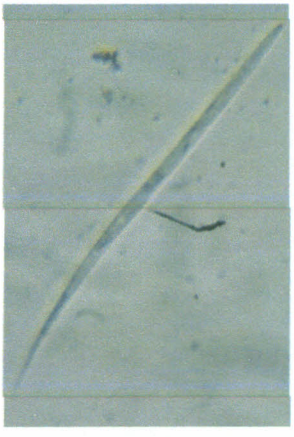
Fourteen (14) genera of plant parasitic nematodes were observed in this study. These included *Meloidogyne, Pratylenchus, Helicotylenchus, Ditylenchus, Tylenchorynchus, Tylenchulus, Criconema, Hirshmaniella, Rotylenchus, Paratylenchus, Trichodorus, Paratrichodorus, Hoplolaimus* and *Tylenchus* spp. (plate 4.1). Their range of diversity on the Shannon-Weinner diversity index was between 0.6 and 1.2 across the 7 sites surveyed (Table 4.1).



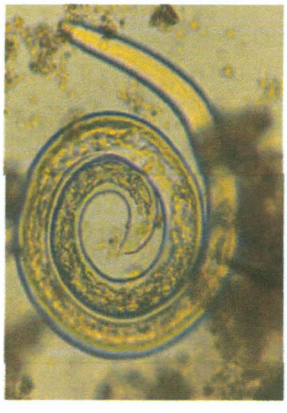
Meloidogyne spp.



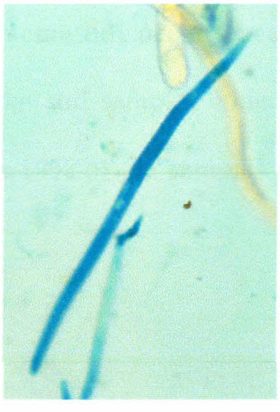
Pratylenchus spp.



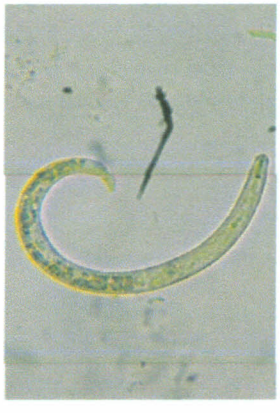
Tylenchus spp.



Helicotylenchus spp.



Ditylenchus spp.



Rotylenchus spp.

Plate 4.1. Major plant parasitic nematodes observed in Mwea (Wendot, 2011)

Riambogo and Kiumbu had indices of 0.69 and 0.82, respectively. All the other five sites had indices above 1. Three sites; Mbombaini, Ndindiruku and Kiumbu had an equal number of PPN genera but their diversity varied among the sites (Table 4.1).

Species evenness ranged between 0.3 and 0.6 among most of the sites except for Riambogo where an evenness of 1 was observed. Gathigiriri, Mbombaini and Ngurubani which are rainfed sites had close levels of species evenness (Table 4.1).

Table 4.1 Nematode diversity and evenness in Mwea

Site	H'	J	No. of genera
Riambogo	0.69	1.00	2
Kiamanyeki	1.27	0.65	7
Gathigiriri	1.21	0.55	9
Mbombaini	1.06	0.46	10
Ndindiruku	1.22	0.52	10
Kiumbu	0.82	0.36	10
Ngurubani	1.06	0.54	7

Key; H'- Species diversity index, J- Species evenness

4.2 Incidence of root-knot nematode occurrence in samples

From the survey, 81% of the soil samples collected in Mwea were observed to be infested with RKN whereas 19% of the samples collected were not infested (Figure 4.1) with RKN.

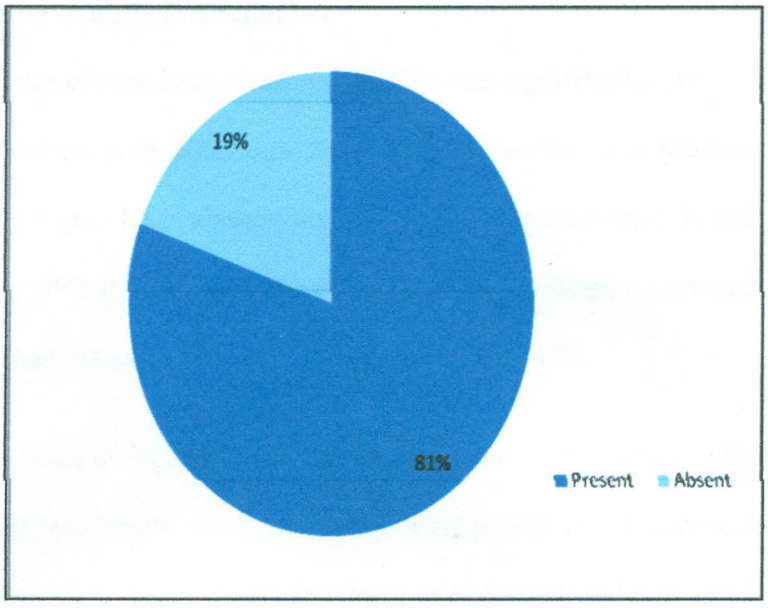


Figure 4.1: Root-knot nematode incidence in Mwea

Within each site, the incidence of nematode species infestation varied widely. Ngurubani and Ndindiruku had significantly high root-knot nematode infestation incidence. Gathigiriri, Mbombaini, Kiamanyeki and Kiumbu had incomparable levels of RKN infestation (Fig. 4.2). Moreover, all sites exhibited relatively high incidence of RKN infestation with over 50% of the samples collected in all the sites being infested (Fig. 4.2).

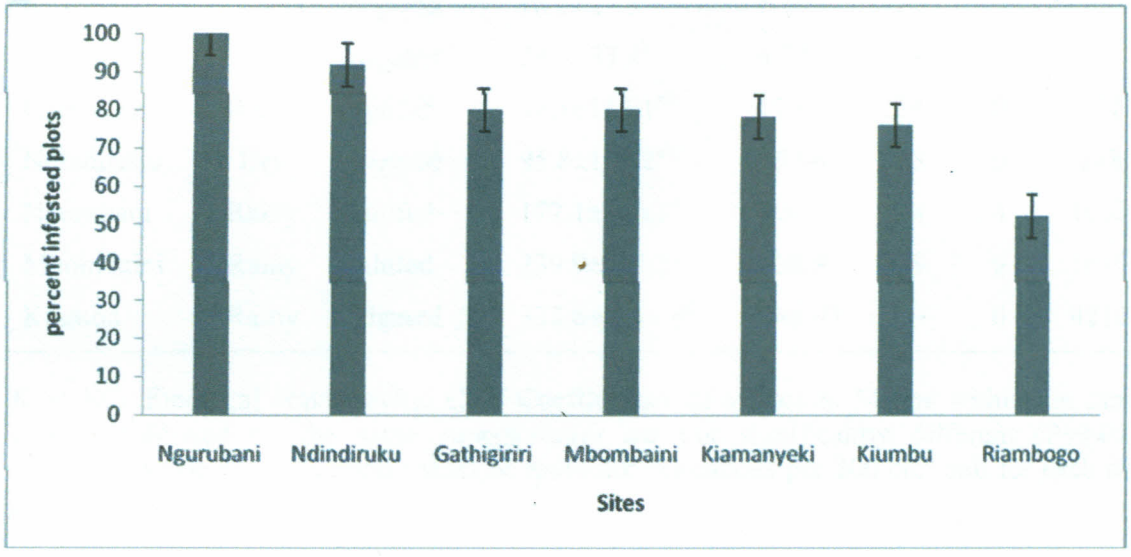


Figure 4.2: Root-knot nematode infestation incidence in different sites

4.3 Root-knot nematode abundance

The abundance of root-knot nematodes (RKN) was significantly ($P<0.05$) different among the tomato production sites. Ngurubani, Mbombaini and Kiumbu had significantly higher RKN abundances compared to the other sites. Riambogo had significantly ($P<0.05$) RKN abundance as compared to Kiamanyeki and Gathigiriri which also had incomparable RKN abundance (Table 4.2).

Spatial distribution of RKN among the sites was significantly different ($P<0.05$) ranging over two orders of magnitude for most groups. For example, populations of RKN in Riambogo and Kiamanyeki ranged from 0 to 60 and 0 to 140 individuals per 200 cm^3 soil, respectively while populations of RKN in Mbombaini and Kiumbu ranged from 0 to 1630 and 0 to 4214 individuals per 200 cm^3, respectively (Table 4.2).

