EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI (AMF) INOCULATION AND MANAGEMENT OF INDIGENOUS AMF POPULATION ON THE EX-SITU PERFORMANCE OF MAIZE AND BEAN IN EMBU AND TAITA DISTRICTS, KENYA

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Effect of arbuscular mycorrhizal fungi (AMF) inoculation and management of indigenous AMF population on the ex-situ performance of maize and bean in Embu and Taita districts, 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

I dedicate this work to my late Mom, Lydiah Wanjiru, ‘Thanks for the love and care for the days we shared. You are my daily motivation! Love you.’

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LIST OF ABBREVIATIONS

AMF	Arbuscular Mycorrhizal Fungi
ATC	Agricultural Training Centre
BGBD	Below-Ground Biodiversity
C.A.N	Calcium Ammonium Nitrate
CSM	Conservation and Sustainable Management
DAP	Days after Planting
DEMO	Demonstration Block
FP	Farmer practice (Triple Super Phosphate + C.A.N)
GEF	Global Environment Facility
GOK	Government of Kenya
HCl	Hydrochloric Acid
ISFM	Integrated Soil Fertility Management
KARI	Kenya Agricultural Research Institute
KOH	Potassium Hydroxide
MOALDM	Ministry of Agriculture, Marketing and Livestock Development
PPM	Parts Per Million
TSBF-CIAT	Tropical Soil Biology and Fertility Institute of CIAT
TST	Test Strip
UNEP	United Nations Environment Programme

ABSTRACT

Many experiments examining the effect of Arbuscular Mycorrhizal Fungi (AMF) on crops are ambiguous and many of those demonstrating positive effects have been carried out in the greenhouse using simplified systems rendering the results not easily reproducible in the field. Therefore field experiments become necessary considering gaps in understanding of the Arbuscular Mycorrhizal (AM) association, importance of AMF species diversity and the effect of different agronomic practices on the ecology and function of AMF. The study demonstrated the importance of mycorrhizae in agricultural production systems in tropical landscapes, through establishing effects of various soil fertility management practices such as use of different fertilizers, use of manure, and slow releasing rock phosphate (mijingu) on AMF. Effect of direct AMF inoculation in the field and management of indigenous population and the performance of maize and bean were also evaluated. Soil from Below Ground Biodiversity (BGBD) test strips and demonstration blocks under FP (combination of TSP and CAN), mijingu, manure and mavuno (organic fertilize) application were sampled. The effects of existing AMF and AMF introduced across management practices were evaluated and compared to plant growth and yield. Mycorrhizal density and prevalence was determined over a period of two cropping seasons and the experiment replicated in the two benchmark sites namely Embu in the highlands of central Kenya and the coastal highlands in Taita-Taveta. This constituted the on-farm experiments of the project. On-station experiments were also set up and direct inoculation of AMF was done on common bean *(Phaseolus vulgaris* L*)* and maize *(Zea mays* L*)* intercrop; effects on crop performance were determined. Field inoculation with AMF has been demonstrated to positively affect the

yield of maize and bean at Embu experimental site though not significantly different with application of the different soil fertility amendments. The use of inorganic and organic fertilizers enhanced AMF utilization; the addition of these fertilizers to AMF led to higher crop yield as well as root colonisation compared to plots under AMF applied alone. A total of 15 AMF morphotypes were isolated and described from both Taita and Embu sites, majority being Gigasporaceae (9), followed by Acaulosporaceae (4) and Glomaceae (2). The highest species count was obtained from 0-10cm depth. Inoculation of plots with AMF was found to increase the total AMF abundance in the soil. However there was no significant ($p \geq 0.05$) difference in spore abundance at on-station experiments with use of different soil fertility amendment practices in the first season but varied less significantly ($p \leq 0.05$) after the second season but a marked reduction in AMF population was recorded with passage of each cropping season. On-farm experiments (test strips) also recorded a reduction in AMF population with subsequent season. The spore abundance showed no significant difference with application of the different soil amendments. This was also the case with species richness in the soil during the two seasons. In demonstration plots, there were significant ($p \leq 0.05$) differences in spore abundance among the different soil fertility amendment practices. Also a marked decrease in AMF population in subsequent cropping season was recorded. There was higher root colonisation as well as spore abundance in the soil under manure application with subsequent average maize and bean production. Manure application was found to be the best method to conserve AMF population in the soil and thus recommended as a cheap and an environmentally friendly method of soil fertility management.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

Soil organisms contribute a wide range of essential services in the sustainable function of all ecosystems, such as regulating nutrient cycles and the dynamics of soil organic matter, soil carbon sequestration and greenhouse gas emission; modifying soil physical structure and water regimes; enhancing the amount and efficiency of nutrient acquisition by the vegetation through mycorrhizal fungi and nitrogen fixing bacteria; and influencing plant health through the interaction of pathogens and pests with their natural predators and parasites. These services are not only essential to the functioning of natural ecosystems but constitute an important resource for the sustainable management of agricultural ecosystems. Soil organisms constitute what is now referred to as "below-ground biological diversity" (BGBD) or, sometimes, "soil biodiversity". Below-ground biodiversity is dramatically affected when forests are converted to agricultural land, and when agricultural land use is intensified (CSM-BGBD, 2007). Reduced below ground biodiversity may decrease agricultural productivity and reduce the sustainability of agricultural systems, which then become more vulnerable to adverse climatic events, erosion, pests, diseases, and other threats (CSM-BGBD, 2007).

Mycorrhizal fungi are part of the important component of soil microorganisms that is vital in sustainable development of agricultural lands. Mycorrhizal symbiosis between plants and fungi is one of the most well-known plant-fungus associations and is of significant importance for plant growth and persistence in many ecosystems; over 90% of all plant species engage in some kind of relationship with fungi and are dependent

upon this relationship for survival. The mycorrhizal symbiosis is ancient, dating to at least 400 million years ago. It often increases the plant's uptake of inorganic compounds, such as nitrate and phosphate from soils having low concentrations of these key plant nutrients. In some mycorrhizal associations, the fungal partners may mediate plant-to-plant transfer of carbohydrates and other nutrients (Jeffries *et al*., 2003).

1.2 AMF taxonomy and nomenclature

The first report that root fungi may be beneficial to plants was observed on Indian pipe plant (Kamienski, 1881). Frank (1885) named the symbiosis between fungi and roots "Mykorrhizen", from the Greek meaning "fungus root". Amongst the mycorrhizal associations, the AM association is the most common. Arbuscular mycorrhizal fungi belong to the fungal phylum Glomeromycota (Schuessler *et al*., 2001). The Glomeromycota is divided into four orders, eight families and ten genera. The genera which include most of the described species are *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora*. The AM fungi obtain their energy through an obligate symbiosis with vascular plants; the AM, although non-vascular plants also are reported to form the AM (Russell and Bulman, 2005).

The AM fungi are named by their formation of highly branched intracellular fungal structures or "arbuscules" which are the site of phosphate exchange between fungus and plant. Vesicles, which contain lipids and are carbon storage structures, are formed commonly in most genera of Glomeromycota, although this will depend on environmental conditions (Smith and Read, 1997). They have a very broad host range,

which makes them definitely different from the biotrophic fungal plant pathogens as well as other root symbionts (Gianinazzi-Pearson, 1996). Fossil records suggest that the AM symbiosis dates back to the Ordovician age, 460 million years ago. These fossils indicate that Glomeromycota-like fungi may have played a critical role in facilitating the colonisation of land by plants (Redecker *et al*., 2000).

As AM fungi are obligate symbionts, they are not yet successfully cultured in the absence of plant root. The symbiosis is normally mutualistic and based on bi-directional nutrient transfer between the symbionts. However, the mycorrhizal associations may vary along a symbiotic continuum from strong mutualism to antagonism (Carling and Brown, 1980; Modjo and Hendrix, 1986; Howeler *et al.*, 1987; Johnson *et al*., 1997).

More than 150 species are described within the phylum Glomeromycota on the basis of their spore development and morphology, although recent molecular analyses indicate that the definite number of AM taxa may be much higher (Daniell *et al.*, 2001;Vandenkoornhuyse, *et al.*, 2002). However, the biological knowledge is lacking for some of the described species and others are synonyms (Walker and Trappe, 1993; Walker and Vestberg, 1998). All members of the AM fungi are asexual and the vegetative mycelium and intraradical structures are aseptate and multinucleate. Most spores are between 50 and 500 µm in diameter depending on the species.

1.3 Colonisation of host by AMF

There are three important components of the mycorrhizal root system namely; the root itself, the intraradical mycelium (the fungi within the root) and the extraradical mycelium (the fungi within the soil). Colonisation of roots by AM fungi can arise from spores, infected root fragments and/or hyphae. The spores are formed on the extraradical hyphae, but some species also may form spores inside the roots. Soluble exudates or extracts from the roots of host species stimulate the growth and branching of mycelium growing from spores (Graham *et al*., 1982; Elias and Safir, 1987; Gianinazzi-Pearson *et al*., 1989), while the exudates from a non-host has no effect (Gianinazzi-Pearson *et al.,* 1989). The main hypha approaches a root often giving rise to a fan-shaped complex of lateral branches, which is thinner and may be septate, and colonisation of the root usually occurs from these hyphae (Mosse and Hepper, 1975; Giovannetti *et al*., 1993a,b). Hyphal contact with the root is followed by adhesion and formation of swollen appressoria preceding the penetration (Bécard and Fortin, 1988; Giovannetti *et al*., 1993b).

The symbiotic performance of AM fungal isolates depends on two main parameters, colonization ability and efficiency. The rate of colonization is influenced by the ability of AMF to spread rapidly and extensively in plant roots, and is affected by factors linked to spore germination, presymbiotic mycelial growth and appressorium formation (Giovannetti, 2000). Efficiency is correlated with the ability of different isolates to promote plant growth by improving mineral nutrition and increasing tolerance to biotic and abiotic stresses (Giovannetti and Avio, 2002; Jakobsen *et al*., 2002).

1.4 Benefits of AMF impact to the host plant

The host benefits from the association in a number of ways and this is mainly through increased uptake of relatively immobile phosphate ions. AMF produce extraradical mycelium forming an extensive network within the soil. The mycelia grow beyond the phosphate depletion zone that usually develops around the root (Smith and Read, 1997).

Other benefits of AMF to the host plant include; improved drought resistance (Auge` *et al*., 1994), increased tolerance to toxicity caused by heavy metals as well as tolerance from extreme salinity (Feng *et al*., 2002). Other than phosphorus, AMF also help in uptake of other macronutrients such as nitrogen (N), potassium (K) and magnesium (Mg) (Hodge *et al*., 2001) and also micronutrients (Azaizeh *et al*., 1995).

Higher than normal carbon dioxide concentrations help to promote soil aggregation by increasing the production of glomalin (Riling *et al*., 1999). These findings could have important future implications in the use of mycorrhizal fungi to promote the production of stable soil aggregates, improve water infiltration, and soil carbon sequestration in agricultural systems.

AM fungi are recognized as potential agents in plant protection and pest management (Quarles, 1999). In several cases direct biocontrol ability has been demonstrated, especially for plant diseases caused by *Phytophtora*, *Rhizoctonia*, and *Fusarium* (Abdel-Aziz et *al*., 1997; Vigo *et al*., 2000).

Though the AM association can offer multiple benefits to the host plant, it may not be obviously mutualistic at all points in time, and it is possible under some conditions that

the AMF may 'cheat' their host plant into supplying carbon with no apparent benefit to the plant. In some cases, this can cause a decline in growth (Lerat *et al.,* 2003). However, demonstrating that AMF are actually 'cheating' is difficult not least because of the wide range of benefits to the host, which may only become obvious at specific times or under certain environmental conditions or stresses (Fitter, 2001).

1.5 Dynamics of AMF in the soil

Several factors have been shown to affect AMF spore abundance and species diversity. This is directly through damaging or killing AMF and indirectly, by creating conditions either favourable or unfavourable to them. In general, farming practices have a negative impact on the AM association and agricultural soils are AMF impoverished, particularly in the number of species (Helgason *et al*., 1998; Menendez *et al*., 2001). These include, use of excess phosphorus fertilizer which may lead to reduced AM colonisation of roots and AMF spore density in soil (Kahiluoto *et al.,* 2001). Use of other readily soluble fertilizers, particularly, nitrogen fertilizers has also been reported to have a negative impact on AM colonization and diversity in some cases (Treseder and Allen, 2002) but not in others (Jumpponen *et al*., 2005). Farm yard manure and slow release mineral fertilizers such as rock phosphate do not seem to suppress AMF and may even stimulate them (Joner, 2000; Alloush and Clark, 2001). Agricultural production also leads to low diversity of AMF compared to a natural ecosystem and tends to propagate *Glomus* species due to low diversity of hosts which is severe in case of monoculture (Oehl *et al.,* 2003). Previous research on AMF abundance in Embu revealed that least spore

abundance is found in land under crop food production compared to cash crop and undisturbed forests (Jefwa *et al*., 2006).

Soil tillage causes severe disruption to the common mycorrhizal network resulting in delayed or reduced root colonization and a reduction in the volume of the soil that is exploited by the AMF leading to reduced plant nutrient uptake, consequently crop growth and yield (Evans and Miller, 1990; Kabir *et al*., 1998)

1.6 Crop choice

1.6.1 Maize (*Zea mays* L.)

Maize being a staple food in Kenya and a source of carbohydrates to a large proportion of people is one commodity that has undergone these structural reforms. As a food commodity, maize provides a large proportion of calorie needs to a majority of consumers in urban and rural areas (Nyoro, 2002). A large proportion of maize production comes from small-scale producers although most of them retain part of their produce for consumption. About 3.5 million small-scale farmers are involved in maize production producing about 75 percent of the total maize crop. Large–scale farms account for the remaining 25 percent of the production and are estimated to be just about 1000 farmers (Nyoro, 2002)

Maize accounts for more than 20% of all agricultural production and 25% of agricultural employment. Smallholders produce about 70% of the nation's maize;

although large-scale farmers also contribute a significant proportion of commercial maize production (Pingali 2001).

There has been constant maize production deficit in Kenya for the past 10 years (2000 to 2010). Thus the domestic consumption has not been met by domestic production. These can be attributed to a number of factors including land fragmentation due to increased pressure on land occasioned by ever increasing population. As the land sizes decrease, the use of improved technologies through mechanization is drastically reduced. Declining soil fertility and lack of proper management practices such as crop rotation and intercropping (Owuor, 2010). There is a drastic soil nutrient depletion countrywide as increasing land pressure from a still burgeoning population, nutrient losses (from leaching and erosion) and off-takes from crop harvests removals often exceed additions from biological processes and application of organic and inorganic fertilizers (Omamo, 2002). Over-reliance on rain-fed agriculture in the wake of global climate change has also contributed to negative maize production figures

The average yield for maize in Kenya is about 1.5 t/ha (Pingali, 2001). Most smallholders produced under 1 t/ha, well below the potential average of 4.7 t/ha (Hassan *et al.,* 1998). Intercropping with beans is usually practiced by small scale farmers.

1.6.2 Common bean (*Phaseolus vulgaris* L.)

Common bean is an important component of the production systems and a major source of protein for the poor in Eastern and southern Africa. Crop production trend has not kept pace with the annual growth rate (estimated above 2 percent) in population (Katungi *et al.*, 2009).

In terms of production, Kenya comes second after Uganda in Africa. Common bean yields are higher in Uganda than in Kenya because of a relatively favourable biophysical environment (such as weather condition) in Uganda compared to Kenya. Bean production in Kenya has moved above 500,000 tonnes (FAO, 2007)

Although Kenya has two seasons for common bean, a significant number of farmers grow the crop once a year because of adverse climatic conditions. The Rift valley and the Western region which respectively produces 33 percent and 22 percent of the national outputs allocates land to common beans once a year, during March- May season (also refereed to as long rains) while farmers in the Central and Eastern regions grow twice a year but only 70 percent of the farmers in the Eastern region grow it in the long rains. Almost all farmers in these two regions grow common bean in short rains (October to December). Nationally, average yield fluctuates between 0.35 ton/ha to 0.54 ton/ha (Katungi *et al.*, 2009).

It is produced in areas between 800 and 2300 m above sea level with average rainfall of 500 to 2000 mm and temperature of 16 to 24ºC (Allen and Edja, 1990; Wortmann *et al*., 1998). Bean producing areas are Eastern, Central, Rift valley and Western Provinces of Kenya (MOALDM, 1994). Small scale farmer are the main producers of beans in Kenya. They intercrop with other crops such as maize (*Zea mays* L.), sorghum

[*Sorghum bicolor* (L.) Moench], cowpeas [*Vigna unguiculata* (L.) Walp.] cotton (*Gossypium sp*.), cassava (*M. esculenta* Crantz) and potatoes (*Solanum tuberosum* L.) (Mwaniki, 2000). The beans varieties grown in Kenya include Rosecoco (GLP-2), Canada Wonder (GLP-24), Red haricot (GLP-585), Mwezi Moja (GLP-1004), Mwitemania (GLP-92) and Zebra (GLP-806). These varieties were released by Grain legume project of the National Horticulture Research Station, Thika, for different agro-ecological zones (Origa, 1992).

1.7 Statement of the problem

Micro organisms in the soil are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in such key processes as soil structure formation, decomposition of organic matter, cycling of carbon, nitrogen, phosphorus and sulphur. Microorganism's such as mycorrhizae also play key roles in suppressing soil borne plant diseases and promoting plant growth

Various soil fertility amendment and management practices greatly alter the soil environment under which soil microorganism function. This greatly alters the occurrence as well as their survival. Their efficiency may thus be interfered with leading to a possible total elimination of vital bio-components before their potential is realized. Awareness in environmental degradation by agricultural chemicals has also highlighted the need to seek environmentally friendly farming practices that improve soil fertility and use less fertilizer and chemical inputs. Pesticides are also expensive and thus unavailable to most African subsistence farmers. The use of biological agents such as AMF has been shown to protect plants against pathogenic root-infecting pathogens. However, field inoculation under prevailing environmental conditions and under different soil fertility amendment practices is necessary to determine the success or failure of possible AMF utilization.

1.8 Justification

Although much soil nutrient studies in different soil fertility regimes are being conducted, there is a lack of basic knowledge on how these practices affect below-ground biodiversity in subsistence farming and which of the practices applied would be optimum to maintain the biodiversity. Most crops are established to benefit from below ground biodiversity, particularly the mycorrhizal symbioses and these include; increase in growth vigour, increased productivity (crop yield) as well the ability of the crop to resist attack or minimize the detrimental effects caused by pathogenic attack. Understanding how these microorganisms are affected by various soil fertility amendment practices is the first step towards integrated soil fertility management (ISFM). Also proper and sustainable utilization of these organisms can only be possible with quantification of their individual benefits in crop production under various soil fertility regimes and prevailing environmental conditions. This study will provide data on the effect of AMF inoculation on maize and bean growth as well as on the yield in Embu and Taita districts. Also, data on effects of various soil fertility amendments practices on AMF abundance and diversity will be obtained. Maize and bean production in Kenya is a highly relevant activity due to its importance as it is a dominant food crop. It is wholly produced under rain fed conditions and the bulk of the small-scale farmers who do not apply fertilizers or manure obtain low yields ranging between 1.1 and 2.5t ha-1. Traditional farming practices are no longer capable of meeting Kenya's maize production requirements; consequently, widespread application of scientific methods is essential.

1.9 Objectives

1.9.1 General objectives

To determine the effect of AMF inoculation and management of indigenous AMF population on the ex-situ performance of maize and bean.

1.9.2 Specific objectives

1. To investigate the effect of direct AMF inoculation on the growth and yield of maize and bean in the field.
2. To determine the effect of soil fertility management practices on AMF diversity and abundance.
3. To carry-out an impact assessment on the best soil fertility management practice recommended for farmers adoption on AMF diversity and abundance.

1.10 Hypotheses

- Soil fertility amendment practices affect the abundance and diversity of AMF in Embu and Taita districts.
- Inoculation of maize and beans with AMF has a positive affect on their performance.

CHAPTER 2: MATERIALS AND METHODS

2.1 Site description

The studies were conducted in Taita Taveta District (Taita Hills) and Embu District of Mount Kenya region (Fig 2.1). The benchmark sites have high biodiversity, as they are known to contain a number of endemic plant and animal species. They are designated among the twenty-five globally recognized biodiversity "hotspots" (TSBF-CIAT BGBD GEF-UNEP Project, 2002). The communities in both study areas are mainly smallholder subsistence farmers. The sites provided an interesting framework for the proposed ecological studies of belowground biodiversity.

Land use intensification due to prolonged cultivation culminating to nutrient mining and use of pesticides is evident in the regions (Muya and Mutsotso, 2009). The most commonly used practices of replenishing soil fertility are through use of fertilizer. This does not take into consideration of the life in the soil systems that are paramount in nutrient cycling processes. The major factor contributing to low soil quality and productivity in the research area is high acidity, which is far much above the critical limit of 0.2% in all the sites whereby Taita soil had an average of 0.26% and that of Embu had a value of 0.61%.

