EFFICACY OF SELECTED MICROBIOLOGICAL PRODUCTS ON GROWTH, HEALTH AND NUTRIENT UPTAKE OF TISSUE CULTURED BANANA IN DIFFERENT SOILS IN KENYA

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Efficacy of selected microbiological products on growth, health and nutrient uptake of tissue cultured banana in different soils in kenya

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DECLARATION

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

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DEDICATION

To my beloved husband, John, my children, Joseph, Neema and Baraka for their patience, support, prayers and encouragement

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LIST OF ABBREVIATIONS AND ACRONYMS

AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of variance
BCE	Before the Common Era
CE	Common Era
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
ICRAF	International Centre for Research in Agroforestry
INIBAP	International Network for the Improvement of Bananas and Plantain
ISFM	Integrated Soil Fertility Management
KARI	Kenya Agricultural Research Institute
MOALD	Ministry of Agriculture and Livestock Development
PGPB	Plant Growth Promoting Bacteria
RMD	Relative Mycorrhizal Dependency
TC	Tissue culture
VAM	Vesicular Arbuscular Mycorrhiza

ABSTRACT

Banana and plantains are important food crops whose production is constrained by disease and soil fertility. The use of clean planting materials such as tissue cultured banana could mitigate the losses caused by diseases and pests. However, the low survival rate of plantlets during the acclimatization phase is as a major constraint. Inoculation with beneficial microorganisms is one way of protecting tissue cultured plantlets against environmental stresses, thus improving their growth, health and survival. Green house and field experiments were conducted to evaluate the efficacy of selected commercial microbiological products on survival, growth, nutrition and health of tissue cultured banana. Plantlets were grown in soils sampled from three banana growing regions in Kenya; a Vertisol (Western Kenya-Bondo), Rhodic Ferralsol (Coastal Kenya-Kilifi) and a Humic Nitisol (Central Kenya-Meru south) and inoculated with *Bacillus*, mycorrhizal and *Trichoderma* based products at the acclimatization and nursery phases of growth. A conventional nursery media from Jomo Kenyatta University of Agriculture and Technology was used as a positive control. Survival of inoculated plantlets was recorded at the 8 weeks after deflasking. Growth parameters were assessed every two weeks under green house conditions and every two months under field conditions up to the flowering stage. A destructive harvest was carried out at the end of the nursery phase. Shoots were analysed for plant nutrient uptake using the inductively coupled plasma method. Root dry weights, root number and total root length were assessed. Soils from the harvested pots were used for assessing the suppression of *Fusarium*. Inoculation with *Bacillus* enhanced survival of plants in the Vertisol; mycorrhiza and *Trichoderma* inoculation in the Rhodic Ferralsol and mycorrhiza in the Humic

Nitisol and conventional media. Application of *Bacillus* based products significantly (p 0.05) increased plant growth in the Vertisol and Rhodic Ferralsol in the nursery phase. Application of multiple species mycorrhiza and *Trichoderma* under field conditions significantly (p 0.05) increased apparent volume and leaf surface area in the Vertisol by over 100% and 25% compared to the control and conventional practice respectively. Mixed species *Bacillus* products significantly (p 0.05) enhanced uptake of Phosphorous by over 160 and 400% in the Vertisol and Rhodic Ferralsol respectively. Mycorrhiza inoculation enhanced P uptake by over 170% in the Rhodic Ferralsol and Vertisol respectively. Mixed species mycorrhiza and *Bacillus* significantly (p 0.05) inoculation enhanced potassium, zinc, iron, manganese, sulphur, copper and magnesium uptake in the Rhodic Ferralsol and Vertisol by over 100%. Suppression of *Fusarium oxysporum* f.sp. *cubense* by *Bacillus*, mycorrhizae and *Trichoderma* inoculation differed significantly at p 0.05 within soil types. PHC Biopak, ECO-T and Rhizatech reduced *F. oxysporum* f.sp. *cubense* CFU g^{-1} by 47, 68% and 55% respectively in the Humic Nitisol. ECO-T reduced *Fusarium* CFU g^{-1} by 6% in the Rhodic Ferralsol and PHC Biopak by 50% in the Vertisol compared to the non-inoculated soils. Under nursery conditions, combining Rhizatech and Mavuno and ECO-T and manure increased plant growth by over 100% in the Rhodic Ferralsol and Vertisol respectively. Under field conditions, the combined application of Rhizatech and minjingu rock phosphate enhanced plant growth by 57% in the Rhodic Ferralsol while both sole and combined application of products did not significantly affect plant growth in the Vertisol. This study reveals that tissue cultured (TC) banana plant survival, growth, nutrition and health could be improved by commercial microbiological inoculation.

This is however, dependent on the prevailing soil conditions. The findings from this study could highly benefit nursery management of TC banana plants particularly when nutrient limited soils are used for post flask management. The efficacy of biological inoculants and their interactions on tissue culture banana growth, yield and disease suppression should be investigated in the long term so as to establish the residue effects of the inoculants and determine the timings for repeat applications. This investigation should be done under integrated soil fertility management options that promote proliferation and functioning of microbe-based inoculants, especially under field conditions.

CHAPTER ONE

1.0 INTRODUCTION

Banana is an important crop globally, with the world annual production estimated to be 55 million metric tonnes (FAOSTAT, 2011). It is a source of staple food and critical income to more than 30 million people living in densely populated areas (Karamura *et al.,* 1999). In Kenya, the area under banana increased from 43,000 hectares in 1996 to 82,518 hectares in 2006 (Mbaka *et al*., 2008) increasing the total production to 1,058,018 metric tones valued at over 9 billion Kenya shillings ($ 0.15 billion) were produced (MoA, 2006). Over the decade, however, banana production in Kenya has been on the decline (HCDA, 2008). The crop is faced with several challenges including diseases such as the panama disease, pests such as nematodes, and decline in soil fertility with the depletion of macro and micro nutrients. To mitigate the losses caused by these challenges, several interventions have been employed. These include use of hot water treated suckers, macropropagation through stimulation of lateral growth of latent buds in a corm, micropropagation, use of chemical, organic and biological fertilizers (Kloepper, 1992; Tenkouano *et al.*, 2006; Mbaka *et al.*, 2008).

Micropropagation (synonymously used with the term tissue culture) for example is a successful technique used in the mass-production of plants for commercial and research purposes (Nowak, 1998; Nguthi, 1999; Wambugu and Kiome, 2001; Kahangi, 2003; Mbaka *et al.,* 2008). Tissue cultured banana plantlets are increasingly replacing conventional planting material for the establishment of new or replacement of existing plantations (Wambugu and Kiome, 2001).

Micropropagation offers the advantage of rapid multiplication and production of high quality, disease-free, uniform plants. Nevertheless, micropropagated plants present some inconveniences such as more probability of somaclonal variations (Damasco *et al.,* 1996), poor physiology (George, 1996) and the lack of soil microbiota. It is well accepted that for most micropropagated crops, acclimatization, which is the hardening phase, is the most critical period in the micropropagation process (Vestberg *et al.,* 2002). Symbiotic organisms such as arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) are known to play a fundamental role in sustainable agroecosystems, improving plant performance under environmental stress conditions (Nowak, 1998) and may facilitate plant adaptation to the nursery. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that colonize the roots of most cultivated plant species (Smith and Read, 1997). Mycorrhizal symbiosis is found in nearly all types of ecological situations and most plant species are able to form this symbiosis naturally (Smith and Read, 1997). This association, which normally occurs naturally when plantlets are transplanted into the field, favors plant establishment, enhancement of nutrient uptake and protection against cultural and environmental stresses (Barea *et al.,* 1997). Plant growth-promoting rhizobacteria (PGPR) are able to colonize the root surface, survive and multiply in microhabitats associated with the root surface and promote plant growth (Kloepper, 1996). Their positive effects on plant development or establishment of seedlings have been described for different herbaceous and woody crop species (Caesar and Burr, 1987; Polonenko *et al.,* 1987). Other important contributions such as protection against soilborne plant pathogens or nutrient cycling have been attributed to plant growth-promoting rhizobacteria (Barea *et al.,* 2005). Some

authors have frequently described some strains belonging to *Bacillus*, *Pseudomonas*, *Azospirillum*, *Azotobacter, Enterobacter* and *Serratia* as plant growth-promoting rhizobacteria (Kloepper, 1992). Arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria have been shown to interact during their processes of root colonization (Frey-Klett *et al.,* 2007). Soil microorganisms, particularly plant growth-promoting rhizobacteria, can influence arbuscular mycorrhizal formation and function and consequently, mycorrhizas can affect plant growth-promoting rhizobacterial populations in the rhizosphere (Barea *et al.,* 2004).

Some products, such as rhizobial and mycorrhizal inoculants have been proven to substantially enhance the productivity of specific crops (Handelsman and Stabb, 1996; Giller, 2001; Filion *et al.,* 2003). The development of commercial mycorrhizal inoculants has now become a reality (Blal, 2003). However their integration in agriculture, forestry and land reclamation is not complete. Commercial formulations of a number of inoculants with differing efficacy exist in the market (Kloepper, 1996; Smith and Read, 1997; Nowak, 1998). Though the benefits of application of arbuscular mycorrhizal fungi and *Bacillus* on growth and nutrition of micropropagated banana plants have been reported (Jaizme-Vega *et al.,* 2004; Rodríguez-Romero *et al.,* 2005) in the nursery phase, there is little information on microbiological inoculation of TC banana under field conditions. Further, research has mainly focused on microbiological inoculation of TC banana in special commercial nursery media (Juma, 2011) thus limiting the distribution of TC banana from commercial nurseries. This study was therefore aimed at evaluating the efficacy of microbiological products that are already available in the market and

unveiling the soil-root-microorganism interactive processes that affect TC banana growth, nutrition and health.

1.1 RESEARCH PROBLEM

Decline in soil fertility and increase in soil-borne pests and diseases (Akyeampong and Escalant, 1998; Frison and Sharrock, 1999) are some of the constraints that face tissue culture (TC) banana production. Tissue culture plants are likely to succumb to disease and roots may not establish well in low fertility soil environments. Biological hardening of TC is therefore recommended before transplanting (Smith and Read, 1997; Nowak, 1998).

Some commercial products that enhance plant growth and production exist in the market and the conditions for effective functioning of the products vary (Kloepper, 1996; Smith and Read, 1997; Nowak, 1998). Some products, such as rhizobial and mycorrhizal inoculants have been proven to substantially enhance the productivity of crops such as maize and beans (Handelsman and Stabb, 1996; Giller, 2001; Filion *et al*., 2003). In Kenya, for example, commercial strains of mycorriza are already in use by commercial flower growers and a few maize farmers. Little is reported on the utilization of commercial microbiological products for aclimatization, growth and performance of tissue culture banana under both nursery and field conditions. There is therefore need to evaluate the efficacy of existing commercial products to harness the potential for enhancing tissue culture banana growth, health and nutrition.

1.2 JUSTIFICATION

Inoculation with beneficial microbes is one way of protecting tissue cultured plantlets against environmental stress, thus improving their growth, health and survival (Azcon-Aguillar and Barea, 1997; Monticelli *et al*., 2000; Vestberg *et al*., 2004). The benefits of application of arbuscular mycorrhizal fungi (AMF) and *Bacillus* on growth and nutrition of micropropagated banana plants have been reported in the nursery phase (Jaizme-Vega *et al*., 2004; Rodríguez-Romero *et al*., 2005). Rhizospheric microorganisms such as mycorrhiza, plant-growth promoting bacteria and fungi such as *Bacillus* and *Trichoderma* have the ability to colonize the rhizosphere and help plants to acquire nutrients (Shamsuddin *et al*., 2000; Jaizme-Vega *et al*., 2004; Harman, 2006). Biological control agents such as rhizospheric bacteria (Handelsman and Stabb, 1996) and other saprophytic fungi *(Trichorderma harzianum*, AMF) have been identified as promising candidates to combat various deleterious soil-borne pathogens on different crops (Jaizme-vega *et al*., 1998; Steyaert *et al.,* 2003; Thangavelu *et al*., 2010; Sun *et al*., 2011).

Various isolates of *Bacillus*, *Trichoderma*, mycorrhiza and non-pathogenic endophytes have been demonstrated to promote plant growth, increase nutrient uptake and combat various diseases (Jaizme-Vega *et al.,* 2004; Rodríguez-Romero *et al*., 2005). However, a lot of these strains have not been formulated and packaged into products that can be made available for farmers to apply into agricultural systems, especially in Africa. Further, evaluation of microbes for banana growth has mainly been conducted using sterile nursery media to which special nutrients such as

the Hewitt nutrient mixture (Hewitt, 1966) is added thus limiting decentralization of commercial nurseries.

Banana requires large amounts of nitrogen and potassium followed by phosphorus, calcium and magnesium to maintain high yields (Robinson, 1996; Abdullah *et al.*, 1999). To fulfill the plant demand for nutritional attributes, it is essential to apply those elements in the soil, which mostly comes from inorganic chemical sources. The increased use of chemical fertilizer is undesirable because its production is an energetically costly process and considerable pollution is caused through both the production and use of mineral N-fertilizers. This is exacerbated by the relatively low efficiency of their uptake by plants due to non-extensive root system and may also delete soil organic matter in the long term (Ladha and Reddy, 1995; Ladha *et al.,* 1997; Khan *et al.,* 2007).

Efficacy of some rhizospheric microbes used for constituting biofertilizers such as AMF, *Bacillus* and *Trichoderma* is greatly influenced by the prevailing soil conditions. Nitrogen (N), phosphorous (P) and other nutrients such as zinc, copper and sulphur levels in the soil affect the functioning of these microorganisms (Schubert and Hayman, 1986; Fargasova, 1992; Griffin, 1994; Score and Palfreyman, 1994; Okoth, 2007). Mycorrhizal symbiosis benefits, for example, have been suggested to be optimal at P levels of 50mg kg^{-1} (Schubert and Hayman, 1986). Organic and inorganic fertilizers are used primarily to increase nutrient availability and the type or amount of fertilizer added to soil could directly affect the function performed by the various microbial groups in the soil (Marschner *et al.,* 2003; Ge *et*

al., 2008; He *et al.,* 2008). Mineral nutrition is essential for growth, sporulation and stimulation of fungal secondary metabolism (Griffin, 1994) and combining of mineral and bio fertilizers could greatly benefit both the added microbes and inoculated plant as well as improve soil quality (Jeyabal and Kuppuswamy, 2001; Bedini *et al.,* 2007; Atul-Nayyar *et al.,* 2009; Javaid, 2009). Previous studies have focused on the effect of long term fertilization on indigenous microbial diversity and efficiency (Mäder *et al.,* 2002; Hijri *et al.,* 2006) but little is reported on the effect of combining commercial microbiological strains with fertilizers in nutrient poor soils that have little or no history of fertilization.

There is a wide range of mycorrhizal commercial products in the market, however, additional biological and chemical products are available and can be used to compliment and/or supplement mycorrhizal products (Spadaro and Gullino, 2005). There is however need to validate the efficacy of these products under different soil conditions and integrated soil fertility management (ISFM) options to optimize establishment, growth, nutrition and health of TC bananas.

1.3 OBJECTIVES

1.3.1 Overall objective

To evaluate the efficacy of selected microbiological products on overall performance and growth of tissue culture banana

1.3.2 Specific Objectives

1. To determine the efficacy of selected microbiological products on the survival and growth of tissue cultured banana under three different soil conditions (Vertisol, Humic Nitisol and Rhodic Ferralsol)
2. To determine the effect of selected microbiological products on nutrient uptake of tissue cultured banana plantlets.
3. To determine the effect of selected microbiological products on *Fusarium spp*. associated with banana
4. To determine the effect of integrating microbiological products with fertilizers on growth and performance of tissue cultured banana

1.4 HYPOTHESES

1. Microbiological products have no effect on survival and growth of tissue cultured banana irrespective of soil conditions
2. Inoculation of tissue cultured banana with microbiological products does not affect nutrient uptake
3. Microbiological products have no effect on *Fusarium spp*. associated with banana.
4. Integration of microbiological products with fertilizers does not improve growth and performance of tissue cultured banana

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BANANA

2.1.1 TAXONOMY AND ORIGIN

Bananas belong to the family *Musaceae* in the order *Scitaminaceae* (*Zingiberales). Musaceae* contains only two genera, *Musa* and *Ensete* (Karamura, 1998). The genus *Ensete* differs from *Musa* by being monocarpic, non-suckering with a distinctively swollen base, and having large-sized seeds while *Musa* produces suckers and has small seeds (Cobley and Steele, 1976). The genus *Musa* is divided into five series, based mainly on the chromosome numbers, orientation and arrangement of flowers in the inflorescence. The series are *Musa, Rhodochlamys, Callimusa, Australimusa* and *Ingentimusa* (Argent, 1976; Simmonds and Weatherup, 1990). Series *Musa* is the largest with 13–15 species, the most diversified and considered the most ancient (Karamura, 1998). Among the 15 wild species are *Musa acuminata* and *M. balbisiana* both of which have contributed to the origin of the majority of edible bananas (Karamura, 1998).

The genus *Musa*, originated from the Malaysian region of Southeast Asia, from where it dispersed to other parts of the world (Masanza, 2003). The crop is believed to have reached Africa between the 1st and the 19th century A.D (Masanza, 2003). Simmonds and Shepherd (1955) suggested that edible bananas originated from two wild and seedy species *Musa acuminata* Colla ($2n = 22$), and *Musa balbisiana* Colla ($2n = 22$), resulting in a series of diploid, triploid and tetraploid bananas. The resulting genome groups were classified as AA, AB, AAA, AAB, ABB, AABB,

AAAB, and ABBB with letters A and B representing the contributions of *M. acuminata* and *M. balbisiana,* respectively.

2.1.2 BOTANY

The banana plant is a large herbaceous perennial with an underground stem known as the corm, bulb or rhizome (Karugaba and Kimaru, 1999). The banana roots reach a depth of between15-60cm (Masanza, 2003).

The stem has two main parts: the pseudostem which is an overlap of leaf sheaths and the true stem (flower stalk) which is in the middle of the pseudostem. Each leaf consists of a sheath, petiole and the midrib, which bears the leaf blades. The number of leaves borne on the plant depends on the variety, stage of growth and type of field operations undertaken. Leaf sheaths of successive leaves closely encircle each other and form a cylindrical compact structure, which is the pseudostem (Swennen and Vulysteke, 2001).

After the banana has formed a number of leaves (6 – 8 months), the terminal bud of the corm develops and rises to the top of the pseudostem to form the inflorescence from the centre of the leaf cluster. This then forms the bunch after a few days. The ovaries of the female flowers develop into fruits. The female and male flowers of banana are sterile, thus the ovules do not develop into seeds therefore making them pathenocarpic (Karugaba and Kimaru, 1999). A banana bunch possesses between 6 –12 hands, each of which can have at least 12 fingers.

2.1.3 HISTORY OF CULTIVATION

Bananas were first domesticated by South East Asian farmers. Recent archaeological and palaeoenvironmental evidence at Kuk Swamp in the Western Highlands Province of Papua New Guinea suggests that banana cultivation there goes back to at least 5000 Before the Common Era (BCE), and possibly to 8000 BCE (Denham *et al.,* 2003). It is likely that other species were later and independently domesticated elsewhere in south East Asia. Southeast Asia is the region of primary diversity of the banana. Areas of secondary diversity are found in Africa, indicating a long history of banana cultivation in the region. There is linguistic evidence that bananas were known in Madagascar around that time (Zeller, 2005). The earliest prior evidence indicates that cultivation dates to no earlier than late 6th century Common Era (CE). (Lejju *et al.,* 2006). It is likely, however, that bananas were brought at least to Madagascar if not to the East African coast during the phase of Malagasy colonization of the island from South East Asia century 400 CE (Randrianja, 2009).

2.2 PRODUCTION OF BANANA

Banana is the fourth most valuable food crop in the world after rice, wheat and potatoes (INIBAP, 2005). In 2009, India led the world in banana production, representing approximately 28% of the worldwide crop, mostly for domestic consumption (FAOSTAT, 2009). The leading exporting countries (India, Philipines, China, Ecuador, Brazil and Indonesia) together accounted for about two-thirds of the world's exports with each contributing more than 6 million tons (FAOSTAT, 2009).

In Africa, banana cultivation is mainly in the western, central, east and southern parts of the continent. The leading countries in banana production in Africa include:

Kenya, Uganda, Cameroon and South Africa (FAOSTAT, 2010) and the production quantities are shown in Table 1.

Table 1: World's top banana producing countries

Region	Country	Production (tones)
Latin America and the Caribbean	Ecuador	7,931,060
	Brazil	6,978,310
Asia and the Pacific	India	31,897,900
	Philipines	9,101,340
West and central Africa	Cameroon	950,200
	Côte d'Ivoire	265,000
East and southern Africa	Kenya	791,570
	Uganda	600,000

Source: FAOSTAT, 2010.

In Kenya, the area under banana increased after the mid 1990s (MOA, 2005). Between 1996 and 2007, the area under banana in production in Kenya increased at a compound growth rate of 7.6%/year (HCDA, 2008). The increase in the area under banana production in the later half of the 1990s was accompanied by improvements in average banana yields (MOA, 2005). The main banana producing provinces are Nyanza and Western (64.4 %), whereas Central and Eastern Kenya regions account for 26 % of the total banana production (KARI, 2005). Since early 1990's, banana production in Kenya has been on decline (Mbaka, 2008). This decline has been brought about by the infestation with Panama disease caused by *Fusarium oxysporum f. sp. cubense (Foc)*, Black and Yellow sigatoka caused by *Mycosphaerella fijiensis* (Morelet) and *Mycosphaerella musicola* (Leach) respectively, weevils (*Cosmopolites sordidus*) nematode (*Radopholus similis*) complexes and environmental degradation (Wambugu *et al.,* 2000).

In Rwanda, banana is grown on 213, 000 ha and occupies 23% of the arable land (Mpyisi *et al.,* 2000). However, crop yield is continuously declining throughout the

country (NISR, 2007; MINAGRI, 2008). Banana yields are low (about 8t/ha per year) (MINECOFIN, 2001) within the subsistence production systems which is characterized by low inputs and outputs.

In Uganda, the crop is a staple food in many parts which do not experience a pronounced dry season, such as the areas within 80 km of the shore of Lake Victoria, the south western highlands, the slopes of Mt. Elgon in the east and the well watered areas of the western part of the country (Masanza, 2003).

In Tanzania, banana is a staple food in the high rainfall areas, located in higher altitudes and low lying places around L. Victoria in addition to valley bottoms where they are only a minor constituent of the local diet (Karamura, 1998). There is a wide range of different varieties, with factors such as local tastes, eating habits, marketing considerations, and environmental conditions influencing their distribution (Masanza, 2003).

2.3 IMPORTANCE AND UTILIZATION

Banana and plantain are of major importance to food security as well as providing a valued source of income through local and international trade (Chandler, 1995). In terms of gross value of production, banana and plantain are the fourth most important global food crops (Masanza, 2003). Besides being an important food source, banana also provides fibre and starch. Because they contain vitamin A, banana and plantain act as aids to digestion, and it is reported that boiled, mashed ripe fruit can be good for alleviating constipation, especially when mixed with other recommended plants (Gowen, 1995).

The juice from the male bud provides a remedy for stomach problems, while there are reports of the ripe fruit being used in the treatment of asthma and bronchitis (Gowen, 1995). The peels of ripe bananas have anti-septic properties and can be for treating wounds (Gowen, 1995).

In countries such as Uganda, Burundi and Rwanda, per capita consumption has been estimated at over 200 kg per year, the highest in the world (INIBAP, 1998). Banana and plantain grow in a range of environments and will produce fruit all year-round, thus providing a source of energy during the hungry-period between other crop harvests. As well as being a cheap and easily produced source of energy, they are also rich in vitamins A, C and B6.

The highland bananas reduce soil erosion on slopes and are principal sources of cover crop for maintaining and improving soil fertility (Masanza, 2003). This is because the giant herb (2-8 metres high) with its large leaves easily creates closed canopies, which assist in arresting rain impact and direct insolation, both of which are important in soil conservation (Karamura, 1998). The plant roots also bind soil particles thus reducing soil erosion. The leaves as well as the stems decompose as they are being broken down by microorganisms, which give good aeration to the soil, and this also adds organic matter to the soil.

Banana is classified into four types based on use of their end products as dessert, cooking, beer and roasting banana cultivars. The dessert bananas are consumed raw at ripeness and are usually distinguished by the sweet flavour of the fresh fruit when ripe, for example, Gros Michel, Giant Cavendish group (Karamura, 1998). These

belong to the AAA genome group. The cooking banana cultivars in this group belong to the AAA genome group (for example, Nakitembe, Mbwazirume). Mature fruits are harvested green peeled and cooked before eating. They constitute the majority of the East African highland bananas (Karugaba and Kimaru, 1999). The beer bananas are those whose pulp is bitter and astringent. They cannot be eaten raw or cooked (Karugaba and Kimaru, 1999). However, juice and alcohol can be made from this type of bananas, hence the name. Cultivars in this group belong to the AAB and ABB genomes, e.g. 'Kisubi' and 'Kabula' (Masanza, 2003).

The roasting bananas are plantains in the AAB genome group. There are two types of roasting bananas and these are horn and French plantains. The horn plantains have few fingers on a bunch, fingers are shaped like a horn and the fingers are big in size. The French plantains have many fingers on a bunch and the fingers are small (Karugaba and Kimaru, 1999).

2.4 BANANA PROPAGATION

2.4.1 Conventional propagation

Bananas and plantains (cultivar of *Musa*) are propagated vegetatively rather than sexually because nearly all cultivated varieties are seedless, and fruits develop parthenocarpically. The principal method of banana propagation by small-holder farmers is divison of suckers or pups which arise from the base of the main stem or from the underground corm. Farmers from different regions prefer different size suckers for optimal planting material. Very small pups are called buttons. Large suckers are the preferred planting material. These are removed from vigorous

clumps of banana trees with a spade when at least three feet tall, during warm months.

In Africa, expansion of banana cultivation is greatly hindered by scarcity of high quality seedlings for establishing new and expanding existing plantations. Most farmers depend on natural regeneration of existing banana plants to obtain seedlings, which is a slow process and quite often does not yield adequate suckers. Suckers are also sources of pests and diseases that reduce yield and the life span of newly planted orchards (Robinson, 2007).

2.4.2 Macropropagation

Macropropagation is a relatively new technology for banana seedling production. A banana corm contains several axillary buds with meristems at different ages and stages of development (Tenkouano *et al.*, 2006). Macro propagation involves stimulation of lateral growth of latent buds in a corm within a chamber where temperature and humidity are controlled. Scarification of buds further increases suckering by a factor of 2-10 (Tenkouano *et al.,* 2006). The macropagation technology has only been recently introduced in East Africa.

2.4.3 Plant tissue culture

Plant tissue culture has an important role in the production of many plants. The advantage of this technique is that a small piece of plant can produce hundreds of new *in vitro* plantlets which can be identical to the mother plant. Tissue culture is a successful technique used to establish mass-production of plants for commercial and research purposes (Nowak, 1998; Nguthi, 1999; Wambugu and Kiome 2001; Kahangi 2003; Mbaka *et al.,* 2008). Tissue cultured bananas is increasingly

replacing conventional planting material for the establishment of new or replacement of existing plantations (Wambugu and Kiome, 2001). Micropropagation offers the advantage of rapid multiplication and production of high quality, disease-free, uniform plants (Nowak, 1998). It offers exciting prospects to future improvements in crop and land productivity (Punja, 2001). Nevertheless, micropropagated plants present some inconveniences such as more probability of somaclonal variations (Damasco *et al.,* 1998), poor physiology (George, 1996) and the lack of soil microbiota. It is well accepted that for most micropropagated crops, acclimatization, which is the hardening phase, is the most critical period in the micropropagation process (Vestberg *et al.,* 2002).

Plant propagules produced *via* tissue culture tend to be free from fungal and bacterial diseases (Mink, 1991). While this does not eliminate disease once the plants are in the outdoor environment, it does give the plants a healthier start and a better chance of defence (Hwang and Ko, 1992). However, micropropagated plants are also free of any beneficial microbial inhabitants of roots such as arbuscular mycorrhizal fungi (AMF). These symbiotic organisms are known to improve plant growth and performance under environmental stress conditions (Smith and Read, 1997) and may facilitate plant adaptation to nursery. Early mycorrhiza inoculation during the hardening phase of micropropagated plantlets is beneficial to several tropical species, such as papaya, avocado, pineapple (Jaizme-Vega and Azcón, 1991), and banana (Declerck *et al.,* 1995; Jaizme-Vega and Azcón, 1995; Yano-Melo *et al.,* 1999; Jaizme-Vega *et al.,* 2002).

2.5 BANANA PRODUCTION CONSTRAINTS

Diseases and pests are the major constraints to the productivity of banana and plantain (Elsen, 2002). Major banana pests include the banana nematodes and weevil which attack bananas and plantain and cause damage in neglected plants. Nematodes cause important yield losses in East Africa (Elsen, 2002). Heavy infestation may kill young plants and older ones are easily blown over by the wind (Griesbach, 1992).

Panama disease (*Fusarium* wilt / vascular wilt) is the most serious banana disease. All leaves, even the young ones, die but the pseudostem remains standing until it decays (Elsen, 2002). In Cigar-End Rot disease, the fungus infects the dry flower parts and from there it penetrates the fruit skin. It develops into a dry rot which affects mainly the tip of a fruit (Griesbach, 1992).

2.6 IMPACT OF SOIL AND CLIMATIC FACTORS ON BANANA PRODUCTION

Bananas have been regarded as an adaptable crop within certain, well-defined climates and soil types. The recommended soils for banana are slightly acidic, deep, rich and well drained, such as clay loam or loam soils (Borges *et al.,* 1999). Soil type, compaction, drainage, and variations in climate have a strong influence on the development of the root system (Avilán *et al.,* 1982; Stover and Simmonds, 1987; Robinson, 1996; Barber, 1992; Klepper, 1992; Blomme *et al.,* 2002; Dorel, 2002). Heavy, compact, poorly-drained soils and water tables at depths of less than 1 m severely limit root extension, and yields are depressed accordingly (Stover, 1972; Lassoudière, 1978; Stover and Simmonds, 1987; Robinson, 1996).

Texture, compaction and drainage are the main physical soil characteristics that influence banana growth and development; they can limit effective soil depth or the conditions of water supply and aeration in the rhizosphere. According to Stover and Simmonds (1987), studies carried out in bananas indicate that root distribution, in relation to soil depth, is determined by soil type and drainage conditions and that root growth is stopped or reduced by hardpans, impermeable layers, high clay content or waterlogged areas. Horizons in the soil profile that indicate high water saturation, either permanent or temporary, are indicators of conditions that restrict root growth and can cause necrosis and rotting during very wet spells (Stover and Simmonds, 1987).

Soil fertility is very important for successful cultivation, as banana is a heavy feeder. Since banana is one of the few fruit crops with a restricted root zone, planting depth and drainage are important considerations in selecting the soil type for banana. The soil suitable for banana should be 50cm in depth, rich, well drained, fertile, with good moisture retaining capacity and high in organic matter (Karugaba and Kimaru, 1999). Banana performs well in an acid soil with a pH between 5.5 and 6.5 but is not tolerant to salty soils (Karugaba and Kimaru, 1999).

