EFFECT OF SOIL AMENDMENT WITH MANURE ON NODULATION, BIOMASS AND YIELD OF SOYBEANS AND CLIMBING BEANS IN MERU COUNTY, KENYA

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for degree in any other University or any other award.

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DEDICATION

I dedicate to my mother Esther Karimi, my late brother Robert Mbaabu, my husband Nicholas Mawira and our lovely daughters Brenda Kanana and Brencil Rima.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
BNF	Biological Nitrogen Fixation
BTB	Bromothymol Blue
CR	Congo Red
DAP	Diammonium Phosphate
EAI	Enterprise Application Integration
FAO	Food and Agriculture Organization
FTC	Farmers Training Centre
GOK	Government of Kenya
GTZ	German Technical Cooperation
Ha^{-1}	Per hectare
ICRISAT	International Crop Research Institute for the Semi – Arid Tropics
IITA	International Institute of Tropical Agriculture
K	Potassium
KARI	Kenya Agricultural Research Institute
LSD	Least Significance Difference
MT	Metric Tones
N	Nitrogen
P	Phosphorous
RCBD	Randomized Complete Block Design
rRNA	ribosomal Ribonucleic acid
TGx	Tropical Glycine Cross
YEMA	Yeast Extract Mannitol Agar
YEMB	Yeast Extract Mannitol Broth

ABSTRACT

The consequence of intense permanent cultivation of land has been severe loss of soil nutrients through removal of harvested produce, removal of crop residue, leaching and gaseous losses. Experiments were designed to investigate the effect of soil amendment with cow manure, chicken manure and fertilizer on nodulation and nitrogen fixation in climbing beans (MAC 64) and soybeans (Gazelle). Isolation and characterization of isolates together with greenhouse experiments were carried out at Kenyatta University laboratory and greenhouse respectively. Field experiments were carried out at Kaguru Farmers Training Centre and Munithu Girls Secondary School farm. The experimental design was a randomized complete block design with four replications. Each of the legume was planted in soils amended with cow manure, chicken manure, fertilizer and other soils left unamended for control. Data was obtained at 75 % podding and full physiological maturity. Laboratory experiments were carried to isolate and characterize the rhizobia nodulating and fixing nitrogen with climbing beans and soybeans. This was carried out using congo red, litmus milk, Bromothymol blue and peptone agar. Greenhouse experiments were carried out to authenticate the rhizobia obtained from the field. Inocula was prepared from the root nodules of each of the legumes. Isolates from climbing beans inoculated radicles of soybeans and those of soybean inoculated climbing beans. Uninoculated climbing beans and soybeans were used as controls. Cross inoculation was also established in the greenhouse. Rhizobia nodulating and fixing nitrogen with soybeans nodulated and fixed nitrogen with climbing beans. Data obtained from the field experiments was analyzed and significance difference determined in nodule number, root dry weight, stover dry weight, pod number and dry weight of 100 seeds. Results were subjected to standard statistical analysis and presented using both descriptive and quantitative statistical procedures. The effect between amendments was determined using Genstat software for the analysis of variance (ANOVA) and mean separated using least significance tests at 5% level. Indigenous bradyrhizobia do not nodulate effectively in soybean and climbing beans in soils with low levels of nitrogen. Nodules did not form in beans planted in the soils amended with nitrogen fertilizer. Cross inoculation of rhizobia was observed between soybean and climbing beans in the greenhouse. Chicken manure, cow manure and nitrogen fertilizer application had effect on nodulation, root dry weight, stover dry weight, pod number and dry weight of 100 seeds on both soybeans and climbing beans. Climbing beans at Munithu Girls secondary School farm showed better performance in soils amended with chicken manure in the parameters assessed. BNF should be exploited with the view of decreasing overdependence on nitrogen fertilizer for sustainable agriculture. There is need for continuous screening of large numbers of native bradyrhizobia to understand their specificity in nodulation and nitrogen fixation.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Biological nitrogen fixation is the most importance contribution to the world's supply of nitrogen to plants (Hamad *et al*., 2003). It is the only process that can effectively alleviate adverse environmental impacts imposed by nitrogen fertilizer at the same time benefiting both plants and the farmer (Carlsson and Huss-Danell, 2003). There has been tremendous interest in BNF as a source of nitrogen in agricultural systems (Giller *et al*., 2009). Adaptation of BNF technology is still low (Collins *et al*., 2002). In Kenya 8 % of farmers are aware of its function and yet lesser than 15 % are aware of inoculants (Collins *et al*., 2002). Biological nitrogen fixation from legumes can sustain tropical agriculture at moderate levels of input (Giller, 2002). As much as 30-60 kg Nha^{-1} Yr^{-1} is added to the soil by legumes (Carsky *et al.,* 2006). although legumes contribute to soil nitrogen in different cropping systems, the amount of nitrogen fixed is influenced by various factors (Maingi *et al*., 2006) such as, compatibility of the rhizobia in the soil with the legume, soil moisture, soil pH, soil temperature, soil nitrogen, soil mineral phosphorus and mycorrhiza colonization (Aliye and Hagman, 2011) .

Among symbiotic nitrogen fixing systems, nodulating legumes have been used in cropping systems for centuries. Soybean legumes improve the field where they are grown through weed suppression, soil erosion control and fast decomposing residues that release nutrients at the subsequent crop (Vanlauwe *et al*., 2003). Most of the best known legumes that form symbiosis with rhizobia belong to the subfamily papilionoideae where soybeans belong. Soybean plants are known for their high nitrogen fixing ability (Aliye and Hagman, 2011). They contribute to soil nitrogen through BNF, some of which is available to subsequent crops (Carsky *et al*., 2006). Soybeans can fix more nitrogen than that which can be removed, thus making a net contribution to the soil (Alves *et al*., 2003). Soybean is grown from the equator to latitude 55^{o}N or 55^{o} S, and from sea level to altitudes close to 2000 M. The leading producers of soybeans in the world are the United States of America, Peoples Republic of China and Brazil with 74 %, 12 % and 8 % respectively of the total production (www.fao.org., 2002).

Soybeans were introduced in Kenya in the early 1960's (Abate *et al.*, 2008). Although the growing of soybeans never gained popularity then, the scenario has changed tremendously after two popularization programmes by GTZ (2012) and the food and Agriculture organization (FAO). Soybeans have various uses, both domestically and industrially. Apart from providing income for the betterment of the socio-economic status of farmers, soybeans are a human food. The biochemical composition of soybeans is unique among the pulses. It has the highest protein content of all pulses (94 g per 100 g), double of the other popular African pulse, cowpea and common beans (typically 22 g per 100 g), after groundnut. They also have the highest fat content (20 g per 100 g) much higher than cowpea and common bean (1.4 per 100 g).

Apart from soybeans, common bean is another commonly grown legume in Kenya, with production of over 414,000 metric tons per year (Garcia *et al*., 2004). It comes second to maize as a subsistence crop and is a major source of protein in human diets. In East Africa, a major problem with common bean production is low yield. Yield average is between 400-600 Kg per hectare (Schauser *et al*., 2006). Common bean (*Phaseolus vulgaris)* was one of the first plants to have been domesticated by man between 8,000 – 10,000 years ago (Santalla *et al*., 2003). The crop was first discovered in the 16th century by Portuguese in South America from where it spread to other parts of the world. The spread was into the United States, Europe, tropical Africa and secondarily North Africa and Asia (Baudoin *et al*., 2001). Africa supplies between 10 -20 % of the world bean production (Ramirez – Bahena *et al*., 2008) like many legumes it prefers well aerated, sufficiently drained soil with a pH of 6.0 – 7.5, critical thresholds being 5.0 and 8.1. Good soils for bean cultivation are characterized by the presence of a humus–containing layer (Mostasso *et al*., 2002). The optimal amount of organic carbon in the soil should exceed 2.4 % (Baudoin *et al*., 2001).

Common beans are very important legumes in agricultural production systems because of their ability to form symbiotic relationship with nitrogen fixing bacteria (Palmer and Young, 2002) and inherent ability to utilize atmospheric nitrogen in association with rhizobia (Hardason and Atkins, 2003). These are facts that are determined by good soil management which can very easily be achieved by soil amendment with environmentally friendly farm use like use of organic manure (Bernal and Graham, 2001). It is therefore timely to assess the potential of organic manure to complement or replace fertilizer inputs and to consider their contributions to the nitrogen fertility

Soybeans and climbing beans are currently demanded by food processors and animal feed millers in Kenya (Vanlauwe *et al*., 2003 and GTZ, 2012). Therefore they have a ready market. They are also used secondarily to enrich the soil, preserve moisture and prevent soil erosion. They can also be used for wind break, ground cover, trellis and shade or a source of resins, gums, dyes and some oils. Most of the ornamental plants in the tropics are legumes (Sanginga *et al.,* 2009).

However the level of awareness of the benefits of soybeans and climbing beans among Kenya's small scale farmers which has reached only very small proportions (Hornetez *et al.,* 2006) and even smaller proportions in terms of their benefits to the soil fertility (Vanlauwe *et al*., 2003). A very small proportion of small scale farmers in Kenya are aware of the phenomena of biological nitrogen fixation (15 %) (Sanginga *et al.,* 2009) thus it is important to create awareness of environmental friendly inexpensive alternatives to nitrogen fertilizer. Organic manure offers the best alternative to nitrogen fertilizer as it creates favourable conditions for soil biota that consequently enhances high nitrogen fixation (Charman and Roper, 2007) hence increased plant biomass and yield production. The ability of soil microorganisms to fix high amounts of nitrogen is governed by the symbiotic association between *Rhizobium* and the host plant. Hence it may be necessary to introduce a superior method of soil amendments that will encourage good soil aeration, improve soil structure and soil texture and improve soil holding capacity that favours the multiplication of *Rhizobium* as well as providing nutrients to the plants for maximum growth and yield. (Mostasso *et al.,* 2002). Rhizobia in the soil nodulating with soybeans can nodulate with climbing beans providing farmers at all agroecological zones an opportunity to access available nitrogen in the soil by growing either of the legumes (Hornetz *et al.,* 2006).

1.2 Problem statement and justification

The consequence of intense cultivation of land has been severe loss of soil nutrients through removal of harvested produce, removal of crop residues, leaching and gaseous loses. It is estimated that soil nutrient losses in Africa rose from 8.3 million tonnes in 1993 to about 13.2 million tonnes in 2000 (Hungria *et al*., 2006). Biological nitrogen fixation (BNF), a microbiological process which converts atmospheric nitrogen into a plant-usable form offers an alternative to nitrogen fertilizers (Hamad *et al*., 2003). Symbiotic systems such as that of legumes and *Rhizobium* are major sources of nitrogen in most cropping systems. Nitrogen fertilizer rank first among the external inputs to maximize output in agriculture. However, input of nitrogen fertilizer is one of the lowest among the plant nutrients and in turn contributes substantially to environmental pollution. Maximum benefit can be realized by integrating BNF systems and application of organic manure to farming situations and adoption of the technology by farmers. In the light of the aforementioned challenges the study is aimed at assessing how soil amendment with chicken manure, cow manure, nitrogenous fertilizers and BNF systems accompanied by cross inoculation of different legumes with rhizobia nodulating with each of them, would promote sustainable soil fertility.

1.3 Research questions

i) Are soybeans and common beans nodulated by diverse indigenous nitrogen fixing rhizobia?

ii) Does cross inoculation of rhizobia exist between common beans and soybeans?

iii) Does the amendment of soil with chicken manure, cow manure and nitrogen fertilizer affect nodulation and nitrogen fixation in soybeans and common beans?

