EFFECTS OF DUAL INOCULATION WITH MYCORRHIZA AND RHIZOBIUM ON GROWTH PERFORMANCE OF SOYABEANS IN ACIDIC SOILS IN GATANGA, KENYA

BY

NAOMI NJERI KAMAU

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DECLARATION BY CANDINDATE

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

SIGN _____ _____
NAOMI NJERI KAMAU DATE

DECLARATION BY SUPERVISORS

This thesis has been submitted with our approval as supervisors.

SIGN _____ _____
PROF JAMES B. KUNGU DATE
DEPARTMENT OF ENVIRONMENTAL SCIENCES
KENYATTA UNIVERSITY

SIGN_____ _____
PROF DANIEL MUGENDI DATE
DEPARTMENT OF ENVIRONMENTAL SCIENCES
KENYATTA UNIVERSITY

DEDICATION

This thesis is dedicated to my parents, Mr. and Mrs. Ephraim Kamau, my husband, Fredrick Maina, and to my two children, Irene the first born and Anne who was born in the course of this work.

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List of acronyms

AM	Arbuscular Mycorrhiza
BNF	Biological Nitrogen Fixation
C	Carbon
Ca	Calcium
CEC	Cation Exchange Capacity
GRM	Grams
KEFRI	Kenya Forestry Research Institute
NFT	Nitrogen Fixing Trees
P	Phosphorus
S+R	Soyabeans Inoculated with Rhizobium
S+R+M	Soyabeans Inoculated with Rhizobium and Mycorrhiza
S+P+R	Soyabeans Planted with Phosphatic Fertilizers and Inoculated with Rhizobium
S+P+M	Soyabeans Planted with Phosphatic Fertilizers and Inoculated with Mycorrhiza
S	Soyabeans Planted without Fertilizer or Inoculation
S+M	Soyabeans Inoculated with Mycorrhiza
S+P	Soyabeans Planted with Phosphatic Fertilizers
LR	Long Rains
SR	Short Rains

ABSTRACT

Small land holdings and poverty in Central Kenya have made it difficult for farmers to adequately conserve and replenish soil nutrients in their farms. Soil erosion and leaching of nutrients leading to soil acidity have been the inevitable outcome. This study was designed to determine the effect of inoculating soyabeans (*Glycine max)* with both mycorrhiza and rhizobium as a biological means of improving soil fertility in the acidic soils in Gatanga, Thika District. Field experiments were carried out in Gatanga and at Kenyatta University (on-station) in sterilized and non-sterilized soils collected from Gatanga. The field experiments were laid out in complete randomized block design while the on-station ones were laid out in complete randomized design. Analysis of variance (ANOVA) was conducted on the data and means separated using LSD at 5% significance difference using Genstat for Windows Version 8.11. The growth parameters; height, root collar diameter, shoots and root dry weight all increased as a result of dual inoculation with mycorrhiza and rhizobium. Dual inoculation also led to increased nitrogen fixation by soyabeans, evidenced by increased nodulation, and grain yields. Dual inoculation with mycorrhiza and rhizobium did not have any significant effect ($p<0.05$) on germination of soyabeans. Height of soyabeans as a result of dual inoculation increased significantly over the control by 88% in the long rains while in the short rains the increment was not significant. In the on-station experiments, height increment over the control in sterilized and non-sterilized soil was not significant. Dual inoculation increased root collar diameter by 80% and 8.6% in the long and short rains respectively. Shoots dry weight in the on-farm long rains 2005 season increased by140% as a result of dual inoculation while in the short rains season and the on-station experiments, the differences were not significant. Dual inoculation increased grain yields by 356% in the on-farm long rains 2005 season, while in the on-station experiments grain yield increased by 76% and 107% in the sterilized and non-sterilized soils respectively. Though nodulation was poor in all the experiments, the number of nodules increased significantly by 676% over the control in the long rains 2005 season. In the on-station experiments the control (S) had no nodules. The short rains crop performed poorer than the long rains crop as a result of insufficient rains. In conclusion, the biological organisms; mycorrhiza and rhizobium, could be utilized to increase productivity of the legume soyabean, in acidic soils. However, technologies to avail the microorganisms to the farmers need to be developed as the obligate nature of mycorrhizal fungi makes it difficult to culture and commercialize while the low shelf life of rhizobium at room temperature is a hindrance for its use by resource poor farmers.

CHAPTER 1

INTRODUCTION

1.1 Background Information

In tropical countries, crop production is limited by the availability of one or more nutrients with nitrogen and phosphorus being the most limiting ones under continuous cultivation and in degraded soils (Giller, 2002). Fertilization is, therefore, necessary for increasing crop yields.

In Kenya, a third of the land area (7.5 million hectares) is acidic (Kanyanjua et al., 2002). These are deep well-drained soils with low activity clays characteristic of old landscape with very high rainfall. They are highly leached and as such have low cation exchange capacity arising from leaching of bases. The dominant clay fraction is kaolinite with a structure of 1:1 silica: aluminium layer and carries an inherently small negative charge as compared to the 2:1 clay minerals (Giller, 2002). Where the parent material contains much aluminium, it can become the predominant cation when the soils have been leached of other bases. Percentage aluminium can be as high as 80-90% and the base saturation that is, proportion of cation exchange capacity occupied by cations that predominate most soils, notably calcium, magnesium and potassium is low (Giller, 2002).

Biological nitrogen fixation (BNF) by legumes provides an alternative and cheaper source of nitrogen for legume grain crop production. The process of nitrogen fixation is hampered by soil acidity especially in sub-humid countries, which limits the availability of phosphorus thus limiting root development of plants and aggravating the problem of

insufficient nutrient supply from the soil (Harter, 2002). In acidic soils, a large proportion of applied phosphorus is fixed to iron and aluminium oxides and is then not available for plant uptake (Schroth et al., 2003).

Phosphorus is required in BNF for the supply of energy. The problem of phosphorus (P) supply to plants can be circumvented by use of arbuscular mycorrhiza symbiosis. Inoculating with AM (arbuscular mycorrhiza) improves phosphorus uptake and thus increases nitrogen fixation (Young, 1997). Mycorrhiza absorbs carbohydrates from the host plant. In return they improve the extraction of nutrients from the soil having the effect of expanding the extent and surface area of the plant's root system (Sieverding, 1991). Legume plants are course rooted and hence greatly benefit from this association.

The availability of nitrogen to the plants too is a limiting factor in degraded acidic soils (Giller, 2002). Legumes can form a symbiotic relationship with the soil bacteria, rhizobim. Rhizobium bacteria supply ammonia or amino acids through nitrogen fixation to the plant and in return receive organic acids as a carbon and energy source (Leigh, 2002).

Most legumes are simultaneously in symbiotic relations with both rhizobium and arbuscular mycorrhizae fungi (Barea et al., 2005). Rhizobium fixes nitrogen which benefits plant growth and the fungi benefits the host by increasing the efficiency of uptake of mineral elements and water from the soil as well as altering host metabolisms and other physiological parameters. Plant growth in this tripartite relationship is generally

much greater than when the plant is in a symbiotic relationship with either of the microorganisms alone (Arola et al., 1991).

Mycorrhiza and rhizobia inoculated legumes will decrease the need for mineral fertilizers and hence save on energy. It has been established that, 50% of the total energy consumed in agricultural production in the tropical regions is consumed in the production and consumption of nitrogen fertilizers (Chikowe, 2004). Plants harbouring two mutualistic symbioants such as rhizobium nodulated and mycorrhizal plants, are well adapted to habitats with low availability of both nitrogen and phosphorus (Bagyraj, 1996). Once established, both mycorrhiza and rhizobium symbionts are likely to be self-replenishing unlike mineral fertilizers.

The presence of Nitrates in ground water is a major health concern in intensively cultivated areas capable of causing methemoglobinemia in infants, cancer and respiratory illness (Comly, 1987). It is also a major cause of environmental pollution and will result in problems of eutrophication and stratospheric ozone depletion (Bohlool et al., 1992). BNF will provide an economically attractive and ecologically sound means of crop production, reducing external inputs and improving internal inputs.

1.2 Statement of the Problem and Justification

Due to increased poverty levels in the Central Kenya Highlands, small-scale farmers are not able to adequately replenish their soils with either organic or inorganic fertilizers. Soil erosion too, is a serious problem resulting from inadequate soil conservation

measures in the steep terrain. This leads to land degradation and hence reduced soil fertility. Leaching of bases has resulted to the soils becoming acidic.

In degraded acidic soils, since phosphorus is low due to fixation by aluminium and/or iron and also, due to its low mobility, the nutrient is depleted very fast around the plant roots (Sanchez et al., 2003). Soil acidity causes problems of plant growth due to iron, aluminium and/or manganese toxicity as well as molybdenum, calcium and/or magnesium deficiency. In legumes such as soyabeans, molybdenum is essential in nitrogen fixation while calcium requirement is high, hence deficiency of these two nutrients will lead to low biomass production (Giller, 2001). Phosphorus is essential for the high-energy requirement in BNF.

Soil fertility could be replenished either through biological means or by use of fertilizers (organic or inorganic). Inorganic fertilizers are expensive and out of reach for the smallholder farmers and hence could result to cheaper biological means like the use of microorganisms such as mycorrhiza and rhizobium. Mycorrhizal fungi form symbiotic relationships between themselves and plant roots and are reported to significantly increase plant nutrient uptake of especially the less mobile nutrients like phosphorus, zinc, copper and molybdenum (Sieverding, 1991; Marschner, 1992). Rhizobium bacteria fix atmospheric nitrogen thereby availing it for protein synthesis by the plant. Dual inoculation may therefore, address the problem of soil fertility in acidic soils.

It is well documented that soil degradation can reduce the populations of mycorrhiza ((Brundrett et al, 1994) and rhizobium symbionts and either limit the amount of available natural inoculum or eliminate beneficial strains all together. When indigenous populations have been depleted, it becomes necessary to reintroduce specific strains to ensure that population of beneficial symbionts are numerous enough to support plant growth (Bagyraj, 1996).

Dual inoculation of legumes with arbuscular mycorrhiza and rhizobium can, therefore, help circumvent the problem of low soil fertility and hence low biomass and grain production of legume plants in acidic soils and hence help solve the problem of food insecurity and poverty. It will help solve the problem of land degradation in acidic soils through revegetation of affected areas and will minimize nitrate toxicity, which arises from excessive use of nitrogen fertilizers. However research in this area is lacking. The current study tested the effect of inoculating the legume plant *Glycine max* (Soya beans) with arbuscular mycorrhiza (AM) and rhizobia on the growth performance in acidic soils both in the screen house and in the field.

1.3 Research Questions

The research sought to answer the following questions:

1. What is the effect of inoculating soyabeans with AM fungi and rhizobium bacteria on the germination of soyabeans in acidic soils?
2. How does inoculating Soyabeans with AM fungi and rhizobium bacteria on acidic soils affect biomass production and grain yield?
3. Is there any effect of inoculating soyabeans with rhizobium and AM fungi in acidic soils on nitrogen fixation?

1.4 Hypotheses

The study aimed at testing the following hypotheses:

1. Inoculating Soyabeans with both AM fungi and rhizobium will result in increased germination.

2. Inoculating soyabeans with both AM fungi and rhizobium bacteria in acidic soils will result in increased grain and biomass production.

3. Inoculating Soya beans with AM fungi will result in higher nitrogen fixation.

1.5 Objectives of the Study

The overall objective of the study was to explore the potential of dual inoculation of AM fungi and rhizobium on the growth performance of the legume *Glycine ma*x in acidic soils. Specifically the research sought to:

1. Assess the effect of inoculating *Glycine max* with AM and rhizobium bacteria on its establishment germination in acidic soils.

2. Assess the effect of inoculating *Glycine max* with AM and rhizobium bacteria on grain yield and biomass production in acidic soils

3. Quantify the impact of inoculating *Glycine max* with AM and rhizobium on nitrogen fixation in acidic soils.

1.6 Significance of the Study and Anticipated Output

In most tropical soils, growth and production of crops is limited by the unavailability of one or several nutrients such as nitrogen as is the case in sandy soils or phosphorus in most acidic soils. Nutrients are lost from the soils in the form of nutrient export from the farm in harvested products, soil erosion, leaching and fire resulting in the widespread occurrence of negative nutritional balances in tropical farming systems. In the humid climates nutrient deficiency is often associated with soil acidity and aluminium toxicity,

which may reduce the root development of plants, further aggravating the problem of insufficient nutrient supply from the soil.

Unfavourable economic conditions such as unavailability of credit, poor access to agrochemicals at reasonable prices and lack of a lucrative market for agricultural products means that, small scale farmers are not in a position to correct soil nutrient deficiencies and acidity through fertilizing and liming. Hence, productive agriculture is not possible on infertile soils. Small-scale farmers are hence forced to continue cultivating their land without adequate replenishment of lost nutrients eventually leading to land degradation.

Small-scale farmers usually use small quantities of mineral fertilizers or none at all while sufficient amounts of organic fertilizers such as farmyard manure are rarely available, leading to low crop yields. To address the problem of low biomass and grain production, farmers can result to using inexpensive sustainable alternatives. One way of achieving this, is through the use of beneficial microorganisms that can help in the uptake of applied low amounts of nutrients or can fix atmospheric nitrogen. Some of these microorganisms are mycorrhiza and rhizobium. An understanding of the effects of these organisms on the growth performance of plants especially nutrient uptake and nitrogen fixation is, therefore, very important.

