FFECT OF RHIZOBIA INOCULATION AND STARTER-NITROGEN APPLICATION ON NODULATION, BIOMASS AND GRAIN YIELD OF FOOD LEGUMES

BY

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DECLARATION:

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

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To my beloved wife Elizabeth Nyawira and children Dennis and Fiona who gave me all the support and encouragement I needed during the time of doing this work.

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ABSTRACT

A field experiment was conducted at the University of Nairobi's Faculty of Agriculture farm in 2004 long rains (LR) and short rains (SR) to determine the effect of rhizobia inoculation and starter-N dose on the performance of grain legumes. Common bean (*Phaseolus vulgaris* L.), lima bean (*Phaseolus lunatus* L.), cowpea *(Vigna unguiculata* L), green gram (*Vigna radiata* L.), pigeon pea (*Cajanus cajan* L.) and lablab (*Lablab purpureus* L.) were the tested in the field experiment. The test crops were either uninoculated, inoculated with rhizobia, or supplied with 26 kg N ha^{-1}. The treatments were laid out in a randomized complete block design (RCBD) in a split plot arrangement with three replicates. Nodule numbers, nodule biomass, shoot biomass, root biomass and grain yield were determined. Rhizobia inoculation had no significant effect on nodule numbers $plant^{-1}$ in both seasons at 4 weeks after emergence (WAE) and 6 WAE. In most cases, common bean had significantly higher nodule numbers and nodule biomass than most of the other legumes while lima bean generally registered the fewest nodules. At 6 WAE, rhizobia inoculation improved nodule biomass in the short rains (SR) but not in the long rains (LR). Starter-N dose had no significant effect on nodule biomass in both seasons. Inoculation and starter-N had no significant effect on shoot biomass accumulation and grain yield in both seasons. In the LR, lima bean produced the highest grain yield followed by common bean while the converse was the case in SR. Pigeon pea and green gram performed the poorest in the SR. Mean grain yield varied from 148 kg ha^{-1} to 1472 kg ha^{-1} in LR and from 213 to 4398 kg ha^{-1} in SR. It was concluded that inoculation and starter-N application was not necessary for the purpose of improving grain yield under the prevailing field experimental conditions.

A follow-up experiment was conducted in a greenhouse to determine the abundance of

indigenous soil rhizobia nodulating common bean (*Phaseolus vulgaris* L.) and cowpea *(Vigna unguiculata* L.) in soils sampled from Kabete (cultivated and uncultivated land), Machakos, Nyeri and Kajiado sites. The population size of indigenous rhizobia was determined using the most probable number technique. Cowpea and common bean were used as "trap'' hosts for *Bradyrhizobium* spp and *Rhizobium* spp, respectively. Nodule numbers, nodule biomass, shoot biomass, plant tissue-N and the number of rhizobial bacteria cells g^{-1} dry soil were determined. The soil samples varied in chemical characteristics with the Nyeri site having a low pH. Indigenous rhizobia nodulating cowpea in the sites ranged from 78.5 to more than 900 bacterial cells per gram of dry soil. Common bean nodulating rhizobia had more than 900 bacterial cells per gram of dry soil at each of the sites. The Nyeri site had the lowest rhizobia population. In most sites, common bean produced significantly more nodules per plant than cowpea. A similar trend was observed with respect to nodule biomass, though this was only significant with Kabete soils. Inoculation with commercial inocula produced more nodule and shoot biomass than inoculation with soil inocula. It was concluded that indigenous rhizobia were widespread in central Kenya.

CHAPTER ONE: INTRODUCTION

1.1: Background information

Despite global food adequacy since 1974, sub-Saharan countries continue to suffer from food insecurity (Cohen, 2003). Limited availability of additional land for crop production, decreasing soil fertility, and declining yields of major food crops have been cited as the major constraints for agriculture's ability to provide nourishment for the increasing population (Sinclair and Gardener, 1998). Other factors that contribute to low production of food crops in developing countries include lack of technical knowledge and economic incentives. Future strategies for increasing agricultural production have to focus on sustainable use of natural, human and available capital resources (Altieri, 1998).

Kenya is faced by severe constraints of good agricultural land although 80% of its population lives in the rural areas and relies entirely on agricultural production (Ministry of Agriculture (MOA), 2004; Mureithi *et al.*, 2003). Only 17% of the country's land, which is comprised of medium and high potential areas, is suitable for agricultural production. A high rate of population growth estimated at 2.8% per annum and great reliance on rain-fed crop production have aggravated the food insecurity situation in Kenya (MOA, 2003).

The central highlands of Kenya, found within the administrative districts of Central and Eastern Provinces, are some of the most densely populated regions of Kenya. The region has a high population density ranging from 230-730 persons per km^2, with an average of 450 persons per km^2. Most of the population (70%) derives its livelihood from crop production practices (Gitari *et al.*, 1999; Micheni *et al.*, 1999; Mureithi *et al.*, 1998). The altitude of the area ranges

from 1000 to 1800 m above sea level. The main soils in these areas are nitosols and ando-humic nitosols (Jaetzold and Schmidt, 1983 a, b). Rainfall is bimodal and averages between 1000 and 1600 mm per year (Jaetzold and Schmidt, 1983). There are two distinct cropping seasons in the region, March-August for the long rains and October-December for the short rains. Farming in this region is in the hands of smallholders. Farms generally range from 0.5 to 4.0 ha with an average of 1.5 ha (Mureithi *et al.*, 2002). Legumes are grown in mixtures (polyculture) with other crops such as maize, sorghum, cassava, cowpea, and cotton (Mwaniki, 2000) and are only grown as a monocrops during the short rain season (MOA, 2003, Jaetzold and Schmidt, 1983 a, b).

The most commonly consumed and grown food legumes in central Kenya are common bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*), green gram (*Vigna radiata*), hyacinth bean (*Lablab purpureus*) and chickpea (*Cicer arietinum*) (MOA, 2003). Nationally, the average yields of these grain legumes under farmers' field conditions are generally low, ranging from 300 to 500 kg ha^{-1} $year^{-1}$ (MOA, 2002). The potential yields of these legumes range from 5,000 to 7,000 kg ha^{-1} $year^{-1}$ when grown under favourable conditions in the tropics and temperate regions (Rowland, 1993). The average yields of some of these legumes in central Kenya are: 345 kg ha^{-1} (common bean), 400 kg ha^{-1} (pigeon pea), 375 to 445 kg ha^{-1} (cowpea) and 450 kg ha^{-1} (lablab) (MOA, 2001 and 2002).

Legumes are an important source of cheap protein, compared to animal protein, for many people living in the rural areas in Kenya hence are referred to as the poor man's meat. They are rich in amino acids such as lysine and are complemented by cereals, which contain tryptophan that is lacking in legumes to form a common source of protein for the local staple diet.

Legumes are also an important source of vegetable oils and play a key role in long-term soil fertility maintenance in agricultural systems (Gachene and Kimaru, 2003; Amarger, 2001; Mureithi *et al.*, 2000; Rowland, 1993; Smaling, 1993; Hymowitz, 1990).

Legumes fix more than 60% of their own nitrogen requirements hence reduce reliance on commercial nitrogen (N) fertilizers, which are expensive and hence beyond the reach of the resource poor farmers in the tropics (Chemining'wa, 2002; Gachene and Kimaru, 2003: Mureithi *et al.*, 2003; Smaling, 1993). Their ability to fix nitrogen through symbiotic partnerships with root nodule bacteria, collectively referred to as rhizobia, gives them a special value in low input agriculture in developing countries in the tropics (Rowland, 1993). Legumes can fix up to 200 kg N ha^{-1} $year^{-1}$ in a sole crop where pests and diseases are controlled and adequate phosphorous supplied (Giller 2001; Rowland, 1993; Ssali and Keya, 1984). Compared to cereals, legumes are less dependent on soil nitrogen status and their response to N-fertilizer is weak and often negative when appropriate nodulating rhizobia strains are present in the soil (Giller, 2001; Hansen, 1994; Somasegaran and Hoben, 1994; Rowland, 1993). These characteristics enable them to produce well even when grown in intercropping systems. Incorporation of N-fixing legumes with multipurpose uses in the subsistence crop production systems could provide a means for food provision, fodder, soil fertility improvement, soil erosion control, weeds, pest, and disease control.

The main constraint facing production of grain legumes in central Kenya is low soil fertility hence low yields ha^{-1} (MOA, 2003). The gap between food production and population growth is widening, and the yield of legumes has shown more than 20% decrease over a period of 15 years in Africa (Acland, 1971). Despite the escalating food insecurity, production of

legumes is becoming unstable over time, due to the low yields per hectare compared to other staple food crops such as cereals (Rowland, 1993; MOA, 2002 and 2003). According to Food Agricultural Organization (FAO) 1996 world production report, some of the commonly grown food legumes such as common bean, garden pea, and soybean were found to produce 15, 14, and 96 million metric tonnes, respectively, of grains per year compared to 536, 481, and 476 million metric tonnes of wheat, maize, and rice, respectively, under similar conditions.

Crop production in central Kenya is characterized by continuous cultivation coupled with low input use and poor conservation practices which have led to gross nutrient mining from the soil (Gachene, 2003; Mureithi, 2001; Gitari *et al.*, 1999, Lynam *et al.*, 1998; Smaling, 1993). Land scarcity has made it uneconomical to maintain the traditional soil fertility maintenance practices such as fallowing. Central highlands have steep and hilly landscapes which are prone to soil erosion. Most subsistence crops in these areas are grown either without inorganic fertilizer or with sub-optimal levels. The practice has resulted in depletion of native soil fertility and decline in soil productivity (Ojiem *et al.*, 2000). The most depleted soil nutrients by continuous cultivation are usually nitrogen (N) and phosphorous (P) (Muchena and Nyandat, 1980).

Farmyard manure obtained from integrated livestock and crop production systems, could offer low cost plant nutrients, but is of low quality to achieve any reasonable crops yields. It is also inadequate due to competing demand between cash and subsistence crops beside being labour intensive (Mureithi, 2002; Kihanda, 1996; Ikombo, 1984).

1.2: Problem statement and justification

The production levels of legumes compared to cereals have been declining over the years due to decline in agricultural land as a result of rapid population growth in Kenya (MOA, 2004). This has been aggravated over time by compounding recalcitrant effects of soil fertility depletion due to continuous cultivation, reliance on inadequate and erratic rainfall in crop production and increased pest and disease incidence (Gachene and Kimaru, 2003; MOA, 2003; Hymowitz, 1990). Inorganic fertilizers can be used effectively to replenish the deficient soil nutrients, restore fertility, and improve crop productivity (Mureithi *et al.*, 2002). However, although farmers are aware of the potential of inorganic fertilizers for improving soil fertility hence productivity, the adoption has remained low mainly due to their prohibitive prices and in some case inaccessibility.

Nitrogen and phosphorous are the most frequently limiting nutrients in the soils, due to either leaching, volatilization, soil erosion or fixation (Gachene and Kimaru, 2003; Giller, 2001; Rowland, 1993; Peoples and Herridge, 1990). Most crops' optimal N requirement is greater than 0.4% while the amounts of N in most cultivated soils are in the ranges of 0.06% to 0.5% or less (Gachene, 2003; Giller, 2001; Hansen, 1994; Rowland, 1993). N-fertilizers are a substantial cost component of crop production systems in sub-Sahara Africa; so any strategy that can reduce the amount of inorganic-N used by maximizing the availability of legume-N should be encouraged (Garside and Berthelsen, 2004). Use of inorganic N-fertilizers in crop production systems is uncertain hence not a sustainable option to alleviate soil N deficiencies in the long run (Gachene, 2003; Peoples and Herridge, 1990; Power, 1987). Studies have shown that, biological nitrogen fixation process is an efficient way of supplying large quantities of

nitrogen needed by legumes to improve their productivity and protein content (Gachene, 2003; Rowland, 1993). However, knowledge of organic source of soil N remains limited under farm conditions, as research is recent and limited.

The demand for proteinaceous food is currently high due to the high population growth hence the need to use nodulating legumes able to fix nitrogen on poor agricultural soils. There is need to select for the future use of more effective and competitive *Rhizobia* strains, which are compatible with most domesticated food legumes. Enhancement of artificially inoculated rhizobia-legume relationship in fixing the atmospheric nitrogen should be emphasized (Steyn, 2004). Studies on availability of artificial rhizobia inoculants and demonstration on their use by the resource poor farmers are necessary. In order to improve the effectiveness of inoculated rhizobia, information on native rhizobia population levels in legume growing areas is also important. The population level of indigenous rhizobia in many legumes growing areas in central Kenya, and their compatibility with food legume species is not well documented.

Rhizobial inoculation studies conducted in Kenya have focused mostly on common bean and soybean. Studies on underutilized grain legumes such as lima bean, cowpea, pigeon pea and lablab are generally lacking yet these legumes have the potential to overcome the ever-increasing food insecurity and provide large quantities of dietary proteins needed at low cost (Chrispeels and Sadava, 2003).

1.3: Study objectives

The broad objective of this study was to develop sustainable and cost-effective legume production systems, which, are stable, integrative and that utilize low cost input technologies. The specific objectives of the study were:

1. To determine the effect of rhizobia inoculation on nodulation, growth, and yield of six grain legumes.
2. To determine the effect of starter-N application on nodulation, growth, and yield of six grain legumes.
3. To determine the presence and population level of indigenous rhizobia in soils collected from five field sites, and their effectiveness in nodulating grain legumes grown in central Kenya.

1.4: Hypotheses

1. Rhizobia inoculation and starter-N fertilizer application will improve nodulation, growth, biomass accumulation and yield of grain legumes grown in central Kenya.
2. Grain legumes can effectively nodulate with indigenous rhizobia in Central Kenyan soils.

CHAPTER TWO: LITERATURE REVIEW

2.1: Ecology and importance of food grain legumes

One of the most important plant families to the world agriculture is the leguminosae. The plants in this family provide the greatest part of human dietary proteins in the rural areas (Rowland, 1993; Hymowitz, 1990). The contribution of legumes to maintenance of soil fertility has been recognized for centuries (Amarger, 2001). Legumes consist of 650 genera and 18000 species, and are the most utilized plant family in the world (Giller, 2001; Allen and Allen, 1981). The leguminosae family is composed of 3 subfamilies namely: caesalpinoideae, mimosoideae, and papilionoideae (Somasegaran and Hoben, 1994). The subfamily papillionoideae (fabaceae) has three tribes namely: *Viceae*, *Heysareae*, *Phaseoleae* (Smartt, 1976).

Legumes can be divided into three categories namely primary, secondary, and tertiary grain legumes based on their domestication process and how they coevolved with other domesticated crops such as cereals (Giller, 2001; Hymowitz, 1990). The primary legumes belong to the tribe *Viceae* (*Lens* and *Pisum*) of the family papilionoideae while the secondary legumes constitute crops of the tribe *Phaseoleae* (such as *Canavalia*, *Lablab*, *Macrotyloma*, *Phaseolus*, *Vigna*, *Psophocarpus*) that are widely spread in the tropics and subtropics. The tertiary grain legumes are rarely grown outside their native habitats and are not domesticated but are used during times of environmental stress especially during droughts (Steyn, 2004).

The commonly grown legumes under tropical conditions are *Phaseolus* spp. although they are also adapted to other conditions. In developing countries, legumes are grown in agricultural

systems such as in plantations as cover crops, food crops, or shade trees; grazing systems under natural vegetation in semiarid areas and cut-carry systems in high potential areas (Peoples and Herridge, 1990).

Legumes require favorable conditions for plant growth and N_2-fixation (Giller, 2001; Hansen, 1994). Favourable temperatures are necessary to allow seed germination and reduce risk of attack by pathogens. Extreme temperatures cause growth distortion, stunted growth, and failure of chlorophyll development. Rainfall should be adequate to allow crop growth and provide adequate moisture for the symbiotic N_2-fixation to take place (Acland, 1971). Soils should be deep, well drained, fertile and well aerated for nodule formation. Toxic soil nutrients such as aluminum that alter soil pH, fix phosphorous and kill roots should be kept at minimum levels because they affect supplies of other nutrients such as molybdenum required for nodulation and seed development (Hansen, 1994; Rowland, 1993).

Some legumes such as pigeon pea, lablab, lima bean and cowpea are drought tolerant, heat resistant, and have variable growth and maturity periods (Rowland, 1993). They are valuable crops for diversification in both high and low rainfall areas. Other species such as pigeon pea can withstand drought between two bimodal rainfall seasons (Rowland, 1993). They are therefore important food crops in the marginal rainfall areas of central Kenya during the dry periods of the year.

Legumes are used for their aesthetic value, timber, as cooking fuel, browse trees and shrubs, forage crops, pasture crops, cover crops, green manures, and as human food (Power, 1986; Peoples and Herridge, 1990). They are also used as vegetables (leaves and fresh young pods), mature grains, or whole plants after harvest as forage (Shurtleff and Aoyagi, 2004).

Grain legumes are those leguminous plants used as food in form of unripe pods, immature seeds or mature dry seeds directly or indirectly (Steyn, 2004; Smartt, 1976). Legumes are also good sources of proteins, vitamins such as E and C, phosphorous, potassium, iron and dietary fiber (AVRDC, 2005). They are believed to be better than cereals in combating the protein deficiency prevalent in developing world.

Legume grain yields in the tropics are low ranging from 220 kg ha^{-1} to 670 kg ha^{-1} against a potential of more than 2,500 kg ha^{-1} (Acland 1971). The yield potentials of legumes in temperate areas range from 5,000 kg ha^{-1} to 7,000 kg ha^{-1} (Rowland, 1993). The current national average production level in Kenya is between 300 kg ha^{-1} and 500 kg ha^{-1} (MOA, 2002). The average grain yield for the common bean, which is the most important pulse in Kenya, is 250 kg ha^{-1} and 700 kg ha^{-1} in intercrop and pure stand respectively (Songa *et al.*, 1995). These yields are low compared to potential yields of 3-4 tonnes ha^{-1} in the tropics (Abate and Ampofo, 1996). Average grain yields for cowpea, which is a multi-purpose crop (Ortiz and Crounch, 2002), remain low averaging 0.3 tonnes ha^{-1} due to biotic and abiotic stresses (MOA, 2003; Henriet *et al.*, 1997).

A number of biotic and abiotic constraints are responsible for the low production of legumes in central Kenya. These include a wide range of insect pests and diseases, low soil fertility and drought (Wortmann *et al.*, 1998; Grindley and Danial, 1995; Wortmann and Allen, 1994). A host of pests and diseases such as nematodes (especially in dry areas), bean flies, aphids, whiteflies, leafhoppers, thrips, bugs, beetles and moth larvae infest legumes in Kenya (MOA, 2003). The most common legume diseases include the damping off diseases, root rot, halo blights, common bacterial blights, rusts, anthracnose, powdery mildews, cercospora fungal

disease, and viral diseases (MOA, 2002 and 2003, SMP, 1995). Control of pests and diseases especially at reproduction stage has been found to increase yields of legumes by more than 100% (Rowland, 1993).

Legumes also have a wide biodiversity of enormous variations that are not commercially exploited (Hymowitz, 1990). Intensifying production of underutilized legume crops could help to increase food security and provide the needed vitamins, minerals and other nutrients (Chrispeels and Sadava, 2003). Legumes, especially the deep-rooted ones are known to be good extractors of phosphate in the subsoil, which is an important nutrient in N_2-fixation process (Rowland, 1993). Legumes also provide a stable and sustainable source of nitrogen, aid in soil erosion control, improve soil fertility, offer potential for improving surface and ground water quality (Gachene and Kimaru, 2003).

2.2: Role of biological nitrogen fixation in crop production systems

Biological nitrogen fixation is a microbial process by which inert atmospheric N_2 is reduced enzymatically, through a complex biochemical reaction into N utilizable by plants (Steyn, 2004; Giller, 2001). The process of biological nitrogen fixation is important to the natural ecosystems as well as the agroecosystems since it has implication on forage, forest, and food production (Amarger, 2001). The process of N_2-fixation is accomplished by prokaryotes and no eukaryote is known to fix nitrogen on their own (Giller, 2001). Many eukaryotes derive direct benefit from intimate associations with N_2-fixing bacteria. Rhizobia-bacteria that form symbiotic associations with legume plants contribute the greatest amounts of biologically fixed N in agriculture (Amarger, 2001; Rowland, 1993; Power, 1987). Cyanobacteria (commonly

known as blue-green algae) have been known to fix atmospheric nitrogen both in association with eukaryotes such as *Azolla* (an aquatic fern) and fungi (constituting lichens) and as free-living species (Giller, 2001; Ssali and Keya, 1984). They contribute significant amounts of fixed N to rice crop production when introduced in paddy fields.

Non-leguminous flowering plant species such as *Casuarinas* and *Allocasuarinas* in symbiotic co-association with actinomycete *Frankia* species also fix atmospheric nitrogen (Gachene, 2003; Giller, 2001; Hansen, 1994). Free-living microorganisms, such as *Azospirillum*, *Azobacter*, *Beijerinka*, *Derxia*, *Klebsiella*, and *Nostoc*, also accomplish the process of biological nitrogen fixation. Free-living prokaryotes lack adequate sources of energy to support the capacity to fix nitrogen hence supply very little fixed nitrogen to the soil (Amarger, 2001; Giller, 2001; Peoples and Herridge, 1990). Some free-living N-fixing bacteria are mostly heterotrophic and their capacity is limited due to insufficient availability of organic residues. Their contribution to the terrestrial ecosystems is therefore relatively low (1 to 2 kg N ha^{-1} $year^{-1}$) (Hansen, 1994). Agriculture production can be made more sustainable by expanding biological nitrogen fixation in developing countries' farming systems (Peoples and Herridge, 1990).

