OCCURRENCE, ABUNDANCE AND DISTRIBUTION OF NEMATODES OF BANANA LINKED TO ALTITUDE IN SELECTED BANANA PRODUCTION AREAS WITH FOCUS ON PATHOGENICITY OF *Pratylenchus goodeyi* IN KENYA

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DEDICATION

I dedicate this thesis to my kids Zindzi and Jabulani and the memory of the late Dr. George M. Kariuki. May his soul find repose.

DECLARATION

I **NYANG'AU N. DOUGLAS**, declare that this thesis is my original work and has not been presented for a degree examination in any other university or for any other award.

Signature…… Date…….

Supervisors' Approval

We confirm that the work reported in this thesis was carried out by the candidate under our supervision has been submitted with our approval as the University supervisors.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
EAHB	East African Highland Banana
GPS	Global Positioning Systems
IPM	Integrated pest management
KG	Kilograms
Ksh	Kenya Shilling
MASL	Meters above sea level
MM	Millimeters
PPN	Plant parasitic nematode
RF	Reproductive factor
SDI	Simpsons' diversity index
TC	Tissue Culture
USD	United States Dollar

ABSTRACT

Banana (*Musa* spp.) is a popular fruit crop in Kenya, where it is largely cultivated by smallholder farmers. However, plant pathogens, such as plant parasitic nematodes, have been blamed for the fall in output (PPN). A survey of banana-based subsistence farming systems in Kenya's banana-growing counties was done with the objective to a) assess farmers' awareness regarding PPN, b) explore the variability regarding PPN species associated with banana genotypes in Kenya and if the prevalence and abundance of these PPN species vary with altitude and c) differential ability of *Pratylenchus goodeyi* populations to infect banana. One hundred and eighty (180) farms from 12 major banana producing areas with varying altitudes ranging from 1100-2000 m above sea level were surveyed. Farms were selected following a purposive sampling method. A structured questionnaire was administered to every farmer whose orchard was selected for sampling. From each production area, 30 soil and 30 root samples were randomly collected from 15 banana farms. On each farm, one soil and one root sample were collected from both dessert (Cavendish) and cooking (EAHB) banana cultivars. A total of 720 samples were collected from the survey study. Nematode damage was scored on five functional roots randomly selected and scored for the extent of necrosis in the root cortex as a percentage. Nematodes were extracted from 5g of roots and 100 ml soil using a modified Baermann technique and identified to the genus level. Besides, two pot experiments were set up to assess the differential ability of *Pratylenchus goodeyi* derived from diverse altitudinal gradients to infect banana. Mean values of root necrotic indices (RNI %) from the survey and pot trials were arcsine ($\sqrt{x}$) transformed. Nematode relative abundances and genera diversity were computed. The nematode counts were subjected to $\log_{10}(x+1)$. Data were subjected to a two-way ANOVA using R- version 3.5.1 system statistical software and means separated by the Tukey's test at $P \leq 0.05$. Results showed that only 2.3% of the farmers were aware of nematode damage and symptoms, with none applying any management measures. The highest abundance of PPN was recorded at an altitude range of 1601-2000 masl with *Pratylenchus, Meloidogyne* and *Helicotylenchus* spp. as the predominant genera. Across mid and high altitudes, EAHB showed higher numbers of nematodes than Cavendish. Screening tests on *P. goodeyi* revealed that populations from Embu had higher plant infectivity as they recorded the highest reproduction rates. Ng'ombe showed higher infectivity than Sukari Ndizi banana in both trials. The results revealed that nematode damage is more common at higher altitudes and on the EAHB genotype. The findings suggest that strengthening farmers' awareness of pathogen dissemination mechanisms and increasing their availability to disease-free planting supplies should be part of Kenya's banana nematode management strategy. The findings from our study can be used to advise farmers on nematode management techniques suitable for different altitudes.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

The Musaceae family includes bananas (*Musa* spp.). Its origin and domestication is proposed to be South East Asia, an area considered as the center of diversification (Li *et al*., 2013). Banana is a popular fruit worldwide with consumers spending more than 10 billion pounds (£) a year on it as a natural product making it the most prevalent organic product on the planet (Trade Fair, 2013). After rice, wheat, and maize, bananas are the world's fourth most important food crop (Soko Directory, 2018). The projection that banana produces fruits across all months of the year makes it highly significant in Africa as a food stability crop.

Inclusion of banana into human diet is due to its nutritive and therapeutic value as an ideal supplier of vitamin A, B6 and C and potassium (Haslinda *et al*., 2009; Caballero, 2012). Banana is known to be distributed and consumed in developing and developed countries alike and for long, it has been considered a basic weaning food (Haslinda *et al*., 2009). About 38% of the land in the East African highlands, comprising Kenya, Uganda, Tanzania, Rwanda, Burundi, and the Democratic Republic of Congo, is used for banana production (Obaga, 2018). In this region, banana is regarded as a staple food for over 20 million people, with over 20 million tons being produced annually (Obaga, 2018).

As an important food crop, banana accounts for about 35.6 % of all the fruits produced in Kenya (Joachim *et al*., 2018). Kenya is estimated to have 2% of its arable land under banana production which translates into 74,000 ha of land with a total

production of 1.43 million tons valued at USD 0.18 billion annually (Kneoma, 2018). Banana production is majorly practiced by small holder farmers (Ng'ang'a *et al*., 2011) who use low levels of farm inputs resulting in low and sub-optimal levels of production (Soko Directory, 2018).

Bananas can be produced in diverse environments and yield fruits all year, making them a cost-effective source of food energy when other crops are out of season (Joachim *et al*., 2018). Globally, Kenya is ranked as the 16th highest producer of bananas (Kneoma, 2018). By 2018, banana production in Kenya was 1.41 million tonnes with an approximate 6.53% growth rate yearly. Surprisingly, in 2017 production was 1.43 million tonnes (Kneoma, 2018). The main genotypes under cultivation in Kenya include the dessert and the cooking cultivars rated at 40% and 60% of production respectively (Soko Directory, 2018). The leading areas in banana production are: Meru (19%), Kirinyaga (14%), Embu (12%), Bungoma (5%), Taita Taveta (9%), Kisii (6%), and Murang'a (7%) (Soko Directory, 2018).

The rapid growth of the rural and urban populations in Kenya and changes in food behavior with technology improvement provides ideal opportunities for improved banana production for local consumption and demand for export markets (Soko Directory, 2018). Therefore, to meet the standards of the banana export market and compete favorably, there is a desire for the production of good quality fruits. Despite the crop showing great potential, banana production has declined significantly in Kenya for the last twenty years (FAOstat, 2015). This decrease is due to an increase in disease and pest outbreaks (Seshu *et al*., 2007; Kasyoka *et al*., 2011), which is exacerbated by a lack of efficient control techniques (Inzaule *et al*., 2005).

In Kenya, a complex of nematodes remain one of the damaging pathogens affecting banana yields in major areas (Seshu *et al*., 2007; Waweru *et al*., 2014). Previous survey findings conducted in Kenya to define banana production constraints have shown that a banana nematode complex that is *Pratylenchus goodeyi* (Sher and Allen), *Pratylenchus coffeae* (Goodey), *Radopholus similis* (Cobb) Thorne, *Helicotylenchus multicinctus* (Cobb) and *Meloidogyne* spp. were commonly recovered from several farms (Gichure and Ondieki, 1977; Seshu *et al*., 1998; Inzaule *et al*., 2005). *Pratylenchus goodeyi* was found to be the most common species on both exotic and East African highland bananas in a similar investigation conducted in Kenya in 1993 (Seshu *et al*., 2007).

Globally, PPN are reported as major pests parasitizing bananas (Pattison, 2011) resulting in loss of yields of between 30-60% (Gowen *et al*, 2005; Kamira *et al*., 2013). Highland bananas in Africa have been observed to have a yield drop of up to 50% (Gaidashova *et al*., 2009). The nematodes attack banana roots, causing losses and exposing plant tissues to secondary; fungal, bacterial, and viral infections (Rotimi, 2003; Okafor *et al*., 2015) that compromise banana growth, fruit yield and overall quality of *Musa* spp. in plantations.

Roots that have been parasitised by PPN are unable to provide enough water and nutrients to the plant (Risède *et al*., 2010). As a result, root damage slows plant growth, prolongs the fruiting season, diminishes bunch weight, and reduces production life (Risède *et al*., 2010). Due to the weakened root system's poor anchoring, heavy plants may topple (Waweru *et al*., 2014).

Traditionally, PPN species that harm bananas have been dispersed along a dermacated gradient where *R. similis* occurred in lower altitudes (Elsen *et al*., 2000; Blomme *et al*., 2012), while the *P. goodeyi* has been found infecting bananas at higher altitudes (Kashaija *et al*., 1994; Yu *et al*., 2012; Coyne *et al*., 2013; Luambano *et al*., 2018).

Of particular note, these two species have distinct damage potentials due to their varied aggression levels, with *R. similis* being thought to be more damaging than *P. goodeyi* (Price, 2006; Plowright *et al*., 2013; Chitamba *et al*., 2014). There was a need therefore, to conduct an extensive survey to determine the incidence, abundance and distribution of plant parasitic nematodes associating with bananas across different altitudes on different cultivars in major production areas. Information from the study will be useful in developing strategic nematode management protocols for improved banana production in Kenya.

1.2 Statement of the Problem

The rural communities produced bananas exclusively for consumption purposes until the 1980s when farmers resorted to increased production of bananas for commercial purposes (Nguthi, 2007). However, the production of bananas has been constrained by plant parasitic nematodes, out of which, yield losses ranging between 30-60 % annually have been reported (Gowen *et al*., 2005). The significant damage is largely attributed to the distribution of planting materials of low phytosanitary standards (Rahman *et al*., 2014).

Scarcity of nematology knowledge largely nematode biology and inadequate sustainable control measures (Talwana *et al*., 2016), particularly for small-scale farmers are the major constraints to controlling parasitic nematodes in Africa. Management of nematodes in commercial banana production is chiefly by chemical nematicides which is the ideal option despite their ban due to environmental degradation and human health-related issues (Chávez and Araya, 2010). Chemicals are not affordable to most subsistence producers in the developing world. Besides, their excessive use results in nematodes developing resistance constraining their management (Noling, 2012).

The distribution of these nematodes has been stable in the past. However, owing to the shift in temperatures along altitudinal gradients linked to change of climate in the region, there is a possibility of the shifting of *Radopholus similis* to higher altitudes, resulting in an overlap with *Pratylenchus* spp. (Coyne *et al*., 2018). This may have a detrimental impact on the East African Highland banana genotypes (Coyne *et al*., 2018). Besides, Kenya's geographical positioning in the tropical highlands where climate favors nematode reproduction and survival aggravates nematode challenge. *Pratylenchus goodeyi* has been the dominant nematode across banana production systems in the region across high altitudes (Kashaija *et al*., 1994; Gaidashova *et al*., 2004; Seshu *et al*., 2007; Kamira *et al*., 2013) and its recent observation in lower altitudes (Luambano *et al*., 2017; Mgonja *et al*. 2019).

Apart from decreasing banana root effectiveness (Wang *et al*., 2009) and exposing plants to secondary infections at high altitudes (Smiley, 2015), it is yet unknown how *P*. *goodeyi* affects bananas at lower altitudes with higher temperatures.

As a result, little is known about how environmental variables affect the nematode and the damage it causes to banana growth.. There was a need therefore, to conduct an extensive survey to explore the variability regarding PPN species associated with Cavendish and East African Highland banana genotypes in Kenya, evaluate the effect of altitude on the incidence, prevalence and abundance of the plant parasitic nematode species and thirdly, assess the differential ability of *Pratylenchus goodeyi* derived from diverse altitudinal gradients to infect banana.

1.3 Justification of the Study

Banana producers can benefit from these insights and knowledge in banana production Counties of Kenya. The study sought to improve our understanding of nematodes associated with bananas in different banana production agro ecologies as affected by increase in temperatures owing to climate change for sustainable nematode management. The findings pique policymakers' and farmers' interest in banana nematodes, while also laying the groundwork for future research. Furthermore, the research lays the basis for future generation of enhanced and resistant banana types, as well as providing data and suggestions for developing effective management strategies.

This research raises awareness among banana growers about the effects of climate change and the spread of banana nematodes, as well as providing a chance to develop new technology that can be harnessed and integrated into the banana value chain. Farmers will benefit from the knowledge of cultivar tolerance of *P. goodeyi* as a major nematode across different altitudes for better decision making. Eventually,

this could lead to increased banana output, which would boost the country's nutrition and food security.

1.4 Objectives of the Study

1.4.1 General Objective

To determine the occurrence, abundance and distribution of nematodes of banana to establish a knowledge base for sustainable management of plant parasitic nematodes in banana in Kenya.

1.4.2 Specific Objectives

The specific objectives for this study were;

i. To assess farmers knowledge of plant parasitic nematodes of banana in selected banana production areas.
ii. To identify the plant parasitic nematodes associated with Cavendish and East African Highland banana genotypes.
iii. To assess population densities and distribution patterns of plant parasitic nematodes associated with Cavendish and East African Highland banana genotypes.
iv. To assess the pathogenicity of three populations of *Pratylenchus goodeyi* isolated from different altitudes on dessert (sweet banana) and cooking banana (Ng'ombe) genotypes.

1.5 Hypotheses

i. Farmers have no knowledge of nematodes as a pest of banana.

ii. Plant parasitic nematodes are not associated with Cavendish and East African Highland banana genotypes at different altitudes in banana production areas in Kenya.

iii. Population densities and distribution patterns of plant parasitic nematodes associated with Cavendish and East African Highland banana genotypes does not vary with altitude in banana production areas in Kenya.

iv. Pathogenicity of *Pratylenchus goodeyi* isolated from different altitudes does not vary with dessert (sweet banana) and cooking banana (Ng'ombe) genotypes.

1.6 Significance of the study

The study established the status of plant parasitic nematodes (PPN) associated with Cavendish and East African Highland banana genotypes at diverse altitudinal gradients in major banana production areas in Kenya. The study quantified damage levels caused by PPN on banana genotypes in Kenya. Recommendations have been made to improve management regimes with the aim of improving banana productivity and hence revitalize the banana production industry.

1.7 Conceptual Framework

The relationship studied is presented in figure 1.1 which states that PPN associated with different banana varieties depends on availability, population densities and distribution patterns of PPN at the banana producing regions.

