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Role of plant parasitic nematodes (pratylenchus goodeyi sher and allen) on fusarium wilt disease incidence and severity on banana

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ROLE OF PLANT PARASITIC NEMATODES (*Pratylenchus goodeyi* Sher and Allen) ON FUSARIUM WILT DISEASE INCIDENCE AND SEVERITY ON BANANA

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ABSTRACT

A study to examine the status of Fusarium wilt disease (FWD) caused by *Fusarium oxysporum* f.sp. *cubense (Foc)* and plant parasitic nematode (PPN) (*Pratylenchus goodeyi* Sher and Allen*)* on banana was conducted in January to August 2017 at Meru District in Arusha and Rungwe District in Mbeya regions Tanzania. Forty eight farms, twelve villages in major banana growing areas were assessed for FWD incidence, severity and PPN damage using standard protocols. A pot culture experiment was also conducted to study the role of PPN on incidence and severity of FWD on banana in selected susceptible and resistant cultivars. The results indicated that highest (72%) FWD severity was at Sing'isi ward (Meru District) and the lowest (25.83%) was at Mpuguso ward (Rungwe District). The highest FWD incidence (11.48%) was at Nkoaranga ward followed by Akheri ward (8.95%) in Meru District and the lowest incidence (0.83%) was at Mpuguso ward in Rungwe District. The highest PPN damage (37.5%) was at Kimo ward in Rungwe District and the lowest (17.5%) was at Lufingo ward in Rungwe Disrtict. Such results indicated that FWD incidence and severity and PPN damage are a problem in the study area. The results for pot culture experiment revealed that, nematode inoculated 14 days prior to *Foc* and combined inoculation, showed higher FWD disease incidence and severity with a reduction in plant growth compared with untreated control. Such results suggest that PPN play a positive role in the incidence and severity of FWD on banana by acting as a predisposing factor for the fungal pathogen infestation causing injuries on the root surface as well as weakening the root tissues by causing root lesions.

DECLARATION

I, **HASSAN SHABNI,** do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

HASSAN SHABANI _____ _____

(Name and signature of candidate) **Date**

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of a dissertation entitled**; *Role of plant parasitic nematodes on Fusarium wilt disease incidence and severity on banana.*** To be accepted in partial fulfillment of the requirements for the Degree of Master' in Life Science of the Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania.

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LIST OF ABBREVIATIATIONS

BBTV	Banana Bunch Top Virus
BBW	Banana Bacterial wilt

Bsv	Banana streak virus
BXW	Banana Xanthomona Wilt
EAHB	East African Highland Banana
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database
Foc	*Fusarium oxysporum* f.sp. *cubense*
FWD	Fusarium wilt disease
FWDI	Fusarium wilt disease incidence
FWDS	Fusarium wilt disease severity
IITA	International Institute of Tropical Agriculture
INIBAP	International Network for Improvement of Banana and Plantain
ITC	International Transit Centre
M. incognita	*Meloidogyne incognita*
MT	Metric ton
P. coffeae	*Pratylenchus coffeae*
P. goodeyi	*Pratylenchus goodeyi*
PDA	Potato Dextrose Agar
R. similis	*Radopholus similis*
Xcm	*Xanthomonas campestris* pv. *Musacearum*

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Bananas (*Musa spp.*) is a giant herbaceous perennial plant belonging to the genus *Musa*. The genus is divided into four groups; Callimusa, Australimusa, Eumusa and Rhodochlamys (Simmonds and Shepherd, 1955). The origin of Banana is believed to be South East Asia (Jones, 2000). There are more than 1000 cultivars of banana worldwide which are primarily triploids (2n = 3x = 33), seedless, often sterile and parthenocarpic (Heslop-Harrison, 2011). Most of these cultivars are derived from two wild diploid species (2n = 22); *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) (Shepherd and Ferreira, 1982) through intra and interspecific hybridization (Simmonds, 1995) with *M. acuminata* being the most widespread of the Eumusa species (Horry *et al.,* 1997). Generally, modern classifications of banana cultivars follow Simmonds and Shepherd's system in which cultivars are placed in groups based on the number of chromosomes and from the diploid species they are derived from (Karamura *et al.,* 2012). In East Africa, bananas especially the East African highland bananas (EAHB) such as "Matooke" (AAA-EA), the "Illalyi" (AAA) and "Mchare" (AA) (Karamura *et al.*, 2006) are important staple as well as cash crops for majority living in the plateaus (Karamura *et al.,* 2012).

Bananas and plantains rank fourth after rice, wheat and maize in terms of importance globally (Sharrock and Frison, 1999; FAO, 2016). It is believed to be grown in more than 130 countries occupying an area of over 10 million hectares and an annual production of more than 143 million tones (FAOSTAT, 2016). Those that enter international commerce are worth more than $5 billion per year and locally consumed fruit are major staples for over 400 million people in Africa and Latin America (Ploetz, 2006). The Great Lakes region of Africa has remained to be the largest producer and consumer of bananas in Africa where per capita consumption of banana ranges from 230 to 450 kg person^{-1} year-^{1} (FAOSTAT, 2012; FAOSTAT, 2016; Smale, 2006). Nutritionally, Bananas are excellent source of potassium. A single banana can provide 23% of the potassium that is required by human body on a daily basis (Kumar *et al*., 1992). It is a rich source of carbohydrate 23%, fibre 2.5%, fat 0.5% and protein 1% (Mohapatra *et al*., 2010). Banana is also a good source of the following vitamins: carotene, vitamin E, thiamine (B1), riboflavin (B2), niacin, pyridoxine (B6), folic acid, pantothenate, biotin and vitamins C (Kumar *et al.,* 1992). It is also rich in other mineral elements such as sodium, calcium, magnesium,

phosphorus, iron, copper, zinc, chloride, manganese and iodine (Robinson, 1996). Banana leaves and dry pseudo stems are used as animal feeds and for wrapping food stuffs and in thatching houses, handcrafting of mats and as agricultural mulch (Frison and Sharrock, 1999).

Tanzania cultivates about four hundred and three thousand hectares of land that produces about 3.7 MT of which the biggest proportion (i.e. 2.5 million MT) comes from Kilimanjaro and Kagera regions (Kilimo Trust, 2012). Other regions which produce banana in the country include Kigoma, Mbeya, Arusha, Tanga, Mara and Morogoro (Mgenzi and Mkulila, 2004*)*. Despite its importance, banana yield is unceasingly declining in Tanzania (Van Asten *et al*., 2005). For example, in Kagera region the production has declined from 18 tonnes/ha in the 1960s to less than 6 tonnes/ha in 2000 (Walker *et al*., 1984; Sikora *et al*., 1989; Mgenzi *et a*l., 2005). Such decline in production has been associated to various abiotic and biotic factors including soil fertility problems, drought, insect pests and diseases (Swennen *et al*., 2013). Of the recorded constraints, plant parasitic nematodes such as *Pratylenchus goodeyi* and *Radopholus similis* are among destructive pests of banana plant (Coyne *et al.*, 2014). These organisms are small, worm-like and members of the animal kingdom with length ranging from 0.5-1.0 mm (Mbega and Nzogela, 2012). They are usually found in almost every habitat, in fresh or salt water, and in soil. Plant parasitic nematodes have stylets, spear-like mouthparts that pierce cells and allow nematodes to feed on their contents (Weischer and Brown, 2000). Strategies used by plant parasitic nematodes to conquer the host have been clearly illustrated by Mbega and Nzogela (2012). If successful entry into plant is achieved, these nematodes feed, migrate and multiply inside banana roots and corms causing root-tissue necrosis and root system reduction which then cause damage to plants, impaired transport and uptake of water and nutrients resulting in reduced plant growth and yield (Viljoen *et al.,* 2016). In addition, the anchorage function of the root system is adversely affected resulting in plant toppling (Gowen and Queneherve, 1990).

Globally, nematodes have been reported to reduce agricultural production by approximately 11% (Viljoen *et al.,* 2016). The wounds resulting from nematode attack can also provide avenue for entry and infestation by soil-borne fungal organisms such as *Fusarium oxysporum* f.sp *cubense* (*Foc*) a causal agent of Fusarium wilt disease of banana (Inagaki and Powell, 1969). This disease is also known as Panama disease because it first became epidemic in Panama in 1890 and proceeded to devastate the Central American and Caribbean banana industries that were based on the Gros Michel (AAA) variety in the 1950s and 1960s (Perez, 2004). Fusarium wilt disease is in the same rank with some most devastating plant diseases of other crops such as wheat rust

and potato blight in terms of crop destruction (Dean *et al.*, 2011). As of 1995, Panama disease has been reported from all banana-growing regions including East Africa except the Mediterranean, Melanesia, Somalia, and some islands in the South Pacific (Pegg *et al.*, 1996; Ploetz, 2006). Once *Foc* is present in the soil cannot be eliminated (Davis *et al.,* 2000). It disrupts the plant's water conducting vessels resulting in yellowing and wilting of leaves (progressing from older to younger leaves). Inside a vertical section of the pseudostem, brown, red or yellow lines have been reported to be a characteristic attribute of the disease, the lines which also appear as rings in pseudostem cross-section (Viljoen, 2002; Ong *et al.,* 1996). As a result of infestation, the internal section of the pseudo stem rot extensively (Ong *et al.*, 1996).

There are four recognised races of the pathogen which are separated based on host susceptibility as follows: race 1 affects Gros Michel, Lady Finger (AAB) and Silk (AAB), race 2 affects cooking bananas such as 'Bluggoe, race 3 affects *Heliconia* spp., a close relative of banana and is not considered to be a banana pathogen and race 4 which is a more virulent form of the pathogen cause disease in Cavendish bananas (Viljoen, 2002; Ploetz, 2005).

1.2 Problem Statement

Fusarium wilt disease of banana caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*) is ranked as the most devastating disease of banana in Tanzania. It affects common banana varieties including Mchare and Sukari ndizi causing loss of up to 78%. The disease is difficult to manage and once present in the soil cannot be eliminated. The only possible recommendation at this stage is the use of resistant varieties. However, these resistant varieties do not always remain resistant if preceded with nematode infection. Complex interrelationship between nematodes and *Foc* in bananas is believed to produce a combined effect which is greater than the sum of their separate effects (Dinesh *et al.*, 2014). Many farmers find it difficult to discern these interactions therefore any management strategy to *Foc* in the field must therefore also be integrated with nematode management.

1.5 Problem Justification

Banana production in Tanzania has been declining with insect pests and diseases being the major causal factors. For example, in Kagera region (one of the major banana producing regions in the country), the average yield of banana has declined from 18 tons/ha in the 1960s to less than 6 tonnes/ha in 2000 (Walker *et al*., 1984; Sikora *et al*., 1989; Mgenzi *et al*., 2005). Of the pest problems, nematodes are the most frequently mentioned while in terms of diseases, Fusarium

wilt caused by *Foc* has been mentioned to be the most destructive disease of banana in the region (FAO, 2009).

1.6 Significance of the Study

This study will generate information on the status and interaction between nematodes and *Foc* in different Tanzanian banana genotypes. This information can be used as a tool kit in advising management of both nematodes and *Foc*. Furthermore, it can be used in recommending banana varieties to be adopted especially in areas with *Foc* and nematode in Tanzania**.**

1.7 Rationale

Interaction between parasitic nematodes and *Foc* is clear from studies carried outside East Africa. However, this has not been elucidated in Tanzania. Furthermore, the agro-ecologies and banana genotypes in Tanzania are different. It is therefore important to clearly understand well the status and interaction of plant parasitic nematodes and *Foc* in banana genotypes so that recommendations on management options can be made to small scale farmers and to improve resistance screening work during breeding activities in the country.

1.8 Objectives

1.8.1 General Objective

To determine the effect of lesion nematode (*Pratylenchus goodeyi*) on incidence and severity of Fusarium wilt disease on banana so that recommendations on developing management options can be made to small scale farmers in Tanzania.

1.8.2 Specific Objectives

i. To determine the incidence and severity of Fusarium wilt disease and nematode damage on banana in Northern and Southern Highlands of Tanzania.
ii. To examine the effect of lesion nematode (*Pratylenchus goodeyi)* on incidence and severity of Fusarium wilt disease on banana under screen-house conditions.

