EFFECT OF DIFFERENT SOIL FERTILITY AMENDMENTS ON THE NODULATION AND YIELD OF TWO SOYBEAN VARIETIES

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DECLARATION

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

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DEDICATION

This work is dedicated to my late father Stanley Muthuri, who was an ardent believer in the value of education and to my mother Isabel Muthuri for her financial and moral support. Lastly to my husband Simon and my two lovely daughters Lavine and Leayah for their understanding during the entire period of the study.

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LIST OF ABBREVIATIONS AND ACRONYMS

AC	Acre
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
BNF	Biological Nitrogen Fixation
CR	Congo Red
BTB	Bromothymol Blue
DAP	Di-ammonium Phosphate
DF	Degree of freedom
ERS	Economic Recovery Strategy
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistical Database
FYM	Farm Yard Manure
GTZ	German Technical Cooperation
Ha	Hectare
Ib	Pound
IITA	International Institute of Tropical Agriculture
K	Potassium
KARI	Kenya Agricultural Research Institute
KMA	Kenya Ministry of Agriculture
KMD	Kenya Meteorological Department
MT	Metric Tons
N	Nitrogen
NHI	Nitrogen Harvest Index
P	Phosphorous
PM	Poultry Manure
PRC	Peoples Republic of China
RCBD	Randomized complete block design
RDF	Refuse Derived Fuel
SSA	Sub Saharan Africa
SSP	Single Super Phosphate
TGx	Tropical Glycine Cross
YEM	Yeast Extract Mannitol
YEMB	Yeast Extract Mannitol Broth
UAN	Urea Ammonium Nitrate
USDA	United States Development Agency

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ABSTRACT

Soybean (*Glycine max (L.) Merrill*) is referred to as the golden crop of the future. There is a concern in Kenya on its production due to the fact that the country remains a net importer of this vital food to the tune of 100,000 tons annually yet the country has the potential to produce that capacity locally. The major impediments in local production are singled out as: Expensive farm inputs in form of fertilizers and use of inferior soybean varieties in terms of effective nodulation. The nodulation of soybean is influenced by inoculation, soil fertility, agro-ecological zones and soybean varieties. This study was carried out to achieve the following objectives, to investigate the effect of different soil fertility alteration on soybean nodulation and yield and to analyze symbiotic effectiveness between fast and slow growing rhizobia in nitrogen fixation with the soybean. Two soybean varieties (promiscuous and non-promiscuous) were planted in experimental plots at Kisii, KARI during the months of April to August 2008. There were five main treatments: Control, poultry manure, farmyard manure (FYM), di-ammonium phosphate (DAP) and inoculant strain USDA 1011. Sub-treatments were the two soybean varieties, the promiscuous variety was SB19 TGx 1740 2F while the non promiscuous was Gazelle. The experiment was carried out in a split plot design and replicated four times at Kisii, KARI Station. The greenhouse experiments were carried out to analyze the symbiotic effectiveness of fast and slow growing rhizobia isolated from Kisii while laboratory experiments were used to characterize the rhizobia isolated from the field. Nodulation status, plant biomass production, height of the plant and yield were used to generate data for the main field experiments. For greenhouse experiment, nodulation status, plant dry weight and acetylene reduction activities were used to generate the data. The transformed data was subjected to analysis of variance (ANOVA) and means were separated using Tukey's high significant difference (HSD). The effects due to soil amendments on nodulation status were significantly different at $P \leq 0.05$.There was no significant difference for grain weight for both varieties on different treatments. There was a significant difference in terms of nodule numbers and acetylene reduction assay for both soybean varieties when symbiotic effectiveness of the isolates was assessed. From the study it was concluded that the soybean treated with the inoculants had high nodulation and yield. The study also showed that FYM has slow mineralization. Fast growing rhizobia were more effective in nitrogen fixation in TGx variety than in Gazelle variety while slow growing rhizobia were more effective in Gazelle.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Soybean (*Glycine* max (L) Merr) has attained a prominent place in modern agriculture and has a remarkable place in both the domestic and industrial markets in most countries. The soybean is a crop with many uses. It provides human food, animal feeds and materials for many industrial uses. As a source of protein, oil and fats, it complements the contribution of most other major crops (Borget, 1992). Soybean plant contains all amino acids required by the human body except methionine, usually found in cereals such as maize (Osho, 1995). Of all grain legumes, soybean has the highest concentration of protein. While most other grain legumes contain about 20% protein by volume, soybean contains about 40% protein (Greenberg and Hartung, 1998, BIDCO, 2005). Soybeans can produce at least twice as much protein per acre as any other major vegetable or grain crop, 5 to 10 times more protein per acre than land set aside for grazing animals to produce milk and up to 15 times more protein per acre than land set aside for meat production as shown in Table1.1 (USDA, 2011).

Significant progress has been made in developing in the scientific understanding of the crop, its production and in improving yields, but the gains in the yields are less striking compared to crops like corn, wheat, rice and sorghum in Africa (Mpepereke *et al.,* 2000). More land has been diverted to soybean production to meet the increasing demand. With emphasis on soybean research, further progress in understanding the crop and increasing yield can be expected (Osborn and Riedell, 2004). In terms of production, soybean yield 803 kg/ha on average in Sub-Saharan Africa (SSA) compared to 665 kg/ha of cowpea. In addition, the crop has a short (85 days) to medium (125 days) maturation period (Mpepereke *et al.*, 2000)

Soybean thrives in climatic regions characterized with annual mean temperatures of 20 to 30°C and annual rainfall of between 800-1500 mm (Thairu and Shakoor, 1985). Rainfall amount and distribution is of paramount importance to soybean production. Moisture availability is particularly critical during germination, flowering and pod filling stage (De Mota, 1978). It also does well in alluvial soils with good organic content. Being a legume, soybean fixes nitrogen by establishing a symbiotic relationship with the bacterium *Bradyrhizobium japonicum* (Jordan, 1982). Best nitrogen fixation results are achieved when an inoculum of the correct strain of bacteria is integrated with the soybean seed before planting. The plant reaches a height of 1 meter and takes 80 to 120 days from sowing to harvesting.

Soybean grows in areas where maize and common beans are grown. It grows to a height of 60 to 120 cm, maturing in 3 to 6 months depending on variety, climate, and location. Soybean is a drought tolerant plant. Depending on the variety, the crop can be grown from sea level to 2200 m altitude and under rainfall ranging from 300 to 1200 mm and temperatures of about 25-30ºC. Altitude influences temperature that in turn affects the initiation of flowering and maturity. At very high altitudes, flowering may not occur and the crop remains vegetative. Soybean is therefore a crop that requires warm climates and is suitable for low to medium altitudes (Ogema *et al.*, 1988). It grows best when planted in pure stands. It improves soil fertility by fixing nitrogen from the atmosphere (Kasasa *et al.*, 2000; Sanginga *et al.*, 2003).

Table: 1.1 Nutrition content of different parts of soybean plant

Component	**Protein%**	**Fat%**	**Carbohydrate%**	**Ash%**
Whole plant	40	20	34	4.9
Hull	43	23	29	5.0
Cotyledons	8	1	86	4.3
Hypocotyls	41	11	43	4.4

Source Osho, 1995.

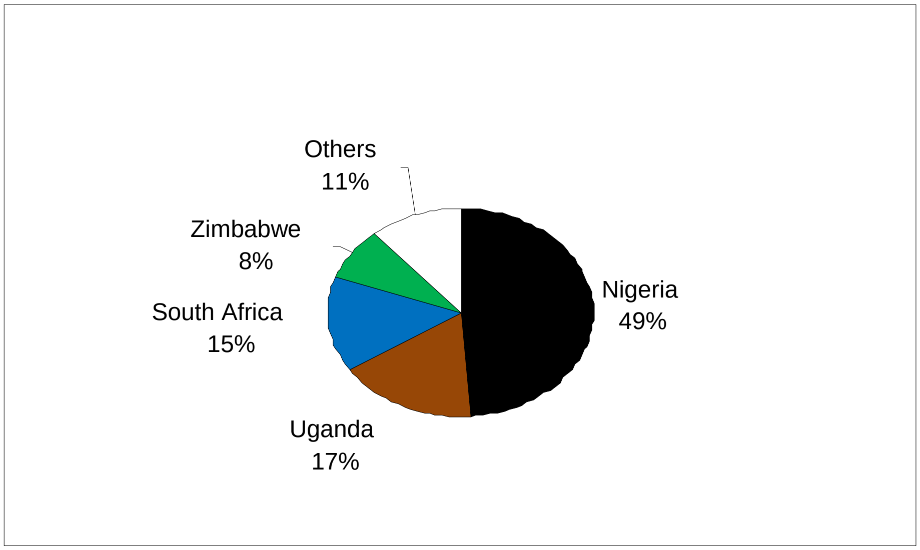
Globally, the main producers of soybean are the United States (35%), Brazil (27%), Argentina (19%), China (6%) and India (4%) (USDA, 2011). Food and Agriculture Organization statistics indicates that total world production of soybean increased from 136.5 million MT in 1994 to 189.2 million MT in 2003, this represents 0.16% annual increase in production. World soybean trade is a big business that amounted to nearly US $11 billion in 2002. Soybean growers in leading producing countries have been using biotechnological innovations to boost soybean production. The use of biotechnology modified planting materials confers the advantages of higher crop yields and greater tolerance to soybean diseases and pests (Jagwe and Nyapendi, 2004). High crop yield increases the profits that farmers make from soybean production and marketing enterprises. Table 1.2, shows the international soybean quality specifications preferred in the world market.

Table: 1.2 Quality specifications for soybean in the world market

Characteristic	%Minimum	%Maximum	%Basis
	Test weight: 54 lbs/bu		
Protein	35		
Oil content	18		
Moisture content		14.0	
Splits		20.0	
Foreign matters		2.0	
Soybean other color		2.0	
Heat damaged kernel		0.5	
Total damaged kernel		3.0	
Oil content			18.0

Source: Jagwe and Nyapendi, 2004

Universally Africa is a very small producer of soybean accounting for 0.4 to 1% of total world production of soybean (Jagwe and Nyapendi, 2004). The main producers within the continent include Nigeria, South Africa, Uganda and Zimbabwe. Nigeria, accounted for a mere 0.3% of the world soybean output in 2003. Soybean production in Sub Saharan Africa (SSA) has been spreading steadily but unevenly. In 1961 soybean production in SSA was 70,000 tones, 80% of this production was from Nigeria. This increased eleven fold within a span of 41 years to 770,000 tones in 2002 (FAOSTAT, 2008). In the 1990s, production in Uganda reached 166,000 tones per annum (Mpepereke *et al.*, 2000). These success stories cannot be told about Kenya yet (Mpepereke *et al.*, 2000, Chianu *et al.*, 2008). The figure 1.0 shows the production of soybean in Africa. Kenya does not feature among the 19 producing countries (FAOSTAT, 2008).



Source. FAOSTAT, 2008

Figure 1.1: Soybean production in Africa

Significant progress has been made in developing scientific understanding of the crop, its production and improving yields, but the gains in yields for soybeans have been smaller and less striking than for crops like corn, wheat, rice and sorghum in Africa (Mpepereke *et al.*, 2000). Along with higher yields, more land has been diverted to soybean production to meet the increasing demands for the crop with increasing emphasis on soybean research (Osborne and Riedell, 2004). In terms of production, soybean yields 803 kg/ha on average in Sub-Saharan Africa (SSA) compared to 665 kg/ha cowpea productivity, (Stanton, 1996). In addition, the crop has a short (85 days) to medium (125 days) maturation period (Mpepereke *et al.*, 2000).