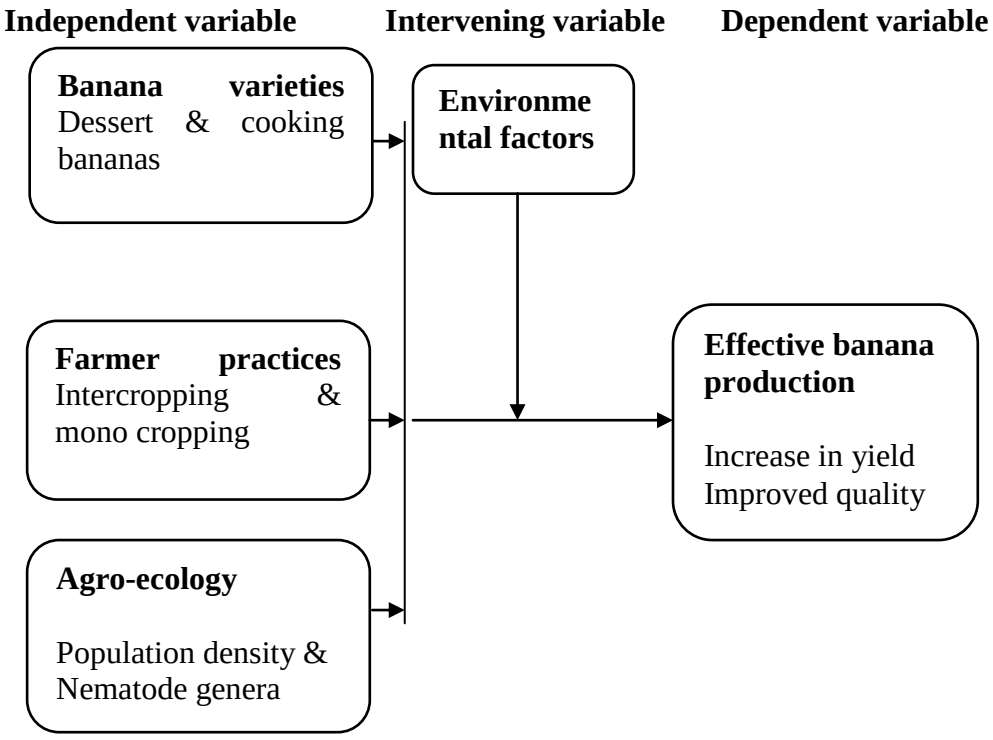


Figure 1.1: Conceptual Framework

CHAPTER TWO

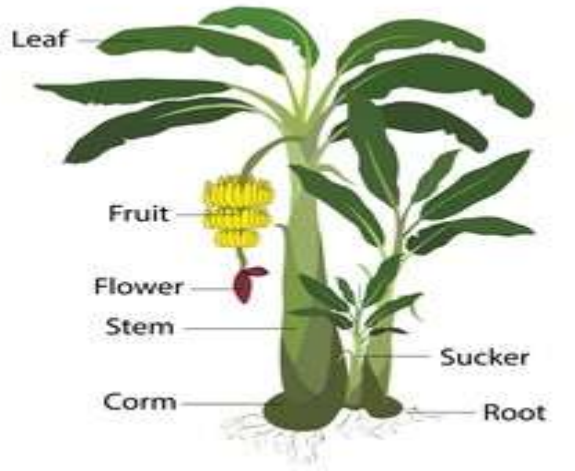
2.0 LITERATURE REVIEW

2.1 Botany of the banana plant

Bananas are not native to Africa and are presumed to have originated in the Asian tropics from the Southwest India East of the island of New Guinea (Blomme *et al*., 2013). Within the order Zingiberales and the Musaceae family, banana is a monocotyledonous, perennial herb. There are two Genera within the Musaceae family; *Musa* and *Ensete*. Besides, there are four sections; Eumusa, Callimusa, Rhodochlamys and Australimusa within the *Musa* genus (Simmonds, 1966). Of the four divisions within *Musa*, Eumusa is the most common and includes the largest number of species and forms, including consumable seedless bananas (Simmonds, 1966). The banana fruit develops parthenocarpically. Banana is a berry despite developing out of an inferior ovary. The epidermis and the aerenchyma layer build up the exocarp, with the mesocarp being the skin (Heuvelink, 2005).

Depending on the variety, climate, soil and management conditions (Kasyoka, 2013), the banana plant grows up to approximately 2-9 m tall (INIBAP, 2000). The trunk is a pseudo-stem consisting of overlapping leaf sheaths in concentric layers. A mature banana plant consists of a corm with roots and suckers, a pseudo-stem with leaves and a bunch with fruits (Figure 2.1). The corm is the underground part of the banana plant (true stem) and its tip bears an apical meristem from which leaves emerge (INIBAP, 1997).

The formation of new leaves is usually every 7-10 days and majority of the plants produce between 35 - 40 leaves.



(Photo courtesy: Robinson and Sauco, 2010)

Figure 2. 1*:* Banana plant showing a corm and a pseudostem

2.2 Requirements for banana production

Bananas can be cultivated in a variety of environments (Wanja, 2010). The average annual rainfall requirements of 1000mm annually and temperatures of 27˚C are optimal for its growth (Bosire, 2013). The pH level should be between 5.5 and 6.5 for optimal performance (Amugune *et al*., 2007). The minimum rainfall needed for optimal banana performance depends on soil type, place of planting, exposure to light and variety. Bananas should be planted in protected areas but open to the sun since they are prone to wind knockdowns (De Waele and Nnennaya, 2011).

To achieve optimal plant growth and performance, manure and fertilizer application is recommended and on average, the banana plant requires soils of organic carbon ranging from, 400 g of potassium and 30 g of phosphorous for optimum growth (Noor *et al*., 2010). Well decomposed Farm Yard Manure should be applied at a rate of 20 kg to 40 kg per stool every year (Kimenju *et al*., 2013). In intense banana production areas, moderate winds of 20 to 50 km/h cause mild to

severe leaf plucking. Leaf pulling may decrease yields, but it does not typically have a pronounced impact if there is no substantial loss of foliage. Also, as maximum daily water tension happens, tearing will further decrease plant transpiration, whereas photosynthesis is impaired at its lowest (Heuvelink, 2005).

Bananas are grown in various agro-ecological conditions in Eastern and Southern Africa, ranging from lowlands at sea level to highlands above 2000 m asl (Karamura *et al*., 1998). For orchard development, farmers primarily use suckers, which are prepared by cutting the pseudostem foliage and paring the corm. However, these type of plantlets are considered of low phytosanitary standards due to soil born pest contamination, in addition to being bulky with poor multiplication rate (Vuylsteke and Ortiz, 1996). Banana genotype distribution is influenced by attributes such as local preferences, eating patterns, consumer demand and prevailing environmental conditions (Mbaka *et al*., 2008).

2.3 Banana crop cultivation

Bananas are grown internationally in tropical Africa, Asia and Latin America, but mainly in emerging economies (Friesen, 2016). Banana genotypes under cultivation are predominantly triploids (3x) which are derivatives of intra- and inter-specific crosses, *Musa acuminata* and *Musa balbisiana*, two diploid species (Hartman *et al*., 2010). Globally, banana production stands at approximately 97 million tonnes yearly (Tripathi, 2003) whereby, dessert types comprise 55 %, while the cooking types make up 45 % (Viljoen *et al*., 2004). Bananas have a 15 to 18 month life cycle (Nyombi, 2013).

About 35 percent of the world's bananas and plantains are grown in Sub-Saharan Africa. In East and Central Africa, the Great Lakes region, which includes Kenya, Uganda, Rwanda, Burundi, Tanzania, and Congo, produces 15 million tonnes of bananas every year (Bioversity International, 2009). Worthy to note, the region has the highest per capita banana intake of 200 – 250 kg per year (Tripathi, 2013). Uganda is the world's largest banana consumer, rated at 1kg per person on daily basis (Tripathi, 2013). Bananas are produced in more than 100 countries worldwide, with 23 of them producing more than one million tonnes annually, of which 42 % of world production is realized by the top five producing countries (Sheth, 2017; Kneoma, 2018) as shown in table 2.1.

Table 2. 1: Table showing banana production data in five leading countries

Rank	Country	Production (in tonnes)
1	India	30,808,000
2	China (mainland)	11,577,938
3	Indonesia	7,264,383
4	Brazil	6,752,171
5	Ecuador	6,505,635
16	Kenya	1,414,176

(Kneoma, 2018)

Bananas are mainly grown in multi-cultivar systems in relatively small parcels close to the homestead (Wanja, 2010) as a stand alone crop or as an intercrop with other crops. In Kenya, bananas are grown in the highlands of Central, Rift Valley, Nyanza and some parts of the Eastern region. Smallholder farmers comprise the majority of banana producers on 74,000 ha of land, which translates to 2 % of total land under cultivation (Daneel *et al*., 2015; Soko Directory, 2018). About 1,000,000 tons are produced each year, with an annual value of more than US $80 million,

accounting for half of Kenya's domestic horticultural value (Kasyoka, 2013; Soko Directory 2018).

2.4 Economic importance of bananas

At a global level, production and trade in bananas is a pillar of food security that provides about 400 million people worldwide with access to staple food and more than 50 million people with a critical source of income (Stoian *et al*., 2018). In the developing world, bananas constitute the fourth most important crop (Bhanusree, 2015). Bananas form a significant subset of most rural farming systems and project a gross value greater than maize, rice, sweet potato or cassava in Sub-Saharan Africa. In most developing countries, a lot of bananas produced are consumed in the household or sold in village markets, thus playing a pivotal role in ensuring the masses are food secure besides revenue generation (Kumar *et al*., 2011).

Bananas are a perennial plant that plays an important part in the economies of many underdeveloped countries with an approximate overall foreign trade value of US$ 4.5 and 5 billion annually (Kirimi *et al*., 2021). The trade of bananas is also key in generating revenue for local councils. A study predicted weekly revenue returns exceeding Ksh. 0.5 Million (US$ 6,250) for over 50,000 banana bunches traded in central and Eastern Kenya (Goswami *et al*., 2008). In addition to its consumption as a food commodity, the fruit may be used for alcohol processing, while the leaves are used as a roofing material, food cooking and transport and for basket making (Carter *et al*., 2010). Dry banana leaves, leaf sheaths, and petioles are also utilized as ropes, cassava fermentation covers and nesting materials for egg laying farm birds (Akinyemi *et al*., 2010; Kamira *et al*., 2015).

In major banana producing regions of Kenya, farmers earn over a billion shillings income from banana proceeds per year (Soko Directory, 2018). Banana plant exhibit soil conserving properties, which are displayed by canopy formation, root system, and mulching a common practice in *Musa* orchards is important for soil conservation as it lowers run-off levels by 30 % in fields cropped with bananas in comparison to fields with annual crops (Lufafa *et al*., 2003). Socio-economically, bananas are suitable crops for growing, consuming and selling and can provide stable comprise the majority in enterprise production (Soko Directoy, 2018).

2.5 Nutritional value of banana

More than 20 million people in underdeveloped countries rely on bananas for 75 percent of their carbohydrates (Dotto *et al*., 2018). Behind rice, wheat and maize, banana is the fourth most significant food crop in the world (Viljoen, 2010; Tripathi, 2013). Bananas are preferred by the masses because they constitute a primary nutritional source, minerals and vitamins (Dotto *et al*., 2018) and it is reported to be a widely produced and consumed fruit globally (Rahman *et al.*, 2014; Affognon *et al*., 2015).

2.6 Banana production constraints

Globally, banana productivity and yields are constrained by low production efficiency worsened by pests and diseases (Swennen *et al*., 2013) such as black leaf streak (*Mycosphaerella musicola*), banana bunchy top disease (BBTV), banana *Xanthomonas* wilt (*Pseudomonas solanacearum*), *Fusarium* wilt (*Fusarium oxysporum f.*sp. *cubense*), weevils (*Cosmopolites sordidus*) and plant parasitic

nematodes (Akankwasa *et al*., 2008; Talwana *et al*., 2016). Farmers in banana-producing areas primarily rely on banana planting materials from their own or neighboring orchards to develop plantations (Ocimati *et al*., 2013) which inherently carry disease pathogens thus aggravating the incidence of banana pests and diseases (Fujardo, 2010). Nematodes are regarded as a significant threat to bananas as they interfere with productivity and cause loss of yields (Van Asten *et al*., 2005; Sasser, 2013).

Previous findings in Uganda and elsewhere have revealed that the burrowing nematode (*R. similis*), the root-lesion nematode (*P. goodeyi*), the spiral nematode (*H. multicinctus*) and the root-knot nematode (*M. incognita*) are the most prevalent nematode species parasitizing bananas (Waele & Elsen, 2014). *Radopholus similis* has been found as the most damaging species (Plowright *et al*., 2013), causing severe root injury, substantial plant toppling, and lower bunch weight than *P. goodeyi* and *H. multicinctus* (Chitamba *et al*., 2013). Damage by *R. similis* and *H. multicinctus* have been linked to 15 to 50 percent production losses in East African highland bananas per crop cycle (Chitamba *et al*., 2013). Under poor crop management, *P. goodeyi* can cause serious damage to East African highland bananas (Waele & Elsen, 2014).

Soils that are compacted are a major constraint to banana productivity as they hamper penetration of soft, fleshy adventitious roots hence becoming physically restricted (Robinson and Galan, 2000). Banana production is further constrained by poor storage facilities that expose the product to damages during transport in addition to insufficient information on production (Hossain, 2014). Furthermore, restricted availability to and usage of improved planting materials has proven to be a major

impediment to the banana crop's full potential production along the value chain (Kikulwe *et al*., 2007; Kirimi *et al*., 2021). From a social standpoint, women confront a number of gender-based limitations, including a lack of authority over property (land) and a meagre portion of the income generated from banana sale (Ajambo *et al*., 2017).

2.7 Feeding in plant parasitic nematodes

The transition of nematodes into phyto-parasites has taken place many times, resulting in different modes of interaction with the banana plant (Smant *et al*., 2018). Nematodes are obligate biotrophic parasites that derive nutrition from living cells (Palomares *et al*., 2017). The action of several genes during host infection causes nematode parasitism (Gregory *et al*., 2017). Plant parasitic nematodes derive nutrition from living plant tissues with the aid of a stylet that punctures the host cell wall (Helder *et al*., 2014). Before feeding, the saliva containing enzymes is injected into the host cells to digest cell contents into a free-flowing nutritious liquid that can pass with ease through the extremely narrow stylet opening and channel (Esser, 1999).

2.7.1 Burrowing nematode (*Radopholus similis*)

The burrowing nematode is a migrating endo-parasitic nematode that causes cortical deterioration (Sekora and Crow, 2012) and toppling disease in *Musa* spp. (Tholkappian and Rajendran, 2011). *Radopholus similis* is thought to have arrived in Africa via contaminated banana suckers (Blomme *et al*., 2012). *Radopholus similis* is considered Australasian in origin (Hahn *et al*., 1996). In Kenya, *R. similis* predominates smallholder farms in the coastal areas and across the Lake Victoria belt

through Busia and Kakamega (Kashaija *et al*., 1994; Price, 2006). The nematode is confined to lower altitude areas (Elsen *et al*., 2000; Coyne *et al*., 2018).