Table 4.2: Root-knot nematode abundance across the tomato production sites in Mwea.

Site	Season	Production System	Abundance Mean±Std. Dev RKN/200cm^3 soil	Std. Error	C.V (%)	Min. value	Max. value
Riambogo	Dry	Irrigated	18.5±22.3^{a}	6.19	121	0	60
Kiamanyeki	Dry	Irrigated	29.3±33.4^{b}	4.72	114	0	140
Gathigiriri	Rainy	Rainfed	41.3±113.1^{ab}	17.67	274	0	574
Ndindiruku	Dry	Irrigated	95.8±194.2^{abc}	36.06	203	0	888
Ngurubani	Rainy	Rainfed	172.1±316.5^{c}	100.1	184	4	1053
Mbombaini	Rainy	Rainfed	239.9±502.5^{c}	158.9	209	0	1630
Kiumbu	Rainy	Irrigated	338.8±1014.6^{c}	246.07	299	0	4214

Key: EC- Electrical conductivity, C.V- Coefficiency of variation. Means within the same column followed by the same superscript(s) are not significantly different ($P<0.05$). Minimum value and maximum values of root-knot nematodes per 200 cm^3 soil for each site are also shown

Root-knot nematode spatial distribution varied significantly ($P<0.05$) among the seven (7) sites. Kiumbu, an irrigated site sampled in the rainy season exhibited a significantly higher average RKN density (339 RKN/200 cm^3 of soil) while in Gathigiriri sampled during the same season had a significantly ($P<0.05$) lower RKN abundance (41 RKN/200 cm^3 of soil) (Table 4.2).

4.4 Spatial and temporal distribution of RKN in rainfed and irrigated tomato fields

The RKN densities in Ngurubani and Mbombaini were not significantly different ($P>0.05$) yet these sites were under the rainfed system of production. However, the two sites had significantly higher RKN abundances compared to Gathigiriri which was under the same system of production (rainfed) but had a significantly lower RKN abundance of 41 RKN in 200 cm^3 of soil (Table 4.2).

There was a significant difference ($p<0.05$) in RKN densities between the rain fed tomato fields and the irrigated fields (Fig. 4.3) with higher RKN densities observed in the rainfed system compared to the irrigated system.

Overall, no significant ($P>0.05$) differences between the dry season and rainy season RKN population densities were observed (Fig 4.4) although RKN densities in the dry season were insignificantly ($P>0.05$) lower compared to the rainy season.

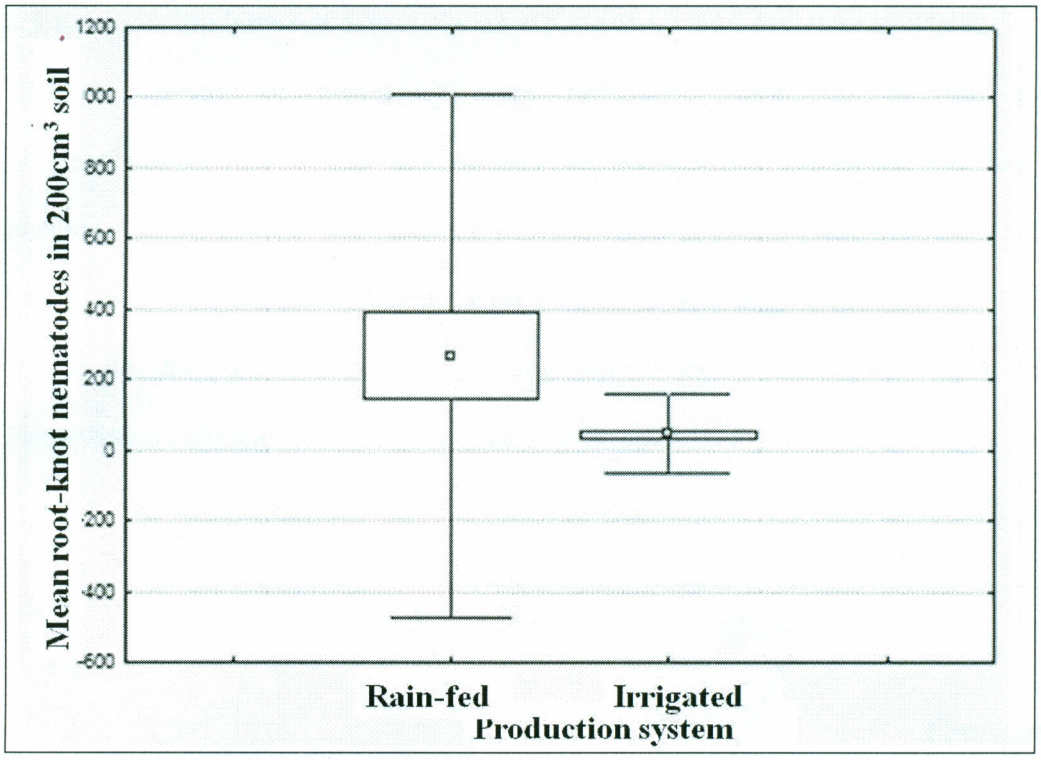


Figure 4.3: Mean density of RKN between rain-fed and irrigated systems

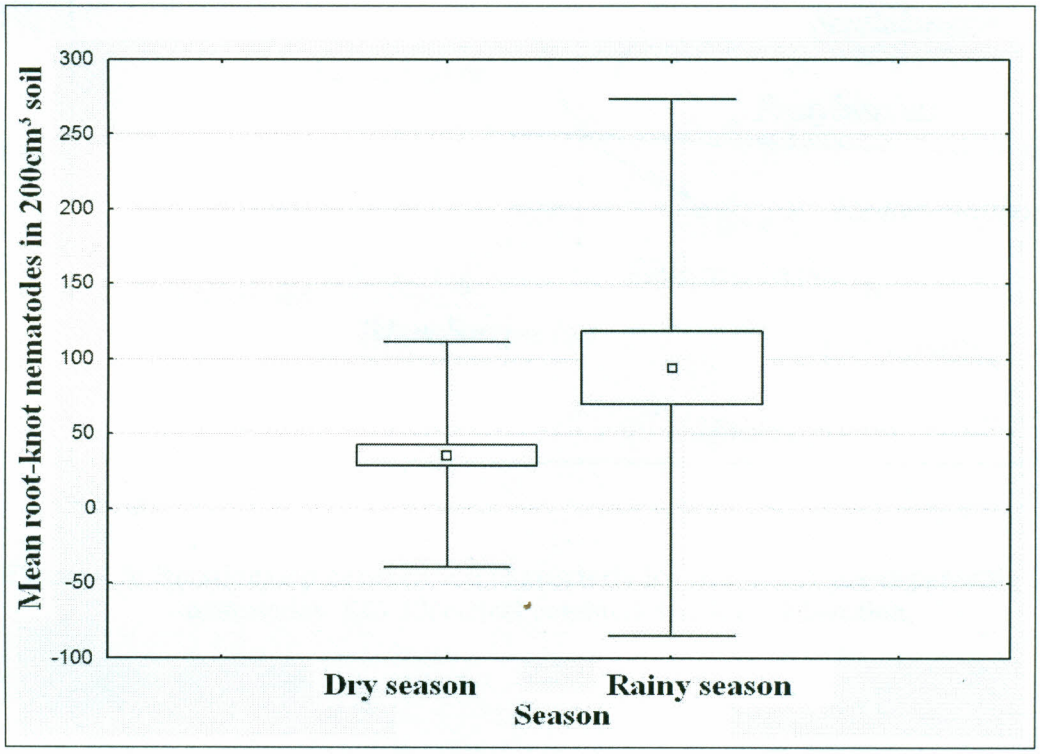


Figure 4.4: Mean RKN densities between dry and rainy seasons

4.5 Relationship between nematode infestation levels and soil characteristics

Different genera of nematodes were influenced differently by the soil physicochemical factors (Fig 4.5). *Aphelenchoides* spp. and *Criconema* spp. had a positive correlation with elevation while *Heterodera* spp. and *Helicotylenchus* spp. had a positive correlation with soil texture (Fig 4.5). *Meloidogyne* spp. was inversely related to the soil pH whereas Lesion nematodes (*Pratylenchus* spp.) and *Tylenchorrynchus* spp. were directly related to the soil pH (Fig 4.5).

