EFFICACY OF SELECTED BIOLOGICAL AND SYNTHETIC AGENTS IN THE MANAGEMENT OF PLANT PARASITIC NEMATODES IN TISSUE CULTURE BANANA

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DECLARATION

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

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DEDICATION

This thesis is dedicated to my late Father Isaiah Mwathi, my late Sister Prof. Cecilia Mwathi, my Sons; Joe-Kevin Muiga, Immanuel Mwathi, Promise Irungu, my Mother Beatrice Ngonyo and my Niece Angela Amani.

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LIST OF ACRONYMS

0^c	-	Degrees Celsius
AMF	-	Arbuscular Mycorrhiza Fungi
ANOVA	-	Analysis of Variance
CRD	-	Completely Randomized Design
CV	-	Co-efficient of Variation
DAT	-	Days after Treatment
DW	-	Distilled water
FAO	-	Food and Agriculture Organization
GM	-	Grand Mean
HCDA	-	Horticultural Crops Development Authority
INIBAP	-	International Network for the Improvement of Banana and Plantain
LSD	-	Least Significant Difference
MOA	-	Ministry of Agriculture
SC/ML	-	Spore Count per Millilitre
SDA	-	Sabouraud Dextrose Agar
US$	-	United State Dollar

ABSTRACT

Pratylenchus spp. and *Meloidogyne spp.* are key pests of banana in Kenya. Tissue cultured banana plantlets are considered clean for planting but infestation with these pests has been increasing due to the state of the soil in the farms. This has contributed a lot to huge losses from toppling disease which occurs at production stages of banana just before the banana bunches are ready for harvesting. Biological control agents in nematodes management may be used separately or can be combined with synthetic products to bring down the number of nematodes and to impart resistance to the plant. In this experiment, evaluation of selected biological agents and synthetic agent on *Pratylenchus spp* and *Meloidogyne spp* damage was conducted. Seedlings of tissue cultured banana were inoculated with biological agents and a synthetic agent and allowed to grow for one month. The variety of banana used in this experiment was grand nain variety. Plantlets were transplanted in clean forest soil. This soil was analyzed for nematodes and was found free of the nematodes. Nematodes were extracted from roots exhibiting root lesions and root galls. These two nematode species were used for inoculation of the clean forest soil after which damage caused by plant parasitic nematodes and their populations were evaluated after the fifth week of treatment. Plant response to biological agent and synthetic agent treatments was assessed from plant height, leaf width and leaf length for the entire duration of the experiments. There was a significant difference in height for banana plantlets inoculated with nematophagus fungi in rice bran substrate (63.6 cm) compared to control treatment. Those inoculated with nematophagus fungi without substrate followed closely with a mean height of 60.2 cm. There was a significant difference in height for plantlets treated with nematophagus fungi (15.1 cm) without substrate compared to those not treated at all. A significant difference in leaf length was also realized in seedlings that were inoculated with nematophagus fungi without substrate (36.6 cm). Nematophagus fungi in rice bran

substrate produced seedlings with the least number of galls (1.5 galls) compared to control treatment. Combined treatment had the least number of root lesions (4.2 lesions). Seedlings inoculated with calcium (27.1 roots) exhibited a significant difference in the total number of primary roots formed compared to control treatment. Similarly, seedlings inoculated with calcium treatment recorded the least number of *Pratylenchus spp*. (95.5 nematodes) while those inoculated with nematophagus fungi in rice bran carrier recorded the least number of *Meloidogyne spp*. (20.2 nematodes). In this experiment, barley exhibited the highest spore count of 3.38×10^{10} on the 28^{th} day of the experiment. Barley was followed closely by rice bran with a spore count of 2.99×10^{10}. With time there was an increase in the number of spores from week one of inoculation to the fourth week. Growth of this fungus was significant in barley carrier. The findings of this study show that barley can be considered as a suitable carrier for production of *Paecilomyces lilacinus* fungi for effective management of nematodes in banana plants. *Paecilomyces lilacinus* positively played a key role towards growth and establishment of the banana seedlings.

CHAPTER ONE

1.0 INTRODUCTION

Banana is an important source of income and food security among small holders and large scale farmers (HCDA/MOA, 2012). It is an important staple and income generating crop for millions of people in the tropical and subtropical parts of the world (Ssebuliba *et al*., 2005; The available data collected in the year 2015 indicate that global production of banana was recorded at 117.9 tonnes and approximately 5.5 million hectares of land put under cultivation (FAO, 2015).

Banana is ranked fourth worldwide after rice, wheat and maize as far as food security is concerned (Tripathi *et al*., 2008). According to Macharia *et al.* (2010), banana offer a reliable source of income to small scale farmers. Well maintained banana orchards remain productive for a long period of time of over fifteen years. During dry seasons, banana offer an alternative source of fodder for domestic animals while the dry pseudostems are used in art industry for making decorative items like table mats, baskets and earrings. Banana pseudostem offers an abundant natural resource in subtropical and tropical regions for providing profitable products like manure (Ultra *et al*., 2005) and feeds (Ulloa *et al*., 2004).

Banana is attacked by a number of pests and diseases (Poornima, 2007) which contribute to significant food and income losses. These losses are caused by; nematodes, weevils, black sigatoka and panama disease (Svetlana *et al*., 2004). Nematodes contribute to 20% -30% yield losses in banana annually (Yu *et al*., 2012). The lesion nematodes cause

lesions that coalesce to produce large necrotic areas of tissue (plate 1) as nematodes continue to migrate and feed within the roots (Davis and Mac-Guidwin, 2000). Root lesion nematodes cause damage and reduction of the root system which leads to stunting of plants, decreased bunch weight, lengthening of the production cycle, and toppling. The toppling disease caused by lesion and burrowing nematodes namely; *Radopholus similis* and *Pratylenchus spp* respectively is a serious problem whose direct effect is not readily quantified. However, it has been estimated that its combined impact on reduction of yields and quality of banana is more than 55% of total productivity (Talwana *et al.*, 2003). Plant-parasitic nematodes are a threat to crops and cause annual crop losses worth about $100 billion worldwide (Oka *et al*., 2000; Li *et al.,* 2014).

Management of the lesion nematode can be achieved by reducing the initial nematode densities by growing resistant crops or cultivars. Mycorrhiza inoculated plants have increased accumulation of phenols which cause resistance to pathogens according to a research conducted on groundnuts (*Arachis hypogaea)* colonized by *Glomus fasciculatum* (Thangaswamy and Padmanabhan, 2006). Mycorrhiza inoculated plants have the following benefits; stimulation of the production of growth regulating substances, increased photosynthesis, improved osmotic adjustment under drought and salinity stresses and also increasing resistance to pests and soil borne diseases (Al-Karaki, 2006). These benefits are mainly attributed to improved phosphorous availability (Plenchette *et al.,* 2005). *Paecilomyces lilacinus* fungus has been reported to reduce nematode population densities. This fungus has been considered as one of the most promising and practical biocontrol agents for management of plant parasitic nematodes (Khan *et al.,* 2003; Shanthi and Rajendran, 2006*;* Gortari *et al.,* 2008; Mucksood and

Tabreiz, 2010). Calcium has been referred to as the plants first line of defense due to its ability to increase resistance to diseases by plants (Usten *et al* .,2006). Strengthening of the plants cells by use of calcium also enables them to withstand impact of nematodes attack.

1.1. Problem Statement

Banana farmers have been affected by yield decline in most of the growing areas in East Africa (INIBAP 2003). This has been caused by a number of factors including soil pests and diseases. The root lesion and root knot nematodes are known cause for weakening of root systems thus destroying anchor roots, reduction of yields, and toppling of plants especially at fruiting stage before harvest. They make banana plants prone to lodging, reduce uptake of fertilizer and utilization and reduce the lifespan of banana orchard (Chitamba *et al*., 2013). This problem has contributed to huge production and economic losses (Tripathi *et al*., 2015). Nematodes cause 20% -30% yield losses of banana annually (Yu *et al*., 2012). Plant parasitic nematodes impose losses of up to 70% on plantains and cooking banana in Africa (Tripathi *et al*., 2015). Banana plants, especially the cavendish types, are sensitive to biotic and abiotic constraints like; pests and diseases, stresses like droughts, salinity, soil moisture deficit and extreme temperatures. Losses caused by *Meloidogyne spp.* in the world are estimated around US $157 billion annually (Nur, 2014).

The losses are in terms of reduced banana production due to breakages of immature banana bunches (Coyne ,2009) and lowered quality which brings down the market value of banana. The affected banana orchards have a reduced lifespan and production of very small banana bunches thus resulting to reduced yields. Banana yields have gone as low

as < 20 T ha^{-1} $year^{-1}$ (FAO, 2009). Currently, plant parasitic nematodes are controlled by use of nematicides which are expensive and toxic to the users and the environment (Diogo *et al*., 2009). Most of the nematicides lose their efficiency with continued use. There is need to carry out research on possible methods of managing the nematodes specifically the root knot and root lesion nematodes which cause toppling disease of banana.

1.2. Justification

Use of chemical nematicides has been restricted and others withdrawn from the market (Wesemael *et al.,* 2011). Methyl bromide was the most widely used nematicides before its ban in 2005. There has been a public concern over the use of chemical nematicides due to their toxicity and loss of efficacy after their prolonged use (Janja *et al.,* 2013). Recently, the need for healthy food and awareness of environmental hazards has led to a shift of research toward alternative pest and disease control strategies by focusing on biological control agents (Janja *et al.,* 2013) to replace chemical methods. Biological control of nematodes has not been studied widely especially in developing countries (Nnennaya, 2011) but has been considered in the present past due to various reasons. These reasons include; concerns about environmental hazards resulting from continued use of chemical nematicides, limited alternative crops for use in crop rotations, and shortage of land that can be left fallow for some time to reduce the nematode population densities.

An effective biological management method of root lesion and root knot nematodes will reduce the potential losses caused in banana hence increase production that will translate to higher profits. The advantage of utilization of biological control agents is twofold;

first biological control methods reduce the use of conventional chemicals which are unfriendly to human beings the environment and secondly they are sold at affordable prices to resource - challenged farmers. Farmers will also access banana plantlets biologically treated at nursery level to boost resistance. This will make the plantlets perform well even in nematode infested soils. The method will also give an assurance of production of chemical free food which is safe to the producers and the users.

1.3. Objectives

1.3.1. General objective

To establish the best combination of biological control and synthetic compounds for plant parasitic nematode management in tissue culture banana and contribute to increased banana productivity.

1.3.2. Specific objectives

i. To identify substrates that stimulates multiplication of a selected bio-control agent.

ii. To determine the efficacy of mycorrhiza fungi, nematophagus fungi and calcium in suppressing nematodes in banana.

iii. To determine the efficacy of combined treatments multiplied in appropriate substrates as bio-control agents in management of plant parasitic nematodes.

1.4. Hypotheses

- Biocontrol agents can enhance build-up of resistance in tissue banana against lesion nematodes.

- Calcium, a synthetic compound can contribute in the strengthening of the cells of banana plantlets thus making them resistant to nematode attacks.
- A combination of synthetic compounds and biological control methods can boost resistance of banana seedlings against nematode infestation.

CHAPTER TWO

2.0. LITERATURE REVIEW

Banana (*Musa spp*.) belongs to the family *Musaceae* and genus *Musa* and is closely related to plantains.

2.1. Botany of banana

Banana plant has a cylindrical, succulent pseudo-stem with a cylindrical leaf petiole sheath reaching a height 6-7.5m and arising from a fleshy rhizome or corm. Suckers spring up around the main plant. The eldest sucker replaces the main plant when it fruits and dies and this process of succession continues indefinitely. Leaves are arranged spirally. The inflorescence commonly referred to as the male bud is a terminal spike, shooting out from the heart in the tip of the stem. Banana production systems can be classified into; backyard garden, subsistence and commercial plantation systems (Karamura *et al*., 1999).

2.2. Ecological requirements of banana

Banana grows well in warm, humid and frost free climate with optimum temperatures between 22^0C and 31^0C and an altitude below 1800m above sea level. Plant grows slows down below 16^0C and stops at 10^0C. They require a well-drained, deep soil with high organic matter (Zake *et al*., 2000) with a pH of 5.5-7.5. They require an average annual rainfall of 1500-2500mm.

2.3. Economic importance of banana

Banana is ranked fourth among agricultural crops in the world and first among fruits. In terms of volumes of food consumed by human, banana is ranked fourth in the world after rice, wheat and maize (Tripathi *et al*., 2008). The available data collected in the year

2015 indicate that global production of banana was recorded at 117.9 tonnes and approximately 5.5 million hectares of land put under cultivation (FAO, 2015). East Africa is the largest banana producing region in Africa (Smale and Groote, 2003). The region leads with per capita consumption of banana in the world. Banana crop is of great importance to small-scale farmers in the developing countries of the tropics and sub-tropics. Banana and plantains provide food to over 100 million people in the sub-Saharan Africa (FAO, 2004). The crop offers an ecological advantage of protecting the ground against erosion by means of its large leaves, root system that holds the soil together and rotting leaves and trunks that add humus to the soil (Ocimati *et al.,* 2013). The crop is also grown for its economic gains with the highest percentage share being traded in the local markets and about 20% being export share (FAO, 2006). Banana fetches good prices in Kenya and other parts of the world (HCDA/MOA, 2012).