1.4 Hypotheses

i) Soybeans and common beans are not nodulated and fix nitrogen with diverse indigenous nitrogen fixing rhizobia.

ii) Cross inoculation of rhizobia does not exist between soybeans and common beans.

iii) Application of chicken manure, cow manure and nitrogen fertilizer has no effect on nodulation and nitrogen fixation in soybeans and common beans.

1.5 Objectives

1.5.1 General objective

To establish the effectiveness of indigenous rhizobia in nodulation and nitrogen fixation in soybeans and climbing beans.

1.5.2 Specific objectives

i) To isolate and characterize indigenous rhizobia that nodulate and fix nitrogen in soybeans and common beans at Kaguru Farmers Training Centre in Nkuene Division and Munithu Girls secondary school farm in Munithu location, Meru County.

ii) To determine whether there is cross inoculation of rhizobia between soybeans and common beans.

iii) To assess the effect of cow manure, chicken manure and nitrogen fertilizer amendment on nodulation and nitrogen fixation in soybeans and common beans at Kaguru Farmers Training Centre in Nkuene Division, Meru County and Munithu Girls secondary school farm in Munithu location, Meru County.

1.6 Significance and anticipated output of the study

It is expected that improvement of agricultural land by soil amendment using organic manure would favour the production of soybeans and climbing beans offer environmental protection. Soybeans and climbing beans have potential for soil fertility improvement through biological nitrogen fixation. Existing evidence points to increasing worldwide food and protein deficiency (Hirsch, 2005). In developing country such as Kenya animal protein is in short supply and expensive for the majority of low- income groups. Soybeans and climbing beans have been reputed at the world's largest source of cheap and readily available protein of high nutritional value. Improvement of soybeans and climbing beans would economically empower farmers through additional income accruing from the sale of the crop. This can assist in poverty alleviation through increased income to farmers. Enhanced family income can cater for improved health and educational needs of the household.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nitrogen fixation

The problem facing farmers everywhere is that the capacity of their soils to supply nitrogen declines rapidly once agricultural activities commence and nitrogen derived from the breakdown of soil organic matter must be supplemented from other sources (Hungria *et al.,* 2005). As a result of losses in soil nutrients, Africa has the unfortunate reputation of being chronically hungry and ever in need of food aid. The amounts of nitrogen removed from farms have been calculated to exceed fertilizer input and biological nitrogen fixation in agricultural systems in Kenya by 112 kg N ha^{-1} (Russelle and Birr, 2004). External nitrogen inputs in the form of fertilizer and biological nitrogen fixation must therefore be increased if farmers are to have any prospects of meeting the food requirements of a growing world population. The economic and environmental costs of the heavy use of chemical nitrogen fertilizer in agriculture are of global concern. Sustainability considerations mandate that alternatives to nitrogen fertilizer must be urgently sought (Hamad *et al*, 2003). Nitrogen fixing systems offer an economically attractive and ecologically sound means of reducing external inputs and improving internal resources (Gage, 2004).

The value of leguminous crops lies in their ability to fix atmospheric nitrogen thus reducing the use of expensive nitrogen fertilizer and enhancing soil fertility. Development of suitable farming systems rely more heavily on the fixed nitrogen from the soil. The ability of legumes to fix nitrogen from the atmosphere arises because of a symbiotic association between the legume and soil borne bacteria of the genus *Rhizobium.* In nodule formation rhizobia infect the plant root, multiply and fix nitrogen to form ammonia within the plant cells (Hirsch, 2005). The ammonia formed in the nodules is assimilated within the root nodules as amides and ureides that are translocated to the shoot hence both form a complementary relationship (Mosier *et al.,* 2000).

Nodulated legumes have the potential to provide entire nitrogen requirement for their growth. In this way, they influence the nitrogen balance in the soil and the availability of nitrogen to the accompanying or subsequent crops. By reducing the inputs of nitrogen fertilizers, legumes reduce the costs of production and the potential for nitrogen contamination of water resources as well as producing pulses of high nutrition value.

2.2 Biological nitrogen fixation

Biological nitrogen fixation comprise 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 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 form 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 (Ruiz *et al.,* 2007). Estimates show that the symbiotic system contributes 40 million tons of nitrogen annually to grain legumes (Russelle and Birr, 2004). Biological nitrogen fixation which enables legumes to use atmospheric nitrogen, contribute to nitrogen for legume growth and grain production under different soil conditions (Gupta *et al.*, 2006).

2.3 Legume-*Rhizobium* symbiosis

The *Rhizobium* legume symbiosis form a unique system among plant microbe interactions. This leads to nodule formation on the roots of the host plant. Nodules are associated with many legume genera and are all formed by the symbiosis between the host plant and rhizobia (Mathesius, 2003). In this relationship plants gain a constant supply of reduced nitrogen from rhizobia and the rhizobia in return are supplied with photosynthate and other nutrients by the host plant. The host plant also provides a suitable environment for rhizobial growth and metabolism (Bernal and Graham, 2001). Legume-rhizobia symbiosis forms a system that has been exploited as a substitute for the chemical nitrogen fertilizer. It provides nitrogen through nitrogen fixation not only for legumes but also for the subsequent crops, as residues returned to the soil are rich in nitrogen and therefore readily mineralized (Hungria, 2006).

Rhizobia are soil saprophytes which proliferate in the rhizosphere. They are also considered to be beneficial symbionts specific to legumes (Giller *et al*., 2002). Rhizobia bacteria belong to six genera: *Rhizobium, Bradyrhizobium*, *Azorhizobium Sinorhizobium*, *Allorhizobium and Mesorhizobium* (Hardarson and Atkins, 2003). Soybeans (*Glycine max*) nodulates with *Bradyrhizobium* spp. while common bean (*Phaseolus vulgaris)* nodulates with *Rhizobium* species (Bergesen, 2011). In *Rhizobium*-legume symbiosis, the infection and nodulation processes involve several developmental steps which have been described by Mpepereki *et al*., (2000). *Rhizobium* entry into legume roots can occur through root hair infection, cracks or wounds at points of lateral root emergence or even through undamaged epidermal tissue cases exist where a single rhizobial strain can infect different leguminous hosts via one or more entry points (Hungria *et al.,* 2006).

In many leguminous species, nodule development commences with the rhizobial infection of a root hair followed by formation of infection thread and mitotic cell division in the hypodermis (Giller, 2002). These early events are coordinated in the plant by the activity of a nodule inception gene product protein whose absence arrests the formation of infection thread and nodule primodial (Madsen and Alexander, 2008). Rhizobia may recognise compatible legume host initially via energy-rich nutrients released from or sloughed off the roots and become attached to a root hair by host plant proteins called Lectins. These proteins bind polysaccharides present on the cell surface of rhizobia species. Lectins and polysaccharides are thought to be involved in the recognition process (Mosier *et al.,* 2004).

Infection proceeds via an infection thread through which the rhizobia penetrate to the cells of the cortex of the host root. The infected cortical cells increase in size and divide to form a ball surrounded by uninfected cells and an outer fibrous layer. Within the infected cells, the rhizobia differentiate into bacteroids, which always remain confined in vesicles bound by the host plant (Baron, 2012). Different nodule structures are formed on infection of different plants, varying from cylindrical to spherical and from annular to irregular. Two broad classes are recognized. These are determinate and indeterminate. Determinate nodule do not have persistent meristems, the vascular system becomes more or less closed, investing the nodule in a continuous system of vascular traces and there is usually little or no involvement of infection threads in the distribution of bacteria to the nodule cells. Nodules tend to be spherical in shape. Soybeans develop this type of nodule (Bergesen, 2011).

Indeterminate nodules have persistent meristems with open vascular system. Growth occurs at the distal end of the nodule by cell division. Infection threads are major mechanisms for distribution of bacteria to the nodule cells. A typical indeterminate nodule tends to be branched and cylindrical in shape initiating new growth from the tip of the old nodules. This type of nodules occurs in peas, clover and alfalfa (Feng *et al.,* 2002).

2.4 Rhizobia strains that nodulate beans

A broad range of rhizobia species are able to nodulate and fix nitrogen with beans. These are *Rhizobium etli* (Salvagiotti *et al.,* 2008) which has multiple copies of the structural, nif genes, *Rhizobium tropici* (Martinez-Romeo *et al.,* 2000), *Rhizobium leguminosarum* (Sawada *et al.,* 2003), *Rhizobium gallicum* and *Rhizobium giardinii* (Armager, 2001). Other bacteria that are able to nodulate beans have been identified but, they show distinct phylogeny positions in relationship to these described species (Broos *et al*., 2005). Biochemical tests such as growth of isolates on YEMA plus BTB, growth of isolates on YEMA and congo red, growth of isolates on litmus milk, Gram staining and growth of isolates on peptone agar describes cultural and morphological characteristics of *Rhizobium* nodulating with common beans and soybeans (Beck *et al*., 2006).

This grouping of rhizobia has been possible due to emergence of new, powerful tools of systematic bacteriology. Molecular sequencing technology in particular, has been influencing radical changes in rhizobial classification over the last few years (LaRue *et al.*, 2007). The technique has mainly been applied to fast growing rhizobial species with the effect that there is now less reliance on host range in classifying these bacteria. With the slow growing rhizobia bacteria, especially in "cowpea miscellany group" host range continues to be the most important determinant in naming species. However, this classification continues to be mired in confusion. It is hoped that if the new tools of systematic bacteriology are applied to the wide range of slow growing rhizobia, it will be a more effective way of classifying them. One method would be to use phylogenetic relationships based on 16S rRNA sequence diversity to ascribe them into genera as suggested for rhizobia (Collins *et al.,* 2002). The use of phenotypic variations to ascribe the slow growing rhizobia isolates into distinct species has also been proposed (Hungaria, 2006). However, this requires a significant amount of research to establish and understand diversity on a wide range of the slow growing rhizobial isolates from various legume species before any effective classification system can be developed (Carsky *et al.*, 2006).

2.5 Cross-inoculation of rhizobia

Cross-inoculation groups in nodulated legumes are based on the ability of rhizobia to nodulate a group of legume species. Earlier studies were carried out by inoculating plants with nodule suspensions (Chen *et al.,* 1988). In general, plants from a given species are nodulated effectively by inocula prepared from crushed nodules of the same species in the green house. The principle of cross-inoculation grouping is based on the ability of an isolate of *Rhizobium* to form nodules in a limited number of species of legumes related to one another (Abaidoo, 2001).

Studies on cross inoculation of rhizobia reveals that indigenous rhizobia nodulating with a particular legume can consequently nodulate with other legumes (Bergesen, 2011). All rhizobia that could form nodules on roots of certain legume types have been collectively taken as a species (Cappucino, 2001). Serologically, it is known that a single nodule contains a homogeneous population of a single strain of *Rhizobium* although it is not uncommon to find more than one strain on the same plate (Javaid *et al.*, 2008).

2.6 Soybean

Soybean is becoming a very important grain legumes in Kenya due to its recognized potential as food, livestock feed, for soil fertility improvement and income generation for smallholder farmers (Giller *et al.*, 2001). As human food, it is regarded as one of the most important sources of high quality protein, edible oils and vitamins (GTZ, 2012). High level awareness of soybeans has been created and several African countries have become interested in soybean production. Nigeria accounts for 56 % (GTZ, 2012) followed closely by Uganda (22 %) and Zimbabwe (11 %) in terms of production in Sub Saharan Africa.

In vision 2030 soybean has been identified as one of the crops that will contribute to a pillar for the economic growth (GOK, 2012). Currently, about 8,000-10,000 MT of soybean is being produced in Kenya against an annual local demand of 60,000 MT (Hungaria *et al.*, 2006). The deficit is met through imports. Agronomic experience has shown that soybean, can successfully be grown in Kenya using low agricultural input. Kenya Agricultural Research Institute released five soybean varieties in 2009 (Hill, Black Hawk, EAI 3600, Nyala and Gazelle) for specific growing areas with a yield potential of up to 2.0 tons per hectare (Boyer, 2005).