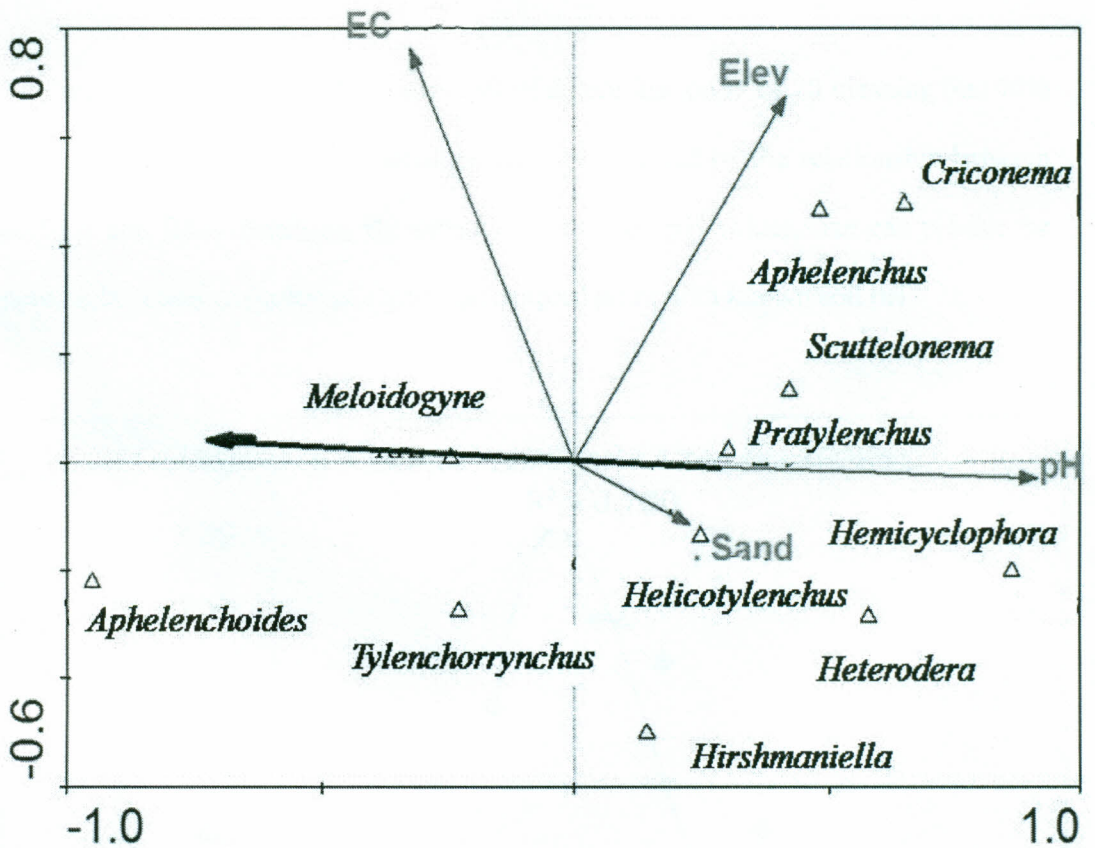


Figure 4.5: Relationship between soil characteristics and major plant-parasitic nematodes. EC- Electrical conductivity, Elev- Elevation,

The relationship existing between the RKN and soil pH is described by the regression model:

$$y=147.1x^3-2786x^2-17453x-36085,\ R^2=0.700,\ (Fig.\ 4.6)$$

Where: y is the expected RKN density

x is the known soil pH value

R^2 = Coefficient of determination

The regression model had the coefficient of determination r^2 of 70 meaning that 70% of the total variation in RKN densities can be explained by the relationship between soil pH and RKN densities. By substituting the soil pH values, one can predict the nematode counts expected at a given unsampled point with known soil pH.

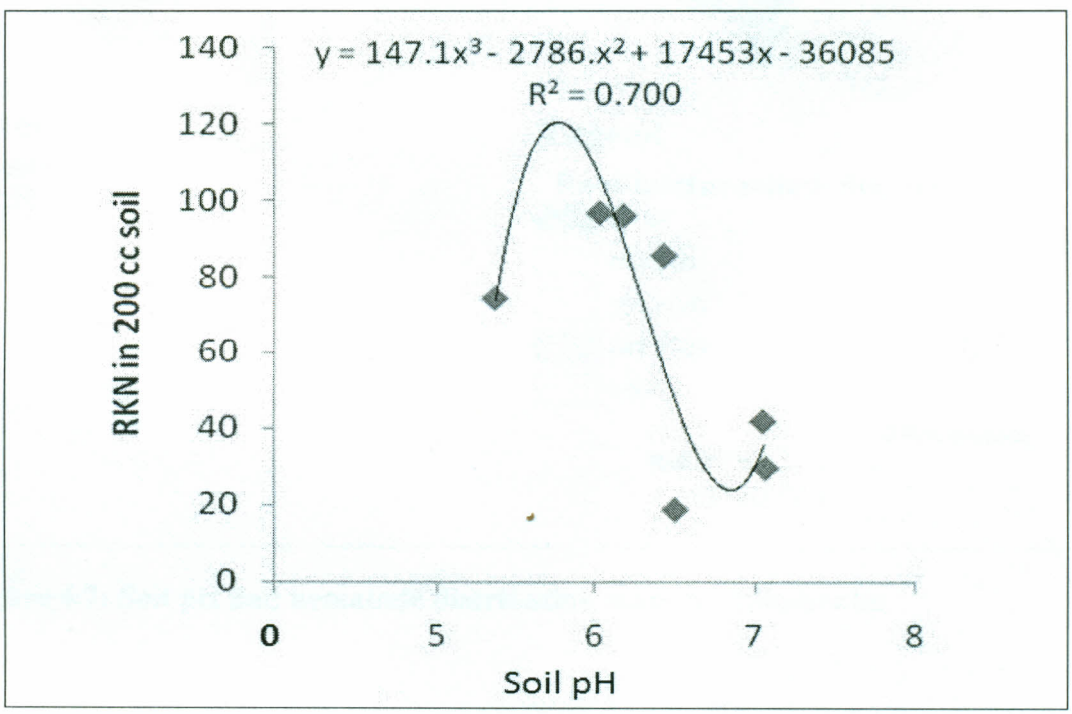


Figure 4.6: Relationship between soil pH and RKN densities in Mwea

4.6 Nematode-density-distribution maps for individual sampled sites in Mwea.

Following the establishment of a strong inverse relationship between RKN densities and soil pH, soil pH and RKN population density distribution maps were created for the tomato production sites in Mwea (Fig 4.7 – fig. 4.13). Although the soil electrical conductivity had a positive correlation with RKN population density, it could not be used because it is an unpredictable and slightly structured variable, with a strong variability even at very short distances requiring a high density of sampling to predict it.