The organic carbon level is very high, being above the critical limit (2%) in Embu (3.74%) but lower in Taita (1.56%). Phosphorus level is generally lower than the critical limit (20 ppm) for both sites. Embu site has a value of 16 ppm while Taita site had a value of 11 ppm. Nitrogen level at Taita was 0.18 which was lower than critical limit of 0.2 but higher in Embu with a value of 0.33. Potentially, these soils are rich, but farmers

must still apply increasing quantities of fertilizers to sustain the production (Muya and Mutsotso, 2009). There were marked differences in soil characteristics between the two benchmark sites reflected in the differences between the major soil types. In Taita Taveta, the major soil types were classified as Plinthic Lixisols, Plinthic Acrisols, Dystric Cambisols and Chromic Luvisols, while in Embu they were classified Rhodic Nitisols, Humic Nitisols, Haplic Acrisols and Chromic Luvisols (Muya and Mutsotso, 2009).

2.1.1 Location, topography and human activities

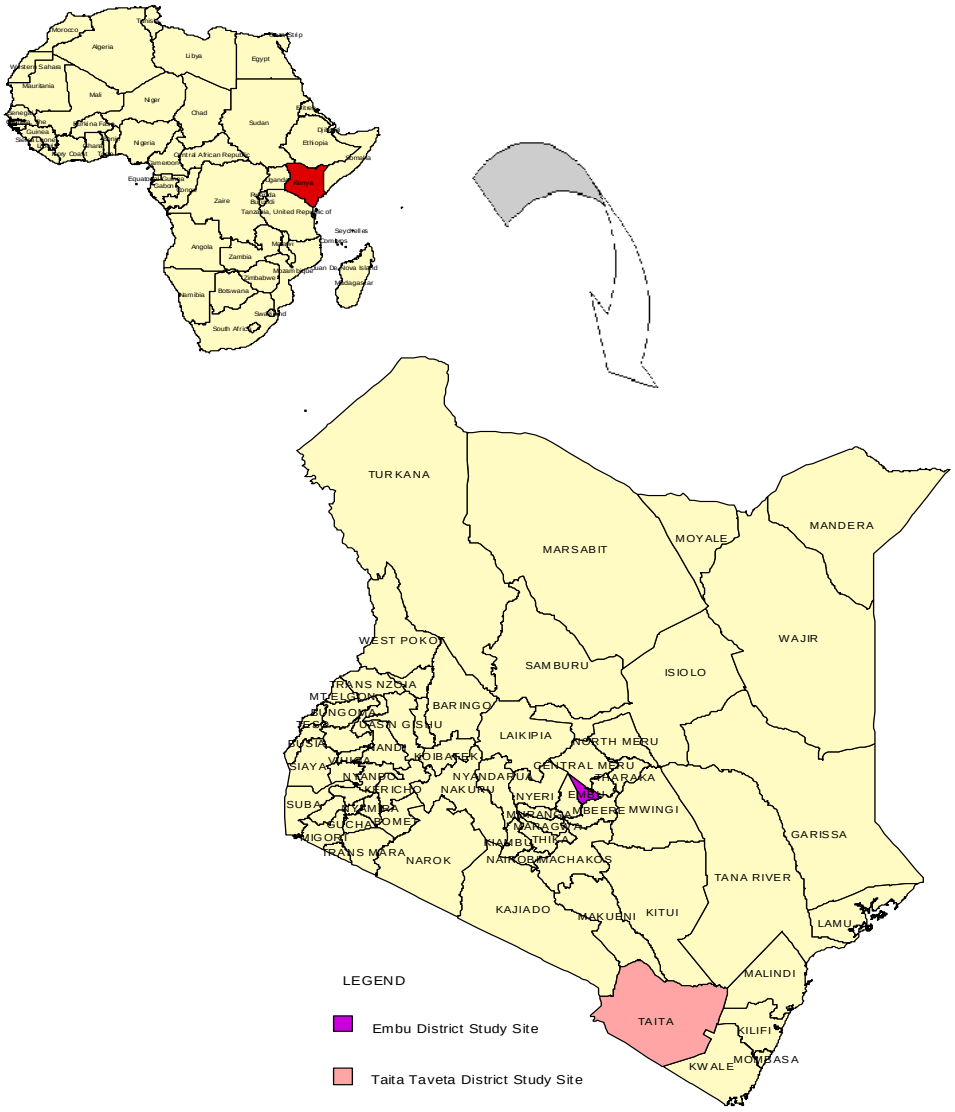


Fig 2.1: Location of Embu and Taita-Taveta Study Sites in Kenya

Embu District is in the Eastern Province of Kenya (latitude: 03o 30′ S, longitude: 37^{o} 30′ E, and altitude 1480 m above sea level). Embu is characterized by typical highlands, midlands and other features which include hills and valleys. Altitudes for highlands range between 1500m and 4500 m at the foot of Mt. Kenya. The population is currently projected at 293,144 persons with more people towards the upper zones of the district. Most of the agricultural areas of Embu falling within the study site have population densities of 538-727 persons/ Km^2.

The second benchmark site was in Taita Hills (lat 3°25´; long 38°20´), situated in Taita-Taveta District in South-Eastern Kenya (Coast Province) at an altitude of 2228 m above sea level). The Taita Hills cover an area of 1000 Km^2 and they form the northernmost part of the Eastern Arc Mountains. Voi, the biggest town in the area (population 60 000) is situated on the plains near the border of eastern section of Tsavo National Park.

The population of the whole Taita-Taveta district has grown from 90,146 (1962) persons to over 250,000 (approximation based on projection 1993). The spatial distribution of the population in the area closely follows the climatic and other ecological conditions. The land use in Taita Hills is dominated by intensive agriculture. Extensive agriculture and grazing are dominant land use types on the foothills and plains surrounding the hills. The largest, still fragmented, forests are located in the most remote areas. Only a very small portion of the land area in the district has agricultural potential and much of it lies in the higher altitudes where the forests are found. The increase in human population with its associated demands continues to place significant pressure on the remaining ones.

2.1.2 Climate and vegetation

Embu district receives a total annual rainfall of between 1200 and 1500 mm in two rainy seasons, 'long rains' (March to June) and 'short rains' (mid October to December).

Mean monthly temperature ranges between 14ºC and 19.5ºC. This site is along the slopes of Mt. Kenya remarkably better known for the mountainous catena of features including Forest resources on the lower slopes, the Bamboo vegetation the upper middle slopes, the Afro-alpine grasslands (moorland) in the upper slopes and snow cap on top of the mountain (Wokabi, 1995)

The climate at Taita experimental site is under the influence of Inter-Tropical Convergence Zone (ITCZ). The area receives an average annual rainfall of 1500 mm in the highlands and 250 mm in the lowlands and the mean monthly temperature ranges between 17.4ºC and 34.5ºC.

2.1.3 Soils

The soils at Embu experimental site are mainly Humic Nitisols (FAO, 1989) derived from basic volcanic rocks (Jaetzold and Schmidt, 1982). They are deep, well weathered with friable clay texture with moderate to high inherent fertility. The soils also follow a similar catena. The area below the forest belt is utilized for agriculture with tea on the upper slopes and coffee on the lower slopes. The rest of the area is under subsistence agriculture with agroforestry being widely practiced in many parts as a means of soil conservation.

The soils at Taita experimental site are sandy loam with high infiltration rates, a low pH, a low water holding capacity, and they are low in nutrients due to excessive leaching. The soils are also characterized by the presence of high aluminium levels, low calcium levels and unavailable potassium, causing a low cation exchange capacity (TSBF-CIAT BGBD GEF-UNEP Project, 2002).

2.1.4 Experimental design

2.1.4.1 On- station experimental plots

At Embu, on-station trials, 80 plots with sixteen (16) treatments replicated five (5) times separated by 1m wide strips were established at the Agricultural Training Centre (ATC) and. The plots measured 3m x 3m in randomized block design (Appendix 1). Only a total of eight treatments were evaluated for mycorrhizal status. The remaining plots had other soil microorganisms being evaluated such as *Bacillus* and *Trichoderma*. The treatments comprised control (maize and bean intercrop without any soil fertility amendment), AMF + Manure, AMF + Mavuno, AMF, AMF + Farmer Practice, Manure, Mavuno and Farmer Practice (FP). FP is a mixture of TSP and CAN according to the KARI recommendation

At Taita, the on-station trial was established at the Agricultural Training Centre (ATC). A total of 55 plots with 11 treatments replicated five times separated by a 1m strip were demarcated. The plots measured 3m x 3m in a randomized block design (Appendix 2) and the treatments applied were similar to those established at Embu experimental site except the omission of plots treated with *Bacillus*.

2.1.4.2 On-farm test strips experimental layout

At Embu, the experiment comprised 11 test strips with 10 treatments receiving the following rates of fertilizer: cattle manure at 9 Kg per plot, Mavuno at 0.9 kg. Farmer practice (KARI Embu recommendation) TSP at 0.8 kg and 0.5 kg of CAN (All fertilizer applications are expressed in kg/ha) (Appendix 3).

At Taita experimental site 12 test strips were set up and various treatments initiated as described in Appendix 4. Fertilizer were applied in the following rates, Manure at 9 Kg, Mavuno at 0.9 kg and Farmer practice at TSP 0.8 kg and 0.5 CAN. These were set up at 12 different farmers' plots with the 12 farms being the replicates. The treatments were similar to those set up at Embu except the omission of *Bacillus* treatments.

2.1.4.3 On-farm demonstration plots

Demonstration plots with practices likely to be adopted by farmers were established alongside the strip at both experimental sites. Three plots were established measuring 29 meters long, consisting of 5x10 meters plots divided by a 1m strip in different farms. At Taita, each plot had five (5) treatments and the following rates of fertilizer were applied in the 3 demonstration blocks: Manure at 50 Kg, Mavuno at 0.9 kg, Farmer practice (KARI recommendation) at TSP at 4.5 kg, CAN at 3.9 and Mijingu at 4kg, CAN at 0.4 Kg (Appendix 5)

At Embu, the following fertilizer rates were applied in all the 3 demonstration blocks: Manure at 50 Kg, Mavuno at 0.9 kg, at Farmer practice (KARI Embu recommendation) TSP at 4.5 kg and 3.9 CAN, Mijingu at 4kg plus CAN at 0.4 Kg per plot (Appendix 5).

2.2 On-station inoculation with mycorrhizae

The source of mycorrhizae inoculant used was derived from indigenous species from the two respective sites. This was applied in mixed form with each site receiving AMF inoculum that had been cultured one and a half years in sorghum then transferred to leek four weeks before planting to generate infective mycelia and infected root fragments. The cultures were initiated and maintained at the National Museums of Kenya and inoculum as produced by Munro *et al.,* (1999). The inoculum consisting of indigenous species was applied in crude state comprising of spores, mycelia and infected root fragment at planting. An average value of 62 spores per 50grammes of soil samples and colonisation intensity of over 90% on the roots fragment were the constituent values of the composite inoculum. Leek was used in culturing and bulking up of the inoculum.

2.3 Maize and bean establishment

At Embu benchmark site, Hybrid 513 (maize) and Mwitemania (GLP 92) beans were planted being the common varieties in use by local communities at the beginning of the project. At Taita benchmark site Hybrid 513 and Mwezi moja (GLP-1004), maize and bean varieties were planted, respectively. Each plot had row spacing of beans at 45cm apart with interspacing of 30cm with 2 seeds per hill. Also, each plot had intra row spacing of maize planted at 90x30cm with 2 seeds per hill (seed rate; 20-25 kg/ha). Each plot (3x3) had 4 rows of maize with 10 hills per row (plant density of 4x10x2= 80 seeds per plot). Wet planting was done at the depth of 2.5-4cm. Thinning was carried

out and single plant per hole was retained: a total of 40 plants per plot were allowed to grow to maturity.

Each plot (3x3) had 3 rows of beans with 10 hills per row; the plant density of 3x10x2= 60 plants per plot. Thinning was carried out and single plant per hole was retained, a total of 30 plants per plot are allowed to grow to maturity. Germination rate of maize and bean was recorded for all plots and compared with plots under AMF inoculation, two weeks after germination. A maize and bean guard row around the farms was also established.

2.4 Amendments of plots with manure, fertilizer and inoculation with mycorrhizae

Manure was broadcasted at 9 kg per plot (equivalent of40-60tons /ha) and maize-bean intercrop planted as described. Farm Practice (CAN + TSP), the Triple Super Phosphate (TSP) was broadcasted at 0.8 kg per plot (200kg /ha) and Calcium Ammonium Nitrate (CAN) was applied at 0.5 kg per plot (150-200 kg /ha).The control treatment had no application of AMF or fertilizer while Mavuno fertilizer was spread evenly at a rate of 0.9 kg /plot.

CHAPTER 3: EFFECT OF DIRECT AMF INOCULATION ON THE EX-SITU PERFORMANCE OF MAIZE (*Zea mays*) AND BEAN (*Phaseolus vulgaris*) AT EMBU AND TAITA DISTRICTS, KENYA

3.1 Abstract

In a two-year study 2008-2009, mycorrhizal treatments in conjunction with 8 soil fertility amendment practices (SFMP) i.e. control, AMF, manure, mavuno, FP (A combination of CAN and TSP), AMF + manure, AMF + mavuno and AMF + FP, were tested on their effect on growth of maize *(Zea mays* L*)* and common bean *(Phaseoli vulgaris* L) under field experiments in a continuous cropping. The treatments were established by the GEF Belowground Biodiversity project, Kenya. Maize and bean performance were determined over a period of two planting seasons and the experiment was replicated in two benchmark sites namely Embu in the highlands of central Kenya and the coastal highlands in Taita-Taveta district.

Field inoculation with AMF has been demonstrated to positively affect the yield of maize and bean at Embu experimental site though not significantly different among the different soil fertility amendments. The use of inorganic and organic fertilizers enhanced AMF utilization; the addition of these fertilizers to AMF led to higher crop yield compared to plots under AMF applied alone. Thus, AMF inoculation on crop has effect on maize and bean growth and is dependant on climatic conditions as well as other farming practices.

3.2 Introduction

AMF forms associations with over 80% of crop plants. The host benefits from the association in a number of ways and it's mainly through increased uptake of relatively immobile phosphate ions. AMF produce extraradical mycelium forming an extensive network within the soil. The mycelium grows beyond the phosphate depletion zone that usually develops around the root (Smith and Read, 1997). Other benefits of AMF to the host plant include, improved drought resistance (Auge` *et al*., 1994), increased tolerance to toxicity caused by heavy metals as well as tolerance from extreme salinity (Feng *et al*., 2002), increased resistance to foliar feeding insects (Gange and West, 1994; Mohammad *et al*., 2003). Other than phosphorus, AMF also help in uptake of other macronutrients such as nitrogen (N), potassium (K) and magnesium (Mg) (Hodge *et al*., 2001) and also micronutrients (Azaizeh *et al*., 1995). However, many of these experiments have been conducted in the greenhouse which does not explain their relevance to the field situation. The study demonstrate the importance of mycorrhizae inoculation in agricultural production systems in tropical landscapes, through establishing effects of various soil fertility management practices such as use of different fertilizers, use of manure, and AMF on growth and yield of maize and bean. This is demonstrated through direct AMF inoculation at the on-station experiments at Embu and Taita Agricultural Training Centres (ATC). Data collection was done over a period of two planting seasons. Field inoculation with AMF has been demonstrated to positively affect the yield of maize and bean at Embu experimental site though not significantly different with application of different soil fertility amendments.

3.3 Materials and Methods

The study was carried out from both on-station and on farm experiments. In the on-station experiment, maize and bean in plots under various soil fertility amendment practices (Appendix 1 and 2) were sampled. The treatments were laid out as described in Chapter 2 above and maize-bean intercrop established. Common farming practices were applied to the plots and these included, land preparation, planting of crops with onset of rains, gapping so as to replace dead seedlings 14 days after germination, weeding and thinning so as to uproot excess plantlets.

3.3.1 Crop growth

Under on-station experiments, the rate of flowering and pod formation for beans were monitored and recorded after every three days from onset. The rate of seedling emergence and survival rates from both the on-farm and on-station experiments was also monitored and recorded. Rate of maize tussling was collected after every three days from onset. This was monitored for two continuous cropping seasons (the short rains and long rains).

3.3.2 Measurement of grain and stover yield at harvest

At harvest, the weight of maize stovers (above ground biomass minus grains) was obtained for each plot and the average computed. This was done on plots treated with mavuno, mavuno plus AMF, AMF only, manure, manure plus AMF, farmers practice, farmers practice plus AMF, control (maize and crop intercrop without the application of soil fertility amendments) treatments. The yield of maize and beans in each plot was assessed at the end of the cropping seasons. Measurements included the number of cobs,

total bean litter and maize stover weights, bean and maize grains dry weights in kilograms per plot. Weight of 10 randomly sampled maize stovers and maize cobs plus grains per plot were also determined and recorded.

3.3.3 Data analysis

Maize and bean yield, rate of bean podding and flowering data was analysed by parametric (ANOVA) method using Genstat (Genstat Discovery Edition 3) statistical package.

3.4 Results

3.4.1 Maize growth and harvest in long rain (April 2008 to August 2008)

3.4.1.1 On-station maize yield in Embu experimental site

Generally, AMF had no effect on yield as evidenced by combination of AMF with mavuno which reduced the positive effect of mavuno. The plots under mavuno had the highest weight of maize grains with an average of 2.78 tons ha^{-1}. For plots under AMF inoculation, the highest average obtained was from AMF in combination with mavuno fertilizer with an average of 2.4 tons ha^{-1} and this was the second highest value. The plots with AMF applied alone had an average of 1.77 tons ha^{-1}. Use of AMF in combination with inorganic (TSP + CAN and organic (manure) amendment practices resulted in decreased maize yield in all the treatments compared to treatment with inorganic or manure applied alone (Fig 3.1).

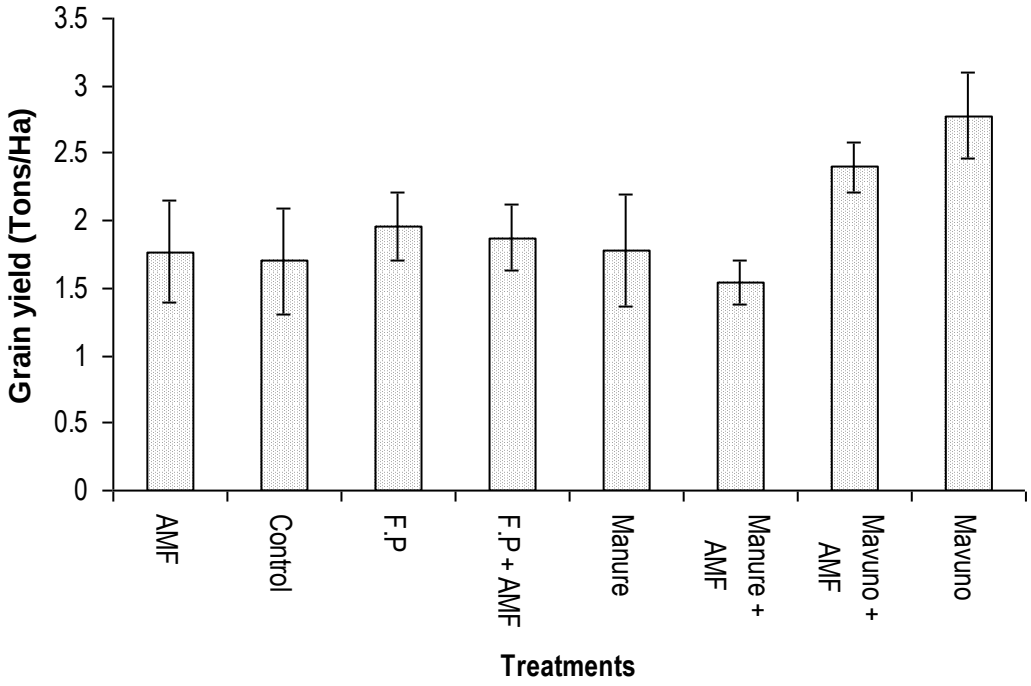


Figure 3.1: Average grain yield (Tons ha-1) of maize (*Zea mays*) stovers in plots treated with AMF, control, FP, FP + AMF, manure, manure + AMF, mavuno and mavuno + AMF at Embu district during the 2008 long rain season (April to October).