2.4. Biotic and abiotic constraints in banana production

Banana plants are attacked by several biotic and abiotic constraints. Biotic factors that affect banana plants are soil moisture deficit, salinity, extreme temperatures and strong winds (Ravi and Vaganan, 2016). The biotic factors include pests and diseases which contribute to significant food and income losses (Poornima, 2007). These pests and diseases include; nematodes, weevils, black sigatoka and panama disease among others (Svetlana *et al.,* 2004). Among the biotic factors that affect banana, plant parasitic nematodes constitute one of the major threats due to the extensive root damage they cause (Shanthi and Rajendran, 2006) Plant parasitic nematodes contribute in weakening of the roots and pseudostems thus reduced banana bunches size (Gowen *et al*., 2005). Soil pests like weevils and nematodes interfere with the root system and the smooth flow

of nutrients and water resulting to stunted plants (Davis and Mac-Guidwin, 2000). The affected roots are also weakened and at times some die completely and this can lead to toppling disease (Ouma, 2009). This reduces anchorage of the plants and during slight wind movements the whole banana plants especially those in fruit fall down destroying the banana bunches (Svetlana *et al*., 2004; Osei, 2013). Broken banana bunches have lowered quality and spoils fast and fetches very poor prices in the market. Among several biotic stresses inflicting damage to banana, plant parasitic nematodes constitute one of the major challenges for profitable cultivation. Nematodes are small worm-like members of the animal kingdom ranging from 0.5-1.0mm in length found in almost every habitat in fresh or salt water and in soil (Kimenju *et al.,* 2013). They are migratory endoparasites of the root cortex in corms and roots of banana plant. They use a hollow, protrusible stylet to penetrate the wall of a plant cell, inject secretions into the cell and withdraw nutrients from the cytoplasm frequently causing cell death (Javaid *et al.*, 2009). They also cause reddish brown lesions in the cortex which lead to death of roots.

Plant parasitic nematodes are known to cause high economic losses in a range of agricultural crops contributing to huge yield losses in a variety of crops (Sikora and Fernandez, 2005). Nematodes are of serious concern in banana cultivation (Matiyar and Mohammed, 2010). They limit banana production because of the extensive damage they cause to the roots (Shanthi and Rajendran, 2006). This reduces water and nutrients uptake and also results to poor anchorage of the plant (Ouma, 2009). The most economically important species of banana nematode destroy the primary roots disrupting the anchorage system and resulting in toppling of the plants. These include the burrowing nematode, *Radopholus similis*, the lesion nematode, *Pratylenchus goodeyi*

and the spiral nematode, *Helicotylenchus multicintus* (Gowen *et al*., 2005; Osei *et al.*, 2013). Nematode infestation of banana fields has various effects on the plant. *R. similis* attack leads to lengthening of the vegetative cycle and toppling of plants especially those bearing fruits (Osei *et al.*, 2013).

The lesions caused by lesion nematodes coalesce to produce large necrotic areas of tissue as nematodes continue to migrate and feed within the roots (Davis and MacGuidwin, 2000). Lesion nematodes produce characteristic necrotic lesions on the surface and throughout the cortex of the infected roots (Davis and MacGuidwin, 2000; Pablo and Nicola, 2007). The name of these nematodes is derived from the conspicuous necrotic lesion they cause on host roots (Pablo and Nicola, 2007). The lesions turn from reddish-brown to black and are initially spotty along the root surface (Davis and MacGuidwin, 2000). Root lesion nematodes cause damage and reduction of the root system which leads to stunting of plants, decreased bunch weight, lengthening of the production cycle, reduced plant anchorage and eventually toppling (Gowen *et al.*, 2005) which results to total loss of unripe fruit.

The toppling disease caused by lesion nematodes like; *Radopholus similis* and *Pratylenchus spp* is a serious problem whose direct effect is not readily quantified, but it is estimated that its combined impact on reduction of yields and in the reduction in quality could be as much as 55% of total productivity. *Radopholus similis* cause rhizome rot or toppling disease or black head disease of banana (Brooks, 2008) and has posed a great challenge in banana production. This shows a clear demonstration of the impact of nematodes on banana plants.

An increase in nematode population leads to invasion and destruction of roots immediately after they are formed. Roots heavily infested by *P. coffeae* show black or purple necrosis of epidermal or cortical tissue. *P. coffeae* cause root lesion disease. Heavy infestation of nematodes to plants makes them stunted, reduced in size, with reduced number of leaves and decrease in bunch weight (Osei, 2013). Research on nematodes has been hampered by the fact that in the field, nematode infestation is invariably caused by more than one species and very little work has been done on partitioning losses between different species when they are present (Gowen *et al.,* 2005).

2.5. Methods of controlling nematodes

2.5.1 Cultural methods

Cultural practices of nematode control have been employed in the past. Among them are those measures that help in restoring and maintaining healthy soils. These include removal of plant debris, solarization of soil, crop rotation with plant species immune to pathogens that harm other rotation crops, fallow cultivation that gives land time to rest to reduce nematode densities, addition of organic amendments, use of pathogen free seeds and resistant varieties (Collange *et al*., 2011). Adjustment of planting time, application of organic amendments and biological control have been tested and proved effective in reducing various nematode population though they are genera and species specific (Mc Donald and Nicol, 2005).

2.5.2 Biological control of nematodes

In the past there have been reports on negative environmental impact associated with chemical control methods (Nico *et al*., 2004). The ban on the use of methyl bromide as a soil fumigant (Collange *et al*., 2011) has contributed a lot in research on alternative

control methods of nematodes (Hashem and Abo-Elvousr 2011; Li *et al*., 2014). Use of chemical nematicides to control nematodes is hazardous to human health and too expensive for small scale farmers serving local markets in Africa (Langat *et al.*, 2008). For instance, Furadan has been widely used for the control of nematodes but its adverse role in environmental degradation has led to it being phased out. Environmental problems associated with nematicides have introduced a sense of urgency into the search for alternative methods of nematode management (Javaid *et al.*, 2009). There is therefore a great need to develop mechanisms that can make banana resistant to lesion nematodes.

Since 1951, when Duddington pioneered the use of biological control against nematodes, research has contributed to production of various commercial biological control products that contain live microorganisms or their metabolites that target specific nematode hosts. Those microorganisms belong to bacteria, fungi and actinomycetes and include *Pasteuria penetrans,* nematode trapping fungi, entomopathogenic nematodes, *Rhizobacteria, Trichoderma* spp, *Fusarium* among others (Janja *et al.*, 2013). Biological control has been gaining popularity as a sustainable strategy in nematode management (Mostafa, 2001; Kiewnick *et al.*, 2004; Langat *et al.*, 2008). However, the availability of these fungal endophytes has been hampered by inadequate materials for mass multiplication. There is, therefore, a need to establish a suitable material for mass multiplication of the endophytes (Jagadeesh *et al.*, 2008). Reliable materials for mass multiplication have not been fully researched on and therefore remains an area to be addressed (Amala *et al.*, 2012).

Biological control has attracted a lot of attention in the management of pests and diseases as an alternative to the chemical control. It has long been considered a good

alternative to nematicides for controlling nematodes due to their safety in usage; their adaptability and also possibility of multiplication of biological agents in soils rich in organic matter (Shanthi and Rajendran, 2006). Fungi are considered as a suitable biological control agents as they can penetrate through the insect cuticle. This aspect enhances control of insect pests (Gonzalez *et al*., 2016). Fungal endophytes have the potential as effective biological control agents for management of various plant diseases and pests. They colonize the tissues of plants as endophytes (Arnold, 2007).

2.5.2.1 Use of nematophagus fungi (Paecilomyces lilacinus) as a biocontrol agent

Nematophagus fungi (*Paecilomyces lilacinus)* are soil fungi with a good potential for biological control and has been described as being efficient as the commonly used nematicide (Diogo *et al*., 2009). Nematophagus fungi or predatory fungi can infect, kill and digest nematodes in each of the three development phases like egg, larva and adult (Mostafa *et al*., 2012). It is also known as predatory or predaceous fungi and refers to a type of carnivorous fungal species that use their spores or mycelial structures to capture vermiform nematodes. They use their hyphal tips to parasitize the eggs and cysts of nematodes (Mostafa *et al.,* 2012), or produce toxins to attack nematodes (Li *et al.,* 2000). This soil filamentous fungi is reported to infect eggs of different types of nematodes (Basualdo *et al.,* 2000; Olivares-Bernabeu and Lopez-Llorca, 2002; Khan *et al.,* 2003). This soil fungus has a good potential for biological control of nematodes (Amala *et al*., 2012). A lot of research interest has been geared towards *Paecilomyces lilacinus* as a biological control agent following its discovery as a biological control agent in 1979. It has been described as being as efficient as the commonly used nematicides (Schenck, 2004) and also in the control of greenhouse insects and mite pests (Fiedler and Sosnowsk,

2007). Several countries have been conducting research on *Paecilomyces lilacinus* adaptability to various climatic conditions and of its efficacy in control of root knot nematodes (Holland *et al.,* 2001).

Paecilomyces lilacinus has been reported to reduce nematode population densities and has been considered as the most promising and the most practicable biocontrol agent for the management of plant parasitic nematodes (Mucksood and Tabreiz, 2010). Mucksood and Tabreiz (2010) further reported that *Paecilomyces lilacinus* could act as a potential biocontrol agent causing a reduction of root knot nematodes thus improving the various plant growth parameters. The fungi colonizes nematode eggs preventing them from hatching and leaving fewer juveniles to penetrate root tissues (Mucksood and Tabreiz, 2010). Nematode egg shell plays a major role against adverse conditions of environment and action of chemical and biological control methods (Huang *et al.,* 2004; Gortari *et al.,* 2008). However, little is known of its efficacy when used in combination with synthetic compounds.

2.5.2.2 Use of mycorrhiza as a bio-control agent

Mycorrhizal use as a biological control dates back over four hundred years ago after the discovery of arbuscules in aglaponian major, an early Devonian plant (Thangaswamy and Padmanabhan, 2006). Mycorrhiza is a mutualistic association between fungi and higher plants (Turk *et al.,* 2006). The plant provides sugars in form of carbon for the fungi while the fungi provide nutrients like phosphorus, water and protection to the plant (Jayaa *et al.,* 2012). The symbiosis benefit plants in uptake of phosphorus nutrients, production of growth hormones, increase of proteins, lipids and sugar levels, helps in heavy metal

binding, salinity tolerance, disease resistance, and also uptake of radionuclides (Thangaswamy and Padmanabhan, 2006).

Arbuscular mycorrhizal fungi (AMF) play a major role in plants by improving growth of many plant species. This has been achieved by increased nutrients uptake, production of growth promoting substances tolerance to salinity and transplant shock, tolerance to drought and synergistic interaction with other beneficial soil microorganisms such as N-fixers and P-solubilizer (Turk *et al.*, 2006). Mycorrhizal symbioses, therefore, facilitates plant uptake of soil nutrients that include phosphorus, nitrogen, calcium and water (Akthar and Siddiqui, 2008). This helps in keeping the plant hydrated in dry soil conditions. The hyphae of the fungi has some advantages over the plant root hairs in that the hyphae reach further out into the soil, they are more attracted to nutrients than root hairs and that they are smaller than root hairs which helps them get into spaces in the soil that the root hairs cannot. The symbiotic root-fungal association improves the uptake of less mobile nutrients (Ortas *et al.*, 2001; Ortas, 2010). There are two types of mycorrhiza that are of major economic and ecological importance namely ecto -mycorrhiza and endo-mycorrhiza (Omid, 2011). AMF is the most widespread root fungal symbiosis and it is associated with the vast majority of the higher plants and helps in soil management leading to low-cost sustainable agricultural systems (Ortas, 2010; Nedorost *and* Pokluda, 2012). Arbuscular symbiosis can improve a soil structure and protect host plants against the detrimental effects caused by drought stress (Nedorost and Pokluda, 2012). Arbuscular mycorrhizal (AM) fungi have emerged as potential bio-fertilizers which are cheap, environmentally friendly alternative to expensive chemical fertilizers (Jayaa *et al.*,

2012). Jayaa *et al*., (2012) concluded that these associations help to maintain the general plant vigor under a variety of adverse and inhospitable ecological conditions.

There are many benefits associated with inoculating a wide array of agronomic plant species with AMF which have been documented in numerous studies including date palms and forest trees (Al -Karaki, 2013). AM or AMF have been reported to increase the survival rate of transplanted seedlings, control of root pathogens (Azcon-Aguilar *et al*., 2002) increased tolerance to salinity and increased aggregation by the external hyphal network (Hrishikesh *et al.*, 2010). Previous research indicates that mycorrhizal roots encourage greater populations of native bacteria that fight root pathogens hence promoting plant growth (Tahat *et al*., 2010). Inoculation at the nursery stage makes very efficient use of the fungi and gives best results compared to inoculating an already established crop or an infected crop (Fernandez *et al*., 2014).

2.5.3 Use of synthetic material

Calcium is a divalent cation that enhances the strength of stems and stalks of plants (Jeremy, 2007). It plays a major role in growth and structure (Mary, 2008). According to Mary, (2008) lack of calcium can result in structural changes of intracellular organelles, a decrease in cell elongation, and affect cell walls and the permeability of cell membranes to solutes and other ions. Low levels of calcium ion leads to several defects in plants such as poor root development, leaf necrosis and curling, blossom end rot, bitter pit, fruit cracking, poor fruit storage and water soaking (Hepler, 2005). Most pathogens like fungi and bacteria invade and infect plant tissue by producing enzymes that dissolve the middle lamella (Ismail, 2014). Such enzymes include polygalacturonases and pectolytic enzymes such as pectate transeliminase.

Increasing the amount of tissue calcium content lowers polyglacturonases and pectolytic enzyme activity (Cakmak, 2014) .It is reported that low calcium levels in plants make the cell wall more susceptible to rupturing compared to high concentrations that increase its rigidity. From researches carried out in the fifties, it was clear that modifying the calcium levels produced pronounced effects on cell growth (Hepler, 2005). Preformed physical and chemical barriers of plants like cuticles, cell walls, and constitutively produced antimicrobial compounds protect the plant against most attempted invasions (Angela *et al.*, 2006).

