EFFECTIVENESS OF PROMISING COMMERCIAL BIOFERTILIZERS ON SOYBEAN PRODUCTION IN BUNGOMA COUNTY, WESTERN KENYA.

BY

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AGR/PGS/06/10

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August, 2013

DECLARATION

Declaration by candidate

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DEDICATION

This work is dedicated to my late parents (Caroline Agut Omondi and Peter Majengo Kadikiny) and my uncle Fred Otieno Omondi who both made great effort in my education.

ABSTRACT

The study was conducted to compare the performance of promising commercial bio-fertilizers that have been evaluated under the green-house conditions at TSBF-CIAT, in farmers' conditions through the use of promiscuous soybean variety (SB19). The trials were laid out on small scale farms in Bungoma County, situated in Western Kenya. The experiment was established in March 2010 during the long rains (LR) and repeated during the short rains (SR) of 2010; laid out in multi-locational one farmer field one replicate design. Treatments were not replicated within each field. During LR 2010, 50 farms were researched on and 100 farms in the second season (SR 2010). A promiscuous medium-maturity soybean variety TGx1740-2E (SB 19) was inoculated with Legumefix (Rhizobia) or/and Rhizatech (mycorrhizae) inoculants. The mycorrhizae inoculum was applied to the soil in the seed furrows at the recommended rate of 30 kg ha^{-1}. Nodulation was examined at mid-podding (50% podding) by carefully uprooting all plants with their entire root system from a 1 m^2 section in each plot. Nodules were counted and weighed; the root and shoot parts separated, and fresh and dry weights assessed. Analysis of variance was conducted to determine the effects of (and interactions between) the two inoculants on plant parameters using a mixed linear model (MIXED procedure, SAS). Rhizobial inoculation resulted in significantly ($p<0.01$) higher nodule biomass (0.93 g plant^{-1}) compared to the control (0.27 g plant^{-1}) across many farms. Mycorrhizal inoculation had no significant effect on nodulation when applied solely (0.38 g plant^{-1}), but co-inoculation of Rhizobia and mycorrhizae increased nodule biomass further by 0.09 g plant^{-1}. There was a significant difference ($p<0.01$) in terms of biomass yield between treatments. Rhizobial inoculated plants had the highest biomass production of 2086 kg/ha. Rhizobial inoculation resulted in higher grain yields of 1116 kg/ha above the control. Soybean inoculation increased both nitrogen and phosphorus uptake in the biomass. Rhizobial inoculant had the highest soybean N uptake of 48.6 N kg/ha which was significantly different ($p<0.05$) from control and sole application of mycorrhizae. Statistical analysis showed that soil factors (pH, P, C, N) significantly ($p<0.001$) affected soybean grain yields during both seasons. It is concluded from this study that rhizobial inoculants have a high potential as commercial bio-fertilizers and can substitute the need for mineral N fertilizer in the legume farming systems. However, there is need to target these inputs to the most responsive fields. Further studies are needed to elucidate the conditions under which synergism between both inoculants may occur, with specific focus towards soil P availability and management of P inputs.

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LIST OF ACRONYMS

AMF	Arbuscular Mycorrhizal Fungi
BNF	Biological Nitrogen Fixation
CIAT	Centro Internacional de Agricultura Tropical
CFU	Colony Forming Unit
COMPRO	Commercial Products Research Project
C.P	Crude Protein
FAO	Food and Agriculture Organization of the United Nations
GOK	Government of Kenya
IITA	International Institute of Tropical Agriculture
LR	Long Rains
Ndfa	Nitrogen derived from atmosphere
SED	Standard Error of Difference
SR	Short Rains
TSBF	Tropical Soil Biology and Fertility

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GOD BLESSES YOU ALL.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Soybean [*Glycine max* (L.) Merrill] is an annual legume that belongs to the legume family *Fabaceae*. It is a strictly self-pollinating legume. World demand for soybean has been able to absorb ever-increasing production at prices that are profitable to producers. Since 1970, world consumption of soybean has grown at an annual rate of 4.8% on average and since the 1990s it showed an annual increase of 5.4% on the average (Okalebo *et al.*, 2005).

In Western Province, mixed cropping, with minimal nutrient inputs are the norm and crop rotation is secondary to continuous maize cropping. Few farmers recognize the benefit of improved soil fertility through nutrient recycling. Leguminous intercrops and improved short fallows contribute nitrogen (N) to the soils through litter falls and biological nitrogen fixation, but this process is not widely recognized as beneficial by farmers. On the other hand, mineral fertilizers and livestock manure are considered important inputs, but are usually in short supply (Chianu, 2009).

The high cost of chemical fertilizers and other inputs has not favored increased food production. One way of increasing food production without degrading the environment is through bio – intensive farming (Chianu, 2009). An indirect benefit of growing soybean is the change they introduce in crop rotations, by acting as break-crops to slow down the build-up of cereal pests, diseases and weeds thus reducing the need for pesticides in subsequent cereal crops (Mahasi *et al*, 2009). Due to lack of alternative crops, most farmers practice continuous cropping (mostly maize, cassava, sweet potatoes and cotton).

Soybeans that nodulate effectively with diverse indigenous Rhizobia are considered as promiscuous (Kuneman *et al*, 1984). Hence, promiscuous genotypes of soybean form symbiotic association with available *Rhizobium* strains in the soil and thus fix atmospheric nitrogen whilst non-promiscuous genotypes need specific rhizobial strains to fix nitrogen from the air.

In the late 1970s, breeders at IITA observed that most high yielding soybean cultivars from USA have specific requirements for *Bradyrhizobium japonicum* (Pulver *et al*, 1982) and inoculation of these varieties was found to be essential when growing them under tropical conditions of low soil nitrogen. In the early 1980s, it was assumed that most tropical countries did not have the facilities and personnel required for inoculum production, storage, and distribution and were dependent upon importation of the final product. The non abundance of commercial *Bradyrhizobium japonicum* inoculants and nitrogenous fertilizers led to the option of breeding promiscuous cultivars in IITA since soybean genotypes that do form symbiotic association with indigenous cowpea-type Rhizobia were identified. Generally, soybean varieties developed for promiscuous nodulation with the indigenous Rhizobia were considered to increase production of soybean in tropical Africa with minimum cost affordable to small-scale farmers (Giller and Wilson, 1991).

1.2 Importance of soybean

Soybean has larger amounts of protein (40%) and calcium than other leguminous crops (Economic Survey, 2007). It has various health benefits: 1) Healing and disease prevention (Chianu, 2009); eating small amounts of soybean protein daily can prevent or lower the risk of heart diseases, breast, colon and prostate cancer, 2) People who suffer from digestive problems or diabetes also stand to benefit from soybean-based foods (Mahasi *et al.,* 2009). Moreover, soybean can be used as a cash crop because once processed it can be sold just like other crops such as sorghum, cassava, sweet potatoes, ordinary beans, groundnuts, sesame etc (Mahasi *et al*, 2009). Major importers of soybean include countries of the European Economic Community, Japan, and Eastern Europe. The demand for soybean is mainly for oil and meal products, rather than whole beans (Duguma *et al*, 1988). By weight, the protein yield of soybean is about twice that of meat and of most beans and nuts, four times that of eggs and cereals, and twelve times that of milk. In countries with rapidly increasing populations, soybean is viewed as a crop that enhances nutritive value of the local diets and lessens national shortages of vegetable oil. With the diet of many people in the world deficient in protein and calories, soybean seems destined to remain an important commodity.

1.3 Problem statement

Productivity of soybeans in Kenya, and particularly Western Province, is low (250 kg/ha) compared with China, Brazil, USA and other countries with yields of above 4t/ha (Chianu, 2009). This low productivity is a problem because Kenya needs more soybeans to satisfy a growing demand for stock feed and to improve human nutrition. The reasons for poor production in Western Kenya have been clearly identified as the absence of desirable traits such as tolerance to extreme temperatures, resistance to soybean mosaic disease, blight, leaf spots and tolerance to acid soils, low indigenous soil Rhizobia and myccorhizae etc. There is also a proliferation of new rhizobial and mycorrhizae inoculants appearing on the market that claim major impact in increasing crop productivity. Many claim to bring benefits across a wide range of crops including cereals, grain legumes, root crops, vegetables and fruit trees, and to substantially improve both yield and produce quality.

1.4 Justification

Nitrogen plays an important role in restoring soil fertility in small scale low input farming system in sub Saharan Africa. With increasing fuel prices, the cost of mineral fertilizer is rising, making it less available to small scale farmers. Additionally, due to high and further increasing land use intensity in western Kenya, there is a decline in the capacity of soil to supply N (Tittonel, 2002). Consequently, BNF appears to be the only economically and environmentally friendly alternative to these farmers to replenish soil N reserves. However, in Bungoma there is low diversity of legumes in the different cropping systems. In some home gardens, mostly *Crotalaria* spp are found, while in the fields *Phaseoulus vulgaris* L. is the dominant crop. In the cultivated lands, no soybean is currently planted while in the fences mainly *Sesbania sesban* can be found. Hence, there is need to introduce a promiscuous soybean variety with inoculation technology in the farming systems of Bungoma which can contribute to agro-biodiversity and add N to the different components of the farming systems through biological nitrogen fixation (BNF).

1.5 Objectives

1.5.1 Broad Objective

To compare the efficiency of newly introduced commercial biofertilizer products on the performance of a promiscuous soybean variety under farmer management in Bungoma, Kenya.

1.5.2 Specific Objectives

1. To determine the effect of soil nitrogen and phosphorus on the performance of applied Rhizobia and mycorrhizae inoculants on soybean growth and yield.

2. To establish the relationship between selected soil chemical properties and bio-fertilizer effects on soybean production.

3. To do economic analysis when soybean is inoculated with efficient bio-inoculants.

1.6 Hypotheses

1.6.1 Guiding hypothesis

Inoculation of soybean using efficient commercial mycorrhizal and rhizobial inoculants at the optimum soil pH, N, P and organic matter content will improve the performance of soybean to give better yields.

1.6.2 Null hypotheses

1. Soil nitrogen and phosphorus improves the effect of rhizobial and mycorrhizal inoculants.

2. There is a correlation between selected soil chemical properties and biofertilizer effects on soybean yield.

3. Inoculation of soybean seeds using effective bio-inoculant is economically viable.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biological Nitrogen Fixation (BNF)

Most tropical soils are highly weathered, leached and widely deficient in nutrients, especially N and P as a result of declining fallow periods with high land use intensity (Sanginga, 2003), the reduction or absence of the use of chemical fertilizer due to limited financial means (Mapfumo, 2001) and the inadequate return of crop residues after harvesting (Gachengo, 1999). While P can be corrected by mineral fertilizer application or organic matter addition, Biological Nitrogen Fixation (BNF) can contribute to the replenishment of soil N and reduce the need for industrial N fertilizer (Larnier, 2005). It offers economically attractive and ecologically sound means of reducing external N input (Yadvinger-Singh, 2004). BNF is a process by which atmospheric N is reduced to ammonium (NH_4) by nitrogenase enzyme complex. It is not energetically favorable reaction carried out by prokaryotic micro-organisms like bacteria etc in symbiotic or non-symbiotic association with plants (Giller, 2001).

$$N_2 + 8e + 16\,ATP + 8H^+ \xrightarrow{N_2ase} 2\,NH_3 + H_2 + 16\,ADP + 16\,Pi \text{………Eqn 1}$$

2.2 Legume Rhizobia inoculation

Legume inoculation is an environmental benign process through which a given leguminous crop is provided with the compatible effective bacterial strain of the family Rhizobiaceae which results in an effective symbiosis between the plant and the bacteria (Abdelgani *et al*, 2002). The ultimate result is higher amount of N fixed, better growth of the plant, increase in production and improvement of soil nitrogen while the Rhizobia obtains plant phosynthates from the host plants. Many factors affect the response to inoculation; these include soil N and P (Karanja *et al*, 1995), the Rhizobia strain used in the inoculum and many such as environmental factors. Positive response to inoculation has always been attributed to low soil nitrogen content. Further, the presence of high populations of indigenous *Bradyrhizobium* spp in tropical soils that nodulate with commonly grown legumes has also acted negatively on the response of cultivated plants to Rhizobia inoculation (Karanja *et al*, 1995).

2.3 Factors influencing nodulation and BNF

Both environmental factors, as well as soil and crop management limit BNF in legumes. The factors responsible for poor nodulation in extremely acidic soils include high concentrations of protons and aluminium (Al) and low concentrations of Ca and available P (Ngome, 2006). Poor nodulation in extremely acidic soils can also be caused by low survival and persistence of certain strains of Rhizobia and can affect the number and distribution of indigenous Rhizobia (Peoples *et al.*, 1995). In species where root hairs are the dominant infection sites, high accumulation of protons and Al can impair nodulation as the attachment of Rhizobia at the host root surface is impaired (Marschner, 1995). The increase in soil pH by liming can therefore be necessary in increasing nodule numbers and BNF in acid mineral soils (Ngome, 2006). Thus, Buerkert *et al.* (1990) observed an increase in number of nodules in common beans as a result of increased soil pH by liming.