At a temperature of 24 to 32°C, the burrowing nematode's life cycle spans 20 to 25 days from egg to adult (CABI, 2014). As they move via the roots, the female *R. similis* lays four to five eggs per day for two weeks (CABI, 2014). After 8-10 days, the eggs hatch into second-stage juveniles, and the juvenile stages take 10 to 13 days to complete. The second-stage juvenile either completes its life cycle within the root or migrates to additional healthy host plant roots. After entering the roots, the nematodes occupy an intercellular site in the cortex, where they feed on the root cells (CABI, 2014).

2.7.2 Root lesion nematode (*Pratylenchus* spp.)

The lesion nematode is an endo-parasitic migratory cortical feeding nematode (Deimi *et al*., 2009) that causes necrotic lesions on the surface of damaged roots (Davis and MacGuidwin, 2005). Originally, *Pratylenchus* is a Pacific and Pacific Rim species (Bridge *et al*., 1997) but currently with a worldwide distribution. *Pratylenchus goodeyi* is thought to have a limited distribution in East Africa highlands and predominates in altitudes above 1200 m above sea level (Price, 2000). The importance of this nematode on banana across East Africa highlands is gaining attention (Coyne, 2009).

Pratylenchus spp enters the soil from heavily necrotized roots and subsequently reaches the root tip's growth section. Eggs hatch in 15-17 days, juveniles in 15-16 days and adults in 15 days before egg-laying (CABI, 2014). The lesion nematode does not cause considerable damage to the banana plant at low levels of infestation.

However, an infection at high densities, can lead to water and nutrient stress (Talwana *et al*., 2016). A microscopic study confirms the presence of *Pratylenchus* spp. in banana roots (Perry and Ploeg, 2010).

2.7.3 Spiral nematode (*Helicotylenchus multicinctus*)

The spiral nematode is a semi-endoparasitic nematode (Coyne *et al*., 2007) that partly burrows into the roots of banana and extracts its nutrition from cortical cells (Talwana *et al*., 2016). The nematode feeds on the host plant's cell walls before moving on to other feeding sites in the root cells, causing substantial root damage (Coyne *et al*., 2014) that may result in the toppling of banana plants. The nematode feeds from a single food cell, where it also lays eggs. The eggs hatch into a second-stage juvenile, then a third-stage juvenile, and finally an adult (CABI, 2014).

2.7.4 Root-knot nematode (*Meloidogyne* spp.)

The root-knot nematodes *Meloidogyne incognita* and *Meloidogyne javanica* are known to attack the roots of bananas (De Waele and Davide, 1998). *Meloidogyne arenaria* is also a pest of bananas. Root-knot nematode is considered a sedentary endoparasite with a wide host range. The second stage juvenile is the infective stage commonly isolated from roots and soil (Tanimola, 2013). The nematode creates galls on damaged plant root tissues (Helder *et al*., 2014). Between 30 and 40 days, a female produces 300 to 800 eggs, which hatch into the juvenile stage. The juveniles pierce the host plant's root tips, triggering the formation of large cells in the root tissues (CABI, 2014). This obstructs the passage of water and nutrients, resulting in stunting.

The plant finally wilts as the leaves turn yellow. *Meloidogyne* spp. have a diverse range of hosts (Eric and Mellisa, 2005) and it is known to infect all plants causing significant damage, though it has not been considered a major banana pest (Agrios, 2005).

2.7.5 Other nematodes associated with banana

Besides the potentially parasitic nematodes of banana, other nematode species have been reported in association with bananas, of which, some are believed to be damaging to banana but there is scanty research metrics to demonstrate their damage potential in causimg desease. Moreover, these PPN live as a community with species already identified as key banana parasites (Quénéhervé and Forgain, 2005). They include; *Rotylenchus reniformis*, *Hoplolaimus pararobustus* and *Cephalenchus emarginatus* (Adiko, 1988).

2.8 Control and management of Plant Parasitic Nematodes on banana

Three interacting elements primarily determine the completion of the nematode life-cycle; the host plant, the species of the parasite and the environment (Talwana *et al*., 2016). All management tactics are intended to influence this 'pest triangle' by managing one or more of these variables. The two major constraints to controlling plant parasitic nematodes in Africa are inadequate nematological knowledge largely nematode biology and inadequate sustainable control measures (Talwana *et al*., 2016), particularly for small-scale farmers.

Nematode control worldwide has been achieved primarily by the application of nematicides like synthetic carbamate and organophosphates that have been classified as toxic or highly toxic (Tholkappian and Rejendram, 2011). Many of these chemical

compounds have been gradually phased out of the market in recent years due to their deleterious impact on humans, animals, and the environment (Tholkappian and Rejendram, 2011). To cause toxicity against PPN, organophosphates and carbamates must be applied at a reasonable temperature and moisture level (Delcour *et al*., 2014). Temperature fluctuations and irregular precipitation as a result of global warming, on the other hand, could impact the permanence and penetration of nematicides in the soil (Delcour *et al*., 2014).

Demand for nematicides increases once nematode challenges in crop production become more severe as a result of climate change (Gatto *et al*., 2016). Furthermore, research has demonstrated that using synthetic insecticides to control pests in crops boosts productivity (Atungwi *et al*., 2009). However, there is a likelihood of misuse of these synthetic pesticides that may result in pollution of the environment and affect negatively nature's equilibrium (Tholkappian and Rejendram, 2011). Further, the acquisition of nematicides is costly and some are not available to farmers (Talwana *et al*., 2016).

Consequently, alternative integrated PPN management strategies have been developed in *Musa* cultivation systems with the support of different stakeholders who among them are growers, researchers and extension officers. This technology amalgamates preventive and curative alternatives in a harmonious approach. Control of PPN is aimed at maintaining nematode populations below the economic injury levels (Stoian *et al*., 2018). The common IPM strategies utilized include prevention by use of certified clean planting materials, check suspect materials before planting, clean equipment before moving to another farm and avoid using contaminated

irrigation water (William, 2012). Besides, cultural strategies like crop rotation, use of resistant varieties and removing plants with symptoms have been exploited and yielded results (Yang *et al*., 2015).

Further, the incorporation of soil amendments like green manure (Perry, 2014), animal manure, sesame chaff and a mixture of beneficial microbial; steam sterilization and root pruning (Chitwood, 2003) have been utilized successfully and proven to be environmentally friendly. Biocontrol is an appropriate strategy that can be amalgamated with other measures. This strategy utilizes bacteria, fungi and other predators to directly kill by feeding or weaken the parasite from colonizing its host plant (Bridge, 2000). The fungus *Paecilomyces lilacinus* and the bacteria *Bacillus thuringiensis* var. kurstaki served as the foundation for this strategy. Of recent, the bacterium *Corynebacterium paurometabolum* has been included in this mode of control, in addition to mycorrhizae from the *Glomus* genus (Fernandez & Gonzalez, 2005).

2.9 Climate change and nematode distribution

In preserving global food stability, global warming has become a big challenge. Climate change is anticipated to raise the median temperature by 1.4–5.5°C and the median precipitation by 2% to 20% by the end of the twenty-first century (Adhikari *et al*., 2015). Moreover, since 1950, global temperatures have risen by around 0.72°C (Hartmann *et al*., 2013). Despite the fact that agriculture is a significant part of many countries' economies, Sub-Saharan Africa's development processes are heavily reliant on rainwater and hence sensitive to climate change.

The temperature in East Africa is shockingly expected to rise due to climate fluctuations, and this change is predicted to occur sooner that the rest of the planet, which could reach 2°C by the middle of the twenty-first century and 4°C by the end (Niang *et al*., 2014). The dynamics of nematode pests in these agro-ecologies will be affected by rising temperatures, resulting in shorter life cycles and faster pest accumulation (Coyne *et al*., 2018).

Furthermore, global warming has resulted in a shift in the distribution spectrum and the emergence of new hosts (Okulewicz, 2017) due to movements towards higher altitudes as a response to higher temperatures (Walther *et al*., 2002). Moreover, climate warming resulting in increased carbon dioxide and ambient temperature will directly affect plant pathogenic nematodes by interfering with their growth and indirectly by altering the physiology of the host plant (Adhikari *et al*., 2015). Besides, with global warming spreading nematode problems to newer areas, PPN distribution ranges across geographies may extend (Somasekhar and Prasad, 2011). This movement allows a species to overcome a natural barrier, thus colonizing new geographic regions from which it was previously excluded.

Worthy to note, in Eastern African Highlands, a number of PPN species infect banana with *R. similis* found to be the most damaging species and considered predominant up to about 1,400 m above sea level (Coyne and Kidane, 2018). Above this altitudinal elevation, *P. goodeyi*, is considered more damaging replacing *R. similis*, thus, establishing a thermo-demarcated divide between the two species based on their preferred temperatures (Speijer and De Waele, 2001). Moreover, findings elsewhere indicate the altitudinal gradient of *R. similis* is expected to rise

proportionally due to an increase in temperature, subjecting bananas to this aggressive nematode with a temperature increase of 1°C, leading to a change in altitude of 170-m (Coyne *et al*., 2018).

2.10 Factors affecting nematode parasitism

The distribution and densities of banana nematodes vary with cultivars, age of plantation, soil texture and pH due to genetic differences. Soil type has demonstrated to influence nematode community composition. In Ivory Coast, *H. multicinctus* was observed to be predominant both in soil and roots in organic soils while *R. similis* predominated in mineral soils (Gowen and Quénéhervé, 1990). Seasonal temperature and rainfall fluctuations greatly influence banana plant development and nematode reproduction in the roots of the plants (Van den Bergh *et al*., 2005). Notably, *H. multicinctus* has been noted frequently in soils of high clay levels, silt and low PH (Gowen and Quénéhervé, 1990). A great diversity in nematode community structure occur in the soil (Gowen and Quénéhervé, 1990).

Besides, banana genotype of differing ploidy have demonstrated variability in terms of nematode invasion with *R. similis* and *H. multicinctus* invading and reproducing successfully (Barekye, 2000). Elsen *et al*. (2000) observed that EAHB were susceptible to *P. goodeyi* as their bunch weight significantly correlated negatively to its population densities. Further, in mixed populations with *R. similis*, high damage has been reported on roots in addition to plant growth decline in comparison to *P*. g*oodeyi* alone (Gaidashova *et al*., 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study areas

A survey was conducted from November 2018 to July 2019 in 12 major banana producing areas of Kenya with varying altitude ranges as shown in Appendix 1. The study areas were selected based on banana production data (Soko Directory, 2018) and altitudinal elevation. An average of 15 samples of soil and roots were taken for each of the two banana cultivars selected for the study (East African Highland banana and Cavendish cultivar) from every banana production area for laboratory assays.

3.2 Farmers' knowledge of banana nematodes

To gather geophysical, agricultural, and historical information in the context of the sample sites, crops, habitats and production practices, a structured questionnaire (Appendix 2) was provided to randomly selected farmers alongside sample collections. This was aimed at assessing farmers' knowledge regarding nematodes as a pest affecting banana production. The questionnaire was administered to every farmer whose farm was selected for sampling. Banana production data (Soko Directory, 2018) in areas where sampling was done demonstrated a population of 26 farmers planted both Cavendish and East African highland banana cultivars on their farms. Sample size determination was computed through the Taro Yamane (1967) formula, thus; $n = N/ (1+ N (e)^{2})$

Where n = sample size required

N = Population under study

e^2 = Level of precision, e = 0.05

Therefore, with the formula above, each study site (N =26) and a 0.05 level of precision projected a sample size of 15 farmers and thus, from the 12 study sites (12×15) a hundred and eighty (180) farmers were selected and interviewed for this study.

3.3 Research design

Twelve major banana producing areas in Kenya were selected for this study. For every production area, fifteen banana farms were selected for sampling following a purposive sampling technique. In reference to the banana plantations of one farmer, the term farm was used. A farm was selected based on having both dessert banana (Cavendish) and cooking banana (EAHB) cultivars (Figure 3.1 A and B). A farm that lacked either of the cultivar was not considered for sampling. Besides, a farm with at least fifteen banana mats for both cultivars was sampled. Moreover, care was taken such that the selected farms were at least 1 km apart. For each of the farms visited, the latitude and longitude coordinates were recorded using a GPS receiver and the elevation using an altimeter.

Figure 3. 1: Dessert banana (A) and cooking banana (B), sampled during the study (Photo: Nyang'au, 2018)

One hundred and eighty (180) farms were selected for the study. Three hundred and sixty (360) samples were collected for the Cavendish cultivar of which 180 were root samples and the other 180 were soil samples with a similar number for East African Highland banana cultivar

3.4 Sampling Procedure

To assess the occurrence, abundance and distribution of PPN associated with Cavendish and EAHB cultivars across altitudes, rhizosphere soil and root samples were taken randomly from each banana farm selected for the study. On each farm, three random mats per banana genotype were sampled. A hole (approx. 20 × 20 × 20 cm) was dug using a sterilized Jembe on the side of the banana mat to expose the banana roots. For every banana mat, five roots (measuring approx. 10 cm each) were removed mixed homogenously and five roots were picked randomly for nematode analysis. The roots were split longitudinally, with one-half of each of the five roots being graded using the root cortex necrosis technique described by Coyne *et al.*

(2014). Upon necrosis examination, all root and soil samples (from the three banana mats) from the same farm and same banana variety were bulked up to form a composite sample.

Collecting soil and root samples in the same sampling bag helped maintain the roots fresh for a relatively long time before analysis in the laboratory. The root and soil samples were sealed and labeled with orchard information, location and collection date, then transported to the laboratory in a cool box and preserved at 10°C before processing was done.

3.5 Nematode extraction from roots

The five functional root segments that were assessed for necrosis in the laboratory were used to extract nematodes. The roots were put aside from the soil sample, carefully washed, and blotted dry between paper towels before being chopped into 1 cm lengths and mixed randomly. A sub-sample weighing 5 g was drawn and set up for nematode extraction for 48 hours following the adapted Baermann technique (Coyne *et al*., 2014). The sieve and root cuttings were submerged in tap water for 48 hours after being placed on a tray with just enough water to submerge the sieve and materials. The nematodes exited the roots and entered the dish through the tissue paper. The nematodes suspension was then put through a 25 μm sieve and the nematodes were back-washed with a stream of water before being collected in a 25 ml beake with water. The nematode suspension was kept in the fridge at 10°C awaiting further processing and identification.