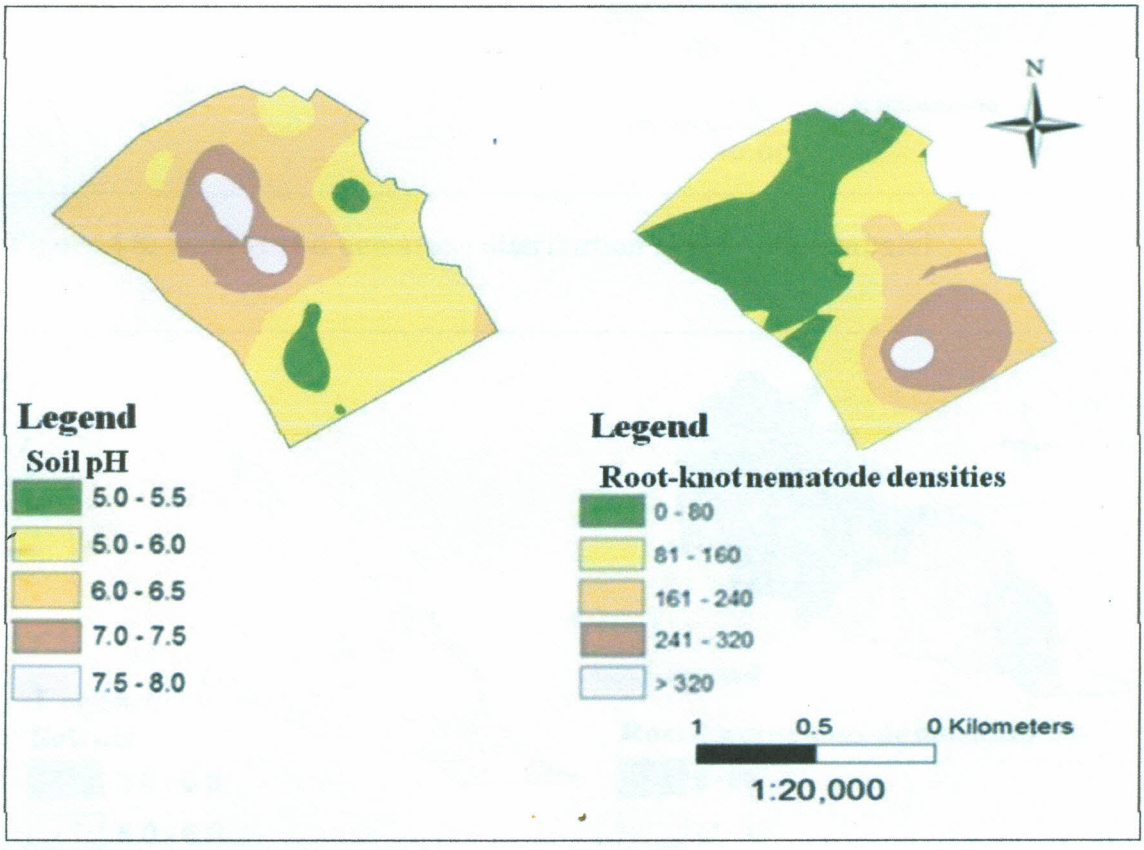


Figure 4.7: Soil pH and nematode distribution maps of Ndindiruku

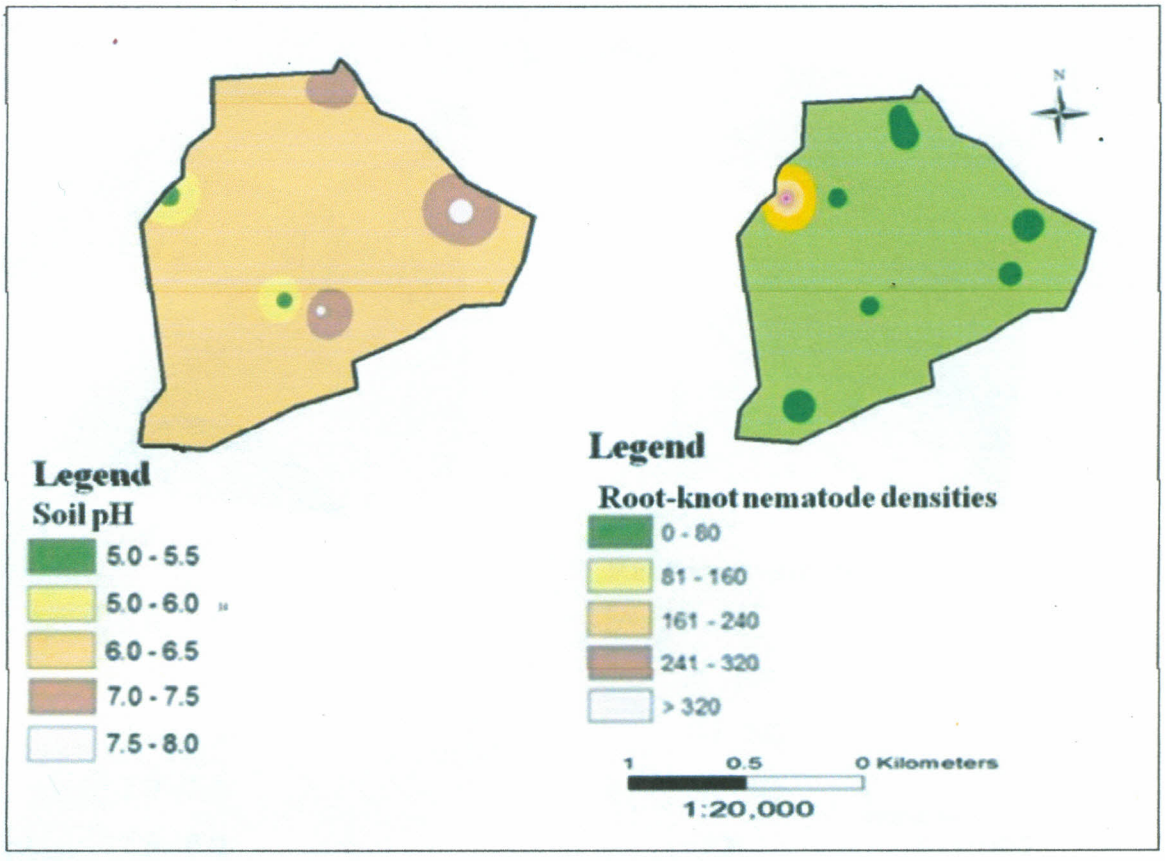


Figure 4.8: Soil pH and nematode distribution maps of Mbombaini

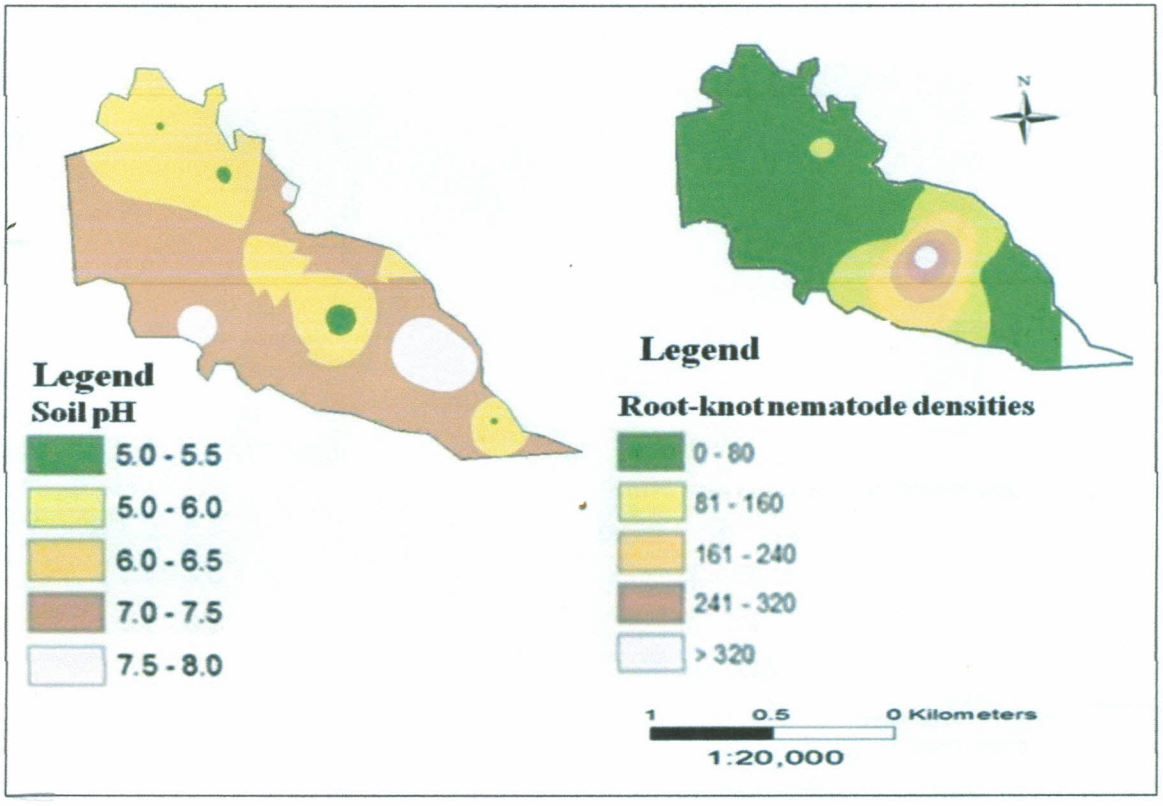


Figure 4.9: Soil pH and nematode distribution maps of Kiumbu

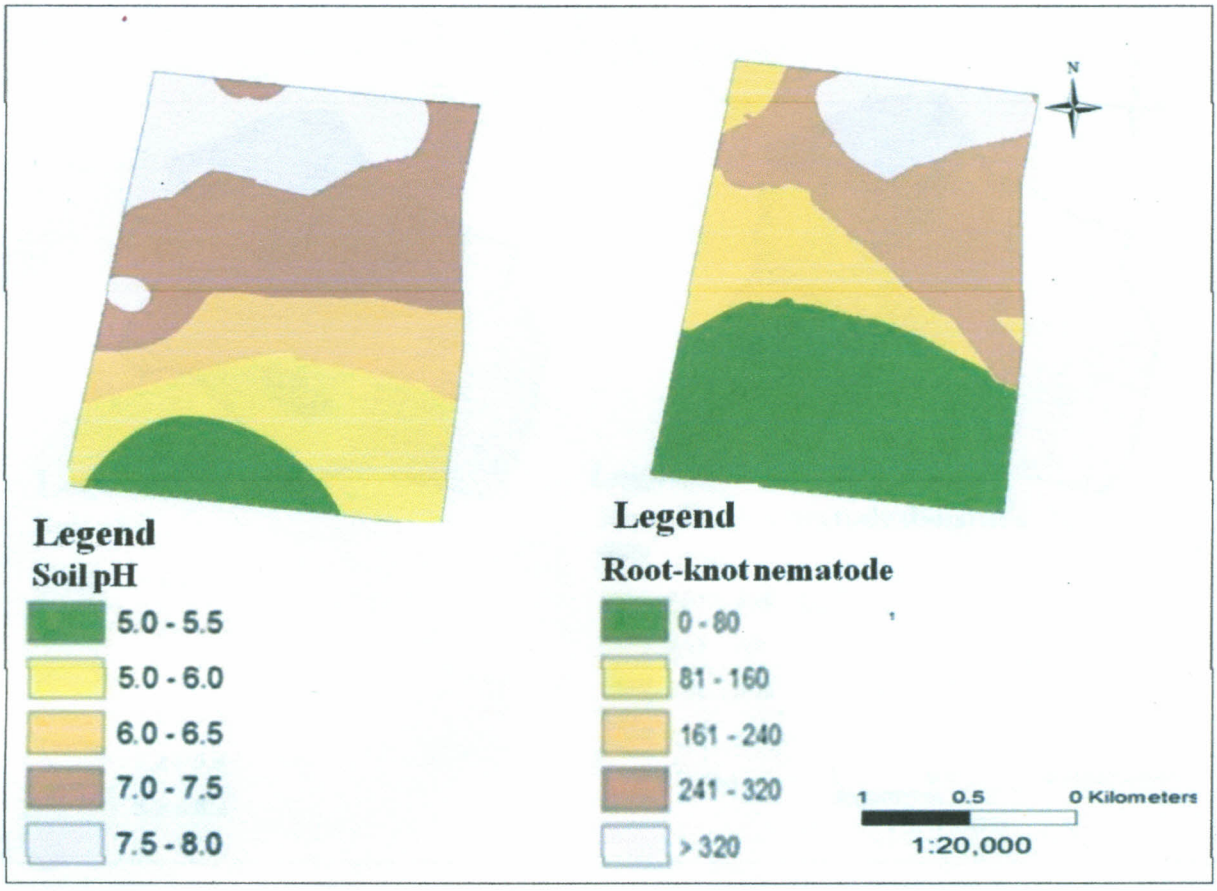


Figure 4.10: Soil pH and nematode distribution maps of Kiamanyeki

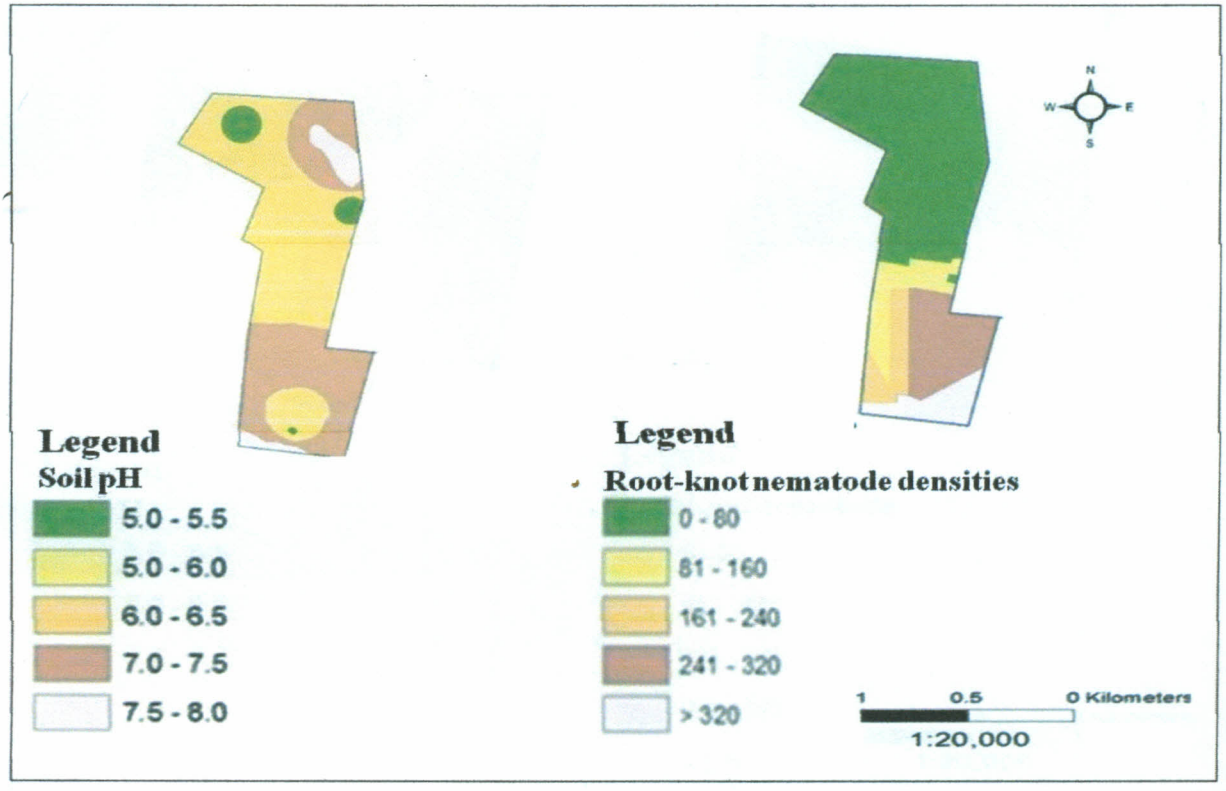


Figure 4.11: Soil pH and nematode distribution maps of Gathigiriri

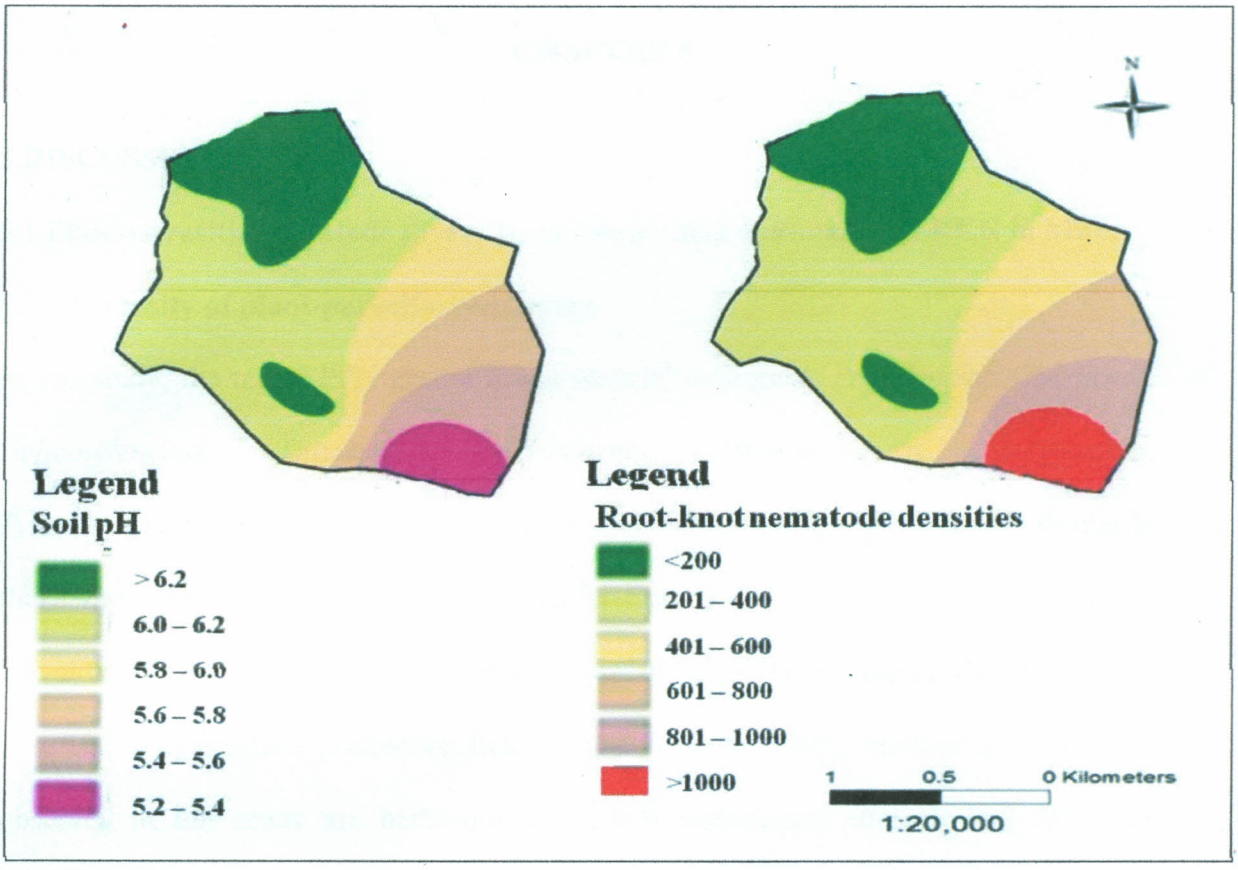


Figure 4.12: Soil pH and nematode distribution maps of Ngurubani

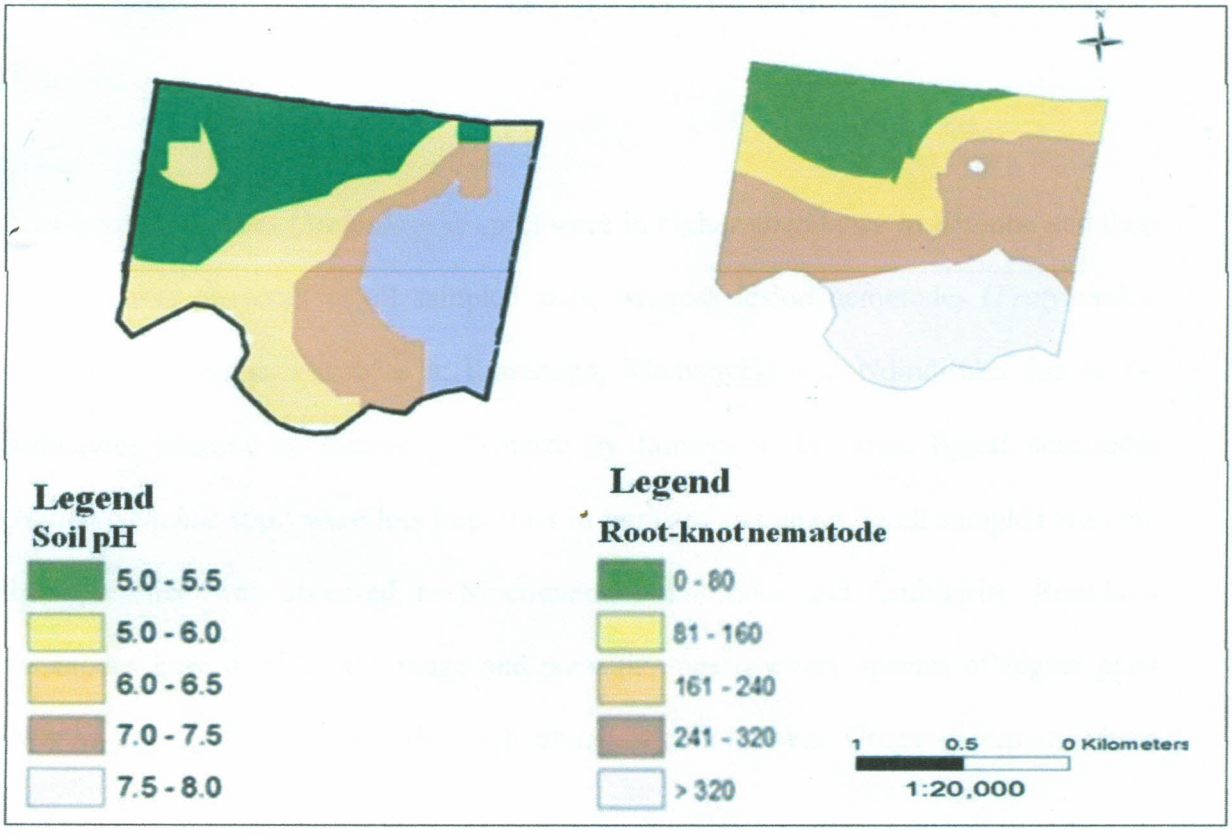


Figure 4.13: Soil pH and nematode distribution maps of Riambogo

CHAPTER 5

5 DISCUSSION

5.1 Plant-parasitic nematode diversity, incidence and abundance of RKN in Mwea

5.1.1 Diversity of plant-parasitic nematodes

In this study, the major PPN genera found were *Meloidogyne, Pratylenchus, Tylenchus, Helicotylenchus, Ditylenchus, Criconema, Hirschmaniella, Rotylenchus, Tylenchorynchus* and *Tylenchulus.* The genera of PPN found in this study are similar to those reported in other studies conducted in Central Kenya (Waiganjo *et al.,* 2006; Kavuluko *et al.*, 2010). Greater abundances of herbivorous nematodes have been observed in agricultural production fields (Kimenju *et al.*, 2009). Most of the nematodes observed in this study are herbivorous and are predominant in soils that are under agricultural production. The increase in the proportion of PPN (herbivores) is associated with an increase in ecosystem disturbance and intensive cultivation of agricultural land (Kimenju *et al.*, 2009).