Apart from providing income for the betterment of the socio-economic status of farmers, small-holders soybean production is expanding rapidly in Kenya (GTZ, 2012). Benefits of soybean production over grain legumes commonly grown by small-holders such as groundnuts (*Arachis hypogea* L.), cowpea (*Vigna unguiculata* L.) and common bean (*Phaseolus vulgaris* L.) includes lower susceptibility to pests and diseases, better grains storage quality and a large leaf biomass which improves soil fertility to subsequent crops (Mpepereki *et al*., 2000).

2.7 Growth habit of the soybean

Soybean is one of the plants in the legume family (Ruiz *et al.,* 2005). It is an erect bushy annual herb that produces many leaves and has a hard stem. Soybean tap root can be up to 2 m long, is branched and spreads side roots horizontally to a distance of up to 2.5 m in the upper 10-15 cm layer of the soil. Depending on the number of the leaves per plant, soybean may be divided into determinate and indeterminate growth habit (Baudoin *et al*., 2001). Indeterminate growth habit, the inflorescences are terminal and axillary on the main stem and have lateral ramifications (Ruiz *et al.,* 2005). The first flowers appear at the top of the plant and flowering is descendant (from the top to the base of the plant). Determinate soybeans produce many beans, have fewer leaves and take a shorter time between planting and harvesting compared to indeterminate soybeans (Peloni, 2006).

2.8 Common bean

In Kenya, the most commonly grown legume is the common beans (*Phaseolus vulgaris*). Common beans are important in all agricultural areas in Kenya except at the Coast and Coastal hinterland up to about 600 m in altitude (ICRISAT, 2011). The total acreages annually covered by the crop is approximately one million acres (Amager, 2001). Common beans are not drought resistant, ideally they need moist soil throughout the growing period. They are annual legumes which have a great variation in their growth habit, most of which is found in the Meru County (Maingi *et al.,* 2006). The required length of wet season depends on growth habit of common bean and the attitude. Most of the common beans are inter-cropped with maize. A broad range of rhizobia species are able to nodulate and fix nitrogen with common beans (Yasmin *et al.*, 2006). In soils lacking rhizobia that nodulate a particular legume, inoculation with efficient strains was found to increase yields (Shah *et al*., 2003). In soils, which contain native rhizobia populations, the introduced strains should be competitive and efficient.

2.9 Growth habit of the common bean

A distinction is made between two growth habits of the common bean each having several forms. These habits and growth forms depend on genetic factors and ecological factors like temperature and photoperiod. The habits are determinate and indeterminate growth (Baudoin *et al*, 2001). Indeterminate growth habit are terminal and axillary on the main stem and have lateral ramifications (Athar and Shabbir, 2008). The first flowers appear at the top of the plant and flowering is descendant (from the top to the base of the plant).This growth habit comprises two types, 1 and 2. Type 1 plants are characterized by a limited number of nodes 3-7 on the main stem before the appearance of the terminal inflorescence. Laterals ramifications are few and grow out from the first nodes on the main stem. The habit is erect. Plant height is 30-50 cm with a very short growth cycle. Type 2 plants are also dwarf with long or short internodes. They have more nodes on the main stem than type 1 plants (7-15). Node production may continue after flowering. Lateral ramifications are limited in number and grow out chiefly from the first nodes of the mains tem. The plant has an erect habit and a height of between 50 and 70 cm (Russel and Jones, 2001).

In East Africa, most farmers grow determinate bean type. The period from sowing to flowering of most bush type beans is about five weeks at an altitude of 1,200 m above sea level, and flowering continues for about two weeks (Osborne and Riedel, 2001). It takes about 2 weeks for a flower to produce a full length pod and another four to five weeks for the pod to mature and dry out. The life of this bean type at medium altitude is approximately three months. At an altitude of 1500 m the maturation period is about four months while at 2400 m it is almost five months (Baudoin *et al.,* 2001).

In the indeterminate growth habit, the inflorescences are auxiliary on the main stem and lateral ramifications. The first flowers appear at mid- height of the plant and flowering is ascendant (from the bottom to the top of the plant) (Garcia, 2004). This growth habit also comprises of two types, 3 and 4. Type 3 plants produce a moderate to high number of nodes after flowering along with a variable number of lateral ramifications starting mainly from the base of the stem. Some laterals are as long as the main stem which is 70-130 cm in length and has long internodes. The plants habit is sometimes erect but it is usually prostrate and or semi-twinning (Unkovich *et al.,* 2008). Types 4 plants are twinning or climbing and produce many nodes after flowering. The lateral ramifications are shorter than the main stem, which is characterized by long internodes and is 160-250 cm in length. The pods are distributed either uniformly along the main stem or concentrated on the upper part of the plant. This type must be staked. The indeterminate beans take longer to mature. At medium altitudes maturation period takes four to five months. In higher altitudes, maturation period is over six months (Stephens and Rask, 2006).

The aim of development of the indeterminate bean genotypes was to increase yield potential and improve yield stability of the determinate beans (Beauregard *et al.,* 2003). It also aimed at facilitating the transfer of plant architecture traits and disease resistance. Indeterminate beans therefore produce greater seed yields than determinate genotypes in low plant populations in order to reduce seed costs (Beauregard *et al.,* 2003). Research directed towards increasing nitrogen fixation in beans has emphasized improving both the plant and the rhizobial strain components of the symbiosis. Genetic variability in nitrogen fixation and traits related to fixation has been reported in bean germplasm (Herridge *et al*., 2000). Athar and Shabbir (2008) reported good progress in breeding beans for nitrogen fixation. He noted that when the bean genotypes differed markedly in nitrogen fixation, only limited gains were realized in crosses involving superior nitrogen fixing genotypes. Nodulation and nitrogen fixation abilities of indeterminate bean cultivars, with a large photosynthetic area, were found consistently superior to most bean cultivars (Athar and Shabbir, 2008). Breeding for improved nitrogen fixation should therefore be incorporated into bean breeding programmes as a standard practice. It should also be given priority equivalent to other objectives such as yield improvements, suitable plant type, disease and insect resistance, adaptability and acceptable food quality (Garcia *et al.*, 2004).

2.10 Cow manure

Animal manures are an excellent source of plant nutrients (Charman and Roper, 2007). Approximately 70-80 % of the nitrogen, 60-85 % of the phosphorus and 80-90 % of the potassium in feed are excreted in the manure (Giller, 2002). The amount of nutrients available for recycling to plants varies widely depending upon the composition of the feed ration, the amount of bedding and water added or lost, the method of manure collection and storage, the method of land application, characteristics of the soil, crop and climate (Charman and Roper, 2007). Manure contains all the plant nutrients needed for crop growth including trace elements. The availability or efficiency of manure utilization by a crop is determined by the method of application (Amager, 2001). Manures have the advantage of supplying essential plant nutrients either directly or indirectly by alleviating aluminium toxicity or by producing organic acids, thereby increasing nutrient availability especially P in high P- fixing soils (Turpin *et al.,* 2003). Other effects of manure include the improvement of soil structure and subsequent soil permeability and water holding capacity (Roper and Gupta, 2007). It also increases the infiltration rate due to its colloidal nature and improves soil aeration. Increased nodulation in legumes is greatly associated with soil amendment with organic manure in legumes (Garcia, 2004). Nitrogen fixation is positively proportional to increased nodulation (Gage, 2004).

2.11 Chicken manure

Poultry manure (chicken in particular) is the richest animal manure in N-P-K (Nitrogen, Phosphorous and Potassium) (Mosier *et al.,* 2004). Poultry manure is an excellent source of nutrients and can be incorporated into most fertilizer programmes. Those using manures must practice sound soil fertility management to prevent nutrient imbalances (Gupta and Roget, 2004). The key to successful management is to match the nutritional requirements of the crop with nutrients available in the manure. The chicken manure provides some good organic material as well and this would actually release some carbon dioxide into the soil which can be picked up by the root and can be an in-soil source of carbon dioxide, which many times lack in fertility programmes (Peloni, 2006). Chicken manure is a good fertilizer, it provides nitrogen, phosphorous and potassium to plants more than that from cow, horse or steer manure (Tatiana and Parniske, 2003). It is a useful source of nitrogen, the main nutrient that plants need for leafy growth web. The soluble nitrogen compounds in the chicken manure helps to enhance the multiplication of soil microbes (*Rhizobium*) which participates in nitrogen fixation in the soil (Shutsrirung *et al.*, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study sites, laboratory and greenhouse experiments

Field experiments were carried out at Kaguru Farmers Training Centre farm and Munithu Girls Secondary school farm. Laboratory and greenhouse experiments were carried out at Kenyatta University

(i) Kaguru Farmers Training Centre

Kaguru Farmers Training Centre (FTC) (AEZ: UM 2-3) is in Imenti South District, Meru County. Kaguru Farmers Training Centre is located at latitude, 0°S and longitude 37^{o}E, with an elevation of 1527 m above sea level (Chianu *et al*., 2009). The soils are well drained clay with high water holding capacity to sustain growth of soybeans and climbing beans. Rainfall is bimodal. The long rains normally start in mid March while the short rains normally start in mid October. The short rains are normally more reliable and give higher yields (Herridge and Peoples, 2002). Mean annual rainfall is about 1765 mm.

(ii) Munithu Girls Secondary School farm

Munithu Girls secondary school farm is in Imenti North District, Meru County. It is located along Meru Maua road North East to Meru town and approximately 7 km from the town. It is along the equator at 0^{o}S latitude, 42.5^{o}E longitude. The area has black cotton soil rich in phosphorus which is essential for the growth of legumes (Kumar *et al.,* 2002). It has mean annual rainfall of about 1,000 to 1,500 mm. Short rains are experienced in October to December and long rains in March to May. Yields are high during short rains (Turpin *et al.,* 2003).

3.2 Seed procurement

Seeds of gazelle soybean and MAC 64 climbing beans were obtained from Kenya Agricultural Research Institute (KARI) Embu.

3.3 Laboratory experiments

Growth media for culturing the isolates were prepared in the laboratory at Kenyatta University.

3.3.1 Preparation of bacterial growth media

For routine culturing of rhizobia in the laboratory, Yeast Extract Mannitol Broth (YEMB) and Yeast Extract Mannitol Agar (YEMA) were used (Somasegaran and Hoben, 2002).

3.3.2 Yeast extract mannitol broth (YEMB)

The composition of YEMB used in the laboratory experiment was as shown in Table 3.1.

Table 3.1: The composition of YEMB

Compound	**Amount (g/L)**
Mannitol	10.0
$KHCO_3$	0.5
$MgSO_4 . 7H_2O$	0.2
NaCl	0.1
Yeast extract	0.5
Distilled water	1.0 litres

Source: Cappucino, 2001.

For preparation of one litre of YEMB, the salts were dissolved in about 250 ml of distilled water in 1 litre volume conical flask. Mannitol and yeast extract, were subsequently dissolved under continuous stirring. The volume of distilled water was topped to the one litre mark. The medium was then autoclaved at 121^{o} C for 15 minutes.

3.3.3 Yeast extract mannitol agar (YEMA)

To prepare 1 litre YEMA medium, YEMB was prepared and 15 g of agar were added. The medium was then autoclaved at 12^{o} C for 15 minutes. The medium was left to cool to a temperature of 50^{o} C before it was dispensed into petri plates, tubes or culture bottles (Broughton, 2003).