CHAPTER 2

LITERATURE REVIEW

2.1 **Introduction**

The word mycorrhiza is the mutualistic symbiotic relationship between soil borne fungus and roots of higher plants and is a Greek word for fungus roots, first coined by A. B. Frank, a German forest pathologist in 1885 (Sieverding, 1991). The plant nourishes the fungus with carbon from the plant photosynthates and the fungus supplies the plant with inorganic nutrients especially phosphorus from the soil at the root level, where individual hyphae extending from the mycelium of the fungus colonize the roots of a host plant and assist in nutrient absorption (Hogberg, 1986).

There are two different types of mycorrhiza: the ectomycorrhiza and the endomycorrhiza. Ectomycorrhizal fungi grow intercellular in the cortex of the plant roots forming a "hartig" net while endormycrrhizal fungi grow inter and intracellular (Sieverding, 1991). The most common group of mycorrhiza is arbuscular mycorrhizal fungi, an endomycorrhiza (Smith and Read, 1997). It lacks host specificity; colonizes over 80% of land plant families (Marschner, 1992; Dodd, 2000) and forms within the cortical cells of many herbaceous and woody plant species specific fungal structures namely arbuscules and vesicles. Arbuscules are finely dichotomously branching hyphal structures that invaginate the cortical cells of plant roots (Doud and miller, 1999), while vesicles are formed by the ballooning of a terminal hyphal tip, intra or inter-cellularly (Jarstfer and Sylvia, 1993). Due to the large surface contact, arbuscules are the most intensive connection between the fungus and the plants increasing the metabolic activity of the host

cell due to the bi-directional transfer of metabolites and nutrients to and from the fungus (Sieverding, 1991; Brundrett et al., 1994). Vesicles contain lipids and are food reserve organs of the fungus utilized during stress situations.

All soil borne fungi that form arbuscules in association with terrestrial plants, mostly herbaceous plants are placed in the order Glomales in the phylum Glomeromycota (Schubler et al., 2001). Some genera however produce only arbuscules, i.e., *Gigaspora* and *Scutellospora*) and are, therefore, named arbuscular mycorrhiza (AM) whereas others also produce vesicles, hence the term vesicular arbuscular mycorrhiza (VAM) (Brundrett et al., 1994). Mycorrhizas are predominantly associated with environments in which phosphorus is the major growth-limiting factor (Read, 1991).

Bacteria that fix nitrogen with legume host plants are collectively referred to as rhizobia, referring to bacteria of several genera that induce and infect nodules on the roots and/or stems of plants of the family leguminosa*e* (Giller, 2001). The bacteria possess the key enzyme nitrogenase, which specifically reduces atmospheric nitrogen to ammonia in the symbiotic root nodules (Postgate 1998, Leigh, 2002). The symbiosis is specific hence there must be recognition between the plant and bacteria. The first step in the establishment of a rhizobial-legume symbiosis is an interaction between a legume species that is susceptible to nodulation and compatible rhizobia through the root hairs (Keyser et al., 1992). The plant root releases chemical signals called flavanoids into the soil as they germinate. These signals are picked by rhizobium in the soil to which they produce a return signal “Nod Factor” to the plant. The root hairs of the plant curl, trapping the

rhizobia inside. The rhizobia multiply and an infection thread which helps the bacteria to move into the root hairs and eventually to other plant root cells develops. The cells of the roots also multiply and form a specialized structure called a nodule. Plants produce leghaemoglobin inside the nodule to absorb oxygen in the root to prevent it from interfering with the nitrogen fixation process. The enzyme nitrogenase produced by the bacteria carries out the process of biological nitrogen fixation (BNF) in which, atmospheric nitrogen is biologically reduced to a biologically useful combined organic form of N-ammonia that can be utilized by plants. The bacteria in return receive organic acids as carbon and energy from plants (Gallon and Chaplin, 1987).

2.2 **Dual Symbiosis of Nitrogen Fixers and Mycorrhiza**

Most legume plants are simultaneously in symbiotic relation with nodule forming rhizobium and arbuscular mycorrhizal fungi. The role of AM fungi in improving nodulation and rhizobial activity within the nodules is a universally recognized process (Barea et al., 2005). Rhizobium fixes nitrogen which benefits plant growth and the mycorrhizal fungi benefit the host by increasing the efficiency of uptake of mineral elements and water from the soil as well as altering host metabolism and other physiological parameters. This tripartite relationship (host – rhizobium – AM) is important in phosphorus deficient soils like the highly acid ultisols and oxisols rich in iron and aluminium oxide, which chemically bind or ‘fix’ the nutrients in a form not available for plant uptake (Jarstfer and Sylvia, 1992).

Due to the extremely low diffusion rate of phosphorus in the form of phosphates (PO_4^-), it is depleted very fast around the growing roots of plants within a distance of a few millimeters, forming a nutrient depletion zone (Sieverding, 1991). Plant roots especially those of legumes that are typically course rooted are not efficient in extracting phosphorus from the soil (Brundrett et al., 1994). Arbuscular mycorrhizal fungi play a critical role in nutrient uptake (phosphorus, copper, zinc and manganese) by plants under most tropical soils by bridging the gap across the zone of nutrient depletion (Bagyara, 1996, Arola et el., 2004). Internal and external myceliums grow to a considerable width of eight or more centimeters (Sieverding, 1991) increasing contact with soil 5-200 times. The extramatrical hyphae increase the rhizospheric soil volume thus increasing the volume of soil from which phosphorus can be exploited (Goldbold and Sharrock, 2003). Phosphorus is utilized in the biological nitrogen fixation process in the provision of energy. Biological nitrogen fixation is energy intensive requiring 16 molecules of adenosine triphosphate (ATP) to break the nitrogen bonds so that it can combine with hydrogen. Rhizobium bacteria fix nitrogen by binding it to hydrogen and making it to ammonia which is then utilized by the plant. Rhizobia benefit as the plant provides it with carbohydrates which it requires as a source of energy.

Interactions of AM fungi with the roots of legumes avail phosphorus for provision of energy in the biological nitrogen fixation process. Through expanded root area, the legume can support sufficient photosynthates to the symbioant. This comes around as a result of enhanced uptake of mineral nutrient. The tripartite interaction between

nodulating legumes, AM fungi and nitrogen fixing rhizobium acts synergistically, frequently resulting in increased nodulation and nitrogen fixation (Dodd, 2000).

Mycorrhizal fungi also exude organic acids and mobilize mineral phosphorus from sparingly soluble sources. Organic acids may also be responsible for the uptake of other nutrients such as magnesium, copper and zinc (Sierverding, 1991). In an experiment to determine rock phosphate and AM effects on growth and nutrient uptake of *Faidherbia albida* seedlings, the seedlings grew poorly without mycorrhizal colonization and without rock phosphate applications. Even without rock phosphate, AM plants achieved better results in terms of biomass (Ba and Guissou, 1996).

2.3 Functions of AM Fungi in Nutrient Cycling

Arbuscular mycorrhizal fungi play a critical role as transport paths for nutrients in nutrient cycling processes. When a nutrient is deficient in the soil solution, the critical root parameter controlling its uptake is surface area. Through extensive exploitation of soil volume by the root external mycelia, AM can efficiently and intensively extract soluble nutrients from the soil thus, solubilised and mineralized nutrients avoid chemical fixation or leaching. This function is concentrated in the uppermost horizon where organic matter is to be found and where most root growth occurs. AM fungi are found in very low densities in deeper soil horizons (Sieverding, 1991). Effective nutrient absorption by mycorrhiza hyphae is their narrow diameter (5-10 um) relative to the root hairs (100 – 500 for plant roots) (Noordwijk et al., 2004). Narrow hyphae can grow into small soil pores inaccessible to roots and root hairs. The ratio of length of hyphae to root

length has been measured in the range of 300 to over 8000 (Jones et el., 1990) and expressed per unit of soil volume, values of 16 to 2000 m hyphae/cm^3 have been measured in plantations and forests (Pampolina et el., 2001).

Arbuscular mycorrhizal fungus has an influence on carbon cycling through allocation of carbon produced by the plant through photosynthesis via the roots to the mycorrhizal hyphae. The carbon is subsequently respired to carbon dioxide, incorporated to the hyphal biomass and exuded in the form of carbohydrates to soil heterotrophs (Wright and Millner, 1994). The exchange occurs in the arbuscles, for endomycorrhiza. Completely dependent on its host plant, the fungus is an obligate symbiont thus, acquires all its metabolic energy via carbohydrate transfer across the plant-fungus interface (Noordwijk et al., 2004). The increased carbon supply to the soil accumulates in patches and at the end of hyphal mats (Finlay and Soderstrom, 1992) and boosts the energy supply to the detrital food web, benefiting saprophytic microbes and other soil organisms (Barea, 2000). Mycorrhizal fungi are estimated to consume 15-50 % of net primary production (Vogt et al., 1982). Johnson et al., (2002) in an experiment using *in situ* $^{13}CO_2$ pulse labeling of upland grassland, found out that mycelium provided a rapid and important pathway of C flux from plants to the soil and atmosphere. Soil temperature was found to increase the speed at which plant photosynthate was transferred to and respired by roots and arbuscular mycorrhiza and also increased the amounts of C respired per unit hyphal length.

Mycorrhiza influence on nitrogen cycle occurs by immobilization of nitrogen in hyphal and spore biomass, uptake and transport of N from micro sites in the soil to host plants,

transformation of inorganic N through nitrogen reductase and indirect enhancement of nitrogen fixation (Ocampo and Hayman, 1981). Stable isotopes labeling experiments have shown that inorganic N (NH_{4+} and NO_{3-}) taken by the fungus outside the roots is incorporated into AA (arginine), translocated from the extraradicle to the intraradical mycelium as arginine but transferred to the plant without carbon (Govindarajulu et al., 2005). In the same experiment, it was found out that the genes for primary nitrogen assimilation are preferentially expressed in the extraradical tissue whereas the genes associated with arginine breakdown are more highly expressed in the intraradical mycelium.

Arbuscular mycorrhizal fungi role in phosphorus cycling is the most important one than that of other nutrients with rates of P inflow to mycorrhizal roots being greater by 17 x 10^{-14} moles cm^{-1} s^{-1} than that of non-mycorrhizal roots which is 3.6 x 10^{-14} moles cm^{-1} s^{-1} (Sanders and Tinker, 1973). A nutrient depletion zone develops when nutrients are removed by plant roots more rapidly than they can be replaced by diffusion. For the poorly mobile phosphate ion, a sharp and narrow depletion zone develops close to the root. Hyphae readily bridge this gap with an adequate supply of nutrients (Smith and Read, 1997). Some AM fungi produce large quantities of oxalic acid, which helps to release inorganic and organic phosphorus. Through mineralization of organic matter, mycorrhiza fungi assist to release inorganic P by phosphatase-mediated hydrolysis of organic phosphate ester bonds. Micronutrients such as zinc and copper are also improved by mycorrhiza in the same way as phosphorus because these elements are also diffusion limited in many soils (Gianinazzi and Schuepp, 1994).

2.4 Transfer of Carbon and Nutrients between Plants

Mycorrhizas form a large network of mycelium and since different plants may be colonized by the same species of mycorrhiza, a common below ground hyphal network may connect different species of trees and other plants. This network forms a pathway for movement of carbon and nutrients between plants and it is particularly important in the transfer of nitrogen from nitrogen fixing trees or crops to receiver plants (Wu et al., 2001; Simard et al., 2002). Secondly the roots and mycorrhizas may take up nitrogen released by decomposition of leaves and root litter or from root exudation of nitrogen compounds (Lindahl et al., 2007). Mycorrhizas are able to transport both organic and inorganic forms of nitrogen both of which may be available in dying roots (Goldbold and Sharrock, 2003). In an experiment to investigate nitrogen exchange through common mycorrhiza network with soyabeans and sorghum, Xinhua (2000) found out that all originally none-mycorrhizal seedlings were colonized and mycorrhiza hyphae penetration through a nylon mesh was directly observed. In this experiment, colonization of roots as high as 80% confirmed that, common AM networks were established between pairs of all combinations. Ingleby et al., (2007) showed that, AM fungal inoculant spread from *Calliandra calothyrsus* to three successive intercrops of maize or beans sown 25, 50 and 75 cm away from the tree once the trees were six moths old.

2.5 Importance of Mycorrhiza for Plant Water Relations

Arbuscular mycorrhizal fungi can improve the water relations of a plant through mechanisms such as increased root hydraulic conductivity, alteration of stomatal regulation through hormone signals, hyphal water transport or improved P and K

nutrition (Sieverding, 1991; Jarstfer and Sylvia, 1992; Goldbold and Sharrock, 2003). When plants are at maximum transpiration, the root diameter shrinks rupturing the film of water around the root. AM fungal hyphae function as physical bridges ensuring contact between the root and the soil water. Hence flow of water to the roots is maintained (Marschrner, 1992). The increased growth rate and an intense deeper root system means that, water is extracted more efficiently, significantly increasing water use efficiency (Smucker and Hopkins, 2002). Further, it has been observed that, AM plants recuperate faster after a period of water stress than plants without mycorrhiza (Awatonye et al., 1992). Mycorrhizal fungi can grow at water potential levels lower than those that plants can tolerate thus remaining metabolically active and scavenging for water and nutrients in conditions when plants would cease to grow (Deacon,1987). Soyabeans inoculated with two species of mycorrhza and the relative water content of leaf taken at flowering and seed maturation stage showed that, all mycorrhizal plants had higher water content than the non-mycorrhizal plants irrespective of soil moisture level (Aliasgharzad et al., 2006).