3.6 Nematode extraction from the soil

A soil sub-sample of 100 ml was used for extraction following the adapted Baermann funnel technique as defined by Coyne *et al*. (2014). A plastic mesh was placed on a plastic tray, which was then covered with a paper towel on the bottom and sides. The soil was evenly spread, then a steady stream of clean water was poured down the inside edge of the collecting tray until the soil layer was just wet, and the tray was left undisturbed for 48 hours (Figure 3.2). The nematodes exited the soil and passed via the paper towel into the dish. The nematodes suspension was then put through a 25 μm sieve and the nematodes were back-washed with a stream of water before being collected in a 25 ml beaker with water.

Figure 3.2: Modified Baermann technique used in the extraction of nematodes from root and soil samples. (Photograph: Nyang'au, 2018)

3.7 Quantitative analysis of plant parasitic nematodes

By allowing the nematode suspension to settle for 1 hour and gently removing excess water with a tiny pipette, the suspension was concentrated to 10 ml in glass vials. A pipette was used to retrieve 2 ml of the homogenous suspension, which was then placed into an open clear plastic counting dish with grids carved on the bottom. Counting of nematodes was done using a Leica MZ12 dissecting stereo microscope with under stage lighting at ×20 magnification. A hand tally counter was used to count nematodes. Counting was conducted three times to acquire the mean used to quantify the total nematodes in a sample suspension. Nematodes counted were represented as the number of nematodes, respectively, in 5 g fresh roots and 100 ml soil.

3.8 Plant parasitic nematode processing and identification

The nematode suspension was concentrated to 10 ml to facilitate identification. Nematodes were killed for 1 minute by a hot water bath technique at 65°C by gently shaking. After heating, the nematode suspension was kept out of hot water to establish a room temperature (Roy *et al*., 2014). Killed nematodes were then fixed in 4 percent formalin (Coyne *et al*., 2014) and kept separately in labeled vials. A 1 ml aliquot of the fixed nematode suspension was removed using a pipette and mounted on a slide.

With a Leica 2500 compound microscope at ×20 magnification set with under stage lighting, nematodes were identified to the genus level based on morphological characteristics according to Hooper *et al.* (2005), Siddiqi (2000) and the University of Nebraska Lincoln nematode identification website (http://nematode.unl.edu/konzlistbutt.htm). Photographs were captured at ×20 magnification.

3.9 Assessment of nematode abundance and distribution patterns

Relative abundance with which each genus was represented in areas sampled was determined by Simpson's index of diversity;

The formula; $(DS) = \sum (ni/N)^2$ (Simpson, 1943).

Where;

DS = Simpson's Diversity index

n*i*= the number of individuals of genera

N = the total number of genera in the sample

Shannon-Weiner index was used to calculate nematode genera diversity to determine genera variations in the areas sampled.

$H^1 = -\sum^{S}_{i=1} PilnPi$

Where;

$\sum$ = "the sum of"

S = the number of genera in the community

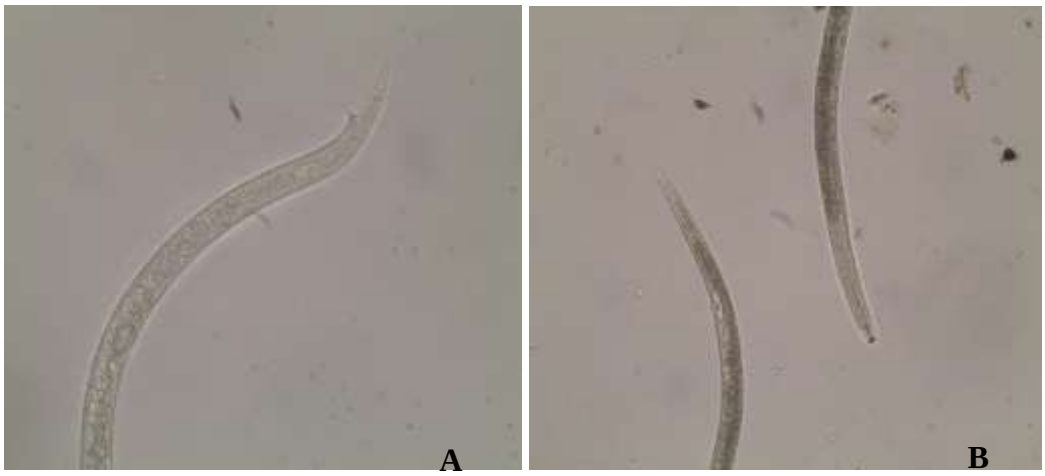
P*i* = Relative abundance (proportion) of the i^{th} genera in the community

ln = Natural log (Shannon and Weaver, 1963).

3.10 Assessment of pathogenicity of *Pratylenchus goodeyi* on banana

A pot experiment was set up under a shade at the International Centre of Insect Physiology and Ecology in Nairobi (1600 masl) to assess the effect of banana type and altitude on pathogenicity of *P. goodeyi* with a repeat experiment set up under a shade on a farmer's field in Kisii (1300 masl). Two banana cultivars were tested (dessert type - sweet banana and cooking type - Ng'ombe) on three *P. goodeyi* populations from three altitude ranges (1100 masl, 1300 masl and 1600 masl).

At the hardening stage, banana plantlets were purchased from a commercial tissue culture (TC) laboratory and transplanted into 1.5-liter pots filled with steam-sterilized soil. As the prominent PPN affecting bananas in Kenya, *P. goodeyi* (figure 3.3) was selected from three populations: Oyugis (1100 masl), Embu (1300 masl) and a farm in Nairobi (1600 masl) areas.



×20 magnification

Figure 3.3: A-Tail of male *P. goodeyi*, B-Head & Tail of female *P. goodeyi* (Photographs: Nyang'au, 2018)

The Nairobi population of nematodes was obtained from a farm maintained by the International Institute of Tropical Agriculture (IITA) for rearing *P. goodeyi*. Banana plantlets were inoculated at the rate of 2,000 nematodes per pot with banana root tissues containing nematodes. The root segments were applied around the plant and covered with soil within a 3 cm radius. Both experiments were replicated 10 times, including control that was not inoculated, and arranged in a completely randomized design. Data on plant height was recorded every 30 days for four months. The 120-day post-inoculation period was reached in both experiments. Plant growth parameters (fresh root weight, shoot weight and number of surviving leaves) and root necrosis data were collected at the end of each experiment. Additionally, the nematode reproductive factor (Rf) was calculated using the formula (Barekye *et al*., 2000).

$$Rf = \frac{\text{Final population}}{\text{Initial population}}$$

3.11 Preparation of *Pratylenchus goodeyi* inoculum

Fresh banana roots were washed and wiped dry with a paper towel before being sliced into 1 cm lengths and mixed. Using a blender, a sub-sample of 10 g was pulled for nematode extraction by the maceration method for 20 seconds (two-10 seconds periods separated by a 5-second interval). Banana root debris together with the nematodes was poured via nested 53 µm sieve and 25 µm sieves. Nematodes trapped by the 25 µm aperture sieve were gently back-washed with a stream of water and the suspension was collected in labeled glass vials. After one hour of settling, the nematode suspension was concentrated to 10 ml by exiting excess water using a micropipette. For nematode quantification, an aliquot of 2 ml was drawn then computed as the nematode population in 10 g of fresh root weight.

Counting was done thrice to obtain the average number of nematodes. The quantified nematodes were used to compute and establish the weight of fresh banana roots in grams, which delivered 2,000 vermiform nematodes from the three populations required for inoculation per pot of a tissue culture banana. Naturally infected banana root segments were used as inoculum due to unavailability of monoxenic cultures of *P. goodeyi* that has demonstrated to be a feasible alternative previously (Coyne *et al*., 2010). Furthermore, employing the traditional approach on carrot discs, monoxenic culturing of a number of *Pratylenchus* species has proven not to yieid success (Santos *et al*., 2012).

3.12 *Pratylenchus goodeyi* inoculation procedure

Plantlets were inoculated with root segments whose weight was similar to the inoculum level (2000 nematodes). *Pratylenchus goodeyi* was introduced after two weeks of establishing the plantlets in the pots. The soil was gently removed to expose the roots during inoculation (Barekye *et al*., 2000). At the base of each banana plantlet, three holes (0.5 cm diam. x 1.5 cm depth) were dug and inoculated with roughly 2,000 nematode-containing banana root segments. After inoculation, soil was spread on banana roots and a uniform watering regime of 1 litre maintained twice weekly for twelve weeks.

3.13 Extraction of *Pratylenchus goodeyi* for analysis

The banana plants were maintained and watered twice weekly to ensure sufficient soil moisture for easy penetration of roots by nematodes. The experiment was terminated 120 days post plant inoculation with nematodes. During termination, plant roots were gently cleaned with a stream of tap water to remove soil and plant growth parameters (height, leaf count, root and shoot weight), root damage expressed as percentages and nematode population density per root system were established. Banana roots were chopped to lengths of 1 cm, and nematodes extracted from 10 g following the adapted Baermann funnel technique for 48 hrs. The nematode suspension was collected in a beaker filled with 25 ml of water and nematodes left to settle for one hour then followed by reduction of suspension volume to 10 ml by removing excess water using a pipette. Nematodes in 2 ml aliquot were taken from a homogenized 10 ml suspension for counting. Counting was repeated three times. Data were means of 10 replicates.

3.14 Assessment of root necrosis

Root necrosis percentages by farm (composite of root sample from three mats) were assessed on five randomly selected functional root pieces and for the trial experiment, on five random selected root pieces, from each banana plantlet as described in Coyne *et al.* (2014). Functional roots were counted per 20 × 20 × 20 cm soil volume. Fifteen functional roots were selected from three mats and mixed homogenously. A total of five functional roots were chosen at random. Their lengths were cut to about 10 cm and they were split longitudinally. The degree of necrosis in the root cortex was rated in half of each of the five roots. The highest root necrosis per root segment was capped at 20%, resulting in a total root necrosis of 100% for one sample of five root halves (Coyne *et al*., 2014).

3.15 Data Analysis

The data from farmer interviews was coded and analyzed using the Statistical Package for Social Sciences (SPSS) version 16.0. Means of various nematode species observed in the survey and from pot trials were computed. Arcsine (x) was used to transform percentage root necrotic indices from the survey and pot trials, while data from nematode counts was log-transformed [$\log_{10}$ (x+1)] to assume normal distribution. R version 3.5.1 was used to perform analysis of variance (ANOVA) on the data (R Core Team, 2015) and MS Excel computer programs. Regression analysis was executed to explore respective causal relationships amongst the nematode population with respect to root necrosis and fresh root weight. The separation of means was computed by the Tukey's test of significance at $P \leq 0.05$. To summarize the data, descriptive statistics were employed.

CHAPTER FOUR

4.0 RESULTS

4.1 Occurrence of plant parasitic nematodes genera in bananas in Kenya

Fourteen (14) genera of plant parasitic nematodes belonging to eight families were found associated with Cavendish and East African highland banana cultivars in mid (1100-1600 masl) and high (1601-2000 masl) altitudes in the 12 major areas of production surveyed (Table 4.1).

Table 4.1: Plant parasitic nematodes genera extracted from roots and of banana and rhizosphere soil in 12 major production areas

Family	**Genus**	**Root**	**Soil**	**Banana cultivar**
		Altitude range[1]	Altitude range[1]	
Pratylenchidae	*Pratylenchus*	Mid, High*	Mid, High*	EAHB*, Cavendish
	Radopholus	Mid	Mid	EAHB, Cavendish
Hoplolaimidae	*Hoplolaimus*	Mid, High	Mid, High	EAHB, Cavendish
	Rotylenchus	Mid	Mid	EAHB, Cavendish
	Helicotylenchus	Mid*, High	Mid*, High	EAHB*, Cavendish
	Scutellonema	Mid, High	Mid, High	EAHB, Cavendish
Aphelenchoididae	*Aphelenchoides*	Mid, High	Mid, High	EAHB, Cavendish
	Aphelenchus	Mid	Mid	EAHB, Cavendish
Meloidogynidae	*Meloidogyne*	Mid, High	Mid, High	EAHB, Cavendish
Tylenchulidae	*Tylenchus*	Mid, High	Mid, High	EAHB, Cavendish
	Filenchus	Mid, High	Mid, High	EAHB, Cavendish
Tylenchorhynchidae	*Tylenchorhynchus*	Mid		EAHB, Cavendish
Longidoridae	*Longidorus*	Mid	Mid	EAHB, Cavendish
Trichodoridae	*Trichodorus*	Mid		EAHB, Cavendish

[1] Mid altitude =1100-1600 masl, High altitude = 1601-2000 masl; * indicates that genus occurrence was significantly higher in the altitude range and banana cultivar

The important PPN found to be associated with bananas were *Pratylenchus goodeyi, Helicotylenchus multicinctus, Meloidogyne* spp. and *Radopholus similis* as indicated in table 4.2. These four parasitic nematode species accounted for 91.7 % of the total PPN population.

Table 4.2: Frequency of occurrence of nematode genera recovered from banana production areas in Kenya

Nematode	Mean Density*	Frequency (%)
Pratylenchus goodeyi	105 ±56.67	66.1
Helicotylenchus multicinctus	26 ±18.91	16.3
Meloidogyne spp.	14 ±12.31	8.9
Hoplolaimus spp.	4 ±3.01	2.6
Filenchus spp.	4 ±2.71	2.3
Longidorus spp.	2 ±1.61	1.3
Trichodorus spp.	2 ±0.87	0.9
Aphelenchoides spp.	1 ±0.54	0.4
Rotylenchus spp.	0.49 ±0.21	0.3
Radopholus similis	0.40 ±0.18	0.3
Tylenchus spp.	0.33 ±0.06	0.1
Aphelenchus spp.	0.31 ±0.07	0.1
Tylenchorhynchus spp.	0.27 ±0.02	0.1
Scutellonema spp.	0.13 ±0.01	0.1

*Mean nematode density in 5 g of roots

4.1.1 Farmers' knowledge of banana nematodes

The study showed that both female and male farmers were involved in banana farming with males being more (51 %). Most farmers in the surveyed areas produced bananas in a mixed system of cultivation (95 %) with other crops majorly annuals like maize, kales and indigenous leafy vegetables while a few produced bananas on pure stands/mono-cropping system (5 %). The study revealed that 98 % of the farmers do not know that PPN attack bananas in their farms. However, a small

proportion of 2 % confirmed to have seen bananas topple in their farms but did not know the cause.

4.1.2 Effect of altitude and banana genotype on banana root necrosis

The *t*-test result on the effect of altitude on root necrosis was shown to be significantly higher ($p<0.05$) at high altitude compared to the mid altitude banana production areas (Table 4.3). Root necrosis at high altitude areas was rated at 14.91 compared to 8.53 in mid altitude areas (Table 4.3). Although root necrosis on EAHB (12.54) was slightly higher than on Cavendish (10.97), *t*-test results showed no significant differences ($p>0.05$) between the banana cultivars (Table 4.3).