1.9 Hypotheses

Ho: Lesion nematode (*Pratylenchus goodeyi*) has no effect on incidence, severity and resistance of Fusarium wilt disease on banana

H1: Lesion nematode (*Pratylenchus goodeyi*) has effect on incidence, severity and resistance of Fusarium wilt disease on banana.

CHAPTER TWO

LITERATURE REVIEW

2.1 Plant parasitic nematode and Fusarium wilt disease transmission

Plant parasitic nematode (PPN) (*Pratylenchus goodeyi* Sher and Allen) is among the migratory endo-parasites of the root cortex and corms of banana, plantain and abaca mostly at higher elevations (Bridge, 1988; Bridge *et al.*, 1997; Sarah, 1989). All life stages and both sexes of *P. goodeyi* invade and feed in the root and corm tissues where the eggs are laid. The life cycle of *P. goodeyi* from egg to egg is less than 30 days at 25-30^{o}C (Bridge, 1988). *Pratylenchus goodeyi* is considered to be indigenous to Africa with much more restricted distribution where it is limited to the higher elevation zones of Central, Eastern and West Africa (Price *et al*., 1995). It is considered as an important pest of highland bananas (*Musa* AAA, Matooke and Mbidde groups) in Uganda, Tanzania, Kenya, Rwanda and Burundi (Kashaija *et al*., 1994).

In a concurrent combination, Fusarium wilt (Panama) disease of banana caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*) which has been comprehensively reviewed by Stover (1962), Ploetz (1990) and more recently by Ploetz and Pegg (2000) is thought to be promoted by the PPN damage. The distribution of the disease is highly related to the introduction of new cultivars and infected plants product or soil basically through human activities to the growing areas (Stover, 1962). The diagrammatic transmission is as shown in Figure 1. The infected plants serves as source of inoculum from which the pathogen can be moved through infected planting materials, cultural practices such as weeding and pruning, and in water through irrigation (Ploetz, 1990).

In Tanzania, the disease is widely distributed throughout all banana growing areas, though it is considered the most destructive in Kagera Region (Mbwana and Rukazambuga, 1999). The *Foc* exclusively attacks several banana cultivars including Mchare and Sukari ndizi. The disease cannot be controlled by using any fungicides and once present in the soil it cannot be eliminated (Stover, 1962).

In most cases development of disease symptoms is not solely determined by the pathogen responsible but it is dependent on the complex interrelationship between host, pathogen and prevailing environmental conditions (Mai and Abawi, 1987). Furthermore, in nature plants are rarely subjected to the influence of only one potential pathogen especially for soil borne pathogens, where there is tremendous scope for interaction with other microorganisms occupying the same ecological niche (Wallace, 1978). In Tanzania, banana interacts with a number of other important pathogens and insect pests as shown in Table 1.

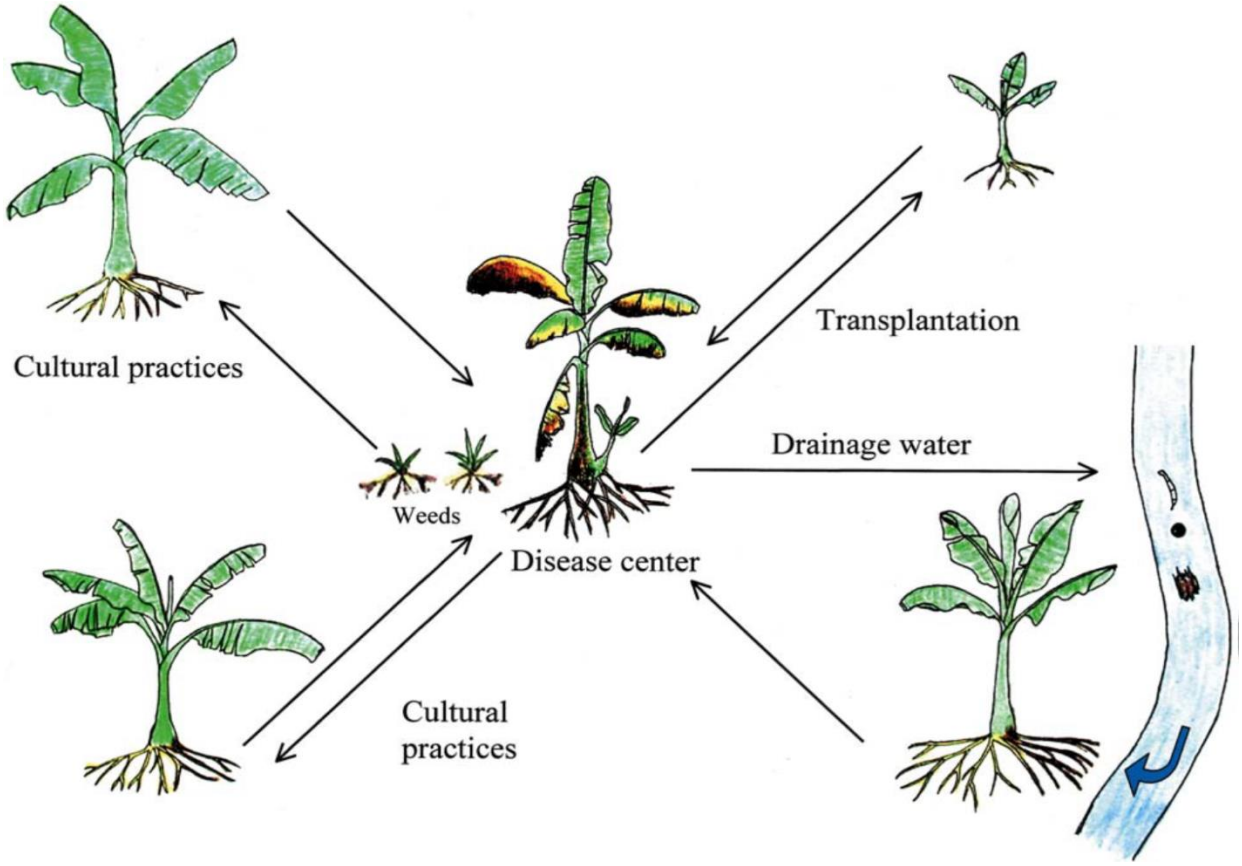

Figure 1: Transmission of Fusarium wilt disease (Cycle adapted from Hwang, 2002)

2.2 Role of nematodes in disease development

The significant role of nematodes in disease development caused by soil-borne pathogens has been demonstrated in many crops (Back *et al.,* 2002). The first recorded case of a nematode-fungus interaction was made by Atkinson (1892) who observed that Fusarium wilt of cotton caused by *Fusarium oxysporum* f.sp. *vasinfectum* was more severe in the presence of root-knot nematodes (*Meloidogyne* spp.) From there onwards, several reports have been published which illustrate that nematode damage has a significant role in the establishment and development of diseases caused by soil borne pathogens. For example, Jonathan and Rajendran (1998) observed that when both pathogens (*Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *cubense)* were inoculated concomitantly and sequentially on the banana cultivar Rasthali, the decline in plant growth was greater than with either pathogen alone. Also the disease development in terms of corm rot during his study was observed to be significantly higher when nematode infection was followed by fungus infection than during in concomitant inoculation. This implied that *M. incognita* predisposes banana plants to *Foc* and enhances the severity of Fusarium wilt disease. Inagaki and Powell (1969) suggested that, the simultaneous introduction of nematode and fungi

pathogens allowed the latter to utilize minute openings created by nematodes in the roots. Orion *et al*. (1999) who studied the roots of banana by using scanning electron microscope revealed that the mycelium of the soil borne fungus was closely and frequently associated with the invasion tracts and lesions created by nematodes. It is also believed that, damage inflicted on plant roots during the process of nematode invasion could result in greater volumes of root exudates which attract fungal invaders (Bergeson, 1972).

Furthermore, studies have shown that there is breakdown of resistance during concomitant infections (France and Abawi, 1994; Marley and Hillocks, 1994; Sidhu and Webster, 1977; Uma Maheswari *et al.*, 1997). Loss of resistance has been tested with the application of split-root methods in crops other than banana. This gives room to researchers to conduct experiments and prove or deny the hypothetic notion that there is also the loss of resistance in banana during synergistic interaction of nematode and fungi. For example, Bowman and Bloom (1966) found that the Tomato cvs. Rutgers and Homestead, previously resistant to *F. oxysporum* f.sp. *lycopersici*, developed symptoms of wilt during split-root experiments with *M. incognita*.

It is not clear yet how the decrease in host resistance happens but some researchers suggest that it occurs as a result of the breakdown of a systemic chemical defense system within the host plant. For example, Marley and Hillocks (1994) demonstrated that, nematode-induced loss of resistance to *Fusarium udum* in pigeon pea (*Cajanus cajan*) was associated with reduced levels of the isoflavanoid phytoalexin cajanol.

Although the mechanism behind soil-borne pathogens - nematode interaction has been described in some systems outside the African environments, there is a critical gap and need to clearly study this interaction in a local context especially on the EAHB systems so that recommendations on management options can be made to small scale banana farmers in Tanzania.

Table 1: Other major important diseases and or insect pests of banana in Tanzania

S/N	Name of disease/insect pest	Causal Pathogen/insect	Characteristic symptoms	References
1	Black leaf streak or black sigatoka	*Mycosphaerella fijiensis*	Necrotic leaf lesions that reduces the photosynthetic capacity of the plants, which result in reduced crop yield	Crous and Mourichon, 2002; Jones *et al*. (2013); Mobambo *et al*. (1993);

			and fruit quality with loses up 50%	Tushemereirwe, 1996; Viljoen *et al*. (2016)
2	Banana xanthomonas wilt or Banana bacterial wilt	*Xanthomonas campestris* pv. musacearum	Yellowing and complete wilting of the plant starting with the most peripheral leaves with yield loss of up to 100%	Aritua *et al*. (2008); Carta *et al*. (2010); Mgenzi *et al*. (2006)
3	Banana bunchy top	Banana bunchy top virus	Dark green, dot-dash flecks along leaf veins adjacent to the midrib; flecks in veins can form characteristic 'hooks' into the midrib from the leaf blade. Loss up to 90%	Fist, 1970; Kumar *et al*. (2011)
4	Banana streak	Banana streak virus	Narrow, discontinuous or continuous chlorotic or yellow streaks that run from the leaf midrib to the margin. It causes yield losses of up to 90%	Daniells *et al*. (2015); Kubiriba *et al*. (2001)
5	Banana weevil	*Cosmopolites sordidus* Germar	The insect makes tunnels into the banana corms resulting into snapping of plants, prolonged maturation rates and reduced yields. Severe infestations can lead to total crop failure resulting into 100% yield loss	Kiggundu *et al*. (2007); Sengooba, 1986

Source: This study

CHAPTER THREE

MATERIALS AND METHODS

3.1 Objective 1: Assessment of the Fusarium wilt disease incidence and severity and nematode damage in Northern and Southern Highlands of Tanzania

3.1.1 Location

This study was conducted in Meru District located in Arusha region in Northern highlands and Rungwe District located in Mbeya region in Southern highlands of Tanzania. Meru is one of the six districts in the Arusha region. It is bordered to the north and west by the Monduli District, to the east by the Kilimanjaro Region and to the south by the Arusha District and the Monduli District. The people are the Meru and the language is Meru. Rungwe is one of the District in Mbeya region located south of the city of Mbeya, at an elevation of around 1250-1500 masl in the highlands. The people are the Nyakyusa and the language is Nyakyusa. The two districts were selected due to their importance in banana production and also based on complains from farmers on *Foc* and nematodes.

3.1.2 Assessment of proportional numbers of banana cultivars

Four wards in Meru District (Shangarai, Akheri, Sing'isi and Nkoaranga) and four wards in Rungwe Districts (Mpuguso, Kimo, Lufingo and Kiwira) with information on existence of different banana cultivars were selected and used in the study (Table 2). To quantify the number of banana cultivars per farm in each ward, six randomly selected banana farms of about 1-2 acres (three farms per village) were used. In every farm, number of each banana cultivars were recorded and their proportion by number were determined.