In an experiment to quantify the contribution of the hyphae to plant water uptake, Khalvati et al., (2005) found out that drought resistance was comparatively increased in mycorrhizal host plants which suffered smaller decreases in leaf elongation, net photosynthetic rate, stomatal conductance and turgor pressure compared to the non-mycorrhizal plants. In the same experiment, quantification of the contribution of Arbuscular mycorrhiza hyphae to root water uptake showed that mycorrhizal plants transferred 4% of water in their compartment through the AM hyphae under drought conditions compared to the non-mycorrhizal treatments.

2.6. Functions of AM in Soil Aggregation

Mycorrhiza fungi aggregate soil particles in two ways; chemical bonding or gluing, and physical binding (Tsidall, 1994). The exudates produced by mycorrhizal filaments, glomalin containing a glycoprotein, glue fine particles of soil together to form microaggregates smaller than 250 μm (Tsidall 1994, Dodd, 2000). Glomalin acts either by coating fine particles with a layer of hydrophobic materials and by gluing the particles together (Miller and Jastrow, 2000). Fungal hyphae and plant roots bind microaggregates to form medium and large sized particles called macro aggregates (Munyanziza et al; 1997). Up to 20 m of hyphae have been found per gram of soil and together with the roots to which they are attached form considerable network through soil (Tsidall, 1994). As a consequence, they contribute to most of the macroaggregation of soil. Mycorrhizal formation in soils results in an increased movement of carbon into roots and rhizosphere via better root growth and respiration (Dodd, 2000). Increase in bulk soil volume is through extra radical mycelium. They provide a physical structure that can entangle soil particles and lead to micro and macro aggregate formation (Dodd, 2000; Munyanziza et al., 1997). This leads to soil stabilization via reduced soil erosion and hence improved ecosystem.

In an experiment to determine the effect of mycorrhizae on the stability of water-soluble soil aggregates and on selected group of microorganisms, water-soluble aggregates stability was significantly correlated with root or hyphal length (Andrade et al., 1998). Riling et al., (2002) used path analysis to show that AMF hyphae and their products

(glomalin) were significant contributors to soil aggregate water stability in a California grassland experiment.

2.7 Arbuscular Mycorrhiza and Plant Pathogen Interactions

Mycorrhizas have been proven to increase the resistance of plants to infection by root pathogens such as *Phytophthor*a parasites and *Fusarium oxysporum* (Newsman et al, 1995). This could be due to morphological and physiological/biochemical alterations in the host plant. Morphological changes may entail lignification of cell walls or increased production of other polysaccharides and increased vascular system of the plant. This results in reduced penetration of the plant tissue by plant pathogens and increased nutrient and water flow in the plant. Biochemical changes result from increased concentration of P and K in the plant tissue and increased uptake of other nutrients, which combine to decrease the susceptibility of plants to diseases. This is especially so in the case when mycorrhizal fungi colonize roots before the pathogen attacks (Sierverding, 1991, Sharma et al., 1992). The mechanisms involved include production of antibiotics by the mycorrhizal fungi, stimulation of host defense mechanism and physical barrier provided by the hyphae. Mycorrhizal fungi have also been shown to reduce damage to plants by sedentary endoparasitic nematodes (Goldbold and Sharrock, 2003).

In an experiment to determine whether mycorrhizal fungi alter plant pathogen interactions, Borowicz (2001) found out that mycorrhizal fungi tended to decrease the harmful effect of fungal pathogens but to increase the harmful effects of nematodes depending on the feeding patterns. Arbuscular mycorrhizal fungi harmed sedentary

nematodes but improved growth of migratory parasites though this outcome was not significant. Mycorrhiza inoculated tomatoes infected with fusarium showed only 9% necrotic roots compared to 32% in non-mycorrhizal plants (Werner, 1992). Ryan et al., (2000). However, this showed that inoculation of potato plants stimulated production of the potato cyst nematode-selective hutching chemical.

2.8 Benefits of Mycorrhiza Symbiosis to the Fungus

The symbiosis between mycorrhiza and plants is mutual and necessary to the survival of the fungi. Quantities of Carbon (C) allocation by the plant to the extraradicle mycelium ranges between 2 and 20% of current assimilate (Pearson and Jakobsen 1993, Smith and Read, 1997). The fungi, for their development and functional activity utilize one to seventeen percent of the carbohydrates, which the plant submits for root biomass production (Sieverding, 1991). Despite the substantial C drain on their hosts, the actual cost of mycorrhizas to plants is said to be negligible because mycorrhizal colonization can increase the rate of photosynthesis (Wright et al., 1998),This would also cause a substantial increase in leaf area arising from improved nutrition (Read and Perez-Moreno, 2003; Ha and Gray, 2008). Carbohydrate flux appears to be regulated by the host plant and it is dependent on the mycorrhiza fungal species (Read et al., 1992). Demand of AM for elemental nutrients such as nitrogen, potassium, phosphorus, calcium and magnesium is low (Sieverding, 1991).

2.9 Soyabeans (*Glycine max*)

Soyabean is a legume whose seed contains 40% proteins, 21% oil, 34% carbohydrate and 5% ash (Keyser and Li, 1992). It is an annual plant that may vary in growth habit and height. It may grow prostrate not growing higher than 20 cm or erect up to 2 metres. Many tropical Soya bean crops are determinate. Soyabeans are classified as oil crops and are the most important source of vegetable oil in the world but they are also used as an animal feed and for human consumption.

Soya beans are nodulated effectively by all the recognized species of *Bradyrhizobium*. The fast growing *Sinorrhizobium fredii* and *S. xinjiangenesis* also nodulate Soya beans but not as effectively as *Bradyrhizobium* which is also relatively host specific (Giller 2001). Biological nitrogen fixation by Soya beans was estimated at 88 kilograms nitrogen per hectare (Schroder, 1992), but can fix up to 300 kg under good conditions (Bohlool et al., 1992). Response to rhizobium inoculation is controlled by levels of indigenous competing *Brandyrhizobium*, nitrogen demand and yield potential of the host, levels of soil nutrients and adverse climatic factors (Olutajo, 1992). Soya beans, being legumes are coarse rooted and are often heavily colonized by AM fungi under natural conditions (Marschner, 1992).

2.10 Knowledge Gaps

For the benefits accruing from the practice of agroforestry farming to be realized, sustainability of the systems is very important. For sustainability to be realized the ability of the soils to provide nutrients namely soil fertility, is of utmost importance. In degraded

acidic farms belonging to resource poor smallholder farmers, the alternative to artificial fertilizer for replenishment of lost soil nutrients is the manipulation of biological processes like symbiotic relations of mycorrhizas fungi and rhizobium bacteria with plants. Utilization of these microorganisms has not been adopted in Kenya due to inadequate knowledge. A multifunctional biofertilizer suitable for use by smallholder resource poor farmers need to be researched on and developed. Once introduced, a study of the microorganism's persistence in the soils will be required. With this information, a basis for effective utilization of the microorganisms by smallholder farmers will have been laid down.

CHAPTER 3

METHODOLOGY

3.1 Site Description

The on-farm experiment was carried out in Gatanga division, Thika district in the Central Province of Kenya. The area is located at 38^{o} 58' 0" E and 0^{o} 55' 59" S and at an altitude of 1680 m above sea level. The area lies on the eastern slopes of the Nyandarua Ranges, and receives 1000 mm rainfall annually in two seasons that start in mid-March to June and in mid-October to December. The average annual temperature is 25^{0} C. The soils are well-drained, extremely deep dusky red friable clays with acid humic topsoil, typically humic Nitisols developed on tertiary basic igneous rocks (according to FAO/UNESCO, 1974). The soils are leached and acidic (Jaetzold and Schmidt, 1983). The topography of the area is undulating and rolling. The population of Gatanga from the 1999 census is 103,048 with a density of 410 persons per KM^{2}. The average farm holding size is 0.25 Ha (Ministry of Agriculture, Thika district).

Kenyatta University lies in Upper Midland 4 (UM4). It is located at 37^{o} 10' 0" E and 0^{o} 34' 0" S and at an altitude of 1650 m above sea level. Average annual temperature is 25^{0}C. It is in a semi-humid climatic zone with a total bi-modal rainfall of 750mm per year received in two distinct seasons, the long rains (LR) mid March to June and short rains (SR) mid-October to December.

3.2 Treatments and Experimental Design

The experiment was conducted both on-farm, in Gatanga and on-station at Kenyatta university. The test crop was soyabean (*Glycine max)*. In the on-farm study, the experiments were laid out in a complete randomized block design (CRBD). Plot size was 7 m by 3.5 m in two blocks and the crop was grown for two seasons, long rains 2005 and short rains 2005. Treatments within the block consisted of:

i) Soyabeans inoculated with rhizobium (S+R)

ii) Soyabeans inoculated with both rhizobium and mycorrhiza (S+R+M)

iii) Soyabeans inoculated with rhizobium plus P fertilizer (S+P+R)

iv) Soyabeans inoculated with mycorrhiza plus P fertilizers (S+P+M)

v) Soyabeans inoculated with mycorrhiza (S+M)

vi) Soyabeans Plus P fertilizers (S+P)

vii) Soyabeans on its own (S) (Control)

Each treatment consisted of a row of 25 plants and the plants were replications of the treatment. Planting was done at the recommended spacing of 45 x 15 cm. Treatments allocated to the rows were determined through random sampling. Mycorrhiza inoculant consisting of three species of AM, namely, *Glomus etunicatum, Glomurus intraradices and Gigaspora albida* mixed together was applied to the treatments S+R+M, S+P+M and S+M by placing 10 grams of it below the seeds in the planting hole. Triple super phosphate fertilizer containing 46 kg of P_2O_5 per 100 kg was applied at the rate of 250 kg per hectare to the treatments S+P+R, S+P+M and S+P. Soyabeans rhizobium inoculant in a sterile carrier was sourced from Kabete campus and was applied at the rate of 50 grams

per 15 kg soyabeans to the treatments S+R, S+R+M and S+P+M.. The control treatment consisted of soyabeans planted on its own.

In the on-station study, the experiments were laid out in a complete randomized design (CRD). Each treatment consisted of three 20cm diameter half litter planting pots, one set with sterilized soil and the other with non-sterilized soil collected from Gatanga filled to the same level. The soil was sterilized in an oven with hot air at 100^0C for 48 hours. There were seven treatments, the same ones as detailed for on-farm experimentation. Each treatment was replicated three times making a total of 42 plots. Treatments were randomly allocated to pots and three seeds of soyabeans per pot planted and later thinned to one plant per pot. The plants were raised in a green house for four months. Plants were watered once per day for 8 days when germination occurred and then once per week for the next three months.

3.3 Data Collection

Top soil (0-20cm) collected from Gatanga was analyzed at a laboratory for pH, soil organic matter, available phosphorus, total nitrogen, exchangeable potassium, magnesium, calcium and cation exchange capacity. Germination percentage was determined at eight days by counting the number of plants that had emerged. Using systematic sampling method, height growth of the plants was measured after every 15 days by measuring the distance from soil level to the growing apex of each plant, starting one month after planting to the onset of flowering, when increase in plant height ceased. Root collar diameter was taken at the end of three months after planting. Using simple

random sampling technique, plants were selected and measured using vanier calipers. At flowering and seed setting stage, five plants and one plant in the on-field and on-station experiments respectively were selected through destructive sampling using a hoe. Soils adhering to the root were washed off using a gentle stream of tap water. The nodules were then detached and counted. At harvesting, 4 plants were selected randomly and their grain yields weighed after hand threshing. For the same plants, their above and below ground biomass was dried in an oven at 50^0C until constant weight was obtained.

3.4 Laboratory Procedures and Analysis

3.4.1 Soil Analysis

Top soil was collected from the study site at Gatanga before planting and was analyzed for soil pH, organic matter content, total nitrogen, potassium and phosphorus. The samples were collected from 5 cores (diagonally and at the center) to a depth of 0-20 cm. The soil was then composited together and mixed thoroughly while removing all visible plant debris to ensure homogeneity after which, it was put in polythene paper and taken to the University of Nairobi, Kabete campus laboratory for analysis.

3.4.1.1 Determination of Soil pH

Soil pH was measured using a pH meter and glass electrode on the ratio of 2.5:1 water to soil suspension. Some 25 ml of deionised water was added with a dispenser to 10 milligrams of the soil collected from Gatanga in a 60 millilitre bottle. The solution was stirred for ten minutes and left to stand for 20 minutes. The soil was then allowed to settle

after which a pH electrode was immersed and readings taken after stabilization of the pH readings.