3.4.1.2 On-station maize yield at Taita experimental site

The same measurements were done at ATC, Taita, and there was no significant difference in average weight of all cobs per plot among the various treatments in Taita on-station experiment. However, the average weight of all stovers was found to be significant ($P \leq 0.05$) with application of various treatments. FP had the highest average weight of stover with an average of 3.07 tons ha^{-1} followed by AMF combined with FP with average value of 3.04 tons ha^{-1}. The plots under AMF applied alone had an average of 2.56 tons ha^{-1} and the lowest value was on plots with AMF combined with mavuno fertilizer. The average weight for all cobs in each plot was also obtained at Taita district

and values recorded. AMF combined with FP had the highest yield of 4.24 tons ha^{-1} and AMF combined with manure had the lowest value of 2.60 tons ha^{-1} (Fig 3.2)

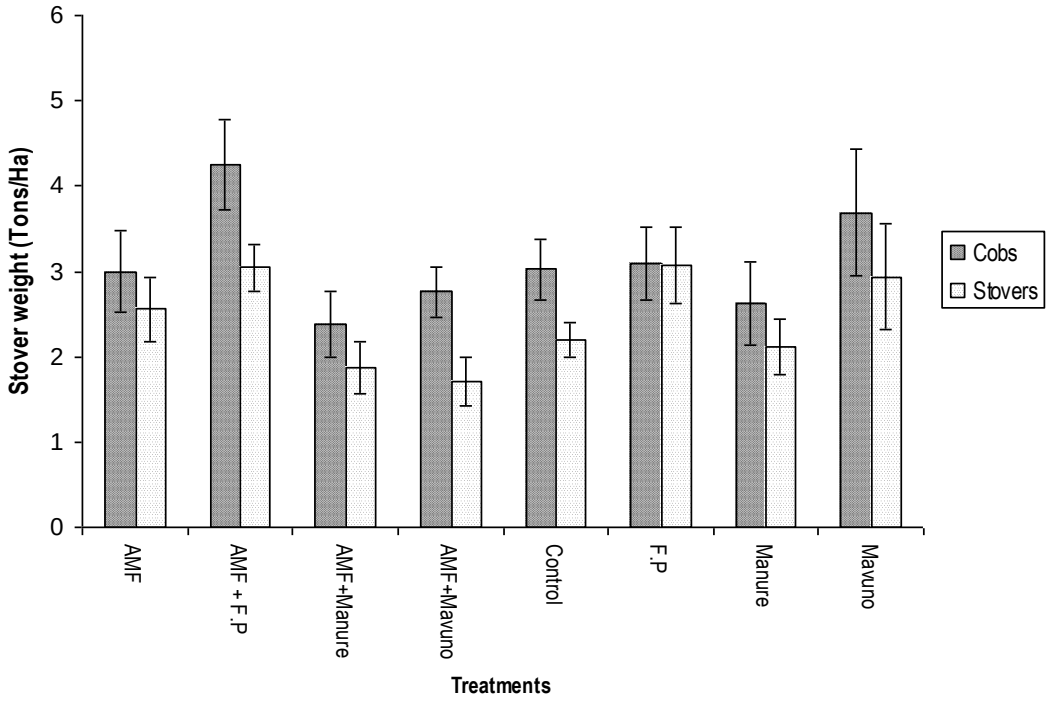


Figure 3.2: Average weights (Tons ha-1) of maize (*Zea mays*) stover in plots treated with AMF, control, FP, FP + AMF, manure, manure + AMF, mavuno and mavuno + AMF at Taita district, during the 2008 long rain season (April to October).

3.4.2 Maize growth and harvest during the short rain season (October 2008 to February 2009)

3.3.2.1 Taita experimental site

There was no significant difference in the average weight of all cobs and also the weight of all stover per plot with application of various treatments at on-station experiment at Taita benchmark site in the short rain season (October 2008 to February 2009). The highest weight of all cobs (an average value of 3.67 Tons ha^{-1}) in Taita on-station experiment was recorded in plots treated with mavuno. The lowest was recorded in plots treated with AMF alone and AMF combined with manure both with an average value of 1.78 tons ha^{-1}. The weights of all stovers in a plot was highest in plots treated with mavuno with a value of 4.67 tons ha^{-1} and lowest on plots under AMF combined with manure treatment with an average of 2.04 Tons ha^{-1}. Combination of fertilizer or manure with AMF had a negative effect on maize production compared to the treatments with either of the fertilizer types applied alone (Fig 3.3). The figure shows that there are no benefits derived from inoculation with AMF compared to fertilizer applied alone. However, the use of AMF in combination with FP and mavuno had higher yield compared to AMF alone. Application of manure with AMF in combination had no such positive effect on maize yield.

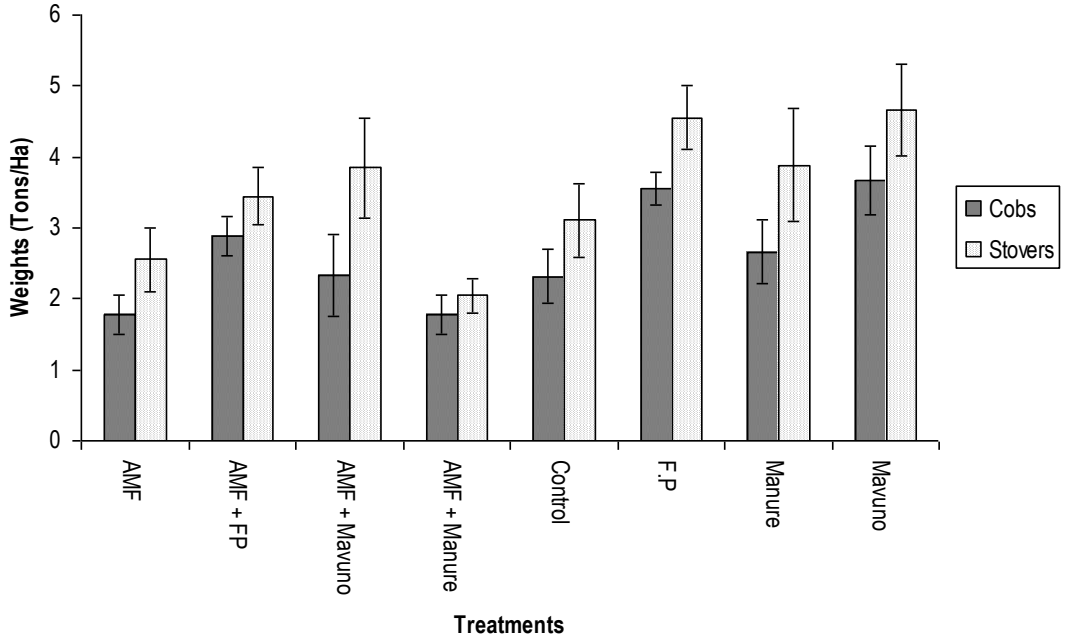


Figure 3.3: Average weight (Tons ha-1) of maize (*Zea mays*) stover and cobs in plots treated with AMF, control, FP, FP + AMF, manure, manure + AMF, mavuno and mavuno + AMF at Taita district, during the 2008 short rain season (October to February).

3.4.3 Rate of bean flowering during the long rain season, April to July 2008

The rate of flowering at on station experiment at Taita was recorded from onset and consecutively after every 3 days. The initial data collection was recorded 34 DAP (Days After Planting). Plots treated with the following, AMF only, AMF plus FP, AMF plus mavuno, FP alone, AMF plus manure also registered early flowering with optimum flowering rate starting at 37 DAP. Plots under mavuno had the highest rate of flowering within 34 to 37 DAP unlike the other treatments which had optimum flowering rate after the 43rd day (Fig 3.4) AMF plus FP treatments showed consistent high level of pods from 34 to 40 DAP. After 40 DAP in plots treated with AMF plus mavuno

treatment had consistent highest number of pods from 40 DAP to 55 DAP. Plots treated with mavuno recorded the lowest number of pods throughout the sampling dates.

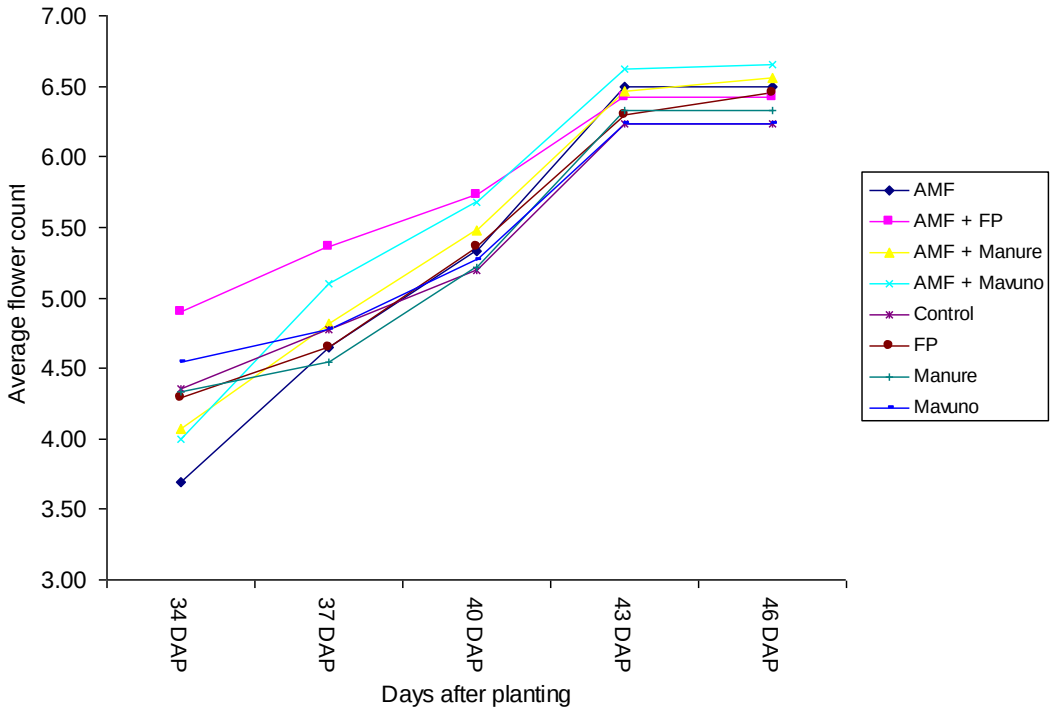


Figure 3.4: Rate of bean (*Phaseolus vulgaris*) flowering in plots treated with AMF, control, FP, FP + AMF, manure, manure + AMF, mavuno and mavuno + AMF at Taita district, April 2008 to July 2008 (long rain season).

3.4.4 Rate of bean flowering during the short rain season (October 2008 to February 2009)

3.3.4.1 Taita experimental site

In the short rain season at Taita, the rate of flowering was optimal between the 1st and 3rd count i.e. 34 to 40 days after planting (Fig 3.5) .Plots under FP treatment had the highest number of flowers recorded as well as a consistent value from 34 to 38 DAP, on the other hand plots under control treatment had the lowest flower count as well as a consistent value as well from 34 to 43 DAP. Plots under AMF plus FP treatment

recorded higher number of flowers compared to plots treated with AMF alone as from 34 to 40 DAP. The count was however lower compared to plots treated with FP alone.

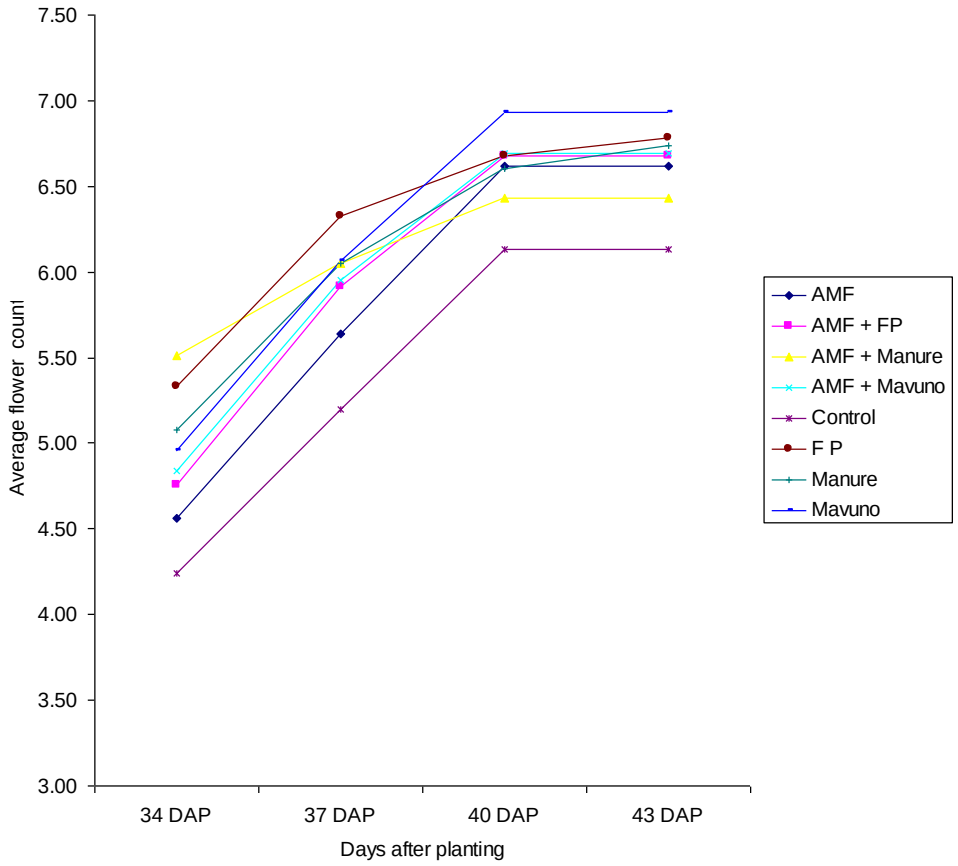


Figure 3.5: Rate of bean (*Phaseolus vulgaris)* flowering in plots treated with AMF, control, FP, FP + AMF, manure, manure + AMF, mavuno and mavuno + AMF at Taita district, during the 2008/2009 short rain (October to February).

3.4.4.2 Embu experimental site

At Embu on-station experiment, plots under FP and mavuno treatment had the highest rate of flowering recorded. The optimum rate of flowering from all treatment was recorded from 20 to 29 days after planting (Fig 3.6). Plots under mavuno treatment had the highest rate of flowering recorded from 29 DAP to 50 DAP. The continuously lowest number of flowers were recorded from the plots under AMF treatment from 23

DAP to 50 DAP. At 20 DAP control treatment had the highest flower count and had the lowest at 50 DAP.

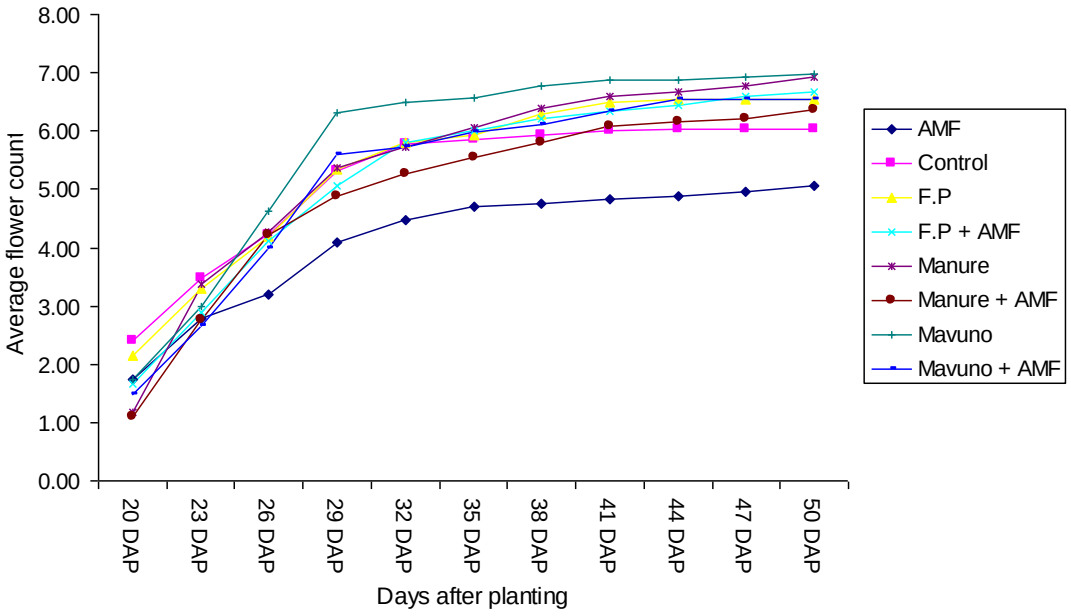


Figure 3.6: Rate of bean (*Phaseolus vulgaris*) flowering in plots treated with AMF, control, FP, FP + AMF, manure, manure + AMF, mavuno and mavuno + AMF at Embu district during the 2008/ 2009 in the short rain season (October to February).

3.4.5 Rate of bean podding during the long rain season, April to July 2008

3.4.5.1 Rate of bean podding at Taita experimental site

The rate of podding of beans at Taita benchmark site was not significant among the different soil fertility amendments at time 1 (50 days after planting: DAP) with application of various treatments. However, at Time 2 (60 DAP) there was significant difference ($P \leq 0.05$) in the rate of podding and the highest average count was recorded in plots under treatment with manure with an average of 36.6 pods count and the lowest

was from plots under treatment with AMF plus FP with a value of 25.2 (Fig 3.7). Treatment under AMF plus mavuno had a consistently high level of podding 56 DAP.

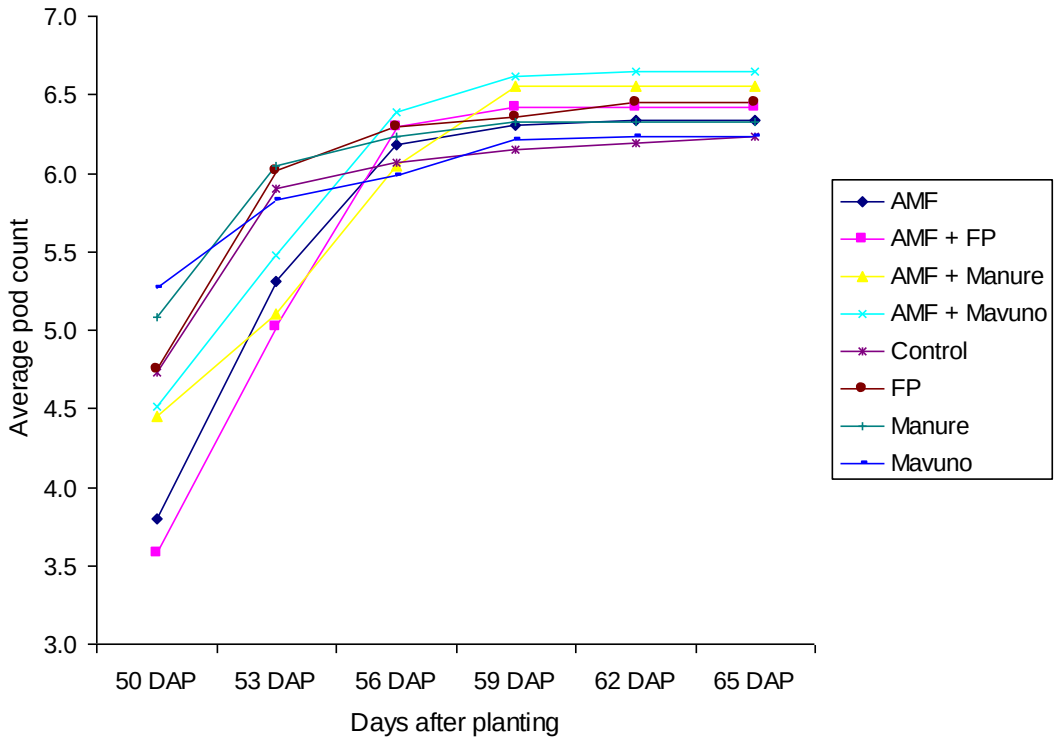


Figure 3.7: Rate of bean (*Phaseolus vulgaris*) pod formation in plots treated with AMF, control, FP, FP + AMF, manure, manure + AMF, mavuno and mavuno + AMF at Taita district, during the 2008 long rain season (April to July).

3.4.5.2 Rate of bean podding at Embu experimental site

At Embu, the rate of bean podding was significantly different among the various treatments at the first count (35 DAP). The highest count was recorded from the plots under FP with an average value of 27 pods per plot. Plots under control treatment had the lowest value of 20 pods per plot. Plots treated with AMF had an average value of 24.8 pods per plot (Fig 3.8). Treatment of manure and FP had a consistently high rate of podding at all sampling dates. There was also no significant increase in the number of

pods as from 35 to 44 DAP. The podding seem to have occurred earlier before initial sampling (35 DAP)

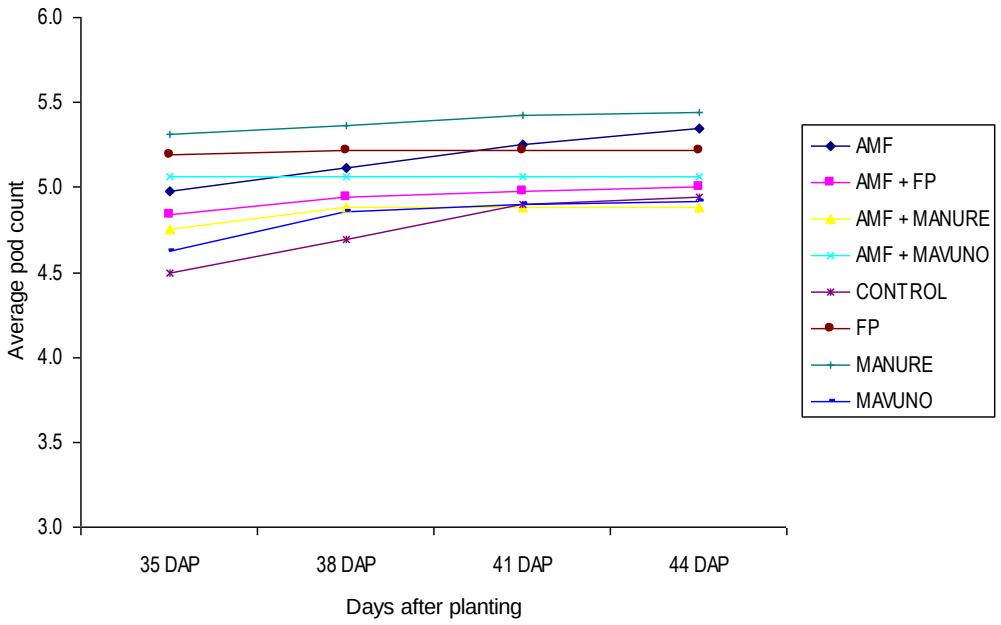


Figure 3.8: Rate of bean (*Phaseolus vulgaris*) pod formation in plots treated with AMF, control, FP, FP + AMF, manure, manure + AMF, mavuno and mavuno + AMF at Embu district during the 2008 long rain season (April to July).

