IMPROVEMENT OF NITROGEN IN FERTILITY DEPLETED SUGARCANE SOILS THROUGH SHORT- TERM PREPLANTING OF LEGUMINOUS PLANTS

BY

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DECLARATION

Declaration by the Student

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To my dear wife, Mary and children Asenath, Fraggie, Garland and Gaylord for their unwavering support, patience and encouragement even when circumstances were not easy.

ABSTRACT

Sugarcane is the main cash crop in the sugar belt of western Kenya and provides income for farmers, particularly poverty stricken smallholders, in the region. However, due to declining soil fertility particularly, soil nitrogen, sugarcane yields have been declining. The increased human population growth resulting in reduced farm sizes and continuous cropping of land has contributed to decline in soil fertility leading to land degradation and serious food insecurity. Leguminous plants are widely used for food, fodder, shade, fuel and constitute part of the cropping systems in rural areas. Other benefits include: attracting beneficial organisms to the cropping system by providing habitat for soil organisms and animals thus improving soil biological and physical structure. The main objective of this study was to screen selected leguminous plants to identify the most effective ones for improving nitrogen fertility depleted sugarcane soils. The study consisted of two field experiments: (i) Pre-plant six legume plants crops to improve the fertility of nitrogen depleted sugarcane soils (Bambara, *Crotalaria*, *Sesbania*, Cowpea, Soybean and Yellow gram) (ii) Sugarcane planted following the harvest of legume plants; in a 3 by 6 factorial experiment laid out with 3 types of starter fertilizer (Control, potassium as KCl and phosphorus as SSP). The study was conducted in Kibos area, Miwani division, Kisumu County ($33^0$20' E and $35^0$20' E and latitudes $0^0$20'S and $0^0$50'S). The trial was for two seasons and the data generated was analyzed using GenStat12th Edition computer package. The results indicated that soils (0-15 cm depth) were acidic to neutral (pH 5.5-7.0); low in organic C, N and P; moderate in Ca. The soil texture was clay loam; soil class, Eutric Vertisol and Dystric Cambisol. There was a significant increment in soil nitrate-N content after harvesting of the legumes compared to initial soil nitrate- N content. The change in soil fertility status particularly increase in soil nitrate-N by legumes had a positive effect on the growth and development of sugarcane in terms of, height, tillering and harvestable fresh biomass ($P \leq 0.05$). There was no significant difference in sugarcane performance with or without application of starter (phosphatic or potassic) fertilizer in soils previously under legume establishment. Cow pea and *Sesbania sesban* improved soil nitrogen content most compared ($P \leq 0.05$). to other leguminous plants by fixing more N hence ideal and best suited for use in improvement of soil nitrogen fertility sugarcane growing soils.

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LIST OF ABBREVIATIONS AND ACRONYMS

KESREF-Kenya Sugar Research Foundation

K-Potassium

Cl-Chlorine

P-Phosphorus

SSP-Single Superphosphate

PH-Hydrogen Ion

C-Carbon

N-Nitrogen

Mg-Magnesium

Ca-Calcium

Na-Sodium

DMRT- Duncan's Multiple Range Test

%-Percent

TSBF-Tropical Soil Biology and Fertility

Kg-Kilogram

Ha-Hectare

ICRAF-International Center for Research on Agro forestry

H-Hydrogen

^{0}C- Degrees Centigrade

M-Meter

Cm-Centimeter

Ml-Milliliter

Mm-Millimeter

Nm-Nanometer

O-Oxygen

AC-Acetate

AAS-Atomic Absorption Spectrophotometer

FP-Flame Photometer

ANOVA-Analysis of Variance

CV- Coefficient of Variation

LSD-Least Significant Difference

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CHAPTER ONE

INTRODUCTION

1.1 Background

Fertilizer use efficiency by most crops and farming systems is very poor and therefore for sustainable productivity with high yields, it's necessary to identify short term and long term needs of the soil. The economic and environmental costs of the heavy use of chemical nitrogen (N) fertilizers in agriculture are a global concern (Ladha *et al.*, 1992). Chemical fertilizers have had a substantial impact on food production in the recent past, and are an indispensable part of modern agricultural practices. The Green Revolution of the past half-century was fueled by technologies heavily dependent on synthetic fertilizers (Eric, 2008). It was estimated that in 1985, the use of 38.8 million tons of N fertilizers on cereals globally resulted in increased world production of 938 million tons which was more than half of the total cereal production in that year (Eric, 2008).

Many small-scale farmers lack the financial means and appropriate incentives to purchase sufficient fertilizer to replenish the nutrients that are removed with harvested plant products. The result has been widespread "mining" of soil nutrients and depletion of soil fertility in Sub-Saharan Africa (SSA) (Buresh and Tian, 1998).

There are, on the other hand, vast areas of the developing world where N fertilizers are neither available nor affordable (Ladha, *et al*., 1992). Furthermore, in most of these countries, removal of N fertilizer subsidies; due to balance of payment problems has resulted in higher price and lower supplies. According to Ladha, *et al*., (1992), even in wealthier nations, economic and environmental considerations dictate that biological alternatives which can augment, and in some cases replace N fertilizers, must be sought.

Biological nitrogen fixation (BNF), a microbiological process which converts atmospheric nitrogen into a plant-usable form by leguminous plants offers this alternative (Ladha, *et al.*, 1992).

1.2 Biological Nitrogen Fixation

The role of legumes, according to Smartt (1990), is very important in the improvement of soil nutrient status in natural vegetation or fallows.

In cultivation, it is hoped that legumes perform soil fertility restorative function, in addition to producing exploitable crops (Dawson, 2008). It is not an exaggeration to say that agriculture would be impossible in vast areas of Africa, for example, were it not for the restorative capacity of indigenous legume plants. Legumes thus have a multipurpose role to play in many subsistence agricultural systems, in helping to maintain nitrogen status of the soil during the cropping phase, where fallowing or long recovery periods occur between cropping phases, to restore soil N status (Smartt, 1990). The wild leguminous species can be trees, shrubs, lianas, or herbaceous plants. Their role is presumed to be of great significance largely on the basis of their abundance in natural re-growth vegetation. In cropping systems where a range of crops can be grown satisfactorily, legumes are usually to be found as a major supplement to carbohydrate containing staple crops such as cereals or tuber and root crops (Smartt, 1990).

1.3 Problem Statement

Continuous cropping of land particularly in sub-Saharan Africa (SSA) region with negligible nutrient inputs contribute to land degradation characterized by low soil fertility particularly low levels of soil nitrogen (N), organic matter and phosphorus.

This has led to decline in soil fertility resulting in low yields of food and cash crops hence serious food insecurity and poverty problems which contribute to development stagnation of the sub-continent (Omotayo and Chukwoka, 2008).

As human population continues to increase, a growing problem in this region, particularly western Kenya, family operated farm decrease in size due to fragmentation (putting up of new homesteads). This leads to intensive cultivation but without replenishing plant nutrients i.e. nutrients mining. The majority of smallholder farmers in western Kenya rely more on nature including biological processes, nutrients cycling to produce crops, including sugarcane without external inputs (Jamoza, J.E., 2003).

Concern has recently been raised regarding the degree of soil degradation that can occur under sugarcane production. Indeed, decline in sugarcane yield per unit area has been linked to soil degradation. Several studies have suggested that the most serious factor associated with soil degradation under sugarcane cultivation is the loss of soil organic matter (Garside, et al., 2004). Sugarcane generates large amounts of waste biomass within a very short time. It is the practice of burning the biomass when harvesting and bringing up ratoon crop that destroys organic matter in sugarcane fields. Furthermore, bagasse, filter-mud, molasses and effluent waste waters are not returned to sugarcane fields thus reducing the organic matter of the soils leading to low sugarcane production.

1.4 Justification

Despite sugarcane farming, most of the smallholder farmers in the western Kenya region are in poverty bracket and therefore need both food and alternative income generating enterprises. Mono-cropping for long periods of time with crops like sugarcane depletes

soil nutrients especially nitrogen (N) and hence the need for fallow cropping with legumes for replenishment. In addition, legumes inhibit and suppress some soil pathogens that affect sugarcane productivity negatively.

Human population continues to rise and over dependence on crop farming without replenishing nutrients removed in harvest continues the depletion of soil nutrients. Use of leguminous plants to replenish soils fertility becomes very important, and especially biological nitrogen fixation (BNF) since nitrogen is the most limiting factor in our soils. By the year 2050, world population is expected to double from its current level of more than 5 billion (*http://www.un.org/esa/population/publications/wpp 2008)*. It is reasonable to expect that the need for fixed nitrogen for crop production will also be at least double. If this is supplied by industrial sources, synthetic fertilizer nitrogen use will increase to about 160 million tons of nitrogen per year from 80 million tons in 1989 in response to the needs of high yielding crops, about equal to that produced by the biological process.

Most sugarcane farmers in western Kenya experience delay in payment of their sugarcane crop and in the process experience food insecurity since most of the land is under sugarcane and generally lack money to buy food (Action Aid International Kenya, 2005). With farming of food cover crops, they will be able to realize returns from the sale of short term food crops to address immediate food and other needs.

It is reasonable to expect that the need for fixed nitrogen for crop production will also be at least double. If this is supplied by industrial sources, synthetic fertilizer nitrogen use will increase to about 160 million tons of nitrogen per year from 80 million tons in 1989 in response to the needs of high yielding crops, about equal to that produced by the biological process *(http://www.un.org/esa/population/publications/wpp 2008)*

1.5 Objectives

1.5.1 Main Objective

To improve soil nitrogen levels of fertility depleted sugarcane soils through short-term pre-planting of leguminous plants.

1.5.2 Specific Objectives

1) To evaluate the effectiveness of selected leguminous plants for short term improvement of nitrogen fertility in depleted sugarcane soils.

2) To evaluate the content of nitrogen and phosphorus in the tissues of different leguminous plants used for short term improvement of nitrogen fertility in depleted sugarcane soils

3) To evaluate the performance of sugarcane in plots previously under short-term different legume plants, with and without starter phosphatic and potassic fertilizers.

1.5.3 Hypotheses

H_A: There are significant differences among legume plants in improving soil nitrogen fertility

H_A: There are significant differences in sugarcane performance with or without application of phosphate or potassium fertilizer in soils previously under short term legumes

$H_{A:}$ There are significant differences in nitrogen and phosphorus tissue contents of leguminous plants used for short term improvement of nitrogen fertility in soils under sugarcane

CHAPTER TWO

LITERATURE REVIEW

2.1 Sugarcane

2.1.1 Origin

Sugarcane belongs to the family Poaceae (formerly *Gramineae*). The commonly cultivated sugarcane is classified as *Saccharum officinarum (http://en.wikipedia.org/Sugarcane).* It belongs to a family of perennial grasses that grow between 3 to 6 m in height and has stems 2 to 5 cm thick. The thick stems contain crystals of sugar that, after a period of refinement, are preserved into what is commonly known as table sugar. Sugarcane is propagated vegetatively from cuttings and on average; the crop is ready for harvesting twelve to eighteen months after planting. The cane stalks are then transported by trucks and tractor-trailers to the sugar mills where the milling process of cane is undertaken beginning with crashing to extract juice (Kampen, 2000).

New Guinea is the home of a cultivated form of sugarcane. In ancient times, people migrating from the Indo-China area to New Guinea encountered different types of wild sugarcane types. Higher-fiber forms were used for construction, softer and juicier forms were propagated in gardens for chewing. Until some 450 years ago, fruits and honey were the most important sweet foods in the world. To date, sugarcane remains the sweetener of choice (Kampen, 2000).

2.1.2 Ecology of Sugarcane

Sugarcane is a tropical plant. It grows more successfully in those regions where the climate is more or less tropical but it can grow in sub tropics too as in north India. Sugarcane can be grown on all types of soils ranging from sandy loam to clay loam.

It, however, thrives best on well drained soils. It can also be raised successfully on lighter soils provided there is adequate irrigation facilities and on heavy clays with proper drainage and addition of organic matter. In India sugarcane is grown in areas with annual rainfall ranging between 600 mm to 3000 mm. Optimum cane growth is achieved with air temperatures between 24 and 30^0 C. Greater incident radiation (Sunshine) favors higher sugarcane and sugar yields (*www.ikisan.com/tg-general-crop-sugarcane.html*).

2.1.3 Importance of sugarcane as an industrial crop in Kenya

Sugarcane is an important industrial crop which plays an important role in agriculture and economic development of Kenya. It contributes significantly to the government policy of self-sufficiency in food production and is a major foreign exchange saver through import substitution. It also contributes direct employment to over 35,000 workers in addition to generating incomes to over 100,000 small scale farmers who contribute 85% of sugarcane supply (Kenya Sugar Research Foundation 2007; Anon 2007).

Sugarcane-growing areas of western and Nyanza provinces in Kenya rely heavily on sugarcane as the main source of income. Sugarcane is the main cash crop in these regions and income from sugarcane farming provides smallholder farmers with financial resources to buy food, pay for health care and educate their children among other household financial obligations. Related to the problem of food security is school enrolment. Sugarcane farming acts as some form of collateral in schools and hospitals, where the schools, the community and friends are sometimes prevailed upon by farmers to offer them credit on the promise that when cane is harvested the sales proceeds will be used to clear fees (Action Aid International Kenya, 2005; Anon 2005). Non-payment for delivered sugar cane literally reduces the rural economies into ′Non-income′ zones.

Smallholder farmers are particularly affected through loss of livelihood, increase of poverty levels and persistent food insecurity.

Sugarcane along with coffee, tea and pyrethrum, play significant roles in raising household incomes, providing employment, earning foreign exchange and enhancing food security to many smallholders and producers (Kenya Sugar Research Foundation, 2007). Sugarcane juice is evaporated after bleaching with sulfur dioxide leaving behind crystals and thick syrup known as molasses. The molasses is sold for use in baking; distillation of rum and manufacture of methylated spirits. The sugar crystal is then packed off to refineries where they are granulated, powdered or lumped into cubes for consumers. The bulk of sugar today comes from sugarcane besides being used as a sweetener. Sugarcane e.g. especially the dry leaves in e.g. Bangladesh are also used in curing tobacco through fire cured tobacco system in a furnace (Nyer, 2008).