3.1.3 Assessment of Fusarium wilt disease incidence, disease severity and nematode damage

The disease incidence was quantified by dividing the number of infected plant units by total number of plants in the field x 100%. Disease severity was quantified based on external symptoms of the disease by inspecting individual plants from each banana variety using a scale of 1 to 5 as established by Viljoen *et al*. (2016) with modifications. Using the established scale, 1 described; no visual leaf symptoms, 2=0-33% of older banana leaves turning yellow, 3=34-66% of older leaves turning yellow with some hanging down the pseudo-stem, 4=67-95% of the leaves turning yellow and necrotic with leaves hanging down the pseudo-stem and 5=96-100% plant dead with brown leaves hanging down the pseudo-stem. Scoring nematode damage was done by estimating visual damage of root (as a percentage) using a scale of 1 to 5 adopted from Speijer and De Waele (1997). Systemic sampling pattern was used in order to accommodate the

patchy nature of nematode distribution where five plants were selected for nematode scoring in every farm. From each plant five functional roots of 10 cm length were randomly selected, washed with water to remove soil and sliced lengthwise. Scoring was done on one half of the root for the percentage of root cortex showing necrosis. As each of the five isolated roots usually carries 20 marks (Speijer and De Waele, 1997) the proportion damage under each root was estimated and the sum damage proportions was used as final score in percentage.

3.2 Objective 2: To examine the effect of lesion nematode (*Pratylenchus goodeyi*) on incidence and severity of Fusarium wilt disease under screen-house conditions

3.2.1 Study area

This study was conducted under screen-house conditions at the Nelson Mandela African Institution of Science and Technology (NM-AIST). The NM-AIST is located 10 km East of Arusha town along the Nelson Mandela road formally known as Old Moshi Road at Latitude $3^0$23' and $3^0$25' South and Longitude $36^0$47' and $36^0$49' East.

3.2.2 Source of planting materials

Planting materials were obtained from the International Institute of Tropical Agriculture (IITA) and Horticultural Research Institute Tengeru (Hort-Tengeru) (Table 2) in collaboration with the International Transit Centre (ITC), Katholieke Universiteit Leuven, Belgium. As soon as the plant material arrived from the ITC, they were multiplied through tissue culture. The isolated tissues were grown in semi-solid multiplication medium (MS) and incubated at 28±2°C for 90 days in a tissue-culture laboratory at the NM-AIST. In total, about 162 plants were cultured. Plantlets remained in *vitro* until they attained a height of 5-10 cm, with three leaves and a well-developed root system before hardening.

Table 2: Banana genotypes used in this study

S/N	Name	Source	Status	Reference
1	Gros Michel	IITA	Susceptible to *Foc* race1	Ploetz *et al.* (2011)

2	Grand Naine	IITA	Resistant to *Foc* race1 Susceptible to Nematode	Fallas *et al.* (1995); Mateille, 1990, 1992; Moens *et al.* (2003, 2005); Ploetz *et al.* (2011)
3	Cv Rose	IITA	Resistant to *Foc* race1	Orjeda, 1998
4	JD Yangambi/Km5	IITA	Resistant to Nematode	Nelson and Javier 2007; Sarah, 1996
5	Sukari ndizi	HORTI-Tengeru	Susceptible to *Foc* race1	Karamura *et al.* (2012); Pérez *et al*. (2002); Rodriguez *et al.* (2014)
6	Mchare Laini	IITA	na	na
7	Huti green	IITA	na	na
8	Nakitengwa	IITA	na	na
9	Kazirakwe	IITA	na	na

*****na=Information not available.

3.2.3 Isolation of the Fusarium wilt-causing pathogen from infected plant material

Corm samples from plants showing symptoms of Fusarium wilt were collected from the field, kept in paper bags and brought to the NM-AIST laboratory. Then the collected corms were paired to a desired size (2x2 cm) and soaked in 15% jik (sodium hypochlorite) for 15 min. The jik was then washed off using sterile distilled water (soaked in sterile water) for 5 min.

The corms were further surface sterilized by soaking in 70% ethanol for 15 min and then ethanol was washed off using sterile water for 5 minutes. Using sterile forceps and blade, the corm was further paired by removing the outer brown sheath that was sterilized. Small cubes (1x1 cm) from the inside of the corm were then sliced and plated on PDA (Potato Dextrose Agar) media supplemented with streptomycin (300 mg/ml). Plates were then sealed and incubated at 24 ^{0}C. After 3 days, pure *Foc* colonies that had grown on the PDA media plates with purplish and whitish mycelium were isolated and plated onto new PDA media.

3.2.4 Preparation and sub-culturing of the pathogen on millet seeds

A fully grown *Foc* plate was cut into small cubes (1.5x4 cm) using a sterile blade and inoculated into sterilised wet millet grains as per Tendo *et al. (*2013). The inoculated millet were incubated

at room temperature and mixed daily for 12 days to ensure uniform fungal growth after which the inoculum was ready for use.

3.2.5 Nematode inoculum preparation

Root samples were collected from infected banana plants and brought to the NM-AIST laboratory for nematode extraction. Root maceration method as adapted from Coyne *et al*. (2014) was used where roots were chopped into small pieces (about 0.5 cm), rinsed with clean water to remove soil and placed in an electric blender with just enough water to cover the blades. Roots were blended twice for 5 seconds, then the blended suspension of roots and water was poured onto tissue paper.

Gently, water was added to the extraction plates to wet the root tissue and left undisturbed for 24 hours. After the extraction period, the sieve was removed and plant tissue disposed. Water from the plate was poured into a labelled beaker and left to settle. The volume of water was reduced by gently siphoning the excess water leaving 20-30 ml at the bottom of the beaker which was then put into vials ready for nematode identification.

3.2.6 Nematode identification and culturing

Extracted suspension was put onto a clean petri dish and placed on a stereomicroscope (Motic K-700). By adjusting the microscope focus, nematodes were kept in view and picked from the water solution with a picking instrument. The tip of the pick was placed into a drop of water on a slide and the nematode was viewed with a fluorescent microscope (B-350 OPTIKA). The nematode of interest (*Pratylenchus goodeyi*) was selected based on its morphology (head, tail, vulva position, and spicule) as indicated by Bridge *et al*. (1997). The selected nematodes were then cultured on a carrot disc following the protocol described by O'Bannon and Taylor (1968) and Pinochet *et al*. (1995). The nematodes were surface sterilised with streptomycin sulphate (0.06 mg) for 1 hour followed by three rinses with distilled water. Carrots were surface sterilised with 96% ethanol and peeled two times. The carrots were then cut into discs of about 5 mm and placed in sterile Petri dishes. About 30 nematodes were placed on each carrot disc. The Petri dishes were then sealed with parafilm and incubated at 26°C. The nematode populations were sub-cultured every 6 weeks for six months. Then nematodes were collected in a test tube by rinsing the petri dishes containing the carrot discs with distilled water.

3.2.7 Inoculation of plants with *Foc* and nematode

Three months old banana plantlet (in triplicates) from the tissue culture laboratory, were planted in plastic pots filled with 1 kg of sterilized mixture of soil, sand and manure (2:1:1) spaced 20 cm apart. During simultaneous inoculation, 50 g of *Foc* inoculated millet were poured into the pot half filled with sterile soil. Plants were placed on top of *Foc* infected millet and nematodes were directly added onto the roots by pouring 2 ml aqueous suspension containing 50 *P. goodeyi* then covered with sterile soil. During inoculation with *Foc* and or nematodes alone, only nematode or *Foc* were added before filling the pot with sterile soil while in sequential inoculation only one pathogen was added followed by the second one after 14 days.

3.3 Experimental design and layout

The study had 6 treatments assigned randomly to 9 experimental plants in three replications in a split plot design. The main plot was banana varieties and subplots were the treatments (Apendix 2). The sub plot treatments were: 1. *Foc*, 2. Nematode, 3. Nematode + *Foc*, 4. *Foc* followed by nematode (14 days later), 5. Nematode followed by *Foc* (14 days later) and 6. Control (no inoculation).

3.4 Data collection

Growth parameters (height, girth, number of leaves) were weekly recorded. Height of the plants was measured using a tape measure from the soil level to the second leaf from the top while plant girth was measured 4 cm from the soil level using an electronic digital caliper. Number of leaves were determined by counting functional leaves (more than 50% green area).

Fusarium wilt disease severity was quantified based on external symptoms of the disease by inspecting individual plants from each banana variety using a scale of 1 to 5 as established by Viljoen *et al*. (2016) with modification. Using the established scale, 1 described; no visual leaf symptoms, 2; = 0-33% of older banana leaves turning yellow, 3; = 34-66% of older leaves turning yellow with some hanging down the pseudostem, 4; = 67-95% of the leaves turning yellow and necrotic with leaves hanging down the pseudostem and 5; = 96-100% plant dead with brown leaves hanging down the pseudostem. For internal symptoms, a scale of 1-6 was used as follows: 1; No internal symptoms, 2; Few internal spots, 3; <1/3 Discoloured, 4; 1/3-2/3 Discoloured, 5; >1/3 Discoloured, 6; Entire inner rhizome discoloured. At the end of experiment, nematode damage was assessed by scoring root necrosis following Speijer and De Waele (1997) protocol whereby five roots from each plant were cut longitudinally and the percentage of visible necrotic

cortical tissue was recorded. For every individual root, necrosis was scored out of 20 and the score was multiplied by five to get the percentage of the damaged root area.

3.5 Data analysis

The data was subjected to two way analysis of variance using Gen-STAT statistical package. Mean separation within the column was performed by Bonferroni multiple Range Test at 5% confidence interval.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Objective 1: Status of Fusarium wilt disease and nematode damage in Northern and Southern Highlands of Tanzania1[1]

4.1.1 Common banana cultivars in the study area

The results showed that, cultivar Mchare was the most common grown banana with proportions ranging from 65.76% in Akeri ward to 72.35% in Sing'isi ward followed by cultivar Grand naine which had a proportional ranging from 16.30% in Shagarai ward to 19.0% in Akeri ward in Meru District (Table 3). The results also showed that the most common grown banana cultivar in Rungwe District was Plantains which ranged from 27.3% at Kiwira ward to 47.65% at Kimo

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ward, followed by Matoke bananas which ranged from 22.30 at Kimo ward to 32.0% at Lufingo ward (Table 3). Information on commonality for other varieties covered in this study are as shown in Table 3.

Table 3: Number (%) of common Banana cultivars in selected wards of Meru and Rungwe Districts as characterized during this study

Ward	District	Banana cultivar and their proportional number (%) per farm				
		Mchare	Matoke	Plantain	Grand Naine	Sukari ndizi
Mpuguso	Rungwe	5.89	28.9	37.28	26.40	0.90
Kimo	Rungwe	2.73	22.35	47.65	29.27	1.19
Lufingo	Rungwe	2.23	34.00	33.33	25.50	1.24
Kiwira	Rungwe	4.20	33.39	27.30	30.20	0.73
Shangarai	Meru	67.39	11.7	1.33	16.30	1.09
Akheri	Meru	65.76	10.69	0.68	19.00	1.89
Sing'isi	Meru	72.35	8.07	1.79	16.14	1.20
Nkoaranga	Meru	71.86	9.24	0.87	17.03	0.87

Source: This study

4.1.2 Fusarium wilt disease incidence, disease severity and nematode damage in the study area

The results showed that Fusarium wilt disease and banana nematodes were found to be present in all villages under study in Arumeru and Rungwe Districts (Table 4). However, there was highly significance difference ($p \leq 0.001$) between districts in the incidence of Fusarium wilt disease in the study area. The highest Fusarium disease incidence (11.48%) was recorded at Nkoaranga ward while the lowest disease incidence (0.83%) was recorded at Mpuguso Rungwe District.