Soil or fertilizer N have often been shown to have a stimulatory effect on legume nodulation and BNF, the so called “starter effect”. This is mainly due to the stimulatory effect of N on growth and plant establishment during the period between root emergence and the onset of active BNF (Giller and Cadisch, 1995). Where soil fertility is high, legumes in the field thrive without fixing N_2.Under such conditions; they may derive all their N requirements from the soil N. But in the majority of Western Kenya soils, levels of plant available N are usually insufficient to satisfy legume N requirement (Gachengo, 1999). Therefore in the presence of effective Rhizobia, the unfulfilled demand of N could be met by BNF (Peoples *et al.*, 1995). Danso *et al.* (1992) noted that low levels of ammonium N can enhance BNF due to improved nodule development. Soils with low levels of soil N and few indigenous Rhizobia have been reported to have measurable benefits in terms of nodulation, N accumulation, plant biomass and grain yield (Hungria and Vargas, 2000). However, it has been widely reported that large amounts of inorganic N, have a prominent influence on reducing BNF (Walley *et al.*, 2005).When the levels of inorganic N increase, nitrogenase enzyme activity declines rapidly as well as the number of nodules. Shoot growth, however continues to increase indicating a shift from BNF to inorganic N nutrition (Becker *et al,* 1991). Fujita *et al.* (1992) reported that BNF was reduced by 55% in groundnut after application of 50 kg N/ha.Warnek and Arndt (2002) observed a significant decrease in number and mass of soybean root nodules with increasing concentration of nitrate. They also noted a decline in BNF from 84% to 3% Ndfa with an increase in nitrate concentration from 0.25 to 25 NO_3 N ($\mu g\ kg^{-1}$).

P bioavailability is a major factor limiting N fixation (Israel, 1987). P deficiency could inhibit the nodule development and the total N_2 fixation (Miao *et al*., 2007). Establishment of Mycorrhizal symbiosis in soybeans offers multiple benefits to plant nutrition, growth, disease resistance and drought tolerance (Moawad and Vlek, 1998). The nutritional aspects of AMF symbiosis have frequently been studied in the past in various plants including common beans. AMF symbiosis usually offers improved acquisition to the plants of P and Zn, particularly under soil conditions when the availability of these elements for plants is low (Rao, 2002) hence stronger correlations (>0.7). These nutritional effects are generally stronger in soybeans than in grasses, most probably due to the differences in the root system architecture.

Legumes obtain nutrients from soil in solution and require water for the translocation of the products of the BNF to the shoot. Hence drought stress or changes in plant water potential can cause a marked reduction in growth and nodulation in legumes due to reduction in nutrient uptake (Fujita *et al*., 1992). This will lead to a reduction in BNF and the translocation of fixed products. Soil moisture can also affect the BNF indirectly by limiting plant growth, nodule formation and functioning (Sanginga *et al*., 1995). Micro-organisms that are responsible for the BNF need water to carry out their activities. Drought stress will definitely have a negative effect on their activities in nodulation and BNF.

2.4 Rhizobial strains that nodulate soybean

Soybeans are nodulated with slow growing Rhizobia *Bradyrhizobium japonicum*, *B. elkanii* (Mpepereki *et al*., 2000) and *B. liaoningese* (Xu *et al*., 1995) as well as fast growing *Sinorhizobium fredii* (Mwakalombe, 1998). On the basis of its ability to nodulate with various Rhizobia strains, soybeans can be grouped into non-promiscuous and promiscuous genotypes. Non-promiscuous genotypes nodulate only with slow growing *B. japonicum* strains, which are considered absent in tropical African soils (Obura *et al*., 2001). On the other hand, promiscuous genotypes such as TGx varieties, naturally form effective nodules with *B. japonicum* strains as well as other *Bradyrhizobium* species indigenous to tropical african soils (Abaidoo *et al*., 2000) and *Sinorhizobium fredii* (Mpepereki *et al.*, 2000).

While a lot of work has been done in many African countries including Zimbabwe, Zambia, and Nigeria on the nodulation of promiscuous soybean varieties with various Rhizobia strains, there is little documentation in Kenya concerning the local field conditions under which the inoculants are efficient.

2.5 Importance of phosphorus nutrition

Microorganisms involved in biofertilizer production can play a major role in increasing nutrient availability for high yield and reducing production cost by limited chemical fertilizer application. Phosphorus biofertilizers could help increase the availability of phosphate for plant growth by solubilization, promoting the plant growth through production of plant growth-promoting substances, and by increasing the efficiency of biological nitrogen fixation (Sanginga N. and Woomer, P.L, 2009). Although the role of P fertilizers in nodulation, N fixation, nutrient-use efficiency and growth of soybean has been reported (Miao *et al.,* 2007), information is scanty regarding the role of phosphate-solubilizing bacteria in P bioavailability, growth promotion and also its interaction with N_2-fixing bacteria.

2.6 Arbuscular mycorrhizal symbiosis

Apart from the association with Rhizobia, soybeans also establish a root association with fungi from the phylum *Glomeromycota*. The mechanisms of establishment of this symbiosis called arbuscular mycorrhizal (AM) show several parallels to the rhizobial association (Sanginga N. and Woomer, P.L, 2009). This knowledge has been established by studies on symbiotic mutants and on the gene expression and signaling events in the different symbioses involving soybeans and other legumes such as peas, medic, and *Lotus japonicum* (Morandi *et al.,* 2005). Although the association with AMF is evolutionarily older, it is more widespread in the plant kingdom than the BNF, and is less understood as compared to the rhizobial association. This is mainly because of unusual (polynuclear) genome organization of the AM fungi, lack of transformation system allowing genetic manipulations of the AM fungi, and their resistance to grow on synthetic media in the absence of a host plant (Jansa *et al*., 2006).

Establishment of mycorrhizal symbiosis in soybeans offers multiple benefits to plant nutrition, growth, disease resistance and drought tolerance (Moawad and Vlek, 1998). The nutritional aspects of AM symbiosis have frequently been studied in the past in various plants including common beans. AM symbiosis usually offers improved acquisition to the plants of P and Zn, particularly under low availability in soils (Rao, 2002). Next to the nutritional effects, the multitude of other benefits have been attributed to AM symbiosis such as more efficient utilization of P fertilizers by the plants, improved root development, increased tolerance of plants to drought and to heavy metal pollution (Auge`, 2004). The mechanisms behind all these effects are not fully understood. They seem to be related to improved mineral nutrition of the plants, changes in internal hormonal balances in the plants, relief from stress through changes in soil properties and selective ion uptake from soil solution, immobilization of heavy metals in the fungal biomass, as well as promotion of development of associated microbes in the mycorrhizal hyphosphere (He *et al.,* 2007). On the other hand, plants supply the AM fungi with reduced carbon originating from their photosynthesis. This means that the observed growth effects are indeed effects of plant performance promotion through mechanisms such as improvement of scarce nutrient uptake combined with associated carbon costs (Lynch and Ho, 2005). Thus the overall greatest net benefits of AM symbiosis are usually seen under stressful environment such as low P and /or Zn availability and drought.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Area

3.1.1 Bungoma County

The trials were laid out on small farms in Bungoma County, situated in Western Kenya. The district lies between latitude 00^{0} $34^{'}$ N and longitude 34^{0} 34’E. Bungoma County falls under two major agro-ecological zones: the transitional upper midland zone UM4 (referred to as the maize-sunflower zone) and the Lower Midland zones which cover a greater proportion of the district (LM1-LM3). LM1 and LM2 are the sugarcane zones and the Marginal sugarcane zones respectively while LM 4 is the cotton zone (Jaetzold and Schmidt, 2006). The topography of the district is characterized by a succession of valleys and higher areas. The altitude ranges from 2000 m above sea level around Mount Elgon to 1100 m at the minor valleys along the Nzoia River, which drains the major part of the district (Jaetzold, and Schmidt, 2006). On the undulating plains of the lower-level uplands (very undulating to undulating, slopes between 2 and 8 %, altitudes between 1200-1900 m) Haplic Acrisols, Ferralic Arenosols, Haplic, Rhodic and Humic Ferrasols, Humic Cambisols and Dystric Planosols are dominant. On the Bottomlands (flat to gently undulating, slopes between 0 and 5%, various altitudes) Dystric Gleysols, Eutric Leptosols and Haplic Nitisols are common (TSBF, 2009). Generally, the soils have good physical properties, but their nutrient levels are low (Ndung'u, 2002). The district has a bimodal rainfall pattern, with the first growing season (long rains) extending from March to August, and the second (short rains) from October to January. The district has generally well-distributed annual average rainfall of 1000-1800 mm, depending on the location (Republic of Kenya, 1993). The temperature in the district ranges from about 20-22°C in the southern part of Bungoma to about 15-18°C on the slopes of Mount Elgon in the northern part of the district. The district is characterized by a rapid population growth (Government of Kenya, 2009). The map of Bungoma district is as shown in Fig 3.1

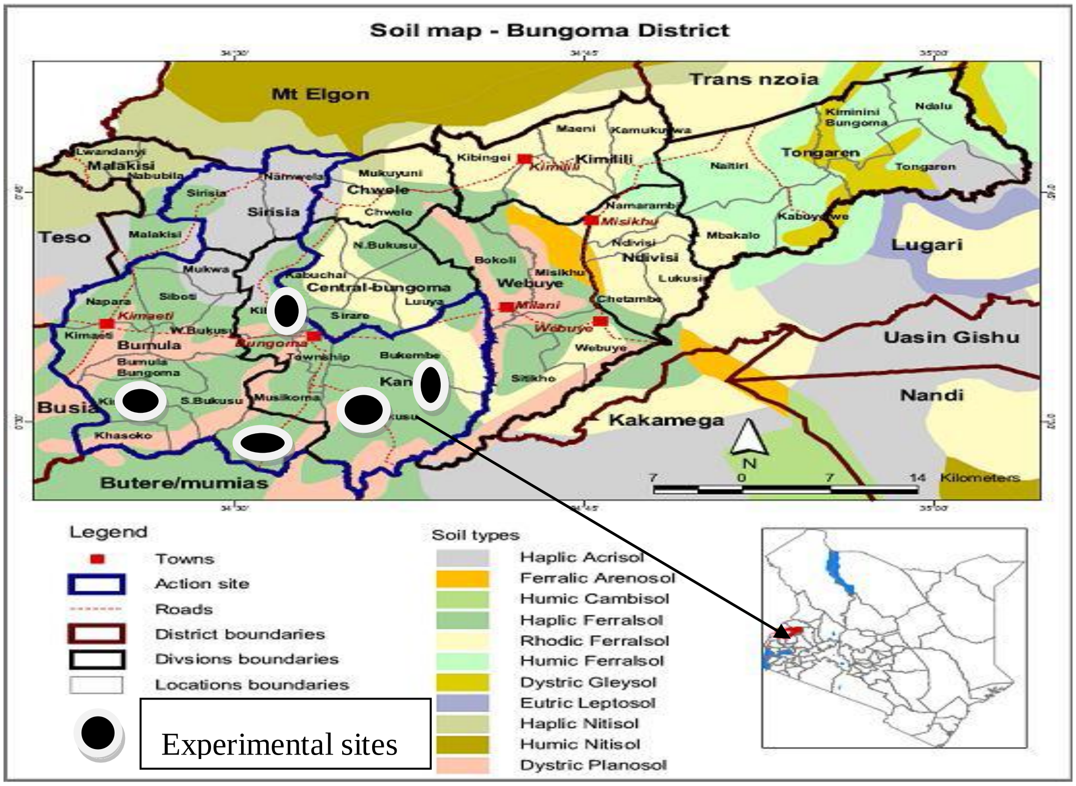

Fig 3.1 The location of the study area in Bungoma County (Source: TSBF-CIAT-IITA-MOI UNIVERSITY COMPRO PROJECT, Document 3, 2009)

3.2 Field layout and design

Performance of soybean was tested with rhizobial and myccorhizal bio-inoculants. The experiment was established during the long rains (LR) and repeated during the short rains (SR) of 2010 laid out in a multi-locational one farmer field one replicate design. Since one of the objective was to assess the correlation between selected soils chemical properties on bio-fertilizers performance within a large geographical area in terms of soybean grain yields, treatments were not replicated within each field or farm: instead, farms and seasons were considered as replicates (Pypers, 2010), with 50 farms in the LR 2010 and 100 farms in the second season (SR 2010). Treatments were allocated in new farms each season to avoid contamination and residual effects of the inoculants. Soil characterization was done on each farm so as to determine the soil types and properties in each farm. The treatments were as given in Table 3.1

Table 3.1 Treatments used in the field study

Plot Number	Code	Treatment
P1	C	Control (no product applied)
P2	R	Rhizatech
P3	L	Legumefix
P4	R+L	Rhizatech and Legumefix

The 4 plots (plot size = 4.5 m by 4.5 m) were separated by alleys of 1 m wide.