2.2 Leguminous (*Leguminosae* family) plants

Legumes are capable of fixing nitrogen from the air through a symbiotic association, called mutualism, with rhizobium bacteria. The amount of fixed nitrogen that leguminous plants can accumulate in the soil ranges between 45 to 224 kg per hectare (Sullivan, 2003). About 40 to 60 percent of this nitrogen will become available to a following crop if the planta are used as a green manure (Sullivan, 2003).

Legumes are widely used for food, fodder, shade, fuel and timber, as cover crops and for green manure. They are a feature of: -cropping systems; grazing systems; plantation

systems and agroforestry systems (Ladha *et al.*, 1992). Leguminous plants can obtain most of the nitrogen they need from the vast supply of gaseous nitrogen in the air.

The family *leguminosae* comprises about 20,000 plant species in about 650 different genera. Only 15% of these have been studied.

Sarrantonio (1991) reported that legumes are also valuable as cover crops in which their purpose is largely to prevent erosion. They protect soil from erosion and can be used year-round to stabilize sloping areas. Leguminous plants have uses as medicines, dyes and fiber plants. Legumes can be used to maintain the kind of internal nutrient cycle found in natural ecosystems. Grazing animals such as cows, sheep and goats are often fed some proportion of leguminous material, such as alfalfa or clover. Soybeans are commonly fed to meat animals to fatten them up (Sarrantonio, 1991).

2.2.1 Yellow grams (*Vigna radiata* L.)

Grams are annual legume crops grown for their seed. Grams could be green, black or yellow in color. The green grams are the most commonly grown in Kenya and are native crops of India often called green gram or golden, and it is cultivated in other several countries of Asia, Africa, and the Americas (*http://www.infonet-biovision.org (2015*).

The dried beans are prepared by cooking or milling. They are eaten whole or split. The seeds or the flour may be used in a variety of dishes like soups, porridge, snacks, bread, noodles and even ice cream. Yellow gram also produces great sprouts, which can be sold in health food shops or eaten at home. Crop residues of *V. radiata* are a useful fodder. Yellow gram is sometimes specifically grown for hay, green manure or as a cover crop, *http://www.infonet-biovision.org (2015).*

Yellow grams grow best at an altitude of 0-1600 m above sea level and under warm climatic conditions (28 to 30°C). They are well adapted to red sandy loam soils, but also do reasonably well on not too exhausted sandy soils. Grams are not tolerant to wet, poorly drained soils. They are drought tolerant and will give reasonable yields with as little as 650 mm of yearly rainfall. Heavy rainfall results in increased vegetative growth with reduced pod setting and development. *https:/www.kari.org (2015)*

2.2.2 Cowpea (*Vigna unguiculata L.*)

Cowpea is found throughout the tropics with some 150-190 species reported. Wild forms are found in Africa, but are absent from Asia. The pea arose from the domestication of wild *Vigna unguiculata dekindtiana* forms in West Africa. The origin of the cultivated cowpea can, with confidence, be located in a broad sub-Saharan belt (Smartt, 1990). It is adapted to a wide range of soils from sands to heavy, well-drained clays, with a preference for lighter soils that allow good rooting. Cowpea is cultivated in soils with wide range of pH including very acid (pH 4) and low-fertility throughout the tropics and subtropics between 35ºN and 30ºS, across Asia and Oceania, the Middle East, southern Europe, Africa, southern USA, and Central and South America. Cowpea is one of the most widely used legumes in the tropical world. The grain is used widely for human nutrition, especially in Africa. It is one of the most important tropical dual-purpose legumes, being used for vegetables (leaves and flowers), grain, as fresh cut and carry forage, and for hay and silage (Smartt, 1990);

(http*://www.tropicalforages/Vigna_unguiculata 2015*)

2.2.3 Soybean (*Glycine max L.*)

Soybean is grown in many areas of the globe in both tropical and temperate climates. It can yield an average of between 3 to 5 tons grain per hectare, given good soil and water conditions. Soybean seed is rich in protein (about 40 %) and oil (about 20 %). Soybean meal is used as a protein additive to both human (tofutti, soyareni, artificial meat) and animal feeds. The classical uses of soybean in Asia (e.g. bean curd, tofu) have extended to the western world because of increased health conscientiousness (Gresshoff, 1990). Additional expansion may arise as industrialized nations recognize that wildly planted basic crop plants can produce renewable industrial reserves. The current improvement strategy is progressively shifting towards reducing inputs as compared to increasing output. Yield, water use efficiency and salt tolerance are controlled by several gene systems, and therefore it is likely that these characters can be improved easily by new gene manipulation techniques. However, aspects that involve 'input costs' such as fertilizer use, application and transport, pesticide and herbicide costs show the potential for genetic manipulation due to the fact that few genes (often one) can affect these characters. The analysis of the plant's contribution to symbiotic relationship has shown that genetic manipulation to increase nodulation, nitrogen fixation and indirectly yield is possible (Gresshoff, 1990).

2.2.4 Bambara nut (*Vigna subterrenea* (*L.*) Verdc (The ground bean)

The ground bean is widely distributed in Africa and is reported to have originated in West Africa. Very few if any taxonomic studies involving hybridization have been carried out on the ground bean. It has very distinctive features and could well, like the

cowpea, have developed very effective mechanisms to isolate it genetically from even those species in the same section as itself. Some studies of the biochemistry of its seed have been undertaken. The protein content is relatively low for a legume (16-21%), and typically the sulfur amino acid content is limiting, relative to reference protein. In West Africa, there appears to be continuing intercrossing between wild and domesticated populations of the ground bean. This tends to erode the effects of selection and establish a near continuum between the wild and cultivated segments of the species. There is no doubt that it is not a strong commercial competitor to the groundnut. However, it may well persist in cultivation in areas where specific nutrient deficiencies, such as those of calcium and boron, for example, limit groundnut production (Smartt, 1990).

2.2.5 *Crotalaria orchroleuca* (Sun hemp)

Crotalaria orchroleuca is a promising shrub for fertility replenishment since it is an N-fixing and accumulates large biomass quantities that upon the incorporation into the soil and eventual decomposition, releases N that can be utilized by the succeeding maize or other crops.

Crotalaria is highly preferred because of its potential multiple uses as a vegetable and for soil fertility improvement. It is also considered to have medicinal value (Odendo *et al.*, 2004).

2.2.6 *Sesbania sesban* (River bean)

Sesbania sesban (Linn) Merril (Syn. *S. Aegyptica*) is a soft wooded, fast growing, and short-lived, cultivated tree that also grows wildly in nature. It tolerates wide range of acidity, periodic flooding and water logging (Albrecht, 1993). It forms root nodules by symbiotic association with *rhizobium* species capable of fixing atmospheric nitrogen.

It is often grown as a perennial green manure crop. It can endure 0.4 -10 % salt concentration at the seedling stage and 0.9 -1.4 % near maturity. The plant has been reported to grow up to 5 meters in 12 months and the yield recorded in India was up to 75 tons ha^{-1} (10 % moisture) in one year (Commonwealth Science Council, 1986).

In Kenya, the species is widely distributed and found in places like Kakamega, Kisumu, Siaya, Kitale, Uasin Gishu, Kisii, South Nyanza, Kericho, Nyeri and Loitokitok. In these areas, it is planted for the production of fuel wood, shade tree and soil stabilization (Albretcht, 1993). It is an agro-forestry tree especially in western Kenya. It is known to be a good shade tree and soil improver, and grows well on swampy sites (Teel, 1984).

Research on improved fallow in Africa's sub-humid tropics focuses primarily on the indigenous nitrogen-fixing tree, *Sesbania sesban* (ICRAF, 1992).

According to ICRAF, (1996) over the past six years of research in Chipata (Zambia) has shown that short-rotation fallows 1-3 years using *S. sesban* can significantly improve maize yields without additional inorganic fertilizers.

According to ICRAF (1996), *S. sesban* indeed reduced the number of Striga weed in the soil by 34 %, thus enabling land to be more productive when grown to susceptible maize. Sesbania tree are also used for food (leaves), wood, fiber, green manure, ornamental and windbreak in China (Nair, 1993)

2.3 Biological Nitrogen Fixation

Biological nitrogen fixation (BNF) is carried out by a specialized group of prokaryotes. These organisms utilize the enzyme *nitrogenase* to catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). Plants can readily assimilate NH_3 to

produce the aforementioned nitrogenous biomolecules. These prokaryotes include aquatic organisms, such as cyanobacteria, free-living soil bacteria, such as *Azotobacter*, bacteria that form associative relationships with plants, such as *Azospirillum*, and most importantly, bacteria, such as *Rhizobium* and *Bradyrhizobium*, which form symbioses with legumes and other plants (Wagner, 2011**).**

Biological nitrogen fixation (BNF) occurs when atmospheric nitrogen is converted to ammonia by an enzyme called ***nitrogenase***. The overall reaction for BNF is:

$$N_2 + 8\ H^+ + 8\ e^- \rightarrow 2\ NH_3 + H_2,$$ (Postgate, 1998)

The process is coupled to the hydrolysis of 16 equivalents of ATP and is accompanied by the co-formation of one molecule of H_2. The conversion of N_2 into ammonia occurs at a cluster called FeMoco, an abbreviation for the iron-molybdenum cofactor (Chi Chung *et al*, 2014).

Biological nitrogen fixation is an essential natural process in which higher plants and animals ultimately obtain nitrogen from nitrogen-fixing organisms or from nitrogen fertilizers (including nitrogen compounds formed during lightning). Available soil nitrogen, which originates from decomposing plant residues and microorganisms, is normally inadequate for intensive crop production. This is the compelling reason to improve our understanding of BNF for application to agriculture and forestry production worldwide. In addition, the projected doubling of population over the next 50 years will put increasing pressure on food production, the environment, and the need for fixed nitrogen. Growing concerns about the environment, energy, nutrition, and agricultural sustainability make the need for BNF research even more compelling. Legumes thus have a multipurpose role to play in much subsistence agricultural systems, in helping to

maintain nitrogen status of the soil during the cropping phase, where fallowing or long recovery periods occur between cropping phases, to restore soil N status. In cropping systems where a range of crops can be grown satisfactorily, legumes are usually to be found as a major supplement to carbohydrate containing staple crops such as cereals or tuber and root crops (Smartt, 1990).

2.3.1 Importance of Nitrogen

Nitrogen is so vital because it is a major component of chlorophyll, the compound by which plants use sunlight energy to produce sugars from water and carbon dioxide (i.e. photosynthesis). It is also a major component of amino acids, the building blocks of proteins. Without proteins, plants wither and die. Nitrogen is a component of energy-transfer compounds, such as ATP (adenosine triphosphate). ATP allows cells to conserve and use the energy released in metabolism. Finally, nitrogen is a significant component of nucleic acids such as DNA, the genetic material that allows cells (and eventually whole plants) to grow and reproduce. Without nitrogen, there would be no life as we know it, *http://www.cropnutrition.com/efu-nitrogen (2015).*

The nitrogen cycle describes movement of the element from the air into the biosphere and organic compounds, then back into the atmosphere. Synthetically produced nitrates are key ingredients of industrial fertilizers, and key pollutants in causing the eutrophication of water systems (Gray, 2009). Molecular nitrogen (N_2) is the major component (approx. 80 %) of the earth's atmosphere. The element is an essential part of many chemical compounds, such as proteins and nucleic acids, which are the basis of all life forms. However, N_2 cannot be used directly by biological systems to build the chemicals

required for growth and reproduction. Before its incorporation into a living system, nitrogen must first be combined with the element H.

This process of reduction of N_2, commonly referred to as 'nitrogen fixation' (N-fixation) may be accomplished chemically or biologically, (i.e. biological nitrogen fixation- BNF). The significance of BNF as the major mechanism of recycling nitrogen from the unavailable atmospheric form to an available form in the biosphere cannot be over emphasized (Hubbell and Kidder, 2003).

2.3.2 The mechanism of nitrogen fixation by legumes

The *Rhizobium* or *Bradyrhizobium* bacteria colonize the host plant's root system and cause the roots to form nodules to house the bacteria. The bacteria then begin to fix the nitrogen required by the plant. Access to the fixed nitrogen allows the plant to produce leaves fortified with nitrogen that can be recycled throughout the plant. This allows the plant to increase photosynthetic capacity, which in turn yields nitrogen-rich seed. The process begins when the rhizobia are attracted to flavonoids released by the host legume's roots. For legumes like alfalfa, clover, and soybeans (others like lupines and peanuts form nodules in other ways) the bacteria then begin to attach themselves to extensions of root epidermal cells called root hairs. The attachment process is actually a two-step process where the bacteria first attach using a Ca^{2+} - binding protein called rhicadhesin. After the bacteria accumulate and anchor themselves to the root hair surface, a firmer attachment that involves lectins and/or cellulose fibrils and fimbriae produced by the host plant and bacteria, respectively (Wagner, 2011).

The host legume then senses chemicals produced by the rhizobia called Nod factors that cause the colonized root hairs to curl and form what is called a shepherd's crook. Then

rhizobia penetrate the root hairs and typically form a tubular structure called an infection thread. Once the bacteria reach the root itself, they stimulate cortical cell divisions that lead to the formation of a nodule. As the nodule begins to form, the bacteria become surrounded by a plant-derived membrane and are released inside plant cells forming the nodule. The bacteria subsequently lose their cell walls and undergo a profound change in cell morphology to form large, irregularly shaped branching cells called bacteroids. They then are entirely dependent on the host plant for their energy needs. In return, the bacteria fix nitrogen for the plant (Wagner, 2011).