Table 4.3: Effect of altitude and banana genotype on root necrosis

	Mean Necrosis (%)	Std Error	p-value
Altitude			
Mid	8.53	±1.05	0.000
High	14.91	±7.08	
Variety			
Cavendish	10.97	±6.71	0.303
EAHB	12.54	±9.03	

4.2 Population densities and distribution patterns of plant parasitic nematodes associated with two banana varieties at different altitudes in banana production areas

4.2.1 Mean population densities of nematodes recovered from banana production sites

Higher densities of PPN were isolated from Kakamega a high altitude (> 1601 m) area that had 508.08 nematodes associated with EAHB banana than Cavendish that had 462.87 nematodes as shown in Figure 4.1. A similar observation was made for Meru, with more PPN on EAHB that had 316.93 nematodes than on Cavendish that had 145.47 nematodes (Figure 4.1) The results further indicated that Bungoma a high altitude area (>1601 masl) had Cavendish cultivar supporting more PPN than EAHB cultivar though not statistically significant ($p<0.05$) as in figure 4.1

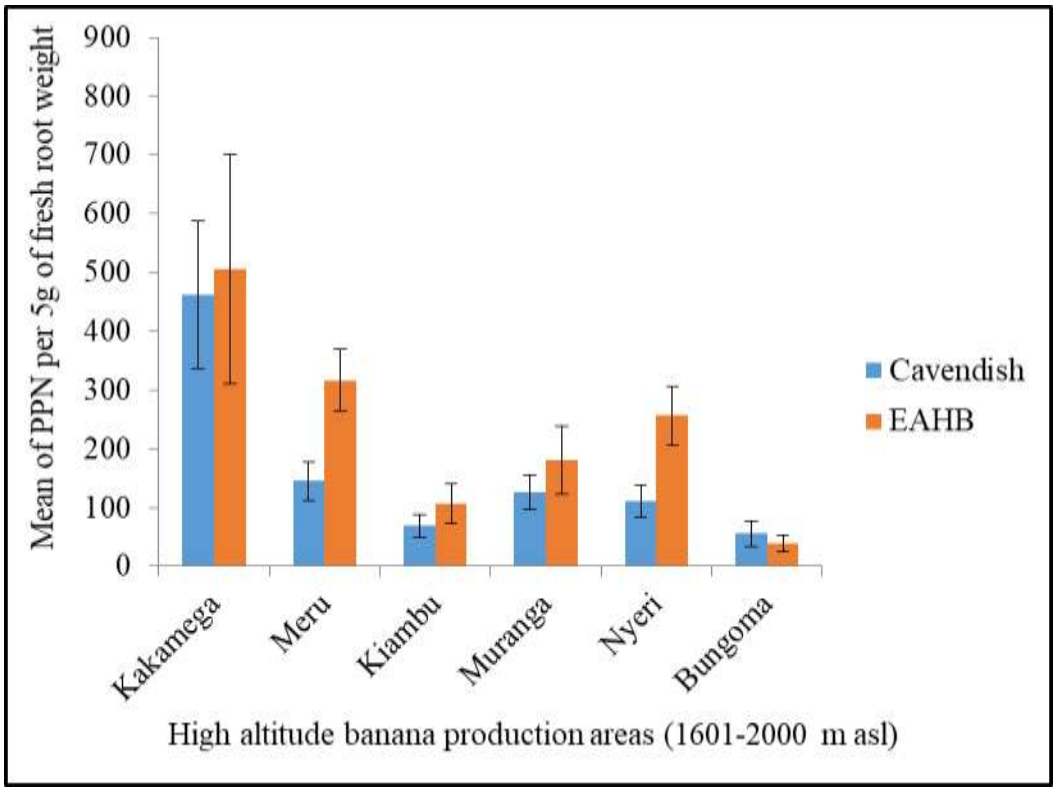


Figure 4.1: Mean population density of nematodes isolated from high altitude (1601-2000 m asl) banana production areas.

Embu banana production area, a mid altitude area (< 1600 masl), demonstrated to have more PPN associated with EAHB 648.47 differed significantly ($p<0.05$) with Cavendish banana 208.74 as indicated in Figure 4.2. Further, in Kisii EAHB supported 238.38 and Cavendish 126.69 (Figure 4.2). In Homabay, a mid altitude area (<1600 masl) more PPN were recovered from the Cavendish cultivar 249.83 while EAHB cultivar had 177.67 though the differences did not differ significantly ($p<0.05$) as shown in Figure 4.2. In Busia, both EAHB and Cavendish supported the least poppulations of PPN.

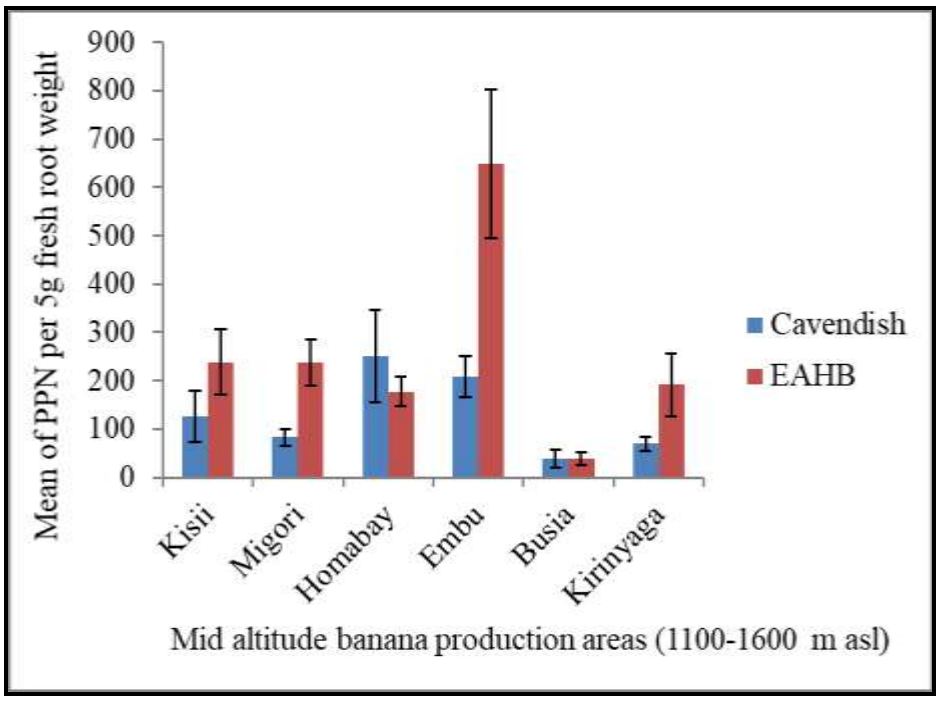


Figure 4.2: Mean population density of nematodes isolated from mid altitude (1100-1600 m asl) banana production areas.

4.2.2 Effect of banana cultivar on Plant parasitic nematodes distribution

4.2.2.1 Mean population density of nematodes in roots of banana across different banana production areas

The study compared the relative population densities of the PPN recovered from banana roots in Cavendish and EAHB cultivars across mid and high altitude areas. It was revealed that EAHB supported more *P. goodeyi* than Cavendish at high elevations (>1601 m) as indicated in Figure 4.3. The *t*-test results showed that the difference between Cavendish and EAHB roots was statistically significant ($p<0.05$). *Helicotylenchus* m*ulticinctus* on EAHB roots were 3.48 and 3.85 on Cavendish

cultivar (Figure 4.3). A *t*-test analysis showed no significant differences (p>0.05) between Cavendish and EAHB roots as indicated in figure 4.3.

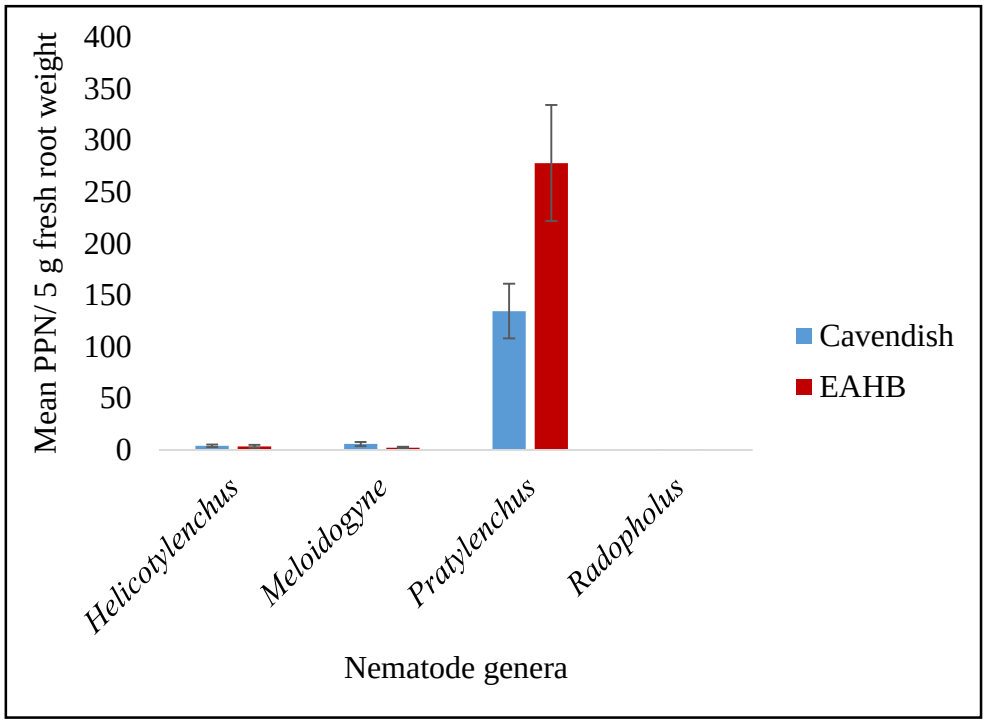


Figure 4.3: Mean density of nematode genera on Cavendish and EAHB roots in high altitudes (1601-2000 m asl)

Although *Meloidogyne* spp. was observed to be slightly more on Cavendish cultivar than on EAHB, it did not differ significantly (p>0.05) as shown in figure 4.3. An independent *t*-test further indicated that this difference in numbers was not statistically different (p>0.05). In addition, *R. similis* was not recovered from high elevations (> 1601 masl) for both Cavendish and EAHB cultivars (Figure 4.3).

In mid altitude banana production areas (1100-1600 masl), a similar and statistically insignificant ($p<0.05$) observation for *P. goodeyi* was made with 173.78 and 104.64 nematodes isolated from EAHB and Cavendish cultivars respectively as in Figure 4.4. *Helicotylenchus multicinctus* was noted to be high on EAHB with 31.72 nematodes compared to Cavendish cultivar at 18.71 nematodes (Figure 4.4). On the other hand, *Meloidogyne* spp. were recovered at uniform densities from EAHB and Cavendish banana cultivars. However, *R. similis* densities were negligible on EAHB roots with 0.49 nematodes while on Cavendish roots the nematodes were absent (Figure. 4.4).

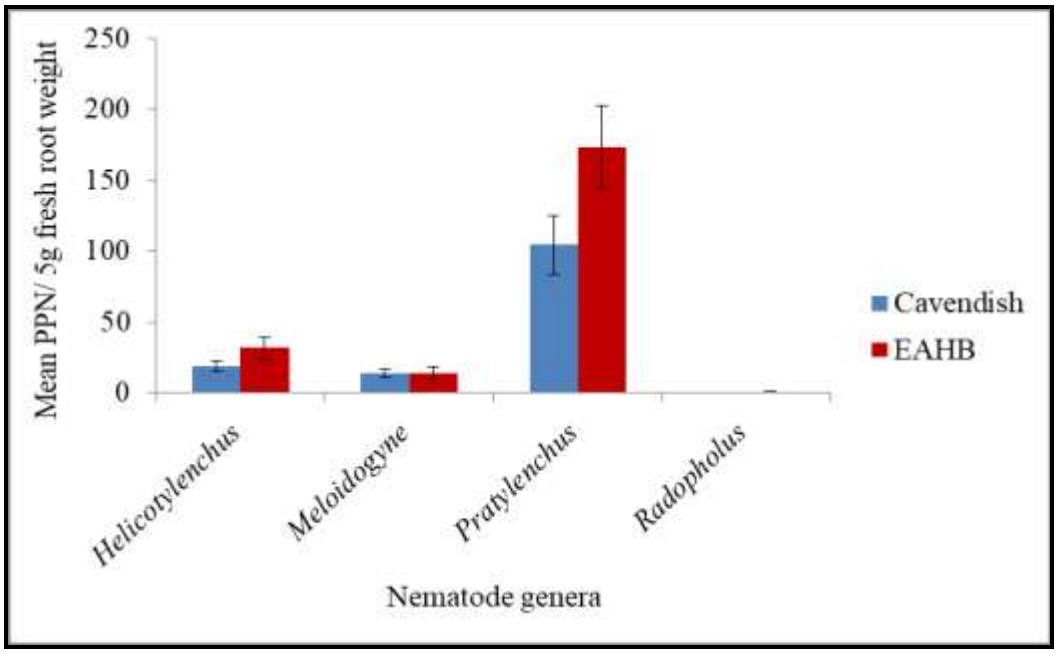


Figure 4.4: Mean density of nematode genera on Cavendish and EAHB roots in mid altitudes (1100-1600 m asl).

4.2.2.2 Mean population density of PPN in rhizosphere soil of banana in different production areas

The study compared the mean population densities of the PPN recovered from banana rhizosphere soils of Cavendish and EAHB across mid and high altitude areas of banana production. The study showed that *P. goodeyi* were more on EAHB compared to Cavendish cultivar at high altitudes (> 1601 masl) as shown in Figure 4.5. A *t*-test analysis showed no differences between soils of Cavendish and EAHB ($p>0.05$). *Helicotylenchus multicinctus* in EAHB soil occurred significantly ($p<0.05$) in low numbers than in Cavendish cultivar (Figure 4.5).