Jeremy, (2007) also reported that calcium mineral also regulates the absorption of nutrients across plasma cell membranes and is a part of the cell wall hence acts as cement that binds cell walls together. Substantial reviews have presented a comprehensive overview of Ca^{2+} role in various aspects of plant growth and development (Reddy, 2011). Ca^{2+} plays a major role as one of the nutrients that strengthens the structure of the cell wall and as a key ion in maintaining the structural rigidity of the cell walls and in membrane structure and function (Kadir, 2004). It enhances resistance to bacterial and viral diseases in plants (Usten *et al*., 2006). A lot of research has been done on calcium in responses of cell cultures or protoplasts to microbial products (elicitors) (Levine *et al.*, 1996). The calcium ion is now firmly established as a second messenger in numerous plant signaling pathways, conveying a wide range of environmental and developmental stimuli to appropriate physiological responses.

For a plant to develop a defense response there is an initial requirement that involves the perception or recognition of the pathogen by the plant. In plants, there are preformed physical and chemical barriers like cuticles, cell walls and constitutively chemical

compounds that protect the plant against most attempted invasions (Angela *et al.*, 2006). Angela *et al*., (2006) further noted that if a putative pathogen gets over the aforesaid barriers, when the plant recognizes the invader, a resistant plant induces a rapid defense response to prevent the bio-aggressor from developing. Calcium has been reported (Cakmak, 2014) as a signal carrier due to its very low cytosolic concentrations in plant cells. Any manner of stress inflicted on plants results to quick rise in cytosolic Ca^{2+} which is a key factor in expression of stress-responsive genes and physiological responses of plant cells to stress conditions such as drought, salinity, and pathogenic attack.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Study area

The study was carried out in two sites, one in Tetu sub-county in a private farm next to Gichira police post and the other in Mathira sub county, Karatina University in Nyeri County, Kenya. The two sites are endowed with varying climatic conditions. The first trial was set in a greenhouse in Gichira village in Tetu sub-county. The Gichira site, where the greenhouse was situated lies at latitude of $0^0$28'0''S and longitudes of $37^0$1'0''E at an altitude of approximately 1683 M above sea level and about 93km from Nairobi. The second trial was set up in Karatina University greenhouse situated at Kagochi area, which has an average temperature of 16^0C. The university neighbors Mt Kenya forest to the south. The University land lies at latitude 0^0 24' 0''S and longitudes 37^0 09' 0''E at an altitude of approximately 1815M above sea level. The average annual mean temperatures are 16.5^0C. The highest temperature is experienced in March at 17.2^0C. The least temperatures are experienced in July at an average of 14.8^0C. The area is classified as a warm and temperate area and it receives significant rainfall throughout the year averaged at 1497mm. The least amount of rainfall is received in September at 53mm. Most precipitation is received in April with an average of 276mm.

3.2. Experimental design and treatment application

Grand nain variety of tissue cultured banana seedlings were used in the study. The experimental design used was completely randomized design (CRD) with five treatments replicated four times in the first trial (Table 1) and seven treatments replicated four times

in the second trial (Table 2). Calcium a synthetic control compound was evaluated amongst two biological control methods namely nematophagus fungi and mycorrhiza fungi. One treatment was an integration of the two biological control agents and the synthetic agent which has been referred to as the combined treatment. In trial two, there was a repeat of all the treatments used in trial one and an addition of two more treatments. The two added treatments were nematophagus fungi in rice bran carrier and nematophagus fungi in barley carrier. These two treatments that were added in trial two were considered after the completion of an experiment that involved selection of the best substrate. The first two substrates were chosen from barley and rice bran. A control treatment was included. This treatment was of the banana plantlets that received no inoculation at all.

Table 1: Treatment quantities for trial one

Treatment	**Rate of biological and synthetic control agents**
Control	Zero (No inoculation).
Calcium	1ml of 1g in 50ml of DW.
Nematophagus fungi	1ml of 12.5g in 50ml of DW per plant.
Mycorrhiza fungi	15 g per plant.
Combined (calcium, Nematophagus fungi and Mycorrhiza	As per above quantities combined together.

Table 2: Treatment quantities for trial two

Treatment	Rate of biological and synthetic control agents
Control	Zero (No inoculation).
Calcium	1ml of 1g in 50ml of DW per plant.
Nematophagus fungi	1ml of 12.5g in 50ml of DW per plant.
Mycorrhiza fungi	15 g per plant.
Combined (calcium, Nematophagus fungi and Mycorrhiza	As per above quantities combined together.
Nematophagus fungi in barley substrate	1ml of 12.5g in 50ml of DW per plant.
Nematophagus fungi in rice bran substrate	1ml of 12.5g in 50ml of DW per plant.

The greenhouse experiment was set on benches and covered an area of 10m by 6m with inter-treatment spacing of 10 cm in between treatments and a spacing of 30 cm to separate the replications.

3.2.1. Experimental set up

The two trials involved use of two week old Grand nain variety of tissue culture banana plantlets. The plantlets were potted in germination trays and allowed to grow for two weeks before biological control agents and synthetic control agent were applied. This

variety is highly preferred for its high yield and has a high local market demand. The variety is highly susceptible to plant parasitic nematodes (Van den Berg *et al*., 2002).

Work in the greenhouse involved a setup of trial one and trial two experiment for inoculation of banana seedlings with the plant parasitic nematodes (*Pratylenchus spp.* and *Meloidogyne spp*.) and application of the various treatments. Those treatments were; calcium for synthetic control agent, nematophagus fungi and mycorrhiza both for biological control agents, combined treatments (calcium + mycorrhiza + nematophagus fungi) that incorporated the first three treatments integrated together (Table 1). A control experiment was also included which involved banana plantlets that did not receive any treatment at all. The banana plantlets used per treatment were five in number. All the treatments were replicated four times. Randomization was also observed in every replication. Potting soil was sourced from Karatina University forest and confirmed to be free from plant parasitic nematodes by sterilizing it by autoclaving at 121^0C for 15 minutes and afterwards conducting soil analysis for nematodes in the laboratory before planting. The plantlets were placed in green house after which they were inoculated with nematodes. Nematode infested soil was obtained from a banana orchard that had toppling disease to provide source of inoculum for inoculation purposes. The infested soil contained roots with lesions and galls as an indication of the presence of root knot nematodes and root lesion nematodes. This soil was analyzed for nematodes and was found to contain both *Pratylenchus spp* and *Meloidogyne spp.* The roots containing galls were cut in small pieces of about 10cm and were mixed together. Similarly those containing lesions were cut into small pieces. A sub-sample of 100g of each type of roots was weighed and prepared by macerating the roots in a blender containing 100ml of

distilled water. The blender was run for fifteen seconds twice. The blended suspension of roots was poured in a beaker rinsing the blender container to clean it of all the debris. Modified Baermann's technique was used to extract the nematodes from the blended suspension. Juveniles were collected using a 38µm pore sieve and rinsed with tap water. The inoculum suspension of 100 nematodes per plant was introduced through a small hole of about 4cm from the base of the banana seedlings at the soil level. The seedlings were allowed three weeks to grow in the nematode inoculated soil to allow infestation on the banana seedling roots.

Laboratory work entailed soil analysis to confirm that the soil was free from nematodes. Soil sample of 200g weight was weighed for analysis. Nematodes extraction was done using modified Baermann's technique. The soil sample was sprinkled on a serviette spread in a sieve that was placed on a plate. Distilled water was put gently in the plate and the extraction apparatus placed in a dark cabinet for 2 days. The filtrate was passed through a 38mµ sieve. The sieve was rinsed and nematodes collected in a petri-dish to be viewed under a compound microscope. There were no nematodes observed under the microscope. Mycology work was also carried out in the laboratory which entailed culturing of *Paecilomyces lilacinus* to obtain pure cultures. Commercial *Paecilomyces lilacinus* traded as Mytech was weighed at (12.5g) and was mixed with 50ml of distilled water resulting to a concentration of 1.0×10^{10}. This mixture of the fungus was cultured in petri-dishes containing molten SDA nutrient. Pure cultures were obtained after re-culturing in the SDA media as outlined in section 3.3. Three substrates were used to conduct the experiment on mass multiplication of nematophagus fungi. The substrates used in the experiment included: rice bran, ground barley and ground rice husks. Barley

and rice husks were ground into fine particles using a blender. The substrates were weighed at 50g per substrate. Sterilization of the substrates was done by autoclaving them at 121^0C for 15 minutes. The substrates were allowed to cool on a laminar flow. Pure cultures of *P. lilacinus* that had been prepared were scooped with a cork borer of 1mm diameter. The discs were plated at equidistant points on the different substrates in the incubator set at 25^0C for 28 days. Rate of sporulation of the nematophagus fungi in the different substrates was used to quantify the best substrate in the experiment. Trial one varied from trial two in that in the second trial, two more treatments were added. The two treatments added in trial two were nematophagus fungi incorporated in the first two substrates that supported the highest growth. Amount used for each substrate in trial two was 200g per pot. The main reason for adding these two treatments was to assess whether the plantlets would perform better if inoculated with nematophagus fungi incorporated in the substrate that supported the highest growth. The two added treatments in trial 2 involved scooping of soil at the base of the seedlings to reveal the roots after which the substrates were incorporated in the pot holding seedlings. Treatment with the *P. lilacinus* was done on the substrate at the rate of 1ml of 12.5g in 50ml of distilled water per plant. After treatment the roots were covered with the soil ensuring that it did not go beyond the initial soil level of the seedling.

3.2.2. Routine management of banana seedlings in the acclimatization chamber

Good agricultural practices were observed in the entire experimental area for the two trials. Watering was done to the potted seedlings to field capacity. Pruning of old yellowing leaves was done appropriately. Hand weeding was done continuously as weeds

appeared in the pots. One spray was done in each trial with an insecticide lambdex (lambda-cyhalothrin as the active ingredient) at the rate of 12 ml in 20 l of water to control aphids and caterpillars in the banana seedlings. The spray was done to all the banana seedlings used in the experiment.

3.3. Assessment of sporulation of *Paecilomyces lilacinus* in three different carriers

Laboratory work was carried out at Karatina University Agriculture laboratory, Mathira sub-county, Nyeri County. In this experiment, 65g of Sabouraud Dextrose agar (SDA) was suspended in 1 liter of sterile distilled water and heated to completely dissolve. The dissolved media was sterilized by autoclaving at 121^0C for 15 minutes. The molten SDA was poured in sterile petri-dishes and allowed to solidify in a laminar flow for about 45 minutes. A commercial nematophagus fungus (*Paecilomyces lilacinus*) marketed as Mytech was weighed (12.5g) and diluted in 50 ml of distilled water and mixed thoroughly. The nematophagus fungi suspension was serially diluted up to the seventh dilution. A micropipette was used to transfer 100µl of the serially diluted suspension in the solidified media. The fungi was allowed to grow in the incubator set at 25^0C and observed after the 5^{th} day to determine the rate of growth of the fungus in the substrate. The three different substrates obtained from barley, ground rice husks and rice bran were weighed to obtain 50 g of each and put in 250 ml conical flasks, autoclaved at 121^0C for 15 minutes.

A cork borer of 1 mm diameter was used to cut discs of the SDA media holding spores. This gave uniform sized discs of 1mm to ensure uniformity in population of the spores growing on the three different types of substrates. The cut discs were placed in 250ml

conical flasks that contained 50g of the sterilized substrates. Conical flasks containing substrates with the seeded 1mm fungus disc were put in an incubator set at 25^0C and observed on the 4^{th}, 7^{th}, 14^{th} and 21^{st} day to assess the rate of growth and sporulation of the fungus in order to identify the substrate that supports the highest rate of growth of the *Paecilomyces lilacinus.* In each day of observation, 10g of media with spores was taken from each of the substrate and suspended in100ml of sterile distilled water. The suspension was filtered using a double layered muslin cloth, placed on a haemocytometer and the spore count was done using a microscope and recorded at weekly intervals for a period of one month.

3.4. Data collection

3.4.1. Growth parameters

The plant heights of the banana seedlings were measured on randomly selected and tagged plants using a tape measure from the soil level to the tip of the freshly formed leaf. The measurements were taken weekly and recorded in centimeters. The leaf widths of the selected plants were measured from the widest part of the leaf. Leaf lengths of the same plants were also obtained from the base of the leaf to the tip of every fourth leaf of the selected plant. Data was collected on weekly basis for a period of five weeks. Data was taken from selected plants from each treatment in every replication and was subjected to analysis.

3.4.2. Destructive sampling of roots

After one and a half months, banana plantlets were sampled for assessment of presence of the root lesions and root galls on the roots of seedlings. From each replication, one banana seedling was selected per treatment. This was done by destructive sampling of the

roots and counting the number of root lesions and root galls found on the primary roots per plant. This helped in assessing the level and extent of damage of banana roots. It also helped in comparing the damage done to the roots and the response of banana seedlings to the damage. The state of the roots was also determined to find out if there was normal growth or not. This was done by counting the total number of primary roots that were alive per plant per treatment in every replication. The presence of nematodes in the soil in the planting pots where the banana plantlets were grown was analyzed in the laboratory. This was to determine the populations that survived after treated banana seedlings were grown in nematode infested soil. The nematode load in the soil was determined in the laboratory after extracting nematodes using modified Baermann's method of nematode extraction (Hooper *et al*., 2005). In summary in destructive sampling, data collected included; total number of lesions per plant of an average size, total number of galls per plant, total number of live primary roots and number of plant parasitic nematodes per 200g of soil from each treatment.