The interaction between the bacteria and host legume is so intricate that a particular *Rhizobium* or *Bradyrhizobium* will only nodulate a select number of plant genera. For example, *Rhizobium melilotii* will only nodulate alfalfa, while *Rhizobium leguminosarum biovar trifolii* will only nodulate clover (*Trifolium*). This host specificity is referred to cross inoculation group cell signaling between the bacteria and the legume host (Wagner, 2011).

2.3.3 The Diversity of BNF Systems

BNF is known to occur to a varying degree in many different environments including fresh salty soils, on or within roots, stems and leaves of certain higher plants, and even within the digestive tracts of some animals. The potential for nitrogen fixation exists for any environment capable of supporting growth to microorganisms. Biological systems, which are capable of fixing nitrogen, are historically classified as nonsymbiotic or symbiotic, depending on the required involvement of one or more organism, respectively, in the process (Hubell and Kidder, 2000).

2.3.4 Environmental factors influencing BNF

2.3.4.1 Temperature

Generally, soil temperature inhibits legume BNF through its control on nodulation, nodule establishment, and *nitrogenase* activity when it is either too high or too low.

2.3.4.2 Soil water status

In a similar manner to soil temperature, soil water content in the root zone controls N fixation through nodule establishment and nodule activity, plus gas permeability. Soil water deficit inhibits N fixation (Goh and Bruce, 2005), and the inhibition is reinforced as drought stress becomes intense.

2.3.4.3 Nitrogen concentration in the root zone

It has been widely reported that soil mineral N in the root zone inhibits legume nodulation, nodule establishment as it costs less energy for legumes to take up N from soil than fix N biologically from the atmosphere (Cannell and Thornley, 2000).

2.3.4.4 Carbon demand for fixation

Even though it is difficult to distinguish the proportion of CO_2 generated by N fixation from that generated by respiration for nodule growth and maintenance, the correlation between the rate of CO_2 produced from either nodulated roots or nodules and N fixation rate may be used to evaluate C consumption by N fixation (Liu, *et al*. 2010).

2.3.4.5 Seasonal regulation of BNF

The rate of legume BNF changes with physiological growth stages. It is low in the early growth stages while nodules are establishing and reaches a maximum value between early flowering and early seed-filling, depending on the species and growing conditions**.**

2.3.4.6 Chemical components of the soil (e.g. Molybdenum)

Molybdenum is involved in enzyme systems relating to nitrogen fixation by bacteria growing symbiotically with legumes. Nitrogen metabolism, protein synthesis and sulfur metabolism are also affected by molybdenum. Molybdenum has a significant effect on pollen formation, so fruit and grain formation are affected in molybdenum-deficient plants. The influence of molybdenum on plant nitrogen metabolism is in nitrogen-fixing legumes. Nodules accumulate significantly more molybdenum than what is required in order to support bacterial nitrogenase activity and symbiotic nitrogen fixation. The mobilization and export of fixed nitrogen out of the nodule requires the activity of the molybdoenzyme XDH (Mendel and Haensch, 2002).

2.4 Plant Nutrients

For any kind of crop or plant to grow well, certain nutrients must be made available to the plants from the soil and/or air. The essential element for plant growth include C, H, O, N, P, K, Ca, Mg, B, Cl, Cu, Fe, Mn, Mo, S, and Zn. In sugarcane production, elements that are of nutritional concern include N, P, K, Mg, B, Cu, Fe, Mn, Si, and Zn. A deficiency or over-abundance of one or more of the above elements may limit yields. Sugarcane production may also be markedly enhanced by the application of silicon (Si). Silicon deficiency may lead to general vigor reduction in sugarcane. Farmers striving to produce high sugarcane yields and quality should pursue management strategies that deliver a balanced supply of nutrients to the plant.

2.4.1 Nitrogen

According to Sanchez (1976), nitrogen is the nutrient element that most frequently limits yields in the tropics as well as in the temperate region. With the exception of some

recently cleared land, most cultivated soils are deficient in this element. Additions of nitrogen to soils originate from rain and dust, nonsymbiotic fixation, symbiotic fixation, and animal and human wastes. Losses of nitrogen from the soil are due to crop harvests, soil erosion, volatilization, leaching and denitrification.

The supply of elemental inert nitrogen is inexhaustible. The inert nitrogen is in dynamic Equilibrium with the various fixed nitrogen forms. Even as nitrogen is fixed by the different processes just indicated, so is there a release of elemental nitrogen to the atmosphere from these fixed forms by microbiological and chemical processes (Brady and Weil, 1999).

2.4.2 Phosphorus

This element is present in plant tissues and in soils in smaller amounts than are nitrogen and potassium and in quantities about equal to that of sulfur (Brady and Weil, 1999).

Tropical soils are commonly short of phosphorus (P) and additions of fertilizer P can increase yields on most soils which have not previously received P fertilizer. Warren (1992) reported that agro-forestry and management of mycorrhizae have the potential to improve the P nutrition of crops in tropical Africa but they cannot, however, replace P removed by crops.

2.4.3 Potassium

Potassium is absorbed by plants in larger amounts than any other mineral element except nitrogen. Although the total potassium content of soil is usually many times greater than the amount taken during a growing season, in most cases only small fraction of it is available to plants (Brady and Weil, 1999).

2.5 Starter fertilizers

Early stimulation of the seedlings is usually advantageous and it is desirable to have NPK near the plant root zone. The early growth of the plant shoot is essentially all leaves. In a crop such as maize, leaf growth is completed in about 60 days. Since photosynthesis occurs in the leaves, the number of leaves produced in this period will influence the grain produced in the next 45 days. It is important to have a small amount of nutrients near the very young plants to promote early growth and the formation of healthy leaves. Starter response to NPK is often independent of fertility level.

Under cool temperatures, the early available nutrient supplies may be inadequate because of slow mineralization of nitrogen, phosphorus, sulfur and so on, from the soil organic matter; restricted leaves of plant nutrients in the soil minerals; reduced diffusion of phosphorus and potassium; or limited absorption of phosphorus, potassium, and other nutrients by the plant. Localized applications of fertilizer at planting are commonly referred to as starter or planting fertilizers. Factors that are considered for this are: resistance to pests; competition with weeds; early maturity and maintenance. This permits for more efficient use as saving a trip over the field (Brady and Weil, 1999).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Sites/Area

The study area is located at the Sugar Research Institute (SRI) formerly Kenya Sugar Research Foundation (KESREF) Field Station in Kibos, Kisumu County and lies on longitude 34^0 48'E and latitudes 0^0 04'S and, in the agro-ecological zone (AEZ), LM2 known as marginal sugarcane zone. The mean annual rainfall varies with altitude and proximity to the highlands along the Nandi escarpment and Tinderet. The area has two rainy seasons, with long rains occurring in March/June, while short rains occurring in September/November. During the short rains, the average annual rainfall ranges between 450 mm to 600 mm. The reliability is low and rains are distributed over a long period, making the cultivation of second crop difficult.

The climate at KESREF Kibos is sub-humid at altitude of 1268 m above sea level with mean annual rainfall of 1490 mm. The mean annual maximum temperature ranges from 25^0C to 30^0C and the mean annual minimum temperature ranges from 9^0C to 18^0C following the altitude variation from 1,144 m to 1,525 m above sea level. The land physiography is piedmont plain containing soils developed on alluvium from undifferentiated basement system rocks (Jaetzold and Schmidt, 1982).

The soil is classified as Eutric Vertisol (upland) and Dystric Cambisol (Lowland) (FAO, 1988) and is described as having a dark grayish brown to dark brown sandy clay loam texture underlain by brownish to grayish brown clay loam to light clay. The soils are deep but poorly drained (Landon, 1991; Jaetzold *et al.,* 2005).

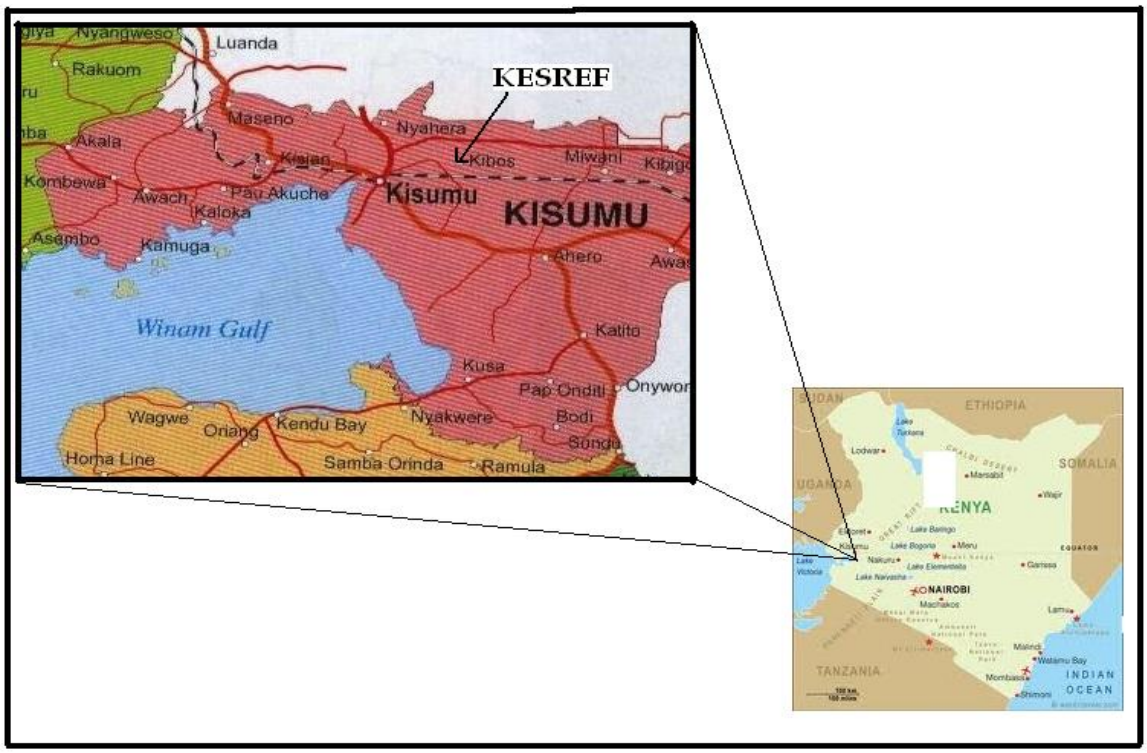

Figure 1: Map showing former Kisumu District (County) and study area in Western Kenya.

Source: Kisumu District Development Plan (1997-2001).

3.2 Experimental design and treatments at KESREF, Kibos, Kisumu

The experiment was a 3×6 factorial experiment arranged (3 fertilizer and 6 legume cover crops) having a total of 18 treatments replicated (blocked) three times.

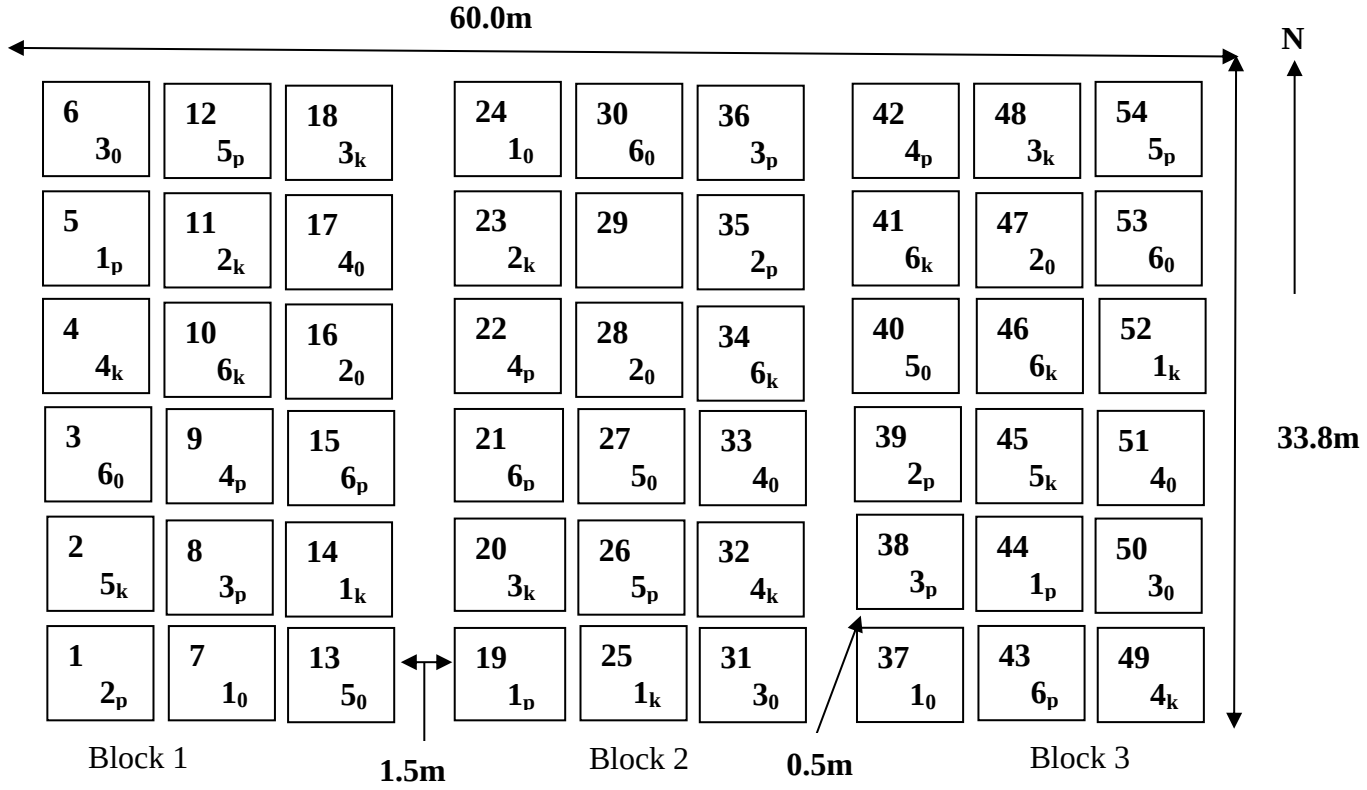

Figure 2: Experimental layout

Key:

$1_{0=}$Yellow grams + Control starter fertilizer; $2_{0=}$ Cowpea + Control starter fertilizer

$3_{0=}$ Soybean + Control starter fertilizer; $4_{0=}$ Bambara nuts + Control starter fertilizer

$5_{0=}$ Crotalaria + Control starter fertilizer; $6_{0=}$ *Sesbania sesban* + Control starter fertilizer

$1_{K=}$Yellow grams + 60 kg K / ha; $2_{K=}$ Cowpea + 60 kg K / ha

$3_{K=}$ Soybean + 60 kg K / ha; $4_{K=}$ Bambara nuts + 60 kg K / ha

$5_{K=}$ Crotalaria+ 60 kg K / ha; $6_{K=}$ *Sesbania sesban* + 60 kg K / ha

$1_{P=}$Yellow grams +30 kg P / ha; $2_{P=}$ Cowpea +30 kg P / ha

$3_{P=}$ Soybean +30 kg P / ha; $4_{P=}$ Bambara nuts +30 kg P / ha

$5_{P=}$ Crotalaria+30 kg P ha; $6_{P=}$ *Sesbania sesban* +30 kg P / ha

Where: 1_0- 6_0 = legumes without starter fertilizer.