The amount of N symbiotically fixed by legume-rhizobia relationships varies widely and is dependent on the type of the rhizobia species, soil, and environmental conditions. The amounts of N-fixed in cropping systems are governed by four principal factors. These factors include the amount of land sown to legumes, the health of the plant, ability of the N-fixing plants to establish their symbioses and achieve their potential rates of N-fixation, and the relative ability of the established symbiosis to fix nitrogen (Hansen, 1994). The ability of the legume-rhizobia

association to fix nitrogen is determined by the genetic potential of the N-fixing bacteria, the plant, and the symbiotic relationship itself (Giller and Cadisch, 1995). The effectiveness of the compatibility between the host legume species and the rhizobia species in symbiotic co-association is of great importance (Giller, 1994; Keya *et al.*, 1982; Chui and Nadar, 1983). Most food legumes can obtain 50–80% or more of their total nitrogen requirements from biological nitrogen fixation process (Steyn, 2004). Studies have shown that tree, forage and grain legumes can fix between 40 kg N and 500 kg N ha^{-1} $year^{-1}$ (Giller, 1994; Rowland, 1993; Ssali and Keya, 1984). It is estimated that legumes contribute globally about 70 million metric tonnes to terrestrial ecosystems per year (Brockwell *et al.*, 1995), which is equivalent to the inputs from N-fertilizers (Amarger, 2001). A critical review on legumes by Rowland (1993) concluded that legumes are responsible for 3 to 4 percent of N fixed at the earth surface per year.

In many countries, biological N-fixation process has not been fully exploited in crop production systems. Present knowledge is inadequate to provide economically competitive alternatives to N-fertilizers although biologically fixed N is environmentally acceptable globally (Peoples and Herridge, 1990). Some of the reasons cited are soil acidity and alkalinity, high soil temperatures, low soil moisture, deficiency of phosphorous in the soil, and lack of other macro and micronutrients in the soils (Gachene and Kimaru, 2003; Giller, 2001; Hansen, 1994; Ssali and Keya, 1984).

2.3: Major characteristics of root nodule bacteria

Rhizobia bacteria belong to the family Rhizobiaceae. The family consists of the genera

Rhizobium, *Bradyrhizobium*, *Allorhizobium*, *Azorhizobium, Sinorhizobium,* and *Mesorhizobium* (Amarger, 2001). *Sinorhizobium,* which is the microsymbiont of alfalfa, and Mesorhizobium, the microsymbiont of chickpea and *Lotus*, were recently recognized as separate from *Rhizobium* (Amarger, 2001; Young and Haukka, 1996; de Lajudie *et al.*, 1994). The genus *Rhizobium* includes the *Rhizobium leguminosarum* bv. *viceae*, *trifolii*, and *phaseoli* (Hirsh, 1996). The genus *Bradyrhizobium* is comprised of *Bradyrhizobium japonicum*, *elkani*, and *liaoningenisis* (Xu *et al.*, 1995).

The species in the genera *Rhizobium* and *Bradyrhizobium* can be differentiated on the basis of their utilization of carbon sources and excretion of metabolites (Werner, 1992). Both genera have several characteristics in common such as growth on organic acids as carbon-source; metabolism of carbohydrate's via Entner-Doudoroff pathway and exhibits no colour of colonies with Congo red (Werner, 1992; Hansen, 1994).

The genera can also be differentiated based on phenotypic and genotypic characteristics (Amarger, 2001). More criteria that are diverse have been adopted to describe new rhizobia species following the development of molecular techniques and proposal of minimum standards (Graham *et al.*, 1991). Polyphasic classifications can be used to delineate new rhizobia taxonomic units (Vandamme *et al.*, 1996). Strains sharing 70% of DNA-DNA relatedness are classified as one species.

The species in the genus *Rhizobium* are motile (have 2 to 6 flagella), fast-growing, acid producing and develop turbidity within 2 to 3 days in a liquid media and their doubling time is 2 to 4 hours (Amarger, 2001; Hansen, 1994; Somasegaran and Hoben, 1994). The species in the genus *Rhizobium* grow on sucrose or mannose as single carbon-source. They are also

effective in temperate and tropical regions and grow on a wide range of carbohydrates (Somasegaran and Hoben, 1994). They are aerobic chemoorganotrophs, and are easy to culture. The optimal growth of most strains occurs in a temperature range of 25 to 30°C and a pH of 6 to 7 (Somasegaran and Hoben, 1994).

The species in the genus *Bradyrhizobium* are slow growing, alkali producing, have mean doubling time of 6 to 8 hours and require 3 to 5 days to produce moderate turbidity in liquid media. The species in the genus *Bradyrhizobium* spp do not grow on sugars such as sucrose or rhamnose. Root nodule forming bacteria of this genus are medium sized, rod-shaped cells, 0.5 – 0.9 μm in width and 1.2 – 3.0 μm in length. They do not form endospores, are gram negative, predominantly aerobic chemoorganotrophs and easy to culture (Amarger, 2001; Xu *et al.*, 1995).

Rhizobia species and strains vary in their ability to infect and fix nitrogen in different host legume species. There are genetic and environmental factors that control the host's ability to support effective nodulation (Giller, 2001). Rhizobia are heterogeneous and genetically diverse bacteria. They can be classified based on their ability to nodulate members of Leguminosae family according to host-based system (Amarger, 2001; Giller, 2001). The legumes are assembled into cross-nodulation groups, which group them with their compatible rhizobial partners (Hansen, 1994, Somasegaran and Hoben, 1994). The cross-nodulation groups are collection of legumes that will form nodules effectively when inoculated with the rhizobia obtained from any member of that legume group. Majority of tropical legumes such as *Vigna* spp, *Lablab purpureus,* and *Cajanus cajan* are capable of nodulation by the *Bradyrhizobium* spp, which is a cowpea cross-nodulating group of rhizobia. Other legumes such as faba bean,

lentils and pea require specific rhizobia strain namely *Rhizobium leguminosarum* bv. *viceae.* Legume-rhizobia association must exhibit compatibility and saprophytic competence over other soil microorganisms, and out-compete other native rhizobia for infection sites on legume roots for successive nodulation to occur (Somasegaran and Hoben, 1994).

2.4: Root nodule formation in legumes

The legume-bacteria infection (nodulation) starts with an exchange of a number of signals, which regulate the expression of genes essential for rhizobia infection and nodule formation. The process begins with adhesion of the specific rhizobia on the root hair surface, which is followed by deformation, and curling of the root hair resulting in a characteristic shepherd's crook appearance (Amarger, 2001; Giller, 2001; Hansen, 1994; Somasegaran and Hoben, 1994). Rhizobia bacteria can enter through the hydathodes, cracks in the root epidermis or through young roots between epidermal cells (Giller, 2001, Hansen, 1994).

The common elements of symbiosis are the stimulation of biochemical activity in the rhizobial strains by flavonoid and isoflavonoids molecule in the plant root exudates, which stimulate nodule formation through activation of nod genes (Giller, 2001; Hansen, 1994). Different flavonoid and isoflavonoid compounds from different legumes activate *nod*D genes (genes essential for nodulation and competitiveness of rhizobia) of their compatible rhizobia preferentially hence specificity in the legume–rhizobial interaction (Werner, 1992; Hansen, 1994)). Others are broad host-range rhizobia species and are activated by a broad range of phenolic compounds (Giller, 2001).

The *nod* factors are formed when the flavonoids and isoflavonoids enter the bacterial wall

and bind with a protein called *nod*D. The *nod* factor triggers response in the plant that leads to rhizobial infection and development of root nodule (Werner, 1992). The nod factors trigger depolarization of the cells in the zone of the emerging root hairs, deformation of root hairs and expression of nodule specific genes (nodullins), induction of the infection thread formation and development of nodules from the purified nod factors (Postgate, 1998; Hansen, 1994; Young, 1992).

The rhizobial nod factors influence the host's root growth by increasing the amount of root hairs and also their deformation into various different shapes (Hansen 1994). The root hair curling or deformation is caused by growth inhibition at the point of infection or change in the direction of the root hair growth at the site of rhizobia attachment. After bacteria penetration, infection threads are developed by lipopolysaccharides and they branch and spread into the cortex. The types of infection threads formed are influenced by surface polysaccharides of rhizobia and legumes species (Hansen, 1994; Werner, 1992).

After infection and thread formation, the cortical cell subdivides commencing the formation of nodules. The threads spread by branching through the root cortex passing close to the host cell nuclei, where they release the rhizobia bacteria into the cytoplasm of the host (Giller, 2001; Hansen, 1994; Somasegaran and Hoben, 1994). The rhizobia divide and differentiate to form the bacteriods and peribacteriod membranes to effectively separate the plant cytoplasm and the bacteriods. The type of the legume species determines the size, shape and the number of bacteriods.

Effective symbiosis is characterized by presence of leghemoglobin proteins that give the interior of the nodules a pink or red colour (Amarger, 2001; Giller, 2001). The enzyme

nitrogenase that facilitates conversion of atmospheric nitrogen to NH_4^+ is a complex of Fe-containing protein and Fe-Mo-protein (Werner, 1992; Hansen 1994). When nitrogenase enzyme is synthesized, repression of glutamate synthesis occurs hence rendering the bacteriods ineffective in assimilating the NH_4^+ into organic compounds hence the reason why rhizobia are not able to freely fix nitrogen outside the host legume plant (Day and Udvardi, 1992; Mellor and Werner, 1990).

There are two types of nodules formed, namely determinate and indeterminate (Hansen, 1994). The type of nodules formed depends on the legume species, the life span of the nodule meristems and not on the rhizobia strains (Hansen, 1994; Somasegaran and Hoben, 1994). The determinate nodules are formed by narrow infection threads, which are spherical and have persistent meristems that differentiate at the same time hence have a finite lifespan. Indeterminate nodules are formed by broad infection threads, which are coralloid or cylindrical and have distal apical meristems, which regenerate every season producing new infection zones (Giller, 2001; Cohn *et al.*, 1998; Brewin, 1998; Hirsch, 1992). Plants such as pea, alfalfa, and clover exemplify the indeterminate nodules (Hansen, 1994). Legumes such as common bean, pigeon pea, green gram, lima bean, lablab, and cowpea have determinate nodules (Hansen, 1994; Somasegaran and Hoben, 1994).

The size, effectiveness, and competitiveness of the indigenous rhizobia against inoculated rhizobia and other soil microbes will affect nodulation level and the process of biological N-fixation. Nodulation level is assessed as counts of nodule number, nodule mass or volume, or simply a visual scoring of relative nodulation success (Sylvester-Bradley and Kipe-Nolt, 1988).

2.5: Ecological factors affecting rhizobial population and the legume-rhizobia symbiosis

The ability of rhizobia bacteria to fix nitrogen in symbiotic relation or in free-living state is strongly influenced by the prevailing environmental condition (Giller, 2001; Amarger, 2001). There are various genetic and environmental factors that affect the biological nitrogen fixation process. Some of these factors include soil N (Sanginga, *et al.*, 1997), population level of indigenous rhizobia (Thies *et al.*, 1991b) and rhizobia inoculant used (Burton, 1985). Efficient and effective symbiotic N-fixation can be achieved when optimal conditions prevail in the soil. It is important to understand these factors in order to optimize them and develop approaches towards enhancement of biological nitrogen fixation process in legume production. Tropical conditions contain some of the most productive environments in the world but stressful conditions are very common (Amarger, 2001; Giller, 2001).

The main environmental stresses that occur in the tropics can be divided into predominantly physical factors (temperature, moisture, and light) and chemical factors, which include toxic effects (acidity, aluminium toxicity) and nutrient deficiencies (Giller, 2001; Hansen, 1994). Genetic variability in tolerance to most environmental stresses has been shown in both host legumes and rhizobial strains (Hungria and Vargas, 2000).

Temperature fluctuations affect both legume growth and rhizobial activities. In order to realize maximum benefits from legume-rhizobial symbiotic co-association, optimal temperatures must be prevalent for the two partners. Optimal growth of root nodule bacteria and legume occurs at temperatures of 25^0 C to 30^0 C (Giller, 2001; Hansen, 1994) but varies among the species and their endosymbiont (Graham, 1992). High temperatures above 50^0 C kill

the bacteria, interfere with cellular metabolic activities, and also inhibit seed germination (Dudeja and Khurana, 1989).

Other effects of high temperatures are prevention of nodulation and inhibition of the N-fixation process (Day *et al.*, 1978). Low temperatures delay plant growth, prevent nodulation, and decrease the rate of biological nitrogen fixation (Hansen, 1994). Varied tolerance to extreme temperatures has been found in both the legumes and the bacteria. Some species of the bacteria have been found to tolerate high temperatures of up to 40^0 C especially fast growing *Rhizobia* spp (Mpepereki *et al.*, 1996; Hansen, 1994). Air temperatures affect photosynthesis and indirectly N-fixation process (Hansen, 1994), while soil temperatures predominantly affect nodule formation and growth of rhizobia (Giller, 2001; Mpepereki *et al.*, 1996; Hansen, 1994).

The response and tolerance of legumes and rhizobia species to soil moisture conditions varies in dry conditions and waterlogged conditions. Low soil moisture content interferes with rhizobia mobility, multiplication and infection thread formation, and may retard nodule formation or cause premature nodule senescence (Giller, 2001; Hansen, 1994). Growth of rhizobia is inhibited under low moisture conditions (Mahler and Wollum, 1981). Slow growing rhizobia have been found to endure seasonal soil moisture deficits better than fast growing rhizobia species (Woomer *et al.*, 1988). The presence of clay and organic matter content in the soil are known to enhance tolerance to adverse low moisture conditions (Giller, 2001; Nazih *et al.*, 1993). Water potential changes affect the rates of N-fixation, photosynthesis, growth of leaves, and N assimilation. Legumes adapt to drought conditions by developing deep rooting systems, adjusting tissue osmotic potentials, periodically closing, and opening the stomata (Serraj *et al.*, 1999; Eaglesham and Ayanaba, 1984; Sprent, 1984; Nilsen *et al.*, 1983).

Dry conditions in the soil can lead to decline in the number of rhizobia but the strains that are able to retain less water within their cells survive longer than the rest (Al-Rashidi *et al.*, 1982; Bushby and Marshall, 1977). The response to dry conditions is dependent on the type of nodules. Amide-exporting indeterminate nodules are known to recover from drought conditions faster than ureide-exporting determinate nodules (Serraj *et al.*, 1999; Eaglesham *et al.*, 1982).

Waterlogging creates anaerobic conditions that are not favourable to rhizobia bacteria and plants roots. Loss of oxygen, due to waterlogging, results in loss of nitrogenase activity and poor root respiration. Waterlogging also aggravates the release of usually insoluble oxide forms of iron and manganese that are toxic to plants and rhizobia (Giller, 2001; Hansen, 1994).

Symbiotic N_2-fixation is strongly influenced in several ways by high concentration of salt, leading to substantial reduction in N_2 fixation activity (Hansen, 1994). Saline conditions cause root hair growth to cease and interfere with rhizobia infection and reduce nodulation (Hansen, 1994; Graham, 1992; Sprent and Sprent, 1990). High salt concentrations, lead to osmotic withdrawal of water from the nodules. Khailova and Lar'kova (1992) made similar findings on alfalfa. Studies have shown that rhizobial strains may be more tolerant to elevated salinity than their host legumes (Singleton *et al.*, 1992).

Macronutrients such as phosphorous, potassium, sulphur, magnesium, and calcium, and micronutrients such as cobalt, molybdenum, boron, copper, and zinc are important to the N-fixation process, legumes, and rhizobial growth (Giller, 2001; Hansen, 1994). Supply of P-fertilizer is essential for effective N-fixation while high soil nitrate-N levels will inhibit nodulation. Molybdenum is required in nodule formation and functioning of the nitrogenase enzyme complex. Mo deficiency results to poorly formed and ineffective nodulates.

Optimal soil pH range of 5.0 to 7.0 is necessary to ensure availability of plant nutrients in the soil. Under acidic soil conditions, resident soil populations of rhizobia are often low and the survival and the persistence of inoculant strains are adversely affected. Most rhizobia strains will not grow at pH below 4.5 (Karanja and Wood, 1988). Variations in tolerance to low soil pH occur between and within species of rhizobia (Graham, 1992).

2.6: Rhizobia inoculation

Legume inoculation is an agricultural practice that has been practiced for more than a century with the aim of introducing rhizobia in the soil at sowing (Brockwell and Bottomley, 1995; Roughley, 1985; Smith, 1992; Brockwell, 1977; Date and Roughley, 1977). The physical process of applying specific N-fixing bacteria to the seed or soil at or before planting is essential because soils sometimes do not have the proper kind of rhizobia capable of nodulating legumes (Adjei *et al.*, 2004). Most commercial inoculants are produced in forms of powder, granules, or liquid. Inoculants are produced based on symbiotic effectiveness, genetic stability, competitiveness with native soil rhizobia, and ability of the bacteria to survive as an inoculant in the soil (Amarger, 2001).

Inoculation can be done directly on the seed, which is the most commonly used method, or can be in form of mineral granules applied on the seedbed (Hynes *et al.*, 1995). Effective nodulation takes places within four weeks after planting and is manifested by vigorous growth of the inoculated legumes (Adjei, 2004; Somasegaran and Hoben, 1994). The aim of seed inoculation is to ensure there is contact between viable N-fixing rhizobia and legume seeds or the seedbed (Hansen, 1994).

Artificial inoculation with effective strains of rhizobia has been tried and practiced in research stations in Kenya and on commercial farming in other countries. The benefits of symbiotically fixed N are usually appreciated when legume responds to inoculation (Somasegaran and Hoben, 1994; Hansen, 1994). The best time to introduce rhizobia inoculation is when you are introducing new legume cultivars in an area or when the soil population level of indigenous rhizobia is low. Inoculation can also be done in fields, which have not been cropped with legumes for more than 3 years. Soils associated with adverse soil conditions such as high temperature; acidic pH and moisture deficit are also likely to have low rhizobial population levels hence recommended for rhizobia inoculation (Giller, 2001; Hansen, 1994).

Rhizobia-legume symbiotic association ranges from promiscuous associations or cross-nodulating group to the specific nodulating group such as *Rhizobium leguminosarum* bv. *viceae*. Most tropical legumes including cowpea, green gram, lima bean, pigeon pea and lablab are nodulated by the promiscuous cowpea cross-nodulating rhizobia (Somasegaran and Hoben, 1994). Other legume species such as faba bean, lentils, and garden pea require specific rhizobia strains for effective nodulation to occur.

In the tropics, adoption of rhizobia inoculation in smallholders' farming has been beset by problems such as perishability of the inocula due to poor handling and storage. Unavailability of the inoculum, lack of trained personnel on use of inoculum at farm level and lack of prior knowledge on inoculum's effectiveness after inoculation also limit adoption of rhizobia inoculation. Other factors include the effects of chemical seed-dressers, or applied fertilizers, abiotic factors (such as low rainfall and high soil temperatures), and lack of adequate

knowledge on population levels of competing indigenous rhizobia in the soil (Styen, 2004). It is widely acknowledged that inoculation of legumes with effective rhizobia can improve yields and provide a substitute to inorganic fertilizers, which are expensive and beyond the reach of resource poor farmers in the tropics. However, the need for inoculation is not universal (Singleton *et al.*, 1992) and inoculation does not always result into positive response (Olsen *et al.*, 1996). Lewin *et al.* (1987) observed that tropical legumes rarely respond to inoculation unless grown in soils where the conditions are not favourable to the survival of rhizobia.

2.7: Effect of soil nitrogen on nodulation and nitrogen fixation in legumes

Nitrogen is the most important nutrient required by plants and is therefore central to plant growth. Studies have indicated that in most tropical soils, nitrogen is often the most limiting plant nutrient under continuous cultivation (Adjei, 2004; Gachene and Kimaru, 2003; Kaleem, 2000). In central Kenyan soils, N occurs in low concentrations in the ranges of 0.02% in the sub-soil to 2.5% in peat high in organic matter (Gachene and Kimaru, 2003). Hence inorganic N-fertilizers have become a cost component in food crop production in Kenya.

There are various nitrogen compounds such as ammonium, nitrate, and amino acids utilized by rhizobia for their growth (Chakrabarti *et al.*, 1981; Elkan and Kwik, 1968). The ability of rhizobia species to utilize various nitrogen sources varies between and within species. Most rhizobia species can utilize amino acid glutamate as a source of nitrogen while only a few strains utilize glycine (Chakrabarti *et al.*, 1981).

In nitrogen depleted soils, a moderate dose of N-fertilizer at planting may have a stimulatory effect on legume nodulation and N-fixation (Chemining'wa, 2002). The applied

nitrogen will promote legume growth during root establishment and before onset of the N-fixation process (Giller, 2001; Giller and Cadisch, 1995). Plants grown in soils deficient in mineral nitrogen and wholly dependent on atmospheric nitrogen require more phosphorous during the biological nitrogen fixation process than those grown in soils rich in mineral nitrogen (Gachene and Kimaru, 2003; Giller, 2001; Giller and Cadisch, 1995). Bushby (1993) observed that soil nitrogen improves the effectiveness of rhizobia inoculation of soybean in nitrogen-depleted soils.

Many studies acknowledge that inorganic forms of nitrate-N, interfere with nodulation and nitrogen fixation in legumes. There have been reports of reduced number of nodules (Rai, 1992), delayed nodulation and even cessation of nodulation in presence of nitrate (Herridge *et al.*, 1984). Havelka *et al.*, (1982) attributed the reduced nodulation to carbohydrate deprivation in the nodules due to energy required for nitrate reduction. It has been pointed out that soil N content can affect rhizobia-legume association by depression of lectin production and attachment of bacteria to the roots (Martensson, 1989; Gibson and Harper, 1985). Stone *et al.*, (1985) reported that sometimes nitrogen supplements result in lowering of crop yield. However, higher levels of soil nitrogen, phosphorous and magnesium contribute to increased seedling growth (Marschner, 1986). Tolerances to soil nitrates by legumes can occur naturally (Herridge and Betts, 1988) but can be induced by genetic manipulation. Growing of super-nodulating legume cultivars that tolerate high soil nitrate levels can overcome the problem of soil nitrates (Herridge and Betts, 1988; Carrol *et al.*, 1985).