3.3.4 YEMA plates

These were prepared by pouring sterilized YEMA into plastic plates (petri dishes). To prevent excessive water condensation in the plates the YEMA medium was cooled down to 50^{o} C before dispensing it into sterile plastic petridishes at the rate of 25 ml of medium per plate. Pouring of medium into plates was carried in a laminar flow to avoid contamination (Tatiana *et al.*, 2003).

3.3.5 YEMA slants

Yeast Extract Mannitol Agar slants were used for routine storage of rhizobia. These slants were prepared by dispensing YEMA into McCartney bottles. Approximately half of the volumes of McCartney bottles were filled with the YEMA media. The media in the bottles were sterilized by autoclaving. After autoclaving, screw caps were tightened and the bottles were allowed to cool to a temperature of about 50^{o} C for ease in handling. They were then transferred and placed on a 45^{o} slanted surface.

3.4 Indicator media

Congo red and Bromothymol blue were used during initial screening of rhizobia (Chianu *et al*., 2009).

3.4.1 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 121^{o} C for 15 minutes and shaken to mix. Shaking the flask mixed the two and the media was then poured into sterile plastic petri dishes (Bala *et al*., 2002).

3.4.2 Bromothymol blue indicator medium

Five ml of bromothymol blue solution (prepared by adding 0.5 g bromothymol blue powder into 100 ml ethanol) were added to one litre of YEMA. The medium was sterilized by autoclaving for twenty minutes at 121^{o} C for 15 minutes. The sterile media was cooled to 50^{o} C and then poured into sterile plastic petri dishes.

3.5 Nodule sterilization

Intact, undamaged nodules were immersed for 10 minutes in 95 % ethanol. This helped to break the surface tension and to remove air bubbles from the tissue. The nodules were transferred into 2.5 % (v/v) solution of sodium hypochlorite, and soaked for 2 minutes. They were then rinsed in five changes of sterile water using sterile forceps for transferring. The pair of forceps was sterilized by dipping it in absolute alcohol followed by flaming. The sterilized nodules were then crushed with the pair of blunt-tipped forceps in a large drop of sterile water in a petri dish.

3.6 Rhizobia

Indigenous rhizobia were isolated from root nodules of Gazelle soybean variety and climbing bean (MAC 64) grown in Munithu Girls Secondary School and Kaguru Farmers Training Centre farms.

3.6.1 Isolation of rhizobia

A sterile inoculation loop full of the resulting suspension was streaked on yeast extract mannitol agar plates. The inoculated petri dishes were incubated for seven days at $28^{o}C$ in the dark for colonies to appear. Colonies which were typical of rhizobia were streaked onto YEMA incorporating Congo red. The plates were incubated and daily observations made for the appearance of colonies typical of rhizobia. Pure isolates were transferred to screw cap McCartney bottles to form stock cultures.

3.7 Broth Culture

Broth cultures used in experiments were grown in 50 ml of YEMB contained in 250 ml flasks. Incubation was carried out at room temperature on an orbital shaker at 120 rounds per minute (r.p.m). The cultures were first sub- cultured from stock cultures in McCartney bottles to YEMA plates before transferring to the YEMB medium. All transfers were carried out in the laminar flow to ensure there was no contamination.

3.7.1 Culture maintenance and preservation

The slants were incubated for 4-7 days. Stock cultures of all isolates were stored on slants in screw cap McCartney bottles at 4^{o} C in a refrigerator. Sub-culturing was carried out after every three months to avoid loss of the isolates (Bala *et al*., 2003).

3.7.2 Characterization of isolates

Presumptive tests were carried out to establish the cultural characteristics of the rhizobia isolated from soybeans and climbing beans grown in the field. The tests carried out were Gram staining, growth on YEMA, growth on YEMA plus Bromothymol blue (BTB), growth of isolates on YEMA plus Congo red, growth on YEMA plus litmus milk and growth on peptone agar medium. The plates were incubated in the dark at 28^{o} C. Bacterial smears from 3-4 days old colonies were prepared on the clean microscope slides. The smears were air-dried, heat fixed by passing the slides over a Bunsen flame and then Gram stained as described by Beck *et al*. (2005). The slides were observed under oil immersion on a compound light microscope. Colonies of the isolates were streaked onto YEMA incorporating Congo red.

3.8 Greenhouse experiments

These experiments were carried out to authenticate rhizobia isolates from soybeans and climbing beans grown at the experimental sites, to establish the effectiveness of isolates and also to assess cross inoculation of rhizobia isolates between climbing beans and soybeans. Seeds of climbing beans and soybeans were pre-germinated in a petri dishes with water agar. The growth medium (vermiculite) was sterilized in the growth container to avoid contamination. Pre-germinated seeds were transplanted into sterile horticulture vermiculite in Leonard jars. To avoid post sterilization rhizobial contamination, aluminium foil was used to cap the open ends of the tubes. Growth medium (vermiculite) was wetted daily with nitrogen–free nutrient solution as described by Giller *et al*. (2009). Four days after transplanting, the root system of each seedling was inoculated with 1 ml of isolates (1 x 10^3) from freshly grown rhizobia cultures diluted with sterile plant nutrient solution. Isolates obtained from soybeans were used to inoculate climbing beans and isolates from climbing beans were used to inoculate soybeans. Sterilized uninoculated soybeans and climbing beans were used as controls. Plants were harvested 28 days after inoculation. Nodulation was observed and recorded.

3.8.1 Rooting medium

The rooting medium which was used in this research was sterilized vermiculite. This material was washed thoroughly for three days by changing the tap water three times a day and stirring frequently. The final rinse was with distilled water and the pH of the medium was adjusted to about 6.8. After attaining the correct pH, water was drained off and the vermiculite was then autoclaved. The sterile vermiculite was packed into the Leonard jars and covered with aluminium foil to reduce contamination.

3.8.2 Seed sterilization and pre-germination

Soybean and climbing bean seeds of good viability (more than 80 % germination), undamaged and uniform in colour and size were selected. The seeds were surface-sterilized by immersing them into a 3 % sodium hypochlorite solution for 10 minutes (3 % sodium hypochlorite solution was prepared by adding 10 parts of commercial bleach (5.25 % sodium hypochlorite) to 7.5 parts of water (Hirsch, 2005). 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 it for one hour. The seeds were transferred aseptically to 2 % water agar plates with a spoon shaped spatula. Twenty five seeds were placed in each plate. The plates with the seeds were incubated upside down at 28^{o} C to enable the shoot to grow away from the water agar. The incubation period was five days. Seedlings whose radicles attained a length of 1-2 cm (Plate 3.1 a and Plate 3.1 b) after the incubation period were considered ready for transfer onto Leonard jars.

Plate 3.1 a: Pre-germinated soybean seeds ready for transfer onto Leonard jars.

Plate 3.1 b: Pre-germinated climbing bean seeds ready for transfer onto Leonard jars.

3.8.3 Planting in Leonard jars

The aluminium foil used to cover the Leonard jars were removed gently and 5 ml of plant nutrient solution added before it was returned. A pair of flame sterilized forceps was used to prepare two holes on the aluminium foil on each Leonard jar. Seeds with a radicle length of 1-2 cm were picked up with a pair of sterile forceps and placed one per hole with the radicle facing downwards. The holes were deep enough to accommodate pre-germinated seeds 0.5 cm below the surface. Four days after transplanting, inoculation was carried out by pipetting 1 ml of 5 day old rhizobia broth cultures onto the radicle base of the seedlings. Each of the two bean types (soybean and climbing beans) was inoculated with all isolates isolated from the field experiments. The nitrogen controls were left uninoculated while the material control was treated with sterile YEMB. These uninoculated controls were used to check for cross contamination. Seedlings were then covered with vermiculite and the Leonard jars arranged on greenhouse benches. Rooting medium was wetted daily with nitrogen free nutrient solution as described by Beauredgard *et al*. (2003). The open ends of the Leonard jars were covered with aluminium foil after emergence of plants to reduce water loss and minimise microbial contamination from the atmosphere (Plate 3.2 a and Plate 3.2 b).

Plate 3.2 a: Soybean (Gazelle) onto a Leonard jar assembly.

Plate 3.2 b: Climbing bean (MAC64) onto a Leonard jar assembly.

3.8.4 Plant growth medium

Nitrogen free plant nutrient solution was prepared as described by Somasegaran and Hoben (2002). Five different stock solutions were prepared as follows:-

Stock 1 - $CaCl_2$. $2H_2O$, 294.1 g/l

Stock 2 - KH_2PO_4, 136.1 g/l

Stock 3 - $MgSO_4.7H_2O$, 123.3 g/l, K_2SO_4, 87.0 g/l, $MnSO_4$. H_2O, 0.3338 g/l

Stock 4 - H_3BO_3, 0.27 g/l, $ZnSO_4.7H_2O$, 0.288 g/l, $NaMoO_2.2H_2O$, 0.048 g/l, $CuSO_4.7H_2O$, 0.56 g/l

Stock 5- Fe Citrate, 5.4 g/l

For each litre of full- strength solution, 0.5 ml was added from each of the five stocks solutions. The pH of the solution was adjusted to 6.8 using NaOH (1.0 M) or HCl (1.0 M). All solutions were sterilized by autoclaving at 121 ^{o}C for 15 minutes. The purpose of using this medium was to ensure that the only nitrogen available for the soybean and climbing bean plants was from nitrogen fixation.

3.8.5 Harvesting of the plants

The plants were harvested forty five days after emergence. They were harvested by emptying the content of the Leonard jars. At harvest, the roots and adhering rooting media were cut at the level of the growth medium and put in a coarse sieve. The rooting medium was washed from the roots using a gentle flow of water. All the nodules were carefully detached from the roots. The number of plants that formed the nodules were recorded.

3.9 Field experiments

Field trials were carried out at Kaguru Farmers Training Centre field and Munithu Girls Secondary School farm, both in Meru County. The experiments were conducted during the short rainy season (October – January) of 2011. The parameters considered were dry weight of plant tissue (stover, nodules, roots, pods and 100 seeds), nodule number, seeds number and assessment of cross inoculation of rhizobia between soybeans and climbing beans isolates.

3.9.1 Experimental layout

The experimental design was a Randomised Complete Block Design (RCBD) with four replications. In each block treatments were as follows:-

1. Climbing beans un amended (control)
2. Climbing beans + Chicken manure
3. Climbing beans + Cow manure
4. Climbing beans + Nitrogen fertilizer (DAP)
5. Soybean un amended (control)
6. Soybean + Chicken manure
7. Soybean + Cow manure
8. Soybean + Nitrogen fertilizer (DAP)

A total of 8 different treatments were represented in 8 plots per block. Each plot occupied 3 m x 3 m area. A total of 32 plots constituted the whole experimental layout. The plant spacing was 75 cm between the rows and 25 cm within the rows (Garcia *et al*., 2004).

3.9.2 Field planting

Land preparation was carried out by hand. It was cleared, dug, harrowed, demarcated and marked into blocks and plots. The seeds were dry planted on October 18th 2011. In manure treated soils for both climbing beans and soybeans at Kaguru Farmers Training Centre and Munithu Girls Secondary School farm, 200 grams of well decomposed manure per planting hole was applied (http://www.kari.org). Manure was then thoroughly mixed with soil. Diamonium Phosphate fertilizer 20:20:0 at the rate of 5 grammes per planting hole (Hungria *et al.*, 2006) was used in the soils amended with inorganic fertilizer. To prevent scorching effect, fertilizer was mixed thoroughly with the soil. Two seeds of climbing beans and two seeds of soybeans were planted per hole (0.75 m inter-row and 0.25 m intra row (Bala *et al*., 2003). Climbing beans grow up to height of 4 metres and therefore they require support materials. Posts and wire were used for staking climbing beans in order to improve aeration and to reduce the incidence of pest attack. Two weeks after germination, the first weeding was carried out and the plants were thinned to one plant per hole in each of the plots. The second weeding was done four weeks after germination while third weeding was carried out seven weeks after germination.