3.4.1.2 Determination of Soil Organic Matter

Soil organic matter was obtained using the Walkle-Black method (Okalebo et al., 2002) where the organic carbon was oxidized with potassium dichromate in the presence of concentrated sulphuric acid. A soil sample that was passed through a 2 mm sieve to remove the coarse fraction was ground to pass a 0.5 mm sieve, to increase homogeneity and to facilitate the oxidation. Two (2) grams of this soil was put in a conical flask and 10 ml of 1 N potassium dichromate added with a pipette and swirled to oxidize the carbon in the soil after which, 20 ml of concentrated sulphuric acid (36 N) was poured in a steady stream into the soil dichromate mixture. The heat of dilution obtained by adding sulphuric acid supplied a standard amount of heat to assist the oxidation. The mixture was then left to cool for 20 minutes. Distilled water was added to bring the volume to 200ml after which 5.0 ml of 85% orthophosphoric acid and 5.0 ml diphenylamine sulphonate indicator were added. The mixture was titrated with 0.1 N ferrous sulphates to reduce the remaining dichromate. The mixture changed to light green colour from turbid dark blue on reaching the end point. Percentage of carbon in the soil was calculated using the following formula which takes into account the fact that 1 ml of dichromate oxidizes 0.39 mg of carbon (the average recovery rate of 77% is taken into account).

$$\% \text{ Carbon} = \frac{(\text{m.e dichromate} - \text{m.e } FeSO_4)}{\text{Weight of soil in grams}} \times 0.39$$

The resultant percentage carbon was then multiplied by 2 to give the percentage soil organic matter (C forms an average of 58% of soil organic matter).

3.4.1.3 Determination of Available Phosphorus

Determination of phosphorus was done using the Olsen method (Okalebo et al., 2002). Some 2.5 grams of sieved soil were put in a polythene-shaking bottle and 50 ml Olsen extraction solution (0.5 M $NAHCO_3$ pH 8.5) was added. The mixture was shaken on a mechanical shaker for 5 minutes then filtered through Whatman No 5 paper after which, 10 ml of P standard solution and 10 ml of the sample and 2 reagent blanks were put in flasks and 5 ml 0.8 boric acid added to each flask. After this, 10 ml of ascorbic acid reagent were added to each flask and distilled water added to fill to the 50 ml mark. The contents were stoppered and shaken well. After one hour, the absorbance of the solution at a wavelength setting of 880 nm was measured. Parts per million (ppm) of the solution was then obtained from the standard P calibration curve. The concentration of phosphorus in the sample expressed as P mg kg^{-1} was calculated using the formula:

$$P = \frac{(a-b) \times v \times f \times 1000}{1000 \times w}$$

Where

a = concentration of P in the sample

b = concentration of P in the blank

v = volume of the extracting solution

f = dilution factor

w = weight of the sample.

3.4.1.4 Determination of Total Nitrogen

Total nitrogen was determined using wet oxidation method based on the Kjeldahl digestion procedure with sulphuric acid and a catalyst (Anderson and Ingram, 1993). A portion of soil sample was ground and passed through a 0.5 mm sieve. Then, 0.3g of it taken and digested using 2.5 ml digestion mixture (dissolved 3.2 g salicylic acid in 100ml of sulphuric acid – selenium mixture.) and digested at 110^0C for 1 hour. After that, the solution was cooled and hydrogen peroxide added and heated at 330^0C until the solution became colourless. Some 25 ml distilled water was added until all sediments dissolved. Total nitrogen was determined calorimetrically, absorbency taken at 650nm and calculated as follows:

$$\% \text{ N in soil sample} = \frac{(a - b) \times v \times 100}{1000 \times w \times al \times 1000}$$

Where:

a = Concentration of N in the solution

b= Concentration of N in the blank

v = Total volume at the end of analysis procedure

w = Weight of the dried soil sample taken

al = Aliquot of the solution taken for analysis.

Determination of Exchangeable Potassium, Sodium, Calcium and Magnesium and Cation Exchange Capacity

The exchangeable potassium, sodium, calcium and magnesium were extracted from the soil sample by leaching with neutral normal ammonium acetate. Acid washed sand (5 grams) was placed in numbered plastic funnels fitted with a plug of absorbent cotton

wool, after which 5 grams of soil mixed with 5 ml sand was added. Another layer of sand was placed on top of the funnel. A Whatman filter paper No.42 was placed in a funnel, which was then placed into the neck of a 250ml flask. Some 10 aliquot of 20 ml ammonium acetate was then added to the funnel and allowed to drain through. This was repeated a number of times after which the flask was withdrawn. The ammonium ions (NH_4^+) replaced the exchangeable cations originally held by the soil. The exchangeable Calcium and Magnesium ions present in the ammonium acetate leachate were determined by titration with EDTA, while the potassium and sodium were determined by using the flame photometry.

Determination of cation exchange capacity was done using the same funnels of soil and sand. Any remaining ammonium acetate was removed from the sample by repeatedly washing with methyl alcohol. The alcohol washed soil was then transferred to a round bottomed flask and 500 ml of water added and connected to a liebig condenser leading to a 500 ml conical flask containing 20 ml of 2% boric acid and a few drops of mixed indicator (methyl red and methyl blue in methanol). Then, 3 spatulas of magnesium oxide (to displace the NH_4 held in by the soil) were added to the sample. The contents of the flask were heated until 300 ml had distilled over and collected in the receiver. The liquid in the receiver changed from blue to green. The contents of the receiver were then titrated with 0.1 N HCl to a pink end point in order to determine the amount of NH_4 in the distillate. Each ml of 0.1 N HCl used in the titration was equivalent to 2 m.e. per 100 g exchangeable capacity as the original soil sample weighed 5.0 g.

3.4.1.6 Determination of Mycorrhiza Spore Count

This was done using the wet sieving and decanting method (Gerdemann and Nicolson, 1963) followed by the sucrose centrifugation method (Daniels and Skipper, 1982). The soil sample was suspended in water and then decanted through a series of sieves (sieves with 0.350 mm, 0.125 mm and 0.045 mm). The contents of the medium and finest sieves were transferred separately with some water to 100 ml centrifuge tubes and 40 ml sugar solution (70 g dissolved in 100 ml water) injected into the bottom of the tube so that a gradient was established. The sample was centrifuged at 2000 revolutions per minute for 2 minutes. During this process, soil particles settled on the bottom and spores remained on the surface. Spores were extracted with a syringe and placed on a clean sieve with 0.045 mm mesh opening and the spores washed with water for about 3 minutes and then transferred in water to a petri dish. The sample was observed through a stereomicroscope at 40X magnification. The spores were then counted.

3.5 Data Analysis

Data was subjected to analysis of variance using Genstat for Windows program version 8.11 computer software (Genstat, 2005). Standard error of differences of means was used to separate treatment means at 95% confidence interval. Mycorrhiza dependency was calculated as the yield of inoculated plants minus the yields of non-inoculated plants divided by the yield of non-inoculated plants multiplied by 100.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

This chapter presents the findings of the study and the discussions. The soils chemical properties and the mycorrhiza inoculant spore count are presented first. Secondly, the effect of dual inoculation of soyabeans with mycorrhiza and rhizobium on its germination, height, root collar diameter, roots dry weight, shoots dry weight, grain yield, and mycorrhiza dependency and on nitrogen fixation is discussed. Each of the six treatments is compared to the control (S) followed by pairwise comparison between the various treatments of interest.

4.1.1 Soil Chemical Properties

The soils chemical properties for the Gatanga study site are as shown in Table 1

Table 1 Soil chemical properties for Gatanga site

Soil Parameter	Level of Nutrient	Optimum Level	Remarks
pH 1:2.5 (soil: water)	5.4	5.5-7.0	Acidic
Phosphorus	8.0 ppm	15-25	Low
% Nitrogen	0.1%	0.2-0.5	Low
%Carbon	0.5%	10-30	Low
% Soil organic matter	1.0%	20-60	Low
Potassium	0.3 Cmol/kg	0.2-0.6	Adequate
Magnesium	0.4 Cmol/Kg	0.5-4	Adequate
Calcium	2.9 Cmol/kg	4-10	Low
Sodium	0.1 Cmol/kg	0.2-0.5	Low
CEC	15.5 Cmol/kg	15-25	Low

4.1.2 Soil pH

The soil in the area can be classified as moderately acidic since it had a mean pH of 5.4, water based (Table 1). This could have resulted from leaching down of most of the base cations leaving behind clay colloids that could be dominated by aluminium and hydrogen ions (Giller, 2001). At pH levels below 5.5, H^+ are released when high levels of aluminum ions (Al^{3+}) in the soil react with water molecule (aluminium hydrolysis). Aluminum ions could be concentrated enough to have limited or retarded root development and as a result plants do not absorb water or nutrients. Hence, they become stunted and exhibit nutrient deficiency syndromes (Ball, 1999). Soil pH is one of the most important soil properties that affect the availability of nutrients. Macronutrients like magnesium, calcium and phosphorus tend to be less available in acidic soils while micronutrients like manganese, iron, boron, zinc and copper tend to be available in soils with high pH but become less available when pH is raised above 8 (Muriuki and Quareshi, 2001). Molybdenum availability, on the other hand, decreases as soil pH decreases. This fact is especially important for legume production. The nodules of leguminous crops contain the enzyme nitrogenase which is rich in molybdenum. Thus, if soil pH is low and available molybdenum is low, legumes will appear N stressed and production of leguminous crops would suffer. The release of major elements from soil organic matter through mineralization could have been regulated indirectly by soil pH through its influence on microbial activity since phosphorus mineralization is greater in near neutral soils than in more acidic soils (Muriuki and Quareshi, 2001).

4.1.3 Available Phosphorus

The available soil phosphorus level was low (8.0 ppm) compared to the optimum level for plant growth (Table 1) and could be attributed to it being chemically bound to iron and aluminium to form a relatively insoluble precipitate rendering it unavailable to plants (Giller, 2001). Phosphorus availability is strongly influenced by soil pH. At low pH (pH < 5.5), phosphates of low solubility are formed after reacting with free aluminum and iron ions in the soil effectively "tying" it up (Wieldetholt and Johnson, 2005). Basic soil conditions (pH > 7.5) cause excessive calcium to be present in soil solution which can precipitate with P decreasing its availability. Phosphorus is reported to be most available for plant uptake at pH 5.5-7.5 (Muriuki and quareshi, 2001).

Phosphorus may have existed in soils of Gatanga as organic or inorganic forms and since the soils in the Gatanga site are low in organic matter, which is an important source of labile or easily mineralized phosphorus, changes in soil organic matter (SOM) are likely to be accompanied by changes in availability of P to plant (Frizano, 1999).

4.1.4 Total Nitrogen

The soil's total nitrogen content was low (0.1%) as shown in Table 1. This could have resulted from the low organic matter status of the soils in the Gatanga site. Soil organic matter is one of the sources of nitrogen, that is, from its mineralization. Differences in the quantity and quality of soil organic nitrogen are known to affect the nitrogen mineralization rates in the field (Schroth et al., 2003). The presence of adequate food in the form of soil organic matter for microorganisms boosts their numbers and contribution

to mineralization (Sande et al., 2001). Soil microorganisms play a major role as a catalyst in the decomposition of soil organic matter (SOM) and release of inorganic nutrients to the soil. The nutrients then become available for plant uptake (Smith, 1994). Due to the low organic matter content of the Gatanga soils, soil microorganisms must have been low resulting to low mineralization of organic nitrogen and hence, low total nitrogen level.

4.1.5 Soil Organic Matter

The soils in the Gatanga site had low soil organic matter (1%) as shown in Table 1. Soil organic matter consists of living microbes, partially decayed plant materials and humus (Griffin, 2006). Crop removal and little addition of organic material in the form of manure in the area may have contributed to the low level of organic matter in the soil. Inadequate or no use at all of fertilizers could have also resulted to low crop yields and consequently, little amounts of residues being returned to the soil thus contributing to the low levels of organic matter. Soil organic matter acts as a revolving bank account for nutrients and improves soil structure and minimizes soil erosion through the binding of small soil particles into larger aggregates by crop residues and some microorganisms like mycorrhiza (Perucci et al., 2000). In soils dominated by low activity clay as is the case with the Gatanga site, the cation exchange capacity, and thus the ability of the soils to retain nutrients against leaching in a plant-available form, depends strongly on its organic matter content (Schroth et al., 2003)

4.1.6 Potassium

The soils at the Gatanga site had adequate potassium as shown in Table 1. This could be explained from the possibility that, the rocks that formed the soil in Gatanga had adequate amount of potassium. The result is in agreement with the findings of other researchers who have reported that potassium is not a major limiting nutrient in Central Kenya (Gikonyo et al., 2000). Potassium is one of the essential nutrients for plant growth and it is classified as a macronutrient (Muriuki and Quareshi, 2001). It is associated with the movement of water, nutrients and carbohydrates in plant tissue. Potassium (K) is not an integral part of any major plant component, but it does play a key role in a vast array of physiological processes, vital for plant growth such as protein synthesis and maintenance of plant water balance (Beegle, 1990).

4.1.7 Magnesium

Magnesium levels in the soil were low as shown in Table 1 but not inadequate since it was at 0.4 Cmol/kg against the optimum for plant growth, that is, 0.5-4 Cmol/kg. This situation could have been caused by the soil's low organic matter status. The soil's ability to hold cations like magnesium is thus low and the nutrient is easily leached. Magnesium is the central core of the chlorophyll molecule in plant tissue, thus its deficiency results in poor and stunted plant growth (Maryland, 1983).