3.4.6 Rate of bean podding during the short rain season (October 2008 to February 2009)

3.4.6.1 Rate of bean podding in Taita experimental site

At Taita, the rate of podding was not significant among the various soil amendments treatments within the first and the second count i.e. 34 and 37 DAP respectively. However, there was significance difference ($P \leq 0.05$) on the rate of podding for the various treatments in the third and fourth count i.e. 40 and 43 DAP respectively. The level of significance reduced with subsequent count i.e. count fifth and sixth. It however increased in the last two counts; count 7 and 8 i.e. 52 and 55 DAP respectively. The rate

of podding was minimal within 34 and 37 DAP (Fig 3.9). It is however optimum between count 2 and count 3 (37-40 DAP) for all the treatment except control treatment which had a rather late podding with optimum rate within the 3rd and 4th count.

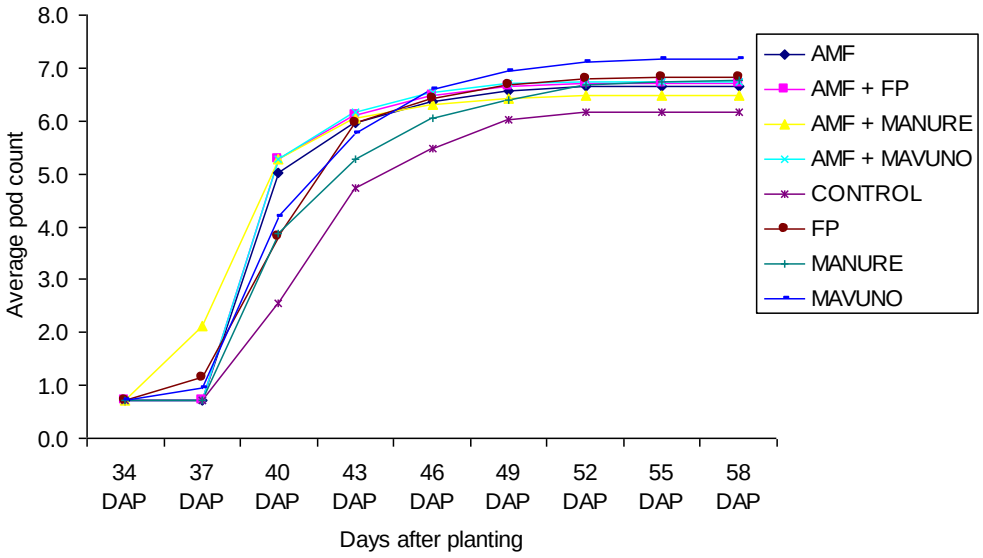


Figure 3.9: Rate of bean (*Phaseolus vulgaris*) pod formation in plots treated with AMF, control, FP, FP + AMF, manure, Manure + AMF, mavuno and mavuno + AMF at Taita district, during the 2008/2009 short rain season (October to February).

3.4.6.2 Rate of bean podding in Embu experimental site

At Embu benchmark site the rate of podding was significantly different ($P \leq 0.05$) among the different soil fertility amendments within the first five counts i.e. 26 to 38 DAP, with application of various soil fertility amendment practices. There was rapid increase in the number of bean plants podding within the first five counts. The plots under application of AMF alone recorded the highest number of average count. The optimum rate of podding was recorded from 25 to 36 days after planting (Fig 3.10). Plots under AMF applied alone had the highest rate of podding recorded while that

treated with AMF combined with either manure or FP had the lowest rate at all counting days.

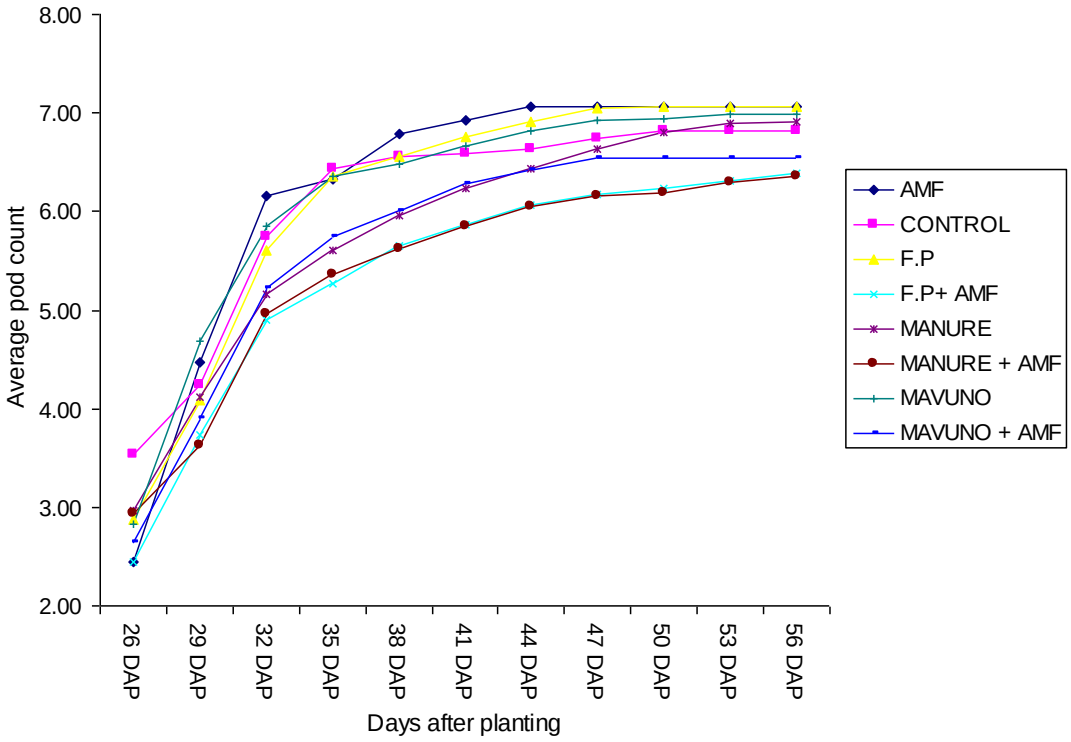


Figure 3.10: Rate of bean (*Phaseolus vulgaris*) pod formation in plots treated with AMF, control, FP, FP+AMF, Manure, manure + AMF, mavuno and mavuno + AMF at Embu districts, during the 2008/ 2009 short rain season (October to February).

3.4.7 Bean growth and harvest in long rain season, April to July 2008

Bean growth parameters and harvest data was also recorded from on-station experiments in Taita and Embu site. In Taita, the average weight of beans was highest on treatment under AMF plus manure with a value of 0.68 tons ha^{-1} and lowest on control treatment with a value of 0.30 tons ha^{-1}. The weight of litter was highest with the plots under FP treatment with an average of 0.86 tons ha^{-1}. The lowest average

weight of litter was recorded on plots under control with a value of 0.22 tons ha^{-1}. Use of AMF in combination of either mavuno or FP fertilizers recorded lower bean harvest compared to fertilizers applied singly. However, use of manure combined with AMF had higher bean yield compared to treatment under manure applied alone. (Fig 3.11) The highest bean litter weight was recorded from plots under the inorganic fertilizer (TSP + CAN) that is FP and lowest from plots under control treatment.

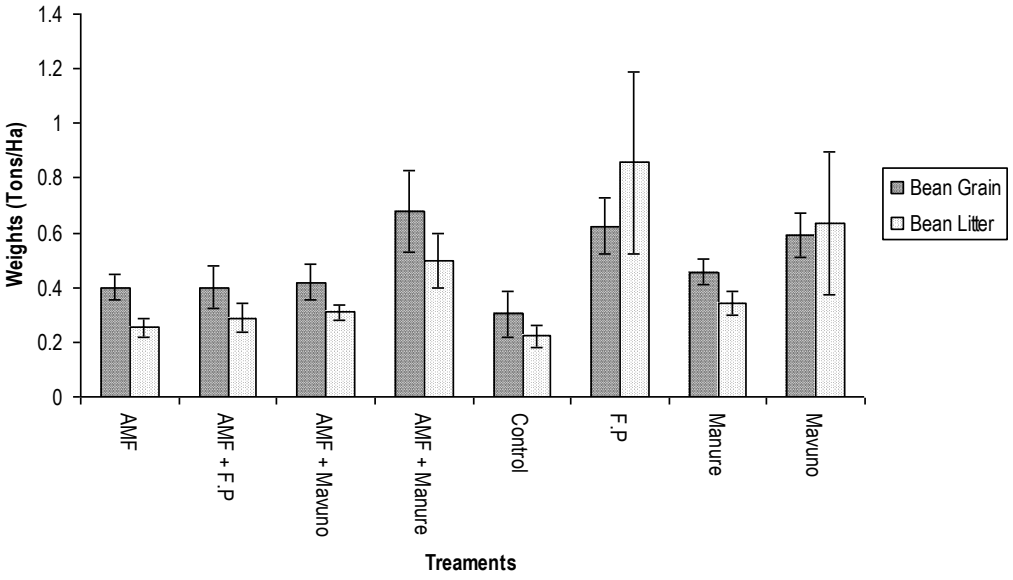


Figure 3.11: Average weights (Tons ha-1) of bean (*Phaseolus vulgaris*) grain and litter in plots treated with AMF, control, FP, FP + AMF, manure, manure + AMF, mavuno and mavuno + AMF at Taita during the 2008 long rain season, (April to July).

3.4.8 Bean growth and harvest in 2008/ 2009 short rain season (October to February)

3.3.8.1 Embu experimental site

The highest grain weight from on-station experiment at Embu in the second season was recorded from plots under mavuno plus AMF with average of 0.21 tons ha^{-1}. Plots under mavuno fertilizer had the highest weight of bean litter recorded with an average of 0.19 tons ha^{-1}. The lowest grain measurement was obtained from plots under AMF applied singly with an average of 0.06 tons ha^{-1}. The average weight of bean litter varied significantly ($P \leq 0.05$) with application among the soil amendment practices unlike the average weight of bean grain. The use of fertilizer in combination with AMF resulted to lower bean yield compared to fertilizers applied singly but higher yield compared to AMF applied singly. This observation was differed from use of manure combined with AMF which had higher yield compared to manure or AMF applied alone (Table 3.1).

Table 3.1: Average weight of bean (*Phaseolus vulgaris*) grain and litter in plots treated with different soil amendments at Embu district, during the short rain season 2008/2009.

	Weight (tons ha^{-1})	
Treatments	Bean grain	Bean litter
AMF	0.056	0.028
Control	0.084	0.04
F.P	0.184	0.124
F.P + AMF	0.106	0.118
Manure	0.09	0.14
Manure + AMF	0.092	0.184
Mavuno	0.19	0.196
Mavuno + AMF	0.168	0.212
F-probability	*2.29*	*1.34*
P-value	*0.052*	*0.26*
Grand mean	*0.10*	*0.15*
s.e	*0.015*	*0.018*

3.4.8.2 Taita experimental site

At Taita, the weight of bean grains varied significantly ($P \leq 0.05$) among the different soil fertility amendments. There was no variation recorded among the weights of dry bean litter. The lowest average weight of beans was recorded from plots treated as control with a value of 0.198 tons ha^{-1}. The highest average was recorded on plots under F.P treatment with a value of 0.64 tons ha^{-1}. Mavuno treatment had the second highest value of 0.55 tons ha^{-1} and in plots treated with AMF plus FP had the third highest value of 0.50 tons ha^{-1}. Plots under mavuno also had the highest weight of bean litter recorded with an average of 0.58 tons ha^{-1} and the lowest value was recorded from the plots under control treatment with a value of 0.21 tons ha^{-1}. The scenario observed in earlier experiments was repeated again whereby use of AMF combined with fertilizer had lower yields compared to treatments with fertilizer applied alone. But the reverse was true for the use of manure where a combination of manure plus AMF had higher yield compared to manure alone (Fig 3.12).

Table 3.2: Average weight (Tons ha-1) of bean (*Phaseolus vulgaris*) grain and litter in plots treated with AMF, control, FP, FP + AMF, manure, manure + AMF, mavuno and mavuno + AMF at Taita district, during the 2008/ 2009 short rain season (October to February).

	Weights (Tons/Ha)	
Treatments	Bean Grain	Bean Litter
AMF	0.47	0.31
AMF + F.P	0.50	0.30
AMF + Mavuno	0.37	0.36
AMF + Manure	0.41	0.26
Control	0.20	0.21
F.P	0.64	0.46
Manure	0.34	0.27
Mavuno	0.55	0.58
F-probability	*3.55*	*1.16*
P-value	*0.01*	*0.35*

Grand mean	*0.43*	*0.34*
s.e	*0.03*	*0.04*

3.5 Discussion

In the on-station experiments, use of fertilizers (FP and mavuno) was shown to tremendously increase crop yield for maize and beans as well as the rate of podding and flowering of bean crop in both short and long rainy seasons at both sites. This is with comparison to use of farmyard manure or use of AMF. This was the case for both experimental sites.

Inoculation of maize crops with AMF combined with FP had higher maize yield compared to FP applied alone. This was however reversed with use of manure and mavuno combined with AMF with yields higher compared to AMF applied alone indicating that use of AMF in combination of inorganic fertilizers is necessary so as to boost AMF activities in the soil. The weight of all stovers (grain plus cobs) was not consistent in the two seasons with the long rainy season showing higher stover compared to the short rainy season. This may be attributed to changes in moisture a major factor that determines growth. Arbuscular mycorrhizal fungi are known to alleviate water stress (Auge` *et al*., 1994). Inoculation with AMF gave positive results with the rate of podding higher compared to control treatment in the short rain season. This was consistent in the two benchmark sites as well as the two seasons. Bean harvest was higher for both seasons at Taita with AMF inoculation compared to control treatment. This is with agreement to earlier work by Mohammad *et al*. (2004), who noted positive effect of AMF inoculation on growth of barley.

Maize harvest data was highest under mavuno treatment across the two experimental sites as well as within the two cropping seasons. This can be concluded to be the best soil fertility amendment practice for maize crop growing within the two experimental sites.

The rate of bean flowering was favoured by application of FP, AMF alone, AMF plus mavuno, AMF plus manure and AMF plus FP at Taita experimental site for the two cropping seasons. At Embu, the results were different whereby the highest rate of bean flowering in season one was recorded from in plots treated with control and mavuno.

The rate of bean podding was favoured by application of mavuno, FP fertilizer and AMF plus mavuno at Taita experimental site in the first season while in the second season plots under AMF plus manure, AMF plus mavuno and mavuno applied singly showed the highest rate of podding.

The weight of bean litter was highest from the plots under FP at Taita in the first season while treatment under mavuno had the second highest value. In the second season mavuno treatment recorded higher value of litter weight compared to FP treatment. At Embu in the second season, plots under mavuno plus AMF had the highest value followed by treatment under application of mavuno singly.

For the grain weight at Taita experimental site in the first season, plots under AMF plus manure recorded the highest value while in the following season the highest value was recorded from plots under FP treatment. At Embu site, the highest value was obtained from plots under mavuno treatment in the second season.

3.6 Conclusion

Field inoculation with AMF has been demonstrated to positively affect the yield of maize and bean though not significantly different among the soil amendment practices and can constitute an environmentally friendly method of soil fertility amendment over time. The inoculum can easily be made by farmers upon training. However, it cannot be used as substitute to use of inorganic or inorganic fertilizers.

Use of AMF inoculum plus either mavuno or FP (A combination of TSP and CAN fertilizers) led to lower maize and bean yields in terms of total biomass of the crops at harvest compared to individual fertilizers. Though not so exquisite pattern, the negative effect was recorded for both maize and beans in the two cropping seasons and from both experimental sites except from Taita benchmark site in the first cropping season and Embu in the second cropping season. Combination of AMF and FP had higher weight of maize stovers compared to treatment under FP only. Also at Embu, in the second season the use of mavuno plus AMF had higher maize yield compared to treatment under mavuno used singly.

In most cases from the both experimental sites and from both seasons, the use of AMF inoculum lead to relatively increased maize and bean growth rate as well as crop harvest compared to control treatment and hence a clear indication that utilization of indigenous AMF species can positively impact on crop growth and to increase efficiency inoculation with indigenous species can be incorporated.

CHAPTER 4: EFFECT OF SOIL FERTILITY MANAGEMENT PRACTICES ON ARBUSCULAR MYCORRHIZAL FUNGI (AMF) ABUNDANCE AND COLONISATION OF MAIZE (*Zea mays)* AND BEAN (*Phaseolus vulgaris*) IN EMBU AND TAITA DISTRICTS, KENYA

4.1 Abstract

The study demonstrate the effect of different soil amendment practices such as use of different fertilizers, use of manure, soil nutrients enhancing organisms (such as *Bacillus* and *Trichoderma*) and slow realising rock phosphate (Mijingu) on the occurrence, abundance of AMF and the subsequent colonisation of maize *(Zea mays* L*)* and common bean *(Phaseoli vulgaris* L) under field experiments. Soil from BGDB test strips and demonstration blocks under Farmer Practice (FP) (combination of TSP and CAN), Mijingu, Manure and Mavuno (organic fertilizer) application were sampled. Mycorrhizal density and prevalence was determined over a period of two planting seasons and the experiments were replicated in two benchmark sites namely Embu in the highlands of central Kenya and the coastal highlands in Taita-Taveta and constitute the on- farm experiments of the project. On- station experiments were also set up and direct inoculation of plots with AMF was done on common bean and maize intercrop. Maize and bean roots samples were obtained and AMF colonisation assessed. A total of 15 morphotypes were isolated and described from both Taita and Embu site, majority being Gigasporaceae (9), followed by Acaulosporaceae (4) and Glomaceae (2). The highest species count was obtained from 0-10cm depth. Inoculation of plots with AMF was found to increase the total AMF spore abundance in the soil. However there was no significant difference in spore abundance among the different soil fertility amendments

at on-station experiments with use of different soil fertility amendment practices in the first season but varied significantly ($p \leq 0.05$) after the second season. A marked reduction in AMF population was recorded with passage of each cropping season. On-farm experiments also recorded reduction in AMF population with subsequent season but the spore abundance had no significant difference among the different soil fertility amendments. At demonstration plots, there was significant ($p \leq 0.05$) difference among the different soil fertility amendment practices with a marked decrease in AMF population with subsequent cropping season.

The use of different soil fertility amendment practices affect the prevalence and abundance of AMF spores in the soil which in turn affects the intensity of host crop colonisation.

4.2 Introduction

Crop plants form symbiotic association with mycorrhizae fungi and it is widespread in a natural ecosystem. In Agriculture, it is the arbuscular mycorrhizal fungi (AMF) of phylum Glomeromycota (Schussler *et al*., 2001), that are most important. AMF forms association with over 80% of all crop plants.

The host benefits from the association in a number of ways and it's mainly through increased uptake of relatively immobile phosphate ions. AMF produce extraradical mycelium forming an extensive network within the soil. The mycelium grows beyond the phosphate depletion zone that usually develops around the root (Smith and Read, 1997).

Other benefits of AMF to the host plant include: improved drought resistance (Auge` *et al*., 1994), increased tolerance to toxicity caused by heavy metals as well as tolerance from extreme salinity (Feng *et al*., 2002), increased resistance to foliar feeding insects (Gange and West, 1994; Mohammad *et al*., 2003) Other than phosphorus, AMF also help in uptake of other macronutrients such as nitrogen (N), potassium (K) and magnesium (Mg) (Hodge *et al*., 2001) and also micronutrients (Azaizeh *et al*., 1995)

A glycoprotein produced by AMF that promotes soil aggregation, "glomalin," has been discovered. Furthermore, higher than normal carbon dioxide concentrations help to promote soil aggregation by increasing the production of glomalin (Riling *et al*., 1999).