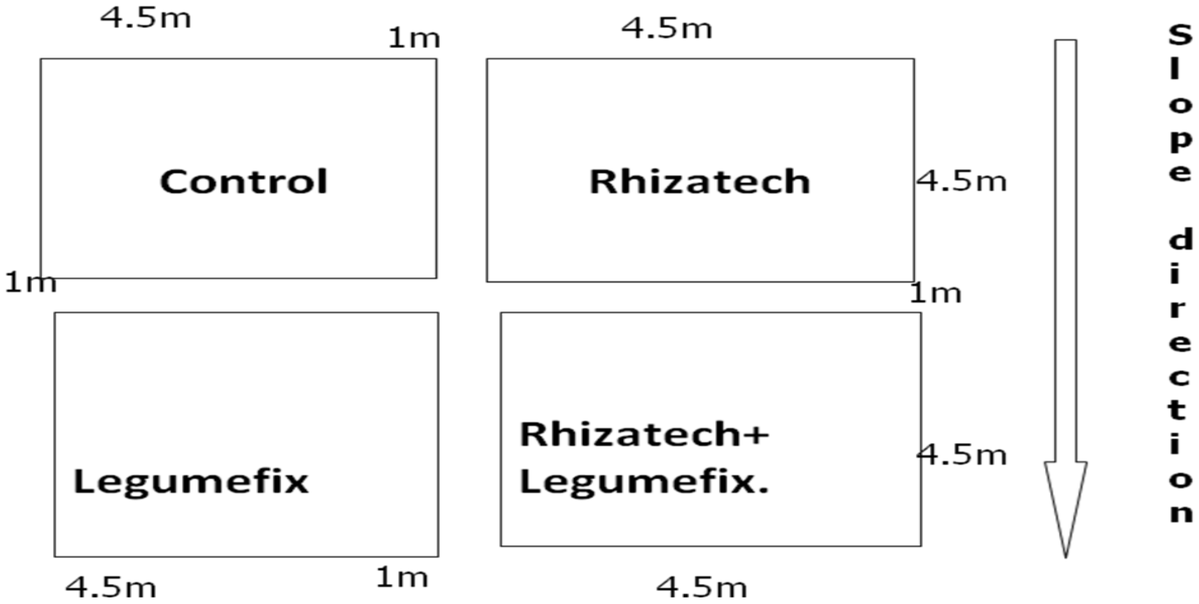

Fig 3.2 Experimental field layout

3.3 Land preparation

Land preparation was done in February 2010 for long rains and September 2010 for short rains using hand hoes. Fine-seedbed preparation was also done by hand prior to demarcation of plots. All the initial land preparations for the two cropping seasons were done by farmers themselves to facilitate the adoption of technologies through their participation in the experimentation.

3.4 Soil sampling

Plots of 10 m by 10 m area were demarcated and zigzag method used to sample the soils giving a total of 9 sub-samples per plot. The top 0-2 cm soil layer was removed to avoid sampling excess debris and samples taken up to 15 cm depth with a soil auger. The sub-samples were thoroughly mixed and 500 g composites were packed in polythene bags for laboratory analysis. The samples were analyzed for pH, organic carbon (C), total N and available P, according to Okalebo *et al.,* (2002). Other routine analyses on cations, micronutrients were not performed due laboratory limitations.

3.5 Planting

The treatments were administered into plot sizes of 10 m by 10 m (Fig 3.2). A promiscuous medium-maturity soybean variety TGx1740-2E (SB 19) that was recommended across locations in Western Kenya by Mahasi *et al.*, (2009) was inoculated with either or both inoculants and planted at 50 cm between rows and 7.5 cm between plants in the rows to give a soybean population of 266,667 per hectare. Each experimental plot had nine rows. Rhizobial inoculation (Legumefix, Legume Technologies, UK, containing 532c strain of *Bradyrhizobium japonicum)* was done by thoroughly mixing 125 g of damp seed with 2 g of inoculum (1 x 10^{-9} CFU g^{-1}) as per the manufacture's recommendation. The mycorrhizal inoculum (Rhizatech, Dudutech Ltd., Kenya, containing spores and mycelial fragments of *Glomus intraradices* (50 propagules/cm^3)) was applied to the soil in the seed furrows at the recommended rate of 30 kg ha^{-1} by the manufacture. The germination and emergence were uniform in all the treatments and there was no visual observation on detrimental effects from the treatments. Apart from the technical operations such as treatment application and data collection; all the other operations were managed by the individual farmers.

3.6 Plant sampling for biomass and tissue N analysis

Nodulation was examined at mid-podding (50% podding) by carefully uprooting all plants with their entire root system from a 1 m^2 section in each plot. Nodules were washed, counted, put in zip lock bags and weighed. The root and shoot parts were separated as depicted in Plate 3.2, and fresh and dry weights assessed. Pods were also separated from the shoots, fresh and dry weights assessed.

Plate 3.1 Biomass and nodule sampling in the field (Source: Author, 2010)

3.7 Harvesting

Soybean was harvested at physiological maturity when the pods were dry but not yet shattered in August 2010 for the first crop and second crop was harvested in January 2011.

All the plants in the entire plot were harvested and grain yields measured by weighing the dry soybean grain yields produced from each plot.

3.8 Laboratory analysis

Plants and soil analyses were carried out using the procedures in Okalebo *et al.*, (2002) as described below.

3.8.1 Soil pH

Measurement of pH is expressed as the inverse log of the hydrogen ion concentration. The pH of the soil solution controls the form and solubility of many plant nutrients. Soil pH was measured on 2.5:1 soil / water suspension.

3.8.2 Organic carbon

Organic carbon was determined using the sulphuric acid and aqueous potassium dichromate ($K_2Cr_2O_7$). After complete oxidation from the heat of solution and external heating (Nelson and Sommers, 1975), the unused or residual $K_2Cr_2O_7$ (in oxidation) was titrated against ferrous ammonium sulphate. The used $K_2Cr_2O_7$, the difference between added and residual $K_2Cr_2O_7$, gave a measure of organic C content of soil. The chemical reaction in the method is;

$$2Cr_2O_7^{2-} + 3C + 16\ H^+ \rightarrow 4Cr^{3+}\ 3CO_2 + 8\ H_2O$$

3.8.3 Extractable soil phosphorus: the Olsen method

The soil was extracted with 0.5 M solution of sodium bicarbonate at pH 8.5. In calcareous, alkaline or neutral soils containing calcium phosphate, this extractant decreases the concentration of Ca in solution by precipitating Ca as $CaCO_3$. The result is an increase of the P concentration in the solution. The Olsen method is suitable for a wide range of soil types and pH values. In acid soils containing Al and Fe phosphate, the P concentration in the solution increases as the pH rises. Precipitation reactions in acid and calcareous soils are reduced to a minimum because the concentrations of Al, Ca and Fe remain at a low level in this extractant. It is to be recognized that many other extraction techniques for plant 'available' P also exist (Okalebo *et al.*, 2002). Phosphorus was measured calorimetrically in the soil extract, using the method of Murphy and Riley (1962).

3.8.4 Total Nitrogen in plant tissues and soils

The content of total N and P was measured in a digest obtained by treating soil and plant samples with hydrogen peroxide plus sulphuric acid; selenium and salicylic acid. The principle takes into account the possible loss of nitrates by coupling them with salicylic acid in an acid media to form 3-nitrosalicylic and or 4-nitrosalicylic. The compounds are reduced to their corresponding amino acid forms by the soil organic matter. The hydrogen peroxide oxidise the organic matter while the selenium compound acts as catalyst for the process and the H_2SO_4 completes the digestion at elevated temperatures brought about by the addition of Lithium Sulphate. The main advantages of this method are that single digestion is required (for either soil or plant material) to bring nearly all nutrients into solution; no volatilisation of metals takes place. Acid digestion of the plant or soil material is followed by either distillation-titration or by colorimetry. The choice is dependent on local facilities; but colorimetry procedures are more rapid and accurate (Okalebo *et al.*, 2002).

3.9 Plant tissue analyses

Wet digestion procedure used in soils above was used followed by colorimetric determination of N and P (Murphy and Riley, 1962).

3.10 Statistical analysis

Analysis of variance was conducted to determine the effects of the inoculants and their interactions on plant parameters using a mixed linear model (MIXED procedure, SAS Institute Inc., 2003). The effects of different treatments were compared by computing least square means and standard error of difference (SED): significance of difference was evaluated at $p<0.05$ level of probability. In the mixed model analysis, farmer group nested within site and season were considered as random factors (Pypers, 2010) while the treatment effects (biofertilizers) were evaluated as fixed factors as shown in the SAS model below.

$$Y = X\beta + Z\gamma + \varepsilon$$

Where: Y = Yield (observation), β = treatment (biofertilizer) effect with known design matrix X, γ = denotes the farmer group within site and season which are considered as a random - effects parameters with known design matrix Z, and ε is an unknown random error vector whose elements are no longer required to be independent and homogeneous (MIXED procedure, SAS Institute Inc., 2003). Pearson correlation analysis was done to determine the effect of selected soil chemical properties on soybean grain yields.

3.11 Economic analysis

Bradyrhizobium inoculation was done by treating the seed, which requires only a minor investment in terms of labour. The main additional cost arises from the purchasing of the product. To treat 80 kg of soybean seed (required to plant 1 hectare), 320 g of peat-based soybean inoculant is needed, which costs \$ 13.4. As soybean in Bungoma on markets is sold at a unit price of about 0.8 \$ per kg, application of the product needs to increase yields by at least 54 kg ha^{-1} above 500 kg/ha to obtain a benefit-cost ratio (BCR) that exceeds \$ 2 \$$^{-1}$(Jefwa *et al.,* 2012). During the two seasons of 2010, 150 individual smallholder farmer households evaluated the products in their own fields. They were trained on how to apply the product, received seed and a small-pack of product, and installed and managed a trial. The yields at harvest were assessed.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Soil characterization of the study area

The major soil type in the experimental sites was Haplic Ferralsols as shown in Fig 3.1. These soils are characterized by deep yellowish or reddish colour, highly weathered, high permeability and stable micro-structure, with very low CEC. They are also chemically poor, with low pH and nutrient reserves, high P fixation, easily depleted by agricultural practices (TSBF, 2009).

The soils in Bungoma are developed from acidic parent materials, mainly volcanic materials i.e. the basalt. Initial soil characterization of the study area indicated strong to moderate acidity (pH 4.5-5.9) in most farms (Fig 4.1) as classified by the National Agricultural Research Laboratories (NARL) – Kabete in Nairobi (Kanyanjua *et al.*, 2002),(Appendix 2). The soils were acidic and below the critical level for sufficient production of most food crops. The soils are generally infertile in the highly weathered Ferralsols of Bungoma district. Thus the pH of the soils in surface (0 - 15 cm) ranged from 4.4 to 7.8 in the 44 farms with a mean of 5.46 during Long rains of 2010 and a pH of 5.39 in 63 farms in second rains of 2010. The high rainfall in this area and continuous cropping have also led to losses of soluble cations (K,Ca,Mg,Na) hence low soil pH. In the moderately acid to strongly acid soils, the availability of some essential nutrients (e.g. P and Mo) for adequate plant nutrition are reduced. It is possible that the long term leaching of cations followed by their replacement by Fe and Al in the exchange complex, probably favored soil acidity. However, in this study there were some farms with pH level above 7.0. These high levels of pH may be explained in terms of “hot” spots from previous to current human settlements whereby the ashes and boma manure from kraals/homesteads are added to croplands.