4.1.8 Calcium

Calcium levels in the soil were low as Table 1 shows. This could have been as a result of low organic matter in the soil leading to inability of the soils to retain the cation and

consequently, leaching results. Calcium is an integral part of plant cell walls (Muriuki and Quareshi, 2001).

4.1.9 Sodium

Sodium levels were low at 0.1% compared to the optimal levels for crop growth as shown in Table 1. High sodium levels of over 0.5% would result to soil dispersion, poor water infiltration, and possible sodium toxicity in plants (Muriuki and Quareshi, 2001).

4.1.10 Cation Exchange Capacity

Cation exchange capacity is the value given to a soil's ability to hold cation nutrients. Cations are positively charged elements (Muriuki and Quareshi, 2001). The soil's most abundant exchangeable cations are calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), sodium (Na^{+}) and aluminium (Al^{3+}). Cations are held by negatively charged particles of clay and humus colloids (Sachs, 1999). The soils at the Gatanga experimental site had low cation exchange capacity (15.5 Cmol/kg) as Table 1 shows. This might have arisen from the soils low organic matter status. The implication is that the soil nutrients and water holding capacity is low. Soluble elements like potassium sulphate cannot be held efficiently because the cation reservoir (humus and clay) is low. Microorganisms such as mycorrhiza are inhibited by lack of water since they are obligate symbionts relying on plant roots for their survival. These soils are also prone to leaching since the cation nutrients are in the soil water. CEC may be improved by adding lime and hence raising pH or by adding organic matter.

4.2 Mycorrhiza Inoculant's Spore Count

The mycorrhiza inoculant sourced from Kenya Forestry Research Institute (KEFRI), used in the on-station and on-field experiments was analyzed for the determination of spore count. The results are tabulated in Table 2. Three species of mycorrhiza, namely *Glomus intraradices, Glomus etunicatum and Gigaspora albida* were used to inoculate the soyabeans. In the long rains experiments, *Glomus etunicatum* had the highest spore count (205) while the other two, *Glomus intraradices* and *Gigaspora albida* had an equal number (140) of spores per 30 grams of the inoculant. In the short rains, and for the screen house experiments *Glomus etunicatum* had the highest number of spores (220) followed by *Glomus intraradices* (180) and *Gigaspora albida* (135) per 30 grams as shown in Table 2. Species and strains of mycorrhiza fungi have been known to differ in the extent to which they increase nutrient uptake and plant growth (Gracy Sailo and Bagyaraj, 2005) hence, the need to mix the three species of mycorrhiza in the trial.

In an experiment to study the efficacy of eleven mycorrhiza fungi on Kalmegh (*Andrographis paniculata*), it was observed that *Glomus leptotichum* and *Glomus intraradices* were the best AM symbioants in increasing plant biomass compared to the others (Chiramel et al., 2006). The two had higher root colonization and sporulation allowing more fungal-host contact and exchange of nutrients hence, better plant growth.

Table 2 Mycorrhiza Spore Count in the Inoculant Sourced from KEFRI Used in the On-field and On-station Experiments

Mycorrhiza Species	Number of Spores Per 30 Grams Pure Inoculum	
	Long Rains 2005	**Short Rains 2005 and On-Station**
Glomus intraradices	140	180
Glomus etunicatum	205	220
Gigaspora albida	140	135

4.3 Effect of Mycorrhiza and Rhizobium Inoculation on Soyabeans Germination

Soyabeans germination was significantly different for the various treatments ($p < 0.05$) in both the on-farm and on-station experiments as Table 3 shows. In the LR 2005 season, only the dual inoculated plants and the mycorrhiza treated plants (S+R+M and S+M) had significantly higher germination rates than the control (S). Soyabeans inoculated with mycorrhiza (S+M) had the highest germination percentage of 97.5% while soyabeans inoculated with rhizobium and planted with phosphatic fertilizers, (S+P+R) had the lowest germination percentage at 70%. The performance in decreasing order of the germination rate was S+M>S+R+M> S> S+P+M> S+R> S+P>S+P+R.

Pair wise comparison between the mycorrhizal plants and the control (soyabeans alone with 80% germination) in the on-farm experiments 2005 LR revealed that, inoculation significantly increased germination in the S+M (97.5) and S+R+M (87.5) %) treatments

but not in S+P+M where the rate of germination decreased though not significantly to 77.5%.

Table 3 Germination of Soyabeans On-field in Gatanga and On-station at Kenyatta University under Different Treatments

Treatments	On-farm		On-station	
	Long Rains (%)	Short Rains (%)	Sterile Soil (%)	Non-sterile Soil (%)
Soyabeans + Rhizobium	75.0cd	79.5a	77.7c	100.0a
Soyabeans + Rhizobium + Mycorrhiza	87.5b	67.0d	100.0a	89.0b
Soyabeans + P fertilizer + Rhizobium	70.0d	79.0a	77.7c	89.0b
Soyabeans + P fertilizer + Mycorrhiza	77.5c	54.0d	100.0a	100.0a
Soyabeans	80.0c	73.0b	89.00b	67.0c
Soyabeans + Mycorrhiza	97.5a	77.0a	67.0d	56.0d
Soyabeans + P fertilizer	70.5d	77.0a	67.0d	67.0c
SED	2.7	1.5	0.22	0.31

NOTE: Numbers in each column followed by the same letter are not significantly different at p = 0.05

Dual inoculation of soyabeans with mycorrhiza and rhizobium (S+R+M) resulted to a significantly higher germination (87%) compared to the rhizobium treated plants, S+R (75%) but was significantly reduced over the mycorrhiza inoculated plants S+M, (97.5%).

In the second season, short rains 2005, S+R had the highest germination percentage of 79.5% while soyabeans planted with phosphatic fertilizers and inoculated with mycorrhiza (S+P+M) had the lowest germination percentage (54%). The performance in decreasing order of the germination rate was S+R, S+P+R, S+M and S+P, S, S+R+M and S+P+M.

Treatments S+R, S+P+R, S+M and S+P had a germination rate that was significantly higher than the control (S), while S+R+M and S+P+M which are both mycorrhizal had significantly lower rate than the control (S) ($p < 0.05$). The singly inoculated plants S+R (79.5%) and S+M (77%) had significantly higher germination rates than the dual inoculated ones S+R+M (67%).

In the on-station sterile soils experiment, S+R+M and S+P+M had the highest germination rate of 100% while S+P and S+M had the lowest at 67%. The performance in decreasing order of germination was S+P+M=S+R+M>S>S+R=S+P+R> S+M=S+P. Only S+P+M and S+R+M (both mycorrhizal) had significantly higher germination rate than the control ($p < 0.05$).

The singly treated plants S+M and S+R had significantly lower germination rates than the dual inoculated ones (S+R+M) suggesting that, dual inoculation with mycorrhiza and rhizobium could have increased the rate of germination in sterile soils. S+P+R had significantly higher germination percentage than S+P indicating that, inoculation with rhizobium could have increased the rate of germination in P applied plants while with S+R the rate was the same. S+P+M had a significantly higher germination rate than S+P and S+M indicating that, mycorrhiza inoculation could have increased the rate of germination.

In the non-sterile soil, S+R and S+P+M had the highest germination rate of 100% while S+M had the lowest at 56%. The performance in order of decreasing rate of germination

was S+R and S+P+M, S+R+M and S+P+R, S and S+P, SM. Pair wise comparison between the treatments and the control (S) plants revealed that, there were significantly higher germination rates in S+R, S+R+M, S+P+M and S+P+R but not in the S+M and S+P treatments ($p < 0.05$). Dual inoculated plants (S+R+M) had significantly lower germination rates than the rhizobium inoculated (S+R), but significantly higher than mycorrhiza inoculated (S+M) treatments showing. This was an indication that, mycorrhiza could have played a role in enabling germination but not rhizobium.

The findings of the experiment were inconsistent thus failing to prove that inoculation with micorrhizal fungi and rhizobium bacteria could increase the germination rate of soyabeans. This can be explained by the fact that at germination, the plants had not developed roots and hence had not started the symbiotic relationship with the two microorganisms. Association starts when mycorrhiza hyphae respond to the presence of a root by growing towards it, establishing contact and growing along its surface (Brundrett et al., 1994). Many studies have shown that there is a lag phase between mycorrhizal inoculation and the time period when its effect is manifested in the plant (Brandon and Shelton, 1993). At germination phase, the fungus is at lag stage and it is autotrophic (Sieverding,1991)

This is in contrast to the findings by Kikuchi et al.(2007) who found out that, flavonoids contained in root exudates as the plant germinates, play a role in signaling molecules in symbiotic relationships between woody plants and ectomycorrhizal fungi. Likewise, the rhizobium bacteria is in its saprophytic phase and will only infect and gain entry into the root (infective phase), and eventually cooperate in the formation of a functioning root nodule (symbiotic phase) on receiving chemical (Flavanoids) signals from the plant as

they germinate. Brandon and Shelton (1997) came to the same conclusion when working with *Leucaena leucocephala* to determine the factors affecting its early growth.

Phosphorus extraction efficiency increased with time as AM fungi progressively increased root colonization thereby concluding that high rates of P would have to be used to compensate for slow early colonization. In their experiments, large effects of mycorrhiza inoculation were realized 41 days after sowing

Germination involves mobilization and utilization of food reserves (Howell 1960) and is affected by environmental factors such as temperatures, soil moisture, nutrients and oxygen supply (Fagena et al., 1997). Other factors affecting seed germination are the internal seed physiology for example seed vitality, genetic potential and seed maturation as well as hormonal or chemical changes that occur when the seed is accumulating its food reserves (Bennet, 2004). Any or a combination of these factors could explain the differences in the germination rates among the different treatments.

4.4 Effect of Inoculating Soyabeans with Mycorrhiza and Rhizobium on Height Increment

There were significant differences between the various treatments at all the three stages of height measurements that is at 30, 45, and 60 days after planting in the on field experiments (Table 4).

In the LR 2005 season, at the 30^{th} day, only treatments S+R+M and S+P+M (both mycorrhizal) had height increments significantly higher than the control S, while at the 45^{th} day, S+R+M, S+P+R, S+P+M and S+M were significantly higher than the control.

On the 60th day, S+R, S+R+M, S+P+R, S+P+M were significantly higher than the control. The enhanced height increment in the rhizobium treated mycorrhizal (S+R+M) plants could be attributed to enhanced nutrient absorption and greater rates of photosynthesis. Mycorrhiza colonization is known to enhance plant growth by increasing nutrients uptake and use (Marschner and Dell, 1994, Clark and Zeto, 2000). AM fungi, through its hyphae could have shortened the distance nutrients diffused through the soils to the roots.

Table 4: Height of Soyabeans during the Long and Short rains 2005 under Different Treatments at the On-field Experiment at Gatanga, Kenya

	Long Rains 2005 (cm)			Short Rains 2005 (cm)		
Treatment	30 days	45 days	60 days	30 days	45 days	60 days
Soyabeans + Rhizobium	9.20b	10.80d	19.90b	7.70c	10.70cd	14.20a
Soyabeans + Rhizobium + Mycorrhiza	14.90a	18.50a	26.90a	8.85a	12.65a	15.25a
Soyabeans + P fertilizer + Rhizobium	10.75b	14.55bc	21.95b	8.65ab	12.20ab	15.15a
Soyabeans + P fertilizer + Mycorrhiza	15.45a	16.00b	23.50ab	8.00ba	10.60d	14.95a
Soyabeans	7.90b	10.45d	14.30c	8.25abc	11.65bc	14.10a
Soyabeans + Mycorrhiza	10.55b	14.03c	16.30c	7.90bc	11.70ab	14.35a
Soyabeans + P fertilizer	9.00b	10.35d	15.75c	7.95bc	11.55bcd	13.75a
SED	1.90	1.24	1.71	0.39	0.50	0.84

NOTE: Numbers in each column followed by the same letter are not significantly different at p= 0.05.

In the on-field LR 2005 season experimentation, soyabeans height was significantly increased ($p < 0.05$) over that of the control (S) by dual inoculation with mycorrhiza and rhizobium (S+R+M) at all the three stages. Dual inoculation with mycorrhiza and

rhizobium (S+R+M) resulted to a height increment that was significantly higher than S+R and S+M (singly inoculated plants) at all the stages. This could have arisen from benefits accrued from the tripartite symbiosis of legume-AM fungi- rhizobium through stimulation of host nutrition (Barea et al., 1992).The extraradical mycelium of AM fungus grow beyond the phosphate depletion zone reaching a new pool of soluble phosphates (Smith and Read, 1997). The nitrogenase enzyme of the rhizobia fixes atmospheric nitrogen in the nodules and fungal hyphae facilitate the uptake of ions mainly phosphates in mycorrhizal roots (Postgate 1998, Leigh, 2002). The result is increased rate of photosynthesis and hence height increment.

Inoculating with rhizobium and applying phosphatic fertilizer (S+P+R) had a significantly higher height increment over inoculating with rhizobium singly (S+R) only on the 30^{th} day. Inoculating with rhizobium and applying phosphatic fertilizer (S+P+R) led to a significantly higher height increment over the plants with phosphatic fertilizers (S+P) on the 45^{th} and 60^{th} day. Provision of P fertilizer which was low in the soils contributed to increased height growth.