AM fungi are recognized as high potential agents in plant protection and pest management (Quarles, 1999). In several cases direct biocontrol potential has been

demonstrated, especially for plant diseases caused by *Phytophtora, Rhizoctonia*, and *Fusarium* pathogens (Abdel-Aziz *et al*., 1997; Vigo *et al*., 2000). However many of these experiments have been conducted in the greenhouse which does not explain AMF relevance to the field situation, although, a number of field experiments showed a positive results such as increased nutrient uptake and yield or reduced disease severity (Mohammad *et al*., 2004; Douds *et al.*, 2005)

Several factors have been shown to affect AMF spore abundance and species diversity; this is directly through damaging or killing AMF and indirectly, by creating conditions either favourable or favourable to them. In general, agricultural practices have a negative impact on the AM association and agricultural soils are AMF impoverished, particularly in the number of species (Helgason *et al*., 1998; Menendez *et al*., 2001). This include, use of excess phosphorus fertilizer which may lead to reduced AM colonisation of roots and AMF spore density in soil (Kahiluoto *et al.*, 2001).Use of other readily soluble fertilizers, particularly, N fertilizers has also been reported to have a negative impact on AM colonization and diversity in some cases (Treseder and Allen, 2002) but not in others (Jumpponen *et al*., 2005). Farm yard manure and slow release mineral fertilizers such as rock phosphate do not seem to suppress AMF and may even stimulate them (Joner, 2000; Alloush and Clark, 2001). Soil tillage causes severe disruption to the common mycorrhizal network resulting in delayed or reduced root colonization and a reduction in the volume of the soil that is exploited by the AMF leading to reduced plant nutrient uptake, consequently crop growth and yield though not always (Evans and Miller, 1990; Kabir *et al*., 1998)

Agriculture production also lead to low diversity of AMF compared to a natural ecosystem and tends to propagate *Glomus* species due to low diversity of hosts which is severe in case of monoculture (Oehl *et al.,* 2003). Previous research on AMF abundance in Embu revealed that least spore abundance is found in land under crop food production compared to cash crop and undisturbed forests (Jefwa *et al*., 2009).

4.3 Materials and Methods

The experiments were carried out at on-station and on farm. From on-station experiment, plots under various soil fertility amendment practices (Appendix 1 and 2) were sampled. Sampling was also done from on-farm experiment under different soil amendment practices (Appendix 3 and 4). The demonstration blocks were set up to evaluate and show the best farming practices which farmers can adopt (Appendix 5). AMF abundance and diversity was evaluated from the demonstration blocks.

AMF inoculum was obtained from an existing culture of AMF isolate that was effective on the growth of beans. Arbuscular mycorrhizal fungal comprising of infective propagules: spores were bulked up on leeks.

4.3.1 Soil Sampling Method

Sequential soil sampling at planting and at maturity of maize crop was carried out and soil samples used to determine AMF population. The change in AMF population was monitored for two cropping seasons. Soil collection under different soil fertility amendment practises (manure, control, AMF + mavuno, mavuno, AMF + manure, FP and AMF + FP) helped in determining their effect on AMF population in the soil.

Sampling of both the experimental plots and the on-station areas was done by sub-dividing plots into 3x3 m sampling areas which formed a grid from which random sampling of the soils was done. In all the sampling points, soil samples were collected using a soil corer 5 cm diameter and 5 cm deep. From each sampling point 5 sub-samples were taken and mixed to form a composite sample from each plot and collection was done in two depths (0-10 cm and 10-20cm). Each soil sample was placed in bag and labelled accordingly.

Soils were transported to the National Museums of Kenya, Nairobi where AMF spores isolation and root processing and evaluation was carried out. The data obtained assisted in determination of abundance and diversity of AMF across the two benchmark sites.

4.3.2 Separating mycorrhizal spores from soil

Spores were extracted from the soil by sucrose centrifugation method (Jenkins, 1964), modified by using 710 µm and 45 µm mesh sieves (Walker *et al*., 1982) and sucrose concentration of 50% w/v. Spores were examined using a stereomicroscope (40x), with an addition of a transmitted light to aid in morphological character recognition in addition to colour determination. Spore data was obtained by counting spores in soil samples.

Selected spores were used to make semi-permanent microscope slides using polyvinyl alcohol-lacto-glycerol (PVLG) and Melzer's reagent (5:1 v/v) (Koske and Tessier 1983; Walker 1983; Morton 1988). Spores on slides were squashed to reveal inner-wall layers,

for identification purposes. Morphological characters were used to separate the spores into different morphotypes.

4.3.3 AMF colonisation assessment

Five (5) plants per plot were randomly selected and roots obtained for AMF assessment. The roots were stored in plastic bottles in 70% v/v ethanol preservation until assessment was done. Assessment was carried out at the National Museums of Kenya.

The roots were washed and rinsed in several changes of tap water until all soil particles were removed, 10% KOH was then added and left at 90^{o}C for one hour in the oven or autoclave at 120^{o}C for 15 minutes. Alkaline hydrogen peroxide was added to bleach the roots as they were highly pigmented. The roots were then acidified by adding 1% HCL for 5 minutes. The HCL was decanted and 0.05% trypan blue in lacto glycerol added and allowed to simmer for 10 minutes and later decanted. Root pieces were stored in glycerol acidified with a few drops of HCL until they are examined for AMF colonisation. The roots were assessed for mycorrhizae by placing root pieces on a glass slide followed by application of few drops of glycerol and covering by a cover slip.

4.3.4 Analysis of AMF colonization

Slides were examined under the compound microscope and the frequency and intensity of AMF colonization (arbuscles, coils, vesicles, internal and external hyphae) was recorded for each slide. Care was taken to distinguish artefacts from AMF structures. The frequency of AMF was recorded as the number of root fragments infected with AMF expressed in percentage. The intensity of

AMF colonization was recorded as the percentage cover of AMF colonization in each root fragment as described in McGonigle *et al*. (1990). The percentages obtained from the quantification of intensity were categorized into classes (1-20, 21-40, 41-60, 61-80 and 81-100). The number of roots in each category was multiplied by median number in each class (10v + 30w + 50x + 70y + 90z) where v, w, x, y, z are the number of roots in each class. The AMF intensity was expressed as (10v + 30w + 50x + 70y + 90z)/ (v + w + x + y + z).

4.3.5 Data analysis

The spore count data was all collected and stored in excel spreadsheets and square root transformation carried out. Species frequency and ANOVA from the different treatments were computed using SPSS statistical package. Non-linear regression model, the species accumulation curves, was used to estimate species richness and rank abundance of species. The proportion of the soil fertility amendment type with most abundant AMF were computed by Rènyi diversity profiles, Hα (H-alpha).

Extrapolating technique (species accumulation) curve was used to estimate the number of species that would be found in a complete survey. Similarity index was used to derive dendrogram that establish similarities between soil fertility amendment type while the redundancy analysis (RDA) for constrained ordination was used to test for relationships with explanatory environmental variables or factors of a multifactorial analysis of variance model (Legendre and Gallagher, 2001).

4.4 Results

4.4.1 Abundance of AMF spore at on-station experiment

The highest spore count was recorded from plots under FP plus AMF and this was in February 2009, (after the second crop harvest). Plots inoculated with AMF recorded no increase in AMF abundance after the first cropping season (October 2008; Long rain season) but there was marked increased after the second season (short rain season). Similar results were obtained from plots under control, manure and FP + AMF treatments.

Plots under mavuno only, mavuno plus AMF and manure plus AMF recorded the highest spore count after the first harvest (long rain season) then declined after second crop harvest (short rain season).

Analysis of variance (ANOVA) showed no significant differences in spore abundance among the different soil fertility amendment practices in the initial spore count and after first cropping season but varied significantly ($P \leq 0.05$) after the second season. Soils collected in plots treated with FP + AMF had the highest spore abundance after the second cropping season, February 2009 (2.61±0.39). The second highest value was obtained fin plots treated with manure with a value of 2.21±0.24. The lowest value was obtained in plots treated with mavuno treatment with a value of 1.69±0.2 and the second lowest value from plots under FP only treatment with a value of 1.73±0.33 (Fig 4.1).

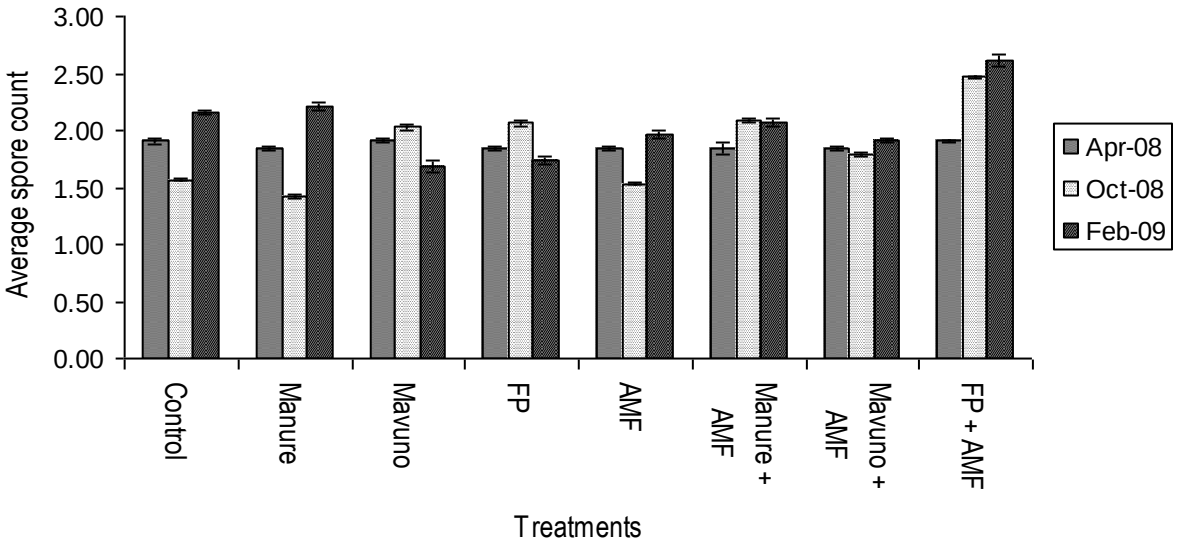


Figure 4.1: Changes in Arbuscular Mycorrhizal Fungi abundance in plots treated with AMF, control, FP, FP + AMF, manure, manure + AMF, mavuno and mavuno + AMF at ATC (on-station experiment), Embu between April 2008 and February 2009.

The plots under FP combined with AMF and manure combined with AMF treatments had continuous increase in spore abundance over the two cropping seasons and thus a build up of AMF in the soil. Plots under control, manure, AMF and mavuno combined with AMF showed reduced spore reduction after the first season and eventual increase as well as general increase in spore abundance after the second season with comparison to initial spore count. Plots under mavuno and FP treatments recorded a reduction in spore count over the cropping season. The reduction in spore count under FP and mavuno treatment compared to the others could signify that these practices do not conserve AMF well. Though high colonization is indicative of good conditions, hence low spores may mean less disturbed conditions that trigger less sporulation. Under extremely low fertility like the control AMF may also not be conserved.

4.4.2 Abundance of AMF spore at on-farm experiments

Analysis of variance (ANOVA) showed no significant differences in spore abundance among the soil fertility amendments in all seasons on the plots under test strips. There was a general decrease in mean spore count for all treatments (Fig 4.2)

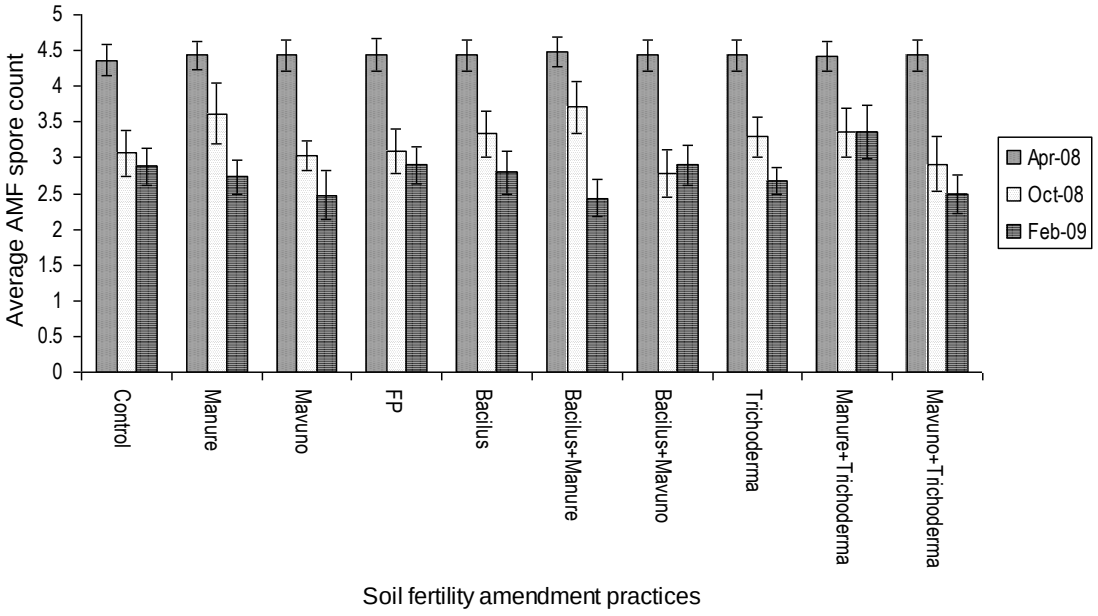


Figure 4.2: Changes in Arbuscular Mycorrhizal Fungi abundance in on-farm test strips experiments treated with various soil fertility amendments (control, manure, mavuno, FP, *Bacillus*, *Trichoderma* and combination of microorganisms with manure or mavuno) at Embu, April 2008 to February 2009.

At the beginning of the season, April, the spore abundance was high for all practices, with seasons, and the abundance was less in plots under *Bacillus* plus manure treatment followed by mavuno plus *Trichoderma*. Thus the recovery of AMF and subsequent conservation is less in the two treatments. Though high colonization is indicative of

good conditions, low spores count in the soil may mean less disturbed conditions that trigger less sporulation.

4.4.3 Abundance of AMF and colonisation intensity

Roots samples from maize crops were assessed for AMF colonisation as described above (Section 4.3.4). There was no significant difference on the severity of AMF colonisation among the different soil fertility amendments. The treatments under AMF + FP had the highest level of AMF intensity with a value of 79.5 as well as the highest average spore count with a value of 49.4 spores per 1000 grammes of soil (Fig 4.3), plots under mavuno only had the second highest value of 79.7 followed by manure treatment with a value of 72.8. The lowest value was recorded from plots under control treatment with a value of 60.0 while the lowest average spore count was recorded from plots under manure with an average value of 28.5 spores per 1000 grammes of soil. Plots under AMF only treatment had a value of 69.9, which was slightly higher than plots under mavuno + AMF treatment with a value of 65.

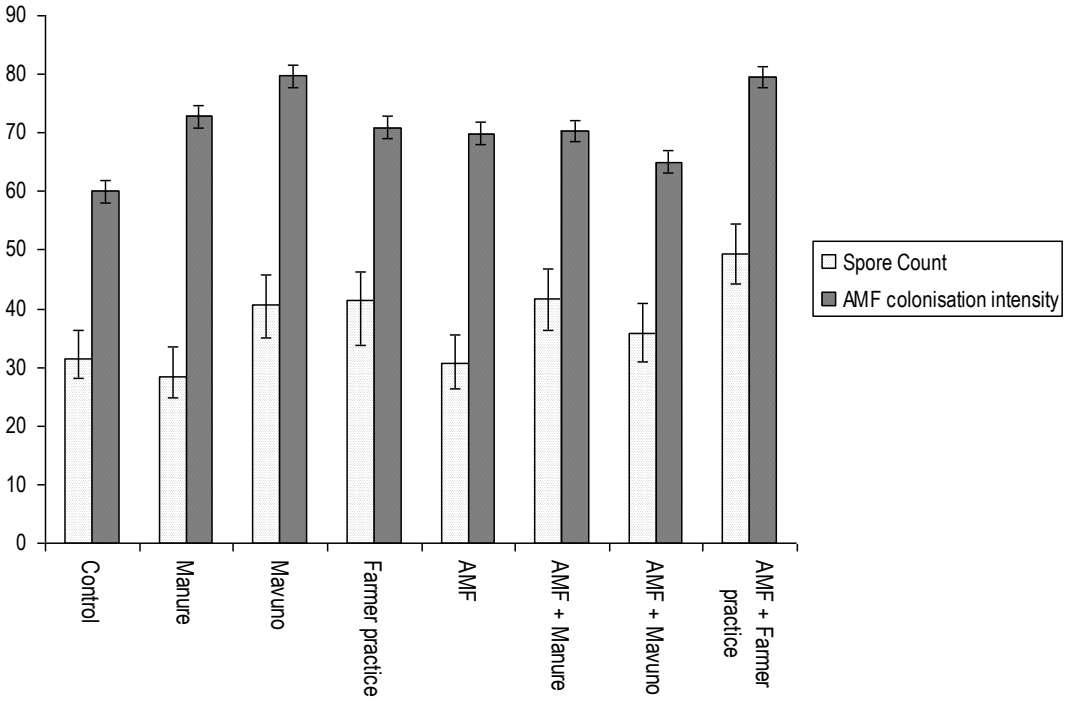


Figure 4.3: Average spore count from 1000g of soil samples collected from on-station experiment at Embu districts in October 2008 and the relative AMF colonisation intensity of the maize roots collected from the maize crop**.**

A positive correlation between the number of spores in the soil and the level of AMF colonisation was found to exist; the number of AMF spores in the soil was directly proportional to the AMF colonisation intensity of maize roots. This is proof for host (maize) response to AMF presence in the soil and subsequent colonisation.

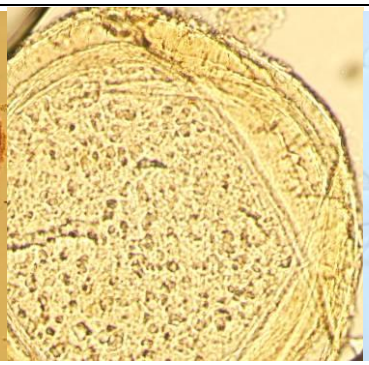
4.4.4 Composition of AMF species

A total of 15 AMF morphotypes were isolated and described from both Taita and Embu site, majority being Gigasporaceae (9), followed by Acaulosporaceae (4) and Glomaceae (2) (Plate 4.1). There were variations in spore proportions of individual species after the first and second cropping seasons. The morphotypes with remarkable decrease with all forms of farm use include *Scutellospora sp a*, *Acaulospora sp1*, *Scutellospora sp b*, *Scutellospora nigra* and *Scutellospora sp e*. Others recorded an increase in species count with application of different soil amendment practices over a period of two cropping seasons include *Acaulospora sp3*, *Scutellospora sp c*, and *Scutellospora pellucida* (Table 4.1). The highest proportion after the first cropping season was that of *Scutellospora sp a* (30.5%) followed by *Acaulospora sp1* with a proportion of 15.9% each. After the second cropping season, *Scutellospora sp a* had the highest proportion as well with a value of 30.2 followed by *Scutellospora sp b* with a value of 11.3 %.

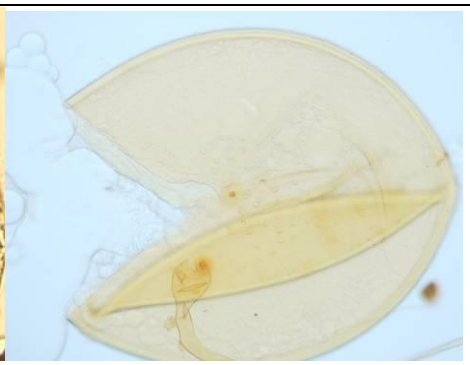
Table 4.1: AMF species rank after the first and second cropping seasons.