Root-knot nematodes (*Meloidogyne* spp.) were in higher abundance in Kiumbu and their presence was observed in all sampled sites, whereas lesion nematodes (*Pratylenchus* spp.) were in higher densities in Riambogo, Kiamanyeki and Ndindiruku due to the continuous rotation of tomato and maize by farmers in the area. Spiral nematodes (*Helicotylenchus* spp.) were less important in terms of frequency in all sampled sites but their presence was observed in Mbombaini, Ndindiruku and Gathigiriri. Root-knot nematodes have a wide host range and parasitize nearly every species of higher plant (Moens *et al.*, 2009) hence the high abundance in Mwea. Crops grown in Mwea

including French bean, onions, tomatoes, maize and bananas are grown in rotation. These crops are important hosts of RKN and this might have led to their detection in all the sites. Spiral nematode (*Helicotylenchus* spp.) is commonly found in vegetable crops (Sikora and Fernandez, 2005) and many *Helicotylenchus* spp. are native to natural systems which have been converted to agriculture but are most damaging to grasses and that could have led to their reduced frequencies since most of the tomato production fields sampled had a long history of intensive cultivation except the fields in Riambogo which had been cultivated for a few seasons.

This study found various patterns of distribution of PPN and a lack of consistency concerning RKN distribution. Root-knot nematodes and lesion nematodes (*Pratylenchus* spp.) were the most abundant PPN genera throughout the region. The free living nematodes (FLN) were present