Inoculating with mycorrhizas and applying phosphatic fertilizers (S+P+M) resulted to height increment significantly higher than the mycorrhiza singly inoculated plants (S+M) and phosphatic fertilizer applied plants (S+P) at all the stages. The mycorrhizal inoculated plants (S+M) had the highest height at all the stages indicating the efficiency of mycorrhiza fungi in extracting nutrients especially P from the soil. Mycorrhizas are known to increase growth increment by increasing nutrients uptake notably P and other essential nutrients (Marschner and Dell, 1994; Clark and Zeto, 2000). This was achieved

through wider physical exploration of the soil by mycorrhizal fungi than the roots. The ratio of length of hyphae to root length has been measured in the range of 300 to over 8000 (Read and Boyd, 1986; Jones et al., 2001).

During the short rains, 2005 season, just as during the long rains, the differences between the various treatments were significantly different ($p< 0.05$) at all the stages. All the treatments except the dual inoculated plants (S+R+M) on the 45^{th} day were not significantly higher than the control (S) at all the stages. Dual inoculation with mycorrhizal fungi and rhizobium bacteria (S+R+M) resulted to a growth increment that was significantly higher than rhizobium singly inoculated plants (S+R) on the 30 and 45^{th} day but not on the 60^{th} day. Inoculating with mycorrhiza and rhizobium (S+R+M) resulted to a significant height increment over (S+M) only on the 30^{th} day but not the other stages. The results indicate that the short rains crop did not benefit from the soyabeans-rhizobium-mycorrhiza symbiosis which would have led to increased rate of photosynthesis and hence height increment. In the long rains 2005, season when moisture was not limiting, the rhizobium bacteria fixed atmospheric nitrogen to ammonia which could have been assimilated by the plants and converted to amino acids and hence the growth increase. In the short rains 2005 season, moisture stress could have affected the efficiency of these bacteria in fixing atmospheric nitrogen since P and other nutrients can only be absorbed in solution. Soyabeans planted with P alone (S+P) showed non-significant height increment over the control (S) in the field experiments. Although P was provided, plants roots could not adequately absorb it without the benefit of increased surface area that could have arisen from the mycorrhiza association. Inoculating with

mycorrhiza and applying P fertilizer (S+P+M) resulted in a height increment over S+M and S+P that was not significant at any of the stages in the short rains. Inoculating soyabeans with rhizobium and applying P fertilizer (S+P+R) resulted to height increment over planting with P alone (S+P) that was not significant at all the stages in the short rains.

In the on station experiments, the differences between the various treatments in both the sterile and non-sterile soils were significantly different ($p < 0.05$) except on the 60^{th} day in the sterile soils (Table 5). In these experiments, dual inoculation with mycorrhiza and rhizobium (S+R+M) resulted to a height increment that was significantly higher ($p < 0.05$) from the control (S) on the 30th and 45th day but not so on the 60th day in the sterile soil. In the non sterile soil, there was significant height increment of the dual inoculated plants (S+R+M) over the control (S) only on the 30th day. There could have been indigenous microorganisms which contributed to some improved nutrient absorption resulting to height increment in the non sterile soils. Dual inoculation with rhizobium and mycorrhiza (S+R+M) in the on-station sterile soils experiment resulted to a height increment that was significantly higher than inoculating with rhizobium alone (S+R) on the 30^{th} and 45^{th} day and not on the 60th day. In the non-sterile, dual inoculation (S+R+M) resulted to a height increment that was significantly higher than inoculating with rhizobium alone (S+R) only on the 30^{th} day.

Table 5: Height of Soyabeans in the On-station Sterile and Non-sterile Soils under Different Treatments at Kenyatta University

	Sterile soil (cm)			Non-sterile soil (cm)		
Treatment	30 days	45 days	60 days	30 days	45 days	60 days
Soyabeans + Rhizobium	16.2cd	41.7bc	58.7	27.9c	51.0b	75.7b
Soyabeans + Rhizobium + Mycorrhiza	25.4a	49.3a	61.7	34.7a	53.0b	73.7b
Soyabeans + P fertilizer + Rhizobium	14.0de	20.0d	59.7	12.1d	25.0c	56.7c
Soyabeans + P fertilizer + Mycorrhiza	23.7ab	53.0a	75.3	35.0a	54.0b	74.7b
Soyabeans	15.7d	36.0c	56.7	28.9c	46.3b	62.bc
Soyabeans + Mycorrhiza	21.7b	52.3a	68.3	30.3bc	65.7a	88.7a
Soyabeans + Rhizobium	11.3e	41.3bc	62.7	8.9d	23.7c	62.0bc
SED	1.5	3.6	4.6	2.1	5.0	6.4

NOTE: Numbers in each column followed by the same letter are not significantly different at $p \leq 0.05$.

Dual inoculation with rhizobium and mycorrhiza (S+R+M) in the sterile soils resulted to height increment that was significantly higher than S+M only on the 30th day while in the non-sterile soil there was a significant height increment on the 30th day but a significant reduction on the 45th and 60th day. The results failed to demonstrate that, dual inoculation with mycorrhizal fungi and rhizobium would lead to height increment over the singly inoculated plants (S+R) and (S+M). In these experiments, soyabeans growth lacked the benefits of the legume rhizobium mycorrhiza symbiosis. Without the benefits of mycorrhiza, the plants may not have been able to access phosphorus and other relatively immobile nutrients from the soils. The process of BNF was hampered by insufficient supply of these nutrients.

The difference between the (S+P) treated plants and the mycorrhiza (S+P+M) ones, were significantly higher for (S+P+M) in all the trials (sterile and non-sterile). Mycorrhizal fungi could have played a critical role of acquiring more P and other nutrients from distances beyond the phosphorus depletion zone. The nutrients must have been used for BNF eventually leading to increased photosynthesis.

In an experiment to determine rock phosphates and mycorrhiza effects on growth and nutrient uptake of *F albida* seedlings in an alkaline soil, even without the rock phosphates, mycorrhiza inoculated plants achieved better results in terms of biomass. Mycorrhiza dependency decreased as the phosphates applied levels increased (Ba and Guissou, 1996).

The soil in the experimental site was highly weathered, acidic and leached of bases and hence of low fertility as is the case with most tropical soils (Giller, 2001). Phosphorus is known to be depleted very fast around the growing root within a distance of a few mm (Sieverding, 1991). Due to the extremely low diffusion rate, this zone cannot be adequately replenished with P. Root external mycorrhiza mycelium grows far beyond this zone and increases the soil volume that is exploited for P uptake (Sieverding, 1991). In acidic soils, there is induced enhanced excretion of organic acids, which increase the solubility and acquisition of mineral nutrient P and micronutrients Zn, Fe, and Mn in particular, (Marschner, 1992). Phosphorus is a critical limiting nutrient for nitrogen fixation. Rhizobium is particularly sensitive to mycorrhiza and requires their association to satisfy the high P demand for nodulation and nitrogen fixation (Bhatia et al., 1998).

Dual inoculation with micorrhiza, *G caledonius*, and rhizobium is reported to have increased the performance of transplanted *P. Juliflora* in a semi-arid wasteland. Similarly, *Leucaena leucocephala* inoculated with 3 strains of rhizobium, at a field trial in Jammu India increased height growth significantly (Dutt and Palhania, 1983). Marques et al., (1999) came to the same conclusion when inoculating *Cetrolobium tomentosum*, a woody legume with both mycorrhiza and rhizobium. Plants inoculated with rhizobium improved their height only when associated to mycorrhizal fungi.

4.5 Effect of Mycorrhiza and Rhizobium Inoculation of Soyabeans on Root Collar Diameter

There was significant difference ($p < 0.05$) between the various treatments in the on-farm experiments during the long and short rains of the year 2005 seasons, but not in the on-station experiments as shown in Table 6.

In the on-farm LR 2005 experiments, there was significant root collar diameter increment over the control and each of all the other treatments as Table 6 shows. Inoculating soyabeans with mycorrhiza (S+M) and with rhizobium (S+R) increased root collar diameter over the control by 54.8% and 30% respectively

Dual inoculation of soyabeans with mycorrhiza (S+R+M) resulted to a root collar diameter increment over the control (80%) that was significantly higher than that of S+R. This suggested that mycorrhizal fungi may have had played a role in increasing growth of the root collar.

Table 6: Effect of Mycorrhiza and Rhizobium Inoculation of Soyabeans on Root Collar Diameter in Gatanga and Kenyatta University

Treatments	On-farm (cm)	On-station (cm)		
	Long Rains	Short Rains	Sterile	Non-sterile Soil
Soyabeans + Rhizobium	3.2d	2.3d	2	2
Soyabeans + Rhizobium + Mycorrhiza	4.5ab	3.8ab	2	3
Soyabeans + P fertilizer + Rhizobium	4.1abc	3.4c	1	2
Soyabeans + P fertilizer + Mycorrhiza	4.6a	3.9a	2	2
Soyabeans	2.5 e	3.5bc	2	2
Soyabeans + Mycorrhiza	3.9acd	3.8ab	1	2
Soyabeans + Rhizobium	3.5cd	3.5bc	2	2
SED	0.4	0.2	0	0

NOTE: Numbers in each column followed by the same letter are not significantly different at p=0.05.

The treatment S+R+M had a larger root diameter than S+M though the increment was not significant. The dual inoculated plants (S+R+M) thus, had a root collar diameter larger than when soyabeans were planted with only one of the microorganism inoculants (S+R and S+M). The higher root collar diameter in mycorrhizal plants could be attributed to enhanced inorganic nutrients absorption and greater rates of photosynthesis (Marschner, 1992). The larger diameters in the dual inoculated plants (S+R+M) could have resulted from increased photosynthetic rates arising from provision of P and other nutrients from wider mycorrhiza exploration of soils and provision of plant available N through biological nitrogen fixation by the rhyzobium bacteria (Allen et al., 1981).The treatment

S+P+R had an increment that was not significantly higher than S+P and was so to S+R in the long rains. Providing P and inoculating with rhizobium could have resulted to higher rates of photosynthesis and hence the larger root collar diameter.

In the short rains, dual inoculated plants (S+R+M) had the same root collar diameter with the mycorrhiza singly treated plants (S+M), but was significantly higher than the rhizobium inoculated ones (S+R). Dual inoculation increased root collar diameter by 8.6% over the control S. Mycorrhiza fungi could not have played the role of nutrient acquisition effectively due to drought.

In the on-station experiments though the differences between the treatments were not significant, soyabeans inoculated with both mycorrhiza and rhizobium (S+R+M), had the biggest root collar diameter (3 mm) in both the sterile and the non-sterilized soil as shown in Table 6.

Inoculation with mycorrhiza has been shown to increase root collar diameter of plants. Gorsh and Verma (2006) inoculated *Acacia mangium* with three VA-mycorrhiza fungi *(Glomus occultum, G. aggregatum and G mosseae*) and found out that all inoculations enhanced growth with respect to shoot, height, root collar diameter, chlorophyll and biomass compared to uninoculated control seedlings. Inoculation with mycorhizal fungus and rhizobium bacteria does not always lead to consistently better growth in plants.

In an experiment to assess the effect of dual inoculation with rhizobium and mycorrhiza on the growth of *Calliandra calothyrsus,* it was found out that, although plants inoculated with both symbionts grew better than the inoculated controls, the results were not statistically significant and inoculation did not have a long lasting effect on the growth of trees even when majority of the nodules were occupied by the inoculated rhizobia and the roots infected by mycorrhiza (Lesueur and Sarr, 2008).

4.6 Effect of Mycorrhiza and Rhizobium Inoculation of Soyabeans on Root Dry Weight

There were significant differences among the different treatments in the root dry weight ($p<0.05$) on-farm 2005 long rains season experiments. However, in the on-farm short rains 2005 season, and the on-station experiments, the differences were not significant as Table 7 shows.

In the on-field long rains 2005 season experiment, the differences between the control (S) and each of the other treatments were significant except in S+R and S+P. In particular, soyabeans planted with phosphorus and inoculated with mycorrhiza (S+P+M) followed by soyabeans planted with P fertilizer and inoculated with rhizobium (S+P+R) had the highest roots dry weight. The control (S) had the lowest root weight as Table 7 shows.

The high weight realized in the mycorrhizal plants could have been attributed to the effect of the mycorrhizal fungi in improving nutrient uptake through increased surface area for absorption. The increased nutrient absorption must have resulted to an increase in

photosynthetic rate giving rise to an increase in plant growth. The roots increased in growth suggesting there could have been increased flow of the photosynthates to the roots and the fungal hyphae. Association of plant roots with mycorrhiza could have led to an increase in the amount of phosphorus available to the plants and subsequently to an increase in the root biomass.

Table 7: Effect of Mycorrhiza and Rhizobium Inoculation of Ioyabeans on Rot Dry Weight in On-farm and On-station Experiments in Gatanga, Kenya

Treatments	On-farm (grm /plant)		On-station(grm/plant)	
Long Rains	Short Rains	Sterile	Non-sterile soil	
Soyabeans + Rhizobium	0.522d	0.345	0.04	0.09
Soyabeans + Rhizobium + Mycorrhiza	1.146bc	0.761	0.5	0.21
Soyabeans + P fertilizer + Rhizobium	1.44abc	0.459	0.05	0.08
Soyabeans + P fertilizer + Mycorrhiza	1.96a	0.470	0.32	0.12
Soyabeans	0.477d	0.414	0.07	0.11
Soyabeans + Mycorrhiza	1.034c	0.705	0.16	0.2
Soyabeans + Rhizobium	0.781d	0514	0.08	0.05
SED	0.3114	0.1693	0	0

NOTE: Numbers in each column followed by the same letter are not significantly different at p=0.05.