Morphotypes	Proportion (Season 1)	Proportion (Season 2)
Scutellospora sp a	30.5	30.2
Acaulospora sp1	15.9	6.0
Scutellospora pellucida	2.8	3.5
Scutellospora sp c	0.0	0.2
Scutellospora nigra	10.1	10.2
Scutellospora sp e	11.8	10.2
Glomus sp1	0.0	0.0
Scutellospora sp b	30.2	25.3
Glomus sporocarpic	0.3	0.2
Scutellospora sp d	0.0	0.0
Acaulospora sp2	0.0	0.0
Acaulospora denticulata	0.0	0.0
Scutellospora sp f	0.1	0.1
Acaulospora sp3	0.6	1.8
Gigaspora sp1	0.0	0.0

Scutellospora sp a



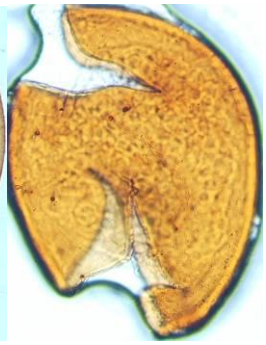
Acaulospora sp1



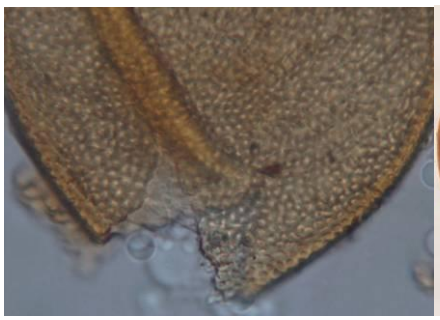
Scutellospora sp b

Scutellospora nigra

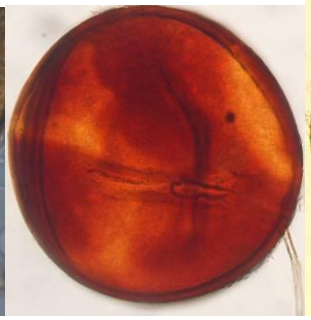
Scutellospora sp c



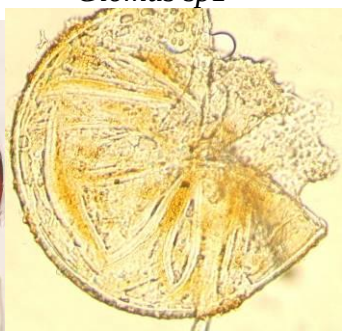
Glomus sp1



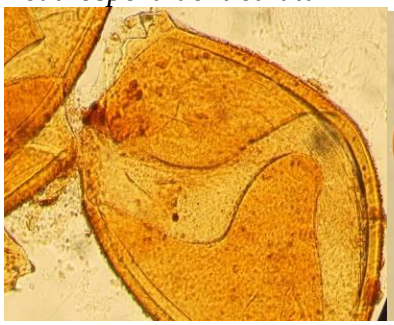
Acaulospora denticulata



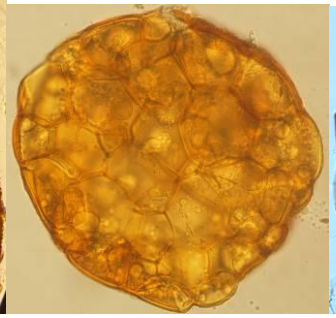
Scutellospora sp d



Acaulospora sp2

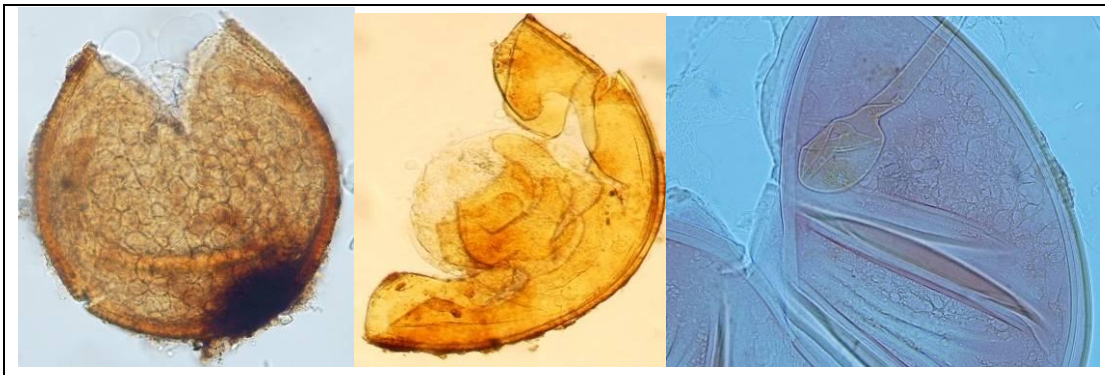


Gigaspora sp1



Glomus sporocarpic

Scutellospora sp f



Acaulospora sp3 *Scutellospora sp e* *Scutellospora pellucida*

Plate 4.1: Pictures of selected morphotypes of AMF species representing the 4 genera isolated from Embu and Taita experimental sites.

4.4.5 AMF species richness

A higher species count was obtained from 0-10 cm depth compared to 10-20 cm below the ground in the first season. Non-linear regression model, the species accumulation curve, showed soil at 10-20 cm depth to reach a plateau with fewer sampling points than 0-10 cm soils suggesting more intensive sampling required at 0-10 cm to record species for the entire area (Fig 4.4). Soils from 10-20 cm depth reached a plateau with 20 species, suggesting 100 points to be adequate for sampling entire species of the area while 200 samples were still not adequate for depth of 0-10 cm to capture the entire species for the area.

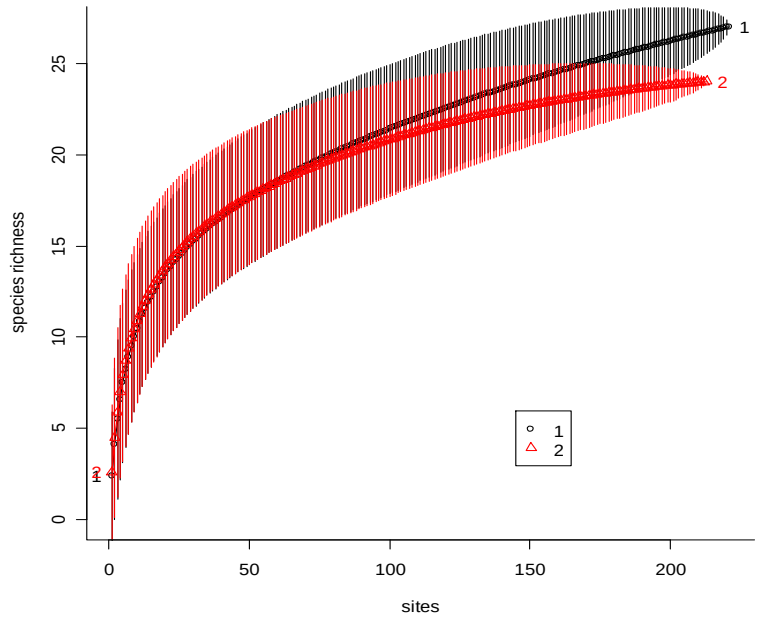


Figure 4.4: Species accumulation curves by depth after the first cropping season (October 2008) (Key: 1 represents 0 to 10cm and 2 represents 10 to 20cm below ground).

Species richness for individual soil fertility amendment practice was also extrapolated (Fig 4.5). Soil samples collected from plots under AMF, mavuno + AMF, mijingu + CAN, FP + *Trichoderma*, FP + AMF and AMF + manure did not plateau suggesting more variations within these treatments and hence the need for intensive sampling.

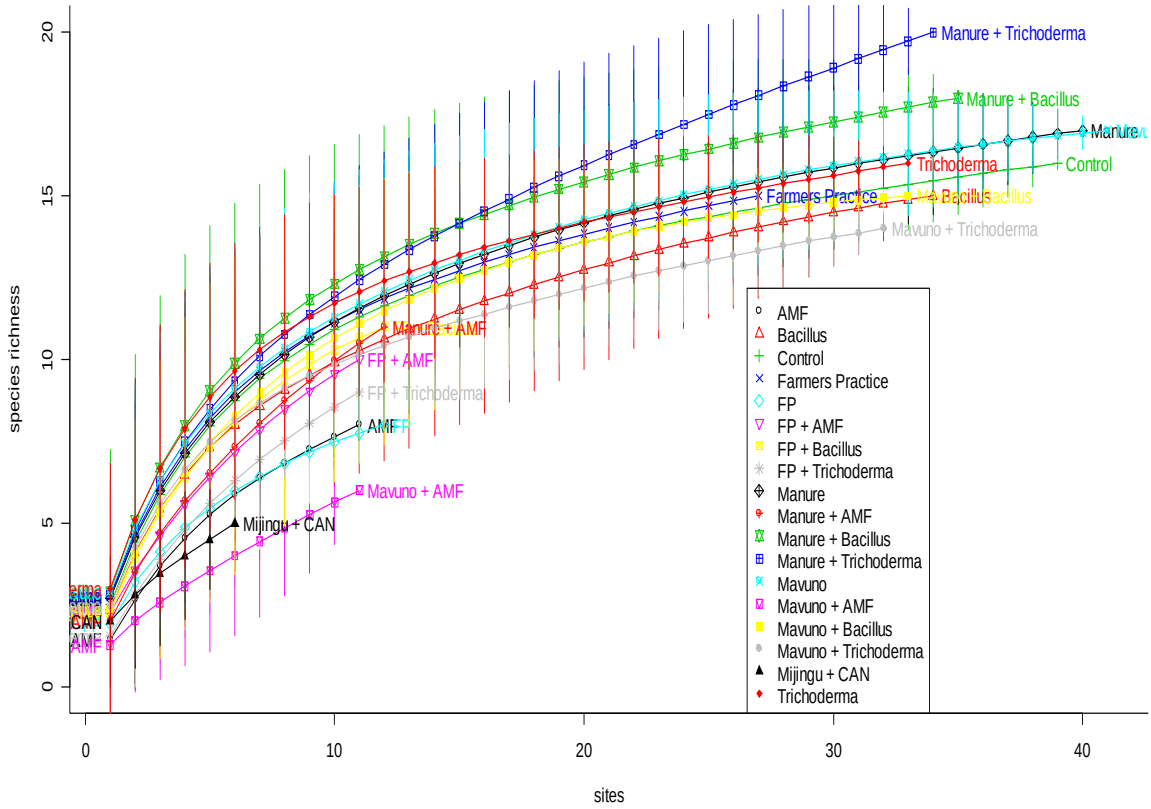


Figure 4.5: Species accumulation curves by soil fertility amendments treatment after first cropping season (October 2008).

For soil samples obtained from treatments such as manure + AMF, FP + *Bacillus*, *Trichoderma*, mavuno and *Trichoderma* + manure levelled with samples collected indicating less variation and thus enough sampling (Fig 4.6). However, others including soil samples from *Trichoderma* + FP, mavuno+ B*acillus*, control, AMF and manure + AMF require more intense sampling.

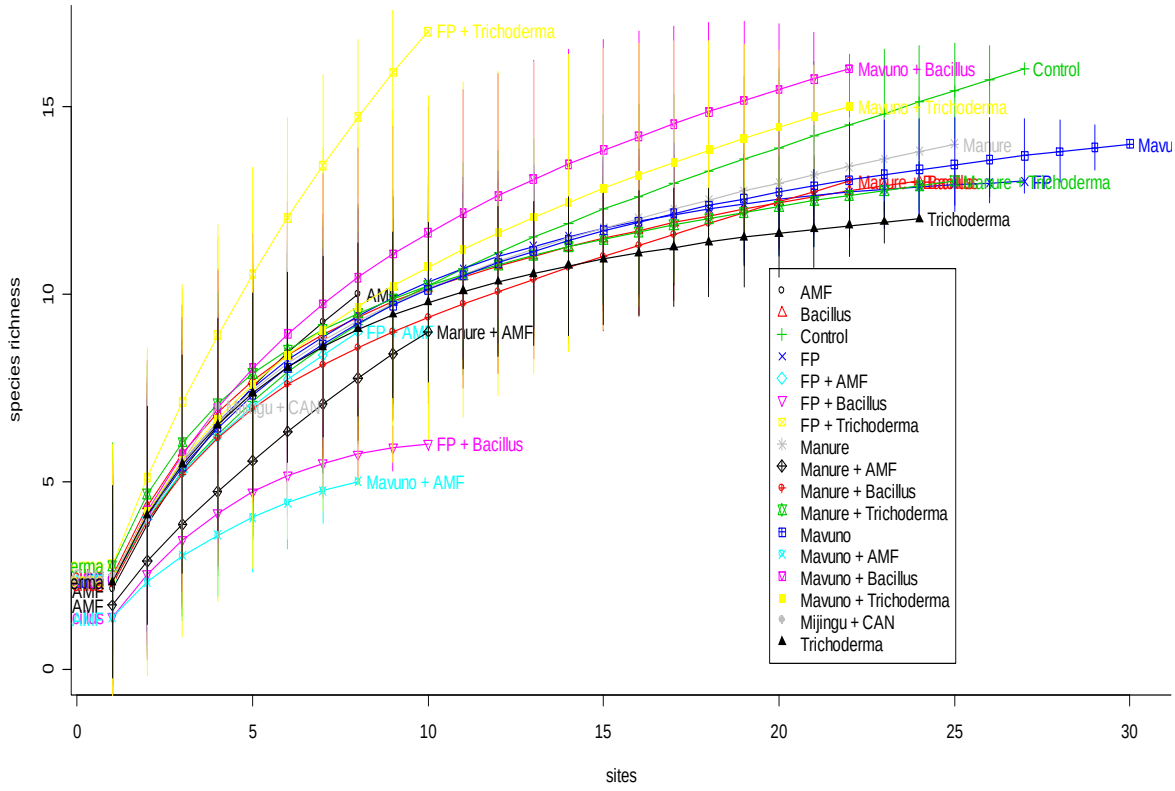


Figure 4.6: Species accumulation curves by soil fertility amendment treatment after second cropping season (February 2009).

4.4.6 AMF species diversity

Species diversity was less variable in soil sample collected after the first cropping season (Fig 4.7) and also after the cropping season two (Fig 4.8) soil samples. This was evident for field soils as shown by the Rènyi profile curve of AMF species for individual soil fertility amendment practices. Rènyi profile curves for both seasons' soils showed slight differences in diversity amongst different soil fertility regimes.

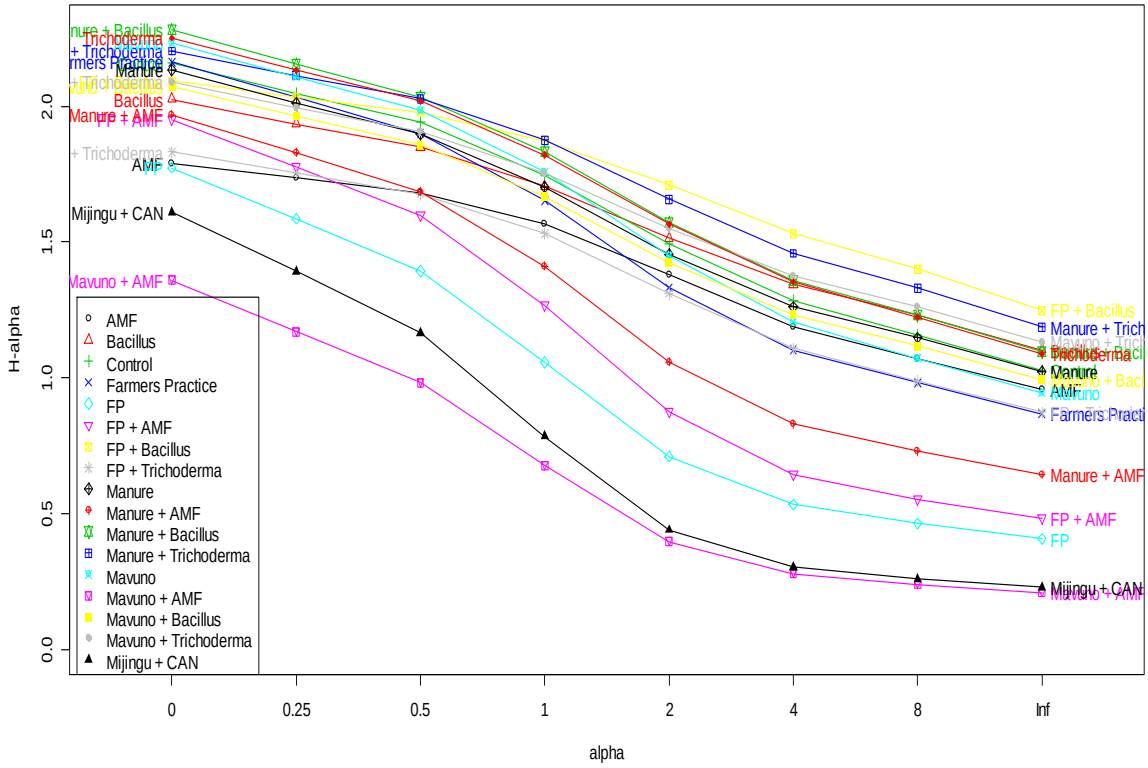


Figure 4.7: Rènyi diversity profile for the differences in AMF species diversity among the different soil fertility amendments after first cropping season (October 2008).

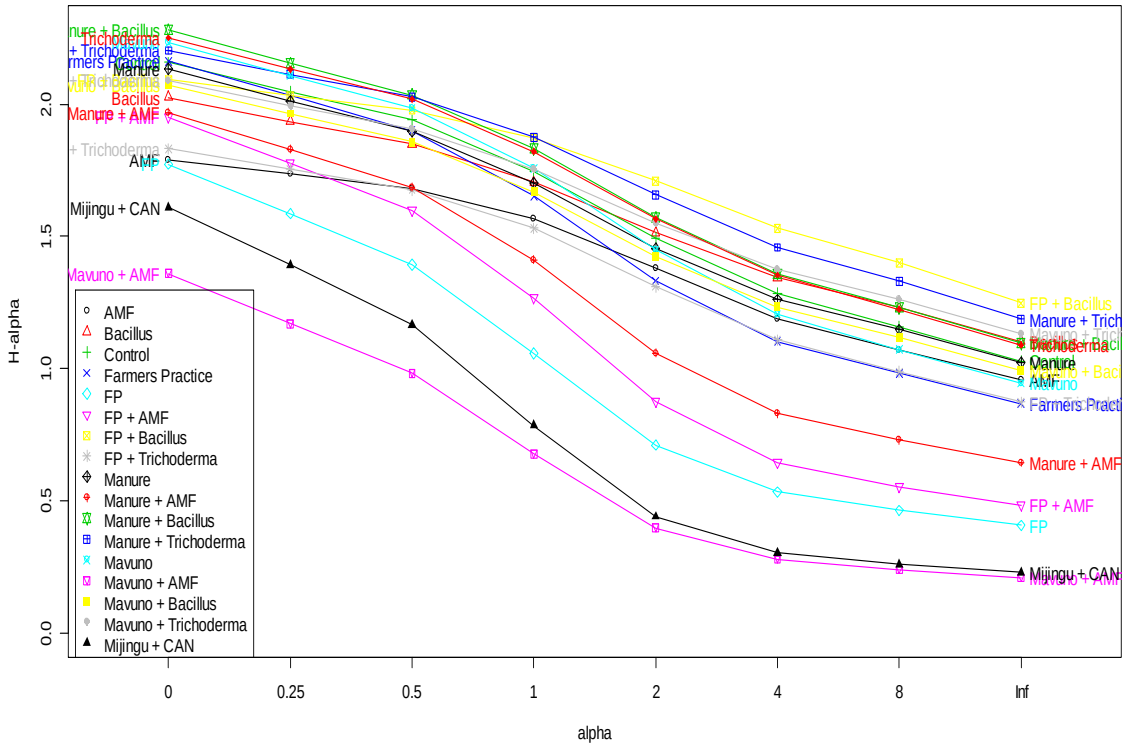


Figure 4.8: Rènyi diversity profile for the differences in AMF species diversity among the different soil fertility amendments after the second cropping season (February 2009).

Species similarities within the different treatments were considered by use of cluster dendrogram (Fig 4.9). Plots under AMF treatment showed close similarity with plots under FP application with regard to AMF species. The control treatment showed close similarity to plots under soil fertility enhancing microorganism such as *Trichoderma* and *Bacillus*. FP and AMF clustered on one side of the dendrogram.

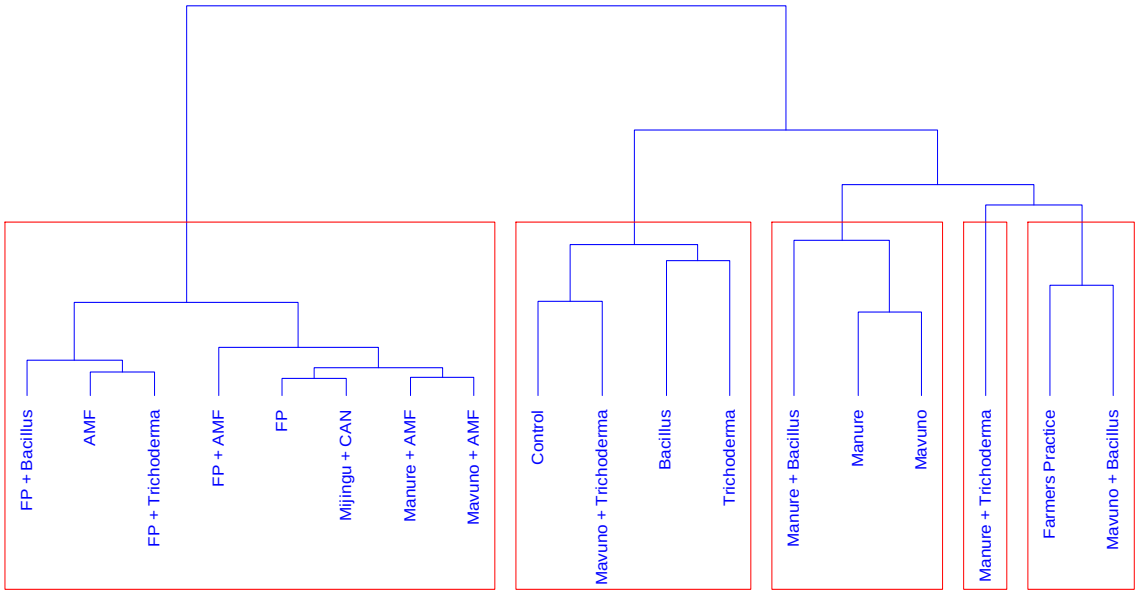


Figure 4.9: Similarities among the different soil fertility amendments in respect to AMF species.

4.5 Discussion

A total of 15 morphotypes were isolated and described from both Taita and Embu site, majority being Gigasporaceae (9), followed by Acaulosporaceae (4) and Glomaceae (2). The numbers of species isolated were low compared to 40 species recorded by Mason *et al*., (1992) and also compared to 17 species isolated from Embu, Kenya by Jefwa *et al*., (2009).