Table 4: Fusarium wilt disease incidence, disease severity and nematode damage in Meru and Rungwe Districts as established in this study

Ward	District	Fusarium wilt disease Incidence (%)	Fusarium wilt disease severity (%)	Nematode damage (%)
Mpuguso	Rungwe	0.83a	25.83a	22.50bc
Kimo	Rungwe	1.50a	52.50abc	37.50a

Lufingo	Rungwe	1.57a	32.17ab	17.50c
Kiwira	Rungwe	2.72a	54.67abc	30.83ab
Shangarai	Meru	7.94b	63.33bc	34.17ab
Akheri	Meru	8.95b	55.83abc	35.00a
Sing'isi	Meru	9.30b	52.00abc	30.83ab
Nkoaranga	Meru	11.48b	48.33abc	30.00ab
Mean	na	5.53	50.58	29.80
LSD	na	3.82	32.88	11.97
F-statistics	na	***	*	**

Means followed by the same letter(s) are not significantly different based on the Bonferroni multiple test a p=0.05., na= not applicable. ns=non-significant., *=significant at P≤0.05, **= significant at p≤0.01 and *** significant at p≤0.001

However, such disease incidences were based on overall evaluation in each surveyed field regardless of existence of resistant banana varieties. Thus results for specific cultivar related disease incidences indicated that only Mchare and Sukari ndizi cultivars were susceptible to Fusarium wilt disease with incidences of 46.84% on cultivar Sukari ndizi at Lufingo ward to 59.9% on cultivar Mchare at Kiwira ward both in Rugwe District (Fig. 2).

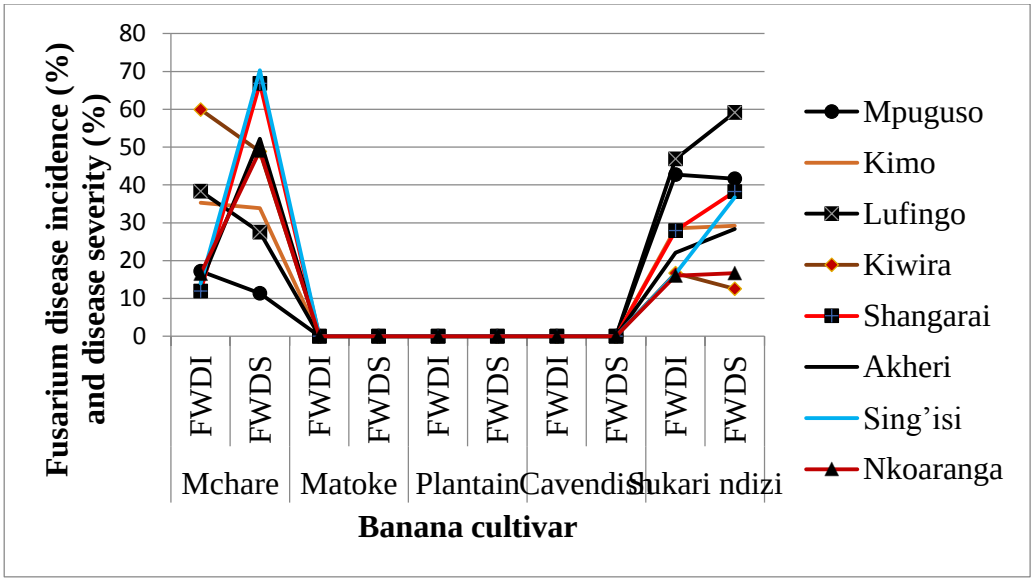

Figure 2: Fusarium wilt disease incidence (FWDI) and disease severity (FWDS) on common banana cultivars in the study area

The results also showed there was significance difference (P≤0.05) between Fusarium disease severity on banana cultivars in the study area (Table 4). Fusarium wilt disease severity ranged from as high as 59.17% on cultivar Sukari ndizi at Lufingo ward in Rungwe District to 70.33%

on Mchare at Sing'isi ward, Meru District (Fig. 2). The results also showed that all banana cultivars in the study area were susceptible to nematode and the damage was significantly different ($p \leq 0.01$) (Table 4 and Fig. 3).

The highest score for nematode damage (37%) was recorded at Kimo ward in Rungwe District followed by Akheri (35%) and Shangarai (34.17%) in Meru District while the lowest score (17.5%) was recorded at Lufingo ward in Rungwe Disrtict (Table 4). On different cultivars, the results showed that Matoke bananas was the most susceptible of all with damage levels of as high as 50.35% at Shangarai ward in Meru District and 65.12% at Kimo ward in Rungwe District (Fig. 3). Other banana cultivars with high nematode damage levels were Grand Naine (50.26%) at Akeri ward, Meru District, Plantains (46.62%) at Kiwira, Rungwe District and cultivar Mchare (40.83%) at Shangarai ward, Meru District (Fig. 3)

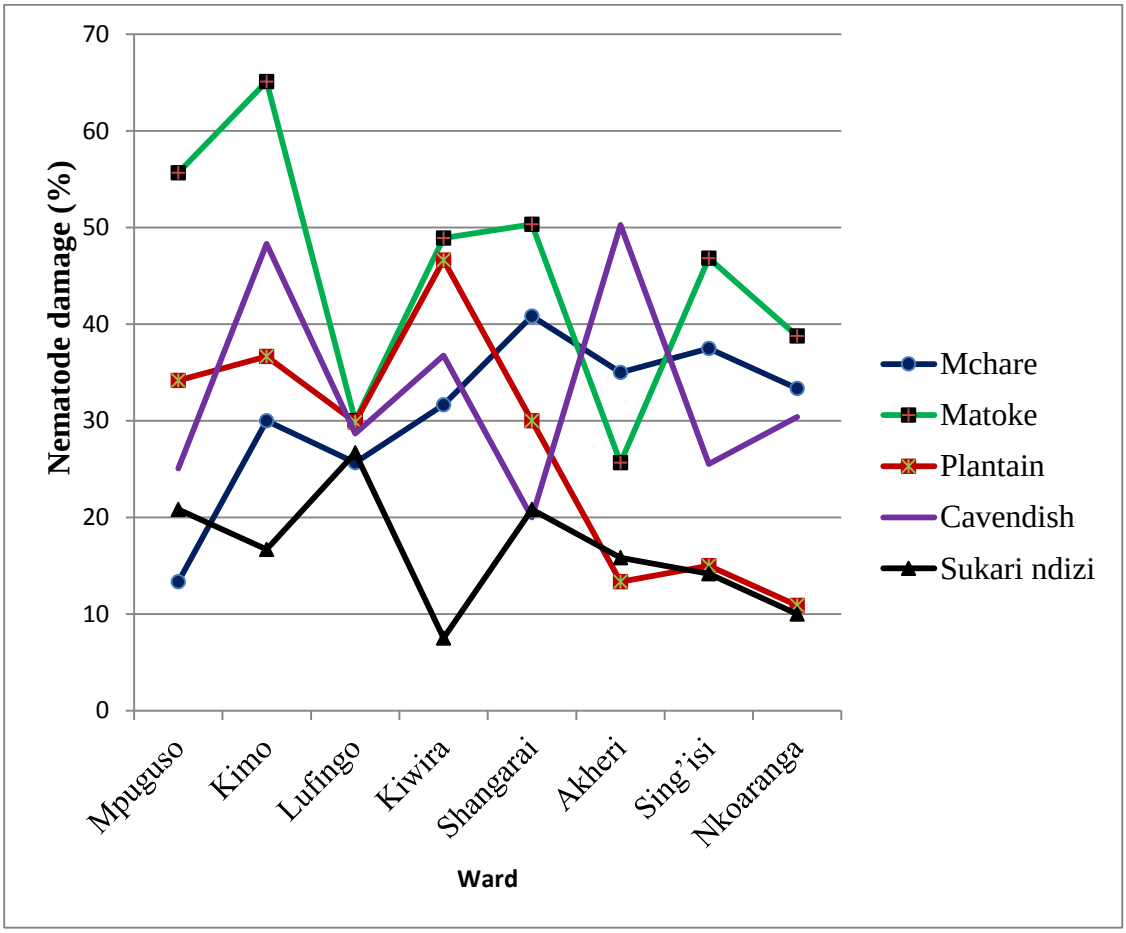

Figure 3: Nematode damage on common banana cultivars in the study area

4.1.3 Discussion

This study has revealed that common grown banana varieties under the study area are Mchare, Matoke, Plantain, Grand Naine and Sukari ndizi. However, the importance of each cultivar differs between the two sites. While Mchare cultivars are highly grown in Meru District, important banana cultivars in Rungwe District are Plantains and Matoke bananas. This was also reported by Karamura (2006) who mentioned Meru-Kilimanjaro axis to be dominated by Mchare banana. Similarly, Maruo (2007) reported plantain to be playing a key role in consolidating the development of the Nyakyusa (ethnic group in Rungwe district) rural community. In this study, Fusarium wilt disease and nematode were reported to be present in both Meru and Rungwe districts. The highest score for Fusarium wilt disease incidence and severity was recorded in Meru District. One of the reasons for high incidence and severity of Fusarium wilt disease in this region might be due to high cultivation of susceptible cultivars which are Mchare Koka and Swennen (2017) and Sukari ndizi (Viljoun *et al*., 2016) compared with Rungwe District. In addition, farming systems in Meru District differ from those found in Rungwe. During dry season, farmers do irrigate their banana field using surface/furrow irrigation method, which essentially causes movement of pathogen from infected plants to the health ones through running water (Ploetz, 2006). Farmers in Arumeru also use banana plants (leaves and pseudostem) as feeding material to their cattle. Pruning of functional leaves for animal feed with the same machete across the field without using disinfectants is another way of spreading the disease (Ploetz, 2006), which leads to the increase of disease incidence. However, the proportion of incidence to the total number of plants is low because farmers normally do not keep diseased plants in the field for a long period. They destroy diseased plants as soon as they see signs of Fusarium wilt unless the symptoms occur at a later stage when the bunch is close to maturity. Plantains, Matoke, and Grand Naine which are highly grown in Rungwe district are all resistant to *Foc* race 1 (Kashaija *et al*., 1994; Speijer *et al*., 1994) hence the low disease incidence and severity compared with Meru District where they are not popularly cultivated. Relative to Meru district, there is no furrow irrigation in Rungwe and therefore, spread of pathogen through irrigational water is avoided. Also, most of the farmers do not keep animals therefore no extensive pruning of leaves which reduces disease severity and incidence.

The highest nematode damage between the two sites was reported to be in Rungwe District. However, the difference observed was small when compared with Meru District. The small difference in nematode damage might be contributed by the presence of similar susceptible

varieties to nematode i.e. Matoke and Grand naine (INIBAP, 1997) in both regions and similarity in climatic conditions. For example, Nkwamansa village in Arumeru District located 1406 meters above sea level is similar to Kalalo in Rungwe District which has an altitude of 1415 meters above sea level. Normally nematodes distribution is much influenced by altitude (Price 2000). For example, the occurrence of *Radopholus similis* rapidly declines at elevation above 1300 meters above sea level while *P. goodeyi* decreases below 1200 meters above sea level. *Helicotylenchus multicinctus* and *Meloidogyne* spp. are high at lower altitude (Speijer and Fogain, 1999; Elsen *et al*., 2000). However, there are some nematode species like *P. goodeyi*, with unique characteristics. They have much more restricted distribution and are said to have a lower temperature preference than others and its distribution is closely linked to altitude and the higher latitudes of the cooler banana growing areas of up to 1500 meters above sea level. (Bridge *et al.,* 1997). When looking on the specific crop, Matoke bananas has been affected more by nematode compared with other varieties. This is because Matoke, the East African Highland Bananas (AAA-EA) is more prone to *Pratylenchus goodeyi* which is a more prominent nematode specie in East African Highland Bananas and found in many banana growing areas (Elsen *et al*., 2000).

4.2 Objective 2: Effect of lesion nematode (*Pratylenchus goodeyi*) on incidence and severity of Fusarium wilt disease on banana used in this study

Screen house results showed that there was considerable variation in appearance of Fusarium wilt disease (FWD) symptoms in different treatments and also among the cultivars of banana (Table 7-appendix 1). The earliest symptoms were recorded on Sukari ndizi with combined (*Foc* + nematode) treatment at 28^{th} day after inoculation. On cultivar Huti white initial symptoms appeared 42 days after inoculation in sequential inoculation involving nematode followed by *Foc* after 14 days while other susceptible cultivars such as Gros Michel and Mchare laini, initial symptoms appeared on the 56^{th} day with the same treatment. (Table 7-appendix 1). In plants inoculated with *Foc* alone, FWD symptoms developed 42 days after inoculation on Sukari ndizi, 56 days on Mchare laini and Huti green while Gros Michel developed symptoms on 70^{th} days after inoculation (Table 7-appendix 1).