Banana is essentially a tropical plant requiring a warm and humid climate. However, the crop can do well at a temperature range of 25 – 32°C (Robinson, 1996). Banana grows at sea level up to 2200 meters altitude.

2.6.1 Nutrient requirements

Nitrogen (N) is a key element in banana nutrition and extra nitrogen must be frequently applied on infertile soils since growth and development are promoted by

N (INIBAP, 1998). Nitrogen enhances growth by increasing leaf size and gives the leaves their green colour. Nitrogen therefore enhances fruition (INIBAP, 1998).

Banana plants accumulate the phosphorus (P) they require over a long period and loose relatively little through the fruit (Robinson, 1996). Phosphorus is essential for proper root development and thus better utilization of water and nutrients. It enables the plant to resist diseases. Potassium (K) is the most important element in banana nutrition as it plays an important role in the growth and development of fruits. Its deficiencies are closely correlated with yield reductions. A fully-grown banana plant contains more K than all other minerals combined (Robinson, 1996).

Potassium influences plant growth by regulating water use through regulating stomatal opening and closing. It also regulates protein utilization hence the growth of the plant (Robinson, 1996) and also improves the resistance of plants to diseases and pests by enhancing the thickness of the sclerenchyma tissue. Calcium is important for cell wall formation, absorption of N and reduction of soil acidity. Magnesium is essential for photosynthesis and cell division (Karugaba and Kimaru, 1999).

2.7 BANANA AND MYCORRHIZAL SYMBIOSIS

Banana is a highly mycotrophic plant (Declerck *et al.,* 1995) which forms symbiotic association with many arbuscular mycorrhizal fungal (AMF) species (Adriano-Anaya *et al.,* 2006). The major functions of AMF in the rhizosphere are: (i) nutrient transfer from soil to plant via the extraradical mycelium, (ii) soil structure

improvement via aggregation, and (iii) protection of plants from drought and abiotic stresses and soil root pathogens (Dodd, 2000). Studies on AMF in banana cropping systems have been rare (Adriano-Anaya *et al.,* 2006). Very little research has been conducted to document the presence of AMF especially in East Africa (Jefwa *et al.,* 2008).

Effect of AM fungi on banana plant growth was studied almost entirely in controlled conditions and on young plants (mostly Cavendish cultivars) derived from tissue culture (Yano-Melo *et al.,* 1999; Jaizme-Vega *et al.,* 2002; Thaker and Jasrai, 2002). Banana plants inoculated with AMF had increased growth rate and/or foliar nutrient concentrations. Inoculation with AMF decreased the impact of Al toxicity (Rufyikiri *et al.,* 2000). Inoculations of banana plantlets during the hardening phase (i.e. using micropropagated plantlets) increased root and shoot biomass (Jaizme-Vega and Azcon, 1995; Reyes *et al.,* 1995; Yano-Melo *et al.,* 1999; Jaizme-Vega *et al.,* 2002; 2003; Thaker and Jasrai, 2002), photosynthesis and respiration (Yano-Melo *et al.,* 1999). In many cases, banana plants responded better in terms of biomass increase to the inoculum consisting of *Glomus* species as compared to other genera (*Acaulospora scrobiculata* (Yano-Melo *et al.,* 1999) and *Scutellospora heterogama* (Jaizme-Vega and Azcon, 1995). Identically, within the *Glomus* genus, differences were noted among species (Declerck *et al.,* 1995; Reyes *et al.,* 1995; Jaizme-Vega *et al.,* 2003). Under controlled pot culture conditions, bananas inoculated with *G. macrocarpum* had the highest dry plant biomass increase as compared to those of plants inoculated with *G. mosseae* (Declerck *et al.,* 1995). The effect of the AM fungi on foliar nutrient concentration increase in banana was not always observed.

Jaizme-Vega and Azcon (1995) reported increase of foliar nitrogen (N), phosphorous (P) and potassium (K) in banana plants due to inoculation, while Jaizme-Vega *et al.,* (2003) observed only increase of foliar P. Since the increase of foliar nutrient concentrations in banana is not always achieved, the magnitude of the AMF inoculation effect is measured by biomass increase of the inoculated plant.

In many tropical soils, phosphorus (P) availability is limited due to its fixation (Nogueira *et al*., 2007). Upon root infection and colonisation, mycorrhizal fungi develop an external mycelium which is a bridge connecting the root with the surrounding soil (Toro *et al*., 1997). One of the most dramatic effects of infection by mycorrhizal fungi on the host plant is the increase in P uptake (Koide, 1991) mainly due to the capacity of the mycorrhizal fungi to absorb phosphate from soil and transfer it to the host roots (Asimi *et al*., 1980).

2.7.1 Mycorrhiza and micropropagated plants

A wide range of banana cultivars of all genomic constitutions has been found amenable to *in vitro* culture (Cronauer and Krikorian, 1984). However, micropropagated plants have problems concerning their survival and development, particularly during their acclimatization phase as demonstrated by poor root, shoot and cuticle development (Varma and Schuepp, 1995). Such plantlets however, are delicate and, by their nature, free from pathogenic and beneficial microbes (Nowak, 1998; Dubois *et al*., 2004). They are therefore ideal candidates for study with beneficial microbes.

Arbuscular mycorrhizal fungi are known to increase the vigor of plants by increasing absorption of water and mineral nutrients, especially phosphorus (Schubert and Hayman, 1986). Moreover, AMF can protect host plants from root pathogens and mitigate the effects of extreme variation in temperature, pH and water stress (Dixon and Max, 1987). The mycorrhizal symbiosis is important in the cycling of and access to nutrients (Smith and Read, 2008) and transfer of nutrients from one plant to another (Whittingham and Read, 1982; Haystead *et al*., 1988).

Mycorrhizal symbiosis can also increase plant access to water and nutrients from the soil (McGonigle and Miller, 1996), play an important role in the formation of soil aggregates (Hamel *et al*., 1997), influence plant biodiversity (van der Heijden *et al*., 1998), help protect against pests and diseases (Azcon-Aguilar and Barea, 1997) and improve fitness of plants in polluted environments (Hildebrandt *et al*., 1999).

2.8 BENEFITS OF SOIL-MICROBIOTA INTERACTIONS

Microorganisms living in the rhizosphere can have a neutral, pathogenic or beneficial interaction with their host plant (Whips, 2001; Raaijmakers *et al*., 2008). Mycorrhiza are regarded as tripartite symbioses since they commonly interact with bacteria and other soil organisms producing beneficial effects on plant nutrition and health as well as on soil structure and stability (Frey-Klett *et al*., 2007). It has been shown that mycorrhization has a qualitative effect of plant exudation, which affects the associated microflora and the soil adjacent to the roots (Jones *et al.,* 2004). Although little detailed information is available on the direct impact and interaction of bacteria on mycorrhizal fungi, it has been shown that the germination of mycorrhizal spores can be affected by the presence of some bacteria (Daniels and

Trappe, 1980; Mayo *et al*., 1986; Mosse, 1995). Some of the bacteria associated with arbuscular mycorrhiza fungi (AMF), can improve the mycorrhizal colonization (Barea *et al*., 1998; Budi *et al*., 1999), improve root branching (Gamalero *et al*., 2002), or present antifungal properties (Budi *et al*., 1999).

Plant growth promoting bacteria (PGPBs), as well as AMF, are able to enhance biomass production during the first stages of crops and can contribute to plant adaptation from the in vitro to the post vitro phase (Carletti, 2000). This benefit should be harnessed and utilized for post flask management of micropropagated crops which are increasingly being used for commercial purposes (Nowak, 1998; Nguthi, 1999; Wambugu and Kiome 2001; Kahangi 2003; Mbaka *et al*., 2008). Direct mechanisms such as nutrient solubility or fixation and siderophore or phytohormone production have been reported for some bacterial strains (Kloepper *et al*., 1989 and 1991). It is known that some auxin-type phytohormones (indol-acetic acid, IAA) produced by PGPB strains, promote lateral and primary root elongation and production, with positive effects on root system development (Kloepper *et al*., 1989 and 1991).

Since they share common microhabitats, AMF and PGPBs must interact during the colonization process and/or as rhizosphere microorganisms (Barea, 1997). PGPBs can have a great influence on the establishment of mycorrhizal symbiosis (Barea *et al*., 1998; Budi *et al*., 1999; Toljander *et al*., 2007). Toljander *et al*., 2007, showed that mycorrhizal fungi through their exudates had a direct impact on the soil bacterial community. In the same way, AMF can affect PGPB populations in roots (Rillig *et al*., 2006). Studies by Rillig *et al*. (2006), reviewed differential influence

of different taxa of AMF on associated bacteria. Some of the bacteria associated with AMF can improve the mycorrhizal colonization (Barea *et al*., 1998; Budi *et al*., 1999), improve root branching (Gamalero *et al*., 2002), or present antifungal properties (Budi *et al*., 1999). For example, from the mycorhizosphere of sorghum, a bacterial strain, *Paenibacillus* spp. B2 was isolated and found not only to be able to stimulate mycorrhizal colonization but also produces a molecule with biopesticide properties (Budi *et al*., 1999). Thus, microbial interactions should be taken into account in studies on both the single and cooperative effects of these organisms on root systems (Barea, 1997; 2000).

Banana (*Musa acuminata* Colla AAA), is a monocotyledonous herbaceous species, that shows a great ability to establish mycorrhizal symbiosis (Rizzardi, 1990; Jaizme-Vega *et al*., 1991, 1998, 2002; Declerck *et al*., 1994; Yano-Melo *et al*., 1999). Banana-PGPB associations have also been reported as beneficial (Rodríguez-Romero *et al*., 2003, Jaizme-Vega *et al*., 2004). However, despite the great number of studies on banana, there are not many references concerning the effect of rhizosphere microbiota on banana root health (Jaizme-Vega *et al*., 1994; García-Pérez and Jaizme-Vega, 1997) and their consequences on plant growth and health.

2. 9 MICROBIOLOGICAL INOCULANTS AS BIOCONTROLS, BIOFERTILIZERS AND BIOENHANCERS

2.9.1 Microbiological inoculants as biocontrols

Several microbe-based inoculants have been used as biofertilizers/bioenhancers and biocontrols amongst them *Trichorderma harzianum*, Arbuscular Mycorrhizal Fungi (AM Fungi), *Bacillus, Azotobacter, Azospirillum* (Kloepper, 1992). Recognizing the hazards of fungicides and pesticides to man and the environment, many countries in the world are considering biological control as the best alternative to chemical control of plant diseases and pests (Souto *et al.*, 2004). Plant roots growing in soils are a major source of carbon and energy to microorganisms in the form of root exudates, cells detached from old parts of the root, or the root itself after plant death (Cook and Baker, 1983). Competition for nutrients, primary carbon, nitrogen, and iron may result in biological control of soil borne plant pathogens (Scher *et al.*, 1984). Elad and Baker (1985) and Elad and Chet (1987) reported that carbon sources, either provided by synthetic substances or excreted by plant roots might be involved in the chlamydospore and oospore germination of *Fusarium oxysporum* and *Pythium aphanidermatum*, respectively. Proliferation along the developing rhizosphere is one of the most important trails for antagonistics applied to seed (Cook and Baker, 1983). Most studies in this field have dealt with antagonistic rhizobacteria (Kloepper *et al.*, 1980; Whipps and Lynch, 1983; Ordentlich *et al.*, 1987), but there is relatively little information involving fungi.

Biological control of soilborne plant pathogens has been achieved by seed treatment with antagonists. Harman *et al.*, (1980), reported the biocontrol of *Rhizoctonia*

solani and *Pythium* spp. by coating radish and pea seed with *Trichoderma hamatum* (Bain). Also, Hadar *et al*., (1979) and Elad *et al*., (1980) reported that the application of wheat bran colonized by *Trichorderrma harzianum* to soils infested by *Rhizoctonia solani* and *Sclerotium rolfsii* reduced the incidence of disease caused by these pathogens in beans.

Several reports have also been made on *Bacillus subtilis* potential as a biological control agent against plant pathogenic fungi and bacteria (Ferreira *et al*., 1991). Baker and Cook, (1982) reported that *B. subtilis* was able to reduce the incidence of bean rust caused by *Uromyces appendiculatus* up to 74 % under field conditions. It has also been reported that some *Bacillus* strains showed significant inhibition activity against *Botrytis cinerea* (Walker *et al*., 1998), *Puccinia pelargonii-zonalis* germination (Rytter *et al*., 1989) and *Fusarium oxysporum* (Lang *et al*., 2002) under greenhouse experiments. The proposed mechanisms resulting in biocontrol are competition for substrate (Sivan and Chet, 1989), ability to colonize the niche favored by the pathogen, antagonism by antibiotics, antibiosis (McKeen *et al*., 1986) and action of cell-wall degrading enzymes (Chet *et al*., 1998). Fiddaman and Rossall (1993) suggested that another potential mode of action might lie with the production of antifungal metabolites which was antagonistic to the growth of *Sclerotium rolfsii*.

Trichoderma spp are well documented as effective biological control agents of plant diseases caused by soilborne fungi (Sivan *et al*., 1984 and Coley –Smith *et al*., 1991). Applications of *Trichoderma koningii* (Tsahouridou and Thanassoulopoulos, 2002), *T. harzianum* (Lima *et al*., 1999) and *Serratia marcescen* (Ordentlich *et al*., 1987) were found to be effective in controlling *S. rolfsii* via secretion of chitinase.

Pesticides and organic compounds are widely used to control plant pathogens in many countries. However, the degradation of such compounds is very difficult and the concentration and/or accumulation of them in food chains are leading to higher toxicity in animals (Chet, 1987) and Lynch, 1990. *Trichoderma* spp have recently been used as biological control agents and their isolates have been commercially available of late (Elad *et al.*, 1980; Basim *et al.*, 1999; Küçük 2000). *Trichoderma* spp, have therefore been studied as biological control agents against soil-borne pathogenic fungi (Henis 1984; Chet and Baker, 1980; Sivan and Chet, 1993; Küçük 2000). Results from different studies showed that several strains of *Trichoderma* had a significant effect in reducing plant diseases caused by pathogens such as *Rhizoctonia solani*, *Sclerotium rofsii*, *Pythium aphanidermatium*, *Fusarium oxysporum, F. culmorum* and *Gaeumannomyces graminis* var. *tritici* under green house and field conditions (Chet and Baker, 1981; Whipps 1987; Sivan and Chet 1993; Chet and Inbar, 1994; Inbar *et al.*, 1994; Basim *et al.*, 1999; Küçük 2000). Knowledge concerning these fungi as antagonists is essential for their effective use because they can act against pathogens in several ways (Chet, 1987; Küçük 2000). Isolates of *Trichoderma* can produce lytic enzymes (Haran *et al.*, 1996; Küçük 2000) and antifungal antibiotics (Dennis and Webster 1971; Brewer *et al.*, 1987; Almassi *et al.*, 1991) and they can also be competitors of fungal pathogens (Whipps, 1987), and promote plant growth (Inbar *et al.*, 1994).

2.9.1.1 Biocontrols and suppression of *Fusarium* wilt of banana

Rhizospheric and endophytic microorganisms employ various mechanisms to combat *Fusarium oxysporum* f.sp. *Cubense (Foc)* and reduce disease incidence and severity. Mycorrhizal mode of action mechanisms that mediated bioprotection have

been reported as direct competition (Xavier and Boyetchko, 2004), alteration in plant growth, nutrition and morphology (Copetta *et al*., 2006), biochemical and molecular changes in mycorrhizal plants that induce plant resistance to pathogen infestation (Vierheilig 2004; Hause *et al*., 2007; Pozo and Azcon-Aguilar, 2007), alterations in the soil microbiota and development of pathogen antagonism (Garcia-Garrido and Vierheilig, 2007; Meixner *et al*., 2007) and systemic alterations of root exudates thus affecting microconidia germination of soil-borne *F. oxysporum* (Scheffknecht *et al*., 2006; 2007)

Bacillus strain, (KY-21) isolated from the soil of banana's rhizosphere and tested against *Foc* both under *in-vitro* and *in-vivo* conditions affected *Foc* mycelium growth by deforming the tips of the hypha into spherical structures that were remarkably constricted by dual culture *in-vitro* and reduced disease severity *in-vivo,* 60 days after *Foc* inoculation (Sun *et al*., 2011). Some of the suggested mechanisms of *Bacillus* antagonistic mechanisms against *Foc* and which could lead to disease suppression include increased activities of polyphenol oxidase and peroxidase (Sun *et al*., 2011), production of a wide range of antifungal compounds which can degrade fungal cell wall (Berg *et al*., 2001; Leelasuphakul *et al*., 2006), competition for nutrients and space and the induction of plant resistance (Van loon *et al.,* 1998; Guerra-Cantera *et al*., 2005). *Trichoderma* spp, have been studied as biological control agents against soil-borne pathogenic fungi (Chet and Baker, 1980; Henis, 1984; Küçük 2000; Thangavelu *et al*., 2004). Control of plant pathogens including *Fusarium* spp. by *Trichoderma* spp. has been reported to be as a result of induced systemic or localized resistance which occurs due to the interaction of bioactive molecules such as proteins avr-like proteins and cell wall fragments released by the

action of extracellular enzymes during mycoparasitic reaction (Thangavelu and Musataffa, 2010). Thangavelu and Musataffa, (2010) reported that the application of *T. viride* as rice chaffy grain formulation and challenge inoculation with *Foc* in cv. *Rasthali* resulted in the induction of defense related enzymes such as Peroxidase and Penylalanine Ammonia lyase and also the total phenolic content significantly higher (>50%) as compared to control and *Foc* alone inoculated banana plants and the induction was maximum at 4-6th day after treatment. Morpurgo *et al*. (1994) reported that the activity of peroxidase was at least five times higher in the roots and corm tissues of *Foc* resistant banana variety than in the susceptible variety. Technological advancement in production of commercial products of AMF, *Trichoderma* amd *Bacillus* are in place. Commercial products exist in the African market but have never been evaluated for their ability to suppress *Foc*.

2.9.1.2 Molecular characterization of *Fusarium*

Recent advent of multilocus phylogenetic methods has allowed for the objective identification of species boundaries in *Fusarium* (Taylor *et al.,* 2000). Relationships among well-defined *Fusarium* species have been inferred, showing a great deal of species diversity that was vastly under-estimated by all previous morphological treatments (O'Donnell *et al.,* 1998a, b, c; Aoki and O'Donnell, 1999; O'Donnell, 2000; Geiser *et al.,* 2001; Ward *et al.,* 2002).

Species newly identified using phylogenetic methods are generally difficult or impossible to identify using conventional morphological traits (Aoki *et al.,* 2003). The markers of choice for species-level phylogenetics in fungi are intron-rich portions of protein coding genes (Geiser, 2003). These gene regions tend to evolve

at a rate higher than that which is observed at the species level in more commonly applied markers such as the internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA gene repeat (O'Donnell et al., 1998a; O'Donnell, 2000). Moreover, many *fusaria* within the Gibberella clade possess non-orthologous copies of the ITS2, which can lead to incorrect phylogenetic inferences (O'Donnell and Cigelnik, 1997; O'Donnell et al., 1998a). The translation elongation factor 1-*a* (TEF) gene, which encodes an essential part of the protein translation machinery, has high phylogenetic utility because it is (i) highly informative at the species level in *Fusarium*; (ii) non-orthologous copies of the gene have not been detected in the genus; and (iii) universal primers have been designed that work across the phylogenetic breadth of the genus. This gene was first used as a phylogenetic marker to infer species- and generic-level relationships among *Lepidoptera* (Cho *et al.,* 1995; Mitchell *et al.,* 1997). Primers were first developed in the fungi to investigate lineages within the *F. oxysporum* complex (O'Donnell *et al.,* 1998c). The ef1 and ef2 primers were designed based on sites shared in exons between *Trichoderma reesei* (Hypocreales/Sordariomycetes/Pezizomycotina/ Ascomycota) and *Histoplasma capsulatum* (Eurotiales/ Eurotiomycetes/Pezizomycotina/Ascomycota), and they can be applied to a wide variety of filamentous ascomycetes. These primers amplify a ~700 bp region of TEF, flanking three introns that total over half of the amplicon's length, in all known *fusaria*. This gene appears to be consistently single-copy in *Fusarium*, and it shows a high level of sequence polymorphism among closely related species, even in comparison to the intron-rich portions of protein-coding genes such as calmodulin, beta-tubulin and histone H3. For these reasons,

TEF has become the marker of choice as a single-locus identification tool in *Fusarium*.

2.9.2 Influence of microbiological inoculants on nutrient uptake and plant growth

Microbes such as Arbuscular mycorrhizal fungi (AMF) and plant-growth promoting bacteria (PGPB) help the host plant under limiting conditions provoked by abiotic and/or biotic factors (Egamberdiyeva, 2007; Schubert and Hayman, 1986). Mycorrhizal symbiosis significantly improves plant nutrition under low fertility soil conditions such as low P (Schubert and Hayman, 1986). Mycorrhizal hyphae are more efficient than roots alone in nutrient uptake, especially of elements with low mobility in soil such as P. Studies have also reported changes in phytohormone balance (Drüge and Schönbeck, 1992) and the ability of AMF to change root architecture Jaizme-Vega *et al*., 2004). These changes lead to more efficient nutrient uptake in mycorrhizal plants (Hooker and Atkinson, 1992). In many tropical soils, P availability is limited due to its fixation (Nogueira *et al*., 2007). Upon root infection and colonisation, mycorrhizal fungi develop an external mycelium which is a bridge connecting the root with the surrounding soil (Toro *et al*., 1997) and helps the host plant to take up P from the soil (Koide, 1991; Asimi *et al*., 1980).

Direct mechanisms such as nutrient solubility or fixation and siderophore or phytohormone production have been reported for some bacterial strains (Kloepper *et al*., 1989 and 1991). Enhanced mineral uptake in inoculated cereal plant was proposed as a possible mechanism of plant growth enhancement by plant growth promoting rhizobacteria (Lin *et al*., 1983). The major element involved was

suggested to be N in the form of nitrate in wheat, sorghum and corn plants (Lin *et al*., 1983; Kapulnik *et al*., 1985a; Ferreira *et al*., 1987; Boddey and Dobereiner, 1988; Kucey, 1988) or ammonium in rice plants (Murty and Ladha, 1988). Other elements such as P and K were also suggested to play a key role in this plant-bacterium interaction. Shamsuddin *et al*. (2000) found increased amounts of P and K uptake in banana plants inoculated with PGPB. Jaizme-vega *et al*. (2004), evaluating the effect of the interaction between *Glomus manihotis* and with a mix of rhizosphere *Bacillus* spp on root morphology of three micropropagated banana cultivars ('Grande Naine', 'Dwarf Cavendish' and ITC 283) in the nursery stage concluded that either mycorrhizae and *Bacillus* or their interactions promote positive banana root system development that leads to an improvement in plant nutrition and health.

Altomare *et al*. (1999) and Yedida *et al*. (1999) noted that *Trichoderma harzianum* increases the solubility of phosphate and micronutrients such as Zn, Cu, Fe and Mn ions, all plant nutrients with low solubility that have important role in plant growth (Altomare *et al*., 1999). *Trichoderma* are also known for secretion of exogenous enzymes, siderophores (Jalal *et al*., 1987) and vitamins (Kleifeld and Chet, 1992; Inbar *et al*., 1994), as well as for their indirect control of root infesting pathogens (Harman *et al*., 2004) in the rhizosphere. Some of these mechanisms indicate multiple modes of action (Harman *et al*., 2004; Harman, 2006) that lead to increase nutrient availability and uptake by the plant and thereby developing the root system.

2.9.3 Integration of microbiological inoculants with fertilizers

Inoculant biofertilizers are more environmentally sound and their introduction in agricultural production systems could be one of the means to mitigate the onset of global warming as well as the reduction in fertilizer input costs of farmers (Kennedy *et al*., 2004).

Arbuscular mycorrhizal (AM symbiosis) provides numerous services to crops (Gianinazzi *et al*., 2010), including efficient use of fertilizer and soil nutrients (Javaid 2009), protection against drought stress (Porcel *et al*., 2007; Aroca *et al*., 2008; Yin *et al*., 2010) and diseases (Shaul *et al*., 1999; Liu *et al*., 2007), increased N-fixation in legumes (Barea and Azcón-Aguilar, 1983; Haselwandter and Bowen, 1996), and improved soil physical properties (Hallett *et al*., 2009). The AM symbiosis is best known for improving plant access to mineral nutrients, including N, P (Liu *et al*., 2000a), K, Ca, Mg (Liu *et al*., 2002), Fe, Cu, Zn, and Mn (Liu *et al*., 2000). Plants tapping into soil AM hyphal networks access a pool of nutrients mobilized by extraradical AM hyphae (Atul-Nayyar *et al*., 2009).

Organic and inorganic fertilizers are used primarily to increase nutrient availability and yield of crops. Any changes in the type or amount of fertilizer entering the soil could directly affect the soil bacterial and fungal community structure and the function performed by the various microbial groups in the soil (Marschner *et al*., 2003; Ge *et al*., 2008; He *et al*., 2008). However, the direct influence and response pattern of AMF to such changes has not been well understood thus far. One study revealed that spore density and diversity of AMF were significantly higher in organic than in conventional systems (Oehl *et al*., 2004); another study showed that

colonization of roots was increased by repeated use of phosphorus (P) fertilizer (Graham and Abbott, 2000); while it was recently reported that the AMF community was not affected by long-term P fertilization, although soil fungal and bacterial diversity changed greatly (Beauregard *et al*., 2009). Long-term heavy application of mineral nitrogen (N) under field conditions has been shown to reduce the AM colonization of roots, soil microbial activity (Mäder *et al*., 2002), and spore abundance of some study indicated that N-fertilization increased soil AMF hyphae, Glomalin-related soil protein (GRSP) pools and water-stable macro-aggregates (Wilson *et al*., 2009). GRSP, as an alkaline-soluble protein material linked to AMF (Wright and Upadhyaya 1996), is a component of the hyphal wall (Driver *et al*., 2005) that accumulates in soils (Rillig *et al*., 2002). Based on the good correlation between GRSP and spore biovolume, it has been suggested that it could be used as a representative biochemical parameter for the assessment of biological soil fertility in sustainable agriculture (Bedini *et al*., 2007). Considering that AMF may be influenced by different agricultural management systems, whether GRSP can be used as a common parameter for assessing soil fertility still needs to be further examined.

Though agricultural operations represent intensive disturbance to agro-ecosystems in general, a previous study of different management intensities in five agricultural field sites suggested that the diversity of AMF is not always low in arable soils, and low-input agriculture involving crop rotation is important for AMF diversity preservation (Hijri *et al*., 2006). Recent studies have provided some fascinating insights into the structure and diversity of AMF communities in farmland, and have distinguished a wide range of biotic and abiotic factors influencing AMF diversity

and community such as crops, locations and seasons (Oehl *et al*., 2005; Mathimaran *et al*., 2007; Toljander *et al*., 2008).

Mineral nutrition is essential for growth, sporulation and stimulation of fungal secondary metabolism (Griffin, 1994). High total N availability increased sporulation, production of antifungal anthroquinone pigments, hyphal growth rate (Fargasova, 1992), and antagonistic activity of *Trichoderma spp*. against wood rot fungus *Serpula lacrymans* (Score and Palfreyman, 1994). Soil nitrate levels were positively correlated with cellulose production (Widden and Breil, 1988) and may favor competitiveness of the bio-control agent with the pathogen. Magnesium increased growth of *T. viride* (Shukla and Mishra, 1970), and copper enhanced conidiogenesis and biomass nitrogen in other hyphomucetes (Ismail *et al*., 1991; King *et al*., 1982).

A lot of studies have focused on the effect of longterm fertilization on indigenous AMF diversity and efficiency but little is reported on the effect of combining commercial strains of AMF with fertilizers in nutrient poor soils that have no history of fertilization. Although several formulations of mycorrhiza and *Trichoderma* are available in the market, their efficacy on plant growth is variable depending on the soil nutrient levels. Low nitrogen (N) and phosphorous (P) and other nutrients such as zinc, copper and sulphur levels in the soil may limit the functioning of these microorganisms. This study aimed to validate the efficacy of selected microbe-based commercial products on survival, growth, health and nutrient uptake of tissue cultured banana cv. Gros michel under differing soil conditions in Kenya.

CHAPTER THREE

SURVIVAL AND GROWTH OF TISSUE CULTURED BANANA INOCULATED WITH MICROBIOLOGICAL PRODUCTS IN THREE SOIL TYPES

ABSTRACT

The effect of inoculation of microbiological products on post-flask management and field establishment of tissue cultured (TC) banana plantlets was investigated. Tissue cultured banana cv. Gros Michel plantlets were inoculated with *Bacillus*, mycorrhizal and *Trichoderma* based products in a Vertisol, Humic Nitisol, Rhodic Ferralsol and conventional nursery medium as a positive control. Initial inoculation of plants with products was done at the acclimatization phase and subsequently at the potting phase. Survival of inoculated plantlets was recorded at the end of the acclimatization phase and 8 weeks after deflasking. The effect of these products on growth was evaluated as the ability to enhance height and girth of pseudostem, leaf length, leaf width, number of functional leaves and root and shoot biomass yield. The efficacy of products on survival of plants at hardening was variable and depended on soil type. Inoculation with *Bacillus* enhanced survival of plants in the Vertisol, mycorrhiza and *Trichoderma* inoculation in the Rhodic Ferralsol and mycorrhiza in the Humic Nitisol and conventional medium. Performance of inoculated plants was dependent on soil type. Application of *Bacillus* based products significantly increased plant growth (leaf length, leaf width, plant height, dry shoot weight) in the Vertisol and Rhodic Ferralsol in the nursery phase. Application of multiple mycorrhizal species and *Trichoderma* under field conditions significantly

increased plant growth (apparent volume and leaf surface area) in the Vertisol by over 100% and 25% compared to the control and conventional practice, respectively. Results demonstrate that tissue cultured bananas can benefit from application of arbuscular mycorrhizal fungi, *Trichoderma* and *Bacillus* to improve survival and growth during the nursery phase as well as enhance plant performance under field conditions. The vertisol was most receptive to mixed *Bacillus* species inoculation under nursery conditions and mixed mycorrhizal species and *Trichoderma* inoculation under field conditions as evidenced by plant growth.

