# EFFECTIVENESS OF MACROPROPAGATION TECHNIQUE IN PRODUCTION OF HEALTHY BANANA SEEDLINGS IN EASTERN AND CENTRAL REGIONS, KENYA

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## DECLARATION

I, Njeri Njau, declare that this thesis is my original work and has not been presented for the award of a degree in any other University or any other award.

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# DEDICATION

This work is dedicated to my parents Samuel Njau and Jane Wanjiku Njau for their love, support and encouragement during the period of study.

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## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of variance |
| BXW | Banana Xanthomonas Wilt |
| FAO | Food and Agriculture Organization |
| FaCT | Farm and Community Technologies |
| FHIA | Foundation Hondurena Internationalo AgricoIa. |
| *Foc* | *Fusarium oxysporum* f.sp. *cubense* |
| INIBAP | International Network for Improvement of banana and Plantain |
| IPM | Integrated Pest Management |
| K.U | Kenyatta University |
| LSI | Leaf Symptom Index |
| Masl | Meters above sea level |
| NA | Nutrient Agar |
| PDA | Potato Dextrose Agar |
| PPN | Plant parasitic nematodes |
| RDI | Rhizome Discoloration Index |
| TC | Tissue Culture |
| WA | Water Agar |
| *Xvm* | *Xanthomonas vasicola* pv. *musacearum* |

## ABSTRACT

Banana (*Musa* spp.) is one of the most important food and cash crops in Kenya. It provides food security, nutrition and income for many smallholder farmers. Bananas are eaten ripe or cooked depending on the variety. Despite its economic importance, banana production faces major challenges including scarcity of high quality seedlings, insect pests and diseases. Demand for pest free and high quality planting materials has been on the increase. Naturally regenerated suckers that are preferred by farmers are more likely to carry pests and diseases leading to reduced productivity and a short lifetime of new plantations. Tissue Culture has been introduced but its adoption has been low due to the high costs and skills involved. To address this constraint, macropropagation has been introduced as an alternative propagation technology. The technology can be implemented with little capital and skill. The study was aimed at assessing whether macropropagation technology can produce healthy banana seedlings. A survey was conducted to identify the current important diseases and insect pests of bananas in Eastern and Central Kenya. Macropropagation nurseries were established at Kenyatta University and in farmers' fields at Mathioya, Kirinyaga, Embu East, Meru Central and Mitunguu and Ntharene in Imenti South District. These sites represent different agroecological zones, including high, mid and low altitudes. Corms obtained in accordance with established quality assurance protocols were propagated and the health of the macropropagated banana seedlings monitored. Macropropagation was done in two cycles, the first in Feb - June 2011, and the second from July - November 2011. The importance of the isolated organisms in banana health was determined through pathogenicity tests carried out under controlled conditions. To determine the effectiveness of macropropagation protocol in eliminating pathogens, corms were artificially inoculated with *Radopholus similis*, *Fusarium oxysporum* f. sp. *cubense* (*Foc*) and *Xanthomonas vasicola* pv. *musacearum* (*Xvm*) followed by monitoring health of the seedlings from the inoculated corms. Fusarium wilt and Sigatoka were recorded as the most important diseases with 66 and 50% incidence respectively, while nematodes and banana weevil were the most important pests with 21 and 17% incidence, respectively. An average of 98 and 100% healthy seedlings resulted from the first and second cycles of macropropagation. Under simulated infection, macropropagation did not eliminate *Foc* and *Xvm* but completely removed plant parasitic nematodes (PPN). The study found that macropropagation technique is an effective method to produce healthy banana seedlings however precaution is needed to ensure quality of the seedlings where *Xvm* and *Foc* are present. The key control points for safeguarding health and quality of the macropropagated seedlings is during certification of corm sources to ensure selection of healthy corms, the process should be done aseptically and the corms should be treated before planting.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background information

The banana, *Musa* spp belongs to the Musaceae family. Bananas originated from South East Asia, a region considered as the primary centre of diversification of the crop and where the earliest domestication occurred (Simmonds, 1962). In East Africa, bananas have evolved into an important zone of secondary genetic diversity for the East African highland bananas (*Musa* EA-AAA) (Smale, 2006). The crop is grown mostly by small scale holders and constitutes a major crop for food, nutrition, security and income generation. Bananas have a high nutritional value (Wall, 2006), and are a valuable source of Potassium, vitamin A, B6 and C. They are perennial and are a source of steady income all year round. Bananas can be eaten fresh when ripe or after cooking or processing.

Commercial cultivation of banana is greatly hindered by biotic and abiotic factors (Seshu Reddy *et al*., 1999). These include scarcity of high quality seedlings, diseases and pests, lack of agricultural inputs and limited land space for farming. Farmers mostly rely on natural regeneration of existing plants for propagation (Faturoti *et al*., 2002). These seedlings are likely to carry pests and diseases leading to reduced production. Tissue Culture (TC) is one of the available propagation methods that produce seedlings free from diseases and pests, with genetic purity and uniform growth (Sheela and Ramachandran, 2001). However, adoption has been low due to high capital investments and subsequent high cost of seedlings. This has led to the plantlets being too expensive for majority of the small holders to acquire. Macropropagation technique can be used as a cost effective method to produce a large number of high quality seedlings. In Kenya, it has not yet been evaluated whether macropropagation technique can be used to produce seedlings that are free of pests and diseases, considering the distribution of pests and pathogens in different localities and the key local varieties that are widely grown by farmers. This study was therefore aimed at determining the effectiveness of macropropagation technique to produce healthy banana seedlings in Eastern and Central regions of Kenya.

### 1.2 Statement of the problem

Bananas play a major role in the diets of the people and the economy of Kenya. It is an important food crop providing carbohydrates, vitamins and a source of income for the majority of smallholder growers (Seshu Reddy *et al*., 1999). In the period between 1970 and early 1990's banana production in the Sub-saharan region declined quite significantly below its potential (Karamura, 1998; Viljoen, 2010). The decline was attributed mainly to an increase in the prevalence of pests and diseases combined with lack of effective control strategies (Kahangi *et al*., 2002). The major diseases have been Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*); Black and Yellow sigatoka leaf spot caused by *Mycosphaerella fijiensis* and *Mycosphaerella musicola* respectively, weevils

(*Cosmopolites sordidus*) and plant parasitic nematodes. Other constraints include declining soil fertility, poor crop management, and lack of clean planting material, poor marketing infrastructure, post harvest losses, genetic erosion and high cost of inputs (Seshu Reddy *et al*., 1999). The various constraints threaten food security and are associated with increased poverty due to the reduced yields.

The area under banana and plantain cultivation in Kenya was 115,500 ha in 1989 and increased to 125,000 ha in 1997 with a corresponding production of 520,000 and 595,000 metric tons, respectively. This resulted in the production output being between 4.5 to 4.8 tons/ha which was quite low (Wabule, 1998). Banana production rose to 620,000 metric tons in 2006 (FAOSTAT, 2008) and was attributed to the introduction and adoption of tissue cultured seedlings. Indeed great potential could be realized if more farmers adopted banana farming and followed proper practices to ensure maximum yield.

Across Africa, commercial banana production has been hindered by scarcity of high quality seedlings (Nkendah and Akyeapong, 2003). Most farmers rely on natural regeneration to obtain seedlings for expanding or establishing new farms which is a slow process and often does not yield adequate amounts (Farouti *et al*., 2002). These suckers are also associated with higher risks of pests and disease spread between farms or regions (Mwangi and Muthoni, 2008) leading to reduced productivity and short life time of new plantations. Although tissue culture is efficient in producing large numbers of high quality seedlings, its adoption is constrained by high capital and skills required leading to high cost of seedlings, hence not affordable to most farmers. Somaclonal variations which arise from repeated subculturing of tissue cultured banana plants also lead to low adoption of the technology (Sahijram *et al*., 2003).

### 1.3 Justification of the study

Banana production in Kenya can be improved by increasing availability of affordable clean planting material. Macropropagation technique increases seedling availability and the propagation method is farmer friendly (Sengendo *et al*., 2006). It relies on simple cost effective methodology that could be easily implemented with good training and fewer resources than are required with other propagation methods. The nurseries can be located near the farmers thus eliminating transport costs and reducing cost of seedlings significantly.

This research was therefore aimed at determining the effectiveness of macropropagation protocol in producing healthy banana seedlings.

### 1.4 Research Questions

i. What is the current status of pests and diseases constraining banana production in Eastern and Central regions of Kenya?

ii. Does macropropagation eliminate pests and diseases to produce healthy banana seedlings considering variations in agro-ecologies and varieties?

iii. Does treating corms in hot water, pesticides or curing improve quality of macropropagated banana seedlings?

## 1.5 Hypotheses

i. Pests and diseases are the most important constrains to banana production in Eastern and Central regions of Kenya.

ii. Macropropagation eliminates pests and diseases to produce healthy banana seedlings regardless of agro-ecology or banana variety.

iii. Treatment of corms in hot water, pesticides or curing enhances quality of macropropagated banana seedlings.

## 1.6 Objectives

### 1.6.1 General Objective

To determine the efficiency of macropropagation technique in producing healthy banana seedlings.

### 1.6.2 Specific Objectives.

i. To determine the important current pests and diseases constraining banana production in Eastern and Central Regions of Kenya.

ii. To assess whether macropropagation method produces healthy seedlings comparing agroecologies and varieties.

iii. To evaluate the effect of corm treatment with hot water, pesticides and curing on quality a of macropropagated banana seedlings.

## 1.7 Significance of the study

The study established the current important banana pests and diseases in Eastern and Central regions of Kenya. The key pathogens and pests were determined and factors favoring their spread identified for developing more effective Integrated

Pest Management (IPM) strategies. Efficiency of macropropagation technology to generate healthy banana seedlings was investigated and demonstrated to farmers. Recommendations have been made to improve the macropropagation protocol to ensure production of high quality seedlings and hence revamp banana production. Recommendations from the study shall contribute to sustainable banana production in Kenya and other countries of similar socioeconomic status.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 The banana plant, its importance and production

Banana is a major food crop globally and is grown and consumed in more than 100 countries throughout the tropics and sub-tropics (INIBAP, 2000). Many small scale farmers grow the fruit for food, fodder and income generation in local and regional markets. In developing countries, banana is the fourth most important food crop after rice, wheat and maize (INIBAP, 2000). The highest consumption globally is in Uganda, estimated at 1 kg per person per day (Edmeades *et al*., 2007). It fruits all year round thus ensuring food security. Intercropping can be carried out to increase land use efficiency of smallholder farms and contributes to a balanced diet for producers.

The banana is a tall 2-9 m perennial monocotyledon (INIBAP, 2000). The whole trunk is a pseudo-stem and consists of concentric layers of leaf sheaths rolled into a cylinder of 20 - 50 cm diameter (Pillay and Tripathi, 2007). The true stem is a large underground corm, which produces aerial shoots that arise from the lateral buds, which develop into eyes and later suckers. The continuous vegetative growth of suckers perpetuates the corm's life and hence the perennial status of bananas. Bananas have adventitious roots which extend laterally to 5m around the plant. They are predominantly clumped in a stool or a mat composed of the parent plant, its suckers and some followers. Flowering usually begins around 7 - 9 months after planting, depending on climatic conditions and type of soil (Augstburger *et al*., 2001). At emergence the inflorescence is usually erect but points downward due to its weight (Robinson, 1996). Development of the bunch takes 3 - 4 months depending on the weather. After full formation of the bunch, the parent plant dies off. Edible bananas do not have seeds but seed formation is still visible as blackened bands at the centre (Augstburger *et al*., 2001).

Bananas grow well in soil rich in organic material with good drainage and a pH range of 5.6 - 7.5. They also require considerable amounts of nitrogen (N) and potassium (K) to maintain high yields. The temperature required for growth is 13 - 38° C with the optimal being 27° C and annual mean rainfall of 1500 - 2500 mm. Banana production systems vary from backyard garden systems, subsistence systems and commercial plantation systems with the subsistence system being the most common in the tropics (Karamura *et al*., 1998). Plantation systems have high levels of management and reduced pest and disease incidence.

Bananas can be processed into flour and can be fermented for production of vinegar, wine and beer (Pillay and Tripathi, 2007). The trunk and leaves can be fed to livestock. Banana leaves have a variety of uses. They can be used to wrap food, polish floors, as thatch and are also a good material for mulch. Fibers from the pseudostem and leaves are used for making cloth and ropes (Nelson *et al*., 2006). Bananas have been found to have medicinal properties (INIBAP, 2003). The root sap can be used to treat mouth thrush in children and skin warts and banana peel has been found to have antibiotic properties (Nelson *et al*., 2006). Cultural events such as births, marriage, deaths and other rituals and ceremonies are associated with specific banana varieties by some communities in Africa.

Banana plantations are established on land that has been cleared manually or with herbicides. The recommended spacing is 3m by 3m within and between rows. On sloping land the rows follow contours and terracing can be done to prevent soil erosion (Tushemereirwe *et al*., 2001). Weeding is done to prevent competition and mulching to preserve moisture. Old dry leaves and surplus shoots are removed periodically from the plant. Banana varieties with tall trunks should be propped using a forked stick to prevent the plant from falling due to the weight of the bunch. Bunches are harvested and transported carefully to prevent post harvest diseases (Augstburger *et al*., 2001).

## 2.2 Banana production constraints

Banana production in Kenya has not reached its full potential. The average yields are less than 10 tons per hectare compared to potential yield of up to 60 tons per hectare (Karembu, 2002). This is mostly due to pests and diseases that have increased considerably; leading to reduced life span of plantations from 50 up to 4 years. Scarcity of clean planting material has contributed to disease spread due to farmers using infected suckers. There are no formal standards to regulate banana planting material (Macharia *et al*., 2010) with many farmers using naturally regenerated suckers due to their availability and low cost. In addition, there are limited seedling production facilities that provide quality banana seedlings. Thus there is need for farmers to have access to certified, healthy and affordable planting material. In addition, rising population pressure on land has led to shortened fallow periods leading to decline in soil fertility. Limited opportunity for rotation also has led to planting in infected soil thus further compounding the problems. Low levels of farm inputs, lack of credit facilities and poor management due to lack of information by farmers result to poor yields. Other constraints include; poor marketing infrastructure, perishability, postharvest losses, competition with other crops for land, lack of labour and capital, genetic erosion and drought (Tushemereirwe *et al*., 2001).

### 2.2.1 Pests affecting banana plantations

A number of pests infest banana plantations causing damage and significant yield loss. Plant parasitic nematodes (PPN) puncture cells using a hollow stylet in their mouth cavity causing root necrosis, root rot and breakage and reduce root growth in a variety of crops. The degree of damage and loss in yield of banana varies depending on the nematode species involved, interaction between nematodes and other soil pathogens, the susceptibility of the cultivar and the climatic environment in which it is grown (Sarah, 1989; Davide, 1996). The burrowing nematode (*Radopholus similis*) (Cobb, 1893 and Thorne, 1949) is the most damaging nematode in banana, especially on Cavendish types (Sarah, 2000), that are mostly produced for export markets. *Radopholus similis* occur in most banana growing regions of the world (Gowen *et al*., 2005). It is an endoparasite, completing its entire life cycle within the root and causing necrosis and decay of the whole root cortex. The pest is a common cause of banana plants falling over, a condition known as "toppling disease" and can cause losses of 30 to 80%. Other important PPN of banana include *Pratylenchus goodeyi, Pratylenchus coffeae, Helicotylenchus multicinctus* and *Meloidogyne* spp (Gaidashova *et al*., 2008). Vegetative propagation using infested corms or suckers has disseminated the pest throughout the world. Nematodes can be managed through crop rotation, mulching, organic amendments, nematicide application and use of clean planting material.

The banana weevil, (*Cosmopolites sordidus* Germar) is the most important pest of banana and plantain throughout the tropics (Gold, 1998; Gold *et al*., 2001). The weevil has a narrow host range and attacks only plants in the genera *Musa* and *Ensete*. It has four main stages; the egg, larvae, pupae and adult. Banana weevils oviposit in the base of the plant (Abera *et al*., 2000) where the eggs hatch into larvae within 5 and 8 days. The larvae pass through 5 or 6 instars lasting between 30 and 50 days depending on temperature. They feed actively and develop into pupa which lasts 5 – 9 days. Adults are slow, nocturnal and negatively phototrophic. Damage to banana is caused by larvae feeding within the corm and pseudostem, causing galleries that weaken the plant and provide entry points for other pests and pathogens. Weevils also impede water and nutrient uptake (Gold *et al*., 2001).

Damage eventually leads to plant toppling after snapping at the base. The affected plants have retarded and stunted growth, leaf drop, little or no roots, reduced bunch size and reduction in the number and vigor of suckers (Mwangi, 2007). Weevil attack in newly planted banana stands can lead to poor crop establishment (McIntyre *et al*., 2002). Besides, banana weevil damage can reduce yield and heavy infestation is likely to result in crop failure in newly planted fields. They can be controlled by trapping, mulching, intercropping and use of entomopathogens (Gold *et al*., 1998).

Other banana pests include aphids (*Pentalonia nigronervosa*), moth (*Opogana sacchari*), mealy bugs, white grubs and thrips. These affect plants directly by sucking sap and indirectly as vectors of diseases. They are controlled by chemical means (Carlier *et al*., 2000).

### 2.2.2 Diseases of banana

Fusarium wilt (panama disease) is the most destructive disease of bananas in the world (Ploetz, 1997). Dessert banana varieties Gros Michel (AAA) and Bluggoe (ABB) are most susceptible. The causal organism is *Fusarium oxysporum* schlecht f. sp. *cubense* (*Foc*). It has different races known to cause panama disease in banana and these differ in ability to infect different varieties. The pathogen has been characterized into pathogenic races, 1 - 4. Race 1 is pathogenic to cultivars in the AAB ‘Silk’ and ‘Pome’ subgroups and on AAA ‘Gros Michel’. Race 2 is pathogenic to ABB ‘Bluggoe’ and other closely related cooking bananas. Race 3 has been recorded in Honduras, Costa Rica and Australia on *Heliconia* species and has little to no effect on banana (INIBAP, 2009). Race 4 attacks Cavendish (AAA) types such as Williams, Grand Nain and Dwarf Cavendish which are of major importance to the international export trade while Gros Michel and sweet banana cultivars are attacked by races 1 and 2. Fusarium races 1and 2 have been confirmed to be present in Kenya (Ploetz, 1993). Race 4 is generally confined to subtropical areas and is problematic in South Africa and the Canary Islands (Ploetz, 1990) leading to many farmers in the region abandoning banana cultivation. This poses a threat with likelihood of the disease spreading to East Africa from imports. *Foc* survives in the soil and on plant debris from where it enters the roots of the banana plant often through bruises (Ploetz, 1993). It spreads through the tissue of the plant upward throughout the pseudostem and the disease can spread very quickly throughout the entire stool. Suckers may look healthy even when they are infected depending on strain virulence and environmental conditions. The main characteristic symptom of the disease is brown-reddish discoloration of the internal vessels of the pseudostem (Ploetz and Pegg, 2000). The leaves exhibit chlorosis progressing from the oldest to the youngest leaves. Leaves gradually collapse at the petiole and commonly towards the base of the midrib and hang down to form a “skirt” of dead leaves around the pseudostem. The youngest leaves are the last to show symptoms and often stand unusually erect giving the plant a “spiky” appearance (Daly and Walduck, 2006). Growth does not cease in an infected plant and leaves which emerge are usually paler in appearance than those of the healthy plant. The lamina of emerging leaves may be markedly reduced and exhibit wrinkling and distortion. Longitudinal splits may also develop in the pseudostem. A susceptible banana plant infected with *Foc* rarely recovers. However, poor growth of the clump may continue and infected suckers may be produced before the clump finally dies. No disease symptoms have been observed in the fruit. *Foc* can be spread through infected planting material and contaminated farming tools. The fungus can survive in soil for long as chlamydospores in infested plant debris or in the roots of alternative hosts.The disease can be managed through use of resistant cultivars, fumigation of soil, sanitation and use of clean planting material (Seshu Reddy *et al*., 1999).