3.4.3. Assessment of growth of *Paecilomyces lilacinus* in three different carriers

In this experiment, *Paecilomyces lilacinus* was cultured in sterile rice-husks, rice bran and barley. Barley and rice husks were blended using a blender to reduce them into finer particles. These substrates were autoclaved for 15 minutes at 121^0C. The three substrates acted as carriers for the *P. lilacinus* fungi. Isolation and enumeration of the spores was done on the 7^{th}, 14^{th}, 21st and 28^{th} day of incubation. The conidia was taken from each of the substrate by pulverizing and suspended in 100ml sterile distilled water. The suspension was filtered using a double layered muslin cloth, placed on a haemocytometer

and the spore count was done using a microscope and recorded at weekly intervals for a period of one month. The data was subjected to Analysis of Variance (ANOVA).

3.5. Data analysis

Data was subjected to analysis of variance (ANOVA) using Genstat Release 12.1(PC/ Window). Treatment means were separated using LSD at $P \leq 0.05$ level.

CHAPTER FOUR

4.0. RESULTS

4.1. Growth parameters

Various growth parameters were assessed in this experiment. These parameters included; plant height, leaf width, leaf length, sporulation of *Paecilomyces lilacinus* in various substrates, population of *Meloidogyne spp.* and *Pratylenchus spp.* after treatments, number of live primary roots after destructive sampling. All treatments were compared with the control plants in the experiment.

4.1.1. Assessment of sporulation of *Paecilomyces lilacinus* in three different carriers

In this experiment, *Paecilomyces lilacinus* performed differently depending on the type of substrate. Among the different substrates tested, there was observed a difference in all the sampling times except on day 14 where rice bran and barley substrates did not differ much. Rice bran recorded the highest spore count in the first 7 and 14 days with $2.45x10^{10}$ spores /ml and $2.65x10^{10}$ spores /ml followed by barley with $1.29\ x10^{10}$ spores /ml and $2.56x10^{10}$ spores /ml and lastly rice husks with $1.23x10^{10}$ spores /ml and $1.41x10^{10}$ spores /ml respectively. In the first two weeks, rice bran recorded a higher spore count than barley and rice husks. In the 3rd day of observation (day 21) and 4th day of observation (28) barley recorded the highest spore count ($3.06x10^{10}$ spores /ml and $3.38x10^{10}$ spores /ml) which was followed closely by rice bran ($2.90x10^{10}$ spores /ml and $2.99x10^{10}$ spores /ml) but rice husks recorded the least. From the results obtained, barley performed better than the other two substrates on the 28th day (Table 3). Barley substrate

was ranked first in supporting the highest growth of nematophagus fungi after culturing it in the lab while rice bran was ranked second.

Table 3: Mean sporulation of *P.lilacinus* in different substrates

	Spore count x 10^{10}spores /ML (Days after inoculation)			
	7th	**14th**	**21st**	**28th**
Substrate	**SC/ML**	**SC/ML**	**SC/ML**	**SC/ML**
Rice husks	1.23*a*	1.42*a*	2.56*a*	2.57*a*
Rice bran	2.45*c*	2.65*b*	2.91*b*	3.0*b*
Barley	1.29*b*	2.57*b*	3.07*c*	3.38*c*
GM	1.66	2.21	2.84	2.98
LSD	3.49	8.29	7.40	10.00
CV	1.00	2.70	1.80	2.10
PV	<0.001	<0.001	<0.001	<0.001

Means followed by the same letter in the same column are not significantly different ($P < 0.05$). **SC/ML**-Spore count per Milliliter, **GM**-Grand means, **LSD** -Least significant difference, **CV**-Co-efficient of variation. Data are means of four replications.

4.1.2. Effect of treatments on the width (cm) of the 4th leaf of banana plants

Banana plantlets treated with nematophagus fungi recorded the highest leaf width (15.10 cm) at 42 DAT in both trials. Trial one recorded the highest leaf width in all the treatments. At 42 DAT, in trial one nematophagus fungi (15.10 cm) was followed by combined treatments (calcium, mycorrhiza and nematophagus) (14.53 cm), mycorrhiza (11.93 cm), calcium (11.73 cm) and control (4.70 cm). At 14 DAT, means recorded were as follows; control 4.15 cm, combined treatment (calcium + mycorrhiza + nematophagus

fungi) 8.15 cm, calcium 7.73 cm, mycorrhiza 7.56 cm and nematophagus without substrate 9.48 cm. At this sampling time, nematophagus without substrate recorded the highest leaf width while control plants recorded the least leaf width growth. Similarly, at 21 DAT, the treatment that exhibited the highest leaf width was nematophagus without substrate which recorded 11.00 cm an increase of 1.52 cm from 14 DAT. It was followed by combined treatment (calcium + mycorrhiza + nematophagus fungi) 10.55 cm, mycorrhiza (9.65 cm), calcium (9.28 cm), and finally control (4.28 cm). At 28 DAT, nematophagus without substrate recorded 11.00 cm and emerged as the best treatment at this particular time. It was followed by combined treatment (calcium + mycorrhiza + nematophagus fungi) 11.03cm, mycorrhiza (10.40 cm), calcium (10.10 cm) and finally control treatment (4.35 cm). At 35 DAT, a similar trend of performance was observed. Nematophagus without substrate recorded 12.75 cm followed by combined treatment (calcium + mycorrhiza + nematophagus fungi) 12.73 cm, mycorrhiza (11.50 cm), calcium (10.65 cm), and finally control (4.40 cm). In trial one in all sampling times, nematophagus fungi maintained the lead in performance while control plants were last in performance. In trial 2, at 42 DAT, seedlings treated with nematophagus without substrate exhibited the highest leaf width (9.43 cm) followed by nematophagus in rice bran carrier (8.53 cm), nematophagus in barley carrier (8.03 cm), combined treatment (calcium + mycorrhiza + nematophagus fungi) (7.63 cm), mycorrhiza (7.45 cm), calcium (6.90 cm), and control (4.88 cm). At 14 DAT, nematophagus in rice bran carrier (7.13 cm) recorded the highest leaf width followed by nematophagus in barley carrier (6.43 cm), combined treatment (calcium + mycorrhiza + nematophagus fungi) (6.18 cm), nematophagus without substrate (6.10 cm), mycorrhiza (5.38 cm), calcium (5.28 cm) and

calcium (4.18 cm). At 21 DAT, there was a slight variation in performance of the treatments. Nematophagus in rice bran carrier maintained the lead in performance (7.78 cm) followed by nematophagus without substrate (6.98 cm), which was followed by combined treatment (calcium + mycorrhiza + nematophagus fungi) (6.85 cm), nematophagus in barley carrier (6.60 cm). Mycorrhiza and calcium recorded similar mean 5.63 cm and finally control treatment recorded a mean of 4.38 cm. Similarly at 28 DAT, nematophagus in rice bran carrier produced plantlets with the highest leaf width of 8.08 cm, nematophagus without substrate followed with 7.25 cm, nematophagus in barley carrier (7.13 cm) combined treatment (calcium + mycorrhiza + nematophagus fungi) (7.00 cm), calcium (6.58 cm), mycorrhiza (5.98 cm) and finally control with 4.43 cm. Means for nematophagus fungi in rice bran carrier recorded at 35 DAT exhibited a variation from all the other previous sampling periods in this experiment. At this period, nematophagus without substrate recorded the highest leaf width of 8.58 cm followed by nematophagus in rice bran carrier which recorded a mean of 8.20 cm. This mean was followed by nematophagus in barley carrier with a mean of 7.28 cm. combined treatment (calcium + mycorrhiza + nematophagus fungi) (7.20 cm) followed, then mycorrhiza followed closely with a mean of 7.18 cm. Calcium recorded a mean of 6.40 and finally control treatment attained a mean of 4.45 cm. Generally, in both trials there was a notably positive influence of the treatments on the leaf width. However, in control treatment, leaf width increment was minimal at any sampling period. It was also observed that seedlings treated with nematophagus fungi either alone or in combination or in a carrier contributed to a high leaf width in both trials. Control treatment exhibited minimal increase in leaf width from 14 DAT to 42 DAT in both trial one and trial two (Table 4).

Table 4: Mean leaf width (cm) of banana plantlets from various treatments (Trial 1 and 2)

Trial one (cm)						**Trial two (cm)**				
	Days after treatment (DAT)									
Trmt	**14**	**21**	**28**	**35**	**42**	**14**	**21**	**28**	**35**	**42**
Con	4.15*a*	4.28*a*	4.35*a*	4.40*a*	4.70*a*	4.18*a*	4.38*a*	4.43*a*	4.45*a*	4.48*a*
Comb	8.15*b*	10.55*c*	11.03*c*	12.73*d*	14.53*c*	6.18*c*	6.85*c*	7.00*cd*	7.20*c*	7.63*cd*
Cal	7.73*b*	9.28*b*	10.10*b*	10.65*b*	11.73*b*	5.28*b*	5.63*b*	6.58*c*	6.40*b*	6.90*b*
Myco	7.56*b*	9.65*b*	10.40*b*	11.50*c*	11.93*b*	5.38*b*	5.63*b*	5.98*b*	7.18*c*	7.45*c*
NWS	9.48*c*	11.00*c*	11.80*d*	12.75*d*	15.10*d*	6.10*c*	6.98*e*	7.25*d*	8.58*e*	9.43*f*
B + N						6.43*c*	6.60*c*	7.13*b*	7.28*c*	8.03*de*
RB + N						7.13*d*	7.78*d*	8.08*e*	8.20*d*	8.53*e*
GM	7.40	8.90	9.50	10.40	11.60	5.80	6.30	6.60	7.00	7.50
LSD (0.05)	1.14	0.50	0.57	0.43	0.49	0.39	0.39	0.46	0.33	0.53
CV%	1.00	3.60	3.90	2.70	2.80	4.00	4.20	4.70	3.20	4.70
PV	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means followed by the same letter in the same column are not significantly different ($P < 0.05$).**Com** –Combined (calcium + mycorrhiza + nematophagus fungi), **Con** -control, **NWS** -Nematophagus fungi without substrate**, Myco**-Mycorrhiza, **Cal**-Calcium, **B + N** – Nematophagus fungi in barley, **R. B + N** –Nematophagus fungi in rice bran, **G.M**-Grand Mean, **LSD**-Least significant difference, **CV**- Co-efficient of variation**, Trmt** -Treatment. Data are means of four replications.

4.1.3. Effect of various treatments on the length of 4th leaf of banana plantlets

All banana plantlets inoculated with various treatments recorded a higher increase in their means at any sampling period compared to control plantlets which exhibited the lowest growth in leaf length. Comparing both trials, plantlets treated with calcium in trial one exhibited the highest growth of 30.95 cm, followed by those treated with nematophagus fungi without substrate with a mean of 36.60cm, mycorrhiza (30.43cm), combined treatment (calcium + mycorrhiza + nematophagus fungi) with a mean of 29.70 cm. At 14 days after treatment (DAT) trial one recorded the following means of leaf length; control 11.62 cm, combined treatment (calcium + mycorrhiza + nematophagus fungi) 22.02 cm, calcium 17.82 cm, mycorrhiza 19.50 cm and nematophagus without substrate 21.10 cm. At 21 DAT, the following means were recorded; 11.68 cm, 23.68cm, 23.88cm, 23.10 cm and 24.00 cm for control, combined (calcium + mycorrhiza + nematophagus fungi), calcium, mycorrhiza and nematophagus without substrate respectively. At 21 (DAT), plantlets treated with calcium in trial one had an increase of 6.06 cm while those in control treatment had an increase of 2 cm only. At 28 DAT, control, combined (calcium + mycorrhiza + nematophagus fungi), calcium, mycorrhiza and nematophagus fungi without substrate recorded the following means 11.78cm, 27.22 cm, 26.6 cm, 25.8cm, and 29.9 cm respectively. At 35 DAT, control, combined (calcium + mycorrhiza + nematophagus fungi), calcium, mycorrhiza and nematophagus fungi without substrate recorded the following means 13.10 cm, 28.40 cm, 29.20 cm, 28.62 cm, 30.15cm. The final data was collected at 42 DAT and recorded means as follows; control, combined (calcium + mycorrhiza + nematophagus fungi),calcium, mycorrhiza and nematophagus fungi without substrate 13.85 cm, 29.70 cm, 30.95 cm, 30.43 cm, and 36.60cm

respectively. In trial two, control, combined (calcium + mycorrhiza + nematophagus fungi), calcium, mycorrhiza and nematophagus fungi without substrate, nematophagus fungi in barley and nematophagus fungi in rice bran had the following means of leaf length; 12.15 cm, 16.08cm, 14.03 cm, 16.10 cm, 19.12 cm, 15.70 cm and 17.08 cm respectively. At 21 DAT, there was a slight growth of leaf length and the recorded means were control 12.60 cm, combined (calcium + mycorrhiza + nematophagus fungi) 17.25 cm, calcium 15.73 cm, mycorrhiza 18.15 cm, nematophagus fungi without substrate 19.98 cm, nematophagus fungi in barley 16.88 cm and nematophagus fungi in rice bran 19.28 cm. At 28 DAT, all the other treatments exhibited a higher increase in leaf length except control treatment 12.88 cm, combined (calcium + mycorrhiza + nematophagus fungi) 19.18 cm, calcium 16.15 cm, mycorrhiza 19.00 cm, nematophagus fungi without substrate 23.40 cm, nematophagus fungi in barley 18.43 cm and nematophagus fungi in rice bran 21.15 cm. At 35 DAT, means recorded were as follows; control experiment 13.10cm, combined treatment (calcium + mycorrhiza + nematophagus fungi) 19.82 cm, calcium 17.18 cm, mycorrhiza 19.60 cm, nematophagus fungi without substrate 24.82 cm, nematophagus fungi in barley 19.65 cm and nematophagus fungi in rice bran 21.93 cm. At 42 DAT, banana plantlets treated with nematophagus fungi without substrate recorded the highest leaf length followed by nematophagus fungi in rice bran with 23.35 cm, nematophagus fungi in barley attained the same leaf length as combined treatment (calcium + mycorrhiza + nematophagus fungi) of 21.62 cm, mycorrhiza followed with 20.77cm, followed by calcium with 18.55 and lastly control with 13.30 cm. All the treatments in trial one recorded a significant difference from control treatment. In trial two, the highest leaf length recorded at 42 DAT was 26.6cm, observed in banana

plantlets treated with nematophagus fungi without substrate. These mean was followed by the mean of plantlets treated with nematophagus fungi in rice bran (23.35 cm), followed by nematophagus fungi embedded in barley carrier (21.62 cm) and combined treatment (21.62 cm), mycorrhiza (20.77 cm), calcium (18.55cm) and lastly control experiment (13.30cm). Generally, there was an increased leaf length observed in all the treatments in both trials at 42 DAT. All observations made in both trials recorded significant growth from 14 DAT to 42 DAT (Table 5).