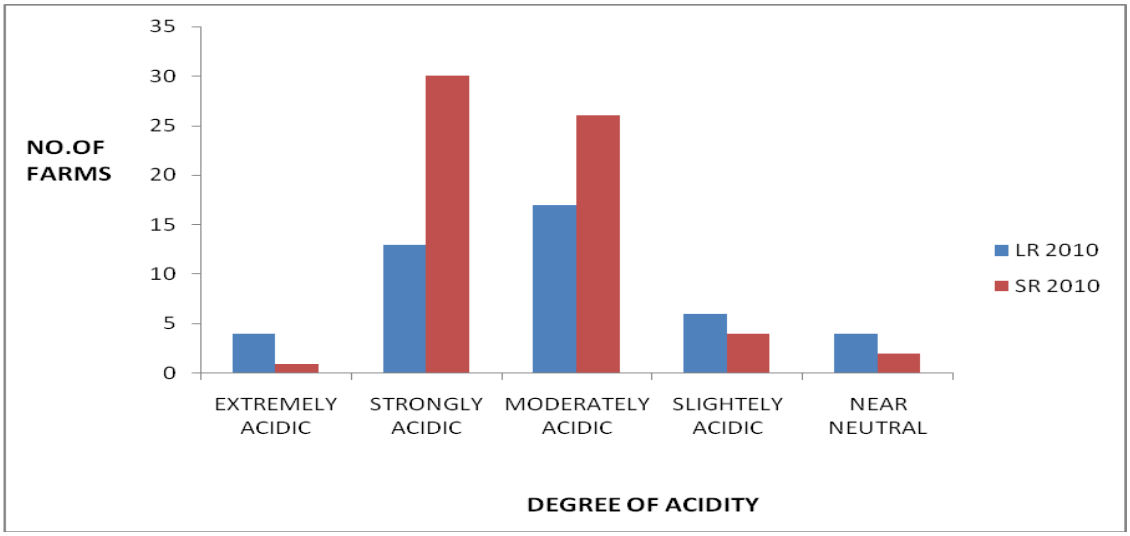

Fig.4.1: Distribution of farms based on soil pH during the experimentation period

The farms were grouped according to available phosphorus (Olsen) based on Marx *et al.*, (1999) classification as shown in Appendix 3. Available phosphorus in surface soils (0-15 cm) by the Olsen *et al.*, (1954) sodium bicarbonate extraction, ranged from 1.31 to 34.64 mg Pkg^{-1} during LR 2010 and from 1.1 to 40 mg Pkg^{-1} during SR 2010 (Fig.4.2). Soils testing below 10 mg P kg^{-1}, are likely to respond to fertilizer P application (Okalebo *et al*, 2002). There has been a positive correlation between crop yield and P uptake and levels of P extract in acid soils of Western Kenya by the Olsen method (Ndung'u, 2002).

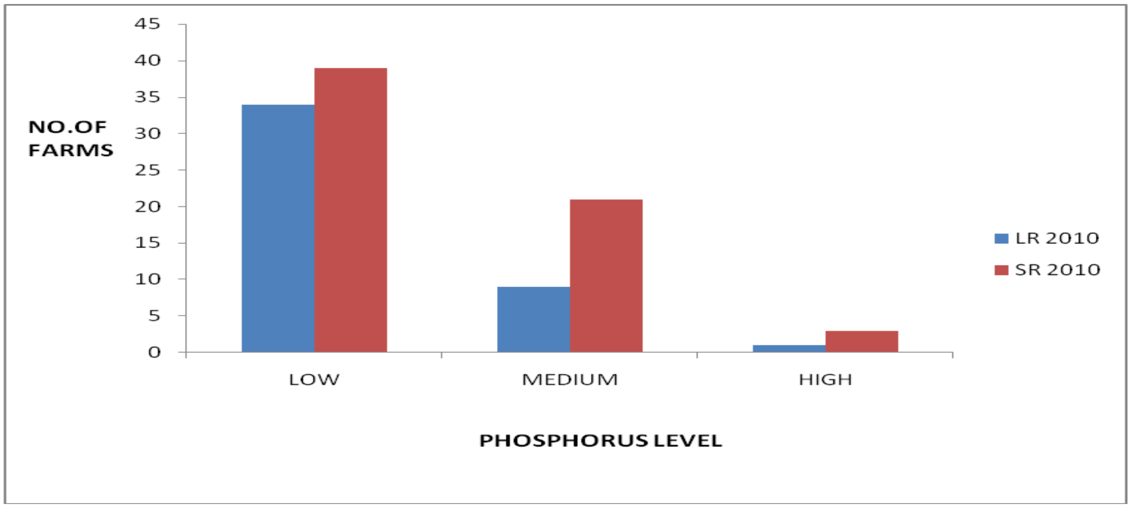

Fig.4.2. Distribution of farms based on soil available P (Olsen) during the research period.

The total N content in soils was low to moderate (0.05 to 0.25 %N) as per Okalebo *et al.*, (2002) in Bungoma farms during both seasons (Table 4.1).

Table 4.1 Distribution of farms during LR 2010 and SR 2010 in Bungoma based on soil total N data (0-15cm)

Level of Soil N	Soil N range	LR 2010	SR 2010
Very low	< 0.05	1	0
Low	0.05 – 0.12	11	40
Moderate	0.12 – 0.25	29	20
High	> 0.25	3	3
Total		44	63

The carbon (or organic matter) contents of soils were low to moderate (0.5 – 2 %C) according to Okalebo *et al*., (2002) with a mean of 1.32%C during LR and 1.42%C during SR seasons of 2010 (Table 4.2).

Table 4.2 Distribution of farms during LR 2010 and SR 2010 in Bungoma based on Soil Organic Carbon data (0-15cm)

Carbon levels	Carbon range	LR 2010	SR 2010
Very low	< 0.5	3	2
Low	0.5 – 1.5	10	37
Moderate	1.5 – 3.0	31	24
High	> 3.0	0	0
Total		44	63

In general, the soil test data presented for the Bungoma district suggest the need to use N and P inputs for improved and sustained crop yields.

4.2 Treatment effects on nodule weights

4.2.1 Long rains 2010

Rhizobial inoculation resulted in significantly ($p<0.001$) higher nodule weights (0.93 g plant^{-1}) compared to the control (0.27 g plant^{-1}). Mycorrhizal inoculation had no significant ($p>0.05$) effect on nodule weight when applied solely (0.38 g plant^{-1}), but co-inoculation of

Rhizobia and mycorrhizae increased nodule weight further by 0.09 g plant^{-1}, relative to sole application of the rhizobial inoculant (Fig 4.3).

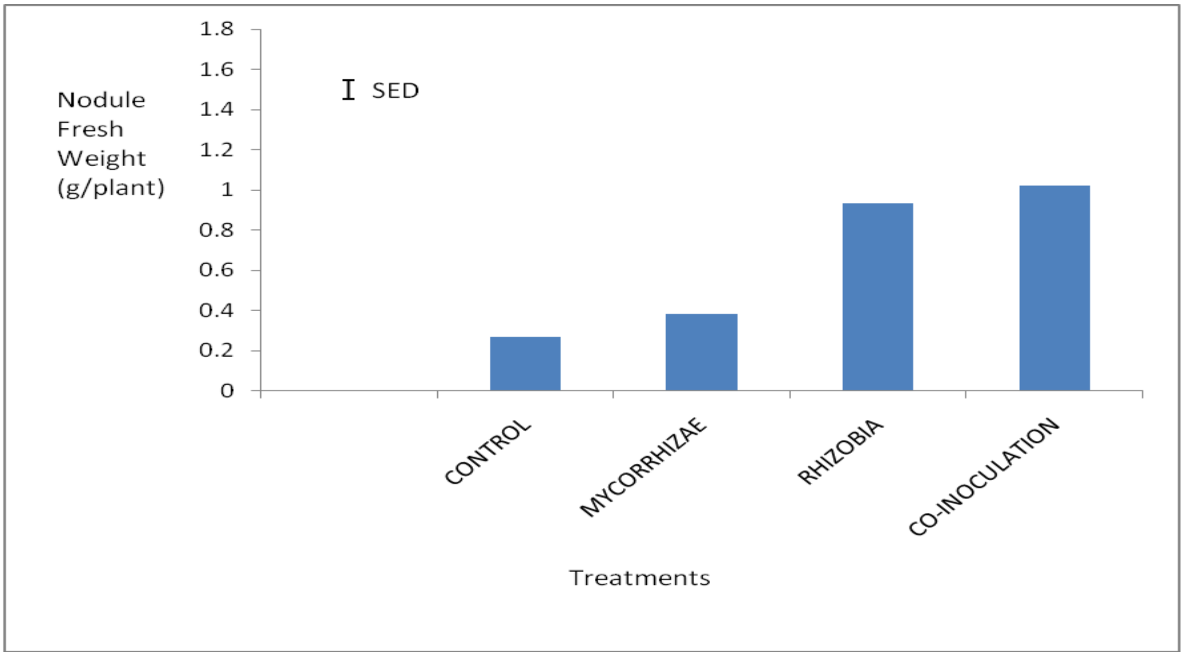

Fig 4.3: Treatment effect of commercial bio-inoculants on nodule fresh weights during LR 2010. The SED stands for standard error of difference and is used to determine the significant difference between treatments.

4.2.2 Short rains 2010

During the short rains of 2010, rhizobial inoculation resulted in significantly ($p<0.001$) higher nodule weight (0.57 g plant^{-1}) compared to the control (0.27 g plant^{-1}). Mycorrhizal inoculation had no significant ($p>0.05$) effect on nodule weight when applied solely (0.26 g plant^{-1}) as shown in Fig 4.4.

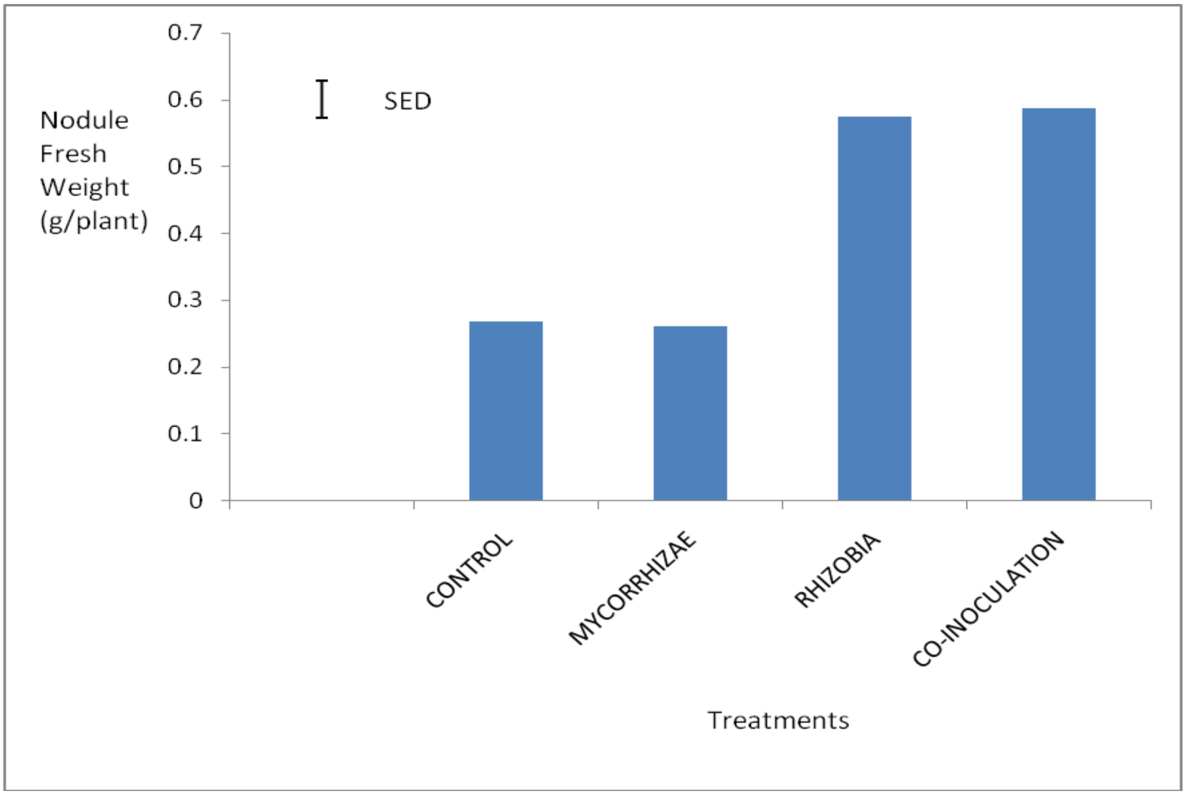

Fig 4.4: Treatment effect of commercial bio-inoculants on nodule fresh weights during SR 2010

Results showed that rhizobial inoculation significantly increased the nodule weights in soybean. There was no significant ($p>0.05$) difference between rhizobial and the co-inoculation treatments. This can be explained in terms of the poor quality of the myccorhizae inoculant. The number of nodule per plant formed is dependent on the amount of assimilate available to them. High nodule per plant weights in the co-inoculation plots could be due to the fact that mycorrhizal endophyte could be stimulated in quantity, efficiency and longevity by metabolic product released from the inoculated bacteria (Zaidi *et al*. 2005). The mycorrhizae inoculant assists the plant in acquiring the scarce available soil P since it is needed for root formation and the BNF process. Myccorhizae inoculant may be of poor quality hence no significant difference between the control and the mycorrhizae inoculant (Majengo *et al*. 2011).