3.1 INTRODUCTION

Banana and plantains are important food crops whose production is largely constrained by disease and soil fertility (Akyeampong and Escalant, 1998; Frison and Sharrock, 1999). The use of clean planting materials such as tissue cultured banana could mitigate the losses caused by diseases and pests (Mink, 1991; Robinson 1996; Kahangi 2003; Mbaka *et al.*, 2008). However, the low survival rate of plantlets during the acclimatization phase is a major constraint (Cassalls and O'Herlihy, 2003). Unlike suckers that have a corm which sustains growth at establishment, tissue cultured bananas have inadequate food reserves, delicate root systems and are devoid of beneficial microbial associations to sustain their initial establishment, hence predisposing the plantlets to water stress and inadequate nutrient uptake. Inoculation with beneficial microbes is one way of protecting tissue cultured plantlets against environmental stresses, thus improving their growth, health and survival (Azcon-Aguillar and Barea, 1997; Monticelli *et al.*, 2000; Vestberg *et al.*, 2004). The benefits of application of arbuscular mycorrhizal fungi (AM fungi) and *Bacillus* on growth and nutrition of micropropagated banana plants have been reported in the nursery phase (Jaizme-Vega *et al.*, 2004 and Rodríguez-Romero *et al.*, 2005). The inoculation in banana nursery showed better growth and seedling health and consequently increased seedling survival (Jaizme-Vega *et al.*, 2004). AM fungi are known to improve plant growth and performance under environmental stress conditions (Smith and Read, 1997) and may facilitate plant adaptation to nursery (reviewed by Rai, 2001).

It is known that some auxin-type phytohormones (indole-acetic acid, IAA) produced by plant growth promoting bacterial (PGPB) strains, promote lateral and primary root elongation and production, with positive effects on root system development (Kloepper *et al*., 1989 and 1991) and promotion of plant growth (Kloepper, 1996). Significant increases in crop yields following application of PGPB have been documented under diverse field conditions (Bashan, 1998). *Trichoderma* spp. have been reported to promote plant growth (Inbar *et al*., 1994) in addition to their biocontrol activities (Dennis and Webster 1971; Whipps, 1987; De La Cruz *et al*., 1992; Haran *et al*., 1996). In a field experiment, an indigenous *Trichoderma harzianum isolate* promoted growth of primary root length and root branching in maize and beans by inducing lateral root growth (Okoth *et al*., 2011).

Previous research has focused on isolation and evaluation of indigenous microbes on their efficacy on banana plant growth, health and nutrition. Further, evaluation of microbes for banana growth has mainly been conducted using sterile nursery media in to which special nutrients such as the Hewitt nutrient mixture (Hewitt, 1966) is added thus limiting decentralization of commercial nurseries. A wide range of commercial products that enhance plant growth and production exist in the markets and the conditions of effective functioning of the products are variable (Kloepper, 1996; Smith and Read, 1997; Nowak, 1998). The emerging commercial products need to be evaluated under different soil conditions to understand their functionality in post flask management and subsequent field establishment and performance of TC banana.

This study aimed to validate the efficacy of selected microbe-based commercial products on survival and growth of banana cv. Gros Michel under different soil types.

3.2 MATERIALS AND METHODS

3.2.1 Source of tissue cultured materials and soil properties

Tissue culture banana plantlets cv. Gros Michel, were obtained from Jomo Kenyatta University of Agriculture and Technology (JKUAT) Biotechnology laboratory. Gros Michel is the most preferred banana cultivar and one of the most affected by *Fusarium* wilt disease in Kenya (Kungu, 1998) and was therefore used in this study.

The experimental soils (Vertisol, Humic Nitisol and Rhodic Ferralsol) were sampled from three banana growing regions in Kenya namely, Western Kenya (Bondo), Central Kenya (Meru South) and Coastal Kenya (Kilifi) (Table 2) at a depth of 0-20cm and used for hardening and potting of tissue culture plantlets. The 0 to 20 cm soil depth was chosen for microbiological considerations since it contains the majority of soil microbiota (Skujins, 1984). The three banana growing regions were also selected based on reported occurrence of *Fusarium* wilt of banana (Kung'u, 1998). Soil nutrient composition (Nitrogen, Phosphorous, Potassium, Carbon, Magnesium, Calcium, Sodium), Cation Exchange Capacity (CEC), pH and soil texture composition (% Clay, % Sand and % Silt) were determined according to standard procedures (Anderson and Ingram, 1993; Okalebo *et al*., 2002). The initial soil characteristics are described in Table 3.

Table 2: Description of soils used for hardening and potting of tissue culture banana

Site of soil collection	Description of soil
Western Kenya (Bondo)	Vertisol: dark montmorillonite with swelling properties, heavy cracking when dry; poorly drained, leaching of weathering minerals limited, high in Ca and Mg, pH. Low hydraulic conductivity; N, P and micronutrient deficient; no P-fixation (FAO, 2006).
Coastal Kenya (Kilifi)	Rhodic Ferralsol: developed on limestone, reddish brown to weak red top soil, weak to moderate texture silty clay texture with low nutrient status, especially low in N and P. Deeply weathered soil, moderately fertile, texture loamy sandy with slightly acid pH (FAO, 2006).
Central Kenya (Meru South)	Humic Nitisol: deep, well weathered with moderate to high inherent fertility (FAO, 2006).
Conventional medium	Solarized forest soil with manure, Diammonium phosphate (DAP), and Morcarp.

Table 3: Initial soil characteristics (0-20 cm) of Rhodic Ferralsol, Vertisol, and Humic Nitisol used for establishing tissue culture banana under greenhouse conditions

Soil property*	Units	Rhodic Ferralsol	Vertisol	Humic Nitisol	Conventional nursery medium
pH (H_2O)		6.9	5.87	5.68	6.36
OlsenP	(mg kg^{-1})	7	3	8.5	56
K	($cmol_ckg^{-1}$)	0.6	0.82	0.88	5.96
Ca	($cmol_ckg^{-1}$)	4	25.99	6.98	7.38
Mg	($cmol_ckg^{-1}$)	1.7	12.53	2.54	2.71
Na	($cmol_ckg^{-1}$)	0.1	0.19	0.08	0.35
ECEC	($cmol_ckg^{-1}$)	7	52.5	15	19
N	(%)	0.1	0.25	0.23	0.611
C	(%)	1	3.67	2.63	5.757
Clay	(%)	19.7	54.7	37.7	
Sand	(%)	76.3	20.3	42.4	
Silt	(%)	4.0	25	19.9	

*Determined according to the procedures of Anderson and Ingram, 1993 and Okalebo *et al.*, 2002. $cmol_ckg^{-1}$- centimoles of charge per kg of soil.

3.2.2 Green house experiment

A factorial experiment comprising of six commercial products and four soil media was set up under green house conditions. Each treatment was replicated 3 times (10 plants per replicate measured separately). Control treatments (no product applied) also consisting of 3 replicates for each soil condition were also included. The experimental units were arranged in a completely randomized design in a green house. The microbe-based products are described in Table 4. Application of products was done as recommended by the manufacturers. Application rates per plant corresponded to the recommended rate in the field, converted using the medium planting density for banana (2500 plants ha^{-1}). The area of pots used for establishing plants was considered when determining the exact amount of inoculum to be added per pot. The mode of application of products is described in Table 4. ECO-T was applied at 1.25g/L, Subtilex at 0.16g/plant, Rhizatech at 2g/plant, MYCOR at 0.29g/plant, PHC Colonize AG and PHC Biopak at 0.8g/plant. The products were added to three soil types (Vertisol, Humic Nitisol and Rhodic Ferralsol) and a conventional nursery media (sterilized forest soil with manure, di-ammonium phosphate (DAP) and Morcarp). The conventional nursery medium was mixed as three parts forest soil plus one part manure plus 100g DAP plus 30g MORCAP. Three replicates consisting of 10 plants per replicate were considered per treatment.

Table 4: Description of microbe-based commercial products used in the experiments

Product	Manufacturer	Composition	Asserted non-active ingredients	Dose	Mode of application
PHC Biopak	Plant Health Care, Inc., USA	*Bacillus azotofixans*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus polymyxa*, *Bacillus subtilis*, *Bacillus thuringiensis* (7.5 x 10^9 CFU/g each)	Maltodextrin (13.5%), yeast extract (5.5%) soluble seaweed extract (24%), humic acids (31%), precipitated silica (11%), leonardite extract (14%), polyethylene glycol (1%)	2 kg ha^{-1}	Drench application to planting soil
PHC Colonize AG	Plant Health Care, Inc., USA	*Paenibacillus azotofixans, Bacillus licheniformis, B. megaterium, B. polymyxa, B. subtilis, B. thuringiensis* (5 x 10^7 CFU/g each), *Trichoderma harzianum* (5 x 10^9 CFU/g)	Maltodextrin (50%), yeast extract (5%), soluble seaweed extract (14%), humic acids (19%), precipitated silica (2%), yucca plant extract (1%), Non-humic acid components of leonardite (7%), polyethylene glycol (1%)	1.5 kg per 100 litres of water (4 L ha^{-1})	Drench application to soil.
ECO-T	Plant Health Products (Pty) Ltd. South Africa Distributed by Lachlan Ltd. Kenya	Spores of *Trichoderma harzianum* strain Rifai KRL AG2 (4.8%)	Kaolin clay (95.2%)	0.25g per $3m^2$	Dry powder applied to soil
Rhizatech	Dudutech (K) Ltd. Kenya	Spores and mycelial fragments of arbuscular mycorrhizal fungi species: *Glomus mosseae*, *G. etunicatum, G. intraradices and G. aggregatum).* (50 propagules/cm^3)	Not specified	60 kg ha^{-1}	Granules applied to soil
MYCOR	Iftech France	*Glomus intraradices* 200,000 propagules per gram	Un-specified carrier	15g per tray	Dry powder applied to soil
Subtilex	Becker Underwood USA	*Bacillus subtilis*, MBI 600 strain (5.5 x 10^{10} spores/mL, 2.75%)	Inert ingredients (97.25%)	400g per 200 m^3	Dry powder applied to soil

CFU- colony-forming units; PHC Colonize AG coded as PHC Colonize.

3.2.3 Inoculation process

Inoculation of plantlets with products was done at the hardening and subsequently at potting phase. Plantlets with three fully developed leaves in nutrient agar (Murashige and Skoog, 1962) were transferred to small 8 cm^3 pots in sterilized soils (Vertisol, Humic Nitisol and Rhodic Ferralsol) and conventional nursery media at hardening stage. Planting pots were three quarter filled with soil and inoculants added before planting. Pots were then filled up with soil to avoid exposing the delicate plantlet roots. Soils were sterilized at 80°C for 30 minutes following the mycorrhizal training manual prepared from the Centre for Ecology and Hydrology, Peniciuk, UK (Ingleby, 2007). Soils were left to cool for one hour before repeating the procedure. The plants were maintained under high humidity conditions of 60-80%. Average minimum and maximum temperatures recorded in the greenhouse during plant growth were 38°C during the day and 18°C during the night. The plants were maintained under natural lighting conditions only. To minimize contamination, each treatment was planted in separate trays. The trays were placed under high humidity boxes. Misting was done regularly to maintain the humidity. After a month, the boxes were gradually opened for a period of two weeks after which the cover was completely removed.

Eight weeks after deflasking, plantlets were transferred to 2L pots, re-inoculated and maintained under normal open nursery conditions at an average minimum and maximum temperature of 18°C and 38°C respectively. Plants were watered when necessary to field capacity. Three replicates were considered per treatment and each consisted of 10 plants planted in separate pots.

3.2.4 Assessment of survival and growth parameters

The number of surviving plantlets per treatment was recorded at 8 weeks after deflasking. Plant height and number of functional leaves was evaluated at 4, 8, 12, 16, 18 and 22 weeks after deflasking. The number of functional leaves was evaluated by physically counting the number of leaves which had no signs of chlorosis and necrosis. Leaf length and width of the second leaf from the sword shoot were evaluated at 12, 16, 18 and 22 weeks after deflasking. Plant girth was measured at 2cm from the base of the plant using a vernier caliper at 22 weeks after deflasking.

3.2.5 Assessment of shoot and root biomass

Three plants were destructively harvested from each treatment at 22 weeks after de-flasking (end of nursery phase). The roots were separated from the shoots using a sterile scalpel. Fresh weight of shoots were taken using a weighing balance before being oven dried at 70°C for 72 hours. Roots were carefully rinsed and excess water removed using a paper towel before recording fresh weights and number of secondary roots. Root length was measured according to the procedure described by Tennant (1975). To determine the root biomass dry weight, a sub sample of the fresh roots was oven dried at 70°C for 72 hours. The dry weights of the sub samples were used to calculate the total root dry weights.

3.2.6 Assessment of mycorrhizal colonization

The portion of the root preserved in 70% ethanol was assessed for mycorrhizal colonization according to the procedures of Koske and Gemma (1989). Estimation of percentage root mycorrhizal fungi colonization frequency and intensity was done using the subjective visual technique by Kormanik and McGraw (1982) commonly

referred to as the slide method. The roots were cleared with 2.5% KOH (25g KOH in 1000 ml water) by heating in an oven at 70ºC for one hour and then rinsed with tap water. To remove phenolic substances, alkaline hydrogen peroxide (60 ml of 28-30% NH_4OH, 90 ml of 30% H_2O_2 and 840 ml distilled water) was added and roots placed in the oven at 70ºC for 20 minutes. The process was repeated for all the samples. The roots were rinsed with tap water and acidified with 1% hydrochloric acid (HCl) for 30 minutes. The HCl was decanted and without rinsing the roots, 0.05% Trypan blue in acid glycerol (500 ml glycerol, 450 ml water, 50 ml of 1% HCl and 0.5g Trypan blue) staining reagent was added and roots placed in the oven at 70ºC for 1 hour. The stain was decanted and de-staining solution comprising of acid glycerol (500 ml glycerol, 450 ml distilled water, and 50 ml of 1% HCl) was added. Fine root segments were cut into 1 cm-long pieces and 30 pieces randomly picked, mounted on slides and observed under a compound microscope to assess the frequency and intensity of mycorrhizal colonization. Presence of arbuscules, vesicles, internal and external hyphae was examined. The frequency of mycorrhizal colonization was recorded as the number of root fragments infected with mycorrhizal fungi and expressed as a percentage of total number of root fragments observed. The intensity of mycorrhizal fungi colonization was recorded as percentage cover of mycorrhizal fungi infective propagules in each 1cm root fragment.

3.2.7 Experimental design, management and assessment of growth parameters under field conditions

After the nursery phase (22 weeks after deflasking), twelve plants from each treatment under green house conditions were established under a Vertisol, Rhodic Ferralsol and Humic Nitisol field soil conditions at Nyanza, Coast and Central

regions of Kenya, respectively. The three sites have contrasting soil and climatic conditions.

3.2.8 Description of study sites

This study was conducted in three Agro-ecological zones (AEZ). In Western Kenya, the trial was conducted in Nyanza province at KARI Kibos in Kisumu Central district. The area lies at a mid altitude level (1137m above sea level, Low Midland agro-ecological zone 3) with minimum and maximum temperature of 15.4 and 28.9 C respectively. The soil is a Vertisol, black heavy clay with low soil fertility (low soil phosphrous, sulphur and zinc). Plant diseases and high fertilizer input prices are also common (Khan *et al*., 2008). In Central Kenya, the trial was set up in Chuka division of Meru South district. The area lies in the Upper Midland Zone (UM2-UM3) (Jaetzold *et al*., 2006) on the eastern slopes of Mt Kenya at an altitude of 1500 m, with an annual mean temperature of 20°C and a total annual rainfall of 1200–1400 mm. The soils are mainly Humic Nitisol (Jaetzold *et al*., 2006), which are deep and well weathered with moderate to high inherent fertility, but their fertility has declined due to intensive cultivation without adequate replenishment of soil nutrients. In coastal Kenya, the trial was conducted at the Kenya Agricultural Research Institute (KARI), Matuga. The area is coastal lowland 3 (CL3), as described by Jaetzold and Schmidt (1983). The site lies at 3°50 S, 39°44 E, at 15 m above sea level. The Centre receives an average annual rainfall of 1200 mm and the mean monthly minimum and maximum temperatures are 22°C and 30°C respectively. The relative humidity is high (>80%) according to Jaetzold and Schmidt (1983). Soils have a pH-H_2O of about 6.9, are moderately well drained, sandy clay to clay with sandy surface, classified as Ferralsols (Batjes, 1980). These soils are characterized by very low levels of macro-nutrients, especially nitrogen and

phosphorus, low organic matter, low cation exchange capacity and are prone to erosion.

3.2.9 Experimental design

The experimental design followed a randomized complete block design with three replicates per treatment. Plants were planted in 60 x 60 x 60 cm sized pits spaced at 3m x 3m on fields not tilled at the on-set of the trial. Inorganic fertilizer,_DAP, was applied only at planting. Six kg dry weight of manure per pit was applied at planting by incorporating around the banana plant in the pit followed by topping with sub-soil. Hand weeding was done when needed. Desuckering of plants was carried out in order to have a 1-2-3 system (mother, first ratoon, second ratoon), preferably with a circular movement of plants, so that original planting density is maintained. Deleafing of leaves that have <50% of functional leaf surface area was done whenever necessary. Plants were watered when necessary, especially during periods of severe water stress. Plant growth parameters (plant height, leaf length, leaf width and girth) were evaluated at the on-set of flowering, seven months after field establishment. Leaf surface area was determined by as leaf length x leaf width x a calibration factor of 1.94. The calibration factor was determined by measuring the total surface area of a leaf (excluding the petiole) at JKUAT. Plant apparent volume was determined as pseudostem height x girth.

3.2.10 Data analysis

Data on survival were scored as a percentage proportion of the total number of surviving plants per product at 8 weeks after deflasking. To assess the effects of products, soils and their interactions data for all measured variables were subjected to analysis of variance (ANOVA) and differences between means were separated

using the PDIFF option of the LSMEANS statement at P 0.05. Percentage mycorrhizal colonization root colonization data were arcsine-square root transformed before being subjected to ANOVA to bring about normal distribution. All data analysis was performed using the SAS 2006 statistical software (SAS Institute Inc., 2006).

3.3 RESULTS

3.3.1 Survival of plantlets

Survival of tissue cultured plantlets (TC) was highest in plants treated with MYCOR (80.0%) in the Humic Nitisol; MYCOR, Rhizatech and Subtilex (90.0%) in the Rhodic Ferralsol; PHC Biopak and Subtilex (90.0% and 93% respectively) in the Vertisol. Survival of plantlets in the conventional media was highest with plants treated with MYCOR (73.0%) and Rhizatech (63.0%) (Table 5).

Table 5: Survival (%) of tissue-cultured banana plantlets treated with microbiological inoculants, 8 weeks after deflasking

			Soil types	
Treatment	Humic Nitisol	Vertisol	Rhodic Ferralsol	Conventional
Control	73a	87b	80b	57bc
PHC Biopak	73a	90b	40a	0a
PHC Colonize	47b	40a	30a	27ab
ECO-T	63a	70ab	83b	57bc
MYCOR	80a	77ab	90b	73c
Rhizatech	73a	73ab	90b	63c
Subtilex	70a	93b	90b	57c
SED	11.2	12.04	11.06	9.37
p-value	0.017	0.013	<0.001	<0.001

Means followed by the same letter in each column are not significantly different according to PDIFF option of LSMeans statement at P 0.05. SED-standard error of difference between means; >70% = good; 50-70% moderate; <50% not suitable for hardening. Means were for 30 plants

3.3.2 Mycorrhizal colonization

Arbuscular mycorrhizal fungi (AMF) intensity and frequency was not significantly ($p<0.05$) affected by product, soil or soil and product interactions in all the soil substrates (Table 6). Percent intensity of colonization was highest with Rhizatech inoculation (31.9%) and least with non-inoculated control (12.9%) in the Rhodic

Ferralsol; highest with ECO-T inoculation (37.1%) and least with PHC Colonize inoculation (12.9%) in the Humic Nitisol; highest with PHC Biopak (37.8%) followed by Rhizatech (28.9%) and least with ECO-T inoculation (16.2%) in the Vertisol and highest with MYCOR (24%) and least with ECO-T (12.4%) in the conventional medium.

Table 6: Percent mycorrhizal colonization of tissue cultured banana plantlets inoculated with commercial microbiological products, 22 weeks after deflasking

Soil	Treatment	Frequency	Intensity
Humic Nitisol	Control	87.8	23.7
	ECO-T	93.3	37.1
	MYCOR	50.0	17.6
	PHC Biopak	71.1	17.8
	PHC Colonize	48.9	13.1
	Rhizatech	62.2	26.7
	Subtilex	34.4	34.2
***p*-value**		**0.186**	**0.558**
Rhodic Ferralsol	Control	34.6	12.9
	ECO-T	63.3	21.6
	MYCOR	58.3	17.7
	PHC Biopak	55.0	14.9
	PHC Colonize	55.6	15.1
	Rhizatech	76.1	31.9
	Subtilex	52.2	20.2
***p*-value**		**0.977**	**0.023**
Vertisol	Control	61.1	25.1
	ECO-T	63.3	14.7
	MYCOR	38.9	19.8
	PHC Biopak	87.4	37.8
	PHC Colonize	85.6	21.3
	Rhizatech	68.3	28.9
	Subtilex	63.3	19.1
***p*-value**		**0.465**	**0.664**
Conventional	Control	47.0	18.9
	ECO-T	22.0	12.4
	MYCOR	73.3	24.0
	Rhizatech	60.0	16.0
	Subtilex	37.0	12.7
***p*-value**		**0.217**	**0.255**

The effect of products on percent frequency of mycorrhizal colonization was variable and was highest with PHC Biopak (87.4) and PHC Colonize (85.6%) inoculation in the Vertisol. In the Rhodic Ferralsol, Rhizatech inoculation had the highest percent frequency of mycorrhizal colonization (76.1%) while the control had the least (34.6%). Similar to the observations made on percent intensity of colonization, inoculation of plants with ECO-T had the highest percent frequency of colonization (93.3%) in the Humic Nitisol and MYCOR inoculation had the highest percent frequency of colonization (73.3%) followed by Rhizatech (60%) while ECO-T had the least colonization (22%) in the conventional medium.

3.3.3 Plant growth at end of nursery stage (22 weeks after deflasking)

The parameters evaluated for growth were height, girth, leaf length and width and number of functional leaves.

3.3.3.1 Plant height

Plant height within Vertisol and the Rhodic Ferralsol was significantly (p 0.05) affected by product inoculation (Table 7). Plants inoculated with PHC Biopak, PHC Colonize, Rhizatech and ECO-T in the Rhodic Ferralsol had significantly higher plant height (108.0, 86.0, and 16.0%, respectively) relative to the control. PHC Biopak, PHC Colonize, Rhizatech, MYCOR and ECO-T inoculated plants in the Vertisol had significantly higher height than the control by 40.5 and 45.2, 21.4, 14.3 and 13.9% respectively. Products had no significant effect on height of plants in the Humic Nitisol and conventional media (Table 7).

3.3.3.2 Girth

Effect of products on girth of plantlets was soil dependent with significant increase in girth observed in the Rhodic Ferralsol and Vertisol (Table 7). In the Rhodic Ferralsol, all products increased girth of plantlets by a magnitude of 75.0% (PHC Biopak), 62.5% (PHC Colonize), 50.0% (MYCOR), 25.0% (ECO-T) and 12.5% (Rhizatech) relative to the control. In the Vertisol, the highest increase in girth relative to the control was observed with the plantlets inoculated with ECO-T (57.1%) followed by PHC Biopak (42.9%) and PHC Colonize (35.7%).

3.3.3.3 Number of functional leaves

The number of functional leaves was increased by product inoculation within the Vertisol, Rhodic Ferralsol and conventional media (Table 7). Plantlets inoculated with the mycorrhizal products (Rhizatech and MYCOR) in the Vertisol had higher number of leaves (1.1 times) compared to the non-inoculated control and all other treatments. In the Rhodic Ferralsol, all products except Subtilex increased number of leaves by 1.2 times relative to the control. Only Rhizatech inoculated plantlets in the conventional media had higher number of leaves (1.1 times) than the control plants. The number of leaves produced across all soils ranged from 6 to 10 leaves irrespective of the product used (Table 7).

Table 7: Growth of tissue cultured banana plantlets treated with microbiological inoculants, 22 weeks after deflasking

Soil	Treatment	Height (cm)	Girth (cm)	Leaf length (cm)	Leaf width (cm)	NOFL
Humic Nitisol	Control	9.8	1.3	20.2	7.7	9a
	PHC Biopak	7.9	1.3	17.5	5.7	8b
	PHC Colonize	7.5	1.4	19.4	7.1	7b
	ECO-T	9.5	1.3	17.7	6.9	9a
	MYCOR	8.4	1.3	18.0	6.5	9a
	Rhizatech	9.3	1.3	19.3	6.5	9a
	Subtilex	9.8	1.3	19.7	6.8	9a
***p*-value**		**0.163**	**0.99**	**0.142**	**0.2911**	**<0.0001**
Rhodic Ferralsol	Control	5.0a	0.8a	14.1a	4.1a	6
	PHC Biopak	10.4b	1.4b	18.8b	6.0b	7
	PHCColonize	9.3b	1.3b	18.8b	6.7	7
	ECO-T	5.8c	1.0c	14.1a	4.1a	7
	MYCOR	5.0a	1.2b	12.3c	3.6ac	6
	Rhizatech	5.0a	0.9ac	12.9ac	3.9a	7
	Subtilex	5.2ac	0.9ac	13.9a	4.4ad	7
***p*-value**		**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.17**
Vertisol	Control	8.4a	1.4a	20.4a	7.6a	9a
	PHC Biopak	11.8b	2.0abcd	23.7b	8.4ab	9a
	PHC Colonize	12.2b	1.9ab	24.2	8.6	8
	ECO-T	9.5a	2.2bc	21.8ac	8.8b	9a
	MYCOR	9.6c	1.5ab	22.2c	8.5b	10b
	Rhizatech	10.2c	1.5ab	22.0c	8.4b	10b
	Subtilex	8.6a	1.3ab	19.5d	7.3ac	9a
***p*-value**		**<0.0001**	**0.03**	**<0.0001**	**0.0021**	**0.0059**
Conventional medium	Control	23.8a	3.2	38.6	14.9	9
	ECO-T	23.7a	3.2	37.3	14.7	9
	MYCOR	20.8b	3.0	36.6	13.7	9
	Rhizatech	23.4a	3.2	36.8	16.3	10
	Subtilex	23.7a	3.2	36.5	14.8	9
***p*-value**		**0.0001**	**0.47**	**0.35**	**0.1187**	**0.87**
	Treatment	**<0.001**	**0.005**	**<0.0001**	**0.1033**	**<0.0001**
	Soil	**<0.001**	**<0.0001**	**<0.0001**	**0.0004**	**0.1751**
	Treatment x Soil	**<0.001**	**0.005**	**<0.0001**	**0.0055**	**<0.0001**

Means of 30 plants. Means followed by the same letter in each column are not significantly different according to PDIFF option of LSMeans statement at P 0.05. Means not followed by a letter in each column are not significantly different at P 0.05. NOL- Number of functional leaves. Height, leaf length, leaf width and girth were measured in centimeters.

3.3.3.4 Leaf length and leaf width

Effect of products on leaf length was highly significant (p 0.05) in the Vertisol and Rhodic Ferralsol (Table 7). In the Vertisol, PHC Biopak recorded significant

increase in length compared to the non-inoculated control (23.7cm vs 20.4cm), Subtilex (19.5cm), ECO-T (21.8cm), MYCOR (22.2cm) and Rhizatech (22.0cm). In the Rhodic Ferralsol, similar observations were made with plantlets inoculated with the PHC Biopak and PHC Colonize (18.8cm) performing significantly better than the non-inoculated plants (14.1cm) as well as plants inoculated with ECO-T (14.1cm), MYCOR (12.3cm), Rhizatech (12.9cm) and Subtilex (13.9cm). Similar observations were made on leaf width with plants inoculated with PHC Biopak and PHC Colonize in the Rhodic Ferralsol increasing leaf width by 1.5 and 1.6 times compared to the control. All products except Subtilex increased leaf width in the Vertisol by a range of 0.8-1.2 times compared to the control (Table 7).

3.3.3.5 Trends of growth of inoculated banana plants

The effectiveness of products to sustain tissue cultured banana growth was confirmed by assessing banana growth at different times through out the nursery phase. The results showed PHC Biopak and PHC Colonize to significantly enhance banana growth in the Vertisol; PHC Biopak in the Rhodic Ferralsol, Subtilex in the Humic Nitisol and ECO-T in the conventional media through out the nursery phase. The trends of growth in height are presented in Fig. 1, 2, 3, and 4 respectively.

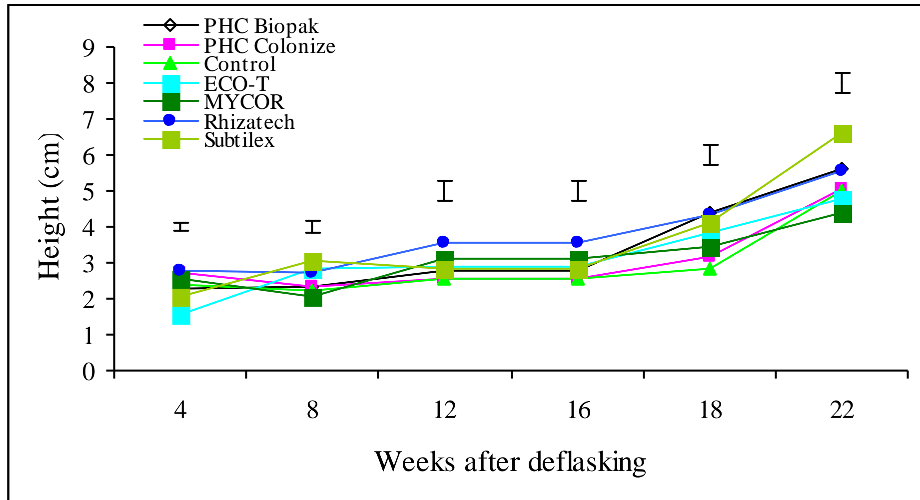

Figure 1: Height of banana plants in Humic Nitisol.
Error bars are standard errors of difference between means at each data collection time. Data points are means of 10 plants replicated 3 times.

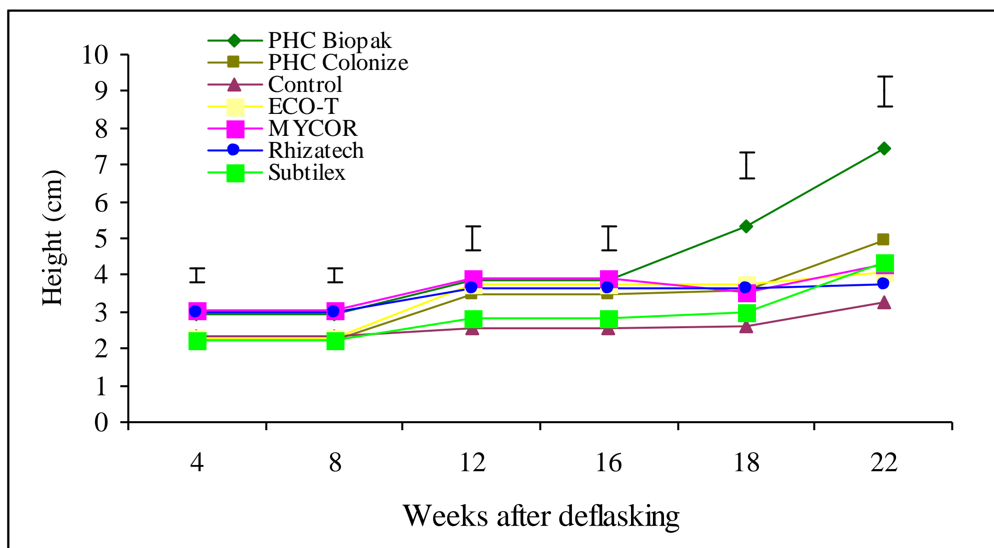

Figure 2: Height of banana plants in Rhodic Ferralsol.
Error bars are standard errors of difference between means at each data collection time. Data points are means of 10 plants replicated 3 times.