The results also showed that there was significance difference ($P \leq 0.001$) between the diseases incidences in different banana genotypes used in this study (Table 5). The highest Fusarium disease incidence (66.67%) was recorded on banana cultivars (Sukari ndizi, Gros Michel, Huti green and Mchare laini) and the lowest (0.00%) was recorded on banana cultivars JD Yangambi,

Kazirakwe, Grand Naine, cv Rose and Nakitengwa (Table 5). There was significance difference (P≤0.001) between the disease severity in different banana genotypes used in this study (Table 5). The highest disease severity was recorded on cultivar Sukari ndizi (48.54%) followed by cultivar Gros Michel (43.11%) and the lowest disease severity was recorded on cultivar Mchare laini (41.39%) (Table 5). The results also showed that there was significance difference (P≤0.01) between the effect of treatments on plant girth among studied cultivars. The highest size of the girth was recorded on Grand Naine (19.75%) while the lowest recorded on Sukari ndizi (13.75%) (Table 5). There was significance difference (P≤0.05) also on the height of plants among cultivars due to treatments with the highest height recorded on Grand Naine (28.37 cm) while the lowest height was recorded on banana cultivar Sukari ndizi (20.49) (Table 5).

Table 5: Mean response of banana cultivars on treatments

Cultivar name	**Response**						
	Disease incidence (%)	**Disease severity (%)**	**Girth (cm)**	**Height (cm)**	**Number of leaves**	**Root lesion (%)**	**Corm rot (1-6) scale**
Grand naine	0.00a	0.00a	19.75c	28.37a	8.89a	0.000a	1.00a
CV-Rose	0.00a	0.00a	17.07abc	25.82ab	7.39b	0.68a	1.00a
Huti green	66.67b	41.89abc	16.36abc	25.74ab	7.39b	1.94ab	3.00b
Mchare laini	66.67b	41.39abc	16.49abc	25.14ab	6.94b	2.22abc	3.28b
JD Yangambi	0.00a	0.00a	16.77abc	24.15ab	6.72b	2.50abc	1.00a
Kazirakwe	0.00a	0.00a	17.00abc	22.76b	6.72b	3.06abc	1.00a
Gros Michel	66.67b	43.11ac	15.96ab	21.98b	6.22b	5.00bc	3.06b
Nakitengwa	0.00a	0.00a	18.88bc	21.46b	6.111bc	5.56c	1.00a
Sukari ndizi	66.67b	48.54c	13.75a	20.49b	5.00c	5.56c	3.94b
Mean	29.60	23.41	16.89	23.99	6.82	2.95	2.031
LSD	21.44	96.57	7.357	11.88	2.836	7.481	0.9915
F-statistics	***	***	**	*	*	**	**

Means followed by the same letter(s) are not significantly different based on the Bonferroni multiple comparison test p=0.05., *=significant at P≤0.05, **= significant at p≤0.01 and *** significant at p≤0.001

In terms of number of leaves, root lesion and corm rots, significance differences (P≤0.05, p≤0.01 and p≤0.01, respectively) among different banana cultivars were observed (Table 5). Respective values scored under each variety in terms of the three parameters (leaves, root lesion and corm rots) were as indicated in Table 5.

The results also showed that, different treatments used in the current study caused significance effect (P≤0.001, P≤0.001, P≤0.05, P≤0.01, P≤0.001 and P≤0.001) in all parameters namely disease incidence, disease severity, height of plant, plant girth, corm rot and root lesions respectively (Table 6). Of these, control treatment resulted in overall plants with the largest girth (8.86 cm), highest plant height (26.83 cm) and no root lesion (Table 6). Effects of other treatments on the assessed parameters was as well variable. For instance, treating different banana genotypes with nematode followed by Fusarium resulted into the highest (36.67%) FWD severity compared to inoculation with *Foc* alone (31.07) (Table 6). More details for each treatments are as summarized in Table 6.

Table 6: Mean effect of different treatments on Fusarium wilt disease severity and incidence, plant girth, height, number of leaves, root damage and corm rot of banana in the study area

Cultivar name	Effects						
	Disease incidence	Disease severity (%)	Girth (cm)	Height (cm)	No of leaves	Com rot (1-6)	Root lesion (%)
Control	0.00a	-0.02a	18.86a	26.83b	8.89a	1.00a	0.00a
Nematode alone	0.00a	0.00a	18.63a	25.17b	7.04a	1.00a	9.44c
Foc alone	4.44b	31.07ab	16.14ab	22.95a	6.48a	2.93b	0.00a
Foc followe by nematode	4.44b	26.89ab	17.12ab	23.59b	6.94a	2.48b	0.56ab
Foc + Nematode	4.44b	20.19b	17.07ab	22.95a	6.93a	2.85b	1.53ab
Nematode followed by *Foc*	4.44b	36.67b	14.16ab	21.35a	6.72a	1.93b	6.48ac
Mean	29.6	19.1	16.99	23.99	6.82	2.03	3.00
LSD	22.37	16.37	4.20	2.72	2.84	0.88	2.84
F-statistics	***	***	**	*	ns	***	***

Means followed by the same letter(s) are not significantly different based on the Bonferroni multiple comparison test p=0.05., ns=non-significant. *=P≤0.05, **= significant at p≤0.01 and *** significant at p≤0.001

4.2.1 Discussion

The current study showed that banana cultivar Sukari ndizi, Gros Michel, Huti green and Mchare laini are susceptible to *Foc* race 1 as they appeared to have FWD symptoms with higher incidence and severity. Such results implying that, cultivation of these varieties may increase FWD problem and consequently affect overall banana production in the study area and locations with similar environmental conditions in Tanzania. Susceptibility to FWD on these banana cultivars might be due to failure of the plant immune system to resist infection by the *Foc* race 1, a common pathogen that is present in the country (Jonathan *et al*., 2006). Banana cultivars JD Yangambi, Kazirakwe, Grand naine, cv Rose and Nakitengwa appeared to be resistant to FWD implying that these materials can be used in areas with FWD problems in the study area and other locations in Tanzania.

This study also revealed that in FWD susceptible banana cultivars, there was reduction in plant girth size, height and number of functional leaves, and also had highest root lesions and corm rots compared with the banana cultivars which appeared to be resistant. Effect on these parameter on FWD infected plant materials was caused by the fact that the FWD is a vascular tissue disease, which affect transportation of food material to other parts of the plant therefore affecting general plant growth (Smith *et al*., 2014). In addition to that infection, *Foc* has been associated with severe wilting of plant leaves which lead to reduction on number of leaves (Ploetz, 2006). These results are in agreement with report by Jonathan and Rajendran (1998) who observed similar findings with the susceptible banana cultivar Rasthali in India.

The study revealed that, treatments which involved nematode followed by *Foc* after 14 days showed the highest FWD incidence and or severity compared with other treatments. Although this is the first time that these findings are revealed in Tanzania, previous study by Jonathan and Rajendran (1998) showed similar trend when a different nematodes, *M. incognita* was used in inoculation followed by *Foc* as it resulted in higher FWD incidence compared with *Foc* or nematode alone, or when *Foc* followed by nematode treatments were used. Under this treatment, nematodes acted as a predisposing factor for the fungal pathogen by causing injury on the root surface as well as weakening the root tissues by causing root lesions and support the *Foc* damage (Cook, 1983; Jensen, 1971). Similar findings were also reported by Pathak *et al.* (1999) who found that inoculating nematodes 10 days prior to fungus infection resulted to maximum FWD development. In other nematode-*Foc* systems such as *P.coffeae* inoculation followed by *Foc*, similar effects have been reported (Thangavelu, 2009).

To find individual effects of either *Foc* alone or nematode alone inoculation, it was interesting that this study has shown that inoculation with *Foc* alone resulted in overall high corm lesion index. This might be due to presence of *Foc* alone which provides free access to all parts of the roots without limitations. Presence of a single pathogen avoids competition for space and food resources or any other toxins produced by a partner pathogen. Also higher root lesions was observed to be in plants inoculated with nematodes alone during this study. This might be due to the presence of a single species in the rhizosphere with no competition for space and resources (Dinesh *et al.,* 2014). Inoculation of nematodes alone gave the organism free access to all parts of the roots without hindrances from other pathogens hence enhancing nematode multiplication irrespective of the host (Rao and Krishnappa, 1994). The higher score for root lesions observed indicated that the rate of reproduction on susceptible cultivars was high implying that they provided refuge for the multiplication and growth of *P. goodeyi*. Presence of the fungal prior to nematode infection is a blocking factor to nematode growth since the fungal organisms are capable of producing toxins that affect cell membranes, mechanical plugging of sieve plates or air embolisms that cause vascular occlusion and consequent water deficits and plant wilting and thus decline in nematode population (Dinesh *et al*., 2014).

CHAPETR FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The present findings give insights on the status of Fusarium wilt disease and nematodes on banana in Rungwe and Meru District. Both sites have shown to have Fusarium wilt and nematode but differ in incidence primarily due to susceptibility and resistivity of the varieties toward Fusarium wilt disease and nematode and management system. Rungwe District grow more of the resistant varieties to Fusarium wilt disease compared with Meru therefore has low disease incidence. The data suggest that, the best way to reduce incidence and severity of both Fusarium wilt disease and nematode is by the use of resistant varieties and good management practices. Also, the current screen house study has revealed that, nematode (*P. goodeyi)* inoculated 14 days prior to *Fusarium oxysporum* f.sp. *cubense* increased incidence and severity of FWD and seriously affected plant growth parameters in all susceptible banana cultivars. The nematodes seemed to act as a predisposing factor for the fungal pathogen infestation as they caused injury on the root surface as well as weakening the root tissues by causing root lesions.

5.1 Recommendations

Nematode population can be reduces by normal management practice like mulching as reported by Talwana *et al.* (2003) who observed that population of nematode (*R. similis*) was less in mulched mat compared with non-mulched mats. However, chemical control by the use of different types of nematicides exists. It is not possible to control Fusarium wilt by using any chemical method because the pathogen can survive for long periods in the soil and cannot be eliminated by any fungicides (Stoffelen *et al*., 2000). The best way is the use of resistant

varieties. Since the results pointed out that nematode damage is associated with increased FWD incidence and severity, management of nematode is strongly advised in the study area and other locations with similar FWD problem in order to reduce severity of the disease. Cultivars JD Yangambi, Kazirakwe, Nakitengwa, cv Rose, Matoke bananas and Grand Naine are recommended for use in the *Foc* infected areas. More studies are required to identify *P. goodeyi*-resistance cultivars and incorporate into the *Foc* resistant banana cultivars indicated in this study so that proper nematode and *Foc* management can be achieved in the study area and similar locations in Tanzania.

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APPENDICES

Table 7 Appendix -1: Response of banana on single, sequential and co-inoculation with *Fusarium oxysporum* f. sp. *cubense* and *Pratylenchus goodeyi*

Treatments		Number of wilted plants (Out of 3 plants)						
		Days after inoculation						
		14	28	42	56	70	84	98
Kazirakwe	**C**		0	0	0	0	0	0
	N	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0
	F→N	0	0	0	0	0	0	0
	F+N	0	0	0	0	0	0	0
	N→F	0	0	0	0	0	0	0
Gros Michel	**C**	0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
	F	0	0	0	0	3	3	3
	F→N	0	0	2	3	3	3	3
	F+N	0	0	1	3	3	3	0
	N→F	0	0	0	2	0	2	3
JD Yangambi	**C**	0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0
	F→N	0	0	0	0	0	0	0
	F+N	0	0	0	0	0	0	0
	N→F	0	0	0	0	0	0	0
Graand Naine	**C**	0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0
	F→N	0	0	0	0	0	0	0
	F+N	0	0	0	0	0	0	0
	N→F	0	0	0	0	0	0	0
Mchare Laini	**C**	0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
	F	0	0	0	2	3	3	3
	F→N	0	0	2	2	3	3	3
	F+N	0	0	2	2	2	3	3
	N→F	0	0	0	3	3	3	3
Sukari ndizi	**C**	0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
	F	0	0	3	3	3	3	3
	F→N	0	0	2	2	3	3	3
	F+N	0	1	3	3	3	3	3
	N→F	0	0	2	2	2	3	3
Nakitengwa	**C**	0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0
	F→N	0	0	0	0	0	0	0
	F+N	0	0	0	0	0	0	0
	N→F	0	0	0	0	0	0	0
Huti green	**C**	0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0
	F	0	0	0	2	3	3	3
	F→N	0	0	1	1	2	2	3
	F+N	0	0	2	2	2	3	3
	N→F	0	0	2	3	3	3	3
C v R o se	**C**	0	0	0	0	0	0	0
	N	0	0	0	0	0	0	0

F	0	0	0	0	0	0	0
F→N	0	0	0	0	0	0	0
F+N	0	0	0	0	0	0	0
N→F	0	0	0	0	0	0	0

Appendix 2-Table 8: Experimental layout.