2.8: Indigenous populations of rhizobia in soil

Rhizobia are saprophytic bacteria representing only a small fraction of soil microflora (Amarger, 2001). The number of rhizobia in the soil depends on indigenous bacteria population, soil properties, climate, inoculation, and cropping history (Rahman and Rastin, 2002; Lawson *et al.*, 1987; Yousef *et al.*, 1987). Their population sizes in agricultural soils varies widely and usually appears to be correlated to abiotic factors such as soil pH, base saturation, soil texture, organic matter content, mean annual rainfall, and temperature (Giller, 2001; Amarger, 2001). Acidic soil conditions or related factors may impair the growth of rhizobia and displace them in the competition struggle to colonize suitable niches (Lowendorf *et al.*, 1981; Rovira, 1961). In most soils, an average of 10^2 to 10^4 rhizobial bacteria cell gram^{-1} of dry soil average density of different populations have been found to coexist and reach up to 10^7 g^{-1} in soil under legume crop (Amarger, 2001). However, soils populations of rhizobia that nodulate *Phaseolus* spp. commonly range in population size from 10^2 to 10^6 g^{-1} of soil (Aguilar *et al.*, 1998; Anyango *et al.*, 1995; Kucey and Hynes, 1989).

Great diversity in rhizobial populations is common no matter which legumes they nodulate. Lower levels of genetic diversity found in some populations are generally associated with specific soil conditions such as acid pH (Harrison *et al.*, 1989) or high nitrogen content (Souza *et al.*, 1994; Caballero-Mellado, 1999). The effectiveness of indigenous rhizobia depends on the soil population density and host plant selectivity of the compatible rhizobia strain under the prevailing environmental conditions.

The survival and abundance of rhizobia will depend on their ability to locate and colonize sites of suitable nutrition under pressure from other competing microorganisms (Coventry and

Evans, 1989). In the absence of host legumes, the number of a particular rhizobia species can be substantially less than 0.1% of the total aerobic bacteria but can increase from 8 to 9% when legumes are cultivated (Mahler and Wollum, 1981; 1982). Population size of effective indigenous soil rhizobia can be used as a reliable index of a legume to derive sufficient nitrogen through biological nitrogen fixation whether the legume responds to inoculation or not (Turk *et al.*, 1993; Thies *et al.*, 1991a). Due to the great variability that exists in soil population levels of indigenous rhizobia, their benefits towards N-fixation are site specific (Singleton *et al.*, 1992). Legume introduced in areas remotely separated from their centre of diversity will only form nodules and fix nitrogen if compatible indigenous rhizobia are present in the soil (Bala and Giller, 2002; Perez-Ramirez *et al.,* 1998).

CHAPTER THREE: MATERIALS AND METHODS

3.1: Determination of the effect of rhizobia inoculation and N-fertilizer on growth, nodulation and yield of grain legumes

3.1.1: Experimental site

The field experiments were conducted at University of Nairobi's Faculty of Agriculture Field Station, Kabete Campus. The farm is situated near the equator at latitude 01°, 15'S and longitude 36° 44' E. The area is in the upper highland zone three (UH_3) at altitude of about 1,850 m above sea level (Nyanga, 1997). It experiences bimodal rainfall pattern, with long rains received between March and May and the short rains received between October and December every year. The average annual rainfall is about 1000 mm $year^{-1}$ with a range of between 700 mm $year^{-1}$ and 1500 mm $year^{-1}$. The soils of the area are deep, well-drained, dark reddish brown to dark red friable clay with acidic humic topsoil (humic NITOSOLS) of moderate fertility developed from Limuru Trachite (Michieka, 1977). The diurnal temperature ranges from 12° C to 28° C with a daily mean of about 20° C (Anon, 1985).

3.1.2: Experimental design and treatments

The field experiments were conducted for two consecutive seasons: long rains (March to August 2004) and short rains (October 2004 to January 2005). The test legumes were lima bean (local variety), pigeon pea (Mbaazi 1), green gram (N26), cowpea (M66), hyacinth bean (DH1002), and common bean (GLP-2). The treatments were rhizobia inoculation, starter-N fertilizer application at a rate of 26 kg N ha^{-1} and a control (0 kg N ha^{-1} and no rhizobia

inoculation). The treatments were laid out in a randomized complete block design (RCBD) in a split plot arrangement and replicated three times. The six test legume species were assigned to the main plots while the sources of N were assigned to the subplots.

The rhizobia inocula were prepared and supplied by the MIRCEN project, Department of Soil Science, University of Nairobi. Triple super phosphate (46% P_2O_6) was used as the source of phosphorous and was applied in all the plots while calcium ammonium nitrate (27% N) was used as starter-N and was applied in appropriate treatment plots. The size of each experimental plot was 3.0 m x 2.0 m with an inter-plot spacing of 1 m. The blocks were spaced 2 m apart and a buffer zone of at least 3.0 m was maintained between the experimental plots and the neighbouring field plots. The total number of plots was 54 in each season. The legume seeds were obtained from the Kenya Agricultural Research Institute's National Dryland Research Station, Katumani and the fertilizers from a locally appointed agrochemical stockist.

The first field experiment was conducted during the long rains of April to August 2004 and the second experiment during the short rains of October 2004 to January 2005. Prior to planting, soils were sampled to a depth of 30 cm. Soil pH, nitrate-nitrogen content, carbon content and electrical conductivity were analyzed using Kjeldahl method (A.O.A.C., 1990) and available phosphorous using "Mellich 1" method at Kenya Agricultural Research Institute's National Agricultural Research laboratories, Kabete (Appendix 1).

3.1.3: Crop production practices

Land preparation for the field experiments was carried out early before the on-set of the rains. The optimum plant population per plot was maintained by planting the legumes at their

respective recommended spacing as follows: common bean (30 cm by 15 cm), pigeon pea (50 cm by 20 cm), lima bean (40 cm by 25 cm), green gram (30 cm by 10 cm), lablab (80 cm by 50 cm), and cowpea (75 cm by 20 cm). At planting, triple super phosphate (46% P_2O_5) was applied to all plots at a rate of 45 kg P_2O_5 ha^{-1} while calcium ammonium nitrate (27% N) was supplied as starter-N treatment in appropriate plots at rate of 26 kg N ha^{-1}. The fertilizers were applied along the rows and thoroughly mixed within the top 15 cm of soil to avoid direct contact with the seeds or the rhizobia inocula.

Rhizobium spp strain USDA 2674 was used to inoculate common bean while cowpea cross-nodulating *Bradyrhizobium* spp strain USDA 3456 was used to inoculate pigeon pea, cowpea, lima bean, green gram and lablab at rates recommended by the manufacturer before planting. Seeds were dusted with peat-based inoculants containing gum Arabic adhesive in a flask, wetted with tap water and then planted immediately to prevent excessive drying which reduces the viability of the inocula. Care was taken to avoid cross contamination of uninoculated seeds and plots with rhizobia, by planting the uninoculated seeds before the inoculated seeds.

Two seeds of each legume were planted per hill in respective plots and later thinned to one plant per hill two weeks after planting. Gaping was done during the first week of emergence for all the species to achieve optimum plant stand per plot. Initially the long rains were high but subsided towards the middle of the season while short rains were low at the beginning of the season but increased toward the middle of the season (Appendix 3).

There were no serious disease incidences except for common bacterial blight diseases, Fusarium root rot, and powdery mildew during alternate moist and dry conditions of the

seasons. Pests such as bean flies, aphids, stripped beetles (pod borers), and systate weevils were also observed at different stages of plant growth and at different levels of infestation among the legume species. The fungal diseases were controlled using Antracol 70% Wettable Powder (WP) at a rate of 40 g per 20 L of water while pests were controlled using dimethoate 40% Emulsifiable Concentrate (EC) at a rate of 40 ml in 20 L of water. The legume species mostly affected by pests were lablab, cowpea, and pigeon pea while those mostly affected by diseases were green gram, cowpea, common bean, and lablab. All the plots were kept weed-free by hand weeding using a hoe throughout the growing season.

3.1.4: Measurements and observations

The parameters assessed in this study were number of nodules, nodule dry matter, shoot dry matter, root dry matter, grain yield and yield components.

3.1.4.1: Nodulation and biomass accumulation

Sampling for number of nodules, nodule dry matter, shoot and root dry matter for all the treatments was done at 4 and 6 weeks after emergence (WAE) in both seasons. To avoid creation of irregular gaps within the field, harvesting for the number of nodules and biomass was stratified and done sequentially from the outer rows inwards leaving the necessary guard plants each time. Three plants were randomly selected from each experimental plot. All the roots were then carefully uprooted by digging 15 cm around the plant using a spade and washed with clean tap water to remove all attached soil from the roots and the nodules. The nodules were counted, and then carefully picked using a pair of tweezers, oven dried at 60^0 C for 48

hours and weighed. The vegetative top parts and the subterranean parts (roots) of the plants were separated, oven-dried at temperature of 60^0 C for 48 hours and their respective dry matter weights determined.

3.1.4.2: Yield and yield components

On reaching physiological maturity, pods were harvested from each experimental plot excluding the outer rows and the outer guard plants in each row. After shelling, grains from each experimental plot were separated from the husks, sun-dried to a constant dry weight and their total grain weight determined. The average number of pods per plant was determined at harvest for each experimental plot from 10 randomly selected plants per plot. The average number of grains per pod per plant of each experimental plot at harvest was determined from five randomly selected pods from 10 plants per plot.

3.1.5: Data analysis

Analysis of variance (ANOVA) was performed on the number of nodules, nodule dry matter, shoot dry matter, root dry matter, and grain yield using General Statistics (GENSTAT®) package (Rothamstead Experimental Station, 1995). When the F test was significant, treatments were separated by the least significant difference (LSD) test at 5% probability level (Steel and Torrie, 1980; Matata *et al.*, 2001).

3.2: Determination of abundance of indigenous rhizobia in soils from the field sites.

3.2.1: Soil sampling and analysis

The soil samples were collected from four cultivated field sites (Kajiado, Machakos, Nyeri, and Kabete) and one uncultivated field site at Kabete. The cultivated field sites had no known history of cultivation of inoculated legumes. Soils were sampled to a depth of 30 cm from five points (the center and at the four corners) within the selected fields. The soil samples from the five points were thoroughly mixed to make a representative sample for each field site. The representative soil samples were divided into two sub-samples. One soil sub-sample was used to determine the abundance of soil rhizobia while the other one was used for the analysis of physicochemical properties. The bulked samples were analyzed at Kenya Agricultural Research Institute's National Agricultural Research Laboratories. Soil pH, percent carbon, nitrate-nitrogen and electrical conductivity were analyzed using Kjeldahl method (A.O.A.C., 1990) while available phosphorous was analyzed using "Mellich 1" method (Appendix 2).

3.2.2: Growth media preparation

The growth media was prepared according to the procedure described by Somasegaran and Hoben (1994). The nitrogen-free plant culture solutions comprised four stock solutions. Stock solution 1 comprised of 294.1 g litre^{-1} of $CaCL_2$. $2H_2O$ while stock solution 2 was made from 136.1 g litre^{-1} of KH_2PO_4. Stock solution 3, was made from 6.7 g litre^{-1} $FeC_6H_5O_7$, 123.3 g litre^{-1} $MgSO_4.7H_2O$, 87.0 g litre^{-1} K_2SO_4 and 0.338 g litre^{-1} $MnSO_4.H_2O$. Stock solution 4 comprised 0.247 g litre^{-1} H_3BO_3, 0.288 g litre^{-1} $ZnSO_4.7H_2O$, 0.100 g litre^{-1} $CuSO_4.5H_2O$, 0.056 g litre^{-1} $CoSO_4.7\ H_2O$ and 0.048 g litre^{-1} $NaMoO_2.2H_2O$. The four stock solutions were

stored separately in a refrigerator at 4^0C. Ten litres of full-strength plant culture solution was made by dissolving 5 ml of each stock solution in 5 litres of water, mixed and then diluted further by adding 5 liters of water. The pH was adjusted to 6.6 – 6.8 with 1N NaOH.

3.2.3: Assembly of Leonard jars

The Leonard jar, which was used as the experimental unit consisted of a 200 ml bottle with the lower portion cut-off and inverted into a 1-litre reservoir jar. A cotton mop head secured by absorbent foam material was used as a wick material to conduct nitrogen-free nutrient solution from the reservoir through the sterilized sand growth medium to the planted legume "trap" host. The complete Leonard jar assemblies were sterilized by autoclaving for 1.5 – 2.0 hours at 121^0C and at 15 ib in^{-1} (1 bar) pressure then cooled overnight. The Leonard jars were then moistened with 800 ml of nitrogen-free nutrient solution until saturated and the excess drained into the reservoir jars before planting the sterilized legume seeds. Three pre-germinated legume seeds were planted per jar but thinned to two plants per jar 7 days after establishment. The Leonard jar assemblies were then wrapped with white moisture proof paper, which acted as an insulating sheath to prevent contamination before the seedlings were inoculated with the whole soil inocula made from the soil samples.

3.2.4: Legume planting, whole soil inoculation and crop husbandry practices

Common bean and cowpea were used as the "trap" hosts for the indigenous rhizobia in the soil samples. The legume seeds were first pre-tested to determine the germination period, which was then used to stagger pre-germination time of seeds to ensure synchronized

germination. After pre-testing, seeds were surface sterilized in 3% sodium hypochlorite solution for 3 –5 minutes, then rinsed in 95% alcohol to remove waxy material on the surface and trapped air, followed by rinsing in at least 6 changes of sterile water. The seeds were then soaked in water and placed in a refrigerator for four hours to imbibe water. Thereafter the seeds were placed in a germination chamber for pre-germination. Overcrowding during pre-germination was avoided to reduce the risk of cross-contamination of neighbouring seeds. Three to four healthy seedlings were planted in every Leonard jar then inoculated with the diluted soil samples and later thinned to 2 plants per jar seven days after emergence.

The whole soil inocula was diluted to 10^{-1} by suspending 20 grams of each soil sample in 80 ml of sterile water and then shaking for 15 to 20 minutes with a wrist shaker. Other dilution series ranging from 2^{-1} to 2^{-6} were prepared from the lowest dilution of 10^{-1}. An aliquot of 1 ml of each diluent per seedling was used to inoculate the seedlings grown in the Leonard jars after emergence. Application and checking of levels of nitrogen–free nutrient solutions, was done on daily basis to ensure that the seedlings received adequate moisture.

3.2.5: Experimental design and treatments

The materials comprised Leonard jars filled with sterilized sand used as the growth medium, sterilized common bean and cowpea seeds used as the "trap" hosts, nitrogen-free nutrient solutions and soil inocula (soil sample diluents). The treatments comprised common bean (GLP-2) and cowpea (M66) used as "trap" host plants and five soil samples each with 7 dilution levels (from 10^{-1} then 2^{-1} to 2^{-6}). A positive control (rhizobia inoculation) and a

negative control treatment (no inoculation) were also included. The treatments were laid out in a randomized complete block design (RCBD), in a 2 x 5 x 9 factorial arrangement with three replicates. Cowpea (M66) was used as the trap host to check the abundance of indigenous cross-nodulating *Bradyrhizobium* spp which nodulate lablab, cowpea, pigeon pea, green gram, and lima bean while common bean (GLP-2) was used to determine the abundance of common bean *Rhizobium* spp. in the soil samples. A total of 90 Leonard jars spaced 15 cm apart were arranged in each of the three blocks. The blocks were spaced 1 m apart. The total number of experimental units was 270 Leonard jars.

3.2.6: Data collection

The experiment was terminated 35 days after planting. The roots were carefully washed with tap water to remove sand, and then the attached foam material and the wick carefully removed taking care not to destroy the roots and nodules. Nodules were counted then carefully removed using a pair of tweezers, oven dried at 60^0C for 48 hours and then weighed. The vegetative parts of the plants (shoot) were separated from the subterranean parts of the plants (root) and separately oven-dried at 60^0C for 48 hours and their respective biomass determined. The amount of cowpea tissue-N in the shoots and the roots was determined using Kjeldahl method at the Kenya Agricultural Research Institute's National Agricultural Research Laboratories.

3.2.7: Data analysis

Analysis of variance (ANOVA) was performed on the number of nodules, nodule dry matter, shoot dry matter and root dry matter using General Statistics (GENSTAT®) package (Rothamstead Experimental Station, 1995). When the F test was significant, treatment means of the parameters were separated by the least significant differences (LSD) test at 5% probability level (Steel and Torrie, 1980; Matata *et al.*, 2001). Pearson correlation coefficients, determined using SPSS statistical package, were used to assess the relationship among soil physicochemical properties, number of nodules, nodule dry matter, the number of rhizobial cells per gram of dry soil and cowpea tissue nitrogen.

The abundance of indigenous rhizobia in the soil samples was determined by the most probable number technique (Somasegaran and Hoben, 1994). The most probable number (MPN) per gram of dry soil was determined by the formula:

$$MPN = \frac{m \times d}{v}$$

Where:

v = volume of aliquot used or applied to the plant;

m = value from MPN table based on the number of replications (n) and the number of dilution steps used (s);

d = the lowest dilution used.

CHAPTER FOUR: RESULTS

4.1: Effect of rhizobia inoculation and N-fertilizer on nodulation, growth, yield and yield components of grain legumes

4.1.1: Effect on nodulation

At four weeks after emergence (WAE), N-fertilizer application suppressed nodule number per plant while rhizobia inoculation had no significant effect ($P \leq 0.05$) on this parameter in the long rains (Table 1a). However, both N-fertilizer application and rhizobia inoculation had no significant effect on this parameter in the short rains (Table 1b). At 6 WAE, the two treatments had no significant effect on the legume species nodule numbers in the two seasons (Tables 2a and 2b). Legume species significantly affected number of nodules per plant in both seasons at 4 and 6 WAE, but their interaction with the sources of nitrogen was not significant (Appendices 4-7).

Common bean had the highest number of nodules and nodule dry matter per plant while lima bean had generally the least nodule count and nodule biomass per plant compared to the other legumes in the long rains (Tables 1a and 2a). On average, common bean had at least 11 times the number of nodules recorded in lima bean and about 2-3 times as many nodules recorded with lablab, green gram and cowpea during the long rains at both 4 and 6 WAE. The number of nodules per plant recorded in common bean was not significantly different from number of nodules per plant recorded in cowpea, lablab and pigeon pea in the short rains.

Table 1a: Mean nodule count per plant of grain legumes inoculated with rhizobia and supplied with N-fertilizer sampled at 4 WAE during the long rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	7.6	4.5	5.4	1.5	5.6	13.3	6.3_x
Fertilizer	4.9	3.0	4.0	0.4	2.7	7.9	3.8_y
Control	5.3	4.9	8.0	1.1	6.2	12.6	6.4_x
Mean	5.9^b	4.1^{bc}	5.8^b	1.0^c	4.8^b	11.3^a	5.5

$LSD_{0.05}$ (Legume) = 3.4 CV = 23.3 % $Lsd_{0.05}$ (N source) =1.5 CV = 33.6 %

Within each row, means followed by the same superscript letter (a, b, c), and within each column, means followed by the same subscript letter (x, y, z), are not significantly different at P≤0.05.

Table 1b: Mean nodule count per plant of grain legumes inoculated with rhizobia and supplied with N-fertilizer sampled at 4 WAE during the short rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	6.6	5.7	1.1	0.0	5.0	7.9	4.4
Fertilizer	3.3	2.6	2.3	0.0	6.0	6.4	3.4
Control	6.9	2.8	0.8	0.0	5.6	4.4	3.4
Mean	5.6^a	3.7^{ab}	1.4^b	0.0^b	5.5^a	6.3^a	3.7

$LSD_{0.05}$ (Legume) = 3.8 CV = 7.7 %,

Within each row, means followed by same superscript letter (a, b, c) are not significantly different at P≤0.05.

Table 2a: Mean nodule count per plant of grain legumes inoculated with rhizobia and supplied with N-fertilizer sampled at 6 WAE during the long rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	7.2	4.0	2.9	0.4	7.6	13.9	6.0
Fertilizer	4.0	4.1	2.9	0.3	3.1	8.7	3.9
Control	3.7	3.6	5.3	0.3	7.0	8.7	4.8
Mean	5.0^{b}	3.9^{bc}	3.7^{c}	0.4^{d}	5.9^{b}	10.4^{a}	4.9

$LSD_{0.05}$ (Legume) = 2.0 CV = 13.2 %,

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at P≤0.05.

Table 2b: Mean nodule count per plant of grain legumes inoculated with rhizobia and supplied with N-fertilizer sampled at 6 WAE during the short rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	32.4	17.6	22.6	1.6	22.6	40.7	22.9
Fertilizer	32.1	11.7	15.9	2.6	20.6	21.6	17.4
Control	29.1	18.9	19.4	1.4	20.6	40.0	21.6
Mean	31.2^{a}	16.0^{b}	19.3^{b}	1.9^{c}	21.2^{b}	34.1^{a}	20.6

$LSD_{0.05}$ (Legume) = 7.7 CV = 3.9 %,

Within each row, means followed by same superscript letter (a, b, c) are not significantly different at P≤0.05.

At 4 and 6 WAE, the differences among the legumes in nodule dry matter per plant were highly significant ($P \leq 0.05$) in the two seasons (Appendices 8-11). The effect of sources of nitrogen was significant at 4 WAE during the long rains (Appendix 8) and at 6 WAE during the short rains (Appendix 11). However, the differences among the interactions in nodule dry matter were significant at 4 WAE in both seasons (Appendices 8 and 9). Rhizobia inoculation significantly improved nodule dry matter per plant for common bean compared to control and N-fertilizer in both seasons at 4 WAE (Tables 3a and 3b). At 6 WAE, rhizobia inoculation significantly improved nodule dry matter in the short rains but had no effect in the long rains (Tables 4a and 4b). Starter-N fertilizer had no effect on nodule dry matter of legumes in both seasons.