3.9.3 Phenological observations

After planting, phenological changes in nodule formation, stover establishment, pod formation and full physiological maturity were observed. A phenological phase was considered to have occurred when 75 % of the crops had attained the characteristics typical of that phase. For example, pod formation occurred when 75 % of the plants had their pods fully filled. Physiological maturity occurred when 75 % of the plants had their pods filled with seeds and hardened (Musiyiwa *et al*., 2005).

3.9.4 Plant sampling and harvesting

Plants were sampled at 75 % podding and at full physiological maturity. Ten (10) plants were randomly sampled from each plot and various growth parameters determined. For first sampling, the plant was dug up carefully and repeatedly washed with clean water to remove any soil particles and any contaminants. Nodules were detached from the roots and counted to determine their numbers. Plant materials were then partitioned into nodules, root and stover. The samples were then air dried, before oven drying at 72^{o} C to a constant weight (Bala *et al.*, 2003). The dry weight of the various parts of the plant samples taken were determined using a Sartorius CPA 225D Semi-Microbalance (Sartorius AG). During the final harvest, dry weight of 100 seeds was assessed.

3.10 Data analysis

Analysis of variance (ANOVA) was carried out using Genstat statistical programme version 6.1, to test significance between the treatments. Means were separated using Least Significant Difference (L.S.D) test at 5% level.

CHAPTER FOUR

RESULTS

4.1 Laboratory and greenhouse experiments

The experiments involved characterization of isolates, authentication and cross inoculation.

4.1.1 Characterization of isolates

After streaking on various differential media, colonies appeared on the media within 3-4 days and attained full size in 5-6 days. All the isolates were found to be Gram negative rods. On Congo red medium, all colonies were milky to translucent. On Bromothymol blue medium, growth of some colonies was accompanied by colour change of the medium from deep green to blue. On peptone agar medium, there was no observable growth of the bacteria. The slow growing and alkaline producing rhizobia were one third (25 %) while fast growing and acid producing *Rhizobium* species constituted 75 % (Figure 4.1 a and 4.1 b).

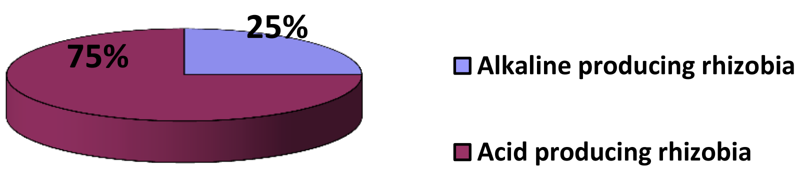

Figure 4.1 a: Acid and alkaline producing rhizobia on bromothymol blue medium

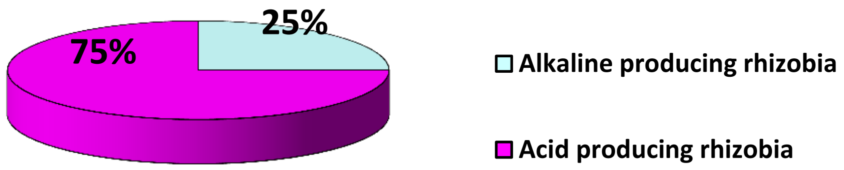

Figure 4.1 b: Acid and alkaline producing rhizobia on litmus milk medium

4.1.2 Cross inoculation and nodulation assessment

Soybean isolates nodulated effectively with isolates from climbing beans in the greenhouse. Soybean isolates had a higher nodulation ability on climbing beans. Climbing bean isolate had nodulation on soybeans (Figure 4.2).

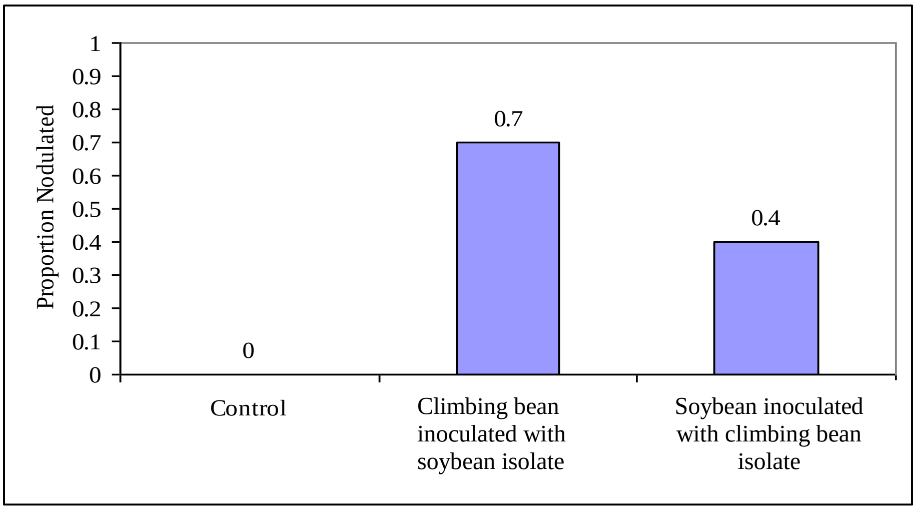

Figure 4.2: Cross inoculation between soybean and climbing bean rhizobia isolates.

Climbing beans and soybeans grown in the greenhouse nodulated with their respective rhizobia isolates obtained from the field effectively (Plate 4.1 a and 4.1 b). Soybeans nodulated with isolates obtained from climbing beans in the field (Plate 4.1

c). Similarly, climbing beans grown in the greenhouse nodulated with isolate obtained from soybeans in the fields (Plate 4.1 d).

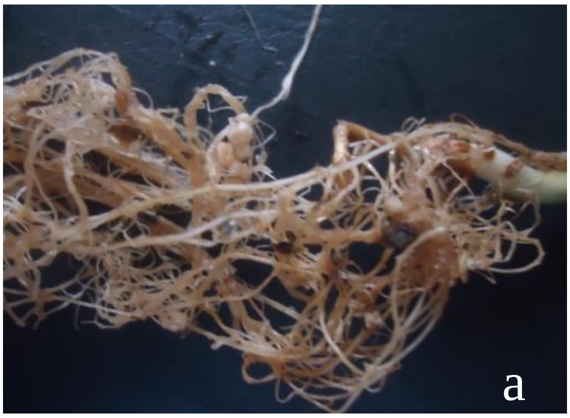

Plate 4.1 a: Climbing bean inoculated with climbing bean isolates.

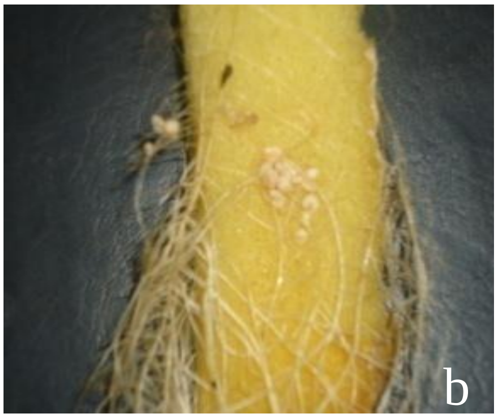

Plate 4.1 b: Soybean inoculated with soybean isolates.

Plate 4.1 c: Soybean inoculated with climbing bean isolates.

Plate 4.1 d: Climbing bean inoculated with soybean isolates.

4.2 Field experiments

4.2.1 Nodulation

Climbing bean (MAC 64) in Munithu Girls Secondary School farm had higher number of nodules (37 nodules) compared to that in Kaguru Farmers Training Centre farm. Similarly, soybeans in Munithu Girls Secondary School farm had higher number of nodules than in Kaguru Farmers Training Centre farm. Nodulation in soybean beans occurred mainly on the lateral and finer roots and very few were located on the tap root in both soybeans and climbing beans (Plate 4.2 a and 4.2 b). Most of the nodules were pink in colour inside while others were white in colour. The nodules which were pink in colour in both climbing and soybeans were large in size. Gazelle soybeans at both sites formed fewer number of nodules as compared to climbing beans at both sites.

At Munithu Girls Secondary School farm the climbing beans planted in soils amended with chicken manure formed the highest number nodules (58 nodules). This was followed by climbing beans planted in soils amended with cow manure (55 nodules). However, climbing beans planted in soils amended with nitrogen fertilizer did not form any nodules. The least number of nodules (20 nodules) were formed in climbing beans planted in control soils. Results indicated that amendment had a significant effect on the number of nodules formed by climbing beans planted in soils amended with chicken and cow manure at Munithu Girls Secondary School farm.

At Kaguru Famers Training Centre the climbing beans planted in soils amended with chicken manure formed the highest number nodules (55 nodules). This was followed by climbing beans planted in soils amended with cow manure (48 nodules). However, climbing beans planted in soils amended with nitrogen fertilizer did not form any nodules. The least number of nodules (18 nodules) were formed in climbing beans planted in control soils. Results show that there was no statistically significant difference in the number of nodules in soil amended with chicken manure and cow manure at Kaguru Famers Training Centre Farm (Table 4.1 a).

Table 4.1 a: Number of nodules per plant formed by climbing beans in different soil amendments at Kaguru Farmers Training Centre farm and Munithu Girls Secondary School farm

	Mean number of nodules	
Treatment	Munithu farm	Kaguru farm
Chicken manure	58.25±7.30a	55.00± 6.20b
Cow manure	55.50±6.08b	48.75±4.92b
Fertilizer	0d	0d
Control	20.00±4.69c	18.25± 3.74c

Mean values within a column followed by the same letter are not significantly different by L.S.D at 5 % level

The number of nodules formed by soybeans planted in soils amended with chicken manure formed higher number of nodules (13 nodules) compared to all the other treatments at Munithu Girls Secondary School Farm. Soybeans under control treatment formed the least number of nodules (2 nodules). Soybeans planted in soils amended with nitrogen fertilizer did not form any nodules. The number of nodules formed by soybeans planted in both soils amended with chicken manure and cow manures were not statistically significantly different (Table 4.1 b).

At Kaguru Farmers Training Centre the soybeans planted in soils amended with chicken manure formed the highest number nodules (12 nodules). This was followed by soybeans planted in soils amended with cow manure (9 nodules). However, soybeans planted in soils amended with nitrogen fertilizer did not form any nodules. The least number of nodules were formed in climbing beans planted in control soils (2 nodules). Results shows that there was no statistically significant difference in number of nodules formed by soybeans planted in soils treated with chicken manure and cow manure at Kaguru Farmers Training Centre farm (Table 4.1 b).

Table 4.1 b: Number of nodules per plant formed by Soybeans in different soil amendments at Kaguru Farmers Training Centre farm and Munithu Girls Secondary School farm

	Mean number of nodules	
Treatment	Munithu farm	Kaguru farm
Chicken manure	13.75±0.85a	12.50±0.65b
Cow manure	12.25±1.31a	9.75±0.85b
Fertilizer	0d	0d
Control	2.55±0.25c	3.05±0.50c

Mean values within a column followed by the same letter are not significantly different by L.S.D at 5 % level

Nodule

Plate 4.2 a: Climbing bean nodules on the roots

Nodule

Plate 4.2 b: Soybean nodules on the roots

4.2.2 Root dry weight

Climbing beans planted in soils amended with chicken manure had the highest mean root dry weight (5.75 g) followed by those planted in soils amended with cow manure (4.96 g) at Munithu Girls Secondary School farm. Root dry weight of climbing beans amended with chicken manure, cow manure and fertilizer at Munithu Girls Secondary School farm were not significantly different with (Table 4.2 a). At Kaguru Farmers Training Centre farm climbing beans planted in soils amended with chicken manure had the highest mean dry root dry weight (4.96 g). Climbing beans planted in soils amended with cow manure had the second highest mean root dry weight (3.87 g) followed by those planted in soils amended with fertilizer (3.61 g) at Kaguru Farmers Training Centre. Root mean dry weight of climbing beans grown in soils amended with chicken manure, cow manure and fertilizer at Kaguru Farmers Training Centre farm were not significantly different (Table 4.2 a).