3				1				8				
1 **E**	2 **C**	3 **B**		7 **C**	8 **D**	9 **A**		13 **B**	14 **F**	15 **E**		
4 **D**	5 **A**	6 **F**		10 **B**	11 **E**	12 **F**		16 **C**	17 **D**	18 **A**		
7				5				2				
19 **C**	20 **A**	21 **F**		25 **A**	26 **B**	27 **C**		31**A**	32 **E**	33 **D**		
22 **B**	23 **D**	24 **E**		28 **E**	29 **F**	30 **D**		34 **F**	35 **C**	36 **B**		Rep 1
4				9				6				
37 **B**	38 **C**	39 **F**		43 **D**	44 **A**	45 **F**		49 **E**	50 **F**	51 **B**		
40 **A**	41**E**	42 **D**		46 **E**	47 **B**	48 **C**		52**D**	53 **A**	54 **C**		

3				1				8				
1 **E**	2 **C**	3 **B**		7 **C**	8 **D**	9 **A**		13 **B**	14 **F**	15 **E**		
4 **D**	5 **A**	6 **F**		10 **B**	11 **E**	12 **F**		16 **C**	17 **D**	18 **A**		
7				5				2				
19 **C**	20 **A**	21 **F**		25 **A**	26 **B**	27 **C**		31**A**	32 **E**	33 **D**		
22 **B**	23 **D**	24 **E**		28 **E**	29 **F**	30 **D**		34 **F**	35 **C**	36 **B**		Rep 2
4				9				6				
37 **B**	38 **C**	39 **F**		43 **D**	44 **A**	45 **F**		49 **E**	50 **F**	51 **B**		
40 **A**	41**E**	42 **D**		46 **E**	47 **B**	48 **C**		52**D**	53 **A**	54 **C**		

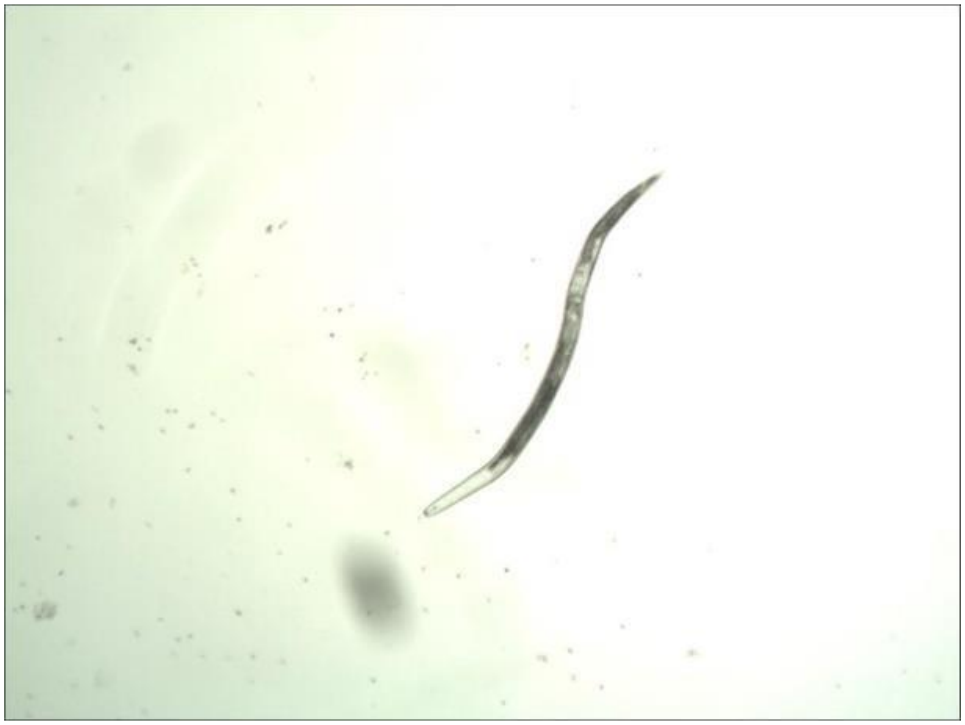
3				1				8				
1 **E**	2 **C**	3 **B**		7 **C**	8 **D**	9 **A**		13 **B**	14 **F**	15 **E**		
4 **D**	5 **A**	6 **F**		10 **B**	11 **E**	12 **F**		16 **C**	17 **D**	18 **A**		
7				5				2				
19 **C**	20 **A**	21 **F**		25 **A**	26 **B**	27 **C**		31**A**	32 **E**	33 **D**		
22 **B**	23 **D**	24 **E**		28 **E**	29 **F**	30 **D**		34 **F**	35 **C**	36 **B**		Rep 3
4				9				6				
37 **B**	38 **C**	39 **F**		43 **D**	44 **A**	45 **F**		49 **E**	50 **F**	51 **B**		
40 **A**	41**E**	42 **D**		46 **E**	47 **B**	48 **C**		52**D**	53 **A**	54 **C**		

*A – B = Treatments, 1 – 54 = Experimental plot

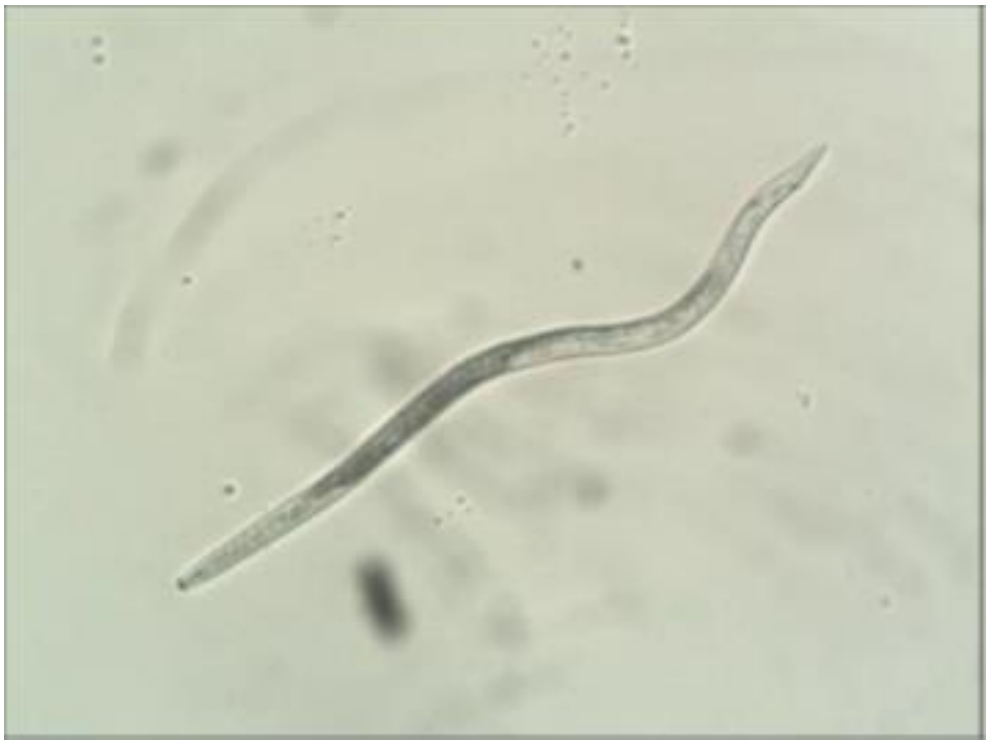
Appendix 3: *Foc* growing on the PDA media plate after 14 days

Appendix 4 - Millet seeds with sliced Foc PDA (Source: This study)

Appendix 5: Foc growing on millet after 12 days (Source: This stud)



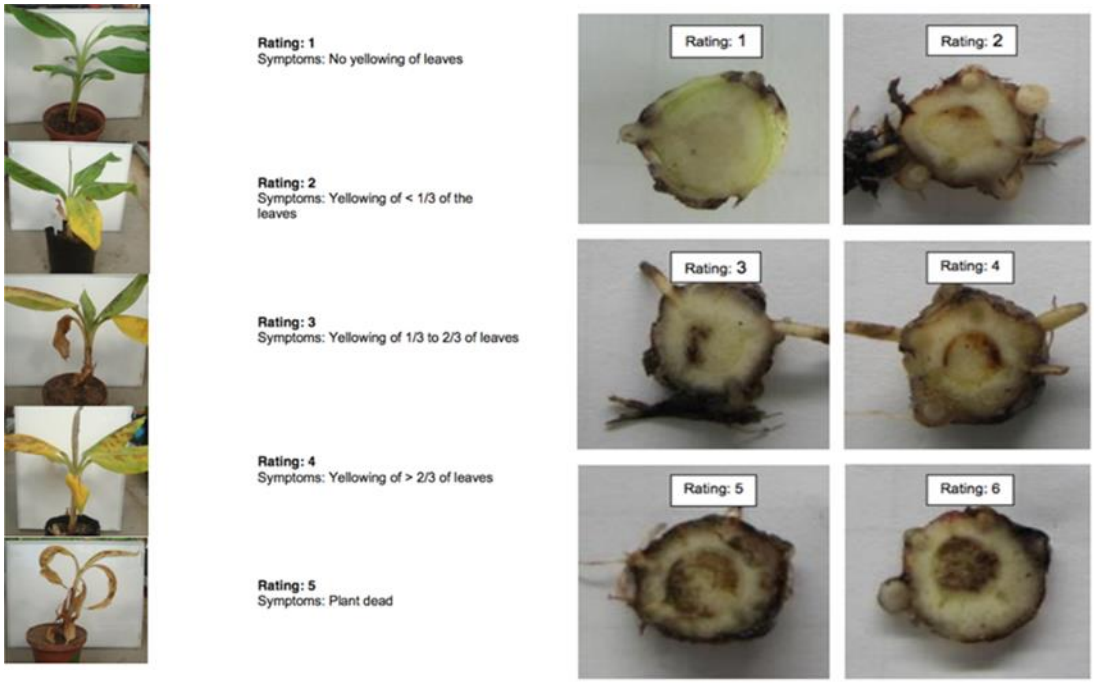
Appendix 6: Female *P. goodeyi* (10x magnification) (Source: This study)



Appendix 7: Male *P. goodeyi* (20x magnification) (Source: This study)

Appendix 8: Foc inoculation with millet seeds (Source: This study)

Appendix 9: Plant inoculation with nematode solution (Source: This study)



Appendix 10: Scale for external and internal Foc scoring (Source: Viljoen et al. 2016).