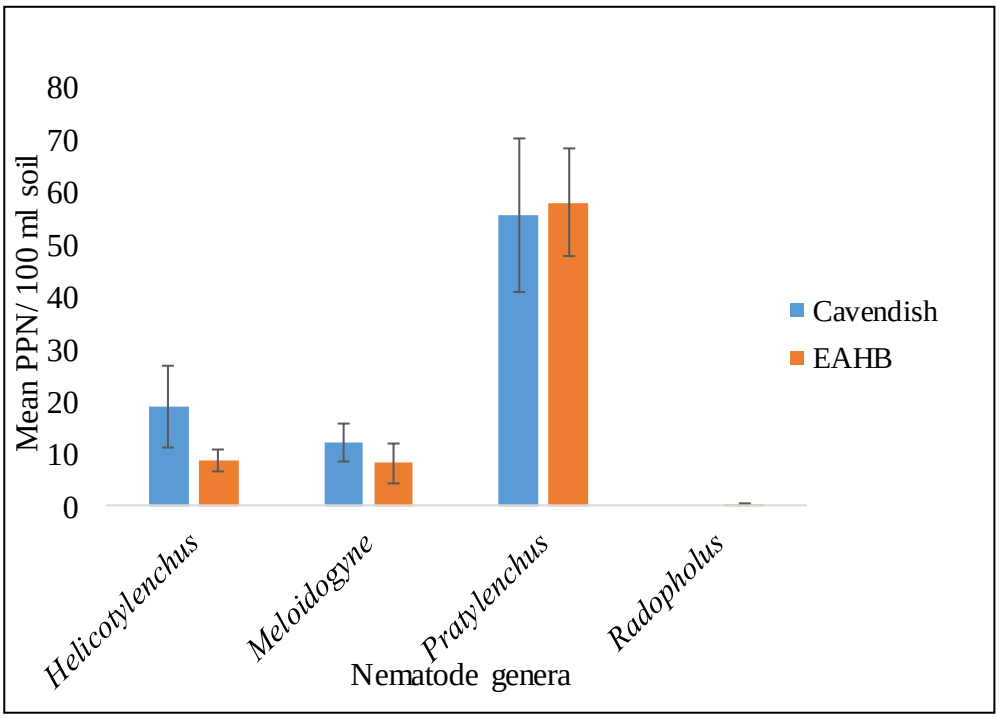


Figure 4.5: Mean density of nematode genera in Cavendish and EAHB rhizosphere soil in high altitudes (1601-2000 masl).

Although *Meloidogyne* spp. were more in Cavendish cultivar soils, there was low recovery reported in EAHB (Figure 4.5). An independent *t*-test indicated the difference to be insignificant ($p>0.05$). Besides, *R. similis* were recorded in low densities from EAHB cultivar while none was recovered from Cavendish genotype from high elevations (> 1601 masl) as indicated in (Figure 4.5).

In mid altitude banana production areas (1100-1600 masl), there were significantly ($p<0.05$) higher populations of *H. multicinctus* compared with the other nematode types on EAHB and Cavendish bananas while *R. similis* had the least nematode densities (Figure 4.6). An independent *t*-test showed the difference between those found in Cavendish and EAHB soil to be significant ($p<0.05$).

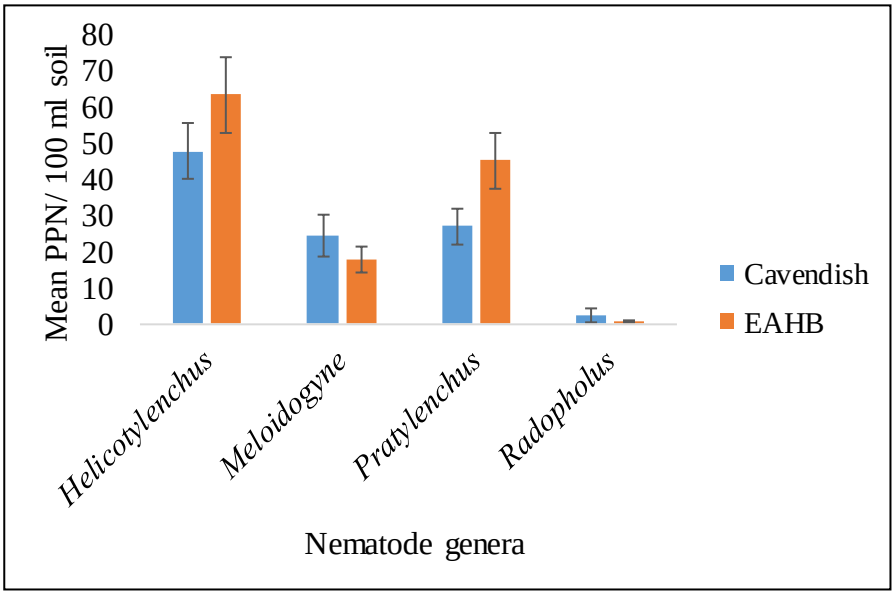


Figure 4.6: Mean density of nematode genera in Cavendish and EAHB rhizosphere soil in mid altitude (1100-1600 m asl)

4.2.3 Effect of altitude on nematode distribution in different banana production areas in Kenya

The population density and frequencies of the PPN was crosschecked against altitude to establish any relationships. There were higher densities of *P. goodeyi* that were isolated from EAHB in high elevations in banana producing areas compared to mid elevations (Figure 4.7). An independent *t*-test on the lesion nematode numbers between the two elevations showed the difference was significant ($p<0.05$). *Helicotylenchus multicinctus* was recorded in significantly ($p<0.05$) higher densities in mid altitudes than in high altitudes while *Meloidogyne* spp. was recovered in relatively higher densities from mid altitude that differed significantly ($p<0.05$) with those from higher altitude areas (Figure 4.7). On the other hand, *R. similis* registered extremely lower numbers in mid altitudes while it was not recovered from high altitudes (Figure. 4.7).

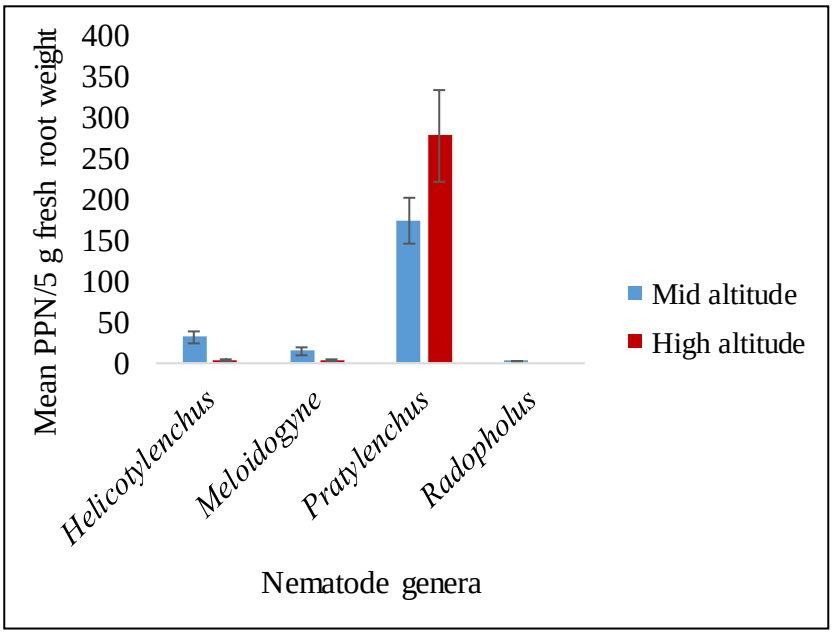


Figure 4.7: Mean density of nematode genera in EAHB roots in mid and high altitude banana production areas

Further, the study revealed that *P. goodeyi* was recovered at higher densities on Cavendish cultivar from high altitude areas compared to those from mid altitudes although the difference was not significant ($p>0.05$) according to an independent *t*-test (Figure 4.8). Further, significantly ($p<0.05$) higher *H. multicinctus* population was recorded in mid altitudes than in the higher altitudes (Figure 4.8). Although the population densities for *Meloidogyne* spp. were significantly higher ($p<0.05$) in mid altitudes compared with those from high altitude areas, lower densities were recorded for *R. similis* in mid altitudes with no recovery of the nematode made from high altitudes (Figure. 4.8).

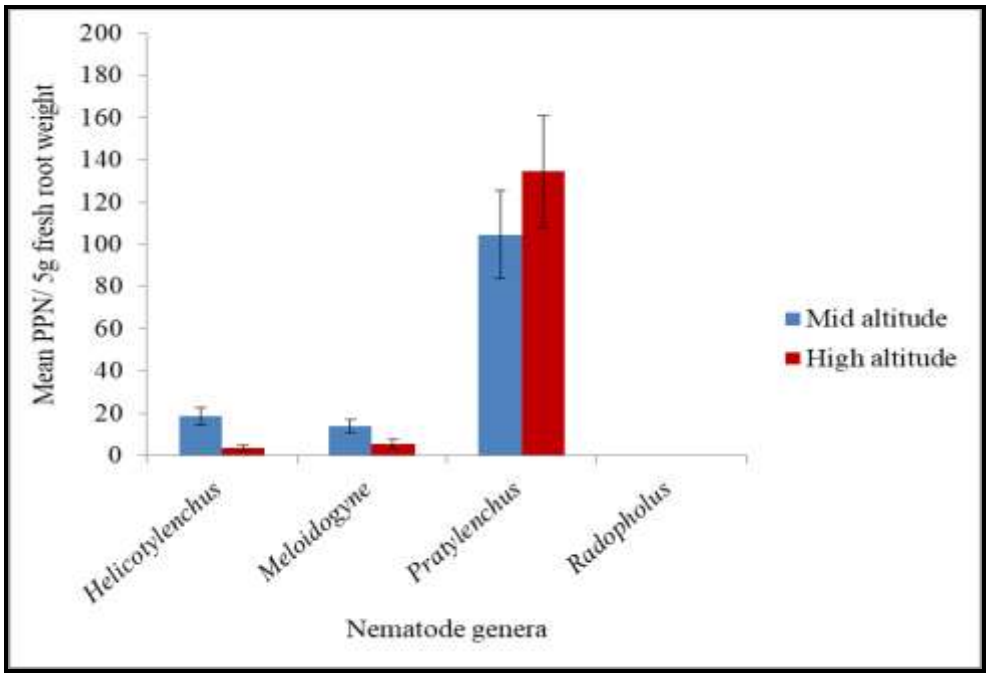


Figure 4.8: Mean density of nematode genera on Cavendish roots in mid and high altitude banana production areas.

Overall, results showed the densities of PPN being more in mid altitude sites (1100-1600 masl) compared to high altitude sites (1601-2000 masl) although the difference was not statistically significant ($p>0.05$) as indicated in Table 4.4. The population of *H. multicinctus* was higher in mid altitude areas compared to high altitude areas while that of *P. goodeyi* was higher in high altitude areas compared to mid altitude areas. The *t*-test further showed that the difference was statistically significant ($P<0.05$) for *Pratylenchus*. The occurrence of *R. similis* population in both mid and high altitude areas was similar and the difference was not statistically significant ($p>0.05$). The mean densities for *Meloidogyne* spp. in mid and high altitude areas of banana production was significantly different ($p<0.05$) with more of the nematodes occurring in mid altitude areas (Table 4.4).

Table 4.4: Effect of altitude on the distribution of plant parasitic nematodes

	Mid altitude	**High altitude**	**Std_Error**	**t-value**	**p-value**
Nematode genera	Mean Density				
Helicotylenchus	40.07	11.9	5.03	5.6	0.000
Pratylenchus	85.09	123.88	16.90	-2.3	0.022
Radopholus	0.783	0.1	0.63	1.1	0.282
Meloidogyne	20.75	7.67	3.00	4.35	0.000

4.2.4 Plant parasitic nematode genera diversity

There was no significant ($P>0.05$) difference in genera diversity across banana production areas. However, Shannon diversity indices indicated a slight variation among genera. The highest Shannon diversity index that did not differ statistically ($P>0.05$) from the other genera was recorded on *Pratylenchus*, while the lowest was recorded in *Radopholus* (Figure 4.9).

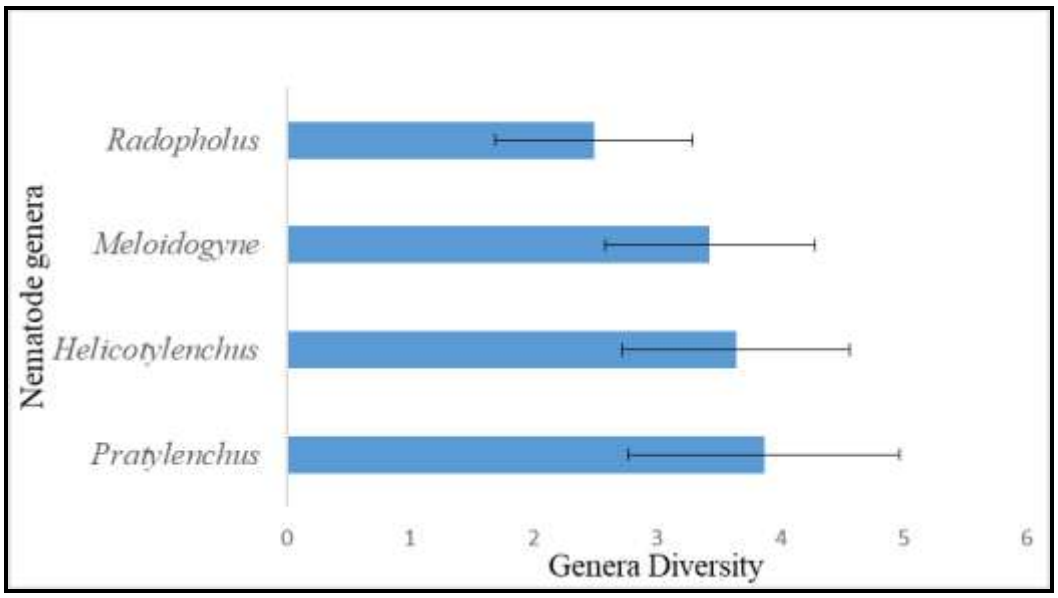


Figure 4.9: Genera diversity for plant parasitic nematodes

4.3 Pathogenicity of three *Pratylenchus goodeyi* populations on two banana cultivars

4.3.1 Banana root necrosis

The study was carried out across two trial sites in Kisii on a farmer's field under a shade (1300 m asl) and in Nairobi at ICIPE under a shade (1600 m asl). The study revealed average root necrosis of 5.8 % in Kisii and 8.5 % in Nairobi which did not differ significantly ($p>0.05$) as indicated in Table 4.5. Further, the average root necrosis did not differ significantly ($p>0.05$) between Ng'ombe cultivar and sweet banana cultivar. A *t*-test revealed that average root damage resulting from the feeding of the three populations was similar ($p>0.05$) as indicated in Table 4.5.

Table 4.5: Mean necrotic indices of two banana genotypes in Kisii (1300 m asl) and ICIPE (1600 m asl) trial sites

	Mean necrosis (%)	t-value	p-value
Trial site			
Kisii	5.8±9.01	1.25	0.218
ICIPE	8.5±11.93		
Banana type			
Ng'ombe	6.9±5.12	0.25	0.817
Sweet Banana	7.4±8.75		
Population		f-value	
Embu	8.3±11.60	9.86	0.273
Nairobi	15.1±14.08		
Oyugis	4.7±10.62		

Control differed significantly (P<0.05) from three *P. goodeyi* populations

A post ANOVA analysis was used to determine root necrosis variation among the three *P. goodeyi* populations. The results showed that the Nairobi population had a significantly (p=0.003) higher value than the Oyugis population, with a difference of 10.43% (Table 4.6). The severity of root necrosis, however, did not differ significantly between the Nairobi and Embu populations, nor between the Oyugis and Embu populations (Table 4.6).