4.3 Treatments effect on soybean biomass yield at 50% podding

4.3.1 Long rains 2010

Biomass yield is an important measure of plant vigor and health. There was a significant difference ($p<0.001$) in terms of biomass yield between treatments in this study. Bradyrhizobium inoculant treated plants had the highest biomass production at 2086 kg/ha. Therefore, the N biofertilizer (Legumefix) can be used as an alternate or as a supplement to N fertilizer to increase agricultural production with less input capital and energy. There was no significant difference ($p>0.05$) between sole rhizobial inoculation and co-inoculation at 2048 kg/ha biomass yield but there was a significant difference ($p<0.001$) between rhizobial and control yielding 1572 kg/ha and/or mycorrhizal inoculation at 1673 kg/ha (Table 4.3). Rhizobium inoculant produced higher quantities of biomass, and likely made highest contributions from N fixation.

Table 4.3: Treatment effect of commercial bio-inoculants on soybean biomass fresh weight in kg/ha during LR 2010 and SR 2010

Treatment	LR 2010	SR 2010	Means
CONTROL	1573	364	969
MYCORRHIAZAE	1673 ^{ns}	385^{ns}	1029
RHIZOBIA	2087 ***	388^{ns}	1238
CO-INOCULATION	2048 ***	382^{ns}	1215
Means	1845	760	1303
SED	75	15	

ns Not Significant

*** p<0.001

Low biomass yields (1673 kg/ha) in the mycorrhizae could be attributed to P defficiency (<10 mgP/kg) since mycorrhizae inoculant was to assist the plant in acquiring the scarce soil P but was not succesful as a result of poor quality. P defficiency is known to undermine nodulation and N_2-fixation processes and limits biomass accumulation by soybean.

Legumes obtain nutrients from soil in solution and require water for the translocation of the products of the BNF to the shoot. Hence drought stress or changes in plant water potential can cause a marked reduction in growth and nodulation in legumes due to reduction in nutrient uptake (Fujita *et al.*, 1992) as observed during short rains. This will lead to a reduction in BNF and the translocation of fixed products. Soil moisture can also affect the BNF indirectly by limiting plant growth, nodule formation and functioning (Sanginga *et al*., 1995). Micro-organisms that are responsible for the BNF need water to carry out their activities. Drought stress will definitely have a negative effect on their activities in nodulation and BNF.

Increased biomass in the mycorrhizal treatment (190 kg/ha) over control treatment during short rains could be due to the fact that AMF improves the root development of the legume that was able to utilize efficiently the little soil moisture that was available and also increased plant tolerance to drought as earlier reported by Auge,'(2004).

4.4 Treatment effects on soybean grain yield

4.4.1 Long rains 2010

Rhizobial inoculation resulted in higher grain yields than control. There was no significant difference between sole application of rhizobial (1116 kg/ha) and the co-inoculation (1027 kg/ha) at ($p>0.05$) (Table 4.4). Low yield in mycorrhizal treatment could be due to the mycorrhizal strain rather than the conditions of the soil and can be attributed to the quality of the strain that might be inferior. Low soybean grain yields in Bungoma even after inoculation (less than 2 t/ha) could be attributed to high soil acidity within the farms (4.5-5.9). Soybeans are very sensitive to soil acidity and prefer a soil pH between 5.8 and 7.8. Rhizobial inoculation also increased the average grain yield by 21% over control treatment (Table 4.4). This is because N fixed due to inoculation resulted into high biomass yield. The high biomass implies increase in the rate of photosynthesis due to high leaf number and leaf area. The photosynthates are transported via phloem and used in grain yield production (Majengo *et al*., 2011). Control plots gave poor results, as well as the mycorrhizal product, though that was not expected, given the good performances observed under greenhouse conditions.

Table 4.4: Treatment effect of commercial bio-inoculants on soybean grain yield during LR 2010

TREATMENT	MEAN YIELDS (kg/ha)	YIELD INCREASE (%)
CONTROL	924	-
MYCORRHIZAE	922 ^{ns}	-0.2
RHIZOBIA	1116 **	21
CO-INOCULATION	1027 **	11

SED	28
ns	Not Significant
**	$p<0.01$

Erratic rainfall in October followed by reduced amount in November (Appendix 1) might have affected the BNF process hence reduced N fixation during short rains. This may have contributed to the low grain yield in rhizobial inoculated treatments as given in Table 4.5. Ajay *et al.*, (1996) reported that small drought stress (about 15%) can reduce production by at least 20% in soybeans grain yield. The yield component affected depends on the reproductive stage when stress occurs.

Different products of BNF and nitrate assimilation may have a differential effect on photo respiratory N metabolism which might be enhanced under drought, which may explain the high yield in the rhizobial treated plants under drought stress.

Table 4.5: Soybean grain yields as influenced by inoculants during SR 2010

TREATMENT	MEAN YIELD (kg/ha)	YIELD INCREASE (%)
CONTROL	454	-
MYCORRHIZAE	489 ^{ns}	0.08
RHIZOBIA	524 *	15

CO-INOCULATION	521 *	15
SED	28	
ns	Not Significant	
*	$p<0.05$	

4.5 Accumulation of plant major nutrients (N and P) by soybean at 50% podding

4.5.1 Long rains 2010

Rhizobial inoculation increased both N and P uptake in the biomass. Rhizobial inoculant contributed to the highest soybean N accumulation of 48.6 N kg/ha (Fig 4.5) and was significantly different ($p<0.05$) from control treatment and sole application of Mycorrhizae. This is because the rhizobial inoculum contains the strains of *Bradyrhizobium japonicum* which are able to fix N through the BNF process hence high N accumulation.

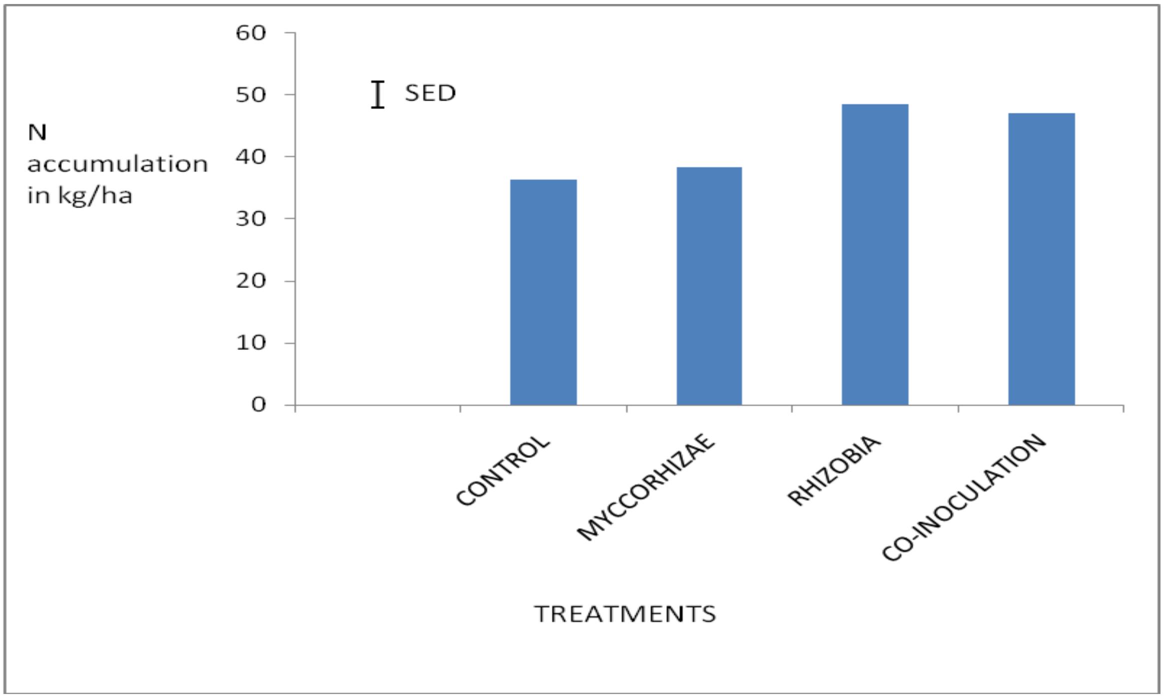

Fig 4.5: Effect of commercial bio-inoculants on N accumulation in biomass during LR 2010 at 50% podding.

Nitrogen accumulation in the soybean biomass supports the observations shown in Plate 4.1 that shows the rhizobial inoculant treatments as dark green, high vigour vegetative growth due to high N accumulation.

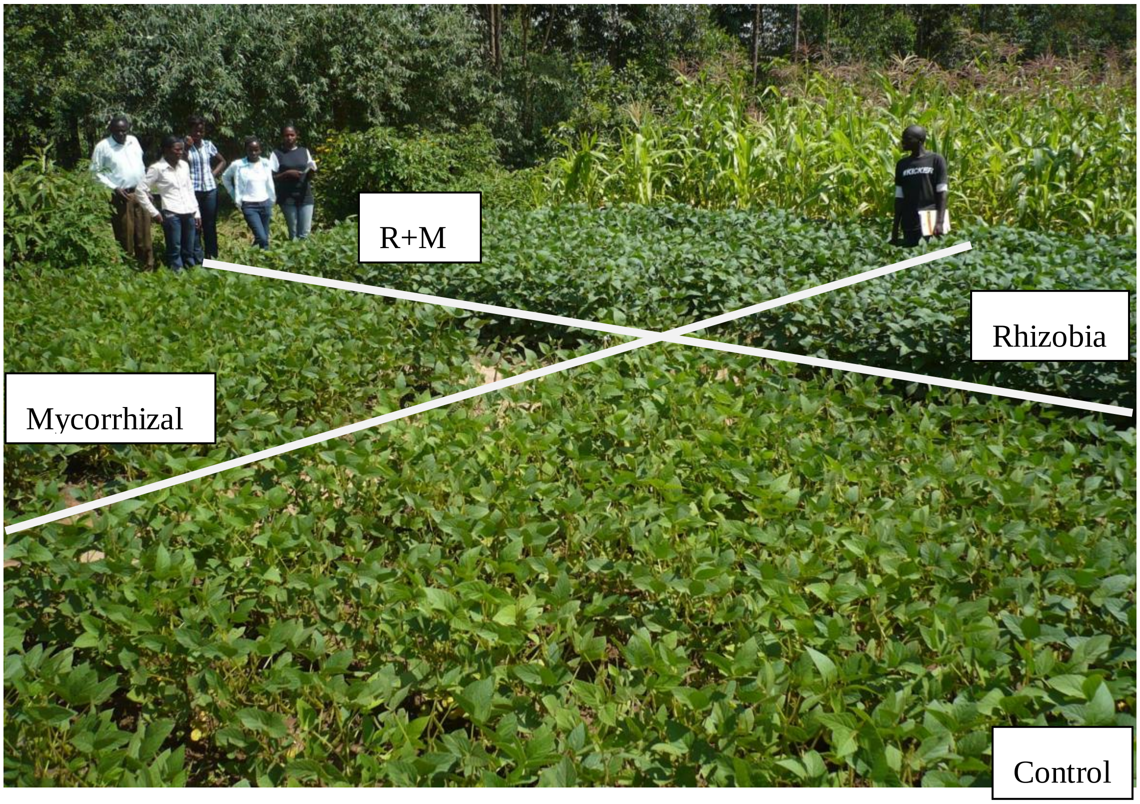

Plate 4.1: Treatment effect on biomass yield during LR 2010 (Source: Author, 2010)

Generally, the addition of AM Fungi to the N_2 fixer increased P accumulation in soybean biomass but not significantly different from single nitrogen fixer as shown in Fig 4.6. The result of this relationship could be due to a negative interaction that may have occurred between AM fungi and nodule bacteria (Zaidi *et al.*, 2004). The *Bradyrhizobium* strain might have P solubilizing effect hence was able to solubilize P fixed in the soil resulting into high P uptake in the rhizobial treated plots.