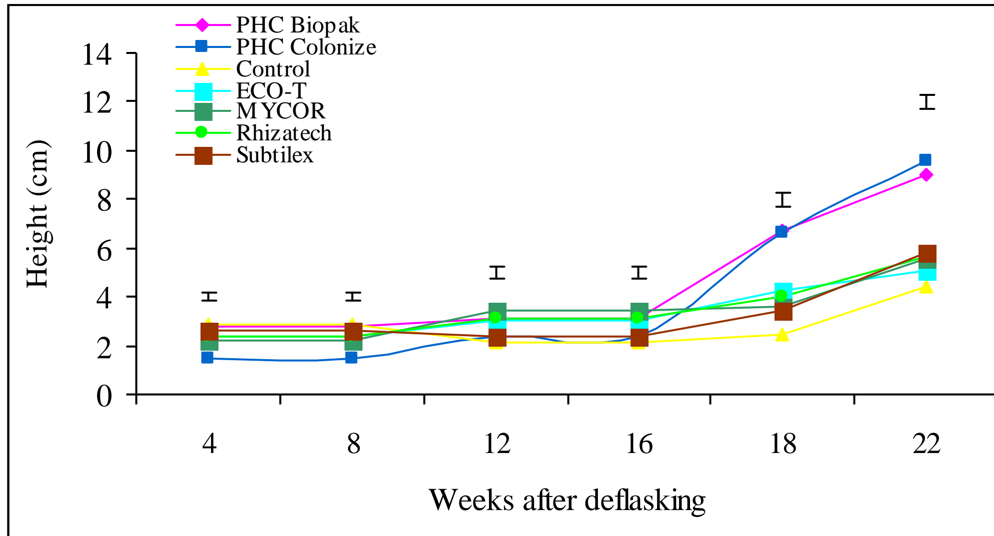

Figure 3: Height of banana plants established in Vertisol.
Error bars are standard errors of difference between means at each data collection time. Data points are means of 10 plants replicated 3 times.

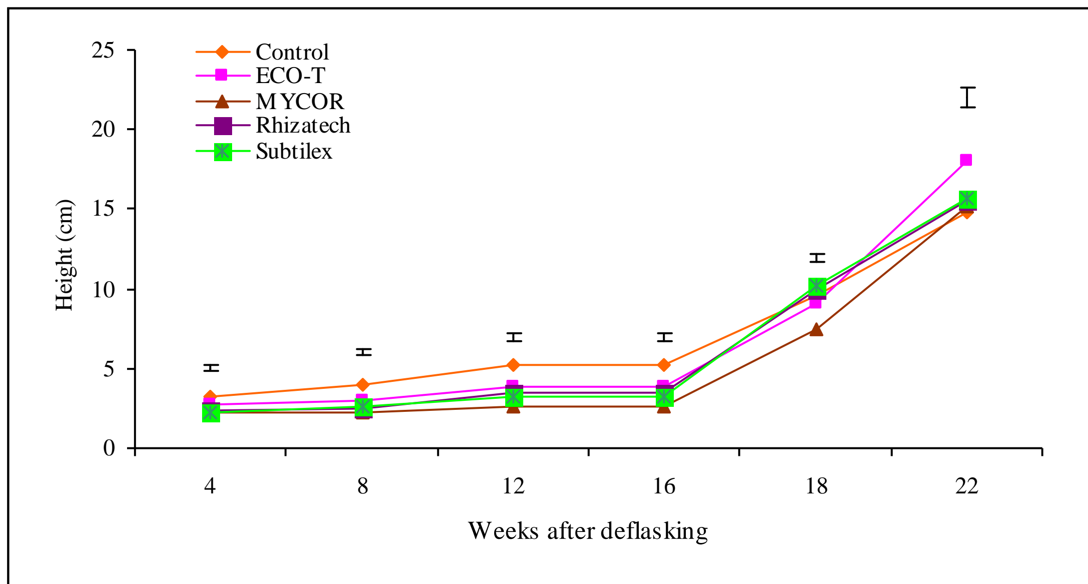

Figure 4: Height of banana plants in conventional media.
Error bars are standard errors of difference between means at each data collection time. Data points are means of 10 plants replicated 3 times.

3.3.3.5 Shoot dry weight

The effect of products on shoot dry weight was highly variable and dependent on soil type (Table 8). All products enhanced shoot dry weight (sdwt) in Vertisols by a magnitude of: 344.9% (PHC Biopak), 333.7% (PHC Colonize), 130.9% (Rhizatech), 100.0% (MYCOR), 87.8% (Subtilex) and 81.1% (ECOT) compared to the non-inoculated control. In Rhodic Ferralsol, only PHC Biopak and PHC Colonize enhanced sdwt by 113.1% and 146.5% respectively. The effect of products was minimal in Humic Nitisol, and this was observed in plants inoculated with Rhizatech (15.0%) and PHC Biopak (5.0%). Similar observations were made in the conventional media with only Rhizatech enhancing sdwt by 11.5 %.

3.3.3.6 Root length

Products had variable effects on root length depending on soil type. Plants inoculated with PHC Colonize, PHC Biopak, ECO-T and Subtilex in the Humic Nitisol had slightly higher root length than the control plants by 2.5, 1.4, 1.1 and 1.2 times respectively. PHC Biopak and PHC Colonize inoculated plants in the Rhodic Ferralsol had the highest root length and this was higher by 3.1 and 3.1 times (respectively) than the control. In the Vertisol, PHC Colonize, PHC Biopak, Mycor, Subtilex and ECO-T inoculated plants had significantly higher root length than the control by 9.5, 3.6, 2.1, 1.9 and 1.5 times respectively. Products had no effect on root length in the conventional medium. The non-inoculated plants had the highest root length of 17,082.0 cm (Table 8).

Table 8: Shoot and root growth of tissue cultured banana plantlets inoculated with microbiological products, 22 weeks after deflasking

	Treatment	Root length (m)	Root number	RDWT (g)	SDWT (g)
Humic Nitisol	Control	31.9	9.7ac	1.2	3.7
	PHC Biopak	44.2	14.0b	1.4	3.9
	PHC Colonize	81.1	11.7abc	1.7	4.8
	ECO-T	34.7	9.3ac	1.2	3.1
	MYCOR	28.0	10.3ac	1.2	3.3
	Rhizatech	32.0	12.7abc	1.9	4.3
	Subtilex	37.2	10.7ac	1.6	3.6
***p*-value**		**0.36**	**0.08**	**0.77**	**0.86**
Rhodic Ferralsol	Control	18.1a	8.7a	0.8a	2.2
	PHC Biopak	56.4b	15.3b	1.8b	13.8
	PHC Colonize	57.0b	9.7a	1.6a	5.3
	ECO-T	14.32a	9.7a	1.6a	2.2
	MYCOR	14.6a	8.7a	0.9a	2.2
	Rhizatech	10.7a	8.7a	0.7a	5.2
	Subtilex	11.4a	8.7a	0.7a	1.5
***p*-value**		**0.0052**	**0.0003**	**0.11**	**0.42**
Vertisol	Control	15.8a	11.0a	0.7a	2.5a
	PHC Biopak	57.2b	22.3bc	2.9b	11.0b
	PHC Colonize	15.0b	21.7bc	4.8c	10.7b
	ECO-T	23.7acd	13.7ac	1.7d	4.5ac
	MYCOR	33.2c	12.0ad	1.4ad	4.9c
	Rhizatech	15.8a	14.0acd	1.7d	5.7c
	Subtilex	30.7c	14.3abcd	1.7d	4.6c
***p*-value**		**<0.0001**	**0.06**	**<0.0001**	**<0.0001**
Conventional medium	Control	170.8a	31.7ac	6.6	23.4ac
	PHC Colonize	137.3a	27.0ab	6.4	15.1b
	ECO-T	19.4b	22.7b	7.2	16.8b
	MYCOR	53.8b	25.7b	5.3	20.2a
	Rhizatech	36.4bc	34.0c	6.9	26.1c
	Subtilex	87.9bc	28.2abc	6.3	20.4abc
***p*-value**		0.0137	0.02	0.87	0.016
Treatment		<0.0001	<0.0001	0.0144	0.0152
Soil		0.0023	<0.0001	<0.0001	<0.0001
Treatment X Soil		<0.0001	0.0169	0.086	0.136

Means of 3 replicates (plants) per soil type. Means followed by the same letter in each column are not significantly different according to PDIFF option of LSMeans statement at P 0.05. Means not followed by a letter in each column are not significantly different at P 0.05. RDWT- root dry weight; SDWT- shoot dry weight.

3.3.3.7 Number of secondary roots

Plants inoculated with PHC Biopak had the highest number of roots in the Rhodic Ferralsol (15) and Vertisol (22). Rhizatech inoculated plantlets had the highest

number of secondary roots in the conventional medium (34) and Humic Nitisol (13) (Table 8).

3.3.3.8 Root dry weight

Product effect on root dry weight (RDWT) was only significant within the Vertisol (Table 8). The PHC Biopak and PHC Colonize treated plants had the highest root dry weight (RDWT) of 2.9g and 4.4g respectively while the non-treated control plants had the least RDWT (0.7g). Plants inoculated with Rhizatech, PHC Colonize, Subtilex, and PHC Biopak in the Rhodic Ferralsol had 1.6, 1.4, 1.3, 1.2 times higher RDWT relative to the control. ECO-T and Rhizatech inoculated plants in conventional nursery medium had higher RDWT than the control by 1.1 and 1.1 times respectively.

3.3.4 Growth response of inoculated banana plantlets under field conditions

Response of inoculated plants under field soil conditions was variable (Table 9). Product effect was best in Vertisol and least in Humic Nitisol. All products enhanced growth of plants in the Vertisol with Rhizatech and ECO-T inoculation significantly enhancing apparent volume by 142.3 and 107.7% and leaf surface area by 73.0 and 60.8% respectively compared to the control plants. In the Rhodic Ferralsol, plants inoculated with single species *Bacillus,* multiple species mycorrhizae, *Trichoderma* and multiple species *Bacillus* enhanced apparent volume by 60.0%, 57.9%, 34.2% and 18.4% respectively than the non-inoculated controls and the single species mycorrhizae. Conventional practice in the Rhodic Ferralsol had the highest apparent volume (7.5cm^3) while plants inoculated with MYCOR had the least (2.0cm^3). Inoculated plants had lower leaf surface area than the control

plants in the Rhodic Ferralsol. Overall, performance of plants in the Humic Nitisol had higher apparent volume, leaf surface area and number of functional leaves compared to the Vertisol and Rhodic Ferralsol. This shows the ability of the Humic Nitisol to support plant growth irrespective of microbiological amendments compared to plants established in the Vertisol and Rhodic Ferralsol.

Table 9: Growth of tissue cultured banana plantlets at flowering

Treatment	Apparent Volume (cm^3)			LSA (cm^2)			Number of functional leaves		
	Humic Nitisol	Vertisol	Rhodic Ferralsol	Humic Nitisol	Vertisol	Rhodic Ferralsol	Humic Nitisol	Vertisol	Rhodic Ferralsol
Control	46.0	2.6a	3.8	52228	9093a	14195	21.0a	11.0	18.8
PHC Biopak	37.7	3.9ab	4.5	41566	11420ab	14060	19.5ab	11.8	17.5
Conventional	51.2	4.3ab	7.3	53123	12525bc	11505	20.8ab	10.6	17.7
ECO-T	41.2	5.4b	5.1	43654	14623c	8785	18.2b	11.6	18.2
MYCOR	49.5	3.1a	2.0	51783	11302ab	10888	20.7ab	11.3	19.3
Rhizatech	43.7	6.3c	6.0	48051	15731d	12494	19.0ab	11.2	18.2
Subtilex	46.9	3.9ab	6.1	46784	12328bc	13206	19.2ab	11.5	19.0
p-value	Ns	0.0004	Ns	Ns	0.0019	Ns	0.0013	Ns	0.0764

Means followed by the same letter in each column are not significantly different according to PDIFF option of LSMeans statement at P 0.05. Ns-means within the same column statistically the same at p 0.05. Means not followed by a letter in each column are not significantly different at P 0.05 LSA-leaf surface area.

3.4. DISCUSSION

3.4.1 Survival of plantlets 8 weeks after deflasking

The study demonstrates variable efficacy of microbe-based products and their interaction with different soil types on survival of tissue cultured (TC) banana plants. The survival of plantlets was dependent on the composition of microbe-based product used for inoculation and the prevailing soil chemical, physical and nutritional conditions. In the Vertisol and Humic Nitisol, the survival of non-inoculated plantlets consistently superseded that in the conventional media indicating the use of non-amended soils as a better option for hardening. Plant growth promoting bacteria (PGPB) as well as mycorrhizae have been reported to enhance biomass production during the first stages of crops and can contribute to plant adaptation from the *in vitro* to the *post vitro* phase (Carletti, 2000). Jaizme-Vega *et al*. (2004) evaluated the effect of a rhizobacteria consortium of *Bacillus* spp. on the first developmental stages of two micropropagated bananas and concluded that this bacterial consortium can be described as a prospective way to increase plant health and survival rates in commercial nurseries.

While the mycorrhizal products and the multiple species *Bacillus* product (PHC Biopak) efficacy on survival was restricted to specific soils, the single species *Bacillus* product (Subtilex) was different in supporting survival of plants in both the Rhodic Ferralsol and Vertisol. This study therefore reveals a differential performance of single and multiple species mycorrhizal and *Bacillus* products. Positive effects of single and multiple plant growth promoting bacteria (PGPB) and

arbuscular mycorrhizal fungi (AM fungi) species have been reported separately. A *Bacillus* consortium containing strains INR7, T4 and IN 937b strains inoculation to different tissue culture (TC) banana cultivars under normal nursery conditions consisting of soil: volcanic ash: peat substrate mixture was reported to improve survival and growth (Rodríguez-Romero *et al*., 2005). *Glomus etunicatum* enhanced growth and nutrient uptake better than the indigenous isolate for plants established in an Andisol mixed with a fungicide and inorganic fertilizer while the indigenous isolates was more effective in sterile sand medium (Juma, 2011).

3.4.2 Plant growth under green house and field conditions

The performance of commercial products was variable and was highly influenced by the composition of the microorganisms, soil conditions and stage of plant development. Plant growth enhancement by *Bacillus* inoculation could be attributed to immediate stimulation effects on root growth by bacterial products, which stimulated longer, larger and denser root systems compared to fungal based products. The plant growth promoting effects of plant growth promoting bacteria (PGPB) are mainly derived from morphological and physiological changes in roots of inoculated plant (Okon *et al*., 1988; Sarig *et al*., 1988; Mia, 2002). The lack of immediate effect of fungal products in growth under nursery conditions could be attributed to the fact that colonization commences after hyphal formation and subsequent colonization of the root. This process may take longer depending on the state of propagules (spores, hyphae), which may take time to germinate and infect the plant.

The effect of products was reversed when inoculated plantlets were established under field conditions with fungal products (Rhizatech and *Trichoderma*) performing better than the bacterial based products. There is evidence that the germination of spores of some species of AM fungi is blocked by a dormancy factor for a certain period of time depending on the species (Tommerup, 1983; Bowen, 1987). Rhizatech, a mycorrhizal product produces hyphae that colonize the root both externally and internally whereas the hyphae produced by *Trichoderma* colonizes externally releasing secondary metabolites in to the rhizosphere at the same time. Several studies have shown that plant growth enhancement by AM fungi is related to colonization level and to the extent of external mycelium, the latter being necessary for P uptake (Sanders *et al*., 1977; Abbott and Robinson, 1981: Tommerup, 1994). Other studies on AM fungi colonization have shown that presence of the root allows development of vegetative mycelium, which, under favorable conditions, can colonize 60-90% of the length of the root system (Becard and Piche, 1989; Bonfante and Bianciotto, 1995). This could have explained the fewer and shorter root systems observed with these products as function is through hyphae and metabolites which were not measured in the experiment. These processes though slow in establishment, the function is more prolonged since it is more intimate, and it is more likely to continue under field conditions and could have contributed to the performance in the field.

Performance of products was affected by soil conditions. The effect of products on growth was more pronounced in Vertisol implying that all the products perform best under low P conditions. There is a lot of existing evidence on the effects of

mycorrhizae at low P levels, solubilization of P by *Bacillus* based products, and limited information on *Trichoderma* and P. Beneficial effects of bacterial inoculation are optimal in nutrient deficient soils than in nutrient rich soil (Egamberdiyeva, 2007). This may explain the low response (for example 15.0% increase in shoot weight) to *Bacillus* inoculation by plants established in the Humic Nitisol and conventional media. Benefits of mycorrhiza are greatest in P-deficient soils and decrease as soil phosphate levels increase (Schubert and Hayman, 1986). In banana cropping systems, soil texture and P, agro-chemical use, and soil wetness affected the soil population density of AM fungi. Sandy soils were associated with lower and clay soils with higher AM fungi soil populations in intensive banana cultivation systems of Martinique (Declerck *et al*., 1999). This may therefore explain the increased plant growth observed with inoculation of plants in the Vertisol which is a clay soil. The P level in the conventional media (56mg/kg) was slightly above 50mg/kg thresh hold (Schubert and Hayman, 1986) thus providing better conditions for the mycorrhizae to function and may explain the enhanced shoot and root growth compared to the control in the conventional media under green house conditions and the enhanced by addition of inorganic phosphate as evidenced by the high apparent volume of plants established in the conventional practice under field conditions in the Rhodic Ferralsol. This prompts further investigation in to the combining of appropriate doses of inorganic fertilizers with microbial inoculants for optimal plant growth and performance.

The increase in plant growth as a result of *Trichoderma* inoculation may also be explained by the high organic carbon level (3. 7%) in the Vertisol compared to the

Rhodic Ferralsol (1.0%) and Humic Nitisol (2.63%). This may explain the over 60% increase in growth observed with *Trichoderma* inoculated plants in the Vertisol compared to the conventional practice which had a C level of 5.8%. Physical and chemical factors influence the occurrence of *Trichoderma* spp. (Okoth *et al*., 2007). Forests soils high in C, N, Fe, organic matter and Mg favored the occurrence of the fungus (Okoth *et al*., 2007) depicting that high organic matter content promotes proliferation of *Trichoderma*.

3.4.3 Mycorrhizal colonization

Positive effects of some microbe-based commercial inoculants on mycorrhizal colonization compared to the non-treated control in the nursery were more evident in the Vertisol, Rhodic Ferralsol and the conventional media than in the Humic Nitisol. Some bacteria associated with AMF can improve the mycorrhizal colonization (Barea *et al*., 1998; Budi *et al*., 1999) and this is confirmed by the high percentage of mycorrhizal colonization observed with plants inoculated with the mixed species *Bacillus* (PHC Colonize and PHC Biopak) in the Vertisol. The high mycorrhizal colonization in the Vertisol that is P deficient could have promoted the positive growth effects observed with the same treatments. This is in agreement with the findings of Schubert and Hayman, (1986) who reported that the benefits of mycorrhiza are greatest in P-deficient soils and decrease as soil phosphate levels increase. Though this was confirmed in the Vertisol, it was not the case with inoculation of the Rhodic Ferralsol with the mixed *Bacillus* products. Mycorrhizal colonization could not explain the plant growth patterns observed with mixed species *Bacillus,* mixed species mycorrhiza and *Trichoderma* in the Rhodic

Ferralsol. Lack of correlation between mycorrhizal infectivity and effectiveness of fungi has been reported in commercial AMF inoculated to banana cultivar Petite Naine (Rodríguez-Romero, 2007) and in strawberry (Bull *et al*., 2005).

The addition of phosphate fertilizers results in a delay in infection as well as a decrease in the percentage of colonization of roots by mycorrhizae (deMiranda *et al*., 1989). Inorganic Phosphorous fertilizer is normally added as a constituent of the conventional media. This may be related to the low mycorrhizal colonization of inoculated plants in the conventional media compared to the Humic Nitisol, Rhodic Ferralsol and Vertisol and may also be associated with the low survival rates of plants treated with the *Bacillus* and *Trichoderma* since mycorrhizal symbiosis is beneficial for *in vitro* plant adaptation (Carletti, 2000).

Tissue culture bananas can benefit from application of arbuscular mycorrhizal fungi, *Trichoderma* and *Bacillus* to improve survival and growth during the nursery phase as well as enhance performance under field conditions. The effect of microbiological inoculation is however dependent on soil type. The result from this research reveals essential information in the management and growth of tissue cultured banana and gives a possible strategy for decentralization of commercial banana nurseries which depend entirely on the use of conventional media.

CHAPTER FOUR

NUTRIENT UPTAKE OF TISSUE CULTURED BANANA INOCULATED WITH MICROBIOLOGICAL PRODUCTS IN THREE SOIL TYPES

ABSTRACT

A green house study was conducted to evaluate the efficacy of commercial microbiological products on nutrient uptake in tissue cultured banana cv. Gros Michel. Tissue cultured plants were established in a Vertisol, Rhodic Ferralsol, Humic Nitisol and conventional nursery medium (positive control) and inoculated with commercial formulations of mycorrhiza (Rhizatech and Mycor), *Trichoderma* (ECO-T) and *Bacillus* (PHC Biopak, PHC Colonize AG and Subtilex) at the deflasking and nursery stages of development. Assessment of plant nutrient uptake was done at the end of the nursery phase (22 weeks after deflasking). Harvested shoots were analyzed for macro-nutrients (nitrogen, phosphorous and potassium), micro nutrients (zinc, manganese, iron, sulphur and copper) and secondary nutrients (calcium, magnesium and sodium) using the inductively coupled plasma analysis (ICP). Data were analyzed using the SAS, 2006 statistical package and significant means separated by Tukey's HSD test. The ability of products to enhance nutrient uptake varied with soil substrate and type of product applied. Mixed species *Bacillus* products significantly enhanced uptake of phosphorous by over 160 and 400% in the Vertisol and Rhodic Ferralsol respectively. Mycorrhiza inoculation enhanced P uptake by over 170% in the Rhodic Ferralsol and Vertisol respectively. Mixed species mycorrhiza and *Bacillus* inoculation enhanced potassium, zinc, iron, manganese, sulphur, copper and magnesium uptake in the Rhodic Ferralsol and

Vertisol by over 100%. Nutrient uptake of all other nutrients except zinc was least affected by product inoculation in the Humic Nitisol and conventional media with slight enhancement of less than 20% increase. Calcium uptake was negatively affected by inoculation with most products (except mycorrhiza) reducing uptake by a magnitude of up to 46% in the Vertisol. Mixed species bacterial and mycorrhizal inoculants were most effective for nutrient uptake in the nutrient deficient Vertisol and Rhodic Ferralsol. This study revealed that TC banana plant nutrition could be improved by commercial microbiological inoculation and that the efficacy of microbe-based inoculants on nutrient uptake is dependent on soil type. These findings could highly benefit nursery management of TC banana plants particularly when nutrient deficient soils are used for post flask management.

4.0 INTRODUCTION

Banana is an important crop globally with the world annual production estimated to be 70 to 88 million tons (INIBAP, 1998; 2005). Over 70 million metric tons of bananas were produced worldwide in 2004 with eastern Africa accounting for over 3.3 million tons (FAOSTAT, 2005). Kenya accounts for about 0.3% of the world banana output (FAOSTAT, 2005). Several methods have been used as sources of planting propagules among them the tissue culture technique which has been successfully used to establish mass-production of uniform, high quality, disease-free plants (Nowak, 1998; Nguthi, 1999; Wambugu and Kiome 2001; Kahangi, 2003; Mbaka *et al*., 2008). However, tissue cultured (TC) plants present some inconveniences such as more probability of poor physiology (George, 1996) and the lack of soil microbiota. In addition, TC plants are delicate, devoid of food reserves, and lack a proper root system that can help plants to establish well in growth media, absorb nutrients and subsequently grow. Root growth and proliferation can be improved by inoculation with microbes that are able to colonize the rhizosphere and benefit the plant host by either causing root proliferation (Smith and Read, 1997) or producing root exudates that are essential for growth, protection, solubilization and acquisition of soil nutrients (Jaizme-Vega *et al*., 2004; Rodríguez-Romero *et al*., 2005).

Commercial nursery management of TC banana plants mainly involves the use of sterile growth media into which essential nutrients such as the Hewitt nutrient mixture (Hewitt, 1966) are added to facilitate growth and development of plants. In Kenya, the increased demand for TC banana plants (MOA, 2007; Kahangi, 2003)

necessitates the purchase of TC plants at the *in vitro* stage and the use of natural soil media. Considering that suckers, which are the conventional planting propagules, have food reserves and are able to establish well in soils, tissue culture plants are devoid of food reserves and may not be able to absorb nutrients efficiently from the natural soil media and this could lead to massive loss of plants at the acclimatization stage. Biological hardening is therefore recommended for acclimatization, nutrient acquisition and subsequent plant establishment. Rhizospheric microorganisms such as mycorrhiza, plant-growth promoting bacteria and fungi such as *Bacillus* and *Trichoderma* have the ability to colonize the rhizosphere and help plants to acquire nutrients (Shamsuddin *et al*., 2000; Jaizme-Vega *et al*., 2004; Harman, 2006). Different rhizosphere microbes have been commercially formulated and are available in the market with variable efficacy (Nowak, 1998; Kloepper, 1996; Smith and Read, 1997) and this could greatly enhance the ability of TC banana to acquire nutrients especially under nutrient deficient soil conditions. This could particularly benefit the decentralization of commercial TC banana nurseries which entirely depend on the use of sterile nursery media and addition of special inorganic nutrients for post flask management of TC banana. This study evaluated the efficacy of six commercial products containing either *Bacillus*, arbuscular mycorrhiza fungal (AMF) and *Trichoderma* species on nutrient uptake of TC banana established in a Vertisol, Rhodic Ferralsol, Humic Nitisol and a conventional nursery media.

4.1 MATERIALS AND METHODS

4.1.1 Source of tissue cultured materials and soil properties

Tissue cultured (TC) banana plantlets were obtained from Jomo Kenyatta University of Agriculture and Technology Biotechnology laboratory. Soils for establishing TC banana plants were obtained from three banana growing regions in Kenya as described in section 3.2.1 of this thesis.

4.1.2 Experimental design and inoculation process of tissue cultured plants

The experiments were set up in a completely randomized design. A factorial experiment consisting of six commercial microbiological products (PHC Biopak, PHC Colonize, Rhizatech, ECO-T, MYCOR and Subtilex) and three soil types (Vertisol, Rhodic Ferralsol and Humic Nitisol) was followed. Inoculation of TC banana plantlets with microbiological products was done at the deflasking stage and subsequently at the potting stage. The description of products, soils and inoculation process was as described in subsection 3.2.1, 3.2.2, 3.2.3 of this thesis.

4.1.3 Assessment of shoot and root biomass

Three plants were destructively harvested from each treatment at 22 weeks after de-flasking. The roots were separated from the shoots using a sterile scapel. Roots were carefully rinsed and excess water removed using a paper towel and a portion of the root preserved in 70% ethanol for further assessment. Fresh weight of shoots were taken using a weighing balance before being oven dried at 70°C for 72 hours and ground in a ball mill and analyzed for macro nutrients (P and K), micro nutrients (Zn, Mn, Fe, Cu and S) and secondary nutrients (Ca, Mg and Na) contents using

Inductively Coupled Plasma method (ICP)-OES at K.U.Leuven, Belgium. Nutrient uptake was standardized per plant based on the total shoot dry weights per plant. Nitrogen analysis was carried out by Kjeldahl digestion (Morries, 1983) and steam distillation using a spectrophotometer (Spectronic Genesys 5 heating digester with programmable increases of time, manufactured by Velp Scientifica, model DK in Europe).

4.2 Data analysis

To assess the effects of products, soils and the interaction between soil and products, data were subjected to analyses of variance (ANOVA) using the mixed procedure of SAS (SAS, 2006). Means found to be significant at P 0.05 were separated using Tukey's HSD test.

4.3 RESULTS

4.3.1 Phosphorous, potassium and nitrogen uptake

Inoculation of tissue cultured banana with microbiological products variably affected the uptake of macronutrients (phosphorous, potassium and nitrogen) in the three soil types (Table 10). The uptake of phosphorous (P) and potassium (K) was positively affected by products in the Vertisol and Rhodic Ferrasol soils. No product effect was observed in the Humic Nitisol. The uptake of P was highest in the Vertisol with Mixed *Bacillus* based products (PHC Biopak and PHC colonize) increasing P uptake by 0.07 g $plant^{-1}$ and 0.08 g $plant^{-1}$ respectively, followed by Rhizatech (0.04), ECO-T (0.029), MYCOR (0.029) and the uninoculated control (0.014 g $plant^{-1}$). In the Rhodic Ferrasol, increase in P uptake was observed with PHC Biopak and PHC colonize and this was by a magnitude of 0.08 g $plant^{-1}$ and 0.09 g $plant^{-1}$ respectively (Table 10).

Table 10: Uptake of macronutrients (g/ plant) in tissue cultured banana plants treated with microbiological products, 22 weeks after deflasking

	Humic Nitisol			Rhodic Ferralsol			Vertisol			Conventional		
	N	P	K	N	P	K	N	P	K	N	P	K
PHC Biopak	1.23	0.02	0.76	1.06	0.03a	1.0157a	0.9a	0.08a	2.54d			
PHC Colonize	1.43	0.02	0.61	1.31	0.05a	1.3017a	0.9a	0.08a	2.29cd	1.39	0.25	5.54a
Control	1.70	0.03	0.75	1.97	0.01c	0.4733b	3.26c	0.01b	0.74a	1.48	0.31	13.00bc
ECO-T	2.12	0.02	0.70	1.67	0.01bc	0.4733b	2.51bc	0.03cd	1.27ab	2.42	0.20	9.74abc
MYCOR	1.27	0.02	0.60	1.35	0.02c	0.6153b	2.55bc	0.03bc	1.40abc	2.05	0.21	11.57abc
Rhizatech	1.19	0.03	0.94	2.68	0.01c	0.6483b	2.2067abc	0.05d	2.03bcd	1.44	0.34	13.50c
Subtilex	1.83	0.03	0.80	2.29	0.01bc	0.4147b	1.6467ab	0.03cd	1.24ab	1.45	0.28	6.90ab
SED	**0.617**	**0.011**	**0.296**	**0.555**	**0.012**	**0.243**	**0.394**	**0.006**	**0.293**	**0.366**	**0.065**	**1.667**
Fpr.	**0.683**	**0.866**	**0.917**	**0.128**	**0.019**	**0.026**	**0.0004**	**<.0001**	**0.001**	**0.100**	**0.264**	**0.004**

Means of 3 replicates. Means followed by the same letter within the same column are not significantly different according to Tukey's HSD test at p 0.05. Means not followed by a letter in each column are not significantly different at P 0.05

Uptake of potassium (K) followed the same trend as P with significant differences between products on K uptake observed in the Vertisol (p=0.0005) and Rhodic Ferralsol (p=0.026). All products enhanced K uptake in the Vertisol and the highest uptake was observed with PHC Biopak inoculation (243% increment) followed by PHC Colonize (209%), Rhizatech (174%), MYCOR (88%), ECO-T (71%) and Subtilex (67%) compared to the uninoculated control. All products except Subtilex and ECO-T enhanced K uptake in the Rhodic Ferralsol with the highest uptake realized with inoculation of plants with PHC Colonize (by 175%) followed by PHC Biopak (by 114%), Rhizatech (37%) and MYCOR (30%) inoculation. Only Rhizatech and Subtilex slightly enhanced K uptake (by 25 and 6% respectively) in the Humic Nitisol (Table 10). The effect of products on N uptake ranged from slightly positive to negative depending on the soil type. In the Humic Nitisol, ECO-T and Subtilex enhanced N uptake by 24 and 8 % respectively compared to the uninoculated control (Table 10).