The banana Xanthomonas wilt (BXW) is caused by the bacterium *Xanthomonas vasicola* pv. *musacearum* (Valentine *et al*., 2006) and the disease endangers the livelihoods of millions of banana farmers in East Africa. This is due to its ability to spread rapidly causing death of entire banana plantations. It was first reported in Ethiopia on a close relative of banana, *Enset ventricosum* in 1968 (Yirgou and Bradbury, 1968). The disease has now spread to Rwanda, Democratic Republic of Congo, Tanzania, Kenya, and Burundi (Mwangi *et al*., 2007). It affects almost all commonly grown banana cultivars except Cavendish varieties that have persistent neutral flowers. However, the pathogen can also be spread through tools. Symptoms are characterized by a progressive yellowing and wilting of leaves, uneven and premature ripening and rotting of fruit, wilting and shriveling of the male bud (Tushemereirwe *et al*., 2004). Cross sections of the pseudostem show yellowish bacterial ooze. All banana cultivars grown in East Africa are susceptible to BXW and farmers usually experience total crop loss when plantations are attacked (Eden-Green, 2005). Disease spread differs between the mid and high altitude agroecological regions. In mid altitudes, the spread has largely been attributed to insect vectors carrying *Xanthomonas* from infected to healthy plants (Tinzaara *et al*., 2006). Insect vectors are of highest importance in mid-altitude agroecological conditions (1100 - 1600 m above sea level), where vectors are more active and abundant (Mwangi *et al*., 2006). In the high altitudes it has been postulated that insect vectors play a reduced role in disease spread, possibly due to the lower temperatures, or lower populations (Ndungo *et al*., 2005). Disease spread is also facilitated by planting infected plants in areas that do not have the disease and failure to disinfect cutting tools. In addition movement of infected plant parts such as pseodostems and banana leaves contribute to disease spread. There are no curative measures for BXW and control measures aim at reducing disease spread (Eden-Green, 2004). The disease can be managed by using clean planting material, restriction of movement and destruction of diseased material, host resistance and genetic engineering. The disease affects banana populations quite rapidly, such that by the time the farmer begins to respond, it may be too late (Tripathi *et al*., 2009).

Black leaf sigatoka caused by a fungus *Mycosphaerella fijiensis*, is the most serious leaf spot disease in banana (Crous and Mourichon, 2002). The disease causes reduced photosynthetic capacity of the leaves eventually resulting to premature drying of leaves which in turn causes incomplete filling of banana fingers leading to yield losses of 30 – 50% (Shotkoski *et al*., 2010). Bananas are most susceptible during the wet season. The fungus is controlled by planting resistant hybrids such as FHIA 17 and 23 (Tushemereirwe *et al*., 2001).

Other diseases include cigar end rot and corm rot. Corm rot is caused by the fungus *Armillaria* spp. and affects plants that are grown on recently cleared forest land. Spread of the disease can be reduced by uprooting and burning infected plants. Cigar end rot gives bananas an ashy appearance at the tip of the fruit. It is air-borne and caused by the fungus, *Verticillium theobromae*. The disease is favoured by high humidity with incidence being highest during the rainy season. It is common in the dwarf Cavendish banana, and incidence is high in Western Kenya and Kisii (BIOVISION, 2008). The pathogen colonizes banana leaf trash and flowers, from where spores are disseminated in air currents to other drying flower parts. Old and badly maintained plantations suffer most damage. The disease incidence can be reduced by removal of flower remains (Tushemereirwe *et al*., 2001) and washing fruits in disinfectant before packaging.

## 2.3 Propagation of banana

Bananas are majorly parthenocarpic (produces fruit without fertilization) due to their triploid nature and have to be propagated through vegetative means. They are propagated traditionally by planting naturally regenerated suckers and also use of seedlings propagated through TC and macropropagation.

### 2.3.1 Natural regeneration

Natural regeneration is where farmers select planting material from existing plantations to start or expand plantations. Farmers make decisions about the cultivar, age and size of the sucker (Staver *et al*., 2010). This method is affordable because obtaining the suckers is relatively easy. It is used widely but has a risk of spreading diseases and pests, though disease spread can be reduced through treatment of suckers. In addition, the seedlings do not have uniformity, the number of seedlings produced is low and a high possibility exists of farmers not being sure of the variety (Sheela and Ramachandran, 2001). These factors can lead to inconsistency in yield.

### 2.3.2 Tissue Culture

Tissue Culture is based on the ability of a plant species to regenerate a whole plant from a shoot tip. It entails using tiny shoot-tips which are dissected into small pieces. They are then placed in a growth medium containing MS basal salts, (Murashige and Skoog, 1962) glucose and other nutrients. A dosage of growth hormones or regulators is added into the medium at different stages to enhance various processes of growth such as shoot initiation, multiple shoots' formation and rooting induction at the final stage. The technology results to rapid production of seedlings in large quantities that are disease free, uniform and of assured genetic purity (Singh *et al*., 2011). However, adoption of tissue culture in Kenya is low due to high costs of seedlings that are associated with high initial investments and the advanced skills needed for implementation. The seedlings also require additional care and improved management which is expensive to farmers (Qaim, 1999). This includes spraying the seedlings with micronutrients (Singh *et al*., 2011) to ensure better establishment in the field. Extra manure and fertilizer are also needed for proper growth.

### 2.3.3 Macropropagation

Macropropagation is a cost effective seedling production technology that was introduced in Africa to address the gap in availability of affordable healthy planting bananas. Banana corms contain several axillary buds with meristems at different stages of development. In macropropagation, apical dominance is suppressed mechanically to stimulate lateral bud development and increase suckering rate (Farouti *et al*., 2002). This leads to high quality and affordable banana seedlings. The technique is inexpensive and materials for constructing the growth chambers can be sourced locally. The seedlings can be selected to have uniform size and they tolerate post establishment stress better than tissue cultured plants (Tenkouano *et al*., 2006). The pioneering trials and validation of the macropropagation technology have been done largely in Cameroon and Nigeria, and recently spread to other West African countries including Ghana and Ivory Coast, and to Uganda, Rwanda and Tanzania in East Africa (Lefranc *et al*., 2010). Macropropagation relies on simple cost effective methodology that could be easily implemented with good training and few resources. In 2007 - 2008, FaCT Limited (a private company) implemented the macropropagation technology on a pilot basis in Kenya. Initial activities were focused on assessing the potential and challenges to implementation as well as to gauge market response (Mwangi and Muthoni, 2008). That research found that uptake of the seedlings was high in all the locations where they were introduced due to the low cost. Farmers had a variation in the preference of varieties and majority of women preferred cooking varieties. However TC seedling entrepreneurs mostly produce desert bananas thus macropropagation can address the need for seedlings of cooking varieties. It was also found that macropropagation has potential to increase access to affordable high quality seedlings (Mwangi and Muthoni, 2008). Therefore research is needed to assess the effectiveness of the technology in producing healthy seedlings to enhance its adoption by farmers.

### 2.3.4 Seed regulation

In Kenya, there is a framework for regulating the seed industry with a committee whose mandate is to develop seed policy and seed certification standards. The Seed and Plant Variety Act 1972, gives seed regulations guidelines. Primary considerations for planting materials is that they should be free from diseases, originate from plants with superior production, resistance to pests and pathogens and with good quality traits (FAO, 2010). The regulations for banana seedlings require that they should also have uniformity and the suckers or corms used should be at least 12cm diameter. If they exhibit symptoms of disease they should be discarded (FAO, 2010). Seedlings produced should be assessed for quality by looking at several traits. Plantlets should be 20cm long with the older leaves being larger than the younger ones, with 1% tolerance to plantlets that do not meet this criterion. Occurrence of off types such as dwarfism, gigantism, mosaic-like, variegated, chlorotic/necrotic leaf patches, droopy leaves have tolerance criteria of 1%. Damage of the container or the substrate should be tolerated up to 2% (FAO, 2010). Banana seedlings should comply with these guidelines.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Study sites

This study was conducted in high altitude (Lower highland - LH), mid altitude (Upper Midland - UM) and low altitude (Lower Midland - LM) agro-ecological zones in Eastern and Central Kenya. In Central region, the study locations were Mathioya (LH 1) in Murang'a County, Kerugoya (UM 3) in Kirinyaga County and Kenyatta University (UM 2) in Kiambu County (Appendix 1a, Appendix 2). The climate in Central Region is generally cool, due to the higher altitude, 1750 to 2400 m above sea level. Rainfall is fairly reliable, falling in two seasons, long rains from early March to May and short rains during October and November ranging from 400mm – 2700mm annually (Jaetzold, 2006). The temperature varies with altitude ranging between 26 to 30 º C in the lower area and between 10 to 18 º C in the upper areas. These areas are good for farming and have great agricultural potential (Imbernon 1997; Ondieki 1999).

In the Eastern Kenya region, the study was carried out in Mitunguu (LM 3), Embu East (LM 3), Ntharene (UM 3) and Meru Central (UM 1) (Appendix 1b, Appendix 2). Eastern is the most diverse and complex topographic region in Kenya. It extends between 38°15'E and 39°30'E and 1° N and 3° S. It contains many agro-ecological zones and subzones. Farming activities in the southern arable parts of the region are strongly influenced by altitude. Below 550 m, it is normally too dry while above 2200 m on the windward side it is too cold and wet. On the leeward side, cultivation may go up to 2800 m, as is the case on the northern slopes of Mt. Kenya. In some parts of this region, the soils have lost fertility; droughts are frequent, leading to crop failures. The fertility of the soils in climatically favorable volcanic areas is decreasing rapidly because of recurrent permanent use over many years with almost no recycling of nutrients back to the soils (Jaetzold, 2006).

### 3.2 Determination of important pests and diseases

A survey was carried out in Mathioya, Murang'a, Kirinyaga, Gichugu, Embu, Meru Central and Imenti South districts in Eastern and Central regions of Kenya in July 2010. The purpose was to confirm the important pests and pathogens and other key banana production constraints. A sample of 84 farms was selected in the study areas. The sample size for each region was arrived at using the following formula,

$$n = NC^2 \div C^2 + (N- 1)\, e^2$$

Where, n = Sample size, N= Population size, C= Coefficient of variation which is $\leq$ 30% and e = Margin of error which is fixed between 2-5% (Nassiuma 2000). District extension officers were contacted to help in identifying survey areas and farmers. Farms surveyed were selected randomly with a distance of 3–5 Km apart. Required information was obtained from District Agricultural Officers (D.A.O.'s) and farmers using a questionnaire (Appendix 3). Further information was obtained through direct observation during a transect walk through each farm that was surveyed. Disease incidences and severity levels were recorded. A photo card (Appendix 4) was used to aid the farmers in description of the diseases and pests. Banana plant parts showing symptoms of infection or infestation were collected and taken to laboratory to isolate and identify pests and pathogens. Farm management in terms of agronomic practices such as weeding, sanitation and manure application among others was rated at a scale of 1-5 with 1 indicating poor management and 5 indicating good management.

## 3.3 Production of banana seedlings using macropropagation technique

### 3.3.1 Establishment of macropropagation nurseries

Nurseries were established at the different sites in Mathioya, Kerugoya, Embu East, Imenti South, Meru Central and Mitunguu. These were selected principally on the basis of agroecological zones and preferred banana cultivars. The nurseries were constructed using locally sourced wooden poles treated with preservative and polythene sheets (1000 cc gauge). The roof was covered using black polythene and the sides using clear polythene to provide 50% shade. The propagation compartments were 0.5 m high and completely covered with transparent polythene sheets to ensure high humidity and temperature. The propagation beds were filled with steam sterilized sawdust as the propagation medium.

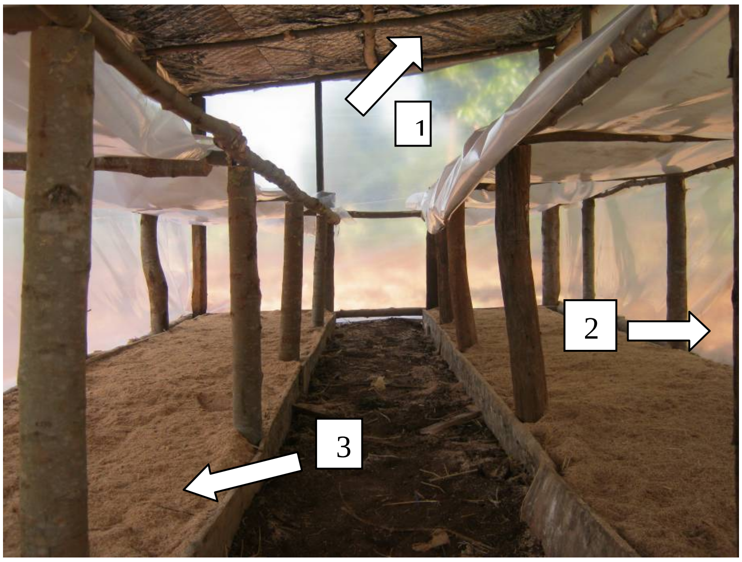



Fig 3.1: Macropropagation chamber. (1- Roof covered with black polythene *2*- Sides covered with clear polythene. 3- Propagation beds filled with steam sterilized sawdust).

### 3.3.2 Macropropagation process

Macropropagation technology was assessed in two cycles. The first cycle was done between February – June 2011, and the second from July – November 2011. Corms of farmer preferred varieties which were Cavendish, Gros Michel, Sweet banana and Uganda green were procured from mature orchards using a farm certification protocol (Njukwe *et al*., 2007). Healthy maiden and sword suckers that were about to flower were selected for macropropagation from visibly suitable mother plants. Corms of recently harvested banana plants that have good yielding characteristics were also selected. The pseudostem was cut off from the suckers and the corms were pared to remove the roots using a sharp knife. The process of macropropagation (Fig 3.2) was carried out systematically using sterilized tools. Farms were inspected to certify freedom from pests and diseases. This was followed by a thorough wash to remove soil and debris. The buds were exposed by removing the sheaths of the pseudostem serially one by one and trimmed 2mm above the corm and from the leaf base. The buds were scarified by making an X shaped incision then disinfected using 10% jik® (Sodium hypochlorite) (Nelson *et al.*, 2006). The corms were placed in steam sterilized sawdust in the humidity chamber (propagation beds) spaced 30 cm apart and fully covered with sawdust. The propagation beds were well watered immediately after planting and subsequently after every 2 days. Care was taken to avoid water logging. The plantlets were allowed to grow and give rise to many new shoots by further scarification. These plantlets were detached from the corm using sterile sharp knives upon attaining a height of 15 cm. They were potted in labeled plastic bags containing steam sterilized soil mixed with manure in the ratio of 3:1. They were placed in a hardening nursery.

1. Selection of corms from healthy plantations.

2. Corms ready for macropropagation.

3. Pared corm

4. Serial removal of the pseudostem sheaths


5. Disinfecting the cutting tools

6. Corms ready for planting

7. Corms in sterile sawdust.

8. Seedlings sprouting from the corms after and ready for transplanting.

Fig 3.2: The process of macropropagation.

### 3.4 Isolation of pests and pathogens from banana corms

Samples were acquired from corms after preparation for macropropagation so as to isolate and identify any pests and pathogens present. Presence of bacterial and fungal microorganisms was detected by culturing the sampled plant tissues on Nutrient Agar (NA), and Potato Dextrose Agar (PDA), respectively. The samples from corms were washed and cut into 2 - 3 $mm^3$ size pieces. They were surface sterilized with 70% ethanol and rinsed with two changes of sterile distilled water. Micro-organisms isolated from each corm were cultured, identified and preserved separately. Identification up to genera level relied on morphological characteristics, such as the shape and size of conidia and appressoria, appearance of colony in culture and presence or absence of setae and sclerotia. After removing samples for culturing, each corm was placed within the macropropagation medium in the nursery, with its position well marked to enable tracking and association of corm health to the eventual health of the suckers it produced.

Roots from the corms used for macropropagation were collected and nematode damage assessed according to Speijer and Gold, (1996). From each study site, five functional roots were selected randomly from 10 corms of each variety after paring. Their lengths were reduced to approximately 10 cm and split longitudinally. One half of each of the five roots was scored for the extent of necrosis in the root cortex (Barekye *et al*., 1999). Scoring was done and rated at 0% for no necrosis, less than 20% for slight necrosis, 21 - 50% for moderate necrosis and 51 - 100% for severe necrosis. Nematode extraction from roots was done using the modified maceration and filtration technique, (Hooper *et al.*, 2005), and observed under a compound microscope for identification using major morphological characteristics such as body shape, stylet, oesophagus and vulva position with the aid of keys (Sarah, 1996). The roots were cut into 1cm pieces and macerated using a kitchen blender for 5 seconds. The suspension was poured on a plastic sieve lined with a double layer of paper towel, placed on a plate. Water was added in the tray to ensure the roots were submerged and was left to stand for 24 hours. The suspension in the plate was poured through 100 µm sieve nested over 75 µm and 38 µm sieves consecutively. The suspension in the 38 µm sieve was backwashed using a wash bottle and collected in labeled vial bottles. The solution was left to stand for 2 hrs and decanted. The nematodes obtained were identified up to genera level and counted.

Weevil damage on the corm was scored based on the percentage of the total exposed tissue occupied by the tunnels according to Bridge and Gowen, (1993). The damage was scored at four levels; 0% - no tunnels, 1-10% - slight damage, 11 - 30% - moderate damage and >30% - severe damage.

### 3.5 Pathogenicity of the isolated pests and pathogens

To determine whether the isolated organisms were pathogenic or endophytic, pathogenicity tests were carried out (Photita *et al*., 2004). Five replicates of the fungal isolates obtained from corms were grown on PDA to get pure cultures. Fresh pieces (10 cm long) of healthy banana leaves from the same variety as initially isolated from were surface sterilized 70% ethanol. Each healthy leaf piece was incubated in sterilized plastic boxes, lined with sterilized tissue paper and moistened with sterilized water. Leaves were wounded by puncturing with a sterile needle before inoculation. Mycelial disks from the actively growing edge were obtained using a sterile cork borer and placed on the healthy wounded banana leaves. In the control treatment, leaves were treated using disks of sterile water agar (WA). These were incubated for 1-2 weeks under high humidity at room temperature. The diameter of necrotic lesions that occurred on the leaves as a result of pathogenic isolates was measured. At the end of the trials, tissue pieces of 1-2 mm were cut aseptically from symptomatic leaves, surface sterilized in 1% NaOCl for 1 minute and placed on PDA. Plates were incubated for 1-2 weeks, and colonies subcultured and identified.