In Kenya research carried out by FAO (2008) it is estimated that an average yield of 800 kg ha^{-1} of soybean has been stagnant since 1990. However, there is regional variability in yield. Between 1999 and 2003, soybean annual average yield ranged from 560 kg ha^{-1} (Western province) to 1100 kg ha^{-1} (Eastern province). The average yields obtained in Rift Valley and Central provinces were between these figures. It has, however, been demonstrated that it is possible to obtain soybean yields of 3000 – 3600 kg ha^{-1} from improved varieties of soybean and with good management practices (FAO,2008). According to FAO (2008) the highest farm-level soybean yield of 1600 kg ha^{-1} in Western province was obtained from Butere/Mumias district. A survey carried out in 1998 on the six soybeans varieties (Ducker, EA13600, Nyala, SCS1, Gazelle and Sable) showed that Nyala, Gazelle and Duicker were most widespread, most probably due to seed availability, rather than the choice of farmers (Kaara *et al.*, 1998).

Other major soybean producing provinces in Kenya after the Western province are Nyanza and Central provinces, which accounted for 11-12% of total smallholder soybean production in 2003. The quantity of soybean produced by large scale holders is not precisely known but is estimated at about 4000 MT per annum. Small scale farmers produce about 1000 MT per annum (GTZ, 1996). The large scale operators include: Hugo Wood (located in Narok), George Nightgale farm (Njoro), Menengai Feedlot (Njoro), Timau soybean production (Meru), Kisima farm (registered seed merchant, Meru), Rumuruti Kisima (Laikipia district), Hortitec Seed Company (Naivasha), Delamere Estates (Naivasha), Elkana Owgeas, Western Seed Company (Kitale), Mwea irrigation scheme managed by National Irrigation Board (Kirinyaga district and KARI (Njoro) (Kaara *et al.*, 1998). The major factors responsible for stifled growth in production of soybean in Kenya include expensive farm inputs in form of commercial nitrogen fertilizer which is out of reach to most small holder farmers and lack of sensitization of the most appropriate soybean varieties to be grown on Kenyan soil.

Table 1.3: Soybean area, yield and production in Kenya: 1990-2007

Year	**Area (ha)**	**Yield (kg ha^{-1})**	**Production (tons)**
1990	2500	800	2000
1991	2500	800	2000
1992	2500	800	2000
1993	2500	800	2000
1994	2500	800	2000
1995	2500	800	2000
1996	2500	800	2000
1997	2500	800	2000
1998	2500	800	2000
1999	2500	800	2000
2000	2500	800	2000
2001	2500	800	2000
2002	2500	800	2000
2003	2500	800	2000
2004	2500	800	2000
2005	2500	800	2000
2006	2513	826.5	2077
2007	2500	840	2100

Source: FAO, 2008

1.2 Statement of the problem

Soybean has become an important crop in Kenya due to its supply of proteins and oil. In spite of its importance as a source of protein and oil, researchers have noted that its production is way below potential (FAO, 2008). In the past, researchers have been emphasizing on inorganic fertilizer to foster the production of crops including soybean. However declining soil fertility and high fertilizer costs are major limitations to crop production in smallholder farms in Kenya (Maobe *et al.,* 2004). This has been augmented by intensification of agriculture coupled with the reduction in farm sizes (Saha and Muli, 2000). Requirements for nitrogen (N) exceed any other major nutrient. Soils in the tropics rarely have enough of this nutrient to produce high sustainable yields (Wringley, 1982). This lack of adequate amounts of nitrogen in most soils puts a limitation on the farmers' goals of increasing yield per unit area. Rebuilding soil fertility in traditional agricultural systems has been achieved through long duration fallow periods (Poubom *et al.*, 2005).

However, with increased human population and land pressure, long fallow period are no longer feasible (Gichangi *et al.,* 2002). The quantity of nitrogen needed for agriculture is projected to increase in the period up to 2030 (Tillman *et al.*, 2001) and would lead to greater environmental degradation. Reduced dependence on fertilizer nitrogen and adopting farming practices that favour the more economically viable and environmentally prudent nitrogen fixation will benefit both agriculture and the environment (Vance, 2003). There are several options which are available to manage nitrogen in farmers' fields with chemical fertilizers often considered to be an immediate answer to the current nutrient deficiencies in soils (Woomer *et al.,* 1997). Unfortunately, commercial nitrogen fertilizers are expensive and out of reach to most small-scale farmers. As a result cheaper sources of nitrogen need to be sought if yields are to be sustained and food security attained. Biological materials may offer a solution in alleviating soil fertility problems and hence increase in crop production. The farm derived sources such as crop residue, compost, manure and household waste has commonly been used by farmers in the management of soil fertility (Kimani *et al.,* 1998). Animal manure and compost are beneficial in soils because they can increase the water holding capacity and cation exchange capacity (Nandwa, 1995). Therefore, the use of organic inputs as external nutrient sources can be used as alternative to expensive fertilizer. In addition, countries where nitrogen fertilizers are imported and the technology for manufacturing them are limited or too expensive to afford (Mwangi, 1994).

1.3 Justification

In the Economic Recovery Strategy (ERS) for wealth creation and employment creation, the Kenyan government identified agriculture as an important vehicle for the realization of its employment creation and poverty reduction objectives. According to this strategy, the government's vision is to transform Kenya's agricultural sector into a profitable economy (Government of Kenya, 2004). This transformation calls for fundamental shift to market oriented production, diversification of soybean agriculture and adoption of greater use of appropriate farming practices. Soybean is one such crop that has the potential to make significant contributions to healthcare (Government of Kenya, 2002). To date, Kenya is an importer of soybean since its production (5,000 MT) cannot meet the ever increasing demand of 100,000 MT (GTZ, 1996).

Greater demand is being made on alternative and inexpensive sources of nitrogen (Mwangi, 1994). Hence use of natural manure is seen as an answer to small scale farmers in soybean production in Kenya as it does not only save on expensive farm inputs but also protects the environment from effects of commercial fertilizers. This research was therefore formulated to assess the effect of soil amendment with manure (poultry and farmyard) on the plant growth, nodulation and yield respectively as compared with inorganic fertilizer and to establish the symbiotic effectiveness of slow growing *Bradyrhizobium* and fast growing *Sinorhizobium* in fixing nitrogen with promiscuous and non promiscuous soybean varieties.

1.4 Hypotheses

(i) Soil fertility amendments have no effect on soybean nodulation and yields.

(ii) Effectiveness of nitrogen fixation in soybeans is not influenced by different rhizobia isolates.

1.5 Objectives

1.5.1 General Objective

To investigate the effect of soil amendment by organic manure on soybean nodulation and yield.

1.5.2 Specific Objectives

(i) To determine the effect of different soil fertility amendments on soybean nodulation and yield.

(ii) To assess the effectiveness of local isolates of slow and fast growing soybean rhizobia in nitrogen fixation.

1.6 Significance of the study

Through increased growing of soybean by the smallholder farmers in Kenya, income generation, poverty alleviation, improved nutritional status and improved quality of life are possible benefits.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Declining soil fertility in smallholder farms is a fundamental impediment to agricultural growth and food production in Sub-Saharan Africa (SSA). Therefore, soil fertility replenishment in SSA is viewed as a critical process of poverty alleviation; this was symbolized by the award of the 2002 World Food Prize to Pedro Sanchez, a pioneer in the field (Place *et al.,* 2003). Limited use of nutrient inputs among smallholder farmers increases soil nutrient deficiency. Nitrogen fertilizers improve soil fertility and result in increased yields but are expensive and often beyond the reach of resource poor farmers. This has resulted in food insecurity in Africa (World Bank, 1996; Omamo *et al.,* 2002).

Solutions to smallholder farmers' soil fertility problems may be found in the strategic combination of organic resources, particularly from nitrogen-fixing legumes (Palm *et al.,* 1997). The increase in the use of N and P fertilizers between 1960 and 2000 by intensive agricultural practices led to degradation of air and water quality (Tilman *et al.,* 2001). The average annual loss in soil nutrients of 42 kg N ha^{-1}, 3 kg P ha^{-1} and 29 kg ha^{-1} in Kenya is among the greatest in Africa (Smaling, 1993). Nutrient depletion at farm level results from imbalance of nutrient inputs and losses over time and reaches critical proportions when land is continuously cultivated without the addition of adequate external nutrients (Woomer *et al.,* 2003). Soil fertility replenishment, should therefore be considered as an investment in natural resource capital (Sanchez *et al.,* 1995). Nitrogen quantities in harvested crops are usually high and therefore a continued supply of N is fundamental to the long-term productivity of any cropping system.

2.2 Rhizobia in soybeans

Rhizobia are rod-shaped, gram-negative and non-spore forming bacteria. They are aerobic and can be found free-living in soils, or cultured in agar (Vincent, 1982). The recent concept of rhizobial classification is based on techniques designed to examine large portions of the bacterial genome. On this basis, Jordan (1984) classified root-nodule bacteria under two genera namely; (i) *Sinorhizobium* and (ii) *Bradyrhizobium*. *Sinorhizobium* consists of all fast growing acid producing rhizobia while *Bradyrhizobium* comprises of the slow growing alkali producing rhizobia. In soil or cultures, the actual densities of nodule bacteria required in the rhizosphere for the successful infection are of

the order of 106 to 109 organisms per ml (Purchase and Nutman, 1957).

Rhizobia are soil bacteria characterized by their unique ability to infect root hairs of legumes and induce effective nitrogen fixing nodules to form on roots. Rhizobia commonly occur in soils but often fail to produce effective nodulation (Jordan, 1982). They multiply by simple cell division and generation time ranges from two to four hours for fast growers which generally form relatively large colonies of 2-4 mm diameter within three to five days and for slow growers takes six to eight hours to form colonies of 1µ in diameter within 7 -10 days (Brockwell *et al.,* 1987). Rhizobia are not fastidious in their nutritional requirements (Brockwell *et al.,* 1987). They can use sugars, alcohols and some acids as sources of energy. Yeast extract provides growth factors and vitamins and usually enhances growth but some species can produce their own growth factors. During infections, the rhizobial cell encounters the root of susceptible legume seedlings, then it increases in number and root hair colonization occurs. The root hair curls and *Rhizobium* enters, multiplies and an infection thread is formed. This thread penetrates the root cortex and then the embryonic nodule develops (FAO, 1994).

2.3 Nodulation of soybeans

Biological nitrogen fixation is the process used by microorganisms living in the soil to fix nitrogen in leguminous plants (Gregoire, 2003). During nodulation, host plants excrete flavonoids and bacteria Nod-protein recognizes proper flavonoids, and initiate synthesis of Nod factor by a series of nod genes products, (Newcomb *et al.*, 1979; Rao and Keister, 1978; Date and Halliday, 1987). Nod factor, in return initiate early processes of nodulation. The first nodules form within one week after seedling emergence and become visible as they increase in size. Ten to fourteen days later, the nodule bacteria are able to supply most of the plant's nitrogen requirements. The nodules allow fixation of atmospheric nitrogen but are energetically expensive to develop and maintain (Shantharam and Mattoo, 1997). Hence the host suppresses the growth of most potential root nodules soon after the initial bacterial invasion of root hairs (Spaink, 1995). It also further regulates nodules number in response to environmental factors such as the presence of nitrate or other sources of fixed nitrogen in the soil (Vandyk, 2003).

The nodules which are bright in colour are effective while the nodules white in colour are ineffective, or have not yet developed to a stage at which they can fix nitrogen.

Soybeans are nodulated by the slow growing *Brandyrhizobium japonicum* (Jordan, 1982), *Brandyrhizobium elkanii* (Kuykendall *et al.,* 1992; Sanginga *et al.,* 1995), *Brandyrhizobium liaoningese* (Abaidoo *et al.,* 2006; Xu *et al.,* 1995) as well as the fast growing *Sinorhizobium fredii* (Scholla and Elkan, 1984). Promiscuous soybean varieties are known to nodulate with a wide range of rhizobial strains and therefore, are likely to be widely adopted by farmers (Okogun and Sanginga, 2003; Fening and Danso, 2002; Okereke *et al.,* 2000; Sanginga *et al.*, 1999; Sanginga *et al.,* 1996). The foregoing researchers have only dealt with the type of *Bradyrhizobium* that fix nitrogen with the soybean, but they have not shown which one is more effective in fixing the nitrogen.