in all the sites except Riambogo. This may be because sampling at Riambogo was done after a flood hit the site. The black cotton soil, cropping sequence and anoxic conditions during the flood could have influenced the patterns of PPN distribution in the area. Furthermore, soil suppressiveness might have influenced nematode distribution in Riambogo.

5.1.2 Incidence and abundance of RKN in Mwea

From the survey, it was observed that root-knot nematodes infested a great proportion of samples with relatively high RKN infestation within the sites being observed. This observation can be attributed to the intensification of tomato production in the sampled fields. The high incidence and abundance of RKN in the tomato fields agrees with Kimenju *et al.* (2009) who observed high incidences of RKN following agricultural activities in disturbed land.

All populations of RKN had very high coefficients of variation (CV > 100%). This is because of the innate susceptibility of the tomato varieties grown in the area and that RKN, being sedentary endoparasites have a highly aggregated spatial pattern because the females lay eggs at the same place (Ferris *et al.*, 1990). The high coefficient of variation confirms the influence of biotic and abiotic factors on the changes in RKN spatiotemporal variability (Ortiz, 2007). High standard deviations observed in the study may have been caused by the high spread nematode densities encountered in the sites. The high variability observed in this study agrees with Dinardo-Miranda and Fracasso (2009) who observed great variability in *Meloidogyne* populations which is related to its biology.