Pure cultures of the pathogenic microorganism, *Foc* isolated from corms were made and inoculated onto healthy banana plantlets of three month old seedlings of cultivar Gros Michel. The fungus was inoculated by cutting the roots at a length of 1 cm from the tip and placing 10, 20 and 30, 7mm mycelia discs at the plant roots and then rooted in polybags containing sterile soil medium. In the control water agar was used. The tests were done under green house conditions. The plants were observed for development of infection over a 14-week period. Evaluation was based on the Leaf Symptom Index (LSI) and Rhizome Discoloration Index (RDI) which were recorded and used to get the Disease Severity Index (DSI). Disease incidence was calculated as percentage of plants showing symptoms against the total experimental plants in a treatment. Disease severity was assessed according to Moore *et al*., (1993). Plants were scored for external symptoms on a 1-5 scale with 1 representing a healthy plant and 5 representing a dead plant. The pseudostem and the corm were also assessed for extent of invasion on a scale of 0 – 5 according to Carlier *et al*. (2002).

The burrowing nematode, (*Radopholus spp.*) isolated from roots from Mitunguu (1071 Masl, LM 3) was used for inoculation. The nematodes were homogenised, and counted using a calibrated nematode counter resulting to approximately 10 *Radopholus spp.* per millilitre (ml) of the suspension. Inoculation was done in polythene bags containing sterile soil and sand mixed in the ratio 2:1. Suspensions of 10 ml, 25 ml and 50 ml of PPN were poured near the roots of 4 months old TC banana seedlings of cultivar Grand Nain. Distilled sterile water measured in 10 ml, 25 ml and 50 ml was used as control. The pots were laid on polythene to prevent the roots from overgrowing and coming into contact with unsterilized soil which can result to re-infestation. Data was recorded for the height, basal circumference, number of leaves and fresh and dry weights of shoots and roots of the inoculated plants and the control. These plants were later used for macropropagation to determine whether the technology can eliminate *Radopholus* spp.

### 3.6 Monitoring health of macropropagated seedlings.

Corms under propagation were well watered to keep the humidity chamber moist and monitored for sucker development for up to 12 weeks. Suckers that wilted or withered soon after emergence were removed and taken to the laboratory for identification of the causal agent. Corms rotting within the propagation medium were noted and the cause determined. After 10 weeks, suckers from different corms in each propagation nursery were sampled randomly to check the presence of pathogens and pests. Organisms detected in suckers were compared to those initially isolated from the mother corms. Data on health of suckers, when compared to that of the corms, gave an indication of the effectiveness of macropropagation protocols in preventing pest transmission to seedlings and also identified adjustments required to improve efficiency of the protocol.

### 3.7 Assessing effect of macropropagation using artificially infected banana corms (Infection simulation tests)

Infection simulation tests were done to determine whether the process of macropropagation is able to eliminate *Foc*, plant parasitic nematodes and *Xvm*. Plantlets that had been artificially infested with *Fusarium oxysporum* f. sp. *cubense* (*Foc*) during pathogenicity tests were used. The experiment was set in a complete randomized design (CRD) with 10 replicates per pathogen treatment. For the control, plantlets that had not been inoculated with *Foc* were used. These were subjected to the process of macropropagation and the resulting seedlings were monitored for disease development.

*Xanthomonas vasicola* pv. *musacearum* culture was obtained from the University of Nairobi, and inoculated in 5 month old healthy banana plantlets of cultivar Giant Cavendish. The corms were injured with a sterile needle and 10ml of $10^8$, $10^6$, $10^4$ Colony Forming Units (CFU) per ml of the bacteria was introduced at the point of injury. For the controls, sterile distilled water was used. The plants were incubated for 2 weeks and severity ratings were recorded using the number of leaves. These corms were then used for macropropagation to check whether the technique is able to eliminate the pathogens. The seedlings resulting were monitored for 12 weeks and observed for symptoms of disease development.

Banana plants infested with 250 *Radopholus* spp. during pathogenicity tests were used for the infection simulation test. Macropropagation technique was carried out with different treatments on the corms to assess the most appropriate for improving macropropagation protocol. A complete randomised block design was used with four treatments, replicated five times and twenty plants per plot. The treatments comprised of; paring of roots combined with treating of corms in a nematicide, Ethroprop (MOCAP ®) before planting, paring of roots combined with exposure in the sun for three days at 25 - 30° C to dry the corms and paring of roots combined with hot water treatment for 20 minutes at 55° C (Hauser and Messiga, 2010). For the control, the macropropagation technique was done in the usual way that is paring and disinfecting the corms with no further treatment. Seedlings that emerged were monitored to determine growth rate and assessed for nematode damage.

### 3.8 Data processing and analysis

Descriptive statistics were used to summarize the data from the survey and comparisons were made between the various districts surveyed using SPSS statistical package. Disease severity and incidence levels were recorded for diseases and pests that were observed in the farms. The means of the scores of roots and corms infested with PPN and weevils recorded during the macropropagation process were computed and damage indices were also calculated. The parameters recorded were subjected to Analysis of Variance using GENSTAT 13th Edition statistical package, with regard to variety and location. Those that were significantly different were separated using the Least Significant Difference at ($P \leq 0.05$).

The Area Under Disease Progress Curve (Campbell and Madden, 1990) was calculated for banana plantlets inoculated with Fusarium wilt and BXW using the LSI and RDI. The following formula;

$$A = \sum_{i=1}^{n} [(x_i + x_{i+1})/2]\, t$$

Where; xi =the number of wilted leaves on date i,
n= the number of assessments
ti = the time in weeks between disease assessment xi and xi+1.

Severity ratings and AUDPC ratings were subjected to analysis of variance (ANOVA).

Means that were considered significantly different ($P \leq 0.05$) were compared using Least Significant Difference post ANOVA test.

# CHAPTER FOUR

# RESULTS

## 4.1 Constraints to banana production in Eastern and Central regions of Kenya

### 4.1.1 Diseases and pests of banana

Fusarium wilt, sigatoka, plant parasitic nematodes and the banana weevil were determined to be the major diseases and pests attacking banana in Eastern and Central regions (Fig 4.1). Fusarium wilt was the most serious disease attacking cultivar Gros Michel (Kampala) and Sweet banana varieties, having 66% incidence level of all the surveyed farms. This disease was highest in Kerugoya at 88% incidence with 20% farms having the whole plantation infected, followed by Embu East (74 %), Mitunguu (73 %) Meru Central (67%), Murang'a (57%) and in Mathioya at 42% incidence. Sigatoka leaf spot disease was observed in 50% of the surveyed farms with the incidence levels ranging from 40 - 75%. This disease was noted in all the areas surveyed and it affected all varieties of banana. Cigar end rot disease had a low incidence level of 4% mostly attacking dwarf cavendish variety, while Armirallia corm rot had the lowest incidence level at 2% across all the areas surveyed. Banana bunchy top disease, Banana xanthomonas wilt and Banana streak virus were not observed in any of the farms surveyed.

Symptoms of toppling from plant parasitic nematodes were observed in 21% of the farms surveyed. In addition, the banana weevil infestation was observed in 17% of the farms, but not in Murang'a. Mineral deficiency was observed in 6% of the farms surveyed.

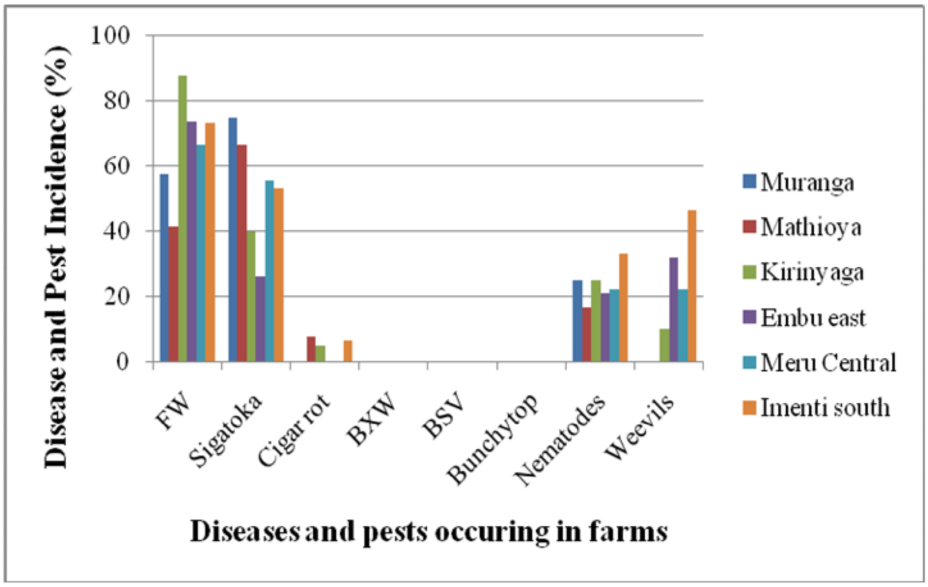



Fig 4.1: Incidence of diseases and pests attacking bananas in Central and Eastern Kenya.

N = 84

Farmers employed different measures to prevent diseases from attacking their plantations. On average these included restriction of entry in the farm 12%, using clean planting material 13%, sanitation measures 50% and applying fertilizer or manure 93% (Fig 4.2). In addition, reporting of outbreaks to extension officers 37%, removing diseased plant parts 43% or removing whole diseased plants 57% and disinfecting tools 14% were adopted to manage any disease noted in the farm (Fig 4.3). There was no significant difference (P=0.300) and (P=0.554) in the proportion of farmers among the different districts restricting entry to their farms and those using clean planting material respectively. Besides, there was no significant difference (P=0.175), in the proportion of farmers disinfecting tools, reporting outbreaks to extension officers (P=0.754) and the farmers practicing sanitation (P=0.342) in the surveyed districts. Moreover there was no significant difference (P=0.628) in the proportion of farmers removing diseased plant parts, using pesticides (P=0.140), applying fertilizer and manure (P=0.090) and those applying amendments (P=0.653) in the districts surveyed. However, there was significant difference (P=0.007) in the proportion of farmers removing diseased plants in Embu East, Meru Central and Imenti South districts as compared to other districts.

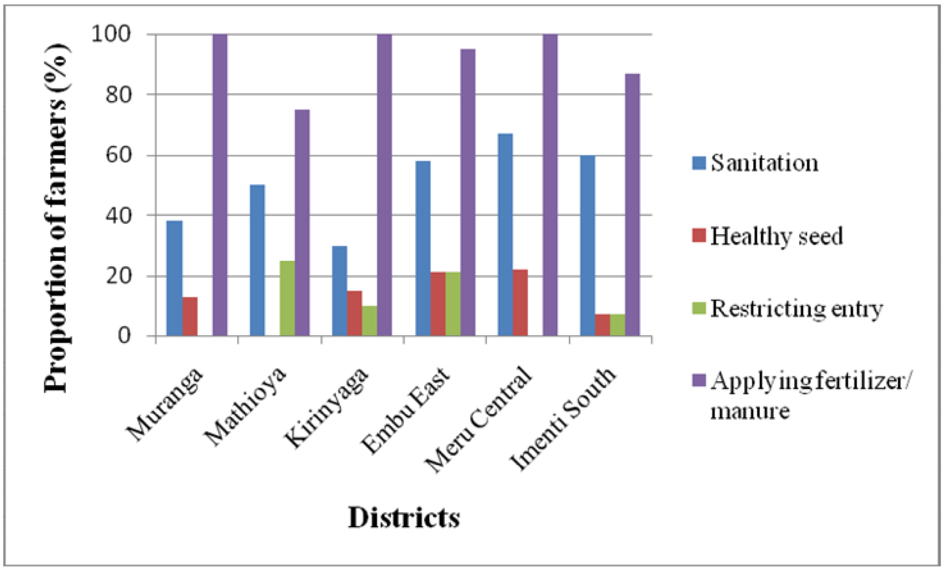



Fig 4.2: Proportion of farmers employing various measures to prevent diseases in banana plantations in the different districts.

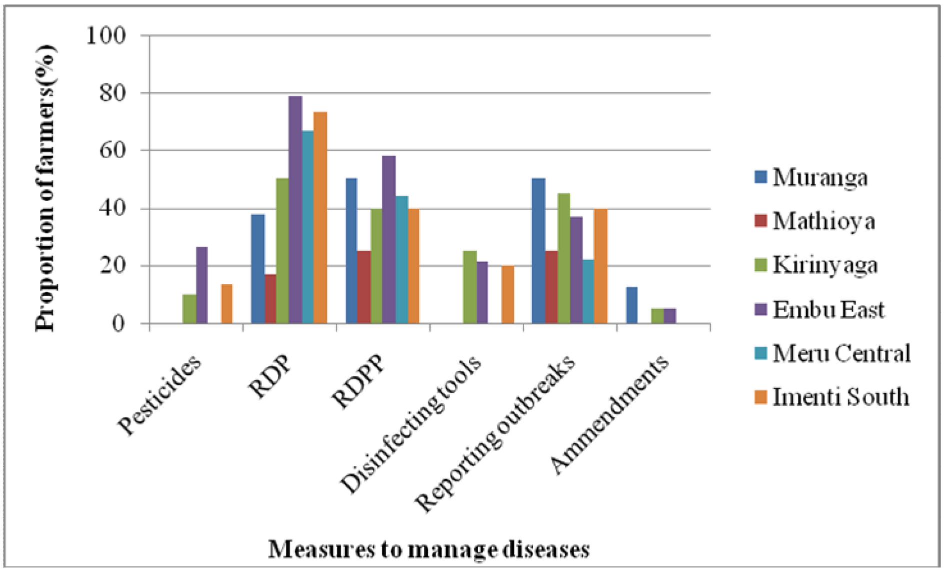



Fig 4.3: Proportion of farmers employing measures to manage diseases in their plantations in the different districts. (Key: RDP = Removing diseased plants; RDPP = Removing diseased plant parts.)

Agronomic practices were carried out by differing numbers of farmers in the districts surveyed (Fig. 4.4). These included, cutting off infected leaves 71%, desuckering 87%, removing the male bud 88%, applying manure 90%, weeding 92% and removing dry leaves 93%. There was no significant difference (P=1.373) in nutrition, sanitation (P=1.449) and general management (P=1.738) in the farm management practices among the different districts surveyed (Fig 4.5).

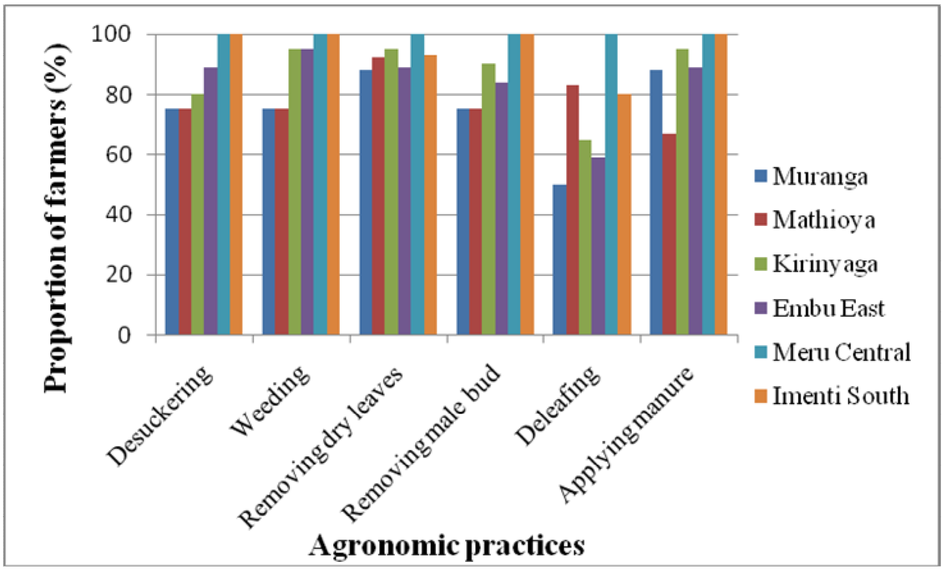



Fig 4.4: Proportion of farmers carrying out agronomic practises in their plantations in the different districts.

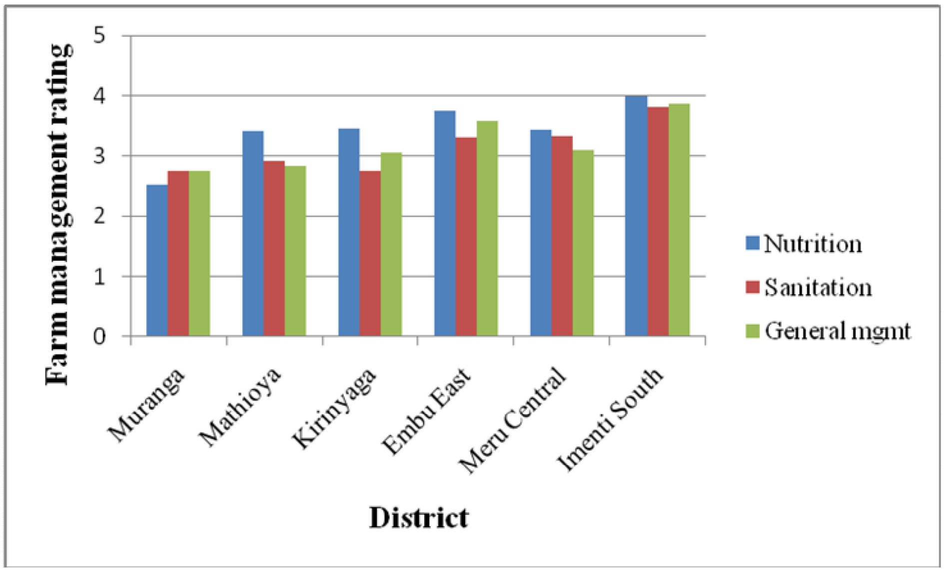



Fig 4.5: Farm management practices in the different distics surveyed.

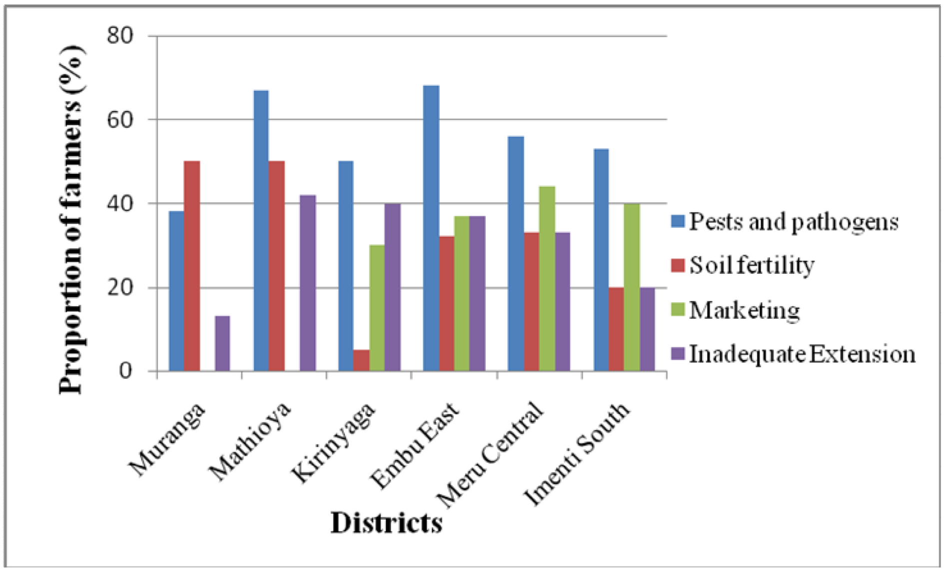



Fig 4.6: Constraints in maintaining banana plantations as perceived by farmers in the different districts.