1_k- 6_k = legumes with 60 kg K/ ha (KCl- Murate of potash).

1_p- 6_p = legumes with 30 kg P/ ha (SSP).

3.3 Experimental plot layout

The gross plot area was 5m×4.8m, effective plot size 4.5 m × 4.3 m with a plant spacing varying from 30 cm× drill to 1m × 1m depending on type and size of the seed as shown in figure 3.

NB: Numbers 1, 2, 3….to 54 represents individual plots within the blocks

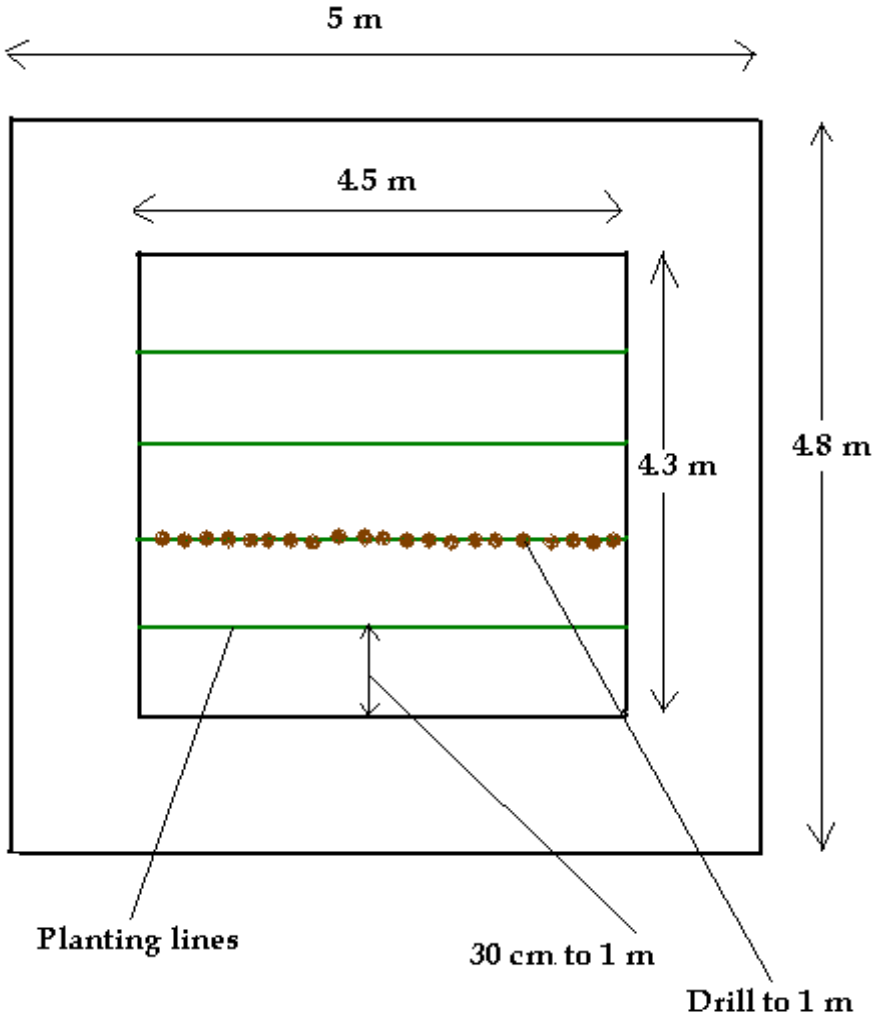

Figure 3: Gross and Effective Plot layout

Sowing of Legumes Key:

Legume pre-plant crops	Spacing
Yellow gram	30 cm x drill
Cowpea	30 cm x drill
Soybean	50 cm x 10 cm
Bambara nut	50 cm x 10 cm
Sun hemp (*Crotalaria*)	30 cm x drill
River bean (*Sesbania*)	1 m x 1 m

NB: There was no combination of fertilizers (P and K), but there was blanket application of the fertilizers to stimulate the growth of legume seeds. Secondly, the experiment was to determine the best leguminous plants out of the six planted.

3.4 Land preparation

All plots were prepared using mechanical (tractor) and manual labor. This activity involved plough and harrow for seedbed preparation. Subdivision of plots was done and checked before seed was sown.

3.5 Planting of sugarcane

A recommended sugarcane variety (KEN- 83-737) for this area was planted in the plots immediately after all legumes had matured and been harvested. Twenty (20) sets were placed per line at a spacing of 1.2 m. Sets each with 3 eye buds were placed in an overlapping manner in each line. Each subplot had 4 lines of sugarcane.

3.6 Starter Fertilizer application

Starter fertilizer was applied using the broadcasting method at uniform rate of 30 kg P ha^{-1} and 60 kg K ha^{-1}, respectively to the earmarked plots. Nitrogen fertilizer was not used because the legumes are nitrogen fixing and if used could have interfered with the BNF process.

3.7 Agronomical practices

3.7.1 Weed control

Weeding was done two weeks after germination and thereafter whenever the plots became weedy until good ground cover was achieved by the crops (legumes and sugarcane).

3.7.2 Pests and Diseases

The sub-plots were monitored all the times and where there was outbreak/attack by pests or diseases, appropriate measures were undertaken to control them.

Karate was used to spray on all legumes especially; Cow peas, *Crotalaria*, Yellow grams and Bambara nuts.

3.8 Data Collection

3.8.1 Initial Soil sampling and after harvesting legumes

First soil sampling for site characterization was carried out before the sowing of legumes while the second sampling was after harvesting the legumes (after N had been fixed) but before introduction of sugarcane. Soils were sampled (initial sampling) at 0-15 cm depth before fertilizer application for the experimental site characterization. Soil sampling at two levels (0-15cm and 15-30cm) were taken from each plot (giving a total of 108 samples) to determine nitrate-N and ammonia-N levels as influenced by the legumes. Three (3) subsequent samples were carried out at an interval of one month after planting of sugarcane. The samples were always put and carried in a cooler box and transferred to a refrigerator before laboratory analysis.

The soils were sampled by grid system and analysis of soil parameters (Soil pH, NO_3-N, Total Soil Nitrogen (N), K, Ca, Mg and organic C (%)) using standard analytical procedures as described by Okalebo *et al.*, (2002).

3.8.2 Plant tissue sampling

Plant tissue sampling for legumes was carried out at harvesting. Four (4) mature plants were randomly sampled from the experimental plots. Samples consisted of plant tops obtained from plants cut at the soil surface then analyzed to determine the N and P plant nutrient contents using standard analytical procedures as described by Okalebo *et al.,* (2002).

3.8.3 Sampling of N fixing nodules

3.8.3.1 Candidate plants

Four (4) plants in the inner two rows were chosen and each excavated from 60cm wide, 30cm long and 30cm deep. Gently, the excavated roots were cut off from the remaining deeper roots. The soil was then removed gently from the roots. The 4 roots were then washed out and assesment for nodule distribution, number, shape, size and colour was done.

3.8.4 Tagging of plants for height assesment

Four (4) sugarcane plants were randomly selected and tagged from the 2 middle lines by folding their leaves. They were used for height, diameter and girth measurements until seed cane stage.

3.9 Harvesting

3.9.1 Harvesting of Légume grain

This was done for each legume species except *S. sesban* on after attainment of physiological maturity. The fresh and dry weights of the grain legumes were taken and recorded. For cowpea, no grain was harvested because the crop was stunted, did not flower and performed very poorly. *S. sesban* was harvested before producing seeds.

3.9.2 Harvesting of fresh and dry biomasses of legumes

The plant fresh weight was obtained by cutting sampled plants using 90 cm x15 cm quadrants in the plot's effective harvest area at the soil surface level. They were then weighed on site using an analytical balance and determined on per ha basis. The weighed plant samples were put into gunny bags and dried in the greenhouse for one week and later oven dried at 40^0C for 24 hours. At 40 ^{o}C we get rid of moisture and retain the nutrients in their natural form to avoid dilution or denaturing them. The dry weight (DM) under the effective sampled area (0.9 m by 0.15 m) was measured and then extrapolated to per hectare basis (1ha = 10000 m^2).

3.10 Sugarcane crop

3.10.1 Harvesting Sugarcane

Sugarcane was harvested at seed cane stage at ground level from the effective plot area (4.5 m by 4.3 m) using a panga, the leaves removed and the cane bundled together.

The harvested sugarcane was then weighed per plot using spring balance. The fresh weight from effective plot area was then extrapolated to per hectare basis. (1ha = 10000 m^2).

3.11 Chemical and physical analyses of soil/plant tissue samples

The chemical and physical analysis of soil and plant tissue was done as per Okalebo et al., (2002).

3.11.1 Soil pH determination

Soil pH was determined by weighing 10 g of air-dry soil (< 2mm sieve) in a beaker. Twenty five (25) ml of distilled water was added, the mixture stirred for 10 minutes using a mechanical shaker and the suspension allowed standing for 30 minutes after which it was stirred again for 2 minutes. The pH of the soil sample suspension was measured using a pH meter after calibration in buffer solutions of pH 4 and 7 (Okalebo *et al.* 2002).

3.11.2 Determination of Nitrates (NO_3^- - N)

Colorimetric determination of nitrates from the soil was based on extraction in 0.5 M K_2SO_4. Ten (10) g of fresh soil in 20 ml of extractant was centrifuged for 30 minutes at 60 rpm. The sample was then filtered using a nitrate-free Whatman filter paper to determine the nitrate in the clear solution.

Soil samples were kept fresh by keeping in a refrigerator to avoid accumulation of nitrates as a result of mineralization. Micro-pippetting with suitably marked test tubes was done. One (1) ml of salicyclic acid solution was added to each test tube and mixed thoroughly immediately (by using a mixer) and left to stand for 30 minutes.

In each test tube, ten (10) ml of sodium hydroxide solution was mixed well and left to stand for one (1) hour for colour development. Each standard and sample absorbance was read at 410 nm (Okalebo *et al*, 2002).

3.11.3 Total Soil Nitrogen (N) determination

Total N was determined by the Kjeldhal wet acid oxidation method followed by distillation and titration (Okalebo*et al*., 2002).

3.11.4 Digestion of soil sample

About 0.3 g of air-dry soil (< 0.25 mm, 60 mesh) was weighed into a digestion tube and 4.4 ml of the digestion mixture added to each tube and to two reagent blanks. Digestion was done at 340^0C for two hours until the solutions became clear and allowed to cool. The solution was made to the 50 ml mark by adding distilled water and mixed well (Okalebo *et al.,* 2002). The total N was determined from the digest using steam distillation-titration method as follows:

3.11.5 Steam distillation-titration

The digest was analyzed for total N using the distillation-titration method (Okalebo *et al*., 2002). The distillation apparatus were set and steam passed through the system for 30 minutes. 10 ml of sample solution was put into the Markam still reaction chamber and 10 ml of 40% alkali mixture (NaOH) added. Steam distillation started immediately into 5 ml of 1 M boric acid containing 4 drops of the mixed indicator. This continued for about 2 minutes until the indicator turned green. The distillate was titrated by N/40 HCl to a pink end-point using a micro-burette.

The titre was then recorded, T. the blank was subtracted from the sample titres (T). N in the soil sample was obtained from the following relationship:

$$\text{N in the soil} = \frac{\text{Corrected ml of N/40 HCl x 0.5}}{\text{W}}$$

Where:

W = Weight of the soil used is 0.3 g

0.1= Molarity of the hydrochloric acid used in titrating the distillates.

3.11.6 Determination of Potassium and Calcium

Potassium (K) and Calcium (Ca) were determined by extraction of the soil samples with 1 M ammonium acetate (NH_4OAc) solution. 5 g of air-dry soil (< 2 mm, 60 mesh) was measured into a plastic bottle and 100 ml of 1 M NH_4OAc solution added. The contents were shaken for 30 minutes and filtered through No. 42 Whatman filter paper. The soil extract obtained was diluted ten (10) times to fall within the measurable range of Flame Photometer (FP) and Atomic absorption spectrophotometer (AAS) that was used to determine K and Ca. 5 ml of the solution was pipette into a 50 ml volumetric flask and 1 ml of 26.8 % lanthanum chloride solution added and the contents diluted to the mark with 1 M NH_4OAc extracting solution. Flame Photometer and Atomic Absorption Spectrophotometer was used to determine the amount of K and Ca respectively (Okalebo *et al.,* 2002).