All inoculated common bean plants were not significantly different in nodule dry matter per plant compared to the other inoculated legume species in both seasons at both 4 and 6 WAE (Tables 3a, b and 4a, b). Lima bean in most cases had significantly the lowest nodule dry matter during the short rains. At 6 WAE, in the long rains, common bean and lablab were not significantly different in nodule dry matter values, which were however, significantly higher than the nodule dry matter values for cowpea, lima bean, green gram and pigeon pea (Table 4a). Similar observations were made in the short rains except that lablab outperformed all the other legumes, and lima bean and pigeon pea had the lowest nodule dry matter (Table 4b). At 4 WAE during the long rains, on average, common bean had over 10 times more nodule dry matter than lima bean and 2½ times more nodule dry matter than either lablab, green grain or pigeon pea nodule dry matter per plant (Table 3a).

able 3a: Mean nodule dry matter (mg) per plant of six legumes inoculated with rhizobia and supplied with N-fertilizer sampled at 4 WAE in the long rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	$16.7^{bc}{}_{x}$	$25.3^{bc}{}_{x}$	$27.3^{bc}{}_{x}$	$6.7^{c}{}_{x}$	$19.0^{bc}{}_{x}$	$98.7^{a}{}_{x}$	32.3
Fertilizer	$18.7^{b}{}_{x}$	$16.3^{b}{}_{x}$	$23.3^{ab}{}_{x}$	$4.3^{b}{}_{x}$	$13.2^{b}{}_{x}$	$39.0^{a}{}_{z}$	19.0
Control	$8.7^{b}{}_{x}$	$26.7^{b}{}_{x}$	$20.0^{b}{}_{x}$	$4.3^{c}{}_{x}$	$15.7^{b}{}_{x}$	$62.3^{a}{}_{y}$	22.9
Mean	14.7	22.8	23.6	5.1	15.7	66.7	24.7

$LSD_{0.05}$ (Legume X N source) = 20.2; CV = 48.3%

Within each row, means followed by the same superscript letter (a, b, c) and within each column, subscript letter (x, y, z) are not significantly different at $P \leq 0.05$.

Table 3b: Mean nodule dry matter (mg) per plant of six legumes inoculated with rhizobia and supplied with N-fertilizer sampled at 4 WAE in the short rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	$86.7^{b}{}_{x}$	$66.7^{bc}{}_{x}$	$56.7^{cd}{}_{x}$	$0.0^{e}{}_{x}$	$33.3^{d}{}_{x}$	$130.0^{a}{}_{x}$	62.2
Fertilizer	$80.0^{x}{}_{a}$	$60.0^{ab}{}_{y}$	$70.0^{ab}{}_{x}$	$0.0^{c}{}_{x}$	$53.3^{b}{}_{x}$	$60.0^{ab}{}_{y}$	53.9
Control	$83.8^{a}{}_{x}$	$90.0^{a}{}_{x}$	$53.3^{b}{}_{x}$	$0.0^{c}{}_{x}$	$53.3^{b}{}_{x}$	$70.0^{ab}{}_{y}$	58.3
Mean	83.3	72.2	60.0	0.0	46.7	86.7	58.1

$LSD_{0.05}$ (Legume X N source) = 24.1; CV = 24.6%

Within each row, means followed by the same superscript letter (a, b, c), and within each column, means followed by the same subscript letter (x, y, z), are not significantly different at $P \leq 0.05$.

Table 4a: Mean nodule dry matter (mg) per plant of six legumes inoculated with rhizobia and supplied with N-fertilizer sampled at 6 WAE in the long rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	32.0	176.7	35.7	6.3	32.3	104.3	64.6
Fertilizer	22.3	111.0	30.3	2.0	36.7	121.0	53.9
Control	20.0	159.0	49.0	3.3	31.3	103.3	61.0
Mean	24.8^{b}	148.9^{a}	38.3^{b}	3.9^{b}	33.4^{b}	109.6^{a}	59.8

$LSD_{0.05}$ (Legume) = 58.2 CV= 30.9%

Within each row, means followed by the same superscript letter (a,b,c) are not significantly different at $P \leq 0.05$.

Table 4b: Mean nodule dry matter (mg) per plant of six legume species inoculated with rhizobia and supplied with N-fertilizer sampled at 6 WAE during the short rain season

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	130.3	257.7	100.3	8.7	34.7	84.7	102.7_{x}
Fertilizer	76.7	166.7	88.0	12.3	26.7	69.0	73.2_{y}
Control	72.3	183.3	103.0	7.7	32.3	63.3	77.0_{y}
Mean	93.1^{b}	202.6^{a}	97.1^{b}	9.6^{c}	31.2^{c}	72.3^{b}	84.3

$LSD_{0.05}$ (Legume) = 39.6 CV =16.7% $Lsd_{0.05}$ (N source) = 16.7 CV = 25.8%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at $P \leq 0.05$.

4.1.2: Effect on biomass accumulation

At both 4 and 6 WAE, rhizobia inoculation or N-fertilizer application had no significant ($P \leq 0.05$) effect on shoot dry matter per plant in both seasons (Tables 5a, b and 6a, b). However, the differences among legume species in shoot dry matter were highly significant in the two seasons (Appendices 12 -15). At both 4 and 6 WAE, common bean produced significantly the highest shoot dry matter per plant compared to the shoot dry matter of the other legume species in the two seasons. At 6 WAE, cowpea, lablab and pigeon pea shoot dry matter per plant was the lowest compared to the other legume species in both seasons (Tables 6a and 6b). At 4 WAE, the shoot dry matter of cowpea, lablab and pigeon pea and those of cowpea, green gram and lima bean were not significantly different in the long rains (Table 5a). Similar observations were made at 6 WAE in the short rains with the exception of cowpea shoot dry matter per plant, which was significantly different from green gram and lima bean (Table 6b).

Table 5a: Mean shoot dry matter (g/m^2) per plant of six legume species inoculated with rhizobia and supplied with N-fertilizer sampled at 4WAE during the long rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	3.10	2.13	8.37	9.27	2.53	48.30	12.28
Fertilizer	3.87	2.43	11.13	9.77	2.30	37.97	11.24
Control	3.10	1.73	9.13	10.13	2.20	32.53	9.64
Mean	3.03^{bc}	2.10^{c}	9.54^{b}	9.72^{b}	2.34^{c}	39.60^{a}	11.06

$LSD_{0.05}$ (Legume) = 7.00 CV = 34.8%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at $P \leq 0.05$.

Table 5b: Mean shoot dry matter (g/m^2) per plant of six legume species inoculated with rhizobia and supplied with N-fertilizer sampled at 4WAE during the short rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	10.40	2.00	32.80	12.67	5.77	57.70	20.22
Fertilizer	7.87	4.07	24.77	12.80	8.50	52.17	18.36
Control	5.00	3.97	29.20	17.60	4.20	40.67	16.77
Mean	7.76^{c}	3.34^{c}	28.92^{b}	14.36^{c}	6.16^{c}	50.18^{a}	18.45

$LSD_{0.05}$ (Legume) = 13.26 CV = 39.50%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at $P \leq 0.05$.

Table 6a: Mean shoot dry matter (g/m^2) per plant of six legume species inoculated with rhizobia and supplied with N-fertilizer sampled at 6 WAE during the long rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	11.60	7.80	26.50	24.87	5.40	86.60	27.13
Fertilizer	9.90	9.23	30.17	29.43	6.17	81.10	27.67
Control	8.93	7.43	31.67	26.37	4.37	73.63	25.40
Mean	10.14^{c}	8.16^{c}	29.44^{b}	26.89^{b}	5.31^{c}	80.44^{a}	26.73

$LSD_{0.05}$ (Legume) = 8.67 CV = 17.8%

Within each row, means followed by the same superscript letters (a, b, c) are not significantly different at $P \leq 0.05$.

Table 6b: Mean shoot dry matter (g/m^2) per plant of six legume species inoculated with rhizobia and supplied with N-fertilizer sampled at 6 WAE during the short rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	92.4	67.3	173.2	162.7	28.3	482.2	167.7
Fertilizer	65.3	75.7	252.8	168.5	23.9	405.1	165.2
Control	88.7	53.5	236.1	167.7	18.3	377.0	156.9
Mean	82.1^{c}	65.5^{c}	220.7^{b}	166.3^{b}	23.5^{c}	421.4^{a}	163.3

$LSD_{0.05}$ (Legume) = 106.14 CV = 35.70%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at $P \leq 0.05$.

During the sampling done at both 4 and 6 WAE, rhizobia inoculation and starter-N application did not significantly ($P \leq 0.05$) influence root dry matter per plant (Tables 7a, b and 8a, b). However, application of starter-N suppressed root dry matter per plant in common bean while rhizobia inoculation improved root dry matter in lablab at 6 WAE in the long rains (Table 8a). The differences among the legume species in root dry matter per plant were highly significant during the two seasons (Appendices 16-19). Common bean yielded significantly the highest root dry matter per plant in both seasons at both sampling times but was not significantly different from lablab root dry matter per plant at 6 WAE in the short rains. Green gram and pigeon pea produced significantly the lowest root dry matter per plant compared to the rest of the legumes in the two seasons but were not significantly different from cowpea root dry matter per plant during the short rains at 4 WAE (Table 7b). The root dry matter values per plant in lima bean and lablab were not significantly different during the two seasons.

Table 7a: Mean root dry matter (mg) per plant of six legume species inoculated with rhizobia and supplied with N-fertilizer sampled at 4 WAE during the long rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	157.7	151.0	46.7	162.3	54.3	278.7	141.8
Fertilizer	94.3	144.3	55.7	168.0	40.0	327.7	138.3
Control	63.3	93.3	61.0	118.7	31.3	296.7	110.7
Mean	105.1^{b}	129.6^{b}	54.4^{c}	149.7^{b}	41.9^{c}	301.0^{a}	130.3

$LSD_{0.05}$ (Legume) = 49.5 CV =16.5%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at P≤0.05.

Table 7b: Mean root dry matter (mg) per plant of six legume species inoculated with rhizobia and supplied with N-fertilizer sampled at 4 WAE during the short rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	20.0	73.4	11.1	34.4	21.1	118.9	46.5
Fertilizer	27.8	41.1	43.3	36.8	6.6	69.7	42.1
Control	20.4	77.8	24.4	46.7	30.0	92.2	48.6
Mean	22.7^{c}	64.1^{b}	26.3^{c}	39.3^{b}	19.3^{c}	102.6^{a}	45.7

$LSD_{0.05}$ (Legume) = 28.9 CV = 23.4%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at P≤0.05.

Table 8a: Mean root dry matter (mg) per plant of six legume species inoculated with rhizobia and supplied with N-fertilizer sampled at 6 WAE in the long rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	$335.0^{c}{}_{x}$	$486.3^{b}{}_{x}$	$34.3^{d}{}_{x}$	$286.7^{c}{}_{y}$	$116.7^{d}{}_{x}$	$624.3^{a}{}_{x}$	313.9
Fertilizer	$271.^{0cx}$	$332.3^{bc}{}_{y}$	$52.3^{d}{}_{x}$	$415.7^{ab}{}_{x}$	$67.7^{d}{}_{x}$	$486.3^{a}{}_{y}$	270.9
Control	$225.7^{b}{}_{x}$	$304.3^{b}{}_{y}$	54.3_{x}	$324.3^{b}{}_{x}$	$63.3^{c}{}_{x}$	$596.7^{a}{}_{x}$	261.4
Mean	277.2	374.3	47.0	342.2	82.6	569.1	282.1

$LSD_{0.05}$ (Legume x N source) = 113.4 CV = 23.8%

Within each row, means followed by the same superscript letter (a, b, c), and within each column, means followed by the same subscript letter (x, y, z), are not significantly different at $P \leq 0.05$.

Table 8b: Mean root dry matter (mg) per plant of six legumes inoculated with rhizobia and supplied with N-fertilizer sampled at 6 WAE in the short rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	382.0	466.0	249.0	311.0	86.0	574.0	345.0
Fertilizer	464.0	459.0	214.0	389.0	107.0	501.0	356.0
Control	450.0	428.0	306.0	412.0	79.0	474.0	356.0
Mean	432.0^{b}	451.0^{ab}	256.0^{cd}	371.0^{bc}	90.0^{d}	517.0^{a}	353.0

$LSD_{0.05}$ (Legume) = 166.2 CV = 7.5%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at $P \leq 0.05$.

4.1.3: Effect on yield and yield components

Rhizobia inoculation and starter-N did not significantly affect the legumes species grain yield in the two seasons (Appendices 20 and 21). However, there were significant differences ($P \leq 0.05$) in grain yield among the legume species in both seasons (Tables 9a and 9b). In the long rains, lima bean produced the highest grain yield followed by common bean which significantly out-yielded cowpea, lablab, green gram and pigeon pea. The latter four legume species were not significantly different in their grain yield in the long rains but cowpea significantly out-yielded lablab, which in turn out-yielded green gram and pigeon pea in the short rains. In the short rains, common bean had the highest grain yield followed by lima bean which in turn out-yielded cowpea grain yield. The mean grain yield varied from 148 to 1472 kg ha^{-1} in long rains and from 214 to 4398 kg ha^{-1} in the short rains.

The number of pods per plant was not significantly ($P \leq 0.05$) affected by rhizobia inoculation or N-fertilizer treatments in both seasons. Only the difference in number of pods per plant among the legume species was significant during the two seasons (Appendices 22 and 23). Lablab out-preformed the other legumes in number of pods per plant in both seasons while common bean registered the lowest number of pods per plant during the long rains, while green gram registered the lowest number of pods per plant in the short rains (Tables 10a and 10b).

There was highly significant ($P \leq 0.05$) difference in number of grains per pod among the legume species in the two seasons (Appendices 24 and 25). However, rhizobia inoculation or N-fertilizer treatments had no significant effect on the number of grains per pod in both seasons (Tables 11a and 11b). Common bean out-performed the rest of the legumes in number of grains per pod in the two seasons, but was not significantly different from green gram in this attribute

ring the short rains. The number of grains per pod was statistically similar among green am, pigeon pea and lablab in the long rains and pigeon pea, lablab and cowpea in the short ins.

able 9a: Mean grain yield (kg/ha) at harvest of six legume species inoculated with rhizobia and supplied with N-fertilizer during the long rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	306.0	394.0	206.0	1350.0	100.0	967.0	554.0
Fertilizer	383.0	500.0	211.0	1689.0	150.0	778.0	619.0
Control	294.0	283.0	172.0	1378.0	194.0	850.0	529.0
Mean	328.0^{c}	393.0^{c}	196.0^{c}	1472.0^{a}	148.0^{c}	865.0^{b}	567.0

$LSD_{0.05}$ (Legume) = 298.2 CV = 28.9%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at P≤0.05.

Table 9b: Mean grain yield (kg/ha) at harvest of six legume species inoculated with rhizobia and supplied with N-fertilizer during the short rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	3050.0	1979.0	304.0	3462.0	228.0	4524.0	2258.0
Fertilizer	2744.0	1760.0	300.0	3706.0	149.0	4518.0	2169.0
Control	2804.0	2547.0	314.0	3429.0	265.0	4151.0	2252.0
Mean	2866.0^{c}	2095.0^{d}	306.0^{e}	3532.0^{b}	214.0^{e}	4398.0^{a}	2235.0

$LSD_{0.05}$ (Legume) = 311.5 CV = 7.7%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at P≤0.05.

Table 10a: Mean number of pods per plant at harvest of six legume species inoculated with rhizobia and supplied with N-fertilizer during the long rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	18.3	57.3	15.3	18.0	10.8	5.7	20.9
Fertilizer	14.7	70.7	15.3	25.3	12.5	4.7	23.9
Control	18.7	53.3	14.1	19.3	14.6	4.3	20.7
Mean	17.2^{bc}	60.4^{a}	14.9^{bc}	20.9^{b}	12.6^{c}	4.9^{d}	21.8

$LSD_{0.05}$ (Legume) = 6.8 CV = 5.2%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at $P \leq 0.05$.

Table 10b: Mean number of pods per plant at harvest of six legume species inoculated with rhizobia and supplied with N-fertilizer during the short rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	21.9	114.7	5.2	17.6	29.2	5.0	32.3
Fertilizer	18.0	128.3	4.8	18.5	26.6	5.2	33.6
Control	18.1	102.7	4.7	14.4	32.1	4.9	29.5
Mean	19.3^{c}	115.2^{a}	4.9^{d}	16.8^{c}	29.3^{b}	5.1^{d}	31.7

$Lsd_{0.05}$ (Legume) = 6.7 CV = 2.7%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at $P \leq 0.05$.

Table 11a: Mean number of grains per pod per plant at harvest of six legume species inoculated with rhizobia and supplied with N-fertilizer during the long rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	2.7	1.2	1.4	2.5	1.5	3.3	2.1
Fertilizer	2.7	1.3	1.2	2.1	1.6	3.4	2.1
Control	2.6	1.1	1.2	2.5	1.7	3.5	2.1
Mean	2.7^{b}	1.2^{c}	1.3^{c}	2.4^{b}	1.6^{c}	3.4^{a}	2.1

$LSD_{0.05}$ (Legume) = 0.5 CV = 19.3%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at $P \leq 0.05$.

Table 11b: Mean number of grains per pod per plant at harvest of six legume species inoculated with rhizobia and supplied with N-fertilizer during the short rains

	Cowpea	Lablab	Green gram	Lima bean	Pigeon pea	Common bean	Mean
Rhizobia	4.2	3.2	5.2	2.6	4.0	5.0	4.0
Fertilizer	4.0	3.0	4.8	2.4	3.4	5.3	3.8
Control	3.9	3.0	4.7	2.5	3.5	4.9	3.8
Mean	4.1^{c}	3.1^{de}	4.9^{ab}	2.5^{e}	3.6^{cd}	5.1^{a}	3.9

$LSD_{0.05}$ (Legume) = 0.8 CV = 0.5%

Within each row, means followed by the same superscript letter (a, b, c) are not significantly different at $P \leq 0.05$.

4.2: Abundance of indigenous rhizobia in the field sites

4.2.1: Population size of indigenous rhizobia in the field sites

All the soil samples collected from the fives field sites contained common bean and cowpea nodulating indigenous rhizobia. The population size of indigenous rhizobia in the field sites varied from 79 to more than 900 bacterial cells per gram of dry soil (Table 12). When common bean was used as the trap host, all the field sites had more than 900 bacterial cells per gram of dry soil (g^{-1} soil). A similar number of bacterial cells g^{-1} soil was observed when cowpea was inoculated with soils from Kajiado and Machakos field sites. The lowest number of bacterial cells per g^{-1} soil was recorded when cowpea was inoculated with the soil from Nyeri field site. Cultivated Kabete field site recorded a higher population size of indigenous cowpea rhizobia than the uncultivated Kabete field site.

Table 12: Number of rhizobial bacteria cells g^{-1} soil in the soil samples collected from five field sites in central Kenya.

	Field sites				
Trap host	Kabete cultivated	Kabete uncultivated	Kajiado	Nyeri	Machakos
Common bean	$>9.0 \times 10^2$	$>9.0 \times 10^2$	$>9.0 \times 10^2$	$>9.0 \times 10^2$	$>9.0 \times 10^2$
Cowpea	3.1×10^2	2.1×10^2	$>9.0 \times 10^2$	7.9×10^1	$>9.0 \times 10^2$

2.2: Nodulation of cowpea and common bean inoculated with soils from the field sites in Central Kenya

The interactive effects of the field sites and the legume species on nodule count per ant were highly significant (Appendix 26). Common bean produced higher number of odules per plant than cowpea when inoculated with soils from Kajiado and Kabete (cultivated nd uncultivated) field sites. However, there was no significant ($P \leq 0.05$) difference in nodule umbers between the two legumes when inoculated with soils from Nyeri and Machakos field tes (Table 13).

Cowpea produced the highest number of nodules per plant when inoculated with soil om Machakos site than when inoculated with soils from the other sites. Common bean noculated with soil from Kajiado site produced more nodules per plant than those inoculated ith soils from Nyeri, Machakos and uncultivated Kabete sites. There was, however, no gnificant difference between common beans inoculated with soils from Kajiado and ultivated Kabete field sites.

Rhizobia inoculated common bean had fewer nodules per plant than those inoculated ith soils from Kajiado and Kabete cultivated sites. However, there was no significant ifference between rhizobia inoculated common bean and those inoculated with soils from Nyeri, Kajiado, Machakos and Kabete uncultivated sites. Inoculation of cowpea with soil from Machakos field site produced more root nodules per plant than cowpea inoculated with soils rom the other four sites. There was no significant difference in number of nodules per plant when cowpea was inoculated with soils from Kajiado, Nyeri and Kabete (cultivated and uncultivated) sites.

The interactive effects of the field sites and the legume species on nodule dry matter per ant were also highly significant at P≤0.05 (Appendix 27). Common bean produced higher odule dry matter per plant than cowpea when inoculated with soils from cultivated Kabete and ajiado field sites (Table 14) while cowpea nodule dry matter per plant was higher than ommon bean when inoculated with soil from uncultivated Kabete field site. However, there as no significant difference in the nodule dry matter per plant between the two legumes when noculated with soils from Machakos or Nyeri field sites.

Table 13: Mean number of nodules per plant of common bean and cowpea inoculated with whole soil inocula made from soils collected from five field sites

	Kabete cultivated	Kabete uncultivated	Kajiado	Machakos	Nyeri	Rhizobia inoculated	Un-inoculated	Mean
Common bean	$36.4^{ab}{}_{x}$	$28.0^{bc}{}_{x}$	$44.8^{a}{}_{x}$	$19.6^{cd}{}_{x}$	$7.3^{de}{}_{x}$	$15.3^{c}{}_{y}$	$0.0^{e}{}_{x}$	21.6
Cowpea	$7.3^{c}{}_{y}$	$3.0^{c}{}_{y}$	$5.7^{c}{}_{y}$	$22.6^{b}{}_{x}$	$0.6^{c}{}_{x}$	$38.0^{a}{}_{x}$	$0.0^{c}{}_{x}$	11.0
Mean	21.8	15.5	25.3	21.1	3.9	26.7	0.0	16.3

$LSD_{0.05}$ (Legume X Site) = 13.71 CV = 50.0%.

Within each row, means followed by the same superscript letter (a, b, c), and within each column, means followed by the same subscript letter (x, y, z), are not significantly different at P≤0.05.