The outcome of these experiments suggests that as a result of enhanced phosphorus nutrition occasioned by the mycorrhizal fungi, the root biomass of the mycorrhizal plants increased due to increased photosynthates to the roots. Gueye (1990) also arrived at the same conclusion when Bambara groundnuts were inoculated with Rhizobium and

mycorrhiza (*Glomulus mosseae*). Inoculation with mycorrhiza was reported to always significantly increase the roots weight. Micropropagated bananas inoculated with mycorrhiza *Glomus interradices* had larger shoots and dry root weight and P content than non-mycorrhiza plants in a greenhouse experiment in Belgium (Declerck et al., 2002).

In the SR season, the roots dry weight differences between treatments were not significant. The crop experienced drought stress at flowering stage resulting to growth retardation. This is in contrast to an experiment where drought stress significantly increased the percentage of mycorrhizal infection by 8-41% in *G sepium* and *Albiza lebbeck* under drought stress (Awatonye et el., 1992).

In the on-station experiments, the root dry weight differences between the various treatments were not significant ($p<0.05$). This was both in the sterilized and non-sterilized soil, though the mycorrhizal plants had higher diameter compared to the non-mycorrhizal ones as Table 7 shows.

4.7. Effect of Mycorrhiza and Rhizobium Inoculation on Soyabeans Shoot Dry Weight

There was significant difference in shoot dry weight ($p<0.05$) between the different treatments only in the on farm experiments during the 2005 long rains season but not during the 2005 short rains season or the on-station experiments as Table 8 shows. In the 2005 long rains season, differences between the control and each of the other treatments

were significant except the S+R and the S+P treatments. The order of performance was S+P+R >S+M> S+R+M> S+P+M> S+R>S and S+P.

The dual inoculated plants (S+R+M) had a shoot dry weight increment over the control S, by 172% while the singly inoculated plants, S+R and S+M, increased by 103% and 185% respectively. This could be attributed to improved absorption of inorganic nutrients especially P and greater rates of photosynthesis occasioned by inoculating with mycorrhiza and rhizobium (Jia and gray, 2004). There was an increment of shoot dry weight of the dual inoculated plants (S+R+M) over S+R though it was not significant. Except for treatments S+P+M, the other mycorrhizal plants, S+M and S+R+M performed better than the non mycorrhizal plants.

Table 8: Effect of Mycorrhiza and Rhizobium Inoculation of Soyabeans on Shoot dry Weight in Gatanga and Kenyatta University, Kenya

Treatments	ON-FARM (grm/plant)		ON-STATION (grm/plant)	
	Long rains	Short rains	sterile	Non-sterile soil
Soyabeans + Rhizobium	3.35bcd	0.7	0.76	0.6
Soyabeans + Rhizobium + Mycorrhiza	4.49b	1.2	1.92	1.2
Soyabeans + P fertilizer + Rhizobium	7.94a	1.0	0.65	0.6
Soyabeans + P fertilizer + Mycorrhiza	3.92b	0.8	1.39	1.3
Soyabeans	1.65cd	0.4	0.51	0.5
Soyabeans + Mycorrhiza	4.71b	0.6	1.42	1.2
Soyabeans + Rhizobium	1.17d	0.8	0.63	0.6
SED	1.097	0.2	0	0

NOTE: Numbers in each column followed by the same letter are not significantly different at p=0.05.

This could be due to the improved absorption of inorganic nutrients, especially of P and greater rates of photosynthesis in inoculated plants. Extensive mycorrhizal hyphal network could have enabled soyabeans to acquire phosphorus from distances beyond the nutrient depletion zone of the roots as well as to solubilize phosphorus from unavailable sources (Marschner, 1992). Mycorrhizal plants could also have obtained P from normally unavailable sources of both inorganic and organic forms (Koide and Kabir, 2000; Feng et al., 2003). The enhanced phosphorus could have been utilized in the nodules as an energy source in biological nitrogen fixation where atmospheric nitrogen is reduced to ammonia (NH_3) which is taken up by the plant and assimilated into amino acids, leading to increased leaf weight (Hogberg, 1986).

In an experiment to investigate the influence of mycorrhiza inoculation in an alley cropping trial, increase in stem and leaf biomass was strongly correlated with increase in P uptake indicating that the improvement was attributed to mycorrhiza inoculation (Atayese et al., 1992). *Faidherbia albida* seedlings grew poorly without mycorrhiza colonization and when colonized, they achieved better results in terms of biomass (Ba and Guissoui, 1996). Inoculation of *Faidherbia albida* and *Acacia nilotica* with mycorrhiza and rhizobium in sterile soil, at the end of a drought stress, increased plant biomass of the two tree species (Onsube et al., 1992).

In an experiment to determine the effect of dual inoculation of black locust (*Robinia pseudoacacia* L) with rhizobia and *glomus* on a desurfaced soil, similar results were obtained. Synergistic effect was observed as double inoculation produced significantly

higher shoot mass by 93% than single inoculation treatment (Ferrari et al., 2008). The authors concluded that, mycorrhiza colonization helps nodulated plants reach their P demand in P limited soils.

In the 2005 short rains season experiment, the order of treatment performance was S+R+M followed by S+P+R, S+P+M, S+P, S+R, S+M and S. Dual inoculation (S+R+M) resulted to higher soyabean dry weight, though not more statistically different than when inoculated with each of the microorganisms alone (S+R and S+M). In this experiment, various treatments may have failed to show any significant difference over the control and with each other because of the drought that was experienced. The number of rhizobia in the soil could have declined drastically due to the soil drying as shown in Table 8. The rate of nitrogen fixation and translocation of the products of nitrogen fixation to the shoots, could have been reduced by a reduction of soil water content (Giller, 2001). In an experiment to determine the response of some tropical nitrogen fixing woody legumes to drought and inoculation with mycorrhiza in a sterile soil, Awatonye et al., (1992) came to the same conclusion on *Acacia auriculiformis*. They concluded that, mycorrhiza inoculated plants survived better and had more dry matter and nutrients content and a large leaf surface area than uninoculated plants.

In the on-station experiments, though the differences were not statistically significant, the mycorrhizal plants had higher shoot weights than the non-mycorrhizal ones. Existence of indigenous rhizobia or mycorrhiza in the non-sterilized soil, may explain the results in

this experiment. Indigenous strains may have competed with the introduced strains for nodule occupation and reduced their effectiveness (Marques et al., 1999).

4.8. Effects of Mycorrhiza and Rhizobium Inoculation on Soyabeans Grain Yields

Inoculating soyabeans with mycorrhiza and rhizobium led to a significant difference ($p < 0.05$) between the different treatments in both the on-farm (long rains 2005 season) and on station sterilized and non-sterilized soils as Table 9 shows. There was no grain yields realized in the second season (short rains 2005) due to drought.

Table 9: Effect of Mycorrhiza and Rhizobium Inoculation on Soyabeans Grain Yield (gm/plant) in On-farm and On-station Experiments

Treatment	On-farm Long Rains 2005	On-station Sterile	No-sterile Soil
Soyabeans + Rhizobium	0.84cd	0.52d	0.37g
Soyabeans + Rhizobium + Mycorrhiza	2.24a	0.86c	0.89a
Soyabeans + P fertilizer + Rhizobium	1.71ab	0.23g	0.62e
Soyabeans + P fertilizer + Mycorrhiza	1.98ab	0.9b	0.76b
Soyabeans	0.49d	0.49e	0.43f
Soyabeans + Mycorrhiza	1.42bc	1.59a	0.74c
Soyabeans + Rhizobium	1.39bc	0.33f	0.67d
SED	0.32	0.20	0.15

NOTE: Numbers in each column followed by the same letter are not significantly different at p=0.05.

In the long rains 2005 season on-field experimentation, soyabeans inoculated with mycorrhiza and rhizobium (S+R+M) had the highest grain yields followed by soyabeans planted with phosphorus and inoculated with mycorrhiza (S+P+M). The rest followed as: S+P+R, S+M, S+P, and S+R and S in that order. All the treatments except soyabeans inoculated with rhizobium (S+R), had grain yields increments that were significantly higher than the control (S).

Dual inoculation of soyabeans with mycorrhiza and rhizobium (S+R+M) increased grain yield over the control (S) by 356%. On the other hand, inoculating with either of the microorganisms alone led to and increase of 71% for rhizobium (S+R) and 189% for mycorrhiza (S+M). Dual inoculation increased grain yield over the singly inoculated plants S+M and S+R by 82% and 166% respectively. Mycorrhizal fungi and rhizobium, thus acted synergistically since combined inoculation enhanced grain yield more than inoculation with either mycrosymbioant alone (Table 9).

Mycorrhiza inoculation could have increased the soil volume explored for nutrient uptake thereby enhancing the efficiency of nutrient absorption from the soil solution. Nutrients such as phosphorus which was in low supply in the site soil, has an extremely lower diffusion rate in soil compared to the rate of its absorption by the growing roots hence getting rapidly depleted from the root zone (Busman et al., 2002).

The extra radical mycelium of the mycorrhizal fungi must have grown far beyond the depletion zone, reaching a new pool of soluble phosphates (Smith and Read, 1997). Phosphorus was thus availed to the plants and utilized in the nodules for biological

nitrogen fixation where, it provided the energy required to convert nitrogen into ammonia. Ammonia was consequently converted to amino acids and proteins leading to increased soyabeans grain yields. In addition to provision of P, mycorrhiza fungi could have contributed through an increase in the absorbing surface to increased uptake of other nutrients (Marschner, 1992; Marschner and Dell, 1994). The overall effect was an increased rate of photosynthesis and consequently, yields.

In an experiment with yard long beans (*Vigna unguiculata sesquipedalis*), Mridha et al., (1992), observed that dual inoculation with rhizobium and micorrhiza (*Glomus clarum*) increased the growth, yield and nutrient content of the beans markedly compared to the non-inoculated control. Sieverding (1991) performed over 50 field trials inoculating cassava varieties with mycorrhiza in acidic soils of varying fertility levels and reported over 20-25% increase in tuber yields. Applying P fertilizer to Mycorrhiza inoculated Soyabeans (S+P+M) resulted to a grain yield increment that was not significantly higher than the plants with fertilizer alone (S+P) or with mycorrhiza alone (S+M). Inoculating soyabeans with rhizobium and applying P fertilizer (S+P+R) resulted to a grain yield increment that was significantly higher than the rhizobium treated plants (S+R) but not the mycorrhiza treated ones (S+M). Mycorrhiza could have increased the volume of soils from which P was sourced from by the roots of the plants.

In the sterilized soils, soyabeans inoculated with mycorrhiza (S+M) had the highest yields. The decreasing grain yield order was S+M> S+P+M> S+R+M> S+R> S>S+P and S+P+R. All the treatments were significantly different from each other. The mycorrhiza

inoculated plants had higher yields than the non-mycorrhizal ones as was the case in the long rains 2005 experiment.

In the non-sterilized soils the decreasing order of the grain yields was S+R+M> S+P+M> S+M, S+P> S+P+R> S and S+R. In both sets of the on-station experiments, the mycorrhizal plants performed significantly better than the non-mycorrhizal ones. In the sterile soil, dual inoculation of soyabeans with mycorrhiza and rhizobium (S+R+M) resulted to a grain yield increment that was statistically higher than when soyabeans was planted with rhizobium (S+R) but led to a significant decline when planted with mycorrhizal fungus alone (S+M). In the non sterile soils dual inoculation (S+RM) led to grain yield increment significantly higher than S+R and S+M. Legume productivity has been shown to improve through the synergistic interactions among the members of the tripartite symbiotic association (legume-rhizobium-mycorrhiza). In an experiment to assess the influence of rhizobium and arbuscular mycorrhizal fungal on nitrogen and phosphorus accumulation by the broad bean (*Vicia faba)*, Jia et al., (2004) found out that plants with the rhizobium and mycorrhizal fungi symbiotic associations were found to have higher photosynthetic rates per unit leaf area. Lizzy (1999) while working with mycorrhiza fungi and rhizobium on pea and lentil found out that, specific mycorrhizal fungi + rhizobia combinations enhanced plant growth and yield. Xavier and Germida (2003) found out that, the yield and N nutrition of pea inoculated with mycorrhizal fungi and rhizobium varied depending on the particular mycorrhiza fungi-rhizobium strain combination. Yield and N nutrition was enhanced in pea inoculated with a superior rhizobium strain and a compatible mycorrhiza fungi species.

4.9. Mycorrhiza Dependency of Soyabeans Inoculated with Mycorrhiza and Rhizobium

Mycorrhiza dependency defined as the degree to which a plant is dependent on the mycorrhiza to produce its maximum growth or yield at a given level of soil fertility (Brundrett et al., 1994) in the on-farm experiment was high (248.9% to312.3%) for all the plants treated with mycorrhiza (Table 10). In the on-station experiments, mycorrhiza dependency ranged from (75.5% to 224.5%) in the sterilized treatments while in the non-sterilized treatments, it ranged from 72.1% to 107.6%. The effect of competition from indigenous mycorrhiza species in the non-sterilized soil suggests the low mycorrhiza dependency in that experiment. In general, soyabeans being legumes and hence with a course root system with a high phosphorus requirement for biological nitrogen fixation, are highly dependent on mycorrhizas (Brundrett et al., 1994).