5.2 Root-knot nematode spatial and temporal distribution in rainfed and irrigated tomato fields in Mwea

Knowledge of the spatial and temporal distribution of RKN is important from the viewpoints of both planning surveys and scientific understanding of parasite transmission and epidemiology (Gyapong *et al.*, 2002).

5.2.1 Root-knot nematode distribution in rainfed and irrigated tomato fields in Mwea

This study established a significant difference ($P<0.05$) in RKN densities between rainfed and irrigated tomato fields. The distribution and occurrence of nematodes has been found to be correlated with irrigation systems (Gul, 1988). However, in this study, higher RKN densities were observed in rain-fed tomato production system compared to the irrigated

system. The results of this study contradict findings by Singh *et al.* (1994) and Pokharel *et al.* (1997) who observed significantly higher RKN in irrigated fields compared to rainfed fields. This was due to the furrow irrigation practiced which favours the mechanical dissemination of RKN. Faulkner and Bolander (1970) demonstrated spread of RKN with irrigation water. The passive dispersal of RKN through irrigation systems is potentially serious to growers using the furrow system. It is expected that soil moisture remains steady during rainfall but frequent weather fluctuations are experienced and though irrigation is practiced in the dry periods in between, high evapotranspiration do occur. However, there were some irrigated sites such as Kiumbu where very high nematode densities were observed.

The high densities of RKN observed in the rainfed production system may be due to the fact that sampling was done during the rainy season when the moisture was high and the larval densities had peaked. When soil moisture increases, egg hatching resumes and larval densities increase. The relatively lower densities of RKN juveniles observed in irrigated systems may be due to the fact that great environmental fluctuations occur within these fields on a daily basis including inconsistent water supply to the tomato fields coupled with very high evapotranspiration taking place in the fields.

Water logging occurring due to poorly controlled furrow irrigation in the fields is also a factor that may have led to the low densities of RKN observed in the irrigated system. Robinson and heald (1991) observed that oxygen at very low concentrations limits the activity of RKN juveniles. Water logged soils are quite undesirable environments for the RKN due to low oxygen concentration and hence the low population density observed

due to CO^2 intoxication typically encountered in water logged soils (Curtis *et al.*, 2009) which is the case in irrigated fields in Mwea.

In the current study, RKN were mainly observed in tomato fields where tomato was intercropped with maize and where producers rotated or intercropped maize with tomato in their fields. It was observed that production fields that had been cultivated for few seasons reported low RKN densities and this could be due to the low amount of inoculum that the fields had been exposed to. In Riambogo where tomato cultivation had been done for two seasons, only two major PPN genera noted were *Pratylenchus* and *Meloidogyne.*

5.2.2 Root-knot nematode distribution in dry and rainy seasons

It was observed from this study that RKN densities in the dry season were relatively lower than the rainy season. However, there was no significant difference between the two seasons. It was only in sites where irrigation is practiced where high RKN densities were detected during the dry season because of the constant supply of moisture in the soil.

The balance between water and air greatly influence nematode activity within the habitable pore space in the soil (Andre *et al.*, 2002). Composition of nematode communities in the soil are impacted by saturation and drought which result in anaerobiosis and dehydration, respectively (Neher, 2010). Greco and Vito (2009) observed that in the presence of a suitable host and favourable soil moisture content, RKN would reproduce continuously in tropical regions where temperatures do not vary greatly between seasons although the reproduction potential of RKN often increase

during the rainy season. The findings of the current study agree with those of Souza *et al.* (2008) who observed population fluctuations due to the alternate rainy and dry seasons.

5.3 Relationship between RKN infestation and soil characteristics

The results from this study indicate that a relationship exists between the measured soil factors and RKN infestation. Moreover, a direct relationship was observed between the soil EC and RKN populations in the detrended canonical correspondence analysis. Increasing RKN populations were detected in areas with high soil electrical conductivity (EC) values. It is observed from this study that the spatial patterns of soil EC and RKN population densities are very similar. This is in agreement with Yavuzaslanoglu *et al.* (2011) who observed positive correlations between soil EC and soil RKN. Subsequently, these findings contradict findings by Ortiz (2007) who found an inverse relationship between RKN and soil EC.

The results from this study indicate that RKN densities are negatively correlated with the soil pH. Correlations between nematode densities and soil attributes have been illustrated in other studies (Noer and Barker, 1985; Monfort *et al.*, 2008; Mueller, 2010). Gorres *et al.* (1998) found significant correlations between nematode densities and soil bulk density and moisture at various times of the year whereas the reproduction of *Meloidogyne incognita* tended to be high in coarse textured soils. Other studies have also shown that organic matter is associated with the highest densities of *Tylenchulus semipenetrans* Cobb (Marshela *et al.*, 1992).

The soil EC and pH had an inverse relationship. Consequently, an increase in soil EC resulted in a decrease in soil pH and an increase in RKN densities. This was in agreement

with Ortiz (2007) who found that areas of high RKN densities had low soil pH. This is likely because some mineral salts including $CaCl_2$ and $Ca(NO_3)_2$ which have relatively high pH have been described as having nematicidal effects on RKN (Castro *et al.*, 1990; Cadet *et al.*, 2004). The soil pH had a negative correlation with soil EC. Subsequently, the soil texture was inversely related to the soil EC. Silt and clay fractions were less important factors impacting the nematode distribution patterns in Mwea because they had no effect on RKN distribution. Coarse-textured sandy soils have been associated with patches of high population density of RKN (Goodell and Ferris, 1980).

Each habitat poses particular challenges for nematode survival. Physicochemical stressors, which include osmotic strength, ion concentration, pH, and O_2 tension that can exceed the levels typically associated with metazoan existence (Lee, 2002) with soil moisture, temperature, and microbial activity being mainly influential. Hatching factors are rapidly inactivated at pH greater than 8; soils with high organic matter content have reduced diffusate mobility and hatching activity (Perry, 2002). These factors might have led to the observed inverse relationship between RKN population and soil pH.

Nematodes profoundly affect the pH of their immediate environment by excreting organic acids across the cuticle (Sims, *et al.*, 1994, 1996) thus lowering pH and hence where RKN density is high, the pH is relatively low. In the present study, it was observed that sites having relatively lower pH values (ranging from 4.0-5.5) had high RKN population values compared to sites that had pH values greater than 6.0. *Meloidogyne* survive and reproduce at pH levels ranging from 4.0 – 8.0 (Ferris and van Gundy, 1979).

Distribution of lesion nematodes (*Pratylenchus* spp.), yam (*Scuttelonema* spp.) and ring nematodes (*Criconema* spp.) seems to be influenced mainly by elevation. Soil texture influences to a greater extent the distribution pattern of free living nematodes, spiral nematodes (*Helicotylenchus* spp.) and cyst nematodes (*Heterodera* spp.) as illustrated in the DCCA ordination.

Soil pH ranges between 4.5 and 6.0 favoured high densities of RKN as observed in the soils harbouring high RKN densities in all the seven sampled sites. Contrary to findings by other workers such as Monfort *et al.* (2007) who found soil texture to be a good predictor of RKN, soil texture in general had a very poor explanatory power in relation to the RKN occurrence and sand was found to be negatively correlated to RKN population densities in the Mwea region. It is to be noted that only EC had some positive correlation to the population density of RKN with soil pH showing a strongly negative correlation. Detrended canonical correspondence analysis showed that soil EC and pH are the most important factors affecting the distribution of RKN. This agrees with Davis *et al.* (2008) who found that populations of RKN and nematode-induced yield reductions in cotton are best predicted by EC and in turn is used to delineate nematode management zones (NMZ).

Other workers have found a positive correlation between *M. Incognita* and *M. Javanica* occurrence with high soil pH (Perry and Evans, 2009). Neumann *et al.* (2006) suggest that nematodes at the root-soil interface could be influenced by gradients formed along the root axis and in the rhizosphere due to chemical strategies for nutrient mobilization which induce modification of pH and redox potential. *Meloidogyne* spp. is attracted to

roots and contact is maintained by the low pH and the lower redox potentials of the root surface (Bird, 1959; Prot, 1980). Following classification of soil, it was observed that the prevalent soil type in Mwea is Clay (Appendix 9) belonging to the vertisol group. The homogenous soil type observed in the area allowed a single sampling per season and production regime.