2.4 Importance of Biological Nitrogen Fixation (BNF)

Biological nitrogen fixation comprises of non-symbiotic and symbiotic systems. Non-symbiotic nitrogen fixation involves free-living organisms like *Azotobacter*, *Klebsiella*, *Clostridium* and many algae which are able to fix atmospheric nitrogen independently. Symbiotic nitrogen fixation, on the other hand, is based on very close physical and physiological associations between rhizobial bacteria and leguminous plants. The bacteria fix atmospheric nitrogen by incorporating nitrogen gas from the atmosphere into forms utilizable by legumes for the synthesis of organic compounds. Although non-symbiotic organisms fix nitrogen, their contribution to the nitrogen economy of the soil is not as great as those of the symbiotic ones (Hardy and Havelka, 1975). According to Meiklejohn (1954) non-symbiotic nitrogen fixation levels ranges from 10 to 15 kg N/ha/year. In contrast, rhizobia in symbiosis with legumes are believed to fix nitrogen at levels varying from less than 100 kg N/ha/year to more than 600 kg N/ha/year (Graham and Hubbell, 1975).

Estimates show that the symbiotic system contributes 40 million tons of nitrogen annually to grain legumes (Hardy and Havelka, 1975). Rhizobia-legume symbiosis is therefore the most important source of biologically fixed nitrogen in agricultural systems.

Biological Nitrogen Fixation contributes to productivity both directly by increasing the production of legume and indirectly by improving soil fertility (Giller and Cadish, 1995; Giller and Wilson, 1997; Giller, 2001; Mpepereki *et al.,* 2000). The 165 tons estimated to be fixed each year by anthropogenic sources, between 25 and 40 originated from intensive legume cultivation, 80 from application of synthetic N fertilizer and 20 from fossil fuel combustion (Smil, 2002). The amount of nitrogen fixed by the legumes is influenced by various factors such as; compatibility of the rhizobia in the soil with the legume, soil temperature, soil pH, soil moisture (Eaglesham and Ayanaba, 1984; Thies 1991; Ankoma *et al.,* 1998; Maingi *et al.,* 2000)), soil nitrogen, soil mineral phosphorous and mycorrhiza colonization (Nwoko and Sanginga, 1999). In East Africa, ninety eight percent of farmers are aware of legume nodules but only 52% are aware of inoculants (Karanja *et al.*, 1995; Woomer *et al.*, 2003).

Lack of knowledge on BNF information on availability and use of inoculants (Woomer *et al.*, 2003), cost of inoculant (Javaheri, 1996) and special storage condition for inoculant (Mabika and Maringa, 1996) have been identified as major factors hindering adaptation of BNF technology in the region. Soil fertility improvements through incorporation of plant residue from N fixing legumes have been reported (Giller and Wilson, 1997; Ankoma *et al.*, 1995; McDonagh *et al.*, 1993; Toomsan *et al.*, 1995). Farmers have traditionally incorporated N_2 fixing legume cover crops as green manure or legume residue to increase soil organic and available nitrogen to subsequent crops (Herridge *et al.*, 1995). However, most grain legumes have been found to remove more nitrogen from soil than they leave behind because of their high nitrogen harvest indices (NHI) (Toomsan *et al.*, 1995). This research is necessitated by the fact that application of rhizobia inoculants is cumbersome and farmers are not aware of it. This calls for the need of naturally nitrogen fixing soybeans coupled with soil amendments to maximize nitrogen fixation.

2.5 Influence of farm yard manure on soybean nodulation

The ability of soybeans to utilize nutrients in the manure has economic and environmental impacts. Soybeans have the potential to utilize nitrogen (N), phosphorus (P) and potassium (K) in the manure (Varel and Peterson, 1992). Soybeans can remove up to 200 kg N/ha (178 lb N/ac) from the manure. Additionally, liquid manure will provide phosphorus and potassium to crops in deficient soils. There is evidence that nitrate uptake is necessary for the initial growth stages of the plant; yet, evidence shows that residual soil nitrogen is usually sufficient to meet this need. However, nitrate uptake and reduction may be necessary during the growing season if the plant is undergoing environmental stressors (Katoch *et al.,* 1983).

It was observed at flowering stage, the effect of farm yard manure (FYM) in the number of nodules, fresh weight of nodules and amount of nitrogen fixed per plant was higher compared to urea (Kumar *et al.*, 2002). However increasing levels of FYM (200, 400 and 1200 g per plot) at flowering stage were unfavorable for nodules formation (Martensson and Witter, 1990). The inhibitory effect of FYM on nodule formation gradually diminished with aging. Application of FYM at harvesting time at a range of 80 g to 400 g per plot led to increase in seed yields accompanied with large amount of fixed nitrogen and higher ratio of nitrogen in the seed to total nitrogen uptake. Application of FYM results in the increase of seed yields through improvements of symbiotic nitrogen fixation (Johnson, 1987). Application of FYM leads to increase in nodules production, nodule dry weight, dry matter production and seed yields of soybeans, but the proportion of total nodules to active nodules falls sharply at application higher than 8 tonnes of FYM (Ganeshamurthy and Sammi, 2002). When nitrogen is available to the crop, a decrease in enzymatic activity by the nitrogen fixing bacteria occurs to reduce nitrogen fixation by the plant; however, studies have shown that enzymatic activity resumes following the depletion of the nitrate solution (Harper, 1987).

Several studies have been performed in the Midwest region of the United States in which positive yield increases related to liquid swine manure application has been reported on soybeans (www.extension.org, 2012). The studies identify yield increases from manure as the potential result of pre-application of soil nitrate deficiency, pre-application phosphorus or potassium deficiency and the provision of nutrients or other unknown factors via liquid manure. One study, from India, identifies the effect of manure in the clay soil (Vertisols) in the improvement of seed yield, including increased organic carbon content and improved water-use efficiency (Kaur *et al.*, 2006). According to Schmidt *et al.* (2001) there is an increase yields in soybeans with varying rates of manure application and stated that the increased yields coincided with sites having low available soil nitrogen during mid-June. Barbazan (2004) found out that sites in which manure was applied had slightly higher and more frequent early plant growth as compared to those in which phosphorus fertilizer was used. However, the early plant growth did not translate to higher yields.

Rakshit (2002) compared soil nitrate levels and soybean yield from various manure application rates and a control. This study showed statistically significant yield increases in the three of the five manured sites compared to the control. DeJong (1995) compared the effect of manure application to soybeans with inorganic nitrogen application in which it was found that there was an increase in yield with increasing application rate while application of increasing urea-ammonium nitrate (UAN) fertilizer did not result in an increase in yields. Increase in yield may be related to increased availability of phosphorus, potassium, or other unknown factors in manure (Dejong, 1995). The results of the studies showing yield responses to manure imply that manure application to soybeans can overcome deficiencies in the soil that might otherwise require amendment with inorganic fertilizers. Additionally, these studies suggest that when manure nutrient application rates are matched to crop removal there is no negative impact on water quality. DeJong (1995) in his study showed that ammonium-nitrogen soil concentration increased with increasing UAN application rates for the plots receiving UAN during the first and second soil sampling dates.

The highest manure application rate also had elevated soil nitrate-nitrogen levels (Dejong, 1995). However, by the third soil sampling date, all fields receiving manure and UAN applications had less than 10 mg NO_3-N/kg at 120 cm in the soil profile. The groundwater quality data collected from the lysimeters in this study were indicated to be semi-quantitative at best. However, the data indicated that the highest rate of UAN application resulted in the highest lysimeter nitrate concentrations peaking at 49 mg/kg in while the manure plot receiving comparable nitrogen rates peaked at 32 mg/kg in early July. According to DeJong (1995).This was the expected result given the availability of the UAN compared to the expected slower release inorganic nitrogen form in manure. Based on soil nitrate-nitrogen concentrations at the 120 cm soil profile, DeJong (1995) summarized that it is possible to apply liquid swine manure at a rate of 240 kg N/ha (214 lbs / acre) on soybeans in a corn-soybean rotation with little or no effect on ground water quality.

2.5.1 Disadvantages of manure application in soybean production

A few studies have documented the environmental impacts of manure application to soybeans to the soil. Schmidt *et al.* (2000) evaluated the impact of liquid swine manure application on nodulating and non-nodulating soybeans. They found that applying manure at greater nitrogen rates than needed for maximum soybean yields did not adversely affect soybean yield. However, they found that application of nitrogen from the liquid swine manure increased post harvest soil nitrate levels. It has also been reported greater increases in soil nitrate levels early in the growing season than post harvest (Randall and Schimitt, 1998 and Schmitt *et al.*, 1996). While direct water quality measurements were not taken as part of the study, the reported increase in residual nitrate could pose a potential risk to the environment.

Two drainage water quality studies in Iowa, U.S.A have evaluated the impact of liquid swine manure application to both corn and soybeans within a corn-soybean rotation. For the four-year study (2001-2004) at the Gilmore City research site in Pocahontas County, applying liquid swine manure at the rate of 168 kg N /ha (150 lb N/acre) for both corn and soybeans did not increase either corn or soybean yields compared to a rate of 224 kg N /ha (200 lb N/acre) of manure applied every other year (Lawlor *et al.*, 2007). In addition, the total of 336 kg-N/ha (300 lbs, two years of 150 lb N/acre versus the 224 kg-N/ha (200 lb N/acre) two-year-rate resulted in nitrate-N concentrations in tile drainage increasing on average from 17 to 23 mg/L, a 35 percent increase that was statistically significant. For the six-year study (2001-2006) at the Iowa State University Northeast Iowa Research Farm in Floyd County, applying liquid swine manure at the rate of 168 kg-N/ha (150 lb-N/acre) to corn and 224 kg-N/ha (200 lb-N/acre) to soybeans increased corn and soybean yields slightly for some years (on average 3 and 2 acre for corn and soybeans respectively) compared to 168 kg-N/ha (150 lb N/acre) of manure applied every other year before corn. The total of 392 kg-N/ha (350 lb-N/acre (one year of 150 lb N/acre and one year of 200 lb N/acre versus the 168 kg-N/ha (150 lb N/acre) two-year-rate resulted in nitrate-N concentrations in tile drainage increasing on average from 21 to 38 mg/L, an 81 percent increase. Both of these studies applied a relatively high nitrogen rate to the soybeans, but at these rates when liquid swine manure was applied every year in a corn-soybean rotation there was an increase in nitrate-nitrogen concentrations in the sub surface drainage water (Lawlor *et al.*, 2007).

2.6 Effect of poultry manure on soybean production

Application of carbonized chicken manure increased soybean seed yield by 23% and 43% when applied at a rate of 50 and 100 kg ha^{-1} respectively. Dried chicken manure applied at a rate of 50 and 100 kg ha increased soybean seed yield by 7% and 30% respectively. There was no difference in the N yield of both manure when applied at the same rate. The percentage N recovery was 17.6% and 8.9% for carbonized chicken manure, 19.2% and 10.5% for dried chicken manure when applied at a rate of 50 and 100 kg ha^{-1} respectively at peak flowering stage of soybean growth (Tagoe *et* *al.,* 2007). Carbonized chicken manure showed positive effect on nodule number and weight of soybean and cowpea while it depressed nodule number of adzuki bean. Biomass total N content of soybean and cowpea seed yield increased by 27% and 43% respectively in response to carbonized chicken manure supply (Tagoe *et al.,* 2010). According to Edward and Daniel (1992), if poultry manure was added in combination with chemical fertilizer, it supplemented all nutrients to crop and increased the productivity of the crop. (Shepherd and Withers, 2009).

2.7 Importance of inoculants on soybean nodulation

Soybean (*Glycine max* (L.) Merrill) production requires good supply of nitrogen (N) for high seed yield. The crop, like many other annual legumes has the ability to meet most of its N requirement through inoculation with bradyrhizobia. Following infection of soybean by bradyrhizobia to form nodules, N from the air is converted into a form readily available to the plant. The International Institute of Tropical Agriculture (IITA) developed promiscuous soybean varieties, which are capable of establishing symbiotic relationship with indigenous bradyrhizobia, as a practical alternative to inoculation by African farmers (Dashiell *et al*., 2002).