Table 14: Mean nodule dry matter (mg) per plant of common bean and cowpea inoculated with soil samples collected from five field sites

	Kabete cultivated	Kabete uncultivated	Kajiado	Machakos	Nyeri	Rhizobia inoculated	Un-inoculated	Mean
Common bean	$120^{a}{}_{x}$	$20^{cd}{}_{y}$	$60^{b}{}_{x}$	$60^{b}{}_{x}$	$10^{c}{}_{x}$	$30^{c}{}_{y}$	$0^{d}{}_{x}$	40
Cowpea	$30^{b}{}_{y}$	$80^{a}{}_{x}$	$30^{b}{}_{y}$	$60^{a}{}_{x}$	$10^{bc}{}_{x}$	$70^{a}{}_{x}$	$0^{c}{}_{x}$	40
Mean	80	50	40	60	10	50	0	40

$Lsd_{0.05}$ (Legume X Site) = 20 CV = 32.3%

Within each row, means followed by the same superscript letter (a, b, c), and within each column, means followed by the same subscript letter (x, y, z), are not significantly different at P≤0.05.

2.3: Common bean and cowpea biomass accumulation, and cowpea shoot tissue-N content

The interactive effects of field sites and legume species were highly significant ($P \leq 0.05$) (Appendix 28). Common bean shoot dry matter per plant was generally higher (about 2 times) than cowpea shoot dry matter per plant but not significantly different (Table 15). However, cowpea inoculated with soils from Kajiado and Machakos sites had significantly higher shoot dry matter per plant than common bean inoculated with the same soils. The highest shoot dry matter per plant of the legume species was recorded when the legumes were inoculated with the commercial rhizobia. Common bean recorded the lowest shoot dry matter per plant when inoculated with soils from Nyeri site but was only significant different from Machakos site (Table 15). The shoot dry matter per plant recorded with cowpea was not significantly different among the five soils samples.

Table 15: Mean shoot dry matter (g) per plant of common bean and cowpea inoculated with the soil samples collected from five field sites.

	Kabete cultivated	Kabete uncultivated	Kajiado	Machakos	Nyeri	Rhizobia inoculated	Un-inoculated	Mean
Common bean	$0.57^{bc}{}_{x}$	$0.57^{bc}{}_{x}$	$0.68^{bc}{}_{x}$	$0.69^{b}{}_{x}$	$0.45^{c}{}_{x}$	$1.23^{a}{}_{x}$	$0.13^{d}{}_{x}$	0.62
Cowpea	$0.34^{b}{}_{x}$	$0.36^{b}{}_{x}$	$0.37^{b}{}_{y}$	$0.37^{b}{}_{y}$	$0.34^{b}{}_{x}$	$0.68^{a}{}_{y}$	$0.22^{b}{}_{x}$	0.38
Mean	0.467	0.465	0.527	0.528	0.392	0.975	0.17	0.50

$LSD_{0.05}$ (Legume X Site) = 0.23 CV = 22.9%

Within each row, means followed by the same superscript letter (a, b, c), and within each column, means followed by the same subscript letter (x, y, z), are not significantly different at $P \leq 0.05$.

The amount of cowpea fixed tissue-N (mg/plant) was highly significant (Appendix 29). The highest amount of accumulated fixed shoot tissue-N (mg/plant) was observed when cowpea was inoculated with rhizobia inoculant and with soil from Machakos site (Table 16). Cowpea inoculated with soils from Nyeri, Kajiado and Kabete (Cultivated and uncultivated) field sites and those that were not inoculated had no significant difference in amount of accumulated fixed shoot tissue-N.

Table 16: Average shoot tissue-N (mg/plant) accumulated by cowpea inoculated with soils collected from four field sites in central Kenya

	Kabete cultivated	Kabete uncultivated	Kajiado	Nyeri	Machakos	Rhizobia inoculated	Control
Tissue-N (mg/plant)	54^{b}	60^{b}	65^{b}	73^{b}	115^{a}	130^{a}	59^{b}
% Tissue N	1.04	1.07	1.11	1.45	2.02	1.27	1.14

$LSD_{0.05}$ =31 CV=21.1%

Within each row, means followed by the superscript letter (a, b, c) are not significantly different at P≤0.05.

4.2.4: Relationship among soil chemical properties, nodulation, biomass accumulation, rhizobia cells g^{-1} soil, and plant tissue nitrogen content

The Pearson correlation coefficients relating rhizobial bacteria cells g^{-1} soil to soil carbon, nitrogen, pH, electrical conductivity, and phosphorous demonstrated non-significant direct and inverse correlations (Tables 17a and 17b). A significant positive correlation (r=0.965, P≤0.05) was demonstrated between cowpea fixed tissue-N and soil carbon (Table 17a). However, the other soil physicochemical properties demonstrated non-significant direct and inverse correlations.

ble 17a: Pearson correlation coefficients relating cowpea nodule count, nodule biomass, shoot biomass, shoot fixed tissue-N, rhizobia cell g^{-1} soil to soil chemical characteristics

	Soil carbon (%)	Soil nitrogen (%)	EC dsm^{-1}	Soil pH	Phosphorous (ppm)
Nodule count	-0.674	-0.159	0.778	0.667	0.823
Nodule dry matter (mg)	-0.556	-0.226	0.809	0.712	0.856
Shoot dry matter (g)	0.444	-0.684	0.438	0.651	0.616
Rhizobia cell	-0.063	0.091	0.678	0.708	0.852
Shoot tissue-N (g)	0.965**	-0.642	0.064	0.214	-0.049

able 17b: Pearson correlation coefficients relating common bean nodule count, nodule biomass, shoot biomass, rhizobial bacteria cell g^{-1} to soil chemical characteristics

	Soil carbon (%)	Soil nitrogen (%)	EC dsm^{-1}	Soil pH	Phosphorous (ppm)
Nodule count	0.714	0.870	0.537	0.628	0.348
Nodule dry matter (mg)	0.220	0.426	0.706	0.665	0.337
Shoot dry matter (g)	-0.124	-0.176	0.293	0.266	0.535
Rhizobia cell	-0.960	-0.962	-0.951	-0.976	-0.885

* r is significant at the P=0.05 level; ** r is significant at the P= 0.01 level.

CHAPTER FIVE: DISCUSSION

1.1: Effect of rhizobia inoculation and N-fertilizer on nodulation

Rhizobia inoculation improved nodule biomass in common bean, lablab and cowpea gume species over the two seasons, but had no effect on nodule count in all the legumes. ack of response to rhizobial inoculation by the legume species implied that there might have een adequate population of indigenous rhizobia in the soil. The improved nodule biomass uggested that the inoculant strain used was more effective than the indigenous soil rhizobia rains. Common bean and cowpea are the most important and widely grown legumes in Kenya Ortiz and Crouch, 2001; GOK, 1998; Gethi *et al.,* 1997; Wortmann and Allen, 1994).

Many studies may have been done on rhizobia inoculations resulting to isolation of ighly compatible rhizobia strains. Bordeleau and Prevost (1994) reported improved nodulation f soybean with inoculation in soils low in rhizobia populations. Similar observations were nade by Abdelgani *et al.* (2002) on inoculated cluster bean grown in soil low in rhizobia opulation. When effective rhizobia are absent or soil population level is low, inoculation of legumes have been found to be necessary (Amarger, 2001 and Giller, 2001). Adjei, *et al.* (2004) reported that rhizobia inoculation was essential to seed or soil at or before planting when proper kinds of rhizobia are absent in the soil. Wallace, (2002) observed that inoculation was necessary when introducing legumes or when the soil conditions are likely to cause decreased rhizobial population levels in the soil.

Lima bean, green gram and pigeon pea leguminous species responded poorly to *Bradyrhizobium* spp inoculation. Lima bean had the poorest nodulation level compared to the

ther two legumes. This suggested that either the soil population level of compatible rhizobia as high or the strain used in the inoculum was not compatible with the legume species. The nteractions between *Bradyrhizobium* strains and the compatible legume species are known to e host specific (Qiang *et al.*, 2003). Thies *et al.*, (1991a) reported that inoculation of legumes n soils containing 10 to 100 indigenous rhizobia cells g^{-1} soil improved nodulation while Houngnandan *et al.* (2000) showed that response to inoculation was achieved when the opulation was greater than 5 rhizobia cells g^{-1} of soil.

The population size of rhizobia in cultivated soil at Kabete field site was estimated at 311.6 hizobia cells g^{-1} of soil. This suggested that Kabete soils had adequate population level of ndigenous rhizobia capable of nodulating these legumes hence the poor response to noculation. The population levels of rhizobia capable of nodulating these legume species may have increased over time due to intensive cropping. Studies done in Southern Alberta, (Kucey and Hynes, 1989) reported 100 to 1000 times *Rhizobium Leguminosarum* bv. *phaseoli* in common bean fields than wheat fields. Most of the rhizobia found in the soil following homologous host legume plants probably come from senescing nodules (Thies *et al.*, 1995; Bottomley, 1992). Legumes have also been reported to produce compounds that encourage proliferation of rhizobia strains in the soil. Hirsch, (1996) reported that one nodule can have up to 10^6 viable bacterial cells.

Starter-N fertilizer generally suppressed the nodule count and nodule biomass of common bean, green gram, pigeon pea and lablab over the two seasons. This implied that the legumes utilized the supplied nitrogen in preference of forming nodules and fixing nitrogen (Allos and Barthlomew, 1959) or the nitrogen level was high in the soil. It has been widely reported that

organic nitrogen especially nitrate-N suppressed nodulation of legumes. Studies have shown at nitrate can delay nodulation (Rai, 1992), reduce number of nodules (Herridge *et al.*, 1984) even prevent nodulation to take place (da Silva *et al.*, 1984). Other studies by Giller, (2001), erridge *et al.*, (1984) and (Agboola and Fayemi, 1972) reported suppression of nodulation by -fertilizer application in soils high in nitrogen. Truchet and Dazzo (1982) also reported ecreased binding of rhizobia to root hairs as well as increase in number of aborted infection vents after application of NO_3^{-1} to field grown legume.

However, under conditions of restricted presence of soil-N, a moderate dose of starter-N as been successfully shown to stimulate seedling growth and subsequently N_2-fixation (Goi *et l.*, 1993). Marrero and Guzmand (1986) while studying common bean, and Herridge *et al.* 984) while studying cowpea reported that legumes require at least 30 kg N ha^{-1} in soils low in ineral-N to achieve full nodulation. Legumes require substantial amount of N during the nitrogen hunger period' for their nodule development, shoot and root growth before the onset f N_2-fixation process (Hansen, 1994; Pate and Layzell, 1990).

The seasonal differences in nodulation level varied with the sampling time. At 4 WAE, the esponse to rhizobia inoculation and N-fertilizer application was better during the long rains han short rains. This could be attributed to the higher temperature (20°C) and rainfall (500mm) xperienced during the first five weeks of the long rains compared to the cooler (18.5°C) and ower rainfall (100mm) experienced during the short rains (Appendix 3).

Many factors affect response to inoculation. These include: soil-N (Sanginga, *et al.*, 1997), native rhizobia population in the soil (Thies *et al.*, 1991b), rhizobial strain used in the

oculum (Burton, 1985) and many environmental factors (Vincent, 1988). Giller, (2001) ported that cooler temperatures delay plant development, and nodule formation. Hansen, 994) indicated that N_2-fixation is more temperature sensitive than assimilation of combined . Hansen (1994) and Somasegaran and Hoben, (1994) also reported that N_2-fixation is subject a specific temperature optimum beyond which water deficits can easily induce additional omplications. The average temperatures at the study site were between 18.5°C and 19°C uring the two seasons (Appendix 3). Optimal growth of most rhizobia strains occurs at a emperature range of 20°-30°C (Somasegaran and Hoben, 1994).

Soil moisture affects rhizobial mobility, multiplication of rhizobia in the rhizosphere, eduction in infection thread formation and retards nodule development (Griffith and Roughley, 992; Worall and Roughley, 1991). Soil moisture depletion can induce premature nodule enescence (Hansen, 1994). If nodules lose more than 20% of their maximum fresh weight, nodule activity is prematurely terminated (Pena-Cabriales and Castellanas, 1993).

At 6 WAE, the differences in the nodulation level between rhizobia inoculated, N-fertilized legumes and control treatments were less than at 4 WAE suggesting nodulation is better done at 4 WAE before flowering and pod-fill stage (Hansen, 1994).

5.1.2: Effect of rhizobia inoculation and starter-N fertilizer on biomass accumulation

Rhizobia inoculation and supply of N-fertilizer had no significant effects on shoot dry matter of the legumes over the two seasons. However, rhizobia inoculation improved root dry matter in lablab but suppressed root dry matter in lima bean while N-fertilizer application suppressed root dry matter in common bean. The results indicated that although rhizobia

noculation generally improved nodulation, this was not translated into improved shoot biomass ccumulation. This implies that the soil mineral-N at the experimental sites could have been dequate and the population level of indigenous rhizobia capable of nodulating these legumes vere also high to support shoot biomass accumulation in legumes. Studies have shown that the oot growth of field grown legumes have been augmented by presence of soil nitrate (Streeter, 998) while inoculation and nodule development reduce root growth (Hansen, 1994). Rahman nd Rastin (2002) reported increase in soybean shoot dry matter, seed yield and total N uptake n the soil poor in N content and low in indigenous rhizobia population after inoculation. Similar findings were reported by Bordeleau and Prevost (1994) on soybean shoot dry matter after inoculation.

Starter-N fertilized lima bean, green gram, cowpea and pigeon pea had relatively higher shoot dry matter than those inoculated with rhizobia but the differences were not significant. The results suggested that legumes require soil-N during growth before the establishment of N_2-fixation process (Hansen, 1994). Harper and Gibson (1984) and Keya *et al.* (1982) also reported that legumes required substantial amount of soil nitrate during growth. Jensen and Sorenson (1988) also reported increase in legume vegetative growth on application of N–fertilizer. Andrew and Eck (1993) observed that N-fertilizer improved vegetative growth which eventually resulted to increased total plant dry matter. A study done on *Phaseolus vulgaris* L. by Dean and Clarke (1980) also reported significant increase in the legume shoot dry matter on N-fertilizer application.

The effects of rhizobia inoculation and N-fertilizer application on legume biomass accumulation were dependent on legume species. Shoot and root dry matter was significantly

igher over the two seasons in common bean compared to other legume species. Pigeon pea ccumulated the least biomass over the two seasons. Pigeon pea is a woody perennial grown as nnual and well adapted to lower altitude areas (Giller, 2001; Rowland, 1993).

5.1.3: Effect of rhizobia inoculation and N-fertilizer on yield and yield components

Rhizobia inoculation and N-fertilizer application had no significant effect on the grain yield of legumes. This implies that improved nodule biomass of legume species was not translated into improved yield. This suggested that the soil nitrogen was high to support yield hence no need to apply N-fertilizer or inoculate legumes with rhizobia for the purpose of improving yield. Olsen *et al.* (1996) observed that inoculation does not always result to positive yield response. Musandu and Ogendo (2002) while studying common bean reported poor grain yield response to rhizobia inoculation. However, significant increase in seed production by 8.7 to 49.5% has been reported on different strain of cluster bean (Abdelgani *et al.*, 2002). Many other factors affect the legume response to inoculation such as soil N (Sanginga, 1998), population size of indigenous rhizobia (Thies *et al.*, 1991b), rhizobia strain used in the inoculum (Burton, 1985) and other environmental factors (Vincent, 1988).

The grain yield was observed to be dependent on the legume species and the season. Lima bean and common bean had the highest grain yield over the two seasons. The high grain yield obtained from lima bean could be attributed to the ability to produce multiple flowers after every onset of rains rather than rhizobia inoculation or N-fertilizer application. Lima bean is also deep rooted hence capable of mining N deep in the soil and also resilient to dry soil moisture conditions (Rowland, 1993). Green gram and pigeon pea produced significantly lower

rain yield compared to the other legumes over the two seasons.

Legume grain yields were higher during the short rains, which received lower but well-istributed rainfall than the long rains, which received higher but poorly distributed rainfall Appendix 3). The temperature differences may have also contributed to the legume species rain yield differences. During the long rains, a dry spell condition was experienced at the lowering of most legumes, which could have caused flower and pod abortion, increased pest nd disease incidences resulting to low grain yield. The higher amount of rainfall and avourable temperatures at flowering could have contributed to the higher yield experienced luring the short rains. Late maturing legumes such as lablab and cowpea produced higher grain ields during the short rains than long rain season. Legumes that produced multiple flowers and were semi determinate such as lima bean, lablab, and cowpea produced higher grain yields during the short rains compared to the other legumes due to improved number of rainy days (Appendix, 3).

5.1.4: Abundance and presence of indigenous rhizobia in the field sites

Soils from all the field sites had more than 900 rhizobia cells g^{-1} soil when common bean was used as "trap" host, while when cowpea was used as "trap" host, the number of rhizobia bacteria cell g^{-1} soil varied from 78.5 for soil from Nyeri to more than 900 for soil from Kajiado and Machakos sites. This provides evidence that indigenous rhizobia nodulating common bean (*Rhizobium* spp) and cowpea nodulating rhizobia (*Bradyrhizobium* spp) are widespread in central Kenya. The high population levels of common bean and cowpea nodulating ingenious rhizobia in soils from these sites can be attributed to the widespread

ıtegration of legumes in the cropping system in central Kenya. Common bean and cowpea are ıe most important widely cultivated legume crop in Kenya (Ortiz and Crounch 2002; Mbene *et l.*, 2000; Gethi *et al.*, 1997; Wortmann and Allen, 1994). Common bean has been found to be reely nodulated by indigenous rhizobia strains (Chilimba, 2002). Mahler and Wollum (1981 nd 1982) reported increase in indigenous soil rhizobia population from 0.1% in absence of egume to 8-9% of the total aerobic bacteria when legumes were cultivated in the field. The urvival and abundance of rhizobia has been found to be dependent on their ability to locate nd colonize sites of suitable nutrition under pressure from other competing soil nicroorganisms (Coventry and Evans, 1989).

Soils samples from Kajiado and Machakos were found to contain more than 900 rhizobia ells g^{-1} soil when cowpea was used as a 'trap' host. However, the soil sample from cultivated and in Kabete had 311.6 rhizobia cells while the soil sample from uncultivated land in the ame site had 207.9 rhizobia cells. The soil sample from Nyeri, which had low pH and low P ıad 78.5 rhizobia cell g^{-1} soil. Under acidic soil conditions, indigenous rhizobia populations are ften low and survival of inoculant strains is adversely affected while low P in the soil will nhibit effective N-fixation process (Karanja and Wood, 1988).

The wide range in population size of indigenous rhizobia nodulating cowpea in the sampled soils from different sites was not uncommon. Trotman and Weaver (1986) when examining soils in Guyana observed variation of indigenous bacteria in soils from different sites. Variation in population sizes of rhizobia bacteria in soils from different areas observed in this study is common. Mpepereki (1994) reported *Bradyrhizobium* spp population size ranging from 0 to 29 x 10^3 cells g^{-1} soil in Zimbabwe, while Abaidoo *et al.* (2002) reported population

izes ranging from 0 to 10^4 cells g^{-1} soil in 63 soils from Africa including Kenya. Similar population size variations were observed by Singleton *et al.* (1992), Thies *et al.* (1991a) and Woomer *et al.* (1988) in other areas.

The high number of rhizobia cells in soils from Machakos and Kajiado suggested high population of indigenous rhizobia that nodulates cowpea in these sites. The relatively lower number of rhizobia cells in soils from cultivated and uncultivated land in Kabete implied lower population of cowpea rhizobia in this site than Kajiado and Machakos field sites. Karanja *et al.* (2002) reported poor nodulation of cowpea and common bean in most soils in Kenya. Rahman and Rastani (2002), Yousef *et al.* (1987), and Lawson *et al.* (1987) reported that the population size of effective rhizobia in the soil might be influenced by climatic, biological, physical and chemical soil factors that affect legume growth and survival of rhizobia. Trotman and Weaver (1986) reported a population of more than 150 *Bradyrhizobium* spp. g^{-1} soil in locations with previous history of cowpea rhizobia inoculation.

Previous cropping of cowpea in Kajiado and Machakos, which are marginal areas, could have increased the population level of rhizobia nodulating cowpea than in Nyeri and Kabete. Cowpea is usually grown in drier regions where rainfall is low and erratic and soils are sandy with low fertility (Singh, 2002). It is cultivated as an intercrop with cereals in marginal environment without inputs (Henriet *et al.*, 1997). Rhizobia populations have been found to increase due to stimulation of nodules by chemicals from the legumes (Amarger, 2001; Thies *et al.*, 1995). The high soil P, low soil N and neutral pH of soil from Machakos could have provided suitable conditions for growth of legume and rhizobia (Appendix 2). Phosphorus availability in the soil has been found to improve nodulation, hence N_2-fixation (Abdelgani *et*

., 2002; Reddel *et al.*, 1996). Neutral soil pH improved cation exchange capacity (CEC) of ie soil hence most plant essential nutrients are available (Gachene and Kimaru, 2003).

Soils from cutivated land in Kabete had higher population of indigenous rhizobia that odulate cowpea than soils from uncultivated land at the same site. This could be attributed to ither the previous cropping of cowpea in the cultivated field site or introduced rhizobia rough inoculation. Studies done by Thies *et al.* (1995) and Wilson (1944) found that *radyrhizobium* spp. that nodulates cowpea and soybean increased in population size when the egumes were planted in the field. The increased population of *Bradyrhizobium* spp. has been eported in previously cultivated fields with soybean (Weaver *et al.*, 1972) and peanuts (Yousef t *al.*, 1987). The facts that soils from uncultivated land in Kabete with no known history of egume cultivation contained high population of rhizobia indicated that rhizobia nodulating owpea are widespread.

Soil samples from Nyeri had the lowest root nodule rhizobia bacterial cells g^{-1} soil ompared to the other areas. The soil sample from Nyeri was low in soil pH and available hosphorous. The low soil pH makes availability of essential soil nutrients to plant and N-ixation difficult due to formation of complex compounds. Establishment and efficiency of a ymbiotic relationship requires a narrower range of soil reaction that is necessary for growth of lants not relying on N_2-fixation (Jackson, 1967).