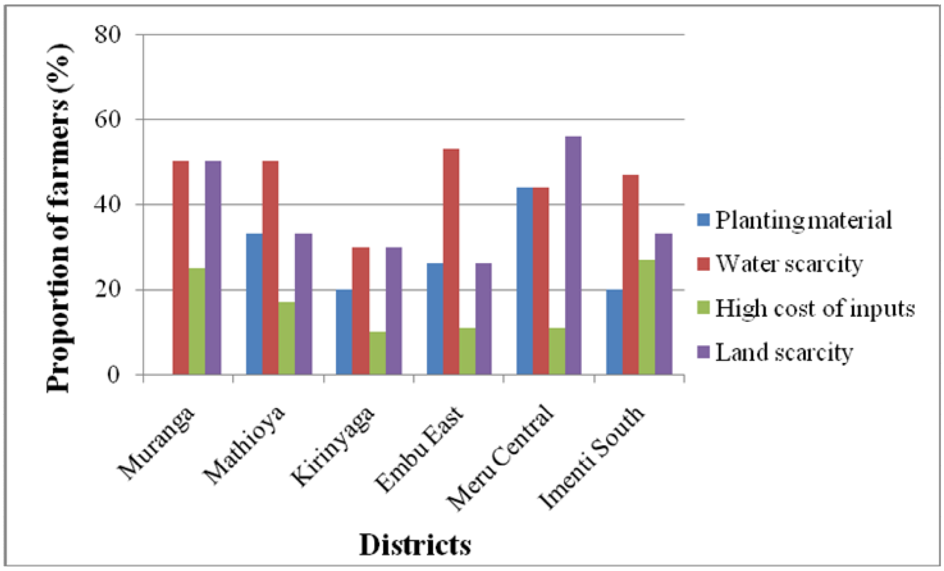



Fig 4.7: Constraints in initiating banana plantations as perceived by farmers.

Major constraints to banana production as perceived by the farmers on average were pests and diseases 57% of farmers, inadequate extension support (33%), soil infertility and marketing (28%) in all the districts surveyed (Fig 4.6). Constraints encountered during initiation of plantations included water scarcity (45%), scarcity of land (35%), scarcity of planting material (24%) and high cost of inputs (16%) (Fig 4.7). Others included lack of information (19%), transport (13%) and lack of pesticides and fertilizer (11%) in the districts surveyed.

### 4.1.2 Source of planting materials

The planting materials used were from natural regeneration and tissue culture. The survey results showed that on average, 92% of the farmers used suckers as the major planting material. Imenti South and Mathioya had 100% of the farmers using sword suckers, followed by Kirinyaga, Meru Central, Muranga, Embu East at 95, 89, 88 and 79%, respectively. An average of 17% of the farmers used tissue culture seedlings for planting, with Embu East having the highest use at 32%. This was followed by farmers in Muranga, Meru Central, Kirinyaga and Imenti South Districts at 25, 22, 15 and 7% respectively. However, none of the farmers were using the corm as planting material. The number of farmers using suckers was highly significant (P=0.000) than those using TC seedlings as planting material in all the districts. However, there was no significant difference (P=0.275) in the number of farmers who were using Tissue Culture seedlings between the districts surveyed. Also, there was no significant difference (P=0.337) in the number of farmers who use suckers between the districts that were surveyed.

The source of planting material was mostly from neighbours (60%) and from their own farms (57%). Of the farmers interviewed, 12% obtained clean planting material from non-governmental organizations such as Africa Harvest Biotech Foundation International and Technoserve. Planting materials were also acquired from research institutions such as Jomo Kenyatta University of Agriculture and Technology by 4.8% of the farmers and 1.2% from Kenya Agricultural Research Institute (Fig 4.8). Out of the farmers who used suckers as planting material, 20% treated them before planting. Paring was done by 1% of the farmers, hot water treatment (1%), use of pesticides (6%) and use of ammendments such as ash and *Tithonia* (12%).

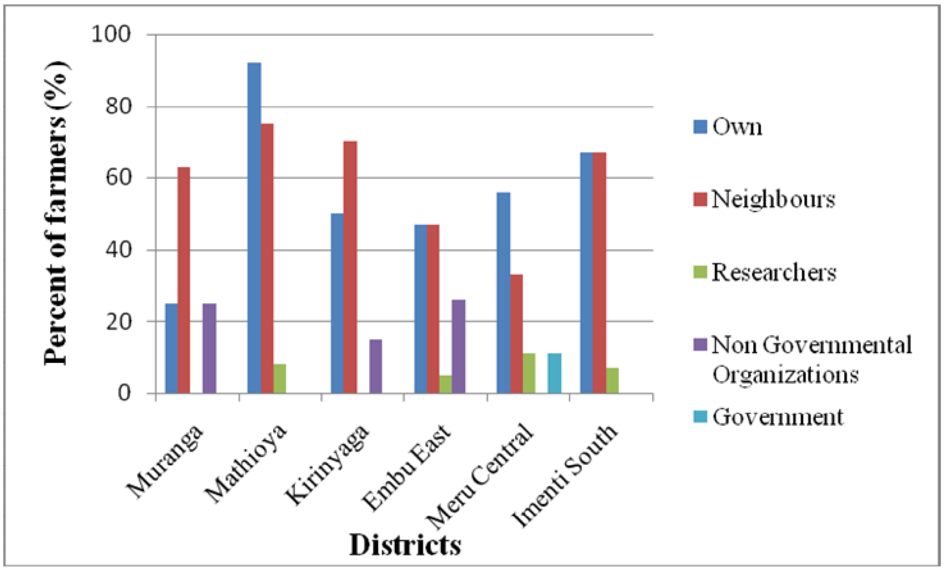



Fig 4.8: Percentage of farmers in the surveyed districts who used planting materials from different sources.

### 4.1.3 Pathogens and pests isolated from symptomatic banana samples collected in farms

The fungal pathogens isolated from samples collected from the field were *Fusarium oxysporum, Mycosphaerella* spp. and *Verticilium* spp. causing cigar end rot. Plant parasitic nematodes that were extracted from roots obtained in plantations that had symptoms of toppling included *Pratelynchus* spp., *Radopholus* spp. and *Helicotylenchus* spp.

## 4.2 Production of banana seedlings through macropropagation technology

### 4.2.1 Certification of farm health status for sourcing corms

In Eastern region, 20% of the farms were not certified as having banana plants that were free from pests and diseases. This was majorly due to weevil infestation causing heavy tunneling in the corm thus rendering majority banana plantations unsuitable for sourcing of corms to use in macropropagation. Weevil infestation was lowest at Mathioya in Central region and highest in Mitunguu in Eastern region (Table 4.1). In addition, some farms were not certified due to poor management practices such as weediness, poor nutrition and low yields. Also, 98% of the farms in Kerugoya that had Gros Michel were infected by Fusarium wilt and therefore corms could not be selected from them.

**Table 4.1: Mean scores ± SE of banana infestation by weevils in the study districts in Eastern and Central Kenya.**

| Location | Cavendish | Gros Michel | Kiganda | Sweet Banana |
|---|---|---|---|---|
| Embu east | 1.9± 0.09$^{ab}$ | 1.70± 0.07$^{a}$ | 2.40± 0.20$^{a}$ | 1.80±0.09$^{a}$ |
| K.U | 1.40± 0.01$^{c}$ | 1.50± 0.06$^{b}$ | 2.10± 0.14$^{ab}$ | 1.60± 0.06$^{b}$ |
| Kerugoya | 1.70± 0.06$^{b}$ | * | 2.30± 0.17$^{a}$ | 1.70±0.06$^{ab}$ |
| Mathioya | 1.50±0.06 $^{c}$ | 1.40± 0.01$^{c}$ | 1.90± 0.09$^{ab}$ | 1.60± 0.06$^{b}$ |
| Meru Central | 1.60± 0.06$^{bc}$ | 1.40± 0.01$^{c}$ | 1.90± 0.09$^{ab}$ | 1.50± 0.06$^{b}$ |
| Mitunguu | 1.90± 0.13$^{ab}$ | 1.70± 0.06$^{a}$ | 2.60± 0.22$^{a}$ | 1.80± 0.10$^{a}$ |
| Ntharene | 2.00± 0.09$^{a}$ | 1.50± 0.06$^{b}$ | 2.40± 0.20$^{a}$ | 1.80± 0.09$^{a}$ |
| P | <0.001 | <0.001 | 0.28 | <0.001 |

Means ± standard error (SE) separated using Fisher's LSD test, means within the column followed by the same letter are not significantly different at P<0.05, * indicates the variety was not present in the study area.

There was a significant difference P≤0.001 between the varieties with regard to weevil attack. The corms of cultivar Kiganda had more tunnels as compared to other varieties and had a greater number of adult and larvae forms of the weevil indicating it was most affected (Fig 4.9) while cultivar Gros Michel was least affected. In addition, there was no significant difference (P=0.28) in the damage caused by weevils in Kiganda variety among the different study areas.

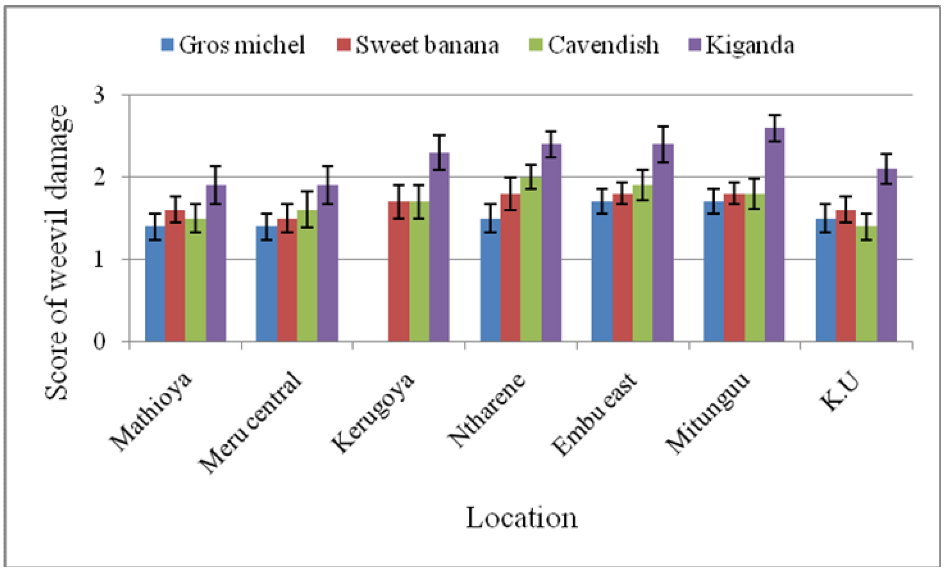



Fig 4.9: Weevil infestation in different banana varieties in the study sites. Overlapping bars are not significantly different at P≤0.05.

### 4.2.2 Severity of nematode damage on banana roots

The plant parasitic nematodes that were extracted from the roots of the banana plants used for macropropagation in the study sites were *Pratylenchus* spp., *Helicotylechus* spp. and *Radopholus* spp. There was a highly significant difference (P<0.001) in infestation of Kiganda and Cavendish varieties from damage assessment through root necrosis across all the districts. Sweet banana variety showed a significant difference (P=0.003) while in Gros Michel variety there was no significant difference (P=0.194) in the study districts (Table 4.2). Cavendish banana variety was most affected by PPN in all the study districts. However, there was no significant difference (P>0.05) between the districts with regard to PPN infestation (Fig 4.10). Gros Michel variety recorded lower levels of infestation by the PPN except in Meru Central. On average, *Helicotylechus* spp was present in

90% of the study sites, while *Pratylenchus* and *Radopholus* spp. were present at 70 and 50%, respectively. The population of *Pratylenchus* was found to be high in Mathioya while in Mitunguu area, *R. similis* population was highest.

**Table 4.2: Mean damage score ± SE of banana varieties infested by plant parasitic nematodes (PPN) in the study districts in Eastern and Central Kenya.**

| | Gros Michel | Sweet Banana | Cavendish | Kiganda |
|---|---|---|---|---|
| Mitunguu | 2.28 ±0.09$^{a}$ | 2.40 ±0.10$^{a}$ | 3.44 ±0.09$^{a}$ | 3.04 ±0.11$^{a}$ |
| Ntharene | 2.14 ±0.09$^{ab}$ | 2.42 ±0.10$^{a}$ | 3.22 ±0.11$^{a}$ | 2.52 ±0.10$^{b}$ |
| Embu East | 2.22 ±0.11$^{a}$ | 2.58 ±0.10$^{a}$ | 2.90 ±0.11$^{bc}$ | 2.78 ±0.09$^{b}$ |
| Kerugoya | 2.08 ±0.10$^{ab}$ | 2.20 ±0.09$^{ab}$ | 2.7 ±0.11$^{c}$ | 2.64 ±0.10$^{b}$ |
| Mathioya | 2.20 ±0.09$^{a}$ | 2.38 ±0.11$^{a}$ | 2.98 ±0.12$^{b}$ | 3.08 ±0.08$^{a}$ |
| Meru Cenral | 2.32 ±0.12$^{a}$ | 2.24 ±0.10$^{ab}$ | 3.22 ±0.11$^{a}$ | 2.72 ±0.12$^{b}$ |
| K.U | 1.98 ±0.07$^{ab}$ | 2.02 ±0.07$^{b}$ | 3.02 ±0.12$^{ab}$ | 3.08 ±0.10$^{a}$ |
| P | 0.194 | 0.003 | <0.001 | <0.001 |

Means ± standard error (SE) separated using Fisher's LSD test, means within the column followed by the same letter are not significantly different at P<0.05

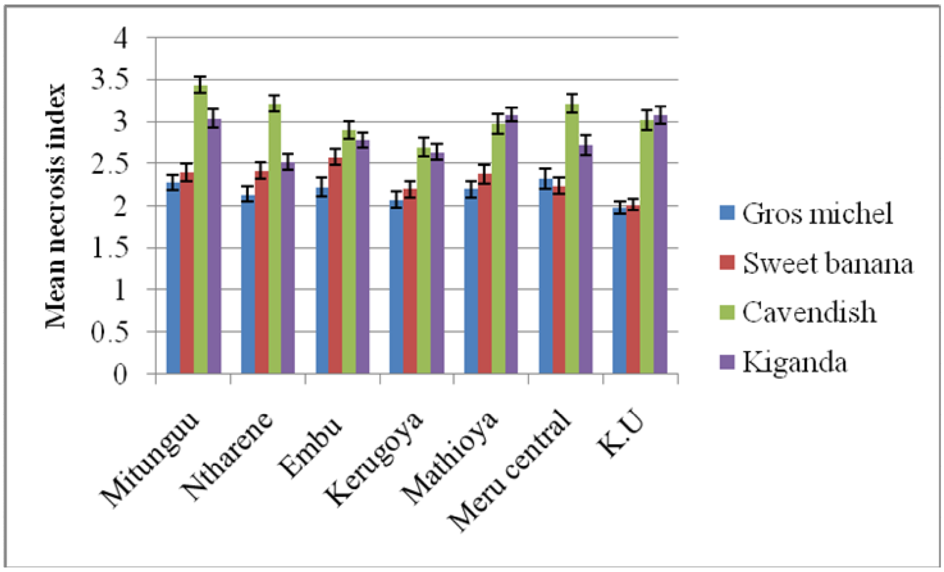



Fig 4.10: Nematode damage on different banana varieties at the seven study sites. Overlapping bars are not significantly different at $P \leq 0.05$.

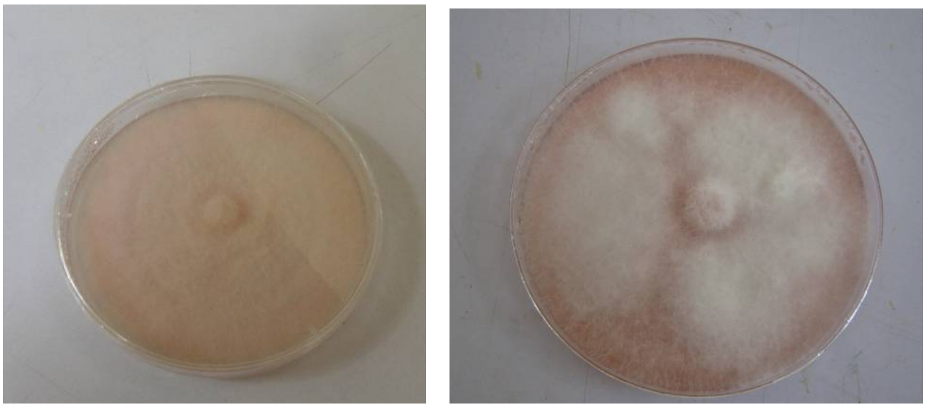
### 4.2.3 Microorganisms isolated from the corms

The microorganisms that were isolated from samples collected from the corms used for macropropagation were mostly non-pathogenic. Endophytes were isolated from over 80% of the corms (Table 4.3). These endophytes included, *Fusarium oxysporum* (Fig 4.11 A), *Penicillium* spp (Fig 4.11 B) *Colletotrichum* spp. (Fig 4.11 C), *Cordana* spp., *Aspergillus* spp. (Fig 4.11 D), *Trichoderma* spp. (Fig 4.11 E). Pathogenic *Fusarium oxysporum* was isolated in less than 1% of the corms. Actinomycetes and endophytic bacteria were also isolated from some corms in less than 10% of the corms in all the study sites. *F. oxysporum* was identified by macroconidia, philiades and chlamydospores. *C. gleosporiodes* was identified by the colony having alternating green and white concentric rings in culture and their ovoid conidia.

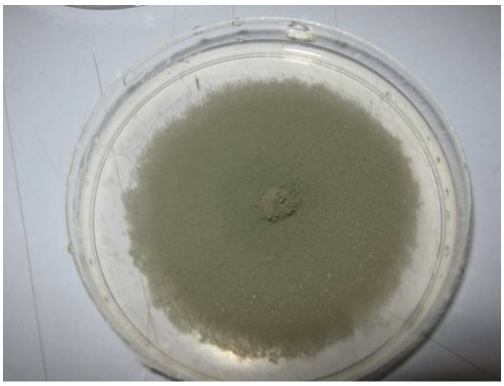
Table 4.3: Occurrence of fungal endophytes in the corms

| | | Meru | | | | Embu | |
|---|---|---|---|---|---|---|---|
| Genera | Mathioya | Central | Ntharene | Kerugoya | Mitunguu | East | K.U |
| *Aspergillus* | + | - | - | - | + | + | - |
| *Colletotrichum* | - | - | +++ | ++ | - | +++ | ++ |
| *Cordana* | ++ | ++ | ++ | ++ | + | ++ | - |
| *Fusarium* | + | ++ | + | ++ | +++ | ++ | ++ |
| *Penicilium* | + | - | - | ++ | - | - | - |
| *Trichoderma* | - | + | - | - | + | - | + |

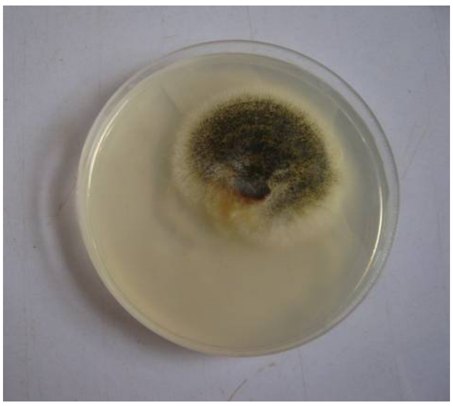
Key: - = Not recorded; + = Low frequency (≤33% corms); ++ = Moderate frequency (34 – 67% corms) ; +++ = High frequency ( 68 – 100% corms).