Table 5: Mean leaf length (cm) of banana plantlets from various treatments (Trial 1 and 2)

Trial one (cm)						**Trial two (cm)**				
				Days after treatment (DAT)						
Trmt	14	21	28	35	42	14	21	28	35	42
Con	11.62*a*	11.68*a*	11.78*a*	13.10*a*	13.85*a*	12.15*a*	12.60*a*	12.88*a*	13.10*a*	13.30*a*
Com	22.02*c*	23.68*b*	27.22*cd*	28.40*b*	29.70*b*	16.08*c*	17.25*c*	19.18*c*	19.82*c*	21.62*c*
Cal	17.82*b*	23.88*b*	26.60*bc*	29.20*b*	30.95*d*	14.03*b*	15.73*b*	16.15*b*	17.18*b*	18.55*b*
Myco	19.50b*c*	23.10*b*	25.80*b*	28.62*b*	30.43*c*	16.10*c*	18.15*d*	19.00*c*	19.60*c*	20.77*c*
NWS	21.10*c*	24.00*b*	29.90*d*	30.15*c*	36.60*e*	19.12*e*	19.98*e*	23.40*e*	24.82*e*	29.60*e*
B + N						15.70*c*	16.88*c*	18.43*c*	19.65*c*	21.62*c*
R B + N						17.08*d*	19.28*e*	21.15*d*	21.93*d*	23.35*d*
GM	18.40	21.30	23.90	25.92	17.10	15.80	17.10	18.60	19.40	20.80
LSD(0.05)	2.98	0.87	0.98	0.91	0.40	0.63	0.77	0.90	0.97	0.86
CV (%)	10.50	2.70	2.70	2.30	0.90	2.70	3.00	3.30	3.30	2.80
PV	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means followed by the same letter in the same column are not significantly different ($P < 0.05$).**Trmt-**Treatment**, Com** – Combined (calcium + mycorrhiza + nematophagus fungi), **Con** -control, **NWS** -Nematophagus fungi without substrate**, Myco**-Mycorrhiza, **Cal**-Calcium, **B + N** –Nematophagus fungi in barley, **R. B + N** –Nematophagus fungi in rice bran, **G.M**-Grand Mean, **LSD**-Least significant difference, **CV**-Coefficient of variation. Data are means of four replications.

4.1.4. Effect of various treatments on the height (cm) of banana plantlets

The heights of banana plantlets were influenced positively by nematophagus fungi (*Paecilomyces lilacinus*) used alone or in combination with other treatments, mycorrhiza or calcium in both trials. There was an increase in height for all treatments at any one sampling period. However, means of height recorded in control plants was increasing at a very low rate compared to means of height of all the other treatments. Trial two recorded the highest height in seedlings treated with nematophagus fungi in rice bran with 63.60 cm in both trials. In trial one, at 14 DAT, combined treatment (calcium + mycorrhiza + nematophagus fungi) recorded a mean of 45.05 cm. Nematophagus fungi without substrate emerged second with a mean of 42.33 cm followed by calcium with 41.55 cm. Mycorrhiza recorded a mean of 40.33 cm and the least in performance was control with 30.60 cm. At 21 DAT, nematophagus fungi without substrate recorded the highest mean of height of banana seedlings, followed by combined treatment (calcium + mycorrhiza + nematophagus fungi) with a mean of 48.70 cm. Calcium recorded a mean of 45.42 cm followed by mycorrhiza (43.25 cm) and the least in performance was control with a mean of 31.38cm.Treatments recorded a similar trend in means of height at 28 DAT. They exhibited the following order; nematophagus fungi without substrate (54.05 cm), combined treatment (calcium + mycorrhiza + nematophagus fungi) (51.50 cm), calcium (47.20 cm), mycorrhiza (46.18 cm) and control (31.75 cm). The highest mean of height attained at 35 DAT was 56.25 cm by nematophagus fungi without substrate, followed by 54.10 cm exhibited by plants treated with combined treatment (calcium + mycorrhiza + nematophagus fungi). Mycorrhiza treated seedlings followed with a mean of 52.10 cm. Calcium treated seedling recorded a mean of 49.22 cm and the least recorded height was

recorded in control plants (32.02 cm). Similarly at 42 DAT, the trend of means recorded followed that one of treatments at 35 DAT. The highest mean of height attained at 42 DAT was 61.20 cm by nematophagus fungi without substrate, followed by 57.30 cm exhibited by plants treated with combined treatment (calcium + mycorrhiza + nematophagus fungi). Mycorrhiza treated seedlings followed with a mean of 54.83 cm. Calcium treated seedling recorded a mean of 52.90 cm and the least recorded height was recorded in control plants (33.53 cm). In trial 2, nematophagus fungi without substrate generally exhibited the highest means of height. This treatment recorded the highest mean from 14 DAT to 28 DAT after which it emerged second in performance. At 14 DAT, the highest height was recorded in seedlings treated with nematophagus fungi without substrate followed by nematophagus fungi in rice bran carrier (29.82 cm). Nematophagus fungi in barley carrier recorded a mean of 28.07 emerging third in performance. Next in performance was calcium (27.60 cm), combined treatment (calcium + mycorrhiza + nematophagus fungi) (25.42 cm), mycorrhiza (24.82 cm) and the lowest mean of height was recorded in control (21.70 cm). At 21 DAT, the treatment that recorded the highest mean of height was nematophagus fungi without substrate which recorded 40.22 cm. This treatment was followed by nematophagus fungi in rice bran carrier (37.95 cm), calcium (32.57 cm), nematophagus fungi in barley carrier (31.50 cm). Combined treatment (calcium + mycorrhiza + nematophagus fungi) recorded a mean of 29.27 cm. Combined treatment was followed by mycorrhiza (28.75cm) and finally control (21.77 cm). There was a gradual increase of the means of height at 28 DAT. Means recorded in this sampling period followed the following order; nematophagus fungi without substrate 44.40 cm, nematophagus fungi in rice bran carrier (44.02 cm),

nematophagus fungi in barley carrier (40.50 cm), calcium (37.20 cm), combined treatment (calcium + mycorrhiza + nematophagus fungi) (35.80 cm). Combined treatment was followed by mycorrhiza (35.52) and finally control (22 .35 cm). At 35 DAT, nematophagus fungi in rice bran carrier recorded the highest height of 51.30 cm. At this level, nematophagus fungi without substrate (49.73 cm) emerged second in performance though it had maintained the lead in performance in the previously recorded means. Nematophagus fungi in barley carrier (46.00 cm) emerged third in performance followed by mycorrhiza (42.60cm), calcium (42.40 cm) and control (22.45 cm). The final sampling period was at 42 DAT. The highest means recorded were 63.60 cm, 60.20 cm, 55.07 cm, 54.80 cm, 54.27 cm, 52.45 cm and 23.20 cm. These means were recorded in, nematophagus fungi in rice bran carrier, nematophagus fungi without substrate, calcium, nematophagus fungi in barley carrier, mycorrhiza, combined treatment (calcium + mycorrhiza + nematophagus fungi) and control respectively. Trial two recorded the highest (63.6 cm) growth compared to trial one (61.2 cm). Trial one and trial two had means that were significantly different. In both trials there was a gradual increase in height in each treatment at any one given sampling period. All the treatments recorded a higher increase in height on weekly basis compared to control experiment which had a minimal increase per week. The lowest means were recorded in control experiment throughout the experiment. In the two trials, nematophagus fungi generally produced seedlings with the highest height means compared to all the other treatments (Table 6).

Table 6: Mean plant height of banana plantlets from various treatments (Trial 1 and 2)

Trial one (cm)						**Trial two (cm)**				
				Days after treatment (DAT)						
Trmt	**14**	**21**	**28**	**35**	**42**	**14**	**21**	**28**	**35**	**42**
Con	30.60a	31.38a	31.75a	32.02a	33.53a	21.70a	21.77a	22.35a	22.45a	23.20a
Com	45.05d	48.70d	51.50c	54.10d	57.30d	25.42b	29.27b	35.80bc	41.60b	52.45b
Cal	41.55bc	45.42c	47.20b	49.22b	52.90b	27.60c	32.57c	37.20c	42.40b	55.07c
Myco	40.33b	43.25b	46.18b	52.10c	54.83c	24.82b	28.75b	35.52b	42.60b	54.27c
NWS	42.33c	51.85e	54.05d	56.25e	61.2e	39.90e	40.22e	44.40e	49.73d	60.20d
B + N						28.07e	31.50c	40.50d	46.00c	54.80c
RB + N						29.82d	37.95d	44.02e	51.30e	63.60
GM	40.00	44.10	46.10	48.70	52.00	27.60	31.70	37.10	42.30	51.90
LSD(0.05)	1.30	1.16	1.67	0.78	0.92	1.28	1.11	1.47	1.08	1.52
CV (%)	2.10	1.70	2.40	1.00	1.10	3.10	2.40	2.70	1.70	2.00
PV	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Means followed by the same letter in the same column are not significantly different (P < 0.05).**Com** -Combined, (calcium + mycorrhiza + nematophagus fungi) Con-control, **NWS** -Nematophagus fungi without substrate**, Myco**-Mycorrhiza, **Cal**-Calcium, **B+N** – Nematophagus fungi in barley, **R.B +N** –Nematophagus fungi in rice bran, **G.M**-Grand Mean, **LSD**-Least significant difference, **CV**-Coefficient of variation, Trmt-Treatment. Data are means of four replications.

4.1.5. Assessment of gall formation, number of lesions and total number of roots formed for various treatments in tissue culture banana

4.1.5.1. Effect of various treatments on gall formation in tissue cultured banana

It was observed that the means of treatments used in both trial one and trial two differed from the means of the control treatment. The overall best performance was realized in trial two in plantlets treated with nematophagus fungi in rice bran carrier with a mean of 1.5 galls. Combined treatment (calcium + mycorrhiza + nematophagus fungi) (1.75 galls) was second best in performance in trial two. Mycorrhiza treatment recorded a mean of 4.00 galls. This treatment emerged third in performance in this experiment. Nematophagus fungi without substrate followed closely with a mean of 4.50 galls followed by calcium (5.00 galls) and nematophagus fungi in barley carrier (5.00 galls). There was no significant difference in the means of galls of these two treatments. Control treatment recorded the highest mean of galls in this experiment. In trial one, combined treatment (calcium + mycorrhiza + nematophagus fungi) (1.75 galls) recorded the least number of galls compared to control treatment (3.75 galls). Mycorrhiza followed in performance with 2.0 galls, calcium (2.5 galls) and nematophagus fungi without substrate (2.75 galls). In both trials, combined treatment (calcium + mycorrhiza + nematophagus fungi) maintained the overall best performance by recording a mean of 1.75 galls which was the least mean attained in this experiment. Plantlets where no treatment was applied recorded the highest mean of galls in both trials (Figure 1).

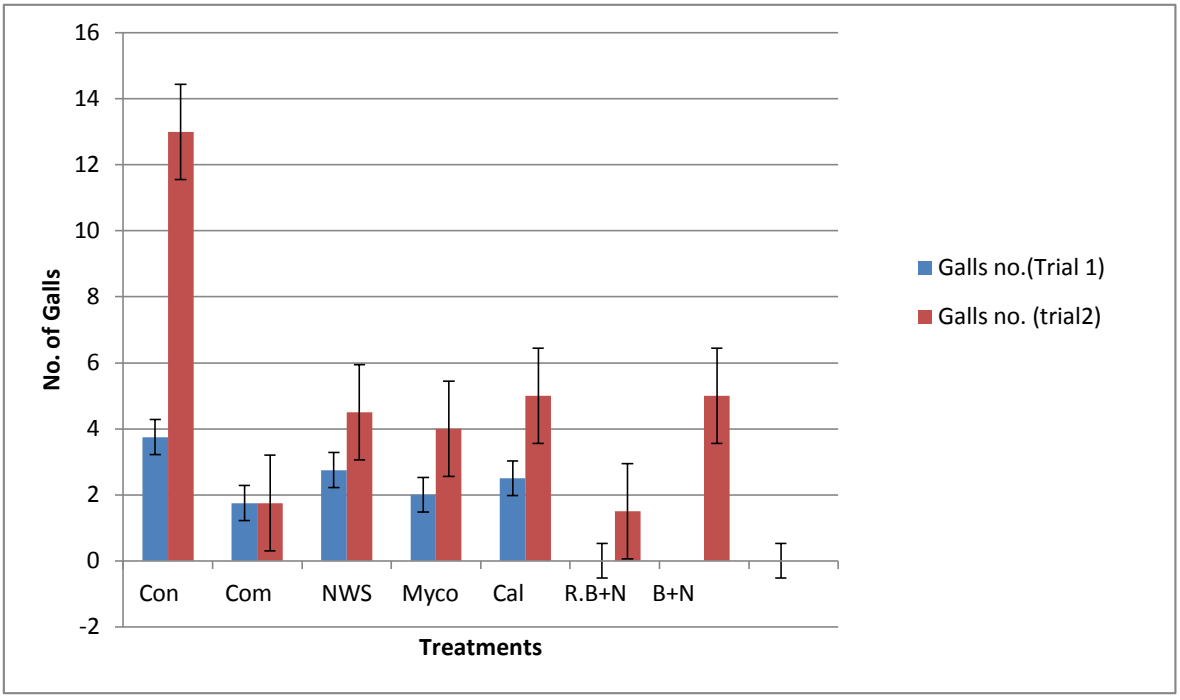

Figure 1: Mean root galls formation on banana seedlings from various treatments

Com-Combined (calcium + mycorrhiza + nematophagus fungi), **Con-**control, **NWS** -Nematophagus fungi without substrate**, Myco-** Mycorrhiza, **Cal-** Calcium, **B + N** –Nematophagus fungi in barley**, R.B +N** –Nematophagus fungi in rice bran.