Nitrogen uptake was significantly reduced by all products in the Vertisol (p=0.0004) in the range of 21-73 % relative to the uninoculated control. Product inoculation in the Rhodic Ferralsol had no effect on N uptake.

Macronutrients uptake in the conventional media was either slightly enhanced or reduced by product inoculation. MYCOR and ECO-T inoculation enhanced N uptake by 39 and 63% respectively while Rhizatech enhanced K and P uptake by 4 and 10% respectively in the conventional medium compared to the non-inoculated control (Table 10).

4.4.2 Uptake of Zinc, manganese, sulphur, copper and iron

Micronutrients (zinc, manganese, sulphur, copper and iron) uptake was significantly affected by product inoculation in the Vertisol, followed by the Rhodic Ferralsol and least affected in the Humic Nitisol (Fig. 5, 6, 7, 8 and 9). PHC Biopak inoculation increased Zn uptake in the Vertisol by the highest magnitude of 430% followed by PHC Colonize (337%), Rhizatech (289%), MYCOR (209%), ECO-T (115%), and Subtilex (93%) relative to the non- inoculated control. In the Rhodic Ferralsol, PHC Colonize increased uptake by 121%, PHC Biopak by 35%, MYCOR by 45% and Rhizatech by 12% relative to the uninoculated controls. ECO-T and Subtilex inoculated plants had lower Zn uptake than the controls in the Rhodic Ferralsol. Rhizatech inoculation had the highest Zn uptake in the Humic Nitisol increasing uptake by 62% relative to the uninoculated control (Fig. 5).

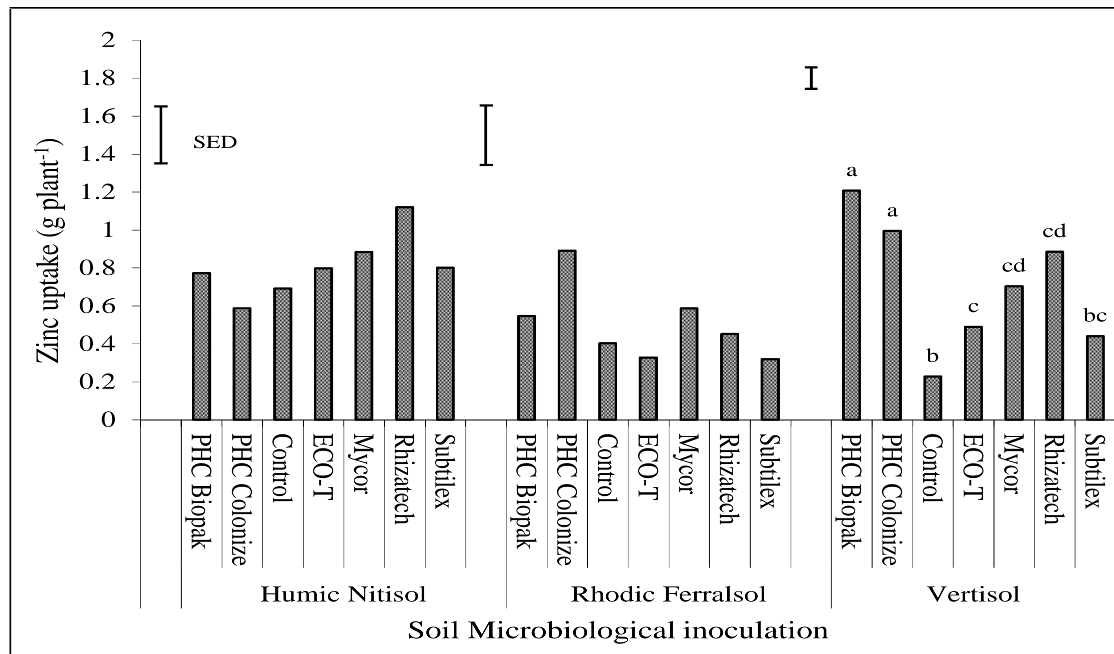

Figure 5: Zinc uptake in tissue cultured banana plants treated with microbiological products in three soil types, 22 weeks after deflasking.
Error bars represent standard error of difference between means. Means followed by the same letter within the same soil type are not significantly different according to Tukey's HSD test at p 0.05. Bars without letters indicate no significant differences between means within a soil type.

Similarly, all products significantly increased Manganese (Mn) uptake in the Vertisol (p=0.0001) with PHC Biopak enhancing uptake by 809%, PHC Colonize by 608%, Rhizatech by 190%, Subtilex by 132%, MYCOR by 107% and ECO-T by 87% relative to the uninoculated controls. In the Rhodic Ferralsol, PHC Biopak inoculation increased uptake by 140%, PHC Colonize by 234%, ECO-T by 18% and MYCOR by 2% relative to the uninoculated control. Rhizatech and Subtilex inoculated plants in the Rhodic Ferralsol had lower Mn uptake than the uninoculated controls. In the Humic Nitisol, enhancement of Mn uptake was observed with PHC Biopak (62% increament), Rhizatech (20%) and MYCOR (6%) relative to the uninoculated controls (Fig. 6).

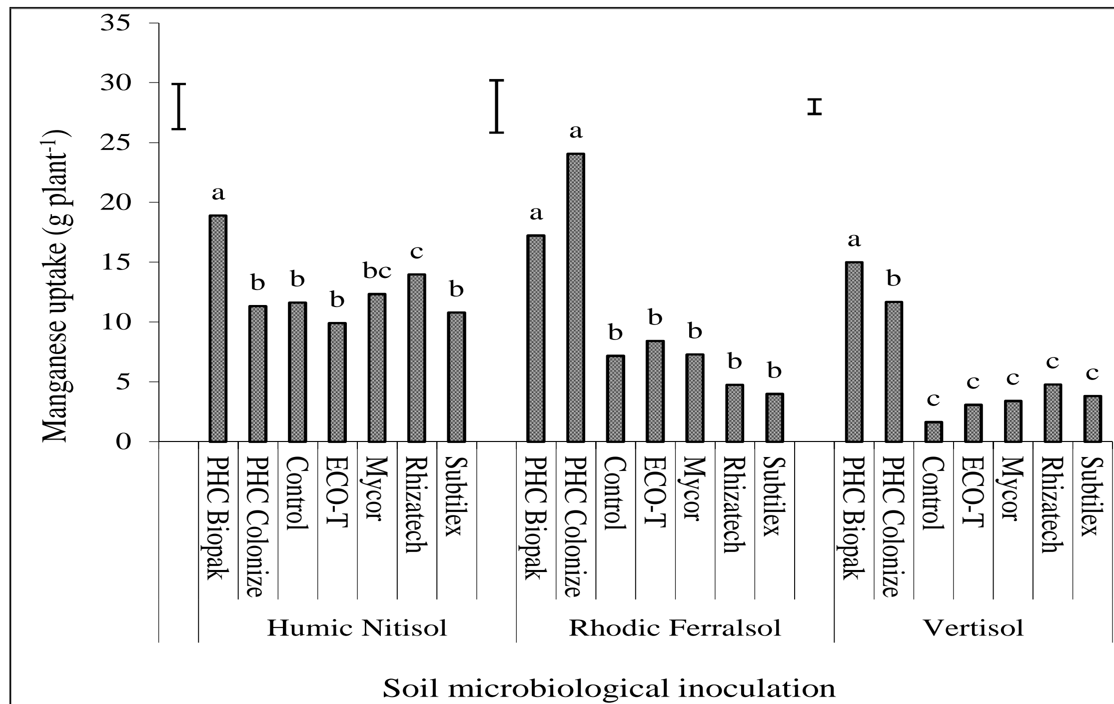

Figure 6: Manganese uptake in tissue cultured banana plants treated with microbiological products in three soil types, 22 weeks after deflasking. Error bars represent standard error of difference between means. Means followed by the same letter within the same soil type are not significantly different according to Tukey's HSD test at p 0.05.

Copper uptake followed the same trend as Zinc and Manganese with PHC Biopak enhancing uptake by 244%, PHC Colonize by 243%, Rhizatech by 156%, ECO-T by 104%, MYCOR by 94% and Subtilex by 61% relative to the uninoculated control. In the Rhodic Ferralsol, only PHC Biopak and PHC Colonize enhanced uptake by a magnitude of 34 and 36% respectively relative to the uninoculated controls. In the Humic Nitisol positive effects on Cu uptake were observed with Rhizatech (20% increment) and Subtilex (1% increment) relative to the control (Fig. 7).

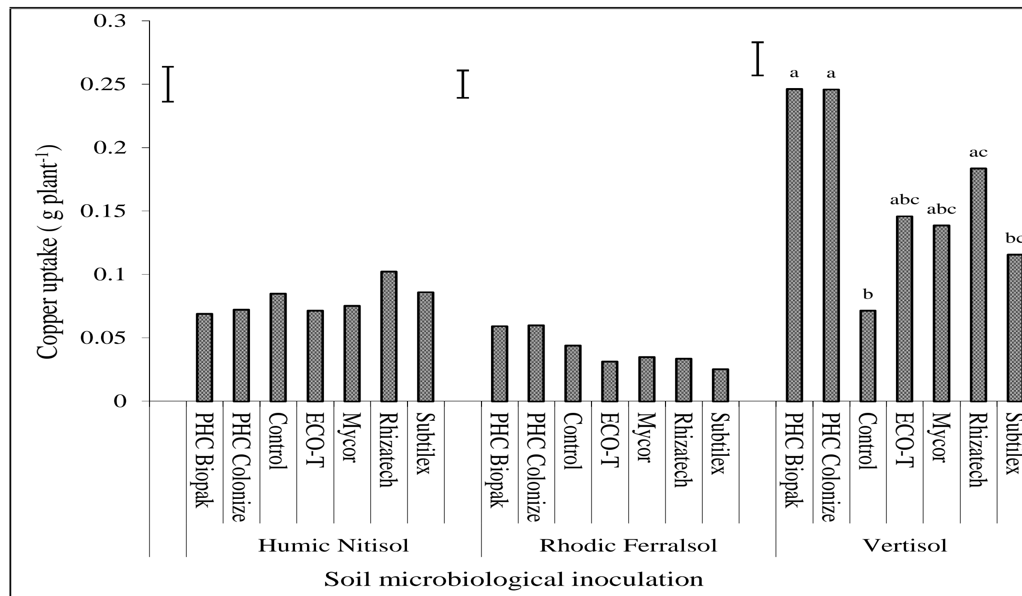

Figure 7: Copper uptake in tissue cultured banana plants treated with microbiological products in three soil types, 22 weeks after deflasking.
Error bars represent standard error of difference between means. Means followed by the same letter within the same soil type are not significantly different according to Tukey's HSD test at p 0.05. Bars without letters indicate no significant differences between means within a soil type.

Significant differences in sulphur uptake by inoculated plants were observed within the Vertisol ($p<0.0001$) and Rhodic Ferralsol ($p=0.016$). In the Vertisol, all products increased uptake by a magnitude of 330% for PHC Colonize, 320% for PHC Biopak, 138% times for Rhizatech, 87% for ECO-T and MYCOR and 76% for Subtilex relative to the uninoculated control. PHC Colonize enhanced S uptake in the Rhodic Ferralsol by 181%, PHC Biopak by 151%, ECO-T by 3% and MYCOR by 11% relative to the uninoculated controls (Fig. 8).

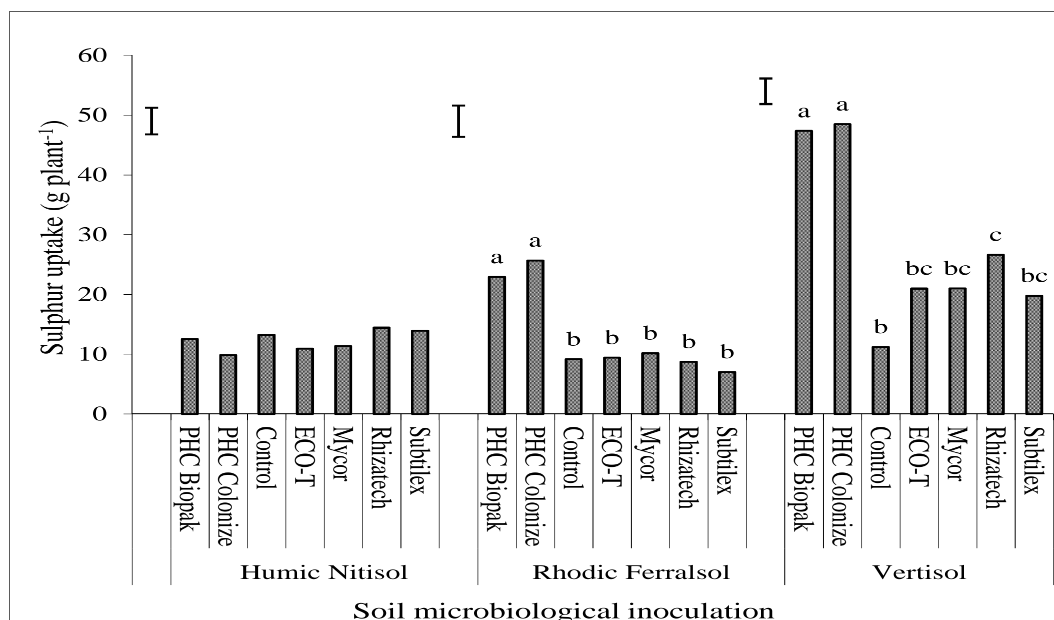

Figure 8: Sulphur uptake in tissue cultured banana plants treated with microbiological products in three soil types, 22 weeks after deflasking.
Error bars represent standard error of difference between means. Means followed by the same letter within the same soil type are not significantly different according to Tukey's HSD test at p 0.05. Bars without letters indicate no significant differences between means within a soil type.

Iron uptake followed the same trend observed with Zn, Mn, Cu and Sulphur, with PHC Biopak and PHC Colonize being the most effective product on Iron uptake in the Vertisol and Rhodic Ferralsol (Fig. 9). In the Vertisol, PHC Colonize enhanced Iron (Fe) uptake by 425% followed by PHC Biopak by 271%, Rhizatech by 183%, Subtilex by 76%, ECO-T by 57% and MYCOR by 45%. In the Rhodic Ferralsol PHC Colonize enhanced uptake by 46%, PHC Biopak by 39%, ECO-T by 10% and Rhizatech by 5% relative to the uninoculated controls. Interestingly all products enhanced uptake in the Humic Nitisol in the following order relative to the uninoculated control: PHC Colonize (158%) > PHC Biopak (99%) > Rhizatech (76%) > ECO-T (43%) > MYCOR (41%) > and Subtilex (10%).

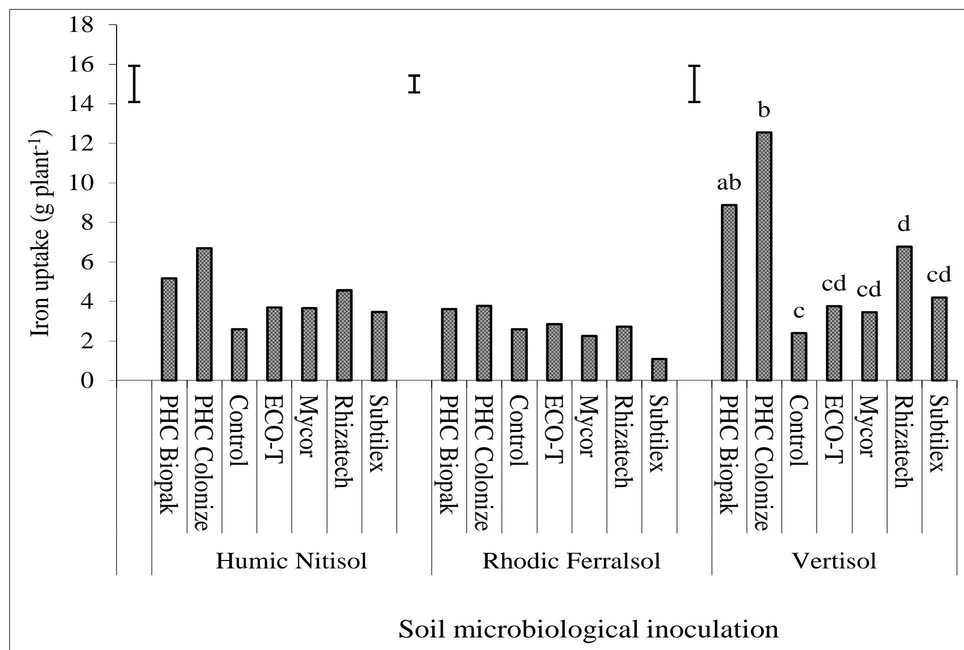

Figure 9: Iron uptake in tissue cultured banana plants treated with microbiological products in three soil types, 22 weeks after deflasking.
Error bars represent standard error of difference between means. Means followed by the same letter within the same soil type are not significantly different according to Tukey's HSD test at p 0.05. Bars without letters indicate no significant differences between means within a soil type.

Micronutrient uptake in the conventional media was only statistically different for Zinc uptake with Rhizatech enhancing uptake by 17% compared to the uninoculated control. Except for Rhizatech inoculation, which enhanced the uptake of Cu (5%), S (7%), Fe (20.6%) and MYCOR which enhanced the uptake of Fe (12.4%), all other products had negative effects on micronutrient uptake (Table 11).

Table 11: Uptake of zinc, manganese, copper, sulphur and iron (g/ plant) in tissue cultured banana plants treated with microbiological products in conventional nursery medium

Treatment	Zn	Mn	Cu	S	Fe
PHC Colonize	4.86a	18.96	0.32	65.31	14.43
Control	8.07ab	67.91	0.58	125.05	17.18
ECO-T	5.74a	43.76	0.32	101.33	14.04
Mycor	8.33b	49.67	0.36	115.21	19.31
Rhizatech	9.45b	66.32	0.61	134.47	20.72
Subtilex	4.02a	64.84	0.46	99.50	18.87
SED	**1.12**	**20.33**	**0.15**	**22.42**	**5.45**
Fpr.	**0.005**	**0.169**	**0.211**	**0.086**	**0.738**

Means followed by the same letter within the same column are not significantly different according to Tukey's HSD test at p 0.05. Means not followed by a letter within a column are not significantly different at p 0.05.

4.4.3 Sodium, magnesium and calcium uptake

Similar to the observations made on the macro and micro nutrients, significant interaction between soil and products were observed in the uptake of secondary nutrients (sodium, magnesium and calcium). Product inoculation enhanced sodium (Na) and magnesium (Mg) uptake in the Vertisol and Rhodic Ferralsol. Variable product effects on Calcium (Ca) uptake were observed in the Vertisol, Rhodic Ferralsol and Humic Nitisol (Table 12).

All products increased Na uptake in the Vertisol with the highest uptake observed with PHC Biopak (141%) followed by inoculation with PHC Colonize (115%), MYCOR (56%), Subtilex (47%), Rhizatech (45%) and ECO-T (12%) relative to the uninoculated control (Table 12). Inoculation of plants in the Rhodic Ferralsol enhanced Na uptake by 195 and 10% respectively compared to the uninoculated controls. Inoculation with all other products reduced uptake in the range of 32 to 56% relative to the uninoculated control. Sodium uptake in the Humic Nitisol was

mostly enhanced by PHC Biopak inoculation by a magnitude of 67% relative to the uninoculated control. Rhizatech, Subtilex and PHC Colonize enhanced Na uptake in the Humic Nitisol by 36, 24 and 22% respectively while ECO-T and MYCOR inoculation reduced uptake by 6 and 4% compared to the control.

Similarly, PHC Biopak inoculated plants had the highest Mg uptake with a 226% increase followed by PHC Colonize increasing uptake by 208%, Rhizatech by 147%, MYCOR by 86%, ECO-T by 81% and Subtilex by 49% in the Vertisolq. In the Rhodic Ferrralsol, PHC Colonize enhanced uptake by 91%, PHC Biopak by 58% and Rhizatech by 8% while MYCOR, ECO-T and Subtilex inoculation reduced uptake by 90, 9 and 24% respectively relative to the uninoculated control (Table 12).

Contrally to the observations made on Mg and Na uptake, all products reduced uptake of Ca in the Vertisol by a range of 12-46% relative to the uninoculated control. Plants inoculated with Rhizatech in the Rhodic Ferralsol had the highest Ca uptake (56%) followed by Subtilex (4%) and ECO-T (1%). Rhizatech and Subtilex inoculation slightly enhanced Ca uptake by 18 and 1% respectively compared to the control in the Humic Nitisol (Table 12).

Secondary nutrients uptake in the conventional media were statistically different ($p<0.05$) for Magnesium uptake with Rhizatech inoculation enhancing magnesium uptake by 12%. All other products reduced magnesium uptake in the range of 7-47%, Sodium uptake by 17-33% and Calcium uptake by 7-33% (Table 12).

Table 12: Uptake of sodium (g/ plant) magnesium and calcium in tissue cultured banana plants treated with microbiological products in three soil types

	Humic Nitisol			Rhodic Ferralsol			Vertisol			Conventional nursery medium		
	Na	Mg	Ca	Na	Mg	Ca	Na	Mg	Ca	Na	Mg	Ca
PHC Biopak	0.004	0.084	0.403a	0.003a	0.123	x	0.012a	0.309a	0.433ab	*	*	*
PHC Colonize	0.003	0.074	0.443ab	0.004b	0.148	0.417a	0.011ac	0.292	0.380a	0.038	0.534a	0.37
Control	0.003	0.123	0.690ab	0.006c	0.077	0.610a	0.005b	0.095a	0.707c	0.057	1.003b	0.547
ECO-T	0.002	0.093	0.637ab	0.003ac	0.070	0.618a	0.005b	0.171cd	0.520abc	0.044	0.728a	0.600
MYCOR	0.003	0.108	0.583ab	0.017ac	0.093	0.520a	0.008bc	0.176cd	0.520abc	0.054	0.933c	0.556
Rhizatech	0.004	0.154	0.817b	0.006ac	0.084	0.937b	0.007b	0.234d	0.623bc	0.051	1.118c	0.547
Subtilex	0.003	0.122	0.700ab	0.003c	0.059	0.637a	0.007b	0.141bc	0.443ab	0.040	0.781a	0.510
SED	**0.001**	**0.039**	**0.104**	**0.0031**	**0.0420**	**0.0836**	**0.001**	**0.168**	**0.003**	**0.01182**	**0.1189**	**0.07869**
Fpr.	**0.357**	**0.473**	**0.020**	**0.0010**	**0.2738**	**0.0004**	**0.0047**	**0.0001**	**0.003**	**0.4503**	**0.0062**	**0.0662**

Means followed by the same letter within the same column are not significantly different according to Tukey's HSD test at p 0.05. Means not followed by a letter in each column are not significantly different at P 0.05
*All plants treated with PHC Biopak in the conventional medium did not survive.

4.5 DISCUSSION

The study showed that commercial microbiological products influence nutrient uptake in tissue cultured banana and this is dependent on soil type. The efficacy of mixed species *Bacillus* and mycorrhiza in nutrient uptake was best in the Vertisol and Rhodic Ferralsol soils which have been previously reported (and also confirmed by the initial soil characterization of experimental soils) as deficient in nitrogen, phosphorous and micronutrients (FAO, 2006). This depicts the ability of bacteria and mycorrhiza to enhance nutrient uptake in nutrient poor soils. The benefits of *Bacillus* and mycorrhizae are best in nutrient poor soils (Schubert and Hayman, 1986; Egamberdiyeva, 2007). Bacterial strains had a much better stimulatory effect on plant growth and N, P and K uptake of maize in nutrient deficient Calcisol soil and their efficiency reduced in relatively rich loamy sandy soil where only root growth and N and K uptake was stimulated (Egamberdiyeva, 2007).

Mixed species mycorrhizal inoculation enhanced P and K uptake in the Vertisol and Rhodic Ferralsol soils depicting the ability of mycorrhizae in acquisition of nutrients that are limited or fixed in soils (Schubert and Hayman, 1986). Mycorrhizae are known to help plants acquire nutrients that are fixed in the soil such as P and also significantly improve plant nutrition under low fertility soil conditions especially low P (Schubert and Hayman, 1986).

Mycorrhizal inoculation enhanced uptake of P and Zn uptake in the conventional media (56 P kg^{-1}) suggesting positive nutrient uptake effects of mycorrhizae in both

poor soils (with P levels as low as 3 mg P kg^{-1} in the Vertisol) and moderately fertile soils. Mycorrhiza functions best in soils with a P level of 50mg/Kg (Schubert and Hayman, 1986). However, the higher levels of nutrient uptake in the conventional media prompts a need for further research to establish the optimal soil nutrient levels that facilitate optimal efficacy of biological inoculation on plant nutrient uptake.

Efficacy of products on N uptake was variable ranginging from negative to positive in the Vertisol, Rhodic Ferralsol, Humic Nitisol and conventional media suggesting that the efficacy of the microbe based inoculants on N uptake does not always follow that of P and K. Arbuscular mycorrhiza (AM) fungi inoculation effect on foliar nutrient concentration increase in banana was not always observed (Jaizme-Vega and Azcon, 1995). In some studies, mycorrhizal inoculation increased foliar N, P and K in banana plants (Jaizme-Vega and Azcon, 1995) while in other studies only increase of foliar P was observed (Jaizme-Vega *et al.,* 2003). *Trichoderma* inoculation was the best for N uptake in the conventional media suggesting the influence of organic amendments on the functioning of *Trichoderma*. *Trichoderma* is known to function best in high organic soils (Okoth *et al.,* 2007) and the enhanced N uptake in the conventional media could be attributed to the high organic N level in the conventional media (0.61%) compared to the Vertisol (0.25%), Rhodic Ferralsol (0.1%) and Humic Nitisol (0.25%).

There was consistency in the performance of products in the uptake of macro nutrients, micro nutrients, and secondary nutrients suggesting a similarity in the efficacy of microbiological inoculation in macro and micronutrient uptake. There is

a lot of information on the uptake of macro and micronutrients by AM fungi but limited information on uptake of macro and micro nutrients by rhizobacteria. Liu *et al*., (2000), reported an interaction between P and micronutrients levels, with the AM fungi extraradical length higher at low P than at high P only when no micronutrients were added. It is widely reported that AM fungi increase plant Cu and Zn uptake (Lambert *et al*., 1980; Clark *et al*. 1999; Liu *et al*., 2000). More Cu and Zn were measured in mycorrhizal soybean plants than in P-supplemented non mycorrhizal soybean plants of the same size, in soil with low levels of Cu and Zn. *Glomus mosseae* contributed from 16-25% of total Zn uptake by maize growth in calcareous soil and from 52-65% of total Cu uptake by clover plants grown in the same soil (Liu *et al*., 2000). This may explain the consistent performance of AM fungi in the uptake P, K, Zn, Mn, Cu uptake in all the experimental soils (Vertisol, Rhodic Ferralsol, Humic Nitisol and Conventional media).

The effect of products on the uptake of secondary nutrients followed the same trend as for macro and micro nutrients except for calcium that was mainly negatively affected by product inoculation. However, mixed species mycorrhizal inoculation enhanced Calcium uptake in the Rhodic Ferralsol and Humic Nitisol soils both of which had lower cation exchange capacity (7 and15 $cmol_c kg^{-1}$ respectively) compared to the Vertisol (52.5 $cmol_c kg^{-1}$) and conventional media (19 $cmol_c kg^{-1}$). This may depict the ability of mycorrhiza to enhance the uptake of fixed soils nutrients (for example Calcium and Phosphorous) in soils that have low cation exchange capacity (Liu *et al.,* 2000; Brady and Weil, 2002).

The effect of products on mycorrhizal colonization was variable and mostly not consistent with the effects of products on nutrient uptake. Mixed species mycorrhizal product (Rhizatech) had the highest percent mycorrhizal colonization in the Rhodic Ferralsol suggesting that mycorrhiza could stimulate mycorrhizal symbiosis in soils with a P level of 7 mg kg^{-1}. Mycorrhizal symbiosis is one of the mechanisms employed by rhizospheric microorganisms for plant nutrient acquisition (Harman, 2006; Shamsuddin *et al.,* 2000; Jaizme-Vega *et al.*, 2004). The enhanced nutrient uptake in the Vertisol inoculated with mixed species may be attributed to the high mycorrhizal colonization observed in the study. Under some conditions, plant growth-promoting rhizobacteria are able to promote fungal spore germination and germinative tube elongation (Andreucci *et al.*, 1999) or even enhance mycorrhizal hyphae density (Ravnskov and Jakobsen, 1999).

A positive relationship between mycorrhizal symbiosis and nutrient uptake was not always observed in the study depicting that some microbes, for example, the *Bacillus* inoculation in the Rhodic Ferralsol may have employed other mechanisms, for enhancing banana nutrient uptake. Similarly, high mycorrhizal colonization with the mixed species mycorrhizal product (Rhizatech) did not correspond to enhanced nutrient uptake in the Rhodic Ferralsol depicting that the soil nutrient levels may have affected the overall plant nutrient acquisition. Lack of correlation between mycorrhizal infectivity and effectiveness of fungi has been reported in commercial AM fungi inoculated to banana cultivar Petite Naine (Rodríguez-Romero, 2007) and in strawberry (Bull *et al.,* 2005). This may explain why the single species mycorrhizal product (MYCOR) was not effective in enhancing nutrient uptake in the

conventional media yet plants inoculated with this product had the highest mycorrhizal colonization in the conventional media.

It is evident from these findings that commercial biological inoculation enhances nutrient uptake in tissue culture banana and this is dependent on the microbial formulations and the type of soil media used for planting. From this study, inherent soil nutrient levels, soil organic content and cation exchange capacity played a major role in influencing the functioning of the microbes used for inoculation. Soils that are poor in nutrients require microbiological inoculation to enhance nutrient uptake as was evident with the Vertisol and Rhodic Ferralsol while inoculation of moderate to rich nutrient soils may have minimal to no effect on nutrient uptake as was the case with the Humic Nitisol and conventional media. Nutrient uptake was affected by the species composition of the commercial products. Multiple species formulations of *Bacillus* and mycorrhiza greatly enhanced macro, micro-nutrient and secondary nutrient uptake especially in the Vertisol and Rhodic Ferralsol compared to the single species formulations of *Bacillus*, mycorrhiza and *Trichoderma* implying differential functioning of single and multiple species product formulations. This implies that it is important to understand the prevailing soil conditions and the mode of action of commercial microbiological formulations before application.