Inoculation ensures successful symbiosis by introducing effective rhizobia strains into soils, in the proximity of seeds, thus enhancing nitrogen fixation by leguminous plants. Remarkable positive response of soybean to rhizobia inoculation has been obtained in many tropical countries. In Tanzania, for example, Bossier variety, which failed to nodulate without inoculation, when inoculated gave an increased yield of

300 percent (Min. Agriculture Tanzania, 1978). Similarly, in experiments carried out in Nigeria using superior strain inoculants, high yielding soybean cultivars like Bossier and TGM 294-4 showed yield increase of up to 100 percent (IITA, 1978). Contribution of seed inoculation in increasing soybean yields has also been demonstrated by the use of most promising Malawian strains of rhizobia on the soybean variety Gedult. Average yields of 3148 kg seed/ha, were obtained compared to 2703 kg/ha for the control (Anonymous, 2002). Gomez (2004) studied soybean(s)-maize (m) cropping sequences namely m-m, s-s, m-s and s-m. He found that maize in rotation with soybean maintained high yields similar to those of sequential maize fertilized with nitrogen. Similarly, Caldwell (1982) obtained 14 percent yield increase above nitrogen treatments for maize following soybean and attributed this to nitrogen fixed by the soybean.

The contribution of soybean rhizobia to soil nitrogen economy was also demonstrated in an intercrop system by Searle *et al.* (1981). They showed that nitrogen uptake by wheat following an intercrop of maize and soybean was about twice that following maize alone without nitrogen and was equivalent to that following maize fertilized with 100 kgN/ha. This could be of great significance in the tropics where intercropping and crop rotations are major crop production systems (Okigbo, 1978). The long term value of rhizobial strains introduced into soils through inoculation will only be realized if the production of soybean crops is substantially supported by nitrogen fixed by these rhizobia. Soybean inoculation is the process of adding rhizobia bacteria to the soybean seed, starting a partnership between rhizobia bacteria and the soybean plant. The rhizobia bacteria colonize the roots of a soybean plant in the form of nodules. In this form, the *Rhizobium* takes nitrogen from the air and converts it into a form that the soybean plant can use. This promotes healthier and more vigorous growth, resulting in higher yields. The better the quality of the bacteria the more efficient they will be in nitrates production.

Inoculation with *Bradyrhizobium japonicum* culture has served to raise the yield of grain in all soybeans producing regions of the world by enabling *Bradyrhizobium* nodules to form on the roots and the symbiotic pair to assimilate atmospheric N (Caldwell and Grant, 1970). The increased N supply and growth causes increased uptake of other soil nutrients. Yield responses up to 31% have been attributed to inoculation of seeds. Inoculation leads to a buildup of soybean rhizobia while a little or no responses are obtained for cowpea, jack bean, lima bean and pigeon pea (Ayanaba and Nangju, 1973). *Bradyrhizobium japonicum* is favourable in the inoculation of soybean in Nigeria (Ayanaba and Nangju, 1973); Ghana (Mercer and Nsowah, 1995), Sierra Leone (Kamara, 1973) and Kenya (Souza, 2009)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The field experiments were carried out at Kenya Agricultural Research Institute (KARI) which is located in Kisii County. The region has two rainy seasons March-May (long rain) and October-November (short rain). The area lies on a highland and has equatorial climate. The cropping seasons in Kisii are medium to long. The altitude of the area is 1760 m above the sea level or 5954 Ft, whereas the latitude is 0^o 41’0’S and longitude 34^o48’0’E. The mean annual rainfall is 2089.14 mm and annual evaporation rate is 1783 mm while the average temperature is between 10-31^{o}C (KMD, 1984). The soils are mainly humic nitisoils (FAO, 2008) derived from basic volcanic rocks. The soils are deep, well weathered with moderate to high inherent fertility (Jaetzold and Schmidt 2006).

3.2 Experimental design

The experiment was laid out in a randomized complete block design in a split plot arrangement replicated four-times for each treatment. The nitrogen sources which were the main treatment were: Poultry Manure (PM), Farm Yard Manure (FYM), Di-ammonium phosphate (DAP) and Inoculants (USDA 1011) while the soybean varieties were Promiscuous variety (SB19 TGx 1740-2F) and Non-Promiscuous variety (Gazelle).

The main plot sizes was 6 m by 4 m and subplots was 3 m by 4 m and 1 m alley between the plots and blocks to minimize inter-plot interference (Appendix 1). The treatment and varieties were randomized for each replicate.

3.3 Seed procurement

The variety TGx 1740-2F was obtained from KARI, Njoro and Gazelle from KARI, Kisii. Seed of uniform size and colour were selected for use in the experiments according to the procedure by Maingi *et al*., 1999.

3.4 Soil sampling

Before planting, soil sampling was done. Soil samples were collected and sorted by removing wood, root and stone particles. The samples were air dried and taken to laboratory of KARI station in Kisii County for fertility test.

3.5 Land preparation and planting

The field with no history of soybean growth and which had not been fallowed for long was selected. It was then cleared of grasses and other prevalent weeds using mechanical methods, followed by demarcation. The fields were ridged at 45 cm intervals to a depth of 30 cm. soybean seeds of high viability and quality were carefully sorted to increase chances of uniform germination.

Organic fertilizers (poultry manure and farm yard manure) were collected from Mogunga Farmers Association in Kisii County. They were packed in 90 kg bags and transported to the KARI Station and applied at the rate of 60 kg ha^{-1}. Inorganic fertilizer (DAP) was applied at the rate of 60kg P_2O_5 ha^{-1}. The plant spacing was 45 cm by 10 cm. Both organic and inorganic fertilizers were drilled into planting furrows then mixed with the soil and soybeans planted in rows, for the inoculant treatment 100 grams of USDA 110 inoculum was mixed with 15 kg of seeds and then wetted with 290-300 ml of sugar solution which acted as an adhesive. The moist seeds were thoroughly mixed with the inoculants in the shade and sown immediately. Then two seeds were placed in furrows and covered with soil immediately in order to minimize rhizobia exposure to the sun. The plants were thinned to one plant per hole after emergence. The crops were also sprayed with Dimethoate 40% EC and copper oxychloride WP for the control of insect, pest and diseases. Weed control was done manually by uprooting the weeds.

3.5.1 Sampling

Nodulation was measured at 50% flowering, 50% podding and 50% leaf senescence, while plant height and number of leaves was carried out at 50% podding. This was done at different dates depending on the maturity class of the two soybean varieties.

3.5.2 Plant height and number of leaves

At 50% podding, one 50 cm long row of plants (10 plants with a space of 5 cm between each plant) was selected from the one but last line of the plot and leaving 50 cm from the end of the line (net plot). The leaves were counted from each plant and there number recorded. Immediately after counting the leaves, the plant was uprooted and by using a ruler it was measured and the measurement recorded.

3.5.3 Nodule assessment

The nodules were collected from ten healthy green plants. A spade was used to dig a circle of 15 cm radius around the plant and at a depth of 20 cm. The plant was lifted slowly from the soil clump and placed carefully in a plastic bag and transported to the laboratory of KARI station in Kisii County. In the laboratory the plant shoot was removed and roots washed under running water with a sieve underneath to catch detached nodules. The root was cut 1-2 mm each side of the nodule and nodules counted. The nodules were then carefully stored in the refrigerator at $4^{o}C$ for isolation and characterization of rhizobia.

3.5.4 Yield components

Yield components were assessed at physiological maturity (when 95% of the pods had turned golden yellow), by cutting all the soybeans in the net plot 5 cm above the ground level. Pods were separated from haulms weighed and then sun dried until they were ready for threshing. After the grain was threshed, the pod walls were combined with the haulms to comprise the crop residue. The threshed seeds were oven-dried to a moisture content of about 8% and the weight determined.

3.6 Laboratory and green house experiments

The experiments were carried out in the laboratory and greenhouse, in the department of Plant and Microbial sciences of Kenyatta University. The laboratory experiments were done to isolate and characterize the rhizobia that naturally nodulate promiscuous soybeans TGx 1740 and non promiscuous soybean variety Gazelle in Kisii County. Greenhouse experiments were carried out to authenticate the isolates as rhizobia and also to assess the effectiveness of local isolates of rhizobium isolated from the field experiment done in Kisii KARI station.

3.6.1 Morphological tests

These tests were carried out to distinguish the rhizobial cultures from other microorganisms by examining the cell morphology and staining.

3.6.1.1 Cell morphology

Wet mounts of 3-5 day old cultures were prepared and examined under a compound light microscope as described by Somasegaran and Hoben (1994). The size and shape of the rhizobia isolates were determined.

3.6.1.2 Motility test

Vaseline jelly was applied around the depression of a hanging drop slide. Using a sterile inoculating loop one drop of the culture was aseptically transferred to the center of a clean cover slip. The hanging drop slide was inverted and centered well over the drop of the culture and pressed down on the edges of the cover slip so that the Vaseline would make a firm seal. Quickly and carefully, the slide was turned to suspend the hanging drop in the well (Somasegaran and Hoben (1994). The culture was then examined under a compound light microscope.

3.6.1.3 Gram staining

Thin smears from 3-5 day old rhizobial cultures were prepared on clean microscopic slides. The smears were air-dried and heat fixed then Gram stained as described by Somasegaran and Hoben (1994). The slides were rinsed with water, blot dried and examined under oil immersion objective in a compound light microscope to check if the bacteria were gram positive or gram negative.

3.6.2 Biochemical tests

These tests were to distinguish rhizobia from other bacteria by growth responses on various media. The tests included growth on yeast extract mannitol agar (YEMA) containing bromothymol blue dye and YEMA containing Congo red dye.

3.6.2.1 Yeast extracts mannitol broth (YEMB)

Yeast extract mannitol broth (YEMB) was prepared as follows; Mannitol 10g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 0.2 g, yeast extract powder 0.5 g and 1 litre of distilled water. The salts were dissolved in 1 litre of distilled water followed by mannitol and yeast extract. The pH was adjusted to 6.8 using 0.1 N NaOH, the YEMB was put in McCartney bottle half-full and autoclaved at 121^{o}C for 15 minutes at 15 psi.

3.6.2.2 (Yeast extract mannitol agar YEMA)

To prepare YEMA, 15 g of agar was added to 1 litre of YEMB. The mixture was shaken to evenly suspend the agar and then autoclaved at 121^{o}C for 15 minutes at 15 psi. After autoclaving the flask was shaken to ensure even mixing of melted agar with the medium. It was then dispensed into the petri dishes and allowed to cool to 45^{o}C to prevent excessive condensation of water in the plates. The slants were used for storage of pure cultures of rhizobia. McCartney bottles were half-filled with YEMA and then autoclaved at 121^{o}C for 15 minutes. The bottles were tightly corked and placed along a slanted surface to allow the media to solidify.

3.6.2.3 Indicator Media

Congo red and Bromothymol blue media were used in the initial stages of rhizobia screening according to recommendation by Vincent (1970), Somesegaran and Hoben, (1994), and Beck *et al.* (1993).

3.6.2.3.1 Congo red indicator medium

One ml aliquot of sterile Congo red solution (prepared by adding 2.5 g of Congo red powder to 100 ml of sterile water) was added to one litre of sterilized YEMA at 45-50^{o}C. The flask was shaken to mix the two and the medium was poured into the sterile plastic petri dishes.

3.6.2.3.2 Bromothymol blue indicator medium

Five mls of bromothymol blue solution (prepared by adding 0.5 g bromothymol blue powder into 100 ml ethanol) were added to one litre of sterilized YEMA. The medium was sterilized by autoclaving for twenty minutes at 121^{o}C. The sterile medium was cooled to 45-50^{o}C and then poured into the sterile plastic petri dishes.