Table 4.6: Mean necrosis indices in relation to the experimental site and banana genotype

Population	Contrast Dif(a-b)	Std. Err.	t- value	P>t	[95%_Conf	Interval]
NRB_vs_EMB	6.88	2.78	2.47	0.069	-.35	14.10
OYG_vs_EMB	3.56	2.86	-1.24	0.602	-10.99	3.88
OYG_vs_NRB	10.43	2.93	-3.56	0.003	-18.03	2.82

Key: EMB: Embu NRB: Nairobi OYG: Oyugis

Control differed significantly (P<0.05) from three *P. goodeyi* populations

4.3.2 *Pratylenchus goodeyi* population densities

For deeper analysis, the densities of three populations of *P. goodeyi* were cross checked against two trial regions and two banana cultivars. From the two trial sites, the density of the *P. goodeyi* was higher in ICIPE compared to Kisii although there was no significant difference ($p>0.05$) established (Table 4.7). The population density in relation to the banana cultivars varied significantly ($p<0.05$) as indicated in table 4.7. The average density of *P. goodeyi* in the Ng'ombe cultivar was significantly ($p<0.05$) higher compared to the sweet banana cultivar. Similarly, the nematode densities among the three populations differed significantly ($p<0.05$) as indicated in table 4.7.

Table 4.7: Densities of three *Pratylenchus goodeyi* populations on Ng'ombe and Sweet banana cultivars

	Mean	**t-value**	**p-value**
Trial region			
Kisii	266±78.28	-0.7	0.479
ICIPE	346±63.01		
Banana genotype			
Ng'ombe	487±62.65	-3.25	0.002
Sweet Banana	134±20.03		
Population		f-value	
Embu	577±49.81	4.76	0.0033
Nairobi	271±51.79		
Oyugis	376±39.97		

A post ANOVA test revealed variations in mean densities of *P. goodeyi* varied across the three populations which did not differ significantly ($p>0.05$) as shown in table 4.8.

Table 4.8: Post Hoc analysis among three populations of *Pratylenchus goodeyi*

P. goodeyi population	Contrast Diff(a-b)	Std. Error.	t	P>t	[95%_Conf	Interval]
NRB_vs_EMB	-285.66	149.56	-1.91	0.228	-674.06	102.73
OYG_vs_EMB	-180.50	153.85	-1.17	0.645	-580.05	219.03
OYG_vs_NRB	105.15	157.27	0.67	0.909	-303.28	513.59

Key: EMB: Embu NRB: Nairobi OYG: Oyugis

Control differed significantly ($P<0.05$) from three *P. goodeyi* populations

4.3.3 Reproductive fitness of three *Pratylenchus goodeyi* populations on two banana types

The degree of infectivity was crosschecked against two trial sites, two banana cultivars and three populations. From the two trial regions, the reproductive factor (Rf) of *P. goodeyi* was on average lower in Kisii compared to that of ICIPE (Table 4.9). However, the Rf differed significantly among the banana types with Ng'ombe recording significantly ($p<0.05$) higher Rf compared to sweet banana type (Table 4.9). A comparison of the reproductive factor of *P. goodeyi* populations was higher with the Embu, followed by the Oyugis while the Nairobi population had the lowest although the differences were not statistically ($p>0.05$) different (Table 4.9).

Table 4.9: Reproductive fitness levels of *Pratylenchus goodeyi*

	Mean	Std Error	t-value	p-value
Site				
Kisii	0.23	0.05	0.95	0.3455
ICIPE	0.35	0.11		
Banana genotype				
Ng'ombe	0.46	0.12	2.87	0.0048
Sweet banana	0.12	0.02		
P. goodeyi population			F-value	
Embu	0.45	0.16	1.78	0.1728
Nairobi	0.19	0.03		
Oyugis	0.23	0.06		

4.3.4 Plant growth with respect to banana cultivar

The study revealed that the corresponding *t*-test results for all the growth parameters did not differ significantly ($p>0.05$) as indicated in Table 4.10 and 4.11. Analysis of Variance (ANOVA) showed that growth of banana plantlets did not vary significantly ($p>0.05$) among the three populations of *P. goodeyi* ($p>0.05$) as indicated in table 4.10 and 4.11.

Table 4.10: Mean FRW, FSW, NSL and HT of banana genotypes inoculated with *Pratylenchus goodeyi* at ICIPE trial

ICIPE														
	Ng'ombe							Sweet banana						
	FRWg	FSWg	HT1	HT2	HT3	HT4	NSL	FRWg	FSWg	HT1	HT2	HT3	HT4	NSL
Control	57.7 ± 14.96	108.6 ± 23.03	18.7 ± 3.04	20.8 ± 3.39	21.9 ± 3.4	25.3 ± 3.75	4.9 ± 0.85	39.4 ± 9.39	171.4 ± 40.66	26.15 ± 1.54	28.15 ± 1.99	30.95 ± 5.14	32.45 ± 5.88	4.7 ± 0.8
Embu	40.9 ± 8.92	103.1 ± 23.01	22.65 ± 3.02	23.2 ± 3.79	26.5 ± 5.53	27.15 ± 6.45	4.8 ± 0.83	49.5 ± 12.22	156.5 ± 41.52	24.8 ± 1.71	26.2 ± 1.98	27.85 ± 5.61	32.78 ± 6.3	4.1 ± 0.94
Nairobi	44 ± 11.52	102.5 ± 25.99	17.55 ± 2.71	19.9 ± 3.2	20.01 ± 3.52	23.04 ± 4	4.7 ± 0.8	43 ± 13.25	178.9 ± 35.78	26.1 ± 1.21	27.34 ± 1.57	31.05 ± 4.72	31.4 ± 5.39	4.8 ± 0.84
Oyugis	55.1 ± 13.68	135.8 ± 31.95	20.2 ± 2.46	20.8 ± 2.98	23.7 ± 4.19	24.51 ± 5.15	4.6 ± 0.83	42.9 ± 7.71	160.1 ± 25.46	21.4 ± 1.66	25.5 ± 2.06	26.05 ± 3.65	29.55 ± 4.18	5.3 ± 0.62
t-value	0.43	0.36	0.16	0.22	0.33	0.37	0.079	0.15	0.08	2.37	2.82	0.09	0.13	0.37
p-value	0.730	0.781	0.921	0.880	0.802	0.778	0.994	0.928	0.970	0.086	0.053	0.963	0.942	0.773

Key:

FSW-g: Fresh shoot weight in grams; FRW- g: Fresh root weight in grams; HT1: Plant height in centimeters for first month; HT2: Plant height in centimeters for second month; HT3: Plant height in centimeters for third month; HT4: Plant height in centimeters for fourth month; NSL: Number of Surviving Leaves

Table 4.11: Mean FRW, FSW, NSL and HT of banana genotypes inoculated with Pratylenchus goodeyi in Kisii trial

	Kisii													
	Ng'ombe							Sweet banana						
	FRWg	FSWg	HT1	HT2	HT3	HT4	NSL	FRW	FSW	HT1	HT2	HT3	HT4	NSL
Control	17.5 ± 6.78	26.2 ± 9.65	8.55 ± 0.85	9.60 ± 1.03	10.7 ± 1.86	11.4 ± 2.69	4.2 ± 0.76	8.8 ± 2.17	13.2 ± 2.91	8.55 ± 0.79	9.75± 1.49	11.1 ± 1.34	11.35 ± 1.89	3.8 ± 0.74
Embu	14.3 ± 3.71	34.2 ± 6.79	8.65 ± 0.81	11.35 ± 0.78	12.45 ± 1.67	13.7 ± 1.69	4.9 ± 0.62	7.2 ± 1.76	9.3 ± 2.3	7.6 ± 0.61	7.9 ± 0.9	9.3 ± 1.76	10.3 ± 2.08	3.4 ± 0.83
Nairobi	8.9 ± 1.48	15.7 ± 3.35	7.3 ± 0.7	9.2 ± 0.74	9.4 ± 1.27	11.1 ± 1.46	4.2 ± 0.55	9.4 ± 1.19	7.9 ± 1.59	7.55 ± 0.81	9.65 ± 0.88	9.95 ± 1.37	11.75 ± 1.57	3.8 ± 0.57
Oyugis	19.4 ± 5.3	33.8 ± 10.23	9.5 ± 1.05	10.65 ± 1.27	11.9 ± 2.3	12.65 ± 2.77	4.1 ± 0.72	9.7 ± 2.39	13.2 ± 3.11	7.5 ± 0.64	9.4 ± 0.81	9.9 ± 1.47	11.8 ± 1.7	4.3 ± 0.78
t- value	0.94	1.18	1.11	1.43	0.5	0.39	0.31	0.33	1.14	0.45	0.68	0.58	0.41	0.25
p- value	0.433	0.331	0.357	0.249	0.684	0.761	0.821	0.801	0.346	0.715	0.57	0.633	0.75	0.860

Key:

FSW-g: Fresh shoot weight in grams; FRW- g: Fresh root weight in grams; HT1: Plant height in centimeters for the first month; HT2: Plant height in centimeters for the second month; HT3: Plant height in centimeters for the third month; HT4: Plant height in centimeters for fourth month; NSL: Number of Surviving Leaves

4.3.5 Correlation between *Pratylenchus goodeyi* densities in banana roots and root damage

The number of nematodes in the root system and the extent of root necrosis had a positive (r^2=0.1766) and significant ($p<0.05$) association, according to the findings. In addition, as shown in table 4.12, there was a positive (r=0.1153) association between fresh root weight and *Pratylenchus* species in the root system that did not differ significantly ($p>0.05$).

Table 4.12: Correlation between *Pratylenchus goodeyi* in roots, root necrosis and fresh root weight

	Root necrosis (%)	**Fresh root weight (FRW-g)**
***P. goodeyi* in root**	0.1766*	0.1153

* Significant at 0.05

CHAPTER FIVE

5.0 DISCUSSION

5.1 Farmers' awareness of plant parasitic nematodes in banana production areas

The findings revealed that the great majority of Kenyan banana growers were unaware of PPN as a pest infesting their fields. In addition, none of the farmers interviewed had ever noted any attack by nematodes despite symptoms posed by nematodes visible such as banana plant toppling observed in the farms during the study. This could be attributed to the general lack of nematology knowledge and weak extension expertise as regards to the understanding of banana nematodes and their management (Talwana *et al*., 2016). These findings corroborate those of Brooks (2004) where it was observed that majority of banana producers were not aware of nematodes as a key contributor to decline in banana production. Besides, it has been shown that farmers have little understanding of banana PPN as a pest of economic importance in areas of production (Chitamba *et al*., 2016). Such knowledge gap, predisposes farmers to fail to associate problems like banana toppling and yield decline to nematode infestation (Wang and Hooks, 2009). In general, the results showed that farmers were incurring and will continue to incur losses however much they intensify production.

5.2 Plant parasitic nematodes associated with banana cultivars across different altitudes

The most abundant nematodes associated with banana recovered in the study areas attacking both EAHB and Cavendish banana cultivars, in order of importance, were found to be *Pratylenchus goodeyi*, *Helicotylenchus multicinctus*, *Meloidogyne* spp., and *Radopholus similis*. These results mirror those reported earlier in Costa Rica (Araya *et al*., 2002), Bolivia (Quispe, 2004), Nigeria (Rotimi *et al*., 2005), Kenya (Seshu *et al*., 2007) and Ecuador (Chavez and Araya, 2010). The current study demonstrated that bananas across mid (Embu, Kisii, Migori, Homabay, Kirinyaga and Busia) and high (Kiambu, Bungoma, Murang'a, Nyeri, Meru and Kakamega) altitudes were infested with parasitic nematodes though at varying densities.

Poor agronomic practices, such as the use of contaminated planting materials during the development of plantations, could be to blame for the incidence of nematodes at mid and high altitudes, as shown throughout the study. Besides, farmers in Kenya were discovered to be getting their planting materials from relatives, neighbors, parents, and friends during a public rural evaluation (Seshu *et al*., 1999; Mwaura, 2008). A practice that consequently aggravates the transfer of nematode pests from one farm to the other. The study further determined that nematodes were present across all farms surveyed. This could be attributed partly to the practice of mixed cropping systems across the surveyed areas besides presence of favourable edaphic and climatic conditions in banana producing areas (Ramclam and Araya, 2006).

Pratylenchus goodeyi, *H. multicinctus*, *Meloidogyne* spp. and *R. similis* are well-known parasites of banana (De Waele, 2000; Gowen *et al*., 2005). From the study, it was observed that important banana nematodes existed in mixed populations, complementing results from elsewhere by Chavez and Araya, (2010) and Chitamba *et al*. (2013). This observation of nematodes in a mixture of different species makes it tricky in determining the species-specific effects of common banana nematodes (Blomme *et al*., 2012; Kamira *et al*., 2013). Further, current results corroborate the findings of Gowen *et al*. (2005) and Chitamba *et al*. (2013) where it was observed that parasitic nematodes in banana were characterized by a community of nematode species feeding simultaneously at different levels.

In addition, nematodes from 10 other genera were identidied throughout the research, albeit their relevance and harmful effect on banana genotypes are unknown (Kamira, 2011). However, other PPN like *Rotylenchus* spp. are recognized as important banana pests in other localities (De Waele *et al*., 2000).

5.3 Population densities and distribution patterns of plant parasitic nematodes associated with banana cultivars across different altitudes

Pratylenchus goodeyi was found to be the most prevalent nematode at high altitudes in Kenya (>1600 masl) compared to its distribution at mid altitudes (<1600 masl) in the current study. This observation is consistent with earlier findings from across Eastern Africa (Kashaija *et al*., 1994; Gaidashova *et al*., 2004; Seshu *et al*., 2007; Kamira *et al*., 2013). However, Luambano *et al*. (2017) found that both *P. goodeyi* and *P. coffeae* occured at low altitudes in the Zanzibar Islands and along the

Tanzanian coast. Luambano *et al*. (2018) also reported *P*. *coffeae* in Tanzania's mainland, where it had previously been unknown. *Pratylenchus* species, particularly *P*. *goodeyi*, appear to be migrating away from their "known" distribution in East Africa and into new areas (Mgonja *et al*., 2019).