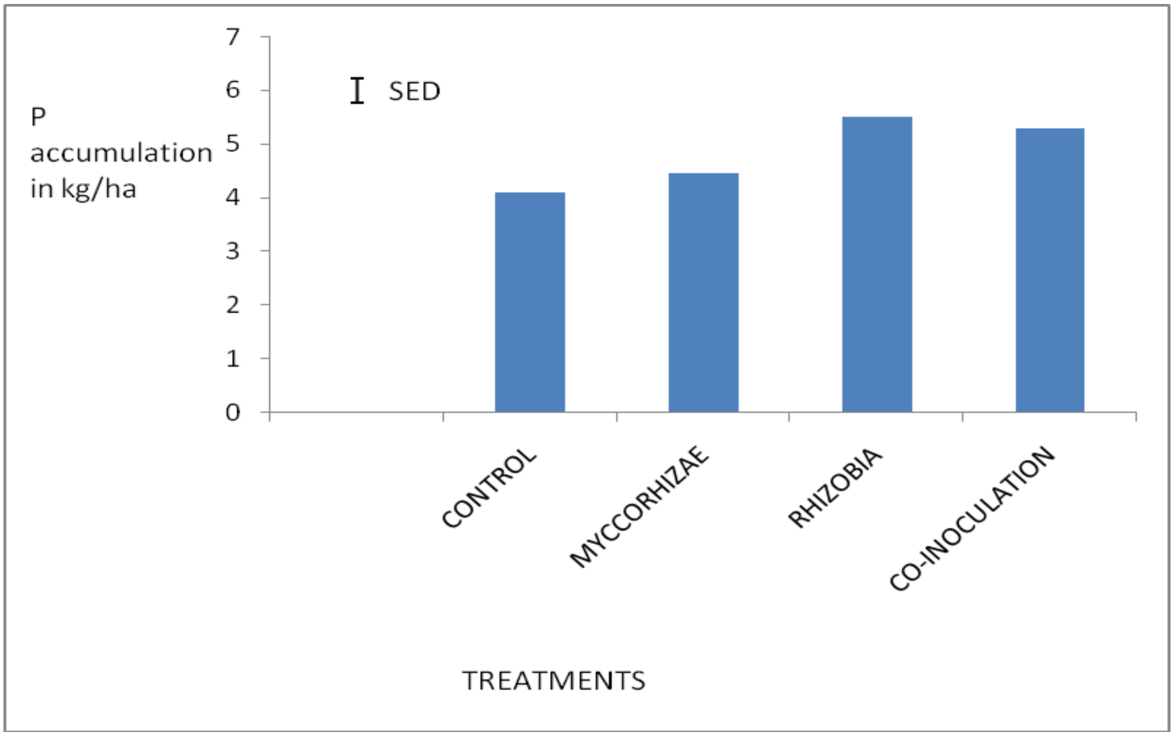

Fig 4.6: Effect of commercial bio-inoculants on P accumulation in biomass during LR 2010 at 50% podding.

N and P accumulation in grains was significantly different between treatments ($p<0.05$) with the rhizobial inoculum giving high N accumulation of 67.2 kg/ha N (Fig 4.7 and Fig 4.8). This could be as a result of N fixed through the BNF process being transferred to the grains. Soybean crop may not significantly contribute to soil N and P when grains are removed for human and animal nutrition. This is due to high N and P stored in grains hence depleting soil N (Majengo *et al*., 2011) as shown in Fig 4.7 for soya grains from trials in Bungoma. From the results, there is an overall response to the rhizobial inoculant but not to mycorrhizal inoculant (independent-no interaction).

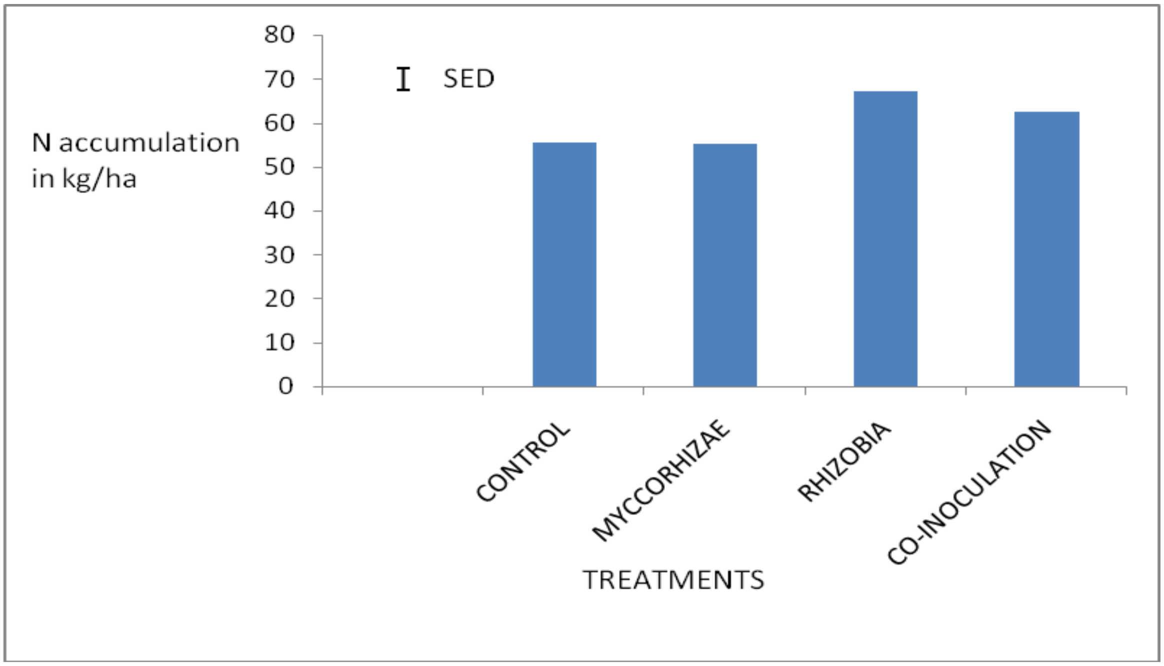

Fig 4.7: Effect of commercial bio-inoculants on N accumulation in grains during LR 2010

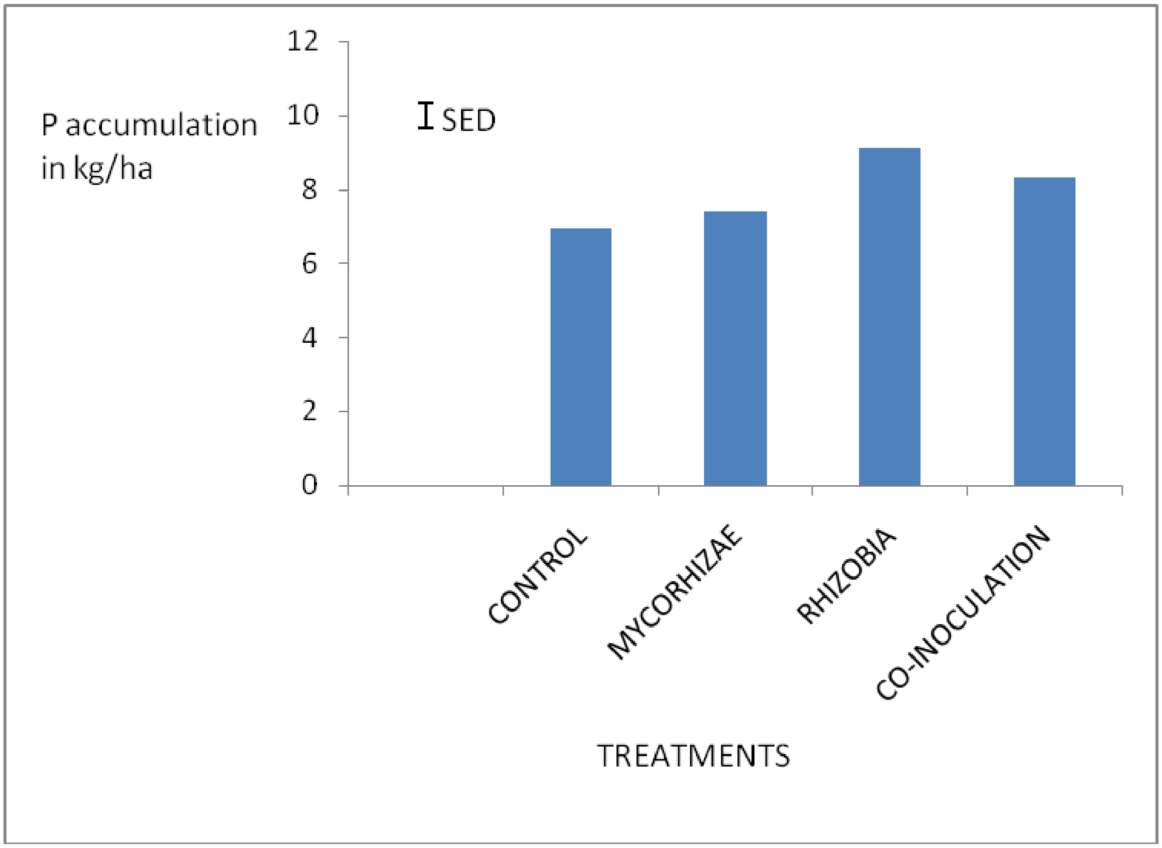

Fig 4.8: Effect of commercial bio-inoculants on P accumulation in grains during LR 2010

4.5.2 Short rains 2010

During short rains, there was reduced N and P accumulation in plant tissue during SR 2010 compared to LR 2010 probably due to rainfall variations (Appendix 1). There was a significant ($p<0.001$) difference between treatments in terms of N and P accumulation in biomass at 50% podding as shown in Fig 4.9 and Fig 4.10. The mycorrhizae inoculant responded when there was reduced rainfall (Appendix 1). Ruiz-Lozano *et al.*, (1994) reported that the effect of AM fungi on drought resistance may be independent of P uptake.

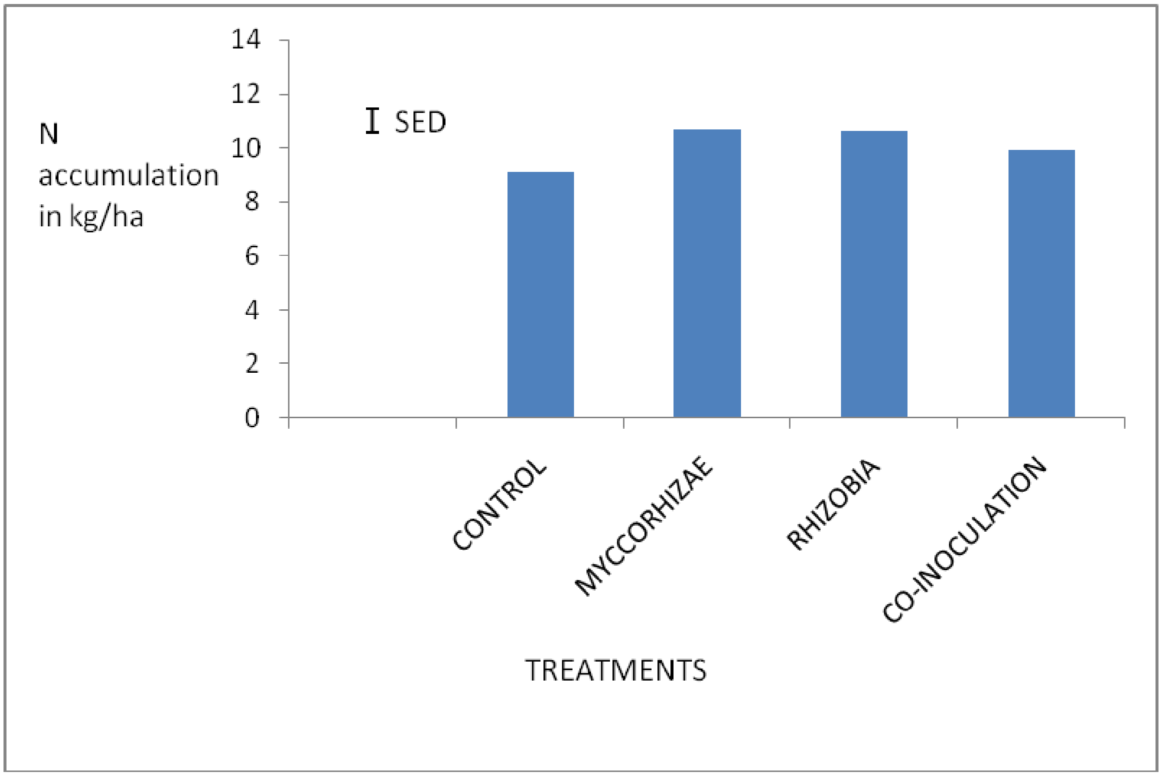

Fig 4.9: Effect of commercial bio-inoculants on N accumulation in Biomass during SR 2010.

Grain P content at harvest was significantly affected by the treatments ($p < 0.05$) as illustrated in Fig 4.12 with control having 3.5 kg/haP and co-inoculation having 4 kg/haP. Low soil P resulted in low P uptake by the plants since the plants were not able to extract sufficient P. N uptake in grains was significantly different ($p<0.05$) with the rhizobial inoculum giving high N uptake (30.1 kg/ha N) as shown in Fig 4.11.