Inoculation of maize and bean with AMF for a period of 2 cropping seasons led to increase in AMF population (abundance) in the soil as well as increase in intensity of root colonisation. Inorganic fertilizers application (FP) and mavuno enhanced AMF abundance within a period of two cropping season. This is in support of earlier

experiments by Joner, (2000); Alloush and Clark, (2001) who observed the use of farmyard manure and slow release mineral fertilizers such as rock phosphate not to suppress AMF and may even stimulate them their multiplication. Fertilizer application such as FP (CAN + TSP) and mavuno reduced AMF abundance except for the combination of AMF and FP. This demonstrates the negative effects of fertilizer application to AMF population in the soil. Use of readily soluble fertilizers was also reported to have a negative effect on AMF colonization and subsequent multiplication in some cases, Treseder and Allen, (2002) but not in others Jumpponen *et al*. (2005).

Arbuscular mycorrhizal fungi and FP (CAN + TSP) enhanced AMF spore abundance in the soil. Inorganic fertilizer has been widely reported to have negative effects on AMF which was not the case in this study Kahiluoto *et al*. (2001). This is in contrast to the experiment whereby he observed reduction of AMF spore density in soil with phosphorus fertilization. The increase in abundance and colonization may be explained by low soil fertility which reached an optimum level for mycorrhizal functioning with application of inorganic fertilizer. There was marked decrease in AMF population with use of FP (TSP + CAN), a contrast to manure, control and AMF treatment. This is in agreement with the experiment carried out by Kahiluoto *et al.* (2001)

Use of different soil fertility managements resulted in significant differences in spore abundance among them. This indicates that use of different soil fertility management practices have an effect on the abundance of AMF. There was a marked decrease in spore abundance with each cropping season. This observation is in agreement with work of Helgason *et al*. (1998) and Menendez *et al*. (2001). Presence of spores in the soil is

indicative of presence of AMF and thus disappearance of spores could therefore mean that the species sporulation is suppressed or the species is eliminated. The first scenario can be confirmed by use of molecular techniques, the second scenario simply means, when the species spores appear, there is some indication of stressful conditions that trigger sporulation. Hence presence of high spore abundance may indicative of some degree of conditions that are not suitable for the fungi. It is only through AMF colonisation assessment that the beneficial or detrimental effect of soil fertility amendment can be quantified in relation to AMF activity outside and inside the host plant. In this experiment, there was a positive correlation between AMF colonisation in the host plant and the spore abundance in the soil. Thus presence of spores in the soil is indicative of AMF presence and not presence of stressful conditions in the soil.

Use of different soil fertility management practices resulted in significant ($p \leq 0.05$) difference in species richness. This indicates that use of different soil fertility management practice has effect on the species of AMF present in the soil. Majority of morphotypes isolated from the soil were *Scutellospora* sp. This is in contrast to what was observed by Oehl *et al*. (2003), where agricultural production led to low diversity of AMF compared to a natural ecosystem and tends to propagate *Glomus* species due to low diversity of hosts which is severe in case of monoculture. The highest spore count was from the depth of 0-10 cm compared 10-20 cm below the ground and can be explained by a number of reasons. First, the sampling was done from a maize and bean intercrop land and hence the majority of mycorrhizal activity is to be expected from

such depth and secondly, the fact that most soil disturbance occur in the region and hence may trigger a lot of AMF sporulation.

4.6 Conclusion

Artificial AMF inoculation should be emphasized as an environmental friendly and sustainable means of soil fertility management. Inoculation with AMF translated into higher spore abundance in the soil and subsequent crop plant colonisation. The crop is thus in a better position to gain from the AMF-plant association.

The use of different soil fertility amendment practices affect the prevalence and abundance of AMF spores in the soil which in turn affects the intensity of host crop colonisation and thus it's necessary to use methods that ensure the conservation of these soil microorganisms that are an integral part of soil fertility management practices.

CHAPTER 5: EFFECTS OF SOIL FERTILITY MANAGEMENT PRACTICES, RECOMMENDED FOR FARMERS ADOPTION, ON ARBUSCULAR MYCORRHIZAL FUNGI (AMF) UTILISATION

5.1 Abstract

A study was undertaken to monitor abundance of AMF spore and diversity in experiments established on-farm at Embu and Taita districts, Kenya. Test strips and demonstration plots under different soil amendment practices were sampled with the aim of identifying the best practice that would conserve AMF and function. The experiments were under demonstration blocks and test strips. A relationship between AMF spore abundance in the soil and their subsequent colonisation of host plant is compared so as to illustrate the impact of each soil fertility management practice on AMF spores abundance and function. The treatments under demonstration blocks were mijingu + CAN, manure, FP, mavuno and control. A significant ($P \leq 0.05$) difference in spore abundance among the different soil fertility amendment practices in demonstration blocks was recorded AMF inoculation in the farms was found to boost AMF colonisation intensity in the host plant i.e. maize and beans. Manure application was found to be the best method to conserve AMF population in the soil. Also, there was better AMF utilization under manure application.

5.2 Introduction

Disturbance of vegetation, soils and associated microflora is increasing with expanding human populations. Various land use practices and soil fertility management practices greatly alter the soil environment under which soil microorganism function. This greatly alters the occurrence as well as the survival of these organisms (Jefwa et al., 2006). Their efficiency may thus be altered possibly leading to a total elimination of vital bio-components before their potential is realized.

Estimation of species of a region is useful for detecting trends in, impacts, or recovery of ecosystems, for selection and management of nature preserves, and for basic understanding of biophysical factors controlling species numbers and hence preserving ecosystem services such as nutrient cycling and control of pests and diseases.

Several factors have been shown to affect AMF spore abundance and species diversity; this is directly through damaging or killing AMF and indirectly, by creating conditions either favourable or favourable to them. In general, agricultural practices have a negative impact on the AM association and agricultural soils are AMF impoverished, particularly in the number of species (Helgason *et al*., 1998; Menendez *et al*., 2001). This include, use of excess phosphorus fertilizer which may lead to reduced AM colonisation of roots and AMF spore density in soil (Kahiluoto *et al.*, 2001). Use of other readily soluble fertilizers, particularly, N fertilizers has also been reported to have a negative impact on AM colonization and diversity in some cases (Treseder and Allen, 2002) but not in others (Jumpponen *et al*., 2005). Farm yard manure and slow release

mineral fertilizers such as rock phosphate do not seem to suppress AMF and may even stimulate them (Joner, 2000; Alloush and Clark, 2001). Soil tillage causes severe disruption to the common mycorrhizal network resulting in delayed or reduced root colonization and a reduction in the volume of the soil that is exploited by the AMF leading to reduced plant nutrient uptake, consequently crop growth and yield though not always (Evans and Miller, 1990; Kabir *et al*., 1998)

Agriculture production also lead to low diversity of AMF compared to a natural ecosystem and tends to propagate *Glomus* species due to low diversity of hosts which is severe in case of monoculture (Oehl *et al.*, 2003).

The objective of this study was to highlight the effects of various soil amendment practices on AMF spore abundance and diversity thus enabling their better utilisation as well as conservation

5.3 Materials and Methods

The experiments were carried out at on-station and on farm. From on-station experiment, plots under various soil fertility amendment practices (Appendix 1 and 2) were sampled. Sampling was also done from on-farm experiment under different soil amendment practices (Appendix 3 and 4). The soil and root samples collection and assessment of AMF colonisation and spore abundance was carried out as described in Chapter 4.3

5.4 Results

5.4.1 Spore abundance

Spore count carried-out on demonstration plots at Embu indicated a significant ($P \leq 0.05$) difference among the different soil amendments in the three sampling times. There was however reduced spore count with every season in all plots (Fig 5.1). The highest reduction in spore count was recorded from the plots under control treatment with a value of 1.18 (average spore count per 50g of soil). The second lowest value was from FP treatment (1.4). The treatment with least effect on the abundance of AMF spore count was mijingu + C.A.N treatment followed by manure treatment.

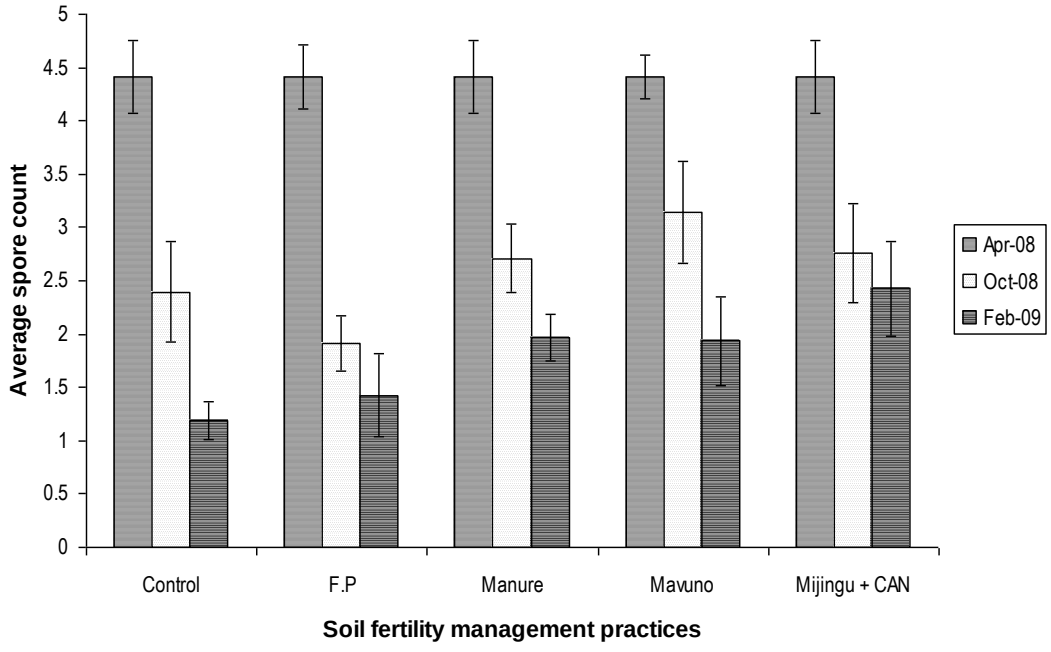


Figure 5.1: Changes in Arbuscular Mycorrhizal Fungi abundance in demonstration plots at Embu district treated with various soil fertility amendments from April 2008 to February 2009.

5.4.2 Effects of Soil fertility management practices on AMF colonisation on maize

5.3.2.1 Taita experimental site

At Taita on-station experiment, there was no significant difference in the level of AMF colonisation intensity among the different soil fertility amendment practices. The highest AMF colonisation intensity was recorded from plots treated with mavuno combined with *Trichoderma* followed by practices with AMF combined with Farmer practice. (Fig 5.2) The lowest level of AMF colonisation intensity was recorded from plots treated with *Trichoderma*. Level of AMF colonisation abundance was highest from the plots treated with control followed by AMF combined FP and the lowest evaluated value was obtained from treatment with AMF applied alone.

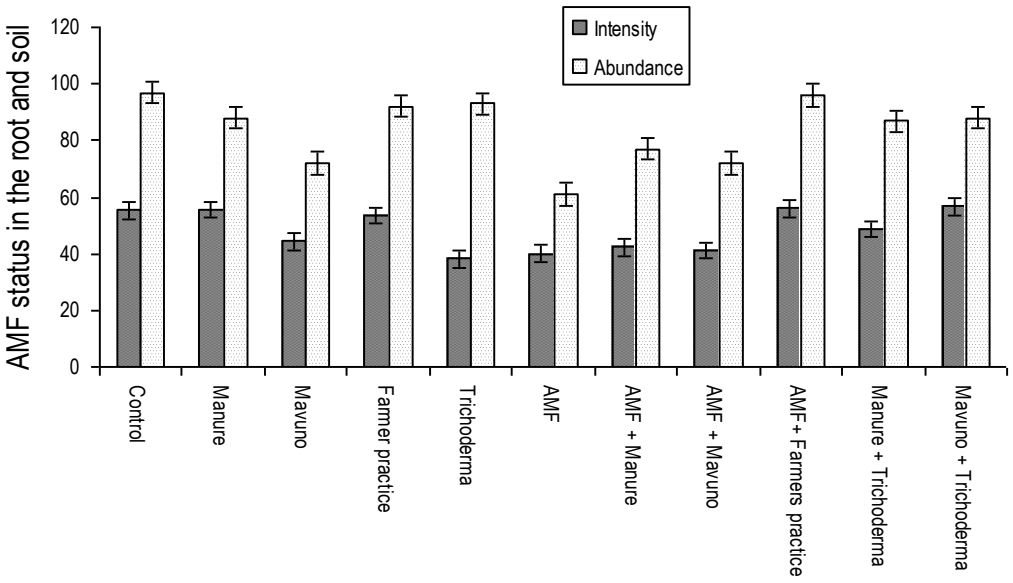


Figure 5.2: AMF colonisation intensity in maize *(Zea mays*) root and spore abundance in the soil in plots treated with different soil fertility amendments at Taita district on-station and on-farm experimental plots in October 2008.

At Taita districts, on-farm experiment, there was no significant difference in the colonisation intensity among the different soil fertility amendment practices. The highest level of AMF colonisation intensity was recorded from plots under mijingu combined with CAN which also had the highest percentage level of spore abundance. Plots treated with control had the second highest colonisation intensity recorded and manure combined with Trichoderma had the lowest (Fig 5.3).

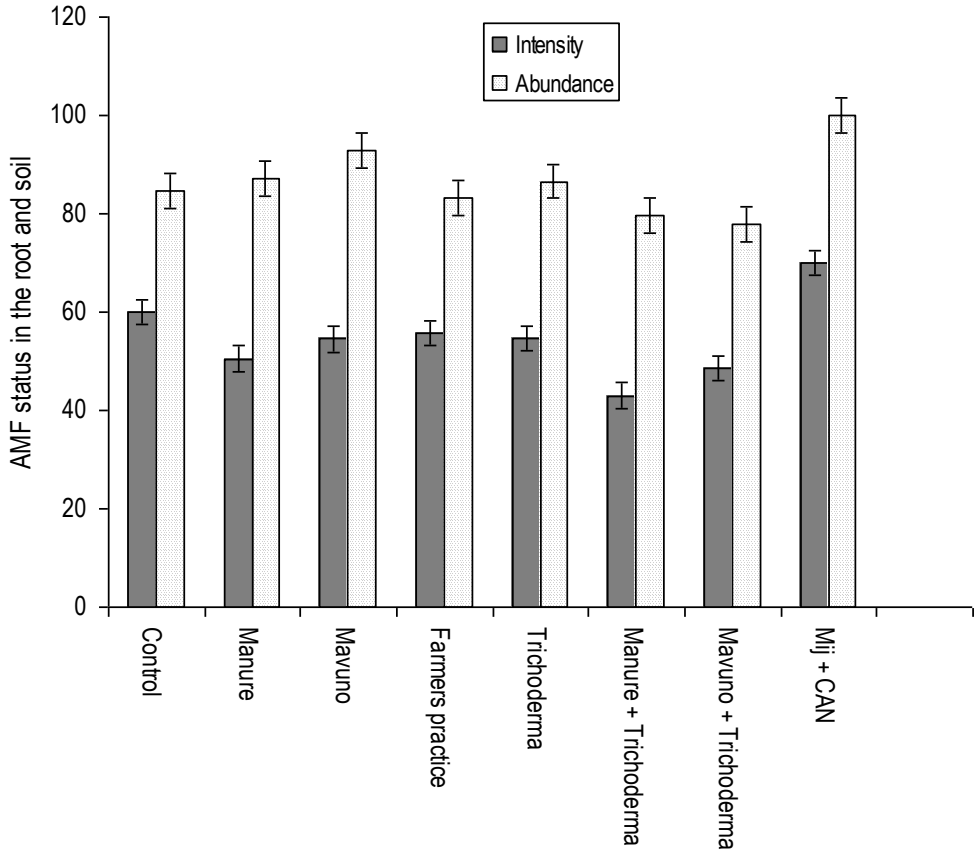


Figure 5.3: AMF colonisation intensity in maize (*Zea mays*) roots and spore abundance in the soil in plots treated with different soil fertility amendments at Taita district on-farm experiment in October 2008.

5.4.2.2 Embu experimental site

At on-station experiment in Embu district, the highest AMF colonisation intensity was recorded from mavuno with an average value of 79.7% followed by AMF combined with FP with a value of 79.5% (Fig 5.4). The lowest value of 60% was recorded from practices with no fertilizer application.

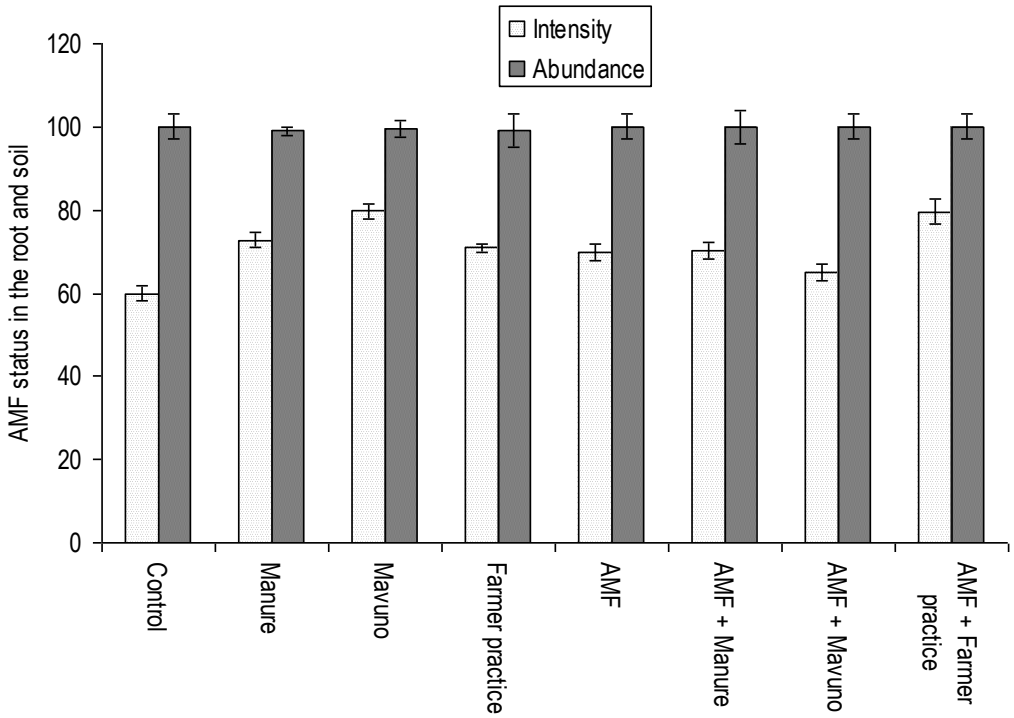


Figure 5.4: AMF colonisation intensity in maize (*Zea mays*) root and spore abundance in the soil in plots treated with different soil fertility amendments at Embu district on-station experiment in October 2008.

On both on-farm and on-station experiment at Embu district there was no significant difference in the intensity of root colonisation by AMF among the different soil fertility management practices. At on-farm experiments from Embu, the highest percentage value of AMF colonisation intensity was recorded from plots under FP treatment with a

value of 72.8. The second highest value was obtained from manure combined with *Trichoderma* with a value of 69.3% (Fig 5.5)

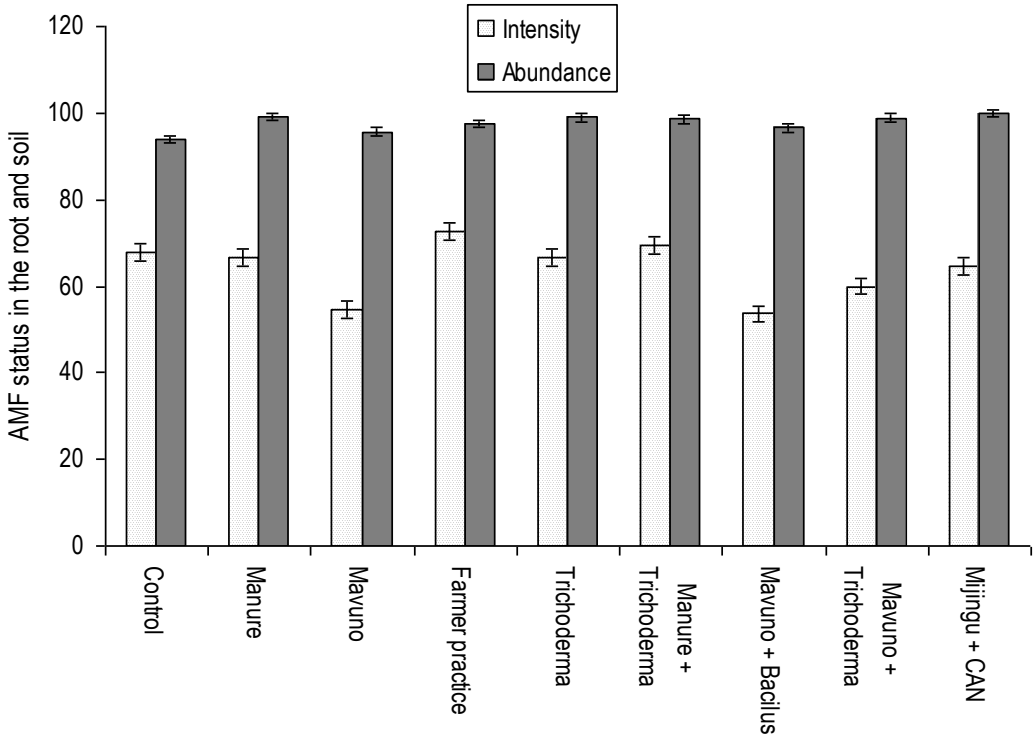


Figure 5.5: AMF colonisation intensity in maize (*Zea mays*) root and spore abundance in the soil in plots treated with different soil fertility amendments at Embu district on-farm experiment in October 2008.