Table 4.2 a: Root dry weight (g) of climbing beans per plant on different soil amendments at Kaguru Farmers Training Centre farm and Munithu Girls Secondary School farm

	Mean root dry weight (g)	
Treatment	Munithu farm	Kaguru farm
Chicken manure	5.75±0.11c	4.96±0 .31c
Cow manure	4.85±0.08c	3.87±0.06c
Fertilizer	4.23±0.22c	3.61±.34c
Control	3.61±0.35c	1.47±0.18d

Mean values within a column followed by the same alphabet are not significantly different by L.S.D at 5 % level

Soybeans planted in soils amended with chicken manure and cow manure had the highest mean root dry weight (2.66 g) followed by those planted in soils amended with fertilizer (2.41 g) at Munithu Girls Secondary School farm. Soybeans planted in soils amended with cow manure recorded second lowest mean root dry weight (2.36 g) while those planted in control soils recorded the lowest mean root dry weight (1.65 g) at Munithu Girls Secondary School Farm. Amendment had no effect on mean root dry weight of soybeans at Munithu Girls Secondary School farm (Table 4.2 b).

At Kaguru Farmers Training Centre, soybeans planted in soils amended with fertilizer had the highest mean root dry weight (2.02 g) followed by those planted in soils amended with chicken manure (1.82 g) at Munithu Girls Secondary School farm. Soybeans planted in soils amended with cow manure recorded second lowest mean root dry weight (1.69 g) while those planted in control soils recorded the lowest mean root dry weight (1.47 g) at Kaguru Farmers Training Centre. Root dry weight of climbing beans grown in the soil amended with chicken manure, cow manure and fertilizer at Kaguru Farmers Training Centre farm were not significantly different (Table 4.2 b).

Table 4.2 b: Root dry weight (g) of soybeans per plant on different soil amendments at Kaguru Farmers Training Centre and Munithu Girls Secondary School farms

	Mean root dry weight (g)	
Treatment	Munithu farm	Kaguru farm
Chicken manure	2.66±0.11c	1.82±0.10c
Cow manure	2.36±0.06c	1.69±0.14c
Fertilizer	2.41±0.20c	2.02±0.21c
Control	1.65±0.21c	1.47±0.18c

Mean values within a column followed by the same letter are not significantly different by L.S.D at 5 % level

4.2.3 Stover dry weight

At Munithu Girls Secondary School farm, amendment with chicken manure resulted in highest mean stover dry weight (202.80 g) of climbing beans followed by amendment with cow manure (142.15 g). Amendment with nitrogen fertilizer resulted in the second lowest mean stover dry weight (116.85 g) of climbing beans while control had the lowest mean stover dry weight (77.95 g). At Munithu Girls Secondary School farm, stover dry weight of climbing beans was statistically significant for soils amended with chicken manure, cow manure and fertilizer (Table 4.3 a).

At Kaguru Farmers Training Centre, amendment with chicken manure resulted in the highest mean stover dry weight (188.66 g) of climbing beans followed by amendment with cow manure (138.17 g). Amendment with nitrogen fertilizer resulted in the second lowest stover dry weight (113.95 g) of climbing beans while control the control plants had the lowest mean stover dry weight (42.50 g). At Kaguru Farmers Training Centre farm, mean stover dry weight of climbing beans was statistically significant for soils amended with chicken manure, cow manure and fertilizer (Table 4.3 a).

Table 4.3 a: Stover dry weight (g) of climbing beans per plant in different soil amendments at Kaguru Farmers Training Centre and Munithu Girls Secondary School farms

	Mean stover dry weight (g)	
Treatment	Munithu farm	Kaguru farm
Chicken manure	202.80±9.08a	188.66± 11.70a
Cow manure	142.15±20.77b	138.17±21.26b
Fertilizer	116.85±33.48c	113.95±33.4c
Control	77.95±8.33d	42.50±8.38d

Mean values within a column followed by the same letter are not significantly different by L.S.D at 5 % level

At Munithu Girls Secondary School farm, amendment with chicken manure resulted in highest mean stover dry weight (86.08 g) of soybeans followed by amendment with cow manure (63.91 g). Amendment with nitrogen fertilizer resulted in the second lowest mean stover dry weight (53.91 g) of soybeans while the control had the lowest mean stover dry weight (22.57 g). At Munithu Girls Secondary School farm, stover dry weight of soybeans was statistically insignificant for soils amended with cow manure and nitrogen fertilizer (Table 4.3 b). However, stover dry weight of soybeans was statistically significant for soils amended with chicken manure.

At Kaguru Farmers Training Centre, amendment with chicken manure resulted in the highest mean stover dry weight (75.02 g) of soybeans followed by that amended with cow manure (53.20 g). Amendment of soil with fertilizer resulted in second lowest mean stover dry weight (49.71 g) of soybeans while the control had the lowest mean stover dry weight (18.54 g) of soybeans. At Kaguru Farmers Training Centre farm, mean stover dry weight of soil beans was statistically significant between the soils amended with chicken manure, cow manure and nitrogen fertilizer (Table 4.3 b).

Table 4.3 b: Stover dry weight (g) of soybeans per plant in different soil amendments at Kaguru Farmers Training Centre and Munithu Girls Secondary School farms

	Mean stover dry weight (g)	
Treatment	Munithu farm	Kaguru farm
Chicken manure	86.08±9.08a	75.02± 11.70a
Cow manure	63.91±20.77b	53.20±21.26b
Fertilizer	53.91±12.12b	49.71±13.42c
Control	22.57±8.33c	18.54±8.38d

Mean values within a column followed by the same letter are not significantly different by L.S.D at 5 % level

4.2.4 Pod number

Soils amended with Chicken manure, cow manure and nitrogen fertilizer had an effect on number of pods in both climbing beans and soybeans (Plate 4.2 c and 4.2 d). At Munithu Girls Secondary School farm the highest mean number of pods on climbing beans was formed from those beans planted in soils amended with chicken manure (104 pods) followed by those from soils amended with fertilizer (57 pods) and cow manure (51 pods) respectively. The least mean number of pods was recorded in the control treatment (36 pods). The differences in the number of pods in climbing beans planted in soils amended with chicken manure and cow manure were statistically significant.

At Kaguru Farmers Training Centre the highest mean number of pods on the climbing beans was formed from those beans planted in soils treated with chicken manure (100 pods) followed by those from soils amended with fertilizer (54 pods) and cow manure (47 pods) respectively. The least number of pods was recorded in the control treatment (32 pods). The differences in the number of pods on climbing beans planted in soils amended with cow manure and fertilizer were statistically insignificant (Table 4.5 a).

Table 4.4 a: Number of pods per plant formed by climbing beans on different soil amendments at Kaguru Farmers Training Centre and Munithu Girls Secondary School farms

	Mean pod number	
Treatment	Munithu farm	Kaguru farm
Chicken manure	104.50±22.77c	100.75± 21.74c
Cow manure	51.00±6.64b	47.75±7.06b
Fertilizer	57.00±10.55b	54.50±11.23b
Control	36.50±4.87a	32.50±4.50a

Mean values within a column followed by the same letter are not significantly different by L.S.D at 5 % level

The number of pods of soybeans planted in soils amended with chicken manure at Munithu Girls Secondary School farm was the highest (165 pods). This was followed by soybeans planted in the soils amended with cow manure (127 pods) and fertilizer (63 pods) respectively. The least number of pods was recorded in soybeans planted in control soil (45 pods). The differences in the number of pods planted in soils amended with chicken manure, cow manure, and fertilizer were statistically significant.

The number of pods of soybeans planted in soils amended with chicken manure at Kaguru Farmers Training Centre was the highest (157 pods). This was followed by soybeans planted in soils amended with cow manure (87 pods) and nitrogen fertilizer (84 pods) respectively. The least number of pods were recorded in soybeans planted in control soil (42 pods). The differences in the number of pods planted in soils amended with chicken manure and cow manure were statistically significant (Table 4.4 b).

Table 4.4 b: Number of pods per plant formed by soybeans on different soil amendments at Kaguru Farmers Training Centre farm and Munithu Girls Secondary school farms

	Mean pod number	
Treatment	Munithu farm	Kaguru farm
Chicken manure	165.25±33.29a	157.00± 33.34a
Cow manure	127.25±23.57b	87.75±8.22b
Fertilizer	63.75±10.47c	84.25±18.7b
Control	45.75±8.22d	42.50±8.38c

Mean values within a column followed by the same letter are not significantly different by L.S.D at 5 % level

Plate 4.2 c: Climbing beans in the field.

Plate 4.2 d: Soybean in the field.

4.2.5 100 seeds dry weight

Soil amendment with chicken manure, cow manure and nitrogen fertilizer had an effect on climbing bean seed dry weight at Munithu Girls Secondary School farm and Kaguru Farmers Training Centres farm. At Munithu Girls Secondary School farm, mean dry weight of 100 seeds of climbing beans was highest for the beans planted in soils amended with chicken manure (126.20 g) this was followed by cow manure (97 g) and fertilizer (87 g) respectively. There was a statistically significant difference in the dry weight of 100 seeds of climbing beans under different soil amendments at Munithu Girls Secondary School farm ($P < 0.05$).

At Kaguru Farmers Training Centre farm, mean dry weight of 100 seeds of climbing beans was highest for those beans planted in soils amended with chicken manure (106.15 g). This was followed by cow manure (93.76 g) and nitrogen fertilizer (89.44 g) respectively. There was no statistically significant difference in mean dry weight of 100 seeds of climbing beans grown in soils amended with chicken manure and cow manure at Kaguru Farmers Training Centre farm (Table 4.5 a).

Table 4.5 a: Dry weight of 100 seeds (g) of climbing beans on different soil amendments at Kaguru Farmers Training Centre and Munithu Girls Secondary School farms

	Mean dry weight of 100 seeds (g)	
Treatment	Munithu farm	Kaguru farm
Chicken manure	126.20±2.20a	106.15± 3.34a
Cow manure	97.03±1.75b	93.76±2.30b
Fertilizer	87.15±2.67c	89.44±.83b
Control	74.21±1.69d	69.15±1.20c

Mean values within a column followed by the same letter are not significantly different by L.S.D at 5 % level

Soil amendment with chicken manure, cow manure and nitrogen fertilizer had an effect on soybean 100 seeds dry weight at Munithu Girls Secondary School and Kaguru Farmers Training Centres farms. At Munithu Girls Secondary School farm, mean of 100 seeds dry weight of soybean was highest for the beans planted in soils amended with chicken manure (77.24 g). This was followed by cow manure (75.84 g) and nitrogen fertilizer (67.44 g) respectively. There was no statistical significant difference in 100 seeds dry weight of soybean grown in soils amended with chicken manure, cow manure and fertilizer (Table 4.5 a). However, seed dry weight planted in control soils was not statistically significant ($p > 0.05$).

At Kaguru Farmers Training Centre farm, mean of 100 seed dry weight of soybeans was highest for the beans planted in soils amended with chicken manure (72.58 g). This was followed by cow manure (73.14 g) and nitrogen fertilizer (61.89 g) respectively. There was no statistically significant difference in the mean dry weight of 100 seeds of soybeans planted in soil amended with chicken manure, cow manure and nitrogen fertilizer ($p > 0.05$). However, there was statistical significant difference in 100 seed dry weight of soybeans planted in control soils (Table 4.5 b).