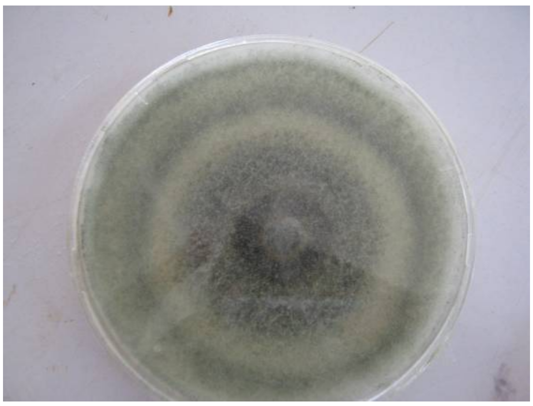


A: *Fusarium oxysporum*



B: *Penicilium* spp. C: *Colletotrichum* spp.





D: *Aspergillus* spp. E: *Trichoderma* spp

Fig 4.11: Two week old plates of microorganisms isolated from banana corms.

### 4.2.4 Effectiveness of macropropagation in eliminating pests and diseases in banana seedlings

During macropropagation cycle 1, less than 2% of the corms rotted in the nursery (Table 4.4). Isolations in the laboratory showed presence of saprophytes from the rotting corms. However, samples from two corms in Mitunguu and Embu East showed presence of pathogenic *Fusarium oxysporum*. During macropropagation cycle 2 no corms rotted in any of the nurseries in the study sites. In cycle 1, there was no significant difference (P=0.936) in the number of corms that rotted between the varieties. Also there was no significant difference (P=0.194) among the locations in the proportion of corms that rotted. Less than 2% of the corms did not give rise to shoots and did not rot.

Considering all the nurseries in the study sites less than 1% of the seedlings wilted in the nursery during macropropagation cycle 1. These were of Sweet banana and Gros Michel varieties in Mitunguu and Embu East and the causal organism was determined to be pathogenic *Fusarium oxysporum*. They were removed promptly from the nursery. Samples collected randomly from the leaves and corms of the healthy seedlings in the nurseries showed no pathogenic microorganisms. Endophytes that corresponded with those from the mother corm were isolated from the seedlings. None of the sampled seedling roots showed symptoms of PPN and upon extraction from the roots, no PPN were recorded.

Table 4.4: Proportion (%) of corms that rotted in the nursery

| Variety | Corms rotted (%) |
|---|---|
| **Kiganda** | 1.67 |
| **Cavendish** | 0.89 |
| **Sweet banana** | 2.63 |
| **Gros Michel** | 1.54 |

### 4.2.5 Health of macropropagated seedlings after transplanting

There was 100% survival of the seedlings after transplanting in all the study sites. None of the seedlings developed symptoms of disease. Isolation from the corm of seedlings showed presence of endophytes of which some were similar to those isolated from the mother corm. Bacterial endophytes were also isolated but more from the new leaves of the seedlings.

### 4.2.6 Pathogenicity tests

Of the microorganisms that were isolated from the corms, 99% did not result in symptom development. However, some *Fusarium oxysporum* species caused disease symptoms resulting to wilting of the leaves and this pathogen was found to be *Foc*.

#### 4.2.6.1 Pathogenicity of *Fusarium oxysporum*

Plantlets of cultivar Gros Michel inoculated with *Fusarium oxysporum* developed wilting symptoms. There was significant difference ($P \leq 0.001$) in the height, basal circumference and number of leaves in the different treatments and the control (Table 4.5). The disease development 14 weeks after inoculation as indicated by the area under disease progress curves (Fig 4.12 and 4.13) was significantly different ($P \leq 0.001$) over time. However, the basal circumference between the different treatments was not significantly different ($P = 1.000$) (Fig 4.14) over time.

**Table 4.5: Mean height, basal circumference and number of functional leaves of banana plants 14 weeks after inoculation with varying inoculum levels of *Fusarium oxysporum*.**

| Treatment (No. of mycelia disks) | Height (cm) | Basal circumference (cm) | No. of functional leaves |
|---|---|---|---|
| 10 | 43.78 ± 1.16[b] | 11.76 ± 0.20[b] | 5.44 ± 0.13[b] |
| 20 | 44.36 ± 0.81[b] | 11.57 ± 0.32[b] | 5.34 ± 0.17[b] |
| 30 | 38.57 ± 2.56[c] | 9.75 ± 0.46[c] | 3.21 ± 0.19[c] |
| 0 | 50.83 ±1.14[a] | 12.33 ± 0.34[a] | 6.43 ± 0.14[a] |
| P | <0.001 | <0.001 | <0.001 |

Data are the mean ± standard error (SE). Means within the column followed by the same letter are not significantly different at $P<0.05$. (Fisher's LSD test)

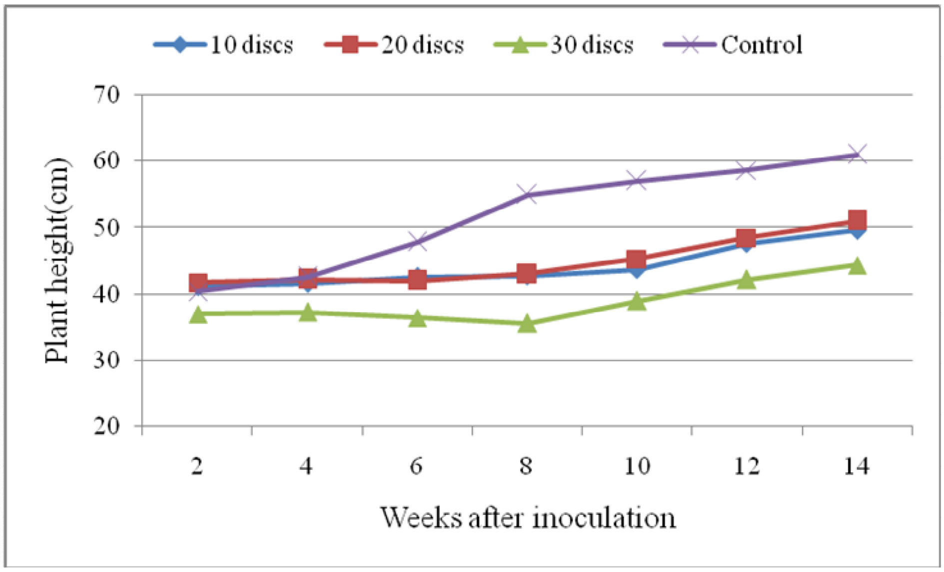



Fig 4.12: Mean height (cm) of banana plantlets of cultivar Gros Michel over 14 weeks period after inoculation with varying densities of *Fusarium oxysporum*.

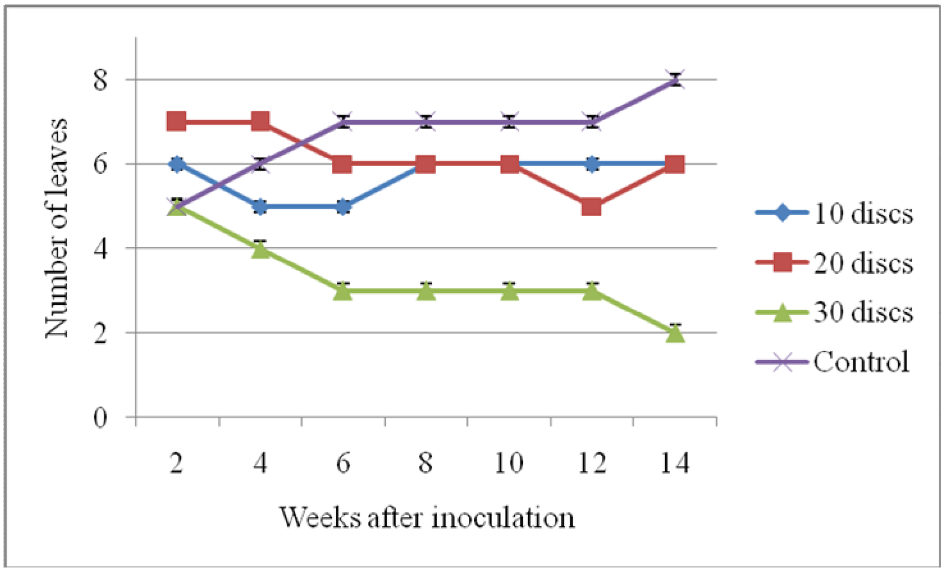



Fig 4.13: Mean number of functional leaves of banana plantlets of cultivar Gros Michel over 14 weeks period after inoculation with varying densities of *Fusarium oxysporum*.

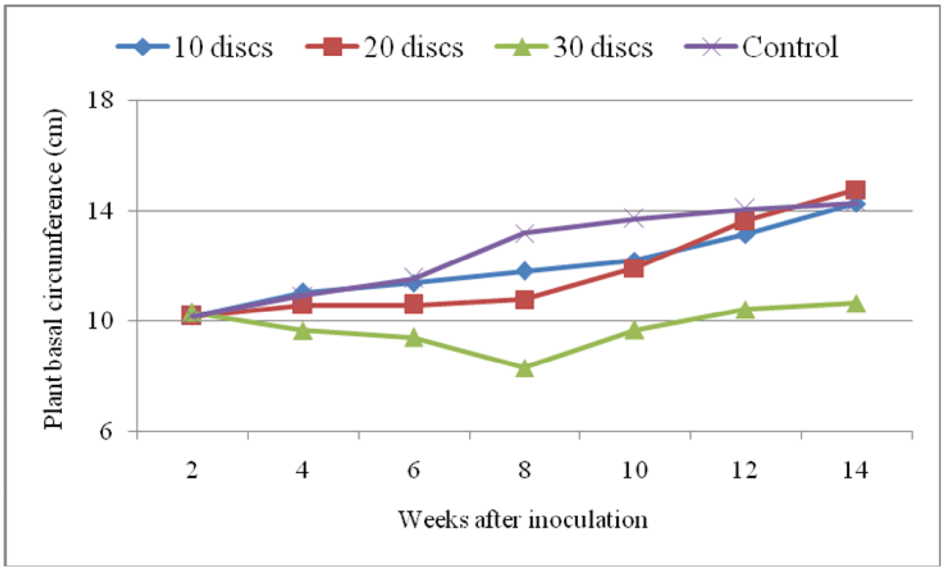



Fig 4.14: Mean basal circumference (cm) of banana plantlets of cultivar Gros Michel over 14 weeks period after inoculation with *Fusarium oxysporum*.

There was a highly significant difference ($P<0.001$) in the Leaf Symptom index (LSI) between plants treated with varying inoculum levels. The LSI was higher in plants where 30 discs of inoculum were used (Fig 4.14). The leaves of the banana plantlets started wilting 2 months after inoculation with the pathogen. The rhizomes also exhibited discolouration in the outer cortex and plants that had more inoculum showed more discolouration in the stellar region. There was significant difference ($P=<0.001$) in the RDI between the different inoculum levels (Fig 4.15). The control plants remained healthy.

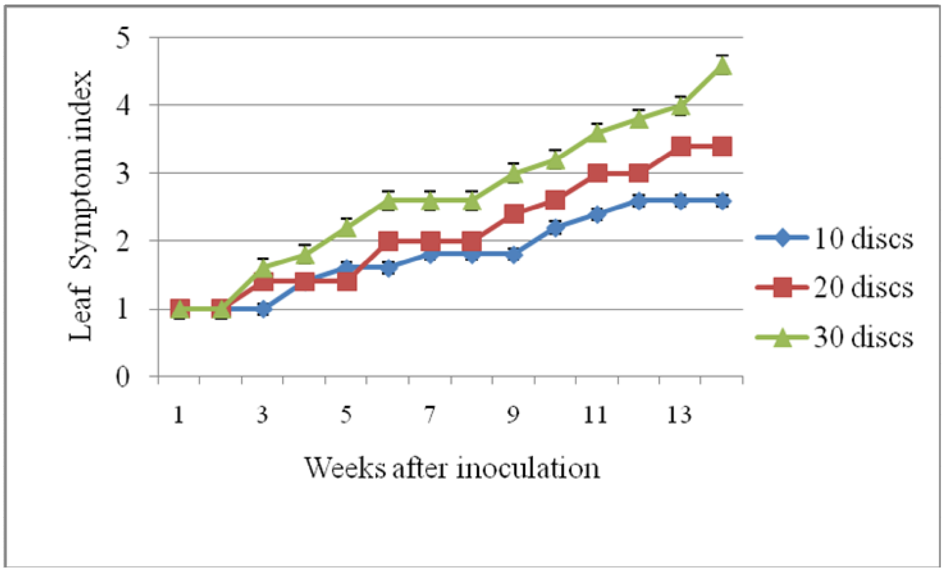



Fig 4.15: Leaf Symptom index of banana plantlets inoculated with varying inoculum levels of *Fusarium oxysporum.*

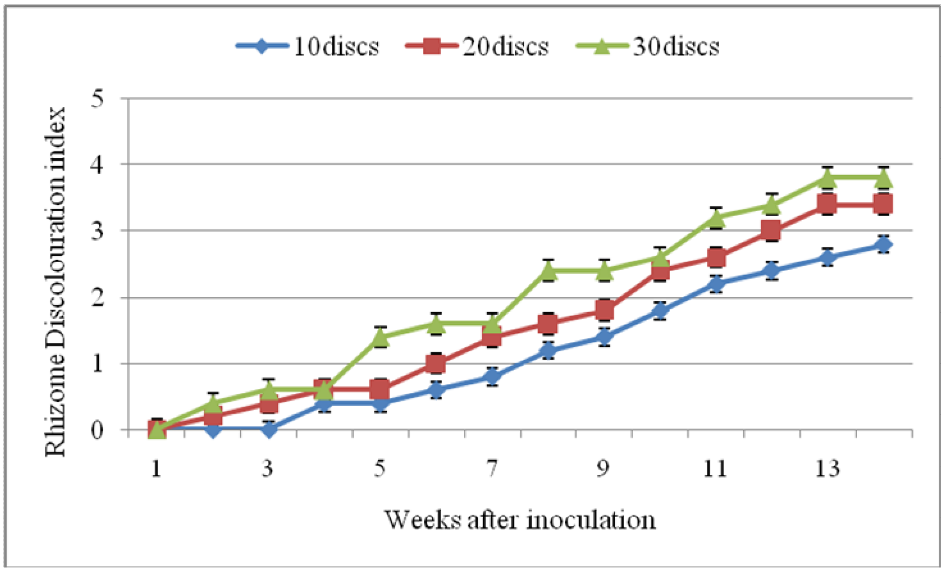



Fig 4.16 :Rhizome Discolouration index of banana plantlets inoculated with varying inoculum levels of *Fusarium oxysporum.*

The fresh and dry weights of roots of the inoculated and control treatments differed significantly (P=0.001), (Table 4.6) as well as the dry shoot weights of the inoculated and the control treatments (P=0.001), (Table 4.7).

**Table 4.6: Fresh and dry root weights of banana plantlets of cultivar Gros Michel inoculated with different inoculum levels of *Fusarium oxysporum* f. sp. *cubense***

| Treatment (No. of mycelia disks) | Fresh root weight ± SE | Dry root weight ± SE |
|---|---|---|
| 10 | 95.6 ±4.25[a] | 10.87 ± 1.59[a] |
| 20 | 83.1 ±2.10[b] | 9.09 ± 0.59[ab] |
| 30 | 68.0 ± 2.25[c] | 6.68 ± 0.48[ab] |
| 0 | 57.6 ± 1.46[d] | 4.32 ± 0.27[c] |
| P | <0.001 | <0.001 |

Data are the mean ± standard error (SE) of fresh and dry root weights. Means separated using Fisher’s LSD test, means within the column followed by the same letter are not significantly different at P<0.005.

**Table 4.7: Fresh and dry shoot weights of banana plantlets of cultivar Gros Michel inoculated with different inoculums levels of *Fusarium oxysporum* f. sp. *cubense***

| Treatment (No. of mycelia disks) | Fresh shoot weight ± SE | Dry shoot weight ± SE |
|---|---|---|
| 0 | 217.4 ±10.384[a] | 23.44 ± 1.738 [a] |
| 10 | 199.8 ±10.382[a] | 20.46 ± 1.737 [a] |
| 20 | 140.8 ±10.375[b] | 14.39 ± 1.736 [b] |
| 30 | 84.9 ± 10.370[c] | 10.88 ± 1.73 [b] |
| P | <0.001 | <0.001 |

Data are the mean ± standard error (SE) of fresh and dry shoot weights.
Means separated using Fisher's LSD test, means within the column followed by the same letter are not significantly different at P<0.05

**4.2.6.2 Pathogenicity of plant parasitic nematodes on banana seedlings**

Scoring of the root damage showed that the non-inoculated plantlets (control) had less dry roots and more functional roots as compared to the inoculated plants (Fig 4.17). There was a significant difference (P=0.001) between the dry roots and the functional (P≤0.005) roots in the inoculated plants and the control. The basal circumference, height and number of leaves of the plantlets were significantly different (P<0.001) after inoculation with different levels of PPN. (Fig 4.18, Fig 4.19 and 4.20).

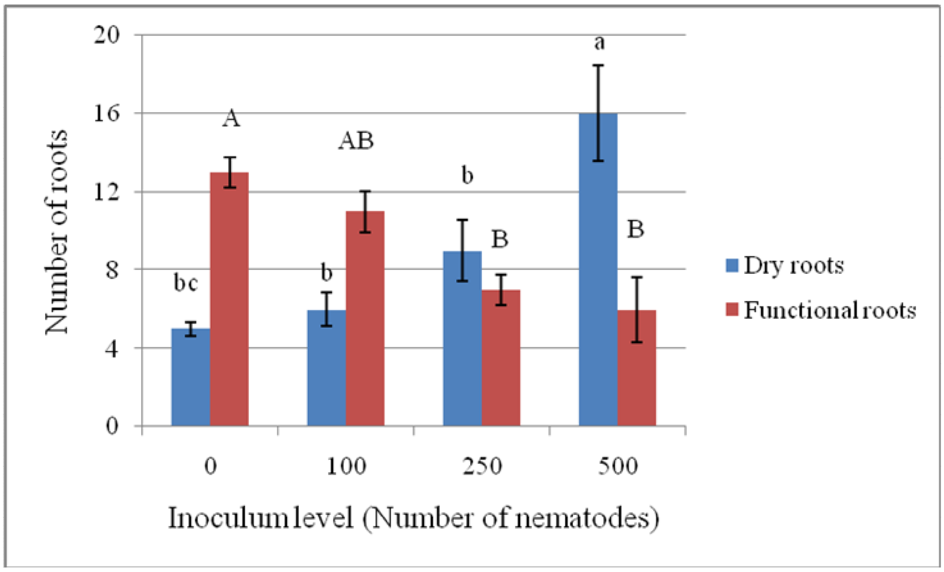



Fig 4.17: Mean number of dry and functional roots in the banana plantlets of cultivar Grand nain inoculated with different levels of *R. similis*. Bars showing the same letters are not significantly different at P<0.05.