3.11.7 Determination of Magnesium

The soil extract was diluted 25-fold (by pipetting 2 ml of soil extract into a 50 ml volumetric flask). 5 ml of 5000 ppm Sr was added and filled to the mark with 1 M NH_4OAc extracting solution.

The solution was sprayed into the flame of the Atomic Absorption Spectrophotometer. The standard working solutions were used in the calibration of the flame photometer (FP) and Atomic Absorption Spectrophotometer (AAS) (Okalebo *et al*., 2002).

Standard curves for Mg^{2+} was constructed from the respective readings. The concentration of Mg in the samples expressed in mg kg^{-1} was calculated using the following formulae:

$$\text{Mg kg}^{-1} \text{ in the soil} = \frac{(a-b) \times v \times f \times 1{,}000}{1000 \times w}$$

Where;

a = concentration of Mg in the sample extract

b = concentration of element in the blank extract

v = volume of the extract solution

w = weight of the soil sample

f = dilution factor

3.11.8 Determination of Organic carbon (%)

Percentage organic carbon (% O.C) was determined using the Nelson and Somers, (1975) oxidation method.

The method involves complete oxidation of soil organic carbon using acid (H_2S0_4) potassium dichromate solution. The excess or unreacted dichromate is then determined by titration using ferrous ammonium sulphate. Thus 0.3 g of air-dried soil (0.25 mm) was oxidized using 7.5 ml of concentrated H_2S0_4 and 5ml-potassium dichromate solution in a

block digester at 145 – 155^0 C for 30minutes. After cooling, the digests were quantitatively transferred into 100 ml conical flasks.

The end point was a colour change from green to brown. The titre was recorded and correction for the mean of two-reagent blanks (T) was made (Okalebo *et al*, 2002).

Calculation:

$$\% \text{ Organic Carbon} = \frac{T \times 0.2 \times 0.3}{\text{Sample weight}}$$

Where:

T = blank titre value

3.12 Statistical Data Analysis

Data was entered in Excel spreadsheet, transferred onto GenStat spreadsheet and subjected to Analysis of Variance (ANOVA) using GenStat12^{th} Edition computer data analysis software, and means separated by the Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984).

Table 1: Skeletal ANOVA

Source of variation	Df	SS	MS	F
Replicates	r-1(3-1=2)	SSr-CF	SSr/r-1	(SSt/r-1)/(SSt/t-1)
Legumes	l-1(6-1=5)	SSl-CF	SSl/l-1	(SSt/l-1)/(SSt/t-1)
Fertilizer type	f-1(3-1=2)	SSf-CF	SSf/f-1	(SSt/f-1)/(SSt/t-1)
Legume x Fert.	(l-1=5)(f-1=2)	(SSl)(SSf)-CF	(SSl)(SSf)/(l-1)(f-1)	(SSt/l-1)/(SSt/f-1)
Treatments	t-1(18-1=17)	SSt-CF	SSt/t-1	
Residual Error	(3-1)(18-1)=34	SS total-SSt-SSr	SSe/(r-1)(t-1)	
Total	3x18-1=53	SSt +SSr +SSe		

Where:

SSr = Sum of squares due to replicates

SSl= Sum of squares due to legumes

SSf = Sum of squares due to fertilizers

$SSlf_{jk}$ = Sum of squares due to legumes* fertilizers

SSt = Sum of squares due to treatments

SSe = Sum of squares due to residual Error

CF = Correction Factor

3.13: General Linear Model of the experiment;

$Yijk = \mu + Bi + Fj + Lk + FLjk + \alpha ijk.$

Where –

Yijk- Parameter

μ- Overall mean

Bi – Block Effect

Fj – Fertilizer type

Lk – Legume type

FLjk – Fertilizer type * Legume type

αijk – Residual Error

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Evaluation of the effectiveness of leguminous plants for short term improvement of nitrogen content in fertility depleted sugarcane soils

4.1.1 Soil Characterization

Results of the initial soil characterization in the experimental site are given in Table 3 below. The soil was found to be low in N, P and soil Organic carbon (OC). However, the soils were found to be medium in K and high in Mg content, while the texture was clay loam.

Table 2: Initial soil chemical and physical characteristics of the experimental site (Kibos) before planting leguminous plants in 2005

Soil parameter	Unit	Value	Remarks
pH (1: 2.5 Soil: Water)		6.55	Slightly acidic to neutral
Organic carbon	%	1.54	Low soil C content
Nitrogen	%	0.11	Low soil N content
Phosphorus	ppm	18	Low soil P content
Potassium	mg/kg^{-1}	0.35	Medium soil K content
Magnesium	mg/kg^{-1}	4.75	High soil Mg content
Calcium	mg/kg^{-1}	20.45	Medium/adequate
Particle size distribution ;			
Sand	%	32.4	
Silt	%	29.3	
Clay	%	38.3	
Texture			Clay loam

4.1.2 Nodulation for different leguminous plants

The results in Table 3 indicate there were no significant differences among leguminous plants in nodulation with soybean recording lowest number of nodules while *S. sesbania* having highest. Soybean nodulation was statistically different from other legumes for control or when no fertilizer was used.

Table 3: Means for number of nodules fixing N for different leguminous plants

Legume	Control	Phosphorus	Potassium	Mean
Sesbania	34.33a	30a	33.20a	32.51
Yellow gram	27.67ab	28.10a	29.10a	28.29
Cowpea	32.67a	29.40a	30.40a	30.82
Bambara	27.33ab	25.30a	28.20a	26.94
Crotalaria	29.33a	27.80a	28.70a	28.61
Soybean	19b	22.40b	20.50b	20.63
Mean	28.33	27.10	28.30	27.91
CV%	3.00	3.60	4.10	
DMRT $lsd_{(0.05)}$	9.20	7.60	5.90	

C.V. = Coefficient of variation, **l.s.d** = Least Significant difference. *Mean values followed by the same letter either along the row or column are Not significantly different at P < 0.05 according to Duncan's Multiple Range Test (**DMRT**)*

Legumes vary significantly in their ability to fix nitrogen. This can be attributed to the physiology, its ability to be nodulated by various species of rhizobia (Sanginga, *et al*., 2000), which enables them to fix nitrogen in association with any given soil rhizobia condition when compared to the other legumes that are host specific in their fixation abilities. The initiation and development of legume nodules induced by compatible *rhizobium* species requires a complex signal exchange involving both plant and bacterial compounds. Phytohormones have been implicated in this process (Brett *et al.,* 2005).

4.1.3 Soil Nitrate-Nitrogen under different leguminous plants

Soil Nitrate N analysis 1st soil sampling (after 30 days)

For Control treatment (no fertilizer applied) cowpea and yellow gram had the highest soil nitrate value followed by *S. sesban* while Soybean, bambara and *Crotalaria* had the lowest value respectively (Figure 4). For K fertilizer application, Soybean had the highest legume plant nitrate value followed by *Crotalaria* and *S. sesban* in that order while Yellow gram had the lowest followed by Cowpea and Bambara respectively. For P fertilizer application, Cowpea had the highest soil nitrate value followed by Soybean and Bambara respectively while Yellow gram had the lowest value followed by *S. sesban* and Crotalaria (Figure 4 and 5).

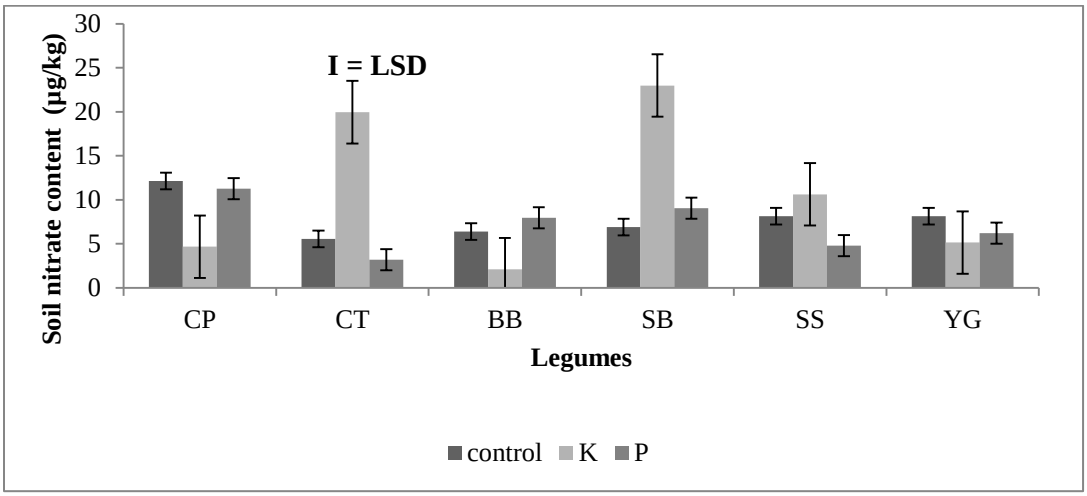

Figure 4: Soil Nitrate N 1st sampling (0-15 cm)

Key: SB- Soybean; CP- Cowpea; BB- Bambara;
CT-*Crotalaria*; YG-yellow grams; SS-*Sesbania*

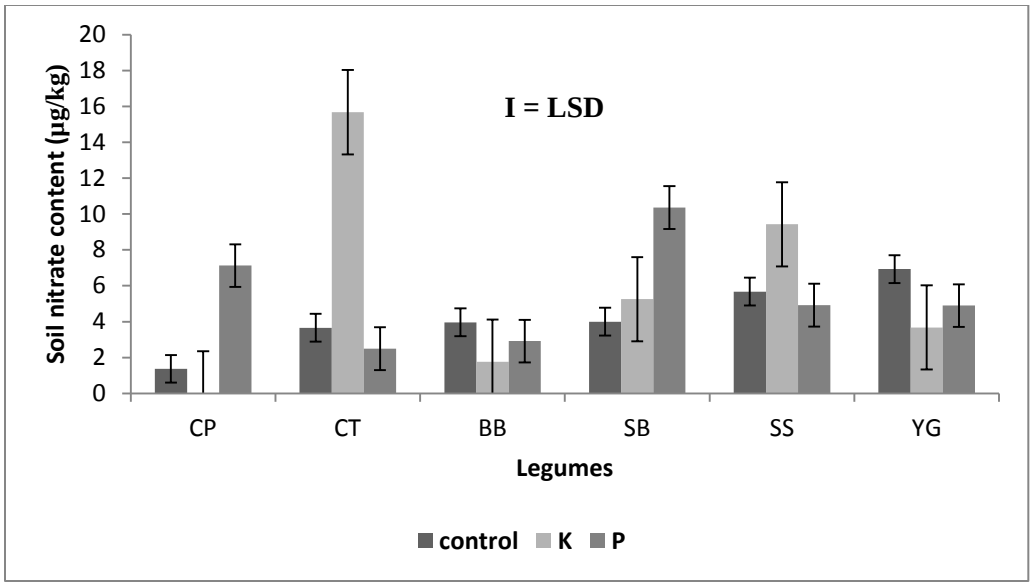

Figure 5: Soil Nitrate N 1st sampling (15-30 cm)

Key: SB- Soybean; CP- Cowpea; BB- Bambara;
CT-*Crotalaria*; YG-yellow grams; SS-*Sesbania*

Soil Nitrate N analysis 2nd soil sampling (after 60 days)

For soil nitrate analysis after 60 days, there were significant differences in legumes for Control, K and P fertilizer applications (Figure 6 and 7) for 0-15cm depth. The same results were also observed for soil nitrate analysis 15-30cm. *Crotalaria* had highest fixed soil nitrate for both depths. Variations between control, K or P were statistically significant. Bambara fixed more N when established under potassic fertilizers. Cowpea and yellow-gram performed better under phosphatic fertilizers.