In the present study, soil samples from Kajiado, Machakos and Kabete sites had pH range rom 6.2 to 6.8 and available P (ppm) from 20.0 to 42.2 compared to the soil sample from Nyeri, which had pH of 4.0 and 3.5 ppm available P. Consequently, soil samples from Kajiado, Machakos and Kabete sites recorded higher nodulation level compared to the soil sample from

Nyeri. Optimal growth of most rhizobium strains occurs at pH range 6.0 to 7.0 (Somasegaran and Hoben, 1994). Correlation between bacterial population and environmental factors such as organic matter, soil fertility and soil moisture has been established (Lawson *et al*, 1987; Faizah *et al*., 1980). Phosphorous availability in the soil has been found to improve nodulation hence N_2-fixation (Abdelgani *et al*., 2002; Reddel *et al*., 1996). Neutral soil pH improves cation exchange capacity of the soil hence most plant essential nutrients are available (Gachene and Kimaru, 2003)

Common bean is the most commonly grown legume in central Kenya hence the high population size of *Rhizobium* spp recorded in all the field sites compared to *Bradyrhizobium* spp. Intensive cropping of common bean may have contributed to the high population size of *Rhizobium* spp.in in soil samples from the four sites. Legumes have been known to produce compounds that stimulate proliferation of rhizobia strains in the soil (Thies *et al*., 1995). The presence of indigenous rhizobia (*Bradyrhizobium* and *Rhizobium* spp) in the uncultivated site suggested other sources of rhizobia other than inoculation. *Rhizobia* bacteria dispersal by agents such as wind, water, man and animals to new sites has been documented (Giller, 2001; Hansen, 1994). Parker *et al*. (1977) suggested that a large number of rhizobia could be dispersed by wind during harvest and on seed after harvest. Agricultural implements and animal forage have also been reported to act as dispersal avenues. Perez-Ramirez *et al*. (1998) reported the establishment of legume symbiosis with rhizobia brought along on the seeds or on soil used to raise seedlings.

1.5: Plant tissue nitrogen accumulated in cowpea inoculated with soil dilutions from four sites in central Kenya.

Cowpea inoculated with soil dilutions from Machakos had the highest shoot tissue-N ontent. This could be attributed to the low sample soil nitrogen; neutral soil pH and high soil hosphorous (Appendix 2). Studies have shown that high quantities of soil N reduce nodulation nd hence symbiotic activity (Pate and Layzell, 1990). The presence of mineral N especially itrates has been known to trigger early events in nodule ontogenesis and reduce nodulation Truchet and Dazzo, 1982). The high nodule count and number of rhizobial cells g^{-1} soil bserved in soil samples from Machakos could be attributed to the low soil-N. However, soil amples from Nyeri, which was low in soil pH and had moderate soil-N had relatively similar mount of shoot tissue-N content to the other soils samples except soil sample from Machakos.

The results indicate that the amount of plant tissue-N may not be necessarily related to nodulation level or the abundance of indigenous rhizobia in the soil. Soil samples from cultivated land in Kabete contained higher amounts of available soil phosphorous than the soil samples from uncultivated land in the same site although the other soil physicochemical properties were similar. However, soil samples from uncultivated land in Kabete produced higher plant tissue-N than the soil sample from cultivated land in Kabete although the difference was not significant. This suggested a relation between plant tissue-N accumulation and soil-N than with available phosphorous.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

: Conclusions

The study results have shown that rhizobia inoculation improved nodule biomass but did translate into biomass accumulation and yield increase of the grain legumes. Common an, cowpea, lima bean and lablab performed better than the other legumes in most of the essed parameters. This could be attributed to the rhizobia that nodulate cowpea and common an being widespread in central Kenya (79 to more than 900 rhizobia cells g^{-1} of soil) even in gin or uncultivated land. The wide range in number of rhizobia bacteria cells signified wide riation in population level among the field sites. Application of starter-N fertilizer suppressed dulation level of grain legumes but had no significant effect on biomass accumulation and eld of legumes. The poor and negative results on application of starter-N fertilizer could be tributed to the high percent soil N (0.38 in cultivated land to 0.4 in uncultivated land) in abete field experimental site. The results suggested that the soils at Kabete probably contain gh levels of rhizobia or high residual nitrogen content such that the plants could not respond small amount of starter-N applied. Consequently, the poor response to inoculation could be tributed to the presence of highly competitive native rhizobia that could have restricted ccupancy of nodules by the inoculant strain.

The effects of rhizobia inoculation and starter-N fertilizer application on nodulation, iomass accumulation and yield varied among the different legume species. Common bean and owpea performed better than the other legumes in all the assessed parameters. Common bean nd lima bean had the highest grain yield compared to the other legume species. Lima bean

sponded relatively poor to rhizobia inoculation and application of starter-N fertilizer and had e least number of nodules compared to the rest of the legumes. However, the legume oduced the highest number of pods per plant and grain yield. This suggested that the rhizobia rain used in the study was not effective in nodulating this legume. N-fertilizer application ay not be necessary for this legume under the prevailing soil conditions.

However, although rhizobia inoculation did not have any significant effects on yield and iomass accumulation in this study, rhizobia inoculation have been known to improve yield and iomass accumulation in soils low in effective indigenous rhizobia. Resource poor farmers who annot afford the expensive inorganic N-fertilizers in central Kenya can inoculate legumes, vith appropriate rhizobia strains, as alternative source of nitrogen. Inoculation of legumes with ffective rhizobia strain will improve the population of indigenous rhizobia in the soil. Application of moderate rates of N-fertilizer during the early stage of legume growth before stablishment of N_2-fixation is also necessary. A combination of starter-N fertilizer and hizobia inoculation with diversification of legume species will improve the depleted soil, ncrease food security, provide the much needed vitamins and minerals, and also control pests and diseases through reduction of pest-host interaction.

The results of present study also showed high population levels of indigenous rhizobia capable of nodulating the test legumes in the field sites. The relatively low population levels of cowpea nodulating rhizobia strain compared to common bean nodulating rhizobia suggest the need to inoculate cowpea, pigeon pea, lima bean, lablab and green gram in the test site soils especially in the Nyeri site, whose soil was acidic and low in available phosphorous. The acidic soil pH and other undesirable soil conditions could be improved by application of lime, use of

non-acidifying fertilizers such as triple super phosphate (TSP), calcium ammonium nitrate and compound fertilizers (NPK). The application of farmyard manure at 7 to 10 tonnes ha^{-1} $year^{-1}$ in Kajiado soils and 15 to 20 tonnes ha^{-1} $year^{-1}$ in Nyeri soil can raise cation exchange capacity and reduce complexing of soil nutrients. This will improve soil reaction, activate microbial activities, and promote legume growth. Application of single super phosphate fertilizer in Machakos and Kabete soils can be used to maintain phosphorous levels in these soils.

6.2: Recommendations

From the findings of this study, the following recommendations are suggested:

1. Conduct a similar study in N-depleted fields prevalent in smallholder production systems.
2. Determine the abundance of indigenous rhizobia in soils from central Kenya using higher dilution levels.
3. Determination of the population level of indigenous rhizobia in soils from other legume growing areas of Kenya.

REFERENCES

aidoo, R.C., Keyser, H.H. and Singleton P.W. (2002). Population and symbiotic characteristics of indigenous *Bradyrhizobium* spp. that nodulate Tgx soybean genotypes in Africa. In: Karanja, N.K and Kahindi, J.P.H. 'Challenges and Imperatives for Biological Nitrogen Fixation Research and Application in Africa for the 21st Century'. Ninth Congress for Africa Association for Biological Nitrogen Fixation 25-29th September 2000, Nairobi, Kenya. 167-188 pp.

ate, T. and Ampofo, J.K.O. (1996). Insect pests of beans in Africa: Their strategy and Management. Annual Review of Entomology **41**, 45-73pp.

bdelgani, M.E., Mohamed, S.S. and Osman, A.G. (2002). The use of rhizobium inoculants for increasing productivity of cluster bean (*Cyamopsis tetragonoloba*) in desert-affected soils. In: Karanja, N.K and Kahindi J.P.H. Challenges and imperatives for Biological Nitrogen Fixation Research and Application. in Africa for the 21st century. Ninth congress for the association for biological nitrogen fixation 25-29th September 2000, Nairobi, Kenya. 1-10 pp.

cland, J.D. (1971). East African crops. Introduction to the field and plantation crops in Kenya, Tanzania, and Uganda. London Longman by arrangement with FAO, 252pp.

djei, M.B., Queensbury, K.H. and Chambliss, C.G., (2004). Nitrogen fixation and inoculation of forage legumes. Http: // edis. Ifas. Ulf. Edu body-ag 152 p (2004).

gboola, A.A. and Fayemi, A.A., (1972). Fixation and excretion of nitrogen by tropical legumes. Agronomy Journal. **64**, 409-412 pp.

guilar, O. M., Lopez, M.V., Riccillo, P.M., Gonzalez, R. A., Pagano, M., Grasso, D. H., Puhler, A., and Favelukes, G. (1998). Prevalence of the *Rhizobium etli* like allele in genes coding for 16SrRNA among the indigenous rhizobial populations found associated with wild beans from the Southern Andes in Argentina. Applied and Environmental Microbiology **64**, 3520-3524pp.

llen, O.N. and Allen, E.L. (1981). "The Leguminosae: A Source book of characteristics, Uses, and Nodulation." The University of Wisconsin Press, Madison.

llos, J. R. and Bartholomew W.V. (1959). Replacement of Symbiotic nitrogen fixation by available nitrogen. Soil Science Journal **87**, 61-67pp.

l-Rashidi, P.K., Loynachan, T.E. and Frederick, L.R. (1982). Desiccation tolerance of strains of *Rhizobium japonicum*. Soil Biology and Biochemistry **14**, 489 – 493pp.

Altieri, M.A. (1998). Agroecology: The science of sustainable agriculture. Westview Press, London, UK.

Amarger, N. (2001). Rhizobia in the field. Advances in Agronomy **73**, 110-148pp.

Amarger, N., Bours, M., Revoy, F., Allard, M.R., and Laguerre, G., (1994). *Rhizobium tropici* nodulates field-grown *Phaseolus vulgaris* in France. Plant and Soil. **161**, 147-156pp.

Andrew, J.J. and Eck, P. (1983). Effects of nitrogen rates and water stress on growth and water relations of young sweet corn plants. Journal of American Society of Horticultural Science **108**, 487-501pp.

Anonymous, (1985). Kenya Meteological Department, Annual report 1985.

Anyango, B., Wilson, K.J., Beynon, J.L. and Giller, K.E. (1995). Diversity of Rhizobia nodulating *Phaseolus vulgaris* L. in two Kenyan soils with contrasting pHs. Applied Environmental Microbiology **61**, 4016-4021pp.

A.O.A.C. (1990). Official Methods of Analysis of the Association of Official Analytical Chemists. 15th ed. A.O.A.C., Washington, D.C.

AVRDC (Asian Vegetable Research and Development Center) (2005). Suggested cultural practices for vegetable soybean. AVRDC Learning Center. (http://www.Avrdc.org/LC/soybean/practices.htm)

Bala, A. and Giller, K.E. (2002). Population size and diversity of rhizobia that nodulate some woody agroforestry legumes in two African soils. In: Karanja, N.K and Kahindi, J.P.H. Challenges and Imperatives for Biological Nitrogen Fixation Research and Application in Africa for the 21st century. Ninth Congress for Africa Association for Biological Nitrogen Fixation 25-29th September 2000 Nairobi, Kenya. 198-204 pp.

Bordeleau, L.M. and Prevost, D. (1994). Nodulation and nitrogen fixation in extreme environments. Plant and Soil **161**, 115-123pp.

Bottomley, P.J. (1992). Ecology of *Bradyrhizobium* and *Rhizobium*. In: "Biological Nitrogen Fixation" (G. Stacey, R.H. and Burris, and H.J. Evans, Eds.), pp. 293-348. Chapman and Hall. New York.

Brewin, N.J. (1998). Tissue and cell invasion by *Rhizobium*: the structure and development of infection threads and symbiosomes. In: Spaink, H.P., Kondorosi, A and Hooykaas, P.J.J. (eds.) The *Rhizobiaceae*. Kluwer, Dordrecht, The Netherlands, 417-429pp

Brockwell, J. (1977). Application of legume seed inoculants. In "A Treatise on Dinitrogen Fixation" (R.W.F. Hardy and A.H. Gibson, Eds.), pp. 277-309. Wiley, Chichester, UK.

Brockwell, J. and Bottomley, P.J. (1995). Recent advances in inoculant technology and prospects for the future. Soil Biology and Biochemistry **27**, 683-697pp.

Brockwell, J., Bottomley, P.J. and Thies, J.E. (1995). Manipulation of rhizobium microflora for improving legume productivity and soil fertility: A critical assessment. Plant and Soil **174**, 143-180pp.

Burton, J.C. (1985). Production and use of legume inoculants in Africa. In: Proceedings of the first conference of the African Association for Biological Nitrogen Fixation. Nairobi 23-27 July 1984 (ed. H. Ssali and S.O. Keya) 119-134pp.

Bushby, H.V.A. and Marshall, K.C. (1977). Water status of rhizobia in relation to their susceptibility to desiccation and their protection by montimorillonite. Journal of General Microbiology **99**, 19-27pp.

Caballero-Mellado, J. and Martinez-Romero, E. (1999). Soil fertilization limits the genetic diversity of *Rhizobium* in bean nodules. Symbiosis **26**, 111-121pp.

Carroll B.J., McNeill, D.L. and Gresshoff, P.M. (1985). Isolation and properties of soybean (*Glycine max* (L.) Merr.) mutants that nodule in the presence of high nitrate concentrations. Proceedings of the National Academy of Sciences, USA **82**, 4162-4166pp.

Chakrabarti, S., Lee, M. S. and Gibson, A. H. (1981). Diversity in the nutritional requirements of strains of various *Rhizobium* species. Soil Biology and Biochemistry **13**, 349-354pp

Chemining'wa, G. N. (2002). The abundance, efficacy, and diversity of *Rhizobium leguminosarum* bv. *viceae* populations in Southern Manitoba soils. Ph.D. Thesis, University of Manitoba, Canada.

Chilimba, A.D.C (2002). Seed pelleting and inoculation of field beans (*Phaseolus vulgaris* L.) "in neutral" and acid soils to enhance nodulation. In: N.K Karanja and J.H.P. Kahindi. 'Challenges and Imperatives for Biological Nitrogen Fixation Research and Application in Africa for the 21st century'. Ninth Congress for Africa Association for Biological Nitrogen Fixation 25-29th September 2000, Nairobi, Kenya. 21-29 pp.

Chrispeels, M.J. and Sadava, D.E. (2003). Plants, genes and crop biotechnology, 2nd Ed. Jones and Bartlett Publishers, London, UK.

Chui, J.N. and H.M. Nadar, (1983). Effect of spatial arrangement on yield and other agronomic characters of maize and legume intercrop. East African Agricultural and Forestry Journal **44**, 127-136pp.

Cohen, M.J. (2003). Food Security: Why do hunger and malnutrition persist in a world of plenty? In: Chrispeels, M.J. and Sadava, D.E. (eds.), Plants, genes and biotechnology, 2nd ed., pp -76-99. Jones and Bartlett Publishers, London, UK.

Cohn, J., Day, R.B. and Stacey, G. (1998). Legume nodule organogenesis. Trends in Plant Science **3**, 105-110pp.

Coventry, D.R. and Evans, J. (1989). Symbiotic Nitrogen fixation and Soil Acidity. In: (A.D. Robson Eds.) pp 103-137. Soil Acidity and Plant growth. Soil Science and Plant Nutrition, School of Agriculture, the University of Western Australia. Academic Press. Sydney.

da Silva, P.M., Tsai, S.M. and Bonneti, R. (1984). Response to inoculation and N-fertilization for increased yield and biological nitrogen fixation of common bean (*Phaseolus vulgaris* L.). Plant and Soil **152**, 123-130pp.

Date, R. A. and Roughley, R.J. (1977). Preparation of Legume Inoculants. In: "A Treatise on Dinitrogen Fixation" (R.W.F. Hardy and A.H. Gibson, Eds.) pp. 243-276. Wiley, Chichester, UK.

Day, J.M., Roughley, R.J., Eaglesham, A.R.J., Dye, M. and White, S.P. (1978). Effects of high soil temperatures on nodulation of cowpeas (*Vigna unguiculata)*. Annals of Applied Biology **88**, 476 – 481pp.

Day, D.A. and Urdvardi, M.K., (1992). Metabolite exchange across symbiosome membranes. Symbiosis **14**, 175-189pp.

Dean, J.R. and Clarke, R.W. (1980). Effect of low nitrogen fertilization on nodulation, acetylene reduction and dry matter on Faba beans and three other beans. Canadian Journal of Plant Science **60**, 121-130pp.

de Lajudie, P., Willems, A., Pot, B. Dewettinck, D., Maestrojuan, G., Neyra, M., Collins, M.D., Dreyfus, B., Kersters, K. and Gillis, M. (1994). Polyphasic taxonomy of Rhizobia: emendation of the genus *Sinorhizobium* and description of *Sinorhizobium meliloti* comb. Nov., *Sinorhizobium saheli* spp. nov. and *Sinorhizobium teranga* spp. nov. International Journal of Systematic Bacteriology **48**, 1277-1290pp.

Dudeja, S.S. and Khurana, A.L. (1989). Persistence of *Bradyrhizobium* spp. (*cajanus*) in a sandy loam. Soil Biology and Biochemistry **21**, 709-713pp.

Eaglesham, A.R.J. and Ayanaba, A. (1984). Tropical stress ecology of rhizobia, root nodulation and legume fixation. In: Subba Rao, N.S. (ed.) Current developments in Biological Nitrogen Fixation. Oxford and IBH, New Delhi, India, 1-35 pp.

Eaglesham, A.R.J.; Ayanaba, A.; Rao, V.R., and Eskew, D.L., (1982). Mineral N effects on cowpea and soybean crops in Nigerian soils. II. Amount of N fixed and accrual to the soil. Plant and Soil **68**, 183-192pp.

Elkan, G. H., and Kwik, I.E.M (1968). Nitrogen, energy, and vitamin nutrition of *Rhizobium japonicum*. Journal of Applied Bacteriology **31**, 399-404pp.

Faizah, A.W., Broughton, W.J. and John, C.K. (1980). Rhizobia in tropical legumes. XI. Survival in the seed environment. Soil Biology and Biochemistry **12**, 219-224pp.

Gachene, C. K. K. and Kimaru, G. (2003). Soil fertility and land productivity. Relma 2003, 146 pp.

Garside, A.L. and Berthelsen, J.E. (2004). Management of legume biomass to maximize benefits to the following sugarcane crop. Protection Australian Society Sugarcane Technology. Australian Agronomy Journal, **225,** 47-55pp

Genstat, 1995. Genstat 5 Release 3.2 for Windows 95. Lawes Agricultural Trust, Rothamstead Experimental Station, U.K.

Gethi, M, Muriithi, F.M. Macharia, N. and Njoroge, K. (1997). Maize/bean intercropping system in medium potential area of Kenya. Farmer's Practice and Research Challenges. African Crop Science Conference Proceedings **3**, 756-770pp.

Gibson, A.H. and Harper, J.E. (1985). Nitrate effect on nodulation of soybean by *Bradyrhizobium japonicum.* Crop Science, **25,** 494-501pp.

Giller, K. E. (2001). Nitrogen fixation in tropical cropping systems. Cabi Publishing, 409 pp.

Giller, K. E. and Cadisch, G. (1995). Future benefits from biological nitrogen fixation: An ecological approach to Agriculture. Plant and Soil **174,** 253-277pp.

Gitari, J. N., F.M. Matiri, I. W. Kariuki, C. W. Mureithi and Gachanja, S. P. (1999). Nutrient and cash flows monitoring in farming systems on the eastern slopes of Mount Kenya. In: Smaling, E. M. A., O. Oeneman and Fresco L. O. Nutrient Disequilibrium in Agroecosystems: Concepts and Case Studies. CABI Publishing International. 211-228pp.

G.O.K. (1998). Government of Kenya. Economic Survey 1997. Central Bureau of Statistics, Nairobi, Kenya.

Goi, S.R., Sprent, J.I., James, E.K. and Jacob-Neto, J., (1993). Influence of nitrogen form and concentration on the nitrogen fixation of *Acacia auriculiformis*. Symbiosis **14**, 115-122pp.

Graham, P.H. (1992). Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under diverse conditions. Canadian Journal of Microbiology **38,** 475-484pp.

Graham, P.H., Sadowsky, M.J., Keyser, H.H., Barnet, Y.M., Bradley, R.S., Cooper, J.E., Deley, D. J., Jarvis, B.D.W., Roslycky, E.B., Strijidom, B.W. and Young, J.P.W. (1991). Proposed minimal standards for the description of new genera and species of root-nodulating and stem-nodulating bacteria. International Journal of Systematic Bacteriology **41**, 582-587pp.

Griffith, G.W. and Roughley, R.J. (1992). The effects of moisture potential on growth and survival of root nodule bacteria in peat culture and on seed. Journal of Applied Bacteriology **73**, 7-13pp.

Grindley, H.E. and Danial, D.L. (1995). Bean production constraints in Africa with special reference to breeding for resistance to common bean mosaic virus. Proceedings of a regional workshop for Eastern Africa held at Njoro, Kenya, October 2-6th, 1994. Wageningen Agricultural University, Wageningen, The Netherlands. 35-41 pp.

Hansen, A.P. (1994). Symbiotic nitrogen fixation of crop legumes (achievements and perspectives). Margraf Verlag, 248pp.

Harper, J. E. and Gibson, A. H. (1984). Differential nodulation tolerance to nitrate among legume species. Crop Science **24,** 797-801pp.

Harrison, S. P., Young, J.P., and Jones, D. G. (1989). Rhizobium population genetics: Host preference and strain competition effects on the range of *Rhizobium Leguminosarum* bv. *trifolii* genotypes isolated from natural populations. Soil Biology and Biochemistry **21**, 981-986pp.

Havelka, U.D., Boyle, M. G. and Hardy, R.W.F. (1982). Biological nitrogen fixation. pp. 365-422. In: F. J. Stevenson (ed.) Nitrogen in agricultural soils. Agronomy **22**. ASA, CSSA, and SSSA, Madison, W1.