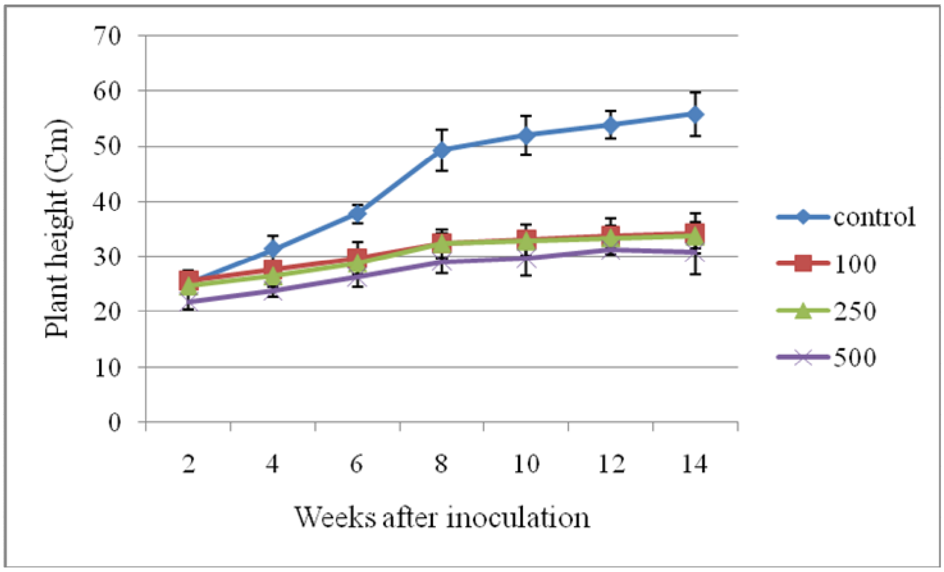



Fig 4.18: Height (cm) of banana plantlets of cultivar Grand nain after inoculation with *R. similis.*

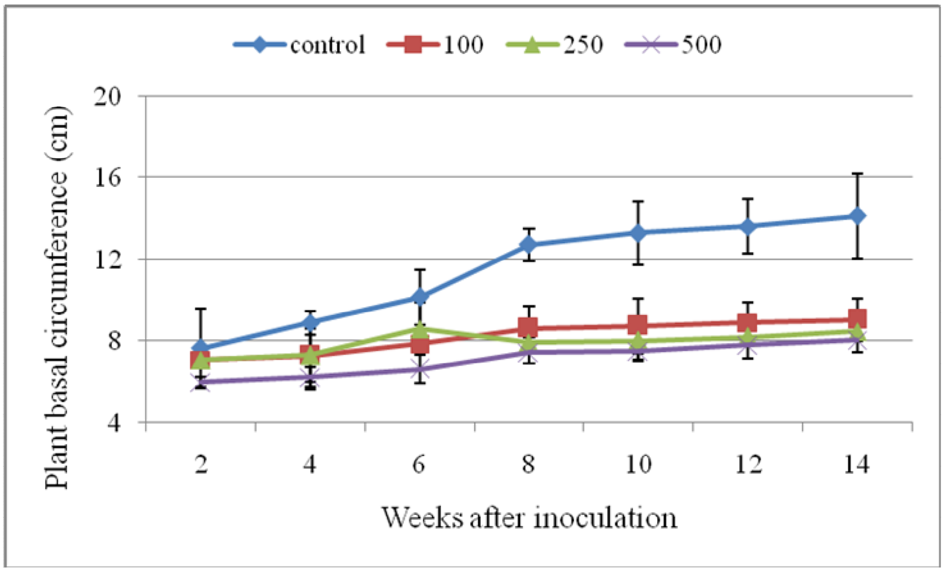



Fig 4.19: Basal circumference of banana plantlets of cultivar Grand nain after inoculation with *R. similis*.

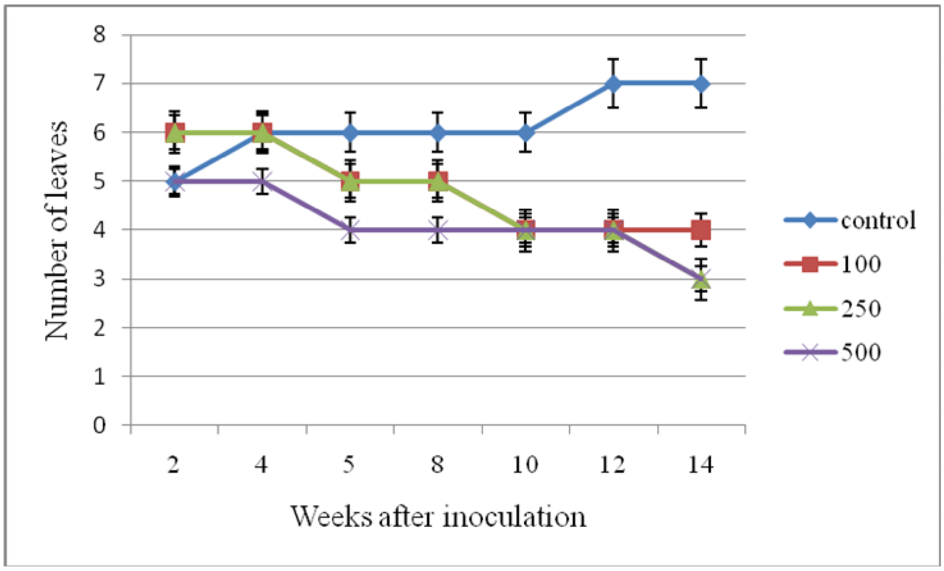



Fig 4.20: Number of leaves of banana plantlets of cultivar Grand nain after inoculation with *R. similis*.

The fresh and dry weights of the shoots and roots differed significantly (P<0.001) in the control and in the inoculated plantlets (Table 4.8). The shoots and roots of plantlets with the highest inoculums levels weighed least.

**Table 4.8: Fresh, dry root and shoot weights (Mean±SE) of banana plantlets of cultivar Grand nain inoculated with varying density of *Radopholus similis.***

| Inoculum level (No. of *R. similis)* | Fresh root weight (gms) | Dry root weight (gms) | Fresh shoot weight (gms) | Dry shoot weight (gms) |
|---|---|---|---|---|
| 100 | 51.8 ± 4.51[b] | 5.96 ± 0.26[b] | 177.5 ± 5.29[b] | 15.76 ±0.99[b] |
| 250 | 20.3 ± 1.43[c] | 3.66 ± 0.26[c] | 126.9 ± 4.03[c] | 11.69 ± 0.28[c] |
| 500 | 12.1± 1.28[c] | 2.55 ± 0.22[d] | 83.9 ± 3.35[d] | 9.93 ± 0.47[c] |
| Control | 85.3 ± 3.27[a] | 8.82 ± 0.28[a] | 211.2 ± 5.77[a] | 22.63 ± 1.75[a] |
| P | <0.001 | <0.001 | <0.001 | <0.001 |

*Means ± SE in the same column followed by the same letter do not differ significantly at P=0.005 (Fisher's LSD test)

### 4.3 Effectiveness of macropropagation technique in eliminating pests and pathogens

#### 4.3.1 Banana seedlings from macropropagation of corms infested with *Radopholus* spp. subjected to various treatments.

The corms responded differently with the different treatments. The corms treated with hot water sprouted before all other treatments that is four weeks after planting while corms cured after paring took six weeks. The tallest seedlings with larger basal circumference and more number of leaves resulted from corms treated with hot water. There were high significant differences ($P<0.001$) in the heights among the different treatments (Table 4.9). In addition, there were high significant differences ($P<0.001$) in the basal circumferences among the different treatments (Table 4.10). Also, there were high significant differences ($P<0.001$) in the Number of leaves among the different treatments (Table 4.11).

**Table 4.9: Heights (Mean ±SE) of plantlets from corms subjected to different treatments over time.**

| Week | Hot water | Paring and pesticide | Curing | Control |
|---|---|---|---|---|
| 4 | $0.85±0.28^{e}$ | 0 | 0 | 0 |
| 5 | $2.4±0.49^{d}$ | $0.30±0.21^{e}$ | 0 | $1.65±0.27^{e}$ |
| 6 | $5.05±0.66^{d}$ | $2.69±0.27^{d}$ | $2.37±0.22^{d}$ | $3.67±0.26^{d}$ |
| 7 | $7.47±0.77^{c}$ | $4.04±0.30^{c}$ | $4.74±0.26^{c}$ | $6.09±0.28^{c}$ |
| 8 | $9.75±0.72^{b}$ | $5.09±0.30^{b}$ | $6.76±0.29^{b}$ | $8.13±0.31^{b}$ |
| 9 | $12.25±0.65^{a}$ | $7.33±0.27^{a}$ | $9.89±0.40^{a}$ | $10.27±0.31^{a}$ |
| P | <0.001 | <0.001 | <0.001 | <0.001 |

*Means ± standard error (SE) in the same column followed by the same letter do not differ significantly at P=0.005 (Fisher's LSD test)
NB: 0 indicates the seedlings had not yet sprouted.

**Table 4.10: Basal circumference (Mean± SE) of plantlets from corms subjected to different treatments over time**

| Week | Hot water | Paring and pesticide | Curing | Control |
|---|---|---|---|---|
| 4 | 0.62±0.18$^{f}$ | 0 | 0 | 0 |
| 5 | 1.26±0.20$^{e}$ | 0.16±0.11$^{d}$ | 0 | 1.18±0.18$^{d}$ |
| 6 | 2.26±0.10$^{d}$ | 1.52±0.09$^{c}$ | 1.41±0.12$^{d}$ | 2.19±0.11$^{c}$ |
| 7 | 2.73±0.12$^{c}$ | 1.84±0.07$^{b}$ | 1.99±0.07$^{c}$ | 2.94±0.14$^{c}$ |
| 8 | 3.49±0.14$^{b}$ | 2.21±0.07$^{b}$ | 2.54±0.07$^{b}$ | 3.32±0.13$^{b}$ |
| 9 | 4.07±0.15$^{a}$ | 3.51±0.17$^{a}$ | 2.99±0.08$^{a}$ | 3.84±0.14$^{a}$ |
| P | <0.001 | <0.001 | <0.001 | <0.001 |

*Means ± standard error (SE) in the same column followed by the same letter do not differ significantly at P=0.005 (Fisher's LSD test)
NB: 0 indicates the seedlings had not yet sprouted.

**Table 4.11: Mean number of seedling leaves of plantlets from corms subjected to different treatments over time**

| Week | Hot water | Paring and pesticides | Curing | Control |
|---|---|---|---|---|
| 4 | 0 | 0 | 0 | 0 |
| 5 | 0.85±0.18$^{d}$ | 0 | 0 | 1.18±0,18$^{d}$ |
| 6 | 1.70±0.24$^{c}$ | 0.85±0.14$^{c}$ | 0.65±0.11$^{c}$ | 2.19±0.11$^{c}$ |
| 7 | 2.60±0.19$^{c}$ | 1.45±0.13$^{b}$ | 1.75±0.14$^{b}$ | 2.94±0.14$^{c}$ |
| 8 | 3.10±0.16$^{b}$ | 2.60±0.18$^{b}$ | 2.65±0.13$^{b}$ | 3.32±0.13$^{b}$ |
| 9 | 3.60±0.11$^{a}$ | 3.15±0.15$^{a}$ | 3.50±0.11$^{a}$ | 3.84±0.14$^{a}$ |
| P | <0.001 | <0.001 | <0.001 | <0.001 |

*Means ± SE in the same column followed by the same letter do not differ significantly at P=0.005 (Fisher's LSD test)
NB: 0 indicates the seedlings had not yet sprouted.

Emergence rates 9 weeks after planting were 100% for corms treated with hot water, 90% for corms that were pared and cured, 96% for corms in which application of nematicides was done after paring and 97% in the control where only paring and disinfecting with sodium hypochlorite was done. The average number of seedlings was 7 for corms treated with hot water, 3 for corms that were pared and cured and 5 for those treated with nematicides and the control. Roots from these plantlets were assessed for nematode damage and there was no indication of lesions. Upon extraction from the roots, no PPN were found.

### 4.3.2 Banana seedlings from macropropagation of corms infected with *Foc*

Corms of plants that had been artificially inoculated with *Foc* were macropropagated and 33% of the corms rotted. *Foc* was re-isolated from the rotting corms. Corms that had been inoculated with 30 mycelial discs gave rise to seedlings that had wilting symptoms and did not survive after transplanting. However 50% of seedlings resulting from corms initially infected with 20 mycelia discs of *Foc* looked healthy but the pathogen was re-isolated from the rhizome. The number of leaves, plant height, basal circumference and number of seedlings in the inoculated plants was significantly different ($P<0.001$) between the different inoculums densities and the control (Fig 4.21, 4.22, 4.23, 4.24).

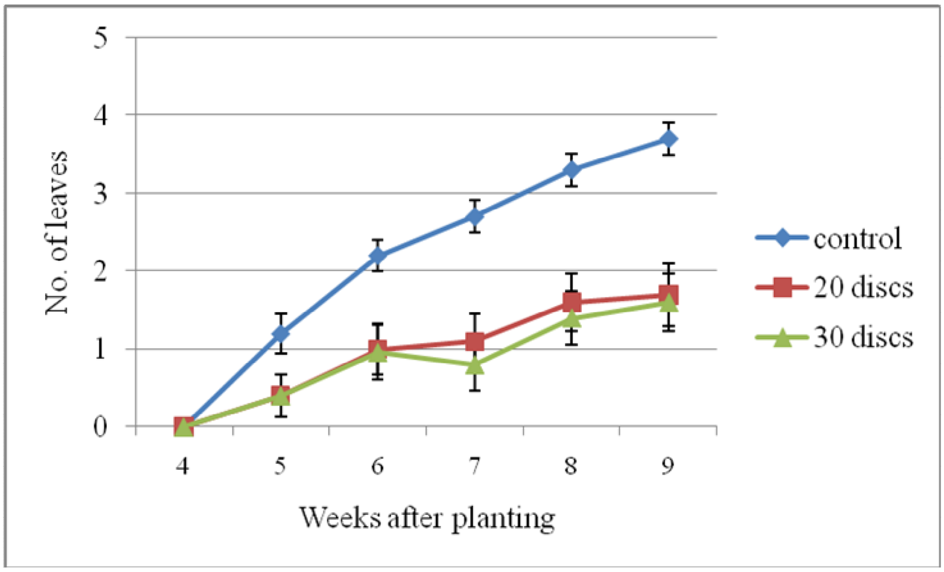



Fig 4.21: Number of leaves of banana plantlets from corms of cultivar Gros Michel inoculated with varying levels *Foc*. Bars that are not overlapping indicate significant differences at P≤0.05.

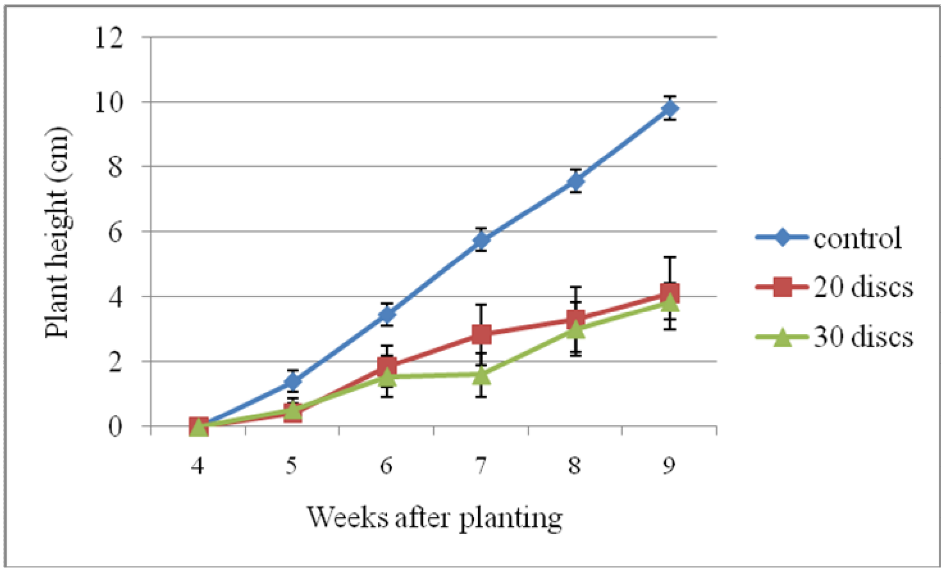



Fig 4.22: Height of banana plantlets from corms of cultivar Gros Michel inoculated with varying levels of *Foc*. Bars that are not overlapping indicate significant differences at P≤0.05.

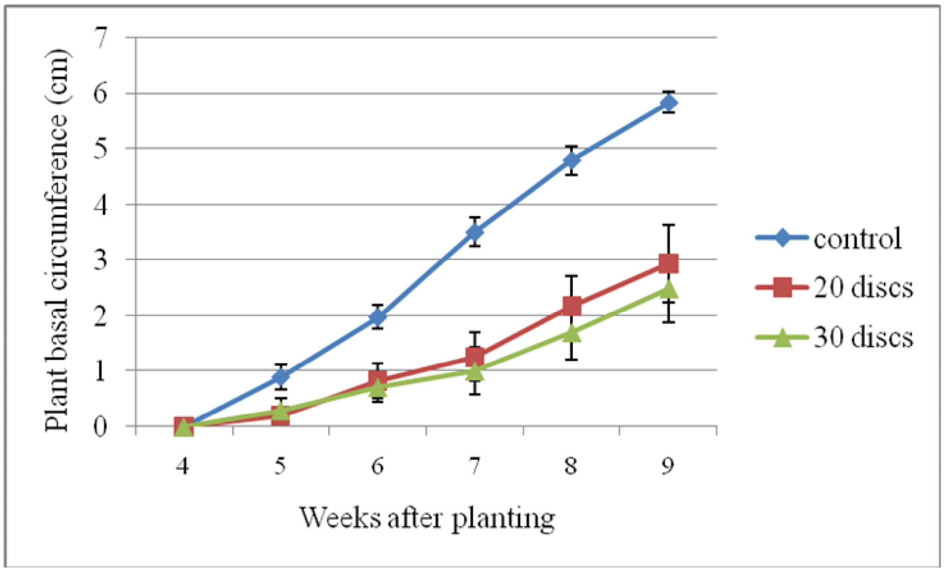



Fig 4.23: Basal circumference of banana plantlets from corms of cultivar Gros Michel inoculated with varying levels of *Foc*. Bars that are not overlapping indicate significant differences at P≤0.05.