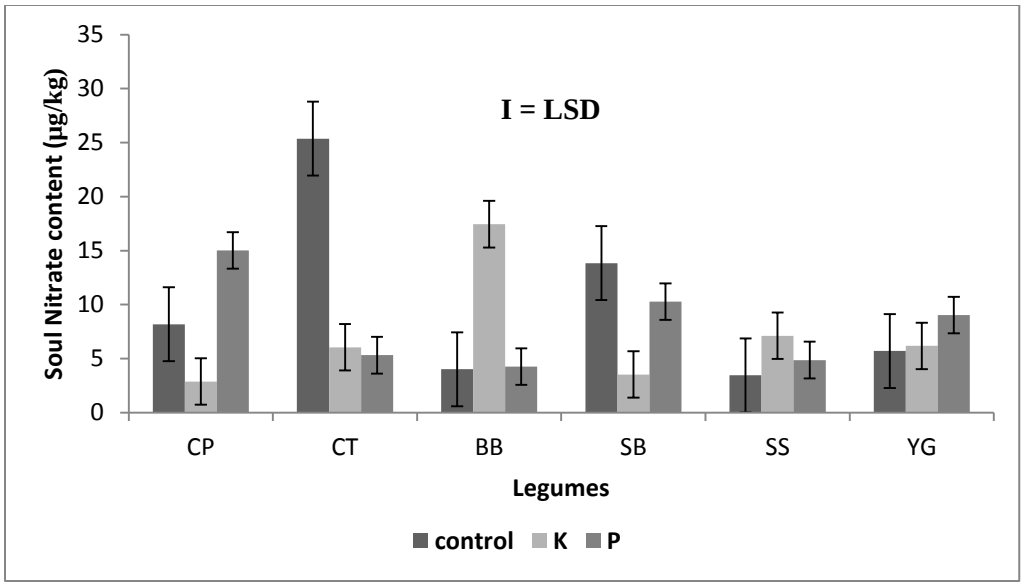

Figure 6: Soil Nitrate N analysis 2nd sampling (0-15 cm)

Key: SB- Soybean; CP- Cowpea; BB- Bambara;
CT-*Crotalaria*; YG-yellow grams; SS-*Sesbania*

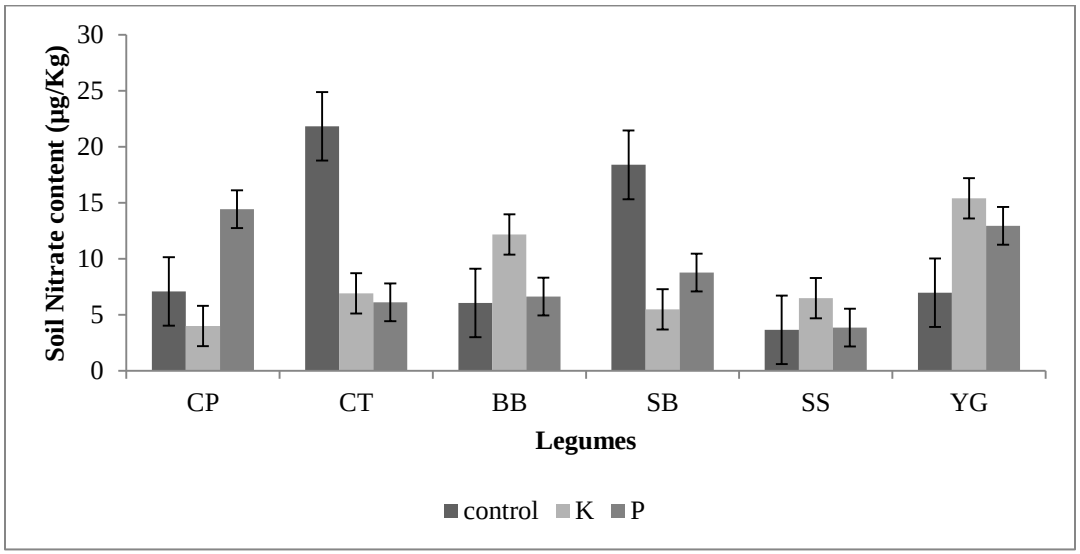

Figure 7: Soil Nitrate N analysis 2nd sampling (15-30 cm)

Key: SB- Soybean; CP- Cowpea; BB- Bambara;
CT-*Crotalaria*; YG-yellow grams; SS-*Sesbania*

Soil Nitrate N analysis 3rd soil sampling (after 90 days)

For nitrate analysis for 3rd soil sampling, Soybean had the highest soil nitrate content for both depths especially when established under no fertilizer (control) (Figure 8 and 9). Cowpea and Yellow gram had high nitrate analysis 3rd bottom. Bambara, yellow gram and cowpea fixed more nitrates in the soil when established under potassic fertilizers with the former being the best fixer for both depths. S. *sesban* had the least amount of fixed soil nitrate N at both depths.

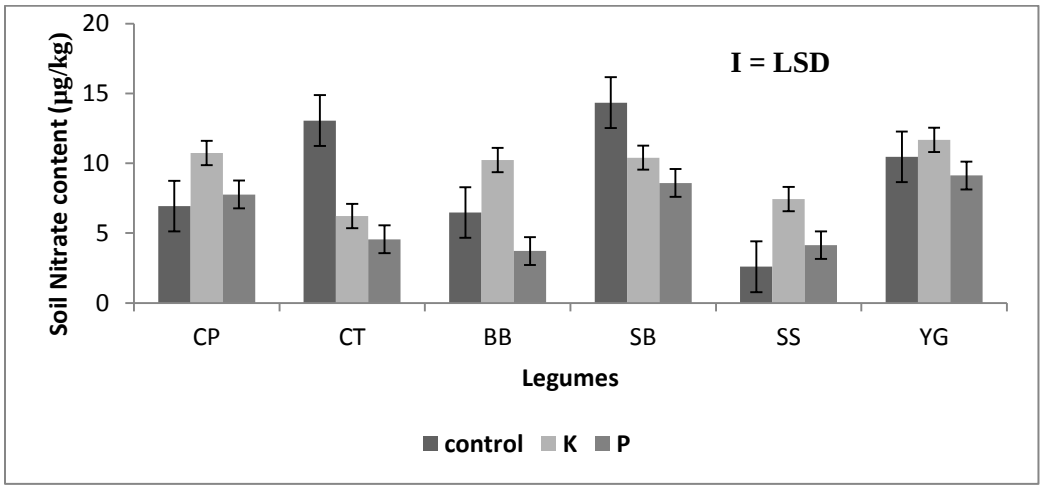

Figure 8: Soil Nitrate N analysis 3nd sampling (0-15 cm)

Key: SB- Soybean; CP- Cowpea; BB- Bambara;
CT-*Crotalaria*; YG-yellow grams; SS-*Sesbania*

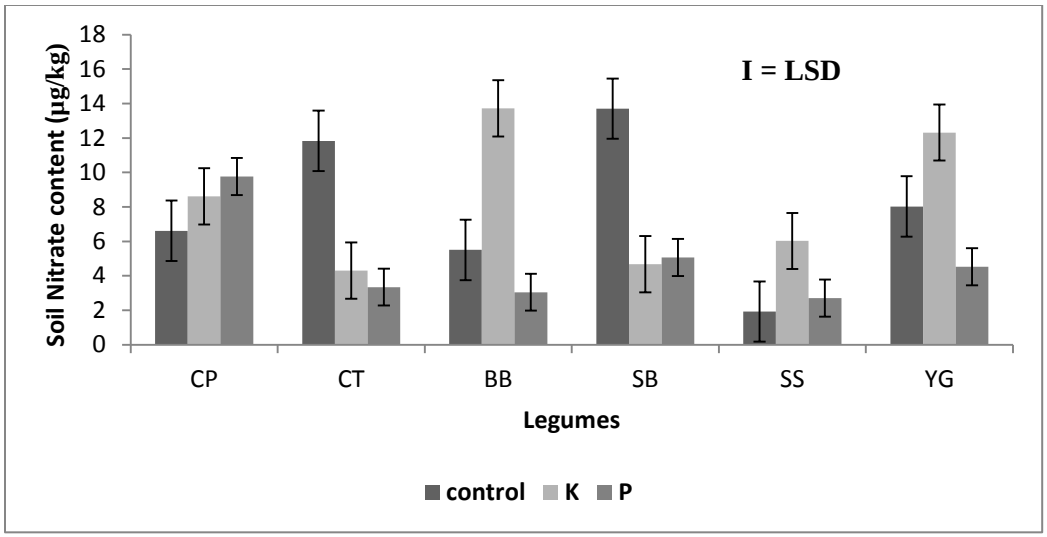

Figure 9: Soil Nitrate N analysis 3nd sampling (15-30 cm)

Key*:* SB- Soybean*; CP*- Cowpea*;* BB- Bambara;
CT-*Crotalaria*; YG-yellow grams; SS-*Sesbania*

Soil Nitrate N analysis 4th soil sampling (after 120 days)

Generally, the amount of soil Nitrate was higher in 15-30cm depth than 0-15cm depth perhaps due to leaching. Yellow gram had the highest soil nitrate (no fertilizer addition) which was statistically significant from other legumes (Figure 10 and 11). For 15-30cm depth, soil nitrate was high with *Crotalaria*, cowpea and yellow gram fixing more N when established under no fertilizer. There were no significant differences in soil nitrates when legumes were established under K or P fertilizers (Figure 10 and 11).

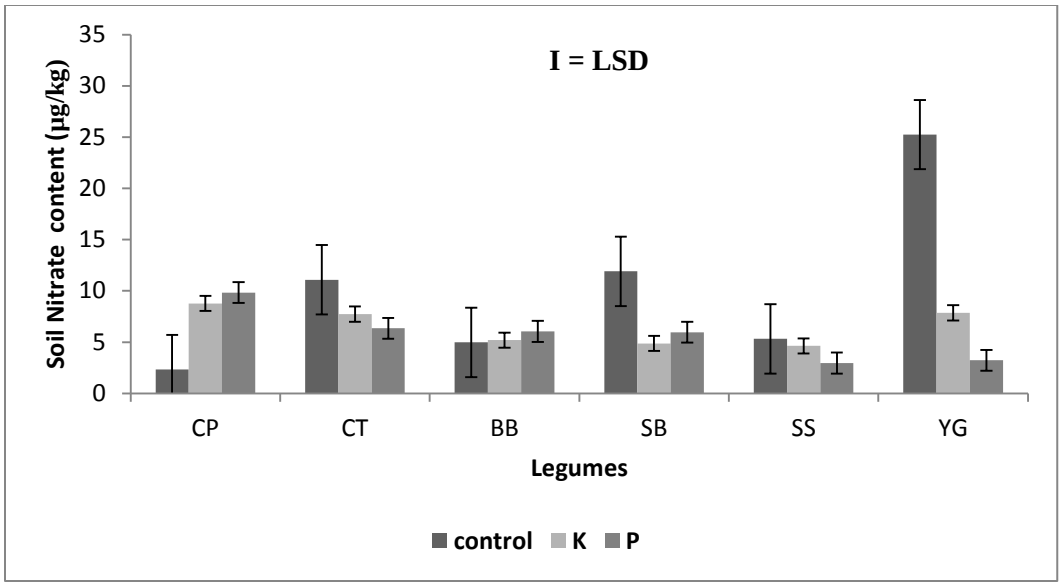

Figure 10: Soil Nitrate N analysis 4th sampling (0-15 cm)

Key: SB- Soybean; CP- Cowpea; BB- Bambara;
CT-*Crotalaria*; YG-yellow grams; SS-*Sesbania*

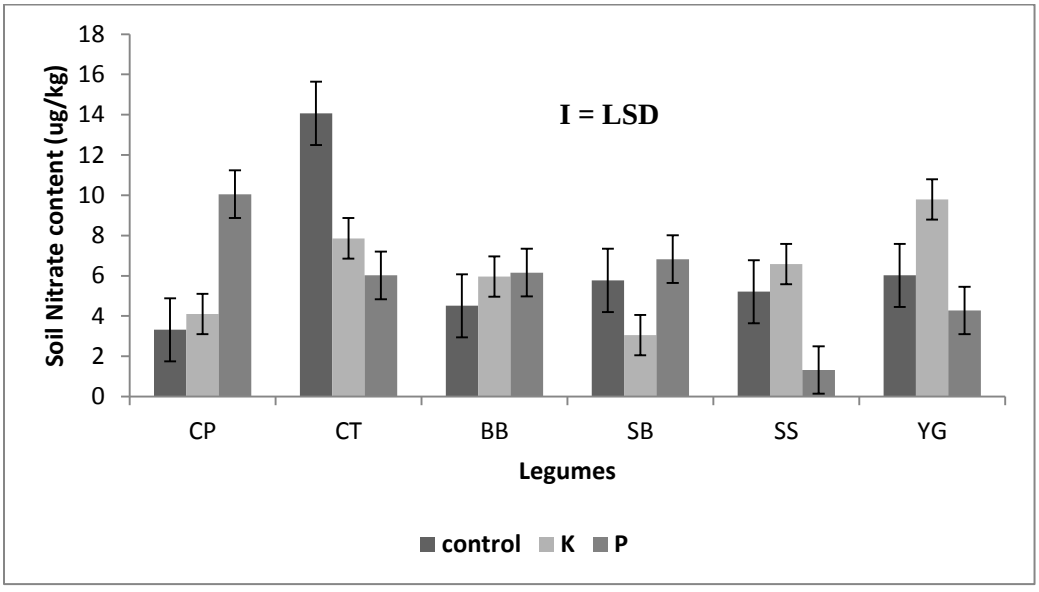

Figure 11: Soil Nitrate N analysis 4th sampling (15-30 cm)

Key: SB- Soybean; CP- Cowpea; BB- Bambara;
CT-*Crotalaria*; YG-yellow grams; SS-*Sesbania*

Leaching and organic matter mineralizations are the key factors affecting the amount nitrates in the soil at any given time. In high rainfall areas like tropical highlands, experience high leaching rates which explains the why the top soil horizons have decreasing trends in nitrates with time. Nitrates are soluble and highly mobile in soils hence leaching. Soil nitrate content at both 0-15 cm and 15-30 cm levels are affected by excessive tillage which increase aeration into the soil that increases the population of microorganism that feed on organic matter to increase the soil nitrates. The same happens when the microorganisms die; increase in soil moisture (provided weeds are controlled) causes un decomposed litter to mineralize thus releasing nitrates; Secondly, roots of *Sesbania sesban* when still young are more efficient in fixing N between 0-30 cm. As the roots grow deeper, they tend to have less nodules which result in less N-fixation as compared to Yellow Grams, Crotalaria and Cow peas whose roots remain shallow and with more nodules, that fix N efficiently (Figure 4-11) (Farm Ahead June, 2009).

4.1.4 Soil Olsen P after harvesting of legumes

Results showed differences in legumes were statistically significant but not for control, K and P fertilizer applications. The results indicated that Bambara and *Crotalaria* were the leading crops with high soil Olsen P values compared to Soya bean, Cowpea, and *S.sesban* (Figure 12). Application of either K or P fertilizers in legumes had no significant effect on available P except in yellow gram where K fertilizer seemed to increase soil available P significantly (Figure 12). Soil Olsen P tends to increase with increase in soil pH. However, leguminous plants have been found not to affect Olsen P in soil. Further, high concentrations of Ca are involved in the decrease of Olsen P values in limed soils and increase in soil pH.