Table 10: Effect of Inoculating Soyabean with Mycorrhiza and Rhizobium on Mycorrhiza Dependency in On-farm and On-station Experiments in Gatanga, Kenya

Treatment		Mean yield (Kg/Ha)	Mycorrhiza dependency (%)
Soyabeans +Rhizobium +Mycorrhiza	Long rains 2005 (on-field)	299.3	312.3
	Sterilized soil	127.4	75.5
	Non-Sterilized soil	131.9	107.6
Soyabeans + Mycorrhiza	Long rains 2005 (on-field)	253.3	248.9
	Sterilized soil	235.6	224.5
	Non-Sterilized soil	109.6	72.1
Soyabeans + P fertilizer + Mycorrhiza	Long rains 2005 (on-field)	293.3	303.9
	Sterilized soil	133.3	83.6
	Non-Sterilized soil	112.6	76.8
Soyabeans (CONTROL)	Long rains 2005 (on-field)	72.6	
	Sterilized soil	72.6	
	Non-Sterilized soil	63.7	

Since the experimental site had soils of low fertility level as Table 1 shows, the mycorrhizal fungi could have played a major role of assisting the plant to source for nutrients and especially phosphorus through their extensive hyphal network depletion thereby availing them for plant uptake and utilization (Bagyara, 1996 and Arola et al., 2004) leading to increased productivity. Gorsh and Verma (2006) while inoculating *Acacia mangium* with three VA-mycorrhiza fungi *(Glomus occultum, G. aggregatum and G. mosseae*) found out that, the growth of *A. mangium* was 57% dependent on *G. occultum*, 47% on *G. mosseae* and 46% on *G.* aggregatum. Ba and Gissou (1996) while investigating the effect of rock phosphate and mycorrhiza fungi on growth and nutrients uptake of *Faidherbia albida* seedlings in an alkaline soil, reported that, growth response and mycorrhiza dependency decreased as the rock phosphate applied levels increased. Mycorrhiza inoculated plants took up more P from the soil and from the rock phosphate than the non-mycorrhizal plants.

4.10: Effect of Inoculating Soyabeans with Micorrhiza and Rhizobium on Root Nodule Numbers

The number of nodules in the various treatments showed differences that were significant, that is, ($P<0.05$) in both the on-farm experiments (both LR and SR 2005 seasons) and the on-station experiments on sterile and non-sterile soils as shown in Table 11.

In the on farm experiment (LR), all the treatments except S+R and S+P had root nodule numbers that were significantly higher than the control S. The treatments put in the decreasing order of the number of nodules were as follows: S+M>S+R+M>S+P+M>S+P>S+R=S+P+R>S.

Table 11: Number of Soyabean Nodules under Different Treatments in the On-farm and On-station Experiments in Gatanga, Kenya

Treatments	On-farm (no/plant)		On-station (no/plant)	
	2005 long rains	2005 short rains	sterile soil	Non-sterile soil
Soyabeans + Rhizobium	6.0b	3bc	3d	4a
Soyabeans + Rhizobium + Mycorrhiza	26.4a	14.4a	7b	4a
Soyabeans + P fertilizer + Rhizobium	19.5b	2.0bc	0f	1c
Soyabeans + P fertilizer + Mycorrhiza	29.4a	6.3b	8a	4a
Soyabeans	3.4b	0.4c	0f	0d
Soyabeans + Mycorrhiza	16.6a	6.3b	5c	4a
Soyabeans + P fertilizer	6.1b	1.6c	1e	2b
SED	6.40	2.20	0.32	0.40

NOTE: Numbers in each column followed by the same letter are not significantly different at p=0.05.

Dual inoculation (S+R+M) resulted to much higher increment on the number of nodules over the control (S) than the singly inoculated plants with rhizobium, (S+R) and mycorrhiza, (S+M).

Dual inoculation with both mycorrhiza and rhizobium (S+R+M) significantly increased the number of nodules over the rhizobium inoculated soyabeans (S+R) but not the mycorrhiza inoculated ones (S+M). Application of P fertilizer to rhizobium inoculated soyabeans (S+P+R) had no significant effect on the number of nodules over the rhizobium inoculated (S+R) or the P applied soyabeans (S+P).

This experiment showed that the plants roots could not effectively source P from the soil without the benefit of mycorrhiza. Phosphorus applied mycorrhiza inoculated soyabeans (S+P+M) did not result to any significance difference on the nodule numbers over the soyabeans inoculated with mycorrhiza only (S+M).

In the short rains trial, only the mycorrhizal plants S+R+M, S+P+M and S+M had nodule numbers significantly higher than the control. Arranged in decreasing order of the number of nodules, the performance was: S+R+M>S+P=M>S+M>S+R>S+P+R>S+P and S. Dual inoculation with both microorganisms (S+R+M) increased root nodules over the control much higher than the singly inoculated plants with rhizobium (S+R) and mycorrhiza (S+M). Phosphorus application to rhizobium inoculated soyabeans (S+P+R), had no significant effect over soyabeans inoculated with rhizobium alone (S+R), while application of P fertilizers to mycorrhiza inoculated plants (S+P+M) significantly increased the number of nodules over S+P but not over S+M.

The results of the number of nodules on mycorrhizal plants of both the LR and SR experiments could be attributed to the role of mycorrhiza fungi in nutrient sourcing. Mycorrhiza infection of the roots of soyabeans is known to stimulate both nodulation and nitrogen fixation especially in soils low in available P. Olsen and Habte (1995) came to the same conclusion when investigating the effect of mycorrhiza inoculation on nodulation and N accumulation in *Cajanus cajan*. Enhanced nodulation at low soil P concentrations was explained by mycorrhiza mediated P uptake. Phosphorus fertilizer application is known to significantly reduced mycorrhiza infection in soyabeans (Hicks

and Loynachan, 1987). The mycorrhiza inoculated plants were able to meet the high P demand for nitrogen fixation by rhizobium, leading to an increase in nodulation and consequently nitrogen fixation.

Similar results were obtained when *Leucaenia leucocephala* was inoculated with mycorrhizal fungi and Rhizobium (Punj and Gupta, 1988). Dual inoculation improved growth, nodulation and nitrogen fixation compared to single inoculation of either organism. Similarly, inoculation of *Acacia auriculiformis* with both mycorrhiza (*Glomus fasciculatum*) and rhizobium resulted in the greatest number of nodules and other growth parameters (Chang et al., 1986). Nodules are the sites where biological nitrogen fixation takes place and an increase in their number is construed to mean that there was an increase in biological nitrogen fixation in the experiment. Olsen and Habte (1995) while investigating the interaction of *Cajanus cajan* with rhizobium and vesicular arbuscular mycorrhiza *Glomus aggregatum* realized that, at low soil P levels, mycorrhiza inoculation significantly increased nodule numbers and shoots dry weight and concluded that, the enhanced nodulation was occasioned by mycorrhiza mediated P uptake. Kumar et al., (1998) while assessing the effect of mycorrhizal fungi, rhizobium and phosphate on nodulation in chick pea came to the same conclusion. The dual inoculated plants significantly increased nodulation over uninoculated control. In *Faidherbia albida*, inoculating the legume seedlings with *G. mosseae* and bradyrhizobium induced profuse nodulation (Diop et al., 2002). Soyabeans inoculated with bradyrhizobium and *Glomulus clarum* had 30% more nodules than those planted with the bacteria rhizobium alone (Antunes et al., 2006). Stancheva et al., (2006) demonstrated that, dual inoculation of pea

(*Pisum sativa*) with mycorrhiza and rhizobium increased plant biomass, nodulation parameters, nitrogen fixation activity compared to single inoculation.

In the non-sterile soil treatments, dual inoculation with rhizobium and mycorrhiza (S+R+M) had the same number of nodules as when each of the microorganisms was applied singly (S+R and S+M) hence, no significant difference. Inoculating with mycorrhiza and applying P fertilizers (S+P+M) led to a significant nodule increment over S+P but not S+M.

In the sterile soils, mycorrhizal plants (S+R+M, S+P+M, S+M) had higher number of nodules than the non-mycorrhizal ones. The number of nodules in decreasing order was S+P+M>S+R+M>S+M>S+R>S+P>S+P+R=S. The control, S had no nodules.

Reduced nodulation in all the control trials (S) may have been due to lack of compatible and effective rhizobia and due to nutrient deficiencies coupled with an insufficiency of mycorrhiza fungi inoculum. Houngnandan et al., (2000) arrived at the same conclusion while working with *Mucuna pruriens* as a fallow plant to restore soil fertility and to control the invasive grass *Imperata cylindrica* in the derived savanna of Benin. The rate of nitrogen fixation of the plant was often limited by low numbers of effective rhizobia and could be boosted by rhizobial inoculation except in very P-poor soils. He concluded that, farmers' management practices that allow for a buildup of mycorrhizal fungi, would alleviate P deficiency and hence increase nitrogen fixation.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The primary objective of this study was to investigate the effect of dual inoculation of mycorrhizal fungi and rhizobium on the growth performance of the legume *Glycine max* in acidic soils. Specifically, the study aimed at assessing the effect of inoculating *Glycine max* with mycorrhiza and Rhizobium bacteria on germination, grain yield and biomass production in acidic soils. It also aimed at quantifying the impact of inoculating *Glycine max* with mycorrhiza and rhizobium on nitrogen fixation in acidic soils.

The results from the study indicate that, all growth parameters except germination are significantly increased when soyabeans are inoculated with mycorrhiza and rhizobium in acidic soils. Plant height, root collar diameter, biomass (shoots and roots) and yields all increased as a result of dual inoculation with mycorrhiza and rhizobium. The effect of inoculation varied depending on the stage of development of the crop.

The study showed that inoculating soyabeans with mycorrhiza and rhizobium had no effect on seed germination. The reason could have been because root colonization by the mycorrhizal fungi and the rhizobium bacteria was still low soon after planting. The soyabean mycorrhiza rhizobium symbiosis had not developed at planting.

The study showed that plant height, when moisture is not limiting, increased when soyabeans is inoculated with mycorrhizal fungi and rhizobium. Height growth is not

increased significantly when soyabeans are planted with only phosphorus fertilizers, rhizobium or mycorrhiza.

Plants inoculated with mycorrhiza had larger root collar diameter, roots and shoots dry weights than non mycorrhizal in the long rains season when moisture was adequate.However this was not so in the short rains and the on-station trials.

Dual inoculation of soyabeans with mycorrhizal fungi and rhizobium bacteria increased grain yield significantly when growth conditions were optimal.

The numbers of soyabean nodules were significantly higher in those treatments that had mycorrhiza, but highest in the dual inoculated plants indicating that dual inoculation with mycorrhiza and rhizobium increased the rate of nitrogen fixation, nodulels being the sites where this activity takes place.

5.2 Recommendations

- Farmers in Gatanga and other areas where environmental degradation has resulted to low levels of plant nutrients in the soil should be encouraged to introduce specific strains of microorganisms like mycorrhizal fungi and rhizobium bacteria to address the problem of soil infertility. Since most of the farmers are resource poor, this is a low input technology that they could adopt to improve their soil fertility. Over the long term, use of mycorrhiza and rhizobium is an efficient method of increasing soil fertility. Once introduced,

the symbioants are likely to be self-replenishing unlike when chemical fertilizers are used. Farmers will hence be able to replenish their soil nutrients cheaply unlike artificial fertilizers which are expensive and out of reach of most farmers.

- Most importantly, farmers should avoid losing their soil through soil erosion as this leads to a decrease in the number of microbial population (Sieverding 1991). Appropriate soil conservation structures and especially the use of biological soil conservation measures like planting of hedgerows of leguminous crops along the contours should be adopted (Cardoso et al., 2003).
- Agroforestry practices known to enhance mycorhiza colonization should be adopted. Such practices are crop rotation that incorporates nitrogen fixing legume plant in the cycle and application of soil organic matter in the form of manures or compost to stimulate proliferation of mycorrhiza (Kale et al., 1992).
- Farmers should have their soils analyzed periodically after every three years to determine the pH and nutrient levels. In acidic soils, liming should be done to avoid loss of cations through leaching and fixation of phosphorus. Where chemical fertilizers are recommended, the right amounts and types should be used to avoid affecting negatively the population of microorganisms such as mycorrhiza and rhizobium.
- Research is needed to come up with effective and competitive multifunctional biofertilizers for a variety of crops suitable for use by smallholder farmers. A study of microbial persistence of biofertilizers in various soil environments,

for example stressful conditions should be undertaken. Mycorrhiza inoculants packages that are appropriate and easy for farmers to use should be studied and developed. Quality control systems for the production of inoculants and their application in the field should be studied and put in place to ensure the benefits of plant microorganism symbiosis are realized. It is noted that the technology for rhizobium inoculant production is in existence and it is being utilized while that of mycorrhiza, due to its obligate nature, is not being utilized as much.

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APPENDIX 1

Rainfall amount and distribution in 2005 long and short rains season at Gatanga, Kenya