CHAPTER FIVE

INFLUENCE OF SELECTED MICROBIOLOGICAL PRODUCTS ON *FUSARIUM* ***OXYSPORUM*** **F. SP.** ***CUBENSE*** POPULATIONS IN FIELD SOILS USED FOR GROWING TISSUE CULTURED BANANA

ABSTRACT

Fusarium oxysporum f. sp. *cubense (Foc)* threatens the survival of tissue cultured (TC) banana worldwide. Control by fungicides has failed and breeding of resistant cultivars is preferred rather than control of the pathogen. A completely randomized design experiment under greenhouse conditions evaluated the potential of three commercially available biological products namely PHC Biopak (*Bacillus*), Rhizatech (mycorrhiza) and ECO-T (*Trichoderma harzianum*) on suppression of *Fusarium oxysporum* f.sp. *cubense* in the rhizosphere of TC banana established in three soil types (Vertisol, Rhodic Ferralsol and Humic Nitisol) from banana growing regions in Kenya. Isolation of *Fusarium* was done before and after inoculation of TC banana with the commercial products under nursery conditions. Using *Fusarium*-selective media three *Fusarium* species were recovered from the soils. *F. oxysporum* f.sp. *cubense* was the most abundant accounting for 60.6% of all the isolates and mainly isolated from the Humic Nitisol. Other isolates were *F. proliferatum* and *F. incarnatum* mainly isolated from the Vertisol and Rhodic Ferralsol accounting for 32.9% and 6.4% of the isolates respectively. Suppression of *F. oxysporum* f.sp. *cubense* by *Bacillus*, mycorrhizae and *Trichoderma* inoculation differed significantly at p 0.05 within soil types. PHC Biopak, ECO-T and Rhizatech reduced *F. oxysporum* f.sp. *cubense* CFU g^{-1} by 47%, 68% and 55% respectively in

the Humic Nitisol. ECO-T reduced *Fusarium* CFU g^{-1} by 6% in the Rhodic Ferralsol and PHC Biopak by 50% in the Vertisol compared to the non-inoculated soils. All products reduced *F. proliferatum* populations by a magnitude of 55-100% in the Humic Nitisol and *F. incarnatum* by 100% in the Vertisol and Humic Nitisol. A positive correlation was observed between soil chemical properties and *Fusarium* populations. Soils with high carbon (C) and nitrogen (N) contents had the highest *Fusarium* population (r=0.268 and 0.25, respectively) and were also the most receptive to inoculation. Soil physical properties had variable effects on *Fusarium* populations with sandy soils showing a negative relationship and clay soil showing a positive relationship with *Fusarium* CFU g^{-1} soil (r=-0.25 and 0.25, respectively). The study demonstrates that there is potential in the use of commercial biological products to suppress *F. oxysporum* f.sp. *cubense* and that the efficacy of the products depends on soil nutrient levels and physical properties.

5.0 INTRODUCTION

Banana is grown in more than 120 countries throughout tropical and subtropical regions and it is the staple food for more than 400 million people (Molina and Valmayor, 1999). Among the production constraints, *Fusarium* wilt (Panama disease) caused by the fungus *Fusarium oxysporum* f.sp *cubense* (*Foc*) is the most devastating disease affecting commercial and subsistence of banana production (Ploetz, 2005). The disease is ranked as one of the top 6 important plant diseases in crop destruction and compares with some of most devastating diseases such as wheat rust and potato blight (Carefoot and Sprott, 1969; Ploetz and Pegg, 1997). The disease almost destroyed the banana export industry, built on the Gros Michel variety, in Central America during the 1950's (Stover, 1962). In addition, the widely grown clones in the ABB 'Bluggoe' and AAA 'Gros Michel and Cavendish' sub groups are also highly susceptible to this disease worldwide.

Presently, *Fusarium* wilt has been reported in all banana-growing regions of the world (Asia, Africa, Australia and the tropical Americas) except some islands in the South Pacific, the Mediterranean, Melanesia, and Somalia (Stover, 1962; Ploetz and Pegg, 2000). *Fusarium* wilt of banana was observed in Kenya for the first time in 1952 in the coastal and central Kenya regions on a cultivar suspected to be Bluggoe (ABB) (Kung'u, 1998). In the mid-1990s the disease was reported in other banana-growing areas in Kenya and the affected varieties include the Gros Michel (AAA), Sukari (AB), Bluggoe (ABB) Pisang awak (ABB) and Muraru (AA) (Kung'u, 1998).

Fusarium oxysporum f.sp *cubense (Foc)* is a soil borne hyphomycete and is one of more than 100 formae speciales of *F. oxysporum* that causes vascular wilts of flowering plants (Domsch *et al*., 1980; Nelson *et al*., 1983). The fungus survives in soil for up to 30 years as chlamydospores in infested plant material or in the roots of alternative hosts (Ploetz, 2000). Management of the disease is mainly through chemical soil fumigation (Herbert and Marx, 1990), fungicides (Lakshmanan *et al*., 1987), crop rotation (Hwang, 1985; Su *et al*., 1986), flood–fallowing (Wardlaw, 1961; Stover, 1962), organic amendments (Stover, 1962) and planting of resistant cultivars (Moore *et al*., 1999). The broad-spectrum biocides used to fumigate soil before planting, particularly methyl bromide are environmentally damaging. The most cost effective, environmentally friendly method is use of resistant cultivars when they are available (Fravel *et al*., 2003). Unfortunately resistant breeding can be very difficult when no dominant gene is known, for example, in bananas (Stover and Buddenhagen, 1996). Furthermore, planting of resistant varieties also cannot be implemented because of consumer preference (Viljoen, 2002).

In cases where there is no treatment for *Fusarium* wilt, the disease is controlled by preventing the introduction of the pathogen, destruction of diseased plants and isolation of susceptible plants from infested sites (Simone and Cashion, 1996). Tissue culture (TC) plantlets offer an excellent means for providing pest- and disease-free planting material to farmers (Mink, 1991, Robinson 1994; Mbaka *et al*., 2008). In micro-propagation, the growth media is nutrient-rich and devoid of microbes, hence predisposing the delicate plantlets to infestation and infection by pests and diseases respectively at nursery and subsequent field establishment.

Biological hardening of TC is therefore recommended before transplanting (Smith and Read, 1997; Nowak, 1998). The use of micro-propagated banana plants led to a reduction in the spread of *Fusarium oxysporum* f.sp. *cubense (Foc)*, but at the same time, resulted in enhanced susceptibility to *Foc*, under field conditions (Smith *et al*., 1998) due to the loss of native endophytes during tissue culture, including beneficial plant growth promoting rhizobacteria and fungi (Nowak, 1998; Smith *et al*., 1998). Therefore, biotization of tissue culture plantlets with native effective non-pathogenic endophytic microbes including mycorrhizal fungi during first or second stage hardening but before planting, enhance plant resistance of tissue cultured plants against *Fusarium* wilt (Nowak, 1998). Lian *et al.,* 2009, reported that reintroduction of naturally occurring endophytes to tissue culture banana plantlets resulted in a substantial reduction in the infection and severity of *Fusarium* wilt disease (67%) as well as increased plant growth parameters (height, girth, leaf area).

Several reports have previously demonstrated the successful use of different species of *Trichoderma, Pseudomonas, Streptomyces*, mycorrhiza and non pathogenic *Fusarium* of both rhizospheric and endophytic in nature against *Fusarium* wilt disease under both glass house and field conditions (Sivamani and Gnanamanickam, 1988; Lemanceau and Alabouvette, 1991; Alabouvette *et al*., 1993; Jaizme-vega *et al*., 1998; Larkin and Fravel, 1998; Thangavelu *et al.,* 2002; Weller *et al*., 2002; Getha *et al*., 2005; Jie *et al*., 2009; Thangavelu *et al.,* 2010). Research has mainly focused on acclimatization of tissue cultured banana plantlets in sterile nursery medium. Seedlings, however, face the challenge of soil-borne pathogens once transplanted to field conditions and this could lead to massive loss of plants. The

potential of *Bacillus, Trichoderma* and mycorrhiza (AMF) to suppress *Fusarium oxysporum* f.sp. *cubense (Foc)* could be harnessed by biological acclimatization. These microbes proliferate in the rhizosphere and may mitigate the challenge possed by soil borne *Foc.* The use of non-sterile field soils for establishment of TC banana in the nursery has not been explored in Kenya despite the opportunity it offers for decentralization of commercial TC banana nurseries to increasing production to meet the demand for plantlets. This study aimed at evaluating the potential of mycorrhizal, *Bacillus* and *Trichoderma* based commercial biological products on suppression of *Fusarium* spp. populations in the rhizosphere of tissue cultured banana grown in field soils.

5.2 MATERIALS AND METHODS

5.2.1. Source of tissue cultured materials and soil properties

Tissue cultured (TC) banana plantlets were obtained from Jomo Kenyatta University of Agriculture and Technology Biotechnology laboratory. Soils for establishing TC banana plants were obtained from three banana growing regions in Kenya as described in section 3.2.1 of this thesis.

5.2.2 Experimental design and inoculation process of tissue cultured plants

The experiment followed a three by three factorial layout with three soil types (Vertisol, Humic Nitisol and Rhodic Ferralsol) and three commercial products (mycorhizae, *Trichorderma* and *Bacillus* based) including ECO-T/T22 (*Trichoderma harzianum* strain Rifai KRL AG2, Plant), PHC Various *Bacillus* spp), Rhizatech (*Glomus mosseae*, *G. etunicatum*, *G. intraradices* and *G. aggregatum*). Application of products was done as per recommendations of the manufacturers. The products are normally recommended for plant growth but due to the compositions of the products, an attempt was made to evaluate whether they also have capacity to reduce soil pathogens. These products also consistently showed positive growth effects especially under green house conditions. Three replicates consisting of 10 plants per replicate were considered per treatment. Control treatments (no product applied) also consisting of 3 replicates for each soil condition were also included. The experimental units were arranged in a completely randomized design in a green house. Details of the experimental design and inoculation process are as described in subsection 3.2.2 and 3.2.3 of this thesis.

5.2.3 Assessment of leaf dryness

Leaf dryness was estimated as a percentage of the total leaf surface affected by wilt and averaged over the total number of leaves affected per plant at 22 weeks after deflasking.

5.3 Soil mycological characterization

Fusarium spp were isolated from soil using the serial dilution plating method (Burgess *et al*., 1988) with 0.1 % Tap Water Agar (TWA), Brayford (1993). Air dried soil samples (10 g) were suspended into dilution blanks (90 ml of sterile TWA) to make a ten fold dilution (10^{-1}) of a microbial suspension. The dilution was shaken to obtain a uniform suspension of microorganisms and 10 ml of resulting suspension were pipetted into a flask containing 90 ml of sterile distilled water. This procedure was repeated up to the third ten-fold dilution. One millilitre aliquots from second and third dilutions, in three replicates, were aseptically pipetted to Petri dishes containing *Fusarium*-selective PCNB-Peptone Agar (PPA) media and spread evenly across the agar surface using a glass applicator. The Petri dishes were kept at room temperature (25°C) and observations were made from the third day onwards for developing colonies. For each of the colonies growing on PPA plates, a well-defined and shaped colony was chosen and a small piece at the edge of the colony was carefully and aseptically transferred onto a separate synthetic nutrient agar (SNA) Petri plate and incubated at 25°C for 5 days. Subsequently, in order to obtain monosporic cultures of each colony formed on SNA, from which identification was based, very dilute inocula, of 5 to 10 spores per drop of suspension (when viewed at low power magnification), were prepared and spread on 2% Tap Water Agar plates.

These were then incubated for 15 hours for germination. Germlings were then subcultured on different media i.e. SNA, Carnation-Leaf- Agar (CLA) and Potato-Dextrose-Agar (PDA) media plates, for growth and identification. Species of *Fusarium* readily formed sporodochia with robust, uniform macroconidia on the CLA that was used for identification. The PDA cultures were used to assess pigmentation and gross colony morphology. Cultures grown on SNA were evaluated for microconidia, which were more abundant and diverse on this medium, and for chlamydospores, which were more common and produced rapidly on this medium. All the pure isolates subcultured on PDA, CLA and SNA were incubated for ten to twenty days at 25°C under fluorescent lamps to enhance sporulation. *Fusarium* was identified to the species level where morphological characters were used as the basis of identification (Nelson *et al.*, 1983; Burgess *et al.*, 1988; Brayford, 1993; Leslie and Summerell, 2006). After identification, the single spore cultures were stored in agar slants of SNA in screw cap bottles at 4°C and in sterilized soil in screw cap bottles.

5.3.1 Molecular confirmation of morphologically identified cultures

In order to obtain DNA from each of the identified *Fusarium* species, single-spore *Fusarium* species were grown for five days at 25°C in Potato Dextrose Agar (PDA) (Difco). Mycelium (~0.1-0.2g) was collected using sterile scapel from PDA media and placed in eppendorf tubes. Culture cells were opened by adding 500µl of CTAB extraction buffer (100Mm Tris Hcl [pH 8], 2% [wt/vol] CTAB, 50Mm EDTA, 0.7 M NaCl, 0.17% [vol/vol] ß-mecarptoethanol and 1% [w/v] PVP), pre warmed to 65°C, two glass beads added and the mixture placed in miller at a frequency of

30/sec for 5 minutes. Samples were incubated at 65°C for 30 minutes in a water bath. Chloroform (500 µl) -isoamyl choloroform) (24:1 vol/vol) was added and the two phases were mixed several times by inverting tubes gently. The tubes were centrifuged at 14,000 rpm for 10 min at room temperature in a microfuge. The supernatant was removed and transferred into new 1.5 Eppendorf tube. Pre-boiled 10 µl of 10mg RNase A was added and mixed gently by inverting the tube five times. The samples were then incubated in a water bath at 37°C for 30 min. Centrifuging and addition of RNase A were repeated. An equal volume of cold isopropanol (pre-chilled in a -20° freezer) was added mixed gently and incubated at -20° in a freezer for 30 min. The samples were then centrifuged at 14000rpm for 10 min at room temperature in a microfuge and supernatant removed by pouring into clean Eppendorf tubes taking care not to loose the pellet. 500µl of 70% ethanol (at room temperature) was added to the tube containing DNA, centrifuged at 14000 rpm for 5 min and the supernatant carefully poured off. The 70% ethanol wash was repeated once, the supernatant carefully poured off and the DNA pellet dried for 60 min by leaving the open tube on its side on the bench. Low salt TE buffer (100µl) was added to the dried pellet. The pellet was dissolved by incubating at 37°C in a water bath for 30min. One µl of DNA was ran on Agarose gel to determine the concentration and stored at -20^{0}C.

5.3.2 DNA amplification and sequencing procedure

The polymerase chain reaction (PCR) procedures were carried out as described by Khalil *et al.* (2003). Forward primer tef 1 (5'-

ATGGGTAAGGA(A/G)GACAAGAC-3') and the reverse primer tef 2 (5'-GGA(G/A)GTACCAGT(G/C)ATCATGTT-3') (O'Donnell *et al*., 1998c) were used to amplify the translation elongation factor 1-*a* (TEF) gene. The TEF region encodes an essential part of the protein translation machinery and has high phylogenetic utility because it is (i) highly informative at the species level in *Fusarium*, non-orthologous copies of the gene have not been detected in the genus and universal primers have been designed that work across the phylogenetic breadth of the genus (Geiser et al., 2004). The amplification reactions were performed in 25 µl volumes in thin-walled PCR tubes after optimization in a PTC-100 (Programmable Thermal Controller), programmed for an initial cycle of 1 minute at 95°C, 5 minutes at 95°C, annealing at 58°C and extension 1 minute at 72°C, followed by 34 cycles of 5 minutes at 95°C, annealing at 58°C and extension 1 minute at 72°C. There was a final extension step of 5 minutes at 72°C followed by a cooling to 4°C until samples were recovered. Amplified products were analysed on a 1.5% agarose gel in 1X TAE buffer (40 mM Tris acetate and 1.0 mM EDTA) and documented using Bio-rad documentation system.

The 700 bp PCR products were sequenced for the DNA region coding for the TEF gene using the BigDye terminator Cycle Sequencer (ABI, Foster City, CA). Seaview4 application (Gouy *et al*., 2010) was used for aligning sequences and building a phylogenetic tree derived from the partial sequences of the TEF gene by neighbor-joining method. Bootstrap values were set at 100 (100 replicates). The phylogenetic analysis was carried out to compare the degree of genetic relatedness of the TEF gene sequences of each isolate with those available in the GenBank

database. Sequences obtained with each primer set were compared to GenBank nucleotide sequences by using nucleotide-nucleotide Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST). Sequences were compared with closest matches in GenBank through BLAST.

5.4 Assessment of Fusarium populations at the end of potting stage

Soils were collected from the rhizosphere of the potted tissue culture (TC) bananas, following a destructive harvest at the end of the nursery stage (22 weeks after deflasking of *in vitro* plants). Three soil replicates from each treatment were thoroughly mixed in sterile containers to constitute a composite sample from which 1 kg soil was taken and placed in sterile paper bags. Soils were stored at 4˚C until they were processed. Isolation and identification of *Fusarium* spp. from the soils inculated with products was carried out as described in section 5.3, 5.3.1 and 5.3.2 above. The soil samples were thoroughly mixed acseptically and air dried for 12 hours before isolation.

5.5 Data analysis

Colony counts data were square root transformed to stabilize variance in the data set and to bring about normal distribution before being subjected to ANOVA using Genstat statistical package version 12. Means found to be significantly different at p 0.05 were separated using Tukey's HSD test. All transformed data were returned to their original scales of measurements for accuracy of interpretation and reporting. Pearson correlation using SPSS version 12 was carried out to establish the

relationship between soil chemical and physical properties with *Fusarium* populations.

5.6 RESULTS

5.6.1 Leaf dryness

Tissue cultured banana plants treated with PHC Biopak, ECO-T and Rhizatech in the Humic Nitisol and Vertisol had less leaf dryness (%) compared to the non-treated control plants (Table 13). ECO-T and PHC Biopak inoculation significantly reduced leaf dryness in the Rhodic Ferralsol by 10 and 17% respectively compared to the non-treated control. Rhizatech inoculated plants in the Rhodic Ferralsol had the highest leaf dryness (27%) compared to all other treatments.

Table 13: Leaf dryness (%) of plants treated with microbiological products in a Vertisol, Rhodic Ferralsol and Humic Nitisol

Soil	Treatment	Leaf dryness (%)
Humic Nitisol	Control	11.67ab
Vertisol	Control	8.38ab
Rhodic Ferralsol	Control	21.67c
Humic Nitisol	PHC Biopak	5.06ab
Vertisol	PHC Biopak	2.18a
Rhodic Ferralsol	PHC Biopak	6.27ab
Humic Nitisol	ECO-T	9.25ab
Vertisol	ECO-T	7.33ab
Rhodic Ferralsol	ECO-T	11.48ab
Humic Nitisol	Rhizatech	10.95ab
Vertisol	Rhizatech	4.05ab
Rhodic Ferralsol	Rhizatech	27.27c
Fpr. Soil		**<.001**
Fpr. Treatment		**<.001**

Means followed by the same letter within the same column are not significantly different (Tukey's HSD test) at p 0.05.

5.6.2 Occurrence of *Fusarium* species

Three species of *Fusarium,* i.e. *F. oxysporum*, *F. proliferatum* and *F. incarnatum*, were isolated from the experimental soils. The species were distinguished by pigmentation on PDA as forming white, white pink and white violet colonies respectively (Plate 1, 2 and 3). The conidia of each of the isolates from Vertisol, Humic Nitisol and Rhodic Ferralsol soils used for growing tissue culture banana plantlets are described in Plate 1, 2 and 3.

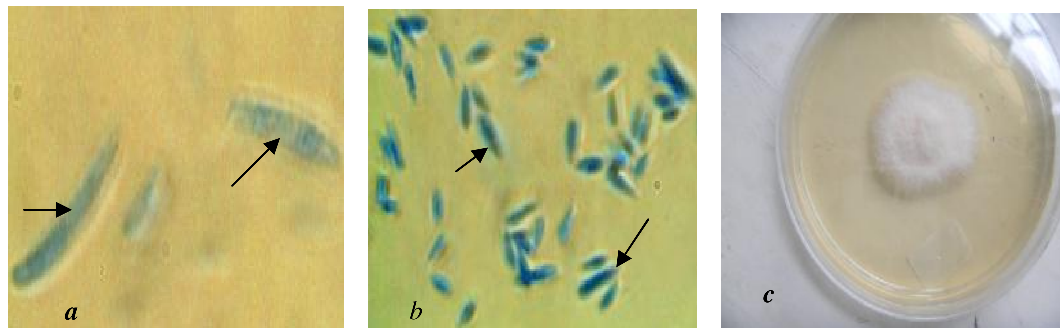

Plate 1: Morphological characteristics of *Fusarium proliferatum* isolated from Vertisol, Rhodic Ferralsol and Humic Nitisol soils used for growing tissue cultured banana plantlets. a) Macroconidia b) microconidia c) colony pigmentation on PDA; Size of macroconidia and microconidia, 0.2µm in width. X1000 magnification for macroconidia and X400 magnification for microconidia.

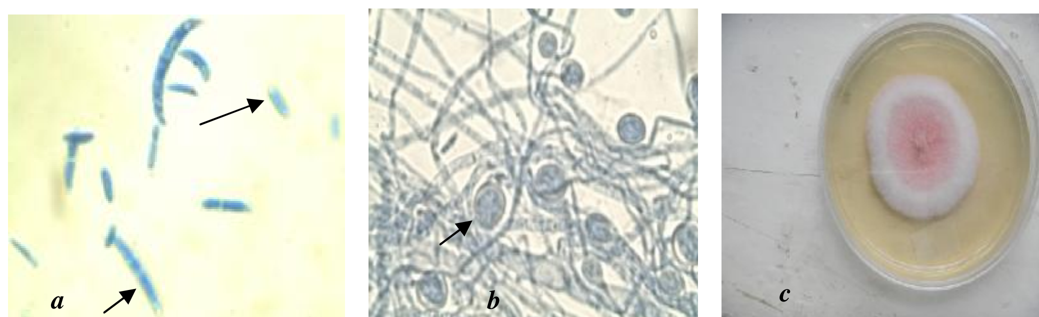

Plate 2: Morphological characteristics of *Fusarium oxysporum* isolated from Vertisol, Rhodic Ferralsol and Humic Nitisol soils used for growing tissue cultured banana plantlets. a) Microconidia and macroconidia b) chlamydospores c) colony pigmentation on PDA; Size of macroconidia and microconidia, 0.4µm in width. X400 magnifications for both macroconidia and microconidia.

Plate 3*: Fusarium incarnatum* isolated from Vertisol and Humic Nitisol soils used for growing tissue cultured banana plantlets. a) Macroconidia b) colony pigmentation on PDA; size of spores, 0.2µm. X1000 magnification for a and X400 magnification for b.

Molecular characterization by sequencing of the translation elongation factor 1-*a* (TEF) gene of the isolates using the NCBI database concurred with the morphological identification. The Polymerase Chain Reaction (PCR) product gel image is shown in Plate 4. DNA database comparison of the TEF-1 sequence showed 100% identity to sequences from the *Foc (*GenBank accession no. AF008486)*, F. proliferatum* (GenBank accession no. FJ538244) and *F. Incarnatum* (GenBank accession no. JF270209). The phylogenetic tree derived from the partial sequences of the translation elongation factor 1 and *Foc*, *F. proliferatum* and *F. incarnatum* standards obtained from the GenBank confirmed the genetic relatedness of the three isolates within the *Fusarium* genus as well as the correct identification of the species (Fig. 10).

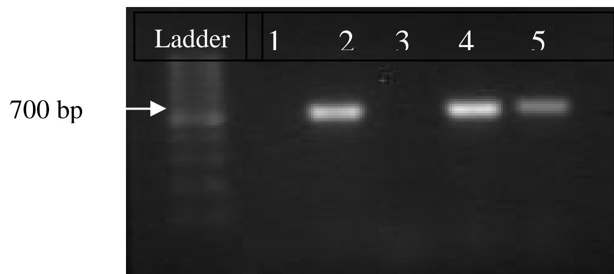

Plate 4: Gel image showing Polymerase Chain Reaction (PCR) product of *Fusarium* isolates. Lanes 1- Other fungi *(Pencillium sp.);* 2-*Fusarium proliferatum;* 3- Other fungi *(Pencillium sp.);* 4- *Fusarium oxysporum;* 5- *Fusarium incarnatum*

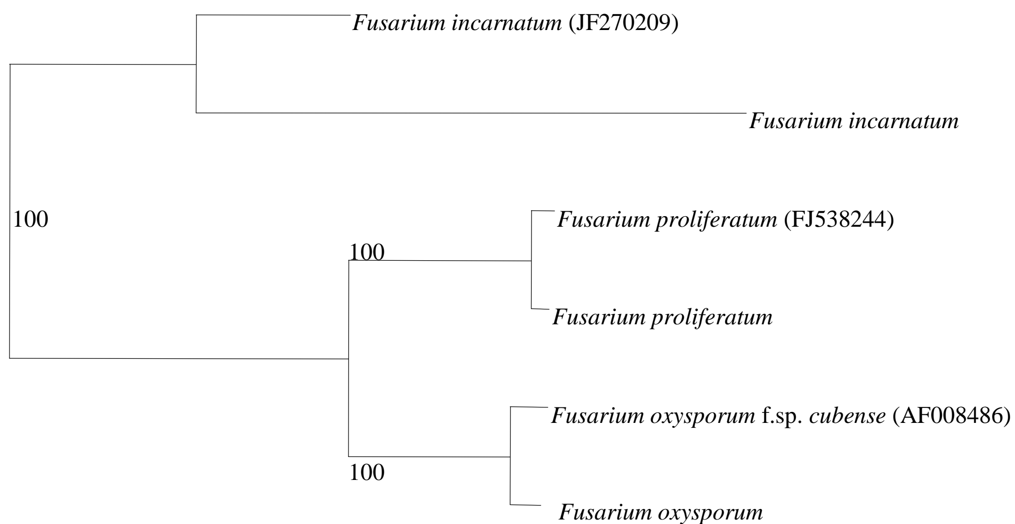

Figure 10: A phylogenetic tree of *Fusarium* isolates from soil derived from the partial sequences of the translation elongation factor 1- (TEF) gene by neighbor-joining method. Bootstrap values were set at 100 (100 replicates). Sequences with accession numbers are standard sequences from the NCBI Genbank.

Preference for soil was observed in the occurrence of isolates with *Foc* prevalent in the central Kenya soil collected from Meru South (Humic Nitisol) and *F. proliferatum* prevalent in soils from western Kenya-Nyanza (Vertisol) and Coastal Kenya-Kilifi (Rhodic Ferralsol) and *F. incarnatum* occurring only in the Humic Nitisol and Vertisol. *Fusarium oxysporum* f.sp. *cubense (Foc)* was the most

abundant in the three soils accounting for 60.6% of all the *Fusarium* colony forming units (Fig. 11). *Fusarium proliferatum* was the second most isolated accounting for 32.9% of total number of isolates and mainly isolated in the Vertisol and Rhodic Ferralsol soils. *Fusarium incarnatum* was least isolated (6.4 %) and was only found in the non-inoculated Humic Nitisol and Vertisol. *Fusarium oxysporum* was frequently isolated in all three soils especially in the non-inoculated Humic Nitisol (Fig. 11).

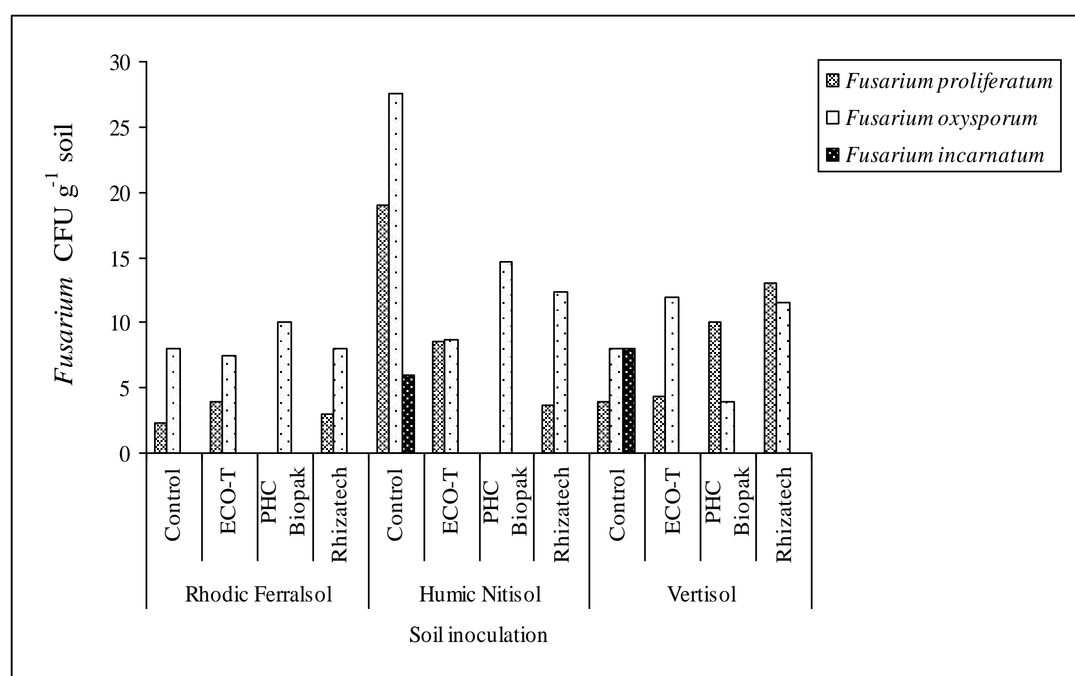

Figure 11: Population of *Fusarium* species in commercial products inoculated field soils used for growing tissue cultured banana.

5.6.3 Suppression of *Fusarium oxysporum* f. sp. *cubense* populations

Statistically, the interaction between soil type and commercial products and its effect on *Fusarium oxysporum* f.sp *cubense* (*Foc*) populations was highly significant at p 0.05 (Table 14). Non-inoculated control soils from Humic Nitisol had the highest *Fusarium oxysporum* f.sp. *cubense (Foc)* (27.5 x10^2 CFU g^{-1}) compared to the non-

inoculated Vertisol and Rhodic Ferralsol ($2.67x10^2$ CFU g^{-1} and 2.33×10^2 CFU g^{-1} respectively). Inoculation of Humic Nitisol soil with PHC Biopak, ECO-T and Rhizatech reduced *Fusarium* CFU g^{-1} by 47, 68 and 55% respectively compared to the non-inoculated control soils. Inoculation of the Vertisol with PHC Biopak reduced *Fusarium oxysporum* f.sp. *cubense* population by 50% while inoculation with Rhizatech and ECO-T enhanced *Fusarium oxysporum* f.sp. *cubense* populations by 50 and 44% respectively compared to the non-inoculated soils. Inoculation of Rhodic Ferralsol with ECO-T reduced *Fusarium oxysporum* f.sp. *cubense* population by 6% compared to the non-inoculated soils while PHC Biopak enhanced *Fusarium oxysporum* f.sp. *cubense* populations by a factor of 25% while Rhizatech had no effect.