5.4 Root-knot nematode density-distribution and pH maps for individual sites

Root-knot nematode distribution varied significantly throughout fields and is negatively correlated to soil pH and other edaphic factors. The availability of GPS and GIS allowed the development of pH and RKN density distribution maps which can be used to project where nematodes are likely to occur within an area or field. The maps show RKN distribution in the production areas as well as the soil pH distributions. They can be used by producers for variable rate application of nematicides and other RKN management strategies. Areas showing high RKN densities require more focus by the farmers. Subsequently, areas that have relatively lower soil pH require intensive RKN management since higher densities of RKN are likely to occur and vice versa.

Nematicide usage reductions of up to 34 – 78% have been achieved with the utilization of NMZ maps as the basis for variable rate application of aldicarb or 1, 3-dichloropropene with cotton yields being equal or greater (by about 5%) than those obtained with single rate nematicide application (Monfort *et al.*, 2008; Mueller *et al.*, 2008) in the control of RKN. Site-specific nematicide application is made possible by the development of accurate, efficient and economical geo-referenced soil pH and nematode density distribution maps which are obtained by use of GPS and GIS technologies (Wyse-pester *et al.*, 2002).

The use of NMZs has yielded promising results across a wide range of soil types and production environments in Arkansas, Georgia, Louisiana and South Carolina (Mueller *et al.,* 2010). According to Wyse-pester *et al.* (2002), once the spatial dependence is known, the value at an unsampled location can be predicted from values at sampled locations and the relative positions of the samples.

Relationships occurring between RKN population densities and soil variables permitted the delineation of nematode management zones in fields based on the mean EC value of the various zones (Overstreet *et al.*, 2009) and in this study, a delineation of management zones in fields is based on the soil pH. This management zone delineation allows variable rate application of nematicides hence high potential economic and environmental benefits.

On the global distribution of plant pests, distribution maps of plant pests are widely respected reference sources of information (CABI, 2013). The distribution maps are the most authoritative source of information available on the presence and extent of specific plant pests. These maps are a vital tool for farmers and those involved in phytosanitary decisions.

CHAPTER 6

6 CONCLUSION AND RECOMMENDATIONS

Based on the results of this study, a significant diversity of plant parasitic nematodes in Mwea is observed. Root-knot nematodes show both spatial and temporal distribution. Their densities vary from site to site, highest in areas with relatively low pH and high EC. The RKN population density was highest during the rainy season in rainfed systems and low under the irrigated tomato production system.

Differences in RKN densities, abundance, spatial and temporal distribution in Mwea were observed, this shows that there is a relationship between soil characteristics affecting RKN density distribution. Important information is provided for scientists and awareness to tomato producers about the occurrence and distribution of RKN infecting tomatoes in the Mwea region.

This study established that soil pH and EC influence the spatial and temporal distribution of RKN in Mwea and a strong spatial correlation between RKN population densities and soil pH was observed, therefore, it is therefore appreciated that there are soil characteristics that influence the RKN spatial and temporal distribution and density in rainfed and irrigated tomato fields in Mwea. The soil pH and electrical conductivity are the key factors determining RKN distribution in Mwea. The relationship observed between these factors and soil texture can be used in relating soil texture with nematode distribution.

Affordable pH maps which delineate the probable high risk nematode density points within a field can be created using the relationship between RKN and soil pH. The maps can be used in management of RKN where a uniform spatial distribution pattern of RKN is lacking.

Recommendations

i. Farmers should avoid uniform application of nematicides in their production fields and test their soil pH as a site-specific management system is validated.

ii. Farmers are encouraged to embrace drip irrigation as furrow irrigation tends to be dispersal means for RKN.

iii. Crop rotations should focus on RKN non-host crops and nematode resistant tropical legumes. Also, the use of green manure plants and organic soil amendments is highly recommended.

iv. The areas for further research include:

   a. Extending the current study to investigate the factors that explain occurrence of other plant pathogens in soil.

   b. Extending the current study to other areas with similar cropping systems.

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APPENDICES

Appendix 1. Description of root knot nematode genera

Abbreviation	Description (Genera)
RKN	Root-knot nematodes (*Meloidogyne* spp.)
Lesion	*Pratylenchus spp.*
Crico	*Criconema spp.*
Aphel	*Aphelenchoides spp.*
Crico	*Criconema spp.*
Scutt	*Scuttellonema spp.*
Tylenc	*Tylenchulus spp.*
Hemi	*Hemicyclophora spp.*
Heter	*Heterodera spp.*
Helic	*Helicotylenchus spp.*
Hirsh	*Hirschmaniella spp.*
Aphel	*Aphelenchus spp.*
Tylerh	*Tylenchorrynchus spp.*
FLN	*Free living nematodes*

Appendix 2. Descriptive statistics for root-knot nematode densities under different production systems in Mwea

Effect	Level of factor	N	RKN Mean	RKN Std. Dev	Std. Err	RKN -95.00%
System	Irrigated	80	93.300	475.109	53.119	-12.430
System	Rainfed	90	95.478	240.083	25.307	45.193
Total		**170**	**94.452**	**368.610**	**28.27**	**38.642**

Appendix 3. Analysis of variance for the root-knot nematodes in different tomato production systems in Mwea

Source of variation	s.s	d.f.	m.s.	F.	P
Intercept	1509334	1	1509334	11.04272	0.01093
System	201	1	201	0.00147	9.97
Error	22962467	168	136681		
Total	**22962668**	**169**			

Appendix 4. Descriptive statistics for root-knot nematode densities in different production sites of Mwea

Effect	Level of factor	N	RKN	RKN	RKN	RKN	
RKN							
			Mean	Std. Dev	Std. Err	-95.00%	+95%
Site	Riambogo	13	18.46	22.30	6.19	4.99	31.94
Site	Kiamanyeki	50	29.30	33.43	4.73	19.80	38.80
Site	Kiumbu	17	338.76	1014.58	246.07	-182.8	860.41
Site	Gathigiriri	41	41.32	113.14	17.67	5.61	77.03
Site	Ngurubani	10	172.10	316.54	100.10	-54.34	398.54
Site	Ndindiruku	29	95.83	194.17	36.06	21.97	169.69
Site	Mbombaini	10	239.90	502.53	158.92	-119.59	599.39
Total		**170**	**94.45**	**368.61**	**28.27**	**38.64**	**150.26**

Appendix 5. Analysis of variance for the root-knot nematode densities in different tomato production sites in Mwea

Source of variation	s.s	d.f.	m.s.	F.	P
Intercept	2111526	1	2111526	16.17914	0.000088
Site	1689670.518	6	281612	2.15779	0.049673
Error	21272997.61	163	130509		
Total	**22962668.12**	**169**			

Appendix 6. Descriptive statistics for soil pH levels in different production sites of Mwea

Effect pH	Level of factor	N	pH Mean	pH Std. Dev	pH Std. Err	pH -95.00%	+95%
Site	Riambogo	13	6.50	0.35	0.09	6.29	6.71
Site	Kiamanyeki	50	7.07	0.87	0.12	6.82	7.31
Site	Gathigiriri	41	7.06	0.68	0.11	6.84	7.26
Site	Ngurubani	10	5.38	0.24	0.08	5.21	5.56
Site	Mbombaini	10	6.35	0.47	0.15	6.02	6.69
Site	Ndindiruku	29	6.18	0.69	0.13	5.92	6.44
Site	Kiumbu	29	5.97	0.53	0.13	5.69	6.25
Total		**170**	**6.62**	**0.86**	**0.07**	**6.49**	**6.75**

Appendix 7. Descriptive statistics for root-knot nematode densities in different seasons in Mwea

Effect	Level of factor	N	RKN Mean	RKN Std. Dev	RKN Std. Err	RKN -95.00%	RKN +95.00%
Season	Dry season	109	93.98	418.20	40.06	14.57	173.37
Season	Wet season	61	95.31	260.58	33.36	28.57	162.05
Total		**170**	**94.45**	**368.61**	**28.27**	**38.64**	**150.26**

Appendix 8. Analysis of variance for the root-knot nematode densities in different seasons in Mwea

Source of variation	s.s	d.f.	m.s.	F.	P
Intercept	1005030	1	1005030	4989699	0.0000
RKN	18	62	0	1	0.0611
Error	22	107	0		
Total	**39**	**169**			

Appendix 9. Classification of soil

Site	% Sand	% Clay	% Silt	Class
Riambogo	16.6	58	25.4	Clay
Kiamanyeki	19.64	59.6	20.78	Clay
Giathigiriri	25	69	6	Clay
Ngurubani	10.2	71.6	18.2	Clay
Mbombaini	18.8	62.4	18.8	Clay
Ndindiruku	17.3	65.8	16.9	Clay
Kiumbu	16.5	60.5	23	Clay