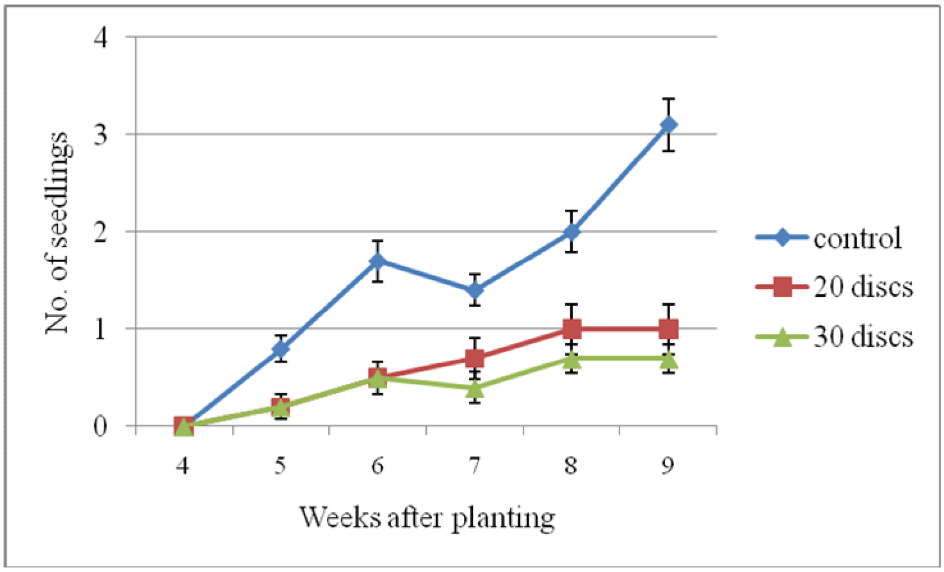



Fig 4.24: Number of banana plantlets emerging from corms of cultivar Grand nain inoculated with varying levels of *Foc*. Bars that are not overlapping indicate significant differences at P≤0.05.

**4.3.3.1 Banana seedlings from macropropagation of corms infested with *Xanthomonas vasicola* pv. *musacearum***

Inoculation with *Xvm* indicated progressive disease development with plantlets that were inoculated with higher inoculum of BXW showing higher disease severity rates. There was significant difference (P≤0.001) in disease development between the weeks except in the control where no disease development occurred (Fig 4.25). There were highly significant differences (P≤0.001) in disease development between various inoculums levels. There was a significantly strong relationship between inoculum level and severity.

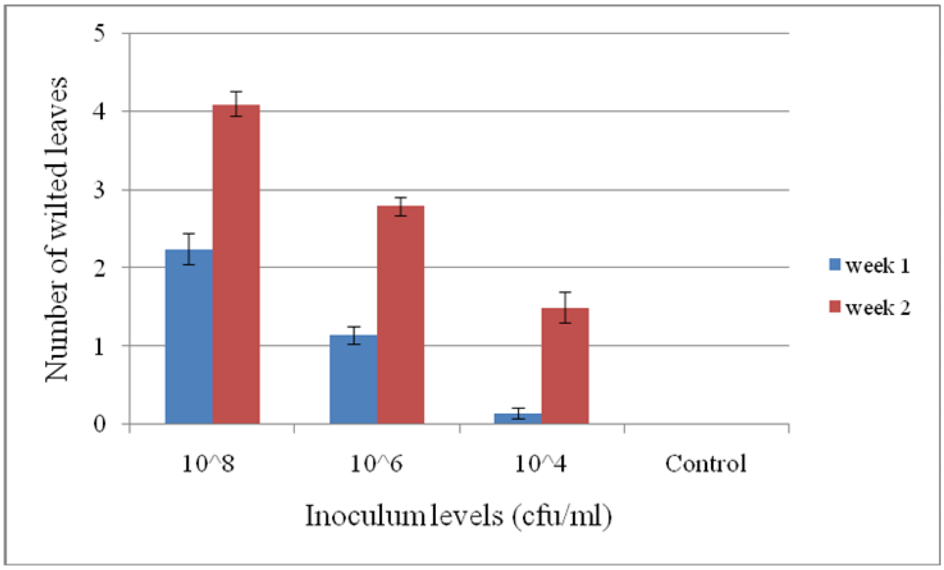



Fig 4.25: Disease severity in plantlets of cultivar Giant cavendish inoculated with varying inoculum levels of *Xvm* in two weeks. Bars that are not overlapping indicate significant differences at P≤0.05.

Majority of the corms (70%) inoculated with $10^8$ cfu/ml did not give rise to seedlings but rotted in the media. There was no significant difference (P=1.000) in disease development among the different inoculums levels of *Xvm* (Fig 4.26). The causal organism, *Xvm* was isolated from the tissues of the seedlings.

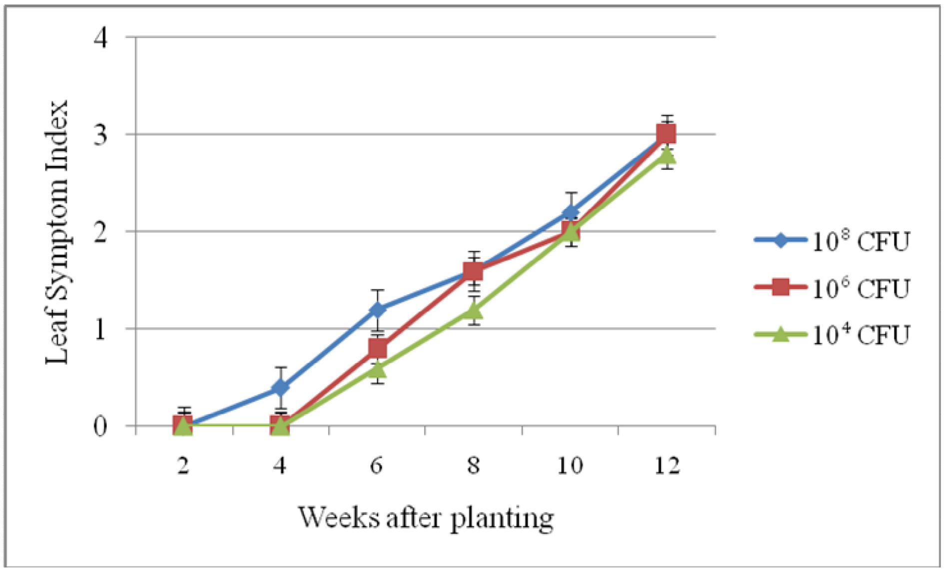



Fig 4.26: Leaf symptom index of banana plantlets of cultivar giant Cavendish 12 weeks after inoculation with different inoculums levels of *Xvm*. Bars that are not overlapping indicate significant differences at P≤0.05.

# CHAPTER FIVE

# DISCUSSION

## 5.1 Important diseases and pests of banana in Eastern and Central Kenya

This study shows that the important diseases and pests of banana in Eastern and Central Kenya are Fusarium wilt (FW), Sigatoka, plant parasitic nematodes (PPN) and the banana weevil (*Cosmopolites sordidus*). The PPN that were found in these areas included *Radopholus* spp., *Pratylenchus* spp. and *Helicotylenchus* spp. Fusarium wilt was found to be more prevelent in Kirinyaga district where Gros Michel and Sweet Banana are preferred by most farmers. In this area, some farms had up to 98% infection. Mitunguu and Embu areas followed in prevalence. However, the disease was not observed in Kiganda and Muraru varieties which are East African Highland bananas (EA-AAA) and are known to be resistant to the disease (Kung'u *et al.*, 2001). The disease was also not observed in cavendish variety (AAA) which is not affeted by race 1 and 2 present in Kenya. These varieties can be planted to prevent potential losses from FW in banana plantations.

Since no longterm measures have been developed to control the disease unless through use of resistant ciltivars (Moore *et al.*, 1999), clean planting material can be used to initiate new plantations. Especially where the farmers prefer the cultivar Gros Michel and sweet banana, which are susceptible to this disease but fetch better prices in the market. Imports from countries whose bananas are affected by race 4 should be restricted through strict measures. Sigatoka disease though found in all the areas surveyed was not considered a major constraint by the farmers because it is usually mostly prevalent during the wet and cold season and its effect on production is through reduction of photosynthesis subsequently declining yield. It is imperative that the disease is noted and measures to prevent disease spread taken early.The disease is controlled effectively through integrated management practises and proper use of pesticides to prevent build up of resistance (Nelson, 2008).

Presence of PPN across all the areas surveyed may have resulted from high moisture in the soil causing increased mobility of the nematodes because the survey was done during a wet season (July) or due to irrigation in areas like Mitunguu that are dependent on water from the river for irrigation. This is consistent with the findings of Barker and Koenning, (1998) who reported that the reproduction and survival of plant parasitic nematodes are depedent upon soil moisture, soil type and temperature. Moisture increases the mobility of PPN thus creating a conducive environment for them to thrive. The banana weevil was more prevalent in Embu East and Imenti South districts because these are warmer areas thus creating a condusive environment for them to thrive. Lescot (1988) surveyed 45 sites in Cameroon and found a negative correlation between weevil damage and elevation; damage was greatest at altitudes below 1000m, very low between 1500 and 1600 m, and absent in areas above 1600m. The weevils have more effect in yield of banana once they affect the central cylinder which is beyond the cortex (Gold *et al*., 2005). However, tunneling in the cortex creates openings through which soil borne pathogens can enter the plant tissues. It has been found that the most important mode of spreading banana pests (nematodes and weevils) to new fields is through use of infested planting materials (Bridge *et al.*, 1995; Sarah, 1989; Seshu Reddy *et al.*, 1999).

Management of diseases can be done using chemical control which involves use of fungicides, nematicides and pesticides. Well established commercial banana farmers can afford pesticides for control of PPN, weevils and black leaf streak. However, this method of disease control is seldom an option for resource-poor farmers (Viljoen, 2011). In addition, chemical fumigation is harzadous to the environment and overuse of pesticides can lead to build up of resistance in some pathogens and pests thus reducing their effectiveness. Biological control involves use of entomopathogenic fungi and fungal endophytes to control diseases and has been considered as a feasible alternative (Niere *et al.*, 1999). Biocontrol has promising potential in disease management.

Susceptible banana cultivars can be replaced with resistant ones to prevent plantations from being attacked by diseases. For instance, Cavendish and East African highland bananas can be planted in regions where FW is prevalent. These varieties can be developed using conventional or unconventional methods of breeding. Resistance against FW and black leaf streak has been introduced through breeding programs however disease-resistant hybrids take a long time to develop and are often not popular at local markets (Viljoen, 2010). Unconventional improvement through mutation breeding and genetic modification can reduce the time and costs of conventional breeding substantially (Crouch *et al*., 1998), but stability of traits against pathogens and pests, and acceptance of such plants by the community is not guaranteed.

It emerged that most farmers used suckers from their farms or from neighbours' farms as the main planting material. They can be obtained at no cost and where they are sold, they cost a minimum of 10 – 30Kshs (Wambugu and Kiome, 2001 ). However, this practise carries a risk of disease and pests spread if the suckers are infected. It has been confirmed that FW, PPN and the weevil are spread widely through the use of infected planting material (Sehu Reddy *et al*., 1999). Use of clean planting material is the most important and feasible way of controlling diseases in banana (Viljoen, 2010). This is because most potentially devasting diseases of banana are introduced and spread through planting material thus it is more practical to prevent introduction of the pests and pathogens. Seedlings from tissue culture are free from pests and diseases but the cost is prohibitive and can only be met by commercial farmers. Tissue culture plantlets are available and can be sourced from NGO's, researchers and Government institutions. However their cost is high ranging from Kshs 100 - 150 (Wambugu and Kiome, 2001). This is prohibitive for the average farmer. Additional expenditure may be required for transport from the multiplication sites and the seedlings need care during the early stages of planting; enough water and nutrients are needed to sustain them and this compounds the cost making it difficult for many farmers to manage. Adoption rate of TC banana seedlings is low despite the associated benefits (Wambugu and Koime, 2001) though farmers would like to use affordable clean planting material. Low cost TC has been introduced but the cost of the seedlings, is still high for most small scale banana farmers. The cost of producing and maintaining macropropagation seedlings is considerably low. Thus the price per plantlet can be 50Ksh and can be further reduced by locating the nurseries close to farmers fields. This technology can increase smallholders access to clean planting material at affordable costs.

It was found that only 12% of the farmers treated the suckers before planting despite the fact that disinfestation of banana suckers can significantly reduce the inoculum levels of pests and pathogens A combination of cultural control measures can be employed to treat suckers and trap pests (Viljoen, 2010). The survey indicated that many farmers are not informed on methods of treating suckers before planting and those who are, find the process labourious. This calls for a cost effective method of producing healthy banana seedlings which are within reach of the farmers. Awareness campaigns can be done to educate farmers on the cultural methods they can employ to control pests and pathogens before planting and in the field.

Banana Xanthomonas wilt was not observed in any farm in Eastern and Central regions of Kenya. However, it has been reported in Western Kenya (Mbaka *et al*.,

2008) at high incidence levels. Many banana varieties are susceptible to the disease and it spreads quite fast especially where infected planting material is used. Transportation of planting material from Western Kenya to other parts of the country should be restricted to prevent introduction of the pathogen. In fields where the disease has been confirmed, the banana plants should be destroyed and allowed to fallow for 5 months. It can then be replanted with disease free banana seedlings.

Viral diseases that affect banana though not observed, are also a potential constraint to banana production. They are transmitted mainly through use of infected planting material and by vectors. To limit the risk of dissemination through infected material, it is vital that phytosanitary regulations be introduced to prevent the movement of diseased planting material from one region to another. A viable solution would be through location of macropropagation nurseries near farmers fields and sourcing for preferred planting material in the same locality to eliminate the need for importation.

Farmers in the area surveyed carried out agronomic practises to prevent and manage diseases from gettting into their farms. Appying manure and fertilizer was widely used in Eastern and Central regions and this aids in the robust growth of banana plants enhancing their ability to withstand disease pressures. Organic matter is vital to the nutrition of banana plants, especially in limited resource farming systems. The benefits of organic matter include improvement of Nitrogen and trace element availability, better soil structure and looseness, lower leaching losses, and enhance proliferation of beneficial mycorrhizae to the root zone (Robinson, 2000).

Disinfection of tools is practiced by only 14% of the farmers because they are not informed on the importance. Most of them disinfect the tools using fire because they consider the bleaches expensive. Upon more sensitization, the farmers can adopt this beneficial practice more widely. Agronomic practices were done widely by the farmers to maintain high yields in their plantations. These increase yield and enhance productivity, by promoting vigorous growth and to eliminate those factors causing weak root growth and development. However, only 13% of the farmers used clean planting material. Most of these farmers are commercial and obtained planting material from Africa Harvest an NGO involved in promoting tissue culture banana seedlings. Removal and destruction of diseased plants or plant parts is employed by farmers to prevent further spread of the diseases.

## 5.2 Health of macropropagated banana seedlings

Macropropagation was found to consistently produce healthy banana seedlings. Farms needed to be certified for selection of mother plants that were true to type and free from pests and diseases. Certification helped in obtaining visibly healthy mother corms thus contributing to the consistent health of the seedlings. However, some banana plants were visibly healthy but were infested with pests which did not manifest through symptoms. This indicated that some banana stools had latent pathogens which were not noted during certification. The study showed that their significance was low because prior certification ensured that visibly healthy plants were selected for macropropagation. However, latent infections are important because they may either cause direct damage or weaken the host plant, predisposing it to infection by other pathogens and pests. The host lacks obvious signs thus, presence of latent pathogens is likely to go undetected and their effect ascribed to other causes. Extra assessment of corms after selection is needed to ensure they do not habour pests and pathogens. The protocol for selection of corms should be modified to include sampling of plants in a plantation. This will allow assessment of the corms for presence of pests and diseases before acquiring of the corms as an additional measure to enhance certification of the mother plants. This saves on labour reducing uprooting corms which may be later rejected.

The major pest encountered during certification was the banana weevil but this pest was not transmitted from the corms to the banana seedlings during macropropagation. Corms had few tunnels on the cortex but none in the central cylinder. The weevils were prevalent in Mitunguu (1071 masl) where annual temperature ranges 26 - 30° C and least in Mathioya (1915 masl) where annual temperature is 15 - 18° C. Weevils are common in lower altitude areas with temperatures ranging from 25 to 30° C (Traore *et al.*, 1993). Temperature influences embryonic development and the eclosion rates of the weevil. According to Schmitt (1993), there is an inverse relationship between stage duration and temperature in the development of the weevil. Thus in areas with higher temperatures, the weevils multiply at a higher rate causing more damage due to increased populations. The variety affected most by the banana weevil was the Kiganda, an East African Highland banana (EA-AAA), which confirms the findings of Gold *et al*., (2001) while the least affected variety was found to be Gros Michel. The weevil can be controlled effectively by Integrated Pest Management (IPM) techniques. This involves trapping, host resistance, planting in deep hole to prevent access by the weevil and crop sanitation to remove possible breeding sites for the weevil. Chemical control can be employed using caborfuran, oxamyl and carbosulfan which can be incorporated in the planting holes to control the larvae (Gold *et al*., 2003). Use of clean planting material can be used but it has been reported that *in vitro* plants are more susceptible to weevil attack in the field (Nuno and Ribeiro, 2002) and are more prone to high mat (Robinson 1996). Macropropagated seedlings could possibly provide a solution to this.

Roots from the corms used for macropropagation showed that there was nematode damage across all the study sites but upon macropropagation of the corms, the seedlings were free from PPN. Cavendish variety was most affected by the PPN indicating higher susceptibility. Gros Michel indicated lower levels of infection and this confirms a previous report by Gaidashova *et al*., (2008) who reported that Gros Michel was considered to be partially resistant to *Pratylenchus goodeyi*. In this study, the altitude was found to influence the distribution of the PPN. *Pratylenchus goodeyi* was found in all the areas but the population increased with elevation being highest in Mathioya (1915 masl). The population of *R. similis* was higher at lower altitude that is Mitunguuu (1071 masl) but were not found at high altitude as reported by Kashaija *et al*., (1994).

Endophytes were isolated from the corms of banana that were used for macropropagation. They are mutualistic symbionts that inhabit the interior of plants asymptomatically for the whole or part of their life cycle in plant tissues, receiving nutrition and structural refuge from the host, while benefiting the host with enhanced growth and health (Faeth and Fogain, 2002; Sikora *et al*., 2003; Paparu *et al*., 2004; Ju, 2006). Mutual association between the endophytes and the host plants has been reported to be beneficial to plant growth (Clay and Schardl, 2002) acting as antagonists to pests and diseases (Clay, 1991; Azevedo, 1998). Non pathogenic *Fusarium oxysporum* was widely isolated across all the areas. This confirms studies done by Griesbach (1999); Pocasangre (2000); and Niere (2001). Griesbach (2000) identified *Fusarium* and *Acremonium* species as the most promising fungal endophytes for the control of banana weevil, with a 25% reduction of larval size and 70% reduction of corm damage, as compared to the uninoculated control. Nematode numbers in endophyte-inoculated plants were reduced by 42–79% and root necrosis by 30–40% (Niere, 2001). *Aspergillus* sp. and *Penicillium* sp. were isolated abundantly from the corms used for macropropagation and have been reported to be the most frequently isolated fungi in soil (Cao *et al*., 2002). *Trichoderma* spp. isolated from the corms have a potential for disease control. According to Howell (2003), enzymes produced by *Trichoderma* spp. usually break down polysaccharides, chitin and ß-glucans, thereby resulting in destruction of the cell wall of *Foc*. They can produce antibiotics and probably inhibit the growth of latent pathogenic fungi. Endophytes are within the banana corm and are not in competition with microrganisms in the rhizosphere and are thus better as compared to commercial biological control agents.