3.6.3 Isolation of rhizobia from nodules

Clean, intact, undamaged nodules obtained during nodule assessment were rehydrated by soaking in the water for one hour at room temperature. The nodules were immersed in 95% ethanol for ten second to break the surface tension and to remove the air bubbles. The nodules were then sterilized by immersing in 3% v/v solution of hydrogen peroxide for four minutes. Immediately after sterilization, the nodules were rinsed in sterile distilled water. The sterile nodules were crushed with a pair of blunt-tipped forceps in large drop of water in a petri dish. A sterile inoculation loop full of the nodule suspension was streaked on YEMA plates containing congo red (CR). Similarly, one loopful of the nodule suspension was streaked on YEMA plate containing bromothymol blue (BTB). The plates were incubated at 28^{o}C and daily observations made for the appearance of water clear, white or cream-colored colonies with transparent or translucent areas. After sub-culturing to obtain pure cultures Gram staining was carried out. The presumptive rhizobia isolates were transferred to McCartney bottles using a sterile transfer loop and stored at 4^oC to form stock cultures.

3.6.4 Nitrogen-free nutrient solution

Nitrogen-free stock solution was prepared as described by Somesegaran and Hoben (1994). To prepare a litre of full-strength plant culture solution 0.5 ml of each stock solution was added to 500 ml of water and mixed. The solution was then topped to one litre. Then pH was adjusted to 6.8 by using NaOH and sterilized by autoclaving at 121^o C for 15 minutes at 15 psi.

3.6.5 Water agar plates

Water agar plates were used for pre-germination of soybean seeds. To one litre of distilled water, 7.5 grams of agar were added. The mixture was shaken to suspended agar evenly. The suspension was autoclaved, allowed to cool to 45^oC and poured to sterile petri dishes.

3.6.6 Sterilization and pregermination of seeds

Soybean seeds of good viability, undamaged and of uniform colour and size were selected. The seeds were surface sterilized by immersing them into a 3% solution of sodium hypochlorite for 5-10 minutes (2.17% sodium hypochlorite solution was prepared by adding 10 parts of commercial bleach to 7.5 parts of water). The seeds were rinsed with 8 changes of sterile distilled water after surface sterilization. They were then soaked in clean sterile distilled water and allowed to imbibe for one hour. They were then transferred aseptically onto 2% water agar plates using a spatula. Ten seeds were placed in each plate. The plates were then incubated upside down at 28^{o}C to enable the radicles grow away from the water agar. The incubation period was four days. Seedlings whose radicles attained a length of 2 cm after the incubation period were considered ready for transferring to Leonard jar assemblies.

3.6.7 Leonard jar assembly

The Leonard jar assembly used was a modification of that described by Vincent (1970). The assembly was composed of a plastic cup, 8 cm mouth diameter which tapered to a bottom diameter of 4 cm. The cup containing the growth medium (vermiculite) was inserted into a larger plastic vessel containing the nutrient solution. A sponge connecting the upper and the lower units of the jar irrigated vermiculite (rooting medium) with the nutrient solution. The whole set up was insulated with a khaki paper bag.

3.6.8 Rooting medium

Vermiculate was the rooting medium which was used in greenhouse experiments. This material was washed thoroughly for three successive days by changing the water three times per day and stirring frequently and finally rinsed with distilled water. The pH of the medium was adjusted to 6.8 by adding 300 g $CaCO_3$ to prevent acidification of rhizosphere (Israel and Jackson, 1982). After attaining the correct pH, water was drained off and the vermiculate packed into small plastic cups of the Leonard jar assemblies. Eight hundred mls of plant nutrient solution was added into the lower container of each Leonard jar assembly. To reduce contamination and entry of water, the top cups were covered with lids and the assemblies steamed for one hour, twice in an autoclave to get rid of microorganisms.

3.6.9 Growth condition in the greenhouse

Plants were grown in a semi-permanent greenhouse at Kenyatta University, Nairobi, Kenya. The growth conditions in the greenhouse were ambient temperatures, 25/15^{o}C, day/night. Natural light with photoperiod of 12/12 hours day/night was used.

3.7 Authentication of the isolates as rhizobia

Authentication test was carried out to assess the ability of the isolates to nodulate soybeans. They were assessed in the Leonard jar assemblies. A set of four Leonard jar assemblies were prepared for each soybean variety. Another set of Leonard jar assemblies served as uninoculated controls. A flame sterilized pair of forceps was used to prepare two holes in the rooting medium in each of the Leonard jar assembly. Pregerminated seedlings of soybean varieties TGx 1740-2F and Gazelle with a radicle length of 1.5 cm were picked up with the sterile pair of forceps and placed one per hole, with the radicle facing downwards. The seedlings were maintained for 8 days in the Leonard jar assemblies in the greenhouse before inoculation. The greenhouse growth conditions were ambient temperatures, 25/15^{o}C and natural light with photoperiod of 12/12 hours day/night. On the eighth day, 1 ml broth culture of rhizobia was inoculated onto each of the seedlings in two growth units. Another set which was uninoculated received plain yeast extract mannitol broth. The plants were maintained in the greenhouse for 45 days after inoculation (Ferraira and Hungria, 2002). Leonard jar were replenished with sterile N-free nutrient solution as required (Odee *et al.*, 1995). In the course of growth from 15 days to 45 days, plants were examined for differences in vigour and colour between the two varieties and between inoculated and uninoculated. At the end the growth period, plants were removed from the rooting medium and the presence or absence of nodules noted.

3.7.1 Assessment of effectiveness in nitrogen fixation of rhizobia

This experiment was carried to assess the effectiveness of local isolate rhizobia isolated from Kisii KARI station in nitrogen fixation. These rhizobia had already been characterized into fast and slow growing rhizobia in section 3.6.3.

3.7.2 Symbiotic effectiveness of isolates

Nodulation, dry weight of nodules, shoot, roots and acetylene reduction were used to compare the symbiotic effectiveness of fast growing *Rhizobium* with that of slow growing *Bradyrhizobium japonicum* isolates with two soybean varieties. The two rhizobia were isolated from the field experiment carried in Kisii KARI station as described in section 3.6.3.

3.7.2.1 Greenhouse experiment for symbiotic experiment

Plant tests were conducted on two soybean varieties: TGx 1740 and Gazelle. A randomized complete block design was used with sampling dates of 30 and 45 days after inoculation and transplanting. Each strain by variety and by sampling date combination was randomized within each of four blocks. The seeds were pregerminated for 72 hours at 30^{o}C and 95% relative humidity. In this experiment sixty Leonard jar assemblies were used and the seedlings were planted in the Leonard jar assemblies as described in section 3.7. The Leonard jar assemblies with the seedlings were set in quadruplicates and inoculated with 1 ml of 5 day old respective rhizobia (approximately 10^{8}cells ml^{-1}) broth culture onto the radicle base following the procedure described by Somasegaran and Hoben (1994). The control was left uninoculated but fed with plain yeast extract mannitol broth. All Leonard jar assemblies were arranged in a randomized complete block design in the greenhouse, where each treatment had 10 Leonard jar assemblies. Jars were replenished with sterile N-free sterile nutrient solution daily as required (Odee *et al.*, 1995). At 14 days after transplantation, the seedlings were thinned to one per Leonard jar assembly. The growth conditions in the greenhouse were as described in section 3.7.1.

3.7.2.2 Acetylene reduction assay

Acetylene reduction assay was performed at Kenya Tea Research Foundation, Kericho, Kenya. The plant roots were packaged in a khaki paper bag to prevent dessication of the nodules. Acetylene reduction was performed on the excised root systems of 30-day-old plants; the roots were cut then washed to remove the sand. The nodulated portion of roots were removed and placed in 60 ml plastic syringes and incubated with a gas mixture prepared according to the method of Hardy *et al.* (1968). The syringe was then connected to the Gas Chromatography (G.C) which was equipped with a hydrogen flame detector. The sampling was taken after every 15 minutes for 1 hour, where 5 ml were injected into the G.C and amount of ethylene released was recorded.

After assay procedure, the syringe was opened and carefully all the nodules were removed from the roots with a razor blade, counted and their number recorded, they were then oven dried at 70^{o}C for 48 hours to obtain their dry weight.

3.7.2.3 Nodule sampling for colouration

At 45-day after inoculation, the plants were harvested and the nodules collected from ten plants in the greenhouse. The plant shoot was removed and the roots washed. The nodules were removed from the roots, sectioned and their inner colouration noted whether dark pink or white and their number recorded for each case.

3.8 Data Analysis

Data collected on the nodule number, nodule dry weight, plant height and yield components stover weight, haulm weight, grain weight and thousand seed weight was analyzed and presented using both descriptive and quantitative statistical procedure. Statistical analyses were carried out using SPSS (Statistical Package for Social

Sciences). The transformed data was subjected to analysis of variance (ANOVA) to determine the main effects of treatments and their interactions. The means were separated by Tukeys Honest significant difference (HSD) test at $P \leq 0.05$.

CHAPTER FOUR

RESULTS

Table 4.1 Soil analytical data for Kisii KARI Station

Soil parameters	Value	Class
Soil pH	4.4	Extreme acid
Acidity me %	0.5	Adequate
Total nitrogen %	0.19	Low
Organic carbon %	2.1	Moderate
Phosphorus ppm	10	Low
Potassium me %	0.18	Low
Calcium me %	1.3	Low
Magnesium me %	1.65	Adequate
Manganese me %	1.2	Adequate
Copper ppm	3.8	Adequate
Iron ppm	35	Adequate
Zinc ppm	8.6	Adequate
Sodium me %	0.45	Adequate

4.2.1 Effect of different soil amendments on soybean nodulation, experiment carried out in Kisii during long rains in the year 2008

4.2.1.1 Nodulation on the 30th day of growth

Numbers of root nodules of two soybean varieties were recorded from 10 plants. The number of nodules in TGx variety significantly differed ($F = 4.22$, $df = 4$, $p<0.05$) at different soil amendments treatments. The TGx variety under inoculants treatment had highest mean nodulation of 7.85 while DAP treatment had the lowest mean of 1.63 (Table 4.2a).

Gazelle variety also significantly differed (F = 23.47, df = 4, $p<0.05$) at different soil amendments. Inoculant treatment had the highest mean nodulation of 10.88 while DAP had the lowest mean nodulation of 1.08 (Table 4.2.b). The T-test showed no significant difference (T= 0.60, P=0.56) in nodulation for the two soybean varieties on the 30th day of growth.

Table 4.2a: Nodulation of TGx plants on the 30th day after planting during long rains in the year 2008

Treatment	Mean number of nodules
Control	4.8 ± 0.55ab
DAP	1.63 ± 0.20b
FYM	2.8 ± 0.54b
Inoculant	7.85 ± 2.48a
PM	2.85 ± 0.55b

Mean values denoted by similar letters are not significantly different at $p\leq0.05$, using Tukey's HSD. FYM- Farm Yard Manure**,** PM-poultry manure DAP- Di-ammonium phosphate.

Table 4.2.b: Nodulation of Gazelle plants on the 30th day after planting during long rains in the year 2008

Treatment	Mean number of nodules
Control	5.15 ± 1.34b
DAP	1.08 ± 0.35c
FYM	1.8 ± 0.29c
Inoculant	10.88 ± 0.6a
PM	3.0 ± 0.99bc

Mean values denoted by similar letters are not significantly different at $p \geq 0.05$; Means separated using Tukey's HSD, FYM- Farm Yard Manure, PM- poultry and DAP- Di-ammonium phosphate.

4.2.1.2 Nodulation at podding stage

Nodulation of TGx variety at podding significantly differed ($F = 9.97$, $df = 4$, $p \leq 0.05$) at different soil amendments treatment. The inoculant treatment had the highest mean number of nodules (36.58) while the DAP and PM treatment had the lowest (5.43 and 11.38 respectively) as indicated in Table 4.3a. Nodulation of Gazelle variety significantly differed ($F = 7.55$, $df = 4$, $p \leq 0.05$) at different soil amendments treatment, both inoculants and controls treatment had the highest mean number of nodules (29.8 and 24.6 respectively) as indicated in Table 4.3b, while the lowest mean number of nodules was recorded in DAP treatment (6.33) as indicated in Table 4.3b. The nodulation of two soybean varieties were not significantly different ($t = 0.03$, $p \geq 0.05$).