Table 14: Suppression of *Fusarium oxysporum* f.sp. *cubense* populations by microbiological products in different soil types

Soil	Treatment	CFU (1 X 10^2 g^{-1} soil)	Suppression of *Fusarium* populations (%)
Rhodic Ferralsol	Control	8ab	0
	ECO-T	7.5ab	6
	PHC Biopak	10ab	-25
	Rhizatech	8ab	0
	Control	27.5c	0
Humic Nitisol	ECO-T	8.67ab	68
	PHC Biopak	14.67b	47
	Rhizatech	12.33ab	55
Vertisol	Control	8ab	0
	ECO-T	12ab	-50
	PHC Biopak	4a	50
	Rhizatech	11.5ab	-44
Fpr.(soil)		0.017	
Fpr. (treatment)		0.099	
Fpr. (soil*treatment)		0.001	

Means followed by the same letter within the same column are not significantly different (Tukey's HSD test) at p 0.05. CFU-colony forming units. Fpr-F propability value.

5.6.4 Relationship between soil properties and *Fusarium oxysporum* f.sp *cubense* populations

A strong positive correlation was observed between soil chemical and physical properties and *Fusarium oxysporum* f.sp. *cubense* (*Foc*) population (Table 15). Soil carbon (C) and Nitrogen (N) had a positive effect on soil *Fusarium oxysporum* f.sp. *cubense* (r=0.268 and 0.25 respectively). Suppression of *Foc* populations was variably influenced by soil physical properties. Sandy soil was negatively correlated with *Foc* CFU g^{-1} showing a strong negative relationship with *Fusarium oxysporum* f.sp. *cubense* CFU g^{-1} and a strong negative relationship between clay soil and *Fusarium* CFU g^{-1} (r=-0.25 and 0.25 respectively).

Table 15: Pearson correlation between *Fusarium oxysporum* f.sp. *cubense* populations and soil chemical and physical properties in soils

SP	pH	P	K	Ca	Mg	Na	CEC	N	C	Clay	Sand	Silt
R	-0.22	-0.15	0.21	0.22	0.21	0.17	0.22	0.27	0.25	0.25	-0.25	0.25
P	0.20	0.38	0.22	0.21	0.22	0.33	0.19	0.11	0.14	0.14	0.14	0.14

SP- soil property; R- Pearson Correlation Co-efficient; P- F probability.

5.7 DISCUSSION AND CONCLUSION

The study showed three *Fusarium* species (*Fusarium oxysporum* f.sp *cubense, Fusarium proliferatum* and *Fusarium incarnatum)* with broad distribution across biogeographical regions and soil types in Kenya. The frequency of occurrence of the *Fusarium* species was variable with some species more dominant in some regions than others. The population of *Fusarium oxysporum* f.sp. *cubense (Foc),* the causative agent of *Fusarium* wilt of banana was also variable. *Fusarium oxysporum* f.sp *cubense* (*Foc)* was more widely distributed than *Fusarium proliferatum* and *Fusarium incarnatum. Fusarium oxysporum* f.sp *cubense* was found in the Humic Nitisol, Rhodic ferralsol and Vertisol soils indicating a high adaptability to soil conditions. The distribution of *Fusarium oxysporum* f.sp. *cubense (Foc)* is determined by the presence of a suitable host and the frequency can be attributed to the intensity of cultivation of the host. The occurrence of *Foc* in the three Kenyan regions can therefore be attributed to cultivation of various types of banana and plantain varieties in the three regions. Fusarium wilt of banana was reported in susceptible cultivars Gros Michel (AAA) and sweet banana (AAA) in central Kenya, sweet banana in western Kenya and Bluggoe (ABB) and sweet banana in coastal Kenya (Kung'u *et al.* 1998). *Fusarium oxysporum* f.sp *cubense* was most frequent in soils from central Kenya (Humic Nitisol) and least isolated from Coast (Rhodic Ferralsol) and Nyanza (Vertisol). This could be attributed to the intensity of cultivation of bananas which is highest in central, where it is driven by market demands from the capital city of Nairobi, compared to the other two regions (Njuguna, 2010; Kung'u *et al.,* 1998). Similalry, the frequency of *Fusarium proliferatum* followed trends explained by the most prevalent host, maize which is

cultivated in all the three regions but at different intensities. It was highest in Vertisol soil where maize and other cereals such as sorghum and millet dominate the landscape (Manyong *et al.,* 2008a). *Fusarium incarnatum* was more frequent in central Kenya and western Kenya soils. There are limited reports on this species with most associating it with cereals (Yli-Mattila, 2010).

This conforms to other studies that have shown *Fusarium oxysporum* to be widely distributed in mostly tropical and subtropical regions accounting for its success as a pandemic pathogen (Stover, 1962; Ploetz and Pegg, 2000). The wide distribution of *Foc* may be attributed to the fact that *Foc* are able to exist in different forms as was reported by Jumjumidang and Soemargono, (2012), in Indonesia who isolated 30 different isolates associated with 7 banana varieties and Leong *et al.,* (2010) who showed 35.7–100% similarity of isolates of *Foc* in Malaysia. The study did not extend to distinction of the *Foc* from the three regions therefore we cannot conclude whether they are in the same *Foc* race, though morphologically, the *Foc* isolates from all three regions were similar.

Based on the observations on the occurrence, frequency and populations observed in these regions, the efficacy of three commercial biological products was evaluated on *Fusarium oxysporum* f.sp *cubense (Foc*) and related species. In this study, commercial biological products affected the composition, abundance and frequency of *Fusarium* spp. Emphasis was placed on the suppression of *Foc* populations. Depending on soil type, *Fusarium* species responded differently to application of commercial products. All products suppressed *Foc* populations in the Humic Nitisol while not all products suppressed *Foc* in the Vertisol and Rhodic Ferrasol soils. The

differential functioning of mycorrhizae, *Trichoderma* and *Bacillus* in the Humic Nitisol, Rhodic Ferralsol and Vertisol may also be attributed to the ability of each soil to support growth and proliferation of the organisms around the rhizosphere and subsequent microbial survival and root colonization. It may be difficulty to suppress soil-borne *Fusarium* in nutrient poor soils by addition of biological control agents as lack of sufficient soil nutrients or root exudates may limit their proliferation and suppressive abilities. Mycorrhizae, for example is known to function optimally in soils with phosphorous (P) levels of 50mg/kg (Schubert and Hayman, 1986). The Rhodic Ferralsol and Vertisol have low available P and this may explain the high populations of *Fusarium* spp. observed with the mycorrhizae inoculated soils.

The lack of suppression of *Foc* by *Bacillus* inoculation in the Rhodic Ferralsol may be related to significant stimulation of root growth and shoot biomass yield. The populations of plant growth promoting microbes and their competitiveness with soil-borne pathogens may decrease with plant growth stimulation. Carbon exudates by plant roots during plant growth may have promoted *Fusarium* germination and proliferation. The numbers of *Azotobacter* decreased as plant growth continued in non-sterile agricultural soils, while the numbers of *Azotobacter* associated with maize roots grown in sterile agricultural soils remained similar to those of the original inoculums (Martinez-Toledo *et al*., 1988). This may explain the high *Fusarium* populations observed in the non-sterile Rhodic Ferralsol inoculated with *Bacillus* but may not explain the suppression of *Foc* by *Bacillus* inoculation in the Vertisol despite stimulating plant growth. However, high percentage of mycorrhizal colonization observed with *Bacillus* inoculated plants in the Vertisol may partly explain this contradiction. Mycorrhizal symbiosis, through colonization of plant roots, protects plants from soil root pathogens (Dodd, 2000). Physical barriers and

physiological responses are essential for resistance to *Fusarium* and temporary blockage of the fungus at the vessel endings in the stele, for example, would provide time for physiological responses to be effective (Beckman *et al*., 1961).

Humic Nitisol soil had the highest P among the three field soils and the highest *Foc* population implying that the P levels in the soils are naturally too low to suppress *Foc*. There is no information on the threshold P levels required to suppress *Foc.* Mycorrhizae, is known to function optimally in nutrient uptake in soils with P levels of 50mg kg^{-1} (Schubert and Hayman 1986). However, Rhizatech was able to function under P level of 8.5 mg P kg^{-1} to suppress *Foc* to a magnitude of 68%. The P level of 7 mg P kg^{-1} in the Rhodic Ferrasol soil was not adequate for functioning of Rhizatech (though positive effects on mycorrhizal infectivity were evident) and worse still, lower P levels of 3 mg P kg^{-1} in the Vertisol soil caused Rhizatech to have a negative effect on *Foc.*

Pearson correlation revealed a strong relationship between soil physical and chemical properties and *Fusarium* populations. There was a negative correlation between sandy soil and Fusarium populations. Clay soil enhanced *Fusarium* CFU. This may explain the high *Fusarium* populations observed in the Humic Nitisol and Vertisol which are clayey soils and low *Fusarium* populations observed in the Rhodic Ferralsol which is a sandy soil. The positive relationship between the *Fusarium* populations and soil carbon and nitrogen content may also explain the high populations of *Fusarium* recovered from the Vertisol and Humic Nitisol. Soil physical and chemical properties have been reported to affect the abundance of

Fusarium species. The levels of *F. solani* f. sp. *phaseoli* are lower when soil pH decreased and the levels of Ca, Mg, K, and P reduced (Beth *et al.*, 2007).

Generally, the suppression of *Foc* by product inoculation followed the same trend observed on leaf dryness. All products reduced wilt in the Humic Nitisol and Vertisol. *Bacillus* and *Trichoderma* products suppressed leaf dryness in Rhodic Ferralsol while mycorrhizae had no effect. This implies that soil nutrients as well as physical properties influence the infestation of banana plants by *Foc*. Physical and chemical properties were correlated with suppression of *Fusarium* wilt of banana in Central American banana plantations (Smith and Snyder, 1971). By manipulating soil amendments, soil pH, and soil water supply, banana wilt caused by *F. oxysporum* f. sp. *cubense* can be suppressed (Peng *et al*., 1999).

It can be concluded that prevailing soil conditions greatly influence the efficacy of the products on *Fusarium oxysporum* f.sp. *cubense* (*Foc*) as it is based on their mode of function. It is evident that Humic Nitisol soil provides the best conditions for suppression of *Fusarium oxysporum* f.sp *cubense* by the products irrespective of the composition (Mycorrhiza, *Bacillus* and *Trichorderma*). This implies that Humic Nitisol soil had conducive conditions for mycorrhiza, *Bacillus* and *Trichorderma,* while Vertisol soil had conducive conditions for only *Bacillus* and Rhodic Ferrasol soil were not conducive for all products. This indicates that it is crucial to understand the soil conditions and mode of action of organisms in the product prior to application.

CHAPTER SIX

INTEGRATION OF MICROBIOLOGICAL PRODUCTS AND FERTILIZERS AND IT'S EFFECT ON GROWTH AND PERFORMANCE OF TISSUE CULTURED BANANA

ABSTRACT

Tissue culture (TC) banana plantlets at the *in vitro* stage are delicate and devoid of microbes and nutrients that are essential for establishment and subsequent growth. Some microbes are known to function best under certain soil threshhold levels of macro and micronutrients and have been associated with growth and performance of TC banana. Green house and field study were conducted to evaluate the effect of combining two commercial biological products [Rhizatech and ECO-T (mycorrhiza and *Trichoderma* based products, respectively) with various sources of nitrogen and phosphorous including Mavuno, Minjingu phosphate rock, CAN, manure and diammonium phosphate (DAP) on growth and performance of TC banana in Vertisol and Rhodic Ferralsol soil conditions. Tissue culture plants were initially inoculated with Rhizatech (mycorrhizal product) and ECO-T at the acclimatization stage and subsequently at the beginning of the potting stage and field establishment. Addition of macro and micro nutrient sources was also done at the same stages of plant growth by mixing with the soil substrates prior to planting. The performance of plants was significantly (at P 0.05) affected by the combinations of macro and micro nutrient sources depending on the soil type and stage of plant development. The growth of plants in the Vertisol increased with *Trichoderma* combined with either organic manure inorganic phosphate diammonium phosphate) or combined with a macro and micro nutrient source (Mavuno) as compared to the sole application of *Trichoderma*. Performance of plants treated with combination of

mycorrhiza and either mavuno (macro and micro nutrients) and minjigu rock phosphate was consistently higher in the Rhodic Ferralsol than either mycorrhiza alone or fertilizer alone. This indicates that tissue culture plants could highly benefit from combined application of microbiological products and inorganic and organic fertilizers. However, a prior knowledge of the product's microbial formulation and prevailing soil conditions is essential in realizing the potential benefits of integrating microbe-based product with inorganic and organic fertilizers.

6.0 INTRODUCTION

Tissue culture banana plantlets offer an excellent means for providing pest- and disease-free planting material to farmers (Mink, 1991; Mbaka *et al.,* 2008). However, tissue culture (TC) plants are fragile, devoid of food reserves and therefore more prone to shock, which may lead to loss of plantlets at establishment. With decline in soil fertility and increase in soil-borne pests and diseases, TC plants are likely to succumb to disease and roots may not establish well in low fertility soil environments (Cassalls and O’Herlihy, 2003).

Banana requires large amounts of nitrogen and potassium followed by phosphorus, calcium and magnesium to maintain high yields (Abdullah *et al.*, 1999: Robinson, 1996). To fulfill the plant demand for nutritional attributes, it is essential to apply those elements in the soil which mostly come from inorganic chemical sources. The increased use of chemical fertilizer is undesirable because its production is an energetically costly process and considerable pollution is caused through both the production and use of mineral N-fertilizers. This is exacerbated by the relatively low efficiency of their uptake by the plants due to non-extensive root system and may also deplele soil organic matter in the long term (Ladha and Reddy, 1995; Ladha *et al.,* 1997; Khan *et al.,* 2007). Inoculant biofertilizers are more environmentally sound and their introduction in agricultural production systems could be one of the means to mitigate the onset of global warming as well as the reduction in fertilizer input costs, prevent depletion of organic matter and increase crop yields (Jeyabal and Kuppuswamy, 2001; Kennedy *et al.,* 2004; Mia and Shamsuddin, 2010).

Inoculation of tissue culture banana with biofertilizers such as mycorrhiza (AMF) and *Trichoderma* has been reported to stimulate root growth, nutrient uptake, enhance plant establishment and increase growth, yield and protection against disease and pest infestation (Jaizme-vega *et al.,* 1998; Benítez *et al.,* 2004; Harman *et al.,* 2004; Rodríguez-Romero *et al.,* 2005; Reino *et al.,* 2008). The growth vigor acquired by biologically inoculated TC banana plants under nursery conditions is expected to give the plants an advantage during field establishment especially in nutritionally and disease challenged soils. However, the functionality of some of the rhizospheric microbes used for constituting biofertilizers such as mycorrhiza (AMF) and *Trichoderma* is greatly influenced by the prevailing soil conditions. Nitrogen (N), phosphorous (P) and other nutrients such as zinc, copper and sulphur levels in the soil affect the functioning of these microorganisms (Schubert and Hayman, 1986; Fargasova, 1992; Griffin, 1994; Score and Palfreyman, 1994; Okoth, 2007). Mycorrhizal symbiosis benefits, for example, have been suggested to be optimal at P levels of 50mg kg^{-1} (Schubert and Hayman, 1986).

Organic and inorganic fertilizers are used primarily to increase nutrient availability and the type or amount of fertilizer added to soil could directly affect the function performed by the various microbial groups in the soil (Marschner *et al.,* 2003; Ge *et al.,* 2008; He *et al.,* 2008). Mineral nutrition is essential for growth, sporulation and stimulation of fungal secondary metabolism (Griffin, 1994) and combining of mineral and bio fertilizers could greatly benefit both the added microbes and inoculated plant as well as improve soil quality (Jeyabal and Kuppuswamy, 2001; Bedini *et al.,* 2007; Atul-Nayyar *et al.,* 2009; Javaid, 2009).

Previous studies have focused on the effect of long term fertilization on indigenous microbial diversity and efficiency (Mäder *et al.,* 2002; Hijri *et al.,* 2006) but little is reported on the effect of combining commercial microbiological strains with fertilizers in nutrient poor soils that have little or no history of fertilization.

Although several formulations of mycorrhiza and *Trichoderma* are available in the market, their efficacy on plant growth is variable depending on the soil nutrient levels. It is therefore essential to add these nutrients in levels that enable optimal functioning of the micro-organisms and consequent promotion of nutrient uptake and plant growth. The integration of microbiological products with fertilizers could adresss the soil fertility constraint faced by TC banana plantlets, especially under field conditions. This study focused on evaluating the potential of integrating microbiological products with inorganic and organic fertilizers on growth and performance of tissue cultured banana in Vertisol and Rhodic Ferralsol soil conditions under nursery and field conditions.

6.1 MATERIALS AND METHODS

6.1.1 Source of tissue cultured materials, soil properties and inoculation process

Tissue cultured (TC) banana plantlets were obtained from Jomo Kenyatta University of Agriculture and Technology Biotechnology laboratory. Soils for establishing TC banana plants were obtained from two banana growing regions in Kenya as described in sub-section 3.2.1 of this thesis. Plants were inoculated with microbiological products at the deflasking and potting stages as described in subsection 3.2.3

6.1.2 Greenhouse experiment

A 2 X 2 factorial experiment comprising of (1) two microbiological products (i) Rhizatech (ii) ECO-T (2) soil substrates (i) Vertisol (sampled from Western Kenya-Bondo) (ii) Rhodic Ferralsol (sampled from coastal Kenya-Kilifi) and (3) Fertilizers (i) Minjingu phosphate rock (MPR) and CAN as sources of phosphorous (P), Mg, Ca and eight more essential micronutrients (Mwaluko, 1996) and nitrogen (N), (ii) Mavuno as a source of N, P, potassium (K) and micronutrients such as calcium Ca, Mg, sulphur (S) and other essential micronutrients including boron (B), manganese (Mn), zinc (Zn), molybdate (Mo) and copper (Cu) (iii) manure and diammonium phosphate (DAP) as a source of P, N and carbon (C). Three replicates consisting of 16 plants per replicate were considered per treatment. Control experimental units (without product addition) were also included with three replicates per soil. The experimental units were arranged in a completely randomized design under greenhouse conditions. Fertilizer application rates followed the recommended fertilizer rates and were applied at the hardening, potting and at field establishment by mixing with the soil substrate.

6.1.3 Study sites description and experimental design under field conditions

The experiment was carried out at two sites with similar soil conditions as those used for hardening and potting of plants under green house nursery conditions. Tissue cultured banana plants established under green house conditions as described in subsection 6.1.1 and 6.1.2 were used as planting materials and established in two agro ecological zones (Western Kenya in Bondo and Coastal Kenya in Kilifi). The description of the study areas is as described in chapter three of this thesis except for the actual location of the farms (Kilifi and Bondo) within the agro ecological zones. The experimental design followed a multilocational complete block design in four farms per study site. Each farm was considered as a replicate. Diammonium phosphate (DAP) and Minjigu rock phosphate fertilizers, used as sources of phosphorous were applied at the rate of 50 kg per hectare. Calcium Ammonium Nitrate (CAN) used as a source of nitrogen was applied at the rate of 120 kg per hectare. Mavuno fertilizer which was used as a source of macro and micro nutrients was applied at the manufacturers recommended rate of 185 kg per hectare. High quality farm yard manure was considered as a source of organic nitrogen and carbon and was applied at 6 tonnes per hectare. Application rates per plant corresponded to the recommended rate in the field, converted using the medium planting density for banana (2500 plants ha^{-1}). The areas of pot used for establishing plants were considered when determining the exact amount of inoculum to be added per pot.

6.1.4 Planting and crop management

Medium planting density of 2500 plants per hectare was adopted hence the plants were spaced at 2m by 2m and established in 60 x 60 x 60 cm planting pits. Guard row plants were established three meters away from the experimental plants only in

edge plots, hence surrounding the entire farm. The inner 2m x 2m inner mats were monitored and evaluated up to the on-set of flowering of the mother plant. Fertilizer was mixed with top soil and incorporated around the banana plant in the pit followed by topping with sub-soil before planting. Hand weeding was done when needed avoiding the use of tools that could mutilate superficial banana roots. Desuckering of plants was carried out in order to have a 1-2-3 system (mother, first ratoon, second ratoon), preferably with a circular movement of plants, so that original planting density was maintained. Deleafing (leaves having <50% of functional leaf surface area) and watering during periods of severe drought stress were also part of the management practices carried out.

6.2 Assessment of growth parameters

Plant growth parameters such as shoot height (from base), length and width of the second leaf from the sword shoot, girth (at two centimeters from the base) of the stem, and number of functional leaves were recorded every two weeks under greenhouse conditions and every two months under field conditions upto the on-set of flowering stage. Only treatments with a high number of surviving plants under green house conditions were considered for field establishment.

6.3 RESULTS

6.3.1 Integration of microbiological products with fertilizers on growth and performance of tissue cultured plants

The effect of combining products with various fertilizers was evident in both Vertisol and Rhodic Ferralsol. Highly significant (p 0.001) differences between treatments were observed on all plant growth parameters evaluated in both soils.

In the Rhodic Ferralsol, the highest plant height was observed on plants treated with combined application of Rhizatech and Mavuno with an increase of 136 and 116.5% compared to the non-treated control and sole application of Rhizatech, respectively (Table 16). The highest number of leaves (10), which was statistically different from all other treatments, was observed with the combined application of Minjigu Rock Phosphate and CAN (MRPCAN) and Rhizatech. Plants treated with combined application of ECO-T and MRPCAN recorded the highest increase in girth of 55% compared to the non-treated control, 44 and 63% compared to the application of MRPCAN alone and ECO-T alone. Leaf length was statistically different between most treatments with the longest leaf length observed on plants treated with combined application of ECO-T and manure and DAP. The combined application of ECO-T and manure and DAP increased leaf length by 71.3, 39.2 and 34.8% compared to the non-treated control, addition of manure and DAP alone and sole application of ECO-T respectively. Combining of Rhizatech with fertilizers also significantly affected leaf length recording an increase of 45.8% (Rhizatech plus MRPCAN), 40.8% (Rhizatech plus manure plus DAP) and 48.3% (Rhizatech plus mavuno) above the sole fertilizer applications. Effect of combining Rhizatech and mavuno on leaf width was statistically different from all other treatments recording an increase of 100, 35.9 and 96% compared to the non-treated control and sole

application of mavuno and Rhizatech respectively. Other treatments with effects on leaf length statistically different from the non-treated control include Rhizatech plus MRPCAN, ECO-T plus manure plus DAP, Mavuno alone, ECO-T plus mavuno and Rhizatech plus mavuno increasing leaf length by 55.7, 62.6, 55.6, 52.7 and 100% respectively (Table 16).

Table 16: Growth of tissue cultured banana plants treated with microbiological products and different fertilizers in Rhodic Ferralsol, 22 weeks after deflasking

Product	Fertilizer	Height	NOL	Girth	LL	LW
None	None	8.0a	7.7a	1.7a	15.6a	5.6a
ECO-T	None	10.9b	7.8a	1.6a	19.8b	7.1abc
Rhizatech	None	8.7a	8.2ab	1.6a	16.9a	5.7a
None	MRPCAN	11.8b	9.1c	1.8a	19.5b	6.8ab
ECO-T	MRPCAN	11.0b	8.7bc	2.6b	20.1b	7.1abc
Rhizatech	MRPCAN	13.7c	9.9d	1. 9ab	24.6c	8.7cd
None	Manure+DAP	13.6c	7.7a	1.84ab	19.2b	6.9abc
ECO-T	Manure+DAP	16.8e	8.6bc	2.1ab	26.7d	9.1d
Rhizatech	Manure+DAP	15.3d	8.9bc	2.0ab	23.6c	6.9abc
None	Mavuno	18.4f	8.7bc	2.2ab	24.3c	8.7cd
ECO-T	Mavuno	16.6de	7.8a	2.1ab	23.8	8.5bcd
Rhizatech	Mavuno	18.9f	8.4abc	2.1ab	25.0cd	11.1e
SED						
Product		0.197	0.112	0.115	0.314	0.275
Fertilizer		0.227	0.129	0.133	0.363	0.318
Product X Fertilizer		0.393	0.224	0.23	0.628	0.551
P value						
Product		<0.001	<0.001	0.147	<0.001	<0.001
Fertilizer		<0.001	<0.001	<0.001	<0.001	<0.001
Product X Fertilizer		<0.001	<0.001	0.068	<0.001	<0.001

Means of 16 plants replicated three times. Within the same column, means followed by the same letter are not significantly different (Tukey's HSD test) at P 0.05. NOL- number of leaves; LL- leaf length; LW- leaf width. Leaf length and leaf width measured in centimeters.

Plants treated with manure alone in the Rhodic Ferralsol had significantly higher number of secondary roots (27) compared to the plants that received neither fertilizer nor product (un-treated control) and plants treated with Rhizatech (Table 17). Effects of all other treatments were not statistically different from the control the untreated control and Rhizatech. Combined application of fertilizer and products

had no significant effects on shoot dry weights (SDWT) in the Rhodic Ferralsol. The highest SDWT (6.07g) in the Rhodic Ferralsol were observed on plants treated with a combination of Rhizatech plus manure plus DAP and the least on untreated control plants. Plants treated with Rhizatech alone had significantly lower root dry weights (RDWT) (1.22g) than plants treated with ECO-T plus manure plus DAP (6.11g) and mavuno alone (6.27g) and statistically not different from the other treatments.

Table 17: Shoot and root growth of tissue cultured banana plants treated with microbiological products and different fertilizers in Rhodic Ferralsol, 22 weeks after deflasking

Product	Fertilizer	SDWT (g)	RDWT (g)	NOSR
None	None	2.1	1.8ab	14.3a
ECO-T	None	4.3	2.1ab	19.3ab
Rhizatech	None	2.5	1.2a	14.7ab
None	MRPCAN	5.4	3.4ab	21ab
ECO-T	MRPCAN	3.3	3.5ab	19ab
Rhizatech	MRPCAN	3.7	2.8ab	18ab
None	Mavuno	7.3	6.3ab	24ab
ECO-T	Mavuno	6.5	5.1ab	24.3ab
Rhizatech	Mavuno	4.9	2.7ab	18ab
None	Manure+DAP	3.4	4.5ab	27.3b
ECO-T	Manure+DAP	5.9	6.1b	20ab
Rhizatech	Manure+DAP	6.1	5.6ab	24.7ab
SED				
Product		0.740	0.643	1.547
Fertilizer		0.855	0.743	1.786
Product X Fertilizer		1.481	1.287	3.093
P value				
Product		0.598	0.178	0.201
Fertilizer		0.006	<0.001	0.001
Product X Fertilizer		0.147	0.343	0.113

Means of 3 replicates. Within the same column, means followed by the same letter are not significantly different (Tukey's HSD test) at P 0.05. SDWT- shoot dry weight; RDWT- root dry weight; NOSR- number of secondary roots.

6.3.2 Growth of tissue cultured banana treated with microbiological products and different fertilizers in a Vertisol soil

Under green house conditions, plant growth (height, girth, no. of leaves, leaf length and leaf width) was significantly affected by sole and combined application of products and fertilizers (Table 18). Effect of combined application of ECO-T plus manure plus DAP on plant height was statistically different from all other treatments increasing height by 110.6, 33.7 and 51.5% compared to the non-treated control and sole application of manure plus DAP and ECO-T, respectively.

Similar observations were made on plant girth, with combined application of ECO-T plus manure plus DAP increasing plant girth by 22.6, 9.3 and 83.9% compared to the non-treated control and sole application of manure plus DAP and ECO-T respectively.

Significant differences between treatments were observed on number of leaves with the highest number of leaves (11) observed on plants treated with sole application of MRPCAN. Most treatments negatively affected number of leaves up to a magnitude of 9.5%.

Leaf length of plants treated with ECO-T and manure plus DAP was statistically different from all other treatments. An increase of 51.6, 25.4 and 25.9% above the non-treated control and sole application of manure plus DAP and ECO-T, respectively was observed. Combined application of Rhizatech and mavuno, ECO-T plus manure plus DAP, Rhizatech plus manure plus DAP, Rhizatech plus MRPCAN had similar effects but statistically higher leaf length than non-treated control, sole application of Rhizatech and combined application of ECO-T and MRPCAN (Table 18).

Table 18: Growth of tissue cultured banana plants treated with microbiological products and different fertilizers in a Vertisol, 22 weeks after deflasking

Product	Fertilizer	Height (cm)	Girth (cm)	NOL	LL (cm)	LW (cm)
None	None	11.7a	2.1bcd	10.7bcd	23.6a	9.1a
ECO-T	None	16.3c	1.4a	10.8cd	28.5cd	10.9bcde
Rhizatech	None	13.7b	2.1bcd	10.6abcd	25.2ab	9.4a
None	MRPCAN	15.1bc	2.0bc	11.2d	26.8bc	9.7ab
ECO-T	MRPCAN	15.6c	2.4def	10.1ab	26.1b	9.2a
Rhizatech	MRPCAN	19.0d	2.3cde	10.5abc	29.1d	11.4de
None	ManuDAP	18.5d	2.4def	10.5abc	28.6cd	10.1abc
ECO-T	Manure+DAP	24.7g	2.6f	10.5abc	35.8f	11.6e
Rhizatech	Manure+DAP	22.4f	2.5ef	10.5abc	33.1e	11.5de
None	Mavuno	19.7de	1.9bc	10.4abc	28.4cd	10.3abcd
ECO-T	Mavuno	20.6e	1.8ab	10.0a	29.7d	11.1cde
Rhizatech	Mavuno	22.5f	2.5ef	10.4abc	33.5e	11.8e
SED						
Product		0.219	0.059	0.099	0.059	0.187
Fertilizer		0.253	0.068	0.115	0.068	0.215
Product X fertilizer		0.439	0.117	0.199	0.117	0.373
P value						
Product		<0.001	<0.001	0.006	<0.001	<0.001
Fertilizer		<0.001	<0.001	0.001	<0.001	<0.001
Product X fertilizer		<0.001	<0.001	<0.001	<0.001	<0.001

Means of 16 plants replicated three times. Within the same column, Means followed by the same letter are not significantly different (Tukey's HSD test) at P 0.05. NOL- number of leaves; LL- leaf length; LW- leaf width.

Combined application of fertilizer and products had no significant effects on shoot dry weights (SDWT) in the Vertisol (Table 19). The highest SDWT was observed with addition of Mavuno alone (13g) and the least with application of Rhizatech plus

MPRCAN) (3.87g). The highest RDWT was observed with combined application of ECO-T plus manure plus DAP (7.45) and the least on the un-treated control plants.The number of secondary roots on plants treated with Mavuno fertilizer alone (31) was statistically different from plants treated with ECO-T (20) and Mijingu rock phosphate plus CAN (MPRCAN) (20) but statistically the same as all other treatments (Table 19).