Appendix 11: Plants arrangements (Source: This study)

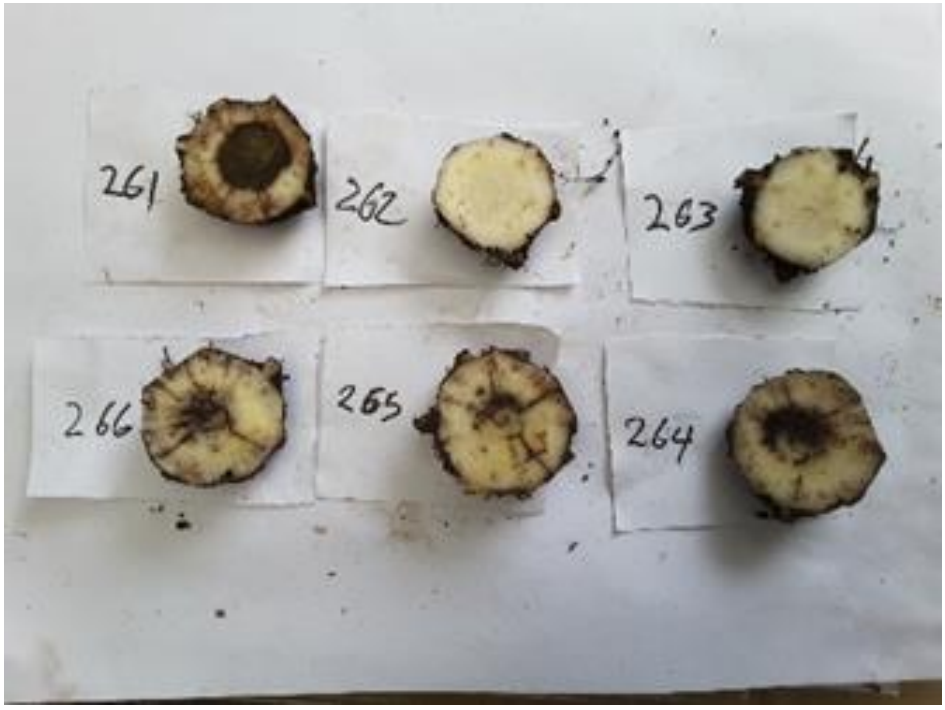
Appendix 12: Reduced plant height (right) on combined and sequential inoculation (Source: This study)

Appendix 13: Nematode damage on banana roots (Source: This study)

Appendix 14: Measuring plant height using tape measure (Source: This study)

Appendix 15: Determining plant girth using digital caliper (Source: This study)

Appendix 16: External symptoms of Fusarium wilt disease on Mchare (Source: This study)



Appendix 17: Internal symptoms of Fusarium wilt disease on Mchare (Source: This study)

RESEARCH OUTPUT

Status of Fusarium wilt disease and plant parasitic nematode on banana in Northern and Southern highlands of Tanzania

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Key words; Incidence, Severity, nematode, Fusarium wilt.

ABSTRACT

A study to examine the status of Fusarium wilt disease and plant parasitic nematode on banana in Northern and Southern highlands of Tanzania was conducted in January 2017 at Meru District in Arusha and Rungwe District in Mbeya region Tanzania. Forty eight farms in twelve villages were scored for Fusarium incidence, severity and nematode damage using scale of 1-5 for severity of Fusarium wilt disease and a scale of 1-5 for nematode root damage (in percentage). Incidence of Fusarium wilt was expressed as the percentage of diseased plants in every field under study. The results indicated that the severity of Fusarium wilt disease was at highest score (72%) at Sing'isi in Meru District while the lowest score (25.83%) obtained at Mpuguso ward in Rungwe District. The highest incidence (11.48%) was found to be at Nkoaranga ward followed by Akheri (8.95%) in Meru District while the lowest incidence (0.83%) were found at Mpuguso in Rungwe District. Highest score for nematode damage (37.5%) was found at Kimo ward in Rungwe District while the lowest score (17.5%) was observed at Lufingo ward in Rungwe Disrtict. The current study shows that Fusarium wilt incidence and severity is high in Meru District while nematode damage is high in Rungwe District.

INTRODUCTION

Bananas (*Musaspp*) is a giant herbaceous perennial plant that belongs to the genus Musa. It is divided into four groups; Callimusa, Australimusa, Eumusa and Rhodochlamys, (Simmonds and Shepherd, 1955). The origin of Banana is believed to be South East Asia (Jones, 2000). There are more than 1000 cultivars of banana worldwide which are mainly triploids (2n = 3x = 33), seedless, often sterile and parthenocarpic (Heslop-Harrison, 2011). Most of these cultivars are derived from two wild diploid species (2n = 22); *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) (Shepherd and Ferreira, 1982) through intra and interspecific hybridization (Simmonds, 1995) with *M. acuminata* being the most widespread of the Eumusa species (Horry *et al.,* 1997). Generally, modern classifications of banana cultivars follow Simmonds and Shepherd's system in which cultivars are placed in groups based on the number of chromosomes they have and species they are derived from (Karamura *et al.,* 2012).

In East Africa, bananas especially the East African highland bananas, such as Matooke (AAA-EA), the Illalyi (AAA), and Mchare (AA) (Karamura *et al.,* 2006) are important staple as well as cash crops for majority living in the plateaus Karamura *et al.,* 2012).

Bananas and plantains rank fourth after rice, wheat and maize in terms of importance globally (FAO, 2016; Sharrock and Frison, 1999). It is believed to be grown in more than 130 countries occupying an area of over 10 million hectares and an annual production of more than 143 million tonnes (FAOSTAT, 2016). Those which enter international commerce are worth more than $5 billion per year, and locally consumed fruit are major staples for over 400 million people in Africa and Latin America (Ploetz, 2006). The Great Lakes region of Africa has remained to be the largest producer and consumer of bananas in Africa where per capita consumption of banana ranges from 230 to 450 kg person^{-1} year^{-1} (FAOSTAT, 2016, 2012; Smale, 2006).

Nutritionally, Bananas are excellent source of potassium. A single banana can provide 23% of the potassium that is required by human body on a daily basis (Kumar *et al*., 1992). It is a rich source of carbohydrate 23%, fibre 2.5%, fat 0.5% and protein 1% (Mohapatra *et al*., 2010). Banana is also a good source of the following vitamins: carotene, vitamin E, thiamine (B1), riboflavin (B2), niacin, pyridoxine (B6), folic acid, pantothenate, biotin and vitamins C (Kumar *et al*., 1992). It is also rich in other mineral elements such as sodium, calcium, magnesium, phosphorus, iron, copper, zinc, chloride, manganese and iodine (Robinson, 1996). Banana leaves and dry pseudo stems are used as animal feeds and for wrapping food stuffs and in thatching houses, handcrafting of mats and as agricultural mulch (Frison and Sharrock, 1999).

Tanzania cultivates about 403,000 hectares of land that produces about 3.7 MT of which the biggest proportion (i.e. 2.5 million MT) comes from Kilimanjaro and Kagera regions (Kilimo Trust 2012). Other regions which produce banana in the country include Kigoma, Mbeya, Arusha, Tanga, Mara n and Morogoro region (Mgenzi and Mkulila, 2004*)*.

Despite its importance, banana yield is unceasingly declining in Tanzania (Van Asten *et al*., 2005). For example, in some locations of the country the production has declined from 18 tonnes/ha in the 1960s to less than 6 tonnes/ha in 2000 (Mgenzi *et a*l., 2005; Sikora *et al*., 1989; Walker *et al.*, 1984). Such decline in production has been associated to various abiotic and biotic factors including soil fertility problems, drought, insect pests and diseases (Swennen *et al*., 2013). Of the recorded constraints, plant parasitic nematodes such as *Pratylenchus goodeyi* and *Radopholus similis* are among destructive pests of banana plant in a variety of environments (Coyne *et al.,* 2014). These organisms are small, worm-like and members of the animal kingdom with length ranging from 0.5-1.0 mm (Mbega and Nzogela, 2012). They are usually found in almost every habitat, in fresh or salt water, and in soil. Plant parasitic nematodes have stylets, spear-like mouthparts that pierce cells and allow nematodes to feed on their contents (Weischer and Brown 2000). Strategies used by plant parasitic nematodes to conquer the host have been clearly illustrated by Mbega and Nzogela (2012). If successful entry into plant is achieved, these nematodes feed, migrate and multiply inside banana roots and corms causing root-tissue necrosis and root system reduction which then cause damage to plants, impaired transport and uptake of water and nutrients resulting in reduced plant growth and yield (Viljoen *et al.,* 2016). In addition, the anchorage function of the root system is adversely affected resulting in plant toppling (Gowen & Queneherve, 1990).

Globally, nematodes have been reported to reduce agricultural production by approximately 11% (Viljoen *et al.,* 2016). The wounds resulting from nematode attack can also provide avenue for entry and infestation by soil-borne fungal organisms such as *Fusarium oxysporum* f. sp. *cubense* (*Foc*) causal agent of Fusarium wilt of banana (Inagaki & Powell, 1969). This disease is also known as Panama disease because it first became epidemic in Panama in 1890 and proceeded to devastate the Central American and Caribbean banana industries that were based on the 'Gros Michel' (AAA) variety in the 1950s and 1960s, (Perez, 2004). Fusarium wilt disease is in the same rank with some most devastating plant diseases of other crops such as wheat rust and potato blight in terms of crop destruction (Dean *et al.,* 2011). As of 1995, panama disease has been reported from all banana-growing regions including East Africa, except the Mediterranean,

Melanesia, Somalia, and some islands in the South Pacific (Pegg *et al.,* 1996; Ploetz 2006). Once *Foc* is present in the soil it cannot be eliminated (Davis *et al.,* 2000). It disrupts the plant's water conducting vessels resulting in yellowing and wilting of leaves (progressing from older to younger leaves). Inside a vertical section of the pseudostem, brown, red or yellow lines have been reported to be a characteristic attribute of the disease, the lines which also appear as rings in pseudostem cross-section (Ong *et al.,* 1996; Viljoen, 2002). As a result of infestation the internal section of the pseudo stem rot extensively (Ong *et al.,* 1996).

There are four recognized races of the pathogen which are separated based on host susceptibility as follows: race 1 affects Gros Michel, Lady Finger (AAB) and Silk (AAB), race 2 affects cooking bananas such as 'Bluggoe, race 3 affects Heliconia spp., a close relative of banana, and is not considered to be a banana pathogen, and race 4 which is a more virulent form of the pathogen cause disease in Cavendish banana (Viljoen, 2002; Ploetz, 2005).

Despite the destructive role that plant parasitic nematode and *Foc* disease have on banana, the status of these pests infestation especially on the East African Highland Bananas (EAHB) which is a unique group of bananas found in East African plateau has not been established in Tanzania. It is clearly known that a complex interrelationship between nematode and *Foc* in bananas is said to produce a combined effect which is greater than the sum of their separate effects (Jonathan and Rajendran 1998). Thus, the aim of this study was to assess the incidence and severity of Fusarium wilt disease and nematode infection on banana growing in Meru and Rungwe Districts located in the main banana growing regions in Northern and Southern Highlands of Tanzania, respectively.

MATERIAL AND METHODS

Location

This study was conducted in two districts one from Northern highland - Meru District located in Arusha region and another from Southern highland, Rungwe District located in Mbeya region Tanzania. These districts were selected due to their importance in banana production in the two regions and also based on complains from farmers on *Foc* and nematodes, the two problems of which their status was unknown.

Assessment of proportional numbers of banana cultivars

Four wards in Meru and four wards in Rungwe Districts with information on existence of different banana cultivars were selected and used in the study (Table 1). To quantify the number of banana cultivars per farm in each ward, six randomly selected banana farms of about 1-2 acres each (two farms per village) were selected. In each farm, number of each banana cultivar was recorded and its proportion by number in the farm was determined by dividing number of its count by total number of banana plants in the field x 100%.

Assessment of Fusarium wilt disease incidence, disease severity and nematode damage

Disease incidence was quantified by dividing the number of infested plant units by total number of plants in the field x 100%. Disease severity was quantified based on external symptoms of the disease by inspecting individual plants from each banana variety using a scale of 1 to 5 as established by Viljoen *et al*. (2016) with modification. Using the established scale, 1 described; no visual leaf symptoms, 2; = 0-33% of older banana leaves turning yellow, 3; = 34-66% of older leaves turning yellow with some hanging down the pseudostem, 4; = 67-95% of the leaves turning yellow and necrotic with leaves hanging down the pseudostem and 5; = 96-100% plant dead with brown leaves hanging down the pseudostem. Scoring nematode damage was done by estimating visual damage of root (as a percentage) using a scale of 1 to 5 adopted from Speijer and De Waele (1997). Systemic sampling pattern was used in order to accommodate the patchy nature of nematode distribution where five plants were selected for nematode scoring in every farm. From each plant, five functional roots of 10 cm length were randomly selected, washed with water to remove soil and sliced lengthwise. Scoring was done on one half of the root for the percentage of root cortex showing necrosis. As each of the five isolated roots usually carries 20 marks (Speijer and De Waele, 1997), the proportion damage under each root was estimated and the sum damage proportions was used as final score in percentage.