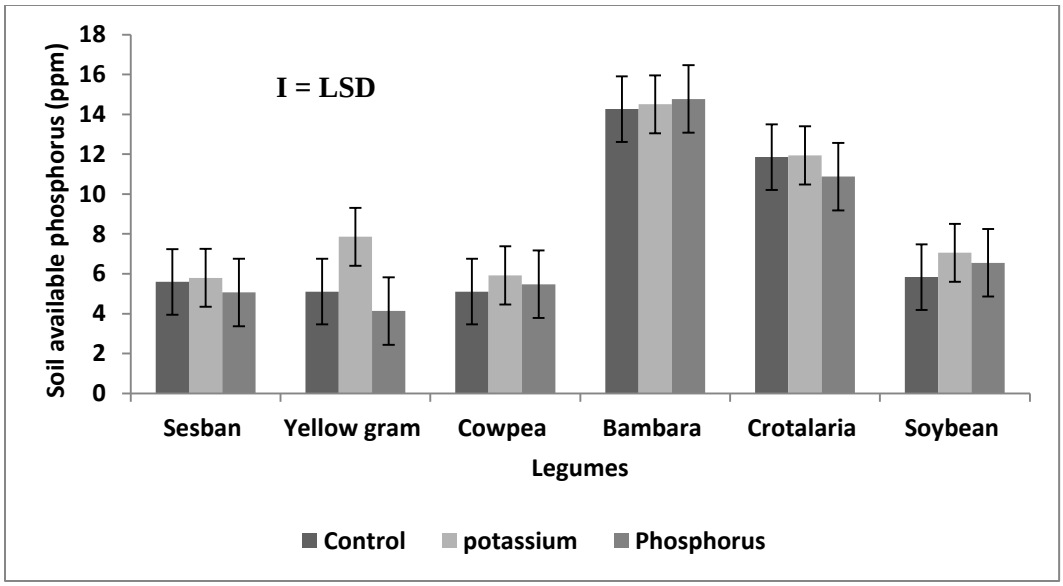

Figure 12: Soil available (Olsen) P after harvesting of legumes

4.2 Evaluation of the content of nitrogen and phosphorus in the tissues of different leguminous plants used for short term improvement of nitrogen fertility in depleted sugarcane soils

4.2.1 Legume tissue total phosphorus (P)

For treatments without fertilizer application (control), *S. sesban* had the highest legume plant tissue total P followed by Cowpea, *Crotalaria* and Yellow gram respectively. Soybean and Bambara had the least values. For K fertilizer application, *S. sesban* and Cowpea had the highest values followed by *Crotalaria* and Yellow gram, while Soybean and Bambara had the least values. For P fertilizer application, *S. sesban* had the highest value followed by Cowpea while Yellow gram and Crotalaria followed. Soybean and Bambara had least tissue phosphorus contents. Use of potassic or phosphatic fertilizers in establishing legume plants had no significant effect on phosphorus accumulation in the legume tissues especially but there was a significant differences on the way different legume plants accumulated tissue phosphorus. Cowpea and *S. sesban* had higher means compared to the rest of the leguminous plants (Figure 13).

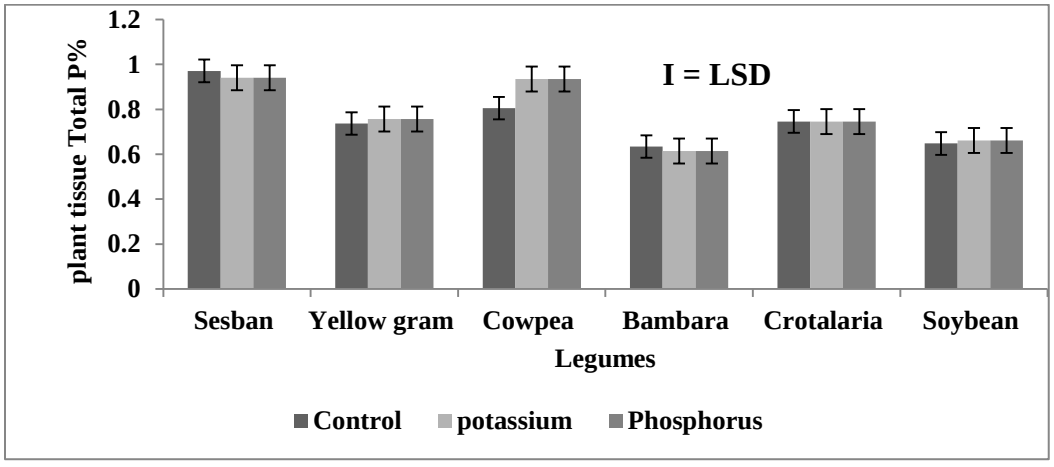

Figure 13: Legume tissue total phosphorus content

S. sesban is a leguminous plant and has the ability to fix atmospheric and soil N into nitrates and nitrites and can facilitate mineralization of organic matter, through enhanced root development, thus promoting P uptake. Secondly, *S.sesban* and Cowpea may probably have numerous nodules on the roots (internal root efficiency), which facilitate N-fixation in soil and P sorption, which contributes to enhanced P uptake (Trolove *et al*., 1996).

4.2.2 Legume plant tissue total Nitrogen (N)

For control treatment (no fertilizer applied), Soybean had the highest tissue total N value followed by Yellow gram and *S. sesban* respectively. Cowpea and Bambara followed and had similar values while *Crotalaria* had the least tissue nitrogen (Figure 14).

For K fertilizer application, *S. sesban* had the highest plant tissue total N value followed by Bambara, Soybean and *Crotalaria*. Cowpea had the least value. For P fertilizer application, Soybean had the highest plant tissue total N value followed by Bambara, *S. sesban* and *Crotalaria* respectively. Cowpea had the least tissue N content (Figure 14).

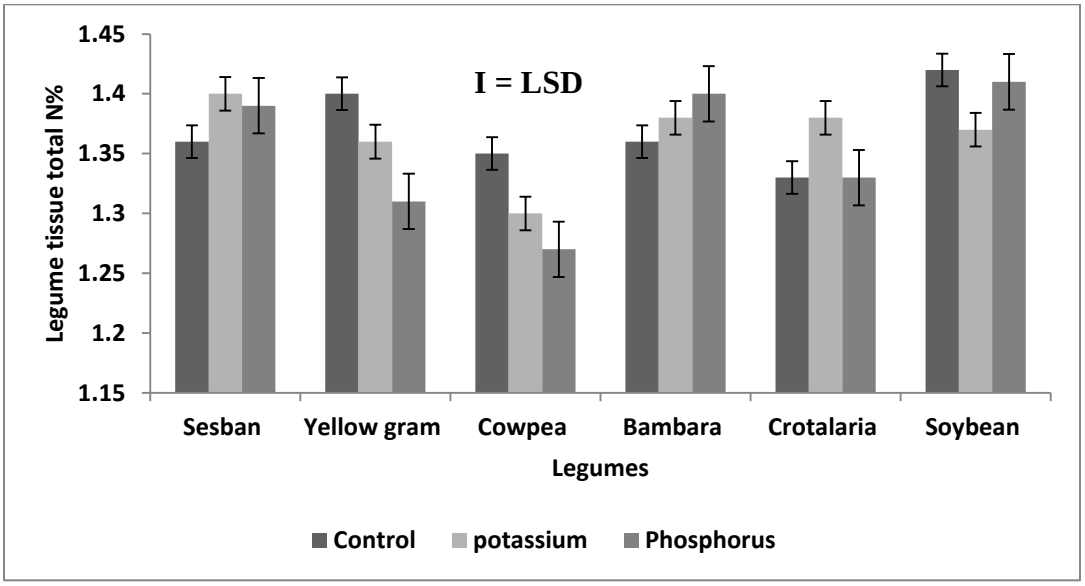

Figure 14: Legume Plant Tissue Total N (%)

The results of analysis indicated that there were significant differences between the fertilizer treatment means, especially where no fertilizer and K fertilizer were applied. However, mean separation showed that the treatment means were not significant for Control and K. It was also observed, that where P fertilizer was applied, there were no significant differences in legumes concerning nitrogen concentration in the tissues,

Legume plants have the ability to fix N and mineralize organic matter thus releasing N into the soil, which is eventually taken up by roots, thus enhancing N uptake. Further, legume roots have numerous nodules on their root surfaces which harbor microorganisms such as bacteria, actinomycetes and cynobacteria which promote biological N fixation, thus contributing to enhanced N uptake (Havlin *et al*., 2005).

4.2.3 Legume grain total P

Application of phosphorus and potassium fertilizers had a significant impact on the grain phosphorus content (Figure 15). There was a statistically significant difference in grain P content when either phosphorus or potassium was applied in legume establishment; Application of potassium fertilizers reduced grain P concentration in *Sesbania* but increased the P content for the other legume plants with significant increments in cowpea and *Crotalaria* (Figure 15). However; phosphatic fertilizers increased grain P content in *Sesbania*, *Crotalaria* and soybean but significantly reduced in yellow gram and no effects in Bambara and cowpea. Results indicated that legume grain P was significantly affected differently for each kind of legume.

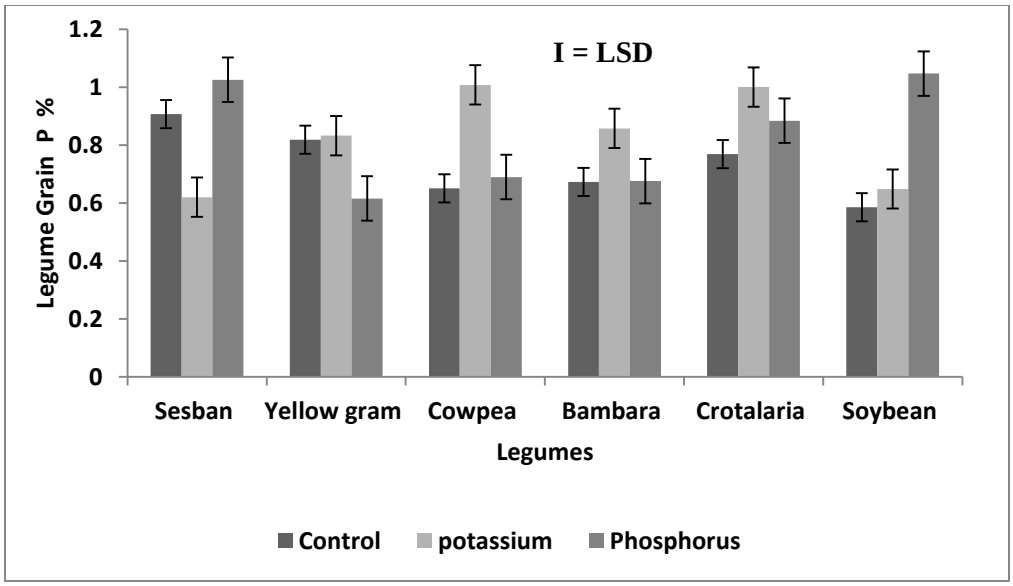

Figure 15: Legume grain total P

When P fertilizer is applied to the soil, the availability of P increases and enters the plant through root hairs, root tips and the outermost layers of root cells. Uptake is also facilitated by mycorrhizal fungi that grow in association with roots of many crops. P is taken up mostly as the primary orthophosphate ion ($H_2PO_4^{-1}$), but some is also absorbed as secondary orthophosphate (HPO_4^{-2}), this latter form increasing as the soil pH increases *(http://www.ipni.net/ppiweb/bcrops)*

4.3 To evaluate the performance of sugarcane in plots previously under short-term different legume plants, with and without starter phosphatic and potassic fertilizers.

4.3.1 Sugarcane height

Sugarcane height 1st measurement (August 2005)

The results indicated that sugarcane heights were not significantly different from Control and K fertilizer applications. Cowpea had higher heights when fertilizer was added while yellow-gram the least height (Table 4).

Table 4: Means for heights of sugarcane grown on plots which had different legumes established with different starter fertilizer (August 2005)

Treatments	Control	potassium	Phosphorus	Mean
Sesbania	93.60 a	101.70 a	89.60 a	94.96
Yellow gram	83.83 a	88.20 a	73.43 a	81.82
Cowpea	97.97 a	84.77 a	81.97 a	88.19
Bambara	86.27 a	87.13 a	90.03 a	87.81
Crotalaria	86.97 a	98.87 a	82.60 a	89.43
Soybean	95.60 a	87.03 a	91.93 a	91.50
Mean	90.71	91.28	84.92	88.95
CV%	11.20	9.90	7.50	
DMRT $lsd_{(0.05)}$	15.37	16.45	16.93	

C.V. = Coefficient of variation, **l.s.d** = Least Significant difference *Mean values followed by the same letter either along the row or column are Not significantly different at P < 0.05 according to Duncan's Multiple Range Test (**DMRT**)*

Sugarcane height 2nd measurement (November 2005)

The differences in heights for Control, K and P fertilizer applications were not statistically different according DMRT. Soybean recorded highest cane height while cowpea the least (Table 5).

Table 5: Means for heights of sugarcane grown on plots which had different legumes established with different starter fertilizers (November 2005)

Treatments	Control	potassium	Phosphorus	Mean
Sesbania	116.90 a	127.60 a	122.50 a	122.33
Yellow gram	121.00 a	113.90 a	123.50 ab	119.16
Cowpea	125.90 a	114.70 a	108.90 a	116.50
Bambara	119.20 a	115.90 a	125.60 ab	120.23
Crotalaria	114.20 a	121.60 a	116.20 ab	117.33
Soybean	126.40 a	123.00 a	128.70 b	126.00
Mean	120.60	119.45	120.90	120.31
CV%	2.70	9.50	6.50	
DMRT $lsd_{(0.05)}$	21.31	21.31	17.27	

C.V. = Coefficient of variation, **l.s.d** = Least Significant difference. *Mean values followed by the same letter either along the row or column are Not significantly different at P < 0.05 according to Duncan's Multiple Range Test (**DMRT**)*

Sugarcane height 3rd measurement (May 2006)

There was a statistically significant difference in sugarcane heights where K and P fertilizers were applied in the legumes but not in the control treatment (no fertilizer applied). Yellow gram had the least effect on height whether established with fertilizer or not while soybean seemed to have a greater effect on height for the three treatments (Table 6).