Table 19: Shoot and root growth of tissue cultured banana plants treated with microbiological products and different fertilizers in a Vertisol, 22 weeks after deflasking

Product	Fertilizer	SDWT(g)	RDWT(g)	NOSR
None	None	4.2	2.6	2.6
ECO-T	None	6.2	4.1	4.1
Rhizatech	None	7.4	3.9	3.9
None	MRPCAN	4.8	3.4	3.5
ECO-T	MRPCAN	7.1	6.6	6.6
Rhizatech	MRPCAN	3.9	3.8	3.8
None	Mavuno	9.2	6.8	6.8
ECO-T	Mavuno	7.7	6.5	6.5
Rhizatech	Mavuno	8.7	5.8	5.8
None	Manure+DAP	5.9	7.1	7.1
ECO-T	Manure+DAP	4.6	7.4	7.4
Rhizatech	Manure+DAP	5.6	5.6	5.6
SED				
Product		1.023	0.676	1.483
Fertilizer		1.181	0.781	1.712
Product X Fertilizer		2.045	1.352	2.966
P value				
Product		0.926	0.11	0.233
Fertilizer		0.041	0.001	0.003
Product X Fertilizer		0.458	0.47	0.023

Means of 3 replicates. Within the same column, means followed by the same letter are not significantly different (Tukey's HSD test) at P 0.05. SWDT-shoot dry weight; RDWT- root dry weight; NOSR- number of secondary roots.

6.3.4 Growth and performance of tissue culture banana plants treated with microbiological products and different fertilizers in Rhodic Ferralsol under field conditions

Combining of microbiological products with fertilizers in Rhodic Ferralsol significantly (<0.001) increased growth of banana plants (Table 20). Plants treated with either fertilizer alone or inorganic fertilizer plus products performed better than plants treated with products only. Combination of ECO-T with manure plus DAP increased plant height by 33.8 and 31.2% above the sole application of manure plus DAP and ECO-T respectively. The effect of combining Rhizatech and MRPCAN on plant height was not statistically different from that of combining ECO-T and manure plus DAP. An increase of 26.5 and 59.7 % above sole application of MRPCAN and Rhizatech respectively was recorded with the combined application of Rhizatech and MRPCAN. Plants treated with Rhizatech alone had statistically lower heights than all other treatments.

Table 20: Growth of tissue cultured banana plants treated with microbiological products and different fertilizers, 12 months after field establishment in a Rhodic Ferralsol

Product	Fertilizer	Height (cm)	Leaf length (cm)	Leaf width (cm)	No. of leaves	Girth (cm)
Rhizatech	None	166.5a	182.6a	63.9	12.bc	18.76
ECO-T	None	205.1ab	204.7ab	63.9	12c	20.8
None	Manure+DAP	206.6ab	203ab	65.1	11abc	18.2
ECO-T	Mavuno	226.7bc	217.8ab	65.1	10a	21.4
None	MRP+CAN	235.8bc	219.2ab	66.4	10a	20.6
None	Mavuno	245.3bc	230.6bc	72.8	11abc	20.9
Rhizatech	MRP+CAN	261.6c	242.4bc	74.5	11abc	23.5
ECO-T	Manure+DAP	273.7c	247.9bc	71.6	116abc	23.2
P value						
Product		0.116	0.193	0.685	0.206	0.160
Fertilizer		0.171	0.496	0.359	0.022	0.287
Product X Fertilizer		0.001	0.019	0.111	0.001	0.146

Means of four replicates. Within the same column, means followed by the same letter are not significantly different (Tukey's HSD test) at P 0.05.

Similar to the observations on plant height, plants treated with the combination of Rhizatech and MRPCAN increased girth by 14 and 27%, respectively compared to sole application of MRPCAN and manure plus DAP, respectively. The highest number of leaves (12) was observed on the plants treated with Rhizatech or ECO-T alone and this was statistically different from MRPCAN and ECO-T plus mavuno-treated plants, which had the least number of leaves (10).

Leaf length was significantly increased by combined application of Rhizatech and MRPCAN (32.7%) and ECO-T plus manure and DAP (21%). All treatments had statistically the same effects on leaf width. The trend of plant growth in the Rhodic Ferralsol is shown in Fig. 12.

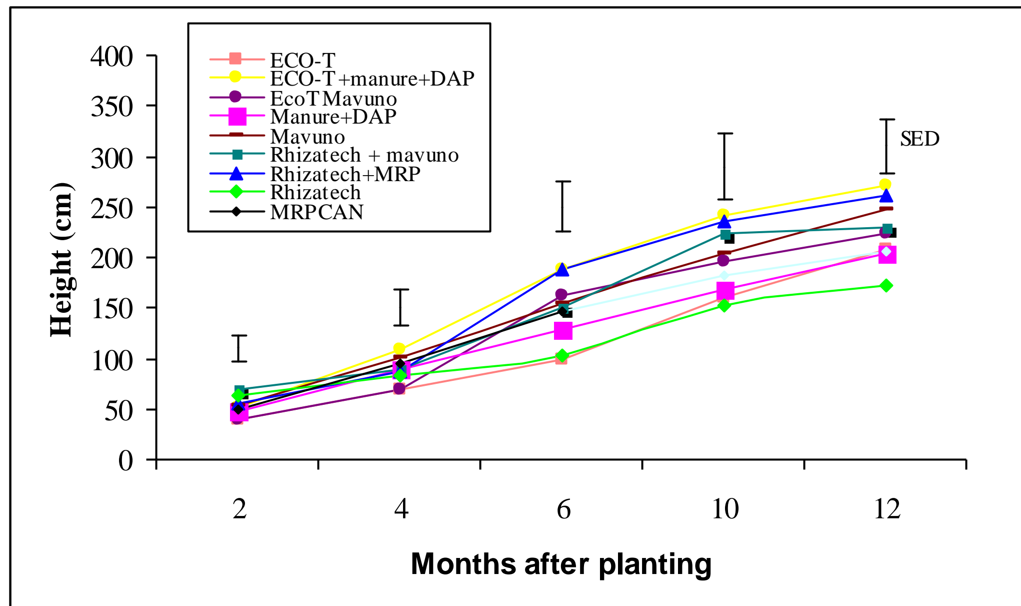

Figure 12: Growth of tissue cultured plants treated with microbiological products and different fertilizers in Rhodic Ferralsol. SED- standard error of difference between means at 12 months after field establishment.

6.3.5 Growth and performance of tissue cultured plants treated with microbiological products and different fertilizers in Vertisol under field conditions.

Plant height in the Vertisol was significantly (p=0.021) affected by the combined application of commercial products with either inorganic or organic fertilizers in (Table 21). Combining of ECO-T with macro and micronutrients (Mavuno) increased growth of plants by 13.5% compared to the sole addition of mavuno and by 2% compared to the addition of ECO-T alone. Combined application of Rhizatech and with P and inorganic N increased plant height by 11.8% compared to sole addition of P and inorganic N and reduced plant height by 7% compared to sole addition of Rhizatech. The trend of plant growth in the Vertisol is shown in Fig. 13.

Plant girth was enhanced by application of Rhizatech plus MRPCAN by 5.1, 37.2 and 16.1% compared to sole application of Rhizatech, MRPCAN and manure plus DAP respectively. This was followed by combined application of ECO-T plus mavuno that increased plant girth by 4.8, 13.8 and 15.8% respectively compared to the sole application of ECO-T, mavuno or manure plus DAP respectively.

Table 21: Growth and performance of tissue cultured plants treated with microbiological products and different fertilizers in Vertisol, 12 months after field establishment

Product	Fertilizer	Height (cm)	Leaf length (cm)	Leaf width (cm)	No. of leaves	Girth (cm)
Rhizatech	None	168.6ab	159.9ab	50.4a	6.6abc	20.6a
EcoT	None	207.3ab	195.8b	62.3b	7.8c	20.1a
None	MRP+CAN	156.7a	140.8a	50.8a	6ab	15.8b
None	Manure+DAP	186.5ab	173ab	58.8ab	6.3abc	18.7a
None	Mavuno	186.2ab	165.6ab	58.6ab	6.1abc	19a
Rhizatech	MRP+CAN	156.7ab	179.5ab	58.9ab	5.5a	17.1ab
Rhizatech	Mavuno	183.1ab	167.6ab	58.9ab	6.7abc	17. ab
ECO-T	Mavuno	211.3b	195.1b	63.1b	7.1bc	21.6a
P value						
Product		0.4	0.3097	0.8957	0.2189	0.169

Fertilizer	0.6896	0.6638	0.592	0.9501	0.7413
ProductXfertilizer	0.0157	<.0001	0.105	0.003	<.0001

Means of four replicates. Within the same column, means followed by the same letter are not significantly different (Tukey's HSD test) at P 0.05.

Leaf length was more affected by combined application of Rhizatech and MRPCAN and ECO-T plus manure plus DAP than leaf width (the effect of the two treatments were not statistically different) (Table 21). Combined application of Rhizatech plus MRPCAN increased leaf length by 17, 35 and 20.8% compared to the sole application of Rhizatech, MRPCAN and manure plus DAP respectively. ECO-T plus mavuno increased leaf length by 14, 13.6 and 17% compared to the sole application of ECO-T, mavuno and manure plus DAP.

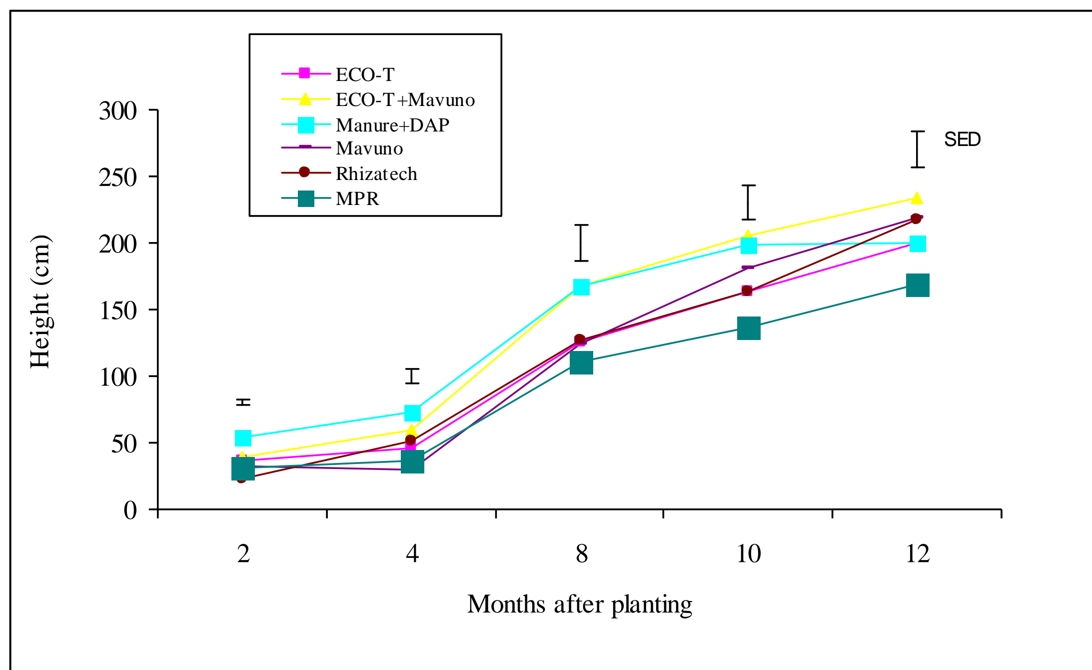

Figure 13: Growth of tissue cultured plants treated with microbiological products and different fertilizers in a Vertisol. SED- standard error of difference between means at 12 months after field establishment.

6.4 DISCUSSION AND CONCLUSION

The performance of tissue cultured banana plants treated with combined application of fertilizers and commercial microbiological products was variable and depended on prevailing soil conditions. Results indicate preference for certain sources of inorganic or organic nutrients by the *Trichoderma* based product (ECO-T) and the mycorrhiza-based product (Rhizatech). The positive effects of combining *Trichoderma* with organic and inorganic fertilizers were more evident in the Vertisol than in the Rhodic Ferralsol suggesting a preference for soils with higher nitrogen levels i.e. 0.25% in the Vertisol versus 0.1% in the Rhodic Ferralsol. The performance of plants was greatly enhanced with *Trichoderma* combined with either organic manure inorganic phosphate (diammonium phosphate) or combined with a macro and micro nutrient source (Mavuno) as compared to the sole application of *Trichoderma*. This indicates that organic and inorganic amendments could enhance the proliferation and efficacy of *Trichoderma* and subsequently enhance plant growth. Okoth (2007), reported higher amounts of *Trichoderma* in soils with organic amendments. The enhanced performance of plants when inoculated with *Trichoderma* combined with mineral nutrients (macro and micro nutrients) could be attributed to availability of nutrients for plant uptake as well as increased proliferation of *Trichoderma* populations with a consequent result of improved plant growth. This finding concurs with the findings of Griffin (1994), who reported that mineral nutrition is essential for growth, sporulation and stimulation of fungal secondary metabolism. Fargasova (1992) reported increased sporulation, production of antifungal anthroquinone pigments, hyphal growth rate with high total N availability and Widden and Breil (1988), reported a positive correlation between soil nitrate levels and cellulase production. Performance of plants treated with

combination of mycorrhiza and either mavuno (macro and micro nutrients) and Minjigu Rock Phosphate was consistently better in the Rhodic Ferralsol than either mycorrhiza alone or fertilizer alone. This could suggest that the efficacy of mycorrhiza is enhanced by addition of mineral nutrients to nutrient poor soils, as is the case with the Rhodic Ferralsol (FAO, 2006). This could also suggest an improved fertilizer use when mycorrhiza is combined with mineral fertilizer. This finding is in agreement with the finding of other authors who have reported that arbuscular mycorrhizal symbiosis provides numerous services to crops (Gianinazzi *et al.,* 2010), including efficient use of fertilizer and soil nutrients (Javaid, 2009), increased N-fixation in legumes (Barea and Azcón-Aguilar 1983), improving plant access to mineral nutrients, including N, P (Liu *et al.,* 2000a), K, Ca, Mg (Liu *et al.,* 2002), Fe, Cu, Zn, and Mn (Liu *et al.,* 2000b). Javaid (2011) evaluating the effect of biofertilizers combined with different soil amendments on potted rice plants reported enhanced 100-grain weight in the NPK fertilizer amendment.

Amendment of both the Vertisol and Rhodic Ferralsol soils with Minjigu Rock Phosphate alone had the lowest effect on plant growth enhancement suggesting minimal solubilization and utilization of rock phosphate by plants. However, plant growth was greatly enhanced with combined application of Minjigu Rock Phosphate and mycorrhiza (Rhizatech) indicating a positive effect of mycorrhiza in the added phosphate (P) solubilization and acquisition of by plants with a consequent increase in plant growth. This finding conforms with the reports of other authors, who have shown that mycorrhiza play an important role in effecting the availability of soil P to plant roots, and increasing P mobilization in soil (Toro *et al.,* 1997; Smith and Read, 2008).

In conclusion, it is evident that tissue culture banana plants could greatly benefit from combined application of commercial microbiological and fertilizers appropriate combinations of mycorrhiza and inorganic macro and micro nutrients or rock phosphate and the combined application of *Trichoderma* and organic sources could greatly enhance plant growth in nutrient poor soils as was observed in the Rhodic Ferralsol and Vertisol. Knowledge of product formulations and prevailing soil conditions is essential prior to inoculation and amendment of the soils.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Tissue culture bananas can benefit from application of commercial formulations of arbuscular mycorrhizal fungi, *Trichoderma* and *Bacillus* to improve survival, growth, nutrition and health during the nursery phase as well as enhance performance under field conditions. The effect of microbiological inoculation is however dependent on soil type. The results from this research reveal essential information in the management and growth of tissue cultured banana and give a possible strategy for decentralization of commercial banana nurseries, which depend entirely on the use of conventional media. The knowledge generated on the effect of microbial inoculation under field conditions is important in devising integrated soil management options that enable optimal functioning of the microorganisms. Soils with low phosphorous and potassium levels limit root colonization, nutrient acquisition and subsequent plant growth. Since no single product was observed to enhance all growth parameters, combining of products should be a necessary investigation. There is little information on combined application of microbial inoculants (e.g. combining of mycorrhiza and *Bacillus* or Mycorrhiza and *Trichoderma*) and the effect of their interaction on performance of tissue cultured banana under field conditions. These interactions should therefore be explored for improvement of banana growth, health and nutrition. The differential functioning of the mixed and single species *Bacillus* and mycorrhizal based products reviewed in this study should be further investigated and considered in the production of inoculum meant for specific soil conditions. It is evident that tissue culture banana plants could greatly benefit from combined application of commercial

microbiological and fertilizers. However, knowledge of the product formulations and composition and prevailing soil conditions is essential prior to inoculation and amendment of the soils. Mycorrhizal formulation works best in soils amended with NPK and micronutrients while *Trichoderma* formulation works best in soils amended with organic nutrient sources.

Recommendations

1. Multiple species *Bacillus* (PHC Biopak and PHC Colonize) and mycorhizal inoculant (Rhizatech) are most suitable for tissue culture (TC) banana growth and nutrient uptake in the Vertisol and Rhodic Ferralsol under green house conditions.
2. Multiple species mycorrhizal inoculum (Rhizatech) is recommended for growth under Vertisol field conditions.
3. Mycorhizal products (Rhizatech and Mycor) and single species *Bacillus* are recommended for enhancing survival, nutrient uptake and growth of TC banana under conventional and Humic Nitisol conditions.
4. Inoculation with *Trichoderma* (ECOT) and mixed species *Bacillus* product (PHC Biopak) is recommended for suppression of *Fusarium* in Humic Nitisol and Vertisol soils respectively.
5. Organic based fertilizers are best for combining with *Trichoderma* while P and micro-nutrient fertilizers are best for combining with AMF

Recommendations for further studies

The differential functioning of the mixed and single species *Bacillus* and mycorrhizal based products revealed in this study should be further investigated and considered in the production of inoculum meant for specific soil conditions. Further, co-inoculation of *Bacillus* and mycorrhizal based products should be investigated to maximize on the microbe-microbe interactions that promote banana survival and growth.

Further investigation should be carried out to establish the appropriate biofertilizer/ biocontrol/bioenhancers dose response and timing of application that maximize on tissue cultured banana survival and growth.

Since prevailing soil conditions affect the functioning of biological inoculants, further studies should be carried out to establish the appropriate soil organic and inorganic amendments that enhance the functioning of biological inoculants for maximizing plant performance. Further studies should be undertaken to determine the optimal P levels that would naturally suppress *Foc* and the levels that would optimize the functioning of Rhizatech.

The efficacy of biological inoculants on tissue culture banana growth, yield and disease and pest control should be investigated in the long term. This investigation could help establish the residue effects of the inoculants and determine the timings for repeat applications. This investigation should be done under integrated soil fertility management options that promote proliferation and functioning of microbe-based inoculants especially under field conditions.

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APPENDICES

Appendix 1: Summary of ANOVA table for height of tissue cultured banana (Greenhouse)

Effect	Num DF	Den DF	F Value	Pr > F
Product	6	458	11.36	<.0001
Soil	3	6	909.26	<.0001
Product*Soil	16	458	7.71	<.0001

Num DF and Den DF, degrees of freedom used in determining the F values; F value; test of group variance against a null hypothesis; Pr > F, p-value associated with the F value of the test statistic

Appendix 2: Summary of ANOVA table for leaf length of tissue cultured banana plants (Greenhouse)

Effect	Num DF	Den DF	F Value	Pr > F
Product	6	458	6.03	<.0001
Soil	3	6	705.18	<.0001
Product*Soil	16	458	3.89	<.0001

Num DF and Den DF, degrees of freedom used in determining the F values; F value; test of group variance against a null hypothesis; Pr > F, p-value associated with the F value of the test statistic

Appendix 3: Summary of ANOVA table for leaf width of tissue cultured banana plants (Greenhouse)

Effect	DF	DF	F Value	Pr > F
Product	6	458	2.43	0.0254
Soil	3	6	472.92	<.0001
Product*Soil	16	458	2.89	0.0002

Num DF and Den DF, degrees of freedom used in determining the F values; F value; test of group variance against a null hypothesis; Pr > F, p-value associated with the F value of the test statistic

Appendix 4**:** Summary of ANOVA table for number of leaves of tissue cultured banana plants (Greenhouse)

Effect	Num DF	Den DF	F Value	Pr > F
Product	6	467	38.59	<.0001
Soil	3	6	11.78	0.0063
product*soil	16	467	1.16	0.2946

Num DF and Den DF, degrees of freedom used in determining the F values; F value; test of group variance against a null hypothesis; Pr > F, p-value associated with the F value of the test statistic

Appendix 5: Summary of ANOVA table for AMF frequency of colonization

Effect	Num DF	Den DF	F Value	Pr > F
Product	6	41	0.87	0.5283
Soil	3	6	0.42	0.7472
product*soil	17	41	1.11	0.3758

Num DF and Den DF, degrees of freedom used in determining the F values; F value; test of group variance against a null hypothesis; Pr > F, p-value associated with the F value of the test statistic

Appendix 6: Summary of ANOVA table for AMF intensity of colonization

Effect	Num DF	Den DF	F Value	Pr > F
Product	6	42	1.92	0.0996
Soil	3	6	1.04	0.441
product*soil	17	42	1.12	0.3724

Num DF and Den DF, degrees of freedom used in determining the F values; F value; test of group variance against a null hypothesis; Pr > F, p-value associated with the F value of the test statistic

Appendix 7: Summary of ANOVA table for N uptake in tissue culture banana

Effect	Num DF	Den DF	F Value	Pr > F
product	6	42	7.02	<.0001
soil	3	6	2.59	0.1485
product*soil	17	42	2.44	0.0095

Num DF and Den DF, degrees of freedom used in determining the F values; F value; test of group variance against a null hypothesis; Pr > F, p-value associated with the F value of the test statistic

Appendix 8: Summary of ANOVA table for P uptake in tissue culture banana

Effect	Num DF	Den DF	F Value	Pr > F
product	6	44	2.72	0.0245
soil	3	6	215.12	<.0001
product*soil	17	44	2.25	0.0159

Num DF and Den DF, degrees of freedom used in determining the F values; F value; test of group variance against a null hypothesis; Pr > F, p-value associated with the F value of the test statistic

Appendix 9: Summary of ANOVA table for leaf surface area of tissue cultured banana plants (Field)

Effect	Num DF	Den DF	F Value	Pr > F
product	6	232	0.84	0.541
soil	2	4	639.08	<.0001
product*soil	12	232	1.75	0.058

Num DF and Den DF, degrees of freedom used in determining the F values; F value; test of group variance against a null hypothesis; Pr > F, p-value associated with the F value of the test statistic

Appendix 10: Summary of ANOVA table for apparent volume of tissue cultured banana plants (Field)

Effect	Num DF	Den DF	F Value	Pr > F
product	6	232	1.97	0.071
soil	2	4	143.42	<.0002
product*soil	12	232	1.01	0.4396

Num DF and Den DF, degrees of freedom used in determining the F values; F value; test of group variance against a null hypothesis; Pr > F, p-value associated with the F value of the test statistic

Appendix 11: Contigs used for constructing phylogenetic tree

>*Fusarium proliferatum* (TEF)
CCACAATTTCACTCCACACAATCAACAATTCCTCTGTCACCAACCATGTT
CACCCATTTCTTTTCACACAAACACATCATTGTGAGGTACCAGTGATCAT
GTTCGTGATGAAATCACGGGTGACCGGGAGCGTCTGAATGATGTGTTAG
TATGAGGAAGTAGGATGAGGTATGAGCGACAACATACCAATGACGGTG
ACATAGTAGCGAGGAGTCTCGAACTTCCAGAGAGCAATATCGATGGTGA
TACCACGCTCACGCTCGGCCTTGAGCTTGTCAAGAACCCAGGCGTACTTG
AAGGAACCCTTACCGAGCTCAGCGGCTTCCTATTGTCGAATGGTTAGTCG
CTGCTTGACACGTGACAATGCGCTCATTGAGGTTGTGGACAGGAAAGGG
CAAAAACGCGCTCATCGCTCGAGTGGCGGGGTAAATGCCCCACCAAAAA
AATTACGGTCATATCGCAAAAATTTTGGTCTCGAGCGGGGTAGCAGGCA
CGTTTCGAATCGCAAGTGAAATCGGTGGGCAGAGGACGCGCGATCGAAG
GGAAAGTGACTAACCTTCTCGAACTTCTCGATGGTTCGCTTGTCGATACC
ACCGCACTGGTAAATCAAGTGACCGGTCTGTGAAGCGATGTCAGCATAT
TGTCTTCCAAGATGTACCCCGCCAGATCTTGGGCAGGATCACGATGGCA
GATAAGCTCAGCGTCCAGGGTAGTACTCACAATGGTCGACTTGCCAGAG
TCGACGGGCCGAAGACGAACGGCATTAAGGTGAATCTGGATCCTTACCC
AATTTTGTTGATGTGATATGTTAATGCTGTGAAAAT

> *Fusarium oxysporum* (TEF)
TTGGAAGTAACCAGTGATCATGTTCCTGATGAAATCACGGTTGACCGGG
AGCGTCTGAGTGATATGTTAGTACGAAGAGAAGTAGAATGAAGCATGAG
CGACAACATACCAATGACGGTGACATAGTAGCGAGGAGTCTCGAACTTC
CAGAGAGCAATATCGATGGTGATACCACGCTCACGCTCGGCCTTGAGCT
TGTCAAGAACCCAGGCGTACTTGAAGGAACCCTTACCGAGCTCAGCGGC
TTCCTATTGTTGAATGGTTAGTGACTGCTTGACACGTGACGACGCACTCA
TTGAGGTTGTGAGAATGGTAAGAGGGCAAACGCTCCCGTCGCTCAAGTG
GCGGGGTAAGTGCCCCACCAAAAAAATTACGGTCATATTGCAAAATTTT
TGGTCTCGAGCGGGGTAGCGGCACGTTTCGAGTCGTAGGGGAAATCGA
TGGGCAAAGGACGCGCGATTGAAGGGAAAGTGACTAACCTTCTCGAACT
TCTCGATGGTTCGCTTGTCGATACCACCGCACTGGTAGATCAAGTGACCA
GTCTGTGAAACGATGTCAGTATGTTGACTTTGAGAAATACCCCGCCAGGT
CTTGGTCGGGATTGACGATGACAGATATGCTCATTGTCGAGGAGAGTAC
TCACAGTGGTCGACTTGCCAGAGTCGACGTGGCCGAAGACCAACCAATC
GGTTGAGGTGAGTCTTGTCCTCCTTTACCCTAAATGTG

> *Fusarium incarnatum (*TEF)
TGATAAACAACACCCCCCAATCCCTCCATCTTACACACTTATAAATCAGC
CTTTTCTTAGTTATCTCTCATAATCATCATTCATTTATTCCCGTCCACCAC
AGCACATGTACTCATCCTTATCTATGGGTAAAAGGTCTCGATTTCATATT
AGTCTCCGTATCGGGCCACGGTCAACTCTGGTCAAGTCAAGCCACTGTG
AGTGTACTAGCCACAATGACCAGATCTATCATGCCACTCGTCAACCCCG
ACGATACATGCGGGGGGAAATTTCACTCAACACATTTGCTGACAAGATT
TTCAGTAAAACGGGCAATTTGTCTACCCAGCCGGCGGGGGATATGACAA
ACGAAGCCACCATCGAAAAGTTGCAAAAAGGTTGGTTTCCCATTTCAAT
CGATCGCACGCCCTCCATGCCCATCGATCTCTTCACCCGAAACAGTCTCG
ACTACCGAACATAGCTCAAAATACCCCGCAATGAATACAAAATTTTGCG
GTTTGGTCAATGCAAATACCCCGTGGGGCTCAGAACCCGATACAGTCGC
CGACAGGCACTTGCTCATCTTCCCATCAAACACATTGCGCATCACGAAGT
CAATCAGTCACTAACCACATGATCAATAGGAAGCAGCCGAGCTAAGTAA
GGGTTCCTTCAAGTACGCCTGGGTTCAAGACAAGCTCAAGGCCGAGCGT
GAGCGTGGTATCACCATCGAAATCGCTCTAGTGAAGGTTCGAGACTCCT
CGAATTACGATGTCACCGTCAATGGGATGTTACCACTTCCACTCATTACC
TTCTCACGTCAAACATGAAATCTAGACCCTACATGTTCACGGCGATTTCA
TCACGAACCATGAACAGTGGTATGGCCACTCCATA

Appendix 12: Morphological and pigmentation characteristics of *Fusarium* isolates three soil types inoculated with Arbuscular mycorrhizal fungi (AMF), *Bacillus* and *Trichoderma*

Treatment	**Soil**	**Isolate**	**Type of conidia present**	**Size of spores, width `(µm)**	**Presence of chlamydospores**	**Colour of colony on PDA**
Control	Humic Nitisol	*Fusarium.proliferatum* and *F. Incarnatum, F. Oxysporum*	Macro and microconidia	0.2 0.4	No Yes	Orange yellow, Red
ECO-T	Humic Nitisol	*F.proliferatum* and *F. oxysporum*	Macro and microconidia	0.2 0.4	No Yes	Orange yellow, Dark brown
PHC Biopak	Humic Nitisol	*F.oxysporum*	Macro and microconidia	0.2	Yes	Dark brown
Rhizatech	Humic Nitisol	*F. oxysporum* *F. proliferatum*	Macro and microconidia	0.4 0.2	Yes No	Dark brown, Orange
Control	Rhodic Ferralsol	*F.proliferatum*	Macro and microconidia	0.2	No	Orange yellow
ECO-T	Rhodic Ferralsol	*F.proliferatum,and F. oxysporum*	Macro and microconidia	0.2 0.4	No Yes	Orange yellow, Dark brown
PHC Biopak	Rhodic Ferralsol	*F. oxysporum*	Macro and microconidia	0.4	Yes	Dark brown
Rhizatech	Rhodic Ferralsol	*F.proliferatum and F. oxysporum*	Macro and microconidia	0.2 0.4	No	Orange yellow, Dark brown
Control	Vertisol	*Fusarium.proliferatum* and *F. Incarnatum, F. Oxysporum*	Macro and microconidia	0.2 0.2 0.4	No Yes	Orange yellow, Red, Dark brown
ECO-T	Vertisol	*F.proliferatum and F. oxysporum*	Macro and microconidia	0.2 0.4	No Yes	Orange yellow, Dark brown
PHC Biopak	Vertisol	*F.proliferatum and F. oxysporum*	Macro and microconidia	0.2 0.4	No Yes	Orange yellow, Dark brown
Rhizatech	Vertisol	*F.proliferatum and F. oxysporum*	Macro and microconidia	0.2 0.4	No Yes	Orange yellow, Dark brown