Table 4.5 b: Weight of 100 seeds (g) of soybeans in different soil amendments at Kaguru Farmers Training Centre and Munithu Girls Secondary School farms

	Mean dry weight of 100 seeds (g)	
Treatment	Munithu farm	Kaguru farm
Chicken manure	77.24±2.82b	72.58±1.18b
Cow manure	75.84±1.99b	73.14±0.45b
Fertilizer	67.44±1.38b	61.89±0.87b
Control	44.69±2.27a	40.98±1.65a

Mean values within a column followed by the same letter are not significantly different by L.S.D at 5 % level

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

Plant nodulation test is the only confirmatory test for rhizobia (Somasegaran *et al.,* 2009). Leonard jars were used for plant nodulation tests. This method has become a standard method for testing nodulation and nitrogen fixation (Rondon *et al.,* 2007). This method is economical in water use and reduction of bacterial contamination (Benehizia *et al.,* 2004). The Leonard jars occupies very little space in the greenhouse and provides good depth for growth and development of roots. Nitrogen production due to algal growth on media and in water has been shown to be minimal (McNeill and Parterson, 2007).

On Congo red, colonies were milky to translucent which meant that they did not adsorb the dye. This further suggested that the bacteria could have been rhizobia as they are very poor absorbers of the dye. Non rhizobial adsorb Congo red and appear as red colonies (Beck, 2005). When the isolates were grown on Bromothymol blue medium, 25 % of the colonies were accompanied by colour change of BTB from deep green to blue indicating production of alkaline substances. This characteristic is typical of the slow growing *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* (Maingi *et al.,* 2006).

All the other isolates representing 75 % were accompanied by colour change of BTB from deep green to yellow indicating production of acidic substances (Figure 4.1 a). These are characteristics of the fast growing rhizobia (Somasegaran and Hoben 2012, Beck, 2005). Production of acid is a characteristic of fast growing rhizobia (Feng, *et al.,* 2002). These were placed in one species of the genus *Rhizobium* by Scholla and Elkan (1984) and in 1988 by Chen *et al.* (cited in Hornetz *et al.,* 2006). On litmus milk, 25 % of colony growth was accompanied by colour change of medium to blue and 75 % of colony turned it pink (Figure 4.1 b).

The 25 % against 75 % of slow growing too fast growing respectively is comparable to the figures obtained by Maingi *et al.* (2006) in experiments carried out in Kaguru and Kiboko in which 22 % to 78 % for slow to fast growing rhizobia respectively was reported. This is an indication that only a small proportion of the indigenous rhizobia in the study area belong to either *B. japonicum* or *B. elkanii*. Peptone agar medium was used to determine whether the bacteria are rhizobia or not. Unlike other bacteria, rhizobia grow very poorly in this medium. The bacteria had no observable growth in the medium which further confirmed that the isolated bacteria were rhizobia. These tests helped in the screening of isolates for contamination (Galloway *et al.,* 2004) and enabled rejection of impure cultures.

Soybeans and climbing beans nodulated with isolates obtained from the field. Climbing beans nodulated with isolates obtained from soybeans and soybeans nodulated with isolates obtained from climbing beans. This showed that cross inoculation exists between rhizobia nodulating with soybeans and rhizobia nodulating with climbing beans. This differs with the results reported by Theuri *et al*. (2010) on the abundance of indigenous rhizobia nodulating cowpea and common bean in Central Kenyan soils carried out at Kabete, Machakos, Nyeri and Kajiado sites in Kenya in which the rhizobia were specific to particular legumes. All the isolates nodulated with the test plant and confirmed that they were rhizobia. The results obtained from Gram staining and growth of the isolates in YEMA conformed with the standard cultural and morphological characteristics of rhizobia as described by Cappucino (2001).

Effect of soil amendment was significant for both climbing beans and soybeans at Munithu Girls Secondary School farm and Kaguru Farmers Training Centre farm in nodulation, stover, root, pod and seed weight. The soil amendment had higher responses at Munithu Girls Secondary School farm compared to Kaguru Farmers Training Centre farm with chicken manure being superior. Cow manure came in second as the most significant amendment followed by DAP. These results concurs with those of Javaid (2008) who reported better growth and yield of soybeans in chicken manure and in cow manure amended soils. This may be attributed to the fact that chicken manure and cow manure have different mineralization rates and nutrient availability (Kautz *et al*., 2006).

In terms of manure application, this study concludes that poultry manure is more effective and appropriate in soybeans and climbing beans production at any agro ecological zone. It is not only cheap in acquisition but also friendly to environment as it does not leave a lot of residual minerals that are detrimental to ground water quality. It also provides highly desirable mineralization of critical nutrient relevant to plant growth. Okereke *et al.* (2000) reported that yields increase in soybeans with different amendments of manure and stated that the increased yields coincided with sites having low available soil nitrogen during mid–June. Gage (2004) reported that sites amended with manure had slightly higher and early plant growth compared to sites only amended with nitrogen fertilizer. These findings conformed to the results obtained from Kaguru Farmers Training Centre and Munithu Girls Secondary School farm.

Osborne and Reidel (2001) compared soil nitrate levels and soybean yields from various manure application and control. This study showed statistically significant yield increase at three of the five manured sites compared to control. These findings agrees with the results obtained from Kaguru Farmers Training Centre farm and Munithu Girls Secondary School farm where climbing beans and soybeans planted in the soils amended with manure yielded more compared to those planted in control soils. Nitrogen fertilizer amended soils at Munithu Girls Secondary School farm and Kaguru Farmers Training Centre farm for both climbing beans and soybeans yielded less than in the soils amended with manure at both sites. This result agrees with studies carried by Baron (2012) who compared manure application and inorganic fertilizer on soybeans. The study noted that yield increase with increasing manure nitrogen application while application of increasing nitrogen fertilizer did not result in yields increase. The results of the study showing yields responses imply that manure application to soybeans and climbing beans is most appropriate for high yields. Broos *et al.* (2005) in the study of inorganic fertilizer soil amendment in soybean production found out that application of phosphorous fertilizers did not significantly influence percentage crop emergence ($p \geq 0.05$) This was attributed to moisture stress caused by fertilizers immediately after planting which may have had a scorching effect on germinating seeds.

Fertilizer application did not significantly influence plant weight, DAP fertilizer had the least effect in climbing beans and soybeans at Kaguru Farmers Training Centre farm and Munithu Girls Secondary School farm in terms of nodulation. In this study it was noted that in nitrogen amended soils there was low root and stover dry weight that contributed to low yields per plot in both sites. This is thought to be attributed to a limited application of DAP, a commercial fertilizer which required substantial increase in application to improve productivity. This effect is counteractive to the overall objective of the study as it only seeks to add to financial expenses in acquiring these commercial nitrogenous fertilizers and secondly which is very critical in large amount as residual nutrient left to the soil which has a negative effect to the environment, especially to the ground water quality.

Mpepereki *et al.* (2000) reported that the application of poultry manure increased soybean seed yield by 62 % over the control. Hungaria (2006) found that soybean seed increased linearly with increasing swine manure rate. The higher the seed yields of both grain legumes in response to the application of carbonized organic materials especially chicken manure suggest that both N and P are important nutrient that influence the growth and yields of soybean and climbing bean as evidenced by the results obtained in the study. Poor nodulation in the field could be attributed to several factors such as inadequate soil moisture, lack of appropriate rhizobia in soil, deficiency or toxicity of a particular nutrient, unfavourable conditions like water logging, unfavourable pH, pests and disease attack among others. Some of these can be solved by soil amendment with organic manure.

In addition to nodulation and BNF, legumes require high amounts of phosphorous for their optimal growth (LaRue *et al.,* 2007). Biological nitrogen fixation itself is a high energy requiring process that utilizes a lot of ATP, thus more phosphorous is required by the process (Hoefsloot *et al.*, 2007). It is against this background that creating a conducive environment for growth and multiplication of rhizobia necessary for nodulation and nitrogen fixation cannot be ignored. Nodulation at flowering in both soybeans and climbing beans at Munithu Girls Secondary School farm and Kaguru Farmers Training Centre farm showed significant difference among soil amendments in the field experiment. Majority of the nodules were pink in colour. This showed that they had leg haemoglobin and were therefore effective in nitrogen fixation. A few nodules were white inside, an indication that they were ineffective or they had not developed enough to a stage where they could fix nitrogen. These results concurred with those obtained by Boyer (2004) that evidenced pink colouration in the nodules of legumes.

Soil amendment had effect on soybeans and climbing beans nodulation at podding stage since there was significant difference between amended soils and unamended soils at both Munithu Girls Secondary School farm and Kaguru Farmers Training Centre farm. At podding all nitrogen amended soils, soybeans and climbing beans in both sites did not produce nodules. Lack of nodules in nitrogen amended soils was expected since high nitrogen content in the soil inhibits nodulation and nitrogen fixation. This influences the amount of nitrogen fixed from the atmosphere negatively. Majority of the nodules were on the lateral and finer roots and were pink in colour. These results agreed with those of Wolyn *et al.* (2004) who established that a large portion of the fixed nitrogen result from fixation by nodules on the lateral roots. The results also agrees with those of Madsen and Alexander (2008) who found out that application of organic manure into the area around the lateral roots may have a significant positive effect on total nitrogen fixation. This is attributed to the lateral movement of rhizobia in the soil which was further supported by Wolyn *et al.* (2004) in which nodulation of the primary root, especially in the crown area was thought to be important, a large portion of the fixed nitrogen in high nitrogen fixing lines of beans result from fixation by nodules on the lateral roots.

There was significant difference in nodule number among the treatments. Soybeans and climbing beans planted in soils amended with chicken manure and cow manure nodulated more than those planted in unamended soils in both sites. Most nodules in soybeans and climbing beans were large in size and had pink colour indicating that they were active in nitrogen fixation (Boyer *et al.,* 2005). Nodulation in climbing beans and soybeans (gazelle plants) was different. This indicates that with improved soil conditions high production in both legumes can be maximized and no ecological zone can stand an advantage over the other in terms of soybean or climbing production (Horntez *et al.*, 2006*).* Nodulation is easily improved by soil amendments with organic manure. In this research gazelle plant growth cycle was short which was 50 days to 75 % flowering as compared to climbing beans which took 70-75 days to 75 % flowering. Studies have shown that gazelle plants' short growth cycle attribute to poor nodulation. This observation is in line with that of Russel and Jones 2011 who noted that early to mature legumes, tend to be weak in nitrogen fixation. These results are also in agreement with that of other researchers who found, that early maturing legumes have lower nodulation compared to the late maturing varieties (Hardarson and Atkins, 2003, Hornetz *et al;* 2006). Current research shows that this can be reversed by good soil management.

Plant dry weight was used to estimate nitrogen fixation. This method is the best for screening large number of plants for nitrogen fixation (Hardason and Atkins, 2003). The method is inexpensive and easy to use. However, it is not sensitive enough to be used in soils with high nitrogen content. This is because other factors besides nitrogen do not permit the nitrogen fixed to be translated into increased dry matter yield (Ferguson and Mathesius, 2003).

Soil amendment with chicken manure, cow manure and nitrogen fertilizer had an effect on root dry weight as evidenced at podding in soybeans and climbing beans. Climbing beans had higher root dry weight at both Munithu Girls Secondary School farm and Kaguru Farmers Training Centre farm compared to soybeans at both sites at podding an indication of high nitrogen fixation (Table 4.2 a and 4.2 b). This results concur with those of Abaidoo (2001) who estimated that nitrogen content in roots to be greater than 10 % of the total above ground nitrogen content.