Table 4.3a: Nodulation of TGx variety at podding during long rains in the year 2008

Treatment	Mean number of nodules
Control	19.38 ± 5.87b
DAP	5.43 ± 1.14c
FYM	16.1 ± 2.99b
Inoculant	36.58 ± 4.27a
PM	11.38 ± 2.51bc

Mean values denoted by similar letters are not significantly different at P≥0.05, using Tukey's HSD, FYM- Farm Yard Manure**,** PM-poultry manure and DAP- Di-ammonium phosphate.

Table 4.2.b: Nodulation of Gazelle variety at podding during long rains in the 2008

Treatment	Mean number of nodules
Control	24.6 ± 5.31a
DAP	6.33 ± 0.7c
FYM	14.45 ± 2.68b
Inoculant	29.8 ± 3.03a
PM	13.48 ± 3.63b

Mean values denoted by similar letters are not significantly different at p≥0.05; using Tukeys HSD, FYM- Farm Yard Manure**,** PM- poultry manure and DAP- Di-ammonium phosphate.

4.2.1.3 Soybean nodulation at leaf senescence

At leaf senescence stage, nodulation of TGx variety at different soil amendments was significantly different ($p \leq 0.05$). The inoculants treatment had the highest mean number of nodules (12.15) while the soil amended with DAP and PM had the lowest mean number of nodules (2.15and 4.23 respectively) as shown in Table 4.4a.

The Gazelle variety significantly differed ($p \leq 0.05$) at different soil amendments. Inoculants soil amendment had the highest mean number of nodules (9.15) (Table 4.4b), while the soil amended with DAP treatment had the lowest mean of nodules (1.9) as shown in Table 4.4b. There was no significant difference ($t=1.89$, $p \geq 0.05$) between the nodulation of two soybean variety at different soil fertility amendments.

Table 4.4a: Nodulation of TGx variety at leaf senescence during long rains in the year 2008

Treatment	Mean number of nodules
Control	6.5 ± 0.87b
DAP	2.15 ± 0.13c
FYM	5.33 ± 0.83bc
Inoculant	12.15 ± 1.62a
PM	4.23 ± 1.37bc

Mean values denoted by similar letters are not significantly different at $p \geq 0.05$; using Tukey's HSD, FYM- Farm Yard Manure**,** PM-poultry manure and DAP- Di-ammonium phosphate.

Table 4.4b: Nodulation of Gazelle variety at leaf senescence during long rains in the year 2008

Treatment	Mean number of nodules
Control	6.95 ± 1.39ab
DAP	1.9 ± 0.43c
FYM	4.35 ± 0.76bc
Inoculant	9.15 ± 0.93a
PM	3.88 ± 0.96bc

Mean values denoted by similar letters are not significantly different at $p \geq 0.05$, using Tukey's HSD, FYM- Farm Yard Manure**,** PM-poultry manure and DAP- Di-ammonium phosphate.

4.3 Effect of soil amendment on the number of leaves

At podding stage, the number of leaves for both soybean varieties had no significant difference ($p \geq 0.05$) at different soil amendments treatments. The mean number of leaves for TGx plant under FYM was the highest (14.30) while the ones under inoculants treatment had the lowest (11.3) as indicated in Table 4.5a. While in Gazelle variety the inoculants had mean number of the leaves (9.35) as indicated in Table 4.5b

Table 4.5a: Number of leaves of TGx variety at podding stage during long rains in the year 2008

Treatment	**Mean number of leaves**
Control	12.08 ± 0.91a
DAP	11.95 ± 2.80a
FYM	14.30 ± 1.74a
Inoculant	11.3 ± 1.0ab
PM	11.7 ± 1.17ab

Mean values were not significantly different at p≥0.05; Means separated using Tukey's HSD, FYM- Farm Yard Manure**,** PM-poultry manure and DAP- Di-ammonium phosphate.

Table 4.5b: Number of leaves of Gazelle variety at podding stage during long rains in the 2008

Treatment	**Mean number of leaves**
Control	12.55 ± 0.83a
DAP	12.65 ± 1.36a
FYM	10.15 ± 1.06b
Inoculant	9.35 ± 1.21b
PM	12.05 ± 1.31a

Mean values were not significantly differently at P≥0.05, Means separated using Tukey's HSD, FYM- Farm Yard Manure**,** PM-poultry manure and DAP- Di-ammonium phosphate

4.4 Plant heights

There was no significant difference ($p \geq 0.05$) on the height of TGx and Gazelle varieties (Table 4.6a and Table 4.6b)

Table 4.6a: Heights of TGx variety planted at podding stage during long rains in the year 2008

Treatment	**Mean height**
Control	52.77 ± 1.39a
DAP	50.99 ± 4.15a
FYM	47.80 ± 1.65a
Inoculant	47.85 ± 1.83a
PM	46.84 ± 2.96a

Mean values were not significantly different at $p \geq 0.05$; using Tukey's HSD, FYM-Farm Yard Manure, PM-Poultry manure and DAP-Diammonium phosphate.

Table 4.6a: Heights of Gazelle variety at podding stage during long rains in the year 2008

Treatment	**Mean height**
Control	55.21 ± 3.55a
DAP	51.15 ± 3.08a
FYM	54.27 ± 3.28a
Inoculant	45.95 ± 4.02a
PM	49.68 ± 2.0a

Mean values were not significantly different at $p \geq 0.05$; using Tukey's HSD, FYM-Farm Yard Manure, PM-Poultry manure and DAP-Diammonium phosphate.

4.5 Effect of different soil amendments on stover weight

There was no significant difference ($p \geq .05$) in stover weight for both soybean variety, however soil amendment with FYM in TGx variety had higher mean weight (7.15 kg) while inoculants amendment resulted to the lowest (3.03 kg) as indicated in Table 4.7a Gazelle variety had the highest stover mean weight of mean (7.33kg) when the soil was amended with DAP (Table 4.7b).

Table 4.7a: Stover weight of TGx variety planted under different soil amendments in Kisii during long rains in the year 2008

Treatment	Mean stover weight (kg)
Control	3.8 ± 1.08a
DAP	5.43 ± 1.1a
FYM	7.15 ± 1.51a
Inoculant	3.03 ± 1.02a
PM	4.98 ± 1.22a

Mean values were not significantly different at $p \geq 0.05$; using Tukey's HSD, FYM-Farm Yard Manure**,** PM- Poultry manure DAP- Di-ammonium phosphate.

Table 4.7b: Stover weight of Gazelle variety planted under different soil amendments in Kisii during long rains in the year 2008

Treatment	**Mean stover weight (kg)**
Control	6.85 ± 1.62
DAP	7.33 ± 1.33
FYM	6.58 ± 0.82
Inoculant	5.65 ± 1.23
PM	5.98 ± 0.97

Mean values were not significantly different at p≥0.05; Means separated using Tukeys HSD, FYM- Farm Yard Manure**,** PM- Poultry manure and DAP- Di-ammonium phosphate.

4.6 Effect of different soil amendments on haulum weight

Haulum weight had a significant difference (p≤0.05) for both varieties at different soil amendments. Farmyard manure had the highest haulum mean weight for both varieties where TGx variety had a mean weight of 4.32. g (Table 4.8a) while Gazelle variety had a mean of 455g (Table 4.8b).

Table 4.8a: Haulum weight for TGx variety planted under different soil amendments in Kisii during long rains in the year 2008

Treatment	**Mean haulum weight (g)**
Control	191.5 ± 63.9b
DAP	298 ± 28.9ab
FYM	432.25 ± 38.4a
Inoculant	364.5 ± 18.4a
PM	282.25 ± 35ab

Mean values denoted by similar letters are not significantly different at p≥0.05; Means separated using Tukeys HSD, FYM- Farm Yard Manure**,** PM- Poultry manure and DAP- Di-ammonium phosphate.

Table 4.8b: Haulum weight for Gazelle variety planted under different soil amendments in Kisii during long rains in the year 2008

Treatment	Mean haulum weight (g)
Control	172.8 ± 43.8b
DAP	283.5 ± 31.6ab
FYM	455 ± 65.3a
Inoculant	328.5 ±79.7ab
PM	348.8 ± 33.6ab

Mean values denoted by similar letters are not significantly different at p≥ 0.05; Means separated using Tukey’s HSD, FYM- Farm Yard Manure**,** PM- Poultry manure and DAP- Di-ammonium phosphate.

4.7 Effect of different soil amendments on grain weight

Grain weight of TGx and Gazelle varieties were not significantly different ($p \geq 0.05$) at different soil amendments as indicated in Table 4.9a and Table 4.9b. There was a significant difference ($t=3.03$, $P=0.007$) between the two soybean varieties on grain weight.

Table 4.9a: Grain weight for TGx variety planted under different soil amendments in Kisii during long rains in the year 2008

Treatment	Mean grain weight (kg)
Control	2.53 ± 0.75
DAP	2.65 ± 0.29
FYM	2.78 ± 0.23
Inoculant	3.1 ± 0.58
PM	2.55 ± 0.45

Mean values were not significantly different at $p \geq 0.05$; Means separated using Tukey's HSD, FYM- Farm Yard Manure**,** PM- poultry manure and DAP- Di-ammonium phosphate.

Table 4.9b: Grain weight for Gazelle variety planted under different soil amendments in Kisii during long rains in the year 2008

Treatment	Mean grain weight (kg)
Control	2.4 ± 0.35
DAP	1.6 ± 0.51
FYM	2.45 ± 0.52
Inoculant	1.70 ± 0.47
PM	2.08 ± 0.14

Mean values were not significantly different at p≥0.05; Means separated using Tukey's HSD, FYM- Farm Yard Manure**,** PM-Poultry manure and DAP- Di-ammonium phosphate.

4.8 Effect of soil amendments on 1000 seed weight

There was a significant difference (p≤0.05) in the 1000 seed weight of TGx variety in different soil amendments. Inoculants soil amendments had the highest mean weight of (348.25 g) while control had the lowest (222.75 g)as indicated in Table 4.10a. There was no significant difference (p≥0.05) in the 1000 seed weight of Gazelle variety at different soil amendments. Farmyard manure had highest mean weight (305.00 g) while PM had the lowest weight (246 g) as indicated in Table 4.10b. There was no significant difference (t=0.30, p≥0.05) in the 1000 seed weight between the two soybean varieties.

Table 4.10a: The 1000 seeds weight of TGx variety planted under different soil amendment at Kisii during long rains in the year 2008

Treatment	**Mean 1000 seed weight (g)**
Control	222.75 ± 6.56c
DAP	296 ± 13.7b
FYM	286 ± 17.4b
Inoculant	348.25 ± 8.55 a
PM	280 ± 11.1b

Mean values denoted by similar letter (s) were not significantly different at p≥0.05; Means separated using Tukey's HSD, FYM- Farm Yard Manure**,** PM- Poultry manure and DAP- Di-ammonium phosphate.

Table 4.10b: The 1000 seeds weight of Gazelle variety planted under different soil amendment at Kisii during long rains in the year 2008

Treatment	Mean 1000 seed weight
Control	289.5 ± 21.4
DAP	301.25 ± 19.7
FYM	305.00 ± 7.91
Inoculant	269.75 ± 24.6
PM	246.00 ± 17.4

Mean values were not significantly different at p≥0.05; Means separated using Tukey's HSD, FYM- Farm Yard Manure**,** PM- Poultry manure and DAP- Di-ammonium phosphate.