5.4.3 Effects of soil fertility management practices on AMF colonisation on bean

5.4.3.1 Taita experimental site

At Taita on-station experiment, there was no significant difference in the intensity of bean root colonisation by AMF among the different soil fertility management practices. The response of bean roots to direct inoculation was also low compared to maize experiment. The highest value was recorded from plots under manure and FP with values of 72.1% and 70.7% respectively. The lowest value was obtained from practices with no fertilizer application a value of 45.6% and FP +AMF had second lowest value of 46.9% (Fig 5.6)

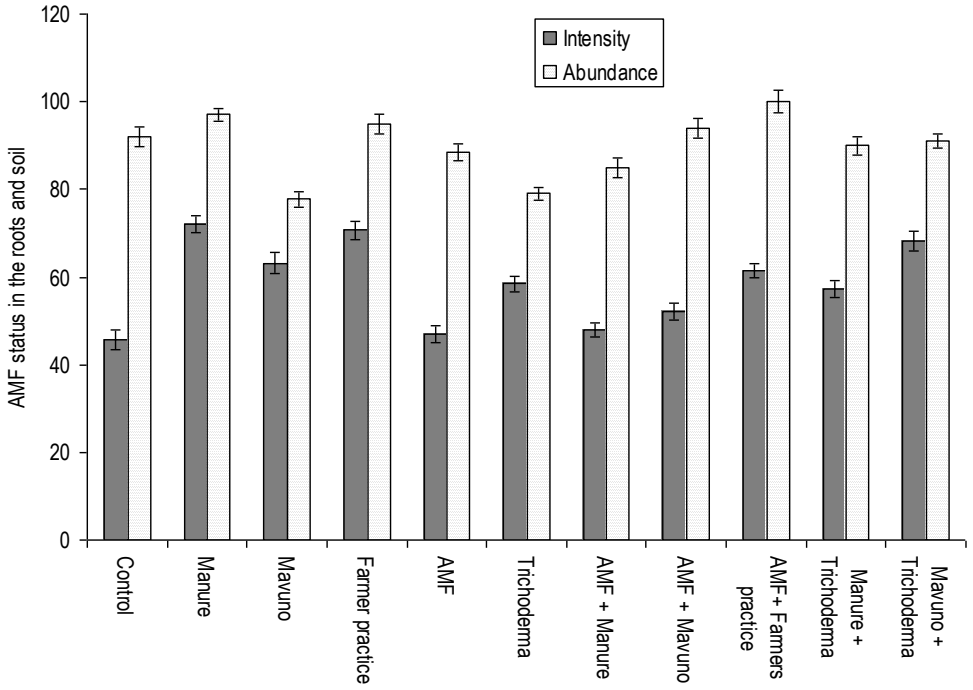


Figure 5.6: AMF colonisation intensity in bean (*Phaseolus vulgaris*) root and spore abundance in the soil in plots treated with different soil fertility amendments at Taita district on-station experiment in October 2008.

5.4.3.2 Embu experimental site

Similar results were obtained from Embu on-station experiment whereby the highest value of AMF colonisation intensity was obtained from practices with manure and the lowest from practices with no fertilizer (Fig 5.7). There was also no significant difference in the colonisation intensity among the different soil fertility amendment practices.

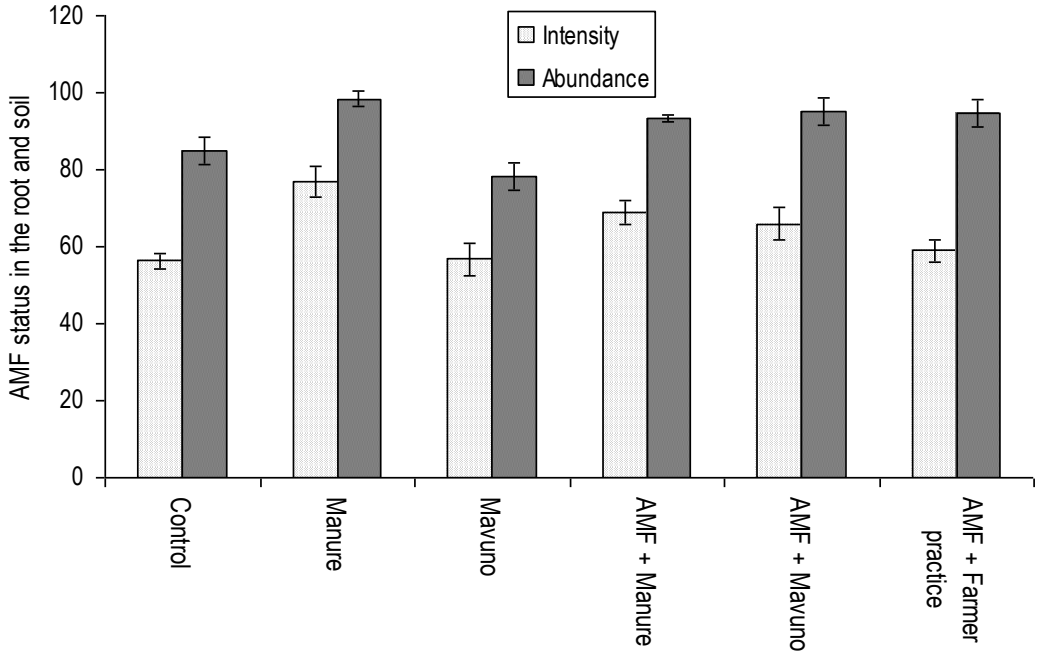


Figure 5.7: AMF colonisation intensity on bean (*Phaseolus vulgaris*) root and spore abundance in the soil in plots treated with different soil fertility amendments at Embu district on-station experiment in October 2008.

5.5 Discussion

Use of different soil fertility management practices resulted in significant difference ($p \leq 0.05$) in spore abundance. This indicates that use of different soil fertility management practice has effect on the abundance of AMF. There was a marked decrease in spore abundance with each cropping season. This is observation is in agreement with work of Helgason *et al*., (1998) and Menendez *et al*., (2001). In April, at the beginning of the season, the spore abundance was high for all practices, with seasons, and the abundance was less in the control followed by FP. Under cultivation this may mean that the recovery of AMF in control treatment is less and same as from FP treatment. The reduction of spore count in FP compared to the others could signify that this practice does not conserve AMF well. Though high colonization is indicative of good conditions, hence low spores may mean less disturbed conditions that trigger less sporulation. Under extremely low fertility like the control AMF may also not be conserved.

There was a general reduction in AMF abundance in all treatments and this shows the negative effects of land use and management on AMF population. This is in agreement with observations by Evans and Miller, (1990); Kabir *et al*. (1998).

In both experimental site application of manure, AMF in combination with FP, mijingu in combination with CAN and no application of fertilizer on maize and bean resulted in high mycorrhizae colonisation intensity in majority of trials. This is indicative of practices leading to high mycorrhizal utilisation by the host as well as the subsequent conservation of AMF.

Use of mavuno, *Trichoderma* and manure with *Trichoderma* recorded a decrease in AMF colonisation intensity in the host plant. Thus low utilisation of AMF and therefore the soil amendment practices are a threat to AMF conservation.

Use of manure, no fertilizer application, mijingu combined with CAN and FP (CAN+TSP) in both experimental sites resulted in high spore abundance in the soil. All of the named practices have also been found to increase the colonisation intensity of the host plant and it can be concluded that the reason for presence of high spore abundance under the practices is as a result of high sporulation and not as a result of presence of stressful conditions in the soil.

Use of AMF, mavuno in combination with *Trichoderma*, mavuno in combination with *Bacillus*, mavuno used singly and AMF in combination with FP resulted in low AMF spore abundance in the soil. Low spore abundance in the soil can be indicative of low AMF sporulation as a result of conducive environmental conditions for mycorrhizal colonisation in the soil or total absence of AMF spores in the soil. The use of mavuno in combination with *Trichoderma* and mavuno used singly resulted in low mycorrhizal colonisation intensity as well. Thus low spore abundance can only mean low AMF population in the soil in the sampled plots and explains to the fact that AMF treatment resulted in low spore abundance in the soil but high colonisation intensity.

5.6 Conclusion

Presence of spore in the soil is indicative of presence of AMF and thus disappearance of spores could therefore mean that the species sporulation is suppressed or the species is eliminated. The first scenario can be confirmed by use of molecular techniques, the second scenario simply means, when the species spores appear, there is some indication of stressful conditions that trigger sporulation. Hence interpretation of high spore abundance may indicative of some degree of conditions that are not suitable for the fungi. It's only through AMF colonisation assessment that the beneficial or detrimental effect of soil fertility amendment can be quantified in relation to AMF activity outside and inside the host plant. In this experiment, there was a positive correlation between AMF colonisation in the host plant and the spore abundance in the soil. Thus presence of spores in the soil is indicative of AMF presence and not presence of stressful conditions in the soil.

AMF inoculation in the farms was found to boost AMF colonisation intensity in the host plant i.e. maize and beans. Manure application was found to be the best method to conserve AMF population in the soil. Also, there was better AMF utilization under manure application.

CHAPTER 6: GENERAL DISCUSSIONS AND RECOMMENDATIONS

The overall maize production at Embu experiments and Taita were lower against a potential of 6t/ha (Makokha *et al.* 2001; Jaetzold, 2006) due to impoverished soils, unfavourable climatic conditions, pests and diseases (Ampofo 1986; Seshu Ready and Sum, 1992). These, among other constraints, present a serious threat to livelihoods and food security in the region. Application of farming practices that would sustain sufficient food production per unit area of land for along time without harming the environment is paramount.

Use of different soil fertility management practices resulted in significant difference (than $p \leq 0.05$) in species richness. This indicates that use of different soil fertility management practice has effect on the species of AMF present in the soil. Majority of morphotypes isolated from the soil were *Scutellospora* sp. This is in contrast to what was observed by Oehl *et al*. (2003), where agriculture production led to low diversity of AMF compared to a natural ecosystem and tends to propagate *Glomus* species due to low diversity of hosts which is severe in case of monoculture. This is however in agreement made by Mathimaran (2007) whereby *Glomus* species were found to be the most dominant in a cropping system.

The highest spore count was from the depth of 0-10 cm compared 10-20 cm below the ground and can be explained by a number of reasons. First, the sampling was done from a seasonally maize and bean intercrop land and hence the majority of mycorrhizal activity is to be expected from such depth and secondly, the fact that most soil disturbance occur in the region and hence may trigger a lot of AMF sporulation.

Field inoculation with AMF has been demonstrated to positively affect the yield of maize and bean at Embu experimental site though not significantly different among the various soil fertility amendments. This is in agreement with earlier work of Siddiqui and Mahmood, 2001. Thus AMF can constitute an environmentally friendly method of soil fertility amendment over time. The inoculum can easily be made by farmers upon training. However, it cannot be used as substitute to use of inorganic or inorganic fertilizers. Also an evaluation on the effect of AMF inoculation on the spore abundance in the soil needs to be carried out.

Use of AMF inoculum plus either mavuno or FP (A combination of TSP and CAN fertilizers) led to lower maize and bean yields in terms of total biomass of the crops at harvest compared to individual fertilizers. Though not so exquisite pattern, the negative effect was recorded for both maize and beans in the Taita experimental site but not at Embu experimental site. This can be explained by other ecological factors such as rainfall, soil conditions as well as interaction of AMF with other microorganism. This may be attributed to changes in moisture, a major factor that determines growth. Arbuscular mycorrhizal fungi are known to alleviate water stress (Auge` *et al.*, 1994)

Combination of AMF and F.P had higher weight of maize stovers compared to treatment under F.P applied singly. The use of AMF inoculum lead to relatively increased maize yield and bean growth rate compared to Control treatment and hence a clear indication that utilization of indigenous AMF species can positively impact on crop growth and to increase efficiency inoculation with indigenous species can be incorporated. The use of inorganic and organic fertilizers also seem to enhance AMF utilization; the addition of these fertilizers to AMF lead to higher crop yield as well as

root colonisation compared to plots under AMF applied singly. Inoculation of maize crops with AMF combined with FP had higher maize yield compared to FP applied singly. This can be explained by action of AMF in helping uptake of macronutrients as observed by Hodge *et al.*, 2001.

The use of fertilizers (FP and mavuno) was shown to tremendously increase crop yield for maize and beans at both sites. This is with comparison to use of farmyard manure or use of AMF applied singly. Organic nutrients increase the abundance of soil organisms by providing organic matter and micronutrients for organisms such as fungal mycorrhiza which aid plants in absorbing nutrients and can drastically reduce external inputs such as fertilizer, at the cost of decreased yield, (Mäder *et al.*, 2010).

Bean harvest was higher for both seasons with AMF inoculation compared to control treatment. This is with agreement to earlier work by Mohammad *et al.*, (2004) on effect of AMF on growth of barley.

Use of indigenous AMF inoculum on maize and beans has been demonstrated to positively affect their growth and yield. Thus, need to be encouraged as a cheap means of enhancing crop productivity by majority of Kenya subsistence farmers, whose crop production in the last decades has dramatically reduced with main reasons being reduced fertility due to over cultivation as well as changing environmental conditions.

Application of farmyard manure was found to effectively conserve AMF population in the soil as well as leading to high utilisation by maize and beans. Therefore, it should be recommended as a cheap and an environmentally friendly method of soil fertility management method.

Field AMF inoculation was also found to boost host crop colonisation intensity and it's therefore an environmentally friendly method that can be encouraged among all agricultural systems so as to maintain a natural and microorganism based soil fertility replenishment methods.

Development of inoculum for different ecological zones need to be done as it was observed the species of AMF vary with these zones; the species isolated from Embu were not similar to those isolated from Taita, though the variation was minimal. This can form basis for evaluation to be done to develop broad based inoculum which can be applicable to all Kenyan situations. Also evaluation of implication on use of exotic AMF species can also be done in Kenyan agricultural ecological systems.

Soil fertility management practices need to be thoroughly investigated for their effect on the population of AMF in the soil. Tools and methods (such as constant evaluation of microorganisms status in the) for monitoring soil health need to be put in place to ensure sustainability of ecosystem services and this is necessary so as to avoid total elimination of these important soil fertility enhancing organisms with capability of supporting crop production.

Also interaction of each soil fertility management practice with AMF need to be individually evaluated with respect to prevailing environmental condition so as to develop an integrated soil fertility management practice best suited for each district/ecological zone in Kenya.

The drastic reduction in spore abundance among the different soil amendment practices need to be investigated further under similar conditions so as to authenticate the cause.

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APPENDICES

Appendix 1: Treatment layout at Embu on-station experiment

Manure + Trichoderma	Control	Manure	Mavuno + Trichoderma	Manure + Trichoderma
Trichoderma	Manure + AMF	Mavuno + AMF	Farmer Practice+ AMF	Farmer Practice + Bacillus
Mavuno + Trichoderma	Manure + Bacillus	Bacillus	Manure	Bacillus
Mavuno + AMF	AMF	Manure + AMF	Control	Manure + AMF
Control	Manure + Trichoderma	Farmer Practice + Bacillus	Farmer Practice	Farmer Practice + Trichoderma
Manure + AMF	Farmer Practice + Trichoderma	Trichoderma	Farmer Practice + Trichoderma	Farmer Practice+ AMF
Mavuno	Mavuno + AMF	Farmer Practice	Manure + AMF	Mavuno + AMF
Farmer Practice + Trichoderma	Farmer Practice + Bacillus	Farmer Practice+ AMF	Farmer Practice + Bacillus	Farmer Practice
Mavuno + Bacillus	Bacillus	AMF	Manure + Bacillus	Mavuno + Bacillus
Bacillus	Mavuno + Trichoderma	Control	Manure + Trichoderma	AMF
Farmer Practice+ AMF	Mavuno	Mavuno + Bacillus	Bacillus	Mavuno
Manure + Bacillus	Farmer Practice	Manure + Trichoderma	AMF	Mavuno + Trichoderma
Farmer Practice + Bacillus	Trichoderma	Farmer Practice + Trichoderma	Mavuno	Trichoderma
Farmer Practice	Mavuno + Bacillus	Mavuno	Trichoderma	Manure
AMF	Manure	Manure + Bacillus	Mavuno + Bacillus	Control
Manure	Farmer Practice+ AMF	Mavuno + Trichoderma	Mavuno + AMF	Manure + Bacillus

Appendix 2: Table showing treatment layout at Taita on-station experiment

Plot 1 Trichoderma	Plot 14 Manure +Trichoderma	Plot 15 Manure +Trichoderma	Plot 28 Control	Plot 29 Farmer Practice
Plot 2 Manure	Plot 13 Mavuno +Trichoderma	Plot 16 Control	Plot 27 Mavuno	Plot 30 Manure
TRASH LINE				
Plot 3 Farmer Practice	Plot 12 Control	Plot 17 Trichoderma	Plot 26 Farmer Practice	Plot 31 Trichoderma
Plot 4 Manure +Trichoderma	Plot 11 Trichoderma	Plot 18 Mavuno +Trichoderma	Plot 25 Manure	Plot 32 Control
TRASH LINE				
Plot 5 Control	Plot 10 Mavuno	Plot 19 Farmer Practice	Plot 24 Manure +Trichoderma	Plot 33 Manure +Trichoderma
Plot 6 Mavuno	Plot 9 Manure	Plot 20 Mavuno	Plot 23 Trichoderma	Plot 34 Mavuno
TRASH LINE				
Plot 7 Mavuno +Trichoderma	Plot 8 Farmer Practice	Plot 21 Manure	Plot 22 Farmer Practice	Plot 35 Mavuno +Trichoderma
GUARD ROW				
TRASH LINE				
Plot 1 AMF +Mavuno	Plot 8 AMF + Farmer Practice	Plot 9 AMF + Farmer Practice	Plot 16 AMF + Mavuno	Plot 17 AMF+ Farmer Practice
Plot 2 AMF	Plot 7 AMF + Manure	Plot 10 AMF+ Mavuno	Plot 15 AMF	Plot 18 AMF
TRASH LINE				
Plot 3 AMF+ Manure	Plot 6 AMF	Plot 11 AMF+ Manure	Plot 14 AMF+ Farmer Practice	Plot 19 AMF+ Mavuno

Plot 4 AMF+ Farmer Practice	Plot 5 AMF +Manure	Plot 12 AMF	Plot 13 AMF+ Manure	Plot 20 AMF+ Manure
TRASH LINE				
GUARD ROW				

Appendix 3: Test strips (TST) treatments layout at Embu

Plot 1 Trichoderma	Plot 2 Manure	Plot 3 Farmer Practice	Plot 4 Manure+ Trichoderma	Plot 5 Manure + Bacillus
Plot 10 Mavuno + Bacillus	Plot 9 Control	Plot 8 Bacillus	Plot 7 Mavuno +Trichoderma	Plot 6 Mavuno

Appendix 4: Test strips (TST) treatments layout at Taita

Plot 1 Trichoderma	Plot 2 Manure	Plot 3 Farmer Practice	Plot 4 Manure+ Trichoderma	Plot 5 Control	Plot 6 Mavuno	Plot 7 Mavuno+ Trichoderma

Appendix 5: Demonstration blocks treatment layout at Embu and Taita

Plot 1 Mijingu + CAN	Plot 2 Mavuno	Plot 3 Farmer practice	Plot 4 Control	Plot 5 Manure

Appendix 6: List of farms and owners name used at Taita on-farm experiment

Demostration block 1	Ms. Megi Mukakina
Demostration block 2	Mr. Mwatika Mwasi
Demostration block 3	Mr. Edman Marasi
Demostration block 4	Mr. Robert Masaka
Test strip (TST) 1	Stephen Kalama
Test strip (TST) 2	Hilton Kilombo
Test strip (TST) 3	Hannah Wakesho
Test strip (TST) 4	Nelson Mwombo
Test strip (TST) 5	Eunice Mwadime
Test strip (TST) 6	Jeremiah Mwafusi
Test strip (TST) 7	Masuvirio Mwangemi
Test strip (TST) 8	Sea Mwakachola
Test strip (TST) 9	Abednego Kitando
Test strip (TST) 10	Josephat Ngoo
Test strip (TST) 11	Stephen Mwadoto
Test strip (TST) 12	Obadia Mwakina

Appendix 7: List of farms and owners name used at Embu on-farm experiments

Test strip (TST) 1	James Ndwiga Ngoroi
Test strip (TST) 2	Nancy Kangethe
Test strip (TST) 3	Njagi Cigiti
Test strip (TST) 4	Kabiru Githinji
Test strip (TST) 5	Abiud Njue
Test strip (TST) 6	Simon Kiura
Test strip (TST) 7	Duncan Ngoroi
Test strip (TST) 8	Mary Wanyaga
Test strip (TST) 9	Samuel Muriithi
Test strip (TST) 11	John Njue
Demonstration block 2	Stephen Kariango
Demonstration block 3	Charity Kiura
Demonstration block 4	Zachariah Njeru