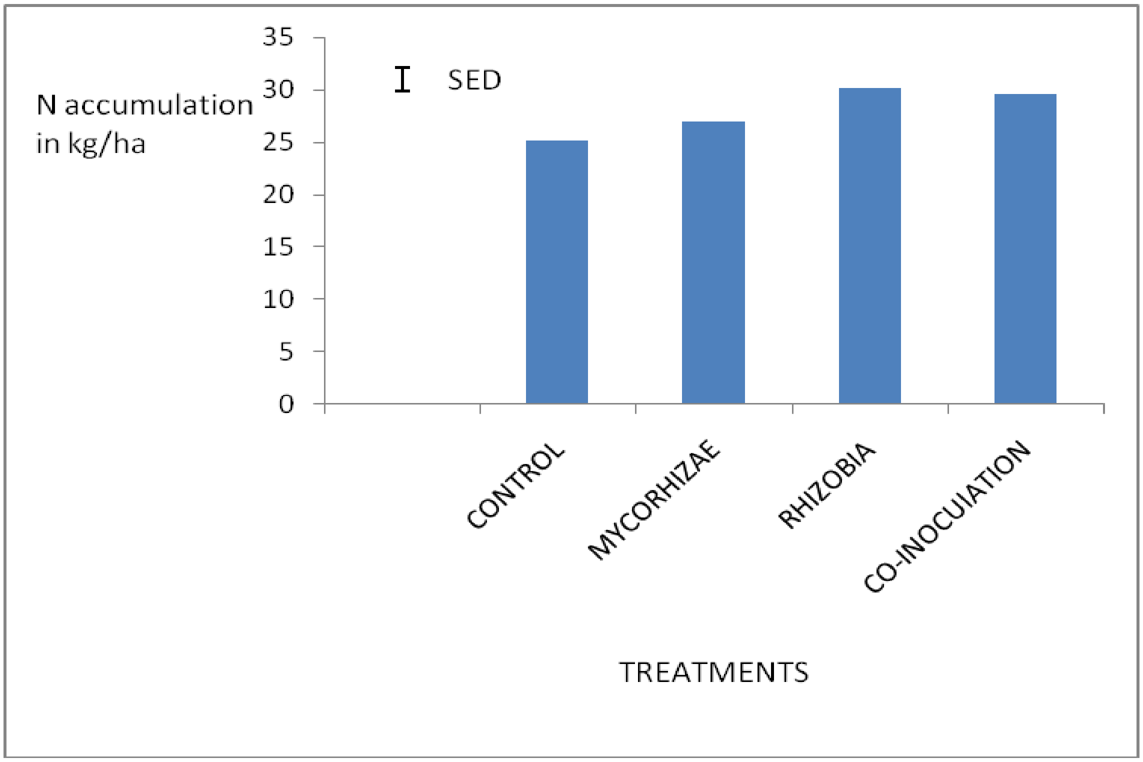

Fig 4.10: Commercial bio-inoculants effect on N accumulation in grains during SR 2010.

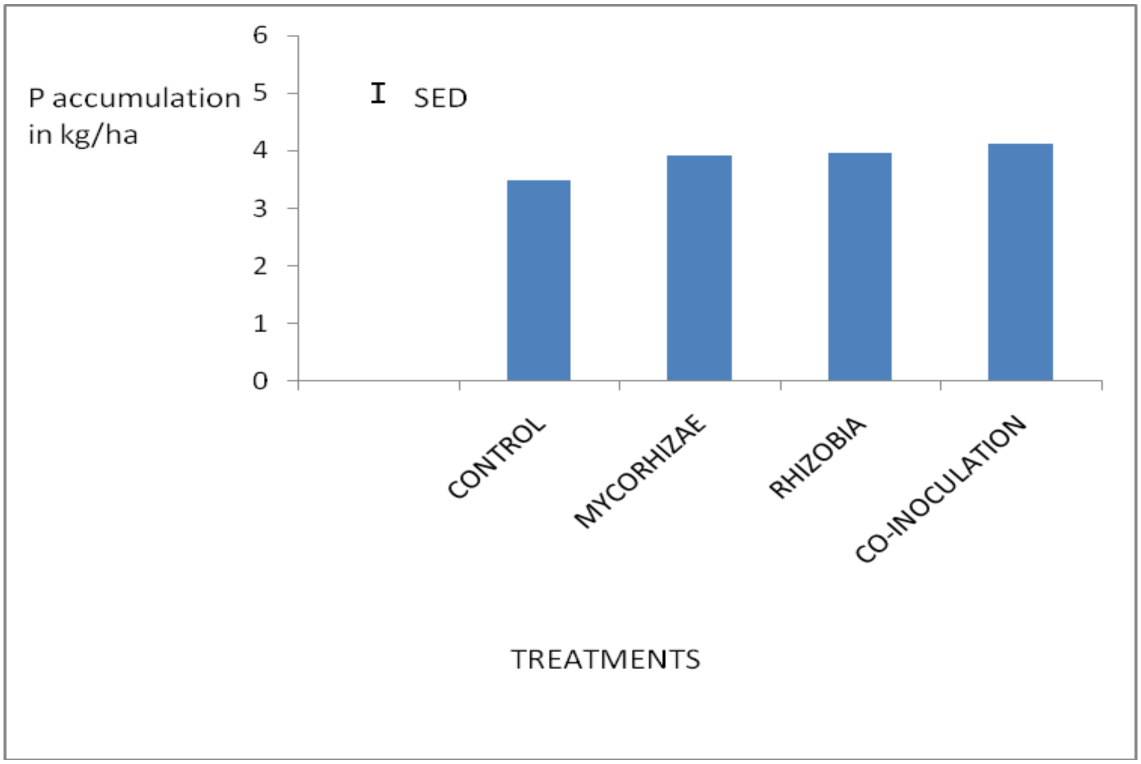

Fig 4.11: Commercial bio-inoculants effect on P accumulation in grains during SR 2010.

4.6 Crude Protein in soybean grain as affected by treatments

The percent of crude protein was not significantly affected ($p>0.05$) by treatments during the LR 2010. During SR 2010, the effect of rhizobial inoculation on grain protein was significant ($p<0.05$) and higher than control or sole application of mycorrhizae. The maximum grain protein of 36.8% was obtained by sole application of rhizobia which was not significantly different ($p<0.05$) from co-inoculation with mycorrhizae treatment. The interaction effect between bacteria and mycorrhizae on grain protein was also absent

($p>0.05$) as compared to sole rhizobial application. This phenomenon indicates that there was no synergistic effect between co-inoculation of bacterial strain and fungus in this regard. Rhizobia inoculation has been reported to increase seed protein content of soybean (Regitano *et al.*, 1995), faba bean (Elsheikh, 1998) and fenugreek (Abdelgani *et al.*, 2002). Low protein content due to mycorrhizal inoculation could be due to low P in the experimental sites (<10 mg P/kg).

4.7 Correlations between selected soil parameters and soybean grain yields

The initial soil pH was positively but weakly correlated ($p<0.05$, $r = 0.19$) with grain yields during LR 2010 season (Table 4.6). This could be attributed to low soil pH that induced deficiency in some essential nutrients, for example P and Mo, thereby leading to a reduction in the number of nodules and BNF (Marschner, 1995). The inhibition of nodulation is a major limiting factor in BNF of many legume species when grown on acid soils (Ngome, 2006). The factors responsible for poor nodulation in acidic soils include high concentrations of protons and aluminium (Al) and low concentrations of Ca and available P hence low yields. Poor nodulation in acidic soils can also be caused by low survival and persistence of certain strains of rhizobia and can affect the number and distribution of indigenous rhizobia (Peoples *et al.*, 1995). In legume species where root hairs are the dominant infection sites, high accumulation of protons and Al can impair nodulation as the adoption of rhizobia at the host root surface is impaired. The increase in soil pH by liming may therefore be necessary in increasing nodule numbers and BNF in acid soils. Buerkert *et al.*, (1990) also observed an increase in number of nodules in common beans as a result of increased soil pH by liming.

Table 4.6: Correlation between selected soil chemical properties and soybean grain yields during LR 2010

Pearson Correlation Coefficients, N = 64
Prob > |r| under H0: Rho=0

	YIELD	pH	Carbon	Total N	Olsen P	NOD WGTS
YIELD						
Ph	0.19*					
Carbon	0.42***	0.26*				
Total N	-0.03	0.16	0.38***			
Olsen P	0.29***	0.43***	0.53	0.21		
NOD Wgt	0.42**	0.24**	0.09**	0.2	0.19*	

Where * represents $p<0.05$, ** represents $p<0.01$ and *** represents $p<0.001$

There was significant correlation ($p<0.001$, $r=0.42$) between soil organic carbon and soybean grain yields. Since the quantity of humus in soil is influenced by the quantity of carbon compounds added, the availabilities of N, S and P compounds is increased in soils with high organic C. At moderate level of carbon, the soil is able to supply the plant with essential plant nutrients hence a high significant correlation with grain yields ($p<0.001$) found in this study. This implies that with increasing level of organic carbon, the plants were able to acquire nutrients from the soil hence increase soybean grain yields. The levels of soil nitrogen were adequate in most farms during long rains hence minimum stress due to N nutrient.Soils high in humus will have high soil N hence the highly significant correlation ($p<0.001$) between total N and soil organic matter as humus is mineralized to release soil N.

Highly significant correlation ($p<0.001$) between soil available P and soybean grain yields could be attributed to the fact that P bioavailability is a major factor limiting N fixation. The nutritional aspects of AM symbiosis have frequently been studied in the past in various plants including common beans. AM symbiosis usually offers improved acquisition of P and Zn, particularly under soil conditions when the availability of these elements for plants is low. These nutritional effects are generally stronger in soybeans than in grasses, most probably due to the differences in the root system architecture (Rao, 2002).

Increase in soil pH results into increase in available soil P upto a near neutral pH (6.5-7.5) range hence the highly significant ($p<0.001$) correlation between soil pH and available P. Similar results were noted in SR 2010.

Table 4.7: Correlation between selected soil chemical properties and soybean grain yields during SR 2010

Pearson Correlation Coefficients, N = 224 Prob > |r| under H0: Rho=0	GRAIN YIELD	SOIL pH	CARBON	TOTAL N	OLSEN P	Fresh Weight nodules
GRAIN YIELD						
SOIL pH	0.2**					
CARBON	0.07**	0.16				
TOTAL N	0.36*	0.45*	0.24**			
OLSEN P	0.12**	0.54***	0.18	0.66*		
Fresh Weight nodules	0.37**	0.17**	0.21	0.33*	0.19	

Where * represents $p<0.05$, ** represents $p<0.01$ and *** represents $p<0.001$

The significant correlation ($p<0.05$) between soil N and soybean grain yields (Table 4.7) attributed to a stimulatory effect on legume nodulation and BNF. This stimulatory effect occurs in the period between root emergence and the onset of active BNF (Giller and Cadisch, 1995).Where soil fertility is high, legumes in the field thrive without fixing N_2. Under such conditions, they may derive all their N requirements from the soil N. But in the majority of Western Kenya, levels of plant available N are usually insufficient to satisfy a legume N requirement (Gachengo, 1999). Therefore in the presence of effective rhizobia, the unfulfilled demand of N could be met by BNF (Peoples *et al.,* 1995). Danso *et al.,* (1992) noted that low levels of ammonium N can enhance BNF due to improved nodule development. Soils with low levels of soil N and few indigenous rhizobia have been reported to give measurable benefits in terms of nodulation, N accumulation, plant biomass and grain yield (Musyoki *et al*., 2011).

However, it has been widely reported that large amounts of inorganic N, have a prominent influence on reducing BNF (Walley *et al.,* 2005). When the level of inorganic N increases, nitrogenase enzyme activity declines rapidly as well as the number of nodules. Shoot growth, however continues to increase indicating a shift from BNF to inorganic N nutrition (Becker *et al.,* 1991). Fujita *et al.,* (1992) reported that BNF was reduced by 55% in groundnut after application of 50 kg N/ha.Warnek and Arndt (2002) observed a significant decrease in the number and mass of soybean root nodules with increasing concentration of nitrate. They also noted a decline in BNF from 84% to 3% Ndfa with an increase in nitrate concentration from 0.25 to 25 µg kg^{-1} (NO_3 N).

4.8 Economic results

Yield was affected by the soil and weather conditions, the management of the crop, and whether the product was applied correctly. Hence, yields obtained varied widely. The average control yield equaled 625 kg ha^{-1}, but varied between 10 and almost 2600 kg ha^{-1}.

The overall average yield increased by 109 kg ha^{-1} due to rhizobium inoculation, which entails a BCR of \$ 4.1$\$^{-1}$. Of all participants, 54 % obtained a yield increase that exceeded 54 kg ha^{-1}, with an average BCR of \$ 11.0 $\$^{-1}$. These participants can be assumed to have correctly applied the inoculant to a well-managed crop in an appropriate soil. However, 27 % of participants obtained yields that differed less than 54 kg ha^{-1} in comparison with the control, and 19 % obtained yields that declined by more than 54 kg ha^{-1}. For these participants, the use of the product entails no financial gain (or even a loss), which may have diverse causes, amongst others: poor rainfall, degraded soil fertility or a high soil N status.

CHAPTER FIVE

CONCLUSIONS AND FURTHER STUDIES

5.1 Conclusions

1. Bradyrhizobial inoculants have a high potential as commercial biofertilizers and can partially substitute the need for mineral N fertilizer in legume farming systems. However, there is need to target these inputs to the most responsive fields. Legumefix inoculant (Bradyrhizobium) was more effective compared to Rhizatech inoculant (mycorrhizae) under local field conditions in Bungoma at moderate soil N and P. Co-inoculation of Bradyrhizobium with mycorrhizae did not result in increased nodulation or soybean yield compared to sole rhizobia inoculation.