4.9 Laboratory and Greenhouse experiments

All the colonies appeared on the media within two days and some attained full size in 3-5 days and others attained full size in 4-7 days. The isolates obtained were Gram negative, rod shaped cells, which were water clear, white or cream colored with transparent or translucent areas. On BTB medium, 75% of the isolate turned the growth medium from deep green to yellow which diffused into the medium while 25% of the isolates turned the medium from deep green to blue which diffused into the medium (Table 4.9). The isolates weakly absorbed Congo red dye. Results from various biochemical tests indicated that 25% of the rhizobia isolated were slow growing while 75% were fast growing (Table 4.11).

Table 4.11 Biochemical tests of rhizobia isolated from the nodules collected from the field experiment carried at Kisii

Isolate number	Bromothymol blue	Congo red	Gram stain
TF1	Y	X	-
TD2	Y	X	-
TP1	Y	X	-
TC2	B	X	-
GF1	Y	X	-
GD2	B	X	-
GP1	Y	X	-
GC2	Y	X	-
TF1	Y	X	-
TD2	Y	X	-
TP1	B	X	-
TC2	Y	X	-
GF1	B	X	-
GD2	Y	X	-
GP1	Y	X	-
GC2	Y	X	-

Y- Yellow colour – characteristic of fast growing rhizobia, B- Blue colour-characteristic of slow growing rhizobia, X- No absorption of Congo red dye, (-) Gram negative, TF1- Nodules from Tropical *Glycine* Cross (TGx) treated with Farmyard Manure, TD2- Nodules from Tropical *Glycine* Cross (TGx) treated with Di-ammonium phosphate, TP1- Nodules from Tropical *Glycine* Cross (TGx) treated with poultry manure, TC2- Nodules from Tropical *Glycine* Cross (TGx) treated with control, GF1- Nodules from Gazelle variety treated with Farmyard manure, GD2- Nodules from Gazelle variety treated with Di-ammonium phosphate, GPI- Nodules from Gazelle variety treated with poultry manure, GC2- Nodules from Gazelle variety treated with control.

Table 4.12 Nodulation status of promiscuous and non promiscuous soybeans inoculated with 1ml of 5 days old rhizobia broth at Kenyatta University greenhouse

Soybean variety	**Nodulation**	**% number of plants nodulated**
TGx 1740 + rhizobia broth	+	90
Gazelle + rhizobia broth	+	95
TGx 1740 non inoculated	-	None
Gazelle non inoculated	-	None

+ indicates nodulation, - Indicates no nodulation, TGx-Tropical *Glycine* Cross.

4.9.1 Symbiotic effectiveness

There was a significant effect ($p \leq 0.05$) on TGx variety in terms of the number of nodules produced and Acetylene reduction assay. The TGx inoculated with fast growing rhizobia had the highest mean number of nodules (34) while the one inoculated with slow growing bacteria had the lowest (11), on TGx variety inoculated with fast growing rhizobia had high ethylene production (18 µmol) while the one inoculated with slow growing rhizobia had high ethylene production (6.57 µmol) as indicated in Table 4.13. However, no significant effect ($p \geq 0.05$) was found in the dry weights of the nodules, shoots and roots of the plants.

There was a significant effect ($p \leq 0.05$) in the number of nodules in Gazelle variety when inoculated with fast and slow growing rhizobia. There were low mean formed when Gazelle variety was inoculated with fast growing rhizobia (17) while the one inoculated with slow growing rhizobia had high number of nodules (38.75) as indicated in table 4.13. Acetylene reduction assay also had a significant effect (p≤0.05) on Gazelle variety inoculated with fast growing rhizobia had low ethylene production (3.55 µmol) while the one inoculated with slow growing bacteria had the high ethylene production (13.78 µmol) as indicated in Table 4.13.

Table 4.13 Symbiotic effectiveness of local rhizobia isolated from the nodules collected from field experiment carried out in Kisii

Variable	Experimental group	Mean and SE
Number of nodules	TGx F.G	34± 2.45a
	TGx S.L	11±3.10b
	Gazelle, F.G	17±1.29b
	Gazelle, S.L	38.75±9.65a
Acetylene reduction (µmol of C_2H_4. $Plant^{-1.}$ h^{-1})	TGx F.G	18.22±1.12a
	TGx S.G	6.57±1.54b
	Gazelle, F.G	3.55± 0.27b
	Gazelle S.G	13.78±2.18a
Dry weight of nodules (mg)	TGx F.G	0.48± 0.11a
	TGx S.G	0.25±0.06a
	Gazelle F.G	0.45±0.12a
	Gazelle S.G	0.57±0.26a
Dry weight of shoots (g)	TGx F.G	1.85±0.28a
	TGx S.G	1.03±0.45a
	Gazelle F.G	0.88±0.14a
	Gazelle S.G	1.3±0.31da
Dry weight of roots (g)	TGx F.G	0.78±0.18a
	TGx S.G	0.58±0.26a
	Gazelle F.G	0.58±0.14a
	Gazelle S.G	1.02±0.22a±

The means with similar letters are not significantly different at p≥0.05; Means separated using Tukey's HSD, TGx- Tropical Glycine Cross, F.G- Fast growing rhizobia, S.G-Slow growing rhizobia.

4.9.2: Number of effective nodules

There was a significant difference ($p \leq 0.05$) on two local isolate rhizobia in terms of nitrogen fixation into two soybean varieties. The TGx variety inoculated with fast growing rhizobia had higher mean number of pink nodules (11.25) while Gazelle variety had a mean of 3.7(Table 4.14).

The slow growing *Bradyrhizobium japonicum* had a significant effect ($p < 0.05$) on the two soybean variety. The Gazelle variety had higher number of pink nodules 10.20 ± 0.40 while TGx variety inoculated with slow growing *Bradyrhizobium japonicum* had a mean of 4.75 ± 0.27 as indicated in Table 4.12.

Table 4.14 Mean number of pink nodules

Treatment	**Mean number of pink nodules**
TGx- F.G	11.25 ± 0.46^{a}
TGx-S.G	4.75 ± 0.27^{b}
Gazelle- F.G	3.7 ± 0.21^{c}
Gazelle- S.G	10.20 ± 0.40^{a}

Mean values denoted by similar letter (s) are not significantly different at $p \geq 0.05$; Means separated using Tukey’s HSD, F.G- Fast growing rhizobia, S.G- Slow growing rhizobia.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Effects of soil amendments to soybean productivity

In the present study, N fertilizer application significantly reduced number of nodules, in both soybean varieties. Many studies have been performed to test the effects of nitrogen on root nodulation and nitrogen production in nodules (Nosheen *et al.*, 2011). Generally when sufficient levels of nitrogen are present in the soil, nodulation is inhibited (Gentili and Huss-Danell, 2002; Laws and Graves, 2005).

Inhibitory effects of added nitrogen fertilizer to nodulation and nitrogen fixation have been reported by other investigators (Floor, 1985; Chemining'wa *et al.,* 2004; Taylor *et al.,* 2005). Terakado *et al.* (2006) showed that French beans plants grown without N-fertilizer had many nodules on their roots. Similarly, and Gentili *et al.*, (2006) reported that N levels inhibited early cell division in cortex of *Alnus incana* there by inhibiting nodulation.

In the present study, fertilizer application didn't improve the plant height dry matter in the long rains and the results disagree with those carried by Otieno *et al.*, (2007). This could be attributed to low rainfall received during that season. Farmyard manure application had no significant effect in the number of nodules for both soybeans varieties compared to control. This was probably due to the slow mineralization of manure hence slow phosphorous release (Otieno *et al.*, 2007). The site also had low phosphorous hence it could be a contributing factor. Perharps the addition of phosphorous to manure could have impacted a positive response. Phosphorous and farmyard manure have been reported to improve both total and active nodules and nodule dry weight of soybeans (Ganeshamurthy and Sammy, 2000). The study has also shown better performance of farmyard manure on yield for both soybeans varieties and this concurs with Javaid *et al.* (2008) who reported better growth and yield of wheat when farmyard manure when compared to green manure amended soil. Similar findings were also reported by Otieno *et al.* (2007) in which manure application did not improve nodulation of Lima beans, Lablab, Common beans and Green grams. The favourable response from farmyard manure application may be due to nutrients availed to plants after mineralization or due to its influence on soil organic matter (Mukindia, 1994). Organic matter increases the moisture retention of soil and improves soil structure and in turn soil porosity which allows better root growth and hence better grain yield (Saad and Tageldin, 2009) Soybean yield increases with increasing manure application rather than increasing the rate of inorganic fertilizer (Schimidt *et al.,* 2001)

The study found that the soybeans planted in the soil amended with poultry manure had poor nodulation and the yield. This could be attributed to the application of raw manure which has high acidity which affects the formation of nodules as compared to dry manure where pH is alkaline. Similar findings were found by Warman (1986) who reported that application of raw poultry manure affected the availability of nutrients to the plants. Similarly, Ramesh *et al.* (2004) explored the effects of two carbonized organic materials, chicken manure and refuse derived fuel (RDF) with or without inorganic K fertilizer on the growth, nodulation, yield, N and P contents of two grain legumes: soybean and cowpea in a greenhouse trial. The application of carbonized chicken manure only increased seed yield by 41% and 146% in soybean and cowpea respectively while the application of carbonized chicken manure and inorganic K fertilizer increased seed yield by 53% and 185% in soybean and cowpea respectively (Tagoe *et al.*, 2008). The application of carbonized RDF and inorganic K fertilizer increased seed yield by 45% and 126% in soybean and cowpea respectively. The trends for haulum and weight of both grain legumes were similar to that of their respective seed yields. The poor nodulation for both types of manure could be attributed to available nitrogen provided by the ammonia found in the farmyard manure and poultry manure which is released during decomposition (Duncan, 2005).

Tropical *Glycine* Cross (TGx) being the promiscuous variety nodulated well with the rhizobia population at the site. However, there was increase in nodulation, dry weight, 1000 seeds dry weight and grain weight for the TGx variety upon inoculation with commercial *Bradyrhizobium japonicum* strain, USDA 110. This shows that, in spite of its promiscuity, it can still respond well to inoculation as evidenced by better performance of the inoculated treatments as compared to uninoculated controls. Positive responses of promiscuous soybeans to inoculation have also been reported in earlier studies in other parts of Africa (Okereke and Eaglesham, 1993; Sanginga *et al.*, 1996; Osunde *et al.*, 2003).

Lack of significant yield improvement by inoculation in Gazelle variety could be attributed to the presence of native effective strains of rhizobia in the soil (Ham *et al.*, 1971), cultivar and strain interactions (Caldwell, 1966). Results of the present work show that the soil had rhizobia that were equally effective even without inoculation. Similar results were reported by Chemining'wa *et al.* (2004).

5.1.2 Symbiotic effectiveness of nitrogen fixation and Laboratory experiments.

The colonies that attained full size in 3-5 days were fast growing rhizobia while the ones that attained full size in 4-7 days are slow growing rhizobia (Somesegaran and Hoben, 1994). All the isolates were Gram negative rods, which is a characteristic typical to rhizobia (Zakhia and DeLajudie, 2001). Rhizobia do not absorb Congo red when incubated in the dark (Maingi *et al.,* 1999). This is the reason why colonies appeared milky in media containing Congo red dye. The change in colour of YEMA containing bromothymol blue from dark green to blue indicated the production of alkaline substances, which diffused into the media hence colour change. This is a typical characteristic of slow growing rhizobia, which is *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* (Xu *et al.,* 1995). The change in colour of YEMA containing bromothymol blue from dark green to yellow indicated the production of acidic substances which diffused into the medium (Somesegaran and Hoben, 1994). The production of acid substances is a characteristic of the fast-growing rhizobia (Beck *et al.,* 1993).

Nodulation and acetylene reduction activity showed that the symbiotic effectiveness of slow growing rhizobia isolate was superior to that of fast growing rhizobia isolatein Gazelle variety. This result is in agreement with another study which showed that the symbiotic effectiveness, as measured by acetylene reduction activities, of USDA 110 was superior to that of USDA 191 (Yelton *et al.,* 1983). Another study conducted by Israel *et al.* (1986) showed that the symbiotic *Bradyrhizobium japonicum* USDA 110 was superior to that of *Sinorhizobium fredii* USDA 191 in terms of nitrogen fixation. The fast growing rhizobia showed high performance in TGx 1740 in terms of number of nodules and acetylene reduction activity as compared to slow growing rhizobia.