4.1.5.2. Effect of various treatments on lesions formed on roots of tissue culture banana

In this experiment, trial one had generally the least recorded mean of lesions. Combined treatment (calcium + mycorrhiza + nematophagus fungi) (4.5 lesions) in trial one recorded the least mean of lesions in both trials. Plants treated with calcium followed with a mean of 5.75 lesions. Mycorrhiza and nematophagus fungi without substrate

followed each other closely with 6.50 lesions and 7.25 lesions respectively. Control seedlings recorded the highest mean of lesions both in trial one (15.75) and trial two (17.75). In trial two, the best performing treatment was mycorrhiza (5.50 lesions), followed closely by combined treatment (calcium + mycorrhiza + nematophagus fungi) with 5.75 lesions. Calcium was third in performance with 7.25 lesions. Nematophagus fungi without substrate recorded a mean of 8.50 lesions, nematophagus fungi in barley carrier (9.25 lesions) and nematophagus fungi in rice bran carrier with a mean of 9.50 lesions. Control treatment (17.75 lesions) in trial two recorded the highest mean of lesions in the two trials. Generally treatments in trial one recorded means which did not differ significantly with means of the same treatments in trial two. For instance control experiment recorded means of 15.75 and 17.75 in trial one and trial two respectively. Combined treatment (calcium + mycorrhiza + nematophagus fungi) recorded means of 4.5 lesions and 5.75 lesions in trial one and trial two respectively (Figure 2).

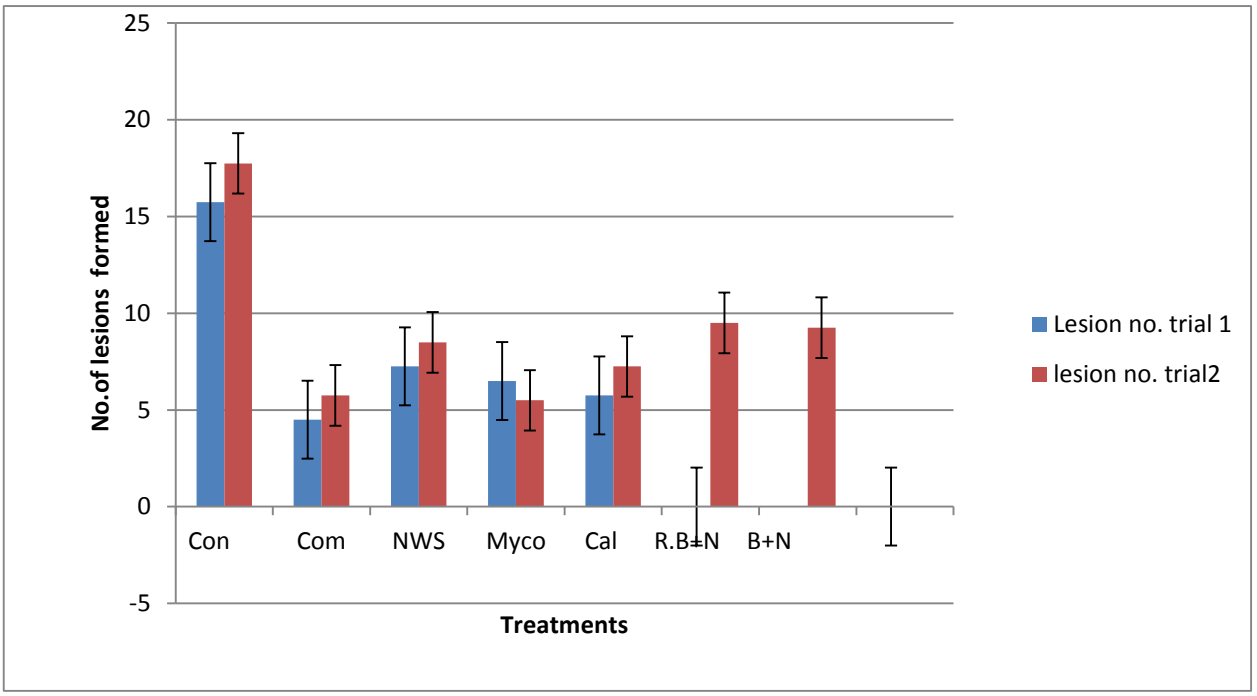

Figure 2: Effect of various treatments on number of lesions formed

Com- Combined (calcium + mycorrhiza + nematophagus fungi), **Con**- Control, **NWS**-Nematophagus fungi without substrate**, Myco**-Mycorrhiza, **Cal** - Calcium, **B + N** –Nematophagus fungi in barley, **R. B + N** –Nematophagus fungi in rice bran.

4.1.5.3. Effect of various treatments on roots formed on tissue culture banana

Trial two experiment recorded the highest means of roots in all treatments compared to trial one. Calcium treated seedlings had the highest (28.25 roots) mean of primary roots followed by mycorrhiza and nematophagus fungi without substrate which recorded a mean of primary roots of 28.0 roots followed by nematophagus fungi in barley (26.25 roots), combined treatments (calcium + mycorrhiza + nematophagus fungi without substrate) with a mean of 25.5 root, nematophagus fungi in rice bran (24.75 roots) and the least (6.5 roots) treatment in performance was control treatment. In trial one the highest (6.5 roots) mean of roots was recorded in calcium followed by mycorrhiza (5.25 roots). Nematophagus fungi without substrate recorded a mean of 4.75 roots. This treatment was followed closely by combined treatment (calcium + mycorrhiza + nematophagus fungi) with a mean of 4.5 roots. Control experiment in trial one recorded the least (2.5 roots) mean of roots comparing the two trials. The two trials differed significantly in their means of treatments recorded (Figure 3).

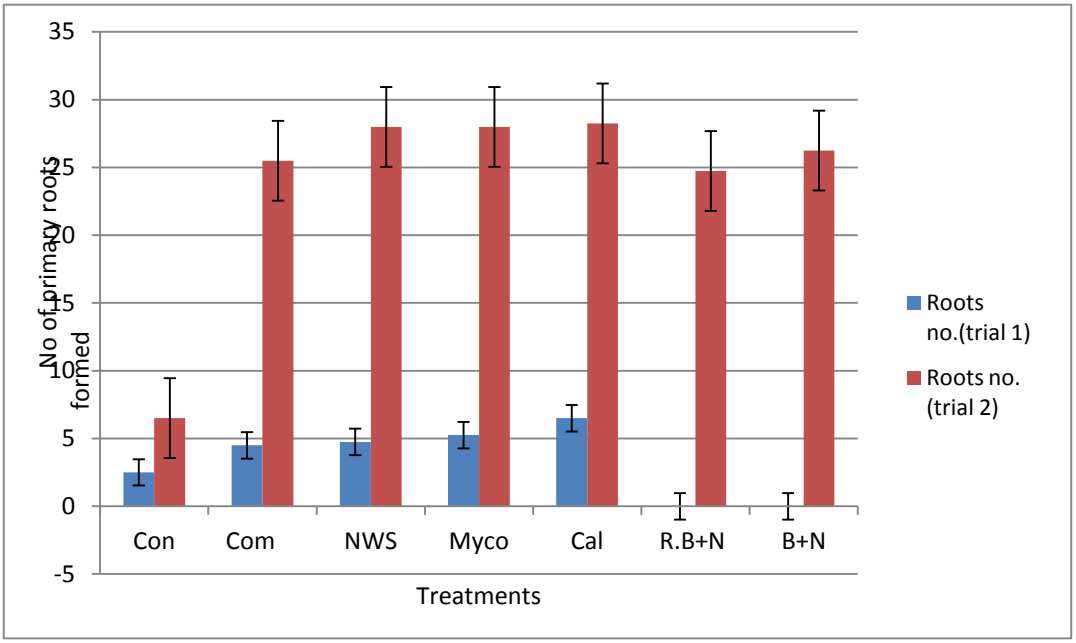

Figure 3: Means of total number of primary **roots formed in banana plantlets after using various treatments (Trial 1 and 2**)

Com-Combined, **Con**-Control, **NWS**-Nematophagus fungi without substrate**, Myco**-Mycorrhiza, **Cal** - Calcium, **B + N** –Nematophagus fungi in barley, **R. B + N** –Nematophagus fungi in rice bran.

4.1.6. Effects of various treatments in management of plant parasitic nematodes in tissue culture banana

In trial one, all means of treatments applied for management of *Pratylenchus spp.* were significantly different from control treatment. In trial one, calcium (95.5 nematodes) recorded the least mean of nematodes compared to all the other treatments used in this experiment. Calcium was followed closely by combined treatment (calcium + mycorrhiza + nematophagus fungi) with a mean of 98.2 nematodes. The rest of the treatments recorded higher (Mycorrhiza 140.5 nematodes and nematophagus fungi without substrate 159.5 nematodes) means of nematodes compared to the first two treatments. Control

treatment recorded the highest (439.0 nematodes) mean of nematodes. In trial two, nematophagus fungi in barley recorded the least (54.8 nematodes), followed by nematophagus fungi in rice bran with a mean of 80.5 nematodes. These first two treatments were followed closely by combined treatments (calcium + mycorrhiza + nematophagus fungi without substrate) with a mean of 80.8 nematodes and calcium (82.0 nematodes). Nematophagus fungi without substrate (134.5 nematodes) and mycorrhiza recorded a slightly higher (154.2 nematodes) mean compared with the earlier reported treatments. Control treatment recorded the highest mean (551.2 nematodes) in both trials. For management of *Meloidogyne spp.* all the treatments used had a significant difference compared to control experiment. Nematophagus fungi without substrate recorded a mean of *Meloidogyne spp*. of 45 nematodes followed by combined treatments (calcium + mycorrhiza + nematophagus fungi) (60 nematodes), calcium (81.8 nematodes) and mycorrhiza fungi (123.5 nematodes). In trial one, control recorded the highest (233.5 nematodes) mean of *Meloidogyne spp.* This mean differed significantly with the rest of the means in this trial. In trial 2, treatments applied for control of *Pratylenchus sp.* also differed significantly from control treatment (551.2 nematodes). Nematophagus in rice bran substrate had the least number of *Meloidogyne spp.* (19.75 nematodes) followed by nematophagus fungi in barley (26.25 nematodes) which was followed by nematophagus fungi without substrate (37.25 nematodes). Combined treatments (calcium + mycorrhiza + nematophagus fungi) recorded a mean of 52.75 nematodes followed by calcium with a mean of 96 nematodes. Mycorrhiza recorded a slightly higher (139.0 nematodes) mean compared to the other treatments. Control treatment recorded a mean of 270.75

nematodes. This was the highest mean of *Meloidogyne spp*. that was recorded in this experiment (Figure 4).

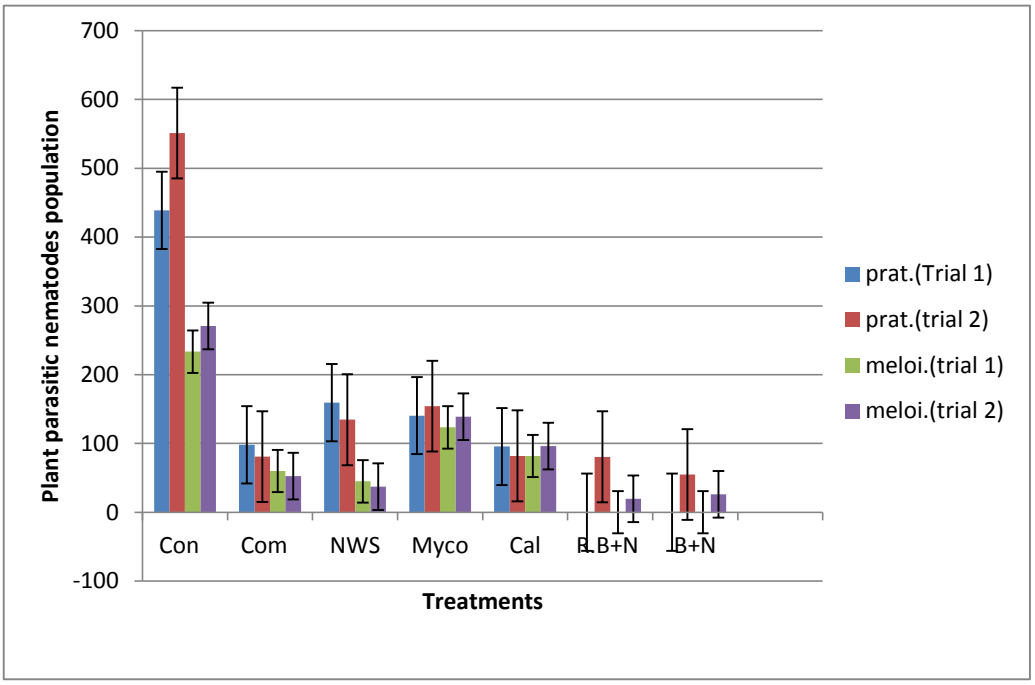

Figure 4: Effect of treatments on plant parasitic nematodes per 200cm^3 of soil after destructive sampling

Prat- *Pratylenchus*, **Meloi-** *Meloidogyne*, **Com-** Combined (calcium + mycorrhiza + nematophagus fungi), **Con-** Control**, NWS-** Nematophagus fungi without substrate, **Myco-** Mycorrhiza, **Cal-** Calcium**, B + N** –Nematophagus fungi in barley, **R. B + N** – Nematophagus fungi in rice bran.