RESULTS

Common banana cultivars in the study area

The results showed that, cultivar Mchare was the most common grown banana with proportions ranging from 65.76% in Akeri ward to 72.35% in Sing'isi ward followed by cultivar Grand Naine which had a proportional ranging from 16.30% in Shagarai ward to 19.0% in Akeri ward in Meru District (Table 1). The results also showed that the most common grown banana cultivar in Rungwe District was Plantains which ranged from 27.3% at Kiwira ward to 47.65% at Kimo ward, followed by Matoke bananas which ranged from 22.30 at Kimo ward to 32.0% at Lufingo ward (Table 1). Information on commonality for other varieties covered in this study are as shown in Table 3.

Table 1: Number (%) of common Banana cultivars in selected wards of Meru and Rungwe Districts as characterized during this study

Ward	**District**	**Banana cultivar and their proportional number (%) per farm**				
		Mchare	**Matoke**	**Plantain**	**Cavendish**	**Sukari ndizi**
Mpuguso	Rungwe	5.89	28.9	37.28	26.40	0.90
Kimo	Rungwe	2.73	22.35	47.65	29.27	1.19
Lufingo	Rungwe	2.23	34.00	33.33	25.50	1.24
Kiwira	Rungwe	4.20	33.39	27.30	30.20	0.73
Shangarai	Meru	67.39	11.7	1.33	16.30	1.09
Akheri	Meru	65.76	10.69	0.68	19.00	1.89
Sing'isi	Meru	72.35	8.07	1.79	16.14	1.20
Nkoaranga	Meru	71.86	9.24	0.87	17.03	0.87

Source: This study

Fusarium wilt disease incidence, disease severity and nematode damage in the study area

The results showed that Fusarium wilt disease and banana nematodes were found to be present in all villages under study in Arumeru and Rungwe Districts (Table 2). However, there was significant different ($p \leq 0.001$) between the incidences of Fusarium wilt disease in the study area.

The highest Fusarium disease incidence (11.48%) was recorded at Nkoaranga ward while the lowest disease incidence (0.83%) was recorded at Mpuguso Rungwe District.

Table 2: Fusarium wilt disease incidence, disease severity and nematode damage in Meru and Rungwe Districts as established in this study

Ward	District	Fusarium wilt disease Incidence (%)	Fusarium wilt disease severity (%)	Nematode damage (%)
Mpuguso	Rungwe	0.83a	25.83a	22.50bc
Kimo	Rungwe	1.50a	52.50abc	37.50a
Lufingo	Rungwe	1.57a	32.17ab	17.50c
Kiwira	Rungwe	2.72a	54.67abc	30.83ab
Shangarai	Meru	7.94b	63.33bc	34.17ab
Akheri	Meru	8.95b	55.83abc	35.00a
Sing'isi	Meru	9.30b	72.00c	30.83ab
Nkoaranga	Meru	11.48b	48.33abc	30.00ab
Mean	na	5.53	50.58	29.80
Lsd	na	3.82	32.88	11.97
F-statistics	na	***	*	**

Means followed by the same letter(s) are not significantly different based on the Bonferroni multiple test a p=0.05., na= not applicable. ns=non-significant., *=significant at P≤0.05,**= significant at p≤0.01 and *** significant at p≤0.001

However, such disease incidences were based on overall evaluation in each surveyed field regardless of existence of resistant banana varieties. Thus results for specific cultivar related disease incidences indicated that only Mchare and Sukari ndizi cultivars were susceptible to Fusarium wilt disease with incidences of 46.84% on cultivar Sukari ndizi at Lufingo ward to 59.9% on cultivar Mchare at Kiwira ward both in Rugwe District (Fig.1).

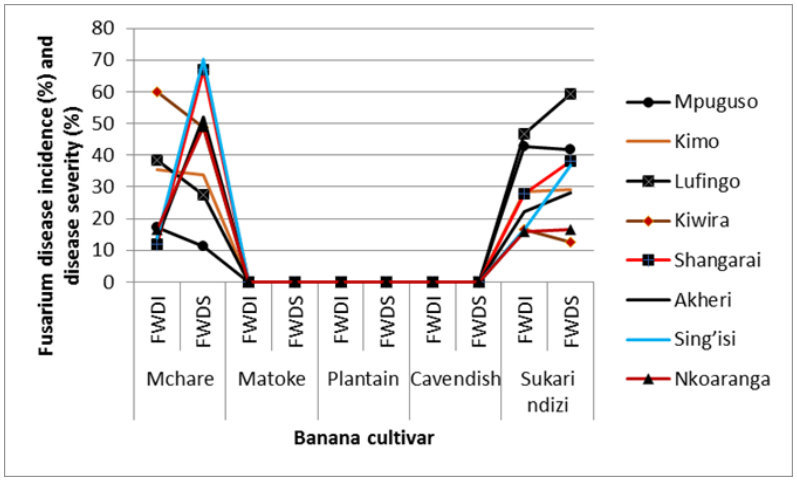

Figure 1: Fusarium wilt disease incidence (FWDI) and disease severity (FWDS) on common banana cultivars in the study area

The results also showed there was significance different (P≤0.05) between Fusarium disease severity on banana cultivars in the study area (Table 2). Fusarium wilt disease severity ranged from as high as 59.17% on cultivar Sukari ndizi at Lufingo ward in Rungwe District to 70.33% on Mchare at Sing'isi ward, Meru District (Fig. 1). The results also showed that all banana cultivars in the study area were susceptible to nematode and the damage was significant different (p≤0.01) (Table 2 and Fig. 2).

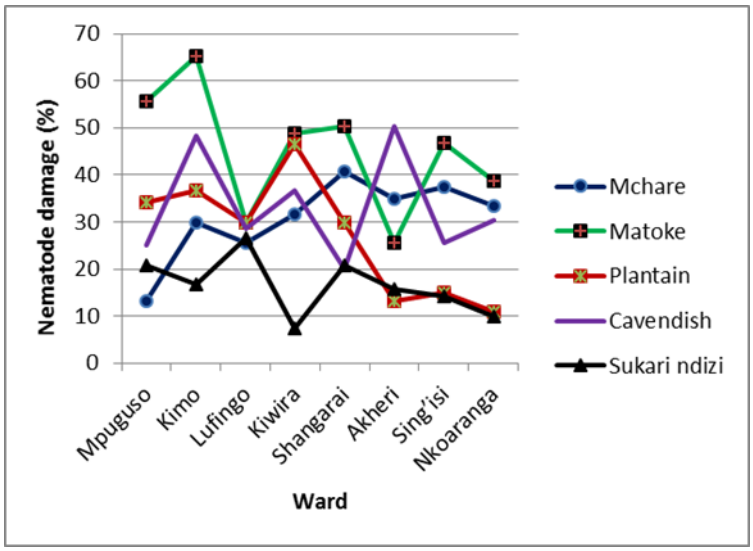

Figure 2: Nematode damage on common banana cultivars in the study area

The highest score for nematode damage (37%) was recorded at Kimo ward in Rungwe District followed by Akheri (35%) and Shangarai (34.17%) in Meru District while the lowest score (17.5%) was recorded at Lufingo ward in Rungwe Disrtict (Table 2). On different cultivars, the

results showed that Matoke bananas was the most susceptible of all with damage levels of as high as 50.35% at Shangarai ward in Meru District to 65.12% at Kimo ward in Rungwe District (Fig 2). Other banana cultivars with high nematode damage levels were Grand Naine (50.26%) at Akeri ward, Meru District, Plantains (46.62%) at Kiwira, Rungwe District and cultivar Mchare (40.83%) at Shangarai ward, Meru District (Fig.2).

Discussion

This study has revealed that common grown banana varieties under the study area are Mchare, Matoke, Plantain, Grand Naine and Sukari ndizi. However, the importance of each cultivar differs between the two sites. While Mchare cultivars are highly grown in Meru District, important banana cultivars in Rungwe District are Plantains and Matoke bananas. This was also reported by Karamura (2006) who mentioned Meru-Kilimanjaro axis to be dominated by Mchare banana. Similarly, Maruo (2007) reported plantain to be playing a key role in consolidating the development of the Nyakyusa (ethnic group in Rungwe district) rural community. In this study, Fusarium wilt disease and nematode were reported to be present in both Meru and Rungwe districts. The highest score for Fusarium wilt disease incidence and severity was recorded in Meru District on Mchare varieties. One of the reason for high incidence and severity of Fusarium wilt disease in this region might be due to high cultivation of susceptible cultivars which are Mchare (Koka and Swennen 2017) and Sukari ndizi (Viljoun et *al*., 2016) compared with Rungwe Didtrict. In addition, farming systems in Meru District differ from those found in Rungwe. During dry season, farmers do irrigate their banana field using surface/furrow irrigation method which essentially cause movement of pathogen from infected plants to the health ones through running water (Ploetz, 2006). Farmers in Arumeru also use banana plants (leaves and pseudostem) as feeding material to their cattle, therefore pruning of functional leaves for animal feed with the same machete across the field without disinfection is another way of spreading the disease (Ploetz, 2006) which lead to the increase of disease incidence. However, the proportion of incidence to the total number of plants is low because farmers normally do not withstand keeping diseased plants in the field for long period. They destroy diseased plant as soon as they see symptoms of Fusarium wilt unless the symptoms occurred at a later stage when the bunch is close to maturity. Plantain, Matoke, and Grand Naine which are highly grown in Rungwe district are all resistant to *Foc* race 1, a causative agent of Fusarium wilt disease (Kashaija *et al*., 1994; Speijer *et al*., 1994) which lead to have low disease incidence compared with Meru District.

Relative to Meru district, there is no furrow irrigation in Rungwe and therefore, spread of pathogen through irrigational water is avoided. Also most of the farmers in Rungwe do not keep animals therefore no extensive pruning of leaves which reduces disease incidence.

The highest nematode damage between the two sites was reported to be in Rungwe District. However, the difference observed was small when compared with Meru District. The small difference in nematode damage might be contributed by presence of similar susceptible varieties to nematode (Matoke and Grand naine) INIBAP, (1997) in both regions and similarity in climatic conditions. For example, Nkwamansa village in Arumeru District located 1406 meters above sea level is similar to Kalalo in Rungwe District which has an altitude of 1415 meters above sea level. Normally nematodes distribution is much influenced by altitude (Price 2000). For example, the occurrence of *Radopholus.similis* rapidly declines at elevation above 1300 meters above sea level while *P. goodeyi* dcreases below 1200 meters above sea level. *Helicotylenchus multicinctus* and *Meloidogyne* spp. are high at lower altitude (Elsen *et al*., 2000; Speijer and Fogain, 1999). However, there are some nematode species like *P. goodeyi*, with unique characteristics. They have much more restricted distribution and are said to have a lower temperature preference than others and its distribution is closely linked to altitude and the higher latitudes of the cooler banana growing areas of up to 1500 meters above sea level. (Bridge *et al.,* 1997). When looking on the specific crop, Matoke bananas has been affected more by nematode compared with other varieties. This is because Matoke, the East African Highland Bananas (AAA-EA) is more prone to *Pratylenchus goodeyi* which is more prominent specie in East African Highland Bananas and found in many banana growing areas (Elsen *et al*., 2000).

Conclusion and Recommendation

The present findings gave insights on the status of Fusarium wilt disease and nematodes on banana in Rungwe and Meru District. Both sites have shown to have Fusarium wilt and nematode but differ in incidence primarily due to susceptibility and resistivity of the varieties toward Fusarium wilt disease and nematode and management systems. Rungwe District grow more of the resistant varieties to Fusarium wilt disease compared with Meru therefore has low disease incidence. The data suggest that, the best way to reduce incidence and severity of both Fusarium wilt disease and nematode is by the use of resistant varieties and good management practices. Nematode population can be reduces by normal management practice like mulching as reported by Talwana *et al.* (2003) who observed that population of nematode (*R. similis*) was less in mulched mat compared with non-mulched mats. However, chemical control by the use of

different types of nematicides exists. It is difficult to control Fusarium wilt disease by using any chemical method because the pathogen can survive for long periods in the soil and cannot be eradicated by any fumigant (Stoffelen *et al*. 200). The best way is the use of resistant varieties.

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