5.2 Conclusions

The field experiment study was undertaken to assess the effect of different soil fertility amendment on nodulation and yield of soybean. The study revealed the following: The soybeans which were inoculated with the *Bradyrhizobium japonicum* had high nodulation and also yield as compared to other soil amendments. Also promiscuous variety performed better than the non promiscuous when treated with inoculants.

The FYM has shown to have slow mineralization; hence soybeans planted in soils amended with FYM had few numbers of nodules which increased with the aging of the manure leading to high yields. The FYM is locally available hence smallholders farmers can easily afford it as compared to expensive N fertilizer, also manure is environmental friendly. The soybeans planted in the soils amended with PM showed poor nodulation as compared to control, this was probably attributed to the status of the manure applied which was raw which made the conditions to acidity hence unfavourable to the rhizobia bacteria.

Promiscuous TGx 1740 had higher nodulation than that of specific variety Gazelle. The higher yield of this variety while uninoculated reflects their promiscuous nature, where they nodulate with other types of rhizobia in the soil. The symbiotic effectiveness has shown that fast growing rhizobia bacteria is more effective in fixing nitrogen in TGx soybean variety than slow growing rhizobia bacteria, while in Gazelle soybean, the reverse appears to be true: *Bradyrhizobia japonicum* is better in nitrogen fixation, thus, the efficacy of nitrogen fixation appears to depend upon the interaction between the type of bacteria and soybean. The soil fertility amendment had no effect on different growth parameters (number of the leaves, height, stover weight and haulum weight).

5.3 Recommendation

1) Farmers should be advised to turn to application of FYM in the production of soybean because it promotes yield, it’s locally available and environmentally friendly.
2) The government through Ministry of Agriculture should avail the inoculants to the farmers and train them through Agriculture Extension services on how to carry the inoculation process, since it has shown to increase the nodulation of soybean hence leading to high yield.

3) There is also a need to establish a better understanding of the persistence of both introduced and indigenous soybean rhizobia populations in soils under different cropping system.
4) More research should be carried on farmyard manure to identify other factors available that lead to soybean yield increase other than phosphorous and potassium.

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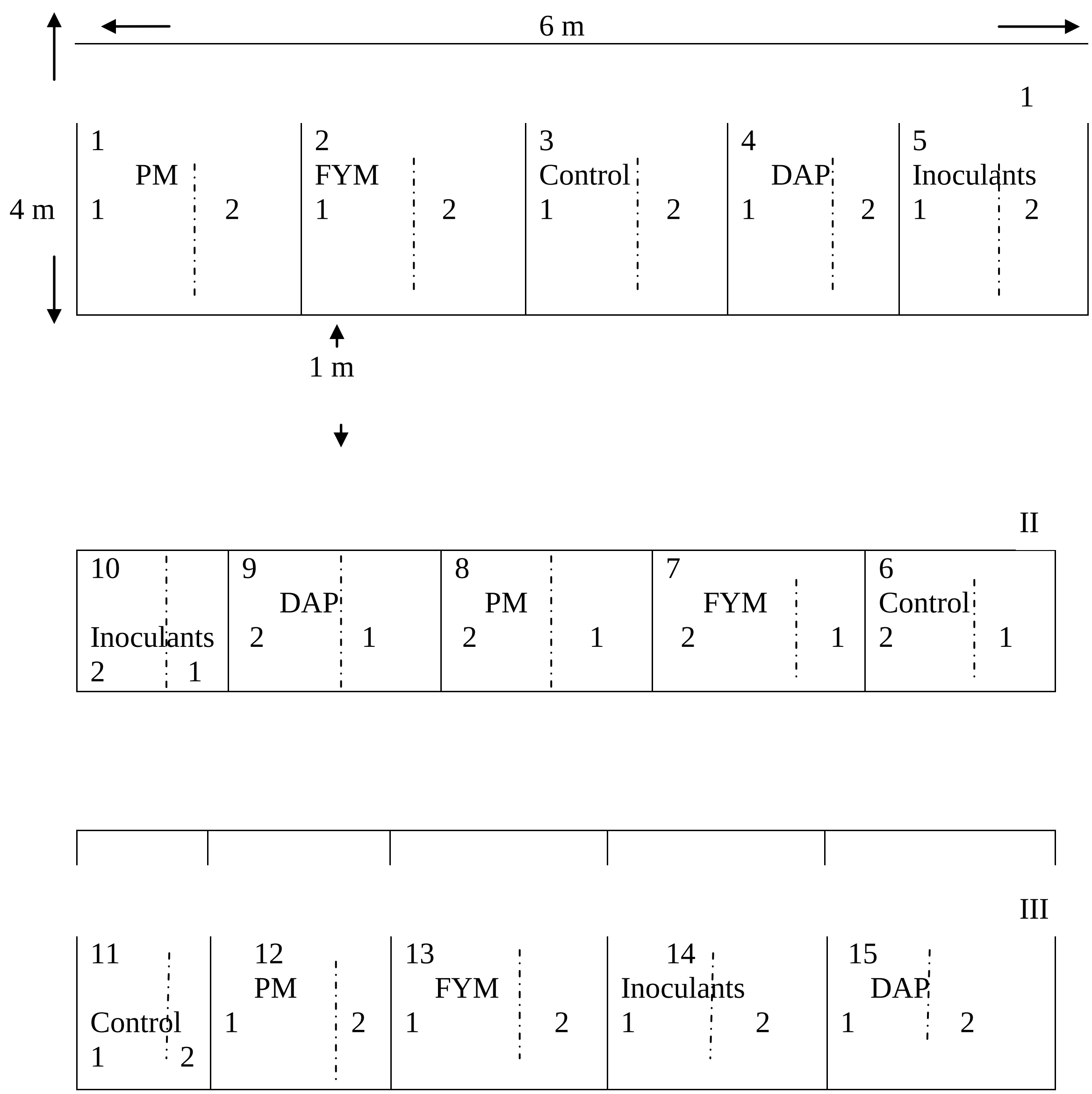
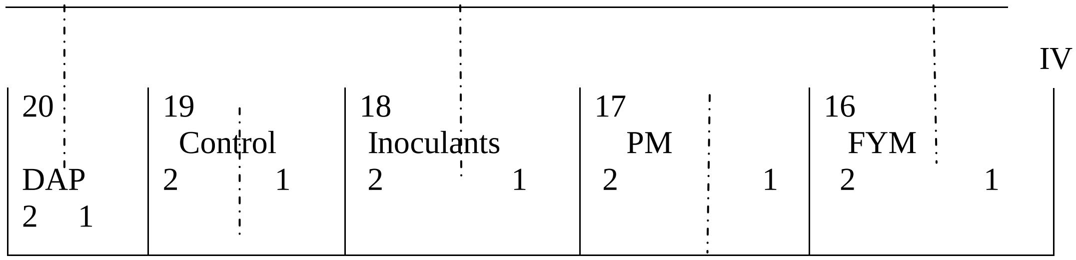
APPENDIX I

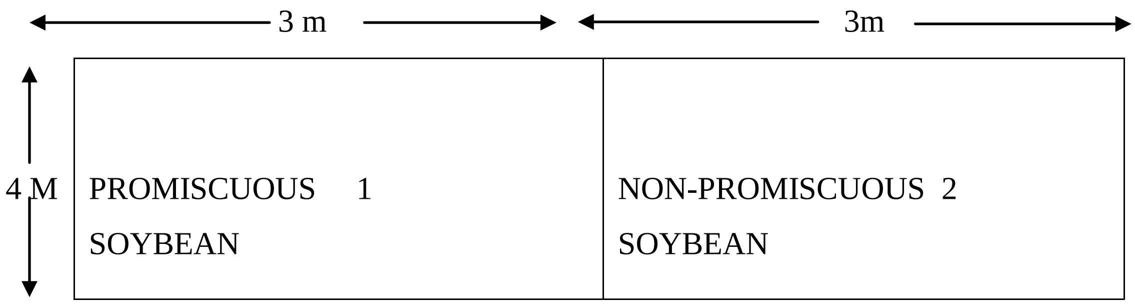
EXPERIMENT LAYOUT

MAIN PLOTS





SUB-PLOT



PM- Poultry manure
FYM- Farmyard manure
DAP- Di-ammonium phosphate
1- Promiscuous soybeans
2- Non-promiscuous soybeans

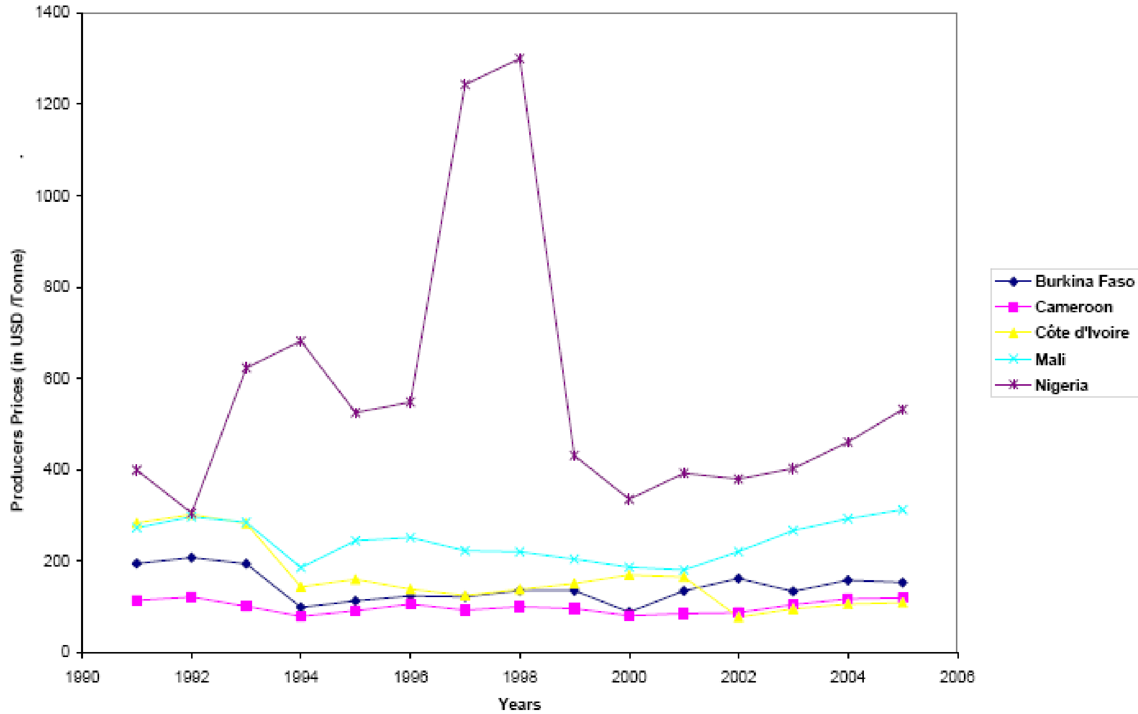
APPENDIX II

SOYBEAN PRODUCTION IN AFRICA

Year	**Area (ha)**	**Yield (kg ha^{-1})**	**Production (tons)**
1990	2500	800	2000
1991	2500	800	2000
1992	2500	800	2000
1993	2500	800	2000
1994	2500	800	2000
1995	2500	800	2000
1996	2500	800	2000
1997	2500	800	2000
1998	2500	800	2000
1999	2500	800	2000
2000	2500	800	2000
2001	2500	800	2000
2002	2500	800	2000
2003	2500	800	2000
2004	2500	800	2000
2005	2500	800	2000
2006	2513	826.5	2077
2007	2500	840	2100

Source: FAO, 2008.

APPENDIX III
TREADS OF SOYBEAN PRICES IN AFRICA



APPENDIX IV

SOYBEAN CONSUMPTION IN KENYA (1961-2003)