Climbing beans planted in the soils amended with chicken manure at Munithu Girls Secondary School farm and Kaguru Farmers Training Centre had higher stover dry weight than those planted in soils amended with cow manure and nitrogen fertilizer. This showed that chicken manure had greater influence on stover dry weight. Statistically, there was a significant difference between the treatments in terms of stover dry weight in both climbing beans and soybeans at Kaguru Farmers Training Centre farm and Munithu Girls Secondary School farm (Table 4.3 a and 4.3 b).

The results indicated that climbing beans produce large amounts of biomass compared to soybeans that contribute to soil organic matter. Late maturing legumes like climbing beans build more biomass thus the possibility of a positive nitrogen balance in the soil unlike the early maturing gazelle plant which have high grain yield as compared to its biomass. High biomass was produced by climbing beans as they were highly branched compared to low biomass producing gazelle plants which were less branched. Soil amendment had effect on pod number in both soybeans and climbing beans at both sites. Soybean grown at Kaguru Farmers Training Centre farm had significantly higher pod number (Table 4.4 b). This could be due to few foliage leaves produced by the soybeans.

In terms of 100 seeds dry weight, there was a statistical significant difference among soil amendments at Munithu Girls Secondary School farm and Kaguru Farmers Training Centre farm. This may have been as a result of the growth cycles favouring the growth of legumes that are early maturing and not allowing the late maturing climbing beans to grow to their full potential by not properly filling their pods. Climbing beans which takes 105 to 120 days to mature would be better for long rains or situations where water may be supplied during seeding (pod filling) stage of development. However, climbing beans had higher 100 seeds dry weight at both sites. Significant differences in seed dry weight per plant in response to different soil amendment in both climbing beans and soybeans were noted at both Kaguru Farmers Training Centre farm and Munithu Girls Secondary School farm. This indicated that soil amendment improved seed yields. Different soil amendments had effect on dry weight of 100 seeds. Although there was no significant difference in dry weight of 100 seeds, climbing beans had higher dry weight of 100 seeds compared to 100 seeds dry weight of soybeans. This is an indication that climbing bean seeds were bigger and heavier compared to those of soybeans. Generally beans (climbing beans and soybeans) planted at Munithu Girls Secondary School farm showed better performance in terms of number of nodules, root dry weight, stover dry weight, pod number and dry weight of 100 seeds as compared to those grown at Kaguru Farmers Training Centre farm. This could have been attributed to warm climatic conditions at Munithu Girls area and the black cotton soil. Legumes tend to do well in soils rich in phosphorous (Sawada *et al*., 2003).

5.2 Conclusions

Soil amendment with chicken manure and cow manure increased nodulation in soybeans and climbing beans. Nitrogen fertilizer affected nodulation negatively since all the plants in nitrogen fertilizer amended soil did not nodulate. The indigenous soil bradyrhizobia were not very effective in nodulation and nitrogen fixation in soybeans and climbing beans on unamended soil. The study indicated that cross inoculation of rhizobia exists between soybeans and climbing beans. Isolates from climbing beans nodulated with soybeans and those from soybeans nodulated with climbing beans. Soil amendment with organic manure affected nodulation and nitrogen fixation in soybeans and climbing beans as it led to increased plant biomass and increased seed yields.

5.3. Recommendations

Adoption of soil amendment when planting soybeans and climbing beans with organic manure would greatly improve their productivity. In this research, differences were noted between soybeans and climbing beans in nodulation and dry matter weight with different soil amendments. In this respect

i. There is need for further research in the evaluation of soybeans and climbing beans germplasm for traits affecting nitrogen fixation.
ii. There is need to establish specific nutrient requirement present in organic manure that is responsible for increased biomass on yields in soybeans and climbing beans.
iii. There is need to apply new tools of systematic bacteriology especially molecular sequencing technology to identify native soil inhabiting bradyrhizobia with the aim of assessing their efficiency in nitrogen fixation in soybeans and climbing beans.
iv. There is need for continuous screening of large number of native bradyrhizobia to understand their specificity, competitiveness, persistence and ability in nodulation and nitrogen fixation.
v. There is need to establish the nitrogen percentage level that is required in the organic manure that increase plant biomass and seed yields.
vi. There is a need to establish the extent of decomposition of the organic manure that would increase legume productivity.
vii. There is need to amend the soil with organic manure to enhance microbial activity.
viii. Farmers should use cow manure and chicken manure in improving soil fertility so as to increase productivity of soybeans and climbing beans in terms of yields and plant biomass.

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APPENDICES

APPENDIX 1: DATA MUNITHU GIRLS SECONDARY SCHOOL FARM

Plot No	Crop type	Amendment	Seeds weight 100 seeds (g)	Number of seeds in 10 pods
1	Soybean	-	50	50
2	Climbing bean	Chicken manure	125	30
3	Climbing bean	Fertilizer	115	20
4	Climbing bean	Cow manure	120	24
5	Soybean	Cow manure	70	60
6	Soybean	Chicken manure	85	65
7	Climbing bean	-	80	25
8	Soybean	Fertilizer	60	48
9	Climbing bean	-	81	22
10	Soybean	Chicken	76	58
11	Soybean	Cow manure	70	52
12	Soybean	Fertilizer	68	55
13	Climbing bean	Cow manure	88	50
14	Climbing bean	Chicken manure	97	32
15	Soybean	-	45	48
16	Climbing bean	Fertilizer	80	26
17	Climbing bean	Chicken manure	132	28
18	Soybean	Fertilizer	69	48
19	Soybean	Cow manure	72	50

20	Climbing bean	Cow manure	100	35
21	Climbing bean	Fertilizer	99	32
22	Soybean	Chicken manure	79	60
23	Soybean	-	60	47
24	Climbing bean	-	77	20
25	Climbing bean	-	80	21
26	Climbing bean	Fertilizer	85	22
27	Climbing bean	Cow manure	92	24
28	Climbing bean	Chicken manure	112	26
29	Soybean	Fertilizer	62	52
30	Soybean	-	42	45
31	Soybean	Cow manure	60	50
32	Soybean	Chicken manure	71	61

Plot No	Crop type	Amendment	Root dry weight (g)	No. of nodule	No. of pods	Stover dry Weight (g)
1	Soybean	-	1.66	0	43	215.3
2	Climbing bean	Chicken manure	5.622	76	78	194.6
3	Climbing bean	Fertilizer	4.529	0	80	199.6
4	Climbing bean	Cow manure	4.892	68	70	174.5
5	Soybean	Cow manure	2.275	16	88	440.6
6	Soybean	Chicken manure	2.531	16	11.1	539.6
7	Climbing bean	-	3.621	32	28	69.8
8	Soybean	Fertilizer	2.185	0	89	440.7
9	Climbing bean	-	4.591	28	150	58.4
10	Soybean	Chicken manure	2.601	13	120	751.1
11	Soybean	Cow manure	2.320	10	97	485.6
12	Soybean	Fertilizer	2.285	0	52	260.3
13	Climbing bean	Cow manure	4.901	58	47	179.5
14	Climbing bean	Chicken manure	5.521	63	76	189.5
15	Soybean	-	3.045	0	54	270.3
16	Climbing bean	Fertilizer	3.723	0	62	54.6
17	Climbing bean	Chicken manure	6.012	42	92	229.6
18	Soybean	Fertilizer	2.173	0	142	710.9
19	Soybean	Cow manure	2.301	0	192	961.2

20	Climbing bean	Cow manure	5.021	28	48	119.7
21	Climbing bean	Fertilizer	4.012	0	57	142.1
22	Soybean	Chicken manure	2.501	2	256	128.1
23	Soybean	-	1.850	1	62	310.4
24	Climbing bean	-	3.011	13	37	92.3
25	Climbing bean	-	3.230	16	31	91.3
26	Climbing bean	Fertilizer	4.675	0	29	71.1
27	Climbing bean	Cow manure	4.621	8	39	94.9
28	Climbing bean	Chicken manure	5.875	17	172	179.5
29	Soybean	Fertilizer	3.012	0	72	861.1
30	Soybean	-	1.050	0	24	107.1
31	Soybean	Cow manure	2.551	9	132	660.9
32	Soybean	Chicken manure	3.015	0	174	871.1

APPENDIX 2: DATA KAGURU FARMERS TRAINING CENTRE FARM

Plot No	**Crop type**	**Amendment**	**Seeds weight 100 seeds (g)**
1	Soybean	-	49
2	Climbing bean	Chicken manure	130
3	Climbing bean	Fertilizer	99
4	Climbing bean	Cow manure	110
5	Soybean	Cow manure	62
6	Soybean	Chicken manure	71
7	Climbing bean	-	70
8	Soybean	Fertilizer	62
9	Climbing bean	-	68
10	Soybean	Chicken manure	70
11	Soybean	Cow manure	69
12	Soybean	Fertilizer	62
13	Climbing bean	Cow manure	91
14	Climbing bean	Chicken manure	95
15	Soybean	-	52
16	Climbing bean	Fertilizer	69
17	Climbing bean	Chicken manure	98
18	Soybean	Fertilizer	60
19	Soybean	Cow manure	65
20	Climbing bean	Cow manure	91

21	Climbing bean	Fertilizer	71
22	Soybean	Chicken manure	70
23	Soybean	-	51
24	Climbing bean	-	71
25	Climbing bean	-	78
26	Climbing bean	Fertilizer	81
27	Climbing bean	Cow manure	99
28	Climbing bean	Chicken manure	101
29	Soybean	Fertilizer	50
30	Soybean	-	45
31	Soybean	Cow manure	61
32	Soybean	Chicken manure	69

Plot No	**Crop type**	**Amendment**	**Root dry weight (g)**	**No. of nodule**	**No. of pods**	**Stover dry weight**
1	Soybean	-	1.44	0	40	172.1
2	Climbing bean	Chicken manure	4.511	69	74	184.5
3	Climbing bean	Fertilizer	3.428	0	78	196.2
4	Climbing bean	Cow manure	3.792	57	67	170.5
5	Soybean	Cow manure	1.265	0	86	386.5
6	Soybean	Chicken manure	1.642	14	10.0	475.5
7	Climbing bean	-	2.821	30	26	67.6
8	Soybean	Fertilizer	1.785	0	87	387.5
9	Climbing bean	-	3.851	26	146	56.2
10	Soybean	Chicken	1.701	10	119	671.1
11	Soybean	Cow manure	1.820	0	92	397.2
12	Soybean	Fertilizer	1.825	0	50	172.5
13	Climbing bean	Cow manure	3.872	56	45	176.4
14	Climbing bean	Chicken manure	4.875	60	74	187.4
15	Soybean	-	2.870	0	52	180.3
16	Climbing bean	Fertilizer	2.854	0	60	52.4
17	Climbing bean	Chicken manure	5.895	0	90	219.7
18	Soybean	Fertilizer	1.875	0	137	666.5

19	Soybean	Cow manure	1.760	0	190	771.8
20	Climbing bean	Cow manure	4.075	26	46	117.6
21	Climbing bean	Fertilizer	3.672	0	56	140.2
22	Soybean	Chicken manure	1.875	1	250	128.1
23	Soybean	-	1.601	1	58	287.2
24	Climbing bean	-	2.921	12	30	87.2
25	Climbing bean	-	2.72	12	28	86.0
26	Climbing bean	Fertilizer	3.825	0	24	67.0
27	Climbing bean	Cow manure	3.784	6	33	88.2
28	Climbing bean	Chicken manure	4.587	16	165	163.0
29	Soybean	Fertilizer	2.661	0	69	672.1
30	Soybean	-	1.00	0	20	102.1
31	Soybean	Cow manure	1.921	7	123	572.5
32	Soybean	Chicken manure	2.102	0	159	689.2