Talwana *et al*. (2003), Gaidashova *et al*. (2009) and Kamira *et al*. (2013) observed a clear altitudinal demarcation for *P*. *goodeyi* distribution, however the current study reveals its occurrence at lower altitudes in the mid-altitude belts. *Pratylenchus goodeyi* was rarely seen in low altitudinal elevations in East Africa until recently (Luambano *et al*., 2017), raising questions as to whether ecotypes that are more tolerant of higher temperatures are forming (Coyne, 2009). Although it has been documented that *P*. *goodeyi* infection reduces banana root effectiveness (Wang *et al*., 2009) and exposes plants to secondary infections (Tanimola *et al*., 2013; Smiley, 2015) at high elevations, it is unknown how this affects bananas at lower altitudes.

The occurrence of *H*. *multicinctus* at elevations above 1600 masl from the current study, unlike earlier reports by Kashaija *et al*. (1994) and Gaidashova *et al*. (2004) where the nematode was observed at all sites below 1600 masl. This might be partly due to banana production in mixed systems because its spread is influenced by agronomic practices that introduce alternate hosts thus predisposing bananas to a greater risk of attacks (Ramclam and Araya, 2006). *Helicotylenchus multicinctus* has been identified as a major limitation in banana production, particularly in West Africa (Coyne *et al*., 2007). Although it is not as aggressive as *R*. *similis*, it is capable of causing significant root damage when found in large populations (Tanimola *et al*., 2013).

Meloidogyne spp. was encountered in mid altitude areas (<1600 masl) at low densities but widely spread in banana fields. The low densities might have resulted from a suppressing effect due to inter-specific competition (Gaidashova *et al*., 2004). These results mirror those by Kamira *et al*. (2013) confirming RKN to be prevalent at mid altitude areas (<1600 masl). Further from this study, *Meloidogyne* spp. was observed at high altitudes (>1600 m asl) but in low densities, contrary to earlier findings by Kashaija *et al*. (1994), Talwana *et al*. (2003) and Seshu *et al*. (2007) where *Meloidogyne* spp. was reported to be restricted to lower altitudes (<1400 m asl) which is customarily its historic range (Elsen *et al*., 2000).

The RKN has not been considered as a major pest of banana in East Africa although the information regarding its contribution to yield decline is still scanty (Tanimola *et al*., 2013). Earlier studies show that *Meloidogyne* spp. have a negative effect on bunch characteristics (Van den Bergh *et al*., 2000), delaying flowering of the crop (Patel *et al*., 1996) besides gall formation in roots, chlorosis and wilting in plants (Coyne *et al*., 2007).

Radopholus similis, a damaging species found throughout the region, was only found infrequently in the current study in Kenya, corroborating findings from an earlier investigation in Kenya by Seshu *et al*. (2007), where it was infrequently observed. This could probably be alluded to its low competitive nature besides the suppressing nature of *Pratylenchus* spp. at high densities since both exhibit similarities in feeding niches (Chavez and Araya, 2010). The use of nematode-infested banana planting materials is mostly responsible for the spread of *R. similis* (Price, 2006; Blomme *et al*., 2012).

Earlier studies reported *R. similis* to be common at altitudes below 1400 masl (Kashaija *et al*., 1994; Seshu *et al*., 2007), while other findings have reported the nematode to occur below 1000 m asl (Gaidashova *et al*., 2004). However, the findings of the current study noted the occurrence of *R. similis* up to altitudes above 1400 masl though infrequently. This observation appears to be unique suggesting the existence of cold-tolerant strains. *Radopholus similis* damage root systems hindering water and nutrient uptake, toppling bananas, renders plants susceptible to wind knockdown and shorten banana production lifespan (Chitamba *et al*., 2013). *Radopholus similis* is a mult-host nematode besides parasitizing banana (Nelson *et al*., 2006).

According to the current study, the average number of PPN on EAHB was higher than on Cavendish cultivar, with higher densities of the dominating *P. goodeyi* on EAHB than on Cavendish cultivar This confirms the earlier findings by Seshu *et al*. (2007) where more lesion nematodes were observed on EAHB in most sub-counties surveyed and considered as a serious pest of EAHB in the region (Gichure and Ondieki, 1977; Elsen *et al*., 2000). This could be ascribed to in part the endemic cultivation of EAHB in cooler highlands unlike lowland areas (Gaidashova *et al*., 2004). According to previous accounts, *P. goodeyi* is not as damaging as *R. similis* (Elsen *et al*., 2000; Talwana *et al*., 2003).

The high densities of PPN that were observed more from the roots than in the soil is a candid indication that farmers are incurring huge losses from nematodes. The spread could be linked partly to the practice of establishing plantations using contaminated suckers as sources of planting materials which is a tradition within

resource strained banana production areas (Tenkouano *et al*., 2006; Kamira *et al*., 2013).

5.4 Pathogenicity of three *Pratylenchus goodeyi* populations on banana genotypes

Analysis of the reproductive fitness of *P*. *goodeyi* across the trial regions revealed that the density of the *P*. *goodeyi* was higher in the ICIPE trial (1600 m asl) compared to that of Kisii (1300 m asl). This could be owing to the fact that there is a natural variation in the degree of aggression and infection between temperatures and within pathogen biology (Talwana *et al*., 2003) as the temperature between the two trial sites varied. Further, the observation confirms that nematodes efficiently moved out of the necrotic root pieces used as inoculum source and effectively infested the healthy plantlets (Hugon and Picard, 1989). Population density has demonstrated over time to be a key variable for determining relations between reduction in the growth of a banana plant and levels of nematode aggressiveness (Hahn *et al*., 1996; Costa *et al*., 2008).

Nematode distribution at various locations is considered to be affected by altitude and soil temperatures (Price, 2000). According to the findings of this investigation, there is a discernible degree of variation in terms of aggressiveness and infection levels between temperatures within sites and among *P*. *goodeyi* populations. At the two trial sites (Kisii and ICIPE), the average percentage root necrosis was observed to be higher in ICIPE than in Kisii. This could be linked to the higher average altitude in ICIPE (>1600 masl) compared to Kisii (<1300 masl). High

elevations are naturally cooler, thus providing ideal conditions for the reproduction of *Pratylenchus* (Gaidashova *et al*., 2009).

Ng'ombe cultivar recorded higher nematode densities with lower root necrosis while sweet banana had lower nematode densities with higher root necrosis. These findings support Marin *et al*. (2009)'s findings that the nematode's reproductive potential and the degree of root necrosis caused on the host are not necessarily connected, implying that these traits may be controlled by distinct genes (Shaner *et al*., 1992). Banana root system destruction demonstrates why nematode-infested plants normally topple (Barekye, 2000). Moreover, for optimal plant performance, root health should be guaranteed. This enables efficient soil exploration towards successful nutrient transfer and maximum water uptake by the plant (Chapman *et al*., 2012).

Pratylenchus feeds on banana roots (Pinochet, 1978) inducing root necrosis (Mateille, 1994; Speijer and Ssango, 1999). Root necrosis on Ng'ombe and sweet banana cultivars varied across sites. This could have been influenced by factors including plant cultivar (Kobenan *et al*., 1997) and climate (Gauggel *et al*., 2005), corroborating prior findings by Talwana *et al*. (2003), who found variation in root necrosis among experimental sites. The percentage of root necrosis differed between the three populations of *P. goodeyi*. These results agree with Speijer *et al*. (1994) and Nega and Fetena (2015) who observed necrosis at different sites in Uganda and Ethiopia to be highly variable. This finding suggests that in nematode resistance breeding projects, banana cultivars that maintain a strong root system under high nematode infestation should be considered (Barekye *et al*., 2000).

Moreover, the current study demonstrated a positive correlation between *P. goodeyi* densities and root necrosis confirming results elsewhere by Gaidashova *et al*. (2009). When compared to Nairobi (24°C) and Oyugis (26°C) populations, the Embu population isolated at 20°C reproduced significantly. These observations allude to differences in soil temperatures (Talwana *et al*., 2003). Furthermore, despite displaying heat sensitivity in aggressiveness, the current study findings suggest that *P. goodeyi* is acclimated to a wide range of temperatures. Callaway *et al*. (2003), argues that innate variability in root system characteristics and phenotypic traits (Osuji *et al*., 1997) affect tolerance levels of cultivars to the susceptibility to nematode infection (Pinochet *et al.*, 1998). Moreover, root damage showed variability among the three populations with the Embu population being slightly higher.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

i. Study results demonstrates a lack of awareness and nematode knowledge by farmers across banana production areas.

ii. The major plant parasitic nematodes attacking bananas in Kenya at both mid and high altitudes are *P. goodeyi*, *H. multicinctus*, *Meloidogyne* spp. and *R. similis*. *P. goodeyi* are abundant and well distributed across altitudes.

iii. From the current study, it was worth noting that the distribution of parasitic nematodes is no longer linked to certain altitudes as previously observed. The EAHB tolerated high densities of major PPN than the Cavendish cultivar at both mid and high altitudes.

iv. *Pratylenchus goodeyi* populations exhibited variability in pathogenicity in both Ng'ombe and Sukari Ndizi banana cultivars. Altitude and temperature differences influenced this observation and therefore a better understanding of the biology and gene makeup of the nematode species could provide insights into its management.

6.2 Recommendations

i. There is need to strengthen extension networks to foster farmer sensitization and training about nematode biology, identification and management of banana nematodes.

ii. Based on the survey results, there is a need for molecular studies to confirm species profiles of the isolated nematodes for better and informed management decisions. Further research is needed to determine yield losses to bananas and measures to control these species.

iii. Climate change has shifted the distribution of parasitic nematodes from their previously known traditional habitats to new areas and this trend is likely to continue. Therefore, more studies are needed to measure nematode adaptation and disease development in these new eco-regions for their effective management.

iv. More *Pratylenchus goodeyi* populations should be collected from diverse altitudinal elevations across the country and conduct pathogenicity studies for longer timelines unlike the current study. These findings present unique implications for the selection, development and dissemination of nematode tolerant banana varieties as nematode resistance is still the best feasible option for sustainable banana breeding programs.

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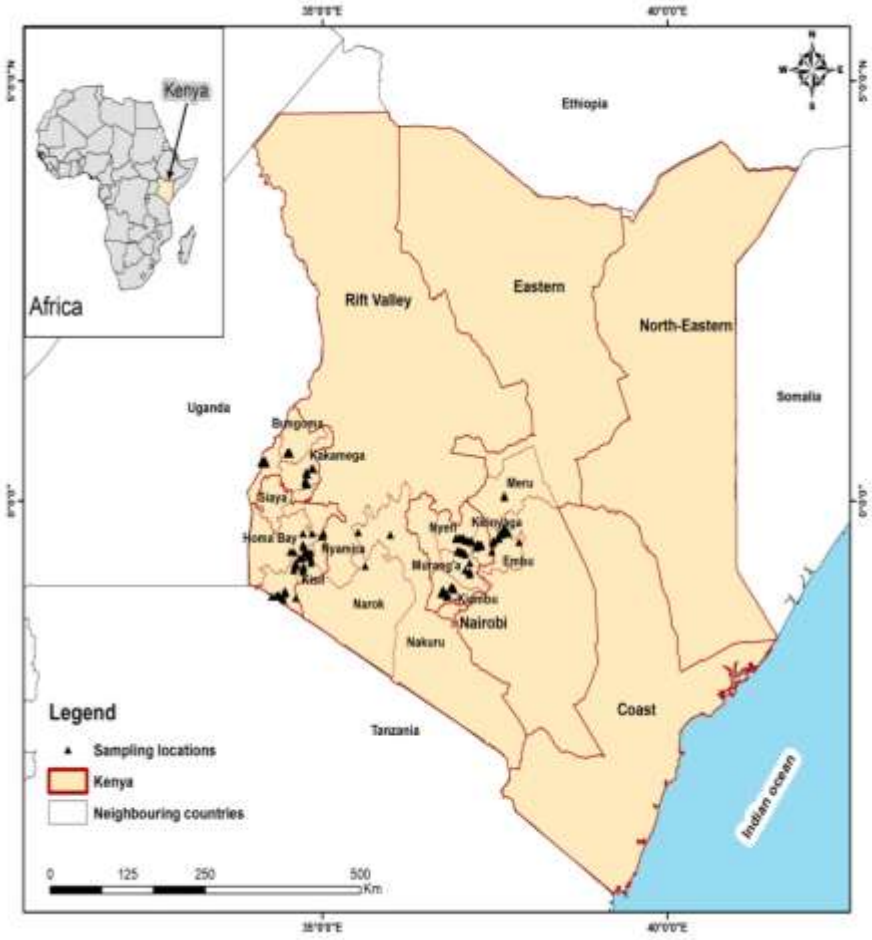
APPENDICES

Appendix 1: Map of Kenya showing sampled areas (▲).



Appendix 2: Questionnaire

SECTION A: DEMOGRAPHIC INFORMATION

Gender: Male [ ] Female [ ]

1. a) District:…… b) Division:……

c) Location: ……d) Sub location…….

e) Village:……..

2. Your occupation

Full time farmer [ ] Part time farmer [ ] Other [ ]

3. Your age bracket (Tick whichever appropriate)

18 – 24 Years [ ] 25 - 30 Years [ ] 31 - 34 years [ ] 35– 40 years [ ] 41 – 44 years [ ] 45 – 50 years [ ] Over 51 years [ ]

4. What is the size of your farm……Acres

5. What cropping patterns do you practice?

Mono-cropping [ ] Mixed cropping [ ]

Others (specify)……

SECTION B: PLANT PARASITIC NEMATODE

1. Do you know about the pests that attack banana in the field?

Yes [ ] No [ ]

2. If YES in question 7, name the pests that are found in your farm

……

……

3. How did you know the Pests that attack banana

Extension [ ] Researcher [ ] Radio/TV [ ]

Other (specify)

4. Have you noticed an attack by nematodes on your farm?

Yes [ ] No [ ]

5. Have you ever seen your bananas topple?

Yes [ ] No [ ]

6. If YES in question 11, do you know the cause of banana toppling

Yes [ ] No [ ]

7. If YES in question 12, please explain

……

……

SECTION C: POPULATION DENSITIES OF PLANT PARASITIC NEMATODES

1. What proportion of bananas are affected in your farm?

less than 10% [ ] up to 25% [ ] up to 50% [ ] up to 75% [ ]

Over 75% [ ]

SECTION D: DIFFERENCE IN DISTRIBUTION OF VARIOUS PLANT PARASITIC NEMATODES

2. Which section of your farm is most affected by banana toppling

Entire farm [ ] Half of the farm [ ] Less than half of the farm [ ]

3. Which section of your farm is mostly affected by banana toppling?

……

……**.....**

.....

4. Which season of the year do you observe most of the damage or more bananas toppling

Dry and hot [ ] Cool and dry [ ] Cool and wet [ ] Warm and wet [ ]

5. What type of bananas are more affected by nematodes?

……

……

……

6. What measures of control do you use for management of the nematodes?

Manure [ ] Chemicals [ ] Resistant varieties [ ]