CHAPTER FIVE

5.0. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

Various biological control agents have been reported to induce resistance in different crops. Biological control of nematodes by use of fungi is an area that has been attracting a lot of attention (Mohammad and Rasoul, 2011). Use of biological control agents for management of plant parasitic nematodes offers a great scope for field application (Brand *et al*., 2010). Nematophagus fungi (*Paecilomyces lilacinus*) refer to a group of fungi with a capability to colonize and parasitize nematodes in a symbiotic relationship. Nutrients from plant roots leak to the rhizosphere as root exudates (Mohammad and Rasoul, 2011). These root exudates increase the population of the fungi in the soil.

Management strategies for nematodes in commercial banana production ought to be reliable, economical, effective and environmentally friendly and must be able to integrate in the overall banana production systems (Mendoza *et al*., 2007).

Among the treatments used in this present study, nematophagus fungi (*Paecilomyces lilacinus*) contributed the most in height increase of the banana seedlings. This was probably due to reduction of plant parasitic nematodes and their egg masses in the planting pots. *P. lilacinus* is a soil fungus with a good potential for biological control and has been described as an efficient nematode control agent just as the synthetic nematicides (Schenck, 2004). Use of nematophagus fungi is a very promising way of nematode management in crops. According to Mucksood (2010), *P. lilacinus* has been considered as one of the most practical biocontrol agent for management of plant parasitic nematodes. Some strains of *P. lilacinus* have been used as biocontrol agents

against nematodes (Khan *et al*., 2003). Similar to the findings of this study, Zareena and Vanita, (2014) recorded an increment of growth of brinjal, reduction of galling, egg masses and number of eggs per egg mass of *Meloidogyne incognita* treated with *P. lilacinus*. Increase in plant growth was contributed by decrease in reproduction rate of nematodes due to inhibition of hatching of eggs. The decreased reproduction of nematodes resulted to reduction of galls of the seedlings. The results of this experiment are in conformity with the study carried out by Mohd *et al.,* (2012) on effect of *P. lilacinus* and plant growth promoting Rhizobacteria on black gram (*Vigna mungo*) inoculated with *Meloidogyne incognita.* The performance of *P. lilacinus* in this present study could have been attributed to its colonization in banana roots that could have reduced nematode numbers and increased defense– related enzymes, phenolics and phenylalanine in the plant. In this experiment, most of the nematodes did not multiply in the presence of the fungi.

Tissue cultured banana either treated with the *P. lilacinus* fungi alone, in combination or with substrate incorporated performed better than the other treatments used in the experiment. Results in this case indicate that *P. lilacinus* embedded in rice bran carrier had a positive impact on the height, leaf width and leaf length of the banana seedlings in this experiment. The fungi also recorded the lowest number of root lesions in the test plants. This observation can be attributed to reduction of nematodes and their eggs in the pot plants. Siddiqui *et al.,* (2000) reported on reduction of *M. javanica* infection on tomato. Cannayane and Sivakumar (2001), reviewed on biocontrol efficacy of *P. lilacinus* and indicated that the fungi contributed to the reduction of root-knot nematodes and potato cyst nematode. Another explanation for the increased heights of seedlings, leaf

width and leaf length is that penetration of the nematodes in the roots is reduced. This is in conformity with results recorded by Mendoza *et al.,* (2007). They reported a decrease of *Radopholus similis* penetration in banana roots after at-plant and post-plant applications of *P. lilacinus* 251. Their results also indicated that the fungus had a significant bio-control effect towards the burrowing nematode (*Radopholus similis*) on young plants when applied during the pre-planting process. Kiewnick and Sikora (2004), also reported a decrease of the number of nematodes penetrating the roots. In a study carried out by Kiewnick and Sikora (2003), it was reported that a population of *P. lilacinus* before transplanting reduced the number of nematodes surviving in the soil hence reduced damage caused by the nematodes. *P. lilacinus* has also been reported to produce chitinase and a basic serine protease which degrades nematodes eggs (Olivares-Bernabeu, 2002; Khan *et al*., 2004).

Results demonstrated that treatment of banana plantlets with various treatments prior to nematode inoculation led to lower nematode populations and associated damage to banana roots. This fungus was capable of colonizing the soil in which the plants were growing (Mendoza *et al*., 2007) and when the plant was transplanted into the field, the fungi had the opportunity to kill nematodes prior to their penetration into the roots of the seedlings. The nematode-trapping fungi develop special mycelial structures that act as traps in response to the presence of nematodes in the soil. This present study revealed that there was a level of management of the nematodes by the fungi in the banana seedlings because their heights increased despite inoculation of the seedlings with the nematodes.

Regarding response of the banana seedlings to treatments in leaf length and leaf width parameters, *P. lilacinus* also demonstrated the highest increase in the two trials compared

to calcium and mycorrhiza. This could have resulted from the reduction of nematode inoculum in the root zone of the banana seedlings due to prior application of the *P. lilacinus*. This is in conformity with results obtained by Kiewnick and Sikora (2006) in their experiment in the control of *M. incognita* by *P. lilacinus* in tomatoes. Their results indicate that there was a reduction of the total number of eggs and juveniles per root. Use of *P. lilacinus* resulted in complete inhibition of juvenile development. Banana seedlings inoculated with nematodes and had not been treated at all exhibited a retarded growth and had the least score in growth parameters in terms of plant height, leaf width and leaf length.

All the seedlings treated with *P. lilacinus* either alone, in combination with the other treatments, incorporated in either barley or rice bran performed well revealing that there was some level of nematode management by use of this fungi in whichever form it was delivered into the seedlings. There was a close relationship between the highest plants height with reduced galls after application of nematophagus fungi embedded in rice bran carrier.

An Arbuscular mycorrhiza fungus (AMF) has been reported to induce resistance in banana to *Pratylenchus coffeae* and *Radopholus similis*, and in grapevine to *Xiphinema* (Elsen, 2008; Hao *et al.,* 2012). Mycorrhiza fungi (AMF) used in this experiment reduced nematode population densities and damage on the roots. Banana plantlets treated with mycorrhiza fungi followed closely in growth increment after those treated with nematophagus fungi. This could have resulted from banana roots colonization by AM fungi. Elsen *et al*., (2008) attributed this to colonization of the roots that acts as the primary system of plant defense against pathogens. Mechanisms that could account for

the protective activity ascribed to AMF include improvement of plant nutrition, root damage compensation, competition for photosynthates or colonization/infection sites, production of anatomical or morphological changes in the root system, changes in mycorrhizosphere microbial populations, and activation of plant defense mechanisms. Another possible explanation for the good performance of AMF in this experiment is that its colonization contributed to increase in phosphorus levels of the banana seedlings making them vigorous and more resistant to pathogen invasion. Earlier work conducted by Greipsson and El Mayas, (2002) on coastal dune systems treated with mycorrhiza revealed that the AMF inoculums protected the dune grass *Leymus arenarius* against migratory endoparasitic nematodes.

Calcium is a synthetic compound that enhances the strength of stems and stalks of plants (Jeremy, 2007). It plays a major role in good growth and structure of plants. Calcium compound used in this experiment contributed in very vigorous seedlings that had many complete primary roots with a high number of root hairs. This possibly enabled the seedlings to continue growing in the presence of the nematodes with very high vigor. The banana seedlings growth was not slowed down by the presence of plant parasitic nematodes. According to Mary Beth (2008), lack of calcium can result in structural changes of intracellular organelles, a decrease in cell elongation, and affect cell walls and the permeability of cell membranes to solutes and other ions. Banana plantlets treated with calcium also had higher means in height than those that were not treated at all. These results are supported by work done by Abdur and Ihsan-ul (2012) on effect of calcium chloride on the height of tomatoes. They recorded an increase in height of tomatoes that were treated with calcium chloride. In the past studies, calcium treated

plants have been reported to have strong stems and stalks. Calcium has also been reported to regulate absorption of nutrients across plasma cell membranes, contributes in plant cell elongation and division (Hepler, 2005), as well as nitrogen metabolism and carbohydrate translocation. It can be concluded from this conclusion that, calcium application resulted in a significant increase in plant height (Mary, 2008).

In the past studies, various types of industrial products have been tested to determine suitable materials for mass multiplication of *P. lilacinus* (Jagadeesh *et al.*, 2008; Amala, 2012). In this study, several naturally available substrates were tested for mass multiplication of *P. lilacinus.* The success of microbial control of pests will highly depend on successful mass production of the microbial agents in the laboratory. High spore numbers of the fungi is one of the main criteria for choosing a fungal pathogen for biological control of pests in the field (Robl *et al*., 2009). Growth and survival of *P. lilacinus* was affected by the type of substrate. In selecting the substrate most suitable for supporting the highest growth of nematophagus, the consistent viability of fungi in the substrate is important. Also the capability of the substrate to support the highest growth of the fungi is important. Substrates used in this study demonstrated their potential to support growth of the fungi. Substrates differ in their capability to support growth of fungi due to their differences in their levels of nutrients which are needed to support growth of the fungi. Past studies indicate different solid substrates had the capability to support growth of fungi differently depending on their nutritional contents (Thet and Saisamorn, 2012). A substrate rich in nutrients will support high mycelial growth compared to one poor in nutrients which will support high sporulation as compared to the rate of mycelial growth (Thet and Saisamorn, 2012). A substrate that supported growth of

P. lilacinus for many days would be preferred most because it would enhance persistence of the fungi in the soil for longer periods. This would offer protection against plant parasitic nematodes attack.

Barley substrate was the best in overall though rice bran substrate followed closely in performance. This is in conformity to observations recorded by Amala *et al., (*2012) who suggested rice bran as a suitable medium for mass multiplication of *P. lilacinus* fungi. That cereal has been reported to be suitable for the mass production of other fungi (Thet and Salsamorn, 2012). Initially rice bran had supported the highest growth of the fungi within the seventh day and fourteenth day but on the twenty first day, it was overtaken by barley which maintained a high performance for the two weeks that followed. According to observations made in this experiment, rice bran can be used for mass multiplication of the fungus in the absence of barley. The high spore count recorded in rice bran for the first two weeks and later in barley for the last two weeks may was attributed to the presence of nutrients in the two substrates. Therefore barley and rice bran can solve the need for an easy and cheap method of mass multiplication of the bio-control agents (Masoud *et al.,* 2013). From the study, it was clear that *P. lilacinus* multiplied in the two substrates obtained from various agricultural by-products and these waste materials can be utilized by nursery operators to culture the fungi under study for its mass production. Study conducted by Thet and Salsamorn (2012) on various entomopathogenic fungi in solid state fermentation revealed that, the tested fungi were able to grow on a wide variety of cereal grains. From observations made in the present study, it was also noted that rice husks supported minimal growth of the *P. lilacinus* in this study compared to barley and rice bran. This could have resulted from the fact that rice husks have minimal

nutrients mainly carbohydrates that support growth of the fungi and might require to be supplemented with materials that could supply carbon (Mucksood and Tabreiz, 2013). Rice husks used in this experiment proved a poor substrate for mass multiplication of the fungi. Humber, (2008) stated that the growth characteristic of the vast majority of entomopathogenic fungi is clearly affected by the supply of nutrients. This supports the findings in this experiment.

In the past studies, *P. lilacinus* was incorporated to the soil using various organic substrates that included wheat bran, oil cakes, and leaf residues among others (Cannayane and Sivakumar, 2001). Effects on substrates can either be positive or negative. In this experiment there was an increment in spore count in barley and rice bran.

Root knot nematodes cause galling in roots of plants. The galls receive nutrients and photosynthates from the plant, which the developing nematodes consume. Infected susceptible banana plants generally get stunted (Bawa *et al.*, 2016) and produce small bunches. Some scientists have conducted research on control of *Meloidogyne spp.* by use of biological control. Sharon *et al*, (2001) reported on the effect of *Trichoderma harzianum* Rifai against *Meloidogyne javanica* after soil pre-plant treatment. A study was carried out (Kerry and Hidalgo Diaz, 2004) on the management of root knot nematodes by use of nematophagus fungus *Pochonia chlamydosporia* (Goddard) Zare and Gams (Hypocreales: *Clavicipitaceae var. catenulata* (synonym: *Verticillium chlamydosporium* Goddard (Hypocreales:*Clavicipitaceae*)). A review by Cannayane and Sivakumar, (2001) on bio-control efficacy of *P. lilacinus* on root knot nematodes and potato cyst nematodes (*Globodera rostochiensis*) indicated several reports of its success. In the current study, it was demonstrated that application of nematophagus fungi resulted in the reduced number

of galls formation in banana seedlings. *Paecilomyces lilacinus* fungus has been reported to reduce *Meloidogyne spp*. populations in various crops. Research carried out on chilli crop indicated a reduction of the nematodes after application of *P. lilacinus* in the crop (Sayed and Tabreiz, 2004). Some trials carried out on pot trials of tomato, barley and tissue cultured banana plantlets by use of *P. lilacinus* fungi indicated that the fungi was effective in the control of plant parasitic nematodes namely *M. javanica, H. avenae* and *R. similis* respectively (Alamgir *et al.,* 2006). Similarly the present study indicated a notable reduction of *Meloidogyne spp.* in banana in both trials mainly in all the treatments that had *Paecilomyces lilacinus* either alone, in combination or embedded in a substrate. All the treatments containing *P. lilacinus* indicated a significant reduction of the *Meloidogyne spp.* compared to control treatment. There was a very high difference between those seedlings that no treatment was applied with those that were treated. Reduction of galls in banana seedlings introduced with *P. lilacinus* was as a result of the antagonistic effect of the fungi on the plant parasitic nematodes (Elvira *et al*., 2009).