2. Selected soil chemical properties (pH, Olsen P, N and C) and nodule weights had significant effect on soybean yields during both seasons.

3. It is therefore of crucial importance that the Bradyrhizobium inoculant (Legumefix) is

used as part of an integrated soil fertility management technology, and applied correctly, accompanied by appropriate crop and soil management for greater economic returns.

5.2 Further studies

1. Further studies are suggested to elucidate the conditions under which synergism between both inoculants may occur, with specific focus towards soil P availability and management of P possible inputs in the low P soils.

2. There is need to seek mechanisms of protein accumulation in soybean grain as influenced by rainfall and its distribution as observed in this study.

3. Further research is needed to determine the contribution of environment and plant interactions in soybean production, especially as soybean yields are still disappointingly low in most Kenyan conditions. Screening of other cultivars is also suggested.

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APPENDICES

APPENDIX I

Cumulative Rainfall data in Bungoma during the year 2010. Source: Nzoia Sugar Factory, Bungoma

Month	Rainfall in mm per month
January	73.1
February	181.7
March	217.6
April	161.5
May	212
June	211.8
July	103.5
August	221.3
September	170.2
October	252.1
November	70
December	37.5

Appendix II

Distribution of farms during LR 2010 and SR 2010 in Bungoma based on soil pH (H_2O) data (0-15cm)

Degree of acidity	pH range	LR 2010	SR 2010
Extremely acidic	< 4.5	4	1
Strongly acidic	4.5 – 5.2	13	30
Moderately acidic	5.3 – 5.9	17	26
Slightly acidic	6.0 – 6.5	6	4
Near neutral	6.5 – 7.0	4	2
Total		44	63

Appendix III

Distribution of farms during LR 2010 and SR 2010 in Bungoma based on soil available P data (0-15cm)

P Level	P range (mg kg^{-1})	LR 2010	SR 2010
Low	< 10	34	39
Medium	10 – 20	9	21
High	20 – 40	1	3
Extremely High	>40	0	0
Total		44	63

Appendix IV

Laying trials in the field

(Source:Author,2010)

APPENDIX V

BIOMASS YIELD AT 50% PODDING DURING LONG RAINS 2010

The SAS System

Class Level Information		
Class	Levels	Values
rep	30	1 10 11 12 13 14 15 16 17 18 19 2 20 21 22 23 24 25 26 27 28 29 3 30 4 5 6 7 8 9
treat	4	CONTROL RHIZOBIA MYCORRHIZAE CO-INOCULATION

The Mixed Procedure

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treat	3	87	5.96	0.0010

Least Squares Means						
Effect	treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CONTROL	1572.67	172.05	87	9.14	<.0001
treat	RHIZOBIA	2086.67	172.05	87	12.13	<.0001
treat	MYCORRHIZAE	1673.33	172.05	87	9.73	<.0001
treat	CO-INOCULATION	2048.00	172.05	87	11.90	<.0001

Differences of Least Squares Means

Effect	treat	_treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CONTR.	RHIZ.	-514.00	150.78	87	-3.41	0.0010
treat	CONTR.	MYCORR.	-100.67	150.78	87	-0.67	0.5061
treat	CONTR.	CO-IN.	-475.33	150.78	87	-3.15	0.0022
treat	RHIZ.	MYCORR.	413.33	150.78	87	2.74	0.0074
treat	RHIZ	CO-IN.	38.6667	150.78	87	0.26	0.7982
treat	MYCORR.	CO-IN.	-374.67	150.78	87	-2.48	0.0149

APPENDIX VI

BIOMASS YIELD AT 50% PODDING DURING SHORT RAINS 2010

The SAS System

The Mixed Procedure

Class Level Information		
Class	Levels	Values
rep	48	1 10 11 12 13 14 15 17 18 19 2 20 21 22 23 24 25 26 27 28 29 3 30 31 32 33 34 35 36 37 38 39 4 40 41 42 43 44 45 46 47 48 49 5 6 7 8 9
treat	4	CONTROL RHIZOBIA MYCORRHIZAE CO-INOCULATION

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treat	3	145	0.26	0.8510

Least Squares Means						
Effect	treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CONTROL	363.61	29.9729	145	12.13	<.0001
treat	RHIZOBIA	387.95	29.9729	145	12.94	<.0001
treat	MYCORRHIZAE	385.38	29.9729	145	12.86	<.0001
treat	CO-INOCULATION	382.01	29.9729	145	12.75	<.0001

Differences of Least Squares Means							
Effect	treat	_treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CONTROL	RHIZOBIA	-24.3469	30.3305	145	-0.80	0.4234
treat	CONTROL	RRHIZOBIA	-21.7755	30.3305	145	-0.72	0.4739
treat	CONTROL	CO-INOC.	-18.4082	30.3305	145	-0.61	0.5449
treat	RHIZOBIA	MYCORR	2.5714	30.3305	145	0.08	0.9326
treat	RHIZOBIA	CO-INOC	5.9388	30.3305	145	0.20	0.8450
treat	MYCORR.	CO-INOC	3.3673	30.3305	145	0.11	0.9118

APPENDIX VII

NODULE FRESH WEIGHTS DURING LONG RAINS 2010

The SAS System

The Mixed Procedure

Class Level Information		
Class	Levels	Values
rep	30	1 10 11 12 13 14 15 16 17 18 19 2 20 21 22 23 24 25 26 27 28 29 3 30 4 5 6 7 8 9
treat	4	CONTROL RHIZOBIA MYCORRHIZAE CO-INOCULATION

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
treat	3	87	29.72	<.0001

Least Squares Means

Effect	treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CONTROL	0.2653	0.09708	87	2.73	0.0076
treat	RHIZOBIA	0.9330	0.09708	87	9.61	<.0001
treat	MYCORRHIZAE	0.3807	0.09708	87	3.92	0.0002
treat	CO-INOCULATION	1.0183	0.09708	87	10.49	<.0001

Differences of Least Squares Means

Effect	treat	_treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CONTROL	RHIZOBIA	-0.6677	0.09893	87	-6.75	<.0001
treat	CONTROL	MYCORR.	-0.1153	0.09893	87	-1.17	0.2469
treat	CONTROL	CO-INOC.	-0.7530	0.09893	87	-7.61	<.0001
treat	RHIZOBIA	MYCORR.	0.5523	0.09893	87	5.58	<.0001
treat	RHIZOBIA	CO-INOC.	-0.08533	0.09893	87	-0.86	0.3907
treat	MYCORR.	CO-INOC.	-0.6377	0.09893	87	-6.45	<.0001

APPENDIX VIII

NODULE FRESH WEIGHTS DURING THE SHORT RAINS 2010

The SAS System

The Mixed Procedure

Class Level Information		
Class	Levels	Values
rep	48	1 10 11 12 13 14 15 17 18 19 2 20 21 22 23 24 25 26 27 28 29 3 30 31 32 33 34 35 36 37 38 39 4 40 41 42 43 44 45 46 47 48 49 5 6 7 8 9
treat	4	CONTROL RHIZOBIA MYCORRHIZAE CO-INOCULATION

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treat	3	145	12.45	<.0001

Least Squares Means						
Effect	treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CONTROL	0.2694	0.05320	145	5.06	<.0001
treat	RHIZOBIA	0.5747	0.05320	145	10.80	<.0001
treat	MYCORRHIZAE	0.2619	0.05320	145	4.92	<.0001
treat	CO-INOCULATION	0.5865	0.05320	145	11.02	<.0001

Differences of Least Squares Means

Effect	treat	_treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CONTROL	RHIZOBIA	-0.3053	0.07291	145	-4.19	<.0001
treat	CONTROL	MYCORR	0.007490	0.07291	145	0.10	0.9183
treat	CONTROL	CO-INOC	-0.3171	0.07291	145	-4.35	<.0001
treat	RHIZOBIA	MYCORR	0.3128	0.07291	145	4.29	<.0001
treat	RHIZOBIA	CO-INOC	-0.01180	0.07291	145	-0.16	0.8717
treat	MYCORR	CO-INOC	-0.3246	0.07291	145	-4.45	<.0001

APPENDIX IX

GRAIN YIELDS DURING LONG RAINS 2010

The SAS System

The Mixed Procedure

Class Level Information		
Class	Levels	Values
rep	43	1 10 11 12 13 14 15 17 18 19 2 20 21 22 23 24 25 26 27 28 29 3 30 31 32 33 34 35 36 37 38 39 4 40 41 42 43 44 5 6 7 8 9
treat	4	CO-INOCU CONTROL MYCORRHI RHIZOBIA

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
treat	3	130	5.53	0.0013

Least Squares Means						
Effect	treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CO-INOCU	1027.25	80.2732	130	12.80	<.0001
treat	CONTROL	924.28	80.2732	130	11.51	<.0001
treat	MYCORRHI	922.04	80.2732	130	11.49	<.0001

Least Squares Means						
Effect	treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	RHIZOBIA	1115.90	80.2732	130	13.90	<.0001

Differences of Least Squares Means							
Effect	treat	_treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CO-INOCU	CONTROL	102.96	55.9402	130	1.84	0.0680
treat	CO-INOCU	MYCORRHI	105.21	55.9402	130	1.88	0.0622
treat	CO-INOCU	RHIZOBIA	-88.6531	55.9402	130	-1.58	0.1154
treat	CONTROL	MYCORRHI	2.2447	55.9402	130	0.04	0.9681

Differences of Least Squares Means							
Effect	treat	_treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CONTROL	RHIZOBIA	-191.62	55.9402	130	-3.43	0.0008
treat	MYCORRHI	RHIZOBIA	-193.86	55.9402	130	-3.47	0.0007

APPENDIX X

GRAIN YIELD DURING SHORT RAIN 2010

The SAS System

The Mixed Procedure

Class Level Information		
Class	**Levels**	**Values**
rep	62	1 10 11 12 13 14 15 17 18 19 2 20 21 22 23 24 25 26 27 28 29 3 30 31 32 33 34 35 36 37 38 39 4 40 41 42 43 44 45 46 47 48 49 5 50 51 52 53 54 55 56 57 58 59 6 60 61 62 63 7 8 9
treat	4	CO-INOCU CONTROL MYCORRHI RHIZOBIA

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
treat	3	187	2.25	0.0838

Least Squares Means						
Effect	treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CO-INOCU	521.18	39.1793	187	13.30	<.0001
treat	CONTROL	453.98	39.1793	187	11.59	<.0001
treat	MYCORRHI	488.92	39.1793	187	12.48	<.0001
treat	RHIZOBIA	523.60	39.1793	187	13.36	<.0001

Differences of Least Squares Means

Effect	treat	_treat	Estimate	Standard Error	DF	t Value	Pr > |t|
treat	CO-INOCU	CONTROL	67.1997	30.8230	187	2.18	0.0305
treat	CO-INOCU	MYCORRHI	32.2556	30.8230	187	1.05	0.2967
treat	CO-INOCU	RHIZOBIA	-2.4221	30.8230	187	-0.08	0.9375
treat	CONTROL	MYCORRHI	-34.9442	30.8230	187	-1.13	0.2584
treat	CONTROL	RHIZOBIA	-69.6218	30.8230	187	-2.26	0.0251
treat	MYCORRHI	RHIZOBIA	-34.6776	30.8230	187	-1.13	0.2620

APPENDIX XI

COMPRO UNIVERSITY OF ELDORET TEAM

(SOURCE:AUTHOR,2010)

APPENDIX XII

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INTERACTION BETWEEN NITROGEN AND PHOSPHORUS MICROBIAL INOCULANTS ON SOYBEAN PRODUCTION IN BUNGOMA, KENYA

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Abstract Nitrogen and phosphorus are the major limiting nutrients for crop production in the western Kenya. Rhizobia and myccorrhizae can be used to enhance N fixation and P solubilisation in low P systems. A trial was conducted in Bungoma in western Kenya to assess the interaction of an effective rhizobial (Legumefix, Legume Technologies, UK) and arbuscular mycorrhizal (Rhizatech,Dudutech Ltd, Kenya) inoculants, earlier screened under greenhouse conditions. A promiscuous medium-maturity soybean variety TGx1740-2E (SB19) was the study crops. Rhizobial inoculation resulted in significantly ($p<0.01$) higher nodulation (0.64 g plant-1) compared to the control (0.29 g plant-1). Co-inoculation of Rhizobia and mycorrhizae increased nodulation further by 30%. Rhizobial inoculation increased soybean dry matter yield ($p<0.01$) by 27% and average grain yield by 15-30%. Grain yield responses to co-inoculation were observed when soil total N content varied between 0.05 and 0.15%. Co-inoculation improves soybean nodulation but no significant ($p<0.01$) effect on grain yields.

Key words: *Glycine max*, mycorrhiza, Rhizobia