Henriet, J., van Ek, G.A., Blade, S.F. and Singh, B.B. (1997). Quantitative assessment of traditional cropping systems in the Sudan savanna of northern Nigeria: (I) Rapid survey of prevalent cropping systems. Journal of Agriculture Research **14**, 37-45pp.

Herridge, D.F. and Betts, J.H. (1988). Field evaluation of soybean genotypes selected for enhanced capacity to nodulate and fix nitrogen in presence of nitrate. Plant and Soil **110**, 129-135pp.

Herridge, D.F., Roughley, R.J. and Brockwell, J. (1984). Effects of *Rhizobium* and soil nitrate on establishment and functioning of the soybean symbiosis in the field. Australian Journal of Agricultural Research **35,** 146-161pp.

Hirsch, A.M. (1992). Transley Review No. 40. Developmental biology of legume nodulation. New Phytologist **122**, 430-431pp.

Hirsch, P. R. (1996). Population dynamics of indigenous and genetically modified rhizobia in the field. New Phytologist **133**, 159-171pp.

Houngnandan, P., Sanginga, N., Woomer, P., Vanlauwe, B. and Van Cleemput, O. (2000). Response of *Mucuna pruriens* to symbiotic nitrogen fixation by rhizobia following inoculation in farmers fields in the derived savanna of Benin. Biology and Fertility of Soils **30**, 558-565pp.

Hungria, M. and Vargas, M.A.T. (2000). Environmental factors affecting N_2 fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crops Research **65**, 151-164pp.

Hymowitz, T. (1990). Grain legumes. pp 54-57. In: J. Janick and J.E. Simon (Eds.) Advances in New Crops. Timber Press, Portland, OR.

Hynes, R. K., Craig, K. A., Covert, D., Smith, R.S., and Rennie, R. J. (1995). Liquid rhizobial inoculants for lentil and field pea. Journal of Production Agriculture **8**, 547-522.

Ikombo, B. M. (1984). Effect of farmyard manure and fertilizer on maize in semi-arid areas of Eastern Kenya. East African Agricultural and Forestry Journal **44**, 266-74pp.

Jackson, W.A. (1967). Physiology effects of soil acidity. pp 49. In: Pearson, R.W. and Adams, F. (eds.). 1967. Soil acidity and liming. No.12 Agronomy series. American Society of Agronomy. Madison, Wisconsin, USA.

Jaetzold, R. and Schmidt, H. (1983a). Farm management handbook of Kenya. Volume IIa. Central Kenya (Central and Eastern Provinces). Ministry of Agriculture.

Jaetzold, R. and Schmidt, H. (1983b). Farm Management Handbook of Kenya.Volume IIb. Central Kenya, (Rift valley and Central provinces). Ministry of Agriculture.

Jensen, E.S. and Sorenson, L. H. (1988). Uptake of soil nitrogen by soybean as influenced by symbiotic N2-fixation or fertilizer supply. Soil Biology and Biochemistry **20**, 921-925pp.

Kaleem, F. (2000). Biological Nitrogen Fixation in a soybean/sorghum cropping system. In:N.K Karanja and J.H.P. Kahindi. 'Challenges and Imperatives for Biological Nitrogen Fixation Research and Application in Africa for the 21st century'. Ninth Congress of the African Association for Biological Nitrogen Fixation 25-29th September, 2000 Nairobi, Kenya. 50-62 pp.

Karanja, N.K., Wangaruro, S. and Anyango, B.M. (2002). Performance of indigenous *Bradyrhizobium* strains isolated from Kenyan soils. In: N.K Karanja and J.H.P. Kahindi. 'Challenges and Imperatives for Biological Nitrogen Fixation Research and Application in Africa for the 21st century'. Ninth Congress of the African Association for Biological Nitrogen Fixation 25-29th September, 2000 Nairobi, Kenya. 198-204 pp.

Karanja, N.K. and Wood, M. (1988). Selecting *Rhizobium phaseoli* strains for use with beans (*Phaseolus vulgaris* L.) in Kenya: Ineffectiveness and tolerance of acidity and aluminium. Plant and Soil. **112**, 7-13pp.

Keya, S.O., Balasundran, V.R., Ssali, H. and Mugane, C. (1982). Multilocational field response of *Phaseolus vulgaris* L. to an inoculation in East Africa. pp 231-234. In: P.H. Graham and S.C. Harris (eds.). Biological Nitrogen Fixation Technology for Tropical Agriculture CIAT March 9-13th 1981. 768pp.

Khailova, G.F. and Lar'kova, T.P. (1992). Effects of chloride salinity on formation and activity of symbiotic system in alfalfa. Soviet Plant Physiology **39**, 205-210pp.

Kihanda, F. M. (1996). The role of farmyard manure in improvement of maize production in the sub-humid highlands of central Kenya. Doctoral Thesis, Soil Science Department. Reading University, United Kingdom.

Kucey, R. M. N. and Hynes, M. F. (1989). Population of *Rhizobium Leguminosarum* bv. *phaseoli* and *viceae* in fields after bean and pea in rotation with non legumes. Canadian Journal of Microbiology **35**, 661-667.

Lawson, K.A., Barnet, Y.M. and Mc Gilchrist, C.A. (1987). Environmental factors influencing numbers of *Rhizobium leguminosarum* bv. *trifolii* and its bacteriophages in two fields soils. Applied biology and Environmental Microbiology **53**, 1125-1131pp.

Lewin, A., Rosenberg, C., Meyer, Z.A.H., Wong, C.H., Nelson, L., Mansen, J.F., Stanley, j., Dowling, D.N., Denarie, J. and Broughton, W.J. (1987). Multiple host-specificity loci of broad range Rhizobium spp. NGR234 selected using the widely compatible legume *Vigna unguiculata*. Plant Molecular Biology **8**, 447-459pp.

Lowendorf, H.S. Baya, A.M. and Alexander, M.M. (1981). Survival of *Rhizobium* in acidic soils. Applied Biology and Environmental Microbiology **42**, 951-957pp.

Lynam, J. M., Nandwa, S. and Smaling, E. M. A. (1998). The African food problem. Agriculture Ecosystems and Environment **71**, 63-80pp.

Mahler, R.L and Wollum, A.G, (1982). Seasonal fluctuation of *Rhizobium japonicum* under a variety of field conditions in North Carolina. Soil Science **134**, 317-324pp.

Mahler, R.L and Wollum II, A.G, (1981). The influence of water potential and soil texture on the survival of *Rhizobium japonicum* and *Rhizobium leguminosarum* isolates in the soil. Soil Science Society American Journal **45**, 761-766pp.

Marero, V. and Guzmand, (1986). Response of bean (*Phaseolus Vulgaris* L.) and cowpea (*Vigna unguiculata*) to nitrogen fertilizer in the presence of indigenous soil rhizobium strains. Field Crop Abstracts 4215.

Marschner, H. (1986). Mineral Nutrition of higher plants. Academic Press, Harcourt Brace Jovanovich publishers.

Martenson, A.M. (1989). Competition between strains of *Bradyrhizobium japonicum* for nodulation of soybeans at different nitrogen fertilizer level. Plant and Soil **117,** 219-225pp.

Matata, J. B. W., Kiriro, F. H., Wandera, E. O., Dixon, J. and Anandajayasekaram, P. (2001). Farming systems approach technology development and transfer. FARMESA, 420pp.

Mbene, F.M., N'Daiye and Lowenberg-DeBoer, J. (2000). Identifying cowpea characteristics, which command price premiums in Senegalese markets, presentation at the World Cowpea Conference, Ibadan, Nigeria, September

Mellor, R.B. and Werner, D. (1990). Legume nodule biochemistry and function. In: The molecular biology of symbiotic nitrogen fixation. In ed. P.M. Gresshoff. CRC Press international, Boca Laton, FL. 111-130pp. 2000.

Micheni, A., D., Rees, I. W., Kariuki, F. R., Manyara, S. K., Gathamba, K., Kaburu, J., N. Ngugi and Gachanja. S. P. (1999). An overview of the farmer participatory regional program of the RRC Embu mandate region. In: the proceedings of KARI/DFD/NARP II end of project conference held at KARI Headquarters on 23-26th March 1999. Nairobi, Kenya. 116-123pp.

Michieka, D.O. (1977). Soils of the Valley Bottoms of Kabete Faculty Farm. National Agricultural Laboratories, Ministry of Agriculture, Kenya. 1-2pp.

Ministry of Agriculture (MOA), (2004). Central province; Annual reports for the year 2004. 1-50pp.

Ministry of Agriculture (MOA), (2003). Central province; Annual reports for the year 2003. 7-11pp.

Ministry of Agriculture (MOA), (2002). Central province; Annual reports for the year 2002. 14-17pp.

Ministry of Agriculture (MOA), (2001). Kiambu district; Annual report for the year 2001. 112 pp.

Mpepereki, S. (1994). Characterization studies of rhizobia in some Zimbabwean soils. Ph.D. dissertation. University of Zimbabwe, Zimbabwe.

Mpepereki, S., Wollum, A. G. and Makonese, F. (1996). Growth temperature characteristic of indigenous *Rhizobium* and *Bradyrhizobium* isolates in Zimbabwean soils. Soil Biology and Biochemistry 28, 1537 – 1539.

Muchena, F. N. and Nyandat, N. N. (1980). Rating of land qualities and the conversion into land suitability in Kenya with particular emphasis on soil moisture storage and climatic conditions. Paper presented at 4th meeting of Eastern African Sub-committee for soil correlation and land evaluation, Arusha, 24th October to 4th November 1980.

Mureithi, J. G., S. N. Maobe, E. Dyck, C. K. K. Gachene, J. N., Gitari, B. Kirungu, B. M. Muli, J. Ojiem, H. M. Saha and Tana, P. (1998). Screening of legume germplasm in Kenya. Effects of *Rhizobia* inoculation on performance of best-bet legumes. Paper presented at the 16th conference of soil science of East Africa held at Tanga, Tanzania on 13-19th December 1998.

Mureithi, G. M., Gachene, C. K. K. and Wamwongo, J. W. (2003). Legumes cover crops research in Kenya: Experiences of Legumes Research Network Project. 267pp.

Mureithi, J.G., Gachene, C. K. K., Muyekho, F. N., Onyango, M., Mose, L. and Magenya, O. (2000). Participatory technology development for soil management by small holders in Kenya. Legume Research Project. Colour Magic Production. 551pp.

Musandu, A.A.O. and Ogendo, J.O. (2002). Effect of *Rhizobium* inoculation and fertilizer nitrogen and phosphorus on the grain yield of beans in Western Kenya. In: Karanja, N.K. and J.H.P Kahindi. 'Challenges and Imperatives of Biological Nitrogen Fixation Research and Application in Africa for the 21st Century'. Ninth Congress for Africa Association for Biological Nitrogen Fixation 25-29th September 2000, Nairobi, Kenya. 86-93 pp.

Mwaniki, A. (2000). Assessment of bean production constraints and seed quality and health of improved common bean seed. M Sc. Thesis. University of Nairobi, Kenya.

h, N., Sen, D. and Weaver, R.W. (1993). Population density of clover rhizobia in Texas pastures and response to liming. Biology and Fertility of Soils **15**, 45-49pp.

sen, E. T., Sharifi, M. R. Rundel, P. W. Jarell, W. M. and Virginia, R. A. (1983). Diurnal and seasonal water relations of the desert phreatophyte *Prosopis glandulosa* (honey mesquite) in the Sonoran desert of California. Ecology **64**, 1381-1393pp.

nga, R.A. (1997). Effect of spatial arrangement and nitrogen levels on yields of potatoes and beans grown as intercrops. M Sc. Thesis. University of Nairobi. Kenya.

m, J. O., Mureithi, J. G., and Okuosa, E. A. (2000). Integrated management of legume green manure, farmyard manure and inorganic nitrogen for soil fertility improvement in Western Kenya. In: Mureithi, J.G., Gachene, C. K. K., Muyekho, F. N., Onyango, M., Mose, L. and Magenya, O. (2000), Participatory technology development for management by Smallholders in Kenya. 97-102pp.

en P.E., Sande, E.S. and Keyser, H.H. (1996). The Enumeration and identification of rhizobial bacteria in Legume Inoculant Quality Control Procedures. NifTAL Center, USA. 96pp.

iz, R. and Crounch, J. (2002). Applied biotechnology in the improvement of the tropical food species.

ker, C. A., Trinik, M. J. and Chattel, D. L. (1977). *Rhizobia* as rhizosphere inhabitants. In: A Treatise on Dinitrogen Fixation. Edited by R. W. F., Hardy and A. H., Gibson. John and Willey, New York. 311-353pp.

e, J.S. and Layzell, D.B. (1990). Energetic and biological costs of nitrogen assimilation. In The Biochemistry of Plants. Ed. Academic Press. Inc. London. 1-42pp.

oples, M.B. and Herridge, D.F. (1990). Nitrogen fixation by legumes in tropical and sub-tropical agriculture. Advances in Agronomy, **44**, 155-223pp.

na-Cabriales, J.J. and Castlellanos, J.Z. (1993). Effects of water stress on N_2 fixation and grain yield of *Phaseolus vulgaris* L. Plant and Soil, 152, 151-155pp.

rez-Ramirez, N.O., Rogel-Hernandez, M.A., Wang, E.T. and Martinez-Romero, E. (1998). Seeds of *Phaseolus vulgaris* bean carry *Rhizobium elti*. FEMS Microbiology and Ecology **26**, 289-296pp.

ostgate, J. (1998). Nitrogen fixation. Cambridge University Press, Cambridge, UK.

ower, J.F. (1987). "Legumes: their potential role in agricultural production." Volume 2, No. 2, 69-73pp.

Qiang, C., Xiapong, Z., Zewdi, T., Seppo, K., Dengyu, L., and Kristina, L. 2003. Diversity of peanut (*Arachis hypogaea*) bradyrhizobia and their host plants. Plant and Soil **255**, 605-617pp.

Rai, R. (1992). Effects of nitrogen levels and *Rhizobium* strains on symbiotic nitrogen fixation and grain yields of *Phaseolus vulgaris* L. genotypes in normal and saline-sodic soils. Biology and Fertility of Soils **14,** 293-299pp.

Rahman, H.A and Rastini, N.S. (2002). Prediction of the necessity of soybean inoculation based on the numbers of *Bradyrhizobium japonicum* and evaluation of N-availability indices. In: Karanja, N.K and Kahindi J.P.H. 'Challenges and Imperatives for Biological Nitrogen Fixation Research and Application in Africa for the 21st century'. Ninth Congress for the Association for Biological Nitrogen Fixation 25-29th September 2000, Nairobi, Kenya. 159-166 pp.

Reddel, P., Yun, Y. and Shipton, W.A. (1996). Do *Casuarina cunninghamiana* seedlings dependent on symbiotic N_2 have higher phosphorous requirements than those supplied with adequate fertilizer nitrogen? Plant and Soil **189**, 123-219pp.

Roughley, R.J. (1985). Effect of soil environmental factors on rhizobia. In: Shibles, R. (ed.) World Soybean Research Conference III: Proceedings. Westview Press, Boulder, Colorado, 903-910pp.

Rovira, A.D. (1961). Rhizobium numbers in the rhizosphere of the red clovers and paspurum in relation to soil treatment and the numbers of bacteria and fungi. Australian Journal of Agricultural Research **12**, 77-83pp.

Rowland, J. R. J. (1993). Dryland farming in Africa. CTA Macmillan. 336pp.

Sanginga, N. (1998). Maximization of biological nitrogen fixation by promiscuous soybean in maize based cropping systems in the Guinea savanna of Nigeria. African Agronomy (special issue) **1**, 3-25pp.

Sanginga, N., Dashiell, K., Okogun, J.A. and Thottapilly, G. (1997). Nitrogen fixation and N contribution in promiscuous soybeans in the southern Guinea savanna of Nigeria. Plant and Soil **195**, 257-266pp.

Serraj, R., Sinclair, T. R. and Purcell, L. C. (1999). Symbiotic N_2 fixation response to drought. Journal of Experimental Botany **50**, 143- 155 pp.

hurtleff, W. and Aoyagi, A. (2004). History of Green Vegetable Soybeans and Vegetable Type Soybeans. A speech Report on the History of Traditional Non-Fermented Soy foods. A Chapter from the Unpublished Manuscript "History of Soybeans and Soy foods: 1100 B.C. to the 1980". Soy foods Centre, Lafayette, California (http://www.the soydailyclub.com/SFC/Nfsoyfoods 30.asp).

inclair, T.R. and Gardener, F.P. (1998). Principles of ecology in plant production CAB International, Walling ford, UK.

ingh, B.B. (2002). Breeding cowpea varieties for resistance to *Striga gesnerioides* and *Alectra vogelii*. In: Fatokun, C.A., Tarawali, S.A., Singh, B.B., Kormawa, P.M. and Tamo, M. (eds.). Challenges and opportunities for enhancing sustainable cowpea production. IITA, Ibadan, Nigeria. 154-166pp.

Singleton P.W., Bohlool, B.B. and Nakao, P.L. (1992). Legume response to rhizobial inoculation in the tropics: myths and realities. In: Myths and Science of Soils of the Tropics. pp. 135-155. Soil Science and Society of America and American Society of Agronomy. Special publication no. 29.

Singleton P.W., Thies, J. and Bohlool, B.B. (1992). Useful models to predict response to legume inoculation. IITA, Wiley-Sayce-Co.

Singleton, P.W., El Swaify, S.A. AND Bohlool, B.B. (1992). Effect of salinity on Rhizobium growth and survival. Applied Environmental Microbiology **44**, 884-890pp.

Smaling, E.M.A. (1993). Nutrient balance as indicators of productivity and sustainability in sub-Saharan African Agriculture. Special edition of Agriculture Ecosystems and Environment, Volume 71. Elsevier, Amsterdam.

Smartt, J. (1976). Tropical pulses. Longman. 348 pp.

Smith, R.S. (1992). Legume inoculant formulation and application. Canadian Journal of Microbiology **38**, 485-492pp.

SMP, (1995). Soil Management Project. Annual Report. Ministry of Agriculture.

Souza, V., Eguiarte, L. Avila, G., Capello, R., Gallardo, C., Montoya, J., and Pinero, D. (1994). Genetic structure of *Rhizobium elti* bv. *phaseoli* associated with wild and cultivated bean plants (*Phaseolus vulgaris* and *Phaseolus coccineus*) in Morelos, Mexico. Applied and Enviromental Microbiology **60**, 1260-1268pp.

Somasegaran, P. and Hoben, H .J. (1994). Handbook for Rhizobia. Springer laboratory, 443 pp.

Bonga, W., Rono, W.K. and Danial, D.L. (1995). Production Constraints of Beans in the semi-arid eastern Kenya with special reference to charcoal rot. Proceedings of a regional workshop of Eastern, Central and Southern Africa held at Njoro, Kenya Oct. 2-6, 1994. Wageningen Agricultural University, Wageningen. 251-255 pp.

Sprent, J.I. (1984). Effects of drought and salinity in heterotrophic nitrogen fixing bacteria and on infection of legumes by rhizobia. In: Veeger, C. and Newton, W. E (eds.) Advances in Nitrogen Fixation Research. Martinus Nijhoff/Dr W. Junk, The Hague. 295-302pp.

Sprent, J.I. and Sprent, P. (1990). Nitrogen fixing organisms. Pure and Applied Aspects. Chapman and Hall

Ssali, H. and Keya, S.O. (1984). Biological nitrogen fixation in Africa. Martinus Press Consultants, Nairobi. 540pp.

Steel, G. D. R. and Torrie, J. H. (1984). Principles and procedures of statistics. A Biometrical Approach/ r.g.d. New York McGraw-Hill Book Company 633 pp.

Steyn, P. (2004). Taxonomy of indigenous rhizobia of South Africa.

Stone, L.R., Whitney, D.A. and Anderson, C.K. (1985). Soybean yield response to residual NO_3-N and applied N. Plant and Soil **84**, 259-265pp.

Streeter, J. (1998). Inhibition of nodule formation and N_2 fixation by nitrate. CRC Critical Reviews in Plant Science **7**, 1-23pp.

Sylvester-Bradley, R. and Kipe-Nolt, J. (1988). Legume-*Rhizobium* Symbiosis. Methods Manual for Evaluation, Selection and Management. CIAT, Cali, Colombia.

Thies, J.E, Singleton, P.W. and Bohlool, B.B. (1991a). Influence of the site of indigenous rhizobial populations on establishment and symbiotic performance of introduced rhizobia on field-grown legumes. Applied Environmental Microbiology **57**, 19-28pp.

Thies, J.E, Singleton, P.W. and Bohlool, B.B. (1991b). Modeling symbiotic performance of introduced rhizobia in the field by use of indices of indigenous population size and nitrogen status of the soil, Applied Environmental Microbiology **57**, 29-37pp.

Thies, J. E., Woomer, P. L. and Singleton, P. W. (1995). The enrichment of *Bradyrhizobium* spp population in soil due to cropping of homologous host legume. Soil Biology and Biochemistry **27**, 633-636pp.

Trotman, A.A. and Weaver, R.W. (1986). Number and effectiveness of cowpea rhizobia in soils of Guyana. Tropical Agriculture (Trinidad) **63**, 129-132pp.

urk, D., Keyser, H.H. and Singleton, P.W. (1993). Response of tree legumes to rhizobial inoculation in relation to the population density of indigenous rhizobia. Soil Biology and Biochemistry **25,** 75-81pp.

ruchet, G.L. and Dazzo, F.B. (1982). Morphogenesis of Lucerne root nodules incited by *Rhizobium meliloti* in the presence of combined nitrogen. Planta **154**, 352-360pp.

andamme, P., Pot, B., Gillis, M., De Vos, P., Kersters, K. and Swing, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiological Review **60**, 407-438pp.

incent, J.M. (1988). The role of legume, Rhizobium and environment in nitrogen fixation: constrains on symbiotic potential and their removal. In: Beck, D.P. and Materon, L.A. (eds). Nitrogen fixation by legumes in Mediterranean Agriculture. Martinus Nijhoff, Dordrecht, The Netherlands, 275-285 pp.