Table 6: Means for heights of sugarcane grown on plots which had different legumes established with different starter fertilizers (May 2006)

Treatments	Control	potassium	Phosphorus	Mean
Sesbania	138.20 a	154.30 b	147.90bc	147.47
Yellow gram	143.20 a	137.60 a	129.70a	136.83
Cowpea	155.80 a	140.70ab	133.80abc	143.43
Bambara	145.20 a	147.30ab	147.30abc	146.60
Crotalaria	151.60 a	151.50ab	139.20abc	147.43
Soybean	154.30 a	150.40ab	153.70 c	152.80
Mean	148.05	146.96	141.93	145.64
CV%	2.60	4.60	6.00	
DMRT $lsd_{(0.05)}$	20.14	14.23	16.53	

C.V. = Coefficient of variation, **l.s.d** = Least Significant difference. *Mean values followed by the same letter either along the row or column are Not significantly different at P < 0.05 according to Duncan's Multiple Range Test (**DMRT**)*

The importance of a balanced nutrition particularly between nitrogen (N) and K in the attainment of the maximum performance of sugarcane in terms of yield height is very essential. Research done elsewhere on the responses of sugarcane to K fertilization reflect to a large extent on the available K status of the soil. Responses have been obtained in soils low in available K (Ng Kee Kwong, 2002). Responses to K fertilizers are frequently not observed in plant cane and often even in first and second ratoons and perhaps this explains why there was no significant differences in sugarcane height in this study for the first two height measurements as shown in (Tables 5 & 6) above. In general sugarcane responds to K fertilizers by an increase in cane yield without any change in sucrose concentration in the cane. As an excessive uptake of K by the sugarcane depresses the recovery of sucrose during milling, K fertilization of sugarcane must be

kept just adequate to produce an optimum yield and to help regulate maturity so that maximum sugar is recovered from the millable canes (Ng Kee Kwong, 2002).

4.3.2 Sugarcane tiller analysis

Sugarcane 1st tiller count (July, 2005)

For Control, legume treatment means were significant, but not when K and P fertilizers applied. Application of either K or P fertilizers in the legumes did not affect the number of tillers produced by sugarcane (Table 7).

Table 7: Means for number of tillers of sugarcane grown on plots which had different legumes established with different starter fertilizers July, 2005

Treatments	Control	potassium	Phosphorus	Mean
Sesbania	51.00 a	66.33 a	63.67 a	60.21
Yellow gram	57.67 a	62.00 a	64.67 a	61.40
Cowpea	58.00 a	58.00 a	56.00 a	57.00
Bambara	58.33 a	60.33 a	54.67 a	57.77
Crotalaria	69.00 a	69.33 a	56.67 a	65.00
Soybean	73.67 a	79.00 a	60.00 a	70.89
Mean	61.28	64.28	59.28	61.61
CV%	19.70	10.90	19.10	
DMRT $lsd_{(0.05)}$	21.28	29.06	45.95	

C.V. = Coefficient of variation, **l.s.d** = Least Significant difference. *Mean values followed by the same letter either along the row or column are Not significantly different at P < 0.05 according to Duncan's Multiple Range Test (**DMRT**)*

Sugarcane 2nd tiller count (September, 2005 Output)

Tiller count for Control (no fertilizer added) and P fertilizer application indicated legume differences were significantly different while for K fertilizer application, there were no significant differences. Soybean performed best when no fertilizer was added while

sesbania the least (Table 8). When P fertilizer was added to legumes, yellow gram had a greater effect on tillering while Bambara the least as shown in (Table 8).

Table 8: Means for number of tillers of sugarcane grown on plots which had different legumes established with different starter fertilizers (September, 2005)

Treatments	Control	potassium	Phosphorus	Mean
Sesbania	74.67 a	101.70 a	88.00 ab	88.12
Yellow gram	85.33 a	104.00 a	115.00 b	101.33
Cowpea	116.00 a	101.30 a	98.33 ab	105.21
Bambara	95.00 ab	118.30 a	79.33 a	97.54
Crotalaria	88.67 a	98.30 a	95.00 ab	93.99
Soybean	112.33 b	107.70 a	109.33 ab	109.78
Mean	95.33	105.83	97.49	99.55
CV%	10.60	8.80	13.00	
DMRT $lsd_{(0.05)}$	21.12	23.32	31.22	

C.V. = Coefficient of variation, **l.s.d** = Least Significant difference. *Mean values followed by the same letter either along the row or column are Not significantly different at P < 0.05 according to Duncan's Multiple Range Test (**DMRT**)*

Tillering is a physiological process of repeated underground branching from compact nodal joints of the primary shoot. Sugarcane tillers growth depends on availability of Substrates (carbohydrates and sugars) stored in seed cane, adequate moisture content (irrigation), light and temperature conditions, variety, and fertilizer practices *(http:/www.sugarcanecrops.com).* Potassium (K) is the most abundant cation accumulating in the cell sap of sugarcane plant. Tiller density leaf area, and number of green leaves per sugarcane plant are not affected, by inadequate K supply but the height of millable stalks at harvest may be impaired (Ng Kee Kwong, 2002).

4.3.3 Sugarcane population

Sugarcane population before harvesting

The influence of cowpea and yellow gram on sugarcane population was statistically significant from other leguminous plants (Table 9). Soybean produced highest population count while yellow gram the least when no fertilizer was added to legumes. Use of phosphatic fertilizer to establish legumes improved cane population significantly in yellow gram (Table 9).

Table 9: Means for Sugarcane population 2006

Treatment	Control	Phosphorus	Potassium	Mean
Bambara	136.30a	128a	158.70a	141.00
Cowpea	134.30a	147a	131b	137.43
Crotalaria	144.70a	135.7a	152.30ab	144.23
Sesbania	124.70a	147a	145.70a	139.33
Soybean	159 b	159.3b	143.70a	154.00
yellow grams	115.70c	149b	141.30a	135.33
Mean	135.78	144.33	145.45	141.85
CV %	14.90	11.00	12.33	
DMRT $lsd_{(0.05)}$	20.52	35.55	35.55	

C.V. = Coefficient of variation, **l.s.d** = Least Significant difference. *Mean values followed by the same letter either along the row or column are Not significantly different at P < 0.05 according to Duncan's Multiple Range Test (**DMRT**)*

These results postulate that fertilizer application had no effect on sugarcane population. Sugarcane population may be a function of planting espacement, good soil nutrient status, favourable climatic conditions, variety and crop management among other factors, Kariaga and Owelle (1992). Tillering may influence the quality of sugarcane due to competition for nutrients and light for high tillering varieties.

4.3.4 Sugarcane fresh Weight

Use of potassic and phosphatic fertilizers to establish legumes did not have a significant effect on sugarcane weight at harvest. Yellow gram had the least effect on sugarcane weight which was statistically significant from other legumes when no fertilizer was used to establish legumes (Table 10). There were no significant differences among the legumes where potassic or phosphatic fertilizers were applied (Table 10).

Table 10: Means for fresh sugarcane biomass (t/ha) at harvesting

Legume	Control	Phosphorus	Potassium	Mean
Bambara nuts	75.19a	67.44a	84.13a	67.44
Cowpea	72.51a	68.99a	66.25a	68.99
Crotalaria	77.52a	70.54a	79.22a	70.54
Sesbania sesban	67.55a	76.33a	74.01a	76.33
Soybean	83.46a	79.17a	71.68a	79.17
Yellow grams	59.84b	79.59a	70.96a	79.59
Mean	72.68	73.68	74.38	73.67
CV%	14.0	16.8	14.10	
DMRT $lsd_{(0.05)}$	11.40	42.59	36.20	

C.V. = Coefficient of variation, **l.s.d** = Least Significant difference. *Mean values followed by the same letter either along the row or column are Not significantly different at P < 0.05 according to Duncan's Multiple Range Test* ***(DMRT)***

According to Abayomi (1987), growth rate, number of green leaves per mother shoot, leaf area, plant height, stalk length and tiller density in sugarcane were significantly affected by nitrogen application but were not significantly affected by potassium.

In addition, cane tonnage was significantly affected by nitrogen but not by potassium. The interaction between nitrogen and potassium on the growth and yield of sugarcane was not statistically significant.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

1. From the results, it was clear that the tested leguminous plants improved soil nitrogen as indicated by the increment in soil nitrate-N content after their harvest. Crotalaria, *Sesbania sesban* and Yellow gram improved the soil nitrogen fertility most compared to other leguminous plants by fixing more Nitrogen. Yellow gram took longer period to improve nitrogen in the soil (90-120 days)
2. There were no significant differences in sugarcane performance (tillering and height) with or without application of starter phosphate or potassium fertilizer in soils previously under legume establishment.
3. The change in soil fertility status by legumes had a positive effect on the growth and development of sugarcane in terms of, height, tillering and harvestable fresh biomass.

5.2 Recommendations

1. Crotalaria, *Sesbania sesban* and Yellow gram as legumes are recommended for improvement of soil nitrogen in fertility depleted in sugarcane growing soils due to their good performance in N fixing and the fact that they offer multiple uses to farmers.
2. Small scale sugarcane farmers using leguminous plants to improve soil nitrogen should use little or no inorganic fertilizers (phosphate or potassium) if not deficient in the soil, as the fertilizers did not have significant difference on sugarcane performance when compared to legumes alone.

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APPENDICES

Appendix I: ANOVA for legume Nodules (Table 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Fertilizer	2	97.6	48.8	0.11	0.899
Legume	5	2046.7	409.3	0.89	0.497
Fertilizer.Legume	10	5636.4	563.6	1.23	0.307
Residual	36	16522.3	459		
Total	53	24303.1			

Appendix II: ANOVA: Height, August 2005 (Table 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Fertilizer	2	457.752	228.876	101.85	<.001
Legume	5	14.577	2.915	1.3	0.287
Fertilizer.Legume	10	37.009	3.701	1.65	0.133
Residual	36	80.901	2.247		
Total	53	590.239			

Appendix III: ANOVA: Height, November 2005 (Table 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Fertilizer	2	6.588	3.294	1.78	0.184
Legume	5	5.494	1.099	0.59	0.705
Fertilizer.Legume	10	9.192	0.919	0.5	0.881
Residual	36	66.705	1.853		
Total	53	87.98			

Appendix IV: ANOVA: Height, May 2006 (Table 6)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Fertilizer	2	444.264	222.132	86.53	<.001
Legume	5	33.702	6.74	2.63	0.04
Fertilizer.Legume	10	86.819	8.682	3.38	0.003
Residual	36	92.415	2.567		
Total	53	657.199			

Appendix V: ANOVA: Tillers, July 2005 (Table 7)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Fertilizer	2	197.904	98.952	46.16	<.001
Legume	5	6.865	1.373	0.64	0.67
Fertilizer.Legume	10	10.338	1.034	0.48	0.89
Residual	36	77.178	2.144		
Total	53	292.285			

Appendix VI: ANOVA: Tillers, September 2005 (Table 8)

Source of variation	**d.f.**	**s.s.**	**m.s.**	**v.r.**	**F pr.**
Fertilizer	2	92.4	46.2	0.11	0.599
Legume	5	3723.9	756.8	1.94	0.161
Fertilizer.Legume	10	4646.4	464.4	1.23	0.201
Residual	36	16322.2	453		
Total	53	23903.1			

Appendix VII: ANOVA: sugarcane population at harvest 2006 (Table 9)

Source of variation	**df**	**ss**	**Ms**	**v.r**	**Fpr**
Fertilizer	2	89.6	44.5	0.75	0.079
Legume	5	2010.7	402	0.89	0.495
legume.fertilizer	10	5604.7	560.4	1.04	0.437
Residual	36	16184	449.6		
Total	53	23808.8			

Appendix VIII: ANOVA: sugarcane fresh weight at harvest 2006 (Table 10)

Source of variation	**df**	**ss**	**ms**	**v.r**	**Fpr**
Fertilizer	2	72.4	36.2	1.1	0.12
Legume	5	2037.7	407.5	0.88	0.502
legume.fertilizer	10	5743.9	574.7	1.04	0.437
Residual	36	16592	460.9		
Total	53	24443.7			

Appendix IX: Guidelines for critical levels / ratings in soil test data

pH Levels and Nutrient ratings / Interpretations			
pH range	Rating	Interpretation	
> 8.5	Very high	Alkaline soil	
7.0 - 8.5	High	Alkaline to neutral	
5.5 - 7.0	Medium	Acid to neutral	
< 5.5	Low	Acid soils	
P	Ratings for exchangeable K, Mg and	P (ppm) Mehlich	
Ratings	K (me/100g)	Mg (me/100g)	P (ppm) Mehlich
Low	0.03 - 0.20	< 0.10	1 – 20
Medium	0.20 - 0.40	0.20 - 0.50	20 -40
High	0.40 - 0.80	> 0.50	> 40
Very high	> 0.80		
Ratings for C and N	% Organic C	% N Content	
Ratings			
Very low	< 0	< 0.1	
Low	(2-4)	0.1 – 0.2	
Medium	(4-10)	0.2 – 0.5	
High	(10-20)	0.5 – 1.0	
Very high	> 40	> 1.0	

Source: **Landon, L.R. 1991.** Brooker Tropical Soils Manual. A handbook for soil survey and agricultural land evaluation in the tropics and subtropics. John and Wiley and Sons, Inc. New York, U. S.A. pp. 474

Appendix X: General guidelines on the interpretation of soil N and C test results

Parameter	**Measured value**	**Rating**
Organic C (%)	> 3.0	High
	1.5 – 3.0	Moderate
	0.5 – 1.5	Low
	< 0.5	Very low
	> 0.25	High
Total N (%)	0.12 – 0.25	Moderate
	0.05 – 0.12	Low
	< 0.05	Very low

Source: **Tekalign, T., Hague, I., and Aduayi, E.A. (1991).** Soil, plant, water, fertilizer, animal manure and compost analysis manual. Plant Science Division Working Document 13, ILCA, Addis Ababa, Ethiopia.