During the process of macropropagation, serial removal of sheaths to expose the buds is key for preventing rotting of the corms in the nursery. Poor scarification resulted to rotting of some corms from which saprophytes were isolated. Scarification has to be done precisely and a sharp cut made at the base of the sheaths to prevent rotting. Disinfestation also needs to ensure that the whole corm is washed with disinfectant after removal of the sheaths. The process has to be done aseptically and the tools need to be disinfected subsequently after handling every corm. Proper training in the skills and precision needed to prepare the corm for rooting needs to be imparted to people carrying out the technology to avoid rotting.

According to the results from the simulated infection of corms using *Foc*, macropropagation technology is not able to eliminate *Foc* if present in the corm. This is further proven by isolation of *Foc* from some corms that had been sourced using the farm certification protocol. It is advisable that corms be selected from disease resistant cultivars such as East African Highland bananas and plantains (Tushemereirwe and Bagabe, 1998) and Cavendish variety which is not affected by race 1 and 2 causing FW in Kenya can also be utilized. Farmer preferred varieties that are susceptible to diseases require proper certification of farms for selection of the mother corms. The most appropriate selection can be done in plantations which had been established using seedlings from TC. This gives assurance that the planting material sourced is healthy. In addition, screening the mother corms is necessary to ensure they are disease free. The seedlings resulting should be planted in fields where *Musa* sp. have not been planted before and preferably fallow land.

The seedlings that were transplanted from the nurseries survived during hardening and 12 weeks after. Although Tenkouano *et al*., (2006) showed that there may be difficulty in survival of plantlets during hardening, this research found that the seedlings established well in the hardening nursery. They however need to be supplemented with fertilizer rich in Potassium (K) which is important because it promotes early shooting and reduces the amount of time the fruit takes to mature while improving size of the fingers and quality. Therefore, soil fertility must be maintained by continuously replenishing the soil with nutrients in form of either organic or inorganic nutrients.

**5.3 The difference in treatment of corms for macropropagation technology**

Hot water treatment of corms after scarification and disinfection, results to vigorous and fast growth of seedlings that are robust. This practise can be incorporated into the macropropagation technology. However, this can be expensive because of the equipment required to regulate the temperatures. Hauser and Messiga (2010) found that boiling water treatment of the corms having a circumference of more than 20cm eliminates PPN from the corm. The process of macropropagation can be enhanced through boiling the corms for 10 seconds before placing them in the propagators. Curing the corms after paring results to slow growth of the seedlings though it contributes to elimination of PPN and any eggs and larvae of weevils that may not be visible. This too can be employed in areas where weevils are prevalent. The disinfected corms are kept in a clean place under the shade for 48 hours so as to kill any eggs or larvae present. Use of nematicides also controls the PPN. However, Coyne *et al*., (2003) and Hauser (2007) reported that nematicides are often impractical or unavailable to small-scale farmers and in addition poses a health hazard to the users. A report from International Institute of Tropical Agriculture revealed that the age of corms does not result to differing quality of banana seedlings. However, younger corms are preferred since they have more sprouts per corm but older non-diseased corms can also be utilized.

# CHAPTER SIX

# CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusion

This study confirmed that pests and diseases are important constraints to banana production in Eastern and Central regions of Kenya. Fusarium wilt was found to be the most damaging disease especially in Kirinyaga County where susceptible varieties such as Gros Michel and sweet banana are most preferred. Black leaf sigatoka was present in all the districts while cigar end rot was not recorded as an important disease. Plant Parasitic Nematodes and banana weevils were found in all banana growing areas. However the population of weevils was higher in the low altitude areas (LM3) as opposed to the high altitude areas (LH 1). These findings show the need to effectively control diseases affecting banana to ensure maximum yield.

Macropropagation technology is efficient in producing banana seedlings that are free from PPN and the banana weevil (*Cosmopolites sordidus*). To ensure that the risk of spreading FW is minimized, resistant cultivars such as Cavendish (AAA) and East African Highland bananas (EA-AAA) should be preferably used. Banana Xanthomonas wilt was not eliminated by macropropagation thus corms should not be acquired from areas where the disease has been reported. Endophytes present in the corm are transferred to the seedlings thus giving them a potential advantage in suppressing diseases in the field.

The protocol for selection of the mother plants for use needs to be followed to ensure that the corms are free from pathogens. Proper training and involvement of the stakeholders is needed for complete understanding of the technology to enable adoption and subsequently increase productivity of banana. Macropropagation can be enhanced through treatment of corms using hot water resulting to robust seedlings.

**6.2 Recommendations**

- There is need for further studies on re-infestation of the seedlings by soil borne pests in the field and the yields of the macropropaged plants. Tests should be carried out to determine persistence of beneficial endophytes in plantlets from macropropagation and percentage of tissue colonization in roots and corms. The endophytes can also be tested whether they protect the macropropagated banana plants from soil borne pests and pathogens in the field. Comparisons of banana fruit yields resulting from fields initiated with seedlings of old corms and those initiated with young corms need to be done to ascertain whether there is any difference.

- A method needs to be developed for enhancing the process of certification further to ensure latent pathogens are noted in corms to avoid selecting diseased corms.

- Further physiological studies need to be done on corms that did not give rise to seedlings and yet did not habour pathogens. This will enhance the process of selection of the corms.

- Proper training needs to be done for persons interested in the seed production process to ensure that the process is done well and the standards of seedling health are maintained.

- Farmers should be educated properly about macropropagation technology to ensure wide adoption across the country and in the region.

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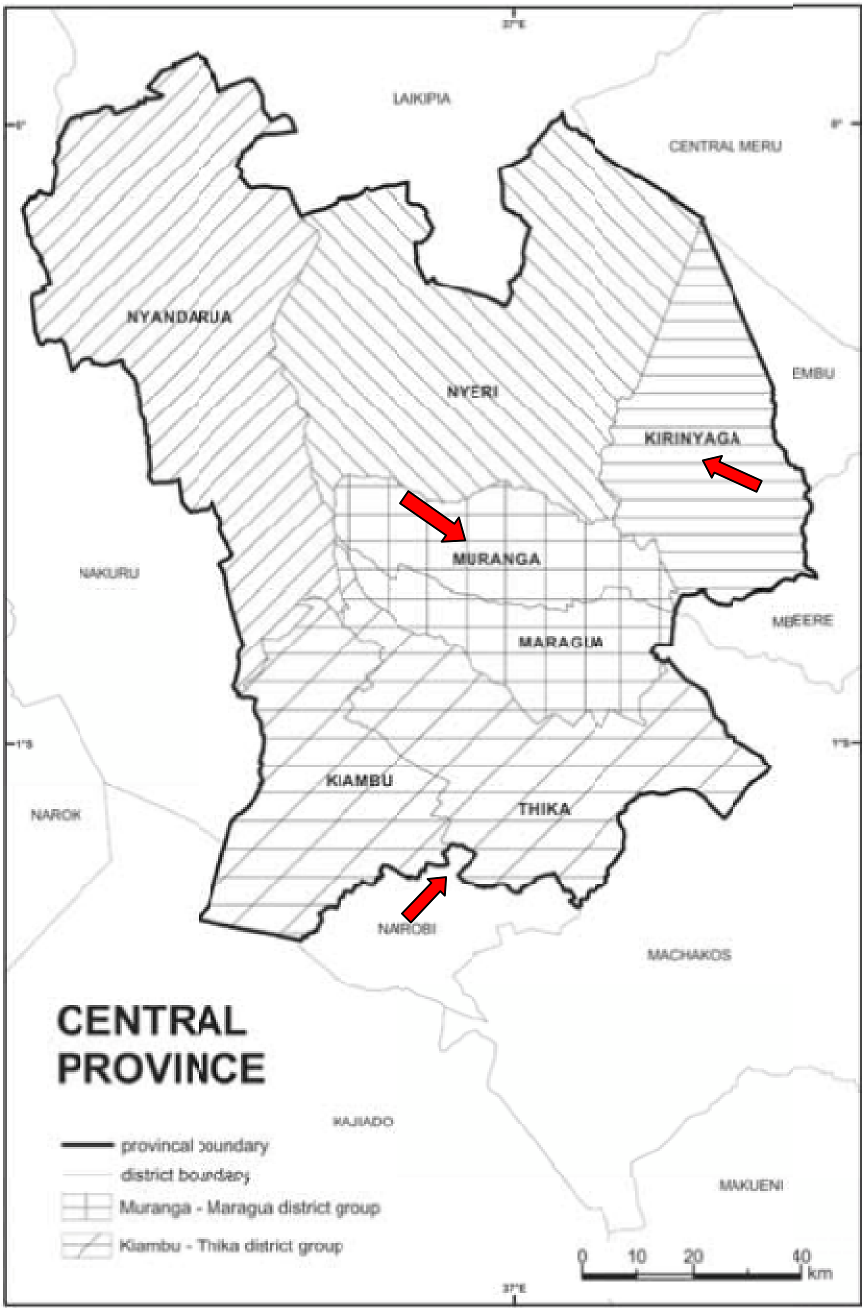
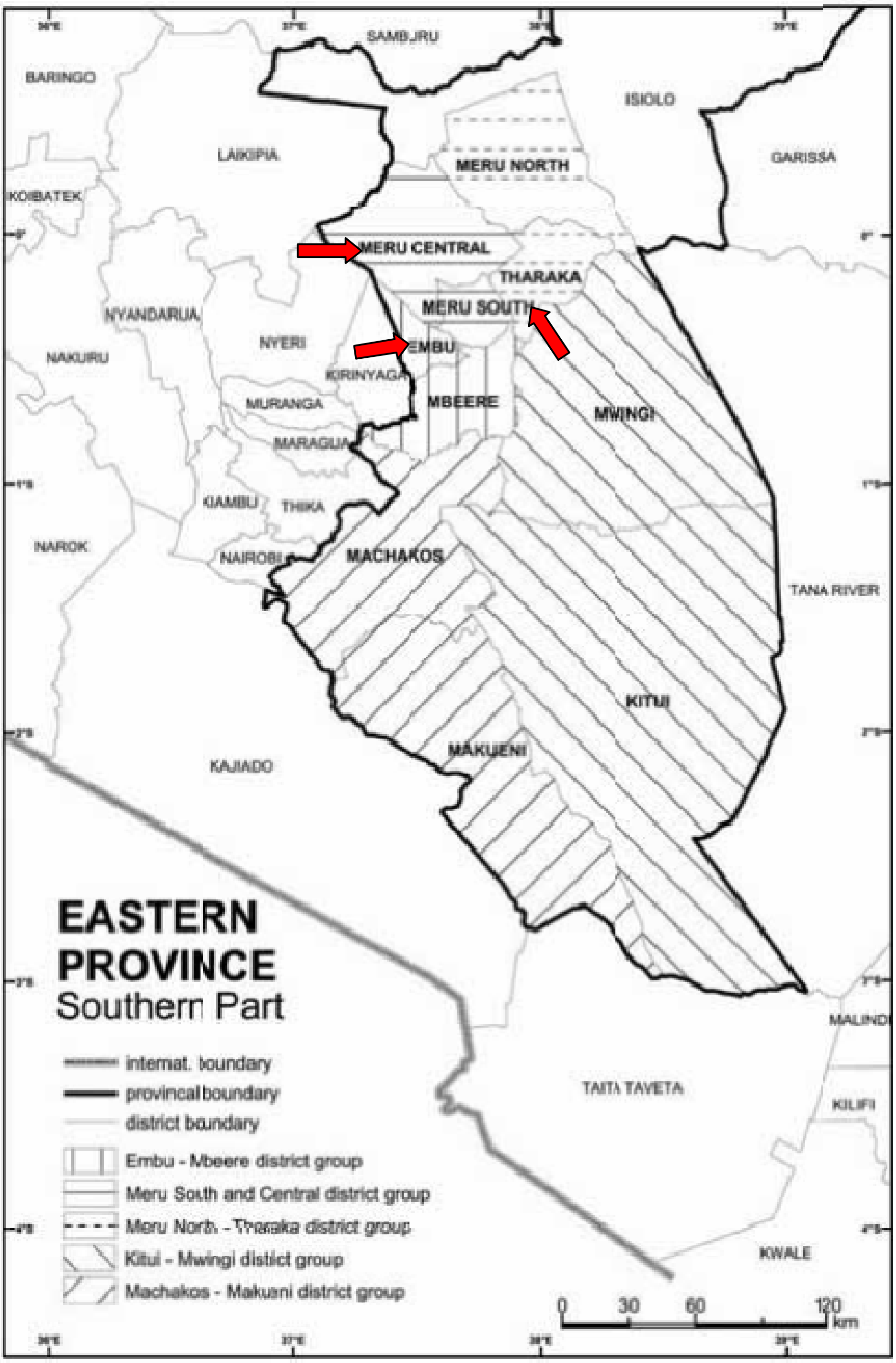
# APPENDICES

## Appendix 1a: Map of Central Kenya




Map showing location of the study areas in Central region of Kenya. Adopted from Jaetzold and Schmidt (2006)

**Appendix 1b: Map of Eastern Kenya**




Map showing location of the study areas in Eastern region of Kenya. Adopted from Jaetzold and Schmidt (2006)

**Appendix 2: Banana production practices at the study sites in Central and Eastern Kenya.**

| Location | Altitude | Production practices |
|---|---|---|
| Mathioya | 1915 M | • Rainfed but use water from the river for irrigation during the dry seasons.<br>• Cooking bananas are most preferred.<br>• The temperatures are low (18-25° C) and bananas take longer (18 months) to grow, mature and produce fruit.<br>• Bananas are intercropped with crops such as maize and sweet potatoes. |
| Kerugoya | 1340 M | • Farmers use irrigation water from bore holes and from the tap for growing bananas.<br>• Farmers prefer Gros michel and Sweet banana varieties. |
| Kenyatta University | 1608 M | • The area is characterized by brown cotton soil.<br>• Irrigation is done using tap water. |
| Ntharene | 1360 M | • Bananas irrigated using river water flowing through gravity from the mountain.<br>• Cavendish variety is mostly grown and traditional varieties such as Muraru is preferred by farmers.<br>• Coffee is also grown as a cash crop as a lone stands and not intercropped with banana. |
| Embu East | 1265 M | • Farmers use irrigation water from bore holes for growing bananas.<br>• Farmers prefer Cavendish variety. |
| Mitunguu | 1071 M | • Bananas are the major crop.<br>• Farmers use irrigation water from a river tapped by a community scheme for growing bananas.<br>• The temperatures are high (25 - 30°C) and the bananas take less time (14 months) to grow, mature and produce fruit. |
| Meru central | 1680 M | • Bananas irrigated using river water that flows from the mountain through the force of gravity.<br>• Cavendish variety is most preferred.<br>• Banana plantations are weeded and manure or fertilizer is added to replenish fertility. |

**Appendix 3: Questionnaire**

**BANANA SURVEY QUESTIONNAIRE**

1. DETAILS OF RESPONDENTS

Date Enumerator

District Division

Location Sub-location

Farmers' name Gender: Male ☐ Female☐

Total farm acreage House hold size

Topography: Steep slope☐ Gentle slope ☐Valley☐ Hill top☐ Flat ☐

GPS Reading Altitude

2. CROPS GROWN

| Crop | Acreage | Rank in order of importance | Uses/Benefits |
|---|---|---|---|
| 1. | | | |
| 2. | | | |
| 3. | | | |
| 4. | | | |
| 5. | | | |

3. ANIMALS REARED

| Animal | Number | Rank in order of importance | Uses/Benefits |
|---|---|---|---|
| 1. | | | |
| 2. | | | |
| 3. | | | |
| 4. | | | |
| 5. | | | |

4. BANANA CULTIVATION

| Clone | Acreage/ Stools | Rank in order of importance | Uses/Benefits |
|---|---|---|---|
| 1. | | | |
| 2. | | | |
| 3. | | | |

| | | | |
|---|---|---|---|
| 4. | | | |

a) Do you intercrop bananas with other crops? Yes ☐ No ☐

| **Crop** | **Order of preference** | **Uses** |
|---|---|---|
| 1. | | |
| 2. | | |
| 3. | | |
| 4. | | |
| 5. | | |

b) Source of planting material

| Clone | Planting material used | Source of planting material | Cost of planting material |
|---|---|---|---|
| 1. | | | |
| 2. | | | |
| 3. | | | |
| 4. | | | |
| 5. | | | |

c) If suckers are planted, how are they treated?

| Treatment method | Tick below |
|---|---|
| 1. Paring and curing | |
| 2. Hot water treatment | |
| 3. Treating with pesticides | |
| 4. Other treatment methods (Explain) | |

5. BANANA DISEASES, PESTS AND NUTRITIONAL DISORDERS

| Diseases/ pests | Observed 0 = No 1 = Yes | % loss based on severity | When noted Month/ Year | Current status Present/ absent | Clone(s) affected |
|---|---|---|---|---|---|
| Xanthomonas wilt | | | | | |
| Fusarium Wilt | | | | | |
| Sigatoka | | | | | |
| Cigar end rot | | | | | |
| Banana streak virus | | | | | |
| Weevils | | | | | |
| Nematodes | | | | | |
| Nutrient stress | | | | | |
| Other diseases/ disorder | | | | | |

a) Impact of pests and diseases on banana production (Quantify where possible)

| Impact | Tick where applicable | Quantify |
|---|---|---|
| Reduction in harvested bunches | | |
| Reduction of eaten bunches in the household | | |
| Change in diet due to shortage | | |
| Reduction of income from banana sales | | |
| Reduction of leaves and stems for fodder | | |
| Cost of buying material for replacement | | |
| Cost of pesticides | | |

b) Measures taken to control banana disease, pests and nutritional deficiencies

| **Measure** | **Tick if done** | **Reason if not done** |
|---|---|---|
| Field sanitation | | |
| Planting healthy seed | | |
| Use of pesticides | | |
| Removal of entire or part of diseased plants | | |
| Disinfecting tools | | |
| Restriction of entry in the farms | | |
| Reporting outbreaks to extension agents | | |
| Applying fertilizer/ manure regularly | | |
| Other measures (Describe) | | |

6. GENERAL AGRONOMIC PRACTICES

| Practice | Tick where applicable | How often | Done by (Owner/ labourer) |
|---|---|---|---|
| Removal of excess suckers | | | |
| Weeding | | | |
| Removal of dry leaves | | | |
| Removing male buds | | | |
| Harvesting green leaves/stem for fodder | | | |
| Manure application | | | |

DISEASE INCIDENCE (%) AND FARM MANAGEMENT SCORING SHEET

| Mat | Clone name | BXW | F W | Sigatoka | Cigar end rot | Weevils | Streak virus | Nematodes | Nutritional status | Sanitation (1-5) | General management |
|---|---|---|---|---|---|---|---|---|---|---|---|
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CONSTRAINTS TO BANANA PRODUCTION

| Constraint | Tick if yes | Propose a solution |
|---|---|---|
| Scarcity of preferred planting material | | |
| Pests and diseases | | |
| Declining soil fertility | | |
| Marketing of banana produce | | |
| Poor transport system | | |
| Inadequate extension support | | |
| Drought and water scarcity | | |
| Lack of information on banana | | |
| Lack of pesticides and fertilizers | | |
| High cost of inputs | | |
| Scarcity of farming land | | |

**Appendix 4: Photo card**

Fusarium wilt (Discoloured stem and chlorotic leaves)

Cigar end rot Armirallia corm rot

Banana xanthomonas wilt (Uneven ripening and bacterial ooze in the fingers and chlorotic leaves)

Tunneling by Banana weevil and adult weevils

Toppling over by parasitic nematodes

Banana streak virus