P. lilacinus fungus is believed to exhibit destructive activities like enzymatic disruption of the worms' structural elements. These structural elements are egg shells larval cuticles and physiological instabilities due to biosynthesis of diffusible toxic metabolites (Elvira *et al*., 2009). *Paecilomyces lilacinus* in rice bran produced the least mean of *Meloidogyne spp*. Banana seedlings treated with *P. lilacinus* alone had the lowest mean of *Meloidogyne spp.* in trial one while in trial two, those treated with *P. lilacinus* incorporated in rice bran had the least. From these findings, it is clear that nematophagus fungi treatment contributed to the reduction of the *Meloidogyne spp.* This is in agreement with work done by Kerry and Hidalgo-Diaz, (2004) when they developed a management

system for control of root-knot nematodes in organic vegetable production based on the nematophagous fungus *Pochonia chlamydosporium* var. *catenulate.* Some researchers also reported a reduction of galling index in potato fields of Peru treated with *P. lilacinus* than those under nematicides treatments (Mohammad and Rasoul, 2011).

Mycorrhiza fungus (AMF) has been reported to play an important role in decreasing soil borne incidences (Harrier and Watson, 2004). Matsubara *et al*., (2001) reported on contribution of *Glomus fasciculatum* and *Gigaspora margarita* in decrease of root rot disease caused by *Fusarium oxysporum f. sp. asparagi* in asparagus. AMF is known to have a symbiotic relationship with host plants (Parniske, 2008; Guether *et al*., 2009). This relationship enables AMF to benefit with carbohydrates from the host plant while the host plants benefits with nutrients like phosphorus and nitrogen. It has been reported that AMF improves the nutrient status of their host plants (Smith and Read, 2008). The improved growth of the seedlings could have resulted from symbiotic role of the fungi and the plants, a relationship which assists the plants in the uptake of phosphorous and other mineral nutrients from the soil (Auge, 2001). The fungi in the cortex of the roots enhance it to obtain carbon from their host plants. This relationship is beneficial to plants because phosphorus (Akthar and Siddiqui, 2008) is a major essential element for growth and development. AMF also contribute in production of growth hormones and protection of host roots from pathogens. This relationship is likely to have enabled the AMF to induce some resistance to the roots of the banana seedlings from the attack by the nematodes. This can be demonstrated by the fact that the number of nematodes was high in AMF inoculated plants as compared to other treatments and yet the AMF inoculated plants remained vigorous and healthy. Christine *et al*., (2012) recorded such an

observation of reduced penetration of nematodes after treatment of tomato roots with AMF. Nematode penetration was reduced in the mycorrhizal treated roots of tomato probably due to mycorrhizal root exudates effects on nematode mortality (Christine *et al*., 2012).

There was improved plant growth due to root colonization of the seedlings by AMF which resulted in alteration of root exudation that can cause a change in microbial diversity in the rhizosphere. This change affects plant-pathogen interactions (Lioussanne, 2010).Azcon-Anguilar *et al*., (2002) reported that AMF is capable of reducing damage caused by soil borne pathogens. Jia *et al*., (2004) reported that biomass production and photosynthetic rates in *Vicia faba* after inoculation with AMF increased due to enhanced phosphorus supply. This relationship helps the plant to cope with stressful conditions (Pozo and Azcon-Aguilar, 2007). Banana seedlings treated with mycorrhiza fungi indicated an increase in height that followed closely after the seedlings treated with nematophagus fungi. The number of galls recorded in current study was reduced by application of mycorrhiza in relation to control experiment. The reduction of galls was as a result of roots colonization by AMF. Elsen *et al*. (2008) indicated that the colonization of the roots acts as the primary system of plants defense against pathogens. This is in conformity with earlier work conducted by Greipsson and El Mayas (2002), on coastal dune systems treated with mycorrhiza revealed that the AMF inoculums protected the dune grass (*Leymus arenarius)* against migratory endoparasitic nematodes. In the present study, there was an increase in plant height of the banana seedlings where AMF was applied. Results obtained from seedlings inoculated with mycorrhiza indicated that AMF performed very closely to those inoculated with *Paecilomyces lilacinus*. A similar

observation was made on results for leaf length and leaf width. The obtained results are in agreement with that of Elsen *et al*. (2008), that application of AMF reduced *Radopholus similis* and *Pratylenchus coffeae* nematodes by more than 50%. Their results further confirmed that AMF has the potential that increased resistance against plant parasitic nematodes. They also reported on reduced nematode population and concluded that AMF has the potential to induce systemic resistance against plant parasitic nematodes in a root system.

Pratylenchus spp. (lesion nematodes) produces necrotic lesions on the root cortex and cause huge yield losses (Brooks, 2008). Extent of plant parasitic nematodes control influences the total number of lesions inflicted on roots. High density populations of root lesion nematodes imply that there is a likelihood of high numbers of lesions on the roots. A reduction of lesions was observed in seedlings treated with mycorrhiza in this study. A possible explanation for reduced number of lesions could be due to the role of symbiotic relationship between the roots of the banana seedlings and the mycorrhiza. This could have introduced an antagonistic interaction (Mukerji *et al.,* 2002) that possibly enabled the AMF to suppress the nematodes and inhibit formation of lesions on the roots. The mycorrhiza fungi treated seedlings recorded the least number of lesions.

There was a notable effect of lesions formation in mycorrhiza treated seedlings possibly due to differences in soil temperatures. These effects of temperature differences have been reported in the past (Hafeel, 2004) to influence and alter the physiology of mycorrhizal symbiosis. This stimulates greatest inoculum production by influencing root morphology and host plant nutrition and growth as well as the general ontogeny of

mycorrhizal roots. Optimal temperatures for germination may be related to the environment to which each endophyte is indigenous (Giovannetti, 2000).

The damage inflicted to the banana seedling roots was minimal compared to the total population density analyzed from the pots where the seedlings were growing. The plantlets grown as control experiment with no inoculation had the highest numbers of the *Meloidogyne spp*. and this reveals that all the other treatments used in this experiment other than control contributed to some extent in *Meloidogyne spp*. reduction.

Calcium is an important nutrient in plants that plays an important role in the structure of cell walls and cell membranes, growth of the fruit and development and fruit quality (Kadir, 2004). Calcium treatment contributed to significant increase of height of seedlings and root growth in this study. Mary (2008) indicated that lack of calcium can result to decrease in cell elongation and affect cell walls and permeability of cell membranes to solutes and other ions. The roots and stems established after treatment of seedlings with calcium could have enabled the plants to access ample water and nutrients with ease in the presence of the nematodes. This could have resulted to resistance of nematode attack by the plants. Hepler (2005) reported that calcium regulates absorption of nutrients across plasma cell membranes, contributes in cell elongation and division. It contributes in enhancing resistance to bacterial and viral diseases (Usten *et al.,* 2006). Calcium taken up from the soil is translocated to the leaves but very little is translocated from the leaves to the fruit (Kadir, 2005). For this reason, plants need a constant supply of calcium for vigorous growth of leaf, roots and canopy development (Del Amor and Marcelis, 2003). In this experiment, calcium treated banana seedlings was third in performance in height parameter resulting from the vigor and strength imparted to the

seedlings by this nutrient. This observation is in conformity with results observed by Juan *et al*, (2008) on poinsettia plants sprayed with calcium at 400mg/L that produced increased stem height. The results of this present study agree with work done by Abdur and Ihsan –Ul (2012), which showed significant increase of height after foliar application of calcium chloride and borax either applied alone or in combination. Calcium has been reported (Cakmak, 2014) as a signal carrier due to its very low cytosolic concentrations in plant cells. Any manner of stress inflicted on plants results to quick rise in cytosolic Ca^{2+} which is a key factor in expression of stress-responsive genes and physiological responses of plant cells to stress conditions such as drought, salinity, and pathogenic attack. This could have been the reason why calcium inoculated plants had a lower mean of galls formed in banana seedlings grown in nematode infested soil.

Banana plantlets treated with calcium recorded the highest number of roots than all the other treatments that were used in banana seedlings in this experiment. This performance was observed in both trials. In the present study, use of calcium was found to increase the number of roots in banana seedlings, reduce the stress level from nematodes attack and played a role in increasing the height of the seedlings. This observation is in conformity with work reported by Cakmak, (2014) on the functions of calcium in plants that it is essential for regulation and developmental processes and to recognize, respond and adapt to wide range of stress conditions. It is also primarily needed for the stability and functions of cell walls. Similar results were obtained by Supanjani *et al*., (2005) by recording an increase in plant growth and yields of *Chrysanthemums coronarium.* Mesbah *et al*., (2010) also observed an increase of plant growth and performance after

application of calcium carbonate up to 5t ha^{-1} but further increases reduced the growth. In the experiment, calcium reduced the level of *Pratylenchus spp.* in banana seedlings.

5.2. Conclusions

Nematophagus fungi is a potential bio-control agent against plant parasitic nematodes and the fungi can be used to protect tissue cultured banana seedlings while still growing in the nursery before they are transferred to the fields. It can also be concluded that there was a level of tolerance that was impacted by the fungi on the banana seedlings thus making them continue growing vigorously in the infested soil. The fungi significantly influenced growth of the banana seedlings in terms of height, leaf width and leaf length. Nematophagus fungi used either alone or in combination with calcium and mycorrhiza or incorporated in rice bran or barley substrates produced the highest means in growth. This is clear evidence that plants grown in nematode infested soil performed best with nematophagus fungi treatment.

From the study, it can be concluded that barley was the best carrier material identified for mass multiplication of *P. lilacinus.* Since barley is readily available in most of the parts in the country, it can be utilized for mass multiplication of the fungi in large quantities. These large quantities of fungi will be incorporated in banana plantlets in nurseries involved in hardening of tissue cultured banana seedlings. Mass multiplication of the fungi will contribute a lot in that biocontrol agents will readily be accessible to nursery operators dealing with tissue culture banana seedlings to enable them produce clean planting materials. Mass production of entomopathogenic fungi will enhance its successful utilization as a biocontrol agent which will contribute to cost saving if locally and readily available materials like rice bran, barley are used for multiplication. This will

also ensure provision of banana seedlings with induced resistance to plant parasitic nematodes.

If culturing of the fungi was to be done for the first fourteen days, rice bran would be the best substrate for use in mass multiplication. Rice husks supported minimal sporulation of the fungi and was found to be a poor substrate. This could be attributed to the fact that rice husks are deficient of plant nutrients. Nematophagus fungi grown on barley and rice bran could be used effectively for a period of one month for the management of the plant parasitic nematodes.

Root galls were also reduced by the treatments evaluated. Combined treatment produced seedlings with the least number of galls followed by mycorrhiza in trial 1 while in trial 2; nematophagus incorporated in rice bran had the least number of galls. This is an indication that nematophagus fungi played a significant role in the reduction of root galls in the seedlings.

In the experiment regarding total number of roots observed per treatment, calcium treatment recorded the highest number of roots in trial one and trial two. Calcium also contributed positively in growth and development of primary roots. The roots were fully formed and this enhanced in proper uptake of nutrients beneficial to the seedlings. This shows that calcium played a major role in growth of seedlings in this experiment. This element also contributed in the reduction of levels of pratylenchus nematodes in the soil. The element increased levels of resistance of plants against nematodes.

In terms of reduction of the populations of the plant parasitic nematodes, nematophagus proved the best and this is a confirmation that use of *P. lilacinu*s contributed a lot in reduction of *Pratylenchus spp.* and *Meloidogyne spp.* The use of nematophagus fungi for

management of plant parasitic nematodes is a strategy likely to contribute greatly in the banana industry in Kenya.

5.3. Recommendations

This study leads to the recommendation that;

- Tissue cultured banana nursery operators should embrace the use of mass multiplied nematophagus fungi and incorporate it in their banana seedlings during hardening stage.
- The seedlings should be treated immediately after they leave the laboratory to give them ample time for the fungi to grow and establish in the plants. This will also enhance reduction in levels of establishment of the plant parasitic nematodes in the banana seedlings at an early stage.
- *Paecilomyces lilacinus* should be mass multiplied in barley carrier and applied directly on the tissue cultured banana seedlings. Nursery operators should adopt inoculation of *P. lilacinus* to tissue cultured seedlings at tray stage and if possible inoculate at the stage just before they leave rooting bottles in the laboratory.
- During establishment of banana orchards, farmers should also embrace the act of mulching them at the base of the pseudostems with barley or rice bran with *P. lilacinus* fungi for further multiplication of the fungi thus providing continuous protection against the nematodes.
- Further work should be conducted on the effects of the substrates used on the efficacy of *P. lilacinus.*

- The biochemical processes taking place during the growth of the seedlings should also be analyzed to determine if there are some that affect the performance of the fungus.
- Further research should be conducted with other carriers to establish the one that gives the highest sporulation of the fungus.
- Nematophagus fungi (*Paecilomyces lilacinus*) in conjunction with calcium element needs to be formulated for management of plant parasitic nematodes.

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Plate 1: Treated banana seedlings after 2 weeks of inoculation

Plate 2: A layout of the treatments six weeks after inoculation

Plate 3: Comparison of the heights of treated- inoculated seedlings

Plate 4: A seedling treated with nematophagus (*P. lilacinus*) and inoculated with plant parasitic nematodes.

Plate 5: Roots from mycorrhizal inoculated plants showing root colonization by the fungi.

Plate 6: Enumeration of root galls, root lesions and total number of primary roots per sample

Plate 7: Banana seedling showing root galls and some lesions

Plate 8: Comparison of seedlings treated with nematophagus fungi and a control plant respectively.

Plate 9: Comparison of seedlings from (left) control and (right) plants treated with combined treatments