Weaver, R.W., Fredrick, L.R. and Dumenil, L.C (1972). Effect of soybean cropping and soil properties on numbers of *Rhizobium japonicum* in Iowa soils. Soil Science **114**, 137-141pp.

Werner, D. (1992). Symbiosis of plants and microbes. Phillips-Universitat Marburg, Germany.264pp.

Wilson, J.K. (1994). Legume bacteria of the soil. Journal of American Society of Agronomy **18**, 911-919pp.

Woomer, P, Singleton, P.W. and Bohlool, B.B (1988). Ecological indicators of native rhizobia in tropical soils. Applied Environmental Microbiology **54,** 1112-1116pp.

Worall, V. and Roughley, R. J. (1991). Vertical movement of *Rhizobium leguminosarum* bv. *trifolii* in soil as influenced by soil water potential and flow. Soil Biology and Biochemistry **23**, 485-486pp.

Wortmann, C.S. and Allen, D.J. (1994). African bean production environments: their definition, characteristic and constraints. Network on Bean Research in Africa, occasional series No. 11 Dar es Salaam, Tanzania.

Wortmann, C.S., Kirkby, R.A., Eledu, C.A. and Allen, D.J. (1998). Atlas of Common Bean (*Phaseolus vulgaris* L.) Production in Africa. CIAT Pan-African Bean Research Alliance. 133pp.

Xu, L.M., Ge, C., Cui, Z., Li, J. and Fan, H. (1995). *Bradyrhizobium liaoningensis* spp. nov. isolated from root nodules of soybean. International Journal of Systematic Bacteriology 45, 706-711.

Yousef, A.N., Al-Nassir, A.S., Al-Azawi, S.K and Abdull-Hussain, N. (1987). Abundance of peanut rhizobia as affected by environmental conditions in Iraq. Soil Biology and Biochemistry **19**, 391-396pp.

Young, J.P.W. (1992). Phylogenetic classification of nitrogen fixating organisms. In: Stacey, G., Burris, R.H. and Evans, H.J. (eds) Biological Nitrogen Fixation.Chapman and Hall, New York, 43-85pp.

Young, J.P.W. and Haukka, K.E. (1996). Diversity and phylogeny of rhizobia. New Phytologist **133**, 87-94pp.

APPENDICES

Appendix 1: Soil chemical characteristics at Kabete Field Station experimental plots

Soil attributes	Levels
Soil pH	6.20
Soil carbon (%)	3.60
Soil nitrogen (%)	0.40
Electrical conductivity (dsm^{-1})	0.36
Phosphorous (ppm)	20.00
C.E.C. (C mol. kg^{-1})	12.60
Copper (ppm)	0.10
Iron (ppm)	1.00
Manganese (ppm)	Trace
Zinc (ppm)	1.00
Potassium (C mol. kg^{-1})	0.50
Sodium (C mol. kg^{-1})	0.20
Magnesium (C mol. kg 1)	3.87

Appendix 2: Physicochemical characteristics of soils from four field sites in Central Kenya

Soil Properties	Kabete cultivated	Kabete uncultivated	Kajiado	Nyeri	Machakos
pH H_2O	6.20	6.20	6.40	4.00	6.80
% Carbon	3.50	4.70	4.70	3.10	1.70
% Nitrogen	0.38	0.40	0.50	0.28	0.21
EC dsm^{-1}	0.30	0.36	0.32	0.12	0.41
Phosphorous (ppm)	25.0	20.0	30.5	3.50	42.2

ppendix 3: Kabete Field Station weather data for the long rains 2004 and short rain 2004

onth	Temperature (^{O}C)	Relative humidity (%)	Rainfall (mm)	Number of rainy days
arch 2004	20.3	65.8	87.2	8
pril	19.2	76.0	411.6	22
ay	18.5	71.5	190.3	10
ne	14.7	69.0	10.4	8
ly	16.3	65.0	6.2	4
ugust	16.7	67.0	0.2	2
eptember	18.5	61.5	16.0	12
ctober	18.9	67.0	82.0	13
lovember	18.6	73.0	118.4	20
ecember	19.1	69.5	58.1	10
anuary 2005	18.7	57.0	77.8	12
ebruary	20.8	51.5	45.7	6
Iean	18.3	66.2	92.5	10.5

Appendix 4: ANOVA Table for the effects of rhizobia inoculation and starter-nitrogen on number of nodules of legumes at 4 weeks after planting during the long rains

Source	df	SS	MS	F value	F pr
Replications	2	58.78	29.39	2.88	
Legumes (A)	5	504.81	101.96	9.91	0.001**
Residual error (A)	10	101.93	10.19	2.08	
Nitrogen source (B)	2	75.58	37.78	7.71	0.003*
Legume X Nitrogen (A x B)	10	42.14	4.21	0.86	0.58 ns
Residual error (B)	24	117.59	4.90		
Total residual error	53	900.83			

Appendix 5: ANOVA Table for the effects of rhizobia inoculation and starter-nitrogen on number of nodules of legumes at 4 weeks after planting during the short rains

Source	df	SS	MS	F value	F pr
Replications	2	2.97	1.49	0.11	
Legumes (A)	5	291.40	58.28	4.37	0.02*
Residual error (A)	10	133.42	13.34	3.74	
Nitrogen source (B)	2	10.71	5.36	1.50	0.24 ns
Legume X Nitrogen (A x B)	10	54.00	5.40	1.51	0.20 ns
Residual error (B)	24	85.71	3.71		
Total residual error	53	578.21			

** Highly significant ns = Not significant * Significant

Appendix 6: ANOVA Table for the effects of rhizobia inoculation and starter-nitrogen on number of nodules of legumes at 6 weeks after planting during the long rains

Source	df	SS	MS	F value	F pr
Replications	2	14.98	7.49	2.02	
Legumes (A)	5	488.55	97.71	26.31	<0.001**
Residual error (A)	10	37.14	3.71	0.49	
Nitrogen source (B)	2	41.78	20.89	2.78	0.08 ns
Legume X Nitrogen (A x B)	10	83.44	8.34	1.11	0.4 ns
Residual error (B)	24	180.43	7.52		
Total residual error	53	846.32			

Appendix 7: ANOVA Table for the effects of rhizobia inoculation and starter-nitrogen on number of nodules of legumes at 6 weeks after planting during the short rains

Source	df	SS	MS	F value	F pr
Replications	2	22.95	11.47	0.22	
Legumes (A)	5	6019.12	1203.82	22.60	<0.001**
Residual error (A)	10	532.55	53.26	0.74	
Nitrogen source (B)	2	296.90	148.45	2.07	0.15 ns
Legume X Nitrogen (A x B)	10	594.65	59.47	0.83	0.60 ns
Residual error (B)	24	1717.07	71.54		
Total residual error	53	9183.24			

** Highly significant ns = Not significant * Significant

Appendix 8: ANOVA Table for the effects of rhizobia inoculation and starter-nitrogen on nodule dry matter (mg) of legumes at 4 weeks after planting during the long rains

Source	df	SS	MS	F value	F pr
Replications	2	2086.30	1043.10	1.20	
Legumes (A)	5	20989.70	4197.90	4.90	0.016*
Residual error (A)	10	8649.70	865.00	6.10	
Nitrogen source (B)	2	1673.80	836.90	5.90	0.008*
Legume X Nitrogen (A x B)	10	4266.90	426.70	3.00	0.013*
Residual error (B)	24	3422.00	142.60		
Total residual error	53	41088.40			

Appendix 9: ANOVA Table for the effects of rhizobia inoculation and starter-N on nodule dry matter (mg) of legumes at 4 weeks after planting during the short rains

Source	df	SS	MS	F value	F pr
Replications	2	11892.60	5946.30	10.40	
Legumes (A)	5	46459.30	9291.90	16.20	<0.001**
Residual error (A)	10	5729.60	573.00	2.80	
Nitrogen source (B)	2	625.90	313.00	1.50	0.237 ns
Legume X Nitrogen (A x B)	10	10796.30	1079.60	5.30	<0.001**
Residual error (B)	24	4911.10	204.60		
Total residual error	53	80414.80			

** Highly significant ns = Not significant * Significant

Appendix 10: ANOVA Table for the effects of rhizobia inoculation and starter-N on nodule dry matter (mg) of legumes at 6 weeks after planting during the long rains

Source	df	SS	MS	F value	F pr
Replications	2	12315.00	6158.00	2.00	
Legumes (A)	5	143248.00	28657.00	9.32	0.002*
Residual error (A)	10	30740.00	3074.00	1.28	
Nitrogen source (B)	2	1062.00	531.00	0.22	0.80 ns
Legume X Nitrogen (A x B)	10	7333.00	733.00	0.30	0.97 ns
Residual error (B)	24	57822.00	2409.00		
Total residual error	53	252556.00			

Appendix 11: ANOVA Table for the effects of rhizobia inoculation and starter-nitrogen on nodule dry matter (mg) of legumes 6 weeks after planting during the short rains

Source	df	SS	MS	F value	F pr
Replications	2	7.70	3.90	0.00	
Legumes (A)	5	204959.90	40992.00	28.84	<0.001**
Residual error (A)	10	14212.70	1421.30	2.42	
Nitrogen source (B)	2	9276.90	4638.50	7.88	0.002*
Legume X Nitrogen (A x B)	10	12324.90	1232.50	2.09	0.07 ns
Residual error (B)	24	14121.60	588.40		
Total residual error	53	254903.60			

** Highly significant ns = Not significant * Significant

Appendix 12: ANOVA Table for the effects of rhizobia inoculation and starter-nitrogen on shoot dry matter (g/m^2) of legumes at 4 weeks after planting in the long rains

Source	df	SS	MS	F value	F pr
Replications	2	47.12	23.56	0.53	
Legumes (A)	5	9353.59	1870.72	42.10	<0.001**
Residual error (A)	10	444.31	44.43	1.98	
Nitrogen source (B)	2	63.62	31.81	1.42	0.26 ns
Legume X Nitrogen (A x B)	10	340.09	34.01	1.52	0.19 ns
Residual error (B)	24	537.89	22.41		
Total residual error	53	10786.61			

Appendix 13: ANOVA Table for the effects of *rhizobia* inoculation and starter-nitrogen on shoot dry matter (g/m^2) of legumes at 4 weeks after planting in the short rains

Source	df	SS	MS	F value	F pr
Replications	2	336.60	168.30	1.06	
Legumes (A)	5	14641.07			0.003*
Residual error (A)	10	1592.66	159.27		
Nitrogen source (B)	2	107.34	53.67	1.15	0.34 ns
Legume X Nitrogen (A x B)	10	570.56	57.06	1.22	0.33 ns
Residual error (B)	24	1124.65	46.86		
Total residual error	53	18372.89			

** Highly significant ns = Not significant * Significant

ppendix 14: ANOVA Table for the effects of *Rhizobia* inoculation and starter-N on shoot dry matter (g/m^2) of legumes at 6 weeks after planting during the long rains

Source	df	SS	MS	F value	F pr
Replications	2	336.60	168.30	1.06	
Legumes (A)	5	14641.07	2928.21	18.39	<0.001**
Residual error (A)	10	1592.66	159.27	3.40	
Nitrogen source (B)	2	107.34	53.67	1.15	0.34 ns
Legume X Nitrogen (A x B)	10	570.56	57.06	1.22	0.33 ns
Residual error (B)	24	1124.65	46.86		
Total residual error	53	18372.89			

Appendix 15: ANOVA Table for the effects of rhizobia inoculation and starter-N on shoot dry matter (g/m^2) of legumes at 6 weeks after planting during the short rains

Source	df	SS	MS	F value	F pr
Replications	2	47676.0	23838.0	2.33	
Legumes (A)	5	950784.0	190157.0	18.62	<0.001**
Residual error (A)	10	102120.0	10212.0	3.70	
Nitrogen source (B)	2	1157.0	578.0	0.21	0.81ns
Legume X Nitrogen (A x B)	10	29429.0	2943.0	1.07	0.42ns
Residual error (B)	24	66239.0	2760.0		
Total residual error	53	1197406.0			

** Highly significant ns = Not significant * Significant

Appendix 16: ANOVA Table for the effects of rhizobia inoculation and starter-N on root dry matter (mg) of legumes at 4 weeks after planting during the long rains

Source	df	SS	MS	F value	F pr
Replications	2	16597.00	8298.00	3.74	
Legumes (A)	5	393473.00	78695.00	35.51	<0.001**
Residual error (A)	10	22161.00	2216.00	0.90	
Nitrogen source (B)	2	10432.00	5216.00	2.12	0.14 ns
Legume X Nitrogen (A x B)	10	18593.00	1859.00	0.76	0.67 ns
Residual error (B)	24	59088.00	2462.00		
Total residual error	53	520343.00			

Appendix 17: ANOVA Table for the effects of rhizobia inoculation and starter-N on root dry matter (mg) of legumes at 4 weeks after planting during the short rains

Source	df	SS	MS	F value	F pr
Replications	2	4111.10	2055.50	2.71	
Legumes (A)	5	46971.70	9394.30	12.40	<0.001**
Residual error (A)	10	7576.20	757.60	1.29	
Nitrogen source (B)	2	400.90	200.40	1.02	0.71 ns
Legume X Nitrogen (A x B)	10	5997.10	599.70		0.45 ns
Residual error (B)	24	14062.70	585.90		
Total residual error	53	79119.70			

** Highly significant ns = Not significant * Significant

Appendix 18: ANOVA Table for the effects of rhizobia inoculation and starter-N on root dry matter (mg) of legumes at 6 weeks after planting during the long rains

Source	df	SS	MS	F value	F pr
Replications	2	92984.00	46447.00	5.01	
Legumes (A)	5	1706497.00	341299.00	36.79	<0.001**
Residual error (A)	10	92774.00	9277.00	2.05	
Nitrogen source (B)	2	28132.00	14066.00	3.11	0.06 ns
Legume X Nitrogen (A x B)	10	111974.00	11197.00	2.47	0.03*
Residual error (B)	24	108604.00	4525.00		
Total residual error	53	2140876.00			

Appendix 19: ANOVA Table for the effects of rhizobia inoculation and starter-N on root dry matter (mg) of legumes at 6 weeks after planting during the short rains

Source	df	SS	MS	F value	F pr
Replications	2	69893.0	34947.0	1.40	
Legumes (A)	5	1091261.0	218252.0	8.71	0.002*
Residual error (A)	10	250437.0	25044.0	3.53	
Nitrogen source (B)	2	1870.0	935.0	0.13	0.88 ns
Legume X Nitrogen (A x B)	10	59004.0	5900.0	0.83	0.60 ns
Residual error (B)	24	170339.0	7097.0		
Total residual error	53	1642805.0			

** Highly significant ns = Not significant * Significant

Appendix 20: ANOVA Table for the effects of rhizobia inoculation and starter-N on grain weight (kg ha^{-1}) of legumes at harvest during the long rains

Source	df	SS	MS	F value	F pr
Replications	2	82740	41370	0.51	
Legumes (A)	5	11777626	2355525	29.23	<0.001
Residual error (A)	10	805768	80577	2.47	
Nitrogen source (B)	2	77357	38678	1.19	0.323ns
Legume X Nitrogen (A x B)	10	290087	29009	0.89	0.557ns
Residual error (B)	24	783293	32637		
Total residual error	53	13816872			

Appendix 21: ANOVA Table for the effects of rhizobia inoculation and starter-N on grain weight (kg ha^{-1}) of legumes at harvest during the short rains

Source	df	SS	MS	F value	F pr
Replications	2	177559	88780	1.01	
Legumes (A)	5	131244104	26248821	298.51	<0.001
Residual error (A)	10	879315	87931	0.25	
Nitrogen source (B)	2	41208	20604	0.06	0.94ns
Legume X Nitrogen (A x B)	10	1539259	153926	0.44	0.91ns
Residual error (B)	24	8466292	352762		
Total residual error	53	14237736			

** Highly significant ns = Not significant * Significant

Appendix 22: ANOVA Table for the effects of rhizobia inoculation and starter-N on number of pods per plant of legumes at harvest during the long rains

Source	df	SS	MS	F value	F pr
Replications	2	137.24	68.62	0.55	
Legumes (A)	5	52178.63	10435.73	83.87	<0.001**
Residual error (A)	10	1244.23	124.42	0.77	
Nitrogen source (B)	2	339.19	169.59	1.05	0.37 ns
Legume X Nitrogen (A x B)	10	1599.23	159.92	0.99	0.48 ns
Residual error (B)	24	3890.77	162.12		
Total residual error	53	19797.31			

Appendix 23: ANOVA Table for the effects of rhizobia inoculation and starter-N on number of pods per plant of legumes at harvest during the short rains

Source	df	SS	MS	F value	F pr
Replications	2	79.50	39.80	0.33	
Legumes (A)	5	237222.10	47444.40	388.71	<0.001**
Residual error (A)	10	1220.60	122.10	1.90	
Nitrogen source (B)	2	474.80	234.40	3.70	3.46 ns
Legume X Nitrogen (A x B)	10	2805.90	280.60	4.37	2.43 ns
Residual error (B)	24	1540.00	64.20		
Total residual error	53	243342.80			

** Highly significant ns = Not significant * Significant

Appendix 24: ANOVA Table for the effects of rhizobia inoculation and starter-N on number of grain per pod of legumes at harvest during the long rains

Source	df	SS	MS	F value	F pr
Replications	2	0.57	0.28	0.36	
Legumes (A)	5	104.71	20.94	26.79	<0.001**
Residual error (A)	10	7.82	0.78	3.36	
Nitrogen source (B)	2	0.11	0.06	0.25	0.78 ns
Legume X Nitrogen (A x B)	10	1.61	0.16	0.69	0.72 ns
Residual error (B)	24	5.59	0.23		
Total residual error	53	40.14			

Appendix 25: ANOVA Table for the effects of rhizobia inoculation and starter-N on number of grain per pod of legumes at harvest during the short rains

Source	df	SS	MS	F value	F pr
Replications	2	2.17	1.08	2.00	
Legumes (A)	5	578.11	115.62	214.11	<0.001**
Residual error (A)	10	68.80	6.88	12.7	
Nitrogen source (B)	2	0.04	0.02	0.04	0.58 ns
Legume X Nitrogen (A x B)	10	17.86	1.79	3.31	0.42 ns
Residual error (B)	24	12.96	0.54		
Total residual error	53	64.16			

** Highly significant ns = Not significant * Significant

Appendix 26: ANOVA Table for the nodule number of common bean and cowpea inoculated with soils collected from five field sites

Source	df	SS	MS	F value	F pr
Replications	2	1712.0	856.0	0.3	
Legumes	2	268209.0	134105.0	44.1	<0.001**
Soil samples	4	50224.0	12556.0	4.1	0.009
Legume X Soil sample	8	10342.0	13418.0	4.4	0.002
Residual error	28	85248.0	3045.0		
Total residual error	44	512735.0			

Appendix 27: ANOVA Table for the nodule dry matter (mg) of common bean and cowpea inoculated with soils collected from five field sites

Source	df	SS	MS	F value	F pr
Replications	2	0.007	0.004	0.93	
Legumes	2	1.490	0.745	192.61	<0.001**
Soil samples	4	0.406	0.101	26.21	<0.001**
Legume X Soil sample	8	0.670	0.083	21.64	<0.001**
Residual error	28	0.108	0.004		
Total residual error	44	2.681			

** Highly significant ns = Not significant * Significant

Appendix 28: ANOVA Table for the shoot dry matter (g) of common bean and cowpea inoculated with soils collected from five field sites

Source	df	SS	MS	F value	F pr
Replications	2	0.01686	0.00843	0.45	
Soil samples	6	1.67632	0.27939	14.92	<0.001**
Legumes	1	1.23875	1.23875	66.13	<0.001**
Legume X Soil sample	6	0.31580	0.05263	2.81	0.03*
Residual error	26	0.48702	0.01873		
Total residual error	4	3.73475			

Appendix 29: ANOVA Table for the shoot tissue-N (mg/plant) of cowpea inoculated with soils collected from five field sites

Source	df	SS	MS	F value	F pr
Replications	2	8.962	4.481	3.28	
Soil samples	6	70.284	11.714	8.59	<0.001**
Residual error	12	16.372	1.364		
Total residual error	20	95.618			

** Highly significant ns = Not significant * Significant

Appendix 30: Mean number of nodules per plant of common bean inoculated with soil dilutions made from soil samples collected from five field sites

Dilution	Kabete cropland	Kabete Pastureland	Kajiado	Machakos	Nyeri	Means
10^{-1}	46.8	35.7	48.7	5.2	21.3	31.5
2^{-1}	72.3	22.2	108.3	23.5	9.5	47.2
2^{-2}	54.5	33.7	66.0	29.7	8.8	36.5
2^{-3}	29.8	26.0	43.8	23.2	4.8	24.6
2^{-4}	28.8	33.3	22.5	15.2	2.2	20.2
2^{-5}	25.0	28.8	15.8	13.2	2.0	16.3
2^{-6}	7.3	24.2	8.7	10.5	1.7	10.5
Rhizobia	25.2	30.0	17.1	40.2	22.0	26.7
Control	0.0	0.0	0.0	0.0	0.0	0.0
Means	33.1	25.5	39.2	15.1	6.3	23.6

Appendix 31: Mean number of nodules per plant of cowpea inoculated with soil dilutions made from soil samples collected from five field sites

Dilution	Kabete cropland	Kabete pastureland	Kajiado	Machakos	Nyeri	Means
10^{-1}	14.7	6.8	7.7	25.2	1.2	11.1
2^{-1}	13.8	8.3	11.7	21.7	0.2	11.1
2^{-2}	6.6	3.5	12.2	22.5	0.8	9.7
2^{-3}	9.0	1.0	4.6	28.8	0.8	8.8
2^{-4}	5.7	0.7	2.2	26.2	0.0	6.9
2^{-5}	0.0	0.0	1.3	16.2	0.0	3.5
2^{-6}	0.0	0.0	0.3	19.8	0.0	4.3
Rhizobia	28.5	36.0	54.0	29.3	27.2	35.0
Control	0.0	0.0	0.0	0.0	0.0	0.0
Means	6.4	2.8	4.9	20.4	0.4	7.0